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Development and evaluation of a fish-based index to assess biological integrity of Mediterranean streams

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ABSTRACT

1. A multimetric Index of Biotic Integrity (IBI) based on fish data was developed to assess the ecological status of Iberian streams, in the context of the European Water Framework Directive.
2. Fish assemblages were determined by electrofishing at 114 sites from 10 basins of the Júcar River Basin District. The sampled streams were typical Mediterranean streams with strong variation in flow, a species-poor and tolerant fish fauna, and low ecological specialization of the fish species. These features make it difficult to employ metrics based on species richness, trophic specialization, and reproductive strategy, which are typical of IBIs and similar indices.
3. The proposed IBI (IBI-Jucar) is composed of five metrics related to fish health, age-structure, and abundance and richness of native and alien species. IBI-Jucar was validated by demonstrating high correlation with various measures of environmental degradation and with several biotic and habitat indices. It was also highly correlated with the European Fish Index (EFI+), despite different methods used for development and contrasting metrics obtained.
4. The results underline the complementarity of different biotic indices and show that indices based on fish can be a valuable tool for determining environmental quality, even in species-poor Mediterranean streams.

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KEY WORDS: ecological status; ecosystem health; index of biotic integrity; Water Framework Directive

INTRODUCTION

Biological assessments are crucial tools for measuring the ecological integrity of freshwater ecosystems and for protecting aquatic life. The European Water Framework Directive (WFD) considers fish to be an essential biotic element for determining the ecological status of running waters (EC, 2000). Indeed, fish are sensitive indicators of the quality of stream habitat because they integrate multiple effects of degraded environments while acting as continuous monitors (Hellawell, 1978); in addition, fish are relatively easy to capture and identify, and they have long been used as indicators of stream ecosystem health (Karr, 1981; Fausch *et al.*, 1990; Angermeier and Davideanu, 2004). A widely used fish-based approach to assess fresh waters is the Index of Biological Integrity (IBI), first developed by Karr (1981) and Karr *et al.* (1986). It is based on the biotic integrity concept, referred to as 'the ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region' (Karr and Dudley, 1981). The IBI is a multimetric index that uses fish assemblage attributes as metrics to assess water quality (Karr, 1981) and ecological condition of streams and catchments (Moyle and Randall, 1998).

The original IBI was composed of 12 metrics that reflected fish species richness and composition, number and abundance of indicator species, trophic organization and function, reproductive behaviour, fish abundance, and condition of individual fish. Most fish-based indices are derived from the original IBI and are popular in the USA (Plafkin *et al.*, 1989; Fausch *et al.*, 1990) and Europe (Oberdorff and Hughes, 1992; Pont *et al.*, 2006, 2007). They use a series of metrics, based on

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assemblage structure and function, integrated into a numerical index scaled to reflect the 'health' of the assemblage. They also use the 'reference condition approach' (Hughes *et al.*, 1998; Bailey *et al.*, 2004), which involves comparing observed fish assemblages with a reference assemblage that is in natural or near-natural state. Although the fundamentals of the IBI are widely accepted, difficulties in developing indices for regions with species-poor, tolerant fish faunas have been noted (Moyle and Marchetti, 1999; Ferreira *et al.*, 2007). The development of an IBI for Mediterranean streams of the Iberian Peninsula thus presents a number of potential problems.

First, an IBI relies on the existence of undisturbed (reference) sites yet stream ecosystems on the Iberian Peninsula have been modified for hundreds if not thousands of years by impoundment, pollution, flow regulation and water abstraction, agricultural and urban development, and introduction of alien species (Elvira, 1995; García de Jalón, 2006; Lacorte *et al.*, 2006; Berzas *et al.*, 2009). Not only are undisturbed sites unlikely to exist but the rate of stream modification has been accelerating in recent decades. For example, the distribution of most native fishes has decreased by more than 50% during the last 100 years in Catalonia, NE Spain (Aparicio *et al.*, 2000). While the 'least disturbed' or 'best available' sites are sometimes used as alternatives to reference sites (Whittier *et al.*, 2006), the WFD requires pristine or near-pristine reference sites (Schmutz *et al.*, 2007). However, it is almost impossible to find stream reaches where native fish assemblages have not been altered.

A second complication for developing a Mediterranean IBI is that the streams naturally support few fish species, most endemic to a few river basins (Almaça, 1995; Doadrio, 2001). In the Iberian Peninsula, most stream reaches typically have a fish assemblage of four or fewer native species (Doadrio, 2001; Ferreira *et al.*, 2007). In contrast, stream reaches where the IBI was originally developed contained 40–50 native species (Karr *et al.*, 1986). This situation makes developing Mediterranean fish-based indices a challenging task (Moyle and Randall, 1998; Moyle and Marchetti, 1999; Ferreira *et al.*, 2007).

A third difficulty is that many IBI metrics rely on classifying species by trophic, habitat, tolerance, and reproductive guilds. However, relatively little is known about the ecology of many fishes in Mediterranean areas, especially the Iberian Peninsula (Doadrio, 2001; Maceda-Veiga and de Sostoa, 2011), and generalizations about their ecology may be confounded by flexible life histories (Wootton, 1990). Mediterranean streams undergo major inter-annual and inter-seasonal flow variations (Gasith and Resh, 1999) and in this harsh environment few fishes show strong habitat or trophic specializations (Poff and Allan, 1995). Most Iberian freshwater fishes are invertivores or omnivores, have wide tolerances to abiotic variability, and are habitat and feeding generalists, well adapted to survive in changing environments (Magalhães *et al.*, 2002). Although several guild classifications exist (Balon, 1975; Welcomme *et al.*, 2006; Noble *et al.*, 2007), standard criteria for guild delineation and selection of appropriate guilds are lacking (Kwak and Peterson, 2007). Maceda-Veiga and de Sostoa (2011) recently provided new tolerance indicator values for fish in NE Spain but many of the species studied are not present in other parts of the Iberian Peninsula, including the area studied in this paper.

Overall, these problems suggest that while a fish-based index can be a useful tool for evaluating stream conditions, each region needs to have a customized index. Each index has to take into account not only local conditions and history but the rationale of the IBI concept. The first IBI index in Spain (IBICAT), developed to assess streams in Catalonia (Sostoa *et al.*, 2003), has been shown to have poor correlation with other biotic indices and inconsistencies among river basins because of a combination of the problems mentioned above (Benejam *et al.*, 2008). Two indices have been developed for the Guadiana basin (Magalhães *et al.*, 2008; Hermoso *et al.*, 2010), which has different species composition and higher richness than the other Mediterranean catchments in the Iberian peninsula (Doadrio, 2001), making it difficult to adapt them to other regions. Another index used in Iberian streams is the European Fish Index (EFI), developed within the FAME project (FAME Consortium, 2004). The EFI is a standardized fish-based assessment method applicable across a wide range of European streams (Pont *et al.*, 2006, 2007). This index is based on a predictive model that derives reference conditions from abiotic environmental descriptors of individual sites, and then quantifies on a statistical basis the deviation of the observed fish assemblage structure from these reference conditions. The metrics used are based on species guilds describing the main ecological and biological characteristics of the fish community. Because of several limitations observed in the performance of the index, a new version (EFI+) was developed to improve performance in Mediterranean ecoregions (EFI+ Consortium, 2009).

The objectives of this study are: (i) to propose a new fish index that is not based on a reference condition for selecting metrics, which does not use metrics based on ecological preferences of species (e.g. guilds), and is capable of assessing assemblages with low species richness, and (ii) to compare the index with the EFI+ and other biotic indices. First, metrics were developed based on different levels of ecological organization to evaluate Iberian streams, and then the index was applied to assess the streams in the Júcar River Basin District (SE Spain).

METHODS

Study area

The Júcar River Basin District (JRBD) comprises an area of 42 989 km^2 in the eastern Iberian Peninsula and consists of 10 basins draining to the Mediterranean Sea (Figure 1). The largest stream of this region is the Júcar River, with a drainage area of 21 600 km^2, a length of *ca* 500 km, and an average flow of 43 m^3 s^{-1}. The average annual precipitation is about 500 mm yr^{-1}, ranging from 200 mm in the south to 1000 mm in the NW mountain ranges (Ninyerola *et al.*, 2005). The climate is Mediterranean, with annual rainfall varying markedly from year to year but following a predictable seasonal pattern, with dry summers and rain precipitation mainly in the spring and autumn. Streams of this region have high seasonal and annual flow variation with severe droughts and floods. The highest flows occur during the wet autumn and spring months and the lowest towards the end of the dry summer months (Robles *et al.*, 2002).

Human population within the JBRD is about 5.16 million inhabitants (year 2009; INE, 2010), concentrated mainly in the coastal areas. Agriculture, livestock, and industry are the main economic activities (Robles *et al.*, 2002). The region is

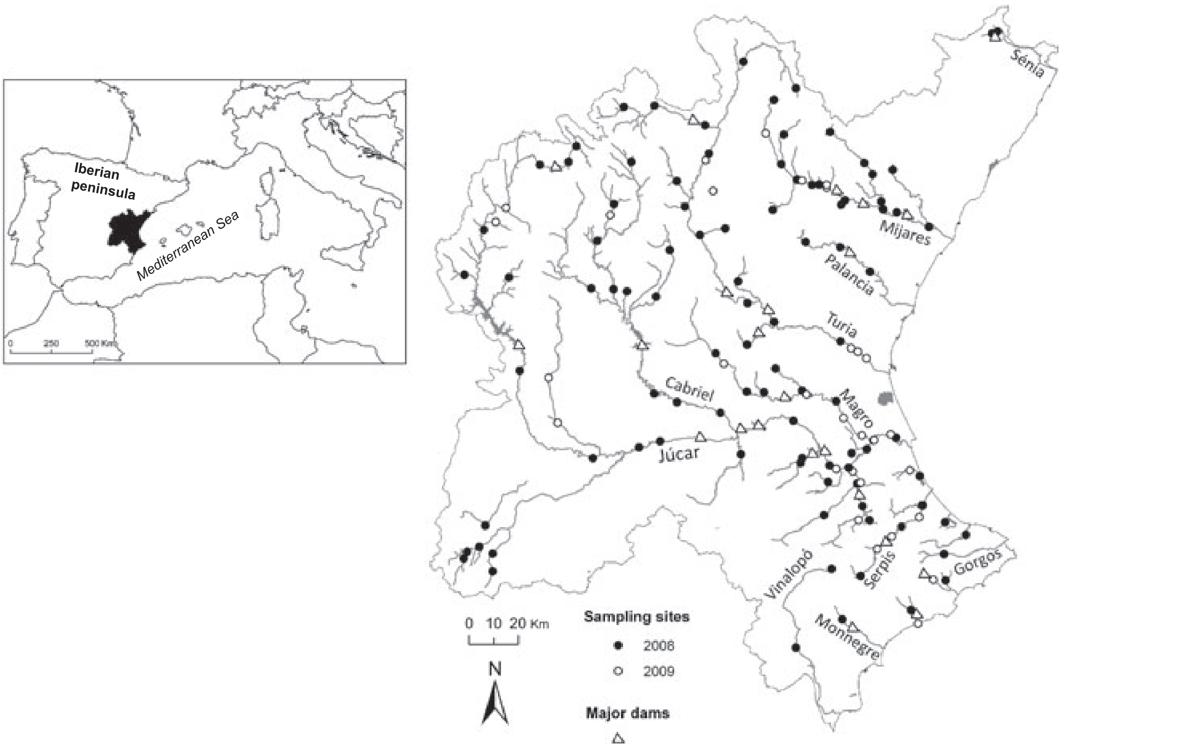

Figure 1. Location of the Júcar River Basin District and the sampling sites ($n = 114$) used for the development of the Index of Biotic Integrity.

under increasing pressure for exploitation of its water resources. The natural flow regimes have been altered considerably through construction of dams and other channel modifications (Sánchez-Navarro *et al.*, 2007). Major streams (i.e. Júcar, Cabriel, Turia, and Mijares) are strongly regulated from their middle reaches to the mouth, although upper parts of their catchments and small tributaries are less affected by water retention structures. In regulated streams, flows below dams are lower than historic flows in winter when reservoirs store water and higher during summer when water is released from the dams for irrigation. Pollution mainly affects the middle and lower reaches of the main streams owing to industrial and urban wastewater discharge. More information on the basin is available elsewhere (http://www.chj.es).

Data sources and sampling protocols

Data on fish assemblages, environmental characteristics and human impacts were collected from 114 sites in the JRBD (Figure 1). Environmental characteristics of the sampling sites are shown in Table 1. Fish were sampled by electrofishing following the CEN 14011 standard protocol (CEN, 2003). A single upstream pass was made and block nets were not used to enclose the sampling area. Sites were electrofished for transects of about 50–200 m in length (5–50 channel widths), which included all geomorphic channel units present in the reach (i.e. riffles, pools, and runs). Fish were identified to species, measured (fork length, mm), weighed (g), examined for disease and anomalies, and then released at the same location. All fish sampling was conducted during summer base-flow conditions from June to September of 2008 and 2009.

Several variables were used to characterize reach level habitat and human disturbance at each site. Data were collected either *in situ* or from national geographical information databases. Reach-level habitat evaluation was conducted using the Rapid Bioassessment Protocol (HABITAT-EPA), which rates 10 habitat parameters on substrate composition, channel morphology, instream cover, riparian conditions and bank erosion to derive a station habitat score (Barbour *et al.*, 1999). Two disturbance variables (land-use change and flow regulation) were used to estimate the extent of human influence on the catchment. The CORINE Land Cover database (available at http://www.eea.europa.eu) was used to quantify land-use variables in a Geographic Information System (ArcGIS 9.2, ESRI, Redlands, California). Land use was categorized as urbanized areas, including urban and industrial units (%ARTIFICIAL), agricultural areas (%AGRICULTURE), and forested areas (%FOREST), and then the percentages of each category were calculated within the drainage basin immediately upstream. To estimate the extent of alteration of the natural flow regime by impoundment, each site was classified as either regulated or non-regulated, based on upstream reservoir capacity and inflows from unregulated tributary streams in the stretch between the dam and the site.

Data from biotic and abiotic indices routinely applied for ecological monitoring in the Iberian Peninsula were gathered to be compared with the new fish index. The indices compared were: the Riparian vegetation quality index, QBR (Munné *et al.*, 2003); the Fluvial habitat index, IHF (Pardo *et al.*, 2002); two indices based on diatoms, IPS (Specific Pollution Sensitivity index; CEMAGREF, 1982) and IBD (Diatom Biological Index; Prygiel and Coste, 1993), and a macroinvertebrate index, IBMWP (Iberian Biological Monitoring Working Party, Alba-Tercedor *et al.*, 2002). Data for these indices during 2007–2009 were obtained from the JRBD's management authority (Confederación Hidrográfica del Júcar) for the same

Table 1. Descriptive statistics of the environmental characteristics of sampling sites ($n = 114$)

Variable	Mean	Median	Standard deviation	Range
Stream width (m)	6.8	5.1	7.4	1.8–60
Water depth (cm)	39	36	16	11–102
River slope (m km^{-1})	8.9	6.2	7.8	0.2–41.4
Elevation (m)	528	530	387	10–1360
Pool (%)	36.2	30.0	27.2	0–100
Run (%)	39.3	37.5	25.0	0–100
Riffle (%)	23.5	21.4	18.1	0–80
Boulders (%)	22.3	16.5	22.3	0–88
Cobble (%)	35.1	34.3	23.6	0–83
Gravel (%)	12.2	6.6	15.4	0–57
Sand (%)	5.2	0.0	8.2	0–34
Silt (%)	20.0	14.1	21.4	0–100
pH	8.0	8.1	0.4	6.3–9.0
Conductivity (μS cm^{-1})	951	852	427	140–2510

sites where fish sampling was conducted. For sites sampled more than once in this period, mean values of the indices were calculated.

The European Fish Index (EFI+) was also computed for the 114 fish sampling sites, using the software available at http://efi-plus.boku.ac.at. EFI+ consists of two different fish metrics that vary with river type (salmonid and cyprinid river types) and ranges between 0 and 1. The EFI+ software requires as input data: site location (ecoregion and river region) and 12 environmental variables (geology, sediment size, altitude, flow regime, lakes, upstream drainage area, air temperature, river slope, distance from source, wetted width, sampling strategy and method, and fished area) to predict the reference values for the fish assemblage. Environmental variables needed for EFI+ calculation were obtained from topographical maps and GIS databases. All study sites were classified by the EFI+ software as ‘cyprinid river type’. Twelve high elevation sites dominated by brown trout (*Salmo trutta*) were reclassified as ‘salmonid river type’, but this had little affect on the results. The cyprinid type uses the metrics ‘richness of rheophilic spawning species’ and ‘density of lithophilic spawning species’. The index for the salmonid type is composed of two metrics: density of species intolerant to oxygen depletion and density of fish ≤150 mm (total length) of species intolerant to habitat degradation. A congeneric surrogate species was used to input data for an endemic species (*Squalius valentinus*) not considered in the EFI+ software.

Development of the IBI

Development of an IBI typically begins with selection of metrics that are likely to differ among disturbed and undisturbed/reference sites. To overcome the need for reference sites when they are not available, an alternative is to construct a set of metrics from expert knowledge that use recognized and accepted interpretations of ecological conditions and ecosystem health (Maitland, 2004; Scardi *et al.*, 2008).

The WFD requires determining the ecological status of streams by using abundance, composition, and age structure of fish assemblages; it was thus considered appropriate to develop a set of metrics following the definition of fish status of Moyle *et al.* (1998). They established three levels of fish ‘health’ (individual, population, and community), each of which adds different information. At the individual level, a healthy fish should have a robust body free of diseases and lesions and reasonable growth rates for the region. Possible metrics for this level are condition (weight–length relationship), percentage of anomalies, and growth rate. Condition and growth rate were not used because they are rarely available from routine biomonitoring, although they could be included in the index when available. At the population level, positive fish status is defined by presence of multiple age classes (evidence of reproduction) and viable population size. Metrics for this level could be the number of age classes and abundance (catch-per-unit-effort or density). Finally, at the community level, the fish assemblage should be dominated by native species, be resilient to extreme environmental events and have persistent composition through time. Possible metrics are loss of native species, number and abundance of alien species, and a measure of similarity of the assemblage at a particular site at different dates. Although the similarity measure could be valuable to test for resilience and persistence of fish assemblages, temporal data are rarely available in Spain and elsewhere, so this metric was not used.

The new fish index (IBI-Jucar) is based on five metrics and is intended to be an indicator of the degree to which Iberian streams have had their capacity to support native fishes reduced, through flow regime and habitat alteration, pollution, and invasions of alien species. It was recognized that the Iberian landscape has been altered by human activity for thousands of years, but the widespread decline of native fishes reflects more recent, accelerated processes of degradation. It was assumed that minimal human disturbance is reflected in an abundant and diverse native fish fauna with complex age-structures and absence of alien species (Kennard *et al.*, 2005). Degraded conditions are reflected in lower native species richness and abundance, with most age classes missing, and high abundance of alien species.

IBI Metrics

Percentage of individuals with anomalies (DELT)

This metric assumes that fish are more likely to develop deformities, eroded fins, lesions, and tumours (DELT anomalies) in degraded conditions, mainly when chronically exposed to stressful conditions (e.g. high temperatures), low water quality, contaminants, or pathogens (Sanders *et al.*, 1999; Benejam *et al.*, 2010a). Parasitic infestations were not included because they are often unrelated to water quality (Simon and Lyons, 1995). This metric was calculated as percentage of total fish captured with evident anomalies.

Age structure of native fish populations (SIZE_CLASS)

The presence of multiple age-classes reflects the persistence of favourable conditions over the years and overall high habitat quality (Munkittrick and Dixon, 1989; Torralva *et al.*, 1997). Age determination is methodologically complex and time consuming, so the number of cohorts in samples was estimated from length–frequency analysis. Five expected size classes were set: fry, juveniles, and three classes of older fish (Table 3). The length groups chosen for the analysis were based on size and longevity data for each species (Doadrio, 2001). This metric was calculated as the number of size classes present for each species at a given site. The metric score for the whole water body was the mean of scores for all native species.

Abundance of native fishes (CPUE)

High abundance of native fish is a good indicator of high-quality water and unaltered stream habitats (Paller *et al.*, 1996; Gafny *et al.*, 2000). This metric was measured as catch-per-unit-effort (CPUE). Catch data among sites were standardized by using the area electrofished (number of fish per hectare). Because multiple-pass electrofishing for quantitative population estimates was not conducted, differential capture probabilities among sites could result in biased abundance data. However, one-pass electrofishing has been shown to be highly correlated with population estimates from multiple-pass depletion sampling (Lobón-Cerviá and Utrilla, 1993; Reid *et al.*, 2009), so it was assumed that the level of accuracy required to detect differences in abundance was achieved. High variability in abundance of young-of-year could disrupt assessment of abundance (Angermeier and Karr, 1986), thus only fish longer than 40 mm FL were used to compute CPUE. This metric was scored independently for each native species at each site and averaged for the final score of the site.

Loss of native species (LOSS_NATIVE)

Loss of native species was assessed by comparing expected ('theoretical') assemblages in the absence of human impact with actual ('sampled') assemblages, based on presence/absence data. Some indices rely on this metric alone to assess ecological status (Kennard *et al.*, 2006; Hermoso *et al.*, 2010). The expected assemblage of fishes can be determined through several approaches. For example, logistic regression models (Oberdorff *et al.*, 2001) and artificial neural networks (Joy and Death, 2004) have been used to predict fish assemblages as a function of one or more independent environmental variables. All these procedures require large datasets to construct the model, including data from a number of reference sites, not available in the JRBD. Hence, a simpler approach was chosen based on the concept of environmental filters (Poff, 1997; Chessman, 2006). In this conceptual model, environmental factors operating at a wide range of scales successively exclude a proportion of a regional species pool, leaving a residual local assemblage to occupy a particular site. This model may be harder to use in regions with a high number of species but seems reliable for streams with low species richness such as Iberian streams. The environmental filters were used to include each species in the expected assemblage of a site if: (i) the species was native to the basin; (ii) the altitude of the site was within the altitudinal range of the species in the region; and (iii) the stream habitat was within the types favoured by the species based on published information and the authors' experiences. Rare species were only included in the expected assemblage if historical records indicated their presence in the specific stream reach. The percentage loss of native species was calculated as the number of species missing at a site divided by the expected total number species, expressed as a percentage.

Alien fish pressure (ALIEN_FISH)

This metric was intended to reflect the number of alien species established as well as the proportional abundance of alien individuals in relation to native fish. Therefore, the percentage of alien species and the percentage abundance of alien individuals were averaged for each site.

Metric scoring

Each metric was scored on a continuous scale from 0 to 10 except for DELT anomalies (Minns *et al.*, 1994; Hughes *et al.*, 1998). For positive metrics, i.e. those that decrease as disturbance increases (SIZE_CLASS, CPUE), minimum values were given a score of 0 and maximum values were given a score of 10, and intermediate metric scores were interpolated linearly. Negative metrics (ALIEN_FISH, LOSS_NATIVE) were scored similarly, with the minimum and maximum values reversed. Minimum and maximum values for CPUE metrics were defined as the 5th and 95th percentile values observed for each individual species in all sites. For species that showed variation in catch-per-unit-effort with catchment area, the 5th and 95th percentile values to score the CPUE metric were set according to the maximum density line approach (MDL; Miller *et al.*, 1988), using quantile regressions (Cade and Noon, 2003). DELT anomalies were incorporated within the index following Lyons (2006). Points were subtracted if sufficient individuals with anomalies (DELT) were found, but the lack of such individuals did not add points to the overall index score. Because of the scarcity of data on fish health in the Iberian Peninsula, guidelines for scoring this metric were based on IBIs developed in North America (Ohio EPA, 1987; Fausch *et al.*, 1990). The metric was scored as follows: frequency of affected fish >6%, score = 10; >4–6%, score = 7.5; >2–4%, score = 5; 0.5–2%, score = 2.5; <0.5%, score = 0. Scores of zero for this metric were not considered for the statistical analyses. The score obtained for DELT anomalies was subtracted from the sum of scores of all the other metrics. Finally, scored metrics were summed and then scaled as necessary to produce an index with scores ranging from 0 (worst) to 100 (best). As a guide to interpreting the final scores, the scoring classes for environmental quality were: <20, bad condition; 20-40, poor condition; >40–60, fair condition; >60–80, good condition; and >80, very good condition.

Index validation and relative contribution of metrics

A biotic index must have a demonstrable empirical relationship with environmental perturbation to be meaningful (Kwak and Peterson, 2007). Validation of IBI scores was obtained by relating the scores to measures of human disturbance and other biotic indices. Bivariate relationships were analysed using Spearman's correlation coefficient, which are Pearson's correlations based on ranks; they have the advantage of being adequate to describe any monotonous relationship and not assuming bivariate normality. Large correlation tables were adjusted for multiple comparisons with Holm's sequential method. Smoothing curves (LOESS) were used to describe the nonlinear relationships in scatterplots. Principal component analysis (PCA) was used to examine relationships among biotic indices. All statistical analyses were performed using the software R version 2.11.0 (R Development Core Team, 2010).

To evaluate the relative contribution of each metric to IBI scores, a reduced index was calculated by removing sequentially one metric from the full IBI, and calculating the percentage difference between each reduced index and the overall index (Minns *et al.*, 1994). Because DELT was incorporated within the index in a different manner from the other metrics, it was not included in this analysis. If all the other four metrics contributed uniformly, the differences would be 25%.

RESULTS

Fish assemblages

In total, 22 species from nine families were collected from JRBD streams. Of these species, 10 (45%) were alien or translocated (Table 2). At the basin scale, richness of native fishes ranged from one species in small basins to nine species in the largest (Júcar). The highest richness of native species per site was six species with a mean of 1.8 species (SD = 1.1), whereas for alien species it was five species with a mean of 1.0 species (SD = 1.2). Fish abundance (CPUE, fish ha^{-1}) varied greatly among sites; the mean for native fish per site was 1295 fish ha^{-1} (SD = 1885; range 0–12 500 fish ha^{-1}) whereas for alien fish the mean was 601 fish ha^{-1} (SD = 1406; range 0–8392 fish ha^{-1}). All streams had a rather similar longitudinal assemblage structure dominated by three widespread and abundant species: brown trout (*Salmo trutta*) in upstream sections, and eastern Iberian barbel (*Barbus guiraonis*) and eastern Iberian chub (*Squalius valentinus*) in middle and lower sections. The most frequent alien fishes were the Pyrenean gudgeon (*Gobio lozanoi*), an Iberian native translocated to the study area, and bleak (*Alburnus alburnus*) (Table 2).

Fish metrics and biotic indices

The 5th and 95th percentile values used to score the fish abundance metric (CPUE) and length-class intervals used to score age-structure metrics (SIZE_CLASS) are shown in Table 3. CPUE data for genera *Parachondrostoma* and *Squalius* were pooled because of the low occurrence of some species. Fish density of *B. guiraonis* and *Squalius* spp. showed variation with catchment area. For both taxa, density decreased as catchment area increased. Therefore, the 5th and 95th percentile values of CPUE at a site were calculated using linear equations obtained by quantile regression of CPUE as a function of catchment area, both $\log_{10}$-transformed, and then back-transforming the dependent variable using anti-logarithms (Figure 2; Table 3). The predicted species richness used to score the loss of native species (LOSS_NATIVE) is shown in Figure 3.

Metrics with the highest mean scores were percentage loss of native species (LOSS_NATIVE) and alien fish percentage (ALIEN_FISH, Table 4), which also accounted for the largest differences between the full IBI and reduced indices (LOSS_NATIVE, 32%; ALIEN_FISH, 30%). CPUE showed the

Table 2. Fish species found at Júcar Basin Water District sites ($n = 114$), percentages of occurrence and abundance, and CPUE statistics. Alien fish refers to species not native to Júcar streams

Species name	Common name	Family	n	% occurrence	% abundance	CPUE (fish ha^{-1})		
						Mean	SD	Range
Native fish								
Anguilla anguilla	European eel	Anguillidae	37	13.8	0.8	76.7	64.5	10–278
Achondrostoma arcasii	Bermejuela	Cyprinidae	125	8.9	2.5	1013.0	2101.6	27–7500
Barbus guiraonis	Eastern Iberian barbel	Cyprinidae	1016	47.2	20.7	728.5	832.0	17–3571
Barbus haasi	Iberian redfin barbel	Cyprinidae	131	12.2	2.7	276.1	280.6	14–1133
Cobitis paludica	Southern Iberian spined-loach	Cobitidae	43	5.7	0.9	390.7	424.1	27–1389
Parachondrostoma arrigonis	Júcar nase	Cyprinidae	7	1.6	0.1	427.5	405.9	22–833
Parachondrostoma miegii	Ebro nase	Cyprinidae	16	0.8	0.3	539	-	539
Parachondrostoma turiense	Turia nase	Cyprinidae	43	5.7	0.9	205.4	347.1	26–1055
Salaria fluviatilis	Freshwater blenny	Blenniidae	36	4.9	0.7	118.3	65.4	50–174
Salmo trutta	Brown trout	Salmonidae	453	29.3	9.2	468.3	627.6	14–3174
Squalius pyrenaicus	Southern Iberian chub	Cyprinidae	36	1.6	0.7	474.2	200.6	274–675
Squalius valentinus	Eastern Iberian chub	Cyprinidae	1441	42.3	29.3	1412.9	1789.4	34–8929
Alien fish								
Alburnus alburnus	Bleak	Cyprinidae	807	22.0	16.4	1541.6	1779.4	5–6532
Carassius auratus	Goldfish	Cyprinidae	59	8.1	1.2	289.8	272.7	22–854
Cyprinus carpio	Common carp	Cyprinidae	41	12.2	0.8	120.3	148.5	20–653
Esox lucius	Northern pike	Esocidae	1	0.8	0.0	14	-	14
Gambusia holbrooki	Eastern mosquitofish	Poeciliidae	56	7.3	1.1	401.1	419.8	14–1444
Gobio lozanoi	Pyrenean gudgeon	Cyprinidae	315	22.8	6.4	679.7	1458.7	10–7333
Lepomis gibbosus	Pumpkinseed	Centrarchidae	67	7.3	1.4	133.4	153.7	37–507
Micropterus salmoides	Largemouth bass	Centrarchidae	109	12.2	2.2	264.6	387.6	12–1566
Oncorhynchus mykiss	Rainbow trout	Salmonidae	45	6.5	0.9	177.8	299.3	10–946
Pseudochondrostoma polylepis	Iberian straight-mouth nase	Cyprinidae	26	0.8	0.5	647	-	647

Table 3. Percentile CPUEs and size class intervals used to score the CPUE and SIZE_CLASS metrics. CPUE data for genera *Parachondrostoma* and *Squalius* were pooled because of the low occurrence of some species. Percentile values of CPUE (y) for *Barbus guiraonis* and *Squalius* spp. were calculated as a function of $\log_{10}$-catchment area (x), and then back-transforming the dependent variable using anti-logarithms

Species	5th percentile CPUE	95th percentile CPUE	Size class interval (mm)				
	(fish ha^{-1})	(fish ha^{-1})	1	2	3	4	5
Anguilla anguilla	10.6	176.2	-	-	-	-	-
Achondrostoma arcasii	24.9	4553.6	≤ 50	50–70	70–90	90–100	>100
Barbus guiraonis	$y = 2.77–0.37x$	$y = 3.69–0.11x$	≤ 100	100–200	200–280	280–360	>360
Barbus haasi	16.2	354.5	≤ 60	60–90	90–120	120–150	>150
Cobitis paludica	65.5	1092.6	≤ 50	50–70	70–90	90–100	>100
Parachondrostoma arrigonis							
Parachondrostoma miegii							
Parachondrostoma turiense	23.4	424.4	≤ 60	60–90	90–120	120–150	>150
Salaria fluviatilis	51.4	213.7	≤ 50	50–70	70–90	90–100	>100
Salmo trutta	16.5	703.9	≤ 80	80–150	150–200	200–240	>240
Squalius pyrenaicus							
Squalius valentinus	$y = 1.83 + 0.01x$	$y = 4.37–0.30x$	≤ 60	60–90	90–120	120–140	>140

lowest contribution to the overall index score (average of differences between full IBI and reduced index = 18%). The mean IBI score for all sites was 62.8, which lies at the lower end of the 'good condition' category of the proposed score classification. The other biotic and habitat indices followed a similar tendency, with mean values located in the fair to good condition ranges (Table 4).

All fish metrics were significantly correlated with one another except for the DELT metric. DELT scores were calculated only when fish with abnormalities were present

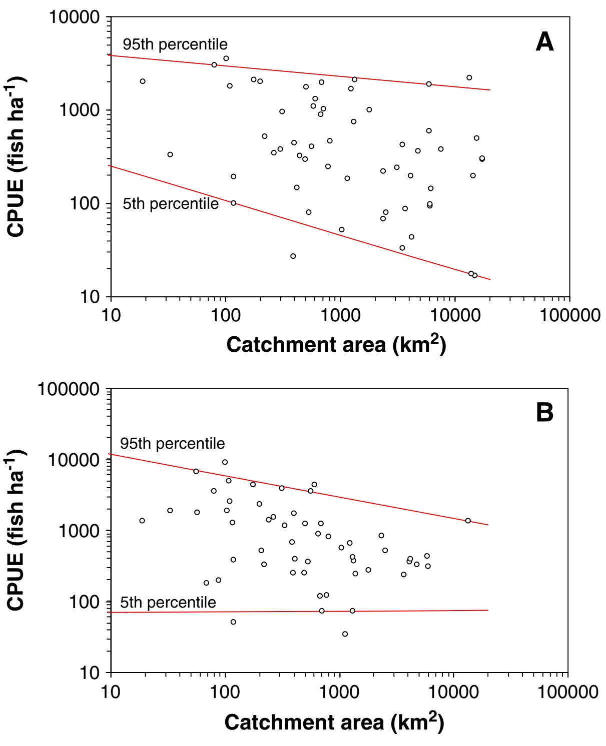

Figure 2. Catch per unit effort of (A) *Barbus guiraonis* and (B) *Squalius* spp. (*S. valentinus* and *S. pyrenaicus* pooled) by catchment area, showing quantile regressions of 5th and 95th percentiles.

($n = 13$) so there are 101 missing values (Table 5). The sign of these fish metrics agreed with the expected response: e.g. sites that have lost more species (high LOSS_NATIVE) had more introduced fish (high ALIEN_FISH) and lower native fish abundance (low CPUE). The metric most correlated with other metrics was LOSS_NATIVE, and the least correlated metric was SIZE_CLASS.

IBI-Jucar scores declined with increased catchment disturbance and were negatively correlated with percentage of agricultural and artificial land-use and positively correlated with the percentage of forest land (Table 6, Figure 4). IBI-Jucar scores were also affected by hydrological alteration: sites strongly regulated yielded significantly lower IBI-Jucar scores than unregulated ones (ANCOVA, $F_{1,\ 109} = 15.2$; $P = 0.0002$), after accounting for differential land-use ($F_{1,\ 109} = 99.5$; $P < 0.0001$) (Figure 4).

All biotic indices were significantly correlated, even after adjusting for multiple comparisons (Table 6). Some of the relationships were clearly nonlinear (Figure 5), with diatom indices having a convex relationship with the rest of the indices (i.e. the average value changed little when other indices were still increasing) and IBMWP having a more concave relationship. The fish indices (EFI+ and IBI-Jucar) were more tightly correlated with habitat indices (IHF, HABITAT_EPA, %FOREST) than with diatom indices (IPS and IBD). The IBMWP was correlated with both habitat and diatom indices (Table 6). EFI+ and IBI-Jucar were highly correlated ($r_S = 0.73$) but the former was slightly better correlated with diatom indices and local habitat indices (IHF, HABITAT_EPA) while IBI-Jucar was better correlated with IBMWP and the landscape indicator (%FOREST). A PCA summarizes these results and the first axis explains most of the variation (56%, eigenvalue = 5.07) demonstrating that it measures overall ecological status and that that all indices are correlated (Figure 6). The diatom indices, particularly the IBD, were less related to the rest and were distinguished by the second axis, which explained 11% of the variation (eigenvalue = 1.01). The PCA also shows that IBMWP is slightly more correlated than other indices with diatom indices, and that biotic indices, except the diatom indices, are well correlated with the habitat indices.

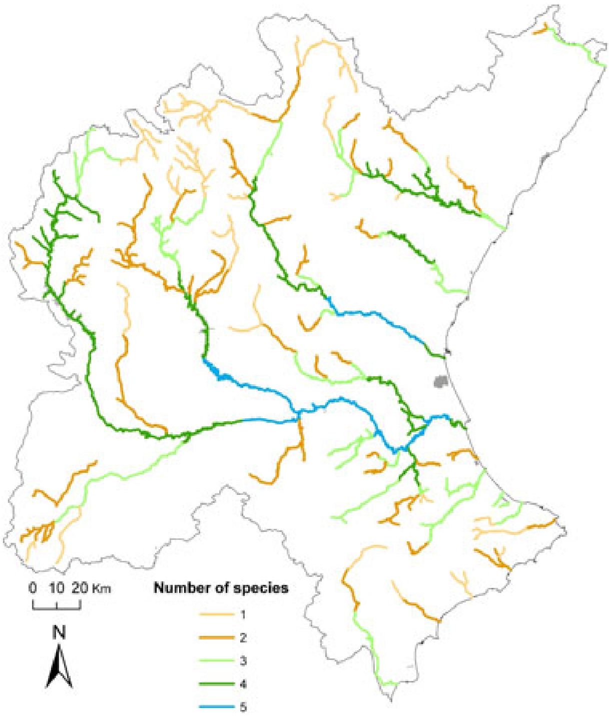

Figure 3. Map showing the predicted fish species richness for the streams of the Júcar River Basin District used to score the LOSS_NATIVE metric.

Biotic integrity of the Júcar streams

IBI-Jucar scores ranged from 0 to 100, with a median of 69. IBI-Jucar classes showed that most of the sites (55%) were below good condition, and overall 27% had a deficient or poor condition. High IBI-Jucar scores were usually associated with the upper reaches and some tributaries of the Mijares, Palancia, Turia, and Júcar streams. Several coastal streams of the southern part also had good values. Low IBI-Jucar scores were mostly linked to middle and lower mainstem reaches affected by regulation, highly altered channels, or close proximity to urban areas (Figure 7).

DISCUSSION

Justification of IBI-Jucar

The lack of suitable reference conditions poses a problem in the development of indices of biotic integrity in regions with a long legacy of human modification. The alternative approach used in developing the IBI-Jucar, which included the selection of metrics based on sound ecological principles, proved to be clearly useful for measuring stream integrity. First, IBI-Jucar was significantly correlated with measures of habitat quality and of human disturbance, such as land use and flow regulation. Land use is linked to biotic integrity of streams by influencing nutrient input, sediment input, hydrology, and channel characteristics (Roth *et al.*, 1996; Allan *et al.*, 1997). Impoundment alters flow regimes, which disrupts the biological performance of native species in many ways, such as changing reproductive cycles, recruitment, and abundance (Freeman *et al.*, 2001; Lytle and Poff, 2004). This leads to declines of populations and reductions in distribution (Aparicio *et al.*, 2000; Clavero *et al.*, 2004). Second, IBI-Jucar is strongly correlated with other biotic indices that have been extensively validated (see Benejam *et al.*, 2008 and references therein), in particular macroinvertebrate indices and the EFI+. Finally, all the IBI-Jucar metrics (except DELT) were significantly correlated with each other, despite being measurements of quite different aspects of fish assemblages, such as species composition (LOSS_NATIVE), abundance (CPUE), size structure (SIZE_CLASS), and dominance of exotic species (ALIEN_FISH). The loss of native species (LOSS_NATIVE) and invasion by exotics (ALIEN_FISH) have been widely recognized as one of the main consequences of degradation of aquatic ecosystems in the Iberian Peninsula (Godinho and Ferreira, 1998; Aparicio *et al.*, 2000; Doadrio, 2001; Hermoso *et al.*, 2011). These two metrics had the greatest influence on the IBI-Jucar scores. Abundance (CPUE) and age structure (SIZE_CLASS) of native fish were more variable among sites but also contributed significantly to final IBI-Jucar scores, because they are affected by a number of human factors. For example, substrate alteration and siltation may affect survival of fry or preclude reproduction, reducing abundance and

Table 4. Descriptive statistics of land-use variables, biotic indices and fish metrics. The statistics of DELT scores do not include samples with no fish anomalies (zero scores)

Variable	Abbreviation	Mean	Median	Standard deviation	Range
Land use					
% catchment forested	%FOREST	57.4	62.8	27.4	0.04–96
% catchment in agriculture	%AGRICULTURE	38.2	37.0	23.5	1–95
% catchment urbanized	%ARTIFICIAL	3.2	0.6	6.7	0–48
Biotic indices					
Fluvial habitat index	IHF	65.1	64.7	9.4	39–87
EPA's Rapid Bioassessment Protocols (Habitat)	HABITAT_EPA	121.3	125.0	31.1	32–177
Riparian Vegetation Quality Index	QBR	64.8	68.3	26.2	8–100
Specific Pollution Sensitivity Index (Diatom)	IPS	15.3	15.6	2.2	9.5–18.8
Diatom Biological Index	IBD	15.7	16.1	1.8	10.7–18.9
Iberian Biological Monitoring Working Party (Macroinvertebrate)	IBMWP	118.8	110.0	53.7	21–245
European Fish Index	EFI+	0.63	0.67	0.21	0.05–1
IBI-Jucar metrics and total					
Presence of individuals with anomalies	DELT	4.8	5.0	2.8	2.5–10
Age (size) structure of native fishes	SIZE_CLASS	5.6	6.0	2.8	0–10
Abundance of native fishes	CPUE	4.9	4.3	3.6	0–10
Loss of native species	LOSS_NATIVE	7.9	10.0	2.9	0–10
Alien fish pressure	ALIEN_FISH	7.4	8.2	3.1	0–10
Index of Biotic Integrity	IBI-Jucar	62.8	68.4	26.0	0–100

Table 5. Correlation matrix (Spearman's coefficient below the diagonal and P values above the diagonal) of fish metrics used in IBI-Jucar. $n = 114$ except for correlations involving DELT, where $n = 13$

	ALIEN_FISH	DELT	SIZE_CLASS	CPUE	LOSS_NATIVE
ALIEN_FISH	—	0.060	0.0006	***	***
DELT	−0.53	—	0.314	0.499	0.081
SIZE_CLASS	−0.32	0.30	—	***	***
CPUE	−0.48	0.21	0.36	—	***
LOSS_NATIVE	−0.66	0.50	0.38	0.59	—

***indicates $P < 0.00005$.
Abbreviations are in Table 4.

Table 6. Correlation matrix (Spearman's coefficient below the diagonal and P values adjusted with Holm's method above the diagonal) of biotic indices in the Júcar streams. $n = 102$–114 (some variables had missing values)

	IPS	IBD	IBMWP	QBR	IHF	HABITAT_EPA	%FOREST	EFI+	IBI-Jucar
IPS	—	***	***	***	***	0.0003	***	***	0.0009
IBD	0.48	—	***	0.029	0.0002	0.014	0.0004	0.0016	0.0018
IBMWP	0.60	0.43	—	***	***	***	***	***	***
QBR	0.44	0.22	0.56	—	***	***	***	***	***
IHF	0.44	0.41	0.67	0.57	—	***	***	***	***
HABITAT_EPA	0.40	0.26	0.62	0.68	0.58	—	***	***	***
%FOREST	0.50	0.39	0.71	0.57	0.51	0.53	—	***	***
EFI+	0.38	0.31	0.57	0.47	0.54	0.52	0.50	—	***
IBI-Jucar	0.32	0.30	0.65	0.39	0.53	0.50	0.64	0.73	—

***indicates $P < 0.00005$.
Abbreviations are in Table 4.

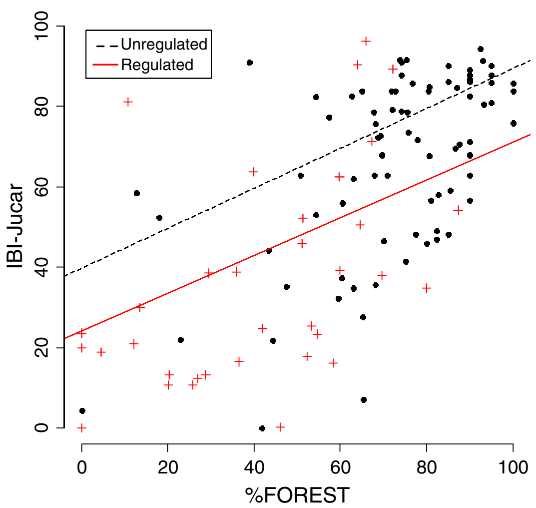

Figure 4. Relationship between the percentage of forest area in the drainage area (%FOREST) and IBI-Jucar for regulated (+) and non-regulated (o) sites of Júcar River Basin district.

removing young age classes (Berkman and Rabeni, 1987); water extraction, pollution and exploitation can increase adult mortality (Matthews and Marsh-Matthews, 2003; Benejam *et al.*, 2010b). Even under natural conditions, populations of native fishes can plunge due to environmental stochastic events, but recovery is also usually rapid because fish adapted to Mediterranean-type streams have evolved the ability to recover quickly from natural events (Bravo *et al.*, 2001; Pires *et al.*, 2008). Therefore, complex age structure and adequate abundance of native fishes can still be used as indicators of ecological integrity in Mediterranean streams. DELT had the lowest influence on IBI-Jucar scores because fish with anomalies were only found in 13 out of 114 sites. This low prevalence is similar for other regions of the Iberian Peninsula (Sostoa *et al.*, 2003). Anomalies have been linked mainly to industrial chemical pollution (Fournie *et al.*, 1996; Sanders *et al.*, 1999; Benejam *et al.*, 2010a), which is low in the study area. However, the DELT metric is interesting because it is one of the few such metrics that measure health at the individual level (Karr, 1981, Karr *et al.*, 1986).

The methodology used to develop IBI-Jucar diverges slightly from other fish indices. Similar to Pont *et al.* (2006, 2007), a type-specific approach, though recommended by the WFD, was not used and the IBI metrics were the same for headwater and lower reaches. Although the more recent EFI+ distinguishes two river types (salmonid and cyprinid types), in practice only one type is used since most streams in the Júcar Basin (like most stream reaches in Mediterranean drainages of the Iberian Peninsula) correspond to the cyprinid river type. Although a type-specific approach might improve sensitivity of the IBI because of natural longitudinal changes of river fish assemblages (Vannote *et al.*, 1980; Lasne *et al.*, 2007), it also imposes artificial boundaries and is probably less useful in small streams such as the ones studied. Another difference from other indices is that the present work did not use explicit 'reference' sites as standards. Many indices try to identify 'reference' or 'least disturbed' sites, developing a predictive model for these sites and identifying metrics through the relationship with measures of human perturbation in more disturbed sites. However, a reference condition approach was implicit in scoring metrics, because they were mostly used with

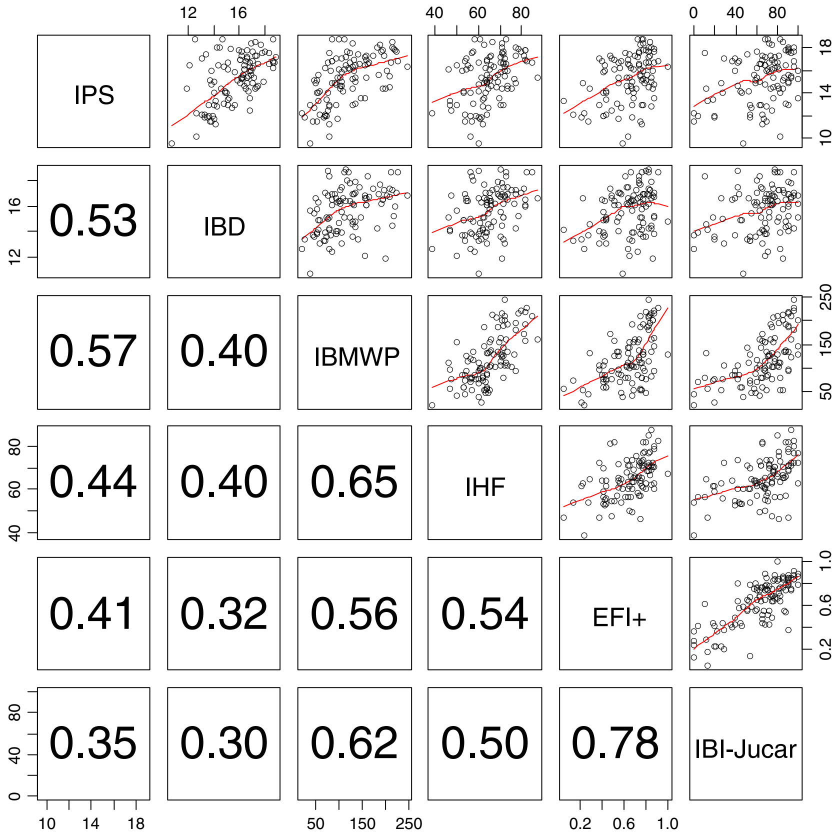

Figure 5. Relationship among selected biotic indices in the Júcar streams. The lower panels show the Pearson correlation coefficients and the upper panels the pairwise scatterplot with a smoothing curve (LOESS). In the scatterplots, the *Y* axis corresponds to the variable in the row diagonal and the *X* axis to the column diagonal (e.g. the scatterplot on the top right has IPS in the *Y* axis and IBI-Jucar in the *X* axis). Abbreviations are in Table 4.

a well-defined expectation of the pristine situation (all native species present, all size classes present, no alien fishes, low incidence of anomalies).

Comparison and validation with other biotic indices

IBI-Jucar was significantly correlated with other biotic indices based on fish (EFI+), macroinvertebrates (IBMWP), diatoms (IPS and IBD) and habitat (IHF, QBR and EPA), indicating that all the indices measure similar aspects of environmental quality of streams. However, correlation coefficients were in general not very high (0.3–0.7), suggesting that each index responds differently to different disturbances. The diatom indices were the least correlated with the IBI-Jucar, as reported for other Iberian regions (Benejam *et al.*, 2008). They seem to be more sensitive to physico-chemical quality and nutrient concentrations (Hering *et al.*, 2006; Justus *et al.*, 2010), while fish indices tend to be more sensitive to long-term habitat and landscape factors (Hughes *et al.*, 2009). Macroinvertebrate indices reflect impairment at intermediate temporal and spatial scales (Hering *et al.*, 2006). The complementary relationships among biotic indices demonstrates the importance of monitoring using multiple organism groups for a comprehensive assessment of the biotic integrity of aquatic ecosystems (Griffith *et al.*, 2005; Hughes *et al.*, 2009; Justus *et al.*, 2010).

We consider IBI-Jucar to be complementary to EFI+, which is the only fish index currently available for use throughout the Iberian Peninsula. The main advantages of EFI+ over IBI-Jucar are its general applicability throughout Europe and its comprehensive statistical validation. The main advantages of IBI-Jucar are: (i) an increased number of

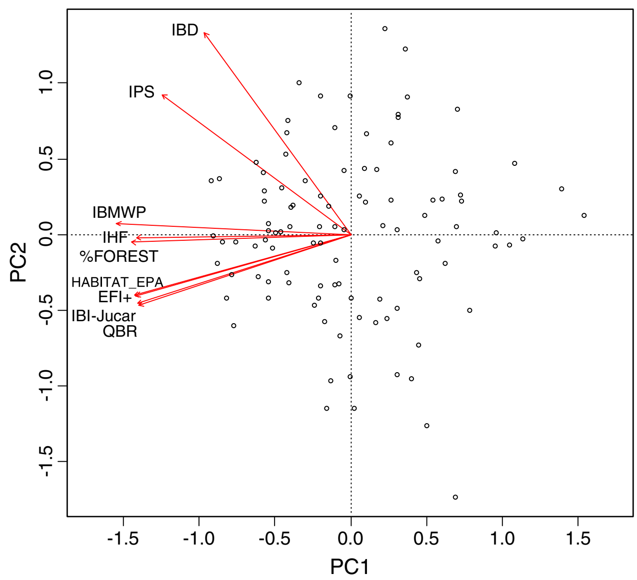

Figure 6. Principal component analysis of the biotic indices in the Júcar streams. Arrows display loadings of the indices while points show site scores. Abbreviations are in Table 4.

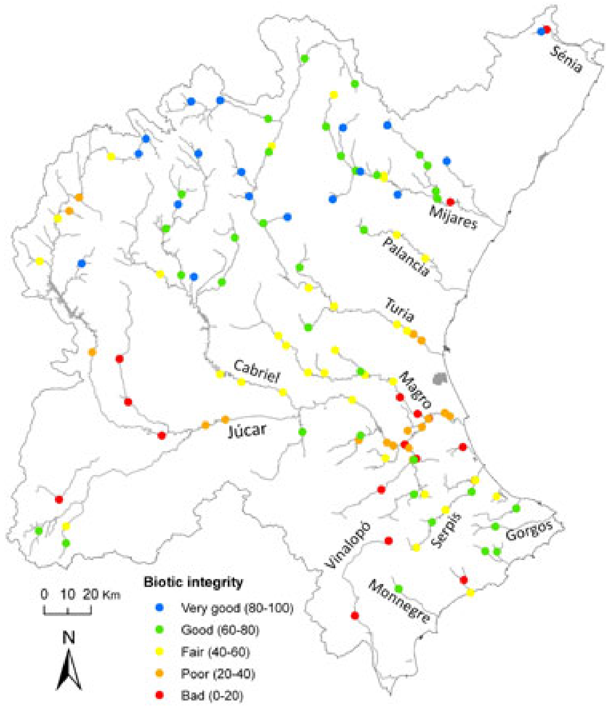

Figure 7. Scores of the Index of Biotic Integrity for the Júcar River Basin District.

metrics, which should respond better to different perturbation types and provide more statistical robustness; (ii) an explicit consideration of alien species, which are an important threat to native fishes in the Iberian Peninsula (Clavero and García-Berthou, 2006; Hermoso *et al.*, 2011); (iii) when impaired conditions are detected, the specific causes of change can be assessed in a *post hoc* diagnostic using individual metric evaluations; and iv) ease of use in species-poor Mediterranean streams. While EFI+ and IBI-Jucar are highly correlated they differ slightly in correlations with other biotic and habitat indices, which suggests that they differ in sensitivity to different environmental perturbations. The high correlation between two quite different fish indices is additional validation for both and supports development of fish-based indices in Mediterranean regions, despite the difficulties imposed by the low species richness, flexible life-histories, and low ecological specialization of fish assemblages (Ferreira *et al.*, 2007).

Potential drawbacks and further developments

Although IBI-Jucar had good performance, the criteria used to score the metrics can still be improved when a larger dataset becomes available to make more precise thresholds for metric score classes. In addition, long-term time series of samples, which are unfortunately still very rare in the Iberian peninsula, would allow testing of the temporal variation of index scores and incorporation of more metrics such as descriptors to test resilience and persistence of fish assemblages, which should improve the overall performance of the index.

IBI-Jucar evaluated streams in a wide area with diverse conditions but was developed only for the Jucar ecoregion. The index, however, can be applied to other ecoregions without basic conceptual changes by adjusting the metrics to reflect region-specific attributes of fish assemblages.

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