Fish-Based Indices in Catalan Rivers: Intercalibration and Comparison of Approaches

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Abstract Freshwater ecosystems are among the most affected by anthropogenic disturbances, and fish have several advantages for monitoring them, such as the response at larger temporal and spatial scales and its visibility to the society. This chapter summarizes our experience in developing fish-based indices in Catalonia. We describe some differences observed among crews in electrofishing captures and habitat assessments. We also analyzed the suitability of a single pass for conventional monitoring in the region and differences in capturability among sites and species by comparison with multiple passes and block nets. Furthermore, we summarize the results of two contrasting approaches, a site- and a type-specific one (IBICAT2a and IBICAT 2b) applied to Catalan rivers. The site-specific was not successful and further data are needed for its improvement. A protocol for the

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computation of a type-specific, multimetric index (IBICAT2b) is given. The IBICAT2b fish index uses 4–8 metrics depending on river type and has been validated with environmental pressures both throughout Catalonia and the whole Ebro River basin. An Excel file is also given as an online supplementary material for the computation of this fish index.

Keywords Biotic integrity, Catalonia, Ecosystem health, Fish biotic index, Rivers, Spain, Water Framework Directive

Contents

1 Introduction

Freshwater ecosystems are severely threatened from human-generated pressures, including water abstraction, pollution, construction of reservoirs, and invasive species. The continuous deleterious effects of human pressures have promoted the need for biological monitoring as well as the development of biological indices [1–3]. Fish are among the taxonomic groups with more longevity in aquatic environments and are excellent ecological indicators for a number of reasons [4]. Fish assemblages have been shown in a number of regions to respond to anthropogenic disturbances including flow regulation (e.g., [5]), habitat fragmentation [6], water pollution [7], land-use change [8], hydrological alteration (e.g., [9]), and acidification [10].

One disadvantage of using fish as ecological indicators is that their population densities are more difficult to estimate accurately and their catchability depends on a number of factors including electrofishing equipment, the characteristics of the river reach [11–13], and species-specific features such as morphology or behavior [14, 15]. The estimation of catchability and intercalibration of data are important to combine data from different fishing teams and to develop protocols for future work or monitoring [12]. Habitat quality is often assessed during fish sampling [16, 17] and inconsistency of habitat assessment among researchers has been also reported by several researchers (e.g., [18–22]).

This chapter summarizes our experience in developing fish-based indices in Catalan Rivers [23, 24] and synthesizes our studies: (1) to estimate the effects of fishing crew and other factors on fish catchability and the resulting fish metrics and on habitat assessments and (2) to attempt to develop type-specific- (i.e., IBICAT2b) and site-specific-based indices (spatially-explicit approach) (i.e., IBICAT2a). We also aim to give a protocol and an Excel for an index (IBICAT2b) that has been validated throughout Catalonia and recently throughout the whole Ebro River basin (Bae et al. unpublished data).

2 Comparison of Electrofishing Crews

Understanding the differences of catchability is particularly important for intercalibration of fish data from various research groups as well as computing fish indices. Several studies have been conducted to balance the compromise between representativeness of fish assemblage in the sampling area and sampling cost (e.g., time, staff, and expenditure), including the comparison of single- vs. multiple-pass electrofishing over various habitats (e.g., [25–30]), and the analysis of electrofishing equipment type (e.g., [31]) and suitable sampling length [30, 32–37]. However, little attention has been paid to assess the differences of catchability among electrofishing crews and equipment and the effects of sampling frequentation in Mediterranean regions.

We compared capture efficiencies based on standard fish descriptors (abundance, observed fish richness and species composition) obtained from four different fishing crews in Mediterranean streams [12]. In eight sites at headwater and middle reaches of a Mediterranean river, we sampled fish in two adjacent stations which had the similar habitat condition at each site using two different methods (single-pass electrofishing without block nets vs. four-pass electrofishing with block nets). During the first fishing day, two different methods were applied, but during the rest of the days only the single pass was applied in order to compare the effects of the consecutive sampling on fish abundance and assemblage structure. We applied a Williams' crossover design, which is based on a Latin square design and is characterized by that (1) all crews are assigned only once to each sampling site during the four consecutive sampling days; (2) all crews are equally distributed; (3) it allows to test for potential carryover effects. We analyzed the differences in species richness, abundance, and proportional abundances due to the different catchability by the four research teams using generalized linear models (GLMs) with Poisson errors and log link functions (species richness and abundance) or binomial errors and logit link functions (proportional abundance). We also applied the software EstimateS (http://viceroy.eeb.uconn.edu/EstimateS) to estimate richness based on the removal estimates (i.e., four-pass electrofishing) using the second-order jackknife richness estimator (Jack 2; [38]), which is one of the most widely recommended estimators. Furthermore, we estimated population sizes and capture probability for the most abundant species in the four-pass electrofishing using program MARK using four different multinomial models (i.e., a model with constant catchability between different electrofishing passes (P), a model with constant catchability between electrofishing passes (P1), a model with nonconstant catchability between electrofishing passes (P1L), and a model with nonconstant catchability between passes and a quadratic function of fish length (P1L2)). These models were compared using Akaike's information criterion [39].

Our results indicated that single-pass electrofishing was effective in the study area. It captured a large percentage of abundance (40–60%) as well as species richness (50–100%). Unsurprisingly, electrofishing was more efficient upstream than downstream and all species were generally captured in sampling sites with few species (i.e., headwaters). Furthermore, even though it is more difficult to detect all species in mid-river sections with higher species diversity, single electrofishing showed also high catchability there. Although observed species richness was not significantly influenced by the use of block nets, average CPUE was significantly higher using block nets. In addition, observed species richness was not significantly influenced by the research team, fishing day, or carryover effects. However, total CPUE depended on fishing day, crew, carryover effects, and site. Catchability varied depending on species, size, and removal passes.

In summary, single-pass electrofishing can be adequate to estimate abundance, species composition, and richness in headwaters and middle courses of this Mediterranean region. However, various methodological factors (e.g., reach length, number of passes, fish size, and species) influence electrofishing capture efficiency. Our results also show that the effectiveness of electrofishing depends on fishing crews because of different personal skills and practice. Therefore, electrofishing sampling protocols (e.g., sampling time and effort and equipment type) should be standardized as much as possible to get comparable data [24].

3 Comparison of Habitat Assessments Among Sampling Teams

The assessment of habitat quality is essential in fish studies because each fish species often has specific habitat requirements [40] and altered habitats are considered a major disturbance in aquatic ecosystems [41]. Therefore, habitat assessment has been developed as an integral part of stream biological monitoring [42–45]. However, because habitat assessments are often based mostly on visual observations or a minimal amount of measurement [45], the variability of assessments frequently occurs among researchers (even experienced ones). We compared the differences in scoring the habitat characteristics among four research teams. Each research team conducted the habitat monitoring with the same protocol at each site after finishing the electrofishing described in the previous section. Each team surveyed hydromorphological descriptors, riparian vegetation, aquatic vegetation, refuge type, observed visual impacts, land use, and habitat based on a

Table 1 Comparison of descriptors of habitat assessment among assessors. Degrees of freedom = 2 and 9

Categories	Variables	Type III sum of squares	F	P	Partial η^2
Hydromorphology (mesohabitat)	% Riffle	756	7.61	0.01	0.63
Hydromorphology (mesohabitat)	% Glide	687	2.84	0.11	0.39
Hydromorphology (mesohabitat)	% Pool	170	1.57	0.26	0.26
Hydromorphology (substrate)	% Bedrock	37	3.65	0.07	0.45
Hydromorphology (substrate)	% Boulder	148	0.62	0.56	0.12
Hydromorphology (substrate)	% Cobble	682	6.37	0.02	0.59
Hydromorphology (substrate)	% Gravel	827	6.57	0.02	0.59
Hydromorphology (substrate)	% Sand	28.5	0.61	0.56	0.12
Hydromorphology (substrate)	% Silt and clay	1.95	0.15	0.86	0.03
Hydromorphology (hydrology)	Average width	0.08	0.43	0.66	0.09
Hydromorphology (hydrology)	Full bank height	0.84	1.43	0.29	0.24
Riparian vegetation	% Marginal riparian cover	309	0.8	0.48	0.15
Riparian vegetation	% Areal cover	78	0.16	0.85	0.03
Riparian vegetation	% Trees	1,315	4.42	0.05	0.5
Riparian vegetation	% Shrubs	1,596	6.33	0.02	0.58
Riparian vegetation	% Grass	3,600	13.5	0.00	0.75
Aquatic vegetation	% Macrophyte cover	160	0.76	0.49	0.15
Aquatic vegetation	% Helophytes	233	1.59	0.26	0.26
Aquatic vegetation	% Hydrophytes	73.6	1.46	0.28	0.24
Aquatic vegetation	% Floating leaves	30.6	1.6	0.25	0.26
Aquatic vegetation	% Floating plants	892	1.95	0.20	0.3
Aquatic vegetation	% Algae	4,988	1.72	0.23	0.28
Refuge type	% Total refuge	5,526	6.78	0.02	0.6
Refuge type	% Structural shelter	67.6	0.1	0.91	0.02
Refuge type	% Caves	523	1.11	0.37	0.2
Refuge type	% Aquatic vegetation	168	2.42	0.14	0.35
Refuge type	% Submerged riparian vegetation	474	3.52	0.07	0.44
Refuge type	% Trunk and branches	45.1	1.08	0.38	0.19
Observed impacts	Muddy water	1.22	4.95	0.04	0.52
Observed impacts	Stones with black bottom	0.06	0.64	0.55	0.13

(continued)

Table 1 (continued)

Categories	Variables	Type III sum of squares	F	P	Partial η^2
Observed impacts	Channelization	0.25	1.5	0.27	0.25
Observed impacts	Erosion	1.06	4.28	0.05	0.49
Observed impacts	Highways, roads, etc.	0.56	1.43	0.29	0.24
Land use	Forest use	0.31	1.6	0.25	0.26
Land use	Agricultural land use	1.95	8.37	0.01	0.65
Land use	Residential land use	0.56	1.43	0.29	0.24
Habitat	Microhabitat score	0.31	0.3	0.75	0.06
Habitat	Habitat diversity (macrohabitat)	0.56	0.2	0.82	0.04
Habitat	Channelization	2.78	1.47	0.28	0.25
Habitat	Channel morphology	0.31	0.07	0.93	0.02
Habitat	Flow	0.62	2.65	0.12	0.37
Habitat	Degree of clogging	6.72	8.01	0.01	0.64
Habitat	Margin erosion, R	10.6	8.36	0.01	0.65
Habitat	Margin erosion, L	12.3	5.62	0.03	0.56
Habitat	Aquatic veg. (macrophytes)	0.06	0.02	0.98	0.01
Habitat	Riparian veg. (R margin)	2.78	0.39	0.69	0.08
Habitat	Riparian veg. (R margin)	9.81	2.9	0.11	0.39
Habitat	Width of riparian veg. (R margin)	15	3.28	0.09	0.42
Habitat	Width of riparian veg. (R margin)	25.5	8.61	0.01	0.66

veg vegetation

modified version of the US Rapid Bioassessment Protocol (RBI) [46] for Mediterranean rivers (Table 1), which was used during the sampling of the project to implement the Water Framework Directive (WFD) in Catalonia [23, 24, 47]. Table 1 shows the list of habitat assessment descriptors as well as the significance of the differences among four assessors and a measure of effect size (partial η^2). Of 49 habitat assessment descriptors, 12 were significantly different among the four research teams that assessed them independently ($P < 0.05$). Percentage of grass in the riparian vegetation showed the highest difference among research groups (Table 1, Fig. 1), and four variables (i.e., degree of clogging, erosion of margins (right and left), and width of riparian vegetation (left margin)) from the Rapid Bioassessment Protocol, which provides a detailed protocol to score these features, were also different among the four assessors. A multivariate test suggested that although overall differences among assessors were not significant (MANOVA Wilks' λ, $F_{2,\ 18.5} = 5.482$, $P = 0.165$), probably due to low power, they were

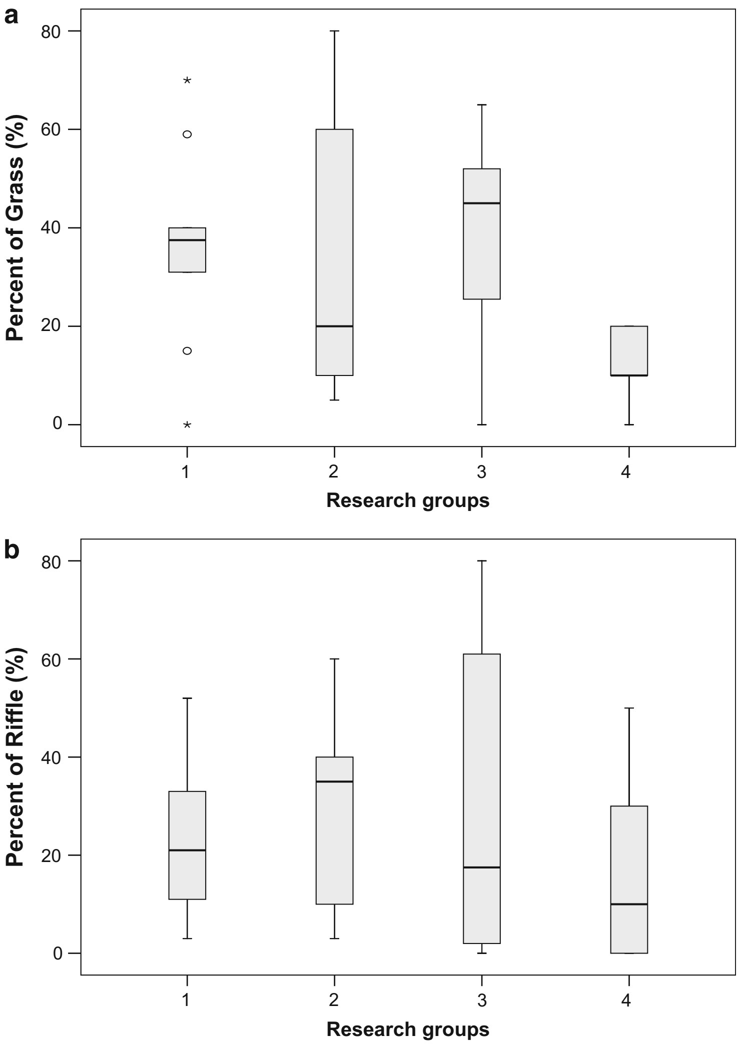

Fig. 1 Box plots of the scoring of % grass and % riffle among four research groups (see Table 1 for statistical analysis). Each box corresponds to 25th and 75th percentiles; the *dark line* inside each box represents the median; error bars show the minima and maxima except for outliers (*open circles* or *asterisks*, corresponding to values >1.5 box heights from the box)

more important (partial η^2 of 0.980 vs. 0.907) than differences among sites, which were significant ($F_{99,\ 18.5} = 3.698$, $P = 0.001$) and very clear.

Roper and Scarnecchia [19] reported that although consistency of habitat quality evaluation is improved with uniform training, inconsistency increases among researchers, as the habitat types to be classified become more diverse. Hannaford et al. [45] showed that even if the evaluation of habitat assessment becomes similar among groups after equal training in a certain type of habitats, large differences are still observed in other habitat types. Our results also suggest that the scoring for habitat assessment can be highly inconsistent among different research groups even using the same habitat assessment protocol. Therefore, habitat assessment requires more clear and detailed criteria and more training to make a similar evaluation among groups.

4 Development and Comparison of Fish Indices: Type- vs. Site-Specific Approaches

In addition to IBICAT$_{2010}$ (see [4] in this book), whose development was led by Nuno Caiola, two other approaches (i.e., a type-specific and site-specific) were attempted in Catalan rivers [23]. Type-specific fish indices are based on a classification of sites in a region on homogenous types based on environmental or faunistic features and use different metrics and scorings in the different areas. On the other hand, site-specific approaches do not use a classification and instead predict the reference fish metrics from the environmental features of the sites [48, 49].

The WFD requests that various biotic assemblage descriptors (e.g., metrics) should be integrated into a single index to assess ecological status [3, 50]. These indices should represent the status of impairment in a research area [51–54]. Community metrics (e.g., number of intolerant species) and trophic guilds (e.g., percentage of piscivores), which group species sharing a common ecological trait into a single variable, have been commonly applied to develop bioassessment metrics based on fish assemblages [52, 55] (Table 2). It is assumed that these traits respond to anthropogenic disturbances consistently across a wide spatial extent [53, 54]. In addition, unlike species composition, which varies strongly across regions and biogeographical areas [56], patterns from functional traits are mainly determined by environmental filtering (e.g., [55, 57–61]).

Most predictive models evaluating ecological status start from comparing the biotic condition at current sampling sites with the expected biota without anthropogenic disturbance or in reference conditions [49, 62, 63]. Thus, changes in biotic condition from anthropogenic disturbance can occur only when the range of variation (or response) in reference (natural) conditions is well known [64, 65].

In this section, we summarize the two approaches (i.e., a site-specific one, IBICAT2a, and a type-specific one, IBICAT2b) based on the same guild classification for the fish fauna of Catalonia (Table 2), which was based on a comprehensive literature review. Fish development was based on a database of 364 sites in Catalonia, visited during 2007–2008, of which 8 sites could not be sampled due to the excessive discharge, 45 sites were dry, 76 sites were sampled but no fish was captured in them, and 235 sites were sampled with fish captured. At the 311 sampled sites, the total number of species (NST) ranged from 0 to 13 (median = 2, mean = 2.3), the number of native species (NSN) was from 0 to 8 (median = 1,

Table 2 Features of the freshwater fish fauna from Catalonia used for development and computation of the indices

Family	Species	Tolerance	Feeding habitat	Habitat	Reproduction	Feeding group	Migration	Longevity	Status
Acipenseridae	*Acipenser sturio*	I		RH	LITH	OMNI	LONG	LL	A
Anguillidae	*Anguilla anguilla*	T	B			PISC	LONG	LL	A
Balitoridae	*Barbatula quignardi*		B	RH	LITH	BENT		SL	A
Blenniidae	*Salaria fluviatilis*		B		LITH	INSV		SL	A
Centrarchidae	*Lepomis gibbosus*	T	WC	LI		INSV		SL	I
Centrarchidae	*Micropterus salmoides*		WC	LI		PISC		LL	I
Clupeidae	*Alosa alosa*	I		RH			LONG	LL	A
Clupeidae	*Alosa fallax*	I		RH			LONG	LL	A
Cobitidae	*Cobitis bilineata*							SL	I
Cobitidae	*Cobitis calderoni*	I		RH		INSV		SL	A
Cobitidae	*Cobitis paludica*	T		RH		INSV		SL	A
Cobitidae	*Misgurnus anguillicaudatus*	T	B	LI		OMNI		IM	I
Cottidae	*Cottus hispaniolensis*	I	B	LI	LITH	INSV		SL	A
Cyprinidae	*Alburnus alburnus*	T	WC			OMNI		SL	I
Cyprinidae	*Achondrostoma arcasii*		WC					SL	A
Cyprinidae	*Barbus meridionalis*	I	B	RH	LITH	INSV		IM	A
Cyprinidae	*Barbus graellsii*	T	B		LITH	OMNI	POTAD	LL	A
Cyprinidae	*Barbus haasi*	I	B	RH	LITH	INSV		IM	A
Cyprinidae	*Carassius auratus*	T	B		PHYT	OMNI		LL	I
Cyprinidae	*Cyprinus carpio*	T	B		PHYT	OMNI		LL	I
Cyprinidae	*Gobio lozanoi*		B	RH		INSV		SL	A
Cyprinidae	*Parachondrostoma miegii*	I	B	RH	LITH			IM	A
Cyprinidae	*Pseudorasbora parva*	T				OMNI		SL	I
Cyprinidae	*Phoxinus bigerri*	I	WC	RH	LITH	OMNI		SL	A

(continued)

Table 2 (continued)

Family	Species	Tolerance	Feeding habitat	Habitat	Reproduction	Feeding group	Migration	Longevity	Status
Cyprinidae	*Rutilus rutilus*	T	WC			OMNI		IM	I
Cyprinidae	*Scardinius erythrophthalmus*	T	WC	LI	PHYT	OMNI		LL	I
Cyprinidae	*Squalius laietanus*		WC	RH	LITH	OMNI		LL	A
Esocidae	*Esox lucius*		WC		PHYT	PISC		LL	I
Gasterosteidae	*Gasterosteus gymnurus*		WC			INSV		SL	A
Gobiidae	*Pomatoschistus microps*		B			INSV	LONG	SL	A
Ictaluridae	*Ameiurus melas*	T	B		LITH	OMNI		IM	I
Mugilidae	*Chelon labrosus*	T					LONG	LL	A
Mugilidae	*Liza ramada*	T					LONG	LL	A
Mugilidae	*Mugil cephalus*	T					LONG	LL	A
Percidae	*Perca fluviatilis*	T	WC			PISC		LL	I
Percidae	*Sander lucioperca*		WC		PHYT	PISC		LL	I
Petromyzontidae	*Petromyzon marinus*	I		RH	LITH		LONG	LL	A
Poeciliidae	*Gambusia holbrooki*	T	WC	LI		INSV		SL	I
Salmonidae	*Oncorhynchus mykiss*			RH	LITH	PISC		IM	I
Salmonidae	*Salmo trutta*	I		RH	LITH	PISC		IM	A
Siluridae	*Silurus glanis*	T	B		PHYT	PISC		LL	I

T tolerant, *I* intolerant, *B* benthic, *WC* water column, *RH* rheophilic, *LI* limnophilic, *LITH* lithophilic, *PHYT* phytophilic, *OMNIV* omnivore, *PISC* piscivore, *INSV* invertivore, *LONG* long migration (diadromous species), *POTAD* short migration, *SL* short longevity, *IM* intermediate longevity, *LL* long longevity, *A* species native from Catalonia, *I* species introduced in Catalonia. Blank means species not classified mean = 1.4), and the number of introduced species (NSI) was from 0 to 10 (median = 0, mean = 0.82).

For selecting candidate metrics, we carefully reviewed the literature including research papers and reports from different countries. In total, for the 311 sites, we computed 199 candidate metrics, which can be classified into four categories as in the original IBI development [51, 66]: species composition and diversity, trophic composition, abundance, and fish condition. All the metrics were in general computed both for native and introduced species separately and for all species together. The native/alien status was considered at the river basin level.

To validate the new indices with gradients of anthropogenic pressure, we used two different anthropogenic disturbance measures. First, we obtained an official statistic of anthropogenic disturbance (the risk of noncompliance measure, RI_AP) from the Catalan Water Agency (document IMPRESS; [67]). It summarizes many different disturbances such as hydromorphological changes, flow regime alterations, changes in land use and the riparian zone, and point and diffuse sources of pollution [47, 67]. Second, a principal component analysis (PCA) was also used to combine this risk of noncompliance with our local measurement at the sampling sites such as the sum of RBI scores, sum of visual impacts, dissolved oxygen concentration, ammonia concentration, and pH. The first PCA axis summarized well a gradient of anthropogenic disturbance (see [47] for details).

The site-specific approach (IBICAT2a) was developed following leading works in Europe [48, 52, 68]. To define the calibration set (low pressure), we followed the usual method (see, e.g., [69, 70]): only sites where none of the pressures (hydrological regime, river connectivity, morphology, toxic acidification, and nutrient organic inputs) was greater than 2, ranging from 1 (no pressure) to 5 (high pressure) were used. Among 369 sites in Catalonia, 49 sites fulfilled all these criteria (of which 34 sites had fish captures). Then, generalized linear models (GLMs), with appropriate error and link functions depending on the types of metrics, were used in the reference condition sites (calibration set) to develop the expected values of fish metrics given numerous natural environmental variables (climatic and topographic) that are not affected by anthropogenic disturbance. A stepwise procedure based on Akaike's information criterion was used to select parsimonious, adequate GLMs. Then the observed values on the rest of sites are compared to the expected values (see, e.g., [71, 72]) to compute an index that ranges from 0 (worst conditions) to 1 (reference conditions).

From the numerous GLMs, we selected 10 metrics considering their significant correlation with anthropogenic disturbance (pressures), their meaningfulness in ecological terms, their complementarity (e.g., different organization levels), and relatively low collinearity. Although the detailed results and a tentative index (IBICAT2a) are given in Sostoa et al. [23], we considered that this index was not suitable because of a number of reasons: (1) the GLMs could not be cross-validated because of low sample sizes and considerable variability in the reference data and probably also because of the considerable environmental heterogeneity of Catalonia; (2) the metrics based on absolute richness and abundance metrics did not behave well (gave unrealistic expected results) probably due to low numbers of reference condition sites (which were mostly at higher elevations) and therefore the index only included relative metrics (i.e., percentages); and (3) dry and fishless sites were not well predicted by predictive models, suggesting many local pressures that are not well captured by available indicators. Therefore, although this approach has been successfully applied in France [48, 52] and across Europe [54, 52, 71] and could potentially be developed in Catalonia, the low sample size available of fish data precludes its current application.

5 IBICAT2b: Development of a Type-Specific Fish Index for Catalonia and the Ebro River Basin

We also attempted a simpler type-specific approach (IBICAT2b), whose results we consider much more reliable than IBICAT2a and that we have validated (through correlation with environmental pressures) throughout Catalonia [23] and the Ebro River (Bae et al. unpublished data). We recommend IBICAT2b as a regional fish index, until further data become available that allow developing a better index. This index uses the official river types based on environmental data that are also used for macroinvertebrate indices and other purposes in Catalonia (e.g., [67, 73, 74]), the whole Ebro River [75], and Spain in general (http://www.chebro.es/; [76, 77]) (Fig. 2, Table 3).

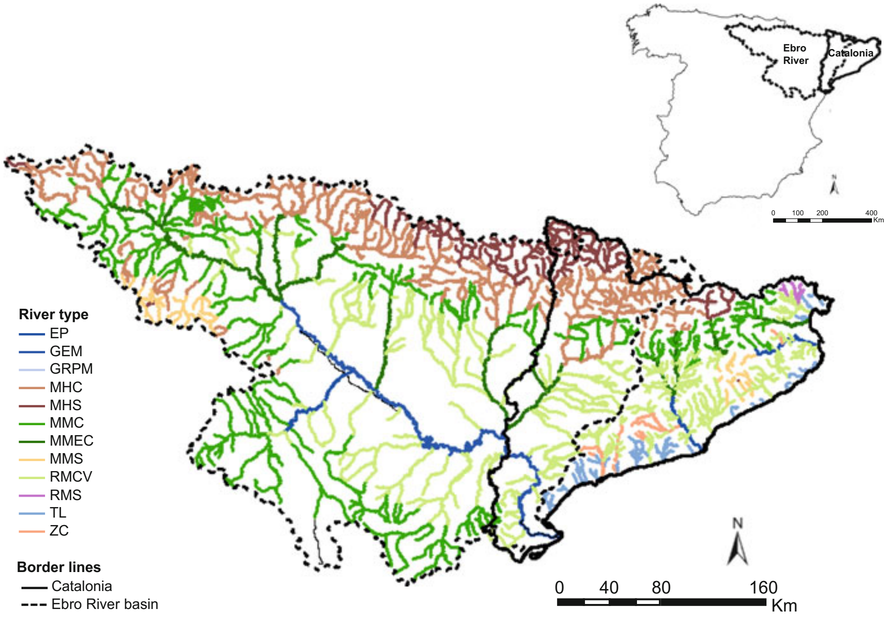

Fig. 2 Official river types in Catalonia and the Ebro River [74, 75]. See Table 3 for the meaning of code abbreviations and further details

Table 3 River typology and number of sites in each river type

Official river type no.	River type	Catalan abbreviation	Number of Catalan sites with fish data used in the study
27	Siliceous wet mountain rivers	MHS	23
26	Calcareous wet mountain rivers	MHC	52
11	Siliceous Mediterranean mountain rivers	MMS	11
12	Calcareous Mediterranean mountain rivers	MMC	47
15	High-flow Mediterranean mountain rivers	MMEC	13
9	Variable-flow Mediterranean rivers	RMCV	147
8	Siliceous Mediterranean lowland rivers	RMS	2
10	Rivers influenced by karstic areas	ZC	16
16	Main watercourses	EP	10
18	Coastal streams	TL	32
17	Large Mediterranean watercourses	GEM	6
15	Large rivers with weak mineralization	GRPM	10

In order to select the metrics that reflected well the gradients of anthropogenic disturbance for each river type, we computed the correlations between PC1 (the anthropogenic disturbance described in the previous section) and all the metrics in each typology separately, which is a classical type-specific approach (see [70]). In this procedure, because the total sampling sites in some of the river types were very low (e.g., EP, GEM, GRPM, MMS, and RMS where the total number of sampling sites were less than 11), we used a coarser statistical criteria ($P < 0.1$). In RMS type, we could not calculate correlations because only two sampling sites were available (Table 3). To select the final metrics for the index in each typology, we considered its diversity (different organization levels and type of metrics), complementarity (as assessed with a principal component analysis, which showed different groups of metrics based on their correlation), and interpretability of results (a few metrics had relationships with PC1 opposite than expected). The final metrics selected are shown in Table 4.

These different metrics were scored following a number of approaches. The number of native species was scored based on expert criteria and the historical records of fish assemblages in Catalonia. For DELT anomalies, we used the traditional IBI scoring: 0–2%, very good; 2–5%, moderate; and >5%, bad

Table 4 Metrics selected for the 10 river types. *Metrics in italics* represent metrics that increase with anthropogenic disturbance (and vice versa for metrics not in italics)

River type no.	Catalan abbreviation	Number of sites	Common metrics	Type-specific metrics
27	MHS	23	NSN, *PSI, PII, PIT_DELT*	PIT_pisciv, PST_pisciv
26	MHC	52	NSN, *PSI, PII, PIT_DELT*	PIT_pisciv, PST_lithophil, PIT_intol, *PST_SL*
11	MMS	11	NSN, *PSI, PII, PIT_DELT*	–
12	MMC	47	NSN, *PSI, PII, PIT_DELT*	NIN_15cmintol
15	MMEC	13	NSN, *PSI, PII, PIT_DELT*	*PST_SL*
9	RMCV	147	NSN, *PSI, PII, PIT_DELT*	PIT_intol, NIN_15cmintol, PST_lithophil, PIT_rheophil
8	RMS	2	NSN, *PSI, PII, PIT_DELT*	–
10	ZC	16	NSN, *PSI, PII, PIT_DELT*	PST_intol
16	EP	10	NSN, *PSI, PII, PIT_DELT*	–
18	TL	32	NSN, *PSI, PII, PIT_DELT*	–
17	GEM	6	NSN, *PSI, PII, PIT_DELT*	–
15	GRPM	10	NSN, *PSI, PII, PIT_DELT*	–

NSN number of native species, *PSI* % of exotic species, *PII* % of exotic individuals, *PIT_DELT* % of individuals with deformities, eroded fins, lesions, and tumors (DELT) abnormality, *PIT_pisciv* % of piscivorous individuals, *PST_pisciv* % of piscivorous species, *PST_lithophil* % of lithophilic species, *PIT_intol* % of intolerant individuals, *PST_SL* % of short longevity species, *NIN_15cmintol* native abundance of individuals <15 cm of habitat intolerant species, *PIT_rheophil* % of rheophilic individuals, *PST_intol* % of intolerant species. See Tables 2 and 3 for further abbreviations

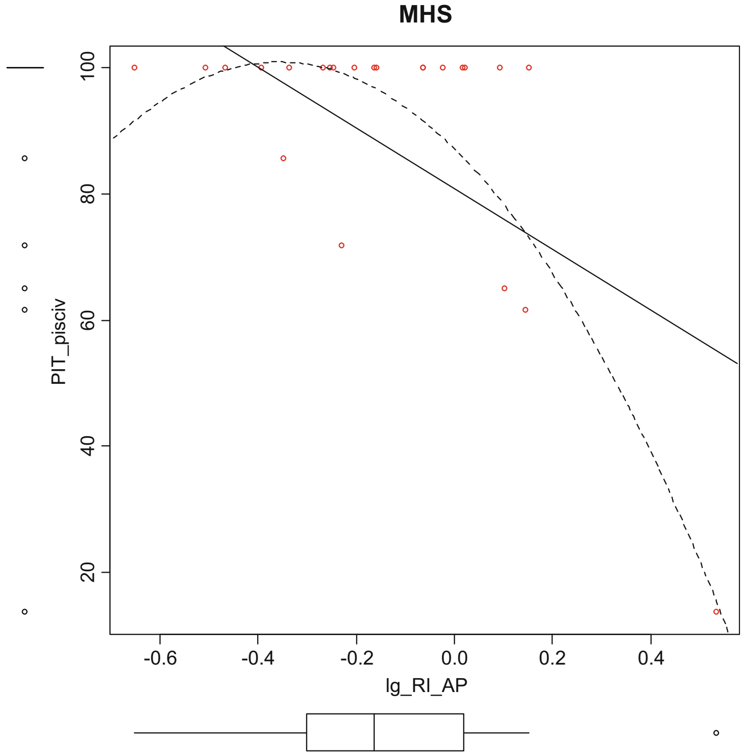

Fig. 3 Relationship between % piscivorous individuals (PIT_pisciv) and anthropogenic pressure (lg_RI_AP: log-transformed RI_AP) in the MHS river type. *Straight line*: linear regression model ($r^2 = 0.375$); *dashed line*: quadratic regression model ($R^2_{\text{adj}} = 0.646$). A likelihood ratio test showed that the quadratic model is significantly better than the linear model ($P = 0.0003$)

[41]. For NIN_15cmintol, only presence/absence was considered, because densities were very low and often null despite a clear relationship with anthropogenic disturbance. For the calibration of the other metrics (i.e., PSI, PII, PIT, PIT_pisciv, PST_pisciv, PST_lithophil, PIT_intol, PST_SL, PTI_intol, PST_lithophil, PIT_rheophil, and PST_intol) (see abbreviations in Table 4), the same approach as in the site-specific approach (IBICAT2a) was used for the scoring of metrics. Figure 3 shows the relationship between one of these metrics (PIT_pisciv) and the anthropogenic pressure index in one of the river types (MHS). As shown in this figure, a quadratic model was often significantly better than a linear model. Using these models and the classes defined for the risk of noncompliance measure (RI_AP < 0.8, no risk; 0.8–1.2, low risk; 1.2–2, average risk; >2 high risk) in the IMPRESS official document for Catalonia [67], we predicted PIT_pisciv values corresponding to each threshold and thus obtained the scoring of metrics.

For all the other metrics, we applied the same procedure as with PIT_pisciv to compute the corresponding thresholds based on RI_AP. Finally, the average of the score for relevant metrics depending on river type was computed to obtain the index and the ecological status.

For large rivers (types EP, GEM, GRPM, and MMEC), we also give a "bad" status, if the study reach is dry or no fish was captured after an adequate sampling. There is published [78] and unpublished (personal observations) evidence that Catalan streams are sometimes dry artificially (due to human water abstraction). Conservatively, we only apply this "bad" status classification to large rivers that should be expected to never run dry or be fishless in natural conditions. For other river types, if the sites are dry or no fish was captured, no status is given, because this might be due to natural causes.

Although both indices (IBICAT2a and IBICAT2b) are very different in terms of the development procedure of indices, both indices showed a similar response to anthropogenic disturbance (i.e., the correlation coefficients were 0.41 for IBICAT2a and PC1, −0.36 for IBICAT2a and lg_RI_AP, 0.40 for IBICAT2b and PC1, and −0.33 for IBICAT2b and lg_RI_AP). There was also high correlation between the two indices ($r = 0.71$), although the relationship was nonlinear because many metrics in IBICAT2a often had values of 0 or 1, indicating that IBICAT2a should be revised with more reference sites to develop further the predictive models and underlying index. Even though IBICAT2a showed relatively high correlation with anthropogenic disturbances, it has several limitations (see section above) and should not be used. A map with the results of IBICAT2b in Catalonia is given in p. 120 of Sostoa et al. [23].

6 Protocol for the IBICAT2b Multimetric Fish Index

An Excel file is given as an online supplementary material to this book chapter (http://invasiber.org/EGarcia/IBICAT2b.html) for the computation of the IBICAT2b index in Catalonia and the Ebro River. The index should not be used in other regions unless it is validated for them (i.e., correlated with environmental pressures) and it should be first adapted for different fish faunas. The following steps should be followed to compute the index. They are automated if the data are imputed in the Excel file.

1. Obtain the river type of your sampling reach.

   River types for this index are the general ones official for the WFD across Spain: there are 12 different river types in Catalonia (Table 3) and 8 in the whole Ebro River basin (all of them also present in Catalonia). Note, however, that there is a minor difference between Catalan and Spanish types: type 15 corresponds to two different Catalan types. Furthermore, there are some reaches declared as heavily modified water bodies and without any official type. Find the river type of your sampling reach in Fig. 2.

2. If your sampling sites are in EP, GEM, GRPM, or MMEC river types and they were dry or fishless, ecological status is “bad” (IBICAT2b = 1, EQR = 0). If the sites were dry or fishless but belong to other river types, the status cannot be defined with this index. Otherwise, proceed to point 3.
3. Score each metric with the fish data from the study site.

All metrics should be independently scored from 1 (bad) to 5 (very good) according to the following tables. Metrics 1–4 are common to all river types. The rest of metrics are for some river types only. If some metrics cannot be computed (e.g., metric 2 has not been measured), they can be omitted from the final average.

Metric 1: number of native species (NSN)

River type no.	Catalan abbreviation	Very good	Good	Moderate	Poor	Bad
27	MHS	>1		1		0
26	MHC	>1		1		0
11	MMS	>1		1		0
12	MMC	>1		1		0
15	MMEC	>2	2	1		0
9	RMCV	>1		1		0
8	RMS	>1		1		0
10	ZC	>1		1		0
16	EP	>3	3	2	1	0
18	TL	>1		1		0
17	GEM	>4	4	3	2	<2
15	GRPM	>3	3	2	1	0

Metric 2: percentage of individuals with deformities, eroded fins, lesions and tumors (DELT) abnormality [41]

	Very good	Good	Moderate	Poor	Bad
DELT	0–2%		>2–5%		>5%

Metric 3: percentage of introduced individuals (PII)

	Very good	Good	Moderate	Poor	Bad
PII	0%		0–5%	5–20%	>20%

Metric 4: percentage of introduced species (PSI)

	Very good	Good	Moderate	Poor	Bad
PSI	0%		0–5%	5–20%	>20%

Other metrics: specific metrics for some river types. See Tables 2 and 3 for further abbreviations.

River type no.	Catalan abbreviation	Specific metric	Very good	Good	Moderate	Poor	Bad
27	MHS	PIT_pisciv	100%	99.99–96.67%	96.66–84.80%	84.79–59.84	<59.84%
27	MHS	PST_pisciv	100%	99.99–90.32%	90.31–82.85%	82.84–68.92%	<68.92%
26	MHC	PIT_pisciv	100%	99.99–44.91%	44.90–39%	39–29.46%	<29.46%
26	MHC	PST_lithophil	100%	99.99–97.38%	97.37–95.51%	95.50–91.77%	<91.77%
26	MHC	PIT_intol	100%	99.99–89.27%	89.26–80.22%	80.21–63.34%	<63.34%
26	MHC	PST_SL	0%	0–32.58%	32.58–39.58%	39.58–45.92%	>45.92%
12	MMC	NIN_15cmintol (presence)	Yes		No		
15	MMEC	PST_SL	0%	0–4.14%	4.14–19.95%	19.95–35.73%	>35.73%
9	RMCV	PIT_intol	>65.78%	65.78–56.65%	56.65–47.28%	47.28–0%	0
9	RMCV	NIN_15cmintol (presence)	Yes		No		
9	RMCV	PST_lithophil	100%	99.99–71.55%	71.55–62.79%	62.79–54.32%	<54.32%
9	RMCV	PIT_rheophil	100%	99.99–80.09%	80.09–71.08%	71.08–62.54%	<62.54%
10	ZC	PST_intol	100%	99.99–65.91%	65.91–55.59%	55.59–43.34%	<43.34%

Therefore, IBICAT2b includes 4–8 metrics depending on river type. Each metric is scored from 1 to 5 (1 = bad, 2 = poor, 3 = moderate, 4 = good, and 5 = very good).

4. The final index is computed as the average of all available metrics. To obtain the ecological status according to IBICAT2b, the following thresholds are used:

	Very good	Good	Moderate	Poor	Bad
IBICAT2b	$\geq$4.5	3.5–4.5	2.5–3.5	1.5–2.5	<1.5
EQR	$\geq$0.875	0.875–0.625	0.625–0.375	0.375–0.125	<0.125

7 Concluding Remarks

Another type-specific index (IBICAT$_{2010}$), quite different from IBICAT2b, was also described in Sostoa et al. [23] (see also [4]). An adaptation of this index (IBIMED), so far (February 2015) not available in published papers, Internet reports, or software, was intercalibrated with EFI+ and the Portuguese fish index [79]. The differences between IBIMED and IBICAT$_{2010}$ include the addition of some of the rest of Spanish fish species with their guild classification (to allow the computation in other river basins) [79] and apparently different thresholds for the EQR classes. IBIMED has only been successfully validated with qualitative environmental pressures in Mediterranean rivers and the Duero and not the rest of Spanish rivers and was only intercalibrated for Mediterranean rivers (excluding the Duero) [79]. Recent unpublished work throughout the Ebro River (García-Berthou and Bae, unpublished data) shows that IBICAT2b and EFI+ are more related to quantitative environmental pressures than IBIMED/IBICAT$_{2010}$, which shows problems mainly in the typology and treatment of fishless or dry sites. However, these three indices are correlated and their values could thus be converted (e.g., IBICAT$_{2010}$ = 0.2099 + 0.1398 IBICAT2b, IBICAT2b = 1.3849 + 2.941 IBICAT$_{2010}$, $r^2 = 0.411$, $P < 0.0005$; EFI+ = 0.2686 + 0.1279 IBICAT2b, IBICAT2b = 1.8573 + 2.2129 EFI+, $r^2 = 0.283$, $P < 0.0005$). Overall, our work suggests that fish indices can be successful in Spain but research is needed to improve them and generalize them. The availability of further fish data, user-friendly software, and extensive validation are essential steps toward the improvement of these fish-based indices.

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References

1. Hellawell JM (1986) Biological indicators of freshwater pollution and environmental management. Elsevier Applied Science, London
2. Rosenberg DM, Resh VH (1993) Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: Rosenberg DM, Resh VH (eds) Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York, pp 1–9
3. Hering D, Johnson RK, Kramm S et al (2006) Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: a comparative metric-based analysis of organism response to stress. Freshw Biol 51:1757–1785
4. Benejam L et al (2015) Fish as ecological indicators in Mediterranean streams: the Catalan experience. In: Munné A, Ginebreda A, Prat N (eds) Experiences from surface water quality monitoring. The EU Water Framework Directive Implementation in the Catalan River Basin District (part I). Springer, Berlin
5. Bain MB, Finn JT, Booke HE (1988) Streamflow regulation and fish community structure. Ecology 69:382–392
6. Morita K, Yamamoto S (2002) Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. Conserv Biol 16:1318–1323
7. Belpaire C, Smolders R, Auweele IV et al (2000) An Index of Biotic Integrity characterizing fish populations and the ecological quality of Flandrian water bodies. Hydrobiologia 434:17–33
8. Snyder CD, Young JA, Villella R et al (2003) Influences of upland and riparian land use patterns on stream biotic integrity. Landsc Ecol 18:647–664
9. Benejam L, Aparicio E, Vargas MJ et al (2008) Assessing fish metrics and biotic indices in a Mediterranean stream: effects of uncertain native status of fish. Hydrobiologia 603:197–210
10. Sandøy S, Langåker RM (2001) Atlantic salmon and acidification in southern Norway: a disaster in the 20th century, but a hope for the future? Water Air Soil Pollut 130:1343–1348
11. Peterson JT, Thurow RF, Guzevich JW (2004) An evaluation of multipass electrofishing for estimating abundance of stream-dwelling salmonids. Trans Am Fish Soc 133:462–475
12. Rosenberger AE, Dunham JB (2005) Validation of abundance estimates from mark-recapture and removal techniques for rainbow trout captured by electrofishing in small streams. N Am J Fish Manag 25:1395–1410
13. Hickey MA, Closs GP (2006) Evaluating the potential of night spotlighting as a method for assessing species composition and brown trout abundance: a comparison with electrofishing in small streams. J Fish Biol 69:1513–1523
14. Dolan C, Miranda L (2003) Immobilization thresholds of electrofishing relative to fish size. Trans Am Fish Soc 132:69–976
15. Mäntyniemi S, Romakkaniemi A, Arjas E (2005) Bayesian removal estimation of a population size under unequal catchability. Can J Fish Aquat Sci 62:291–300
16. Plafkin JL, Barbour MT, Gross SK et al (1989) Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish, EPA 444/4-89-001. U.S. Environmental Protection Agency, Washington, DC, 171 pp
17. MacDonald LH, Smart AW, Wissmar RC (1991) Monitoring guidelines to evaluate effects of forestry activities on streams in the Pacific northwest and Alaska. EPA 910/9-91-001. U.S. Environmental Protection Agency, Seattle, 166 pp
18. Ralph SC, Cardoso T, Poole CG et al (1992) Status and trends of instream habitat in forested lands of Washington: the Timber, Fish, and Wildlife ambient monitoring project-1989–1991. Biennial progress report, University of Washington, Center for Streamside Studies Report to the Washington Department of Natural Resources, Olympia Washington
19. Roper BB, Scarnecchia DL (1995) Observer variability in classifying habitat types in stream surveys. N Am J Fish Manag 15:49–53
20. Wang L, Simonson TD, Lyons J (1996) Accuracy and precision of selected stream habitat estimates. N Am J Fish Manag 16:340–347

21. Roper BB, Kershner JL, Archer E et al (2002) An evaluation of physical stream habitat attributes used to monitor streams. J Am Water Resour Assoc 38:1637–1646
22. Whitacre HW, Roper BB, Kershner JL (2007) A comparison of protocols and observer precision for measuring physical. Stream attributes. J Am Water Resour Assoc 43:923–937
23. Sostoa A, Caiola N, Casals F et al (2010) Adjustment of the index of biotic integrity (IBICAT) based on the use of fish as indicators of the environmental quality of the rivers of Catalonia (in Catalan) Agència Catalana de l'Aigua, Departament de Medi Ambient i Habitatge, Generalitat de Catalunya, Barcelona (in Catalan) 187 pp, http://doi.org/10.13140/2.1.1551.6964. Accessed 24 Mar 2015
24. Benejam L, Alcaraz C, Benito J et al (2012) Fish catchability and comparison of four electrofishing crews in Mediterranean streams. Fish Res 123:9–15
25. Penczak T (1985) Influence of site area on the estimation of the density of fish populations in a small river. Aquac Res 16:273–285
26. Meador MR, McIntyre JP, Pollock KH (2003) Assessing the efficacy of single-pass backpack electrofishing to characterize fish community structure. Trans Am Fish Soc 132:39–46
27. Penczak T, Głowacki Ł (2008) Evaluation of electrofishing efficiency in a stream under natural and regulated conditions. Aquat Living Resour 21:329–337
28. Sályl P, Erős T, Takács P et al (2009) Assemblage level monitoring of stream fishes: the relative efficiency of single-pass vs. double-pass electrofishing. Fish Res 99:226–233
29. Vehanen T, Sutela T, Jounela P et al (2013) Assessing electric fishing sampling effort to estimate stream fish assemblage attributes. Fish Manag Ecol 20:10–20
30. Pritt JJ, Frimpong EA (2014) The effect of sampling intensity on patterns of rarity and community assessment metrics in stream fish samples. Ecol Indic 39:169–178
31. Specziár A, Takács P, Czeglédi I et al (2012) The role of the electrofishing equipment type and the operator in assessing fish assemblages in a non-wadeable lowland river. Fish Res 125:99–107
32. Lyons J (1992) The length of stream to sample with a towed electrofishing unit when fish species richness is estimated. N Am J Fish Manag 12:198–203
33. Hughes RM, Kaufmann PR, Herlihy AT et al (2002) Electrofishing distance needed to estimate fish species richness in raftable Oregon rivers. N Am J Fish Manag 22:1229–1240
34. Meador MR (2005) Single-pass versus two-pass boat electrofishing for characterizing river fish assemblages: species richness estimates and sampling distance. Trans Am Fish Soc 134:59–67
35. Hughes RM, Herlihy AT (2007) Electrofishing distance needed to estimate consistent index of biotic integrity (IBI) scores in raftable Oregon rivers. Trans Am Fish Soc 136:135–141
36. Maret TR, Ott DS, Herlihy AT (2007) Electrofishing effort required to estimate biotic condition in southern Idaho rivers. N Am J Fish Manag 27:1041–1052
37. Fisher JR, Paukert CP (2009) Effects of sampling effort, assemblage similarity, and habitat heterogeneity on estimates of species richness and relative abundance of stream fishes. Can J Fish Aquat Sci 66:277–290
38. Palmer MW (1991) Estimating species richness: the second-order jackknife reconsidered. Ecology 72:1512–1513
39. Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York
40. Barbour MT, Stribling JB, Gerritsen J, Karr JR (1996) Biological criteria: technical guidance for streams and small rivers–revised edition. EPA 822-B-96-001. U. S. Environmental Protection Agency, Washington, DC
41. Karr JR, Fausch KD, Angermeier PL et al (1986) Assessing biological integrity in running waters. A method and its rationale. Illinois Natural History Survey, Champaign, Special Publication, 5
42. Ball J (1982) Stream classification guidelines for Wisconsin. Wisconsin Department of Natural Resources Technical Bulletin. Wisconsin Department of Natural Resources, Madison, Wisconsin

43. OHIO EPA (1987) Biological criteria for the protection of aquatic life: volumes I-III. Ohio EPA, Division of Water Quality Monitoring and Assessment, Surface Water Section, Columbus, Ohio
44. Resh VH, Norris RH, Barbour MT (1995) Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. Aust J Ecol 20:108–121
45. Hannaford MJ, Barbour MT, Resh VH (1997) Training reduces observer variability in visual-based assessments of stream habitat. J N Am Benthol Soc 16:853–860
46. Barbour MT, Gerritsen J, Snyder BD et al (1999) Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, 2nd edn. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, DC
47. Murphy CA, Casals F, Solà C et al (2013) Efficacy of population size structure as a bioassessment tool in freshwaters. Ecol Indic 34:571–579
48. Oberdorff T, Pont D, Hugueny B et al (2001) A probabilistic model characterizing riverine fish communities of French rivers: a framework for environmental assessment. Freshw Biol 46:399–415
49. Roset N, Grenouillet G, Goffaux D et al (2007) A review of existing fish assemblage indicators and methodologies. Fish Manag Ecol 14:393–405
50. Logez M, Pont D (2013) Global warming and potential shift in reference conditions: the case of functional fish-based metrics. Hydrobiologia 704:417–436
51. Karr JR, Chu EW (1998) Restoring life in running waters: better biological monitoring. Island Press, Washington, DC
52. Oberdorff T, Pont D, Hugueny B et al (2002) Development and validation of a fish-based index for the assessment of "river health" in France. Freshw Biol 47:1720–1734
53. Pont D, Hugueny B, Beier B et al (2006) Assessing river biotic condition at a continental scale: a European approach using functional metrics and fish assemblages. J Appl Ecol 43:70–80
54. Pont D, Hugueny B, Rogers C (2007) Development of a fish-based index for the assessment of river health in Europe: the European Fish Index. Fish Manag Ecol 14:427–439
55. Logez M, Pont D (2011) Development of metrics based on fish body size and species traits to assess European coldwater streams. Ecol Indic 11:1204–1215
56. Hoeinghaus DJ, Winemiller KO, Birnbaum JS (2007) Local and regional determinants of stream fish assemblage structure: inferences based on taxonomic vs. functional groups. J Biogeogr 34:324–338
57. Lamouroux N, Poff NL, Angermeier PL (2002) Intercontinental convergence of stream fish community traits along geomorphic and hydraulic gradients. Ecology 83:1792–1807
58. Goldstein RM, Meador MR (2004) Comparisons of fish species traits from small streams to large rivers. Trans Am Fish Soc 133:971–983
59. Statzner B, Dolédec S, Hugueny B (2004) Biological trait composition of European stream invertebrate communities: assessing the effects of various trait filter types. Ecography 27:470–488
60. Bonada N, Doledec S, Statzner B (2007) Taxonomic and biological trait differences of stream macroinvertebrate communities between Mediterranean and temperate regions: implications for future climatic scenarios. Glob Chang Biol 13:1658–1671
61. Logez M, Pont D, Ferreira MT (2010) Do Iberian and European fish faunas exhibit convergent functional structure along environmental gradients? J N Am Benthol Soc 29:1310–1323
62. Wright JF (1995) Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. Aust J Ecol 20:181–197
63. Hawkins CP, Olson JR, Hill RA (2010) The reference condition: predicting benchmarks for ecological and water-quality assessments. J N Am Benthol Soc 29:312–358
64. Osenberg CW, Schmitt RJ, Holbrook SJ et al (1994) Detection of environmental impacts: natural variability, effect Size, and power analysis. Ecol Appl 4:16–30
65. García-Charton JA, Pérez-Ruzafa Á (2001) Spatial pattern and the habitat structure of a Mediterranean rocky reef fish local assemblage. Mar Biol 138:917–934

66. Karr JR (1981) Assessment of biotic integrity using fish communities. Fisheries 6:21–27
67. ACA (Agència Catalana de l’Aigua) (2005) Caracterització de masses d’aigua i anàlisi del risc d’incompliment dels objectius de la directiva marc de l’aigua (2000/60/CE) a Catalunya (conques intra i intercomunitàries) en compliment als articles 5, 6 i 7 de la directiva, http://aca-web.gencat.cat/aca/appmanager/aca/aca?nfpb=true& pageLabel=P1206154461208200586461. Accessed 30 May 2013
68. Pont D, Hugueny B, Roset N, Rogers C (2004) Development, evaluation & implementation of a standardised fish-based assessment method for the ecological status of European rivers - a contribution to the Water Framework Directive (FAME). Final report, WP6-8, 59 s
69. Degerman E, Beier U, Breine J et al (2007) Classification and assessment of degradation in European running waters. Fish Manag Ecol 14:417–426
70. Grenouillet G, Roset N, Goffaux D et al (2007) Fish assemblages in European Western Highlands and Western Plains: a type-specific approach to assess ecological quality of running waters. Fish Manag Ecol 14:509–517
71. EFI+ Consortium (2009) Manual for the application of the new European Fish Index – EFI+. A fish-based method to assess the ecological status of European running waters in support of the Water Framework Directive. June 2009. BOKU, Vienna, 45 pp. http://efi-plus.boku.ac.at
72. Trautwein C, Schinegger R, Schmutz S (2013) Divergent reaction of fish metrics to human pressures in fish assemblage types in Europe. Hydrobiologia 718:207–220
73. Munné A, Prat N (2004) Defining river types in a Mediterranean area. A methodology for the implementation of the EU Water Framework Directive. Environ Manag 34(5):711–729
74. Munné A, Prat N (2011) Effects of Mediterranean climate annual variability on stream biological quality assessment using macroinvertebrate communities. Ecol Indic 11:651–662
75. Munné A. Prat N (1998) Delimitación de regiones ecológicas en la cuenca del Ebro. Asisténcia técnica 1998-PH-08-I. Confederación Hidrográfica del Ebro. Zaragoza. 153 pp (in Spanish)
76. MMA (Ministerio de Medio Ambiente) (2005) Caracterización de los tipos de ríos y lagos. Versión 4.0. Ministerio de Medio Ambiente, Madrid. 251 p
77. MARM (Ministerio de Medio Ambiente, y Medio Rural y Marino) (2008) Orden ARM/2656/2008, de 10 de septiembre, por la que se aprueba la instrucción de planificación hidrológica. BOE 229:38472–38582
78. Benejam L, Angermeier PL, Munné A, García-Berthou E (2010) Assessing effects of water abstraction on fish assemblages in Mediterranean streams. Freshw Biol 55:628–642
79. Segurado P, Caiola N, Pont D, Oliveira JM, Delaigue O, Ferreira MT (2014) Comparability of fish-based ecological quality assessments for geographically distinct Iberian regions. Sci Total Environ 476:785–794