

Article

Strengths and Weaknesses of Different Italian Fish Indices under the Water Framework Directive Guidelines

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Abstract: The ISECI (or F index) has been the first fish index to be recommended by the Italian Ministry of the Environment to assess the rivers ecological status with regard to fish communities, in accordance with the Water Framework Directive 2000/60 EC. In addition to ISECI, other fish indices have been developed such as the Forneris Ichthyic Index (I.I.) and a revised version of ISECI, the so-called NISECI. The latter is nowadays the reference Italian index in the framework of the Water Framework Directive. In this work, we analyzed 30 sampling sites along 18 watercourses in Northern Italy and computed the results of fish monitoring to evaluate the strength of ISECI and NISECI, as well as to assess weak points limiting their application. We detected several issues that undermine the ISECI effectiveness. The weakest point regarded the mismatch between the expected reference fish community and the sampled ones, which decreased the overall algorithm efficiency in the evaluation process. On the other hand, the results confirm the improvements introduced by NISECI. Although with some advancement, all three proposed indices revealed their weaknesses in the overall assessment of the ecological status of the water course, as also highlighted by a pioneering comparison with three expert-based blind judgements.

Keywords: multilevel monitor approach; fish ecology; alien fish species; WFD



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1. Introduction

The Water Framework Directive, or WFD [1], was introduced on 23 October 2000 by the European Parliament and the Council as a legal act aimed at protecting and restoring the European water basins and to promote the sustainable use of the water resource. The WFD requires the classification of surface waters through five different qualitative classes, ranging from the High ecological status (Class I) to the worst one (Class V) [2]. Moreover, following the guidelines stated by the Directive, each member state was supposed to achieve at least the “Good” ecological status (Class II) for its leading water basins by 2015 (2021 after the postponement).

In accordance with the 2000/60/EC legislation, biotic factors acquired a key role as quality indicators, more properly defined as Biological Quality Elements (BQE), with special regard to:

- Phytoplanktonic composition, abundance and biomass;
- Composition and abundance of aquatic vegetation, macrophytes and phytobenthos;
- Composition and abundance of benthic macroinvertebrates;
- Composition, abundance and age structure of fish communities.

Implementing the Water Framework Directive requires the definition of standardized indices to assess the ecological status of water basins through the BQE. This allows the

comparison of results obtained from the application of distinct indices in different European member states. The standardization process constrains values into the range between 0 and 1. In particular, the range is divided into five classes corresponding to those of the BQE.

The ISECI, Index of the Ecological Status of Fish Communities, or F index, is one of the proposed tools established in the application of the WFD. Nowadays, it is the official index used to evaluate the ecological status of Italian watercourses based on the BQE, with regard to fish communities [3,4]. It refers mainly to two specific features: the naturalness of the fish community, referring to the effective presence of expected native species and the absence of aliens, as well as to the good quality status of the indigenous community. The latter translates into a self-sustaining population, which proves to have healthy ecological–evolutionary dynamics as demonstrated by its population age structure. Specific features are useful for assessing the population ecological conditions compared to a putative pristine reference state, characterized by the absence of anthropogenic pressures and consistent with the zoogeographic features of the water basin.

Despite ISECI being the current official index, its application has occurred only in a few Italian regions, yielding fluctuating results. Several previous works published locally [5–8] outlined many issues and highlighted some weak points undermining the index effectiveness. It proved to be often cautious, adequate to evaluate the biological status of local fish communities but at the same time less useful to determine their functional role in evidencing the ecosystem function and energy cycling [5]. More precisely, a common issue is the unsuitableness of the suggested reference fish communities, which often turned out to be too general and inappropriate to assess local ecological conditions. Moreover, it is often difficult to recognize hybrids directly from field surveys, considering only the fish morphological traits.

It must be remarked that the scientific discussion related to the Italian indices application has always been developed at a local scale, and international literature is almost absent about this issue. Starting from this evidence, it is in the intention of this work to present the evolution through time and the present availability of Italian fish indices based on taxonomy and ecological insights. In fact, at the beginning of our sampling campaign ISECI was the only recognized index to assess the ecological status of water basins with regard to fish communities [4]. An updated version named NISECI [9] was developed in 2017, to overcome the weaknesses of the previous one.

Thus, during our research, we first applied ISECI [4] in 18 watercourses covering a wide geographic area between the Apennines and the Po river (Figure 1). The final goal was to test the index effectiveness as well as to identify if the main issues encountered by other researchers in several Italian regions (e.g., Friuli Venezia Giulia, Umbria, Veneto) were also occurring in Emilia Romagna, considering that analyzed sites showed unique hydrographic, biological and environmental features which might have yielded different results compared to those obtained in other regions. Furthermore, the research was extended to two additional Italian indices to make appropriate comparisons among different algorithms. In fact, the different tools were similarly developed to evaluate the ecological status of a water basin through a fish community assessment and putatively proposed by different authors under the WFD. Among them, we chose the Ichthyic Index by Forneris [10,11], hereafter indicated I.I., and the New Index of the Ecological Status of Fish Communities, or NISECI [9]. To our knowledge, NISECI application has officially occurred only once so far [12]. To test the improvements, comparison between outcomes obtained from ISECI [4] and NISECI [12] appeared crucial. Moreover, to perform an appropriate evaluation we decided to make an independent comparison based on a blind expert-based opinion given by ichthyologists, whose extended knowledge of sampled watercourses allowed an effective and reliable evaluation of their ecological status. The latter may overcome issues related to the expected reference fish community. Hence, we performed indices assessment, highlighting their advances and weaknesses, in compliance

with the main targets of the Water Framework Directive and the still controversial dispute about the ecological role of such specific taxa as the brown trout in the index calculation.

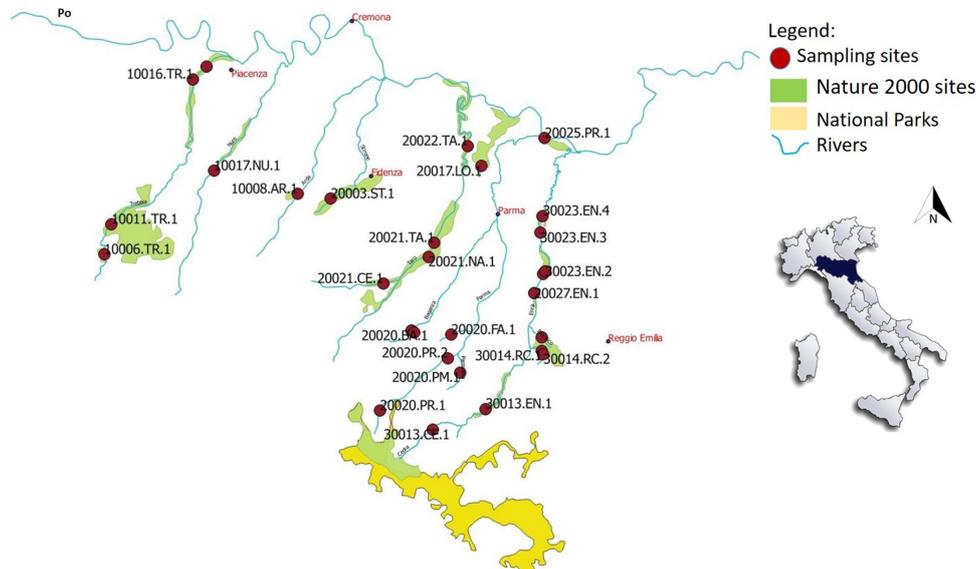


Figure 1. Map describing the locations of sampling sites. Sampling sites along the main Po river tributaries were identified by specific codes attributed in the framework of the Nature 2000 Network.

2. Materials and Methods

2.1. Sampling and Study Area

The sampling campaigns were carried out by a jointed working group team of Parma University and Gen-Tech Academic Spin-Off, in the framework of the European project LIFE13 NAT/IT/001129 Barbie (www.lifebarbie.eu) from August 2014 to August 2016. Fish monitoring was performed using an electrofishing technique, through a backpack electrofisher (ELT60II model; up to 3 amperes; up to 800 volts), following the guidelines for fish sampling reported within the ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale) protocol number 2040 [13]. Environmental data concerning the sampled watercourses are described in a previous work [14].

Fish monitoring was carried out in 30 different sampling sites belonging to the Nature 2000 Network, along 18 watercourses located in the 3 provinces of Parma, Reggio Emilia and Piacenza. According to the “Italian National Strategy for Biodiversity” all sampling sites (Figure 1) belonged to the Pianura Padana (Po plain) ecoregion [15], within ecological areas characterized by either salmonids or lithophilic cyprinid spawners, when referring to the ISECI and NISECI indices. A specific work [4] first attempted to describe the reference fish community of the area useful for an appropriate application of ISECI. However, alternative index proposals created a quite confusing situation. In fact, the alternative I.I. introduced a different biogeographic partitioning [10] and proposed a specific classification of areas following the original works by Bianco [16,17]. Our sampling sites were in the Eastern Apennine area within the Padano–Veneto fish district. Analyzed watercourses and sampling stations were located within the Northern Italian region highlighted in blue on the map (Figure 1). Codes of sampling sites were attributed following the international nomenclature of Nature 2000 Network.

2.2. Application of Fish Indices

The indices computed in our study and their specific formulas described as algorithm 1, 2 and 3 are reported in Table 1. It also describes the sub-metrics characterizing each index and the final score conversion to classify each site into one of the five classes requested by the Water Framework Directive [2]. It is noteworthy to observe that the class

limits of the official NISECI index were submitted to an intercalibration process at the European level. The procedure was carried out separately between alpine (Austria, France, Italy and Slovenia) and Mediterranean (Portugal, Spain, Italy, Greece and Bulgaria) areas. According to the intercalibration results the EQR ranges were partially modified for the alpine area [9].

Table 1. Algorithms applied in the three indices used to rate the quality class of the fish community.

Algorithm 1 Index of the Ecological Status of Fish Communities, ISECI [4]

$$1: \text{ISECI} = p_1 \times (p_{1,1} \times v_{1,1} \times (f_{1,1}) + p_{1,2} \times v_{1,2} \times (f_{1,2})) + p_2 \times \sum_{i=1}^5 (p_{2,i,1} \times v_{2,i,1} \times (f_{2,i,1}) + p_{2,i,2} \times v_{2,i,2} \times (f_{2,i,2})) + 0$$

- 2: f_1 = metric “presence of native species”;
 3: f_2 = metric “biological status of native species detected”;
 4: f_3 = metric “presence of hybrids”;
 5: f_4 = metric “presence of alien species”;
 6: f_5 = metric “presence of endemic species”.

- 7: Each metric is then weighted for a specific “ $p_{(1-5)}$ ”, allowing the conversion within the five classes reported below [4]:
 8: High = $\text{EQR} \geq 0.80$;
 9: Good = $0.60 \leq \text{EQR} < 0.80$;
 10: Sufficient = $0.40 \leq \text{EQR} < 0.60$;
 11: Scarce = $0.20 \leq \text{EQR} < 0.40$;
 12: Bad = $\text{EQR} < 0.20$.
-

Algorithm 2 New Index of Ecological Status of Fish Communities, NISECI [9]

$$1: \text{NISECI} = 0.1 \times x_1^{0.5} + 0.1 \times x_2^{0.5} + 0.8 \times (x_1 \times x_2) - 0.1 \times (1 - x_3) \times (0.1 \times x_1^{0.5} + 0.1 \times x_2^{0.5} + 0.8 \times (x_1 \times x_2))$$

- 2: x_1 = metric “presence/absence of native species”;
 3: x_2 = metric “biological condition of native species population”;
 4: x_3 = metric “presence of alien species or hybrids, relative population structures and numerical ratio with native species”.

- 5: The ecological status assessment, expressed as Ecological Quality Ratio:
 6: $\text{EQR} = (\log \text{NISECI} + 1.1283) / 1.0603$. The final score is in between one of the five classes reported below (boundaries for the sampled area follow [9]):
 7: High = $\text{EQR} \geq 0.80$;
 8: Good = $0.60 \leq \text{EQR} < 0.80$;
 9: Sufficient = $0.40 \leq \text{EQR} < 0.60$;
 10: Scarce = $0.20 \leq \text{EQR} < 0.40$;
 11: Bad = $\text{EQR} < 0.20$.
-

Algorithm 3 Fish Index of Forneris (I.I.) [10,11]

- 1: Each species sampled is given a naturalistic specific attribute called “Intrinsic Value (V)”
 2: $V = \text{AD} \times \text{ST}$ for native species, where:
 3: AD = Value assigned to the species according to the characteristics of its original distribution area in Europe and in Italy;
 4: ST = Value assigned to the species based on the state of its original distribution area;
 5: $V = -1$ for alien species.
 6: For each species detected in the sampling, there is a “Relative abundance index (I_a)”:
 7: “ I_a ” = Abundance index: composed of data relating to demographic consistency and those relating to the age structure of the population.
 8: Each “ I_a ” must be then converted into an “ I_r = Representative Index”, then used to calculate the final value.
 9: Finally, for each species observed, a score is calculated:
 10: $P = V \times I_r$ of each species sampled, where:

$$11: \text{I.I.} = \sum_{i=1}^n P1 \text{ nat. sp.} + P2 \text{ nat. sp.} + Pn \text{ nat. sp.} + P1 \text{ al. sp.} + P2 \text{ al. sp.} + Pn \text{ al. sp.}$$

- 12: *nat. sp.* = native species;
 13: *al. sp.* = alien species.

The result is not standardized, as required by the WFD. However, comparisons between the judgments obtained are allowed, since the final result is divided into 5 equal intervals corresponding to the 5 ecological quality classes required by the Directive [10,11]. High = I.I. > 42; Good = 42 > I.I. > 26; Sufficient = 25 > I.I. > 13; Scarce = 12 > I.I. > 6; Bad = I.I. < 6.

In addition to the standardized approach, we added a pioneering assessment with the aim of evaluating the quality of the fish community and the ecological status of the watercourses through an expert-based judgement to be compared to the indices classification. For this reason, expert-based evaluations were collected in the field involving three ichthyologists selected on the basis of their long-lasting experience in fish sampling and ecology, demonstrated by international publications, as well as by a deep knowledge of the considered sites. The three ichthyologists operated separately in blind conditions (each one unable to view their colleagues' results). Judgements were carried out considering the ecological conditions of the habitats, besides the taxonomic and functional value of the fish species. Afterwards, we made a correlation test to investigate the relationship between the outcomes obtained.

2.3. Brown Trout Determination

During our monitoring sampling campaigns, we found many specimens of trout, difficult to accurately identify without further genetic analysis. Owing to their phenotypes, we classified them as belonging to the *Salmo trutta* complex [18,19], apparently non indigenous or at least hybrids between the Mediterranean and the Atlantic lineages. The correct taxonomy of brown trout is often a common problem for researchers, and it is also nowadays an ongoing debate between ichthyologists. The species is frequently defined as native in the framework of indices calculations [6,20,21] by taking advantage of both trivial considerations about allochthonous strains [22] and practical justifications: (i) it is difficult to recognize the different forms and hybrids from a simple morphological examination; (ii) otherwise, the final result of the ISECI index would be heavily penalized in almost all of the Italian waterways where the reference fish community is represented only by trout. To assess the influence on index final values of possible mismatch in identifying the sampled trout, each index was therefore computed twice for each sampling station with trout: once recording all trout as native and once considering all specimens as aliens.

2.4. Statistical Analysis

Index computations were performed using Microsoft Excel (2016) spreadsheets, specifically designed for each applied index. Afterwards, we chose to perform two statistical tests to compare the distributions of our data. A more powerful Fisher's exact test [23] compared the outcomes of indices obtained considering, alternatively, brown trout either as a native species or as an alien one. This test was computed using R open-source software [24]. The same test was used to compare the results given by expert opinions considering brown trout as an alien species. The same comparisons were also tested with a less conservative approach. On all previous datasets, Pearson correlation tests were computed by using R `cor.test` function to quantify the similarity between pairs of evaluations (brown trout as native vs. alien species) and between pairs of expert judgements. To verify whether the sample size may have influenced our results, we ran simulations assuming to upscale the number of sites up to 2.5 times, maintaining the same frequency distribution of sites in the various quality classes.

3. Results

A total number of 18 fish species, nine of which were autochthonous, were found during samplings completed between 2014 and 2016 (see Tables 2 and 3). In particular, Table 2 illustrates a list of autochthonous taxa while Table 3 reports highly invasive alien species. Species common names are referred to from both Fishbase (www.fishbase.org accessed date 25 February 2021) and the Handbook of European Freshwater Fishes [25].

Table 2. Native species sampled during the field campaigns (endemic species in bold), in accordance with Zerunian et al. [4].

Native Species					
Common Name	Species Name	Order	Family		
Padanian barbel	<i>Barbus plebejus</i> (Bonaparte, 1839)	Cypriniformes	Cyprinidae		
Insubrian barbel	<i>Barbus caninus</i> (Bonaparte, 1839)				
Italian (Cavedano) chub	<i>Squalius squalus</i> (Bonaparte, 1837)				
Italian riffle dace	<i>Telestes muticellus</i> (Bonaparte, 1837)				
Gudgeon	<i>Gobio gobio</i> (Linnaeus, 1758)				
Lasca (South European nase)	<i>Protochondrostoma genei</i> (Bonaparte, 1839)				
Italian spined loach	<i>Cobitis bilineata</i> Canestrini, 1865			Cobitidae	
Padanian goby	<i>Padagogobius bonelli</i> (Bonaparte, 1846)			Perciformes	Gobiidae
Italian spring goby	<i>Knipowitschia punctatissima</i> (Canestrini, 1864)				

Table 3. Alien species sampled during the field campaigns.

Alien Species			
Common Name	Species Name	Order	Family
Goldfish	<i>Carassius auratus</i> (Linnaeus, 1758)	Cypriniformes	Cyprinidae
Pseudorasbora (Stone moroko)	<i>Pseudorasbora parva</i> (Temminck and Schlegel, 1846)		
Common carp	<i>Cyprinus carpio</i> (Linnaeus, 1758)		
Barbel	<i>Barbus barbus</i> (Linnaeus, 1758)		
Bleak	<i>Alburnus alburnus</i> (Linnaeus, 1758)		
Pond loach (Asian loach)	<i>Misgurnus anguillicaudatus</i> (Cantor, 1842)		Cobitidae
Pumpkinseed	<i>Lepomis gibbosus</i> (Linnaeus, 1758)	Perciformes	Centrarchidae
Wels catfish (European catfish)	<i>Silurus glanis</i> (Linnaeus, 1758)	Siluriformes	Siluridae
Brown trout	<i>Salmo trutta</i> complex (Linnaeus, 1758)	Salmoniformes	Salmonidae

Table 4 summarizes the overall comparison among quality class assessments achieved by means of the three indices application in each site. Figure 2 illustrates the percentage of sampling sites falling in the different classes. Outcomes obtained applying the ISECI index and considering brown trout as alien showed the absence of sampling sites belonging to High ecological status (Class I) and only 2 out of 30 sites (6.67%) were in Good status (Class II). However, most of them, 24 out of 30, were classified in the Sufficient ecological status (Class III), representing 80% of the total. Three sampling sites (10%) were assessed as Scarce (Class IV) while only one (3.33%) showed the lowest score (Bad, Class V). Instead, considering brown trout as a native species, these outcomes partially shifted (see Figure 2, column Bt). The sampling sites considered in Good ecological status (Class II) increased from 6.67 to 26.67% while the Sufficient ones (Class III) decreased by 16.67%. There was a slight decrease also for sites classified as Scarce (Class IV) that passed from 10 to 6.67%, while the ones of the lowest (Class V) and highest (Class I) ecological ranks remained constant. Data obtained from both scenarios were compared by using a Fisher's exact test. The p -value obtained computing Fisher's exact test was 0.17, which can be interpreted as a lack of statistical evidence about the existence of two different site distributions, caused by either native or alien attribution of brown trout specimens. Afterwards, the results obtained running the simulation of dataset up-scaling demonstrated that we should have at least increased the dataset by 1.6 times (48 sampling sites) to reach a significant p -value equal to 0.04.

Table 4. Summary table of results obtained applying three different indices (ISECI, NISECI, I.I.); sites where brown trout was present and considered as an alien species in bold.

Id Site	Watercourse	Natura 2000 Site Code	ISECI		NISECI		ICHTHYC INDEX (I.I.)	
			F	Class	RQE _{NISECI}	Class	I.I.	Class
10006.TR.1	Trebbia	IT4010006	0.47	III	0.48	III	17	III
10008.AR.1	Arda	IT4010008	0.50	III	0.58	III	32.5	II
10011.TR.1	Trebbia	IT4010011	0.49	III	0.54	III	19	III
10016.TR.1	Trebbia	IT4010016	0.57	III	0.61	II	38.5	II
10016.TR.2	Trebbia	IT4010016	0.60	III	0.54	III	16	III
10017.NU.1	Nure	IT4010017	0.61	II	0.62	II	20	III
20003.ST.1	Stirone	IT4020003	0.50	III	0.68	II	41.5	II
20017.LO.1	Lorno	IT4020017	0.51	III	0.50	III	14	III
20020.PR.1	Parma	IT4020020	0.41	III	0.28	IV	18	III
20020.PR.2	Parma		0.36	IV	0.32	IV	19.5	III
20021.TA.1	Taro	IT4020021	0.45	III	0.53	III	36.5	II
20021.NA.1	Naviglio	IT4020021	0.41	III	0.35	IV	18	III
20021.CE.1	Ceno	IT4020021	0.56	III	0.63	II	33	II
20022.TA.1	Taro	IT4020022	0.28	IV	−0.23	V	7	IV
20025.PR.1	Parma Morta	IT4020025	0.20	V	NA	V	−5	V
30013.EN.1	Enza	IT4030013	0.41	III	0.37	IV	14	III
30014.RV.1	Rio Vico	IT4030014	0.48	III	0.31	IV	4	V
30014.RC.1	Rio Cerezzola	IT4030014	0.61	II	0.48	III	12	IV
30014.RC.2	Rio Cerezzola	IT4030014	0.51	III	0.44	III	20	III
30023.EN.1	Enza	IT4030023	0.44	III	0.58	III	33	II
30023.EN.2	Enza	IT4030023	0.44	III	0.44	III	22	III
30023.EN.3	Enza	IT4030023	0.50	III	0.54	III	26.5	II

Table 4. Cont.

Id Site	Watercourse	Natura 2000 Site Code	ISECI		NISECI		ICHTHYC INDEX (I.I.)	
			F	Class	RQE _{NISECI}	Class	I.I.	Class
30023.EN.4	Enza	IT4030023	0.43	III	0.52	III	22	III
20020.PM.1	Parmossa	-	0.56	III	0.49	III	23	III
20020.CE.1	Ceno	-	0.49	III	0.28	IV	8	IV
30013.CE.1	Cedra	-	0.50	III	NA	V	-2	V
20020.FA.1	Fabiola	-	0.50	III	NA	V	-2	V
20020.MO.1	Rio Moneglia	-	0.37	IV	0.33	IV	-1	V
20020.BA.1	Baganza	-	0.45	III	0.47	III	20	III
20027.EN.1	Enza	IT4020027	0.49	III	0.67	II	36.5	II

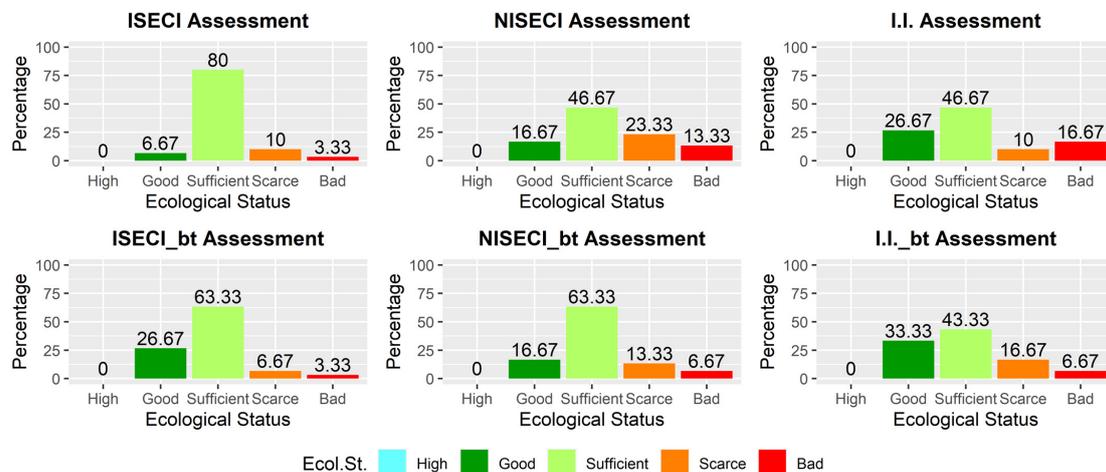


Figure 2. Ecological status of sampling sites; comparison among ISECI (left), NISECI (middle) and I.I. (right) indices considering brown trout either as an alien (top row) or a native species (bottom row).

Moreover, the outcomes obtained through the ISECI index considering brown trout as a native species, and then as an alien, were highly correlated ($r = 0.93$, p -value = 0.020).

The NISECI index application upheld the absence of sites belonging to the High ecological status (Class I) and 5 out of 30 sites (16.67%) were in Good status (Class II). However, most of them, 14 out of 30, were classified as Sufficient (Class III), representing 46.67% of the total. Seven sampling sites (23.33%) were assessed as Scarce (Class IV) while four (13.33%) showed the lowest score (Bad, Class V). Interestingly, if we considered brown trout as a native species, the outcomes slightly shifted (see Figure 2, column Bt). Sampling sites considered to have High and Good ecological status (Classes I and II) remained constant while the Sufficient ones (Class III) increased by 16.67%. Consequently, there was a decrease (10%) also for the sites classified as Scarce (Class IV), while the one of lowest rank (Class V) changed from 13.33 to 6.67%. Data obtained from both scenarios were compared through Fisher’s exact test and the resulting p -value = 0.54 suggested a lack of statistically significant differences between them. The brown trout attribution to alien or native species did not change the overall quality pattern of sites. Even the simulation run strengthened the same conclusion, reporting a p -value = 0.12 notwithstanding the dataset upscaling up to a factor 2.5 (75 sampling stations). Moreover, the outcomes obtained through the NISECI index, considering brown trout as a native species and then as an alien, were highly correlated ($cor = 0.95$, p -value = 0.013).

Results obtained applying I.I. as an alternative choice to the ISECI/NISECI approach are also illustrated in Figure 2. The data show the absence of sampling sites belonging to

High ecological status (Class I) and as many as 8 out of 30 sites (26.67%) were in Good status (Class II). In addition, as for the NISECI index, 14 sites representing 46.67% of the total were classified as Sufficient (Class III). Three sampling sites (10%) were assessed as Scarce (Class IV) while five (16.67%) showed the lowest score (Bad, Class V). Considering brown trout as a native species, the outcomes shifted marginally. In fact, sampling sites of Good ecological status (Class II) increased from 26.67 to 33.33% while the Sufficient ones (Class III) decreased by 3.33%. There was a slight increase for sites classified as Scarce (Class IV), that passed from 10 to 16.67%, as a shift from the ones of lowest rank (Class V) that decreased from 16.67 to 6.67%. A significant difference between both scenarios was not statistically supported by the Fisher's exact test, which released a p -value = 0.60. Indeed, even the simulation run bolstered the same conclusion, reporting a p -value = 0.23 notwithstanding a dataset upscaling up to a factor 2.5 (75 sites). Again, the outcomes obtained considering brown trout as a native species, and then as an alien, were highly correlated ($\text{cor} = 0.92$, p -value = 0.026).

It is noteworthy to observe that in this case the comparison of results obtained applying ISECI and NISECI (considering for both cases brown trout as an alien species) revealed a statistical weak difference with a p -value = 0.07. For this reason, a dataset simulation was run by upscaling results by 1.1 times to reach a significant p -value equal to 0.03, i.e., having only 10% more samples would be enough to reach the statistical threshold of 5%.

Percentages seemed more comparable between NISECI and I.I. than between ISECI and NISECI. However, we could not statistically compare results obtained from these indices, due to the non-standardized nature of the outcomes obtained by the I.I.

Separate expert-based judgements given in the framework of a blind approach performed by researchers having long-lasting experience on the analyzed watercourses are illustrated in Figure 3. Despite all three experimental indices assessing 0% of sites belonging to the High Ecological Status (Class I), the expert-based judgement was consistent in defining a percentage of 16.67, 13.33 and 10%, respectively, of sites belonging to this category. It is also remarkable to observe a clear mismatch for Class II (Good Ecological Status). In fact, considering the 26.67–33.33% range defined by the I.I. as the highest percentage defined by the mathematical approach, this value is distant from the experts' evaluations, which were 60, 53.33 and 50%, respectively. Obtained results were compared by Fisher's exact test. The resulting value $p = 0.82$ can be interpreted as a lack of statistical evidence about the existence of different interpretations and therefore confirmed the consistency of the expert judgements. The simulation run bolstered the same conclusion, reporting a p -value = 0.28 notwithstanding a dataset upscaling up to a factor of 2.5 (75 sites). Moreover, the outcomes obtained by the experts were highly correlated (expert judge 1–expert judge 2 = 0.94, p -value = 0.017; expert judge 1–expert judge 3 = 0.86, p -value = 0.064; expert judge 2–expert judge 3 = 0.98, p -value = 0.004).

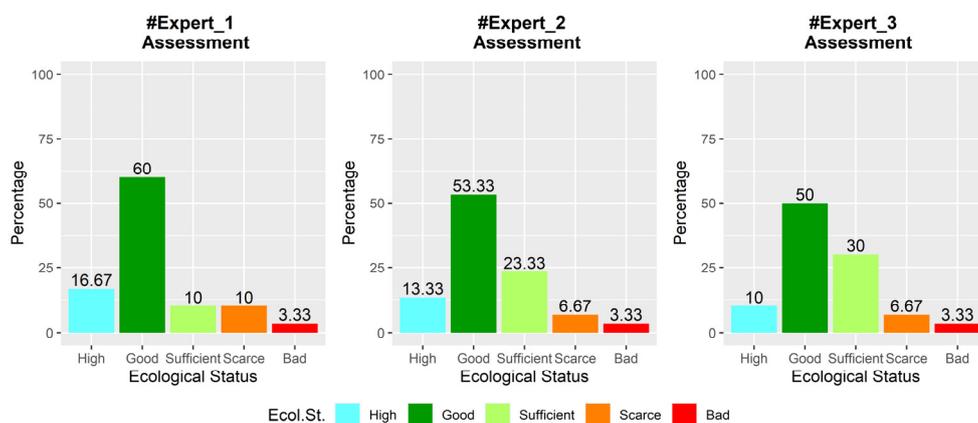


Figure 3. Ecological status of sampling sites assessed by three blind expert judgements.

4. Discussion

The application of multidisciplinary integrated indices is fundamental to fulfil the requests of the European Water Framework Directive. The search for a useful index suitable for the pronounced geographical and ecological variability of Italian water basins has been carried out for a long time, and has opened many disputes about the index efficacy. Notably, considering the ISECI tool as the starting proposal, its application within our study pointed out several critical issues already highlighted in previous works [6,7,26]. The main criticism must be directed to the determination of the reference fish community, considering that the ones suggested by the official guidelines [4] are nowadays inaccurate and ineffective. In fact, many native species of crucial importance for the final score are nowadays absent, and it is difficult to foresee their quick recovery in the near future. That is the case for lampreys or sturgeons, but also for less rare species.

More precisely, two different aspects of this issue should be taken into consideration: (1) the difficulty of finding rare and localized species, such as sedentary lampreys *Lampetra planeri* (Bloch 1784) and *L. zanandreaei* (Vladykov 1955), or other species with similar ecological characteristics; and (2) the present total absence from most of the Italian watercourses of diadromous fishes, such as anadromous lampreys, sturgeons, twaite shad and catadromous eel. It must be remarked that their absence is due to human-caused alterations (i.e., river fragmentation due to man-made weirs and dams). Therefore, a functional tool to assess the ecological quality of watercourses should be able to highlight also anthropogenic pressures.

A further issue deals with the current biogeography of taxa. In fact, the original algorithm expects such subendemic alpine species as the marble trout (shared among Northern Adriatic countries) to be found also in Apennine rivers that actually do not belong to the original biogeographical allocation of this salmonid. Although recent research speculated on a putative ancient presence of the marble trout in Central and Southern Apennine basins [27,28], these data will have to be further confirmed. In any case, the species presence and its role as a resident ecological indicator of this district must nowadays be neglected due to the consistent phylogeographic modifications of river basins since glacial times that make Apennine rivers unsuitable for marble trout acclimation.

Applying the recently modified NISECI index [9], we found improvements compared to the previous form. The removal of the specific parameter regarding hybrid taxa, whose classification based only on morphological traits would produce confusing results, seemed advantageous. This adjustment was pivotal for trout and barbels whose taxonomy is often confused and strongly affected by mixed morphological characters within cryptic phenotypes [27–29]. Despite this, they live in ecologically valuable waters no matter whether or not they are pure lineages or hybrids. The slight importance of hybrids, which are considered together with alien species inside the NISECI computation, had the auxiliary effect to increase the importance of autochthonous taxa. Indeed, the outcomes of our work confirmed that the presence/absence of native species proved to be crucial for the index final score, whereas alien species were less influential. It must be remarked that not all alien species affect biodiversity and the ecological status of freshwater ecosystems in the same way [22,30]. In any case, emphasis gained by native species rather reflected a further enhanced bond between the index and the reference fish community. As mentioned in the previous section, our data demonstrate the unsolved weak point related to the reference communities.

The awaited fish community for index application can be adapted, adding or removing species from the original list, to better describe fish assemblages of analyzed watercourses [6]. Nonetheless, the establishment of fish communities characterizing every waterway threatens the index objectivity, which may result in being too biased, owing to the importance acquired by the single expert assessment. Within our sampling campaigns, we found it particularly difficult to swiftly identify and correctly assign fish belonging to the genera *Barbus* and *Salmo*. This was due to their potential hybridization with the European barbel [29] for the former, and within different lineages of the *Salmo trutta* complex for

the latter [31,32]. For this reason, we made a dual assessment focused on brown trout specimens, considered as an alien and then as a native taxon.

Differences emerged in the ISECI calculation with evident shifts between Class III (Sufficient) and II (Good). In fact, the number of sites belonging to the “Good ecological status” decreased abruptly whenever considering brown trout as an alien species. As a matter of fact, the majority of the Italian brown trout populations are nowadays introgressed [27,28,31,32], and for this reason the index underestimates even high-quality habitats.

The application of NISECI reported slight variations in the categories attribution requested by the Water Framework Directive guidelines, and consequently also when applying the dual assessment of brown trout. These conclusions were proved also by dataset upscaling, which reported no significant statistical difference with Fisher’s exact test, despite an increase equal to 2.5. The assessments obtained applying ISECI [4] displayed greater variations compared to NISECI [14] and to the I.I. [10,11], due to the lesser influence of alien species in the final evaluation of the former and the absence of these species in the reference list of the latter. Indeed, the comparisons between ISECI and NISECI outcomes, both considering brown trout as an alien species, acknowledged a significant statistical difference by Fisher’s exact test following an increase in the dataset by 10%. Therefore, NISECI [9] and I.I. [10] showed their strictness in the final assessments compared to ISECI [4], underlined by the increase in sampling sites belonging to the worse classes of ecological status. It must be remarked that the I.I. considers all salmonid taxa (even the Mediterranean lineages) as alien species in the Apennine district. This is a limiting aspect of the I.I., considering that Mediterranean lineages of brown trout have been described in Adriatic Apennine basins. Regardless of statistical analyses, the morphological classification of hybrid barbels and trout of Northern Italy is often not sufficient to discriminate their taxonomic level. This issue makes difficult their assignment within the reference fish communities currently available, and therefore genetic analyses are the only effective tool able to achieve an appropriate identification.

It is noteworthy to observe that an unexpected result emerged in relation to the ecological status value for each site that had to be expressed in the form of an Ecological Quality Report (EQR) according to the European legislation [2]. Following the index guidelines [9], the conversion from NISECI results to the EQR data should always refer to a positive value, ranging between zero and one. However, a negative EQR value was found at the specific site 20022.TA.1 (SIC IT4020022), determined by a very low NISECI score. This was probably due to the limited number of sampled fish belonging to different species and related unstructured populations affecting the algorithm calculation and generating the unexpected negative value. Such a situation could therefore happen either for sampling biases or for real natural circumstances. This emerging aspect in the application of NISECI metrics will have to be further investigated.

To fulfil an exhaustive evaluation of the above-cited positive and negative aspects, the expert-based judgements added further knowledge despite the metrics application. The three ichthyologists operated individually and in blind conditions. Judgements revealed an astonishing mismatch with the experimental data obtained through indices application. In addition, all three judgements were in good agreement among them. More precisely, the expert judgements upgraded to High and Good status a consistent percentage of analyzed sites previously classified in lower categories. For this reason, it is crucial to consider the knowledge of local experts able to focus on the ecological conditions influencing an expected, not putative, reference population. Thus, it must be remarked the ability of fish to adapt to local conditions also with seasonal variability in many cases (for instance, trout migration according to water temperatures, shads in relation to reproductive homing and cyprinids hiding during winter months), therefore influencing the index calculation especially for sites inhabited by a limited number of species.

5. Conclusions

Results from this study support the hypothesis of the mismatch between the reference fish communities proposed in the application of the official Italian index of the WFD and the observed ones, as well as the pivotal role of brown trout determination. Our conclusions reveal the pronounced influence of the local reference fish community and further aspects bound to ecological conditions. Improvements introduced by the NISECI index [9] are conspicuous, but further investigations are necessary to introduce local correction factors to completely fulfil the European requirements according to local habitat and ecological conditions.

Author Contributions: S.P. carried out the indices computation, statistical analysis, interpreted the data and wrote the first draft of the manuscript; F.N.M. conceptualized the study and edited the advanced draft of the manuscript; A.V. worked on data collection and provided feedback on the research approach; S.L. provided feedback on statistical analysis and edited the advanced draft of the manuscript; P.M.R. and L.M. worked on data collection; F.P. worked on data mapping and provided feedback on the research approach. All authors have read and agreed to the published version of the manuscript.

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