A novel approach to an ecofunctional fish index for Mediterranean countries

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Abstract

The implementation of the European Water Framework Directive, especially regarding the establishment of fish indexes for riverine habitats, has taken different paths in different countries. For example, in Italy previous efforts have been directed towards a taxonomy-based index, contrarily to most other European countries where an ecofunctional approach took place. Taxonomical indexes are particularly hard to apply to Mediterranean countries, where fish taxonomy is often revised causing problems in practical implementation. Alternatively, ecofunctional characteristics of fish communities could be exploited to inform on river habitat quality and to detect anthropogenic impacts, thus reducing the index sensitivity to the taxonomical variability of the fish fauna. We therefore proposed a new, multimetric index based on ecofunctional traits of fish species (EFFI, EcoFunctional Fish Index) and tested it on 208 river sampling stations of the Emilia-Romagna region, northern Italy. Using theoretical reference communities, ecological quality ratios were estimated for the whole area expressing the ecological distance of each site from reference conditions. Perhaps unsurprisingly, this work underlined how fish communities were more degraded at lower altitudes than at higher ones. EFFI scores were remarkably close to two already-established indexes for chemical (LIM) and macrozoobenthos communities (IBE) alteration. Further work should explore the validity of this approach over a wider geographical range as well as investigate the definition of environmental class boundaries and its potential intercalibration with other indexes.

Keywords: ecological indicators; ecological niches; environmental status; WFD

1. Introduction

Fish can be readily used as indicators of aquatic environmental status, as their communities are sensitive to habitat quality and because they respond to anthropogenic pressures such as pollution, eutrophication or habitat alteration (Fausch, Lyons, Karr, & Angermeier, 1990). Based on this characteristic, several indexes have been developed through the years with a variety of approaches (Schmutz, Cowx, Haidvogl, & Pont, 2007). The general aim of these indexes is to provide a measure that summarizes a complex ecosystem and to allow an evaluation of the condition of the environment (Whitfield & Elliott, 2002). A variety of approaches are available to the investigators, but most indexes follow Karr's Index of Biotic Integrity (1981) and use multimetric indexes, exploiting either historical information (Kleynhans, 1999) or relatively undisturbed reference conditions to measure the effects of anthropogenic impacts (Bailey, Kennedy, & Dervish, 1998).

In Europe, directive 2000/60/EC, more commonly known as the Water Framework Directive (WFD), sets indications in its Annex B to build indexes for several biological and chemical parameters of European rivers (EU, 2000). According to these indications, species composition and abundance, as well as age structure of the fish community, should be taken into account when building an index for riverine habitats. WFD has slowly been transposed to national legislation of Member States (e.g. in Italy, with legislative decree 152/06) but several difficulties, mainly related to a lack of systemic approach, were encountered during the implementation of such legislations (Voulvoulis, Arpon, & Giakoumis, 2017) and several different approaches have been elaborated (Birk et al., 2012). Accordingly, the EU has funded research efforts to jointly address the problems that arose in defining indexes: a prime example of these efforts was the FAME consortium, led by France and including a total of 12 EU countries, which developed the European Fish Index (EFI), an index that exploits some ecological characteristics of fish assemblages to infer ecological status (Pont et al., 2006). However, in some countries that were not partners of the FAME consortium, the work on fish indexes has taken a rather different path.

In Italy, for example, two indexes based on taxonomy rather than ecological functionality have been proposed (Forneris, Merati, Pascale, & Perosino, 2004; Zerunian, 2004). Taxonomical indexes measure the deviation of the fish community from a reference community, effectively informing on the fish community status, but focus entirely on the taxonomical units. In Mediterranean countries, where the vast majority of rivers host communities which are altered by anthropogenic actions and conservation biology has been turned into environmental management, a taxonomy-based index poses two major challenges. First of all, the index needs to be continuously revised, as taxonomy is an ever-shifting ground where consensus is hard to reach, particularly in areas rich in endemism (the taxonomy of trouts in Italy is a prime example of such hard-to-resolve controversies, see e.g. Zanetti (2017)). Secondarily, and more generally, freshwaters are impacted also at the taxonomical level, therefore multimetric indexes based on taxonomy tend to assign much lower scores to sites which would be otherwise ecologically sound but host an altered fish community (i.e. host a number of exotic species, often as a result of human-mediated dispersion or intentional management).

Exotic species do constitute a major problem in the Mediterranean region (Bianco & Ketmaier, 2015; Crivelli, 1995) and have been suggested to drive the local extinction of fish species (Castaldelli et al., 2013; Dias et al., 2017). However, not all exotic species are equally capable of altering the habitat they live in or the fish communities they interact with so their relevance for environmental assessment purposes can vary. Furthermore, even though some exotic species (especially successful invaders) are broad generalists, most have their own ecological niches and tolerances which can be exploited to inform on the environmental status of the rivers, similarly to native species.

It has been argued that establishing an ecofunctional index for Mediterranean countries could be extremely challenging (e.g. Pont et al., 2006; Zerunian, Goltara, Schipani, & Boz, 2009)), due to the lack of ecological information on several endemic species. Following the work by Aarts and Nienhuis (2003), Welcomme, Winemiller, and Cowx (2006), Pont et al. (2006) and Noble, Cowx, Goffaux, and Kestemont (2007), we argue that an ecofunctional index, if feasible, could provide significant advantages and inform on the status of both the environment and the fish community. If ecofunctional classes are broad enough, species-specific differences would be downplayed in favor of broad genus or family differences, thus providing more information on the river environmental status and the fish community health compared to a taxonomical indicator. An indicator based on ecofunctional characteristics of fish communities would be most informative on anthropogenic pressures such as hydrological alterations (water flow regulation and migration barriers), chemical and nutrient alterations (pollution and eutrophication), habitat alteration (e.g. changes in spawning substrate) and fisheries (both fisheries pressure and introduction of species (e.g. for recreational fisheries).

This study aimed to define a novel approach to define an ecofunctional fish index for the Mediterranean region, utilizing available information on fish species to assess the status of river stretches. We build a new multimetric index that uses information on fish communities' composition and relative abundance to compare reference and current conditions. This EcoFunctional Fish Index (EFFI) was tested on a dataset of 208 river sampling stations in the Emilia-Romagna region of northern Italy and compared to two already-established indexes for chemical and macroinvertebrate community alteration to preliminarily explore its degree of response to anthropogenic pressures.

2. Materials & Methods

2.1 Ecological functions

A number of ecological functions have been selected to compose the index, following up on the work by Noble et al. (2007). The criteria for selection were dual: ecological functions must cover the available information on species but also have to be relevant for the purpose of inferring the river environmental status.

The ecological functions selected were: Feeding (based on prevalent diet), Reproduction (based on preferred reproduction substrate), Migration (based on the range of movement of the species), Tolerance (to low oxygen or high temperature), Habitat (based on preferred habitat), Native Biodiversity (based on the native/exotic status, and on the potential of the species to alter the fish community or the environment itself).

The different ecological functions inform on fish community status (e.g. Feeding or Native Biodiversity functions, which inform on the community trophic composition and on the potential of species to alter it, respectively) and river habitat ecological status (e.g. Reproduction or Migration guilds, which inform on the available substrates and the habitat fragmentation) with the aim of recording anthropogenic impacts on these components of the ecosystem.

2.2 Ecofunctional guilds

Each ecological function was divided into guilds that would detail characteristics by which single species could be scored, which also followed largely the work of Noble et al. (2007). As with ecological functions, guilds were defined based on their ability to inform on the status of the environment and the availability of information for fish species. For instance, in the Tolerance ecological function, guilds were chosen based on their ability to inform on the river fluctuations of oxygen and temperature or, in the habitat ecological function, to inform on the river current strength and turbidity. All these parameters are affected by anthropogenic disturbances such as nutrient pollution and eutrophication, thermal pollution, damming and water abstraction, and watershed erosion, respectively.

In the feeding ecological function, as most fish species have rather wide trophic niches and exhibit ontogenetic diet shifts, we considered the prevalent diet of adult individuals for the definition of guilds. Fish were divided into planktivores (exhibiting specific adaptations for plankton filtering, such as gill rakers), herbivores (exhibiting specific adaptations for plant feeding, such as pharyngeal teeth), benthivores (exhibiting specific adaptations for bottom feeding, such as downturned mouths or barbels), invertivores (specifically adapted to or predating prevalently on insects and other invertebrates), piscivores (with specific adaptations for feeding largely on fish), parasites (ematophages, limited to lampreys in Italian waters) and generalists (with unspecialized mouthparts and digestive systems, feeding on a broad range of items).

In the reproduction ecological function, fish were assigned to one guild, separated into lithophils (spawning on stones and gravel), phytophils (spawning on submersed vegetation), phytolithophils (spawning both on stones and vegetation), psammophils (spawning on sand or mud), ostracophils (spawning in molluscs), pelagophils or live breeding (pelagic spawners or live spawners) and polyphils (generalist spawners).

In the migration ecological function, guilds were based on the range of movement reported in literature for the species. This included both ranging movements during feeding/life history and spawning migrations. The guilds included short (within the river zones), medium (up and downstream or into flooded areas) and long (true anadromous and catadromous species) ranges of movement.

In the tolerance ecological function, fish species were divided into two mutually exclusive guilds of tolerance/intolerance to low oxygen (indicatively below 3 ppm) and to high temperature (indicatively above 20 ˚C), based on available information.

In the habitat ecological function, fish species were divided into two broad guilds based on current speed and water transparency. Within the first guild, fish were either identified as rheophils (preferring fast flowing water), limnophils (preferring slow or no current) or eurytopic (having no particular preference). Within the second guild, fish were either adapted to clear water, turbid waters or adaptable to a wide range of water turbidity.

In the Native Biodiversity ecological function, fish were divided in mutually exclusive native and exotic (i.e. introduced by human action, irrespective of time) guilds. Exotic species capable of modifying the environment or fish communities were also assigned to a separate guild. Additional remarks in the last column of the matrix (Supplementary Table 1) further detail whether some species native to the national territory have been introduced in areas where they were not formerly present, so that this can be accounted for in specific hydrographic areas within Italy.

Supplementary Table 1 – A full species matrix, including proposed ecological functions and guilds, as well as body classes for each species, is supplied in excel format due to its large size (100 species * 6 ecological functions with 3 or more guilds each).

Furthermore, exotic species that are capable of altering the ecosystem (e.g. common carp, *Cyprinus carpio*, or crucian carp, *Carassius carassius*, increasing turbidity and nutrients through their benthic feeding, Richardson, Whoriskey, and Roy (1995), or grass carp *Ctenopharyngodon idella*, reducing aquatic macrophytes through its herbivorous diet, Shireman and Smith (1983)) or the fish community (e.g. wels catfish, *Silurus glanis*, a large top predator capable of altering fish communities, (Carol, Benejam, Benito, and García-Berthou (2009); Castaldelli et al. (2013))) were separated from species that have low or no impact into another guild. It could be argued that historically introduced and subsequently naturalized species can play an important role in riverine ecosystems as native species (Noble et al., 2007); however, there is no clear and widely accepted time boundary to identify these species (i.e. are 70 years enough to qualify?). Furthermore, when these species are ecosystem or fish community engineers they can induce a change in the habitat of all other species (i.e. higher turbidity or no vegetation) or directly in the biotic community (i.e. local decrease or even extinction of other species) and these changes would persist in time as long as the species are present. Species without these capabilities might still have an impact on singe fish species (e.g. genetic hybridization, displacement through competition) or other components of the ecosystem (e.g. amphibians) but do not change the ecofunctional composition of the community or its habitat, thereby affecting the indicator to a lesser extent.

Each fish species currently present in Italian rivers was assigned to guilds within ecological functions, based on the information from continuously updated online databases such as FishBase (Froese & Pauly, 2017) or Freshwaterecology.info (Schmidt-Kloiber & Hering, 2015), or through peer-reviewed papers when available. When no information was available, expert knowledge was used to fill the gaps, usually assuming that the species would share ecofunctional characteristics with the closest related species for which information was available.

2.3 Scoring principles

Each guild was assigned a score ranging from 0.1 to 1, with the criterion that higher scores would be assigned to guilds that provide the most useful information on environmental status and that reflect higher quality conditions. For example, if a species exhibits long-range migration patterns, its presence indicates that a low degree of habitat fragmentation occurs in the site and thus a score of 1 is assigned to the species that fall within the long-range migration guild. On the contrary, a generalist spawner does not provide much information on the substrate present in the river habitats; therefore the score for species falling within this guild was set to 0.1.

Each ecological function was assigned a weight score to form a total of 5, with the criterion that higher weights would be assigned to ecological functions that were most informative on environmental status (Table 1).

Table 1 – Ecological functions and their guilds with respective weights. The last column offers some examples of species which were assigned to the guilds.

Ecological Function Weight	Ecological function	Explanation	Guild	Guild score	Explanation	Examples
0.5	Feeding	Based on prevalent diet	Planktivores	0.1	Zoo or phytoplankton filterers	*Hypophthalmichthys nobilis/molitrix*
			Herbivores	0.1	Plant feeders	*Ctenopharyngodon idella*
			Benthivores	0.1	Bottom feeders	Cyprinus carpio
			Invertivores	0.75	Mid-water to surface invertebrate feeders	Gambusia affinis, Tinca tinca
			Piscivores	0.75	Over 75% of diet based on other fish	*Esox sp., Silurus glanis*
			Parasites	1	Parasitic feeding	*Petromyzon sp.*
			Generalists	0.25	Unspecialized feeders	*Rutilus sp., Scardinius sp.*
1	Reproduction	Based on preferred reproduction substrate	Lithophils	1	Spawning on stones and gravel	*Barbus sp., Salmo sp.*
			Phytophils	1	Spawning on vegetation	*Esox sp., Perca fluviatilis, Cyprinus carpio*
			Phytolithophils	0.5	Spawning both on stones and vegetation	*Abramis brama* *Micropterus salmoides*
			Psammophils	0.25	Spawning on sand or mud	*Sander lucioperca*
			Ostracophils	0.25	Spawning in molluscs	*Rhodeus sp.*
			Pelagophils or live breeding	0.1	Pelagic spawners or live spawners	*Hypophthalmichthys nobilis/molitrix*
			Polyphils	0.1	Generalist spawners	
0.95	Migration	Range of movement of the species	Short	0.25	Short or very short migrations within the river zones	*Scardinius sp.*
			Intermediate	0.5	Intermediate migration (e.g. considerably up or downstream, from river to flooded areas)	*Esox sp., Abramis brama, Barbus sp.*

			Long (anadromous or catadromous)	1	Long migration, to the sea and back or vice-versa	*Anguilla anguilla, Acipenser sp.*
0.7	Tolerance	Tolerance to low oxygen or high temperature	Low oxygen tolerant or unknown	0.25		*Cyprinus carpio*
			Low oxygen intolerant	0.5		*Salmo sp.*
			High temperature tolerant or unknown	0.25		*Cyprinus carpio*
			High temperature intolerant	0.5		*Salmo sp.*
1.25	Habitat	Preferred habitat	Rheophils	0.5	Fast current	*Abramis brama*
			Limnophils	0.1	Slow or no current	*Squalius sp.*
			Eurytopic	0.25	Adaptable to various current regimes	
			Clear water	0.5		*Esox sp., Salmo sp., Perca fluviatilis*
			Turbid water	0.1		*Sander lucioperca, Ictalurus spp.*
			Wide range of conditions	0.25		
0.6	Native biodiversity	Native/exotic species and their impact	Native	0.5		
			Exotic	0.25		
			Non altering	0.5		
			Altering	0.25	Ecosystem engineering or fish community impact capabilities	*Ctenopharyngodon idella, Silurus glanis*

For example, ecological functions such as Spawning and Migration provide more information on the type of substrate present and on the river connectivity and were assigned higher weights than Feeding or Native Biodiversity, which depend more on and affect/inform less on the environment.

2.4 Index score calculations

A complete and updated list of fish species present in Italian freshwaters, including the matrix of guilds for each species, was created (Supplementary Table 1). Each guild translates directly to a score as outlined in Table 1, so that each species has a set of scores (called species score thereafter). To produce an estimate of the relative abundance of each species in each site, species-specific mass proportions need to be accounted for, as different species have different body sizes. Thus, we assigned species to one of three body-size classes based on their average size (1 = small body up to ~150 g; 2 = medium body ~150-400 g; 3 = large body over ~400g) which were then multiplied by abundance classes (i.e. classes of number of individuals) to obtain an abundance value corrected for mass (i.e. the relative abundance). Following the principle that abundant species are more informative on environmental status than species that occur in low numbers, each species score is then multiplied by the species' relative abundance in the site. This forms a so-called site-specific species score (Equation 1).

$$\textit{Site-specific species scores} = \textit{species score X relative abundance} \qquad (1)$$

As the indicator focuses on the global ecofunctional characteristics of the fish population, the next step involves grouping site-specific scores based on the ecological functions. The sum of all site-specific species scores for each ecological function, forms the site-specific ecological function score (Equation 2).

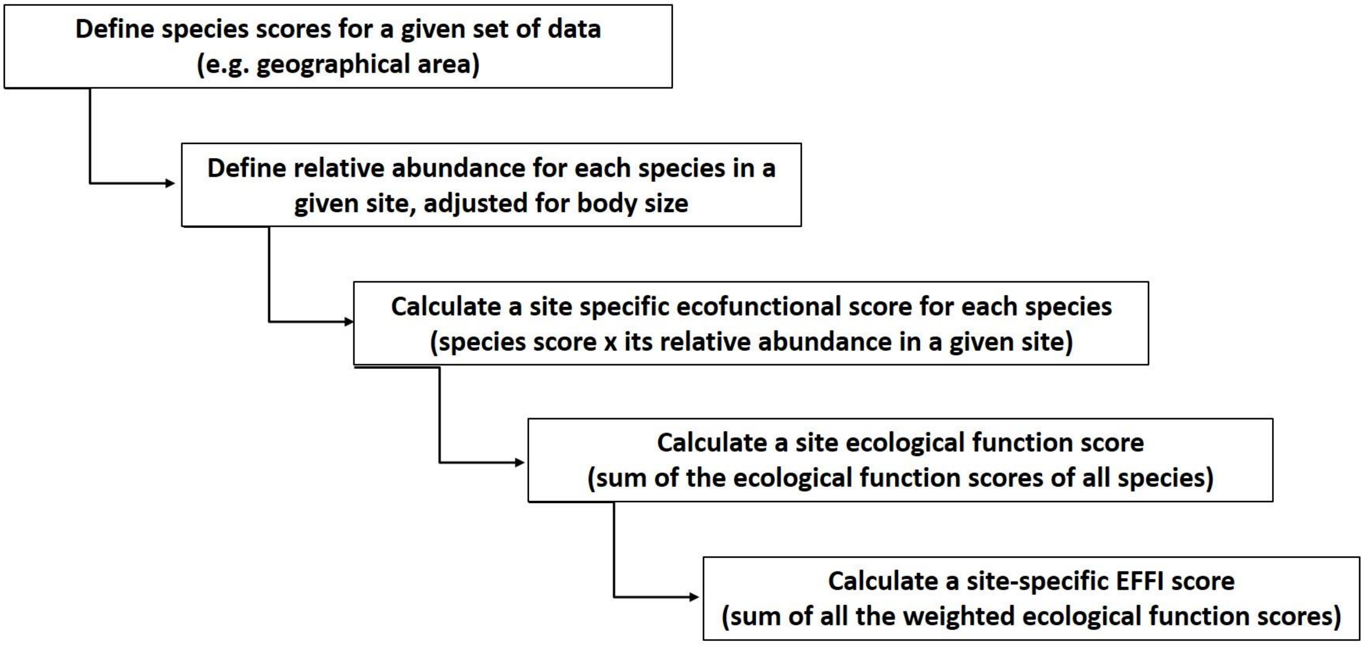
$$\textit{Site-specific ecological function score} = \sum site\text{-}specific\ species\ scores \qquad (2)$$

To adjust for their relative importance towards the final index score, ecological functions have been assigned different weights (Table 1) based on their relative information contribution on the environmental status. The sum of all ecological functions scores and their relative weight, a number that theoretically ranges between 0 and 5, is the EFFI final score (Equation 3).

$$\textit{EFFI score} = \sum site\text{-}specific\ ecological\ function\ score\ X\ ecological\ function\ weight \qquad (3)$$

A summary of the steps needed for the calculation of the index is provided in Figure 1 and an example of score calculation is provided in Supplementary Table 2.

Figure 1 – Stepwise flowchart of index scores calculations

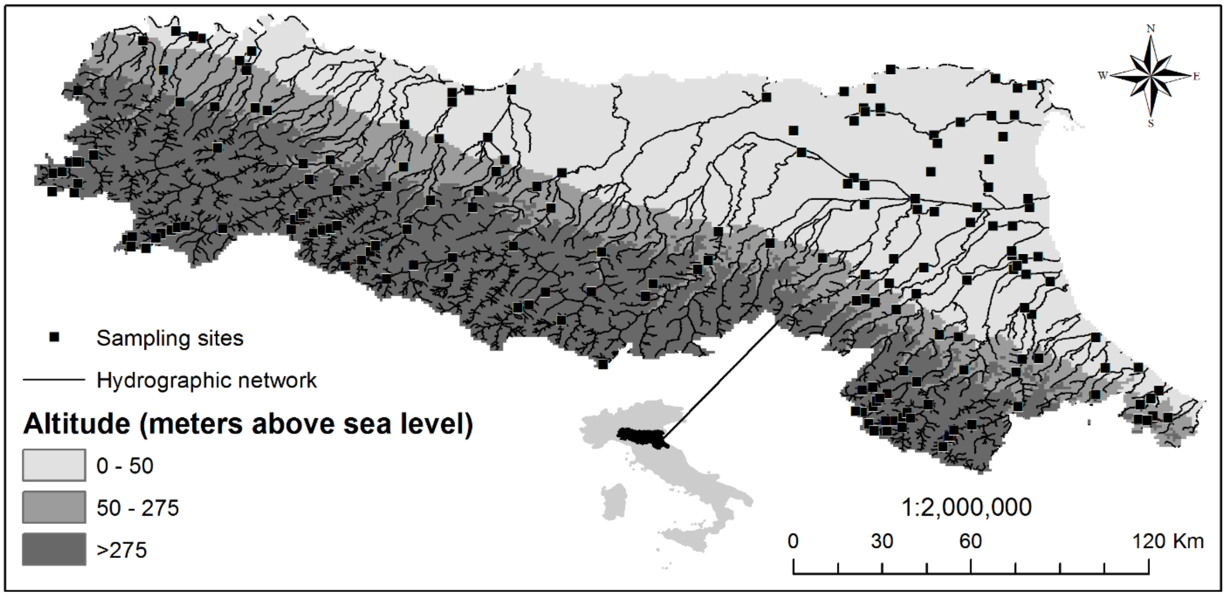


Supplementary Table 2 – Step-by-step practical example of the EFFI score calculation for one site.

2.5 Index score calculation and reference conditions

To cross-validate the EFFI we used a dataset of 208 fish community sampling stations in the Emilia-Romagna region (Northwest Italy, Figure 1). These included river waters over a wide range of stream velocities and altitudes, with different land uses and catchment areas.

Figure 2 – Map of Emilia-Romagna region and its location in the Italian peninsula. Markers indicate the position of the 208 sampling sites used in this study. Shading indicates altitudinal changes within the territory.



This dataset was chosen because it spans the whole spectrum from high-altitude streams to lowland semi-artificial waterways, therefore offering a nearly complete picture of all river types present in Northern Italy and being representative of flowing water bodies found throughout the Mediterranean region. However, this index was not meant to cover transitional waters or lakes, for which an adaptation and further testing of the concept might be needed, but solely riverine habitats.

The dataset included information on the site location and main physical and chemical parameters (e.g. Nitrogen and Phosphorus concentration, BOD, COD, temperature and pH). Sampling covered a rather large timespan, ranging from 1997 to 2005, and was the result of cumulative efforts to map the fish communities of the region. 39% of the sampling (82 sites) occurred post 2002, whereas the remaining 61% (126 sites) occurred between 1997 and 2002. Fish sampling was performed for each site on a 50 m stretch of the river, using multiple passes of a pulsed DC electrofisher according to a standardized survey methodology defined nationally and adopted in the region (Regione Emilia Romagna, 2008). Additionally, traps and trammel nets were used in larger and deeper rivers (about 30% of the sites) to verify the data collected through electrofishing.

Site-specific fish abundances were recorded in Moyle classes (Moyle & Nichols, 1973), which represent the abundance of individuals of each fish species in a given river stretch. Moyle classes range from 1 (low abundance, 1-2 individuals per site) to 5 (high abundance, >50 individuals per site). Site-specific scores were then calculated for each sampling station in order to derive EFFI scores.

Ideally, fish communities in sites that are (to a certain degree) free of anthropogenic impacts should be taken as reference. However, due to the long history of anthropogenic modification in Emilia Romagna (spanning literally millennia), no sites in the area used for cross-validation were suitable for such an assessment, which is a typical problem for Mediterranean countries. Reference conditions had to be thus set theoretically, using reference fish communities derived from the literature (i.e. revising those identified by Zerunian et al., 2009) and unpublished historical data to define the relative abundances of species composing these communities. We thus defined reference fish communities for the Emilia Romagna region, using all the available existing knowledge on undisturbed communities. We also used altitude and hydromorphological characteristics to identify the most appropriate reference community for each site.

Theoretically derived reference conditions were used to express the EFFI index in terms of Ecological Quality Ratio (EQR EFFI), defined as the ratio between parameter values (EFFI scores of actual communities) and the reference value (EFFI scores of reference communities) thus exploring the distance of sites from reference.

2.6 Correspondence with other indicators

Yearly LIM (1993–2002) and IBE (1997–2005) scores were available for nearly each site (LIM n = 200, IBE n = 191, sites without a measure of either index n = 8). LIM (*Livello di Inquinamento da Macrodescrittori*, Pollution Level from Macro-descriptors, in English) measures the environmental status based on the concentration of 7 different parameters representative of the chemical status of the water, sampled at monthly intervals (national legislative decree 152/99). Among these parameters, organic matter, phosphorus and nitrogen dissolved compounds are also measured, therefore LIM does not only measure chemical pollution, but provides also a measure of the eutrophication level. IBE (*Indice Biotico Esteso*, Extended Biotic Index, in English) is an index that uses macroinvertebrate communities (and their deviation from a reference) to measure the environmental status.

Originally developed by Ghetti (1997), IBE has been further modified by APAT-IRSA and CNR (2003). Both LIM and IBE have been nationally adopted for classification of all bodies of water according to the WFD and have been widely used in the peninsula. Their combination, known as SECA (*Stato Ecologico dei Corsi d'Acqua*, River Ecological status, in English), is used as a measure of environmental status for the WFD (sanctioned in national legislative decree 152/2006) and consists of the worst class of the two.

We selected these indexes as a proxy of environmental status for each site and, to validate the fish index, EFFI scores were calculated for each site and then compared to average LIM and IBE scores with the aim to check for coefficients of determination (performing linear regressions) and correlations (performing Spearman rank order tests). As in a few sites LIM measures did not temporally overlap with fish sampling, we checked through a rank sum test that correlation residuals would not differ between sites with and sites without temporal overlap.

3. Results

The extensive review of all available information produced an updated list of fish species present in Italian rivers (100 species), of which 45 were introduced species (Supplementary Table 1). Nearly all species were exclusive of freshwaters, but three species typical of transitional waters (Flathead grey mullet *Mugil cephalus*, Thinlip mullet *Liza ramada* and European flounder *Platichthys flesus*) were also included as they can be sometimes found upstream well beyond the transitional water limit. Some species were excluded from the list because locally extinct (e.g. huchen *Huco hucho*), of dubious taxonomy (i.e. the Volturno spined loach *Cobitis zanandreai*), misreported presence (i.e. yellow bullhead *Ameiurus natalis*, redbreast sunfish *Lepomis auritus*), or because present only in lakes as a result of species introduction (e.g. whitefish *Coregonus* spp., lake trout *Salvelinus namaycush*). Information on nearly all species ecofunctional features was present in online databases; information was lacking for recently established species (e.g. southern pike *Esox cisalpinus*) for which it was borrowed from the closest related taxon. It was thus possible to assign each species to guilds for each ecological function as detailed in the matrix provided in Supplementary Table 1.

3.2 Index score calculation and reference conditions

Based on the ecological functions and guilds matrix, scores were assigned to species sampled In Emilia Romagna and EFFI scores were calculated for each sampling site (see also Supplementary Table 2). A total of 5 reference fish communities were defined for the Emilia Romagna region (Table 2). These communities covered all the water types present in the region, from upper highland streams to lowland rivers.

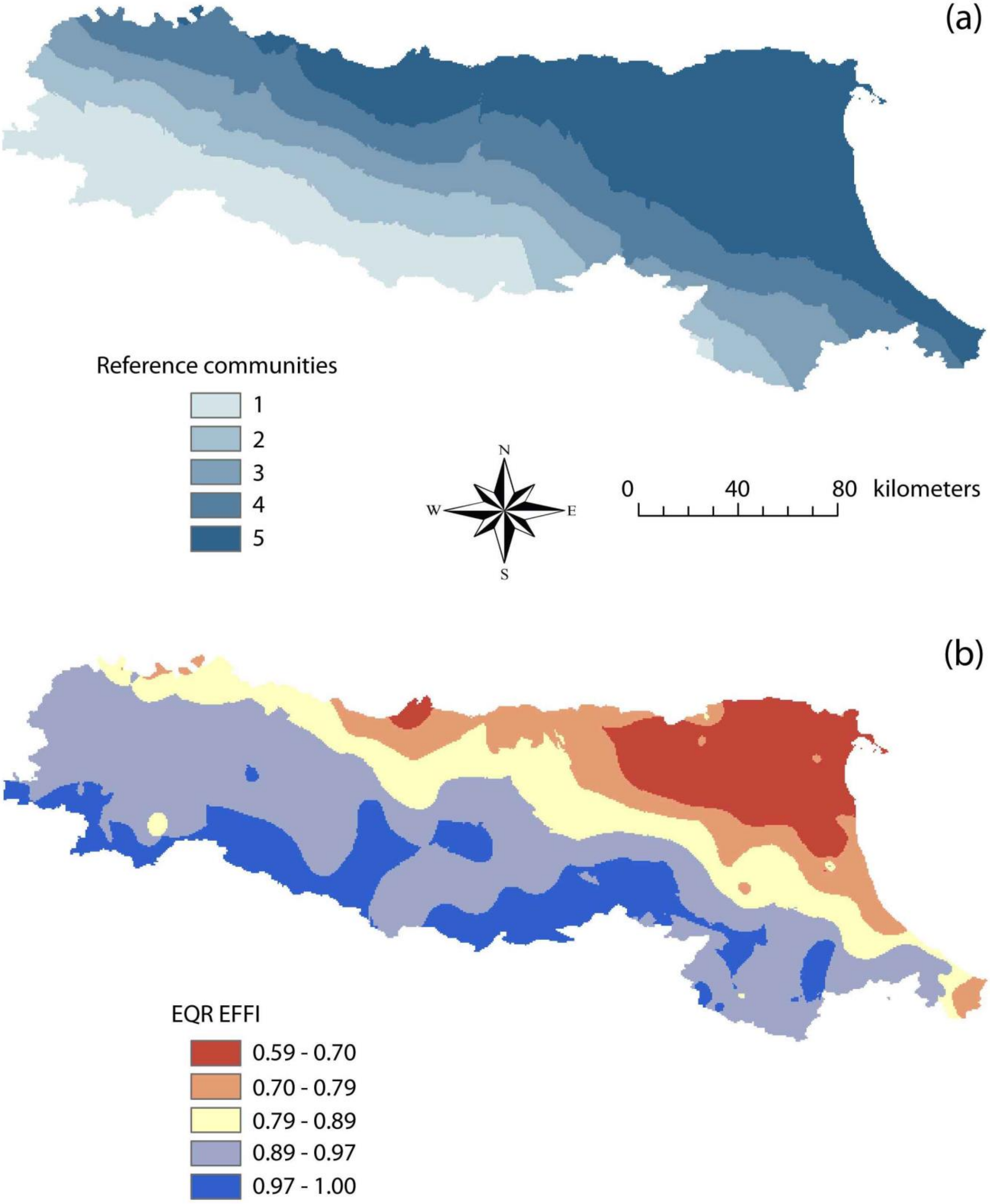
Table 2 – Species composition and Moyle class of abundance of reference fish communities used in the calculation of EFFI reference scores (values for each community in the last column)

Common name	**Family**	**Species**	**Moyle class**	**EFFI reference score**
Zone 1 - upper highland streams				**4.4**
Brown trout	Salmonidae	*Salmo trutta*	3	
Zone 2 - lower highland streams				**4.22**
Brown trout	Salmonidae	*Salmo trutta*	3	
Eurasian minnow	Cyprinidae	*Phoxinus phoxinus*	3	
Bullhead	Cottidae	*Cottus gobio*	3	
Zone 3 - Upper foothills streams				**3.93**
South-european nase	Cyprinidae	*Protochondrostoma genei*	4	
Italian chub	Cyprinidae	*Squalius squalus*	4	
Italian barbel	Cyprinidae	*Barbus plebejus*	3	
Western vairone	Cyprinidae	*Telestes souffia*	3	
Eurasian minnow	Cyprinidae	*Phoxinus phoxinus*	2	
Zone 4 - Lower foothills streams				**3.70**
Italian chub	Cyprinidae	*Squalius squalus*	2	
Western vairone	Cyprinidae	*Telestes souffia*	5	
Eurasian minnow	Cyprinidae	*Phoxinus phoxinus*	2	
Italian gudgeon	Gobiidae	*Romanogobio benacensis*	3	

Brook barbel	Cyprinidae	*Barbus caninus*	2	
Italian barbel	Cyprinidae	*Barbus plebejus*	2	
Po brook lamprey	Petromyzontidae	*Lethenteron zanandreai*	2	
European eel	Anguillidae	*Anguilla anguilla*	3	
Italian golden loach	Cobitidae	*Sabanejewia larvata*	2	
Italian spined-loach	Cobitidae	*Cobitis bilineata*	1	
Stone loach	Nemacheilidae	*Barbatula barbatula*	2	
Padanian goby	Gobiidae	*Padogobius bonelli*	2	
Zone 5 - lowland rivers				**3.66**
Italian red-eye roach	Cyprinidae	*Leucos aula*	4	
Pigo	Cyprinidae	*Rutilus pigus*	3	
Italian nase	Cyprinidae	*Chondrostoma soetta*	3	
Tench	Cyprinidae	*Tinca tinca*	3	
Italian rudd	Cyprinidae	*Scardinius hesperidicus*	3	
Italian	Cyprinidae	*Alburnus arborella*	5	
Italian chub	Cyprinidae	*Squalius squalus*	2	
Sea lamprey	Petromyzontidae	*Petromyzon marinus*	2	
Adriatic sturgeon	Acipenseridae	*Acipenser naccarii*	2	
European eel	Anguillidae	*Anguilla anguilla*	5	
Twaite shad	Clupeidae	*Alosa fallax*	3	
Italian spined-loach	Cobitidae	*Cobitis bilineata*	4	
Southern pike	Esocidae	*Esox cisalpinus*	4	
European perch	Percidae	*Perca fluviatilis*	4	
Three-spined stickleback	Gasterosteidae	*Gasterosteus aculeatus*	1	

Ultimately, EFFI scores for reference fish communities were compared with EFFI scores of sampled communities to derive EQR EFFI values (Figure 3b).

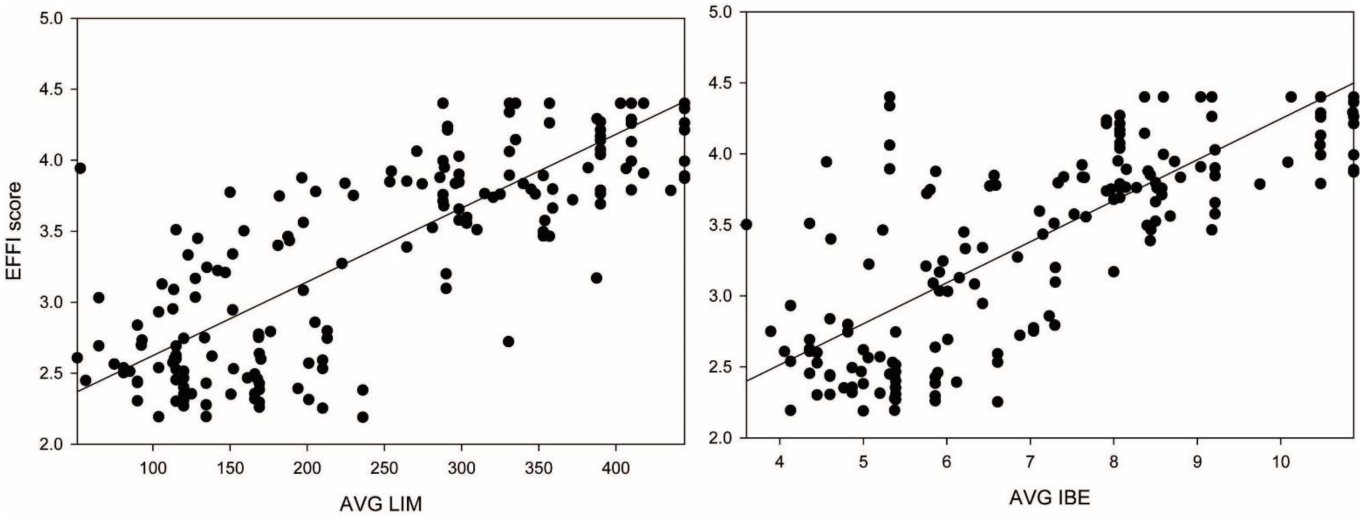
Figure 3 – A map of the reference zones for the Emilia Romagna region (a), where colors represent kriged areas of uniform reference. Kriged spatial distribution of EQR EFFI scores over the same area (b), measuring the distance from reference conditions: values below 1 indicate a deterioration of ecological state from the reference, lower values indicate greater distances from reference. Please note that classes of the score represented in this figure do not correspond to a proposal of valid environmental quality classes.



3.3 Correspondence with other indicators

EFFI scores correlated positively with both average LIM and average IBE scores (Figure 2).

Figure 4 – Plots of EFFI scores versus average LIM (a) and average IBE (b) scores for all sites where measures where available (EFFI vs LIM n = 200, EFFI vs IBE n = 191). Solid lines represent linear correlations between parameters.



Linear regressions between the indices were respectively:

IEFI score = 2.105 + (0.00519 * AVG LIM) (Rsqr = 0.661)

and

IEFI score = 1.362 + (0.289 * AVG IBE) (Rsqr = 0.596)

The Spearman rank order test confirmed that the correlations between EFFI and average LIM scores (correlation coefficient = 0.775) and between EFFI and average IBE scores (correlation coefficient = 0.754) were both positive and significant ($P < 0.05$).

The rank sum test confirmed that there was no difference between residuals in sites where measures of LIM overlapped in time with fish sampling and sites where the fish community was sampled following the LIM measures ($P < 0.05$).

4. Discussion

We succeeded in defining ecological functions and guilds of freshwater fish species which we used to derive a multimetric index of environmental status. Using theoretical reference communities, we defined EQR values for all sites in our dataset, expressing the ecological distance of each site from reference conditions. Perhaps unsurprisingly, this work underlined how fish communities were more degraded at lower altitudes than at higher ones. Ecofunctional fish index scores showed a significant correspondence with other indexes using chemical (LIM) and macrozoobenthos (IBE) measures.

Contrarily to other ecofunctional indexes (e.g. EFI and EFI+), EFFI does not solely rely on the number of individuals sampled, whether expressed as a precise number or a numerical class, recognizing that individual numbers are not very informative in terms of community structure (Begon, Harper, & Townsend, 1996). Depending on the size span, it is clear that large-bodied species with few individuals could represent a larger part of the community due to the biomass of each single individual, while small-bodied species, albeit numerically more abundant, could represent a smaller portion of the total biomass. EFFI attempts to account for this using body-size classes, albeit rather broad and based on assumptions on the average weight of a species individual. However, EFFI does not take into account absolute abundance (only relative abundance) or species richness because, on a wide altitudinal and latitudinal gradient, there are huge variations in productivity and therefore in absolute fish abundances and diversity (Brucet et al., 2013). Mountain streams are typically less productive and species poor, but environmentally sound due to lower rates of human settlement, than lowland rivers which are more impacted but usually highly productive and species rich (see e.g. Milardi et al., 2018). An index that takes into account solely species absolute abundance would risk assigning lower quality scores to the former, unless expected productivity for each stream order is also accounted for. Furthermore, while it is generally recognized that anthropogenic impacts are responsible for a loss in biodiversity (Vitousek, Mooney, Lubchenco, & Melillo, 1997), some impacts are less clear. An increase in eutrophication levels has been known to both reduce (e.g. Seehausen, Van Alphen, & Witte, 1997) and increase species diversity (Brucet et al., 2013). Other impacts specific to fishes, such as biomanipulation of the fish community for recreational fisheries, could temporarily increase diversity but often at the expenses of native species in the long term (Simberloff, Schmitz, & Brown, 1997). Future efforts could attempt to use both species abundance and richness by setting reference values for sites within homogeneous zones, divided based on their productivity and physical characteristics, but this could prove to be a too detailed task for an index meant to work on a large geographic scale.

Despite the explicit request in the WFD to use age structure in the fish index, little information on this parameter is currently collected during fish surveys in Europe, as often fish are released immediately after the survey without collecting sclerochronology samples (i.e. especially for salmonid fish), and our dataset did not contain any information. However, age structure information could be derived from length classes distribution if some sclerochronology analysis could be run for validation (e.g. Pauly & Morgan, 1987). The rationale to include age structure in the WFD indications was to ensure that fish communities have sufficient recruitment and thus would not collapse between assessment cycles. However, the burden of collecting such data falls on the national and local administrations, which are already overburdened making it impractical to fulfill the wishes of the Directive (Dale & Beyeler, 2001) and creating a gap in data collection which prevents the use of this parameter in building fish indexes. However, EFFI could be easily implemented to use both relative abundance and age-structure information to derive its scores, should the information become available in the future.

Most European countries have developed their own approaches to assess environmental quality based on fish communities. As an example, in the alpine region France developed the Fish Based Index (FBI) (Oberdorff, Pont, Hugueny, & Porcher, 2002), Germany the Fish-Based Assessment System« (FiBS) (Dußling, Berg, Klinger, & Wolter, 2004) and Austria the Fish Index Austria (FIA) (Haunschmid et al., 2006). Most of these indexes were based on the IBI concept (Karr, 1981), but some deviated from it and it took a considerable effort to subsequently harmonize them through a series of intercalibration exercises within the EFI+ project (see e.g. Jepsen & Pont, 2007). Out of different proposals, a taxonomical index was ultimately chosen in Italy. Compared to taxonomical indexes of fish fauna, EFFI is closer to the ecofunctional concept used in the rest of Europe and does not only measure changes in the fish community but also provides wider information on environmental quality and on anthropogenic impacts that affect the river environment. While less sensitive to substitutions of native species with exotic species within the same ecological niche, EFFI still takes into account the presence of exotic species and the decline of native species, detecting major fish community structure shifts. WFD indications do not explicitly require accounting for exotic species when building an index; yet other European regulations recognize the threat that such species could pose to the environment (e.g. EU regulation 1143/2014). In EFFI, exotic species are used as a measure of both status (they are a product of human impact, as the introduction and spread of exotic freshwater fish species is largely human mediated) and pressure (they have created or will create a pressure) on the environment and the fish communities. EFFI scores can be readily calculated through a simple excel spreadsheet, providing a continuous measure of habitat quality. This measure is reasonably in accordance with other currently implemented biotic and abiotic indexes and could provide a potential mean to measure future responses of fish communities to human induced changes.

Ideally, references should be derived from sites where no anthropogenic changes have occurred; but this can hardly be accomplished in most countries where human settlement has a long history. Other studies defined reference conditions using data from least impacted sites (Hughes, Howlin, & Kaufmann, 2004; Pont et al., 2006; Schmutz et al., 2007) or using spatially wider references (a whole sea ecoregion for transitional waters, see e.g. Coates, Waugh, Anwar, & Robson, 2007). We used instead historical and scientific data to reconstruct the native communities and identify stream ecologically coherent zones, following the work done by other authors (e.g. Kleynhans, 1999; Zerunian, 2004). Mediterranean countries are characterized by high riverine habitat diversity and strong altitudinal and temperature gradients, which pose a challenge to the definition of reference conditions based solely on stream order, but this is balanced by a wealth of detailed historical records on the fish fauna. Unfortunately, historical and biogeographic information is not equally available to all countries, but both actual and historical data can be used to define EFFI references. While the reference scores provided in our paper are meant solely as a demonstration of the possibility to calculate EQRs with EFFI, EQR EFFI scores provided a spatially significant map of distances from reference conditions, at the very least underlining areas of higher ecological impact on the fish communities. Lowland areas have the highest anthropogenic pressure levels as there are higher rates of human settlement and activities (Castaldelli, unpublished data). Accordingly, EQR EFFI showed that these are the most impacted areas whereas higher streams, in less populated areas, are less impacted.

Like most indexes, EFFI is sensitive to robustness and standardization of sampling methods, as more effort could potentially yield more species per site and different numbers of individuals. However, this paper did not aim to investigate sampling protocols (which are agreed upon at the national level), but merely a way to use the results of sampling to infer ecological quality. As with any multi-pressure assessment index, EFFI could also bear a risk of low detection of some pressures and this is particularly clear when considering the wide spectrum of human-induced pressures. Tackling this could be particularly challenging: for example, the Migration guild is intuitively linked to the presence of long-ranging species in a specific river stretch but, as often there are no clear measures of habitat fragmentation for each site, its correlation to the fragmentation status might be difficult. Moreover, intentional biomanipulation of the fish stocks (i.e. illegal transfers of fish across barriers by “bucket managers” or even authorized restocking programs such as for European eel) might further complicate the validation. The ecofunctional niches of single fish species tend to be broad; therefore it could be counterintuitive that they could provide a precise indication on riverine ecological status. The key is that the sum of ecofunctional niches of the whole community can provide a much more accurate assessment than those of a single species. Therefore, a fish ecofunctional index is fully capable of detecting impacts that go beyond what chemical or macrozoobenthos indexes can detect. That is a keystone motivation of the WFD for the use of different indexes to define the ecological quality of water bodies: different indexes possess different sensitivities to anthropogenic pressures (Marzin et al., 2012). LIM and IBE are indicators mainly geared to gauge the pressure of chemical and nutrient alterations, whereas an ecofunctional fish indicator assesses also hydrological and habitat alterations, as well as the effects of fisheries. For example, if a stream is in good chemical status it can host an undisturbed macroozobenthos community but might host a lower quality fish community due to habitat fragmentation or degradation and uneven flow regime. As fish respond to a wider range of anthropogenic pressures it is not entirely surprising that the correspondence between EFFI, LIM and IBE scores is less than perfect. However, the significant correspondence level could depend on the relative importance of chemical and nutrient alterations (e.g. eutrophication) over other pressures. This information could offer some insight for future research dealing with the interlocking effects of anthropogenic pressures.

Future work will also be needed to link the response of the indicator with the level of anthropogenic pressure, which would be a necessary step to define meaningful ecological quality class boundaries. A limit for this process will likely be the lack of specific information on hydrological and habitat alterations, as several anthropogenic pressures are still not sufficiently quantitatively parameterized over large areas. Further testing and validation might be needed to fine-tune the guild scores and the ecological functions weight, in order to achieve a wider consensus in the scientific community, e.g. through thematic workshops that elicit expert knowledge. However, this expert knowledge should ideally be accompanied by a stronger background on empirical relations between stressors and fish community responses (Mebane, Maret, & Hughes, 2003), which could further help to refine scores and weights in a more objective way. Moreover, further validation is needed on larger datasets covering more than one country which will involve building wider species matrices, after a review of ecofunctional traits across diverse areas and species, and identifying reference conditions, either through historical data or less impacted sites. Similarly, more researchers from different countries need to be involved to investigate the definition of meaningful environmental class boundaries (e.g. through a discriminant analysis over a dataset that spans a wide geographical range, Birk et al. (2012)) which have to be set at the national level. Despite these difficulties, common to most other indexes, EFFI could be employed on a rather wide geographical range across Mediterranean countries, as it covers all relevant ecological functions and does not depend on taxonomical variations.

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