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SEVENTH FRAMEWORK PROGRAMME

WISER

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

DELIVERABLE

Deliverable D4.4-3, Report detailing Multimetric fish-based indices sensitivity to anthropogenic and natural pressures, and to metrics' variation range

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Content

Non-technical summary

The Water Framework Directive (WFD) aims at achieving good ecological status (GES) for surface water bodies 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 of their transitional waters, including fishes.

The present work focuses on the response of fish indicators and indices to anthropogenic pressures and natural factors. For doing that, datasets from the Basque and Portuguese estuaries, in the North East Atlantic, have been used. Hence, biological data from fish (and in some cases, crustaceans), together with different types of pressure (population, industry, ports, dredging, global pressures, pollution, channeling, etc.) and hydromorphological data (flow, estuary volume, depth, intertidal surface, residence time, etc.) have been analyzed. Together with fish assemblages composition and individual metrics (richness, trophic composition, etc.), two fish indices (Basque AFI and Portuguese EFAI) have been investigated. Additionally, the response of five fish indices (AFI, EFAI, ELFI, TFCI, Z-EBI) were tested on a common dataset, within Portuguese estuaries, to check the time lag in the metrics' response to different human pressures and the variability in the strength of responses to those pressures.

This work also focuses on the sensitivity analysis of two European fish-based indices (French ELFI and British TFCI) to changes in their respective metric scores through their observed dynamic range. Sensitivity analyses were run simulating different scenarios of metric score changes, taking into consideration the relationship between metrics. This allowed the metrics with stronger influence in the index score and the resulting water body classification to be highlighted. Importantly, the identification of the most influential metrics could help to guide management efforts in terms of achieving GES by 2015.

In general, the fish metrics and indices tested responded to anthropogenic pressures in the Atlantic estuarine sites, yet at the individual metrics level environmental chemical quality was the main driver for observed differences. Also, some metrics did not respond to pressures as expected, which is most likely related to sampling gear efficiency, namely the low capture efficiency of diadromous species with beam trawl.

The cause-effect relationship study emphasized that fish-based indices developed to assess the water quality of estuarine systems did not detect all the pressures with the same sensitivity in terms of strength and time-lag, and gave more importance to some pressures, namely chemical pollution. The fish-based indices developed to assess the water quality of estuarine systems do not allow the individualization of pressure effects, which may constitute a problem to put forward the correct specific measures for management and rehabilitation of estuaries. On the other hand, some indices also do not seem relevant, in a short time, to detect changes of the ecological quality which may constitute a handicap for management or an indication for their restructuring.

The sensitivity analysis indicates that a number of estuarine resident taxa, a number of estuarine-dependent marine taxa, a number of benthic invertebrate feeding taxa and a number of piscivorous taxa have the greatest influence on the TFCI classification. For the French index ELFI, the most influential metrics are mainly DT (total density) and DB (density of benthic species), followed by RT (total richness). These results suggest a high sensitivity of the quality indication provided by these indices on richness related aspects of the fish assemblages. Management should therefore prioritize efforts to conserve or restore estuarine attributes underpinning abundance and ecological diversity, for example the diversity of fish habitats, food resources and shelter or the hydrological integration between coastal and transitional waters.

1. 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 testing/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, WISER will contribute (i) to make the existing assessment methods more comparable, (ii) to study the response of biological quality elements to human pressures, and (ii) to estimate the uncertainty of the assessments.

Since fish assemblages were first proposed in the 1980s to assess the biotic integrity of freshwater systems (Karr, 1981) a suite of assessment methods based on fish fauna have been proposed (see WISER Deliverable 4.4-1, for an extensive review). This review shows 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 disadvantages. 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. These, together with difficulties posed by the large natural abiotic variability of estuarine systems and the diversity of analytical schemes that can be used, add uncertainty to the assessments and compromise the accuracy and generality of the results.

It is well known that every single ecosystem constitutes a particular case, where the differences observed in the distribution and in the interrelation existing between species and the abundance of their individuals, contribute to put research away from the total understanding of those areas (Franco *et al.*, 2011). Despite this, some indispensable uniformity is used to collect and treat data from those very distinct systems. The characteristics of a community or a population are frequently based on data produced either from relatively homogeneous study strategies (e.g., rigid number of samples, replicates, habitats sampled) or taken from considerably different study strategies which are supposed to produce a more exhaustive collection of information (e.g., complex or multiple sampling strategy). Both can have sound justification for use but difficulties may arise when comparisons between different sites are needed. Independently of further requirements (e.g., analytical procedure), and depending on the aim of the research, it is important to consider firstly which is the sampling technique able to provide the most reliable information on the target community (Watson *et al.*, 2010). Concerning the fish monitoring, it's important to ensure that the different components of the assemblages are captured, not only by the use of complementary methods that are able to cover the different existing niches (Elliott and Hemingway, 2002), anyway comparable, but also through an adequate sampling effort (see WISER Deliverable WP4.4-2).

Since most of the commonly used fish sampling methods are based on traditional fishing gears and techniques, is undeniable that those sampling methods are selective and in some degree regionally adapted (Franco *et al.*, 2011). The catch efficiency of any sampling gear changes when used out of the habitat conditions for which it was developed for (Elliott and Hemingway, 2002). Sampling gears were traditionally developed in response to the fish species present in an area and the habitat type. In particular, a single sampling gear cannot be used with the same catch efficiency in all the habitat types present in these ecosystems (Elliott and Hemingway, 2002). Hence, the choice of the sampling methodology must take into account the aims of the study, as well as the characteristics of the habitat being surveyed.

Additionally, the analytical techniques, concerning the selection and the combination of metrics composing indices, may also contribute to increase variability on results. Although a high number of assessment methodologies, developed during the last years in the scope of the WFD, might be based in a core group of metrics, different results are obtained by those methods, namely a variability of metrics composing indices. These metrics and then indices may have considerable differences in what concerns their ability to evaluate cause-effects relationships between the state of fish assemblages and human pressures.

In a multimetric index it is important to understand the weight that different metrics have on the final index score and thus on the status of a water body (WB) given by the assessment. These analyses can be done by modelling the response of the index to changes in its metrics. This initially provides useful information on the expected dynamic range of composite indices, and also provides insight on the likely effects of improving or worsening ecological conditions on the indices. In the case of fish-based indices, sensitivity analyses help to determine which of the input metrics are driving the results of the index and hence the classification of the water body. This information can be extremely useful to understanding the behaviour of the indices, to facilitate the interpretation of the results and to evaluate which metric will require more effort to reduce the index uncertainty.

The work presented here corresponds to the aim of WISER Deliverable WP4.4-3, detailing multivariate analysis of fish data and metrics against pressures in different European Atlantic transitional waters. The deliverable deals also with the influence on hydromorphological variables in fish assemblages and their responses to fish quality assessment tools, the response of fish community-based metrics against anthropogenic pressures, and the sensitivity in strength and time-lag of indices and their respective metrics in relation to several human pressures.

2. Material and methods

2.1. Case study: Basque estuaries

2.1.1. Fieldwork data collection

Fieldwork research was carried out in 12 estuaries (i.e. Barbadun, Nerbioi, Butroe, Oka, Lea, Artibai, Deba, Urola, Oria, Urumea, Oiartzun and Bidasoa) located at the coast of the Basque Country, in the South-Eastern part of the Bay of Biscay (Figure 1). Surveys, which started in April 2008 and lasted until September 2010, were always carried out during periods of high or rising tide periods.

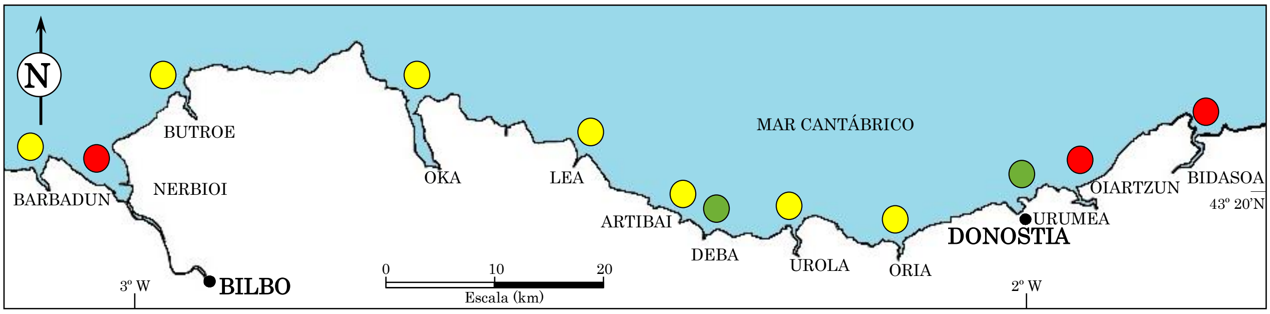


Figure 1: Basque coast graphic representation including the location of the 12 estuaries included in this study. Green: intertidal Atlantic estuary, where fresh water dominates marine water, Yellow: intertidal Atlantic estuary, where marine water dominates fresh water, and Red: subtidal Atlantic estuary.

Hydromorphological and biological benthic surveys were carried out in the inner, middle and outer sections of each estuary. For the Oiartzun and Bidasoa estuaries, two different inner areas were identified, and consequently surveyed. For each estuary section, a transect path was defined and hauled three times in order to obtain replicates..

To collect the samples, a Narwhal zodiac with a towed 1.5 m wide beam trawl, which had a tickler chain and internal and external nets of 8 mm and 40 mm mesh respectively, was used. The beam trawl was dragged along the defined transect path for 10 min at a constant speed of 1.5 knots. Time was reduced down to 5 min when obstacles or minimum depth did not allow for a full 10 min period of survey. At the end of each haul, the beam trawl was brought on board with the samples. Hauls were repeated when the number of individuals in the sample was unusually low for the area or obstacles impeded the adequate use of the technique.

At the start of each sample collection, the date, time, hydrographical and weather conditions were recorded. The position and depth at the start and end of each sample collection were also noted. Furthermore, physical parameters of the water such as temperature, salinity, pH and dissolved oxygen were measured using a YSI566 device.

Each estuary was surveyed at three different seasons: spring, summer and autumn.

2.1.2. Sample management and identification

Once the samples were on board, the number of species and their abundance were recorded both for fishes and crustaceans. Identification of species was carried out according to the European Register of Marine Species (ERMS: www.marbef.org/data/erms.php), the taxonomic code of the National Oceanographic Data Center (NODC: http://www.nodc.noaa.gov) and/or the Integrated Taxonomic Information System (ITIS: www.itis.usda.gov).

Dead organisms and those that were badly preserved were disregarded. To minimize the impacts of this study, organisms were identified *in situ* and returned alive into the system. Only individuals that could not be easily identified were taken into the laboratory for subsequent identification. In the case of crustaceans, they were kept in formosaline solution and taken into the laboratory for their identification (*e.g.* species of the *Palaemon* genus).

To reduce the stress and/or damage to fish during the handling process, fishes (except *Pomatoschistus* sp.) were placed into a bucket filled with a mix of 10 l of marine water and 1 ml of anaesthetic solution. The anaesthetic solution was made out of 2 ml of clove oil and 5 ml of 95% ethanol. This solution does not have a strong anaesthetic effect and only lasts while the fishes are submerged in the solution. Once the fishes had been measured and photographed, they were placed into a different bucket filled only with marine water until the anaesthetic effect disappeared. At that point, fishes were returned into their environment. Since the clove oil anaesthetic properties are not well known (the active molecule of the clove oil varies between 70-90% of the total), caution is recommended in the use of this protocol. Furthermore, experience indicates that species respond differently to this anaesthetic solution, with flat fish being the most sensitive to it and *Anguilla anguilla* the least.

Biological data collected during the fieldwork were used to determine the following parameters: number of taxa (*i.e.* richness at the highest taxonomic separation possible), abundance (net width, speed and length of the surveys were all considered in this estimate), diversity and equitability (note that no estimate for catchability and gear efficiency were included in the abundance estimation).

2.1.3. Statistical analyses

For the purpose of the analyses, the aforementioned four biological variables and 34 abiotic variables, including 18 pressure measures and 16 hydromorphological variables, were considered (Table 1). Information regarding these variables was obtained from previous studies (Borja *et al.*, 2006; Uriarte and Borja, 2009) and current surveys. Variables were transformed using log (1+x) and double square root (e.g. for abundance data) when and as appropriate. This transformation was done to fulfill/add homogeneity and normality data requirements for the analyses and/or reduce the weight of species that were highly abundant.

Table 1. Variables considered in the statistical analyses, including the form (transformation) in which they have been used in the analyses.

Variables	Variable type	Name	Units/measu	Transformation
Biological	Fish	Number of taxa	N	
		Abundance	N	√√
		Diversity	Shannon	
		Equitability	Pielou	
	Crustaceans	Number of taxa	N	
		Abundance	N	√√
		Diversity	Shannon	
		Equitability	Pielou	
Abiotic	Pressures	Population	hab km^{-2}	
		Industrial plants	n	log (1+x)
		Ports	n	
		Port area	km^2	log (1+x)
		Berths	n	
		Dredged volume	m^3 $year^{-1}$	
		Farms in the catchment	n	log (1+x)
		Human Pressures	n	log (1+x)
		Human Pressures	n km^{-2}	
		Human Pressures	n km^{-1}	
		Total pressure index (see Uriarte and Borja, 2009)		
		Global pressure index (as used in NEA-GIG intercalibration group)		
		Water pollution index	%	
		Sediment pollution index	%	
		Channeling in ports	%	
		Channeling out of ports	%	
		Loss of intertidal area	%	
		Nutrient loadings	N kg day^{-1}	
	Hydromorphological	Estuary length	km	log (1+x)
		Average estuary depth	M	
		Estuary volume	Hm^3	log (1+x)
		Estuary subtidal volume	Hm^3	log (1+x)
		Floodplain surface	Ha	log (1+x) → removed
		Subtidal surface	%	
		Intertidal surface	%	→removed
		Average tidal prism	km^2	log (1+x) → removed
		Catchment area	km^2	log (1+x)
		River flow	m^3 s^{-1}	log (1+x)
		Flushing time	Hr	
		Residence time period	days	
		Continental shelf width	km	log (1+x)
		Distance to the estuary mouth	km	log (1+x)
		Orientation of the estuary mouth	degrees	log (1+x)

To avoid multicollinearity, abiotic variables that were highly correlated with others (as shown by Pearson correlation tests; r>0.95 and statistically significant) were removed from the analysis (i.e. estuary subtidal volume, proportion of intertidal surface, floodplain surface and average tidal prism). Creating a similarity matrix, based on Euclidean distances, with the remaining abiotic variables a Multidimensional Scaling analysis (MDS), where distance between estuaries are kept proportional to their hydromorphological and pressure similarities, was created (Table 2).

Biological data were organized into ichthyofauna (fish) alone and ichthyofauna plus crustaceans (fish-crustaceans) and were analyzed separately. This is because the fish quality index, used in the Basque Country, includes both fish and crustaceans in the assessment. The effect of seasonality on biological data was explored using a 2-way nested ANOSIM (ANalysis Of SIMilarities), where season was nested as a factor and the different estuary transects were considered as replicates. Since significant seasonal effect for fish and fish-crustaceans were not found ($R = 0.015$, $p = 0.672$ and $R = -0.003$, $p = 0.501$, respectively), an annual demersal community structure (a unique data set of biological information) was calculated for each estuary for their use in the subsequent analyses.

Table 2. Step-by-step analytical process, which was applied separately to the ichthyofauna and ichthyofauna-crustacean data sets

Analysis	Objective
MDS (Euclidean distances)	Obtain an ordination plot of the estuaries on the basis of their hydromorphological and pressure similarities
2-way nested ANOSIM	Determine the seasonal effect on the biological characteristics of estuaries
MDS (Bray-Curtis)	Obtain an ordination plot of the estuaries on the basis of their similarities in the community composition
Cluster analysis	Obtain a dendrogram plot of estuaries on the basis of their similarities in the community composition
SIMPROF (permutation analysis)	Discriminate estuary clusters on the basis of their similarities in the community composition
BEST	Determine the abiotic variables that best explain the biological characteristics of estuaries
LINKTREE	Determine the abiotic variables that best explain the clusters established by the SIMPROF test
SIMPER	Determine the species that explain similarities and dissimilarities between estuaries

Using the Bray-Curtis similarity matrix of average abundance (of fish and fish-crustaceans respectively), a cluster analysis and an MDS was carried out. The cluster analysis was used to develop ordination dendrograms of samples (estuaries) based on their biological similarities. SIMPROF (SIMilarity PROfile) permutation test was also applied to this analysis with the aim to discriminate estuary clusters. On the other hand, MDS was used to graphically represent the estuaries in a two-dimensional scale, keeping distances between points (estuaries) proportional to their biological similarities.

To determine the abiotic variables that explained the assemblage of estuaries based on their community structure (biological variables), a BEST (Bio-Env+Stepwise) analysis was carried out. Selected abiotic variables were taken into a LINKTREE (LINKage TREEs) analysis with the aim to understand how these selected abiotic variables discriminate different estuary groups that come defined by the community structure.

Finally, SIMPER (SIMilarity PERcentages) analysis was performed to reveal the species that explained most similarities and dissimilarities between LINKTREE estuary groups.

The PRIMER 6 (v.6.1.6.) package, specific to ecological data, was used to perform the described analyses.

2.1.4. Development and improvement of ecological status classification methodologies based on demersal communities

In order to determine the ecological status of estuaries, AZTI´s Fish Index (AFI) was used (Borja *et al.* 2004, Uriarte and Borja, 2009) (Table 3). AFI considers nine metrics: species richness (n), pollution bioindicator species (%), introduced species (%), fish community health (% of affected individuals), flat fish (%), trophic composition (% of omnivores and % of piscivorous) and resident species (n and %) in the estuary. Each metric gets assigned a value (1, 3 or 5), which are added up to generate a general value that ranges from 9 to 45. This value is then associated with an ecological status: very good (39-45), good (31-38), acceptable (24-30), bad (17-23) and very bad (9-16).

Table 3. Key to be used in calculating the AFI Index value. The summary of the values assigned to each indicator defines the ecological status of the water body: very good (39-45), good (31-38), acceptable (24-30), bad (17-23) and very bad (9-16). In estuaries type I and II, both fish (F) and crustaceans (C) are considered, while in estuaries type III only fish (F) are taken into account. Modified from Borja et al. (2004a) and Uriarte and Borja (2009).

Indicator	**Value**		
	1	**3**	**5**
1.- Species richness (fish and crustaceans) (n)	≤ 3	4 to 9	>9
2.- Pollution bioindicator species (F & C) (%)	> 80	30 - 80	< 30
3.- Introduced species (F & C) (%)	> 80	30 - 80	< 30
4.- Fish community health (injured, diseases...)(% affected)	≥ 50	5 to 49	<5
5.- Flat fish presence (%)	<5	5-10 or >60	> 10 to 60
6.- Trophic composition (% omnivorous)	<1 or >80	1<2.5 or 20-80	2.5 to <20
7.- Trophic composition (% piscivorous)	<5 or >80	5<10 or 50-80	10 to <50
8.- Resident species in the estuary (F & C) (n)	<2	2 to 5	>5
9.- Resident species (%) (F & C)	<5 or >50	5<10 or 40-50	10 to <40

Due to the fact that species richness in small estuaries is often very low, the valuation of the ecological status of Basque estuaries of types I and II (small river-dominated estuaries and estuaries with extensive intertidal flats, respectively) were carried out considering both fish and epibenthonic crustaceans. In type III estuaries (Nerbioi, Oiartzun and Bidasoa: estuaries with extensive subtidal areas) this valuation was carried considering fish only (see Borja *et al.*, 2004; Uriarte and Borja, 2009).

Finally, to understand the relationship between the ecological status of estuaries (AFI values) and hydromorphological and pressure variables, a multiple regression analysis was carried out. Only variables that showed a correlation value > 0.5 (i.e. population, industrial plants, dredged area, global pressure index, sediment pollution index, percentage of channeling out of ports, average estuary depth, residence time, and subtidal volume) were considered in this analysis (Colton, 1979). The analysis was carried out using PASW Statistics v. 17.0.2. package.

2.2. Case study: metrics and EFAI response against anthropogenic pressure in Portuguese estuaries

2.2.1. Fieldwork data collection

To help on the purpose of the WISER project, the fish sampling surveys conducted along several years in different estuaries (Transitional Waters) provided the database here used. To test the metrics' response against anthropogenic pressure, the survey was conducted during spring 2009 in five Portuguese estuaries (Ria Aveiro, Tagus, Sado, Mira, Guadiana) (Figure 2). Samples were collected by beam trawl, with 7-8 hauls per site, and performed at ebb tide under dark conditions.

Samples were collected inside each salinity class, following the Venice system (Anonymous 1958): oligohaline (0 – 5); mesohaline (5 – 18); and polyhaline/euhaline (> 18). The length of each beam trawl haul was calculated using the average speed and the duration or computed from the geographic coordinates of the starting and ending points of the haul. The characteristics of the sampling gear are: beam trawl; width 2 m; height 0.5 m; 5 mm mesh size in the cod end; 1 tickler chain.

Figure 2: Sampling sites. The estuaries of Ria Aveiro, Tagus, Sado, Mira and Guadiana.

2.2.2. Sample management and identification

For each fishing event, fishes were identified (whenever possible) at the species level, measured and counted. Beam trawl catches were expressed as individuals per 1000 m^2. Several environmental parameters were also measured during fish surveys, at the bottom or at surface, such as the salinity, temperature, depth and oxygen saturation. Secchi depth was also recorded for some fishing events.

The fish species identification was based on the World Register of Marine Species (WoRMS) database (Appeltans *et al.*, 2011), and was the taxonomic support for the application of the Estuarine Fish Assessment Index (EFAI) (Cabral *et al.*, 2011). The EFAI was here used, together with other single metrics, to analyse the response of indicators (metrics and tools) against the anthropogenic pressure.

The EFAI is a recently developed methodology, compliant with WFD, which includes 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, was used a common assignment to fish species that was previously reached inside this working group (see deliverable 4.4-2 part 1). Although the original definition of the guilds came from Elliott and Dewailly (1995) and Franco *et al.* (2008), the WISER fish working group decided to adapt some of these ecological guilds to have them uniform for the transitional waters inside the geographical working area. 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.

2.2.3. Calculation of pressure indicators

To evaluate the response of the metrics composing EFAI, and the method itself, against anthropogenic pressure, 14 pressure indicators (Table 4) were assessed for each site to produce the site's total pressure level. In order to account for different measurement units, each pressure indicator was standardized, by its maximum and minimum values observed or possible (varying between 0-1), following Vasconcelos *et al.*, 2007. The pressure index (Pi sum) was calculated as the sum of all pressure indicators for each estuarine site. The Aubry & Elliott (2006) adapted method (A&E) was calculated as the sum of 15 environmental integrative indicators (EII) criteria (1,2 re-alignment schemes; 1,3 land claim; 1,4 gross change in bathymetry and topography; 1,5 interference with the hydrographic regime; 2,1 Anthropogenically affected coastline; 2,4 Maintenance dredging – dredging area; 2,5a Maintenance dredging – disposal area; 2,9 Aquaculture; 2,10 fisheries causing nearshore seabed disturbance; 2,11 intensity of marina developments; 2.12 intensity of port developments; 3,1 water chemical quality; 3,2 sediment chemical quality; 3,6 shellfish quality and 3.10 interference with fish migration routes - chemical barrier), according to the values and scales defined by these authors, in order to allow direct comparisons in a common pressure scale.

Table 4. Pressure indicators used to quantify the total pressure present on each site. Type of data used and the source of information used to collect the data. EII – environmental integrative indicators; ERL – effects range low; ERM – effects range medium.

Pressure Indicators	**Type of data**	**Source**
Bank regulation (%)	Percentage of regulated estuarine site bank length	Maps/GE
Dredging	Mean volume and intensity	Port authorities
Interference hydrographic regime	Percentage of area occupied by structures interfering with the hydrographic regime	Maps/GE
River Flow and Dams	Flow (m^3 s^{-1}) and Number of large dams	INAG
Sediment metals concentration	Concentration & ERL and ERM	Long *et al.* 1995
Sediment PAH concentration	Concentration & ERL and ERM	Long *et al.* 1995
Industry	Number of industries in the watershed	INE
Population	Population density of watershed surrounding areas	INE
Shelfish quality	Categories according to national standards	IPIMAR
Agriculture	Used agricultural surface area	INE
Aquaculture	Number and area occupied	IPIMAR/GE
Intensity of port/marina developments	Number of berths in marinas/Port areas	Port authorities
Commercial Fishing	Number of licensed boats/Mean commercial fish landings	DGPA/INE
Recreational fishing	Number of recreational licensed fishermen	DGPA/INE
Pressure index - Pi (Sum)	Sum of all standardized indicators	
Aubry & Elliott (A&E) adapted	Adapted from 15 EII criteria	

2.2.4. Calculation of fish metrics and indices (EFAI)

Biological indicators were also calculated based on the community structure, the ecological guilds, the trophic guilds, and on the vertical distribution and disturbance of sensitive species (Table 5).

Table 5. Biological indicators calculated from the fish community data.

Community Structure	Community Function		
	Ecological Guilds	**Trophic Guilds**	**Vertical distribution and Disturbance Sensitive sp**
Species richness (S)	Ecological Guilds (EG)	Trophic Guilds (TG)	Benthic (B)
Pielou (J)	Estuarine Resident (ER)	Piscivorous (P)	Disturbance Sensitive sp
Simpson (D)	Marine Migrants (MM)	Benthic invertebrate feeders (BIF)	
Shannon (H)	Marine Stragglers (MS)	Omnivorous (OM)	
Fish density (ind 1000m^{-2})	Diadromous (DIA)		
Fish biomass (g 1000m^{-2})		n species	
		density (ind 1000m^{-2})	
		% individuals	
Site **ecological quality assessement** based on a multimetric index – the Estuarine Fish Assessment Index (EFAI) (Cabral *et al. in press*)			

The metrics included in the EFAI are: *(i)* species richness (number of species) (SR); *(ii)* percentage of marine juvenile migrants (%MM); *(iii)* estuarine resident species (ES) (metric score results from a combination of both the number of resident species and the percentage of resident individuals); *(iv)* piscivorous species (P) (metric score results from a combination of both the number of piscivorous species and the percentage of piscivorous individuals); *(v)* diadromous species (D) (assessed based on expert judgment); *(vi)* introduced species (I) (assessed based on expert judgment); and *(vii)* disturbance sensitive species (S) (assessed based on expert judgment). 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. The reference conditions considered for the exercise were based on Portuguese estuaries reference conditions, originally used for the EFAI development.

For the analysis of the response of the metrics against the anthropogenic pressure, after quantification, the pressure data were initially standardized (variation 0 – 1) and then analysed through an ordination analysis (PCA). After the identification of the groups of pressures acting on the study sites, a biological data vs. pressure group table was created and the biological variance associated to each pressure groups was analysed (Non parametric analysis of variance –

Kruskal-Wallis- and *post-hoc* multiple comparisons tests). The Spearman correlation between the EFAI results and the anthropogenic pressure was analysed. Two different pressure estimations were used: a) Pressure index - Pi (sum) (local range of pressures); and b) Aubry & Elliott (2006) adapted pressure index (broader range of pressures).

2.3. Sensitivity in strength and time-lag of indices/metrics to human pressures

The approach chosen to evaluate the sensitivity in strength and time-lag of indices and their respective metrics to human pressures is composed on four steps, detailed in the Figure 3. Firstly, it was elaborated a list of metrics used in the different assessment indices (see Annex 1) and a list of pressures, both from literature and other bibliographic review. The list of metrics was crossed with that one of pressures (see annex 2) to score the cause-effect relationships according to its strength and time lag of response. The scores were attributed from a combination of ecological senses, published literature and expert judgement.

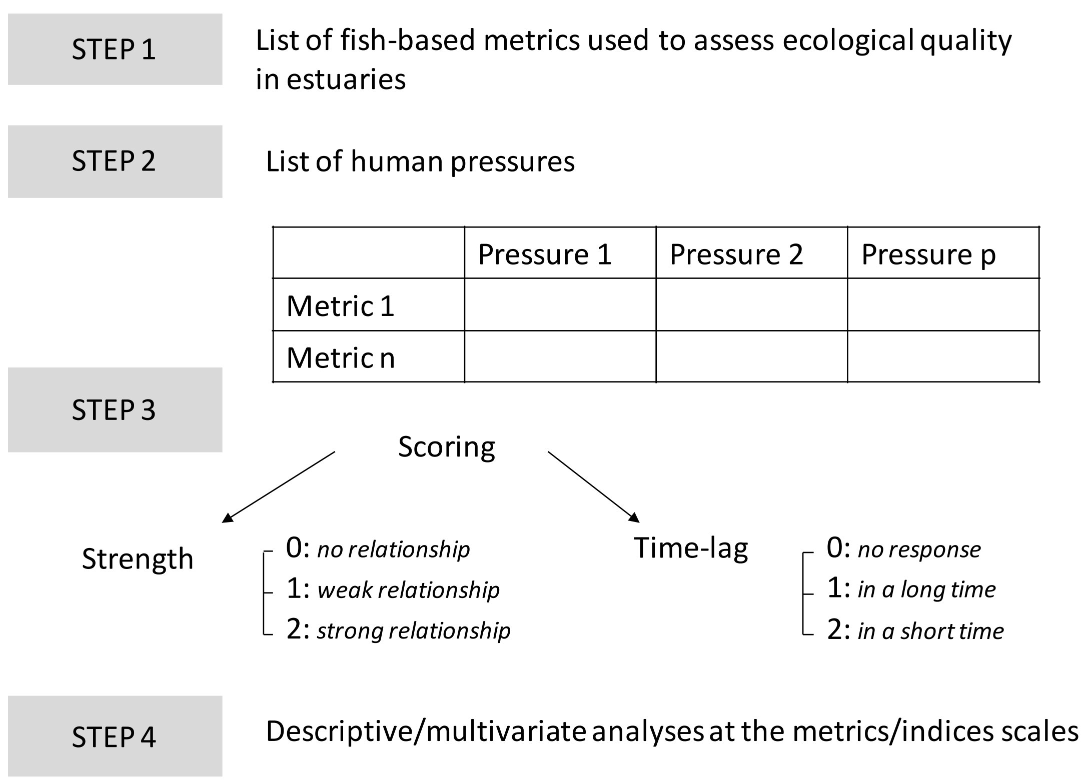


Figure 3. Methodology followed in the analysis of cause-effect relationships strength and time lag in response to human pressures of metrics used to assess water quality of estuarine systems based on fish assemblages.

2.4. Sensitivity analysis of French (ELFI) and UK (TFCI) fish indices to the metrics dynamic range

2.4.1. Data used

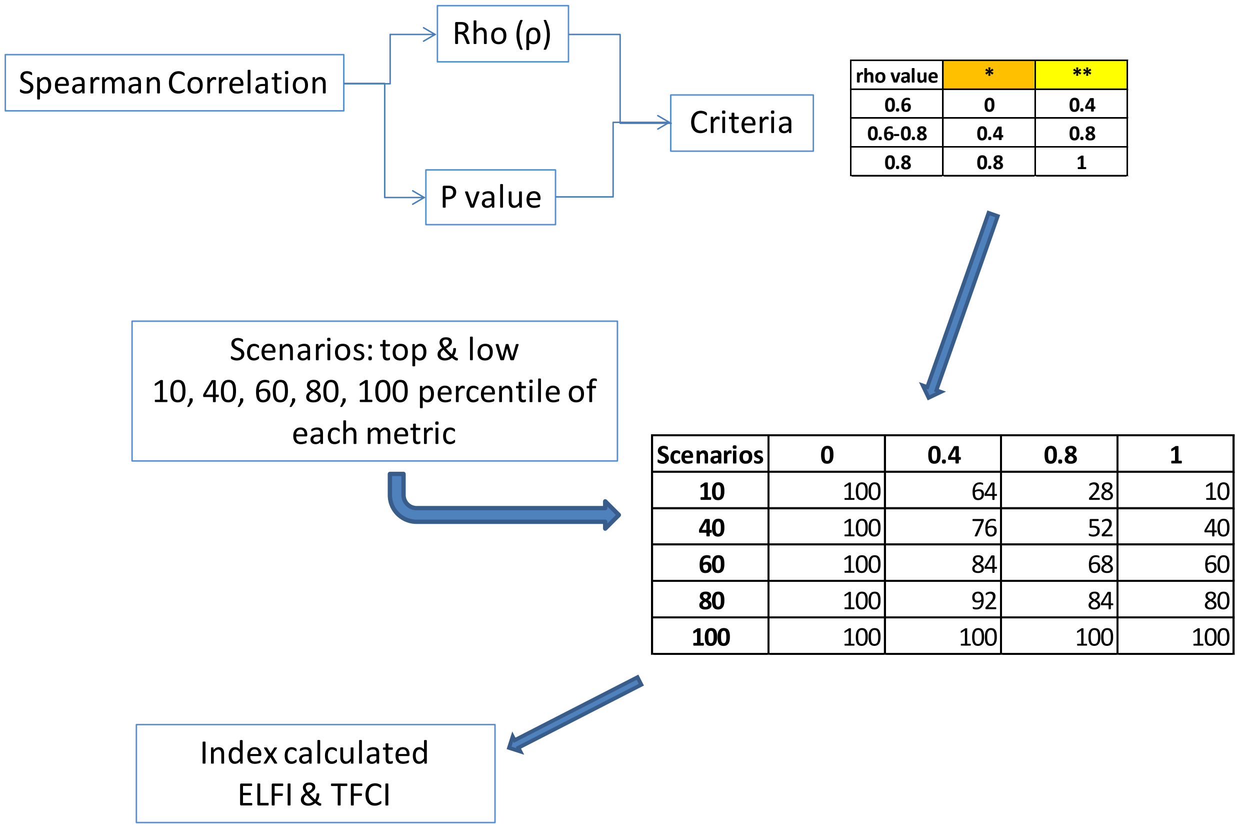
The sensitivity of fish-based indices to metric changes was investigated by using the French ELFI (Estuarine and Lagoon Fish Index) and the TFCI (Transitional Fish Classification Index). The assessment analysed a total of 68 French and 58 British transitional water bodies (WB) as defined by the WFD, covering a period between 2004 and 2010. Data were provided by IRSTEA (formerly CEMAGREF, France) and the Environment Agency (UK) and formed part of the monitoring exercise the French and UK Water Agencies are conducting for the implementation of WFD. The data were organised by water body, sampling year and by scores for the different metrics composing each index. Scores for each metric (6 metrics for ELFI and 10 for TFCI listed in Table 6) were ranked from largest to smallest.

Table 6. Definition of the acronyms of metrics forming the French ELFI and British TFCI indices

ELFI Metrics	*TFCI Metrics*	
DDIA: Density of diadromous species	M1: Species composition	M6: Number of estuarine-dependent marine taxa
DFW: Density of freshwater species	M2: Presence of indicator species	M7: Functional guild composition
DB: Density of benthic species	M3: Species relative abundance	M8: Number of benthic invertebrate feeding taxa
DT: Total density	M4: Number of taxa that make up 90% of the abundance	M9: number of piscivorous species
DER: Density of estuarine resident species	M5: Number of estuarine species	M10: Feeding guild composition
RT: Total richness		

2.4.2. Modelling scenarios

A series of scenarios were chosen to test the sensitivity of the ELFI and TFCI indices to score changes to each of their constituent metrics. Several realistic scenarios were defined based upon the dynamic range of variation of each metric within the investigated dataset by setting each metric score to the average value observed in the 10, 40, 60, 80 percentiles (both top and low percentiles were considered), along with the average value across the entire range (all observations). The option of changing one metric at a time whilst setting the others at their average score value was considered unsatisfactory as it did not take into account relationships among metrics and hence their co-variability. These relationships were explored by using non-parametric Spearman-rank correlations. Based on these relationships, scenarios were defined by changing the score value of each metric and of their correlated metrics, under the assumption that metrics that are correlated with the metric driving the scenario will change more or less according to the strength of the relationship linking them. The results of the correlation tests (the correlation coefficient “ρ” and the p-value) were used to create a relationship criterion to apply when testing the sensitivity of the index to any metric manipulation (see Figures 4, 5 and 6 for details).



rho value	*	**
0.6	0	0.4
0.6-0.8	0.4	0.8
0.8	0.8	1

Scenarios	0	0.4	0.8	1
10	100	64	28	10
40	100	76	52	40
60	100	84	68	60
80	100	92	84	80
100	100	100	100	100

Figure 4. Conceptual diagram of the approach followed to conduct the sensitivity analysis of ELFI and TFCI. Spearman correlations were calculated and a criteria was applied according to the ρ and p-values (≤0.05, **≤0.01) of these correlations. Nine scenarios were selected to understand the behaviour of the indices towards changes in its constituent metrics (see section 2.4.3). Scenario 10 percentile (top and low) represent the more extreme manipulation and scenario 100 percentile indicates the mean value of all recorded scores. A summary of a combination of criterion and scenarios is shown in the table where the percentages needed to calculate for the related metric are shown at the different scenario levels. For example, a scenario at the 40 percentile for a given metric will mean that correlated metrics to a 0.4 level will have a value corresponding to a 76 percentile carried to calculate the index. Indices are calculated with these metric combinations and a percentage change from the average index value is computed. This percentage change is then used to create tornado and radar plots that summarize the sensitivity analysis.*

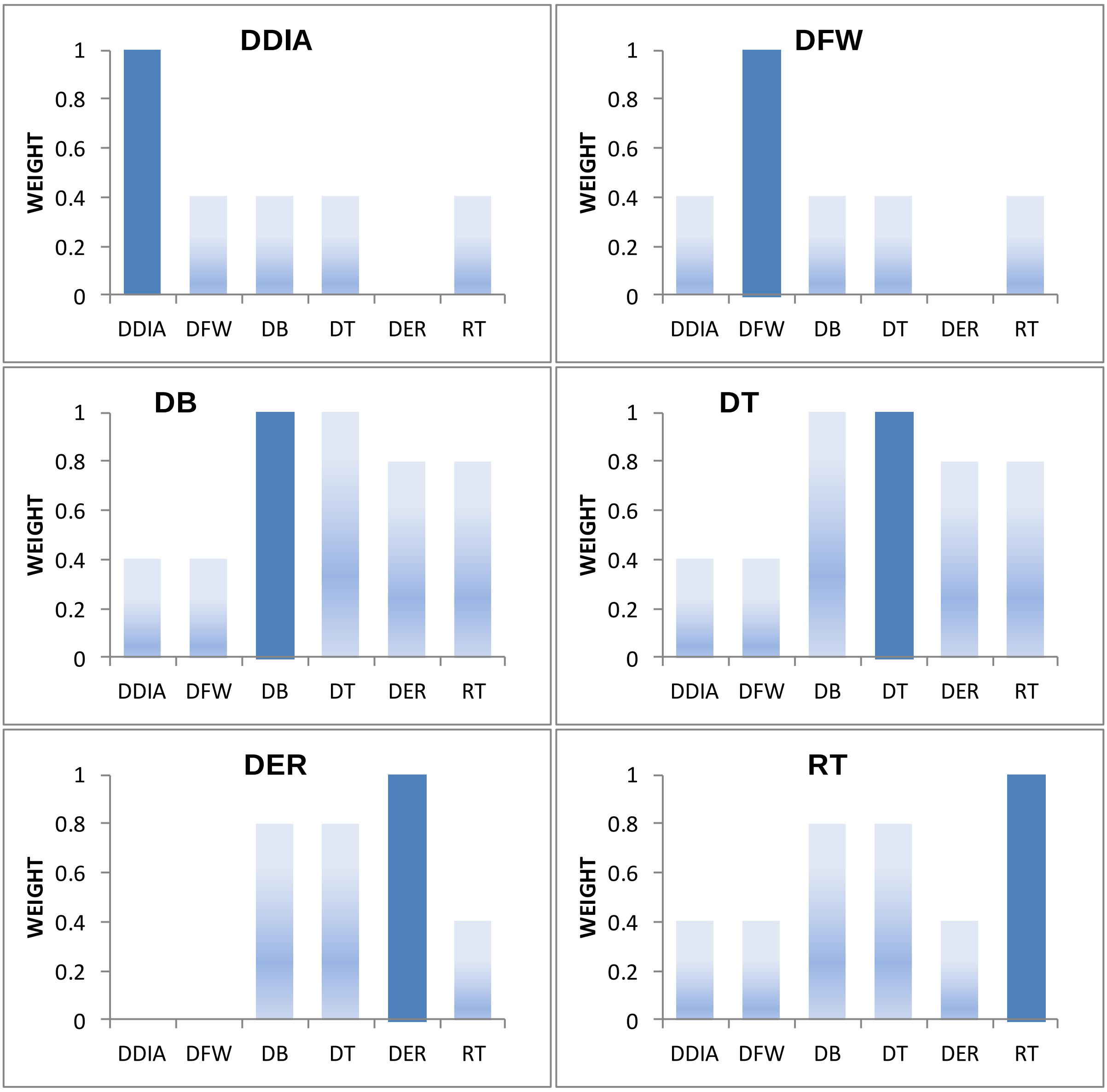


Figure 5. Weight applied for each metric in accordance to their correlation to the tested metric for the French index ELFI. The metric leading each scenario is indicated in the title of each graph and by the solid bar. The absence of bar indicates metrics that are uncorrelated with the metric leading the scenario (for these metrics the average score has been considered in the 4 scenario definition).

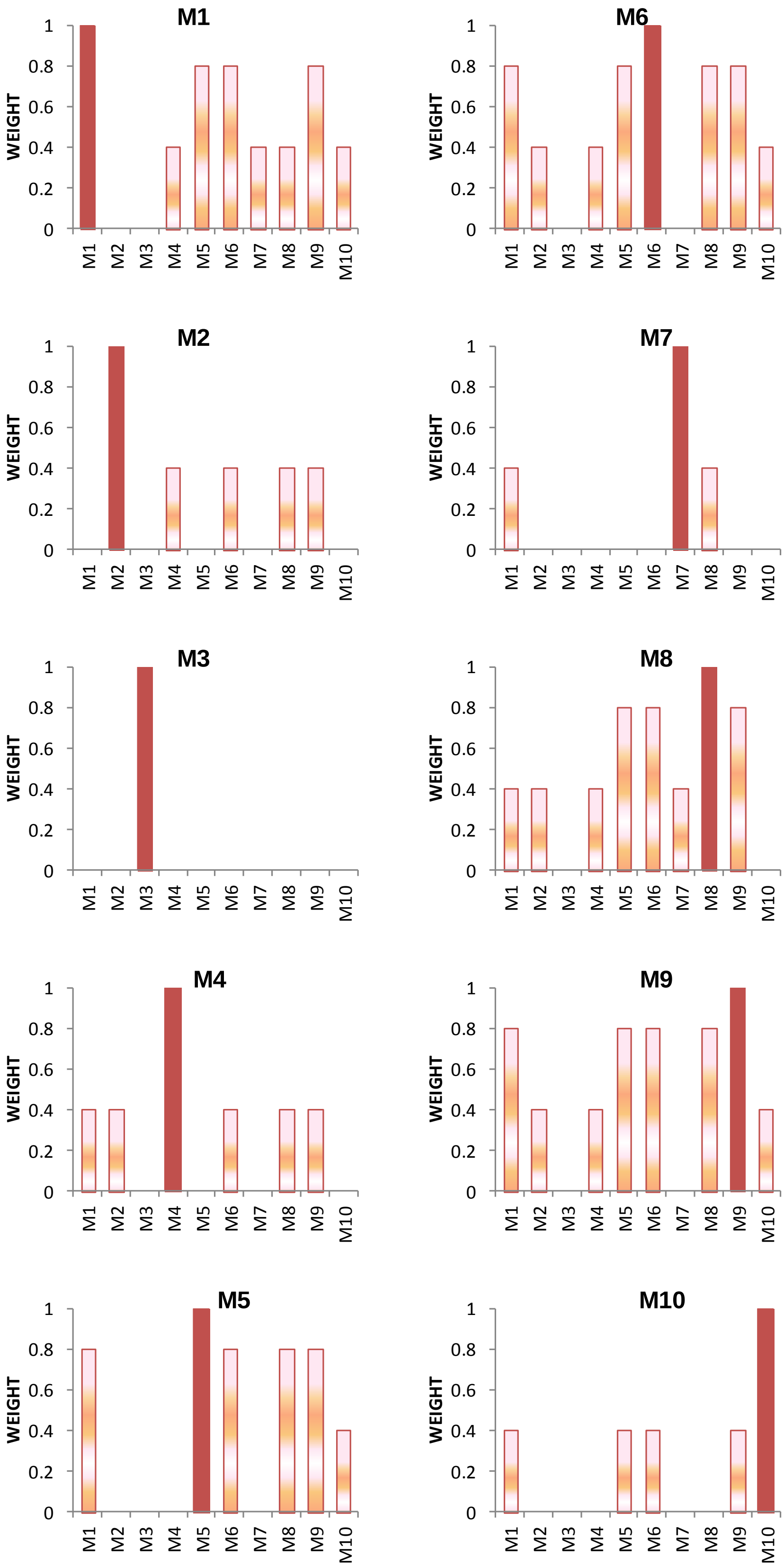


Figure 6. Weight applied for each metric in accordance to their correlation to the tested metric for the British index TFCI. The figure layout is the same as in Figure 5.

2.4.3. Index response

Eight scenarios were selected to conduct the sensitivity analysis on ELFI and TFCI, from the most restrictive extreme cases (top and low 10 percentiles) to the most inclusive (top and low 80 percentile). The metric average (or 100 percentile) was calculated to express the induced change in the composite index as a percentage change from this initial value. The sensitivity analysis can be summarized using different graphing methods. One of the most informative forms are tornado diagrams where the percentage change in the index from its overall average is represented. Another way of representing the sensitivity analysis is by using radar or spider plots where the most influencing variables can be highlighted.

3. Results

3.1. *Case study: Basque Country*

3.1.1. Analysis of abiotic data

The MDS ordination plot below (Figure 7) indicates differences between estuaries based on their abiotic characteristics. For example, the Nerbioi and Lea/Barbadun represent the highest differences and therefore, the more dissimilar estuaries in terms of their abiotic (i.e. hydromorphological and pressure) characteristics.

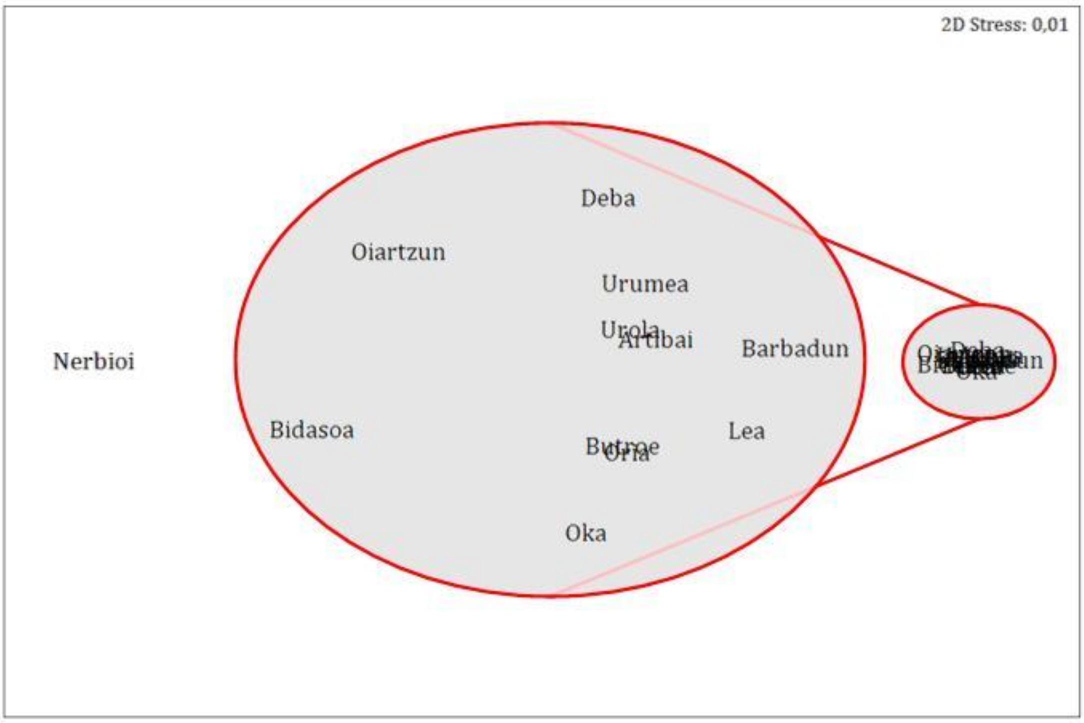


Figure 7. Multidimensional Scaling (MDS) ordination plot, based on Bray-Curtis similarities, establishing the distances (similarities) between estuaries on the basis of their abiotic characteristics.

3.1.2. Ichthyofauna

Structural parameters

Overall, the demersal fish communities at the studied estuaries were relatively poor in terms of abundance and community composition. For example, the average abundance was 8 individuals and ranged from 0 (in several samples) to 129 (Table 7), which was recorded in the inner section of the Butroe, during the autumn survey. Similarly, species richness, Shannon's diversity values and Pielou's equitability values were also low. Zero values were often recorded for these parameters. Due to the high variability in the parameter values between estuaries, it was impossible to determine common patterns within/between estuary sections and seasons.

Table 7. Summary of the structural parameters for the 12 estuaries

	Mean	Minimum	Maximum
Abundance (n)	8	0	129
Species richness (n)	2	0	9
Diversity (Shannon Index, bit ind^{-1})	0.68	0	2.58
Equitability (Pielou Index)	0.46	0	1

Multivariate analysis at the specific level

On the basis of the abundance at the different estuaries, the SIMPROF analysis defined the following statistically different estuary groups (Figure 8): 1. Oiartzun and Bidasoa, 2. Butroe and Oka, 3. Barbadun, Nerbioi, Artibai, Deba, Urola, Oria and Urumea. Lea remained independent.

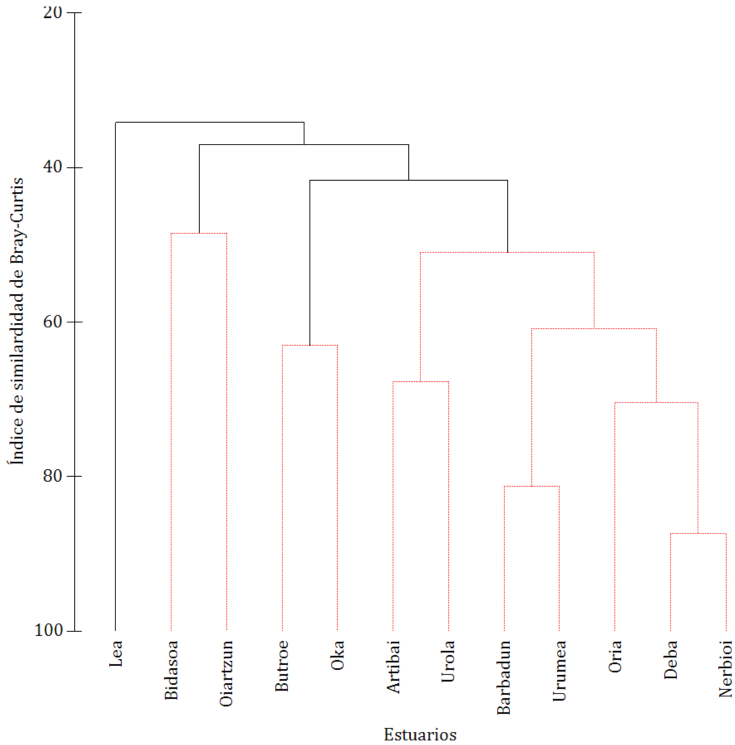


Figure 8. Ordination dendrogram of estuaries, obtained from the application of a cluster analysis to averaged abundance samples and excluding seasonality of the data and estuary sector. Red colour indicates estuary groups for which abundance did not significantly differ.

Similarities and dissimilarities within and between groups (respectively) were explained by the abundance of different species rather than by the species composition (Tables 8 and 9). Hence, Group 1 (Oiartzun and Bidasoa) was defined by the abundance of *Pomatoschistus* sp., *Gobius niger* and *Scorpaena porcus* (Table 8). Group 2 (Butroe and Oka), on the other hand, was defined by *Diplodus sargus*, *Pomatoschistus* sp., *Solea solea* and *Diplodus annularis*, which contributed to approximately 50% of their similarities. Finally, group 3 (all other estuaries excluding the Lea) was mainly defined by species of the *Pomatoschistus* genus and *Solea solea*.

Table 8. Abundance of specific species contributing to the similarities within estuary groups established by SIMPROF analysis. Cont: contribution of each species to the similarity between groups. Cum: Cumulative contributions.

Similarity	Species	Cont. (%)	Cum. (%)
Group 1	*Pomatoschistus* sp.	28.8	28.8
	Gobius niger	19.6	48.4
48.50	*Scorpaena porcus*	14.6	63.0
	Buglossidium luteum	12.5	75.5
	Diplodus sargus	20.9	20.9
Group 2	*Pomatoschistus* sp.	15.2	36.1
	Solea solea	13.9	49.9
63.02	*Diplodus annularis*	12.5	62.5
	Gobius niger	12.0	74.5
	Mugilidae	9.5	84.0
Group 3	*Pomatoschistus* sp.	41.3	41.3
	Solea solea	30.2	71.5
59.63	*Platichthys flesus*	18.4	89.8

Table 9. Species that best explain dissimilarities between the estuary groups established by SIMPROF analysis. Cont: contribution of each species to the dissimilarity between groups. Cum: Cumulative contributions.

Dissimilarity	**Species**	**Mean abundance ($ind^{1/4}$)**				**Cont. (%)**	**Cum. (%)**
		Group 1	**Group 2**	**Group 3**	**Group4**		
	Diplodus sargus	0.00	1.74	-	-	13.0	13.0
Groups 1-2	*Diplodus annularis*	0.00	1.09	-	-	8.2	21.2
	Mugilidae	0.00	0.77	-	-	5.7	27.0
65.82	*Engraulis encrasicolus*	0.00	0.65	-	-	5.1	32.1
	Scorpaena porcus	0.68	0.00	-	-	5.1	37.2
	Scorpaena porcus	0.68	-	0.00	-	8.3	8.3
Groups 1-3	*Buglossidium luteum*	0.59	-	0.00	-	7.2	15.5
	Gobius niger	0.92	-	0.37	-	7.1	22.6
62.19	*Platichthys flesus*	0.33	-	0.76	-	6.6	29.2
	Callionymus lyra	0.54	-	0.08	-	5.8	35.1
	Anguilla anguilla	0.00	-	-	0.69	7.4	7.4
	Syngnathus typhle	0.00	-	-	0.69	7.4	14.8
Groups 1-Lea	*Scorpaena porcus*	0.68	-	-	0.00	7.4	22.2
	Solea solea	0.63	-	-	0.00	6.8	29.0
72.79	*Buglossidium luteum*	0.59	-	-	0.00	6.4	35.4
	Coris julis	0.00	-	-	0.58	6.2	41.6
	Callionymus lyra	0.54	-	-	0.00	5.9	47.5
	Diplodus sargus	-	1.74	0.26	-	16.8	16.8
	Diplodus annularis	-	1.09	0.08	-	11.5	28.2
Groups 2-3	Mugilidae	-	0.77	0.00	-	8.5	36.7
58.34	*Engraulis encrasicolus*	-	0.65	0.00	-	7.8	44.5
	Gobius niger	-	0.95	0.37	-	6.6	51.1
	Dicentrarchus labrax	-	0.58	0.00	-	6.4	57.4
Groups 2-Lea	*Diplodus sargus*	-	1.74	-	0.00	16.1	16.1
	Diplodus annularis	-	1.09	-	0.00	10.1	26.2

72.75	*Solea solea*	-	1.06	-	0.00	9.6	35.8
	Mugilidae	-	0.77	-	0.00	7.0	42.8
	Platichthys flesus	-	0.74	-	0.00	6.9	49.7
	Engraulis encrasicolus	-	0.65	-	0.00	6.4	56.2
	Anguilla anguilla	-	0.00	-	0.69	6.3	62.4
	Coris julis	-	0.00	-	0.58	5.3	67.7
	Dicentrarchus labrax	-	0.58	-	0.00	5.3	73.0
	Solea solea	-	-	0.85	0.00	18.1	18.1
	Platichthys flesus	-	-	0.76	0.00	15.2	33.3
Groups 3-Lea	*Syngnathus typhle*	-	-	0.08	0.69	13.2	46.4
61.93	*Coris julis*	-	-	0.00	0.58	12.1	58.5
	Anguilla anguilla	-	-	0.20	0.69	11.2	69.7
	Gobius niger	-	-	0.37	0.76	9.7	79.5

Some of the species that best explained for differences between groups include: *D. sargus*, *D. annularis*, Mugilidae family, *Engraulis encrasicolus* and *S. porpus*. These species explained for more than 35% of the dissimilarities between groups 1 and 2, being nearly exclusive of group 2. *S. porcus*, *Buglossidium luteum*, *G. niger*, *Platichthys flesus* and *Callionymus lyra* explained for 35% dissimilarities between groups 1 and 3, being these species more abundant in group 1 (except for *P. flesus*, which is more abundant in group 3). Dissimilarities (35% level) between groups 3 and 4 were explained by *Anguilla anguilla*, *Syngnathus typhle*, *S. porcus*, *S. solea* and *B. luteum*. *A. anguila* and *S. typhle* were exclusive to the Lea estuary while the other three species were only identified in group 1.

Dissimilarities between groups 2 and 3 were mainly explained by higher abundances of *D. sargus*, *D. annularis* and the species of the Mugilidae family in group 2, while dissimilarities between groups 3 and 4 were primarily explained by the absence of *D. sargus*, *D. annularis* and *S. solea* in the Lea estuary. Finally, dissimilarities between groups 3 and 4 were explained by the fact that *S. solea* and *P. flesus* were only present in group 3. Opposite, *S. typhlae* was absent in group 3 and present in the Lea estuary.

Characterization of estuaries

The abiotic variables that best explained the ordination of estuaries according to biological data were: water pollution index, percentage of subtidal surface, flushing time and catchment area (BEST analysis: $\rho = 0.476$, $p = 0.004$), with water pollution index being the variable that explained most of this ordination (BEST analysis: $\rho = 0.439$, $p = 0.007$).

Considering the abiotic variables selected by the BEST analysis, LINKTREE grouped estuaries into three groups: 1. Lea and Oiartzun, 2. Oka, and 3. other estuaries (Figure 9). Lea and Oiartzun have a small catchment area (99 km^2 and 86 km^2 respectively, *versus* > 104 km^2). Oka separates from the remaining estuaries due to a flushing time, which nearly doubles that of other estuaries (149 hr *versus* ≥ 78 hr) and a relatively smaller subtidal area (14% *versus* ≤ 16%).

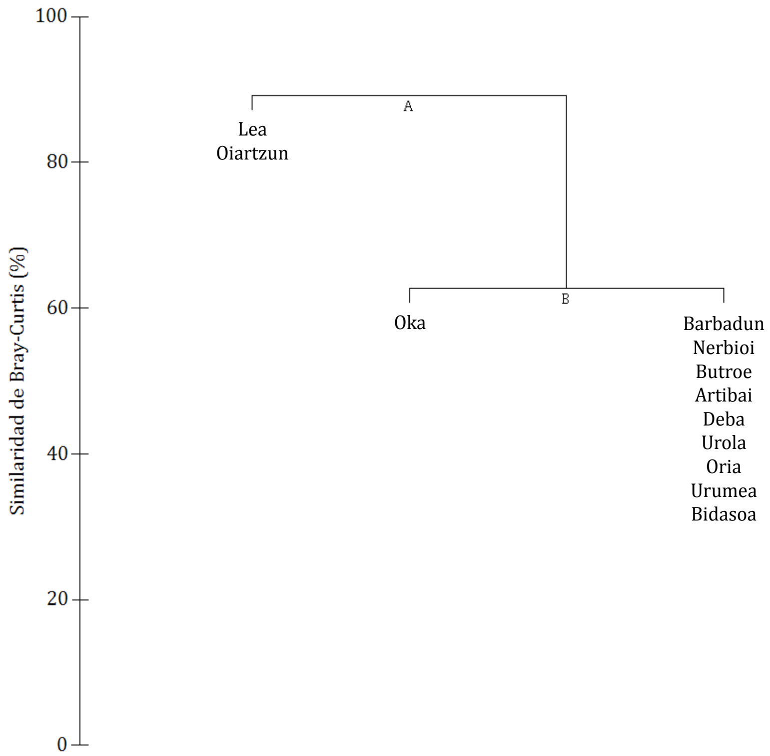


Figure 9. LINKTREE dendrogram based on the Bray-Curtis dissimilarity matrix of biological data and the five abiotic variables selected by the BEST analysis. SIMPROF routine was applied, which limits the number of divisions to those that are significant

On the basis of these new estuary groups, SIMPER results indicate that the Lea and Oiartzun group have relatively low similarities, which are mainly defined by *Pomatoschistus* sp. and *G. niger* (Table 10). Similarities within the other group are higher and come defined by *Pomatoschistus* sp. and *S. solea*.

Table 10. Abundance of specific species contributing to the similarities within estuary groups defined by the LINKTREE analysis. Cont: contribution of each species to the similarity within groups. Cum: Cumulative contributions.

Similarities	Species	Cont. (%)	Cum. (%)
Group 1 27.33	*Pomatoschistus* sp.	56.1	56.1
	Gobius niger	43.9	100.0
Group 3 52.77	*Pomatoschistus* sp.	39.1	39.1
	Solea solea	28.3	67.4
	Platichthys flesus	18.6	86.0

Between estuary groups, dissimilarities were explained by the presence/absence and/or abundance of several species (Table 11). For example, *D. sargus* was the key species explaining for dissimilarities between the Oka estuary and the two estuary groups with a contribution of 11.9% and 12%, respectively, of the dissimilarities. Presence of *P. flesus* and higher abundance of *S. solea* in group 3 explained for most of its dissimilarities with group 1.

Table 11. Species (and their abundances) that best explain dissimilarities between the estuary groups defined by the LINKTREE analysis. Cont: contribution of each species to the dissimilarity between groups. Cum: Cumulative contributions.

Dissimilarity	Species	Mean abundance			Cont. (%)	Cum. (%)
		Group 1	Group 2	Group 3		
Groups 1-2 **68.28**	*Diplodus sargus*	0.00	1.50	-	11.9	11.9
	Diplodus annularis	0.00	0.90	-	7.1	19.0
	Echiichthys vipera	0.00	0.90	-	7.1	26.1
	Solea solea	0.27	1.12	-	7.0	33.1
	Mugilidae	0.00	0.86	-	6.8	39.9
	Hippocampus hippocampus	0.00	0.69	-	5.4	45.3
	Pegusa lascaris	0.00	0.69	-	5.4	50.7
	Pomatoschistus sp.	1.26	1.86	-	5.0	55.7
Groups 1-3 **65.97**	*Platichthys flesus*	0.00	-	0.76	10.5	10.5
	Solea solea	0.27	-	0.85	9.4	20.0
	Gobius niger	0.87	-	0.48	6.7	26.6
	Syngnathus typhle	0.34	-	0.06	6.0	32.6
	Anguilla anguilla	0.34	-	0.15	5.7	38.3
	Coris julis	0.29	-	0.00	5.1	43.5
Groups 2-3 **57.20**	*Diplodus sargus*	-	1.50	0.42	12.0	12.0
	Mugilidae	-	0.86	0.08	7.9	19.9
	Diplodus annularis	-	0.90	0.21	7.9	27.8
	Echiichthys vipera	-	0.90	0.14	7.4	35.2
	Pomatoschistus sp.	-	1.86	1.13	7.1	42.3
	Syngnathus typhle	-	0.76	0.06	6.9	49.2
	Pegusa lascaris	-	0.69	0.00	6.8	56.0
	Hippocampus hippocampus	-	0.69	0.07	6.2	62.2
	Gobius niger	-	1.03	0.48	5.8	68.0
	Lithognathus mormyrus	-	0.58	0.00	5.7	73.7
	Dicentrarchus labrax	-	0.58	0.06	5.2	78.8
	Buglossidium luteum	-	0.58	0.06	5.2	84.0

3.1.3. Ichthyofauna and crustaceans

Structural parameters

In line with the results obtained when analysing the ichthyofauna data alone, it was found that the demersal communities (ichthyofauna and crustaceans combined) in the estuaries were rather poor, both in terms of abundance and species richness (Table 12). For example, the inner section of the Butroe estuary (autumn season) presented the highest abundance of individuals (n = 512) *versus* several sites/fieldwork seasons for which only one individual was found. Despite a low average species richness value (mean = 5), individuals were homogeneously distributed within species (Pielou Index = 0.72).

Table 12. Summary of the ichthyofauna-crustacean structural parameters for the 12 estuaries.

	Mean	Minimum	Maximum
Abundance (n)	34	1	512
Species richness (n)	5	1	14
Diversity (Shannon Index, bit ind^{-1})	1.58	0	3.49
Equitability (Pielou Index)	0.72	0	1

Multivariate analysis at the species level

When considering both fish and crustaceans, the SIMPROF analysis defined two estuary groups (Figure 10): 1. Butroe and Oka, 3. Barbadun, Nerbioi, Artibai, Deba, Urola, Oria, Urumea and Bidasoa. In this case, the Lea and Oiartzun estuaries remained independent from all other estuaries.

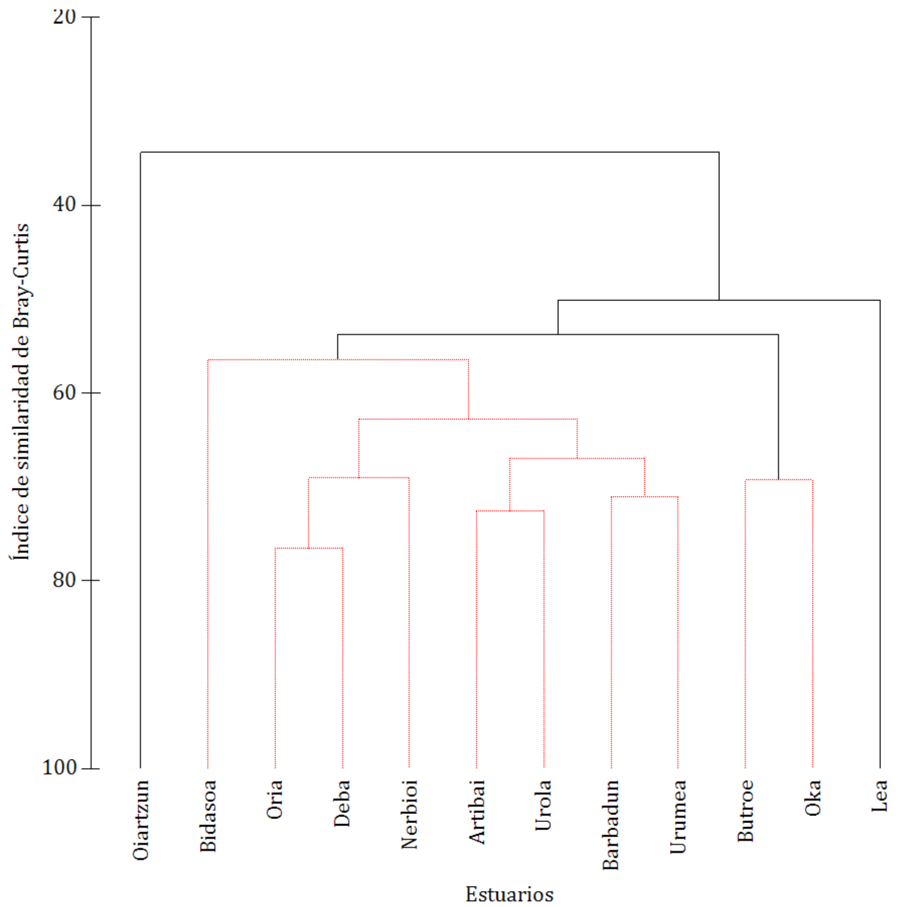


Figure 10. Ordination dendrogram of estuaries, obtained from the application of cluster analysis to averaged abundance samples and excluding seasonality of the data and estuary sector. Red colour indicates estuary groups for which abundance did not significantly differ.

The group formed by Butroe and Oka was determined by *C. crangon*, *C. maenas*, *D. sargus*, *P. longirostris* and *P. elegans*, which contributed to more than 50% of the similarities between these two estuaries (Table 13). *C. maenas*, *C. crangon*, *Pomatoschistus* sp. and *P. marmoratus* explained for more than 50% of the similarities within the other group (excluding the Lea and Oiartzun estuary).

Table 13. Abundance of specific species contributing to the similarities within estuary groups established by SIMPROF analysis. Cont: contribution of each species to the similarity between groups. Cum: Cumulative contributions.

Similarity	Species	Cont. (%)	Cum. (%)
Gruop 1 **69.28**	*Crangon crangon*	13.5	13.5
	Carcinus maenas	11.1	24.7
	Diplodus sargus	9.7	34.4
	Palaemon longirostris	9.2	43.6
	Palaemon elegans	8.5	52.1
	Pachygrapsus marmoratus	7.2	59.3
	Pomatoschistus sp.	7.1	66.4
	Solea solea	6.5	72.9
	Diplodus annularis	5.9	78.7
Group 2 **63.36**	*Carcinus maenas*	17.8	17.8
	Crangon crangon	13.4	31.2
	Pomatoschistus sp.	12.6	43.8
	Pachygrapsus marmoratus	11.3	55.1
	Palaemon longirostris	11.2	66.3
	Solea solea	9.0	75.3

The key species that defined the dissimilarity between the estuary groups are included in Table 14)

Table 14. Species that best explain dissimilarities between the estuary groups established by SIMPROF analysis. Cont: contribution of each species to the dissimilarity between groups. Cum: Cumulative contributions.

Dissimilarity	Species	Average abundance				Cont. (%)	Cum. (%)
		Group 1	Group 2	Lea	Oiartzun		
Group 1-2 **46.28**	*Diplodus sargus*	1.74	0.23	-	-	9.4	9.4
	Palaemon elegans	1.53	0.33	-	-	7.6	17.0
	Diplodus annularis	1.09	0.07	-	-	6.4	23.4
	Crangon crangon	2.32	1.35	-	-	6.1	29.5
	Mugilidae	0.77	0.00	-	-	4.8	34.3
	Palaemon longirostris	1.66	0.98	-	-	4.3	38.6
Group 1-Lea **50.61**	*Diplodus sargus*	1.74	-	0.00	-	10.3	10.3
	Crangon crangon	2.32	-	0.86	-	8.4	18.7
	Diplodus annularis	1.09	-	0.00	-	6.5	25.1
	Solea solea	1.06	-	0.00	-	6.2	31.3
	Palaemon serratus	0.89	-	0.00	-	5.3	36.6
Group 1-Oiartzun **69.92**	*Diplodus sargus*	1.74	-	-	0.00	5.8	5.8
	Palaemon longirostris	1.66	-	-	0.00	5.5	11.3
	Palaemon elegans	1.53	-	-	0.00	5.1	16.3
	Crangon crangon	2.32	-	-	1.00	4.3	20.6
	Pachygrapsus marmoratus	1.30	-	-	0.00	4.3	24.9
	Diplodus annularis	1.09	-	-	0.00	3.6	28.5

	Pisidia longicornis	0.00	-	-	0.90	3.0	31.5
	Mugilidae	0.77	-	-	0.00	2.5	34.0
	Liocarcinus navigator	0.00	-	-	0.76	2.5	36.5
Group 2-Lea 49.73	*Palaemon elegans*	-	0.33	1.21	-	7.5	7.5
	Solea solea	-	0.84	0.00	-	7.0	14.4
	Palaemon longirostris	-	0.98	1.78	-	6.6	21.0
	Macropodia rostrata	-	0.80	0.00	-	6.5	27.6
	Platichthys flesus	-	0.74	0.00	-	6.2	33.7
	Pilumnus hirtellus	-	0.07	0.69	-	5.3	39.0
Group 2-Oiartzun 66.07	*Pachygrapsus marmoratus*	-	1.07	-	0.00	4.9	4.9
	Palaemon longirostris	-	0.98	-	0.00	4.5	9.5
	Liocarcinus navigator	-	0.00	-	0.76	3.5	13.0
	Platichthys flesus	-	0.74	-	0.00	3.4	16.4
	Palaemonetes sp.	-	0.00	-	0.71	3.3	19.7
	Arnoglossus laterna	-	0.00	-	0.71	3.3	22.9
	Arnoglossus thori	-	0.00	-	0.71	3.3	26.2
	Athanas nitescens	-	0.00	-	0.64	3.0	29.2
	Thoralus cranchii	-	0.00	-	0.64	3.0	32.1
	Buglossidium luteum	-	0.07	-	0.64	2.7	34.8
	Scorpaena porcus	-	0.09	-	0.64	2.7	37.5
Lea-Oiartzun 73.32	*Palaemon longirostris*	-	-	1.78	0.00	7.4	7.4
	Pachygrapsus marmoratus	-	-	1.54	0.00	6.4	13.7
	Macropodia rostrata	-	-	0.00	1.25	5.2	18.9
	Palaemon elegans	-	-	1.21	0.00	5.0	23.9
	Pisidia longicornis	-	-	0.00	0.90	3.7	27.6
	Palaemon serratus	-	-	0.00	0.76	3.1	30.8
	Liocarcinus navigator	-	-	0.00	0.76	3.1	33.9
	Palaemonetes sp.	-	-	0.00	0.71	2.9	36.8

Estuary characterization

The abiotic variables (hydromorphological and pressures) that best explained the assemblage of communities of the estuaries were water pollution index, total pressure index, continental shelf width, flushing time, and catchment area (BEST analysis: $\rho = 0.541$, $p = 0.007$). Out all this variables, water pollution index was the most important (BEST analysis: $\rho = 0.421$, $p = 0.081$), followed by total pressure index (BEST analysis: $\rho = 0.397$, $p = 0.113$). None of these variables alone was able to explain for the ordination of estuaries defined by their biological composition.

When considering these five variables only, LINKTREE results indicate four different estuary types (Figure 11): (1) Oiartzun, (2) Lea, (3) Oka and Bidasoa and (4) the remainder estuaries.

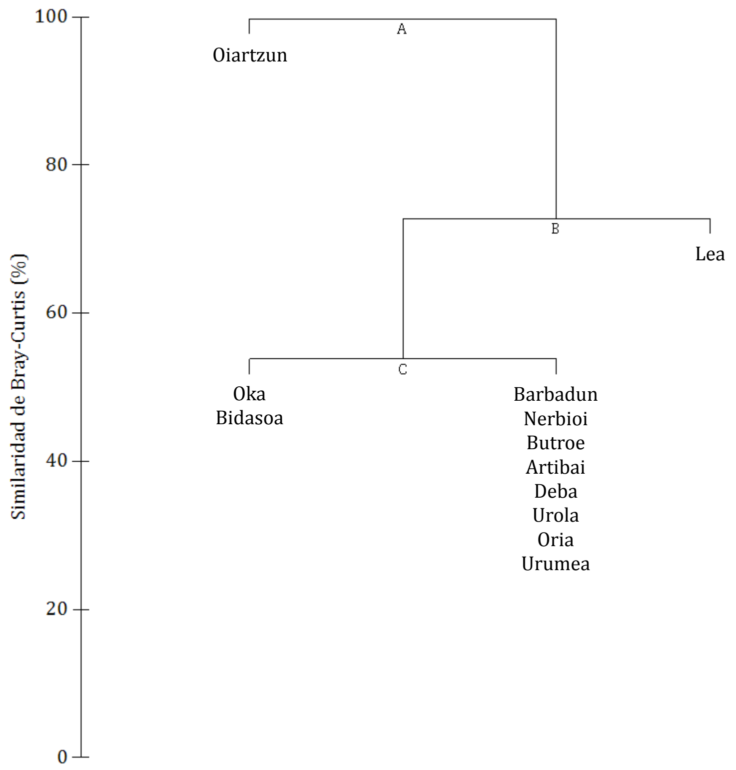


Figure 11. LINKTREE dendrogram based on the Bray-Curtis dissimilarity matrix of biological data and the five abiotic variables selected by the BEST analysis. SIMPROF routine was applied, which limits the number of divisions to those that are significant

In this case, the Oiartzun estuary was characterized by a high water pollution index (39% of the samples were polluted compared to ≤33% in other samples), a high total pressure index (2.9 *vs.* ≤ 2.8), and a smaller catchment basin (86 km^2 *vs.* > 99 km^2). Lea separated from the other estuaries due to its low water pollution index (4% of the samples were polluted *vs.* ≥8% in other samples), low total pressure index (0.8 *vs.* ≥0.9) and a smaller catchment basin (99 km^2 vs. >104 km^2). Finally, Oka and Bidasoa were characterized by a shorter continental shelf width (<16 km) than that of all the estuaries encompassed in group 4.

Having defined these four estuary types, species that characterized the demersal communities of these estuary groups (fish and crustaceans species and abundances) were determined and are now presented on Tables 15 and 16.

Table 15. Abundance of specific species contributing to the similarities within estuary groups defined by the LINKTREE analysis. Cont: contribution of each species to the similarity within groups. Cum: Cumulative contributions.

. Similarity	Species	Cont. (%)	Cum. (%)
Group 3 **55.15**	*Carcinus maenas*	14.8	14.8
	Pachygrapsus marmoratus	10.6	25.4
	Pomatoschistus sp.	10.1	35.4
	Crangon crangon	9.5	44.9
	Palaemon longirostris	8.6	53.5
	Gobius niger	6.8	60.4
	Macropodia rostrata	6.2	66.5
	Solea solea	5.8	72.3
	Hippocampus hippocampus	5.2	77.5
Group 4 **62.70**	*Carcinus maenas*	18.0	18.0
	Crangon crangon	14.2	32.2
	Pomatoschistus sp.	12.7	44.9
	Palaemon longirostris	11.4	56.3
	Pachygrapsus marmoratus	11.4	67.7
	Solea solea	9.5	77.2

Table 16. Species (and their abundances) that best explain dissimilarities between the estuary groups defined by the LINKTREE analysis. Cont: contribution of each species to the dissimilarity between groups. Cum: Cumulative contributions.

Dissimilarity	Species	Average abundance				Cont.	Cum.
		Lea	Oiartzun	Group 3	Group 4	(%)	(%)
Lea-Oiartzun **73.32**	*Palaemon longirostris*	1.78	0.00	-	-	7.4	7.4
	Pachygrapsus marmoratus	1.54	0.00	-	-	6.4	13.7
	Macropodia rostrata	0.00	1.25	-	-	5.2	18.9
	Palaemon elegans	1.21	0.00	-	-	5.0	23.9
	Pisidia longicornis	0.00	0.90	-	-	3.7	27.6
	Palaemon serratus	0.00	0.76	-	-	3.1	30.8
	Liocarcinus navigator	0.00	0.76	-	-	3.1	33.9
	Palaemonetes sp.	0.00	0.71	-	-	2.9	36.8
Lea-Group 3 **53.21**	*Crangon crangon*	0.86	-	1.87	-	5.3	5.3
	Macropodia rostrata	0.00	-	0.97	-	5.3	10.6
	Solea solea	0.00	-	0.92	-	5.0	15.6
	Palaemon serratus	0.00	-	0.80	-	4.4	20.0
	Carcinus maenas	1.33	-	2.06	-	4.0	24.0
	Diplodus sargus	0.00	-	0.75	-	3.9	27.9
	Anguilla anguilla	0.69	-	0.00	-	3.8	31.6
	Hippocampus hippocampus	0.00	-	0.67	-	3.7	35.3
Lea-Group 4 **49.08**	*Palaemon elegans*	1.21	-	-	0.48	7.5	7.5
	Solea solea	0.00	-	-	0.87	7.3	14.8
	Platichthys flesus	0.00	-	-	0.78	6.5	21.2
	Palaemon longirostris	1.78	-	-	1.08	6.3	27.5
	Macropodia rostrata	0.00	-	-	0.70	6.0	33.5
	Pilumnus hirtellus	0.69	-	-	0.00	5.8	39.3
	Syngnathus typhle	0.69	-	-	0.07	5.2	44.5

Oiartzun-Group 3 **60.10**	*Pachygrapsus marmoratus*	-	0.00	1.41	-	5.3	5.3
	Palaemon longirostris	-	0.00	1.25	-	4.7	10.0
	Palaemon elegans	-	0.00	0.93	-	3.5	13.5
	Crangon crangon	-	1.00	1.87	-	3.2	16.6
	Carcinus maenas	-	1.27	2.06	-	3.0	19.6
	Liocarcinus navigator	-	0.76	0.00	-	2.9	22.5
	Diplodus sargus	-	0.00	0.75	-	2.7	25.2
	Palaemonetes sp.	-	0.71	0.00	-	2.7	27.9
	Arnoglossus laterna	-	0.71	0.00	-	2.7	30.6
	Arnoglossus thori	-	0.71	0.00	-	2.7	33.2
	Hippocampus hippocampus	-	0.00	0.67	-	2.5	35.8
Oiartzun-Group 4 **66.07**	*Palaemon longirostris*	-	0.00	-	1.08	4.7	4.7
	Pachygrapsus marmoratus	-	0.00	-	1.04	4.7	9.4
	Platichthys flesus	-	0.00	-	0.78	3.4	12.9
	Liocarcinus navigator	-	0.76	-	0.00	3.4	16.2
	Palaemonetes sp.	-	0.71	-	0.00	3.2	19.4
	Arnoglossus laterna	-	0.71	-	0.00	3.2	22.5
	Arnoglossus thori	-	0.71	-	0.00	3.2	25.7
	Athanas nitescens	-	0.64	-	0.00	2.9	28.5
	Thoralus cranchii	-	0.64	-	0.00	2.9	31.4
	Buglossidium luteum	-	0.64	-	0.00	2.9	34.2
	Scorpaena porcus	-	0.64	-	0.00	2.9	37.1
Group 3-4 **44.23**	*Diplodus sargus*	-	-	0.75	0.48	5.0	5.0
	Crangon crangon	-	-	1.87	1.46	4.8	9.8
	Palaemon elegans	-	-	0.93	0.48	4.5	14.3
	Hippocampus hippocampus	-	-	0.67	0.00	4.3	18.6
	Buglossidium luteum	-	-	0.56	0.00	3.6	22.1
	Palaemon serratus	-	-	0.80	0.39	3.5	25.7
	Gobius niger	-	-	0.94	0.43	3.4	29.0
	Mysidacea	-	-	0.65	0.14	3.2	32.2
	Palaemon sp.	-	-	0.46	0.00	3.1	35.3

3.1.4. AFI value and the ecological status

AFI results

The application of the multimetric AFI to the estuaries allowed determining the ecological status of these water bodies (Table 17).

Table 17. AFI values for each estuary section, total estuary and season, and quality status.

Estuary	Season	Outer	Middle	Inner 1	Inner 2	Total AFI	Status
Barbadun	may-09	0.389	0.389	0.500		0.389	Moderate
	jul-09	0.611	0.444	0.500		0.538	Moderate
	sep-09	0.444	0.444	0.389		0.441	Moderate
Nerbioi interior	may-09	0.556	0.389	0.500		0.477	Moderate
	jul-09	0.389	0.389	0.500		0.422	Moderate
	sep-09	0.333	0.000	0.556		0.293	Poor
Butroe	may-08	0.611	0.389	0.444		0.549	Moderate
	jul-08	0.500	0.389	0.444		0.473	Moderate
	oct-08	0.556	0.778	0.556		0.591	Good
Oka exterior	may-08	0.444	0.444			0.444	Moderate
	jul-08	0.500	0.556			0.525	Moderate
	oct-08	0.556	0.611			0.581	Good
Oka interior	may-08			0.500		0.500	Moderate
	jul-08			0.500		0.500	Moderate
	oct-08			0.556		0.556	Good
Lea	may-08	0.500	0.389	0.444		0.450	Moderate
	jul-08	0.389	0.500	0.500		0.444	Moderate
	oct-08	0.500	0.389	0.444		0.450	Moderate
Artibai	may-08	0.500	0.333	0.444		0.450	Moderate
	jul-08	0.611	0.389	0.500		0.539	Moderate
	sep-08	0.556	0.500	0.389		0.517	Moderate
Deba	may-09	0.667	0.444	0.389		0.461	Moderate
	jul-09	0.556	0.389	0.333		0.394	Moderate
	sep-09	0.667	0.556	0.500		0.550	Good
Urola	may-10	0.500	0.500	0.389		0.487	Moderate
	jul-10	0.444	0.500	0.333		0.443	Moderate
	sep-10	0.389	0.556	0.389		0.426	Moderate
Oria	may-09	0.556	0.444	0.500		0.498	Moderate
	jul-09	0.500	0.444	0.333		0.439	Moderate
	oct-09	0.611	0.389	0.500		0.497	Moderate
Urumea	may-10	0.611	0.556	0.556		0.586	Good
	jul-10	0.444	0.444	0.500		0.453	Moderate
	sep-10	0.556	0.444	0.500		0.514	Moderate
Oiartzun	may-10	0.444	0.389	0.389	0.333	0.397	Moderate
	jul-10	0.556	0.500	0.389	0.389	0.461	Moderate
	sep-10	0.500	0.722	0.389	0.389	0.489	Moderate
Bidasoa	may-10	0.389	0.389	0.333	0.389	0.381	Moderate
	jul-10	0.667	0.444	0.333	0.000	0.448	Moderate
	sep-10	0.611	0.556	0.444	0.444	0.544	Moderate

It is interesting to note that, when we determined the regression between AFI and the pressures, at each of the seasons, only the values of autumn present significant correlation (R^2: 0.556, $p<0.05$).

Multiple regression analysis results

A series of models were created using the BACKWARD regression analysis. Out of these models, the following was the most significant model (Adjusted R^2= 0.859, $p< 0.05$) with the least possible variables:

AFI = 0.013 + 0.017(average estuary depth) – 0.003(global pressure index) – 0.001(residence time) + 0.028(dredged volume) – 0.007(percentage of channeling in ports) + 0.009(percentage of channeling out of ports).

3.2. *Case study: Metrics and EFAI response against anthropogenic pressure in Portuguese estuaries*

The distribution of the pressures, acting into the considered study sites, show that three main groups exist (Figure 12). One group (G1) concerns the human uses and the habitat physical alterations and it includes the recreational and commercial fisheries, the intensity of marina and ports, bank regularization, interferences with the hydrographic regime, and the size of the population and the industry. The fishing activities (commercial and recreational), the intensity of ports and marinas and the direct interference with morphology (bank regularisation) were found as the more important ones. A second group (G2) is composed by environmental and chemical quality, and includes the shellfish quality, metals, PAHs and river flow and dams as the more significant ones. The last group created (G3) included the agriculture as the main pressure here.

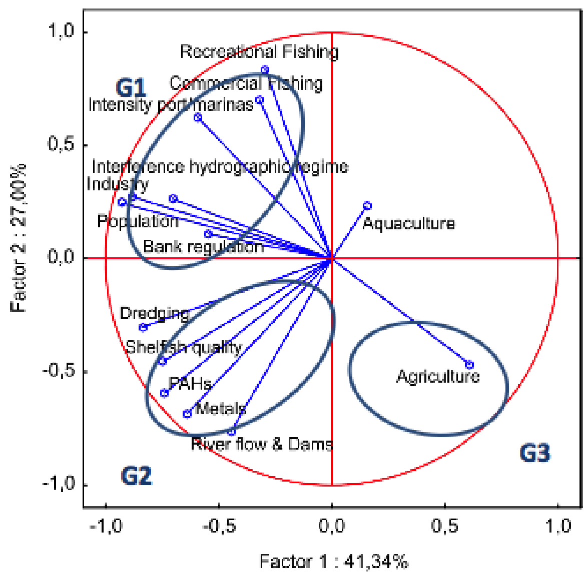


Figure 12. Distribution of anthropogenic pressures into three main groups acting in the considered study sites. G1 - Human uses (Fishing, Population, Navigation) and habitat physical alterations; G2 - Environmental and chemical quality; G3 - Agriculture and low Human uses.

When plotting the sites, based on the same analysis, it is also possible to see which sampling sites / estuaries are more similar to each other in terms of the quantified pressures (Figure 13). Tagus is mainly disturbed by environmental and chemical quality features. The southern estuaries, Mira, Guadiana and one sampling site from Sado, were mainly disturbed by agriculture activities. Concerning the human uses and the habitat physical alterations, they were identified as the main pressure vectors acting in Ria Aveiro and one site from Sado.

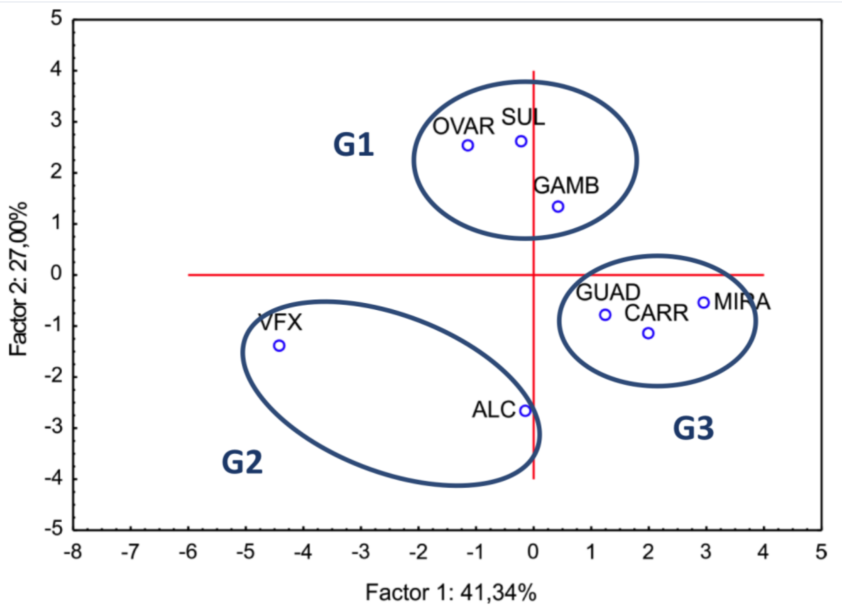


Figure 13. Distribution of sampling sites based on the anthropogenic pressures quantified on each estuary. Ria Aveiro includes OVAR and SUL sites; Sado is composed by GAMB and CARR sites, Tagus includes VFX and ALC sites; and sites GUAD for Guadiana and MIRA for the Mira estuary. G1 - Human uses (Fishing, Population, Navigation) and habitat physical alterations; G2 - Environmental chemical quality; G3 - Agriculture and low Human uses.

After the pressure groups were defined, the hauls were accommodated on each group and the variance of biological metrics calculated for each one of the three pressure groups (Figure 14). Group G2 (environmental and chemical quality) was the one producing more often a distinct result. For the Shannon and Pielou indices, the G2 presented the lower fish species diversity and the lower evenness. For estuarine resident species (ER%) and density of benthic species (B), higher densities were found in estuaries influenced mainly by this type of pressures, but the opposite was observed for the marine migrants (MM%), the number of ecological guilds, the density of piscivorous and the number of trophic guilds, where the lower values were observed for estuaries having this group of pressures as dominant.

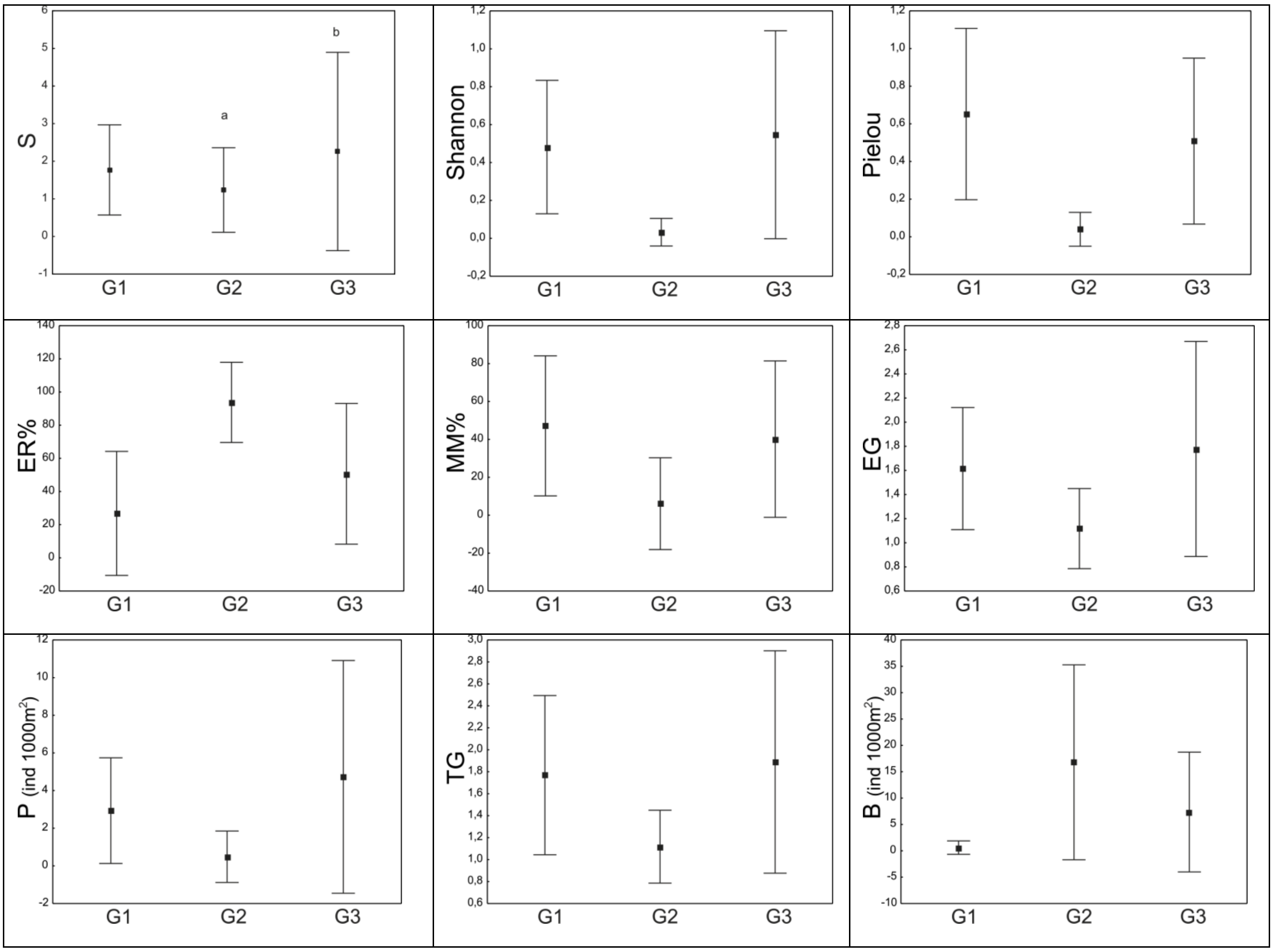


Figure 14. Analysis of variance for the biological metrics according to pressure group. S – number of species; Shannon – shannon-wiener diversity index; Pielou – Pielou evenness index; ER% - estuarine resident fish; MM% - marine migrants; EG – number of ecological guilds; P – piscivorous fish; TG – number of trophic guilds; B – benthic species. G1 - human uses and the habitat physical alteration pressure indices; G2 - environmental and chemical quality pressure indices; G3 - agriculture pressure index. The number of hauls per pressure group was: G1 – 21 hauls; G2 – 15 hauls; G3 – 21 hauls.

The response of EFAI against the pressure indices is shown in Figure 15. For both pressure indices, the total sum and the Aubry & Elliott (2006) adapted index, the response of EFAI was concordant. The EFAI value decreased for higher pressure values.

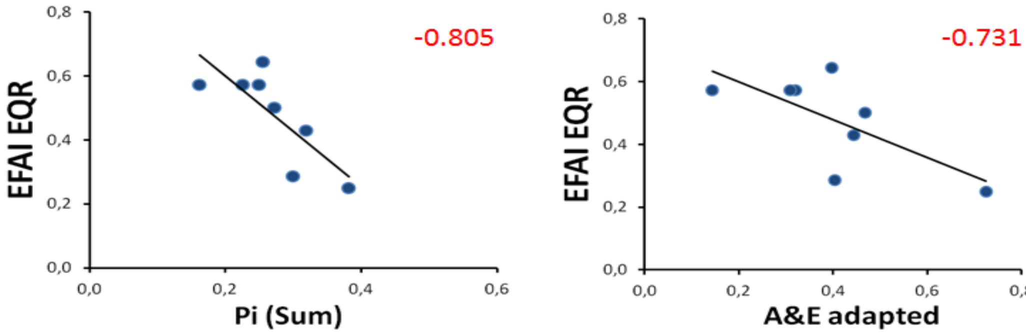


Figure 15. Response of EFAI against the pressure. Pi (Sum) – sum of pressures; A&E adapted – pressure index adapted from Aubry & Elliott (2006).

3.3. Sensitivity of metrics and indices to the cause-effect relationship strength and the time lag in response to human pressures

The list of the metrics and the indices used in this study are synthesized in the Annex 1.

Metrics: The results obtained for the strength of relationships between metrics and pressures are shown in Figure 16. "Chemical pollution" and "loss of habitat" pressures were detected by almost all of the tested fish single metrics, generally with a strong relationship (score 2). On the other hand, "water turbidity" and "habitat fragmentation" pressures presented strong cause-effect relationships but this time with fewer fish metrics than for the previously indicated. The water turbidity is strongly associated with the piscivorous and the trophic guild composition metrics, and the habitat fragmentation with diadromous ones.

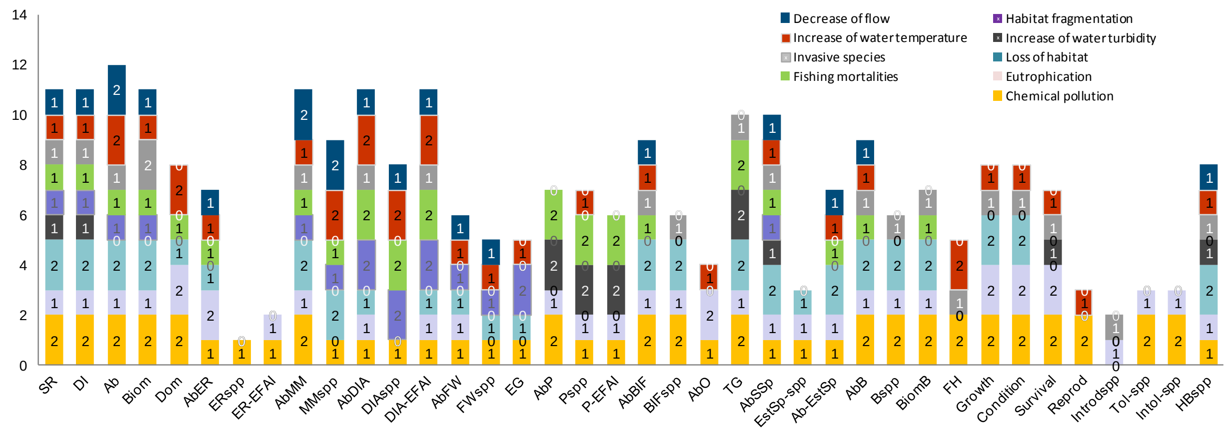


Figure 16. Cause-effect relationships in strength between fish metrics and the different pressures affecting the system. 0: no relationship; 1: weak and/or not well-documented relationship; 2: strong and/or well-documented relationship. See Annex 1 for metrics' abbreviations.

Concerning the fish metrics, the ones relative to diversity, densities (species richness, indices of diversity, total abundance) and certain ecological aspects (habitat use patterns and trophic guilds) presented the strongest relationships with pressures. The metrics detecting less cause-effect relationship with the tested pressures were "Tolerant", "Intolerant", "Introduced", "Estuarine resident species" ones.

The results obtained for the time lag in the metrics' response to human pressure are shown in Figure 17. Diversity (species richness, diversity indices) and density (total abundance, abundance of marine migrants and abundance of diadromous) metrics were the ones detecting several pressures in a short time-lag. For some pressures, i.e. temperature and flow changes, loss of habitat and chemical pollution, most of the metrics were unable to detect their effects in a short-term. However, for other pressures, such as water turbidity, habitat fragmentation, fish mortalities and invasive species, a response in a short time period prevailed.

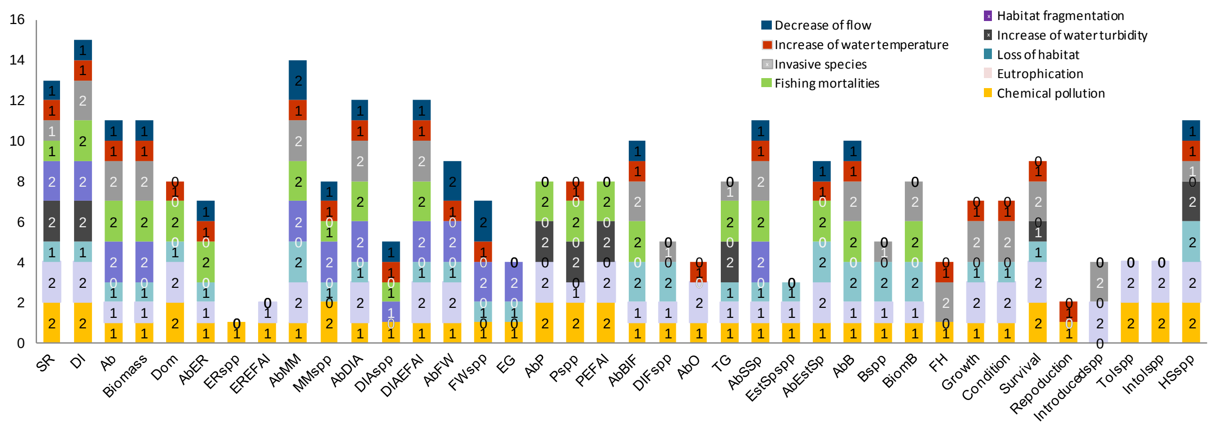


Figure 17. The time lag in the metrics' response in relation to different human pressures affecting the system. 0: no response; 1: response in a long-time (beyond a decade); 2: response in a short time (less than a decade, generally 1 or 2 years). See Annex 1 for metrics' abbreviations

Indices: All the indices considered in this study identified all the pressures assessed but a high variability in the strength of responses was registered (Figure 18). Most of indicators gave more weight to chemical pollution and loss of habitat effects than to the other pressures, with higher average strength compared to those obtained for the other pressures.

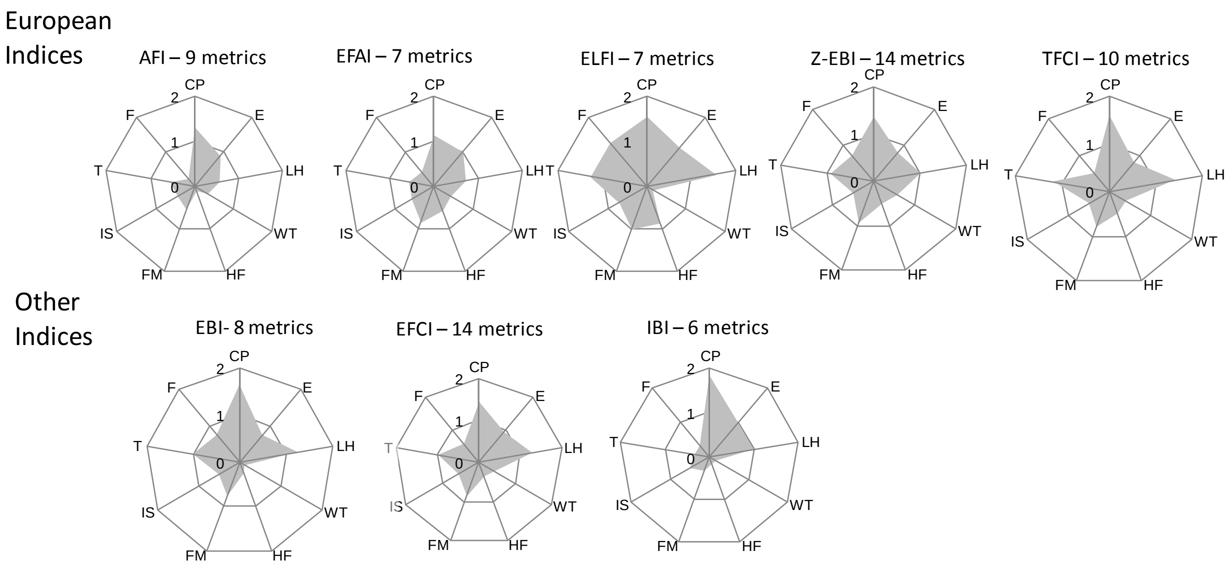


Figure 18. Characteristics of relationships in strength detected by eight indices studied for the 7 pressures considered, i.e. chemical pollution (CP), eutrophication (E), loss of habitat (LH), water turbidity (WT), habitat fragmentation (HF), fish mortalities (FM), invasive species (IS), temperature (T) and flow (F) changes. Axes of the radar plots represent the strength average detected by the metrics for each pressure. Scores range between 0 (no relationship) and 2 (strong strength). Index abbreviations are detailed in the Annex 1.

The time lag in response to human pressures varied for different fish indices (Figure 19). The AFI and EFAI tools respond in a longer time than ELFI and IBI. The more heterogeneous responses according to pressures were shown by AFI, TFCI and EBI. In general, temperature

(T) and river flow (F) changes presented the lowest average time lag of response (i.e. response in a longer time).

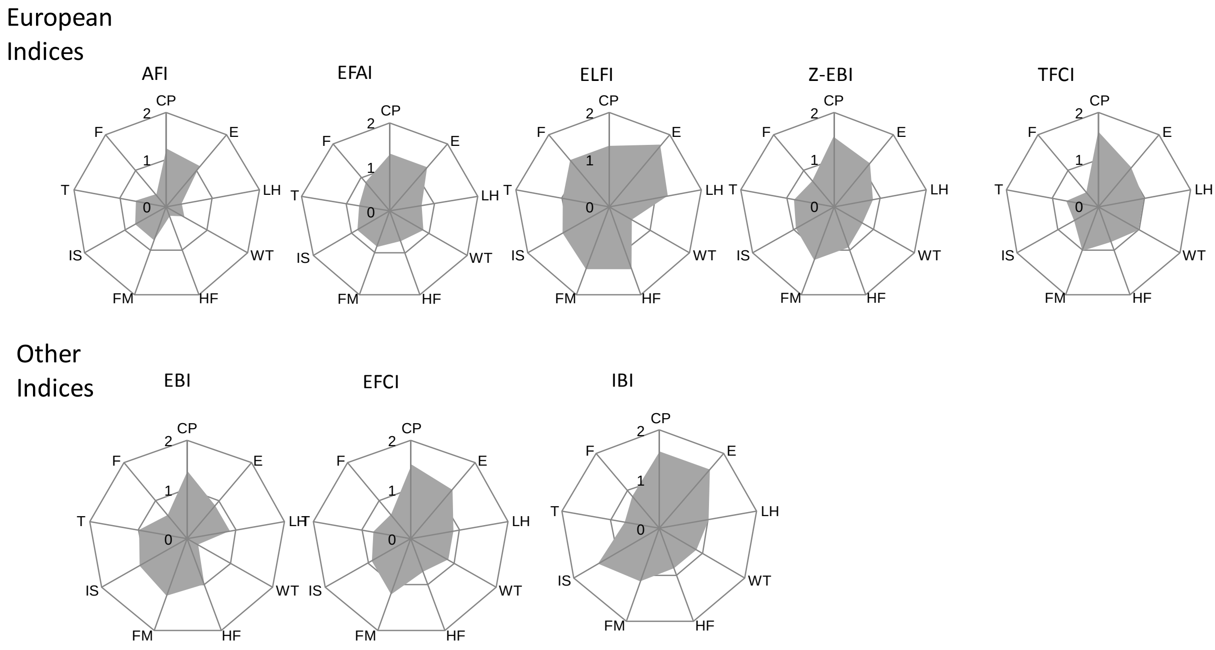


Figure 19. Characteristics of relationships in time lag detected by eight indices studied for the 7 pressures considered, i.e. chemical pollution (CP), eutrophication (E), loss of habitat (LH), water turbidity (WT), habitat fragmentation (HF), fish mortalities (FM), invasive species (IS), temperature (T) and flow (F) changes. Axes of the radar plots represent the average of the time lags detected by the metrics for each pressure. Scores range between 0 (no response) and 2 (response in a short time lag). Index abbreviations are detailed in the Annex 1.

3.4. Sensitivity of ELFI and TFCI indices to the metrics dynamic range

3.4.1. Metric distribution

All ELFI metrics showed a similar frequency distribution in terms of metric scores in the analysed datasets, with the spread of scores being relatively homogenous throughout the range (Figure 20). In contrast, TFCI showed a less homogenous distribution of the metric scores with some metrics strongly skewed towards extreme scores (M1, 2, 7, and 10). In the TFCI indices, not all possible metric scores were represented for metrics M1, 2, 3, 7, and 10 (Figure 20).

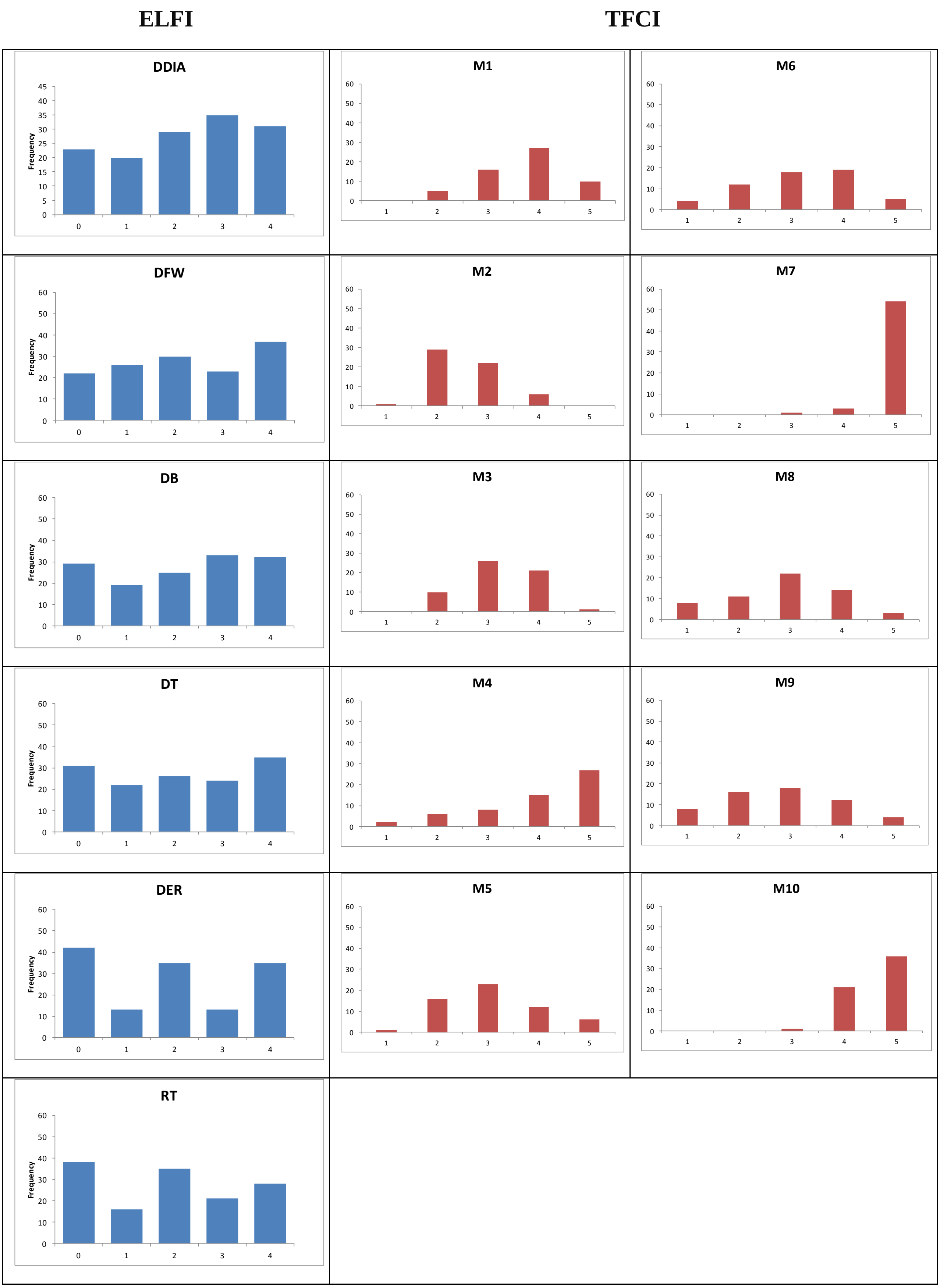


Figure 20. Metric scores frequency distribution for the TFCI and ELFI indices across the investigated datasets.

3.4.2. Metrics correlation

Significant positive correlations were detected between most metrics included in the two indices (Tables 18 and 19). The Spearman rank correlations' parameters were used to apply the four criteria (0 or no change, 0.4, 0.8 and 1 or full value) to the 8 simulated scenarios to understand the index sensitivity to its metrics variability, from the most restrictive cases (when the driving metric was set to values corresponding to the average score in the top and low 10 percentiles) to the most inclusive (top and low 80 percentiles; see section 2.4.3. and Figures 5 and 6).

Table 18. Spearman rank correlation analysis of the ELFI metrics. Values above the diagonal represent the correlation coefficient rho (ρ) and the colour represents the p-level (orange= p<0.05; yellow= p<0.01; white=not significant (p>0.05)). Values below the diagonal indicate the relationship criterion applied (section 2.4.3.) to each pair of metrics based on the strength of their correlation and significance level.

	DDIA	DFW	DB	DT	DER	RT
DDIA	1	0.466	0.511	0.536	0.154	0.580
DFW	0.40	1	0.373	0.539	0.188	0.531
DB	0.40	0.40	1	0.867	0.761	0.700
DT	0.40	0.40	1.00	1	0.671	0.768
DER	0.00	0.00	0.80	0.80	1	0.514
RT	0.40	0.40	0.80	0.80	0.40	1

Table 19. Spearman rank correlation analysis of the TFCI metrics. The table organization is identical as Table 18.

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
M1	1	0.206	0.167	0.399	0.685	0.649	0.372	0.559	0.644	0.445
M2	0.0	1	-0.144	0.346	0.183	0.443	0.184	0.379	0.363	-0.120
M3	0.0	0.0	1	0.030	0.055	0.139	0.147	-0.034	0.125	0.305
M4	0.4	0.4	0.0	1	0.311	0.372	0.271	0.412	0.453	0.161
M5	0.8	0.0	0.0	0.0	1	0.681	0.270	0.683	0.604	0.350
M6	0.8	0.4	0.0	0.4	0.8	1	0.320	0.746	0.628	0.473
M7	0.4	0.0	0.0	0.0	0.0	0.0	1	0.410	0.258	0.267
M8	0.4	0.4	0.0	0.4	0.8	0.8	0.4	1	0.620	0.187
M9	0.8	0.4	0.0	0.4	0.8	0.8	0.0	0.8	1	0.364
M10	0.4	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.4	1

3.4.3. Index response

Tornado diagrams were used to visualise the most influential metric driving changes in the index scores (Figure 21 and 22). For each scenario tested (Top/Low 10%, etc), tornado diagrams show the effect on the index of the different metric changes (identified by the name of the driving metric), from the most influential (top) to the one with smaller influence (bottom) on the index (Figure 21 and 22). The sensitivity of the index to the different metric scenarios can be inferred by the correspondent total range of variability of the index (between top and low percentile), the longer the bars for each percentile range, the stronger the effect of a metric on the index result.

In general the TFCI shows less variation than ELFI to the extreme manipulation of the more influential metrics. TFCI, M2 (presence of indicator species), M3 (species relative abundance) and M10 (feeding guild composition) have little influence while M5 (number of estuarine resident taxa), M6 (number of estuarine-dependent marine taxa), M8 (number of benthic invertebrate feeding taxa) and M9 (number of piscivorous taxa) induce the strongest response, followed by M1 (species composition). Interestingly, M4 (number of taxa that makes 90% of the abundance) appears in the top influential metrics only in the Top/Low 40% scenario. There is greater consistency in the ELFI response, with the metrics rank practically identical across the different scenarios. In particular, the ELFI index shows a high sensitivity to metrics DT (total density), DB (density of benthic species) and RT (total richness), whereas DDIA (density of diadromous species) induces the least amount of change in the index.

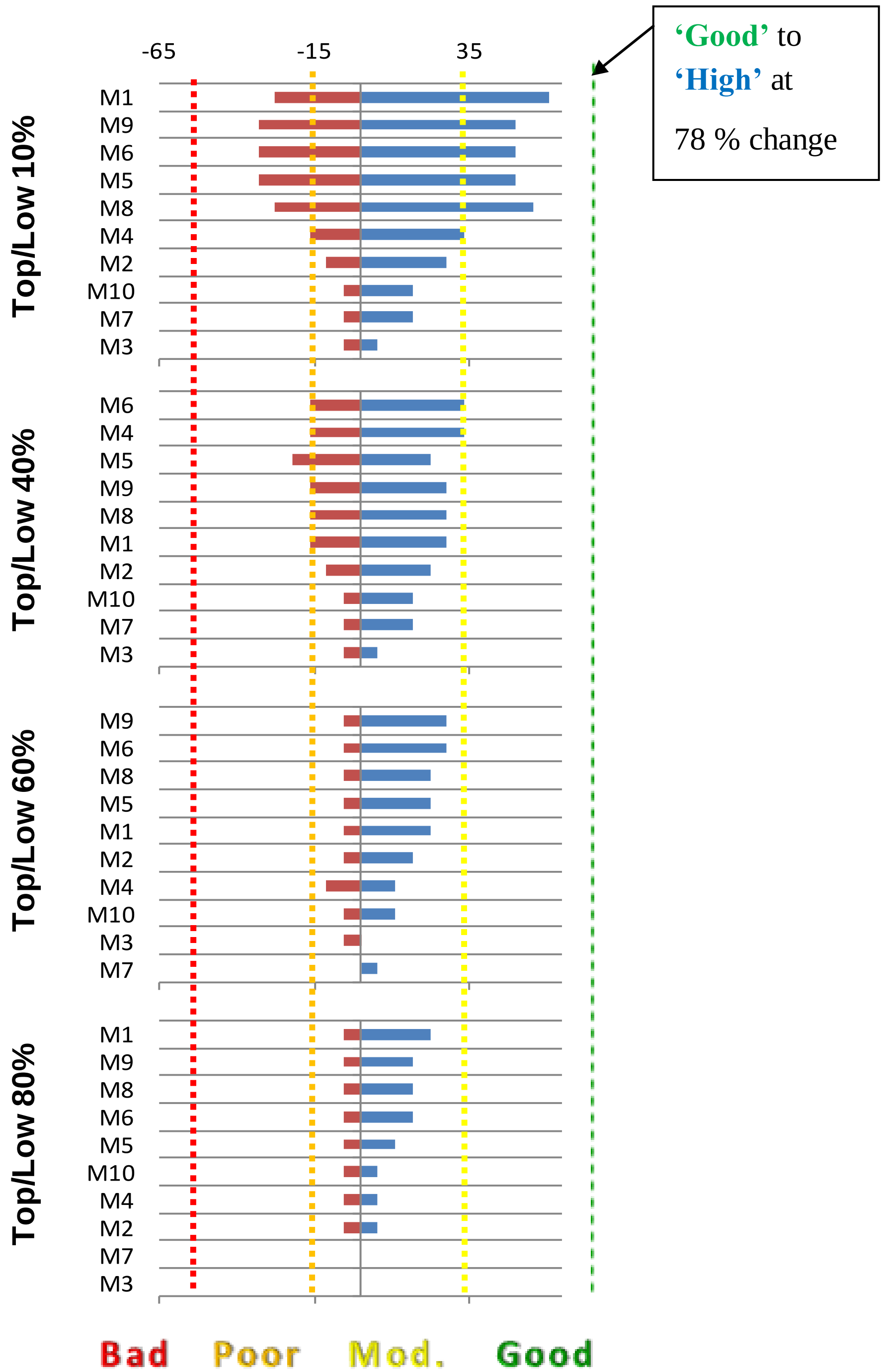


Figure 21 Tornado diagram of the percentage change from the average TFCI value (central axis) under 8 scenarios. The vertical coloured lines indicate the threshold for the different WFD classifications. Threshold for Good to High classification is indicated for reference. Red bars indicate TFCI percent change under the low 10, 40, 60 and 80 percentiles (index impairment) and the blue bars the corresponding top scenario (index improving). The greatest effect is therefore expected for the Top/Low 10% and the lowest for the Top/Low 80%. Top/Low 80% will result in shorter bars closer to the central axis or being absent if no change is detected. A tornado diagram helps to highlight the metrics of larger influence in the index final score and the change required to take an index to a certain classification.

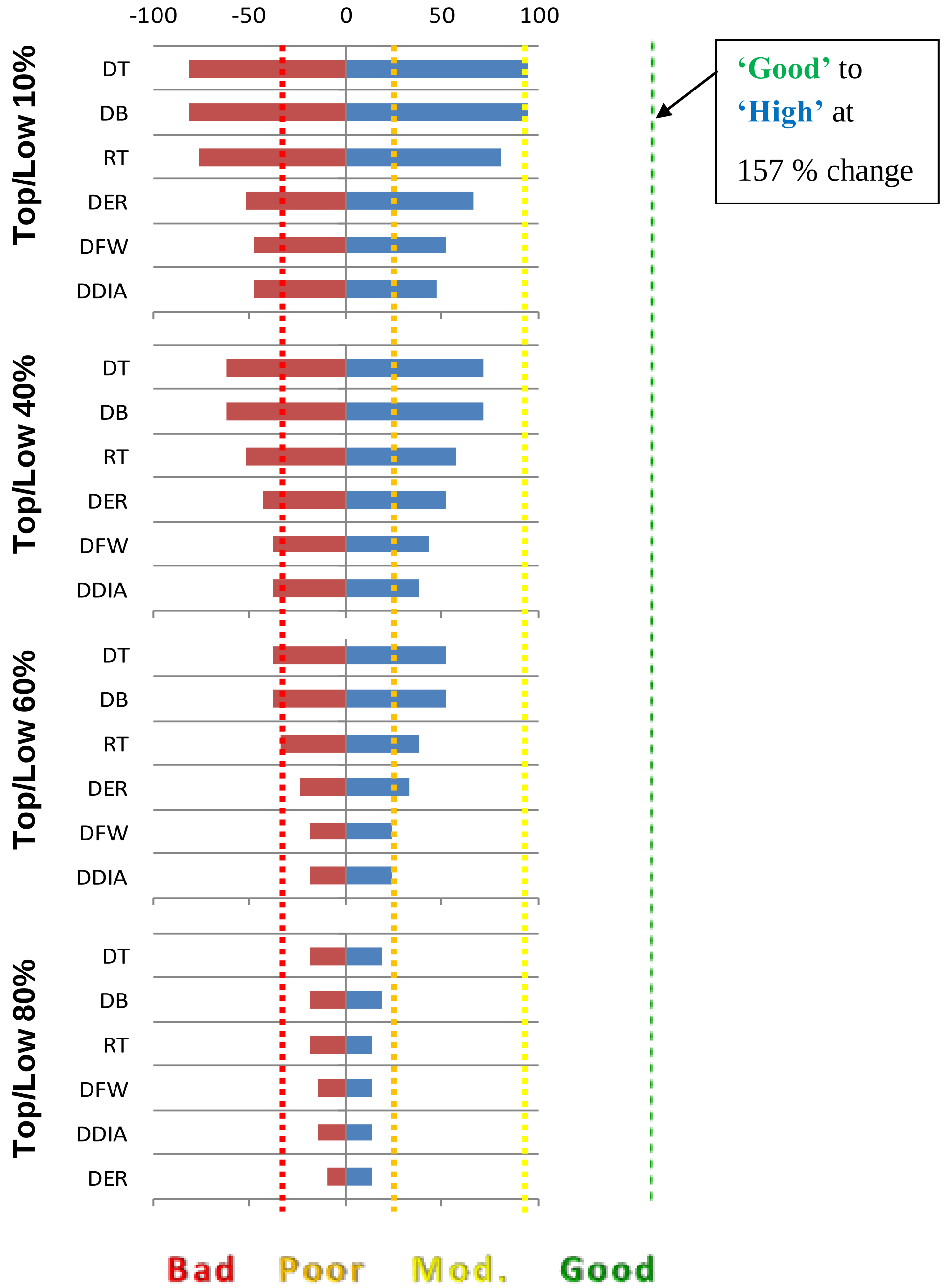


Figure 22. Tornado diagram of the percentage change from the average ELFI value (central axis) under the 8 scenarios. Figure organisation is as figure 21.

Top and low 10 percentiles (best and worst case scenario) for each metric are also presented in radar plots. This is a different way to visually evaluate the sensitivity of the index to changes in its metrics. The larger the separation between top and low 10 percentile for each metric, the higher the influence this metric has in the index result. The TFCI has a more irregular trace suggesting a range of sensitivity to metric extreme values compared to ELFI (Figure 23 and 24). Furthermore, the potential range of the TFCI appears wider with index scores between the Poor and the Good quality classes. In contrast, ELFI scores range from just the Good threshold to Bad.

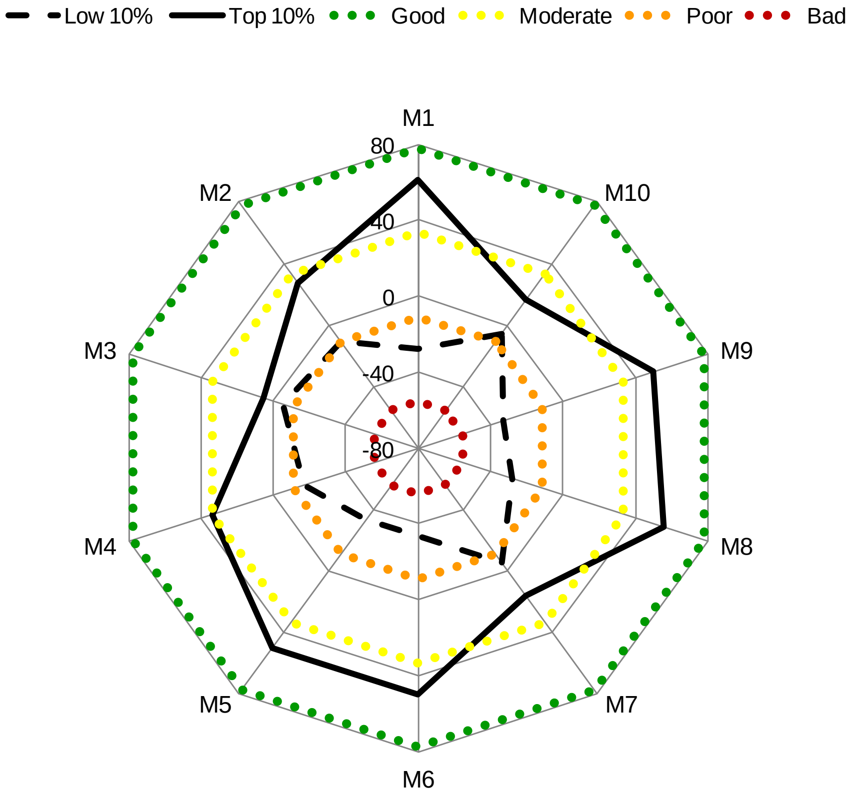


Figure 23. Radar plot showing the % change in the TFCI index value (compared to the value assumed by the index with average metric scores, indicated by 0 in the plot scale) under the two extreme scenarios setting metric values to the top and low 10 percentiles. Lower boundaries for the Good, Moderate, Poor and Bad ecological status classes are indicated.

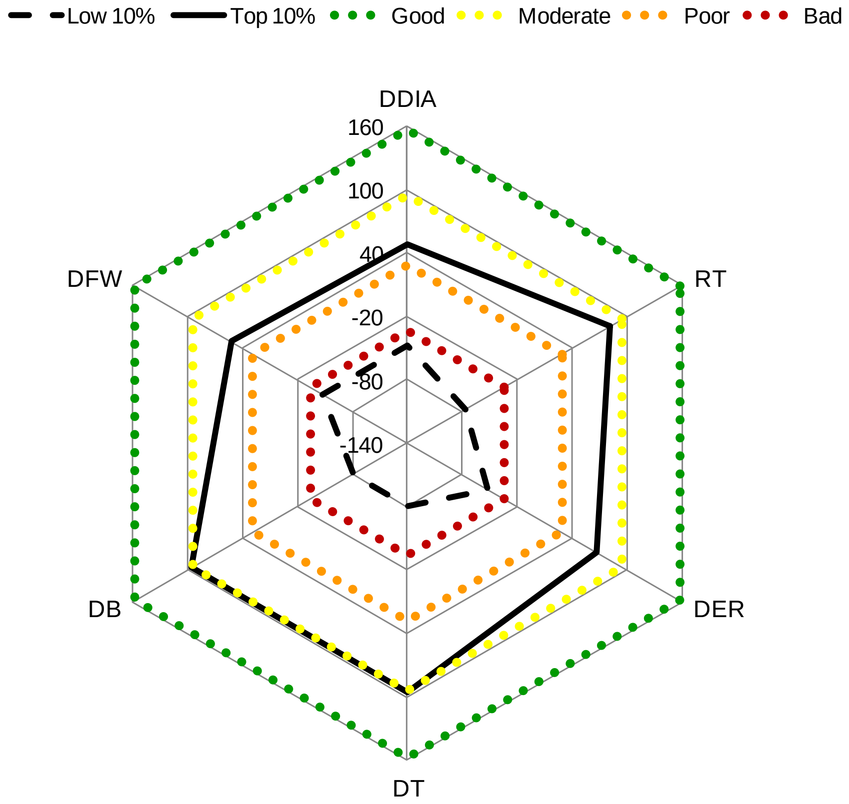


Figure 24. Radar plot showing the % change in the ELFI index value (compared to the value assumed by the index with average metric scores, indicated by 0 in the plot scale) under the two extreme scenarios setting metric values to the top and low 10 percentiles. Lower boundaries for the Good, Moderate, Poor and Bad ecological status classes are indicated.

An indication on the effect of metrics manipulation in the final ecological status assessment obtained with the two studied indices can be also derived from the analysis, by visualising the ecological status classes corresponding to the index values, as shown in Figures 21-24. However, it is of note that the ecological status classification obtained under the simulated scenarios is highly dependent on the analysed dataset and on the resulting classification of the average value of the analysed index. For example, the results show that, on average, the status of the 58 UK transitional water bodies analysed using the TFCI index is classified as Moderate (with the average index score falling in the lower part of this class). Depending on this and on the index sensitivity to the different metrics, an extreme improvement in M1, M9, M5 or M8 (increase to the top 10 percentile average) is required for the index to reach an overall Good status assessment. In turn, a lower improvement (to top 40 percentile average value) is required if M4 or M6 are considered to reach the Good ecological status class. It is interesting to note how a further improvement in M4 (to top percentile average value) does not have a relevant effect on the final index assessment.

In contrast to the TFCI index, the average status of the 68 French transitional water bodies analysed using the ELFI index is classified as Poor (with the average index score falling in the middle of this class). This leads to a lower possibility of improvement of the assessed status to

Good conditions, in spite of the higher sensitivity of this index to metric changes. In fact, a Good ecological status is reached in the studied waterbodies only when metrics DT and DB show extreme high score (top 10 percentile average), whereas no other scenario allows such an improvement. However, asthis is just a theoretical exercise that cannot provide a true indication of change at the level of individual water bodies, the assessment should be taken only to provide a general appraisal on the multimetric index behaviour.

4. Discussion

4.1. Case study: Basque Country

As stated by Whitfield and Elliott (2002), the major physical drivers of ichthyological functioning of the estuaries can be found under geographical and hydrographical categories. The relationship between demersal assemblages and types of estuaries has been studied extensively in other countries (Harrison *et al.*, 2000). From the MDS analysis, it seems that Basque estuaries type III (Nerbioi, Bidasoa, Oiartzun, those with extensive subtidal areas) are separated from the rest (especially Nerbioi). These estuaries have the capacity of supporting stable resident demersal assemblages, with higher richness (see Uriarte and Borja, 2009). Only in the case of Oiartzun, highly affected by pressures, richness is lower. On the other hand, small estuaries (Lea, Barbadún), with extensive intertidal areas, support poorest demersal assemblages, because of their small size (Nicolas *et al.* 2010). From the analysis, it seems that Basque estuaries are very similar to each other, with morphological characteristics, linked to their small size (like in other northern Spain estuaries), which can make difficult for intercalibrating fish quality tools with other large European estuaries. In fact, some analyses at the European level show that small estuaries are separated from the rest (Nicolas *et al.*, 2010). These small estuaries have in general less richness and diversity (Cardoso *et al.*, 2011), as in the Basque estuaries. Given that the diversity of an area is proportionally to, in order of importance, the habitat complexity, the size of volume and the productivity (as long as the latter is not anthropogenic) (Elliott & Hemingway 2002) then these factors need to be assessed.

When studying the variables that best explained the ordination of estuaries, according to fish data, these can be divided into: (i) pressure variables (water pollution index) and (ii) hydromorphological variables (percentage of subtidal surface, flushing time and catchment area). In turn, when including fish and crustaceans the variables are a bit different: (i) pressure variables (water pollution index and total pressure index), and (ii) hydromorphological variables (continental shelf width, flushing time, and catchment area). Hence, the variability of demersal fauna within the Basque estuaries are explained mainly by the size of the catchment and flushing time together with the quality of the environment in which they live. In fact, the variable which explains a higher part of the variability is the water pollution index (the percentage of samples not accomplishing with the environmental quality standards, for priority substances). The influence of pollutants on fish assemblages has been investigated in different countries (Cabral *et al.*, 2001; Whitfield and Elliott, 2002; Courrat *et al.* 2009; Delpech *et al.*, 2010). Normally, river flow largely determines the abundance of fish species in some estuaries (Martinho *et al.*, 2007). Although in our case river flow is not a significant variable explaining the demersal assemblages, this is, at a certain extent, related to the flushing time. In a European scale analysis (135 estuaries), Nicolas *et al.* (2010b) studied the factors explaining patterns of species richness at different scales from local habitat to regional features. They found that the estuarine system size, the entrance width, and also the continental shelf width were identified as the best explanatory variables of estuarine fish species richness at a large scale. Some of these variables have been identified also at small scale in the Basque estuaries.

It is of note that the regression of AFI and pressures is significant only in autumn and not in spring and summer. AFI was designed to be applied using autumn data (Borja *et al.*, 2004), in order to get the fishing period with a stable presence of fishes. Hence, it seems that the application of this index to data from other seasons of the year could be problematic. For other indices (e.g. in Belgium), no significant differences in metric values between the different seasons for the assessed sites were found (Breine *et al.*, 2007). In turn, Martinho *et al.* (2008), applying different indices to a long-term series, found differences among them in the response to seasonal changes.

When calculating the multiple regression between AFI and different pressure indicators and morphological variables, it can be seen that (i) the deeper the estuary, and (ii) the lower the residence time, the pressure index and the channelled ports within the estuary, the higher the AFI values (hence, higher ecological quality). Deeper estuaries will have more vertical niches and so more species; more volume and so more species; and better migration routes and therefore more species. These variables are interesting, since deep estuaries (type III in the Basque typology) can support more resident species and stable populations (in fact, deeper estuaries, such as Nerbioi, show the highest richness in the Basque Country (Uriarte and Borja, 2009)). The same pattern has been described in Portuguese estuaries (França *et al.*, 2009). Another hydromorphological variable, such as the residence time, is related to the capacity of the system to retain pollutants, driving also the levels of dissolved oxygen, which are important for fishes, as demonstrated in the Basque estuaries (Uriarte and Borja, 2009) and others (Jones, 2006). It is clear that the number of pressures must be related to an index measuring the ecological quality, which is the core of the WFD, as detected also in other indices, such as in France (Delpech *et al.*, 2010). In this way, the percentage of the estuary channelled due to the presence of a port is clearly a morphological pressure to which the AFI responds. In this way, channel morphology and habitat niche requirements and niche availability are known to influence fish communities (Hemingway and Elliott, 2002; Coates *et al.*, 2007). However, it is interesting to note that the multiple regression shows also that the more volume dredged and the more channelling out of ports, the higher the AFI values (high quality).This may be a spurious correlation, as dredging is only important in some parts of the deeper estuaries, maybe there is some co-linearity between both variables.

4.2. Case study: Metrics and EFAI response against anthropogenic pressure in Portuguese estuaries

The importance of estuarine areas was sufficiently highlighted by many authors, either because they internally constitute suitable areas as fish nurseries or due to the high importance they have in supporting the offshore stocks of economically valuable species (Marchand, 1980; Costa and Bruxelas, 1989; Blaber *et al.*, 2000; Beck *et al.*, 2001; Gillanders *et al.*, 2003; Able, 2005; Vasconcelos *et al.*, 2007). Despite their ecological importance, estuaries are amongst the most threatened aquatic environments (Blaber *et al.*, 2000) and, as many other coastal regions, they are under severe pressure. Since a long time now, human populations tend to occupy the estuarine surroundings, where the rapid population growth and the uncontrolled development of human activities represent the most serious concerns to the area. Many of the most representative pressures acting into estuarine areas, that endanger the sustainability and health of these habitats (Goldberg, 1995; Costa *et al.*, 2002a; Kennish, 2002), have an human origin and, historically, fisheries have been considered as the most threatening anthropogenic factor impacting estuarine fish populations (Boreman, 1997; Johnson *et al.*, 1998).

Bearing in mind the above mentioned and the WFD demand which outlines that Member States must collect information on the type and magnitude of significant anthropogenic pressures, and to prove if there is a significant relationship between these and the assessment results (EQR), several approaches have been tried. It has been increasing the concern about the role the more traditionally considered anthropogenic factors (e.g., the increase of urban areas, agriculture, industry, general discharges and intensive fishing pressure) play in the decline of commercially and recreationally important marine fish (Haedrich, 1983; Grosse *et al.*, 1997), but also, more recently, the importance of habitat loss and resources use have as greater problems than pollution itself (Cattrijsse *et al.*, 2002; Kennish, 2002).

Evidences from this study confirmed the importance of chemical pollution on estuarine fish populations, and a less clear situation for the habitat loss and the resources use change. The fish community−based metrics responded well to the environmental chemical quality (G2, Figures 13 and 14), where higher chemical contamination resulted in higher presence (% and density) of estuarine residents (ER), benthic invertebrates feeders (BIF) and benthic species. A reduction on species richness (S), Pielou (J), Simpson (D) and Shannon-Wiener (H) indices, as well as for the marine migrants (% and density), piscivorous (% and density), and the number of ecological guilds (EG) and trophic guilds (TG) presence was observed with the chemical quality degradation. On the other hand, the response of fish community−based metrics against the pressure groups G1 (high human use and physical alterations) and G3 (agriculture, lower urban pressure and physical alterations) was not distinct. These results might be due to (1) low site pressure, not sufficiently high to cause detectable response on the fish community, or (2) the metrics are unable to respond or have low sensitivity to these pressure groups.

The existence of a low pressure level along most of Portuguese estuaries is a very plausible situation. Although their importance has not yet been comprehensively assessed, these systems are well known as nursery areas for several commercially important fish species (Cabral and Costa, 2001; Erzini *et al.*, 2002; Martinho, 2005). Some have been studied for several years (Costa and Cabral, 1999) while others have seldom been studied even in terms of their fish assemblages (Bettencourt and Ramos, 2003). This explanation is also sound when the results from the study concerning the sensitivity of metrics and indices to the cause-effect relationship strength and the time-lag in response to human pressures (this deliverable) are considered. There, chemical pollution and loss of habitat pressures are shown as highly detectable by most of metrics and indices, which supports also the idea of a low pressure condition for Portuguese estuaries. A different explanation exists for the lack of response of some metrics, such as the low number of diadromous captured, which can be considered likely related to the low efficiency of the used sampling gear (beam trawl) in relation to that type of fishes.

For the EFAI, although the fish metrics responded to pressure, sustaining the relationship between EFAI and pressure level, the pressure levels used in this study were apparently low, and didn't allow for a clear understanding of the behaviour of the tool for higher degradation levels. In future, sites covering the full pressure gradient (scale of Aubry & Elliott, 2006) and different specific pressures acting in different estuarine typologies should also be considered to include in new perspectives of work. This is considerably important when the results achieved here may be in agreement with findings from other studies (Vascolcelos *et al.*, 2007) where concluded that different pressures may have different impacts in different estuarine typologies. The fish community, and so the vulnerability of the different systems, depends on the intrinsic characteristics of the estuary (e.g., depth, width of entrance) (Uriarte and Borja, 2009; Nicolas *et al.*, 2010b), where the same environmental/anthropogenic pressure condition may represent a different weight into the systems' balance. Identifying pressure sources and recognizing correctly the respective impacts in estuarine fish communities allow classify potential damages, to predict the effects and consequences, and to help on the elaboration of mitigation plans (Vasconcelos *et al.*, 2007).

4.3. Sensitivity of metrics and indices to the cause-effect relationship strength and the time lag in response to human pressures

The cause-effect relationship revealed differences in strength and time lag for both the metrics and indices (tools) here considered.

Metrics: A lot of metrics detect chemical pollution and loss of habitat with strong intensities. The other pressures were more difficult to detect (few metrics responding with a high strength). A high volume of information concerning these pressures and different significant effects on fish (Elliott and Taylor, 1989, McLusky *et al.*, 1992, Gibson, 1994, Johnson *et al.*, 1998, Miliou *et al.*, 1998, Able *et al.*, 1999, Duffy-Anderson and Able, 1999, Robertson, 2000, Hansen *et al.*, 2002, Power and Attrill, 2003, Le Pape *et al.*, 2004, Colclough *et al.*, 2005, Gilliers *et al.*, 2006, Lotze *et al.*, 2006, Cachot *et al.*, 2007, Le Pape *et al.*, 2007, Rochette *et al.*, 2010, Kostecki *et al.*, 2011), is probably the reason why they are commonly used to describe anthropogenic disturbances in estuaries (e.g. Uriarte and Borja, 2009). On the other hand, pressures related to temperature and flow changes were not detected with a strong strength and in a short time lag. To be effective, metrics, and thus indices, are expected to have a low sensitivity to natural environmental variation (EPA, 2000). However, the present work emphasized the difficulty to assess the effects of anthropogenic impacts on temperature and flow changes in a context of high natural variability and long-term climate change.

As a trend, the "generalist" metrics (often selected in the fish-based indices) are apparently good metrics to reflect the global ecological quality of a system and detect most of the pressures. Some of these metrics (e.g., species richness, total abundance, marine migratory and diadromous abundances) provide good responses of cause-effect relationships in strength and time lag with most of the pressures. In opposition to that, this study reveals that few metrics are "specific" ones (detecting few pressures with a good response in strength and in time lag) and show difficulties to individualize conveniently a pressure effect.

Indices: For indices, the variability observed in the detection of pressures in strength and in time lag was probably induced by the methodologies used for the fish-based index construction, as the use of unbalanced combination rules or as a bias occurred during the process of selecting the metrics to incorporate into fish assessment tools. It is frequent to have pressures such as chemical pollution and loss of habitat as proxies of anthropogenic disturbances helping to select metrics to integrate on fish-based indices (e.g. EFI index; Delpech *et al.*, 2010) which leads to that metrics less related to those pressures are less frequently included into fish tools.

Because the metrics' combination rules, often the sum (i.e. AFI, EFAI, Z-EBI, TFCI, EBI, EFCI, IBI; Deegan *et al.*, 1997, Meng *et al.*, 2002, Borja *et al.*, 2004, Harrison and Whitfield, 2004, Breine *et al.*, 2007, Coates *et al.*, 2007, Breine *et al.*, 2010, Delpech *et al.*, 2010, Cabral *et al.*, In Press) or the average(i.e. ELFI; Breine *et al.*, 2010, Delpech *et al.*, 2010), did not take into account for the strength and the time lag of the cause-effect relationships of the selected metrics on the rule's generation, indices gave more importance to some pressures. Other methodological approaches could be developed to overcome this problem, namely metric weighting procedures.

4.4. Sensitivity of ELFI and TFCI indices to the metrics dynamic range

Sensitivity analysis in this context is the systematic test of the effect of each metric change on the index score by setting the metric score to values higher or lower than its average (under different scenarios of change). The analysis also considered linked metrics by changing the value for these other metrics according to their correlation with the metric driving the scenario. The results of this relatively simple mathematical exercise were expressed as tornado diagrams and radar plots which are easy to explain to non specialists. By identifying the most influential metrics determining the index response, the results of this analysis may be used to guide the implementation of management / conservation plans e.g. by prioritising the metrics to be restored / improved to increase the overall ecological status as assessed by the studied fish indices.

The analysis indicated that the TFCI index is especially sensitive to M1 (species composition), M4 (number of taxa that makes 90% of the abundance), M5 (number of estuarine resident taxa), M6 (number of estuarine-resident marine taxa), M8 (number of benthic invertebrate feeding taxa) and M9 (number of piscivorous taxa), whereas the ELFI index showed a higher sensitivity (under all scenarios) to metrics DT (total fish density), DB (density of benthic species) and RT (total species richness). These results suggest that these metrics should be prioritised within management plans aimed at improving the ecological status of transitional water bodies assessed using the two fish-based tools.

Considering the UK WBs included in the analysis, the results for the TFCI index also showed that the minimal changes required to bring the overall WB classification to *Good* will be obtained by increasing M4 (number of taxa that makes 90% of the abundance) and M6 (number of estuarine-resident marine taxa) to the average of the top 40 percentile of the sample population. This has been estimated that an increase in score value of 5 for M4 and 4 for M6 are required from both their current average value scores of 3. It is of note that efforts to improve further M4 (e.g. to top 10 percentile average) would be worthless, given that they would not lead to any relevant additional improvement in the final status assessment. This information can be used to provide immediate targets for management purpose that are, probably, realistically achievable but can also be used to set new and more ambitious management goals when conditions improve.

With regards the ELFI index and the French WBs used in the analysis, the results indicate that a higher effort (hence higher costs) would be required to reach a *Good* status, with metric improvement only being effective when increasing total density of species and specifically the density of benthic species (metrics DT and DB) to their highest possible values (at least to top 10 percentile average value). In other words, the scores of both these metrics need to improve from their average score values of 1.5 and 2 respectively to a score of 4. For this exercise, we used datasets of the countries which the indices were developed for, and it is of note that the results of our analyses are highly dependent on the distribution of the metrics and index scores in the range defined by the data set used. For example, tornado diagrams for the TFCI resulted in a stronger effect towards improvements (top percentiles) compared to equivalent worsening (low percentiles) of metric scores. This is due to a skewed distribution of several metric scores throughout the data set. Nevertheless, this behaviour of the metrics is determined by the actual scores recorded and represents a realistic appraisal of the metrics. It can also be concluded that given the sample size, we expect to have a range of quality scores including the best and worst scenarios. Furthermore, since the indices have a proven response to human pressures, the observed metric distribution scores could be assumed to reflect the dynamic range expected from the fish tool under human pressure gradients. If this is the case, we could use the boundaries of the different scenarios to set realistic targets and also to identify the aspects of the indices that are more likely to affect the outcomes leading to more robust and responsive indices. Further work is necessary to take these aspects into account for new and existing fish indices.

The analysis using two of the currently available fish indices has offered a means of comparing the behaviour of both indices in the classification of WBs. The average classification of WBs by the French ELFI index falls in the *Poor* boundary while the British TFCI average classification falls in the *Moderate* boundary. This indicates that remediation processes to bring WBs to the desired *Good* status will need to be stronger in France than in Britain, assuming the classification obtained by these indices is comparable. This emphasises the importance of the intercalibration exercise currently being undertaken by the EU Member States to support the implementation of the WFD (2000/60/EC).

5. Conclusions

Fish metrics, AFI and EFAI responded to anthropogenic pressures in different Atlantic estuarine sites, yet at the individual metrics level environmental chemical quality was the main driver for observed differences. Also, some metrics did not respond to pressures as expected, which is most likely related to sampling gear efficiency, namely the low capture efficiency of diadromous species with beam trawl. Overall, the individual fish metrics that compose EFAI responded to pressures, sustaining the relationship between EFAI and pressure indices.

The cause-effect relationship study emphasized that fish-based indices developed to assess the water quality of estuarine systems did not detect all the pressures with the same sensitivity in terms of strength and time-lag, and gave more importance to some pressures, namely chemical pollution. The fish-based indices developed to assess the water quality of estuarine systems do not allow the individualization of pressure effects, which may constitute a problem to put forward the correct specific measures for management and rehabilitation of estuaries. On the other hand, some indices also do not seem relevant, in a short time, to detect changes of the ecological quality which may constitute a handicap for management or an indication for their restructuring.

Sensitivity analysis using tornado and radar plots has proven to be a relatively simple way of evaluating the effect of the different composing metrics on the outcome of the index. Furthermore, it has been easy to understand by non-specialists and is a very simple way to answer the 'what if' question that water managers are trying to derive when deciding management options. It helps highlight the metrics on which the restoration efforts will produce the most desirable effects.

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References

Able, K.W., Manderson, J.P., Studholme, A.L., 1999. Habitat quality for shallow water fishes in an urban estuary: the effects of man-made structures on growth. Mar. Ecol. Prog. Ser. 187, 227-235.

Able KW, 2005. A re-examination of fish estuarine dependence: evidence forconnectivity between estuarine and ocean habitats. *Estuarine, Coastal & Shelf Science*, 64: 5–17.

Aubry, A., M. Elliott, 2006. The use of environmental integrative indicators to assess seabed disturbance in estuaries and coasts: Application to the Humber Estuary, UK. *Marine Pollution Bulletin,* 53: 175-185.

Beck MW, Heck KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, *et al.*, 2005. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience*;51:633–41.

Bettencourt A, Ramos L., 2003. Estuários Portugueses. Instituto da Água; 317 pp.

Blaber SJM, Cyrus DP, Albaret JJ, Ching CV, Day JW, Elliott M, *et al.*, 2000. Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. *ICES J Mar*, 57: 590–602.

Blocksom, K., Emery, E., Thomas, J., 2009. Sampling effort needed to estimate condition and species richness in the Ohio river, USA. Environ. Monit. Assess. 155, 157-167.

Boreman J., 1997. Methods for comparing the impacts of pollution and fishing on fish populations. Trans Am Fish Soc,126 :506–13.

Borja Á, Franco J, Valencia V, Bald J, Muxika I, Jesús Belzunce M acute accent]a, Solaun O (2004) Implementation of the European water framework directive from the Basque country (northern Spain): a methodological approach. Marine Pollution Bulletin 48: 209-218.

Borja, A., I. Galparsoro, O. Solaun, I. Muxika, E. M. Tello, A. Uriarte, V. Valencia, 2006. The European Water Framework Directive and the DPSIR, a methodological approach to assess the risk of failing to achieve good ecological status. Estuarine, Coastal and Shelf Science, 66: 84-96.

Breine, J. J., J. Maes, P. Quataert, E. van den Berg, I. Simoens, G. van Thuyne, C. Belpaire, 2007. A fish-based assessment tool for the ecological quality of the brackish Schelde estuary in Flanders (Belgium). Hydrobiologia, 575: 141-159.

Cabral H, Costa MJ. Abundance, feeding ecology and growth of 0-group sea bass, Dicentrarchus labrax, within the nursery areas of the Tagus estuary. J Mar Biol Assoc UK 2001;81:679–82.

Cabral, H. N., M. J. Costa, J. P. Salgado, 2001. Does the Tagus estuary fish community reflect environmental changes? Climate Research, 18: 119-126.

Cabral, H.N., *et al.*, Ecological quality assessment of transitional waters based on fish assemblages in Portuguese estuaries: The Estuarine Fish Assessment Index (EFAI). Ecol. Indicat. (2011), doi:10.1016/j.ecolind.2011.08.005

Cachot, J., Law, M., Pottier, D., Peluhet, L., Norris, M., Budzinski, H., Winn, R., 2007. Characterization of toxic effects of sediment-associated organic pollutants using the lambda transgenic medaka. Environmental Science & Technology 41, 7830-7836.

Cardoso, I., S. França, M. P. Pais, S. Henriques, L. Cancela da Fonseca, H. N. Cabral, 2011. Fish assemblages of small estuaries of the Portuguese coast: A functional approach. Estuarine, Coastal and Shelf Science, 93: 40-46.

Cattrijsse A, Codling I, Conides A, Duhamel S, Gibson RN, Hostens K, *et al.* Estuarine development/habitat restoration and re-creation and their role in estuarine management for the benefit of aquatic resources. In: Elliott M, Hemingway KL, editors. Fishes in estuaries. Blackwell Science Ltd; 2002. p. 266–321.

Coates, S., A. Waugh, A. Anwar, M. Robson, 2007. Efficacy of a multimetric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. Marine Pollution Bulletin, 55: 225-240.

Colclough, S., Fonseca, L., Astley, T., Thomas, K., Watts, W., 2005. Fish utilisation of managed realignments. Fisheries Manag. Ecol. 12, 351-360.

Costa MJ, Bruxelas A. The structure of fish communities in the Tagus Estuary, Portugal, and its role as a nursery for commercial fish species. Sci Mar 1989;53(2–3):561–6.

Costa MJ, Cabral HN. Changes in the Tagus nursery function for commercial fish species: some perspectives for management. Aquat Ecol 1999;33:287–92.
Costa MJ, Cabral HN, Drake P, Economou AN, Fernandez-Delgado C, Gordo L, *et al.* Recruitment and production of commercial species in estuaries. In: Elliott M, Henderson PA, editors. Fishes in estuaries. Blackwella; 2002a. p. 54–123.
Courrat A, Lobry J, Nicolas D, Laffargue P, Amara R, Lepage M, Girardin M, Le Pape O (2009) Anthropogenic disturbance on nursery function of estuarine areas for marine species. Estuarine, Coastal and Shelf Science 81: 179-190. doi:10.1016/j.ecss.2008.10.017
Delpech, C., A. Courrat, S. Pasquaud, J. Lobry, O. Le Pape, D. Nicolas, P. Boët, M. Girardin, M. Lepage, 2010. Development of a fish-based index to assess the ecological quality of transitional waters: The case of French estuaries. Marine Pollution Bulletin, 60: 908-918.
Duffy-Anderson, J.T., Able, K.W., 1999. Effects of municipal piers on the growth of juvenile fishes in the Hudson River estuary: a study across a pier edge. Mar. Biol. 133, 409-418.
Elliott, M., Taylor, C.J.L., 1989. The structure and functioning of an estuarine/marine fish community in the Forth estuary, Scotland, in: Klekowski, R.Z., Styczynska, E., Falkowski, L. (Ed.)^(Eds.), Proceedings of the 21st European Marine Biology Symposium, ed. Polish Academy of Sciences, Gdansk, p.^pp. 227-240.
Elliott, M, Hemingway, K., 2002. Fishes in Estuaries. Blackwell Science Ltd., Oxford.
EPA, 2000. Evaluation Guidelines for Ecological indicators. United States Environmental Protection Agency, EPA-620-R-99-005. Washington, 45 pp.
Erzini K, Bentes L, Coelho R, Correia C, Lino PG, Monteiro P, *et al.* Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (Southern Portugal). Technical Report, Commission of the European Communities DG XIV/C/1. Universidade do Algarve; 2002. 178 pp.
European Council (2000) Directive 2000/60/EC of the European Parliament and of theCouncil of 23 October 2000 establishing a framework for Community action in thefield of water policy. Official Journal of the European Communities: L327/1-72.
França, S., M. J. Costa, H. N. Cabral, 2009. Assessing habitat specific fish assemblages in estuaries along the Portuguese coast. Estuarine, Coastal and Shelf Science, 83: 1-12.
Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. Neth. J. Sea Res. 32, 191-206.
Gillanders BM, Able KW, Brown JA, Eggleston DB, Sheridan PF. Evidence of connectivity between juvenile and adult habitats for mobile marine fauna: an important component of nurseries. Mar Ecol Prog Ser 2003;247:281–95
Gilliers, C., Le Pape, O., Desaunay, Y., Morin, J., Guerault, D., Amara, R., 2006. Are growth and density quantitative indicators of essential fish habitat quality? An application to the common sole Solea solea nursery grounds. Estuarine Coastal and Shelf Science 69, 96-106.
Goldberg ED. Emerging problems in the coastal zone for the twenty-first century. Mar Pollut Bull 1995;31:152–8.
Grosse DJ, Scholz PM, Hirshfield MF, Meaburn GM, Fletcher M. Fisheries and pollution: a conference overview. Trans Am Fish Soc 1997;126:504–5.
Haedrich RL. Estuarine fishes. In: Ketchum B, editor. Ecosystems of the world. Estuarine and Enclosed SeasAmsterdam: Elsevier; 1983. p. 183–207
Hansen, J.A., Welsh, P.G., Lipton, J., Suedkamp, M.J., 2002. The effects of long-term cadmium exposure on the growth and survival of juvenile bull trout (Salvelinus confluentus). Aquat. Toxicol. 58, 165-174.
Harrison, T. D., J. A. G. Cooper, A. E. L. Ramm, 2000. Geomorphology, ichthyofauna, water quality and aesthetics of South African estuaries. Department of Environmental Affairs & Tourism, Pretoria, South Africa: 228.

Jennings, M.J., Fore, L.S., Karr, J.R., 1995. Biological monitoring of fish assemblages in Tennessee Valley reservoirs. Regul. Rivers: Res. Manage. 11, 263-274.

Johnson, L.L., Landahl, J.T., Kubin, L.A., Horness, B.H., Myers, M.S., Collier, T.K., Stein, J.E., 1998. Assessing the effects of anthropogenic stressors on Puget Sound flatfish populations. J. Sea Res. 39, 125-137.

Jones, P. D., 2006. Water quality and fisheries in the Mersey estuary, England: A historical perspective. Marine Pollution Bulletin, 53: 144-154.

Karr JR (1981) Assessment of biotic integrity using fish communities. Fisheries 6: 21-27.

Kennish MJ. Environmental threats and environmental future of estuaries. Environ Conserv 2002;29:78–107.

Kostecki, C., Rochette, S., Girardin, R., Blanchard, M., Desroy, N., Le Pape, O., 2011. Reduction of flatfish habitat as a consequence of the proliferation of an invasive mollusc. Estuarine Coastal and Shelf Science 92, 154-160.

Le Pape, O., Gilliers, C., Riou, P., Morin, J., Amara, R., Désaunay, Y., 2007. Convergent signs of degradation in both the capacity and the quality of an essential fish habitat: state of the Seine estuary (France) flatfish nurseries. Hydrobiologia 588, 225-229.

Le Pape, O., Guerault, D., Desaunay, Y., 2004. Effect of an invasive mollusc, American slipper limpet Crepidula fornicata, on habitat suitability for juvenile common sole Solea solea in the Bay of Biscay. Mar. Ecol. Prog. Ser. 277, 107-115.

Liefferinge, C., Simoens, I., Vogt, C., Cox, T.J.S., Breine, J., Ercken, D., Goethals, P., Belpaire, C., Meire, P., 2010. Impact of habitat diversity on the sampling effort required for the assessment of river fish communities and IBI. Hydrobiologia 644, 169-183.

Long ER, Macdonald DD, Smith SL, Calder FD. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ Manag 1995;19:81–97.

Lotze, H.K., Lenihan, H.S., Bourque, B.J., Bradbury, R.H., Cooke, R.G., Kay, M.C., Kidwell, S.M., Kirby, M.X., Peterson, C.H., Jackson, J.B.C., 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312, 1806-1809.

Marchand J. Seasonality abundance and diversity of the ichtyofauna of the lower Loire Estuary in 1977–1978 (France). Ann Inst Oceanogr Paris 1980;56:127–37.

Martinho F. O estuário do Mondego como viveiro para a ictiofauna: ecologia dos juvenis de Dicentrarchus labrax, Platichthys flesus e Solea solea. MSc Thesis, Universidade de Coimbra, Faculdade de Ciências e Tecnologia, 2005, 66 pp.

Martinho, F., R. Leitao, I. Viegas, M. Dolbeth, J. M. Neto, H. N. Cabral, M. A. Pardal, 2007. The influence of an extreme drought event in the fish community of a southern Europe temperate estuary. Estuarine, Coastal and Shelf Science, 75: 537-546.

Martinho, F., I. Viegas, M. Dolbeth, R. Leitão, H. N. Cabral, M. A. Pardal, 2008. Assessing estuarine environmental quality using fish-based indices: Performance evaluation under climatic instability. Marine Pollution Bulletin, 56: 1834-1843.

McLusky, D.S., Bryant, D.M., Elliott, M., 1992. The impact of land-claim on macrobenthos, fish and shorebirds on the Forth Estuary, Eastern Scotland. Aquat. Conserv.-Mar. Freshw. Ecosyst. 2, 211-222.

Miliou, H., Zaboukas, N., Moraitou-Apostolopoulou, M., 1998. Biochemical composition, growth, and survival of the guppy, Poecilia reticulata, during chronic sublethal exposure to cadmium. Arch. Environ. Contam. Toxicol. 35, 58-63.

Nicolas, D., J. Lobry, O. Le Pape, P. Boët, 2010a. Functional diversity in European estuaries: relating the composition of fish assemblages to the abiotic environment. Estuarine, Coastal and Shelf Science, 88: 329-338.

Nicolas, D., J. Lobry, M. Lepage, B. Sautour, O. Le Pape, H. Cabral, A. Uriarte, P. Boët, 2010b. Fish under influence: A macroecological analysis of relations between fish species richness and environmental gradients among European tidal estuaries. Estuarine, Coastal and Shelf Science, 86: 137-147.

Power, M., Attrill, M.J., 2003. Long-term trends in the estuarine abundance of Nilsson's pipefish (Syngnathus rostellatus Nilsson). Estuar. Coast. Shelf Sci. 57, 325-333.

Robertson, A.I., 2000. The gaps between ecosystem ecology and industrial agriculture. ecosystems 3, 413-418.

Rochette, S., Rivot, E., Morin, J., Mackinson, S., Riou, P., Le Pape, O., 2010. Effect of nursery habitat degradation on flatfish population: Application to Solea solea in the Eastern Channel (Western Europe). J. Sea Res. 64, 34-44.

Smith, K.L., Jones, M.L., 2005. Watershed-level sampling effort requirements for determining riverine fish species composition. Can. J. Fish. Aquat. Sci. 62, 1580-1588.

Uriarte A, Borja A (2009) Assessing fish quality status in transitional waters, within the European Water Framework Directive: Setting boundary classes and responding to anthropogenic pressures. Estuarine, Coastal and Shelf Science 82: 214-224.

Vasconcelos R.P., Reis-Santos P., Fonseca V., Maia A., Ruano M., França S., Vinagre C., Costa M.J., Cabral H. Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: A multi-metric index and conceptual approach. Science of the Total Environment 374 (2007) 199–215. doi:10.1016/j.scitotenv.2006.12.048.

Whitfield, A. K., M. Elliott, 2002. Fishes as indicators of environmental and ecological changes within estuaries: a review of progress and some suggestions for the future. Journal of Fish Biology, 61: 229-250.

Annex 1: List of main composite indices and their metrics used to assess water quality of estuarine systems based on fish assemblages.

Index	Country	Reference	Number of metrics	Metrics	Metric abbreviation
AFI	Basque country	Borja *et al.*, 2004; Uriarte and Borja, 2009	9	Richness (number of species)	SR
				Pollution indicator species (% individuals)	Tol-spp
				Introduced species (% individuals)	Introdspp
				Fish health (damage, diseases) (% affection)	FH
				Flat fish presence (% individuals)	AbB
				Trophic composition (% omnivorous)	AbO
				Trophic composition (% piscivorous)	AbP
				Estuarine resident (number of species)	Erspp
				Resident species (% individuals)	AbER
EFAI	Portugal	Cabral *et al.*, In press	7	Species richness	SR
				Percentage of marine migrants	AbMM
				Estuarine resident species: *% individuals, number of species*	ER-EFAI
				Piscivorous species: *% individuals, spp number*	P-EFAI
				Diadromous species	AbDIA
				Introduced species	Introdspp
				Disturbance sensitive species	Intol-spp
ELFI	France	Delpech *et al.*, 2010	7	Total density	Ab
				Density of Diadromous species	AbDIA
				Density of Marine Juvenile migrants	AbMM
				Density of Benthic species	AbB
				Density of estuarine resident	AbER
				Total species richness	SR
				Density of freshwater species	AbFW
Z-EBI	Belgium	Breine *et al.*, 2010	14	Total number of species	RS
				Total number of individuals	Ab
				Total number of estuarine species	ERspp
				Total number of diadromous species	DIAspp
				Percentage diadromous individuals	AbDIA
				Total number of marine migrating species	MMspp
				Total number of piscivorous species	Pspp
				Percentage of piscivorous individuals	AbP
				Percentage of benthic individuals	AbB
				Total number of pollution intolerant species	Intol-spp
				Percentage of pollution intolerant individuals	Intol-spp
				Total number of specialised spawners	AbSSp
				Percentage of specialised spawners individuals	AbSSp
				Total number of habitat sensitive species	HSspp

Annex list of indices/metrics. Continued

Index	Country	Reference	Number of metrics	Metrics	Metric abbreviation
TFCI	United Kingdom	Coates *et al.*, 2007	10	Species composition (relative to reference assemblage)	SR
				Presence of Indicator species	Intol-spp
				Species relative abundance (relative to reference assemblage)	Ab
				Number of taxa that make up 90% of the abundance	Dom
				Number of estuarine resident taxa	ERspp
				Number of estuarine-dependant marine taxa	MMspp
				Functional guild composition	EG
				Number of benthic invertebrate feeding taxa	BIFspp
				Number of piscivorous taxa	Pspp
				Feeding guild composition	TG
EBI	USA	Chun *et al.*, 1996; Deegan *et al.*, 1997)	8	Number of species	SR
				Dominance (Number of taxa that make up 90% of the abundance)	Dom
				Number of resident species	ERspp
				Number of nursery species	MMspp
				Number of estuarine spawners	Ab-EstSp
				Abundance	Ab
				Proportion of benthic fishes	AbB
				Proportion with physical abnormalities	FH
EFCI	South Africa	Harrison and Whitfield, 2004	14	Total number of taxa	SR
				Rare or threatened species	Intol-spp
				Exotic or introduced species	Introdspp
				Species composition (relative to reference assemblage)	SR
				Species relative abundance (relative to reference assemblage)	Ab
				Number of species that make up 90% of the abundance	Dom
				Number of estuarine resident taxa	ERspp
				Number of estuarine-dependant marine taxa	MMspp
				Relative abundance of estuarine resident	AbER
				Relative abundance of estuarine-dependant marine	AbMM
				Number of benthic invertebrate feeding taxa	BIFspp
				Number of piscivorous taxa	Pspp
				Relative abundance of benthic invertebrate feeding	AbBIF
				Relative abundance of piscivorous	AbP
IBI	USA	Meng *et al.*, 2002	6	Number of estuarine spawner species	EstSp-spp
				Proportion of killifish	Tol-spp
				Number of individuals	Ab
				Proportion of flounder	Intol-spp
				Shannon's diversity index	DI
				Proportion of benthic-associated species	Bspp

Annex 2: List of the pressures considered in this study and their causes connected to driving forces.

PRESSURES		CAUSES	DRIVING FORCES
Chemical pollution	CP	Industrial effluent discharges Pesticides, herbicides, fertilizers Polluted water runoff (boats,…) Contaminant resuspension Sewage discharges, waste treatment Waste discharges	Industry Agriculture Port activities Dredging Population Aquaculture
Nutrient enrichment (Eutrophisation)	E	Fertilizers Sewage discharges, waste treatment Waste discharges	Agriculture Population Aquaculture
Loss of habitat (saltmarsh, eelgrass and intertidal flats destruction)	LH	Bank reclamation Sediment removal Beam trawling	Port activities Agriculture Aquaculture Population Industry Dredging Fishing
Water turbidity change	WT	Sediment removal Industrial effluent discharges Sewage discharges, waste treatment Soil flushing Emptying	Dredging Industry Population Agriculture Dams
Habitat fragmentation	HF	Freshwater flow control, barrier to migratory fish	Dams
Fishing mortalities	FM	Overfishing, Juvenile fisheries, Bycatch, Migratory adults fisheries, Ghost fishing	Fishing
Invasive species	IS	Exotic species introduction by ballast water Genetic introduction and exotic species	Port activities Aquaculture
Water temperature change	T	Increase of air temperature Power plant dredgings	Climatic change Industry

Flow change	F	Fall of the precipitation Irrigation Increase of the water volumes stocked	Climatic change Agriculture Dams