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Abstract: The European Fish Index EFI+ is the only fish-based multimetric index for the assessment of the ecological status of running waters validated and thus applicable across the European Union. Metrics of the index rely on several attributes of the species present in the fish assemblage, irrespective of their native/alien status. The abundance of alien fish, together with other anthropogenic impacts, is one of the most important threats to the conservation of native fish and ecosystem health and is also an indicator of degraded stream conditions. Therefore, to improve the performance and utility of the EFI+ in regions with high incidence of alien species, the EFI+ was adapted to include the alien fish pressure as a new metric that reflects the number of alien species as well as the proportional abundance of alien individuals. The application of the adapted index (A-EFI+) is illustrated with data from several Iberian Mediterranean basins and showed similar or stronger correlations than the original EFI+ with anthropogenic pressure (land-use variables) and with other regional fish indices. EFI+ has been invaluable to intercalibrate fish indices across Europe and A-EFI+ is similar but explicitly includes the alien pressure, thus helping to provide a more comprehensive assessment of ecosystem health and to communicate it to society.

Keywords: ecological status; Index of Biotic integrity; non-native species; Water Framework Directive

Key Contribution: This study developed a modification of the EFI+ fish index to include alien fish pressure to improve its performance in assessing the biotic integrity and ecological status of rivers with high incidence of alien species. The application of the adapted index is demonstrated using data from various Mediterranean basins in the Iberian region.

1. Introduction

The Water Framework Directive (WFD) requires all member countries of the European Union (EU) to assess the ecological status of running waters using biological indicators of several organism groups, including fish [1]. The WFD defines reference conditions (i.e. equivalent to high ecological status) as those water bodies with no or minor presence of anthropogenic changes in which all expected native species are present, populations are in good biological condition, and no alien species exist. Biotic indices are a rapid and cost-effective assessment of the environmental degradation of aquatic systems. It benefits of a standardized approach using a set of metrics or measures that represent various aspects of biological assemblage structure, function, or other measurable

characteristics. This standardization allows for consistent comparisons across different locations and time periods. By combining multiple metrics, the biotic indices provide an indication of the overall biological condition and can help identify and quantify the impacts of human-induced stress on aquatic communities at wide temporal and spatial scales [2]. In aquatic environments, fish are excellent ecological indicators due to their sensitivity to environmental changes and have several advantages as indicator organisms [3]. Fish are found in most of lotic ecosystems and are long-living organisms that reflect the cumulative effects of long-term anthropogenic stressors. Their high mobility allows them to use various habitats within river ecosystems, making them particularly sensitive to disturbances in river morphology and connectivity [4]. Fish-based indices have been used to assess the quality of river ecosystems since the 1980s, when the Index of Biotic Integrity (IBI) was first introduced [2].

The project FAME (Fish-based Assessment Method for the ecological status of European rivers) [5] was the first attempt to develop a pan-European fish index applicable in all the EU member states, resulting in the creation of the European Fish Index EFI [5,6]. The initial formulation of EFI was primarily based on data collected in northern Europe. The index was subsequently improved by expanding the database with data from southern Europe resulting in a new version called EFI+ [7]. EFI+ quantifies the deviation between the predicted fish assemblage (reference conditions) and the observed fish assemblage (sampling data), and is computed as the average of two metrics that vary with river type (salmonid or cyprinid). The river type is assessed automatically by the EFI+ software, based on physical parameters and proportion of salmonid species. The EFI+ model place each species in functional trait categories (guilds). The index for the cyprinid type uses two metrics based on species with rheophilic and lithophilic reproduction habitats, and the index for the salmonid type is composed by two metrics based on intolerant species to oxygen depletion and habitat degradation. The index value is calculated as the arithmetic mean of the two metrics scores. The EFI+ metrics rely on the whole fish assemblage, without any distinction between native or alien species. Therefore, the presence and abundance of alien species belonging to the guilds included in the metrics (i.e. rheophilic, lithophilic or intolerant) positively influences index scores.

The main pressures and impacts that affect surface waters in Europe are eutrophication, chemical pollution, water abstraction and hydromorphological alterations [8]. Alien species also constitute one of the most important threats to the conservation of native fish and ecosystem health and the impact may be as severe as that of other stressors [9,10]. The presence and abundance of alien species reflects biological pollution and causes disturbance to native species, mainly due to predation and competitive interactions [11]. Higher pressure from alien species have been related to a greater loss of native species, reduced density and unbalanced size structures of native fish [12,13]. Furthermore, alien species are also an indicator of degraded conditions because its proliferation is facilitated with increasing eutrophication and the construction of dams with the subsequent reduction of seasonal flooding and stabilization of downstream flows [14,15]. Despite these negative implications, the inclusion of alien fish metrics in the ecological quality assessment of rivers has not been considered in the majority of WFD assessment methods [16]. Thus, only 5 of 25 (20 %) fish assessment methods have an explicit metric for alien species (WISER "Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery"; www.wiser.eu).

EFI+ has been proved effective in determining the ecological status of European rivers [6,17,18] but the absence of negative scoring when alien fish are present may be a serious shortcoming since an ideal indicator should be sensitive to all stressors and impacts [2]. Including this type of impact may be unimportant in European regions with low proportion of alien species (e.g. [19]), but in regions where alien fish are widespread, the inclusion of a negative alien fish metric is considered crucial to properly assess ecosystem health [12,13]. Some European countries such as Poland have already been aware of this limitation and have tried to make some modifications to the index to solve it [20]. In contrast, Spain has chosen the EFI+ as the primary fish index for evaluating the ecological status required by the WFD, despite being a country with a high incidence of alien fish introductions and most basins having more alien than native species [9,21].

The fundamental issue with the EFI+ in relation with alien species lies in the fact that the index's development did not exclude reference sites with alien species, nor did it employ metrics solely based on native species, which is currently recommended to develop multimetric indices [22]. Moreover, it is also recommended to include negative alien species metrics to improve WFD assessments [23]. As the usefulness of a global index at European level is extremely valuable, one way to improve the index without losing its advantages is to include the alien fish pressure. Hence, the objectives of this study were to (1) adapt the EFI+ index to include the alien fish pressure in order to improve its performance in regions with a significant impact of alien fish, and (2) illustrate its application using data from several Mediterranean basins of the Iberian Peninsula.

2. Materials and Methods

The modification of the EFI+ consisted in a weighted combination of original EFI+ metrics with a measure of alien fish pressure to produce an adapted version of the index (A-EFI+). We consider alien species those that occur outside their natural range and have been introduced to new areas by human activity, either intended or unintended. This encompasses species that, while native to a particular country, have been translocated and are now found outside their native range within the same country. Alien fish pressure should reflect the number of alien species as well as the relative abundance of alien individuals in relation to native fish. Thus, the alien fish pressure metric (AFP) was calculated as the average between the proportion of alien species and the proportion of alien individuals in the sample. The scores of the AFP metric range from 0 (absence of alien species) to 1 (all individuals belong to alien species). To calculate the A-EFI+, since a third metric is added to the two metrics of the original index, this additional metric is assigned a one-third weighting on the adapted index. Therefore, A-EFI+ was calculated as follows:

$$\text{A-EFI+} = \text{EFI+} - \left(\frac{\text{AFP} * \text{EFI+}}{3} \right) \tag{1}$$

where A-EFI+ is the adapted index, EFI+ is the original EFI+ index and AFP is the alien fish pressure. When alien fish are present, the A-EFI+ scores are lower than the EFI+ scores, up to a maximum reduction of 33.3% of the original EFI+ score when all individuals are alien. The A-EFI+ ranges between zero and one, like the original EFI+.

The application of the modified index was illustrated using data collected within WFD monitoring programs and available from public databases from 344 sites of the Mediterranean slope of the Iberian Peninsula (230 sites in Catalonia and 114 sites in the Jucar River Basin District; latitudinal range of 38.2–42.8°N). This region was selected because it is severely affected by alien fish introductions [13,24,25]. Most of the streams have a typical Mediterranean hydrological regime, with dry summers and irregular precipitations in autumn and spring. Thus, flow regimes are highly variable, from temporary (seasonal flow) to perennial (continuous flow). A detailed description of the study area can be found elsewhere [13,24].

Fish data originated from samplings by electrofishing during low flow periods, following the CEN 14011 standard protocol [26]. A single upstream pass was made including all mesohabitat channel units present in the reach, with a minimum sampled length of 50 m or minimum area of 100 m². Fish samplings were performed between June-September in 2007, 2008 and 2009. The EFI+ was calculated with the R-package, available upon request at http://efi-plus.boku.ac.at/. Several alien species in this area positively score in the EFI+ metrics (i.e. are considered rheophilic, lithophilic or intolerant) such as *Phoxinus septimaniae* and *Phoxinus dragarum* (included as *Phoxinus phoxinus* in the EFI+ software), *Oncorhynchus mykiss*, *Ameiurus melas* and *Squalius cephalus*. Also, there are some translocated species originated from other Iberian basins, which should therefore be considered alien, that score in the index metrics, such as *Pseudochondrostoma polylepis*, *Luciobarbus graellsii* and *Squalius alburnoides*. Besides the EFI+ and A-EFI+, we calculated two other fish indices used for ecological monitoring in the study region: IBICAT2010 [27] and IBI-JUCAR [13]. The IBICAT2010 utilizes a set of metrics derived from the functional traits and characteristics of fish species, such as feeding guilds, habitat preferences, reproductive strategies and other life history traits. While it distinguishes between native and alien species in some metrics, it lacks a specific metric to negatively score the

presence of alien species [27]. The IBI-JUCAR uses five metrics to evaluate the ecological health of streams based on the loss of native species, the presence of alien species, the abundance of native fish, the age (size) structure of native fish, and the presence of individuals with anomalies [13]. Therefore, this index includes a specific metric for alien species. Other data from biotic and abiotic indices widely applied for ecological monitoring in Spain were also gathered for the same sites and periods with fish data, to be compared with the A-EFI+. The indices compared were the following: the Riparian vegetation quality index, QBR [28]; the Fluvial habitat index, IHF [29]; the Specific Pollution Sensitivity index based on diatoms, IPS [30], and a macroinvertebrate-based index, IBMWP [31]. At each sampling site, land uses were used as indicators of anthropogenic pressure [32]. The Corine Land Cover database (available at http://www.eea.europa.eu) was used to quantify land-use variables. Land use was categorized as urbanized areas, including urban and industrial units (Artificial), agricultural areas (Agriculture), and forested/natural areas (Forest), and then the percentages of each category were calculated within the drainage basin upstream of the site.

To study the performance and behaviour of the A-EFI+, bivariate relationships among biological indices and land-use variables were analyzed using Spearman rank order correlation coefficients (r_s), which are adequate to describe monotonous relationships and do not assume bivariate normality or linearity. We also used multiple regression analyses to also consider all the indicators of anthropogenic perturbation simultaneously. All statistical analyses were performed using the software R version 4.3.2 [33].

3. Results

A total of 39 species were recorded in the compiled dataset, of which 19 (48.7 %) were non-native. Alien species were present in 187 (54.4 %) of the 344 sampling sites, with similar proportions in the two groups of basins studied (55.2 % in Catalonia and 52.6 % in the Jucar River Basin District). The mean percentage of alien individuals at sites with presence of alien species was 63.2 % (range 11.2-100 %). Both the individual alien metrics and its average (AFP, alien fish pressure) were positively correlated with artificial and agricultural land-use and negatively correlated with percentage of forest land (Figure 1), thus supporting their relevance as metrics of stream degradation. Higher pressure of alien species was also related to a lower richness of rheophilic spawning species ($rs = -0.675$) and lower density of lithophilic spawning species ($rs = -0.495$) and thus to the EFI+ index (Figure 2).

After calculating the A-EFI+, the resulting scores were lower than EFI+ in 183 of the 187 sites with alien species. In the remaining four sites with alien fish, the EFI+ was already zero before calculating the A-EFI+ and could not be reduced (Figure 3). In these sites, the mean reduction of the score of the A-EFI+ with respect to the EFI+ was 18.6 % (range: 3.1-33.3 %). This led to a lowering in ecological status classes of the 40.4 % of these sites, mainly from Good to Moderate and from Moderate to Poor. The decrease proportion of the A-EFI+ score was higher as AFP increased, as expected (Figure 2; Figure 3).
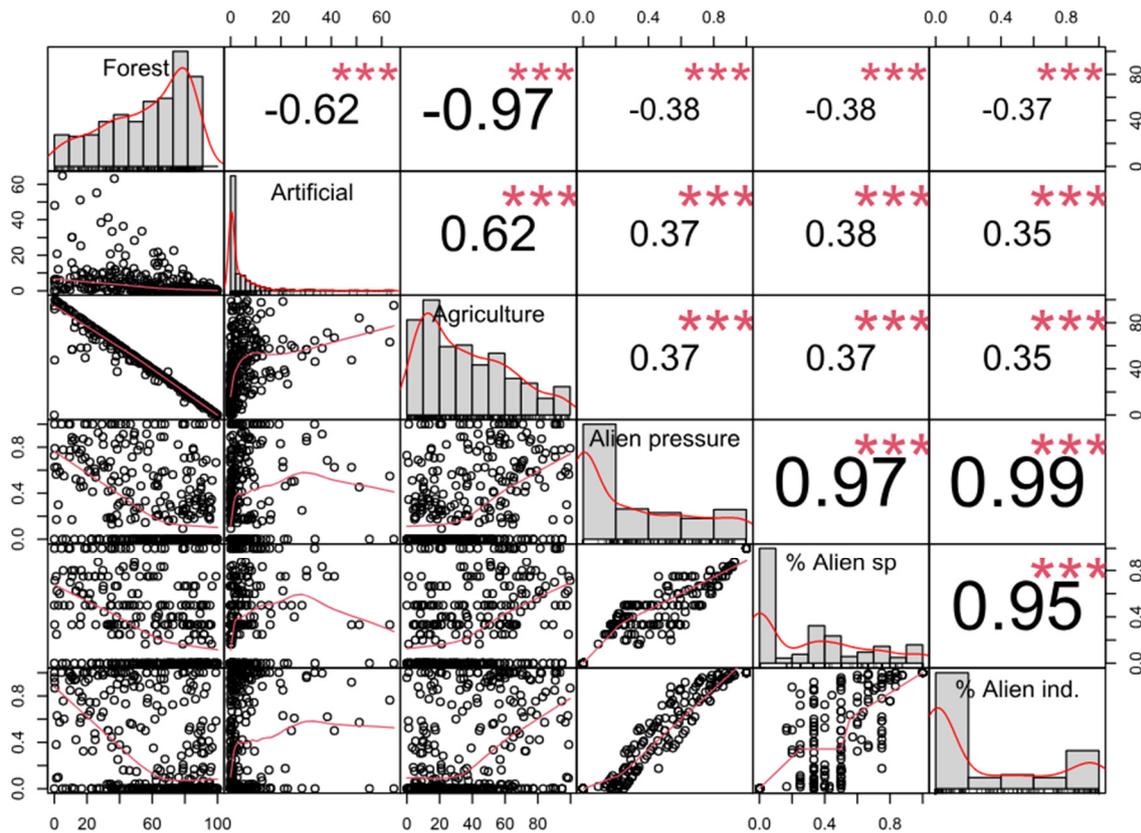
Figure 1. Relationship between fish alien metrics and land-use variables. The panels above the diagonal show the Spearman rank correlation coefficients with significance level (***, $P < 0.001$) and the panels below show the pairwise scatterplot with a smoothing curve (LOESS). In the scatterplots, the Y axis corresponds to the variable in the row diagonal and the X axis to the column diagonal (e.g. the scatterplot on the bottom left has % exotic individuals in the Y axis and % forest cover in the X axis).
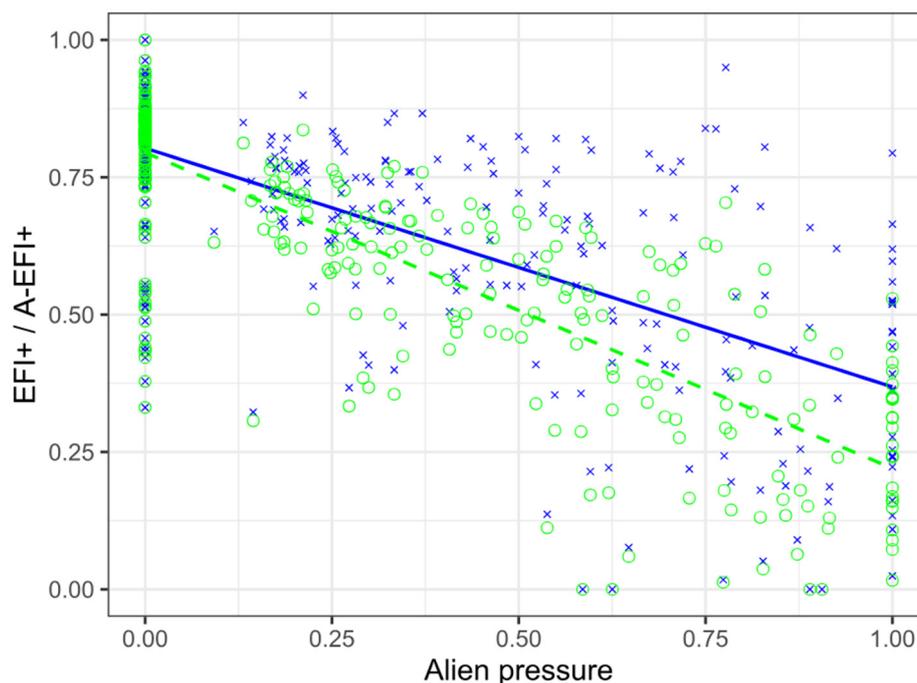

Figure 2. Relationship between the EFI+ and A-EFI+ indices (blue crosses and green circles, respectively) and the Alien fish pressure (AFP) (average of % alien species and % alien individuals)

in the study area. The simple regression lines are also shown (EFI+ = 0.803 - 0.435 AFP, R^2_{adj} = 0.465, P < 0.001; A-EFI+ = 0.795 − 0.575 AFP, R^2_{adj} = 0.683, P < 0.001).
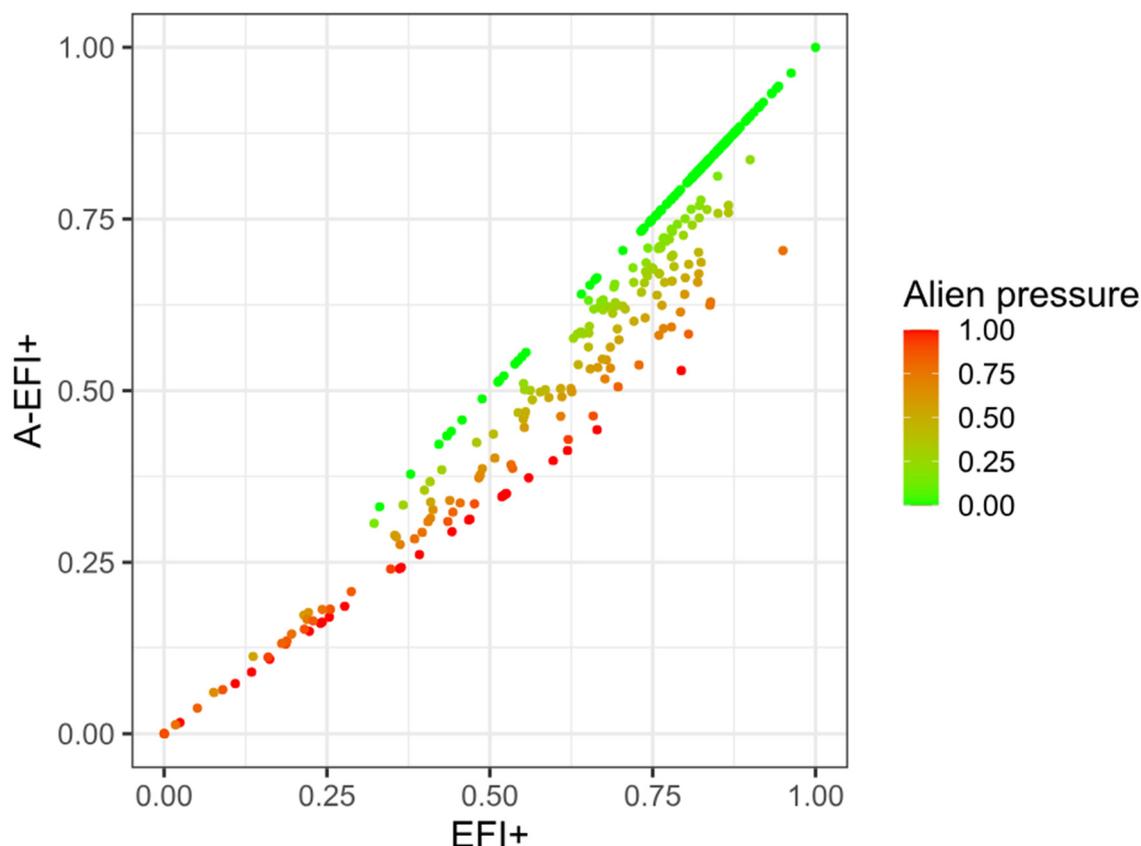

Figure 3. Relationship between the A-EFI+ and EFI+ indices and the effects of the Alien fish pressure. The A-EFI+ index can be easily estimated from EFI+ with the following linear regression functions: A-EFI+ = −0.089 + 1.058 EFI+, R^2_{adj} = 0.940, P < 0.001; A-EFI+ = 0.1308 + 0.827 EFI+ − 0.215 AFP, R^2_{adj} = 0.991, P < 0.001.

The performance of the A-EFI+ was compared with several other biological indices and land-use variables by bivariate correlations and multiple regression. All fish indices (EFI+, A-EFI+, IBICAT and IBI-JUCAR) declined with increased catchment disturbance. They were negatively correlated with percentage of artificial and agricultural land-use, and positively correlated with percentage of forest land (Figure 4). Compared to EFI+, the correlation of A-EFI+ with land use variables (Figure 4) was slightly higher whereas the correlation with other biotic indices, such as the macroinvertebrate index (IBMWP) and the diatom index (IPS), or habitat index (IHF) was slightly lower (Figure 5). A multiple regression model suggested that the relationship with artificial land use was nonlinear but overall very similar for EFI+ and A-EFI+ (Table 1). Unsurprisingly, a regional fish index such as IBI-JUCAR performed slightly better (Table 1, Figure 4, and Figure 5) but was quite correlated with those two indices (Figure 4).
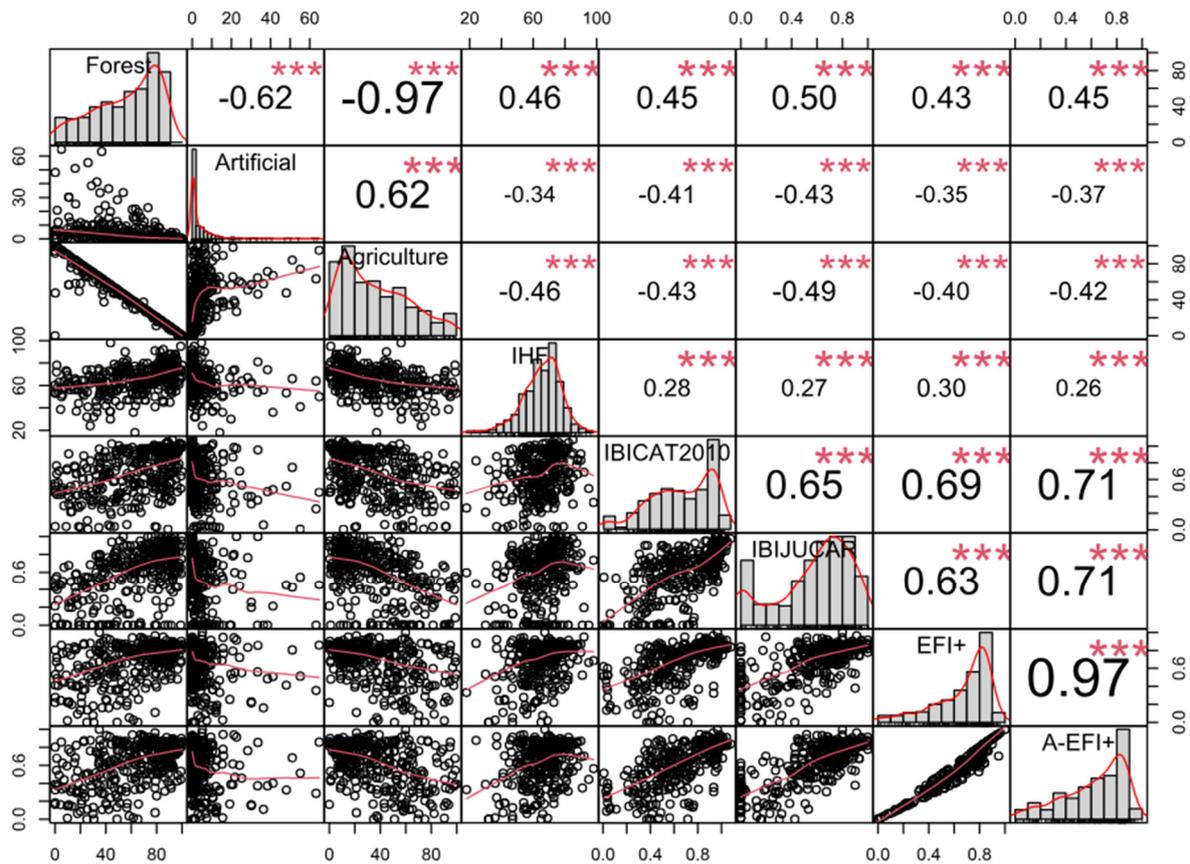
Figure 4. Relationship between fish indices and land-use variables. The panels above the diagonal show the Spearman rank correlation coefficients with significance level (**, $P < 0.01$; ***, $P < 0.001$) and the panels below the pairwise scatterplot with a smoothing curve (LOESS). In the scatterplots, the Y axis corresponds to the variable in the row diagonal and the X axis to the column diagonal (e.g. the scatterplot on the bottom left has A-EFI+ in the Y axis and % forest cover in the X axis).

Table 1. Multiple regression models of four fish indices with a habitat quality index (IHF) and % agricultural and artificial land uses. A quadratic component of artificial land use was also included because it was significant for most models and was supported by Akaike information criteria. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. All models were highly significant ($P \ll 0.001$).

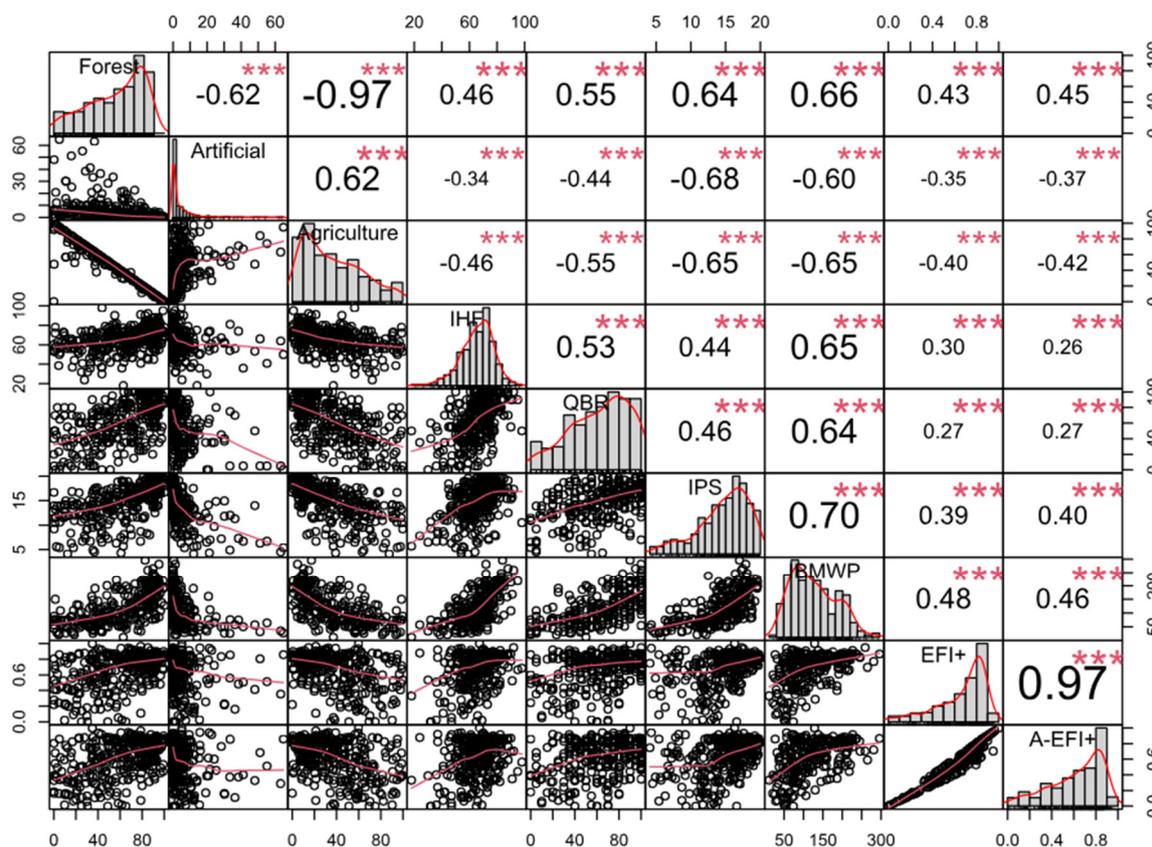|	EFI+	A-EFI+	IBICAT2010	IBI-JUCAR
Intercept	0.5332 ***	0.5947 ***	0.7095 ***	0.7453 ***
IHF	0.0037 ***	0.0024 *	0.0015	0.0005
Agriculture	-0.0022 ***	-0.0029 ***	-0.0030 ***	-0.0044 ***
Artificial	-0.0071 *	-0.0099 **	-0.0082 *	-0.0117 **
Artificial²	0.0001 *	0.0002 **	0.0001	0.0002 *
N	319	319	318	319
Residual standard error	0.1899	0.2079	0.2384	0.2489
R^2_{adj}	0.2193	0.2229	0.1849	0.2552

Figure 5. Relationship between biotic indices and land-use variables. The panels above the diagonal show the Spearman rank correlation coefficients with significance level (***, *P* < 0.001) and the panels below the pairwise scatterplot with a smoothing curve (LOESS). In the scatterplots, the *Y* axis corresponds to the variable in the row diagonal and the *X* axis to the column diagonal (e.g. the scatterplot on the bottom left has A-EFI+ in the *Y* axis and % forest cover in the *X* axis).

4. Discussion

The modification of the EFI+ proposed here is simple and easy to compute and adds an explicit consideration of alien fish pressure in the index, allowing for a wider assessment of stream health and more strictly following WFD guidelines. This modification is only relevant in areas highly impacted with alien species since the index remains unchanged when alien species are absent or reduces the score only slightly when the incidence of alien species is low. Therefore, its applicability should be more important in the western Mediterranean area because of the higher incidence of alien species than in the Eastern or Northern Europe [34].

The positive relationships between the AFP (Alien Fish Pressure) metric and agricultural and urban land uses, used here as a measure of anthropogenic pressures, is consistent with the view that alien fish species are generally more tolerant to environmental alterations than natives [35] and therefore constitute good indicators of stream degradation [34]. Although the two original metrics of the EFI+ were negatively correlated with AFP and, therefore, some alien fish impact is already included in the index, the incorporation of the AFP metric into the EFI+ emphasizes the impact in sites where alien fish pressure is high, resulting in a more appropriate assessment of ecological integrity.

The A-EFI+ scores were highly consistent and proportional to the extent of alien species presence, showing a rapid decline in the quality status classes as the proportion of alien fish increase. Furthermore, the incorporation of the AFP metric into the EFI+ improved the index's performance as an indicator of degradation in comparison with the original version, as demonstrated by its stronger correlation with land-use pressures. The strength of the response to agricultural and artificial land-uses when the alien fish metric was included in the A-EFI+ did not decrease but rather increased. The

A-EFI+ also showed higher correlation values than the original EFI+ with the two fish indices locally developed for the region (IBICAT and IBI-JUCAR), because they also consider explicitly alien species [13].

No important differences were found comparing the correlations of the EFI+ and A-EFI+ with biological indices for other organism groups (IBMWP, IPS) or abiotic indices (QBR, IHF), what suggests that the presence of alien species is not influenced by the ecological quality measured with these indices. Furthermore, the correlations of both EFI+ and A-EFI+ with diatom and macroinvertebrate indices were relatively low, as has been commonly reported [13,36]. This could be related due to the variable response of the different organism groups to degradation and is one of the reasons to consider multiple organism groups for stream health assessment [37]. For example, indices based on macroinvertebrates and diatoms often showed stronger responses to water quality parameters while fish, being more mobile organisms, are more vulnerable to hydrological and habitat alterations [13,36].

Most of EU member countries use fish indices locally developed for their respective territories to account for the specific characteristics of the fish assemblages. Local fish indices often provide a more accurate and precise evaluation of local conditions, but indices that are widely applicable across extensive regions are also desirable to improve the integration of results from various measurement methods and to enhance resource management [22]. Furthermore, national fish indices are required by the WFD for intercalibration to ensure consistency in ecological assessments throughout Europe [38,39]. The EFI+ is the only fish index currently available for use throughout Europe and, therefore, an important application is to be used to convert to a common scale the assessments made with different fish indices and allowing comparison [17]. The modification of the EFI+ proposed here does not affect the utility of this approach, since the transformation of the A-EFI+ to EFI+ and vice versa is simple and straightforward. Moreover, in regions with a significant presence of alien species, the A-EFI+ should provide a more comprehensive tool to assess and communicate the ecosystem health or rivers.

5. Conclusions

The presence of alien species poses a significant threat to the preservation of freshwater native diversity and should be considered an indicator of degraded stream conditions and anthropogenic pressures on aquatic ecosystems. Consequently, any biotic index must be able to effectively assess this type of impact. The EFI+ index lacks a specific metric to weight the presence of alien fish. The adapted version of the EFI+ presented here includes a negative metric for alien species and therefore can assess the extent of their presence and their potential impact on native fish communities. Furthermore, including a metric for alien species improves the accuracy and relevance of the index for a more comprehensive assessment of ecological status, helping to identify areas where conservation efforts should be focused and where management actions are needed to mitigate the negative impacts of alien species. The A-EFI+ is most suitable for assessing ecological status in areas with a high proportion and abundance of alien fish, such as some basins in the Iberian Peninsula, where also host many local endemic species, many of which are severely threatened.

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Institutional Review Board Statement: All the data come from previous research or other databases. In this case, ethical approval is not needed for this article.

Data Availability Statement: The data presented in this study are openly available in FigShare at DOI: 10.6084/m9.figshare.24665571

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