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SEVENTH FRAMEWORK PROGRAMME

WISER

Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery

DELIVERABLE

Deliverable D4.4-2, part 1: Testing the adaptability and behaviour of 6 fish indices on a common dataset composed of multiple gears samplings from 8 estuaries and lagoons

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PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
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Content

Non-technical summary

The Water Framework Directive (WFD) aims at achieving good ecological status for surface waterbodies throughout Europe, by 2015. Consequently European countries are currently developing and intercalibrating methods based on biological, hydromorphological and physico-chemical quality elements for the assessment and the monitoring of their rivers, lakes, coastal and transitional waters. In this context, the FP7 WISER project aims to support the implementation of the WFD by contributing (i) to making the existing assessment methods more comparable, and (ii) to estimating the uncertainty along each step of the assessment.

The present work focuses on fish indicators for estuaries and lagoons (transitional waters in the WFD). Six fish indices (AFI, EFAI, ELFI, TFCI, BHI, Z-EBI) were tested on a common dataset, covering eight estuaries and lagoons throughout Europe. Fish sampling was carried out using several gears in 2009 and 2010. The objectives were twofold: (i) to test the adaptability of fish indices to different gears and different types of transitional waters; and (ii) to compare the behaviour of the indices with regard to their level of agreement.

Five out of the six tested indices were gear-specific and all were specific to some type(s) of transitional waters and have particular data needs. Therefore calculating the indices on a common dataset raised many difficulties, especially regarding the determination of appropriate reference condition values. However, taking some reasonable assumptions it was possible to calculate most of the indices on the majority of the available data, thus showing their relative adaptability. The indication of extreme values for quality (high or bad) was relatively rare and the results of the indices often differed: when used outside of their initial framework, geographical limits or with different sampling methods, fish indices' results are highly uncertain. This shows that the assessment of quality using fish indicators highly depends on the assessment tool, the sampling methodology and the type of transitional water. Despite these results, some statistically significant correlations between pairs of indices' results were found, indicating the possibility of intercalibration between some of the tested indices.

This work corresponds to the first step of WISER WP4.4 Deliverable 2. In a second step, some fish metrics in the indices tested here will be selected for a more in depth study: the effect of several natural sources of variability on these metrics will be studied and uncertainty will be quantified along the assessment process, from the sampling to final EQS formulation using WiserBugs software.

Introduction

The WISER project aims at supporting the implementation of the Water Framework Directive (WFD – Directive 2000/60/EC; European Council 2000) by developing new tools and / or improving existing tools for the assessment of the ecological status of European surface waters such as transitional and coastal waters. These tools are based on phytoplankton, aquatic flora (phytobenthos, macroalgae and angiosperms), benthic invertebrate fauna, and fish fauna. In particular, there is every expectation that WISER contributes (i) to making the existing assessment methods more comparable (*i.e.* WISER is expected to complement the Intercalibration process phase II, presently carried out by European countries), and (ii) to estimating the uncertainty of the assessments.

Since fish assemblages were first proposed in the 80s to assess the biotic integrity of freshwater systems (Karr 1981) a suite of assessment methods based on fish fauna have been proposed. These indices often contain several metrics and as such aim to provide a balanced representation of fish quality features that correlate with the conservation status of the system under evaluation. Although this basic rationale is shared by all fish-based indices, the actual composition of the different indices is not the same. Differences arise in the form of alternate sampling methodologies, constituent metrics, aggregation and scoring of metrics, reference conditions, etc. In most instances indices have been optimized during their development for a particular type of system or even individual estuary(ies), which may have compromised their performance in alternate systems rendering them insensitive or mistaken and open to criticism. The extent to which fish indices are affected by their own developmental method is currently unknown but important to quantify specially for the harmonization of large scale monitoring and restoration programmes, such as those in development for the WFD implementation across Europe.

It is clear that despite the multiple advantages of fish for a high-level quality integration of ecological quality features in bioassessment (Karr 1981) there are also some drawbacks. Especially relevant, due to direct effects on the outcomes of quality assessments, are the often extreme seasonal variability of fish assemblages in estuarine systems and sampling variability. This together with difficulties posed by the large natural abiotic variability of estuarine systems adds uncertainty to the assessments and compromises the accuracy and generality of the results. Inaccurate or uncertain evaluations cast doubts upon the actual status of conservation of the systems under evaluation making effective management plans impossible.

Within this context of current challenges to fish based assessments in transitional waters, WISER Work Package 4.4 (WP4.4) produced a review of existing fish indices for transitional waters throughout the world. The results of this study are presented in WISER Deliverable 4.4-1 (Perez-Dominguez et al. 2010). Deliverable 4.4-2 is the second step toward the contribution of WP4.4 work to both the intercalibration process and the estimation of the uncertainty in the WFD assessments. It has a dual aim: (i) assess the intercomparability between existing assessment methods (present work), and (ii) to estimate the degree of uncertainty built into fish-based assessments.

The work presented here corresponds to the first aim of Deliverable WP4.4-2. Six of the indices reviewed in Deliverable 1 (Perez-Dominguez et al. 2010) were tested on a common dataset obtained by a standardized sampling programme. Fish surveys were carried out in 2009 and 2010, with five different gears in eight transitional sites, including both estuaries and lagoons. From this comparison, several outcomes are expected:

- information on the adaptability of the tested indices for different gears, *i.e.* testing how much each index is dependant on one particular gear and whether it is possible or not to adapt it to other gears;
- a deep insight into the methodological particularities of each index (such as scale of calculation of the metrics, method for the standardisation of the data, etc.);
- information on the correlation between indices' results, both in terms of Ecological Quality Ratios (EQRs) and Ecological Quality Status (EQS).

Difficulties encountered in the application of the indices to the WISER dataset are also analyzed, especially those related to the definition of reference conditions for the corresponding estuaries and lagoons.

Material and methods

WISER surveys

For the purpose of the WISER project, fish sampling surveys were conducted in 5 different transitional water sites, both estuaries and lagoons (Figure 1): Mondego estuary, in Portugal; Lesina lagoon, in Italy; Orwell and Stour estuary, in England; and Varna Bay and Varna Lake, in Bulgaria. Additional data from 3 Basques estuaries (Nervion, Oiartzun and Bidasoa) were also used in this study, as well as supplementary data on the Orwell and Stour estuary that were obtained from the UK Environment Agency (EA). All surveys were conducted in 2009 except for Bidasoa and Oiartzun that were sampled in 2010.

Five different gears were used (Table 1 and Table 2). WISER campaigns were undertaken by WISER researchers to ensure uniform methodology in the operation of the different gears and sample analysis. For Basque and EA data, sampling method was identical to the one used in the WISER field campaigns, for the same gear. The choice of the gear was mainly dependant on technical feasibility and access to the sampling area. The sampling protocol and hence the sampling effort of each fishing event were standardised for all gears except for beam trawls where haul lengths ranged from 200 m to 1100 m (calculated using the average speed and the duration of the hauls or computed from the geographic coordinates of the starting and ending points of the hauls). Table 1 summarizes the principal characteristics of the fish surveys considered in this study: sampling sites, sampling seasons, sampling gear and origin of the data.

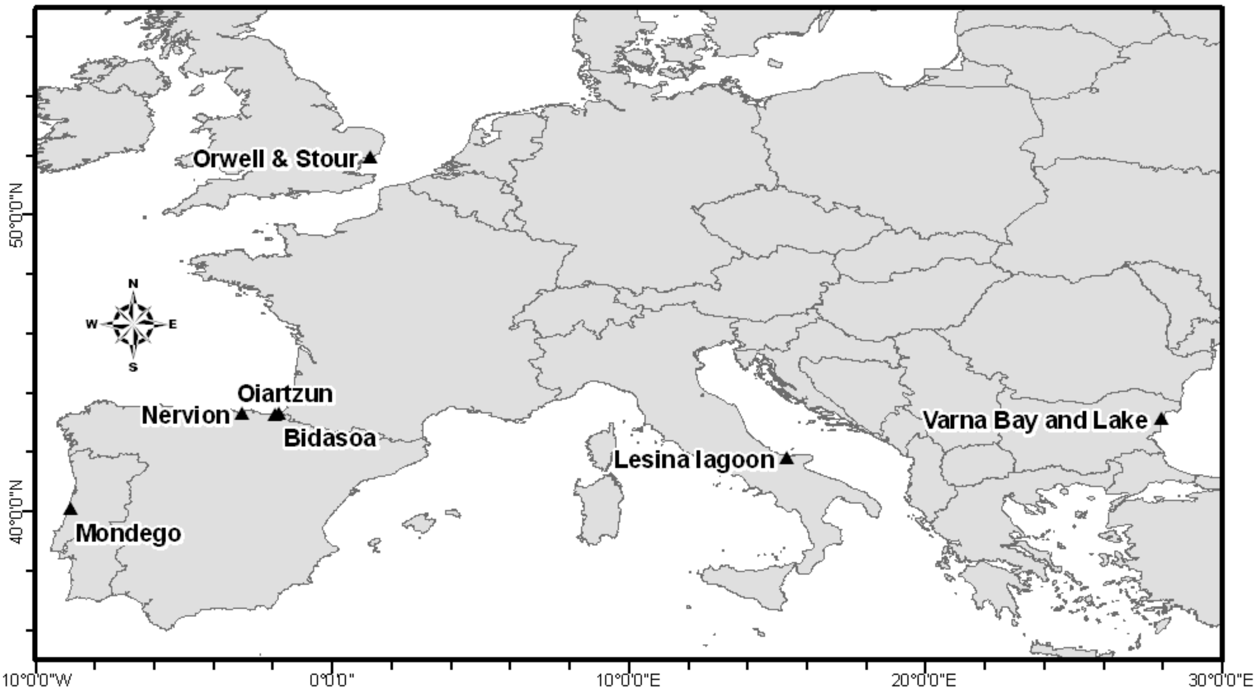

Figure 1: Map of the estuaries and lagoons where fish indices were tested in the present study

Fishing events, corresponding biological and environmental data

Hereinafter a fishing event is defined as a beam trawl haul, a beach seine haul or a fyke net collection.

After removing unsuitable data, *i.e.* fishing events considered as failed, a total of 212 fishing events were finally used to calculate the indices. The number of fishing events per estuary or lagoon, salinity class, gear and season ranged from 3 to 18 (Table 1).

For each fishing event, fishes were identified (whenever possible) at the species level, measured and counted. Several environmental parameters were also measured during fish surveys. Salinity and depth (recorded respectively for 83% and 77% of the fishing events) ranged from 0.16 to 34.8 psu and from 0.5 m to 13.6 m. When salinity data was missing it was possible to get information on the salinity class, hence this data was available for all fishing events. Salinity classes were adapted from the Venice system (Anonymous 1958): class 1: oligohaline (0 – 5); class 2: mesohaline (5 – 18); and class 3: polyhaline/euhaline (> 18). Other environmental variables were sometimes recorded, at the bottom or at surface, such as the pH, temperature and oxygen saturation. Secchi depth was also recorded for some fishing events. However, only data on salinity class were here used since these are the only ones required for some of the tested indices

*Table 1: Overview of the samplings considered in the present work, and number of fishing events per estuary or lagoon, salinity class (1 - oligohaline (0 – 5), 2 - mesohaline (5 – 18) and 3 - polyhaline/euhaline (>18)), season and gear that were used to compute the fish indices.; * Varna lake is here considered as a lagoon though in Bulgaria it is usually considered as a "liman lake", which is a lake formed at the mouth of a river where flow is blocked by a bar of sediments (Violin Raykov, pers. com[1]). Hence, it is a particular type of lagoon.*

Site	Estuary / lagoon	Data source	Salinity class	Season	Gear	Number of fishing events
Varna Bay	Estuary	WISER survey	2	autumn	Beam trawl	10
Varna Lake	Lagoon*		2	autumn	Beam trawl	7
					Fyke net	6
Lesina	Lagoon		2	autumn	Fyke net Cemagref	3
					Fyke net	18
Mondego	Estuary		1	autumn	Beam trawl	3
					Fyke net	4
			2		Beam trawl	6
					Fyke net	6
			3		Beam trawl	6
					Fyke net	5
Nervion	Estuary	AZTI / Basque Water Agency	3	autumn	Beam trawl	9
				spring	Beam trawl	9
				summer	Beam trawl	9
Oiartzun	Estuary		3	autumn	Beam trawl	12
				spring	Beam trawl	12
				summer	Beam trawl	12
Bidasoa	Estuary		1	spring	Beam trawl	3

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			2	spring	Beam trawl	3
			3	autumn	Beam trawl	12
				spring	Beam trawl	6
				summer	Beam trawl	12
Orwell & Stour	Estuary	WISER survey & UK Environment Agency	3	spring	Beam trawl	9
					Fyke net 24 hours	3
					Beach seine EA	15
					Beach seine Wiser	12

Table 2: Gears used in WISER surveys and corresponding characteristics

Gears	characteristics	Type of gear
Beam trawl	Width: approximately 1.5 m. Height: 0.5 m	active
Fyke net and Fyke net 24 hours	Two double fyke nets set in tandem. For each fyke: leader: 20 m; 2 wings: 8 m. Fish catches were collected every 12 hours (fyke net) or every 24 hours (fyke net 24 hours)	passive
Fyke net Cemagref	Fyke net used principally in French lagoons for the purpose of WFD: leader: 20 m; 2 wings: 8 m	passive
Beach seine EA	Beach seine of 45 m x 3.5 m (length x height). Distance sampled from shore estimated to 30 m	active
Beach seine Wiser	Beach seine of 30 m x 2.7 m (length x height). Distance sampled from shore estimated to 20 m	active

Common species list and functional guilds

A common list of fish species was compiled based on the World Register of Marine Species (WoRMS) database (Appeltans et al. 2011). Only these species that were caught in the dataset described previously were considered (Annex 1).

Most of the indices tested here (all except the Biological Health Index – BHI, see Table 3 and 4) include some metrics based on functional guilds, *i.e.* groups of organisms which share their biological characteristics such as nature of reproduction, feeding, spatial and temporal use of an area (Elliott and Dewailly 1995). For the so called “ecological guilds”, “position guilds” and “trophic guilds”, which are used in several fish indices, a common assignment to fish species was reached. In general, definitions of these guilds came from Elliott and Dewailly (1995) and Franco et al. (2008). However for some ecological guilds it was decided to adapt their definition so that it becomes more appropriate to the transitional waters studied here. These modified definitions are detailed hereafter:

- Estuarine resident species (ER): when more than 50% of the population of adults and juveniles is found in transitional waters. In practical terms ER characterizes very small species that are not known to venture outside the transitional water where they reside, such as Gobiidae, *Parablennius*, *Hippocampus*, *Syngnathus*, etc.
- Marine juvenile species (MJ): when a significant shift in juvenile distribution is observed between marine and transitional (or coastal) waters, due to a distinct migration or larval/juvenile dispersal reaching into transitional waters. In practical terms these are marine species when the majority of fishes caught in transitional waters are juveniles;
- Marine seasonal species (MS): species that are entering the transitional system only at a certain periods of the year and where adults and / or juveniles are found in numbers;

- Marine adventitious species (MA): when the main populations of both adults and juveniles are not found in transitional but in coastal waters. These species may be captured with regularity but numbers are low;
- Diadromous species (DIA): species that cross salinity boundaries and are able to survive in freshwater and in sea water.

The guilds commonly agreed by the authors are presented in Annex 1. For all other guilds or fish characteristics that are used in only a few indices (such as introduced species, pollution tolerant species, etc.), no general agreement was necessarily achieved; these guilds were dealt by the partner responsible for the calculation of the corresponding index.

Calculation of the tested fish indices

Six of the indices reviewed in WISER Deliverable D4.4-1 (Perez-Dominguez et al. 2010) were tested: AFI, EFAI, ELFI, TFCI, BHI and Z-EBI (Table 3). Indices were calculated following as far as possible the guidelines specified in the corresponding paper(s) describing the methodology. However, some adjustments were sometimes required to accommodate the WISER dataset and some indices were calculated in their most recent version. Changes are presented hereafter.

Indices were calculated per gear, time of the day, season and salinity class to allow for the comparability of their outcomes (Jurvelius et al. 2011). This approach also avoids bias due to the unbalanced combination of gears between the sampled estuaries and lagoons. Both EQRs and corresponding EQSs were computed.

When necessary for the calculation of fish metrics, beam trawl and seine catches were standardized and expressed as individuals per 1000 m^2. For seine the sampled distance from shore was estimated to 20 m (seine Wiser) and 30 m (seine EA) (Table 2). Fyke net data was directly expressed as catch per fyke tandem arrangement and net visit.

Metrics that compose each index are detailed in Table 4.

Table 3: Indices tested for fish assessment and corresponding references

Acronym	Full name	Country	Sampling method	Ref.
AFI	AZTI's Fish Index	Basque country	Beam trawl	Borja et al. 2004, Uriarte et Borja 2009
EFAI	Estuarine Fish Assessment Index	Portugal	Beam trawl	Cabral et al. Submitted
ELFI	Estuary and Lagoon Fish Index	France	Beam trawl for estuaries and fyke nets Cemagref for lagoons	Delpech et al. 2010
TFCI	Transitional Fish Classification Index	United Kingdom	Seine nets, otter trawls, beam trawls	Coates et al. 2007
BHI	Biological Health Index	South Africa	Multiple gears	Cooper et al. 1994
Z-EBI	Zone-specific Fish-based Biotic Index	Belgium	Double fyke nets	Breine et al. 2010

ELFI

The French index calculated in this exercise is slightly different from the one presented in Delpech et al. (2010). ELFI is continually being improved as new data are getting collected for the purpose of the WFD. It was decided to use the last version of this index, which is the one currently in use to evaluate the ecological status of French transitional waters for the WFD and in the IC exercise.

For estuaries, two more metrics were added to the published version (Table 4): density of estuarine resident (DER) and total species richness (SR) per trawl haul. In the oligohaline zones, the metric “density of marine juvenile migrants” (DMJ) was replaced by the density of freshwater species (DFW). The total species richness is calculated per fishing event and standardised by the log of the sampled surface (Nicolas et al. 2010). Density is the number of individuals per 1000 m^2 and it is log-transformed, as specified in Delpech et al. (2010). Hence it was not possible to calculate ELFI on fyke data in estuaries as they can not be expressed in number of individuals per unit of surface.

The ELFI for lagoons is not finalized yet and several versions are still being tested while the WFD dataset is getting bigger. The version of this index that was tested here has been parameterized on all WFD French lagoons except the Corsican lagoons. It is composed of three fish metrics (Table 4): density of benthic invertebrate feeder species (DIB), density of zooplankton feeders (DZ) and density of diadromous species (DDIA). All three metrics are (log(number of fish)+1). DIB and DDIA correlate negatively with pressure while DZ correlates positively. Metrics are calculated at the scale of each fishing event and then averaged at the scale of the salinity class x season (hence here at the scale of the lagoon) to compute the final result. A description of the data used to compute this indicator, as well as some general information about the method to model the metrics (GLMs), can be found in Drouineau et al. (2012). However, the method used to combine the metrics here is a simple average, hence it is different from the one presented in this last publication.

AFI

All sampled estuaries (or lagoons) were considered as “type III” estuaries (see Borja et al., 2004); hence, only fish data were used to compute AFI (*i.e.* data about crustacean catches were not requested). The Basque estuaries “type I” and “type II” (small river-dominated estuaries and estuaries with extensive intertidal flats, respectively) contain only a small number of estuarine resident fish species, thus in these cases, crustaceans used to be included (as characteristic demersal components of the estuaries – Borja et al., 2004).

Although AFI is only designed for beam trawl surveys with 3 or 4 sampling stations per waterbody and 3 replicates per station, in WISER it had to adapt to new conditions: many sampling stations had no real replicate. AFI has been here used also for fyke nets and beach seine, gears which probably require the use of other metrics. AFI was calculated according to the Basque reference conditions, due to the absence of other reference conditions for the rest of the estuaries and lagoons studied here.

The method incorporates (Table 4): the richness (number of species – metric 1); indicator and introduced species (% of individuals – metric 2 and 3); fish health (% affection – metric 4); flat fish presence (% individuals), trophic composition (% omnivorous and piscivorous – metric 6 and 7); and resident estuarine species (% of individuals and number of species – metric 8 and 9) – see Borja et al. (2004) and Uriarte and Borja (2009) for more details. For metric 2 "pollution indicator species" and metric 3 "introduced species" (Table 4), species were characterized specifically in each of the sampling sites based on the bibliography (sources available on demand). In the case of "fish health" metric (metric 4), it is considered that individuals caught showed good conditions since no operator detected any damage, alteration or illness on them.

Z-EBI

Historical data to compute reference lists were often not available for the studied sites, and, when available, the sampling effort and even sometimes the sampling gears were not stated. This introduces great uncertainty on the relevance of using such data for the present exercise. Thus it was decided to use the reference lists from the Scheldt estuary as presented in Breine et al. (2010). This approach is supposed to be more homogeneous and more easily interpretable. As in Breine et al. (2010) there is no reference list specified for polyhaline waters, Z-EBI was only calculated for salinity class 1 (oligohaline) and salinity class 2 (mesohaline). Freshwater was not considered as in the presently studied dataset, no fishing event was performed in freshwater. Moreover, following advises from Jan Breine (pers. com.[2]) we only calculated Z-EBI on fyke net data; this means beach seine data and beam trawl data were not considered.

TFCI

The TFCI was here tested in its original version, *i.e.* the one described in Coates et al. (2007). Gear-specific reference conditions and metric scoring thresholds were established following Coates et al. (2007). They were derived from EA fish data sampled between April and October 2010 in the Ecotype E4T3 (*i.e.* estuaries within the North Sea, fully mixed, polyhaline, macrotidal, sheltered and with extensive intertidal areas (Coates et al. 2007)). It was not possible to derive such gear-specific reference conditions for each of the estuary or lagoon studied here; hence the ones developed on Ecotype E4T3 were used for all sites considered in the present work.

It is important to note that TFCI has been greatly modified and improved since its very first version (Steve Coates, pers. com.[3]). In particular, the newest version of TFCI includes a whole evaluation of the uncertainty in the assessment followed by important requirements for the assessment to be reliable (such as minimum sampling effort, time of the year, number of years of sampling etc.).

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BHI

BHI was calculated using the formula from Cooper et al. (1994):

$$BHI = 10 * (\text{number of species in the system} / P) * [\ln(P) / \ln(Pmax)]$$

With P: number of species of the reference community and Pmax: maximum potential species richness from all the reference communities.

For Varna Bay and Lake, existing historical data from the literature were used to calculate P and Pmax. Data of P in Nervion and Oiartzun were obtained *via* AZTI from Basque WFD fish data. For all other sites, supplementary data from the French WFD fish dataset were used because no historical data was available. These data of P and Pmax are presented in Annex 2. They were calculated by gear, season and salinity class ("biological segment" – see Cooper et al. (1994)). For Pmax, fish data from 12 lagoons and 30 estuaries were used (year of available data are mentioned in Annex 2). For P, we used supplementary data from French estuaries and lagoons that are considered comparable to WISER estuaries and lagoons (see Annex 2 for detail). As all the sampled estuaries and lagoons are permanently, or at least most of the time, opened to the sea, marine fishes were not removed for the calculation of P and Pmax. Moreover, considering the difficulty to define exotic, rare or uncommon species in a dataset that covers such a large geographical area, no such species were removed from the references.

EFAI

EFAI has been recently submitted for publication hence it was here tested in his submitted version. The metrics included in the index are: *(i)* species richness (number of species); *(ii)* percentage of marine juvenile migrants; *(iii)* estuarine resident species (metric score results from a combination of both the number of resident species and the percentage of resident individuals); *(iv)* piscivorous species (metric score results from a combination of both the number of piscivorous species and the percentage of piscivorous individuals); *(v)* diadromous species (assessed based on expert judgment); *(vi)* introduced species (assessed based on expert judgment); and *(vii)* disturbance sensitive species (assessed based on expert judgment) – Table 4. This index was developed for the overall assessment of transitional waters, with the possibility of being used at the level of water bodies within an estuary, as required by the WFD. Hence, the EFAI is based on 5 trawl hauls per waterbody, salinity class and season. For the purpose of WISER, EFAI was also calculated using all available trawl hauls as well as fyke nets and beach seine data. The reference conditions considered for the WISER exercise were based on Portuguese estuaries reference conditions, originally used for the EFAI development.

Table 4: List of metrics that compose each of the indices tested here. Further information on the metrics is available in the relevant references given in Table 3.

Index	Country	Metrics
ELFI Estuaries	France	1. Total density (TD) 2. Density of Diadromous species (DDIA) 3. Density of Marine Juvenile migrants (DMJ) (only for mesohaline and polyhaline zones) 4. Density of Benthic species (DB) 5. Density of estuarine resident (DER)

		6. Total species richness (SR) 7. Density of freshwater species (DFW) (only for oligohaline zone)
ELFI Lagoons		1. Density of Benthic Invertebrate feeder species (DIB) 2. Density of Zooplankton feeders (DZ) 3. Density of Diadromous species (DDIA)
AFI	Basque country	1. Richness (number of species) 2. Pollution indicator species (% individuals) 3. Introduced species (% individuals) 4. Fish health (damage,diseases) (% affection) 5. Flat fish presence (% individuals) 6. Trophic composition (% omnivorous) 7. Trophic composition (% piscivorous) 8. Estuarine resident (number of species) 9. Resident species (% individuals)
Z-EBI	Belgium	<u>Oligohaline</u>: 1. Total number of piscivorous species (MnsPis) 2. Total number of pollution intolerant species (MnsInt) 3. Total number of diadromous species (MnsDia) 4. Total number of individuals (MnsInd) 5. Total number of marine migrating species (MnsMms) 6. Total number of estuarine species (MnsErs) <u>Mesohaline</u>: 1. Total number of species (MnsTot) 2. Total number of diadromous species (MnsDia) 3. Total number of specialised spawners (MnsSpa) 4. Total number of habitat sensitive species (MnsHab) 5. Percentage of pollution intolerant individuals (MpiInt) 6. Total number of marine migrating species (MnsMms) *Freshwater metrics are not detailed here as no such data were available here*
TFCI	United Kingdom	1. Species composition 2. Presence of Indicator species 3. Species relative abundance 4. Number of taxa that make up 90% of the abundance 5. Number of estuarine resident taxa 6. Number of estuarine-dependant marine taxa 7. Functional guild composition 8. Number of benthic invertebrate feeding taxa 9. Number of piscivorous taxa 10. Feeding guild composition
BHI	South Africa	$BHI = 10(J)[\ln(P)/\ln(P_{max})]$ where J is the number of species in the system / the number of species in the reference community; P is the potential species richness (number of species) of each reference community and P_{max} is the maximum potential species richness from all the reference communities.
EFAI	Portugal	1. Species richness (SR) 2. Percentage of marine migrants (%MM) 3. Estuarine resident species (ES): *Percentage of individuals, Number of species* 4. Piscivorous species (P): *Percentage of individuals, Number of species* 5. Diadromous species (D) 6. Introduced species (I) 7. Disturbance sensitive species (S)

EQR boundaries of the studied fish indices

For the WFD evaluation, the raw values of fish indices are translated into EQS in five classes ranging from “bad” to “high” (Table 5). For 3 out of the 6 tested indices (ELFI, AFI, EFAI) the EQR thresholds used in the present work are the ones currently in use for WFD assessment. The Z-EBI index defines only 4 classes as high quality sites are considered to be absent from the Scheldt estuary (Breine et al. 2010). Hence the scale was modified to accommodate a 5^{th} class

(Jan Breine, pers. com.[4]). BHI was not designed for the purpose of the WFD and it ranges from 0 (poor) to 10 (good); no EQR threshold is mentioned in (Cooper et al. 1994). For the purpose of the present exercise, equidistant thresholds were assigned to the BHI, considering that 0 corresponds to bad ecological status and 10 to high ecological status, similarly to what was done by Henriques et al. (2008). Similarly, the TFCI computed in this study only produces relative scores with respect to the theoretical maximum for the index and values range from 0 to 1 (Coates et al. 2007). To allow comparisons between all indices the relative scores were assigned to the five class system by dividing the index range in five equal parts.

In the end, EQR thresholds were either derived from relationships to different pressure gradients (ELFI estuaries, EFAI, AFI) or maintained equidistant class boundaries (ELFI lagoons, TFCI, Z-EBI, BHI). For example, the response of AFI to environmental variables (oxygen saturation) allows establishing class boundaries. This approach provides an independent way to define the quality class boundaries associated to human pressures, as required by the WFD (Uriarte and Borja, 2009). EQR thresholds are presented in Table 5.

Table 5: Thresholds applied (colour coded) to the EQRs of each of the tested indices

EQS	France ELFI		Basque AFI	Belgium Z-EBI	England TFCI	South Africa BHI	Portugal EFAI	
	Estuaries	Lagoons					Oligohaline	Mesohaline or polyhaline or applied at the whole estuary
high	[0,9 - 1]	[0,8 - 1]	[0,82 - 1]	[0,8 - 1]	[0,8 - 1]	[8-10]	[0,86 - 1]	[0,85 - 1]
good	[0,68 - 0,9[	[0,6 - 0,8[	[0,55 - 0,82[	[0,6 - 0,8[	[0,6 - 0,8[	[6-8[	[0,6 - 0,86[	[0,6 - 0,85[
moderate	[0,45 - 0,68[	[0,4 - 0,6[	[0,34 - 0,55[	[0,4 - 0,6[	[0,4 - 0,6[	[4-6[	[0,43 - 0,6[	[0,42 - 0,6[
poor	[0,23 - 0,45[	[0,2 - 0,4[	[0,17 - 0,34[	[0,2 - 0,4[	[0,2 - 0,4[	[2-4[	[0,30 - 0,43[	[0,31 - 0,42[
bad	[0 - 0,23[	[0 - 0,2[	[0 - 0,17[	[0 - 0,2[	[0 - 0,2[	[0-2[	[0 - 0,30[	[0 - 0,31[

Comparison of indices' results

Fish indices' results were examined both in terms of EQR and EQS. Distinction was made between indices' results computed in conditions considered as appropriate by the authors (*i.e.* approximately equivalent to those for which the index was originally developed) and instances where the index was used clearly outside its development requirements (estuary type, gear and sampling protocol). For example, as AFI was designed for beam trawl data, AFI results on fyke nets data might not be considered reliable. Hence, analyses were made first on all AFI results (including when AFI was calculated outside its initial development requirements), and then in a second step only on AFI adapted results (AFIa).

EQRs and EQS do not follow Normal laws hence a non parametric Kendall τ test was used to check for correlations between indices results. Because the number of pairs of results (respectively pairs of EQR and pairs of EQS) to be compared between indices remains low ($\leq$ 30) and because indices' results contain many tied values (same value is repeated several times

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within each indices' EQR or EQS), Kendall r and corresponding significance levels were calculated using Kendall package (Drouineau et al. 2012) in R software (R Development Core Team, 2009). The Kendall function of this package allows computing more accurate p-values in the presence of ties in the data (McLeod 2009).

Results

Fish assemblages

8687 fishes were caught from 73 identified different species, 50 genera and 32 families. About 5% (432 fishes) could not be identified at the species level and hence were recorded at the genus or family level: 250 Mugilidae, 5 Gobiidae, 1 *Alosa*, 4 *Neogobius* and 171 *Pomatoschistus*. Only 1 fish (fry) was not identified at all. In total 78 taxa (species, genus or family) were recorded. The 3 Basque estuaries showed lower number of taxa, especially the Nervion with only 4 taxa caught (Figure 2). In Basque data, *Pomatoschistus* are not identified at the species level. However, this cannot explain the differences observed, as such lack of precision in the classification occurred also in other sites and for other genus. Overall, these numbers must be considered with caution since the combination of gears as well as the sampling effort differed between sites.

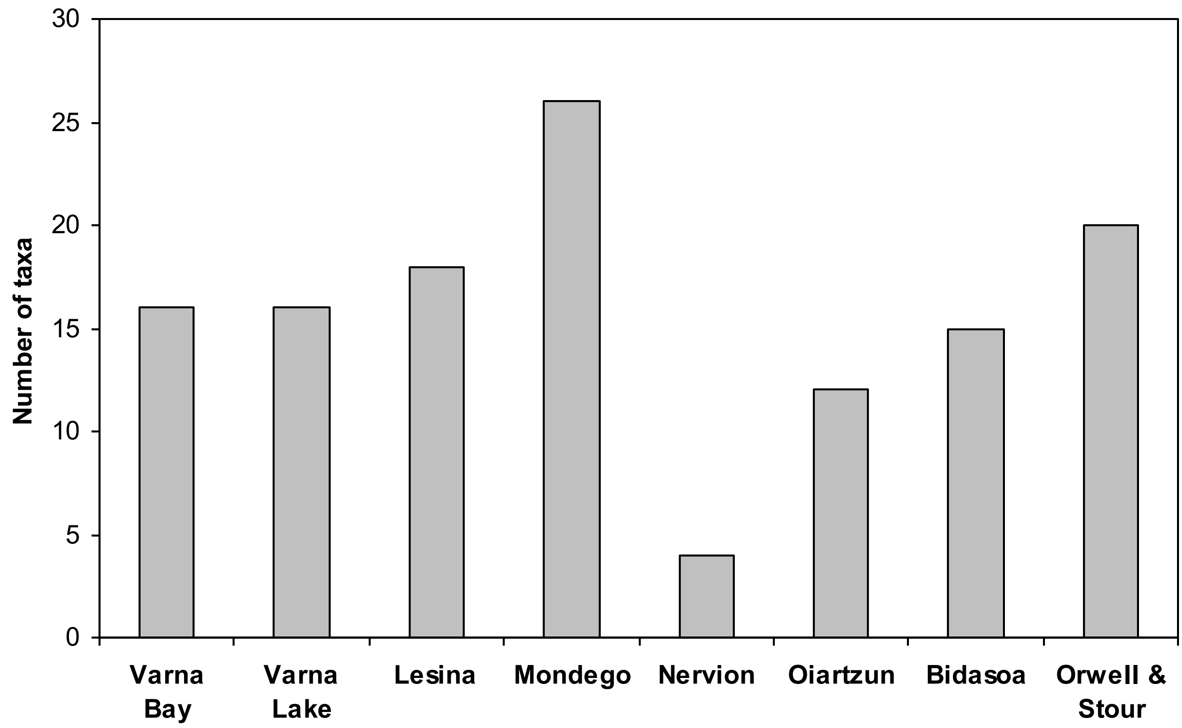

Fig. 2: Number of species caught in the sampled sites

Only 27 out of 78 taxa (35%) were caught in more than one site. When considering Varna Lake and Varna Bay as one single site (to take into account that these two systems are highly linked), this number decreases to 28%. No species was found in all sites. *Gobius niger* was caught in all sites except Lesina lagoon. It is followed by *Platichthys flesus* (caught in all sites except

Oiartzun, Lesina lagoon and Varna Bay) and *Solea solea* (caught in all sites except Lesina, Varna Bay and Varna lake). All other fish species or taxa were caught in 4 sites or less. In total, 40% of genera and 56% of families were found in more than one site. Only the Gobidae family was fished in all sites. Atherinidae, Syngnathidae, Soleidae, Mugilidae and Pleuronectidae were fished in 5 sites. All other families were caught in 4 sites or less.

Catches differed also greatly between gears. For example in Lesina lagoon, more than half (10 out of 18) of fyke nets caught no fish and those with catches contained between 1 and 4 fishes. Conversely, the fyke net Cemagref caught between 410 and nearly 2600 fishes. This difference can be explained mainly by the difference in mesh size between the two gears. As another example, Figure 3 shows the difference in catch between beam trawls and fyke nets in salinity class 2 of Mondego estuary.

A systematic and meaningful analyse of differences in catches between gears was not possible because the number of fishing events per site, salinity class, gear and time of the day was too low.

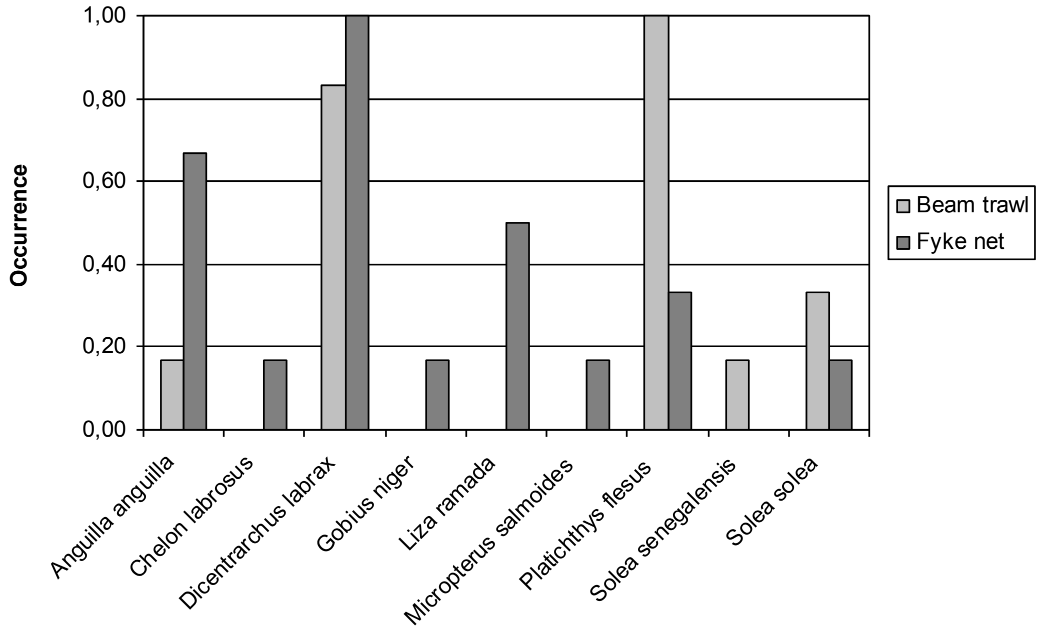

Figure 3: Frequency of occurrence of caught species between beam trawls and fyke nets in Mondego estuary, salinity class 2

Results of fish indices

Indices' results are presented in Table 6. For Z-EBI it appeared that when considering only these species that are part of the reference lists, number of species becomes so low that the metric "Total number of species" directly leads to an EQR of 0.1 in all cases; the calculation of the other metrics composing the index was not even requested (Breine et al. 2010). As the definition of an adapted reference list was not possible here, though it is part of the Z-EBI methodology, and as the use of the reference lists developed for the Scheldt leads to a constant EQR of 0.1 whatever the site, it was decided not to consider Z-EBI results in further analyses because they are assumed not to be representative of the original index.

Leaving aside the particular case of Z-EBI, Table 6 shows the relative adaptability of most of the studied indices as it was possible to calculate them on a great part of fish data. The only index that could not be computed on many data is ELFI (as it could not be calculated on fyke net data in estuaries – see section “Material and Methods”, “calculation of fish indices”). EQR values range from 0 to 1 though the range depends on the index. It is greater for ELFI and smaller for AFI that ranges from 0.29 to 0.72. EFAI assigned the highest scores in average (average EQR of 0.63) while ELFI was the lowest scoring index (average EQR of 0.39). This is especially true for Basque estuaries that are classified as “bad” by ELFI and mostly as “good” by EFAI.

The only index presenting the 5 quality classes is ELFI, while EFAI, TFCI and BHI give 4 quality classes (note that, for BHI EQS range from “bad” to “high” but no “good” was ever recorded). AFI gives only 3 quality classes. The indication of extreme values for quality (high or bad) was relatively rare: only 7 occurrences of “high” EQS and 13 occurrences of “bad” EQS are found among the 147 results (Z-EBI excluded). In general, indices results are very different and they never all agree.

Table 6: Overview of indices’ EQRs and EQS per gear and per day/night or season, for each of the sampled sites. Colours stands for EQS: red: bad; orange: poor; yellow: moderate; green: good; blue: high. White cells: the index could not be calculated for practical reasons or no meaningful reference condition could be estimated. The references for fyke nets used for BHI were obtained from a mix of day and night 12 hours fyke nets, thus BHI results are given for combined day and night fyke nets data.

	Salinity class	Gear	Day / night or season	ELFI (FR)	EFAI (PT)	EFAI (PT) - 5 hauls	AFI (Basque)	TFCI (UK)	Z-EBI (BG)	BHI (S. Africa)
Varna Bay	2	Beam trawl		0,42	0,66	0,66	0,58	0,61		3,81
Varna lake	2	Beam trawl		0,33	0,54	0,54	0,42	0,40		1,68
		Fyke net	Night	0,67	0,66	0,66	0,43	0,65	0,1	
Lesina	2	Fyke net Cemagref		1,00	0,49		0,52	0,69	0,1	9,75
		Fyke net	Day	0,33	0,26	0,26	0,33	0,25	0,1	
			Night	0,33	0,43	0,31	0,41	0,33	0,1	
Mondego	1	Fyke net	Day		0,67		0,56	0,40	0,1	
			Night		0,67		0,67	0,62	0,1	
		Beam trawl (night only)		0,54	0,67		0,50	0,59		2,99
	2	Fyke net	Day		0,71		0,72	0,60	0,1	9,46
			Night		0,66		0,33	0,62	0,1	
		Beam trawl (night only)		0,71	0,66	0,66	0,50	0,47		2,46
	3	Fyke net	Day		0,60		0,67	0,43		
			Night		0,71		0,72	0,80		
		Beam trawl (night only)		1,00	0,83	0,77	0,62	0,77		8,17
Nervion	3	Beam trawl	Spring	0,08	0,66	0,66	0,46	0,35		3,46
			Summer		0,66	0,49	0,42	0,35		
			Autumn	0,17	0,66	0,66	0,29	0,28		2,82
Oiartzun	3	Beam trawl	Spring	0,00	0,60	0,60	0,40	0,26		4,36
			Summer		0,60	0,60	0,46	0,33		
			Autumn	0,04	0,77	0,71	0,49	0,33		5,21
Bidassoa	1	Beam trawl	Spring	0,04	0,40		0,39	0,26		1,39
	2		Spring	0,00	0,49		0,33	0,25		1,29
	3		Spring	0,04	0,66	0,66	0,39	0,28		3,26
	3		Summer		0,77	0,77	0,45	0,26		
	3		Autumn	0,13	0,77	0,71	0,54	0,42		4,92
Stour & Orwell	3	Fyke net 24 hours			0,66		0,38	0,48		8,62
		Beach seine EA		0,75	0,83	0,77	0,52	0,58		
		Beach seine Wiser		0,58	0,71	0,71	0,49	0,47		
		Beam trawl		0,58	0,60	0,54	0,41	0,49		4,17
			Min EQR	0,00	0,26	0,26	0,29	0,25	0,10	1,29
			Max EQR	1,00	0,83	0,77	0,72	0,80	0,10	9,75
			Mean	0,39	0,63	0,62	0,48	0,45	0,10	4,58
			Mediane	0,33	0,66	0,66	0,46	0,43	0,10	3,81

Adaptability of fish indices

Indices' results vary with the gear and some sampling characteristics (such as season or day time). Figure 4a) shows that EQR results for the Basque estuaries tend to present higher variability for autumn data than for other seasons, meaning that ELFI, AFI, TFCI and BHI detect more differences between the 3 estuaries in autumn than in other seasons. However, average EQR values of each index remains relatively stable throughout seasons. Figure 4b) tends to show that TFCI is more sensitive to the fishing time (day or night) for fyke nets than EFAI and AFI. Table 6 and Figure 5 show that all indices give very different results for Lesina lagoon, both in term of EQR and EQS, depending on the gear used (fyke Cemagref or fyke net). In general, all indices appear highly dependant on the gear used, though EFAI results seem to be less dependant on the gear, especially in the Mondego (EQS remains "good" whatever the gear). Table 6 shows that there are few differences in EFAI EQS between the case where EFAI is calculated with 5 trawl hauls (original method for EFAI) and the case where EFAI is calculated with all available data.

Considering the relatively low number of available data and the numerous sources of variability in the dataset (several estuaries and lagoons, seasons, gears…) it was not possible to analyze systematically the response of each index to each of the potential sources of variability. In the same way, the robustness of indices could not be assessed in the absence of anthropogenic pressure data (which would be analysed in Deliverable D4.4-3).

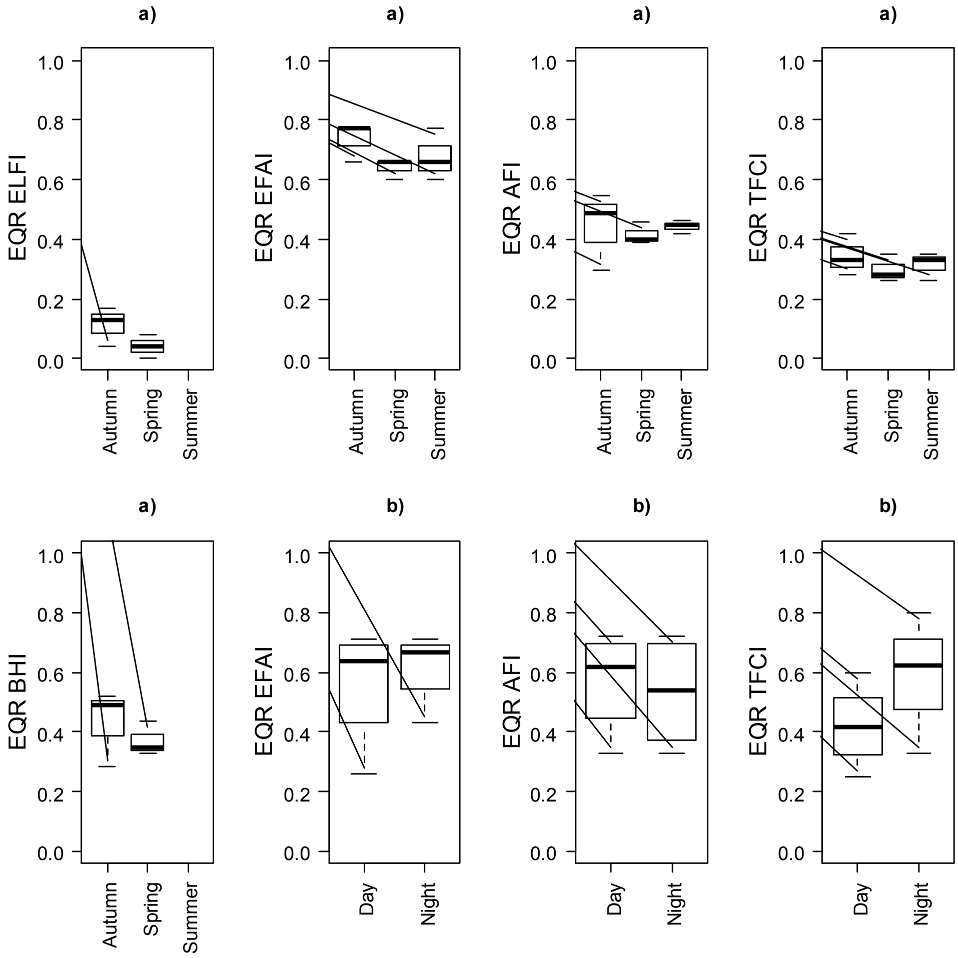

Figure 4: Box plots of EQRs for fish indices; a) EQRs calculated on three Basque estuaries (Nervion, Bidasoa and Oiartzun) on data from three seasons. It was not possible to calculate ELFI and BHI on summer data. b) EQRs calculated on all available fyke nets data, for day and night catches.

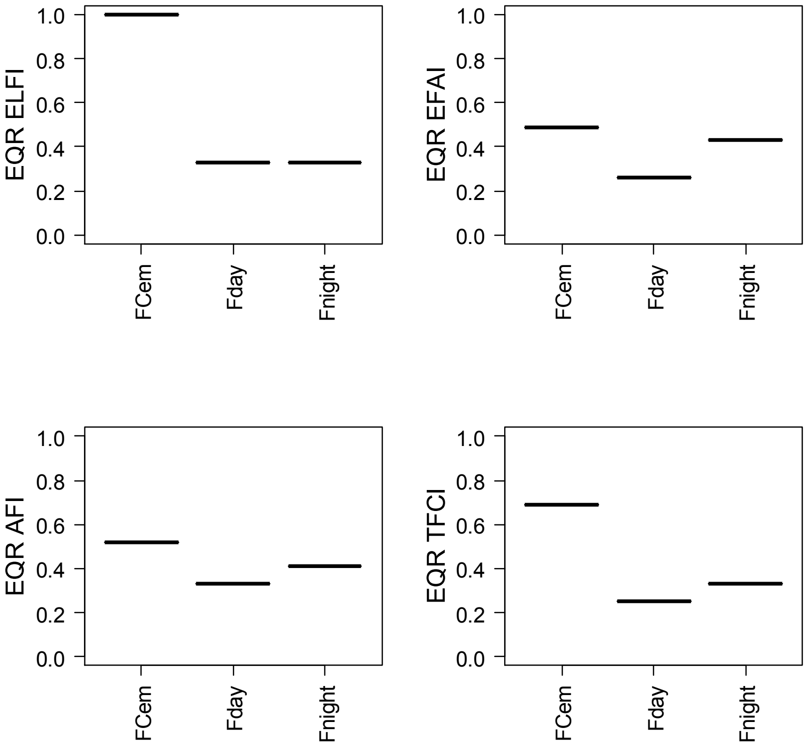

Figure 5: EQRs of four fish indices for the two types of gears used in Lesina lagoon. FCem: fyke net Cemagref; Fday: fyke net day catches; Fnight: fyke net night catches

Correlation between indices' results

Results of BHI were scaled down tenfold to bring the computed values to the same range of all other indices. Table 7 presents the results of Kendall r and corresponding significance levels. Correlation between indices results is generally low, both in terms of EQR and in terms of EQS. When looking at all indices (Table 7a), best correlations of EQRs are obtained between TFCI and ELFI. Only 6 out of 10 possible EQR correlations are statistically significant. EFAI and ELFI, as well as TFCI and EFAI, appear independent, whilst BHI is correlated with only one index (TFCI). For EQS only 4 out of the 6 possible correlations are significant. When looking at indices results when indices are used in adequate conditions (*i.e.* same gear, same sampling protocol and sampling effort as in the original method), only the correlation between ELFI and TFCI EQRs remains significant (Table 7b). EQS from the different indices appear independent except between ELFI and TFCI and BHI and TFCI.

*Table 7: Values of Kendall r (Kendall rank correlation coefficients) between EQRs (white cells) and EQS (grey cells), and corresponding statistical significance. * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001. In Table 7a/ Kendall r was calculated on all indices results. For Table 7b/ only results of indices calculated in appropriate conditions were used. BHI values are the same in both tables.*

a/ Fish indices	ELFI	EFAI	AFI	TFCI	BHI
ELFI	1	0,068	0,337	0,720***	0,325
EFAI	0,26	1	0,352*	0,237	0,394
AFI	0,475**	0,403**	1	0,5**	0,244
TFCI	0,689***	0,332*	0,488***	1	0,514*
BHI	0,254	0,347	0,272	0,502**	1

b/ Fish indices adapted(a)	ELFIa	EFAIa	AFIa	TFCIa	BHIa
ELFIa	1	-0,172	0,372	0,828**	0,404
EFAIa	0,387	1	0,104	-0,064	0,189
AFIa	0,295	0,489	1	0,806	0,301
TFCIa	0,828**	0,151	0,524	1	0,736*
BHIa	0,271	0,443	0,366	0,423	1

Discussion

Testing fish indices: comparison of the present study with the one of Martinho et al. (2008)

Although indices to evaluate ecological quality are routinely developed and improved, there is a lack of studies addressing the applicability to other areas/data sets. Martinho et al. (2008) have tested 5 fish indices on fish data collected monthly in the Mondego estuary from June 2003 to August 2006. At this time they acknowledged a lack of publications in this subject. The pluses of the present work are threefold:

- Since 2008, 6 new publications on fish indices for transitional waters were made available (5 reviewed in Perez-Dominguez et al. (2010) plus Drouineau et al. (2012). The present study tests the more recently published fish indices including, whenever possible, recent improvements so that the indices are often tested in their version currently in use for WFD ecological assessments (except for TFCI).
- While Martinho et al. (2008) tested the performance of fish indices with regards to interannual and seasonal natural variations in a single estuary, the present work includes geographical (*i.e.* inter transitional waters types) and sampling procedures (*i.e.* inter gears) variations. Interestingly, as the WISER surveys were multi-gears, each of the gear-specific indices is tested at least once on data gathered with the gear it was designed for. Whether this has resulted in a more precise assessment is arguably as there is often some other factors that may affect the outcomes (*i.e.* reference conditions, suitability of metrics to the local pressures). Nevertheless, some of the discrepancies found in the study by Martinho et al. (2008) were attributed to the use of gears and guild assignments different from those used in the development of the original indices.
- A common database of fish species and their corresponding guilds was built for the purpose of the present study, as advised in Martinho et al. (2008). Other fish characteristics (such as introduced species or indicator species) were also adapted to each of the studied sites.

Difficulties encountered when applying fish indices to the WISER dataset

Studied sites are located in several ecoregions and are of various types: from lagoon to estuaries, with a great variety in their structural characteristics. Salinity and depth data show that the fish samplings were made in a wide range of different habitats. Nevertheless, all of the tested indices are type specific: in principle they can not be applied to other waterbodies without any adaptations (Breine et al. 2010). Moreover, it appears that all of the tested indices, except for BHI and TFCI, are gear specific. This means they were designed for a (some) particular gear(s) and sampling protocols, and it is not always possible to apply them on data gathered with different gear sets.

In the light of this, one of the main difficulties encountered here was to define appropriate reference conditions for the studied sites and samplings gears in the absence of the requested data to do so. This is a common issue in the implementation of the WFD: data to establish the reference conditions are not always available, especially for transitional waters as no pristine sites remain in Europe. Hence, the characterization of the reference is an important source of bias when assessing the ecological status of transitional waters in Europe (Martinho et al. 2008). For example, Z-EBI reference lists used were the ones designed for the Scheldt estuary and hence it is likely to be less or no relevant for other areas; this alone explains the constant "bad" score obtained with this index across all sites. Also, fixing the reference conditions for TFCI in a single ecotype made it less relevant for others ecotypes and probably inadequate for other ecoregions. ELFI was designed for a wide range of waterbodies hence the reference conditions used were the "closest ones" calculated from the French dataset. Similarly for BHI new reference values were computed based on supplementary data from selected French estuaries and lagoons supposed to be similar to the studied waterbodies. AFI used reference conditions as defined for Basque estuaries type III and EFAI used reference conditions designed on Portuguese estuaries. Hence, we have not systematically taken into account possible changes in reference conditions due to different types across the studied systems or biogeographical differences. In the end most of the indices were obviously tested outside of their limits of validity. This challenges the validity of their outcomes.

Contribution of the present study

Despite the problems encountered to adapt fish indices to the WISER dataset, interesting results raised from this exercise.

Use of guilds

Great differences in fish assemblages appeared between the sampled sites. However, it was possible to calculate most of the fish indices on these data, except Z-EBI that uses list of species. This demonstrates that using ecological guilds allows calculating fish indices on a larger geographical range and on a greater variety of transitional waters.

Methodological differences in fish indices

The present exercise highlights strong methodological differences between the tested fish indices. Letting aside the use of different gear sets, the next main difference is the level of calculation of the metrics. In some indices metrics are calculated after pooling all data per salinity class or per waterbody. This is the case for example for Z-EBI. In others (ELFI, TFCI) metrics are calculated at the fishing event scale. Data may be also pooled per year (Z-EBI) or metrics are calculated for only one season (EFAI, AFI) or two seasons (ELFI). Finally, BHI does not take into account season in its original version.

The second important difference is related to the standardization of fish data. Metrics composing the tested fish indices can be divided in two types: 1/ metrics based on number (absolute or relative) of individuals and 2/ metrics based on number of species. For metrics based on number of individuals, abundances are often considered as relative (percentage of individuals or relative densities) in order to account for variations in sampling effort (Coates et al., 2007; Breine et al., 2010). ELFI consider the abundance as absolute by standardising per the trawled surface for estuaries, and by assuming that the sampling protocol is standardized in lagoons. Z-EBI combines both standardisation per unit of effort and the use of percentage metrics. Depending on the metric the choice of using relative densities can be discussed: as both the denominator and the numerator may vary with pressure, interpretation of these metrics as well as their response to pressure may vary with the level of pressure and be sometimes ambiguous. Metrics of type 2 are mostly number of species. It is important to note that most of the time these metrics do not include any standardisation by sampling effort; this means the sampling must be either perfectly standardised or it must be sufficient to reach the plateau of the rarefaction curves, otherwise the assessment may be strongly dependant on sampling effort. Several approaches were used to address this question in the tested indices: AFI, TFCI and EFAI use standardised data, ELFI standardise number of species by the log-surface of the beam trawl hauls (Nicolas et al. 2010) and Z-EBI has a sufficient sampling effort so that the plateau of rarefaction curves is reached.

It is interesting to note that none of the tested indices give any estimation of the risk of error linked to sampling effort neither to the scale of metric calculation (in time and in space).

Comparison of the fish indices in the light of the WFD requirements: toward intercalibration

All of the tested indices were created specifically for the WFD except BHI that is thus not WFD compliant as such. Indeed, WFD requires that both composition and abundance of fish fauna, along with disturbance sensitive species, are taken into account for the ecological assessment of transitional waters, whilst BHI refers only to species richness. It could be used for WFD purpose but in combination with other fish metrics. Nevertheless, BHI was not created to be used alone as it is part of a wider index, the Estuarine Health Index – EHI, taking into account water quality and aesthetic aspects.

Despite the high degree of disagreement between indices results, the present study showed some common patterns in the diagnostics given by fish indices. TFCI and ELFI are the most strongly correlated indices arguing for a possible intercalibration between them. However, it is important to note that the version of TFCI tested here is different from the most recent version currently in use for WFD assessments (Steve Coates, pers. com.[5]). In particular, this newest version of TFCI includes great improvements such as analyses of the uncertainty in the assessment. It does not use gear-specific reference conditions anymore; instead it uses a global reference for multiple gears sampling and it includes some constraints on the sampling protocol and the sampling effort contributing by each gear, *i.e.* a minimum sampling effort per gear is required for the assessment to be accurate. To compare the outcomes of the new version of TFCI with ELFI is

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an interesting coming task of the intercalibration process. For other indices, correlations are weaker but nevertheless sometimes significant, indicating the possible successful intercalibration between these indices too. It is also important to note that correlations between EQS appear weaker than between EQR. It is hence believed that with an adaptation of EQR boundaries, a better correlation between EQS could be reached.

Conclusions

Not surprisingly, when used outside of their initial framework, geographical limits or with different sampling methods, fish indices' results are highly uncertain. Hence, it is clear that the assessment of the ecological status of transitional waters using fish indicators highly depends on the assessment tool (index) used and the corresponding sampling methods, as shown by Jurvelius et al. (2011). This argues for cautious interpretation and the use of expert opinion to interpret fish indices results, as raised by Henriques et al. (2008). However, caution is needed when considering the conclusions of the present work with regards to the data used (low sampling efforts with regards to the numerous sources of variability included in the dataset).

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Annex 1: Fish species caught and corresponding commonly agreed ecological guilds

Species caught and corresponding guilds for which a common assignment was reached. Ecological guilds: ER: Estuarine Resident species; DIA: Diadromous species; FW: Freshwater species; MJ: Marine Juvenile species; MA: Marine Adventitious species; MS: Marine Seasonal species. Position guilds: P: Pelagic; B: Benthic; D: Demersal. Trophic guilds: F: Piscivorous (exclusively); Z: Zooplankton feeder; IS: Supra benthic Invertebrate feeder; IB: Benthic Invertebrate feeder; O: Omnivorous. Blank: no data.

Species	Ecological guild	Position guild	Trophic guild
Alosa	DIA	P	Z
Ammodytes tobianus	ER	B	Z
Anguilla anguilla	DIA	D	O
Aphanius fasciatus	ER	D	IB
Aphia minuta	ER	P	Z
Arnoglossus kessleri	ER	B	IB
Arnoglossus laterna	MA	B	IB
Arnoglossus thori	MA	B	IB
Atherina boyeri	ER	P	Z
Atherina pontica	MJ	P	Z
Atherina presbyter	ER	P	Z
Barbus barbus	FW	D	IB
Buglossidium luteum	MA	B	IB
Callionymus lyra	ER	B	IB
Callionymus risso	ER	B	IB
Chelidonichthys lucernus	MJ	B	IS
Chelon labrosus	DIA	D	O
Ciliata mustela	ER	B	O
Ciliata septentrionalis	MA	D	IS
Clupea harengus	MJ	P	Z
Conger conger	MA	D	F
Dicentrarchus labrax	MJ	D	IS
Diplodus annularis	MA	D	IS
Diplodus sargus	MJ	D	IS
Diplodus vulgaris	MJ	D	IS
Engraulis encrasicolus	MS	P	Z
Gambusia holbrooki	ER	P	IS
Gasterosteus aculeatus	ER	D	IB
Gobiidae	ER		
Gobius niger	ER	B	IB
Gobius paganellus	ER	B	IB
Hippocampus guttulatus	ER	B	Z
Hippocampus hippocampus	ER	B	Z
Knipowitschia panizzae	ER		
Labrus merula	MA	D	IB
Liza aurata	DIA	D	O
Liza ramada	DIA	D	O
Liza saliens	DIA	D	O
Micropterus salmoides	FW	P	F
Mugil cephalus	DIA	D	O
Mugilidae	DIA	D	O
Mullus barbatus ponticus	ER	B	IB
Mullus surmuletus	MJ	B	IB
Neogobius	ER		IB
Neogobius cephalargoides	ER	D	IB
Neogobius gymnotrachelus	ER	B	IB
Neogobius melanostomus	ER	B	IB
Oreochromis niloticus niloticus	ER	D	O

Osmerus eperlanus	DIA	P	IS
Parablennius tentacularis	ER	B	O
Platichthys flesus	DIA	B	IB
Pleuronectes platessa	MJ	B	IB
Pomatoschistus	ER	B	
Pomatoschistus marmoratus	ER	B	IB
Pomatoschistus microps	ER	B	IB
Pomatoschistus minutus	ER	B	IB
Proterorhinus marmoratus	ER	B	IB
Raja undulata	MA	B	IB
Salaria pavo	ER	B	O
Sardina pilchardus	MJ	P	Z
Scophthalmus rhombus	MJ	B	IB
Scorpaena notata	MA	B	IS
Scorpaena porcus	MA	D	IS
Solea senegalensis	MJ	B	IB
Solea solea	MJ	B	IB
Sprattus sprattus	MJ	P	Z
Symphodus bailloni	ER	D	O
Symphodus cinereus	ER	D	IS
Symphodus roissali	ER	D	IS
Syngnathus abaster	ER	D	Z
Syngnathus acus	ER	D	Z
Syngnathus rostellatus	ER	P	Z
Syngnathus typhle	ER	D	F
Trachurus mediterraneus	MJ	P	F
Zebrus zebrus	ER	B	IS
Zeugopterus punctatus	MA	B	IS
Zoarces viviparus	ER	B	O
Zosterisessor ophiocephalus	ER	B	F

Annex 2: Reference values used for the calculation of BHI (Cooper et al. 1994) **and obtained from French and Basque WFD datasets. BHI references for Varna Bay and lake were calculated on local historical data and are not presented here.**

Values of P_{max}: maximum potential species richness calculated from all the reference community, i.e. here 12 French lagoons and 30 French estuaries; Salinity class 1: oligohaline (0 – 5); salinity class 2: mesohaline (5 – 18); salinity class 3: polyhaline/euhaline (> 18)

Gear	Years	Salinity class	P_{max} in autumn	P_{max} in spring
Cemagref fyke net lagoon	2006	1	17	Not useful here
		2	15	
		3	22	
Fyke net (same as in the WISER samplings)	2005, 2006 and 2007	1	14	18
		2	9	8
		3	11	8
Beam trawl 1.5m wide	2005 to 2010	1	16	12
		2	21	17
		3	32	24

Values of P: number of species in the reference community; Salinity class 1: oligohaline (0 – 5); salinity class 2: mesohaline (5 – 18); salinity class 3: polyhaline/euhaline (> 18);

Site	Supplementary site used as reference	Gear	Years	Salinity class	Season	P
Lesina	Or (French lagoon)	Cemagref fyke net lagoon	2006	2	autumn	14
Mondego	Adour (French estuary)	Fyke net (same as in the WISER samplings)	2005	1	autumn	no available data
				2		8
				3		no available data
		Beam trawl 1.5m wide	2005 to 2010	1		12
				2		20
				3		17
Nervion	Nervion	Beam trawl 1.5m wide	1989-2004	3	spring	3
				3	autumn	9
Oiartzun	Oiartzun	Beam trawl 1.5m wide	1989-2004	3	spring	4
				3	autumn	15
Bidasoa	Bidasoa	Beam trawl 1.5m wide	2005 to 2010	1	spring	4
				2		3
				3		12
				1	autumn	7
				2		4
				3		19
Orwell and Stour	Vilaine (French estuary)	Fyke net (same as in the WISER samplings)	2007	1	spring	6
				2		4
				3		6
		Beam trawl 3m wide	2007 to 2010	1		11
				2		8
				3		24