Report summarising the definitions of reference conditions using predictive models for ecological endpoints for fish in transitional waters: WISER Deliverable D.4.4-4

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SEVENTH FRAMEWORK PROGRAMME

WISER

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

DELIVERABLE

Deliverable D4.4-4: Report summarising the definition of reference conditions using predictive models for ecological endpoints for fish in transitional waters

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PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Content

Non-technical summary

In Europe, the Water Framework Directive aims at reaching good ecological status for surface waterbodies by 2015. Consequently European countries are developing methods based on biological (phytoplankton, macroalgae, angiosperms, macrobenthos and fishes), hydromorphological and physico-chemical quality elements for the ecological assessment and monitoring of rivers, lakes and coastal and transitional waters. In these methods, the value of ecological indicators are transformed into ecological status by comparison to the so called "reference conditions", *i.e.* the conditions of the indicator in pristine areas (absence of human pressure). Hence setting adequate reference conditions is clearly crucial for the sound assessment of ecological status (Borja et al. 2012). The European Framework 7 project WISER is supporting the implementation of the WFD by testing and complementing existing assessment schemes. The development of suitable methods or the improvement of existing methods for the definition of accurate reference conditions is one aim of WISER.

The present work focuses on fish-based quality indicators for estuaries and lagoons (transitional waters under WFD terminology). Fish assemblages highly depend on natural features, both temporal and geographical, at small and large scale. This is especially true in transitional waters where natural abiotic variability is extremely high (Dauvin et Ruellet 2009; Dauvin 2007; Elliott et Quintino 2007)(Elliott et Quintino 2007). Moreover, the measured indicators (or metrics) characterising fish assemblages highly depend on the sampling method and sampling characteristics. For these reasons, any reference condition for fish in transitional waters must take into account these parameters.

There are nearly no transitional waters in Europe that can be considered as being in pristine condition and historical data are not available for all transitional water types. In this context, the aim of the present work is to propose a modelling approach to define type-specific reference conditions for fish assemblages in transitional waters in Europe. The modelling of reference conditions was tested on 13 fish metrics overall, including seven of the most commonly used WFD fish metrics and all the metrics composing the French Estuary and Lagoon Fish Index ELFI. A fish dataset covering 39 estuaries and 14 lagoons distributed across six countries (Bulgaria, Italy, United Kingdom, France, Spain and Portugal) and sampled between 2003 and 2010 was available. For the modelling of reference conditions, two sub-datasets of the best standardized data were selected, one for estuaries and one for lagoons. The dataset for estuaries covers 38 estuaries and contains 1811 trawl hauls. The dataset for lagoons covers 12 lagoons and contains 295 data of fyke net-Cemagref samples collected after a standardised soaking time of about 24 hours. First, selected fish metrics were modelled using Linear Models (LM) and Generalized Linear Models (GLM) taking into account all variables from the sampling protocol, variables from the natural features of estuaries and lagoons and selected pressure indices (Courrat et al. 2009; Delpech et al. 2010; Drouineau et al. 2012), all as fixed effects (Bolker et al. 2009). Pressure indices were calculated from CORINE Land Cover (Commission of the European Communities 1994) data 2006 (http://sia.eionet.europa.eu/CLC2006/), except for Stour and Orwell estuary where only CORINE Land Cover 2000 was available. Land cover indices were calculated on three buffers of 1 km, 1.5 km and 2 km around estuaries and lagoons.

Second, best LM and GLM were translated in mixed LM (LMM) and mixed GLM (GLMM) with adding a random “estuary” or “lagoon” effect to avoid pseudoreplication in data (Bolker et al. 2009). Last, predictions from the best mixed models were used to define reference conditions. Predictions were made both for a theoretical pristine status (absence of pressure) and for the lowest values of pressure indices observed in the datasets.

Preliminary analyses lead to the selection of three pressure indices calculated on a 2 km buffer: the percentage of urban land, the percentage of agricultural land and the percentage of natural land. Only four fish metrics out of the twelve tested for lagoons, and five metrics out of the thirteen tested for estuaries were significantly related to one of the three pressure indices, and effect of pressure indices was always very low (close to zero) in the selected LM and GLM. This may be due to the fact that pressure indices based on land cover and measured on buffers around estuaries and lagoons are not good proxies for human pressure impacting fishes in these transitional waters. When adding a random effect to the LM and GLM, this lead to only three fish metrics responding significantly to some pressure indices. In the end, predictions were computed from the models by linking (respectively) the number of marine migrant species (SR_MM) with the percentage of agricultural land around lagoons, the density of benthic invertebrate feeder fishes (DIB) with the percentage of urban land around lagoons and the percentage of omnivorous individuals (RD_O) with the percentage of natural land around estuaries.

All models (including those where fish metrics were not responding significantly to pressure indices) showed a major effect of the sampling method and of natural features of estuaries and lagoons on fish metrics. In particular, the present work argues for the definition of reference conditions specific to sampling gear, sampling season and salinity class. The approach gives interesting results for number of species. For densities and relative densities, results are promising but improvements to the models are required. Results argues for a lack of robustness of the approach for density metrics, especially in the case of fishing events containing very high numbers of fishes, or, in other words, the approach seems very sensitive to outliers. Careful attention should be paid to fish metrics supposed to vary in the same way as pressure, such as RD_O. Indeed, in this particular case, the present approach leads to predicted null relative densities in pristine status.

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 are developing methods based on biological (phytoplankton, macroalgae, angiosperms, macrobenthos and fishes), hydromorphological and physico-chemical quality elements for the ecological assessment and monitoring of rivers, lakes and coastal and transitional waters. In these methods, the value of ecological indicators are transformed into ecological status by comparison to the so called "reference conditions", *i.e.* the conditions of the indicator in pristine areas (absence of human pressure and assumed to present the highest conservation status). Hence, setting adequate reference conditions is clearly crucial for the sound assessment of ecological status (Borja et al. 2012). The European Framework 7 project WISER is supporting the implementation of the WFD by testing and complementing existing assessment schemes. The development of suitable methods or the improvement of existing methods for the definitions of accurate reference conditions is one aim of WISER.

The present work focuses on fish-based quality indicators for estuaries and lagoons (transitional waters under WFD terminology). Fish are known to be useful ecological indicators as they present multiple advantages for a high-level quality 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. This is especially true in transitional waters where natural abiotic variability is extremely high (Dauvin et Ruellet 2009; Dauvin 2007; Elliott et Quintino 2007). Moreover, the measured indicators (or metrics) characterising fish assemblages highly depend on the sampling method and sampling characteristics. For these reasons, any reference condition for fish in transitional waters must take into account the sampling protocol used for the calculation of the fish indicator (*e.g.* sampling device, sampling season; (Delpech et al. 2010)) as well as the natural features of the sampled transitional water. The identification of transitional water typologies in the WFD (hence the need to define reference conditions that are type-specific) partly accounts for the influence of this latter source of variability on biological communities in transitional waters, although a residual natural variability still occurs among water bodies of a same type, and this might have a relevant influence on fish assemblage characteristics (Courrat et al. 2012).

The WFD proposes three approaches to built type specific biological reference conditions: (i) the spatially based identification of pristine sites, *i.e.* existing undisturbed sites or sites with only very minor disturbance; (ii) modelling by using hindcasting methods (based on historical data and information) or predictive models; or (iii) expert judgement (as a last resort) (European Council 2000; Borja et al. 2004). A combination of these methods can also be used. However, there are nearly no transitional waters in Europe that can be considered as being in pristine ecological status and historical data are not available for all transitional waters types. In this context, the aim of the present work is to propose a predictive modelling approach to define reference conditions for fish in transitional waters in Europe.

Here the modelling of reference conditions was tested on 13 fish metrics overall, including seven of the most commonly used WFD fish metrics and all the metrics that compose the French Estuary and Lagoon Fish Index ELFI. A fish dataset covering 39 estuaries and 14 lagoons distributed across six countries and sampled between 2003 and 2010 was available for this exercise.

Materials and methods

WP44 database: fish data, environmental data and data on estuarine and lagoons features

Five fish datasets were compiled in an Access database called WP44 DB (Table 1). These datasets contain data from fish surveys in 39 estuaries and 14 lagoons distributed in 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 3249 fishing events. A fishing event is described as a beam trawl haul, a seine haul or a fyke net visit.

For each fishing event biological data, data from the sampling protocol and environmental data were recorded. Biological data are the number of fish caught for each species (or family or gender when it was not possible to identify catches at the species level) and their body size. Data from the sampling protocol include date and time of sampling, geographic coordinates of the fishing events, duration of soaking for fyke nets and swept area for beam trawls. Environmental data varied and have irregular coverage. For example, salinity class is available for most (94 %) of the fishing events, while pH was recorded for only 10 % of the fishing events. Other environmental parameters are: depth (85 % of fishing events), temperature (91 %) and oxygen saturation (53 % but with high uncertainty on some measures).

Data on estuaries were also included, as derived from (Nicolas, Lobry, Lepage, et al. 2010): source elevation, littoral substrate, continental shelf width, catchment area, estuarine area, entrance width and entrance depth, mean annual river discharge, wave exposure, tidal range, percentage of intertidal area. Data on lagoons such as area and total cross-section of inlets were obtained from Irstea (formerly Cemagref) and Google Earth. Data on estuaries and lagoons were all collected at the estuary or lagoon level, hereafter referred as “systems”, *i.e.* entire lagoons and estuaries, as it appears more relevant from an ecological point of view than, for example the WFD water body selection criteria which differ between countries and sometimes between systems from the same country. A further benefit is the possibility to make direct geographical comparisons between the whole systems.

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

Dataset (country)	**Data source**	**Years of sampling**	**Number of estuaries and lagoons**	**Number of fishing events**	**Sampling gears**
Basques estuaries (ES)*	Basque Water Agency and AZTI	2008, 2009, 2010	12 estuaries	342	Beam trawl
Mondego estuary (PT)**	IMAR-CMA	2003, 2004	1 estuary	74	Beam trawl
French estuaries and lagoons (FR)***	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

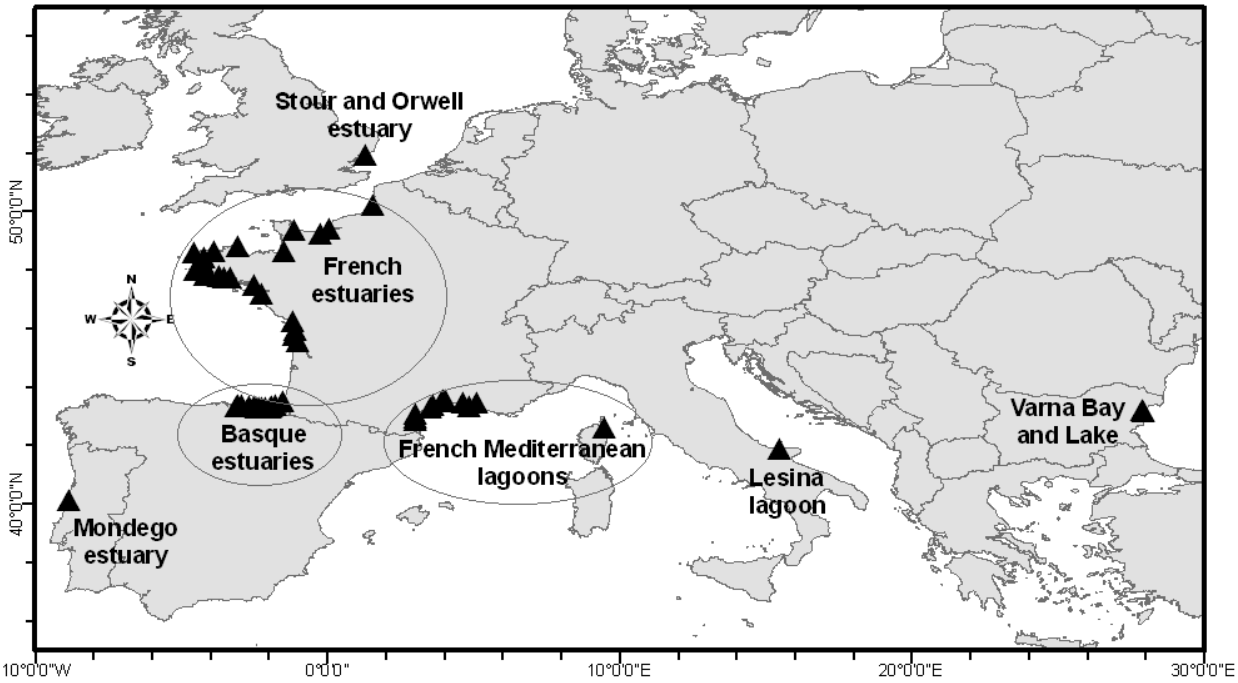


Figure 1: map of estuaries and lagoons where fish data were available in WP44 database for the following analyses

Pressure data

Data of anthropogenic pressure for all estuaries and lagoons were obtained using CORINE Land Cover (Commission of the European Communities 1994) 2006 (http://sia.eionet.europa.eu/CLC2006/), except for Stour and Orwell estuary where only CORINE Land Cover 2000 was available. 3 buffers of 1 km, 1.5 km and 2 km around the estuaries and lagoons were considered. For each buffer, 4 pressure indices based on CLC data were tested (Table 2). Pastures were excluded from agricultural areas because their potential impact on fishes in estuaries and lagoons is considered as low.

Normed Principal Component Analyses (PCA) were computed on sites (active individuals, in row) x pressure indices (active variables, in column) matrices. The aim was to synthesize the data and reduce the number of variables, if possible. PCA were performed separately for estuaries and lagoons because it is believed that relevant pressure indices may differ between these two types of transitional waters.

Table 2: pressure indices calculated from CORINE land cover data (Commission of the European Communities 1994) on 3 buffers of 1 km, 1.5 km and 2 km around the considered estuaries and lagoons.

Pressure indices	CORINE land cover nomenclature
Percent of urban areas	1.1 Urban fabric
Percent of industrial areas	1.2 Industrial, commercial and transport units 1.3 Mine, dump and construction sites
Percent of agricultural areas	2.1 Arable land 2.2 Permanent crops 2.4 Heterogeneous agricultural areas
Percent of natural areas (indicates the absence of pressure)	3. Forest and semi-natural areas 4. Wetlands 5. Water bodies

Fish metrics selection and calculation

The 7 fish metrics selected in Deliverable 2, part 2 (Courrat et al. 2012) were tested in the present work, together with the 6 fish metrics composing the French fish index ELFI (Table 3). These metrics cover the commonly used ways of quantifying fish attributes and some of the characteristics of the fish assemblage that are the most commonly assessed (Perez-Dominguez et al. 2010).

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, salinity or gear type) (Courrat et al. 2009; Delpech et al. 2010). Abundances found in seine net and beam trawl samples were standardised per the sampled surface. For fyke nets, densities are number of fish caught between two fyke net collections (around 24 hours for Cemagref fyke net for lagoons and 12 hours for all other fyke nets). Concerning number of species, for beam trawls and seine nets they were standardised by the log-transformed sampled surface (Nicolas, Lobry, Lepage, et al. 2010), while for fyke nets we used the number of species caught per unit of fyke effort defined as 12h tandem fyke arrangement (2 fykes) deployment, or 24h single Cemagref lagoon fyke deployment for estuaries or lagoons, respectively.

Table 3: Fish metrics tested in the present exercise, origin and expected trend with increasing pressure

Selected fish metrics	Abbreviation	Origin of metric: ELFI Index lagoon / estuary and/or metric commonly used in WFD assessment	Expected trend with increasing pressure (or decreasing natural areas)
Total density	TD	commonly used in WFD assessment & ELFI estuary	decrease
Total number of species	SR	commonly used in WFD assessment & ELFI estuary	decrease
Number of estuarine resident species	SR_ER	commonly used in WFD assessment	decrease
Number of marine migrating species	SR_MM	commonly used in WFD assessment	decrease
Percentage of piscivorous individuals	RD_P	commonly used in WFD assessment	decrease
Percentage of omnivorous individuals	RD_O	commonly used in WFD assessment	increase
Density of marine migrants	DMM	commonly used in WFD assessment & ELFI estuary mesohaline and polyhaline zone	decrease
Density of diadromous species	DDIA	ELFI estuary and lagoon	decrease
Density of benthic species	DB	ELFI estuary	decrease
Density of estuarine resident	DER	ELFI estuary	decrease
Density of freshwater species	DFW	ELFI estuary oligohaline zone	decrease
Density of zooplankton feeder species	DZ	ELFI lagoon	increase
Density of benthic invertebrate feeder species	DIB	ELFI lagoon	decrease

Modelling fish metrics

Choice of appropriate sub-datasets from WP44 database

For the modelling of reference conditions, two sub-datasets of the best standardized data (based on sampling method) were selected, one for estuaries and one for lagoons. The sub-dataset for estuaries covers 38 estuaries and contains 1811 trawl hauls. The sub-dataset for lagoons covers 12 lagoons and contains 295 fyke net collections employing a Cemagref-type fyke net (Table 4). Selected estuaries belong to the North-East Atlantic Geographical Intercalibration Group (NEA-GIG, as defined in the Intercalibration process) and selected lagoons belong to the Mediterranean GIG (MED-GIG) (European Communities 2005). In the NEA-GIG only one type for transitional waters was defined, namely the type TW-NEA11 (van de Bund et al. 2004). For lagoons, a typology was designed by the MED-GIG for the intercalibration process. It is based on two criteria: the size of lagoons (*i.e.* small: < 2.5 km^2 or big: > 2.5 km^2) and the salinity in two classes (> or < to 18) (Mario Lepage, pers. com.). All lagoons selected in the sub-dataset for lagoons belong to the size class “small”. The salinity class is accounted for later in the modelling process, at the scale of the fishing event.

Table 4: Sub-datasets selected for the present 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 in France and Varna Bay in Bulgaria where data of estuarine features are missing)	12 (all except Varna Lake in Bulgaria 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)

Pressure impact models for fish metrics

Fish metrics were first modelled using Linear Models (LM) or Generalized Linear Models (GLM) as advised by (Bolker et al. 2009). Models took into account variability arising from sampling, natural parameters at the fishing event scale or at the estuary or lagoon scale and finally anthropogenic pressure (Courrat et al. 2009; Delpech et al. 2010; Drouineau et al. 2012):

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 features at the local (*i.e.* fishing event) or whole system level. Modelling options depend on data distribution for the different fish metrics (Courrat et al. 2012; Delpech et al. 2010; Drouineau et al. 2012). Numbers of species in lagoons (fyke net data) were modelled using the Poisson distribution, whereas for estuaries (beam trawl data) the normal (or Gaussian) distribution was used on number of species standardised by the log-transformed sampled surface (Nicolas, Lobry, Lepage, et al. 2010). For density metrics, Gaussian models were built on log(n+1)-transformed catch per unit effort data (Drouineau et al. 2012). Relative densities (*i.e.* percentage of omnivorous and piscivorous individuals) were also modelled using a Gaussian law to avoid giving too much weight to fishes caught in school, which would be the case with a Binomial distribution.

Considering available data for lagoons, the following variables were tested in the models as fixed effects (Drouineau et al. 2012): salinity class (*Sal class,* class factor in 3 classes: oligohaline (0-5), mesohaline (5-18) and polyhaline (>18)), temperature measured at the bottom (*Temp*, continuous covariate), *Season* (class factor), lagoon area (*Area*, continuous covariate) and cross-sectional area of the inlets (*Sect*, class factor in 2 classes). Depth was not taken into account because it is relatively constant as it ranges from 0.4 m to 2.5 m only. For estuaries, selected variables are salinity class (*Sal class,* class factor in 3 classes: oligohaline (0-5), mesohaline (5-18) and polyhaline (>18)), *Depth* (continuous covariate), *Season* (class factor) and variables describing estuarine features (Courrat et al. 2009; Delpech et al. 2010; Nicolas, Lobry, Le Pape, et al. 2010; Nicolas, Lobry, Lepage, et al. 2010): latitude (*Lat*, continuous covariate), estuarine area (*Area class*, class factor in 3 classes), entrance width (*Ent width*, continuous covariate), continental shelf width (*Shelf width*, continuous covariate), mean annual river discharge (*Discharge*, continuous covariate) and percentage of intertidal area (*Intertidal area*, class factor in 5 classes). 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). Significance of fixed effect is tested at the level of 5 % (Chi-squared test at 5 % level).

Pressure indices *Pr* are added separately at the end of the models once significant variables linked to sampling and to estuarine and lagoon features are selected (Courrat et al. 2009). The significance of pressure indices is also tested (Chi-squared test at 5 % level). Only fish metrics responding significantly and in the expected way to pressure index (indices) are kept for further analyses.

Best LM and GLM are then translated in either Linear Mixed Models (LMM) or Generalized Linear Mixed Models (GLMM) as appropriate, with adding an estuary or lagoon random factor to take into account that the various fishing events performed in an estuary or in a lagoon are not completely independent, *i.e.* to avoid pseudoreplication in the data (Bolker et al. 2009). Mixed models were computed using the function glmmPQL of library MASS (Venables et Ripley 2002) on R software (R Development Core Team 2009). This function approximates the likelihood using the penalized quasilikelihood (PQL) and tests a null hypothesis of no effect for each effect using Wald tests (Bolker et al. 2009). It was chosen because it both gives p-values on the significance of fixed effects and it can compute GLMM based on non-Normal distributions such as Poisson. However, Wald tests are based on likelihood while in mixed models only approximations of the likelihood can be estimated. Hence it is unclear how reliable is the p-value for the significance of the fixed effects in glmmPQL outputs. For this reason a 10 % level of significance was chosen to take into account possible bias in the p-values.

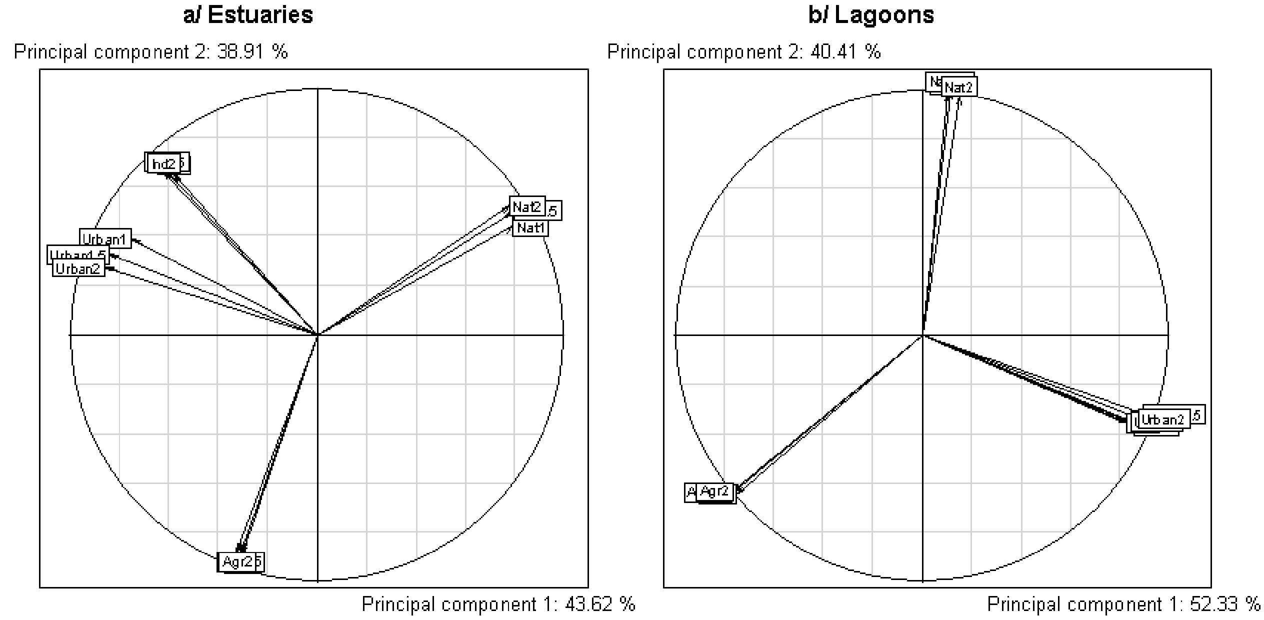
Predicting reference conditions

Fitted values from the mixed models are used to estimate the reference values of each fish metric responding significantly and in the expected way to some pressure index (indices). Two types of references are tested: theoretical reference at zero level of pressure and reference set to the lowest values of pressure indices observed in the datasets (least impacted site approach). Reference values are compiled per combination of variables from the sampling protocol and estuaries or lagoons. Predictions are computed using the function predict of package MASS (Venables et Ripley 2002).

Results

Pressure data

First, PCA were computed on data from the three CORINE buffers to compare them. These PCA show that pressure indices from the three buffers are highly correlated, both for estuaries and lagoons (Figure 2). Hence it was decided to choose only one distance buffer for the modeling exercise.


a/ Estuaries

b/ Lagoons

Figure 2: PCA computed on sites (active individuals, in row) x pressure indices (active variables, in column) matrices for the 3 buffers of 1 km, 1.5 km and 2 km around the estuaries(a/) and lagoons (b/). Nat: Percent of natural areas; Agr: Percent of agricultural areas; Urban: Percent of urban areas; Ind: Percent of industrial areas; 1, 1.5, 2: buffer size in km.

Because they include more information, we chose to work with pressure indices calculated on the 2 km buffer (Table 5). A second PCA was performed on these data in order to decide if pressure indices should be combined or not (Figure 3). The first two principal components of these PCA explain 85.4 % of the total inertia for estuaries and 93.3 % for lagoons. The PCA on estuaries reveals that the percentage of urban areas and the percentage of industrial areas are correlated, thus only one of these pressure indices (percentage of urban areas) was tested in the models. This correlation is even more obvious in the case of lagoons. The PCA analysis also indicates that estuaries (or lagoons) seem to be characterized by one of these pressure indices only, rarely by two or three of them (Figure 3). Based on these results, the three remaining indices (percentage of urban areas, percentage of natural areas and percentage of agricultural areas) were tested separately in the models.

Figure 4a and 4b present the values of the 3 pressure indices selected for being tested in the models and the classification of estuaries and lagoons according to these pressure indices.

Table 5: Overview of the values of the four pressure indices calculated on a 2 km buffer around the studied estuaries and lagoons

		Agricultural areas (%)	Industrial areas (%)	Natural areas (%)	Urban areas (%)
Estuaries	minimum	0	0	4.32	1.08
	maximum	72.81	16.73	69.75	32.66
	median	42.48	1.29	27.11	10.70
Lagoons	minimum	17.18	0	14.12	0.57
	maximum	84.75	14.92	81.71	23.25
	median	29.56	3.80	49.26	10.66

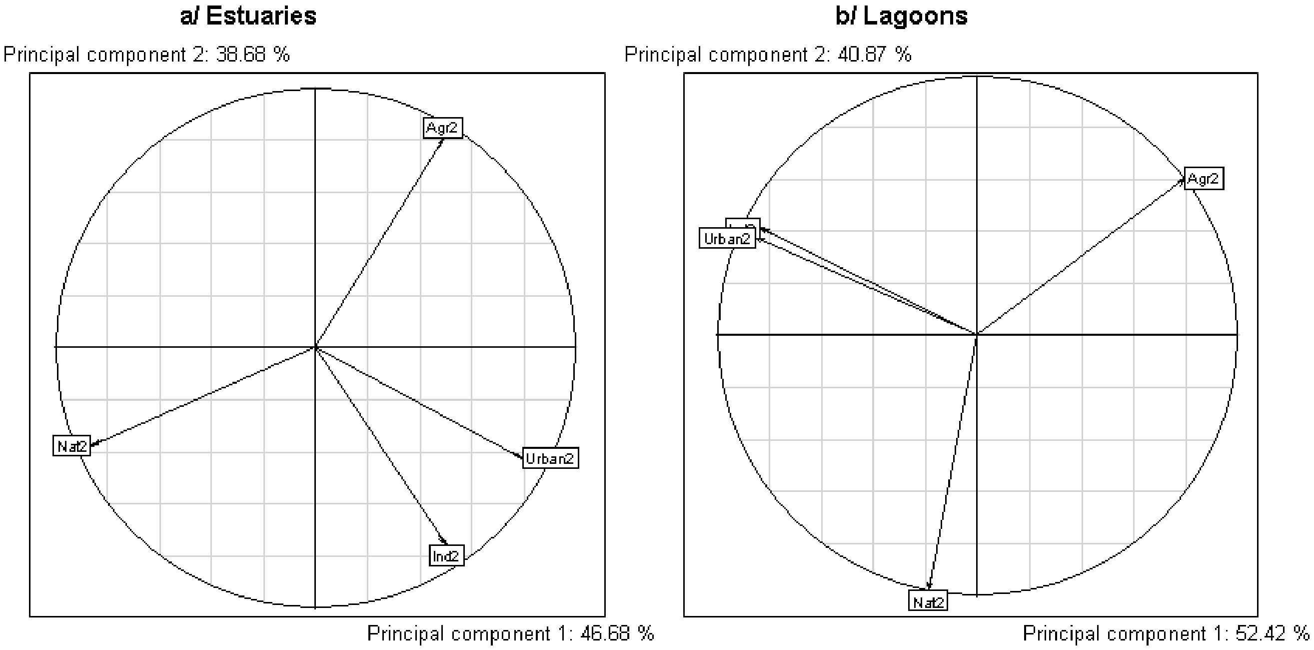


Figure 3: PCA computed on sites (active individuals, in row) x pressure indices (active variables, in column) matrices for a 2 km buffer around the estuaries (a/) and lagoons (b/). Nat: Percent of natural areas; Agr: Percent of agricultural areas; Urban: Percent of urban areas; Ind: Percent of industrial areas; 2: buffer size in km.

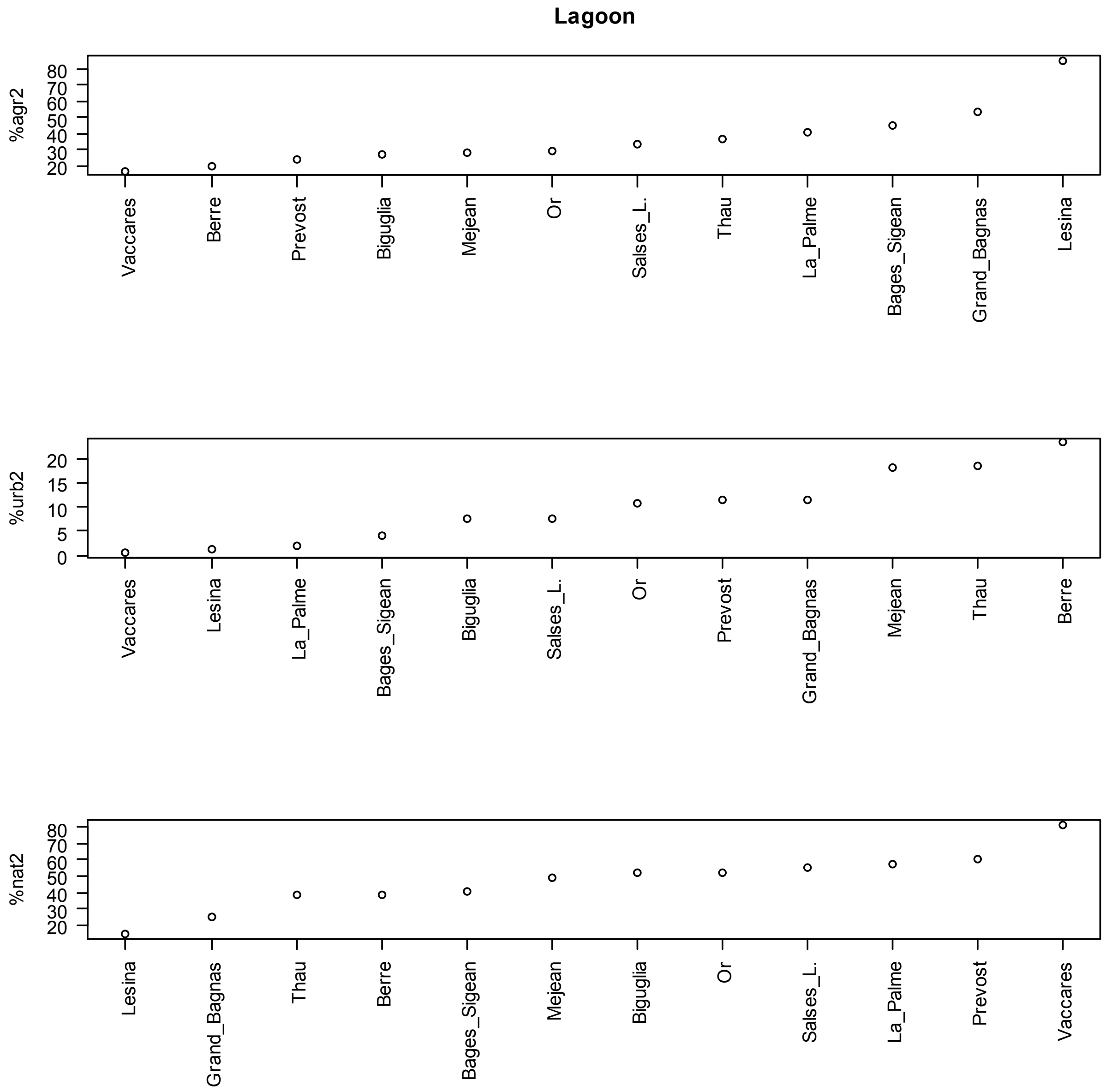


Figure 4a: Percentage of agricultural areas (%agr2), percentage of urban areas (%urb2) and percentage of natural areas (%nat2) calculated on a 2 km buffer around the 12 lagoons studied here and classified along the horizontal axis according to an ascending level.

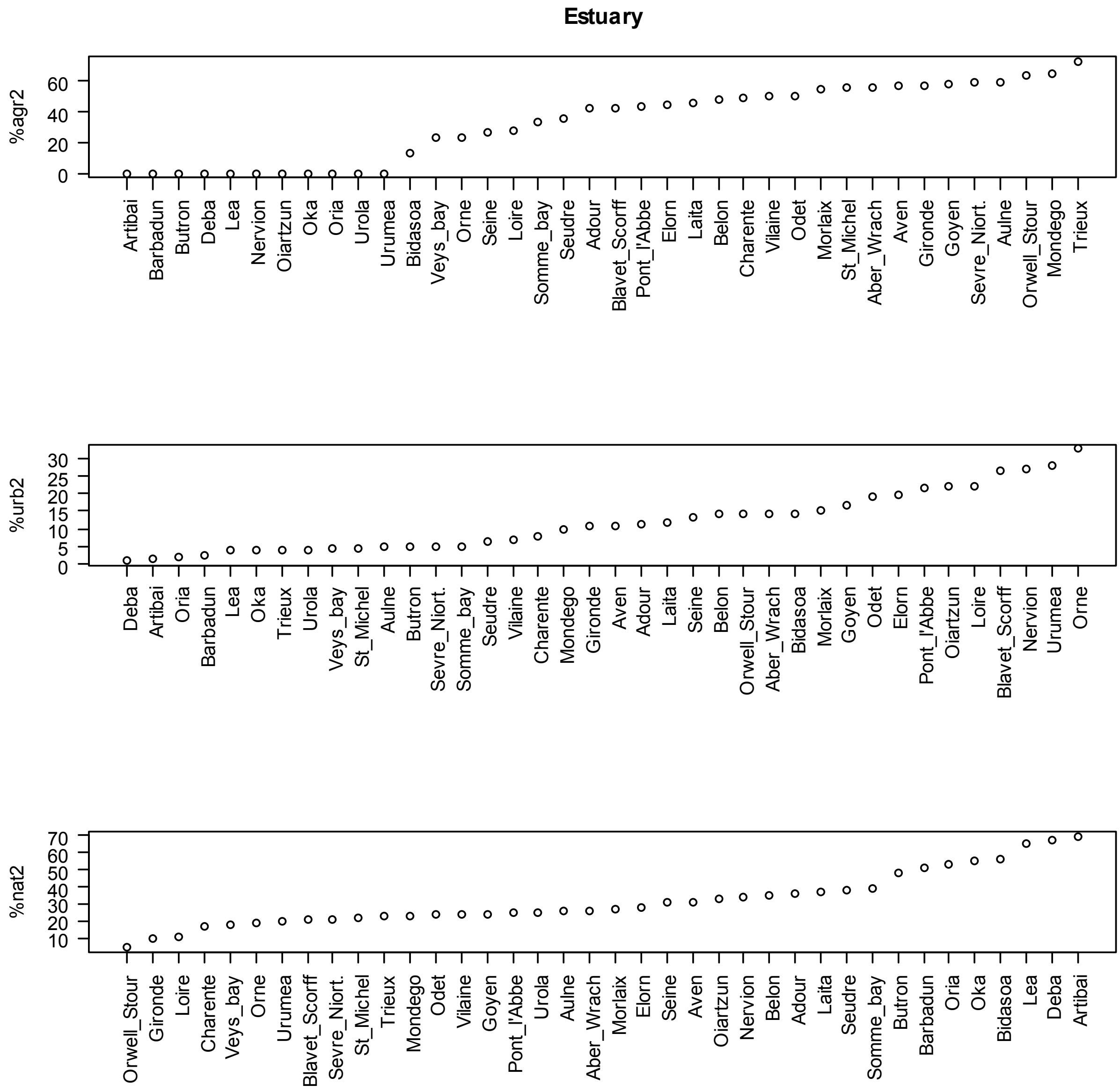


Figure 4b: Percentage of agricultural areas (%agr2), percentage of urban areas (%urb2) and percentage of natural areas (%nat2) calculated on a 2 km buffer around the 38 estuaries studied here and classified along the horizontal axis according to an ascending level.

Pressure impact models

Tables 6 and 7 present the LM and GLM results for lagoons and estuaries, respectively. For lagoons, only 12 fish metrics were tested as the density of freshwater species (DFW) comprised more than 85 % of zeros and it is believed to be more linked to natural characteristics of the studied lagoons (where salinity is superior to 5 in 98 % of fishing events) than to anthropogenic pressure. Models show that many fish metrics did not respond significantly or as expected to pressure indices. Significant effects of pressure indices are always very small as slopes (regression parameter) are always very close to zero. The percentage of natural areas often shows an unexpected effect, when significant, on fish metrics, except for SR_MM and DB in lagoons and RD_O in estuaries.

The best LM and GLM (Tables 6 and 7, in bold) were translated in mixed models (LMM and GLMM) with system (estuary or lagoon) as a random factor. Only three mixed models keep showing a significant (p-value at 10 % level) effect of some pressure indices on fish metric. For lagoons, significant models linked SR_MM to percentage of agricultural areas and DIB to percentage of urban areas, and for estuaries, RD_O to percentage of natural areas.

Table 6: Results of LM and GLM computed on fish data from lagoons: when significant (Chi-squared test at 5 % level) effect of pressure indices (regression parameter) and corresponding model are presented. NS: non-significant; NA: non-applicable. In bold: models selected for mixed model analysis. Highlighted in grey: best models selected after mixed model analysis.

Fish metric	Model	Agr	Urb	Nat
TD	NA	NS	NS	NS
SR	NA	NS	NS	NS
DER	NA	NS	NS	NS
SR_ER	*Sal class + Season + Sect + Pr*	+0.009	NS	NS
DMM	***Sal class + Temp + Pr***	**-0.036**	+0.033	NS
SR_MM	***Sal class + Pr***	**-0.043**	+0.025	**+0.013**
RD_O	*Season + Sect*	NS	NS	+0.304
RD_P	NA	NS	NS	NS
DDIA	NA	NS	NS	NS
DB	***Sal class + Season + Sect + Area + Pr***	**-0.028**	**-0.049**	**+0.036**
DZ	*Sal class + Sect + Area + Pr*	-0.029	**+0.049**	NS
DIB	***Sal class + Season + Sect + Area + Pr***	+0.044	**-0.06**	NS

Table 7: Results of LM and GLM computed on fish data from estuaries: when significant (Chi-squared test at 5 % level) effect of pressure indices (regression parameter) and corresponding model are presented. NS: non-significant. In bold: models selected for mixed model analysis. Highlighted in grey: best models selected after mixed model analysis.

Fish metric	Model	Agr	Urb	Nat
TD	*Sal class + Depth + Season + Lat + Area class + Shelf width + Ent width + Intertidal area + Pr*	+0.014	NS	-0.010
SR	***Sal class + Depth + Season + Lat + Area class + Shelf width + Ent width + Intertidal area + Pr***	+0.003	**-0.003**	NS
DER	*Depth + Season + Lat + Area class + Ent width + Intertidal area + Pr*	+0.010	NS	-0.015
SR_ER	*Sal class + Season + Lat + Area class + Shelf width + Ent width + Discharge + Intertidal area + Pr*	NS	+0.001	NS
DMM	***Sal class + Depth + Season + Lat + Area class + Shelf width + Ent width + Discharge + Intertidal area + Pr***	+0.017	**-0.018**	-0.007
SR_MM	***Sal class + Depth + Lat + Area class + Shelf width + Ent width + Discharge + Intertidal area + Pr***	+0.003	**-0.004**	-0.001
RD_O	***Sal class + Season + Discharge + Intertidal area + Pr***	**+0.041**	NS	**-0.103**
RD_P	*NA*	NS	NS	NS
FW	*NA*	NS	NS	NS
DDIA	*Sal class + Depth + Season + Lat + Area class + Ent width + Discharge + Intertidal area + Pr*	NS	NS	-0.007
DB	*Sal class + Depth + Season + Lat + Area class + Shelf width + Ent width + Intertidal area + Pr*	+0.006	NS	-0.01
DZ	***Sal class + Depth + Lat + Area class + Shelf width + Discharge + Intertidal area + Pr***	**+0.009**	-0.027	+0.007
DIB	*Sal class + Depth + Season + Lat + Area class + Ent width + Pr*	+0.011	+0.011	-0.017

Predictions of reference conditions from the models

Predictions of reference conditions were computed on the three best mixed models highlighted in Tables 6 and 7 (Figure 5, 6 and 7 respectively). These models cover estuaries and lagoons, the three main types of metrics (density, relative density and number of species) and each of the three selected pressure indices.

Predictions were made only for those combinations of system (thus taking into account the natural features) x salinity class x season (if significant in the model) observed in the data, in order to rule out from the definition of reference conditions the significant influence of these natural factors of variability on fish metrics in transitional waters (Figures 5, 6, 7). The mean value of SR_MM, DIB and RD_O in the analysed datasets were also plotted on the graphs. It is important to note that predictions are also given by gear, as models were realised separately for beam trawls data (estuary sub-datasets) and fyke nets data (lagoon sub-dataset). This means that this approach produces different references for different sampling gears, salinity classes and seasons. Moreover, the use of mixed models also leads to different predictions between estuaries (Figure 5 to 7), thus taking into account the residual natural variability that could not be accounted for in the models considering available data.

Figure 5 shows that the number of marine migrating species in the theoretical reference status often appears relatively high compared to the least impacted site. Figure 6 shows that the approach may lead to the prediction of negative densities, although this is mainly ascribed to the data transformation and back-transformation. DIB was modelled after being log(n+1)-transformed. Predictions given in the log scale were back- transformed to the original scale using a simple exp(prediction)-1 transformation, which may lead to the prediction of densities comprised between 0 and -1. One solution to overcome this problem could be to use a delta model coupling the probability of presence and the log density of IB species when present (Courrat et al. 2009; Delpech et al. 2010; Le Pape et al. 2003; Stefánsson 1996) instead of the log(n+ 1) transformation of data. Predictions of DIB for the lagoon Complexe Vaccares are sometimes very high compared to similar predictions for other lagoons (sometimes more than 3 times higher). This is probably due to 3 fishing events containing more than 1500 *Pomatoschistus microps* in Complexe Vaccares. This argues for a lack of robustness of the approach for density metrics, especially in the case of fishing events containing very high numbers of fishes, or, in other words, the approach seems very sensitive to outliers.

Concerning relative density (RD_O, Figure 7a and 7b), predictions for the least impacted site and for the theoretical reference are often inferior to zero. This is because relative densities were modelled using a Gaussian law. The use of a Binomial distribution would allow overcoming this problem but it will lead to overweighting the impact of fishes caught in school. Another bias of the metric RD_O is also linked to the fact that RD_O is supposed to decrease with decreasing pressure (Table 3). This leads to null RD_O in reference status, which appears unrealistic. This argues for caution in the use of fish metrics supposed to vary in the same way as pressure. In this particular case, the present approach of modelling reference conditions is probably not appropriate and should be adapted, for example by setting a minimum threshold to the fish metric.

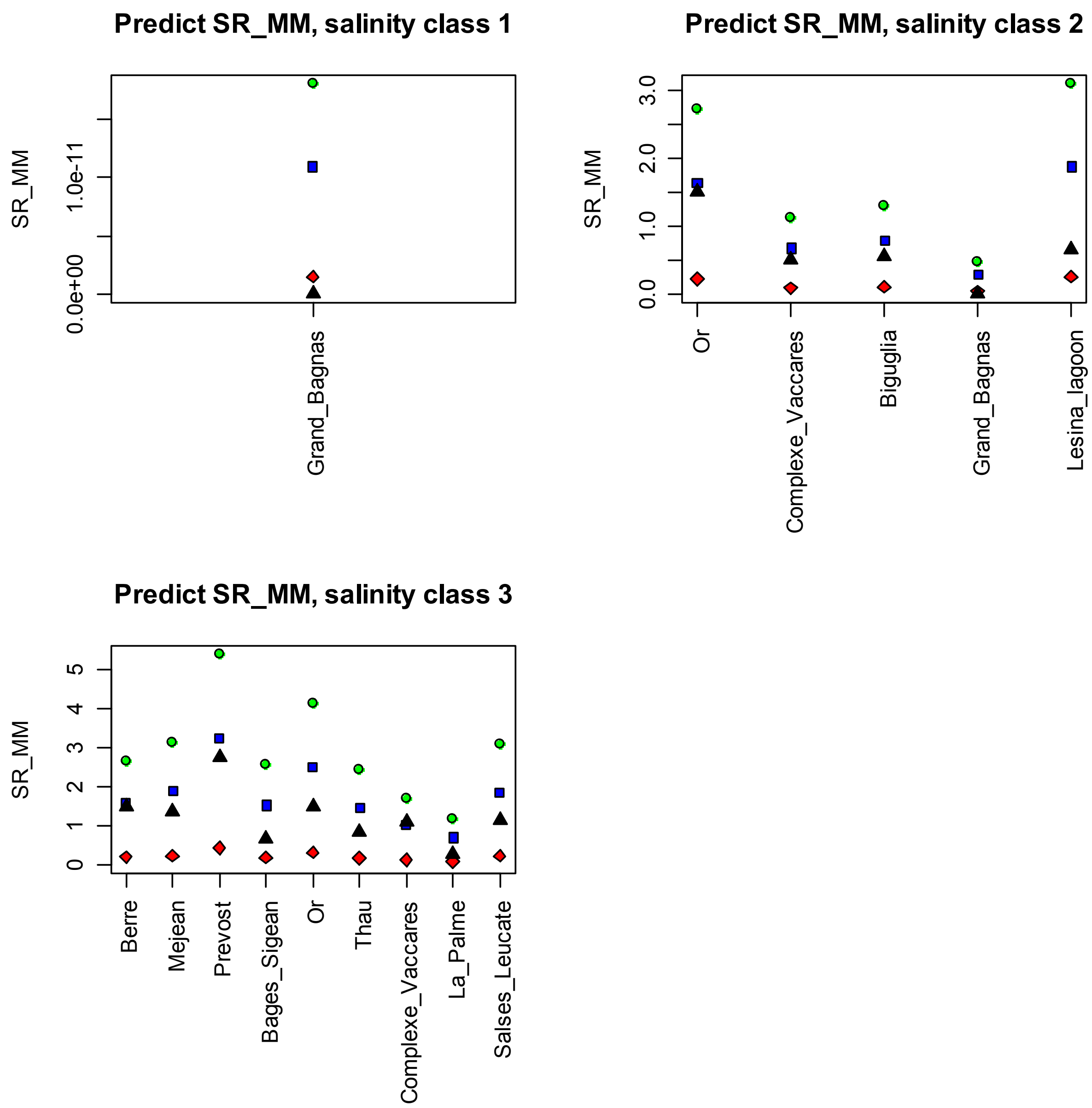


Figure 5: Predictions from the model linking the number of marine migrating species (SR_MM) with the percentage of agricultural land on a 2 km buffer around lagoons, for the 3 considered salinity classes (class 1: oligohaline (0-5), class 2: mesohaline (5-18) and class 3: polyhaline (>18)). Model can be written: ***SR_MM ~ Sal class + Agr****. Green circles: theoretical reference at zero percent of agricultural land; blue squares: percent of agricultural land set to the level of the least impacted site; black triangles: true mean value of SR_MM observed in fish data; red diamonds: agriculture pressure set to the worst level observed among the 12 lagoons.*

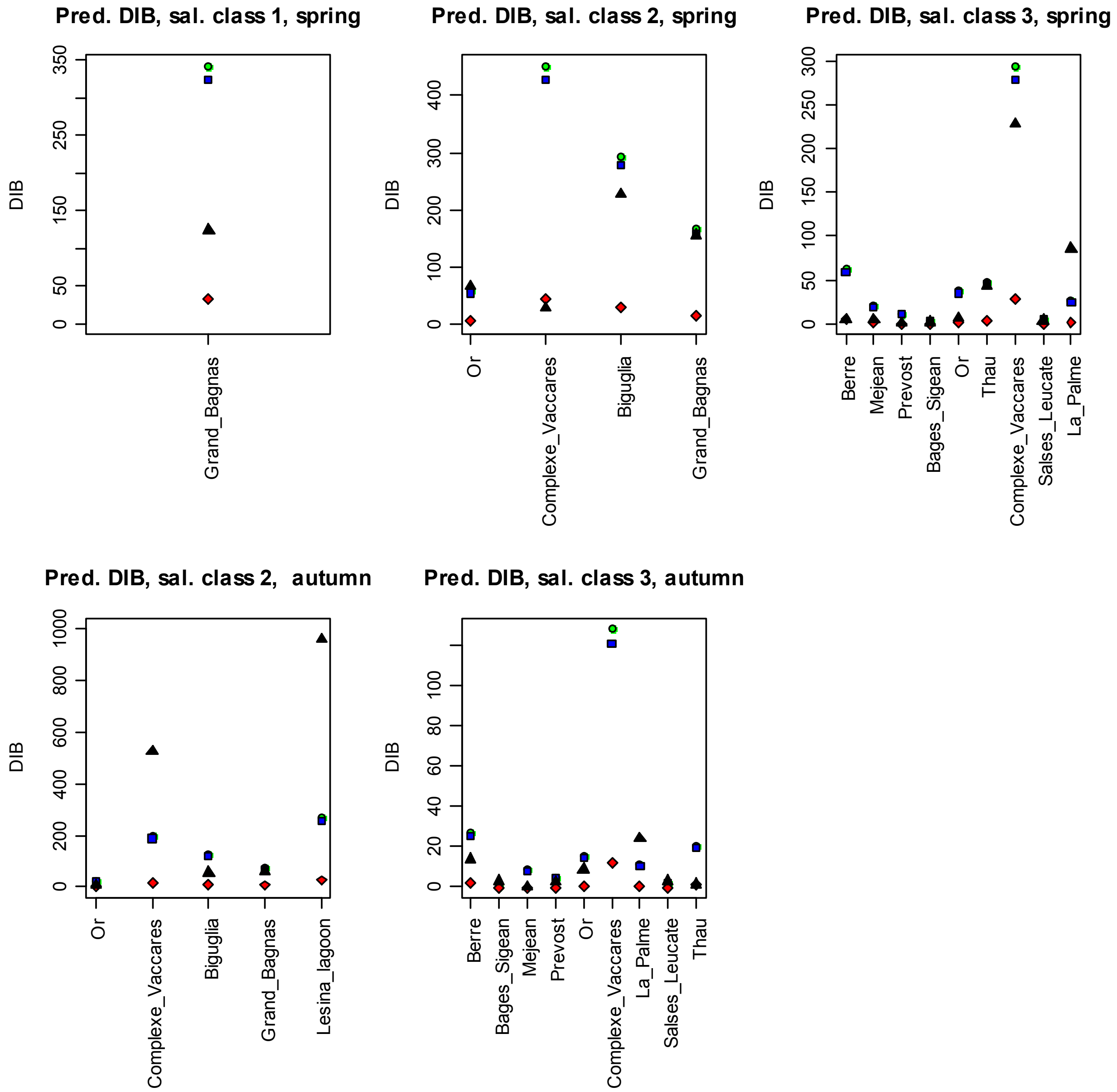


*Figure 6: Predictions from the model linking the density of benthic invertebrate feeder fishes (DIB) with the percentage of urban land on a 2 km buffer around lagoons. Model can be written: **DIB ~ Sal class + Season + Sect + Area + Urb**. Green circles: theoretical reference at zero percent of urban land; blue squares: percent of urban land set to the level of the least impacted site; black triangles: true mean value of DIB observed in fish data; red diamonds: urban pressure set to the worst level observed among the 12 lagoons. Pred. DIB: predict values of DIB; sal. class: salinity class (1: oligohaline (0-5), 2: mesohaline (5-18) and 3: polyhaline (>18)). For the lagoon Complexe Vaccares, salinity class 3, autumn, the true mean value of DIB observed in fish data is 1260.5 (far beyond the maximum value of the y axis).*

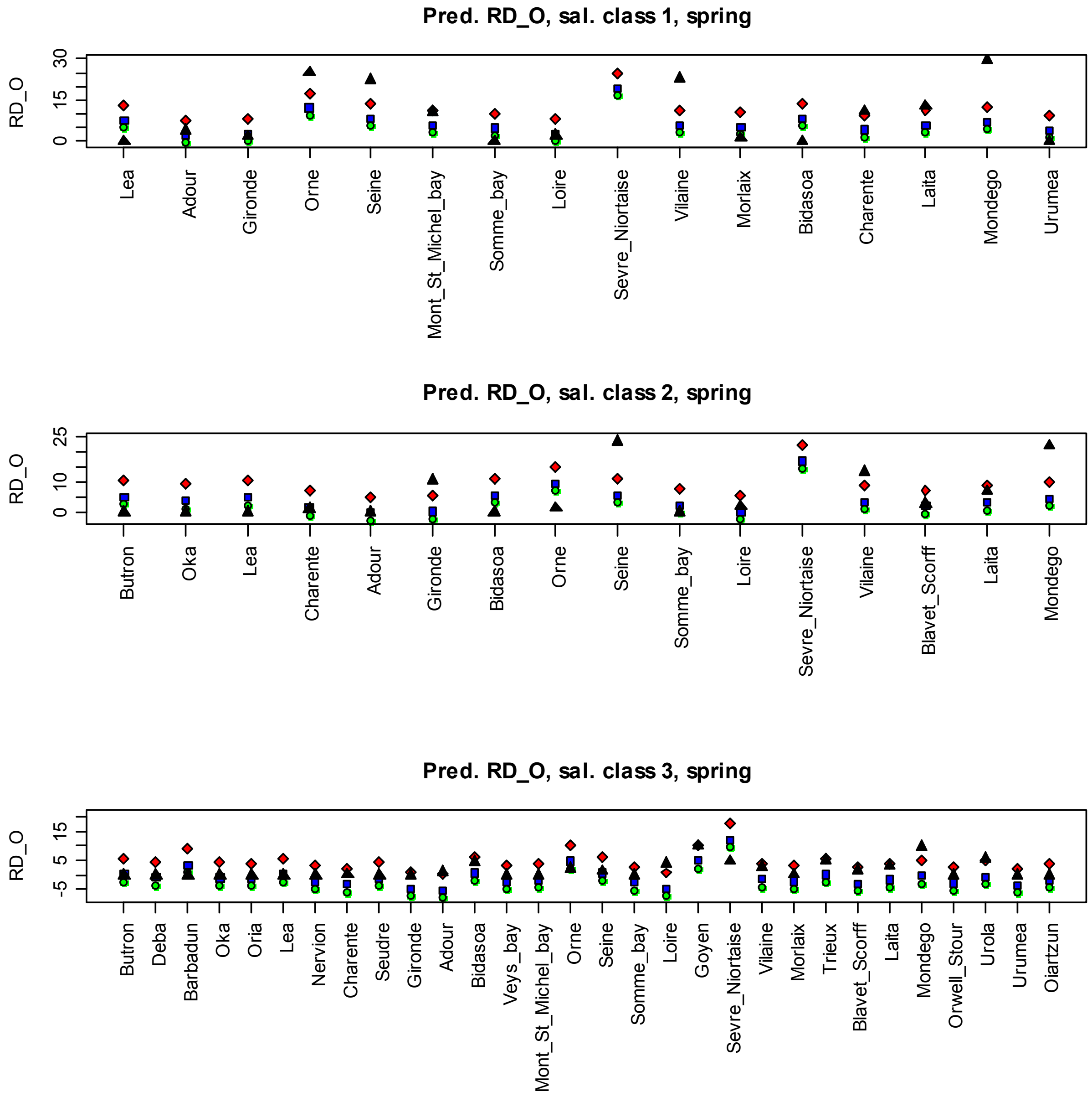


*Figure 7a: Predictions from the model linking the percentage of omnivorous individuals (RD_O) with the percentage of natural land on a 2 km buffer around estuaries, in spring. Model can be written: **RD_O ~ Sal class + Season = spring + Discharge + Intertidal area + Nat**. Green circles: theoretical reference at 100 percent of natural land; blue squares: percent of natural land set to the level of the least impacted site; black triangles: true mean value of RD_O observed in fish data; red diamonds: percentage of natural land set to the worst level observed among the 38 estuaries. Pred. RD_O: predict values of RD_O; sal. class: salinity class (1: oligohaline (0-5), 2: mesohaline (5-18) and 3: polyhaline (>18)). For the estuary Sevre Niortaise, salinity class 1 and 2, the true mean value of RD_O observed in fish data is respectively 62.1 % and 88.5 % (far beyond the maximum values of the y axis).*

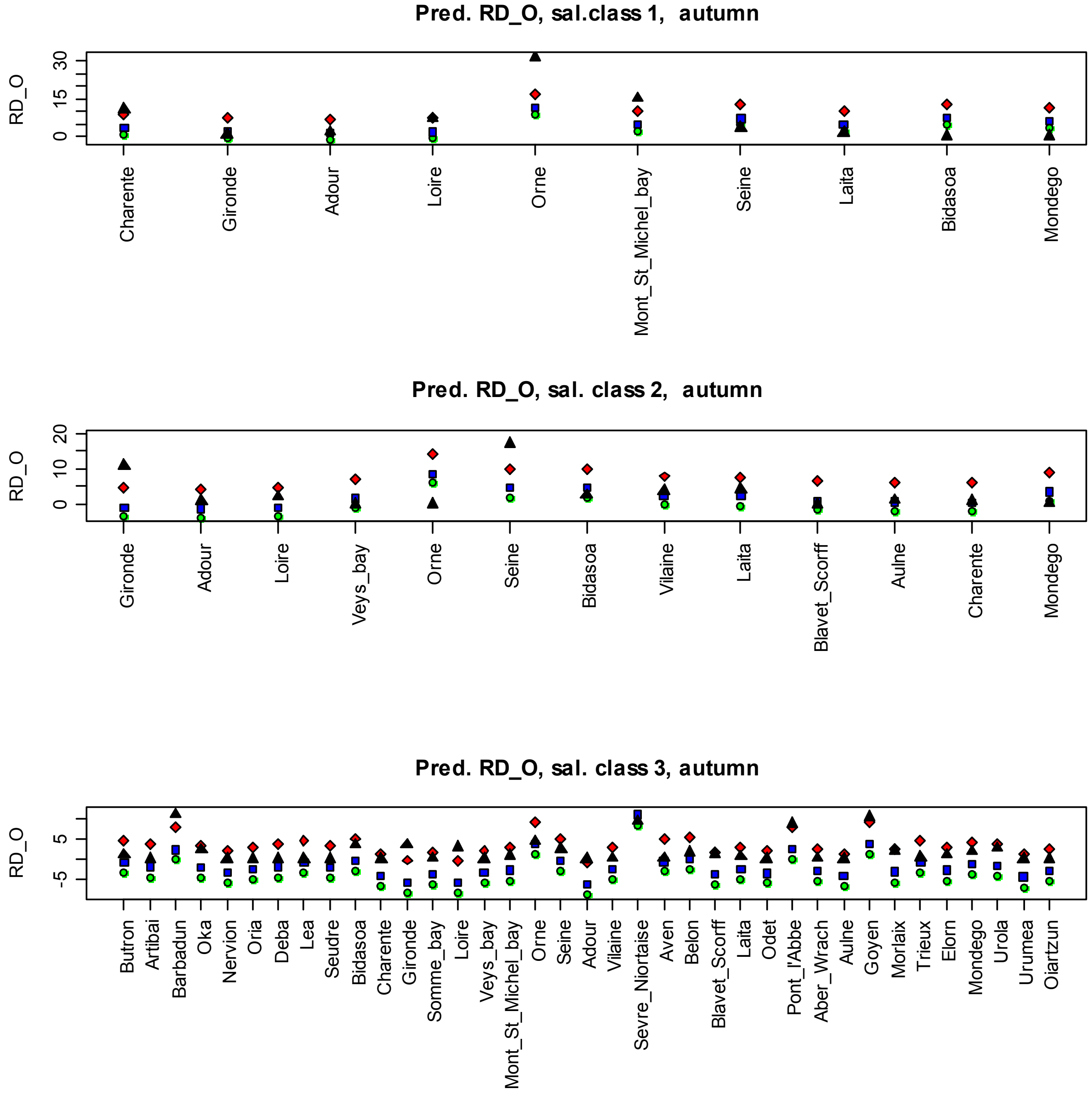


*Figure 7b: Predictions from the model linking the percentage of omnivorous individuals (RD_O) with the percentage of natural land on a 2 km buffer around estuaries, in autumn. Model can be written: **RD_O ~ Sal class + Season = autumn + Discharge + Intertidal area + Nat**. Green circles: theoretical reference at 100 percent of natural land; blue squares: percent of natural land set to the level of the least impacted site; black triangles: true mean value of RD_O observed in fish data; red diamonds: percentage of natural land set to the worst level observed among the 38 estuaries. Pred. RD_O: predict values of RD_O; sal. class: salinity class (1: oligohaline (0-5), 2: mesohaline (5-18) and 3: polyhaline (>18)).*

Discussion

The approach consisting in predicting fish metrics values from fish metric – pressure index models has already been used in (Courrat et al. 2009) and (Delpech et al. 2010). However in these works predictions were computed only for estuarine fish data, from linear and generalized linear models (LM and GLM). Moreover, these predictions were not performed with the aim of defining reference conditions. In the present work, we propose a method based on the use of linear mixed models and generalized linear mixed models, which leads to the prediction of reference conditions for both estuaries and lagoons with specific predictions for each estuary or lagoon, thus allowing to take into account potential residual natural effects that could not be accounted for in the models considering available abiotic data. Moreover, tentative predictions for a pristine status were also computed. The proposed approach leads to interesting and promising results. However, some improvements to the method are required and the testing of the method suffered from the lack of relevant standardised pressure data for estuaries and lagoons at European scale.

Pressure indices were obtained for Corine Land Cover data on buffers around estuaries and lagoons. Preliminary analyses lead to the selection of three pressure indices: the percentage of urban land, the percentage of agricultural land and the percentage of natural land. Then common WFD fish metrics were modelled using fish metric – pressure indices LM and GLM. Only 4 metrics out of the 12 tested for lagoons, and 5 metrics out of the 13 tested for estuaries answered significantly to one of the three pressure indices, and effect of pressure indices was always very low (close to zero). However, several of the selected fish metrics were already shown to be significantly correlate to some proxies of human pressure (Courrat et al. 2009; Delpech et al. 2010; Drouineau et al. 2012). Pressure indices based on land cover in buffers around estuaries and lagoons may not be good proxies for human pressure impacting fishes in these transitional waters. Indeed, other direct pressures (discharges, river load, dredging, etc.) could be locally more important than specific land uses. The fact that a high percentage of natural land lead almost always to poor fish assemblages may also reflect the fact that naturally richer estuaries and lagoons were most likely used by humans while naturally poor systems remained unoccupied.

LM and GLM were then transformed in mixed models, which lead to only three fish metrics responding significantly to some pressure indices. The approach consisting in first selecting fixed effect using LM and GLM and then transforming the best obtained models in LMM and GLMM can be discussed; however it allows overcoming the lack of confidence users can have on the biased p-values given by mixed-models. Moreover this approach is encouraged by some authors such as (Bolker et al. 2009). Several improvements could be made to the models, both mixed models and classical LM and GLM. In particular, in case of zero inflated values, densities could be modelled using delta models instead of simple log(n+1) transformation (Courrat et al. 2009; Delpech et al. 2010; Le Pape et al. 2003; Stefánsson 1996). For relative densities, the use of a Gaussian distribution could be replaced by either a Binomial law or the metric could be transformed before modelling. For modelling the number of species using mixed models considering the low mean number of species per treatment combination, penalized quasilikelihood (PQL) approximation may work poorly (Bolker et al. 2009) and the use of another package of R software may lead to some improvements in the methods. In general, a better reflexion and analysis on the choice of GLMM tools would be necessary to obtain unbiased and reliable estimation of reference points. However, the aim of the present work was more to get a qualitative understanding of what can be done rather than to obtain quantitative reliable predictions from the models. A more complete analysis can then be done for the purpose of WFD implementation, using maybe slightly smaller national WFD datasets but with better descriptors of human pressures.

All models show the major effect of variables from the sampling and from natural features of estuaries and lagoons on fish metrics. In particular, the present work argues for the definition of reference conditions per estuary and lagoon and specific to the gear, the sampling season and the salinity class. These results must be considered with caution considering the limited geographic distribution of the analysed sub-datasets. Indeed, some factors of variability may not have been properly accounted for as they were not relevant at the scale of the dataset, although they might be relevant at a regional scale. For Mediterranean lagoons, for example, longitude was shown to affect some metrics such as species richness (Franco et al. 2008).

However, the approach consisting in modelling such reference conditions separately for each fish metric may lead to unrealistic reference when combining fish metrics. Reference conditions were also modelled to forecast the expected reference at zero level of pressure. However, as there is no site in pristine state in the datasets used to set the parameters of the models, results obtained this way may be inaccurate as they require an extrapolation outside the limits of the models. In particular, such an approach seems unrealistic for metrics supposed to decrease with decreasing pressure as it often leads to predict the absence of the concerned fish in the pristine status. A compromise is to set the reference to the level of the least impacted sites. This increases accuracy but produces a reference condition set at an artificially diminished quality status which may be far from the true reference condition.

Conclusions and recommendations for future work:

From the present study, several conclusions may be highlighted, as well as recommendations for future work:

- The modelling approach proposed here to define type-specific reference conditions for fish assemblages in transitional waters in Europe appears promising.
- Models showed the major effect of variables from the sampling and from natural features of estuaries and lagoons on fish metrics. In particular, the present work argues for considering the definition of reference conditions per estuary and lagoon and specifically to the gear, the sampling season and the salinity class. Other parameters might be relevant depending on the dataset considered.
- Several improvements can be brought to the method. In particular:
  - A lack of relevant standardised pressure data at a European scale was highlighted here. The use of CORINE Land Cover data at a buffer scale lead to some interesting results but the work could be greatly improved by the use of other data such as data on water quality or data at the catchment scale.
  - The results obtained here, both in terms of variability sources affecting the considered fish metrics and in terms of reference conditions, can not be generalized considering the limited geographic distribution of the analysed sub-datasets. Similar analyses are required both at larger and smaller scale, in order to obtain operational models and reference condition values.
  - The models proposed here can be improved. Moreover, other R packages must be used in the future for similar studies and other methods to select the fixed effects in the models must be tested.
- The proposed approach must be tested on more WFD fish metrics and further thought needs to be given on how to combine the reference conditions designed at the fish metric scale to a reference condition at the multimetric fish indicator scale.
- The WFD requires that the ecological assessment is made at the scale of the waterbody. In the present work, we calculated reference conditions at the scale of "system", *i.e.* whole estuaries or lagoons. Further work is required to identify how reference conditions defined at the system level using the proposed approach can be translated to be used at the scale of waterbodies.

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