The ecological status assessment of transitional waters: an uncertainty analysis for the most commonly used fish metrics in Europe: WISER Deliverable D4.4-2, part 2

A. Courrat, Mario Lepage, M.C. Alvarez, A. Borja, Henrique Cabral, M. Elliott, R. Gamito, J.M. Neto, R. Perez Dominguez, V. Raykov, et al.

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DELIVERABLE

Deliverable D4.4-2, part 2
The ecological status assessment of transitional waters: an uncertainty analysis for the most commonly used fish metrics in Europe

Lead contractor: **Irstea Bordeaux (formerly CEMAGREF)**

Contributors: **Anne Courrat (Irstea, formerly CEMAGREF), Mario Lepage (Irstea, formerly CEMAGREF), Maria C. Alvarez (UHull), Angel Borja (AZTI), Henrique Cabral (FCUL), Mike Elliott (UHull), Rita Gamito (FCUL), Joao M. Neto (IMAR), Rafael Pérez-Domínguez (UHull), Violin Raykov (IO-BAS), Ainhize Uriarte (AZTI)**

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Content

Non-technical summary

In Europe, the Water Framework Directive (WFD; Directive 2000/60/EC (European Council 2000)) aims at reaching good ecological status for surface waterbodies by 2015. Consequently European countries have developed methods based on biological (phytoplankton, macroalgae, angiosperms, macrobenthos and fishes), hydromorphological and physico-chemical quality elements for the assessment and monitoring of rivers, lakes, coastal and transitional waters. In addition to the five ecological status classes (high, good, moderate, poor or bad) for each waterbody, the WFD requires that "estimates of the level of confidence and precision of the results provided by the monitoring programmes shall be given in the (monitoring) plan". Such estimates are especially important to avoid misclassification of water bodies in their ecological assessment, which could, *in extremis*, lead to challenges to the final implementation of the Directive. Many factors will affect the final outcome of the assessment exercise, such as sampling design, year(s) of sampling, operator, etc. Therefore the impact of these factors on the assessment must be known and quantified. The European Framework project WISER is supporting the implementation of the WFD by testing and complementing existing assessment schemes, with a focus on the effects of uncertainty on classification strength, in order to make existing assessment methods more reliable and more defendable.

The present work focuses on fish-based indicators for estuarine and lagoon (transitional waters in the WFD) quality. Changes in fish assemblages may not only reflect human impact but also several other sources of variability linked to sampling and natural parameters. This is especially true in transitional waters where natural abiotic variability is extremely high (Elliott and Quintino 2007). For reliable fish-based ecological status assessment, the natural sources of variability impacting fish assemblages need to be identified and their impact on fish metrics and fish indicators must be assessed and, if possible, reduced (Clarke and Hering 2006, Staniszewski et al. 2006). In this context, we focus on the variability sources potentially affecting fish metrics for transitional waters. Hence the present work represents the first step of a global uncertainty assessment with the following three main goals:

- To give an overview of all factors that may affect the value of the most common WFD fish metrics in use for transitional waters and to identify the key sources of variability for these metrics.
- To test the effect of these key sources of variability on individual fish metrics using a European dataset.
- To indicate the general requirements of a sampling protocol that minimizes uncertainty for the fish-based assessment of transitional waters and to highlight main estuarine or lagoon features responsible for natural between estuaries or lagoon variability.
- Finally, a method based on a Bayesian framework is proposed to objectively combine fish metrics in a multimetric indicator

A dataset covering 39 estuaries and 14 lagoons distributed across six countries (Bulgaria, Italy, United Kingdom, France, Spain and Portugal) was available for the analyses. Fish data were collected between 2003 and 2010. Seven of the most commonly used WFD fish metrics were selected for the analyses: total density, total number of species, number of estuarine resident species, density of marine migrants, number of marine migrating species, percentage of omnivorous individuals and percentage of piscivorous individuals. The metrics were selected to cover several metric categories and methods of calculation of current multimetric fish indices. All potential factors that may affect the value of fish metrics were listed based on expert knowledge and bibliographical references. These, when not human disturbance, are considered as potential source of natural variability in the metrics value. Fish metrics and potential sources of variability in fish assemblages were compared in order to highlight the key potential sources of uncertainty for each of the selected fish metrics. Then some of the main potential uncertainty sources were studied using both linear models, generalized linear models and linear mixed models. The sensitivity of fish metrics to some pressure indices based on CORINE land cover, after the effect of sampling and some natural parameters have been taken into account, was also tested.

In addition to this, the influence of sampling effort on fish metrics from the Portuguese Estuarine Fish Assessment Index (EFAI) was tested on data from four Portuguese estuaries (Ria Aveiro, Tagus, Sado, Mira) collected in May-July 2005/2006. Bootstrapping techniques allowed calculating the means and standard deviations of fish metrics for different number of hauls. A cost/bias analysis was also performed in order to provide some evidences on the lowest reliable number of hauls that should be included in monitoring works.

Results showed that potential sources of uncertainty possibly impacting fish-based assessments in transitional waters are numerous. These uncertainty sources occur both within and between estuaries or lagoons. Considering available data, models showed that salinity class, depth, season, time of fishing (day *vs*. night) and year of fishing may influence the values of fish metrics. These parameters must be taken into account in the ecological assessment process. For estuaries, latitude, longitude, source elevation, continental shelf width, size, entrance width, entrance depth, mean annual river discharge, wave exposure at the entrance and intertidal area may affect at least some of the fish metrics tested here. For lagoons, longitude and total cross section of the inlets are the natural parameters explaining some of the between lagoons variability in our dataset. This argues in favour of a typology-based approach in fish-based assessments taking into account these natural parameters. Metrics of relative densities were difficult to model and no satisfactory model was found; moreover, unexplained deviance for these metrics remained very high (between 99 % and 83 %). This questions the suitability of these fish metrics for ecological assessment of transitional waters, unless better models are found in the future. Mixed models showed that for all metrics following a Gaussian distribution, it remains generally much higher unexplained deviance within estuaries or lagoons than between estuaries or lagoons. This observation may be driven by the lack of data on habitat type in the formulation of the models. Without habitat data the variability across samples within a water body due to habitat differences remains unexplained. This within estuaries or lagoons variability must be accounted for to decrease the uncertainty on the values of fish metrics and thus on the assessment. This may be done by increasing the sampling effort or collecting detailed environmental data and habitat characteristics during sampling. In the present study, the relatively high remaining variability within estuaries and lagoons could explain the relatively low sensitivity of fish metrics to the tested pressure indices. Another possible explanation might be that the pressure indices used are not good proxies for the anthropogenic pressures encountered in estuaries and lagoons.

Sampling effort was identified as a major source of uncertainty for transitional fish metrics and indices. Metrics based on the percentage of fish community features (*e.g.* % of marine migrants, % of estuarine residents) revealed to be more robust to changes in sampling effort (varying less with effort) than others based on the number of individuals from a specific category feature (*e.g.* number of species). The case study on Portuguese estuaries showed that sampling effort influences individual metrics and, consequently, also the indices using them (*e.g.* EFAI). For this reason, a minimum number of samples should be defined in sampling protocols for fish-based quality assessment, to ensure a desired level of confidence on the results is realized. Therefore, the robustness of monitoring programs may be highly dependent on the budget managers have available.

Based on pressure-impact statistical models, the Bayesian theorem was applied to estimate probabilities of being at a certain anthropogenic pressure level from fish observation and pressure-impact models outputs. This method allows combining objectively fish metrics in a multimetric fish indicator, taking into account the sensitivity and the variability of the fish metrics. It also provides a rigorous estimate of the uncertainty on the assessments. The method was applied as illustrative example on French lagoons.

Introduction

In Europe, the Water Framework Directive (WFD; Directive 2000/60/EC (European Council 2000)) aims at reaching good ecological status for surface waterbodies by 2015. Consequently European countries have developed methods based on biological (phytoplankton, macroalgae, angiosperms, macrobenthos and fishes), hydromorphological and physico-chemical quality elements for the assessment and monitoring of rivers, lakes, coastal and transitional waters. In addition to the five ecological status classes for each waterbody (high, good, moderate, poor or bad), the WFD requires that "estimates of the level of confidence and precision of the results provided by the monitoring programmes shall be given in the (monitoring) plan". Such estimates are especially important to avoid misclassification of water bodies in their ecological assessment, which could, *in extremis*, lead to challenges to the final implementation of the Directive. Many factors will affect the final outcome of the assessment exercise such as sampling design, sampling year, operator, etc., and so the impact of these factors on the assessment must be known and quantified.

The European Framework project WISER is supporting the implementation of the WFD by testing and complementing existing assessment schemes, with a focus on the effects of uncertainty on classification strength, in order to make existing assessment methods more reliable and more defendable. The present work focuses on fish-based indicators for estuarine and lagoon (*i.e.* transitional waters in the WFD) quality. Fish are known to be useful ecological indicator as they present multiple advantages for a high-level integration of ecological quality features in bioassessment (Karr 1981). However fish assemblages highly depend on natural features, both temporal and geographical, at small and large scale. Moreover, the measures that we make on fish assemblages highly depend on the way they are sampled. Hence changes in fish assemblages may not only reflect human impact but also several other sources of variability linked to sampling and natural parameters. This is especially true in transitional waters where natural abiotic variability is extremely high (Elliott and Quintino 2007). Thus a reliable ecological assessment based on fish requires 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) but also through an adequate sampling effort.

A scientific survey is considerably different from traditional fishing. For the former cost is, or should be, surrogated to other considerations such as the quality of the data, reproducibility of methods or replication to conduct statistical inference. A balanced sampling design is desirable in order to ensure a high representativeness of the assemblage (*e.g.* sampling technique adapted to scientific needs) performed with the lowest reliable sampling effort (*e.g.* adequate number of samples/ replicates) (Blocksom et al. 2009; Jennings et al. 1995; Karr 1981; Liefferinge et al. 2010; Smith and Jones 2005). In general, for a reliable fish-based ecological status assessment, the natural sources of variability impacting fish assemblages and the samples we get from them need to be identified and their impact on fish metrics and fish indicators must be assessed and, if possible, reduced (Clarke and Hering 2006, Staniszewski et al. 2006).

Previous studies highlighted the potential impact of the sampling design and estuarine natural features on the value of some fish metrics (Courrat et al. 2009; Delpech et al. 2010; Nicolas, Lobry, Lepage, et al. 2010). However, these studies only relate to some fish metrics and do not focus on quantifying the degree of uncertainty in them. In this context, we focus on the variability sources potentially affecting most commonly used fish metrics for WFD transitional waters assessment. Hence the present work represents the first step of what would be a global uncertainty assessment. It has three main goals:

- To give an overview of all factors that may affect the value of the most common WFD fish metrics in use for transitional waters and to identify the key sources of variability for these metrics.
- To test the effect of these key sources of variability on individual fish metrics using a European dataset.
- To indicate the general requirements of a sampling protocol that minimizes uncertainty for the fish-based assessment of transitional waters and to highlight main estuarine or lagoon features responsible for natural variability between estuaries or lagoons.
- Finally, a method based on a Bayesian framework is proposed to objectively combine fish metrics in a multimetric indicator.

Material and methods

Data: fish data, environmental data, data on estuarine and lagoons features and pressure data

Sampling protocol and data from fish surveys

Five fish datasets were compiled in an Access database called WP44 DB (Table 1). These datasets contain data from fish surveys performed between 2003 and 2010 in 39 estuaries and 14 lagoons distributed across six countries (Bulgaria, Italy, United Kingdom, France, Spain and Portugal – Table 1 and Figure 1). Three main types of gear were used (Table 1): beam trawls and seine nets (active gear) and fyke nets (passive gear). Datasets are composed of fishing events. A fishing event is described as a beam trawl haul, a seine haul or a fyke net collection. In total, WP44 database contains 3249 fishing events.

For each fishing event were recorded: biological data, parameters of the sampling protocol and some environmental data. Biological data are the number of fish caught from each species (or family or gender when it was not possible to identify catches at the species level) and their size. Data from the sampling protocol include type of gear, date and time of sampling, geographic coordinates of the fishing event, duration of soaking for fyke nets and trawled distance for beam trawls. Some irregularities in the environmental data provided for the fishing events were encountered. For example, while salinity class, temperature and depth were recorded in most of the fishing events (94%, 91% and 85% respectively), oxygen saturation and pH were only recorded in 53% and 10% of the fishing events respectively.

*Table 1: Structure of the datasets used in the sensitivity analyses (dataset description in *(Uriarte et Borja 2009); **(Martinho et al. 2008); ***(Drouineau et al. 2012) and ****(Courrat et al. 2011))*

Dataset	Data source	Years of sampling	Number of estuaries and lagoons	Number of fishing events	Sampling gears
Basques estuaries (Spain)*	Basque Water Agency and AZTI	2008, 2009, 2010	12 estuaries	342	Beam trawl
Mondego estuary(Portugal)**	IMAR-CMA	2003, 2004	1 estuary	74	Beam trawl
French estuaries and lagoons***	French Water Agencies and Cemagref	From 2005 to 2009	12 lagoons 25 estuaries	2414 in estuaries 294 in lagoons	Estuaries: beam trawl and fyke net Lagoons: Cemagref fyke net for lagoons
Stour and Orwell EA data (UK)****	Environment Agency (EA)	2009	1 estuary	23	Beam trawl, fyke net and seine net
Wiser New field data****	Wiser WP44	2009	2 lagoons 3 estuaries	63 in estuaries 39 in lagoons	Beam trawl, fyke net, Cemagref fyke net for lagoons, seine net

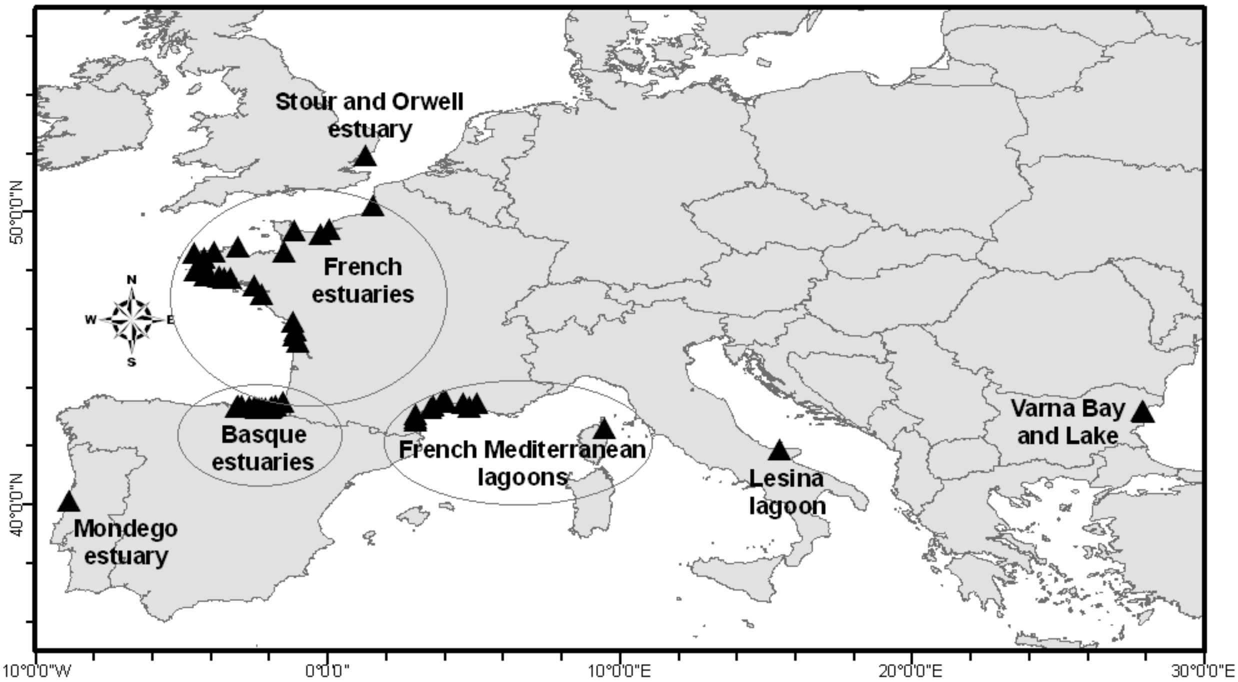

Figure 1: map of estuaries and lagoons where fish data were available in WP44 database for the following uncertainty analyses

In addition to these datasets, four Portuguese estuaries (Ria Aveiro, Tagus, Sado, Mira) were also surveyed between May and July 2005/2006 (Figure 2) to test the influence of the sampling effort on fish-based assessments. Sampling took place during the night, at ebb-tide, using a 2 m wide beam-trawl, with one tickler chain and a stretched mesh size of 5 mm at the cod end. Hauls were performed at a constant speed (approximately 0.7-0.8 m s^{-1}), for 10 minutes, sweeping an average area of 862 m^2. Data were available for the oligohaline (salinity lower than 5), mesohaline (salinity values between 5 and 18) and polyhaline (salinity higher than 18) zones of the Tagus and Mira estuaries and for the mesohaline and polyhaline areas of Ria de Aveiro and Sado estuaries. As previously, 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 such as the salinity, temperature, depth and oxygen saturation. Secchi depth was also recorded for some fishing events.

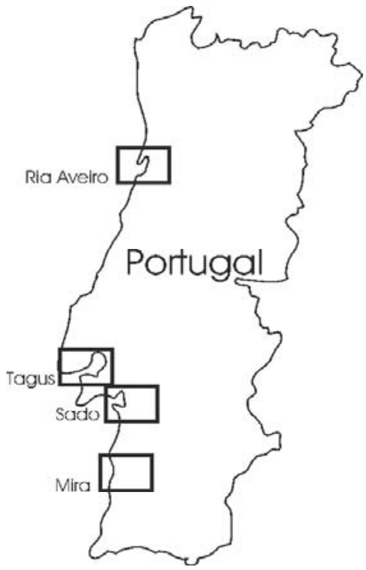

Figure 2: Portuguese sampling sites used to test the influence of sampling effort on fish-based assessment

Three lagoons from Corsica, France, namely Diana, Urbino and Palo (see Drouineau et al. (2012) for details), were added to the dataset to test the Bayesian method to combine fish metrics. Sampling and data characteristics were the same as for all other French lagoons.

Data on estuarine and lagoon features

Data on estuaries from (Nicolas, Lobry, Lepage, et al. 2010) were also included and were updated and completed for the purpose of the present work. For each estuary source elevation, littoral substrate, continental shelf width, catchment area, estuarine area, entrance width and entrance depth, mean annual river discharge, wave exposure, tidal range and percentage of intertidal area were available for inclusion in the analysis. Data on lagoons such as area and total cross-section of inlets were obtained from Irstea and Goggle Earth. Data on estuaries and lagoons were all collected at the estuary or lagoon level, since the criteria used to divide estuaries and lagoons into waterbodies differ between countries and sometimes between water basins. Hence we decided to work at the scale of entire lagoons and estuaries (*i.e.* "systems"), as it was considered more relevant from an ecological point of view.

Pressure data

Pressure indices were defined from CORINE Land Cover (CLC - (Commission of the European Communities 1994) 2006, except for the Stour and Orwell estuary where only CLC 2000 was available. Three pressure indices were selected for the analyses: the percentage of agricultural areas, the percentage of natural areas and the percentage of urban areas in a 2 km buffer around each estuary or lagoon (D4.4-4, Courrat et al. 2012).

To test the Bayesian method to combine fish metrics in a multimetric indicator, an anthropogenic pressure index based on contamination data was estimated using a similar approach to Courrat et al. (2009) and Delpech et al. (2010). This contamination index could be used as the method was tested on French lagoons only and standardised contamination data are available for the considered French lagoons (Drouineau et al. 2012).

Common species list and functional guilds

A common list of fish species was compiled based on the World Register of Marine Species (WoRMS) database (Appeltans et al. 2011). Only the 193 species that were caught in WP4.4 DB were considered (Annex 1). A common assignment of “ecological guilds”, “position guilds” and “trophic guilds” to fish species was agreed by WP4.4 and external experts[1] (see Courrat et al. (2011) for details), based on the definitions of these guilds from Elliott et Dewailly (1995) and Franco et al. (2008). However, for some ecological guilds it was decided to adapt their definition to better approximate the functional quality described by the particular life stages found in the transitional waters studied here. A detailed presentation of these common guilds can be found in Deliverable 4.4-2, part 1 (Courrat et al. 2011). The list of species caught during the surveys contained in WP44 DB and their corresponding common guilds is presented in Annex 1 of the present document.

Uncertainty analysis on fish metrics for transitional waters

The general approach used in the present work is outlined in Figure 3. A total of 7 fish metrics were calculated at the fishing event scale to be able to analyse effects of the sampling protocol and to maximize the sample size for the models (Courrat et al. 2009; Delpech et al. 2010). These 7 fish metrics are commonly used in WFD-compliant fish indices for transitional waters and were selected to provide a representative sample of fish quality attributes. A list of potential sources of variability impacting fish assemblages in estuaries and lagoons was created, based on expert knowledge and bibliographical sources. Fish metrics and potential sources of variability in fish assemblages were compared in order to highlight key potential sources of uncertainty for each of the selected fish metrics. When possible considering available fish data, the uncertainty sources were quantified using either linear models (LM) or generalized linear models (GLM). Models were run separately for estuaries and lagoons and included natural features as well as pressure indices based on CORINE Land Cover (Commission of the European Communities 1994). Only the best LM were transformed to linear mixed models (LMM) by adding estuary or lagoon as random factor. This allowed the separation of the unexplained deviance between and within estuaries or lagoons for these metrics modelled using a Gaussian law.

[1] Steve Coates (Environment Agency, UK) and Jan Breine (INBO, BG)

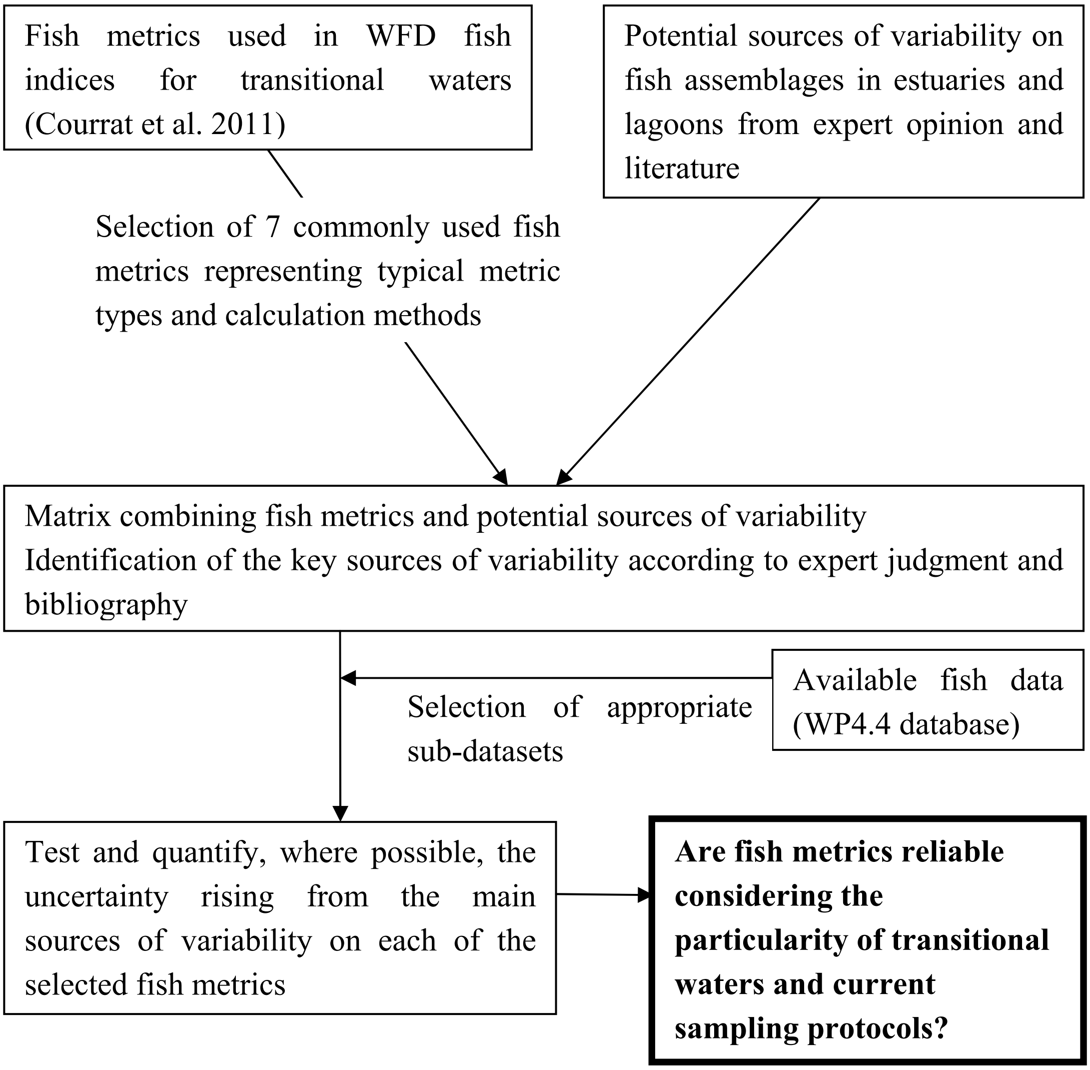

Figure 3: General approach used for the study of uncertainty in fish indices for estuaries and lagoons; bold frame: expected outcomes

Selection of fish metrics

From the WFD fish indices tested in Courrat et al. (2011) (AFI (Uriarte et Borja 2009), EFAI (Cabral et al. in press), ELFI (Delpech et al. 2010), TFCI (Coates et al. 2007) and Z-EBI (Breine et al. 2010)), a list of WFD fish metrics for transitional waters was established (Table 2). However, due to the large number of metrics and the numerous identified sources of variability impacting them, only 7 metrics were selected for the following uncertainty analyses.

*Table 2: Fish metrics that compose WFD fish indices for transitional waters tested in (Courrat et al. 2011): AFI (Uriarte et Borja 2009), EFAI (Cabral et al. in press), ELFI (Delpech et al. 2010), TFCI (Coates et al. 2007), Z-EBI (Breine et al. 2010); in bold: metrics selected for uncertainty analyses. *Some metrics focus only on MJ species however in WP44 common guilds, only 3 species are classified as marine seasonal species (MS) while 38 species are classified as MJ, hence metrics based on MJ are highly correlated with metrics based on marine migrants MM (=MJ + MS). ** Data on fish health is not available in WP44 dataset*

	Number of metrics	Expected trend with increasing pressure
Global density		
Total density	3	decrease
Bray-Curtis similarity of species relative abundance with a reference assemblage	1	decrease
Global species richness and composition		
Total number of species	5	decrease
Number of taxa that make up 90% of the abundance	1	decrease
Bray-Curtis similarity of species composition with a reference assemblage	1	decrease
Habitat use (ecological guilds)		
<u>Estuarine resident (ER)</u>		
Density of ER	1	bell curve
Percentage of ER individuals	2	bell curve
Number of ER species	4	decrease
<u>Diadromous (DIA)</u>		
Density of DIA	2	decrease
Percentage of DIA individuals	1	decrease
Number of DIA species	2	decrease
Loss in diadromous species in 3 classes from expert opinion	1	increase
<u>Freshwater species (FW)</u>		
Density of FW	1	decrease
<u>Marine migrants (MM) : estuarine-dependent marine taxa, including Marine Juveniles (MJ)*</u>		
Density of MM	1	decrease
Percentage of MM individuals	1	decrease
Number of MM species	3	decrease
<u>Global</u>		
Functional guild composition (linked to the number of taxa per guild)	1	decrease
Trophic guild		
<u>Omnivorous</u>		
Percentage of omnivorous individuals	1	bell curve
<u>Piscivorous</u>		
Percentage of piscivorous individuals	3	decrease or bell curve
Number of piscivorous species	3	decrease
<u>Invertebrate feeders</u>		
Density of benthic invertebrate feeders	1	decrease
Number of benthic invertebrate feeding species	1	decrease
<u>Zooplankton feeders</u>		
Density of zooplankton feeders	1	bell curve
<u>Global</u>		
Feeding guild composition (linked to the number of taxa per guild)	1	decrease
Fish condition		
Percentage of damaged, diseased individuals**	1	increase
Benthic and flatfishes		
Density of benthic individuals	1	decrease
Percentage of benthic individuals	1	decrease
Percentage of flat fish individuals	1	decrease
Spawners		

Percentage of specialised spawners individuals	1	decrease
Number of specialised spawners species	1	decrease
Indicator or introduced species		
Indicator		
Percentage of pollution indicator individuals	1	increase
Presence of disturbance sensitive species	1	decrease
Loss in disturbance sensitive species in 3 classes from expert opinion	1	increase
Percentage of pollution intolerant individuals	1	decrease
Number of pollution intolerant species	1	decrease
Number of habitat sensitive species	1	decrease
Introduced		
Percentage of individuals from introduced species	1	increase
Abundance in class of introduced species from expert opinion	1	increase

Fish metrics described eight main characteristics of the fish assemblage (Table 2): global density, global species richness and composition, habitat use by the fish (ecological guilds), trophic aspects (trophic guilds), fish condition, position of fish in the water column (benthic and flatfishes), spawning characteristics, introduced and indicator species. Beside, four major ways of quantifying fish attributes were identified: number of species, number of individuals (global or per unit of effort), relative number of individuals (%) and presence/absence.

Seven fish metrics that cover the four major ways of quantifying fish attributes, and some of the characteristics of the fish assemblage that are the most commonly assessed were chosen for these analyses (Table 3). Total density, total number of species, number of estuarine resident species, number of marine migrating species and percentage of piscivorous individuals were selected as they are commonly used in WFD fish assessment methods for transitional waters (Table 2). Percentage of omnivorous individuals was selected as it is expected to vary following a bell curve with increasing pressure. Finally, density of marine migrants was selected as it can be modelled using delta models combining presence/absence with density when fishes are present (Courrat et al. 2009; Delpech et al. 2010). Fish health was not considered as no such data are available in WP44 database. Indicator or introduced species were not considered as they differ between ecoregions and countries making it difficult to calculate such metrics at large scale.

Table 3: Fish metrics selected for the uncertainty analyses; SR stands for species richness and RD stands for relative density.

Fish metrics selected for the uncertainty analyses	Abbreviation
Total density	TD
Total number of species	SR
Number of estuarine resident species	SR_ER
Number of marine migrating species	SR_MM
Percentage of piscivorous individuals	RD_P
Percentage of omnivorous individuals	RD_O
Density of marine migrants	DMM

Calculation of metrics

Courrat et al. (2011) showed, beside the different metrics that composes each WFD fish indicator, different scale of calculation of fish metrics, both in space and time: at the fishing event scale or at the salinity class / water body scale, data pooled per year, or season, or not pooled. For the present exercise all fish metrics were calculated at the fishing event scale because: (i) it maximizes the number of data for modelling purposes and (ii) some of the main sources of variability occur and were measured at the fishing event scale (*e.g.* depth).

Abundance metrics and number of species were standardised by sampling effort. Beam trawls and seine nets densities were expressed as number of fish per sampled surface. For fyke nets, densities are number of fish caught per effort unit (around 24 hours for one Cemagref-type fyke net and 12 hours for one fyke net). For beam trawls and seine nets, numbers of species were standardised by the log-transformed sampled surface (Nicolas, Lobry, Lepage, et al. 2010), while for fyke nets the catch was not transformed and expressed as number of fish captured per effort unit. Trawl hauls where it was not possible to calculate the trawled surface were discarded from the analyses. In total, fish metrics could be calculated for 3218 fishing events.

Modelling fish metrics to assess uncertainty rising from different potential sources of variability

Fish metrics were first modelled using Linear Models (LM) or Generalized Linear Models (GLM) to test the significance of the effect of the various sources of variability. These models take into account variability between fishing events rising from sampling effects, natural parameters (at the fishing event scale and at the estuary or lagoon scale) and anthropogenic pressure:

Fish metric ~ $S_1 + \ldots + S_n + N_1 + \ldots + N_p$ + anthropogenic pressure

With $S_{[1\ldots n]}$: variables from the sampling protocol and $N_{[1\ldots p]}$: variables from natural parameters. Models options depend on data distribution for the different fish metrics (Courrat et al. 2009; Delpech et al. 2010; Drouineau et al. 2012; Le Pape, Holley, et al. 2003; Le Pape, Chauvet, et al. 2003). Numbers of species in fyke nets were modelled using a Poisson distribution. For beam trawls, standardised numbers of species were modelled using a Gaussian distribution (Nicolas, Lobry, Lepage, et al. 2010). Relative densities were also modelled using a Gaussian distribution to avoid giving too much weight to fishes caught in school, which would be the case with a Binomial law. For densities, the type of model used was chosen considering the percentage of zero values (null densities) in the data. For total densities, the percentage of zero values was low (< 5 %), thus simple Linear Models were built on log-transformed total density + 1. Densities of marine migrants (DMM) were characterized by a large number of zeros: about 40 % of beam trawls or fyke nets caught no marine migrant (MM) fish. In this case, a delta model composed of two sub-models was used: (i) the probability of presence of MM fish was modelled using a Binomial distribution and (ii) the positive densities of MM fish, *i.e.* the densities of MM fish when present, was modelled using a Gaussian distribution after log-transformation to reduce the influence of extreme values in the dataset (Courrat et al. 2009; Delpech et al. 2010; Le Pape, Holley, et al. 2003; Le Pape, Chauvet, et al. 2003; Stefánsson 1996). A stepwise backward procedure based on the Akaike Information Criterion (AIC) was used to select the most relevant and parsimonious models (Drouineau et al. 2012). Pressure metrics (*Pr*) were added separately at the end of the models once significant variables linked to sampling and to estuarine and lagoon features were selected. The statistical significance of each fixed effect (including pressure metric) was tested at the level of 5% (Chi-squared test at 5 % level) and only significant fixed effects were kept in the models. A graphical analysis of the residuals was carried out for each model in order to verify underlying hypothesis (independence and normality of the residuals of deviance).

After relevant and significant fixed effects were selected using LM and GLM, the best LM were transformed in Linear Mixed Models (LMM) by adding an estuary or lagoon random factor. This allows taking into account that the various fishing events performed in an estuary or in a lagoon are not completely independent, this is to avoid pseudo-replication in the data (Bolker et al. 2009).

All analyses were computed on R software (R Development Core Team 2009). Gaussian mixed models were computed using function lme of Package nlme (Pinheiro et al. 2011).

Method for the evaluation of the effect of sampling effort on fish metrics

This analysis was realised on fish metrics composing the Portuguese Estuarine Fish Assessment Index (EFAI). From the data on Portuguese estuaries, pseudo-random samples of 3-100 hauls for each estuary and of 3-25 hauls per salinity zone in each estuary were generated. For each pseudo-random sample, hauls were summed and metrics were then calculated. Finally, 1000 bootstrap cycles were performed and the metrics' means and standard deviations were obtained for each number of hauls considered. The EFAI calculation followed the criteria shown in Table 4. The mean and the standard deviation were used to visualize how important the number of hauls was for the levelling off of the means. Cumulative curves were used to compare the deviation achieved for each group of considered hauls against the overall metric maximum registered for each salinity zone. Bias was also estimated to verify its evolution in relation to the number of hauls. Finally, a cost/bias analysis was performed to evaluate the lowest number of hauls that should be included in monitoring works to obtain a reliable metric value while keeping costs reasonable.

Table 4: Scoring criteria used in the calculation of EFAI metrics (Cabral et al. 2011)

Metrics	Scores		
	1	2	3
Species richness (SR)	≤ 10	11-20	> 20
Percentage of marine migrants (%MM)	≤ 10 %	10-50 %	> 50 %
Estuarine resident species (ES)			
Percentage of individuals	≤ 10 % or > 90 %	10-30 % or 70-90 %	30-70 %
Number of species	≤ 2	3-5	> 5
Piscivorous species (P)			
Percentage of individuals	≤ 10 % or > 90 %	10-30 % or 70-90 %	30-70 %
Number of species	≤ 5	6-12	> 12
Diadromous species (D)	Absent or few species present / Inability to complete life cycle	Several species present but rare	Several species present and common
Introduced species (I)	Present and abundant	Present but rare	Absent
Disturbance sensitive species (S)	Absent or few species present	Several species present but rare	Several species present and common

Bayesian framework to objectively combine fish metrics

This framework is based on two phases. First, pressure-impact statistical models are developed to quantify the impact of pressure on various fish metrics and to select relevant fish metrics. Then the Bayesian theorem is applied to estimate probabilities of being at a certain anthropogenic pressure level from fish observation (*i.e.* selected fish metrics) and pressure-impact models outputs. A full description of the method is available in Drouineau et al. (2012).

The method was applied as illustrative example on 14 French lagoons: the 11 French lagoons included in WP4.4 DB plus 3 lagoons from Corsica. The pressure-impact statistical models linked some fish metrics with a contamination index. For each lagoon, probabilities of being in each of the five ecological status classes were computed using the Bayesian method.

Results

Matrix combining fish metrics and potential sources of variability: identification of the key sources of variability potentially affecting fish metrics in transitional waters

All potential factors that may affect fish assemblages in estuaries and lagoon were identified using expert opinion and bibliographic sources (Tables 5a and 5b). These, when not human disturbance, are considered as potential source of variability for the value of fish metrics that may lead to uncertainty in the ecological assessment. Potential sources of variability occurring within (Table 5a) or between (Table 5b) estuary / lagoon were distinguished. Interactions between sources of variability were not taken into account.

Many parameters may affect the value of fish metrics within estuaries and lagoons (Table 5a). The effects of turbidity and oxygen in transitional waters are supposed to be strong when they reach very high or very low values. All other factors may potentially have a strong effect on fish metric in their "normal" range of values. This effect may be modulated by the nature of the metrics, as those based on guilds depend on functional aspects and are somewhat decoupled from structural (taxonomical) attributes as defined by single species tolerance.

Despite it is sometimes measured during fish samplings, pH is believed to have no or low effect on fish assemblages in transitional waters compared to other factors. The gear effect on fish catches is crucial and greatly documented however it has poorly been studied in the context of WFD fish metrics for transitional waters. Franco et al. (in press) give an overview of such gear effect in lagoons. Gear effect is difficult to quantify because data from several gears at the same site and in sufficient number to perform meaningful analyses can hardly be found. However, most fish indices are gear-specific (Courrat et al. 2011), *i.e.* they are developed specifically for a particular gear or a combination of gears. Hence uncertainty due to gear in WFD ecological assessment based on fish in transitional waters is someway accounted for by the use of gear-specific assessment methods. The level of fish identification may have an effect both within and between estuary/lagoon. Indeed, the percentage of fishes identified at higher level than species level may vary between operators (between year) within site and between sites. The effect of sampling effort was studied for the Portuguese Estuarine Fish Assessment Index (EFAI).

Considering between estuary and lagoon natural variability, all listed factors may potentially have an effect on fish metrics. Courrat et al. (2009), Delpech et al. (2010), Nicolas, Lobry, Lepage, et al. (2010) and Nicolas, Lobry, Le Pape, et al. (2010) have already found a correlation between some fish metrics and some estuarine natural features. With testing the effect of the natural features of estuaries and lagoons on fish metrics, we can define an estuarine or lagoon typology that will minimize uncertainty on fish metrics.

Table 5a: Factors driving the variability of fish metrics within transitional waters systems. Y: likely effect; N: no effect; ?: unknown.

Potential sources of variability		TD	SR	SR_ER	DMM	SR_MM	RD_O	RD_P
Spatial variability	Depth	Y	Y	Y	Y	Y	Y	Y
	Temperature	Y	Y	?	Y	Y	?	?
	Salinity	Y	Y	Y	Y	Y	?	?
	Turbidity	Y	Y	Y	Y	Y	Y	Y
	Oxygen	Y	Y	Y	Y	Y	Y	Y
	Habitat	Y	Y	Y	Y	Y	Y	Y
	Bottom structure	Y	Y	Y	Y	Y	Y	?
Sampling method	Gear (type and characteristics)	Y	Y	Y	Y	Y	Y	Y
	Speed	Y	Y	Y	Y	Y	Y	Y
	Tide	Y	Y	Y	Y	Y	Y	Y
	With or against current	Y	Y	Y	Y	Y	Y	Y
	Operator	Y	Y	Y	Y	Y	Y	Y
	Sampling effort	Y	Y	Y	Y	Y	Y	Y
	Method to chose sampling sites	Y	Y	Y	Y	Y	Y	Y
Temporal variability	Duration	Y	Y	Y	Y	Y	Y	Y
	Day *vs.* night	Y	Y	Y	Y	Y	Y	Y
	Season	Y	Y	N	Y	Y	?	?
	Date (ex. early vs. late spring)	Y	Y	N	Y	Y	Y	Y
	Interannual	Y	Y	Y	Y	Y	Y	Y
Sample processing	Errors in species identification	N	Y	Y	Y	Y	Y	Y
	Fishes not identified at the species level	N	Y	Y	Y	Y	Y	Y
	Subsampling	Y	Y	Y	Y	Y	Y	Y
Note: See Table 3 for metric acronyms								

Table 5b: Natural features of estuaries and lagoons potentially inducing between estuaries (or lagoons) variability in fish metrics. Most environmental data were obtained from Nicolas, Lobry, Lepage, et al. (2010) and Nicolas, Lobry, Le Pape, et al. (2010). Y: probably has an effect; N: no effect; ?: unknown/unclear

Potential sources of variability	TD	SR	SR_ER	DMM	SR_MM	RD_O	RD_P
Estuary / lagoon	Y	Y	Y	Y	Y	Y	Y
Size of estuary / lagoon	?	Y	Y	?	Y	?	?
Latitude	Y	Y	Y	Y	Y	?	?
Longitude	?	?	?	?	?	?	?
Ecoregion	?	Y	?	Y	Y	?	?
Source elevation	?	?	?	?	?	?	?
Catchment area	?	?	?	?	?	?	?
Mean annual river discharge	?	?	?	?	?	?	?
Entrance width	?	Y	?	?	Y	?	?
Entrance depth	?	?	?	?	?	?	?
Tidal range	?	?	?	?	?	?	?
Intertidal area in class	Y	?	?	?	?	?	?
Wave exposure	?	?	?	?	?	?	?
Continental shelf width	?	Y	?	?	?	?	?
Section of inlets for lagoons	?	?	?	?	?	?	?
Littoral substrate	?	?	?	?	?	?	?
Fishes not identified at the species level	N	Y	Y	Y	Y	Y	Y
Note: See Table 3 for metric acronyms							

Potential uncertainty sources that can be studied in the present work considering available data

Several sources of variability listed in Table 5a and 5b could not be studied and quantified in the present work. Turbidity, habitat, tide and bottom structure were rarely recorded during sampling. No data was available about errors in fish species identification. Speed of towing for beam trawls was recorded for about 16 % of trawl hauls and its precision is low as only average speed was recorded. Sampling with or against the current, the method to choose sampling sites and sub-sampling processing were usually mentioned in the sampling protocols but in the available datasets there never are two different approaches of these parameters performed at the same time in the same place. The situation is the same for the operator effect: data from two different operators sampling on the same site at the same time were not available. Hence, for these variability sources, it is not possible to disentangle between an operator or a sampling protocol effect and a time or site effect with available data. It was also difficult to study the impact of the percentage of fish that were not identified at the species level because it is closely linked to an operator effect, including the way the sampling sites were chosen. Natural features distinguishing between an estuary and a lagoon effect could not be assessed as estuaries and lagoons were usually sampled with different gear sets, making the disentangling of the gear effect from the estuary/lagoon effect not possible.

For these reasons, sources of variability that could be studied and quantified considering the available data were: depth, temperature, salinity, day *vs.* night, season, interannual variability and all natural features of estuaries on one side and lagoons on the other side.

Modelling fish metrics to test and quantify the effects of some uncertainty sources

Two sub-datasets, one for lagoons and one for estuaries (Table 6), allowed to test and study most of the potential sources of variability identified in the previous paragraph.

Table 6: Sub-datasets selected for most of the uncertainty analyses and main characteristics

	Estuaries	Lagoons
Gear	Beam trawl	Cemagref fyke net for lagoons collected about every 24 hours
Seasons	Autumn and summer	Autumn and summer
Number of systems	38 (all except Grand-Rhône and Varna Bay where data of estuarine features are missing)	12 (all except Varna Lake that was not sampled with Cemagref fyke net for lagoons)
Total number of fishing events	1811	295
Minimum number of fishing events per system and per season	4 (in Stour and Orwell, spring)	3 (in Lesina, autumn)
Maximum number of fishing events per system and per season	117 (in Gironde, autumn)	30 (in Thau, autumn)

For lagoons, latitude range is believed to be not wide enough to impact fish assemblages (it ranges from 41.88 decimal degrees to 43.58 decimal degrees). Moreover, it is correlated with the percentage of agricultural areas (Pearson correlation coefficient of -0.74) and slightly correlated with the percentage of urban areas (Pearson correlation coefficient of 0.55). For these reasons, introducing latitude in the models for lagoon may hide the pressure effect while in the meantime there is no obvious ecological reason for a latitude effect on fish assemblages in the considered lagoons. Latitude was thus excluded from the models on lagoons. Longitude of lagoons range from 3.00 to 15.44 and it is not correlated to any pressure metric thus it was kept in the models. For estuaries, preliminary analyses tested for the correlation (Pearson coefficients) of the various continuous covariates describing estuarine features. The Pearson correlation coefficients are presented in Annex 2. The catchment area and the mean annual river discharge are highly correlated (Pearson coefficient of 0.95) thus only the mean river annual discharge was tested in the models. Latitude was correlated with the tidal range and the continental shelf width (Pearson coefficients of respectively 0.77 and 0.81), however all these descriptors were kept as they might traduce different ecological mechanisms acting on fish assemblages. The littoral substrate could not be included in the models because data were missing for the Mondego estuary.

Tables 7a and 7b present the results of the best models (LM and GLM) computed on theses datasets on lagoons and estuaries, respectively. For lagoons, the percentage of deviance explained by the models ranges between 11.54 % and 22.24 % except for the model on RD_P where only 3.48 % of deviance is explained. For estuaries, between 20.92 % and 40.02 % of deviance is explained by the models, except for the models on RD_O and RD_P where 8.65 % and 1.09 % of variance explained respectively. Graphical analysis of the residuals for models on RD_O and RD_P showed that modelling these two metrics using a Gaussian distribution was not satisfactory as residuals are not completely normal. However, no better way of modelling these two metrics could be found; several transformation of data were tested but none could normalise the data and modelling these metrics using a Binomial law would lead to a model driven only by the few fishing events with high quantities of fish caught.

For fish data in lagoons (Table 7a), the salinity class has a significant effect on all fish metrics except RD_O and RD_P. The season impacts the metrics SR, SR_ER and RD_O. The total cross section of inlets has a significant effect on SR_ER, the density of MM when present and the two relative densities. Temperature at the bottom only impacts the density of MM when present and the longitude has a significant effect on all metrics expect SR, the density of MM when present and RD_P. Concerning the effect of pressures on fish metrics in lagoons, three of the seven tested metrics does not answer significantly to any of the tested pressure indices. When significant, the effect of the percentage of natural areas is always positive. The percentage of urban areas shows a positive effect the metrics SR_MM and on the density of MM when present. The percentage of agricultural areas has a positive effect on SR_ER but a negative effect on MM fishes. In general, the correlation coefficients associated with pressure metrics are very low.

Table 7a: Best LM and GLM computed on sub-dataset from lagoons: when significant (Chi-squared test at 5 % level) effect of pressure metrics (regression parameter) is presented. NS: non-significant. Only statistically significant fixed effects (Chi-squared test at 5 % level) are included in the models. Sal class: salinity class; Sect: cross-sectional area of the inlets; Pr: pressure index; Temp: temperature; Agr: percentage of agricultural areas; Urb: percentage of urban areas; Nat: percentage of natural areas.

Fish metric		Model	Agr	Urb	Nat
TD		*Sal class + Longitude*	NS	NS	NS
SR		*Sal class + Season*	NS	NS	NS
SR_ER		*Sal class + Season + Sect + Longitude + Pr*	+0.009	NS	NS
DMM	*Probability of presence*	*Sal class + Longitude + Pr*	-0.047	NS	+0.026
	Density when present	*Sal class + Temp + Sect + Pr*	-0.035	+0.043	NS
SR_MM		*Sal class + Longitude + Pr*	-0.042	+0.019	+0.015
RD_O		*Season + Sect + Longitude + Pr*	NS	NS	+0.244
RD_P		Sect	NS	NS	NS

Table 7b: Best LM and GLM computed on sub-dataset from estuaries: when significant (Chi-squared test at 5 % level) effect of pressure metrics (regression parameter) is presented. NS: non-significant. Only statistically significant parameters (Chi-squared test at 5 % level) are included in the models. Sal class: salinity class; Lat: latitude; Long: longitude; Area class: estuarine area in class; Ent width: entrance width; Ent depth: entrance depth; Shelf width: continental shelf width; Discharge: mean annual river discharge; Intertidal area: percentage of intertidal area; Pr: pressure index; Agr: percentage of agricultural areas; Urb: percentage of urban areas; Nat: percentage of natural areas

Fish metric		Model	Agr	Urb	Nat
TD		*Sal class + Depth + Season + Lat + Area class + Ent width + Ent depth + Discharge + Wave exposure + Intertidal area + Pr*	+0.015	NS	-0.027
SR		*Sal class + Depth + Season + Lat + Area class + Shelf width + Source elevation + Ent width + Ent depth + Wave exposure + Intertidal area + Pr*	+0.003	NS	-0.005
SR_ER		*Sal class + Season + Lat + Long + Area class + Intertidal area + Pr*	+0.001	+0.002	NS
DMM	Probability of presence	*Sal class + Depth + Lat + Area class + Source elevation + Ent width + Ent depth + Discharge + Wave exposure + Intertidal area + Pr*	+0.016	NS	-0.048
	Density when present	*Sal class + Depth + Season + Lat + Long + Area class + Shelf width + Ent depth + Discharge + Wave exposure + Intertidal area + Pr*	+0.016	NS	-0.046
SR_MM		*Sal class + Depth + Lat + Area class + Shelf width + Ent width + Ent depth + Discharge + Wave exposure + Intertidal area + Pr*	+0.003	-0.003	-0.004
RD_O		*Sal class + Season + Shelf width + Source elevation + Wave exposure + Intertidal area + Pr*	+0.059	NS	-0.091
RD_P		*Sal class + Area class*	NS	NS	NS

For fish data in estuaries (Table 7b), salinity class, depth and season have a significant effect on most of the fish metrics. Concerning estuarine features, the latitude, the estuarine size and the size of the intertidal area, impact all fish metrics except the two relative densities (RD_P is impacted by estuarine size but it explains less than 1% of the total deviance in the metric values). Other parameters such as the entrance width and depth, the mean annual river discharge, the continental shelf width, the source elevation and the wave exposure at the entrance of the estuary also often have significant effect on fish metrics. Tidal range is never relevant in the models. The effect of pressure metrics is unexpected as the percentage of agricultural land always has a positive effect on fish metrics (when significant), while the percentage of natural land always has a negative effect; this was the relationship initially expected for RD_O only. The only fish metrics responding in the expected way to some pressure indices are RD_O and SR_MM for the percentage of agricultural areas and the percentage of urban areas respectively. Again, the correlation coefficients associated with pressure metrics are very low.

Tables 8a and 8b present the repartition of the residual variance, once the fixed effects are fitted, between and within lagoons and estuaries (random effects), for fish metrics that were modelled using a Gaussian distribution. This information was obtained by transforming the best LM in LMM. Similar information is not presented for metrics modelled using a Poisson or a Binomial law as such information is not easily accessible with GLMM. These tables show that, for all metrics that could be modelled using a Gaussian law, there remains high unexplained variability within lagoons or within estuaries despite the fixed effect. The remaining within estuary or lagoon variance is generally much higher than between estuaries or lagoons, probably traducing the high natural variability in transitional waters (Elliott et Quintino 2007).

Table 8a: Partition of the residual variance for fish metrics modelled using Gaussian models, once the fixed effect are fitted, between lagoons and within lagoons

Fish metric		Pressure metric included in the model	Between/within lagoon	Percentage of variance
TD		None	Between	12,38
			Within	87,62
DMM	*Density when present*	Agr	Between	12,57
			Within	87,43
		Urb	Between	14.95
			Within	85.05
RD_O		Nat	Between	31,40
			Within	68,60
RD_P		None	Between	2,00
			Within	98,00

Table 8b: Repartition of the residual variance for fish metrics modelled using Gaussian models, once the fixed effect are fitted, between estuaries and within estuaries

Fish metric		Pressure metric included in the model	Between/within estuary	Percentage of variance
TD		Agr	Between	12.38
			Within	87.62
		Nat	Between	18.91
			Within	81.09
SR		Agr	Between	22.28
			Within	77.72
		Nat	Between	21.33
			Within	78.67
SR_ER		Agr	Between	21.40
			Within	78.60
		Urb	Between	21.44
			Within	78.56
DMM	*Density when present*	Agr	Between	7.98
			Within	92.02
		Nat	Between	5.72
			Within	94.28
SR_MM		Agr	Between	25.54
			Within	74.46
		Urb	Between	26.78
			Within	73.22
		Nat	Between	24.71
			Within	75.29
RD_O		Agr	Between	6.68
			Within	93.32
		Nat	Between	6.72
			Within	93.28
RD_P		None	Between	0.91
			Within	99.09

Effect of sampling time on the value of fish metrics

The effect of day *versus* night fishing and the year effect were tested on a sub-dataset composed of Mondego data year 2003 and 2004. These data contains 55 trawl hauls. To account for other potential sources of variability, the initial following model was tested:

Fish metric ~ salinity class + depth + day time (night or day) + season + year

As total densities presented no null values in this sub-dataset, they were modelled using a simple log transformation. The densities of MM (DMM) comprised only 5 % of zeros, thus they were modelled using a Gaussian model on log (DMM + 1) data.

The results of the best models obtained using (as previously) a stepwise backward procedure based on the Akaike Information Criterion (AIC) indicate that, when significant, night had always a positive effect compared to day (Table 9), meaning that more fish were caught by night than by day. Again, graphical analyses for RD_P and RD_O models revealed that Gaussian models for these metrics are not completely satisfactory and must be improved. Depending on the fish metric, the year effect was different: in year 2004 higher RD_O were obtained while less SR_MM were obtained in this year.

Table 9: Best LM computed on Mondego data year 2003 and 2004. Only statistically significant parameters (Chi-squared test at 5 % level) are included in the models. NS: no parameter had a statistically significant effect; NA: not applicable

Fish metric	Model	Percentage of total deviance explained by the model
TD	Day time	6.8 %
SR	Sal class + Depth + Day time	51.46 % (of which 5.16 % by day time effect)
SR_ER	Sal class + Depth	46.81 %
DMM	NS	NA
SR_MM	Sal class + Depth + Day time + Year	51.28 % (of which 8 % by year effect and 7 % by day time)
RD_O	Year	6.9 %
RD_P	NS	NA

Effect of sampling effort on the value of fish metrics

The mean value for the metrics related to the structure of the community, for an increasing number of hauls grouped and contributing to the mean per estuary, can be observed in Figure 4. The metrics based on percentages (marine migrants, estuarine residents, piscivorous) show a smaller deviation from the global mean and stabilise with fewer hauls than the ones based on the number of species (species richness, estuarine species, piscivorous species) which either never stabilise or only stabilise with a high number of hauls.

Concerning the mean value of metrics per salinity zone (Figure 5), it was clear that percentage of individuals (marine migrants, estuarine residents and piscivorous) stabilized after a fewer hauls, regardless of the salinity zone. Species richness did not reach a plateau in polyhaline and mesohaline zones, but it levelled off with approximately 20 hauls in the oligohaline zone. The number of estuarine species also stabilized but it required an increasing number of hauls at higher salinities. Concerning the number of piscivorous species, it was not possible to reach a plateau in the polyhaline zone. However, lower number of hauls was required to reach stabilization in the oligohaline zone than in the mesohaline zone.

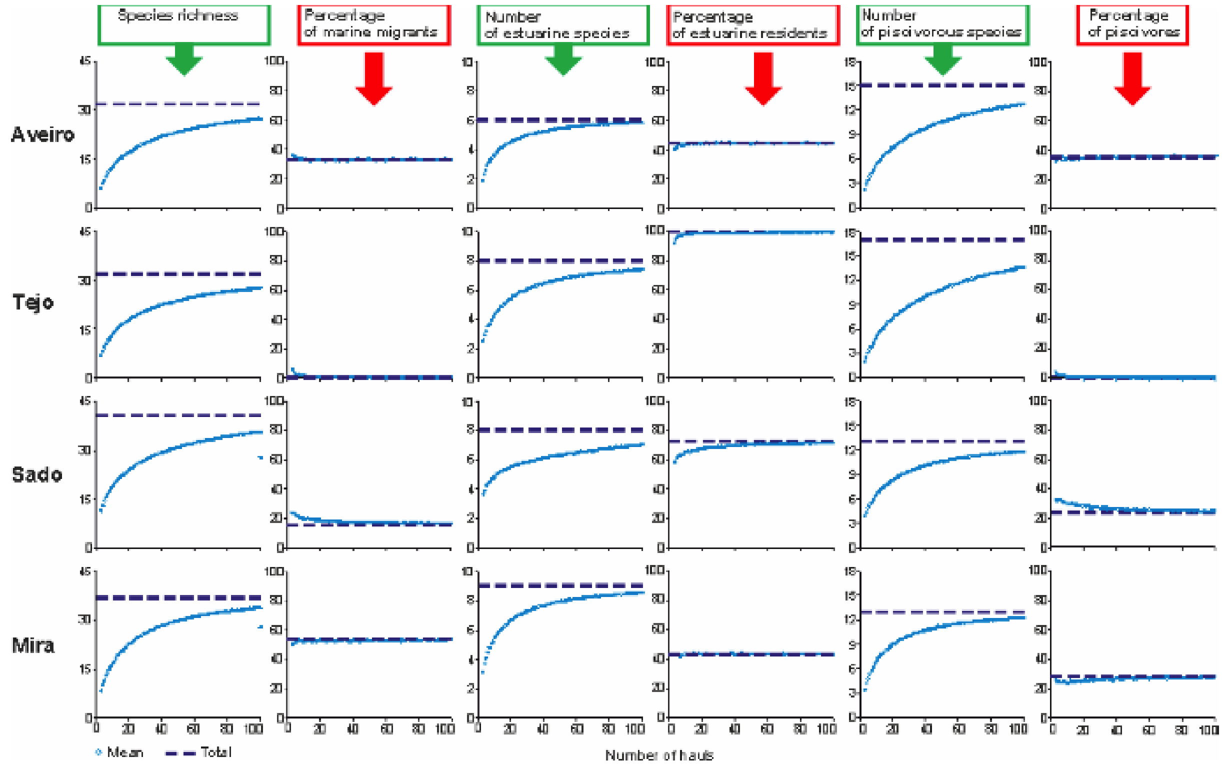

Figure 4: Mean values per estuary calculated for different metrics when a different number of hauls is considered

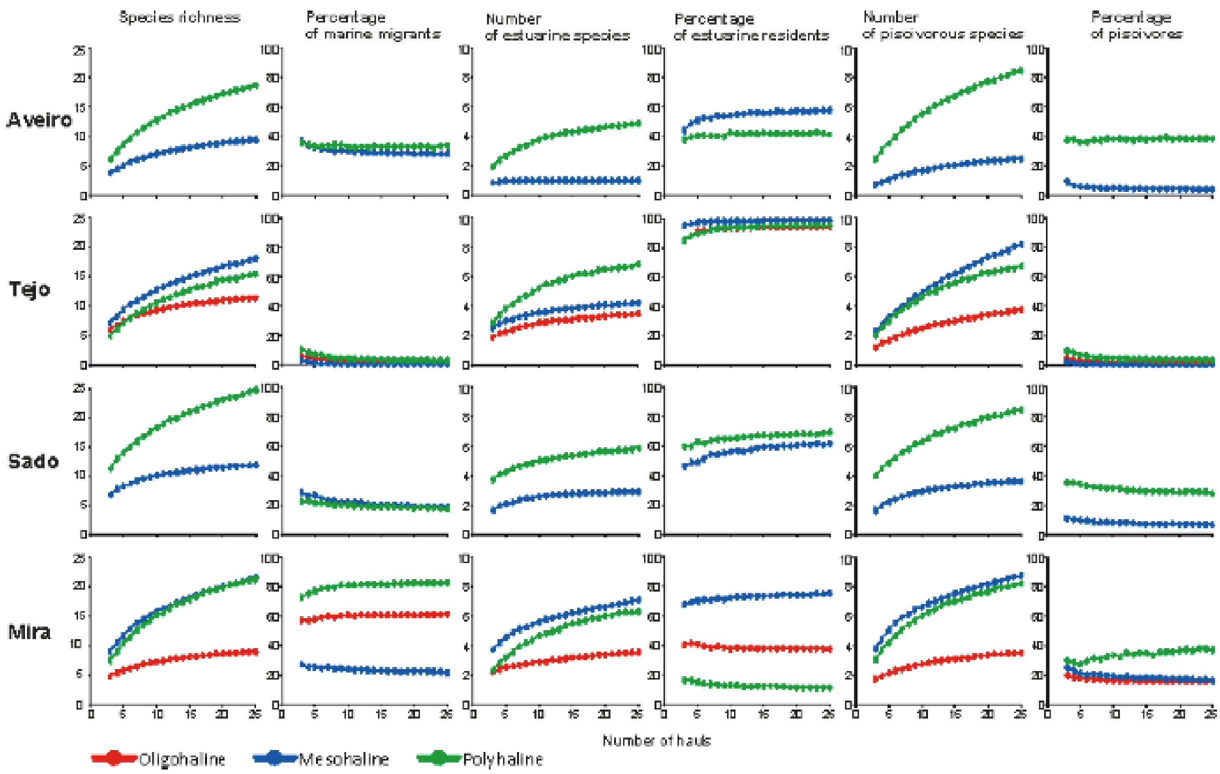

Figure 5: Mean values per salinity zone calculated for different metrics when a different number of hauls is considered

The species richness had similar standard deviation (SD) in the four estuaries, but it was higher in the polyhaline zone (SD ~ 2) than in the mesohaline and oligohaline zones (SD ~ 1). For the number of estuarine species and the number of piscivorous species, SD was approximately equal to 1, regardless the salinity zone. Metrics calculated as percentages of individuals had different SD per estuary and per salinity zone. SDs for the percentage of marine migrants were as follows: Ria Aveiro and Sado 25 ± 5, Tejo 13 ± 0.5, Mira 28 ± 8. Regarding the percentage of estuarine residents, SDS were: Ria Aveiro and Sado 25 ± 5, Tejo 17 ± 0.6, Mira 28 ± 8. Finally, for the percentage of piscivorous fishes SDs were: Ria Aveiro and Sado 25 ± 5, Tejo 10 ± 0.3 and Mira 22 ± 8. Metrics based on percentages showed a smaller bias (percent deviation from the global mean), even using the smallest number of hauls, than the metrics based on numbers of species (Figure 6). For the % MM and % P the distribution was not symmetrical, with Tagus showing a trend opposite to the other systems.

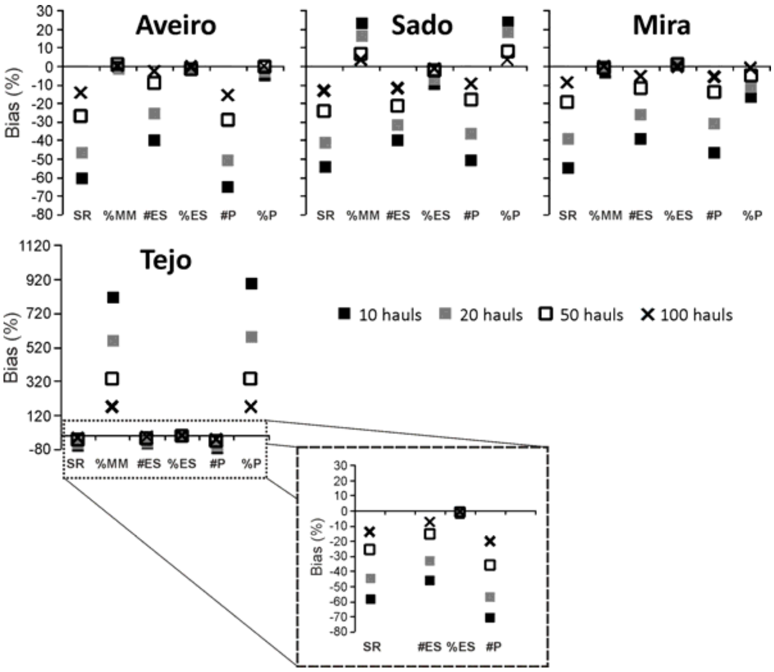

Figure 6: Bias calculated for each metric, per estuary, using 10, 20, 50 and 100 hauls

Reducing the sampling bias to a 10 % level duplicates the cost of the monitoring programme in Aveiro and Mira estuaries while costs increases less in the Sado estuary (Figure 7). Further bias reduction to a 5 % level increases the costs more than 300 % across all estuaries due to the increased in the requested number of hauls and associated processing of the samples.

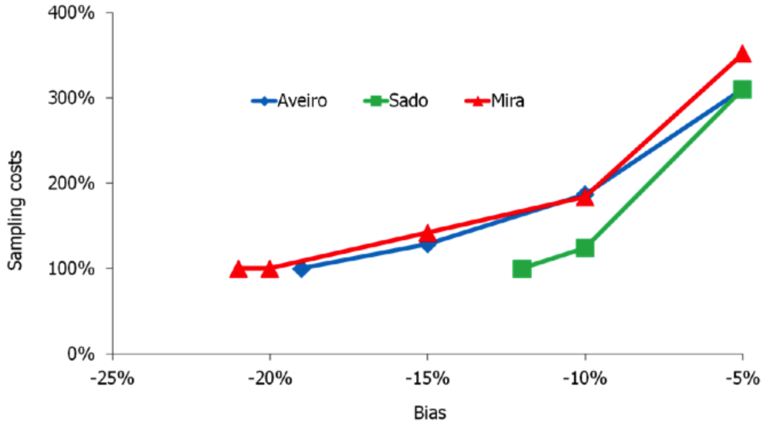

Figure 7: Bias associated to the relation between the number of hauls and the sampling costs. Plots for the estuaries Ria Aveiro, Sado and Mira

Bayesian framework to objectively combine fish metrics

Using the Bayesian method designed by Drouineau et al. (2012), three fish metrics were selected to built the multimetric indicator: the total fish density (TD), the density of marine fishes (DM) and the total number of species (RT). The posterior probability of each French lagoon to be in each of the five quality classes given observed selected fish metrics could then be computed. These probabilities are presented in Figure 8. For each lagoon, uncertainty in the ecological assessment could be evaluated using these probabilities. In the case-study of French lagoons, the fish indicator designed using the Bayesian method gave clear indication of the quality class (*i.e.* the uncertainty in the assessment is low) except for the Berre lagoon where it was not possible to distinguish between the good or the very good quality class.

Probabilities could also be analysed at the scale of the fish metrics (Figure 9). They showed that, in the case of French lagoons, diagnostics based on a single metric are more variable, *i.e.* more uncertain, than diagnostics based on several fish metrics.

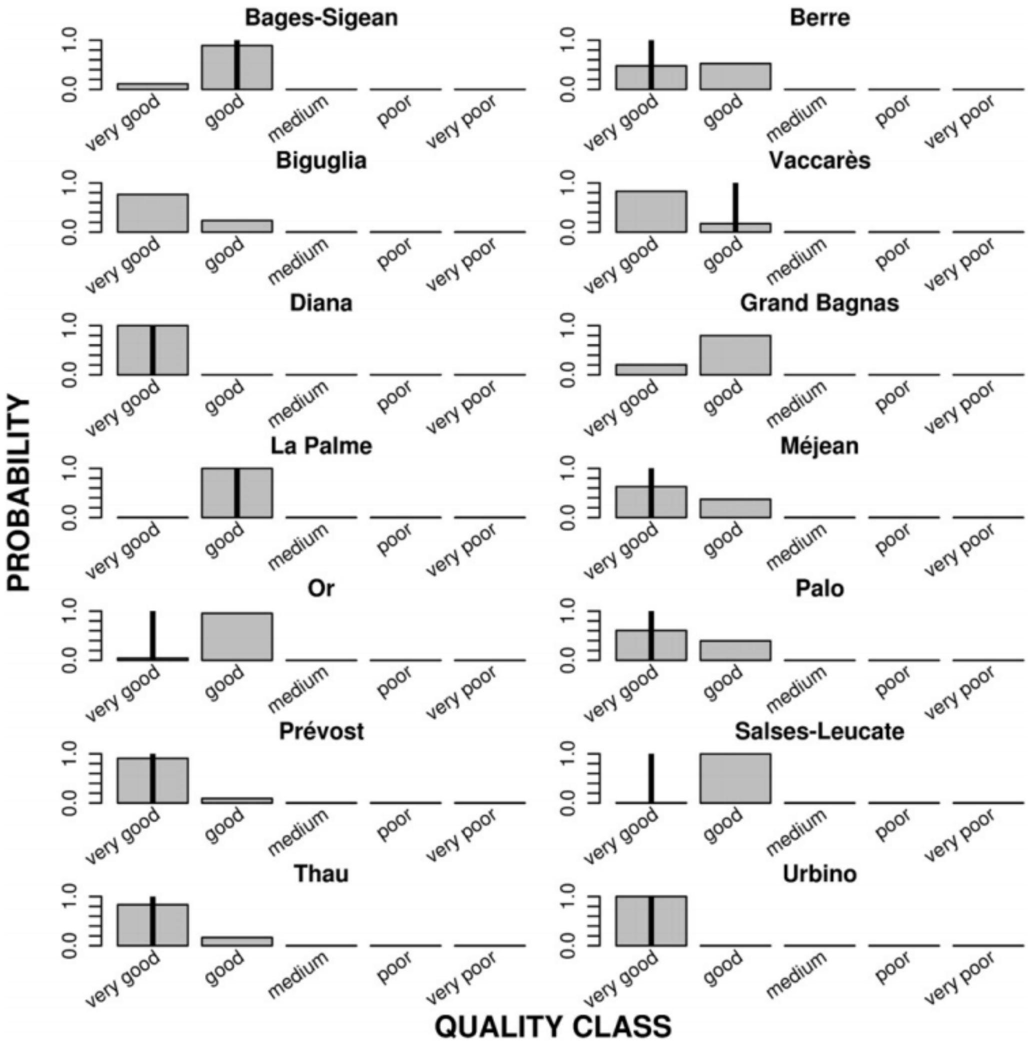

Figure 8: (from Drouineau et al. (2012)) Posterior probability to be in a quality class given the fish observations (barplot) and pressure index quality class based on water contamination (vertical bold line)

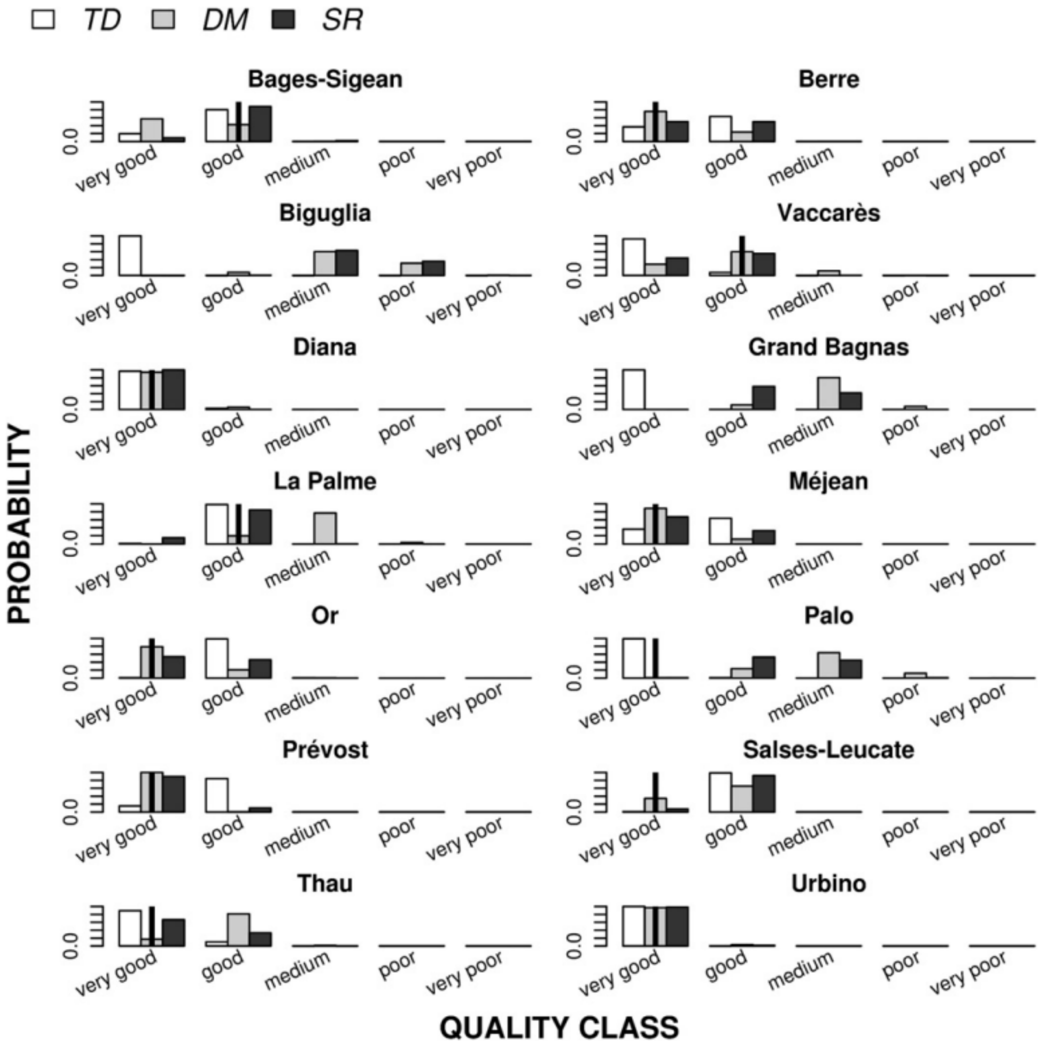

Figure 9: (from Drouineau et al. (2012)) Posterior probability to be in a quality class given TD (total density), DM (density of marine fishes) and RT (species richness) considered individually, and pressure index quality class based on contamination data (vertical bold line).

Discussion

There are important sources of variability that could potentially impact the outcome of fish metrics in transitional waters. Whilst other studies have directly assessed the effect of several factors on the uncertainty at the multimetric-index level (for example the assessment of the effect of some factors on the EQRs obtained from seagrass in coastal waters (Bennett et al. 2011), we decided to conduct the study at the metric level. Indeed, it was believed that some important sources of variability impact fish metrics in transitional waters. The aim of this work was to look at these potential sources of uncertainty in order to give recommendations on how to account for them and decrease their impact on the final assessment.

The present work gives first a list of sources of uncertainty potentially impacting the values of commonly used WFD fish metrics in transitional waters. This first step is followed by an evaluation of the effect of these uncertainty sources using a modelling approach on a European wide fish dataset. Despite the high amount of available data, some sources of uncertainty, including some major ones, such as the habitat effect, could not be studied here. However, some equally important sources of uncertainty could be studied and the present work lead to provide recommendations for the optimization of a sampling protocol and an estuarine typology that minimizes the uncertainty on fish metrics. Concerning the sampling protocol, it is clear in the models that salinity class, depth, season, time of fishing (day *vs.* night) and year of fishing may all impact the values of fish metrics. These parameters must be taken into account in the assessment by standardizing the protocol (*e.g.* fishing only by day or only by night) and/ or by including them in the modelling of fish metrics.

Regarding estuarine and lagoon features, the parameters to take into account in transitional waters typology depend on the range of these parameters in the estuaries or lagoons where the fish metrics have to be used. In the present exercise, available datasets contained a great amount of French data and fewer data from other countries. With a more geographically extended dataset, the natural features of estuaries and lagoons explaining fish metric variability would probably be different. For this reason, the results of the present study must be considered with caution as some factors of variability may not have been properly accounted for as they were not relevant at the scale of the datasets, although they might be relevant at a regional scale. In general, for estuaries, latitude, longitude, source elevation, continental shelf width, size, entrance width, entrance depth, mean annual river discharge, wave exposure at the entrance and intertidal area may affect at least some of the fish metrics tested here (Table 7b). For lagoons, natural parameters explaining some of the between lagoons variability in our dataset are longitude and total cross section of the inlets (Table 7a). The between estuaries or between lagoons variability due to these parameters is natural and it should be accounted for when comparing fish metric values between estuaries or between lagoons. It is important to note that the present results highly depend on the data used. The present work aims at giving clues about the potential sources of uncertainty affecting fish metrics but the significance of their effect on the tested fish metrics can not be generalized. Similar testing should be made on the particular datasets fish indicators are designed for.

The selection of significant fixed effect was here realized using LM and GLM as advised by (Bolker et al. 2009). This approach was chosen because the selection of fixed effect in mixed models is difficult and the method to use is unclear. The p-values given by mixed models are biased and the reliability of the ANOVA function is often discussed and presented as doubtful. Metrics of relative densities were difficult to model (no suitable model could be found, and the unexplained deviance for these metrics remains very high: between 83 % and 99 %). For other metrics, models explained between 11 % and 40 % of the total deviance in the data, the percentage of explained deviance being generally lower in lagoons than in estuaries. In the case of Gaussian models, mixed models allowed to study the repartition of the unexplained deviance between and within estuaries or lagoons. It showed that for all metrics modelled using a Gaussian law, it remains generally much higher unexplained deviance within estuaries or lagoons than between estuaries or lagoons (Table 8a and 8b). This means that some sources of variability impacting fish metrics were not included as fixed effect in the models and this may be linked to the high natural abiotic variability of transitional waters (Elliott et Quintino 2007). It can be due for example to the habitat type where the fish were caught. The high intra lagoon or intra estuary variability must be accounted for to decrease the uncertainty on the values of fish metrics. This may be done by increasing the sampling effort to increase the confidence in the mean values of fish metrics or collecting new environmental data on the places where the fish were caught.

The impact of sampling effort has been identified here as a major source of uncertainty for transitional fish metrics (Figure 4 and 5). The case study on Portuguese estuaries showed that sampling effort influences metrics of the EFAI, especially metrics on number of species, which are common to several other fish-based indices. The effect of all other uncertainty sources was tested on fish metrics calculated at the fishing event scale to enhance the number of data available for the models and to test the impact of some natural variables that were measured at the sampling event scale (such as depth). However, calculation of fish indicators is based on several fishing events and the results are probably closely linked to the sampling effort. Some indices are based on an average of metrics calculated at the fishing event scale (*e.g.* French fish index ELFI), others directly calculate metrics on a combination of several fishing events (*e.g.* AFI metrics are calculated on 3 fishing events and EFAI metrics are calculated on 5 fishing events). Nevertheless, the number of fishing events on which the final multimetric index is compiled may directly influence the results of the assessment. The effect of the different sources of variability on fish metrics may also be influenced by the sampling effort. These findings may have implications for the WFD assessment of transitional waters if the designed monitoring programmes and multimetric fish-based indices used do not take into account the effect of the sampling effort. Therefore, to bring down the risk of misclassification may be simply a matter of budget, where increasing the number of samples used on the assessment as to ensure a desirable low uncertainty level might not be economically viable.

The significance of the effect of pressure indices was also tested via classical LM and GLM. The results are somehow unexpected as correlations between fish metrics and pressures index are often not significant or when significant they often indicate reverse trends than those expected. Moreover, correlation coefficients are always very close to zero. These problems in detecting the effect of pressure indices on fish metrics might be a result of the low percentage of variability explained by the models and the high percentage of remaining variability within lagoons or estuaries. This variability may hamper the detection of pressure indices effects. Another possible explanation could be that pressure metrics based on CORINE Land Cover data and calculated on a 2 km buffer around estuaries or lagoons maybe not be good proxies of anthropogenic pressures. This may be especially true for estuaries where human water quality can be highly affected by anthropogenic disturbances occurring in the catchments explaining why correlations between fish metrics and pressure indices are worse for estuaries than for lagoons.

Mixed models are very effective when working on unbalanced datasets (Venables et Dichmont 2004) and they allow taking into account autocorrelation in the data. For these reasons mixed models are better adapted to our dataset than LM and GLM. However the use of these, and especially of non-Gaussian mixed models, proved to be really difficult in the present study. Further work is needed to maximize the potential of mixed models in further work using the approach.

Following the uncertainty analysis at the fish metric scale, the next logical step is to study uncertainty propagation from fish metrics to multimetric indices. This could have been done using WISERBUGS software, as initially planned; however, we encountered difficulties in this particular exercise due to the fact our fish metrics could not all be transformed to conform to a Normal distribution. WISERBUGS was designed for metrics following normal distributions only. The standard variation required in WISERBUGS is the residual within lagoons or within estuaries variation, after the fixed effects have been taken into account. For non normal models, this data is difficult to obtain. Additionally, WISERBUGS software requires the definition of boundary class thresholds which were not available. No solution was found to overcome these problems and WISERBUGS could not be used.

Instead, a method based on a Bayesian framework was designed (Drouineau et al. 2012). This method allows both to select relevant fish metrics and to combine them taking into account their sensitivity to pressure and their variability. The method can also be a way to integrate data from expert opinion and it finally gives an assessment of the uncertainty of the diagnostic (Drouineau et al. 2012). For all these reasons, this Bayesian method is very interesting and promising. It was tested on French lagoons. In this case, it showed that diagnostics are less variable at the level of the multimetric indicator than at the level of the fish metrics considered individually (Drouineau et al. 2012). As the uncertainty analyses realised at the fish metric scale in the present work suggested that uncertainty on fish metric may be high, this last result is encouraging and further research on the propagation of uncertainty from fish metric to multimetric indicator is required. A good start could be to test the Bayesian method from Drouineau et al. (2012) on other datasets.

Main conclusions and recommendations for future work:

- In the present work, a modelling approach was used to disentangle between the effects of sampling and natural variability and the effect of anthropogenic pressure on fish metrics.
- This modelling approach showed that fish metrics are impacted by many parameters, such as variables from the sampling protocol (including sampling effort), environmental (natural) variables both at the fishing event scale and at the estuary or lagoons scale, and anthropogenic pressures.
- However, the available datasets for the present work were somehow limited as uncertainty analyses were based on a dataset containing mostly French data and the effect of sampling effort was tested on Portuguese estuaries and Portuguese fish metrics only. The present work gives methods and general information about the various factors affecting fish metrics, but such uncertainty analyses should probably be conducted at larger and smaller scales to obtain operational results.
- The modelling approach tested here could explain less than 40 % of fish metric variability. The remaining variability was mainly within estuary or lagoon variability and can probably be attributed, at least in part, to a habitat effect that was not accounted for in the models.
- This great within system variability may hamper the detection of pressure effect on fish metrics and thus bring uncertainty to the assessment. Further work is needed to test how this residual within system variability can be accounted for and/or decreased. In particular, similar testing on sampling effort effect as the one realised on Portuguese estuaries could be an interesting option.
- A Bayesian framework was proposed to combine objectively fish metrics in a multimetric fish indicator, taking into account the sensitivity and the variability of the fish metrics. It also provides a rigorous estimate of the uncertainty on the ecological assessment. The method was applied as illustrative example on French lagoons. It will be very interesting to test this method on other datasets as well as to include expert opinion (as informative prior) in the assessment. This work is currently being conducted on French estuaries (Tableau et al., in prep.).
- The propagation of the uncertainty from the fish metrics to the multimetric indicator is unknown and further research in this field is requested. In particular, it would be interesting to find a way to use WISERBUGS for non-Gaussian fish metrics, in order to perform such study.

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References

Appeltans W, Bouchet P, Boxshall G, Fauchald K, Gordon D, Hoeksema B, Poore G, van Soest R, Stöhr S, Walter T, Costello M (2011) World Register of Marine Species (WoRMS). http://www.marinespecies.org. Accessed 9 mai 2011

Bennett S, Roca G, Romero J, Alcoverro T (2011) Ecological status of seagrass ecosystems: An uncertainty analysis of the meadow classification based on the Posidonia oceanica multivariate index (POMI). Marine Pollution Bulletin 62: 1616–1621. doi:16/j.marpolbul.2011.06.016

Blocksom K, Emery E, Thomas J (2009) Sampling effort needed to estimate condition and species richness in the Ohio river, USA. Environmental Monitoring and Assessment 155: 157–167. doi:10.1007/s10661-008-0425-0

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24: 127–135. doi:10.1016/j.tree.2008.10.008

Breine J, Quataert P, Stevens M, Ollevier F, Volckaert FAM, Van den Bergh E, Maes J (2010) A zone-specific fish-based biotic index as a management tool for the Zeeschelde estuary (Belgium). Marine Pollution Bulletin 60: 1099–1112. doi:10.1016/j.marpolbul.2010.01.014

Cabral HN, Fonseca VF, Gamito R, Gonçalves CI, Costa JL, Erzini K, Gonçalves J, Martins J, Leite L, Andrade JP, Ramos S, Bordalo A, Amorim E, Neto JM, Marques JC, Rebelo JE, Silva C, Castro N, Almeida PR, Domingos I, Gordo LS, Costa MJ (in press) Ecological quality assessment of transitional waters based on fish assemblages in Portuguese estuaries: The Estuarine Fish Assessment Index (EFAI). Ecological Indicators.

Clarke R, Hering D (2006) Errors and uncertainty in bioassessment methods – major results and conclusions from the STAR project and their application using STARBUGS. Hydrobiologia 566: 433–439. doi:10.1007/s10750-006-0079-2

Coates S, Waugh A, Anwar A, Robson M (2007) Efficacy of a multi-metric fish index as an analysis tool for the transitional fish component of the Water Framework Directive. Marine Pollution Bulletin 55: 225–240. doi:10.1016/j.marpolbul.2006.08.029

Commission of the European Communities (1994) CORINE land cover. Technical guide. Luxembourg (Office for Official Publications of European Communities).

Courrat A, Lepage M, Alvarez MC, Borja A, Cabral H, Elliott M, Franco A, Neto JM, Pérez-Domínguez R, Raykov V, Uriarte A (2012) Report summarising the definitions of reference conditions using predictive models for ecological endpoints for fish in transitional waters. WISER Deliverable

Courrat A, Lepage M, Pérez-Domínguez R, Uriarte A, Fonseca VF, Raykov V, Borja A, Neto JM, Cabral H, Alvarez MC (2011) Testing the adaptability and behaviour of 6 fish indices on a common dataset composed of multiple gears samplings from 8 estuaries and lagoons. WISER Deliverable

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, Courrat A, Pasquaud S, Lobry J, Le Pape O, Nicolas D, Boët P, Girardin M, Lepage M (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. doi:10.1016/j.marpolbul.2010.01.001

Drouineau H, Lobry J, Delpech C, Bouchoucha M, Mahevas S, Courrat A, Pasquaud S, Lepage M (2012) A Bayesian framework to objectively combine metrics when developing stressor specific multimetric indicator. Ecological Indicators 13: 314–321. doi:10.1016/j.ecolind.2011.06.029

Elliott M, Dewailly F (1995) The structure and components of European estuarine fish assemblages. Netherlands Journal of Aquatic Ecology 29: 397–417. doi:10.1007/BF02084239

Elliott M, Hemingway KL (2002) Fishes in Estuaries. Blackwell Science Ltd.

Elliott M, Quintino V (2007) The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Marine Pollution Bulletin 54: 640–645. doi:10.1016/j.marpolbul.2007.02.003

European Council (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities: L327/1–72.

Franco A, Elliott M, Franzoi P, Torricelli P (2008) Life strategies of fishes in European estuaries: the functional guild approach. Marine Ecology Progress Series 354: 219–228. doi:10.3354/meps07203

Franco A, Perez-Ruzafa A, Drouineau H, Franzoi P, Koutrakis ET, Lepage M, Verdiell-Cubedo D, Bouchoucha M, Lopez-Capel A, Riccato F, Sapounidis A, Marcos C, Oliva-Paterna FJ, Torralva-Forero M, Torricelli P Assessment of fish assemblages in coastal lagoon habitats: Effect of sampling method. Estuarine, Coastal and Shelf Science: in press. doi:10.1016/j.ecss.2011.08.015

Jennings MJ, Fore LS, Karr JR (1995) Biological monitoring of fish assemblages in Tennessee Valley reservoirs. Regulated Rivers: Research & Management 11: 263–274. doi:10.1002/rrr.3450110303

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

Liefferinge C, Simoens I, Vogt C, Cox TJS, 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. doi:10.1007/s10750-010-0110-5

Martinho F, Viegas I, Dolbeth M, Leitão R, Cabral HN, Pardal MA (2008) Assessing estuarine environmental quality using fish-based indices: Performance evaluation under climatic instability. Marine Pollution Bulletin 56: 1834–1843. doi:10.1016/j.marpolbul.2008.07.020

Nicolas D, Lobry J, Lepage M, Sautour B, Le Pape O, Cabral H, Uriarte A, Boët P (2010) 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.

Nicolas D, Lobry J, Le Pape O, Boët P (2010) Functional diversity in European estuaries: Relating the composition of fish assemblages to the abiotic environment. Estuarine, Coastal and Shelf Science 88: 329–338. doi:10.1016/j.ecss.2010.04.010

Le Pape O, Chauvet F, Mahévas S, Lazure P, Guérault D, Désaunay Y (2003) Quantitative description of habitat suitability for the juvenile common sole (Solea solea, L.) in the Bay of Biscay (France) and the contribution of different habitats to the adult population. Journal of Sea Research 50: 139–149. doi:16/S1385-1101(03)00059-5

Le Pape O, Holley J, Guérault D, Désaunay Y (2003) Quality of coastal and estuarine essential fish habitats: estimations based on the size of juvenile common sole (Solea solea L.). Estuarine, Coastal and Shelf Science 58: 793–803.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2011) nlme: Linear and Nonlinear Mixed Effects Models.

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Smith KL, Jones ML (2005) Watershed-level sampling effort requirements for determining riverine fish species composition. Canadian Journal of Fisheries and Aquatic Sciences 62: 1580–1588.

Staniszewski R, Szoszkiewicz K, Zbierska J, Lesny J, Jusik S, Clarke RT (2006) Assessment of sources of uncertainty in macrophyte surveys and the consequences for river classification. Hydrobiologia 566: 235–246. doi:10.1007/s10750-006-0093-4

Stefánsson G (1996) Analysis of groundfish survey abundance data: combining the GLM and delta approaches. ICES Journal of Marine Science: Journal du Conseil 53: 577 –588. doi:10.1006/jmsc.1996.0079

Tableau A, Drouineau H, Delpech C, Pierre M, Lobry J, Le Pape O, Breine J, Lepage M A fish-based index of estuarine ecological quality incorporating information from both scientific fish survey and experts knowledge. in prep.

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.

Venables WN, Dichmont CM (2004) GLMs, GAMs and GLMMs: an overview of theory for applications in fisheries research. Fisheries Research 70: 319–337. doi:10.1016/j.fishres.2004.08.011

Annex 1: Fish species caught during the surveys included in WP44 database and corresponding commonly agreed ecological guilds

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

scientific_name	Ecological_guild	Position_guild	Trophic_guild
Abramis brama	FW	D	IB
Abramis brama, Blicca bjoerkna	FW	D	IB
Acipenser sturio	DIA	D	IB
Agonus cataphractus	ER	B	IB
Alburnoides bipunctatus	FW	P	IS
Alburnus alburnus	FW	P	Z
Alosa	DIA	P	Z
Alosa alosa	DIA	P	Z
Alosa fallax	DIA	P	Z
Ameiurus melas	FW	D	O
Ammodytes tobianus	ER	B	Z
Anguilla anguilla	DIA	D	O
Aphanius fasciatus	ER	D	IB
Aphia minuta	ER	P	Z
Argyrosomus regius	MS	D	IS
Arnoglossus imperialis	MA	B	F
Arnoglossus kessleri	ER	B	IB
Arnoglossus laterna	MA	B	IB
Arnoglossus thori	MA	B	IB
Atherina boyeri	ER	P	Z
Atherina pontica	MJ	P	Z
Atherina presbyter	ER	P	Z
Balistes carolinensis	MA	D	IS
Barbus barbus	FW	D	IB
Belone belone	MS	P	F
Blennius ocellaris	ER	B	IB
Blicca bjoerkna	FW	D	IB
Boops boops	MJ	P	O
Buglossidium luteum	MA	B	IB
Callionymus lyra	ER	B	IB
Callionymus risso	ER	B	IB
Carassius auratus auratus	FW	D	O
Carassius carassius	FW	D	O
Centrolabrus exoletus	MA	D	IB
Chelidonichthys lucernus	MJ	B	IS
Chelon labrosus	DIA	D	O
Chondrostoma nasus	FW	D	V
Ciliata mustela	ER	B	O
Ciliata septentrionalis	MA	D	IS
Clupea harengus	MJ	P	Z

Clupeidae		P	Z
Conger conger	MA	D	F
Coris julis	MA	D	IS
Crystallogobius linearis	ER	D	Z
Ctenolabrus rupestris	MA	D	IB
Cyprinidae	FW		
Cyprinus carpio	FW	D	O
Dicentrarchus labrax	MJ	D	IS
Dicentrarchus punctatus	MJ	D	IS
Dicologlossa cuneata	MA	B	IB
Diplecogaster bimaculata	ER	B	IB
Diplodus annularis	MA	D	IS
Diplodus cervinus	MJ	D	IS
Diplodus sargus	MJ	D	IS
Diplodus vulgaris	MJ	D	IS
Echiichthys vipera	MA	B	IS
Engraulis encrasicolus	MS	P	Z
Entelurus aequoreus	MA	D	Z
Gadidae		D	
Gaidropsarus vulgaris	MA	D	IS
Gambusia affinis	ER	P	IS
Gambusia holbrooki	ER	P	IS
Gasterosteus aculeatus	ER	D	IB
Gobiidae	ER		
Gobio gobio	FW	D	IB
Gobius cobitis	ER	B	O
Gobius geniporus	ER	B	IB
Gobius niger	ER	B	IB
Gobius paganellus	ER	B	IB
Gobius roulei	ER	B	IS
Gobiusculus flavescens	ER	D	IS
Gymnocephalus cernuus	FW	B	IB
Hippocampus guttulatus	ER	B	Z
Hippocampus hippocampus	ER	B	Z
Hyperoplus immaculatus	MJ	D	IS
Hyperoplus lanceolatus	MA	D	Z
Knipowitschia panizzae	ER		
Labrus bergylta	MA	D	IB
Labrus merula	MA	D	IB
Labrus mixtus	MA	D	IB
Labrus viridis	MA	D	IB
Lampetra fluviatilis	DIA	P	parasitic
Lepadogaster lepadogaster	ER	B	IB
Lepomis gibbosus	FW	D	IS
Lesueurigobius friesii	MA	B	IB
Leucaspius delineatus	FW	P	Z
Leuciscus cephalus	FW	P	O
Leuciscus idus	FW	P	O
Leuciscus leuciscus	FW	P	IS
Limanda limanda	MJ	B	IB

Lithognathus mormyrus	MJ	D	IS
Liza	DIA	D	O
Liza aurata	DIA	D	O
Liza ramada	DIA	D	O
Liza saliens	DIA	D	O
Luciobarbus bocagei	FW	D	IB
Merlangius merlangus	MJ	D	IS
Merluccius merluccius	MA	D	F
Microchirus azevia	MJ	B	IB
Microchirus variegatus	MA	B	IB
Micropterus salmoides	FW	P	F
Mugil cephalus	DIA	D	O
Mugilidae	DIA	D	O
Mullus barbatus	MA	B	IB
Mullus barbatus ponticus	ER	B	IB
Mullus surmuletus	MJ	B	IB
Neogobius	ER		IB
Neogobius cephalargoides	ER	D	IB
Neogobius gymnotrachelus	ER	B	IB
Neogobius melanostomus	ER	B	IB
Neogobius platyrostris	ER	D	IB
Nerophis lumbriciformis	ER	D	Z
Nerophis ophidion	ER	D	Z
Oreochromis niloticus niloticus	ER	D	O
Osmerus eperlanus	DIA	P	IS
Parablennius gattorugine	ER	B	O
Parablennius pilicornis	ER	B	O
Parablennius sanguinolentus	ER	B	V
Parablennius tentacularis	ER	B	O
Pegusa lascaris	MA	B	IB
Perca fluviatilis	FW	P	IS
Petromyzon marinus	DIA	P	parasitic
Petromyzon marinus, Lampetra fluviatilis	DIA	P	parasitic
Platichthys flesus	DIA	B	IB
Pleuronectes platessa	MJ	B	IB
Pleuronectidae		B	IB
Pollachius pollachius	MJ	D	F
Pomatoschistus	ER	B	
Pomatoschistus lozanoi	ER	B	IS
Pomatoschistus marmoratus	ER	B	IB
Pomatoschistus microps	ER	B	IB
Pomatoschistus minutus	ER	B	IB
Pomatoschistus pictus	ER	B	IB
Proterorhinus marmoratus	ER	B	IB
Psetta maxima	MJ	B	IB
Pseudorasbora parva	FW	P	O
Pungitius pungitius	FW	D	IS
Raja	MA	B	IB
Raja clavata	MA	B	IB
Raja undulata	MA	B	IB

Rajella fyllae	MA	B	IB
Rutilus rutilus	FW	P	O
Salaria pavo	ER	B	O
Salmo salar	DIA	P	IS
Salmo trutta fario	DIA	P	IS
Salmo trutta trutta	DIA	P	IS
Sander lucioperca	FW	D	F
Sardina pilchardus	MJ	P	Z
Sarpa salpa	MJ	D	V
Scardinius erythrophthalmus	FW	P	O
Scomber scombrus	MA	P	IS
Scophthalmus rhombus	MJ	B	IB
Scorpaena notata	MA	B	IS
Scorpaena porcus	MA	D	IS
Silurus glanis	FW	D	F
Solea	MJ	B	IB
Solea senegalensis	MJ	B	IB
Solea solea	MJ	B	IB
Sparidae			
Sparus aurata	MA	D	IS
Spinachia spinachia	ER	D	IS
Spondyliosoma cantharus	MJ	D	IS
Sprattus sprattus	MJ	P	Z
Symphodus bailloni	ER	D	O
Symphodus cinereus	ER	D	IS
Symphodus melops	ER	D	O
Symphodus ocellatus	ER	D	IS
Symphodus roissali	ER	D	IS
Symphodus tinca	ER	D	IS
Syngnathus	ER		
Syngnathus abaster	ER	D	Z
Syngnathus acus	ER	D	Z
Syngnathus rostellatus	ER	P	Z
Syngnathus taenionotus	ER	D	Z
Syngnathus tenuirostris	ER	D	Z
Syngnathus typhle	ER	D	F
Taurulus bubalis	MA	B	O
Torpedo marmorata	MA	B	IB
Trachurus mediterraneus	MJ	P	F
Trachurus trachurus	MJ	P	F
Trigla	MJ	B	IS
Trigla lyra	MJ	B	IS
Tripterygion delaisi	ER	B	IS
Trisopterus		D	IS
Trisopterus luscus	MJ	D	IS
Trisopterus minutus	MA	D	IS
Umbrina canariensis	MA	D	IS
Zebrus zebrus	ER	B	IS
Zeugopterus punctatus	MA	B	IS
Zeus faber	MA	D	F

Zoarces viviparus	ER	B	O
Zosterisessor ophiocephalus	ER	B	F

Annex 2: Correlation (Pearson coefficients) between the continuous covariates describing estuarine features and pressure metrics

Pearson correlation coefficients between the various continuous covariates describing estuarine features; annual discharge: mean annual river discharge; agr: percentage of agricultural areas in a 2 km buffer around estuaries; urb: percentage of urban areas in a 2 km buffer around estuaries; nat: percentage of natural land in a 2 km buffer around estuaries

	latitude	longitude	source elevation	continental shelf width	catchment area	mouth width	mouth depth	annual discharge	tidal range	agr	urb	nat
latitude	1											
longitude	0,32	1										
source elevation	-0,59	-0,08	1									
continental shelf width	**0,81**	0,57	-0,38	1								
catchment area	0,11	0,22	0,50	0,17	1							
mouth width	-0,36	-0,56	0,31	-0,08	0,01	1						
mouth depth	-0,18	0,02	0,29	-0,18	0,14	-0,14	1					
annual discharge	0,01	0,19	0,65	0,07	**0,95**	0,02	0,24	1				
tidal range	**0,77**	0,14	-0,45	0,63	0,08	-0,15	-0,17	0,01	1			
agr	0,60	-0,22	-0,23	0,40	0,07	0,22	-0,15	0,11	0,63	1		
urb	0,20	-0,07	-0,06	0,07	0,14	-0,04	0,36	0,12	-0,01	0,06	1	
nat	-0,55	-0,05	0,12	-0,48	-0,32	-0,11	-0,15	-0,31	-0,44	-0,65	-0,45	1