Deliverable 5.A: Report on the comparison of the sensitivity of fish metrics to multi-stressors in rivers, lakes and transitional waters

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Deliverable 5.A: Report on the comparison of the sensitivity of fish metrics to multi-stressors in rivers, lakes and transitional waters

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Dissemination Level		
PU	Public	
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)	X

Content

Non-technical summary

Aquatic ecosystems facing multiple stressors lead to challenging conditions for their management, as stressors can have additive, but also interactive effects on organisms, populations and communities. Accounting for these interactions is important in the assessment of the stressor's impacts and to implement good restoration measures.

Using a comparable modelling approach and large environmental and fish databases, the combined effect of water quality problems and hydrological stressors were assessed, based on characteristics of fish assemblages observed in rivers, lakes, reservoirs and estuaries of Europe. The effects of non-native species in interaction with eutrophication and alteration of hydromorphology were also tested for fish assemblages of natural lakes and reservoirs.

We show that for all the water body types, water quality problems are a major threat that impacts fish assemblages. Similarly, alteration of the hydro-morphology explains a large part of the composition of river and estuarine fish assemblages. Conversely, we fail to demonstrate an effect of this stressor on the fish community of lakes and reservoirs, as sufficient data are not available yet. However, in these standing waters the introduction of non-native species can explain the variability of some characteristics of fish assemblages.

In a second step, we analysed the interactive effect of various stressors. Without interaction, the effect of two stressors on a fish assemblage characteristic corresponds to the sum of the individual effects. This additive effect was compared with the effects really observed in the assemblages to determine the type of interaction. The comparison was done for each fish assemblage characteristic impacted by stressors in each water body type. A large variability of multi-stressor impacts was observed, leading to higher or lower effects than expected in absence of interactions.

These results suggest to consider all potential stressors and interactions in the development of fish-based tools dedicated to ecological status assessment or restoration monitoring whatever the water body type is.

Introduction

To achieve long-term sustainable water resources management and the protection of European aquatic environment, the Water Framework Directive (WFD) was launched in 2000 (European Commission, 2000). To reach the ambitious objectives, i.e. the good ecological status/potential, monitoring of biological, physico-chemical and hydromorphological characteristics of all ground and surface water bodies (rivers, lakes, transitional- and coastal waters) was organized in the different countries of the EU. Currently, these monitoring programs support a large environmental data collection, allowing large-scale analyses of the relationships between biological communities and their environment.

In the framework of the WISER project (http://www.wiser.eu/), the response of biological/ecological characteristics of fish assemblages (i.e. fish metrics) to anthropogenic stressors was studied in rivers, lakes and estuaries. These studies aimed at understanding what the main factors involved in the organisation of fish assemblages are. Based on this knowledge, fish bioindicators/indices have then be developed by aggregating fish metrics in relation with abundance, composition and sensitivity of species, in order to assess the ecological status of the continental water bodies. However, in most of the cases, the analyses focused on the response of fish metrics to a single stressor or to several stressors but resumed by a single index value of pressure.

For rivers, Europe's first River Basin Management Plans (RBMPs) from 2009 indicate that 56% of European rivers failed to achieve good ecological status, as they are affected by a complex set of pressures resulting from e.g. urban and agricultural land use, hydropower generation and climate change (European Environment Agency [EEA], 2012). Along with increasing pressure placed on riverine ecosystems, both scientists and water resource managers need greater understanding of the relationships between multiple anthropogenic stressors and the response of the aquatic community, i.e. if they have synergistic, antagonistic or additive effects, to understand the impact on and the future management of aquatic ecosystem services (Allan et al., 2013). Research projects funded by the EU, e.g. FAME (Fish-based Assessment Method for the Ecological Status of European Rivers, FAME Consortium, 2004) and EFI+ ("Improvement and Spatial Extension of the European Fish Index", EFI+ Consortium, 2007) investigated related issues. Based on the results of EFI+ project (EFI+ Consortium, 2007), Schinegger et al. (2012) first showed that (1) degradation of European rivers is widespread, (2) single water quality pressures (W) are not dominant, but (3) many European rivers are affected by hydromorphological pressures (HMC) or a combination of pressure types (W + HMC).

Similarly, in lakes eutrophication characterised by nutrient concentrations or indirectly by the intensity of non-natural land cover (agricultural and urban) in the catchment of the lake, is recognised as a major threat to achieve the good ecological status. Therefore, most of the bioindicators are dedicated to the assessment of this stressor and fish are often used for this purpose (Argillier et al., 2013; Kelly et al., 2012; Olin et al., 2013). Alteration of hydromorphology is also expected to affect fish communities notably through habitat and reproduction substrate diversity and availability (Drake and Pereira, 2002). Introduction of non-native species, a frequent practice in standing waters (Cowx, 1998; Irz et al., 2004; Welcomme, 1988), highly impacts biodiversity and the ecosystem functioning (Garcia-Berthou and Moreno-Amich, 2000; Whittier and Kincaid, 1999) and can, as a consequence, modify the ecological status of the lakes (Gassner et al., 2003). However regarding these two stressors, clear pressure-impact relationships seem to be difficult to demonstrate by large scale analyses (Brucet et al., 2013). As a consequence, the effects of eutrophication, alteration of hydromorphology and introduction of non-native species in combination are seldom quantified.

In estuaries (i.e. transitional waters), several modelling approaches have been conducted in previous studies to test the sensitivity of fish metrics to a pressure index or specific pressures. Despite the major effect of variables from the sampling and from natural features, most of metrics responded to the gradients of anthropogenic pressures using linear models (GLM, LM). Nevertheless, as for the others water body types, these approaches rarely considered the interaction between stressors and did not evaluate the relative contribution of each stressor.

Current challenges include using the large biological databases generated through the WFD monitoring surveys (http://www.eea.europa.eu/data-and-maps/data/wise_wfd) and for the intercalibration of methods (Birk et al., 2013), to identify and predict the effects of multiple stressors on ecosystems in order to help water managers prioritizing mitigation measures (Hering et al., 2015; Reyjol et al., 2014).

Systems facing multiple stressors are challenging conditions for management because stressors can have additive, but also interactive effects on organisms, populations and communities (Crain et al., 2008). The combined effect of multiple stressors was commonly assumed to be additive, i.e. equal to the sum of stressors' individual effects acting in isolation. However, this model does not seem prevalent in ecological systems compared to antagonistic and synergistic interactions (Crain et al., 2008). Stressors can act in synergy when the combined effect of stressors is greater than the sum of the impacts of individual stressors, whereas antagonistic interactions occur when the combined effect of stressors is less than expected based on their individual effects (Folt et al., 1999). In these conditions, the ecological benefit resulting from efforts to reduce any stressors acting additively can be predicted on the basis of the knowledge of its individual effect, whereas interactive effects could produce some 'ecological surprises' (Paine et al., 1998). Although synergic interactions are expected to be more harmful for ecosystems because of accelerating system degradation, they also provide advantageous opportunities of restoration yielding larger overall benefits than if additive or antagonistic effects are involved (Crain et al., 2008; Piggott et al., 2015). Conversely, the efforts to mitigate stressors often not yield proportional benefits in systems where antagonistic interactions are prevalent, which is often considered as the "worst-case" scenario for ecosystem management (Brown et al., 2013; Folt et al., 1999; Piggott et al., 2015). The development of scheme for prioritizing management actions should thus consider the type and the strength of interactions (Halpern et al., 2008).

The present work is in the wake of the previous analyses conducted in the WISER project. More precisely, it is dedicated to:

- A better understanding of the combined effects of several stressors, on the fish metrics/indices identified as relevant to assess the ecological status of the water bodies. Indeed, water bodies seldom experience a single pressure and it is possible to improve the diagnosis on the ecological status taking into account a larger range of stressors. The stressors can act in interaction or not and impact different facets of the communities.
- The analysis of differences in fish communities responses to multi-stressors in rivers, lakes and estuaries taking into account the specificity of each water body type. Due to differences in ecosystem functioning between lentic and lotic waters and between freshwater and brackish waters, we can expect different responses of the communities to a same combination of stressors.

General approach

In the analyses conducted on rivers and lakes, a metric was defined as a measurable variable or process that represents an aspect of the biological structure, function, or other component of the fish community and changes in value along a gradient of human influence (Karr, 1999). In these studies, metrics tested are related to composition, abundance, sensitivity and size structure of fish communities. These metrics are based on taxonomic or functional guilds. In the analyses conducted on estuaries, some diversity metrics were included in the analyses of a French dataset. However, in estuaries, because sampling methods and strategies were very different from one country to another, calculation of metrics at the European scale is not sound. Therefore, the Ecological Quality Ratios (EQR) of the fish index in application in each country were calculated and used in the present work. The EQR defined as the ratio of the observed fish index value to the expected value under reference conditions was used. These indices are multi-metrics indices supposed to take into account the influence of natural environmental characteristics of estuaries on fish communities.

To achieve the objectives, whatever the water body type, Random Forests models (Breiman, 2001) were implemented to assess and rank the importance of pairwise stressor interactions. This machine learning approach allows freedom from normality and homoscedasticity assumptions and did not require previous data transformation (Mercier et al., 2010). It is suited to identify relevant predictors from a large set of candidate variables even though the number of observations is small (Strobl et al., 2007). The random forest algorithm generates a great number of decision trees involving two specific random features. The first one is a bootstrap resampling used to select approximately 63.2% of the whole dataset to build a given tree (‘in-bag’ data). The second one occurs at each decision node of the tree to select a random subset of predictors from which the predictor minimizing the mean squared error is retained to grow the tree. The remaining 36.8% of data not kept to grow the tree (i.e. ‘out-of-bag’ data) are used to provide independent estimations of the prediction error for each tree. The model estimates are obtained by averaging the predictions from all the individual regression trees of the forest. Using a large number of trees ensure that each metric had enough of a chance to be included in the forest prediction process. The number of metrics randomly selected at each node was fixed at 5 and the minimum number of unique observations in a terminal node was also fixed at 5, as advised for regression analysis. As some predictors contain missing values, the imputation method developed by Ishwaran et al. (2008) was used to attribute data by randomly drawing values from non-missing in-bag data. The percentage of variance explained by the model was estimated based on the out-of-bag observations, which provided a reliable evaluation of the adjustment (i.e. goodness-of-fit).

For all the water bodies, the importance of each stressor in the Radom Forest models, were computed using an approach based on Breiman-Cutler permutations (implemented in the "VIMP" function of "randomForestSRC" R package). For each tree, the prediction error on the OOB data is recorded and then for each stressor, OOB cases are randomly permuted and the prediction error is recorded. The variable importance (VIMP) is then defined as the difference between the perturbed and unperturbed error rate averaged over all trees in the Forest (Ishwaran, 2007). A measure of relative importance (%) expressing the proportion of each VIMP to the sum of VIMP of all stressors was then computed in order to compare the variable importance among different fish metrics.

Analyses were performed with the R statistical software (R Core Team, 2014) and the package 'randomForestSRC' (Ishwaran and Kogalur, 2014).

Multi-stressors analyses on rivers

Data and method

Fish sampling data/fish metrics

In total 3105 European fish sampling sites in 14 countries were available for our analyses, extracted from an extensive database (EFI+ Consortium, 2007). Sites were sampled by electrofishing (wading) considering European standards (C.E.N., 2003) and were associated with four fish assemblage types (FATs, i.e. Headwater streams, Medium gradient rivers, Lowland rivers and Mediterranean streams; Figure 1) based on fish community and environmental characteristics ((Schinegger et al., 2013; Trautwein et al., 2013).

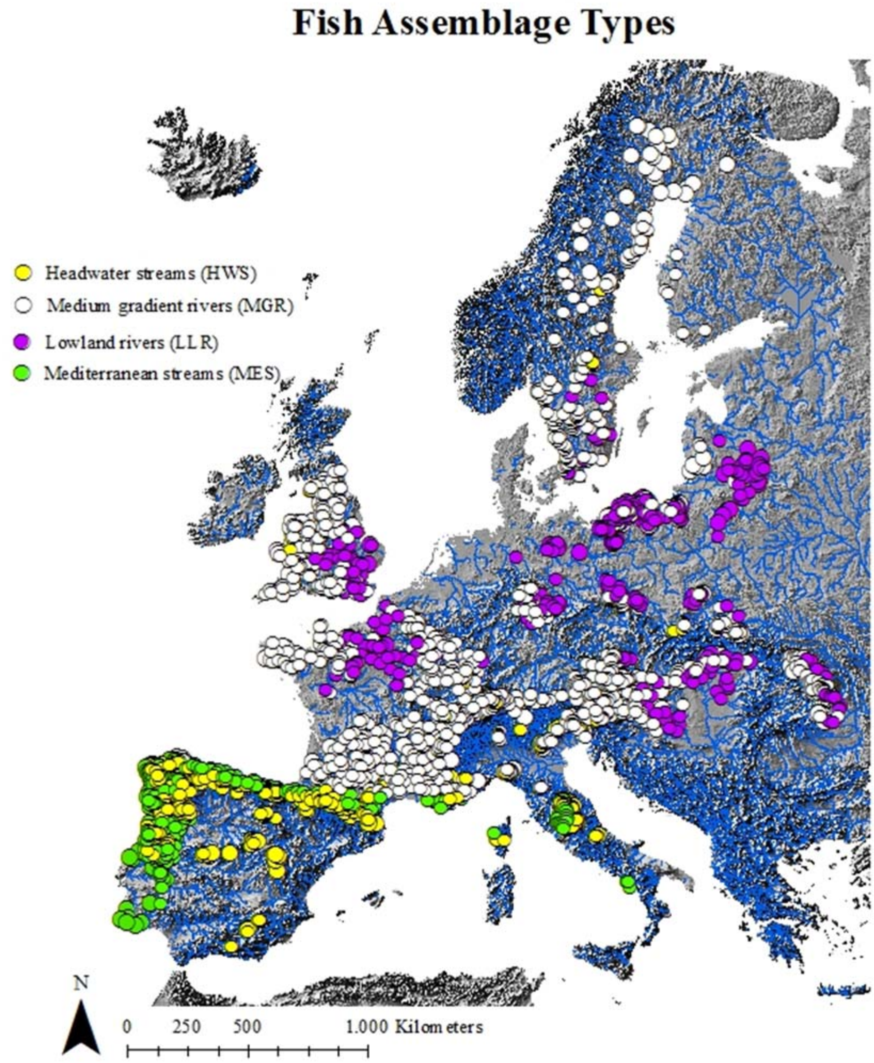

Figure 1 - Spatial location of sites [n = 3105] and associated fish assemblage type (FAT's according to Schinegger et al., 2013).

Overall, 20 fish metrics associated with six structural and functional types (biodiversity, habitat, migration, reproduction, trophic level and water quality sensitivity) were available for further analyses (Table 1), based on the findings of Schinegger et al. (2013), Trautwein et al. (2013) and Segurado et al. (2008) in terms of reaction to single and multiple stressors.

Table 1 - Fish metrics available for river analyses.

Metric name	Definition	Type	Variants	Direction
Nsp_all	Total number of fish species, including native and alien species.	biodiv	nsp	decr/incr
HTOL_HINTOL	Habitat degradation intolerance.	hab	dens juveniles (<150mm)	decr
HTOL_HTOL	Habitat degradation tolerance.	hab	perc_biom	incr
HabSp_RHPAR	Preference to spawn in running waters.	hab	dens	decr
Repro_LITH	Fish spawn exclusively on gravel, rocks, stones, rubbles or pebbles, hatchlings are photophobic.	repro	dens	decr
Atroph_INSV	Insectivorous species.	troph	nsp	decr
Atroph_PISC	Piscivorous species.	troph	perc_nsp	decr
Atroph_OMNI	Food of adult consists of more than 25% plant material and more than 25% animal material. Generalists.	troph	perc_biom, perc_nsp	incr
WQgen_INTOL	In general intolerant to usual water quality parameters.	wq	biom, dens, perc_nsp, pers_dens	decr
WQgen_TOL	In general tolerant to usual water quality parameters.	wq	biom, nsp, perc_dens	incr
WQO2_O2INTOL	Tolerant to low Oxygen concentration. More than 6 mg/l in water.	wq	biom, dens, perc_nsp	decr
WQO2_O2TOL	Tolerant to low Oxygen concentration: 3 mg/l or less.	wq	perc_nsp	incr

Pressure data/pressure combinations

Pre-classification of sites for anthropogenic stressors was available for 13 selected stressor variables (according to Schinegger et al., 2012 and Schinegger et al., 2013), to differentiate unimpacted sites from single- or multiple impacted sites (Table 2).

Table 2 - Stressor variables available for river analyses.

Stressor variable	Abbreviation	Description
Impoundment	H_imp	Natural flow velocity reduction on site due to impoundment
Hydropeaking	H_hydrop	Site affected by hydropeaking
Water abstraction	H_waterabstr	Site affected by water flow alteration/residual flow
Reservoir flushing	H_resflush	Fish fauna affected by flushing of reservoirs upstream of site
Hydrograph modification	H_hydromod	Seasonal hydrograph modification due to hydrological alteration (water storage for irrigation, hydropower etc.)
Morphological alteration	M_morph_instr	Alteration of natural morphological channel plan form, cross section and instream habitat conditions
Embankment	M_embank	Artificial embankment
Flood protection	M_floodpro	Presence of dykes for flood protection
Barriers upstream	C_up	Barriers upstream of site
Barriers downstream	C_do	Barriers downstream of site
Acidification	W_acid	Artificial acidification
Eutrophication	W_eutroph	Artificial eutrophication
Organic pollution	W_opoll	Pollution by organic substances

Pairwise approach – random forests

Random Forests models (Breiman 2001; see brief description of the method in the section General approach of this deliverable) were fitted using each one of the 20 river fish metrics as the response variable and the 13 stressors considered in this study as covariates. Fish Assemblage Type was also included as covariate in the models to control for its effect on the metric response to stressors. According to the cumulative out-of-bag (OOB) error rate as a function of number of trees, a forest of 1000 trees were overall adequate.

The importance of individual stressors was assessed following the method previously described (section General approach). Grown random forests were also used to identify and rank the potential importance of pairwise interactions for all pairs of stressors. This ranking was also based on Breiman-Cutler permutations (implemented for interactions in the "find.interaction" function of "randomForestSRC" R package) but in this case the paired VIMP was calculated instead, referred to as "paired" importance. This measure was then compared with the sum of VIMP of the individual stressors, referred to "additive" importance. A large positive or negative difference between 'Paired' and 'Additive' indicated an interaction worth pursuing in case the univariate VIMP for each stressor was reasonably large. The ranked absolute differences between Paired and Additive VIMP were computed to allow comparing the importance of each pair of stressors among metrics. A relative difference [(Paired – Additive) / Additive] was also computed as an alternative to compare interactions. Pairwise combinations that yielded fewer than 10 sites per pairwise category combination were discarded. Co-plot graphs were plotted for the most responsive metrics to visually identify the most important interactions and assess whether the main deviations from additive effects corresponded either to synergistic or antagonistic interactions.

Multiple combinatory approach – descriptive analysis

Some fish metrics decrease in response to increasing anthropogenic stress (less fish of a guild leading to reduced density and biomass, disappearance of species) but in contrast, several others tend to increase, thus having a reaction in reverse direction, e.g. metrics associated with generalist and tolerant species (Schinegger et al., 2013; Trautwein et al., 2013). We used EQR to identify the degree of metric response between sites impacted by frequently occurring stressor combinations and unimpacted sites. EQRs were calculated for each individual site as follows:

Formula I:

$$\mathrm{EQR_{site\ i}} = \frac{\mathrm{metric_{site\ i}}}{\mathrm{mean(metric_{unimpacted\ sites})}}$$

Where $i = 1 \ldots 3105$

Formula I is applied for metrics expected to decrease under increasing number of stressors as well as metrics expected to increase under increasing number of stressors if they were recorded in percentage rates. For these percentage metrics, a proxy was calculated, based on the percentage not meeting the metric value (i.e. for percentage of species tolerant to habitat degradation, the percentage value not tolerant was considered) (Formula II).

Formula II:

$$\mathrm{EQR_{site\ i}} = \frac{100 - \mathrm{metric_{site\ i}}}{100 - \mathrm{mean(metric_{unimpacted\ sites})}}$$

Where $i = 1 \ldots 3105$

For metrics in absolute numbers, which are expected to increase with increasing number of stressors, the EQR is calculated as follows:

Formula III:

$$\mathrm{EQR_{site\ i}} = \frac{\mathrm{mean(metric_{unimpacted\ site})}}{\mathrm{metric_{site\ i}} \times \mathrm{mean(EQR_{unimpacted\ sites})}}$$

Where $i = 1 \ldots 3105$

These calculations are sensitive to FAT, i.e. separate mean metrics for the unimpacted sites were calculated for each of the four river types to which the metric at a given site of a particular river type is compared. This step was included to compensate for environmental effects that might influence the sampling site. FAT of impacted sites was predicted by the method developed by Schinegger et al. (2013) and Trautwein et al. (2013).

Prediction of combined effects

The combined effects of stressors were predicted from single stressors and were defined as deviation from reference condition by ecological quality ratio (EQR) according to Formula 3 (modified based on Coors and De Meester, 2008). Deviations of observed from predicted EQRs can then be interpreted in the following way: synergistic effects between pressures are indicated by a significantly stronger observed effect of the combined stressors than the predicted one (from single stressors), whereas an antagonistic effect is indicated by a significantly weaker value of observed value than predicted (Coors and De Meester, 2008). No deviation between observed and predicted EQRs can be interpreted as additive effect.

Results

Pairwise combinations - Proportion of explained variance

The proportion of the variance of fish metrics explained by the Random Forest models varied between 36.9% for the metric *% of species intolerant to oxygen depletion* (WQO2_O2INTOL_perc_sp; Fig. 2) and 5.3% for the metric *abundance of rheophilic spawning species* (HabSp_RHPAR_dens; Fig. 2). For most metrics, FAT contributed approximately to half of the total explained variance. Metrics with higher contribution of stressors to the explained variance tended to be also those with the highest total explained variance, and vice-versa. The five metrics with the highest contribution of stressors to the explained variance were: *total abundance of juveniles intolerant to habitat degradation* (HTOL_HINTOL_dens_juv), *% of species intolerant to oxygen depletion* (WQO2_O2INTOL_perc_sp), *total richness* (NSp_all), *% of omnivorous biomass* (Atroph_OMNI_perc_biom) and *% of abundance of intolerant to general water quality degradation* (WQgen_INTOL_perc_dens).

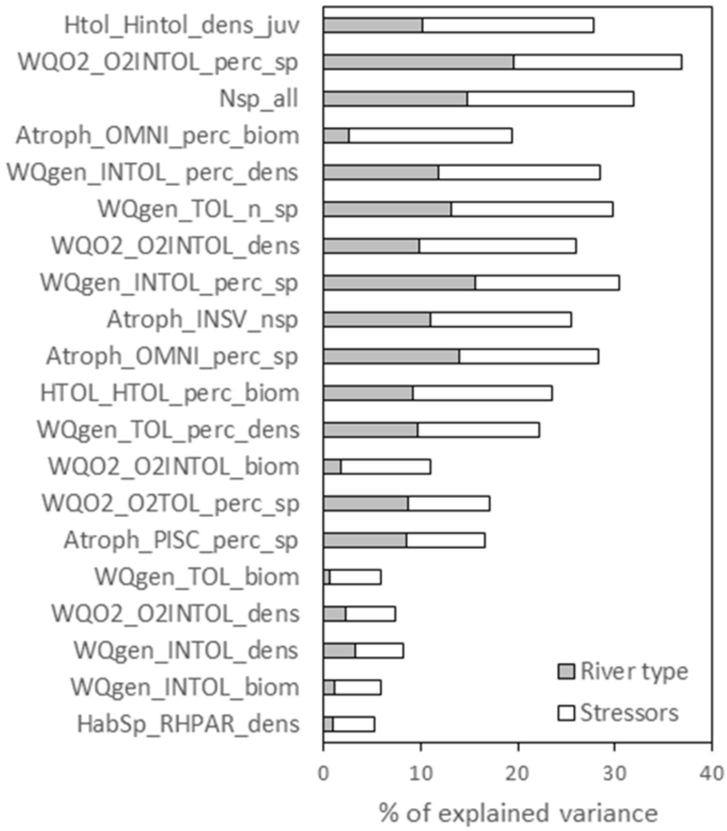

Figure 2 - Total percentage of variance explained by the Random Forest models for each metric. The contribution of FAT and stressors is also represented. Fish metrics are sorted by a decreasing order of stressor contribution to the explained variance.

Stressor importance

Except for four metrics (WQgen_INTOL_biom, WQgen_TOL_biom, WQO2_O2INTOL_biom and Atroph_OMNI_perc_biom), FAT was the most important variable in the random forest models (Table 3, Figure 3). Among the ten metrics that had a higher share of explained variance by stressors, eutrophication was the most important stressor for four metrics, followed by organic pollution for three metrics, in-stream morphology alteration for two metrics and hydrograph modification for one metric (Table 3). These stressors, along with embankment and flood protection, were the stressors showing the highest median importance among the different metrics (Figure 3).

Table 3 – Relative variable importance (%) of each stressor in Random Forest models fitted for each fish-based metric. Metrics are sorted in a decreasing order of the contribution of stressor variables to the explained variance.

Fish Metrics	FAT	C_B_s_do	C_B_s_up	H_hydromod	H_hydrop	H_imp	H_resflush	H_waterabstr	M_embank	M_floodpro	M_morph_instr	W_acid	W_eutroph	W_opoll
Htol_Hintol_dens_juv	36.4	2.1	1.2	5.9	3.1	6.1	0.0	1.5	2.0	0.8	18.5	0.0	18.3	4.1
WQO2_O2INTOL_perc_sp	52.9	1.2	0.8	9.4	1.2	1.2	0.1	0.9	1.3	0.8	4.1	0.3	21.3	4.3
Nsp_all	46.2	0.2	2.9	7.3	0.5	0.4	0.6	1.3	2.3	12.5	3.9	0.7	5.7	15.5
Atroph_OMNI_perc_biom	13.9	0.7	0.5	20.5	1.9	5.4	-0.1	2.3	0.9	1.3	4.4	0.2	25.2	22.9
WQgen_INTOL_perc_dens	41.7	0.8	0.6	10.4	1.3	2.7	0.0	0.8	2.0	0.7	8.6	0.3	23.5	6.7
WQgen_TOL_n_sp	44.5	0.2	1.5	12.3	0.5	2.3	1.1	1.1	2.8	6.6	7.5	0.2	7.4	11.9
Y_MetO2INTOL	38.0	1.5	0.5	7.4	2.8	2.6	0.1	1.2	2.2	0.8	11.2	0.0	28.0	3.6
WQgen_INTOL_perc_sp	51.5	1.2	0.6	9.2	0.9	2.2	0.0	0.5	1.6	0.6	5.4	0.3	20.2	5.8
Atroph_INSV_nsp	43.4	0.4	3.4	2.6	0.5	0.5	0.5	2.1	3.5	17.9	4.3	1.3	2.4	17.3
Atroph_OMNI_perc_sp	49.6	0.8	0.3	7.3	0.3	1.3	0.2	1.4	1.9	1.2	14.2	0.6	12.0	8.9
HTOL_HTOL_perc_biom	39.3	0.5	0.7	10.0	0.5	4.0	0.0	0.4	2.4	0.8	7.9	0.0	11.7	21.6
WQgen_TOL_perc_dens	43.8	3.1	0.3	4.8	0.9	6.9	0.2	1.1	3.0	2.1	16.2	0.2	11.4	6.0
WQO2_O2INTOL_biom	16.1	2.8	3.9	14.4	2.9	-2.1	-0.2	0.6	8.3	29.7	3.6	0.4	14.9	4.7
WQO2_O2TOL_perc_sp	51.3	0.6	0.1	6.1	0.4	1.6	0.1	0.4	2.2	1.5	6.9	0.1	15.4	13.4
Atroph_PISC_perc_sp	51.7	5.1	0.5	2.5	3.2	2.1	0.0	6.1	10.7	1.4	3.4	0.4	1.8	11.2
WQgen_TOL_biom	10.6	7.5	2.2	6.0	-2.2	-2.8	0.0	7.7	5.4	8.4	6.1	0.1	16.2	34.8
WQO2_O2INTOL_dens	31.0	8.2	3.1	3.7	8.3	1.9	0.0	-0.2	16.3	7.6	4.1	-2.1	9.1	9.1
WQgen_INTOL_dens	39.4	2.6	6.3	7.9	12.2	6.5	0.1	0.9	4.8	1.9	5.5	0.0	11.5	0.4
WQgen_INTOL_biom	20.3	-1.6	2.7	4.8	6.3	-3.8	0.0	5.6	16.8	39.0	-0.5	0.4	0.8	9.4
HabSp_RHPAR_dens	19.1	2.8	14.2	4.6	13.4	-1.2	4.3	-2.1	7.5	4.7	8.2	0.2	7.8	16.5

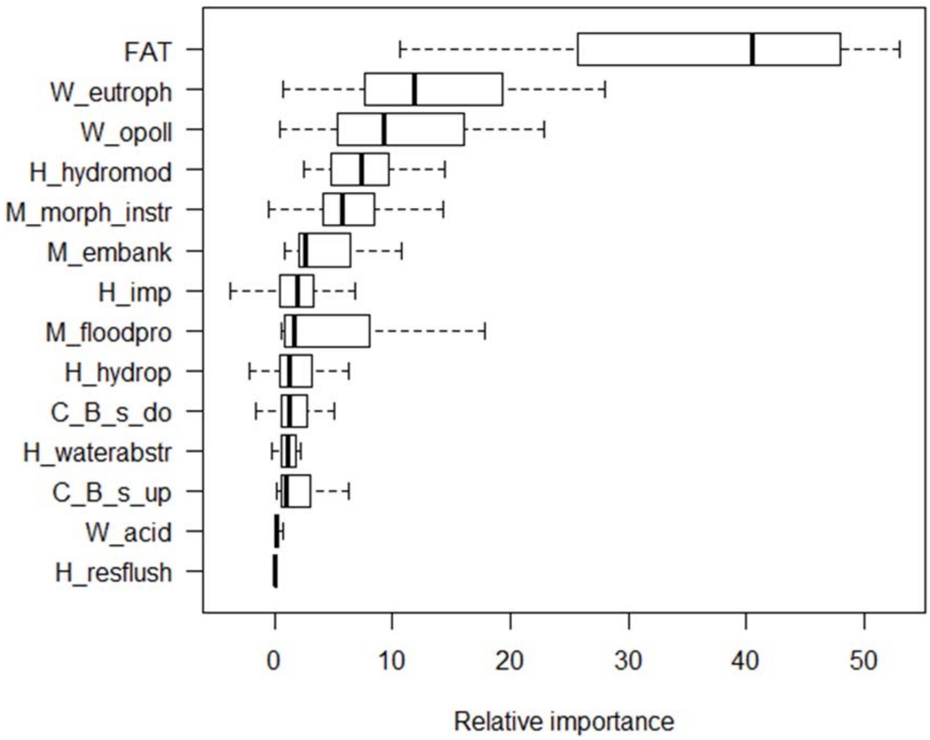

Figure 3 – Boxplot showing the distributions of the relative stressor and FAT (Fish Assemblage Type) importance based on the random forest models for the eighteen metrics analysed.

Importance of pairwise interactions

The boxplots of Figure 4 show the overall importance of stressor interactions according to the Random Forest models, as measured by the ranked absolute difference between paired and additive VIMP values, for the 20 fish metrics. Eutrophication paired with organic pollution showed the overall higher relevance and simultaneously the lowest variability among metrics. Other important interactions included in-stream morphological alteration with eutrophication, in-stream morphological alteration with embankment, flood protection with eutrophication and impoundment with eutrophication.

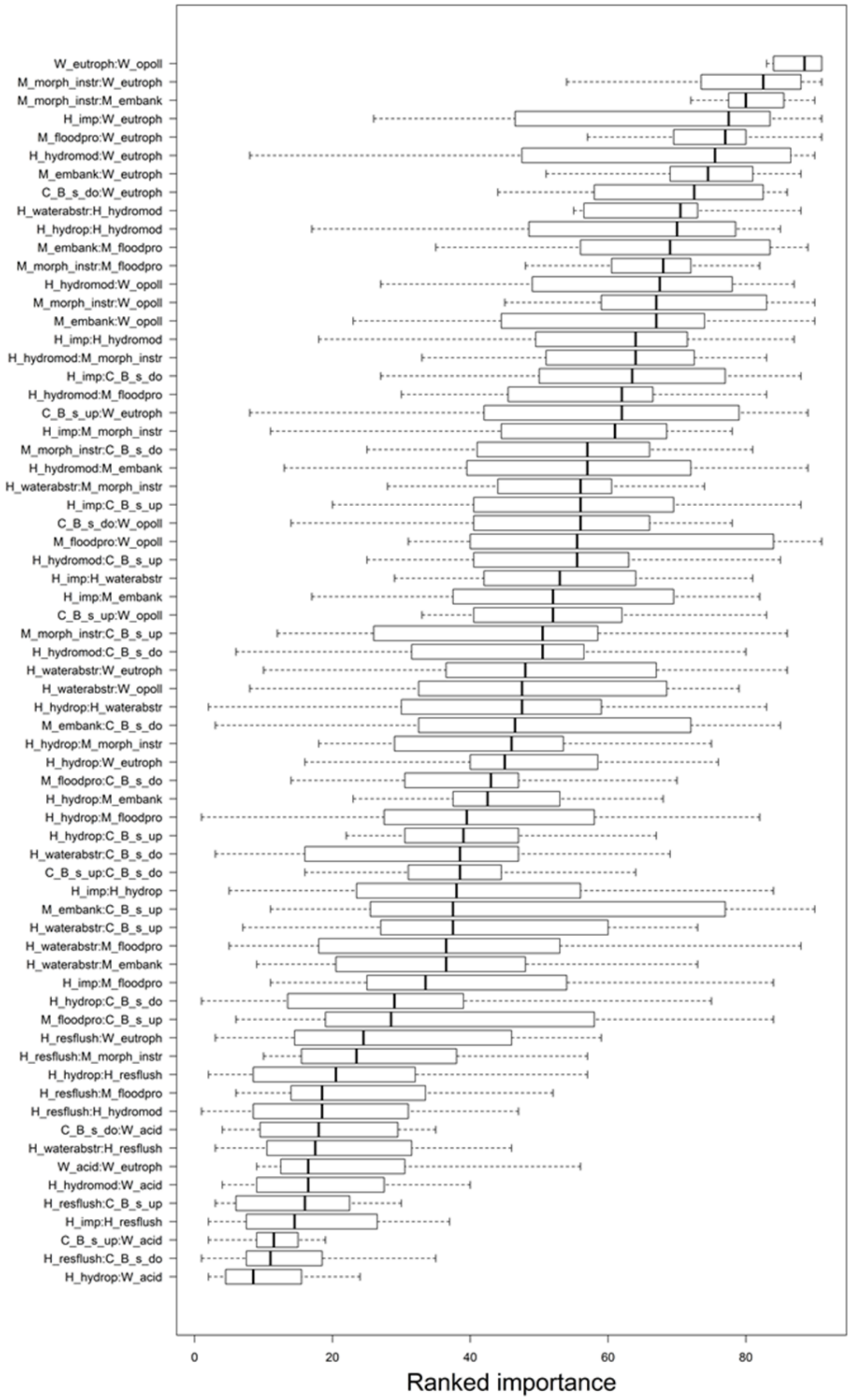

Figure 4 - Boxplot showing the distributions of the ranked interaction importance of each pairwise combination of stressors, based on the random forest models for the twenty metrics analysed.

Type of interactions – deviations from the additive effects

The boxplot of Figure 5 shows the distribution of the relative difference between paired and additive importance in the Random Forest models, which expresses the deviations from the additive effects. The pressure pairs are sorted according to their rank importance for each metric. This figure shows that the rank position of each interaction does not always reflect the magnitude of the deviation from the additive effects, although there is a general trend for pairwise interactions with higher ranks to show more pronounced deviations from additive effects and vice-versa. Eutrophication paired with organic pollution also showed the highest deviations from additive effects. Other pairwise interactions with pronounced deviations from additive effects included impoundment with barriers downstream, in-stream morphological alteration with embankment, flood protection with embankment and in-stream morphological alteration with eutrophication.

The response to the pairwise interaction that deviates the most from additive effects according to the random forest models (eutrophication and organic pollution) was explored with partial co-plots for the five most responsive metrics to stressors: *total abundance of juveniles intolerant to habitat degradation* (HTOL_HINTOL_dens_juv; overall negative response to pressures; Fig. 6a), *% of species intolerant to oxygen depletion* (WQO2_O2INTOL_perc_sp; overall negative response to pressures; Fig. 6b), *total richness* (Nsp_all; overall positive response to pressures; Fig. 6c), *% of omnivorous biomass* (Atroph_OMNI_perc_biom; overall positive response to pressures; Fig. 6d) and *% of abundance of intolerant to general water quality degradation* (WQgen_INTOL_perc_dens; overall negative response to pressures; Fig. 6e). The partial co-plots suggest that both synergistic and antagonistic interactions might be expected for this stressor pair, depending on the metrics. No relationship between the overall direction of the effect (positive of negative) and the type of interaction (synergistic or antagonistic) seems to occur. Synergistic effects - i.e. eutrophication tends to attenuate the effect of organic pollution and vice-versa - are predicted to three metrics (HTOL_HINTOL_dens_juv, NSp_all; Atroph_OMNI_perc_biom) and antagonistic effects – i.e. eutrophication tends to enhance the effect of organic pollution and vice-versa - are predicted for two metrics (WQO2_O2INTOL_perc_sp, WQgen_INTOL_perc_dens).

The co-plots of Figure 7 shows the simultaneous effects of other important pairwise interactions for each five most responsive metrics to stressors. For the metric *total abundance of juveniles intolerant to habitat degradation* (HTOL_HINTOL_dens_juv) the random forest model suggests a potential interaction between instream morphological alteration and eutrophication. According to the co-plot (Fig. 7a), eutrophication tends to attenuate the effect of instream morphological alteration and vice-versa, namely between the categories "no" to "low" of instream morphological alteration, suggesting an antagonistic effect. The same antagonistic effect for the same stressor pair is also suggested by the partial co-plot for the metric *% of abundance of intolerant to general water quality degradation* (WQgen_INTOL) (Fig. 7e). For metric *% of species intolerant to oxygen depletion* (WQO2_O2INTOL), the co-plot suggests a slight synergistic effect between hydrograph modification and eutrophication (Fig. 7b). A slight synergistic effect is also suggested by the co-plot between the partial effects of flood protection and eutrophication for the metric *total richness*

(Nsp_all) (Fig.7c). For metric *% of omnivorous biomass* (Atroph_OMNI_perc_biom), an antagonistic effect is suggested by the co-plot between the partial effects of hydrograph modification and impoundment (Fig. 7d).

In the case of the response of the *total number of species* a synergistic interaction is suggested by the partial co-plot of the simultaneous effects of eutrophication and organic pollution: an increase of eutrophication tends to accentuate the unimodal response to water pollution. The simultaneous response to the pair eutrophication and flood protection suggests also a slight synergistic effect: eutrophication tends to slightly enhance the effect of flood protection (Fig. 7a and 7b).

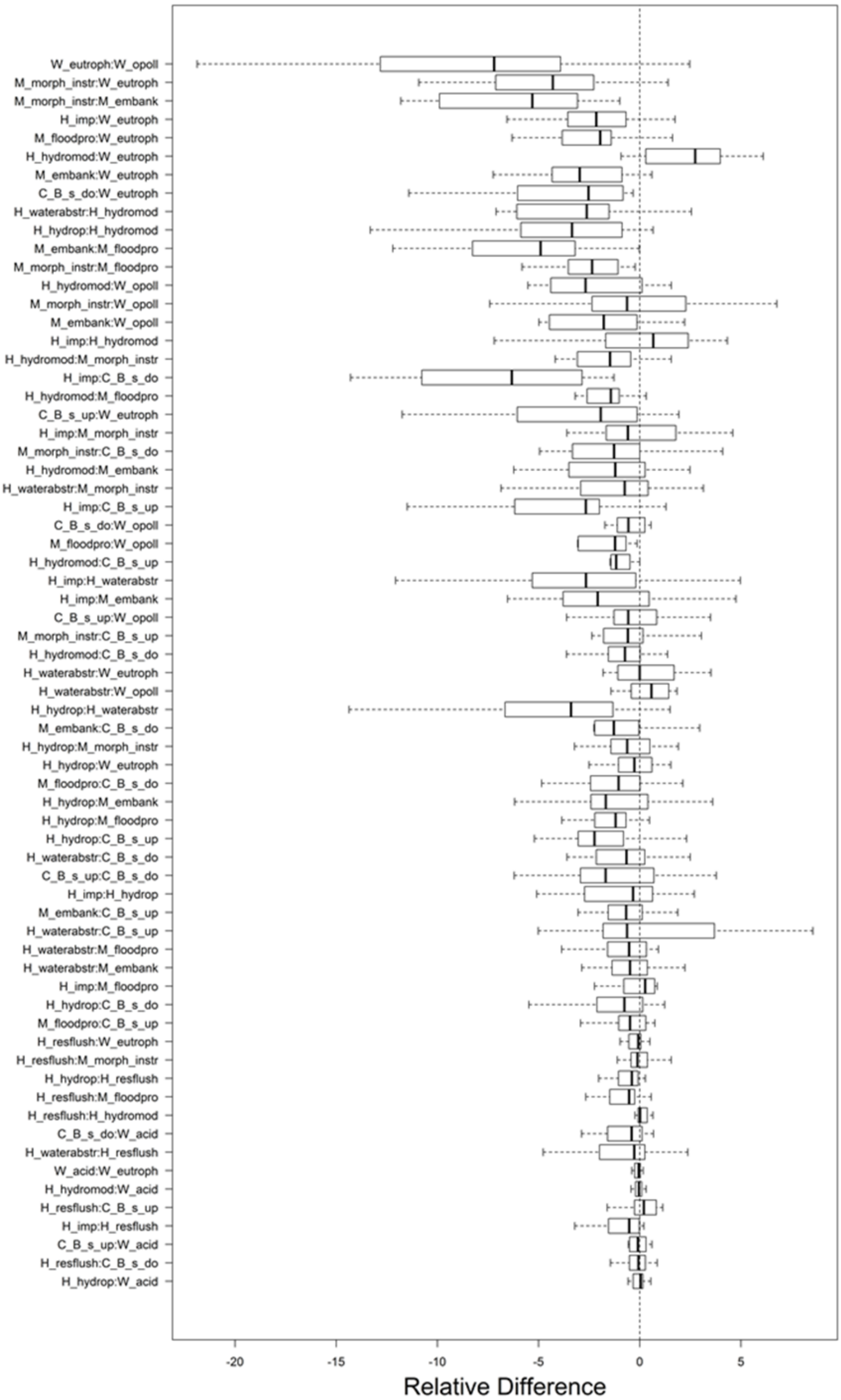

Figure 5 - Boxplot showing the distributions of the Relative difference between the sum of the individual importance values and the paired importance values for each pairwise combination of stressors, based on the random forest models for the eighteen metrics analysed. Values of relative difference that lie either in a more negative or more positive position represent potential interaction effects.

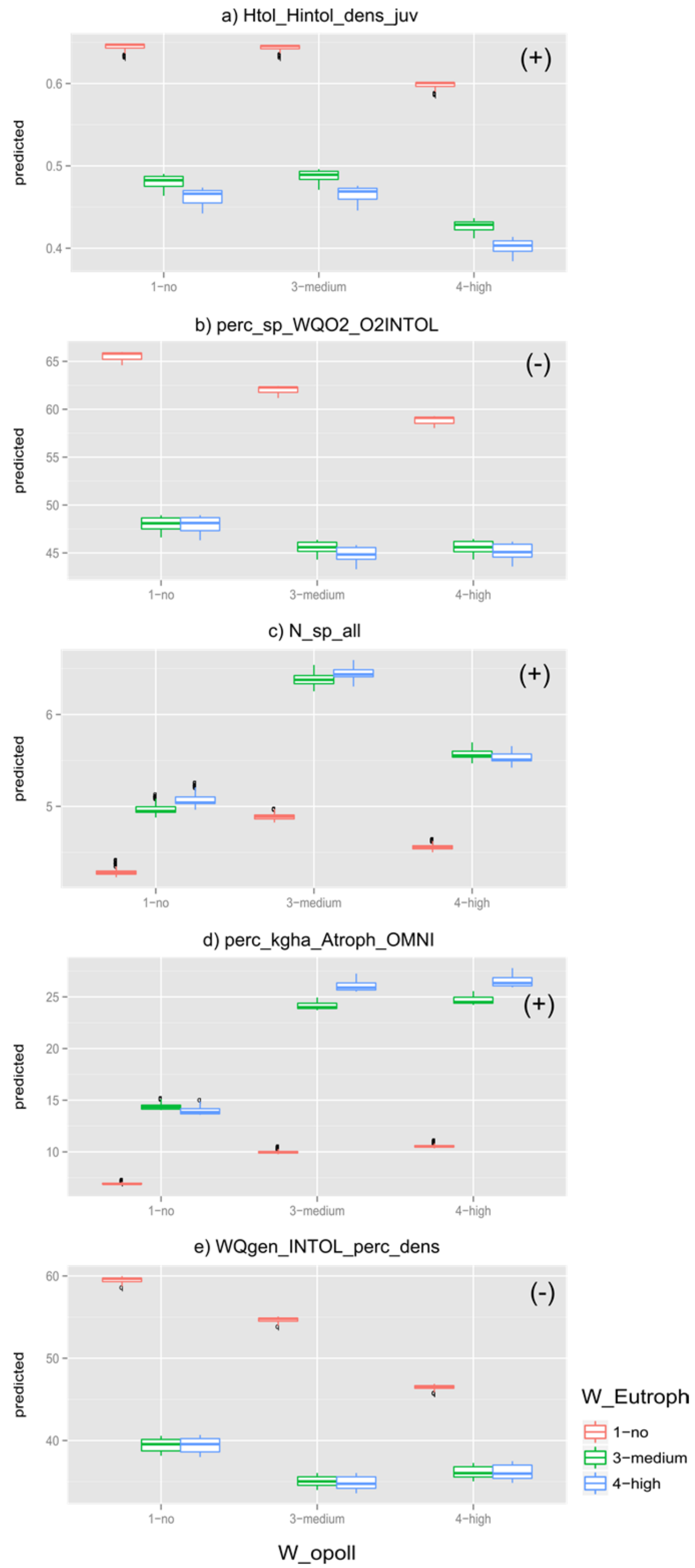

Figure 6 - Partial co-plots showing the simultaneous response of the five most responsive metrics to organic pollution (W_opoll) and eutrophication (W_eutroph). Plots marked with (+) suggest synergistic effects and those with (-) suggest antagonistic effects.

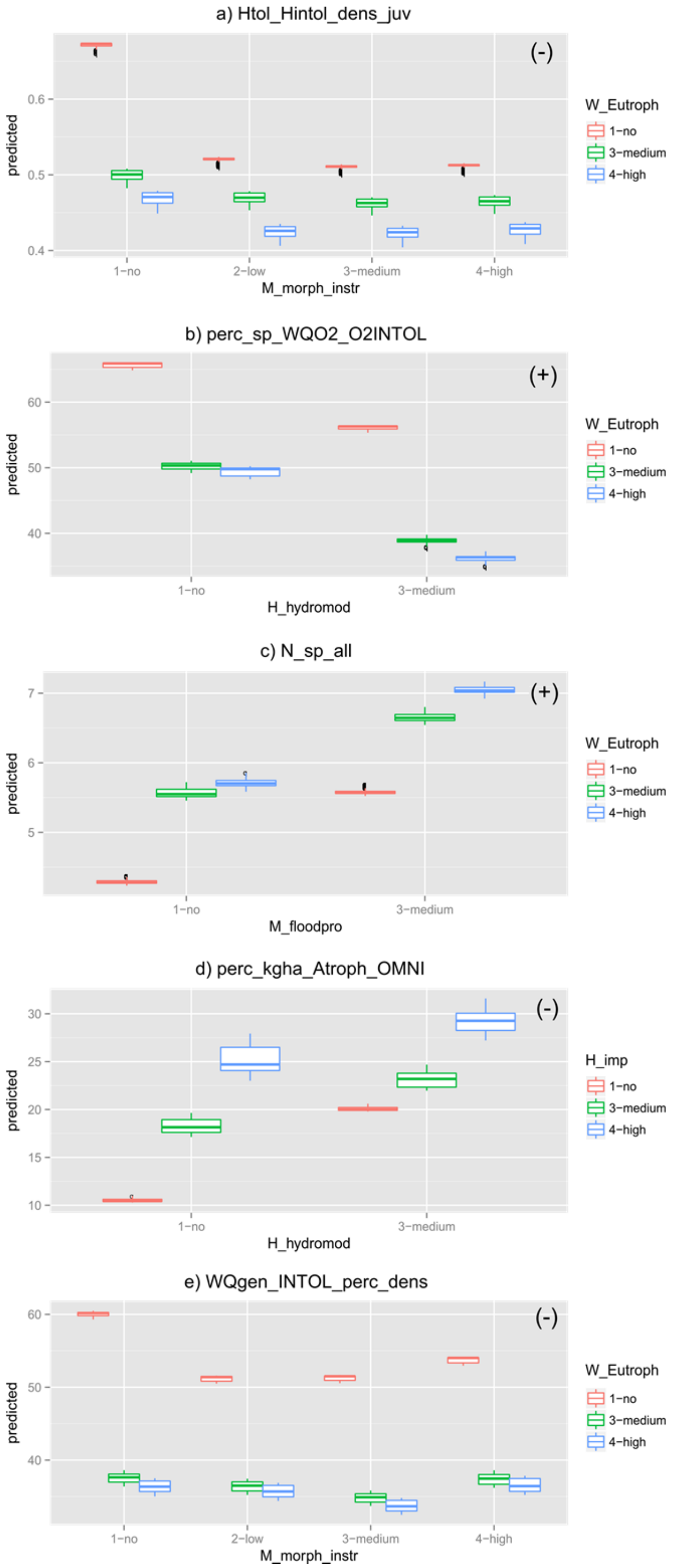

Figure 7 - Partial co-plots showing the simultaneous response of the five most responsive metrics to other important interactions according to the Random Forest models. Plots marked with (+) suggest synergistic effects and those with (-) suggest antagonistic effects.

Stressor distribution and frequent stressor combinations

In terms of stressor distribution and frequent stressor combinations, we found eight single stressors (which affect 611 sites) and eight stressor combinations (which affect 1799 sites) and which are occurring frequently (at more than 20 sites of the overall dataset, in order to be representative for the following analyses). Moreover, 695 sites were unimpacted and thus are available as reference sites. The stressor distribution and frequent stressor combinations are shown in Table 3.

Table 4- Stressor distribution and frequent stressor combinations per Fish Assemblage Type

Stressor combination type	HWS	LLR	MES	MGR	**Total**
no_pressure	194	122	93	286	**695**
C_do	27	9	6	31	**73**
C_up	14	10	9	30	**63**
H_waterabstr	57	2	19	22	**100**
M_floodpro	2	4	2	29	**37**
M_morph_instr	4	44	6	22	**76**
W_acid	10	1	0	26	**37**
W_eutroph	42	3	21	32	**98**
W_opoll	49	8	31	39	**127**
C_do & C_up	19	1	11	12	**43**
W_opoll & C_do & C_up	8	1	4	7	**20**
W_opoll & M_floodpro	0	3	0	18	**21**
W_opoll & W_eutroph	13	18	23	37	**91**
W_opoll & W_eutroph & C_do & C_up	4	1	2	18	**25**
W_opoll & W_eutroph & M_morph_instr	2	14	7	17	**40**
W_opoll & W_eutroph & M_morph_instr & M_embank & C_do & C_up	2	0	0	25	**27**
W_opoll & W_eutroph & M_morph_instr & M_floodpro & M_embank	0	11	0	11	**22**

Figure 8 shows the spatial location of unimpacted sites, sites impacted by single stressors and by stressor combinations.

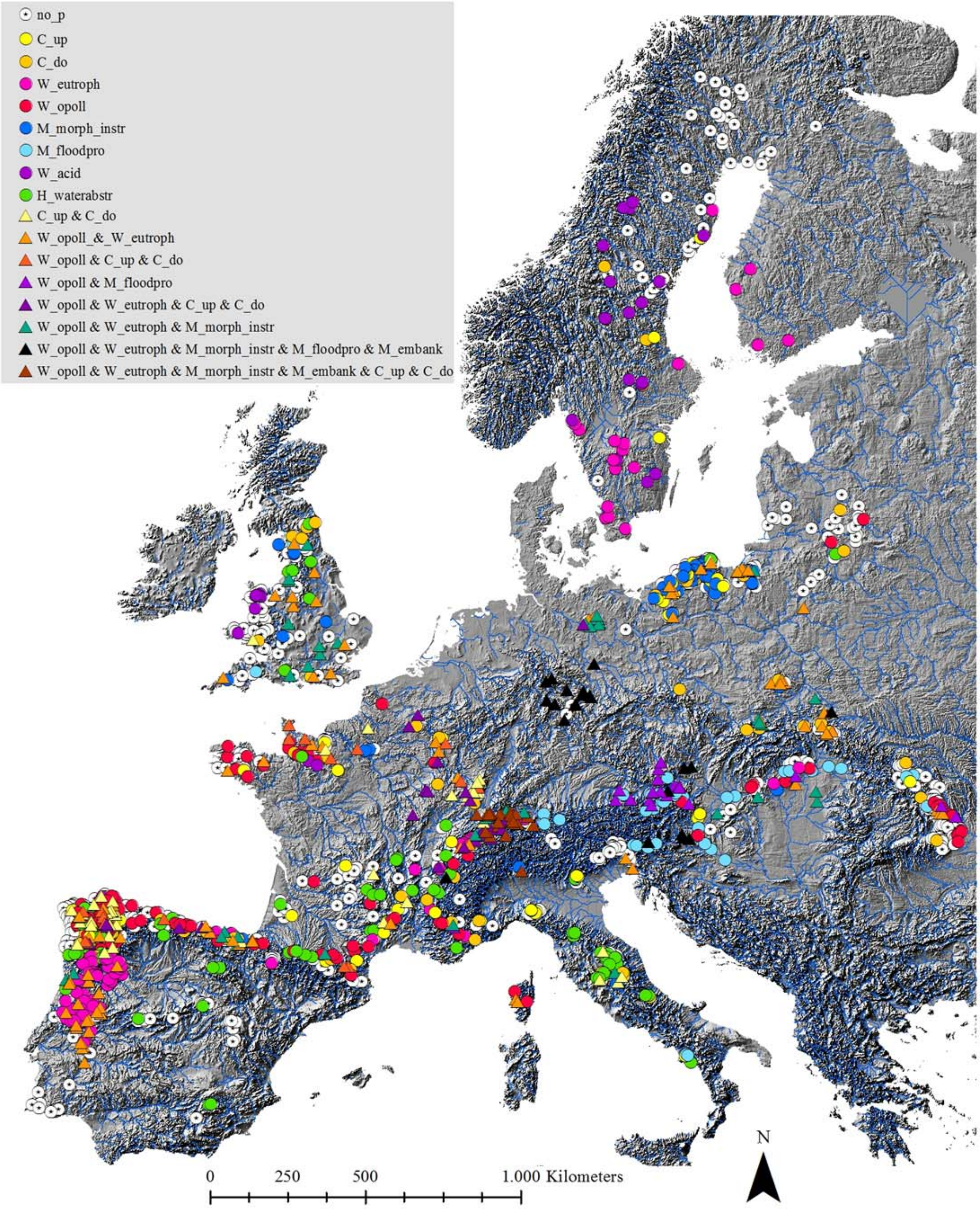

Figure 8 - Location of fish sampling sites affected by single stressors (circle symbols) and combined stressors (triangle symbols).

Reaction of metrics to single stressors and stressor combinations

As shown by Figure 9 A-E, the five most important fish metrics in the random forest model also showed the best response to single stressors and stressor combinations in the boxplots.

These metrics were *total abundance of juveniles (<150mm) intolerant to habitat degradation* (HTOL_HINTOL_dens_juv, Figure 9 A), *% of abundance of intolerant to general water quality degradation* (WQgen_INTOL_perc_dens, Figure 9 C), *% of species intolerant to oxygen depletion* (WQO2_O2INTOL_perc_sp), *% of omnivorous biomass* (Atroph_OMNI_perc_biom, Figure 9 D) and *total richness* (Nsp_all, Figure 9 E). The strongest results where shown for two metrics: For *juveniles intolerant to habitat degradation*, eutrophication as a single stressor and eutrophication in combination with organic pollution and in-stream habitat degradation (Figure 9 A). For this case, the predicted combined stressor was lower than the observed joint interaction, which indicates a synergistic interaction.

Moreover, for *% species intolerant to oxygen depletion*, the strongest deviation was observed for eutrophication as a single stressor, and another strong synergistic interaction with organic pollution and in-stream habitat alteration (Figure 9 B).

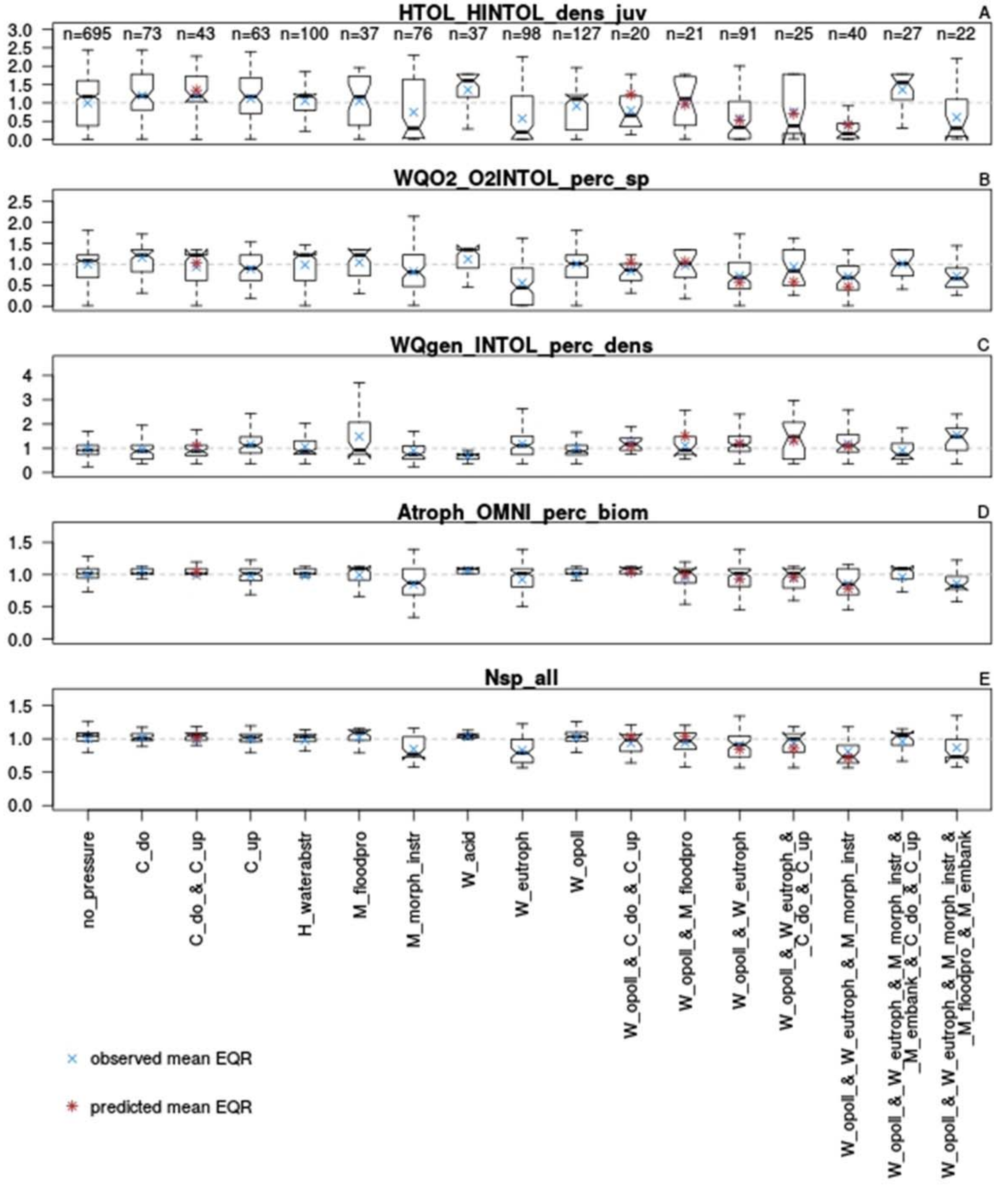

Figure 9 A-E - Boxplots of the five most important fish metrics (displayed as EQR) and their reaction to single and multiple stressors (A – total abundance of juveniles intolerant to habitat degradation, B - % of species intolerant to oxygen depletion, C - % of abundance of intolerant to general water quality degradation, D - % of omnivorous biomass, E - total richness). The blue cross shows the observed mean EQR, the red asterisk the predicted mean EQR.

Multi-stressors analyses on lakes

Data and method

The analyses were conducted on two datasets, one including the natural lakes of seven European countries and another on the Portuguese and French reservoirs.

Natural lakes - The fish data have been collected between 2003 and 2014 in the framework of the intercalibration exercise, then updated during the WISER project. Natural lakes from Estonia, Denmark, France, Germany, Ireland, Norway and Sweden were used (Figure 10). Some French data were collected more recently at the occasion of new fish campaigns. All these lakes were sampled using the Norden gillnet standardised protocol (C.E.N., 2005). This method entails the use of pelagic and benthic nets. In this analysis, only fish data collected with benthic multi-mesh gillnets were used. These benthic nets were 30 m long and 1.5 m high, and composed of 12 different panels with mesh sizes ranging between 5 mm to 55 mm knot to knot in a geometric row. Random samplings were performed in different depth strata during the summer period. The number of nets set in each stratum depended on lake depth and area. A standard fishing period corresponded to one night (setting gillnets at dusk and lifting them at following dawn).

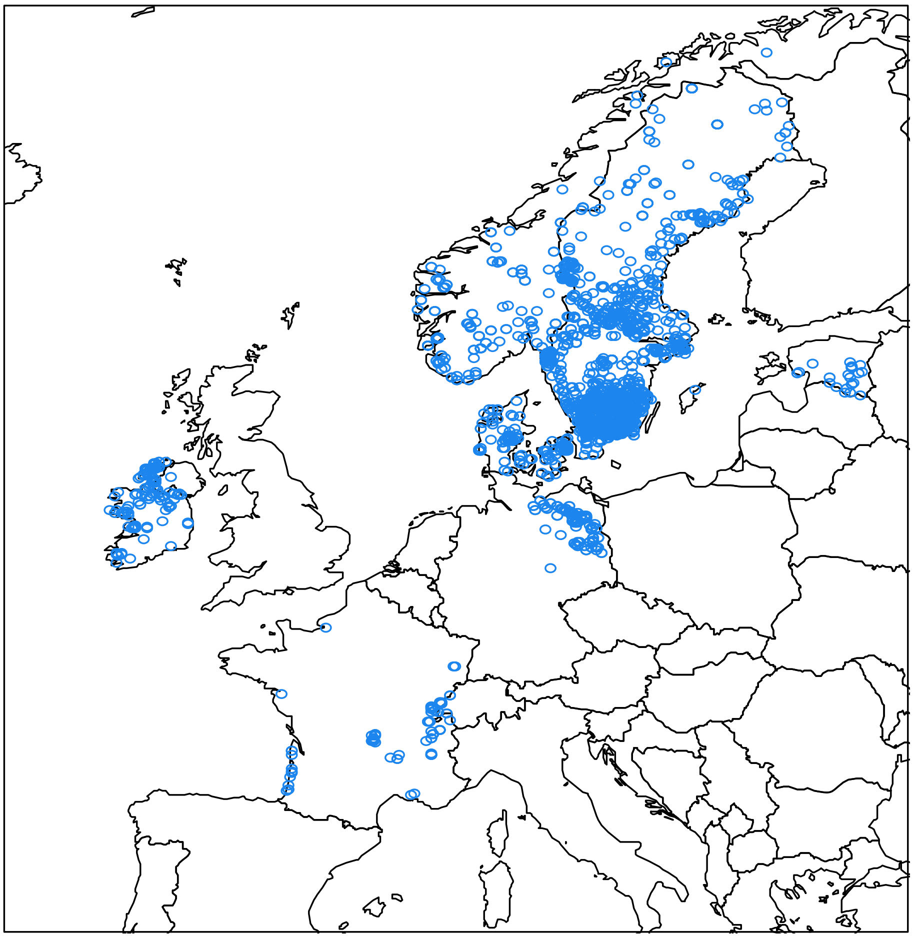

Figure 10 – Location of the 404 natural lakes included in the large scale analysis

French and Portuguese reservoirs (Figure 11) - The French reservoirs were sampled following the same method as natural lakes. Portuguese reservoirs were sampled between 2004 and 2005 using benthic multi-mesh nets measuring 30 x 2.5 m and composed of five panels of 6 m each with mesh sizes ranging from 30 to 95 mm knot to knot. The standard fishing period also covered sunset and sunrise periods and lasted 17 hours on average. As Portuguese reservoirs were sampled using different gillnets, the results of fishing campaigns had to be homogenised. We discarded captured fish individuals smaller than 90 mm and longer than 600 mm in French results to match with Portuguese size ranges.

In both European and South Western datasets, the fish caught were identified to species level, counted and weighed in grams. The fish catches were converted to catch per unit effort (number of fish/m² of net/night) and biomass per unit effort (g/m² of net/night).

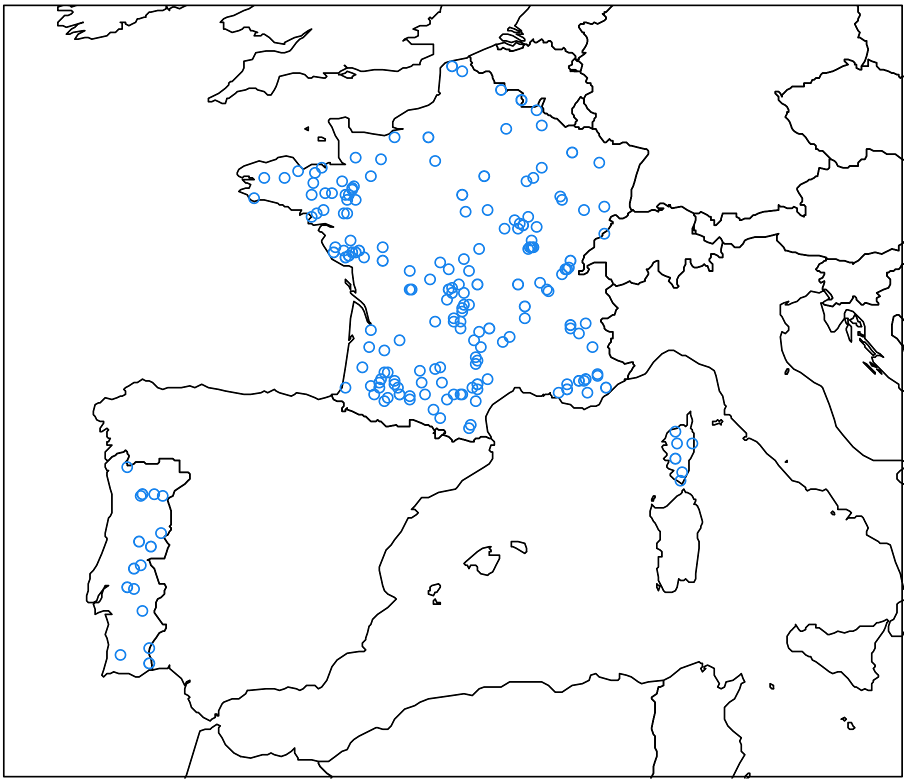

Figure 11 – Distribution of the 236 French and Portuguese reservoirs.

Fish metrics

Traits used to describe species attributes were defined according to a literature survey elaborated from national experts' judgment. Species were assigned to trophic guilds as follows: invertivorous (INV) species whose adult diet consists of more than 75% insects; planktivorous (PLAN) species whose adult diet consists of more than 75% zooplankton and/or phytoplankton; piscivorous species (PISC) feeding on fish, at least partly, as adults. Carnivorous (INV/PISC) species include individuals that are both invertivorous and piscivorous. Species that are both invertivorous and planktivorous (INV_PLAN), invertivorous and herbivorous (INV_HERB), detritivorous and herbivorous (DETR_HERB) or benthivorous (BENT) were not abundant enough and these traits were not used. If plant and animal material both contributed at least 25% to the diet, the species was considered omnivorous (OMNI) (Schlosser, 1982). Species were also classified according to their pelagic (WC) or benthic (BENT) living and feeding habitats. The reproductive guilds considered were phytophilic (PHYT) species spawning on different parts of living or dead vegetation and lithophilic (LITH) species spawning on clean mineral substrate. Species showing indifferent spawning preferences were considered to be both phytophilic and lithophilic (PHLI). Reproductive traits represented by only a few species (ariadnophilic (ARIAD), and ostracophilic (OSTRA), i.e. species spawning in shells; pelagophilic (PELA) species spawning in the pelagic zone) were not taken into account. Species were also classified as being either tolerant (Tolerant) or intolerant (Intolerant) to any stressor related to lake morphology (habitat), hydrology or water chemistry (Karr et al., 1986). Finally, fish species were also sorted in reproductive behaviour guilds according to their guarding and nesting behaviour (Balon, 1981, 1975). The A guild includes non-guarders species, A_1_0 depositing eggs on open substrate while A_2_0 species hide their eggs. The B guild includes species guarding their eggs: B_1_0 species scatter them on plants and B_2_0 species construct nests. A_2_0 and B_1_0 were excluded as these traits were rarely represented in our datasets. All these metrics were expressed as the sum of the biomass and the sum of the abundances of the species they include. Total abundance and total biomass were included (ALL (CPUE) and ALL (BPUE)) and the ratio of roach (*Rutilus rutilus* (L.)) to perch (*Perca fluviatilis* L.). Trait values for each species encountered in European and South Western datasets are described in Appendix 1. It was decided to not integrate unknown species and hybrids in the calculation of metrics for functional guilds (*Abramis* sp., *Coregonus* sp., *Cottus* sp., *Mugilidae* unknown, *Cyprinidae* unknown) because the traits could be different from one species to another, even in the same family.

Thirty metrics were calculated using fifteen traits expressed as biomass (BPUE) or abundance (CPUE) of fish belonging to each guild. Similarly, total individuals and the ratio roach/perch was calculated in occurrence and biomass. A total of 34 metrics were used in the different analyses.

Environment and stressors

The variables given in Table 5 were included in the analyses. Maximum depth (Zmax) and Lake Area (LA) are strong drivers of fish species richness (Barbour and Brown, 1974; Eadie and Keast, 1984). Catchment area (ADB) can be considered as a surrogate for habitat diversity upstream from the lake (Irz et al., 2004).

Latitude and longitude were retained as biogeographical variables (decimal degree (WGS84)). Altitude (Alt) parameter can be related to isolation and climatic data (Godinho et al., 1998; Hinch et al., 1991; Magnuson et al., 1998). No mountain lakes above 1500 m were included because species richness is generally low; moreover, in these lakes, fish communities are generally strongly influenced by human introductions (Argillier et al., 2002a; Argillier et al., 2002b) and fish is not considered as a relevant bioindicator to assess ecological status (Ministère de l'Ecologie et du Développement Durable, 2010).

January to December mean yearly air temperatures ($T_{January}$ & $T_{December}$) were obtained from the climate CRU model (New et al., 2002). January and July mean temperature allowed to derive the following independent variables related to temperature requirements of living organisms (Daufresne and Boet, 2007; Irz et al., 2007; Mason et al., 2008) :

(i) $AveT = (T_{January} - T_{December})/12$

(ii) $AmpT = T_{July} - T_{January}$

Table 5 – Environmental variables in the European natural lakes and South-western reservoirs.

	European dataset		South-western subdataset	
	Mean	**Range**	**Mean**	**Range**
Maximum depth (Z_{max}, m)	15.6	0.6 - 310	28.2	1.2 – 135
Lake Area (LA, km^2)	2.3	0.01 – 577.1	3.3	0.01 – 577.1
Catchment Area (CA, km^2)	82.1	0.05 – 10 628.9	30 001	0.7 – 963 000.0
Latitude (Lat, °)	57.9	43.6 – 69.7	45.9	37.3 – 50.9
Longitude (Long, °)	12.9	-10.2 – 30.8	1.5	-8.5 – 9.5
Altitude (Alt, m asl)	209.6	0 – 1 500	276.3	0 – 1 325.0
Mean temperature (AveT, °C)	5.3	-3.8 – 14.3	11.0	4.3 – 17.6
Temperature amplitude (AmpT, °C)	18.9	8.4 – 29.5	15.1	9.9 – 18.4

These parameters were also retained because of their availability (for example mean depth was excluded because of too many unknown values).

Altitude, maximum depth, lake area and catchment area were log-transformed for graphical display. A correlation between all natural parameters was performed to check their independence.

Environmental variables were grouped and summarized thanks to a PCA method. Groups were Biogeography (2 PCA axes), Climate (1 PCA axis) and Ecosytem size (2 PCA axes), respectively including latitude, longitude and altitude, mean temperature and temperature amplitude, and lake maximum depth, lake area and catchment area.

Stressors: Eutrophication was measured through Total Phosphorous mean annual concentrations. This value was a mean of at least four measurements in a single year for all the lakes. Values were log-transformed to obtain a normal distribution. Non-native species presence was also taken into account as the proportion of non-native species in biomass. Non-native species status was assessed at the basin level thanks to the Fish SPRICH database (Brosse et al., 2013). *Hydromorphological stressors:* In the work done at the European scale, for most of the lakes, the intensity of (hydrological) and mostly morphological modifications were assessed by expert judgement and in some instances in application of the Lake Habitat Survey (Rowan et al., 2005). The lakes were then classified in two classes, one experiencing weak modifications and the other experiencing heavy modifications. In Portuguese and French reservoirs, alteration of the hydromorphology was always measured in the framework of the Lake Habitat Survey. In these analyses, lakes were classified according to six stressor indices: shore zone modification, shore zone intensive use, in-lake use, hydrology, sediment regime and nuisance species (Rowan et al., 2005). Shore zone modification index measures the proportion of the shoreline affected by hard engineering. Shore zone intensive use measures the proportion of natural *vs.* artificial land cover on the shore line (Rowan et al., 2005). In-lake use quantifies the number of in-lake pressures such as dredging, macrophyte control, boat activities, angling, fish stocking etc. (Rowan et al., 2005). Nuisance index indicate the presence of terrestrial (i.e. the Japanese Knotweed *Fallopia japonica*) or aquatic (i.e. Nuttall's pondweed *Elodea nuttallii*) nuisance plants (Rowan et al., 2005). As hydrology index heavily penalizes dammed lakes (i.e. the lowest note was attributed to all the reservoirs) and as in-lake use was not directly related to hydromorphology, we excluded these two indices from the analyses (Rowan et al., 2005).

Modelling

On the two datasets (Europeans lakes and South-western reservoirs), a random forest was grown for each fish metric. As previously described (section General approach), 2500 trees were grown per forest and, the percentage of explained variance and variables relative importance were computed (Breiman-Cutler permutation method). Random forest models were of the form fish metric ~ Biogeography + Ecosystem size + Climate + Eutrophication + Non-native species + Hydromorphological alterations.

We selected pertinent models by keeping only models in which at least one of the stressor variables explains 10% or more of the total variance.

In selected models, the intensity of interactions between stressors was assessed by computing the difference between their "Paired" importance and the sum of their individual importance ("Additive importance") (Ishwaran, 2007). This difference value was expressed relatively to the "additive" importance. This process is described in details in the section "Pairwise approach – random forests". Finally, for each of the selected models, we chose interactions of interests among all possible interactions, notably eutrophication × proportion of non-native species.

Finally, for both natural and artificial lakes, we analysed interactions involving the two stressor variables having the greatest relative importance. The only exception was in artificial lakes, the interaction between eutrophication and shore use for the abundance of B_2_0 species was also taken into account. Interactions were analysed and plotted thanks to co-plots representing the predicted response of fish metrics to a stressor variable (continuous or categorical) for different levels of another stressor variable (categorical).

Results

Effect of stressors on fish metrics of the European natural lakes

At the European level, models explained between 2.3 and 51.4% of the variance (Figure 12). The stressor variables explained at least 10% of the variance in eight models that where studied in details (Figure 12). Total explained variance of these models ranged from 28.2 to 39.5%.

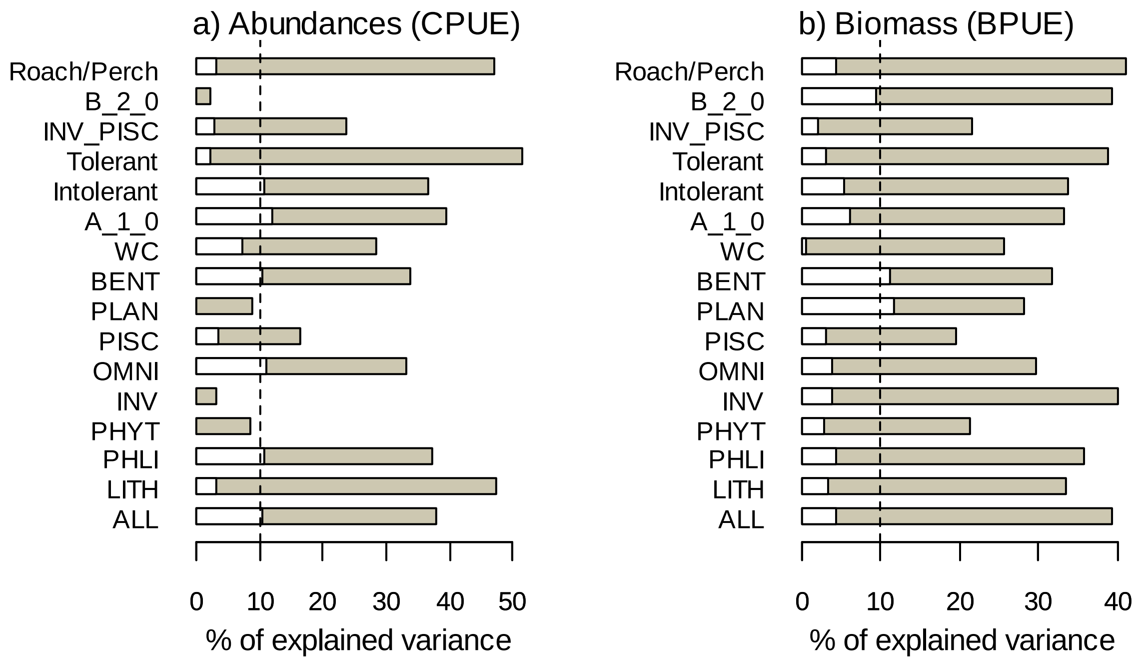

Figure 12 - Total percentage of variance explained by stressor variables (in white) and the environment for each fish metric calculated with the abundance of fish (a) and the biomass of fish (b). The dashed line represents the threshold of variance explained above which the model was selected.

The eight metrics best explained by stressors were in relation with total occurrence (CPUE), tolerance, reproductive guilds (PHLI and A_1_0), trophic guild (OMNI and PLAN) and living and feeding habitats (BENT) (Figure 13). For most of these metrics, biogeography and size of the lakes explained the major part of the variability. However the proportion of non-native species and total phosphorus were the variables explaining most of the variability of the metric CPUE of planktivorous (relative VIMP of 21.1 and 20.3% respectively), and total phosphorus had a relative importance of 29.1% in the model explaining the BPUE of benthic species. Shore Bank Modification index showed only a poor relative importance and added little explanation power to the models (Figure 13). Indeed, in the selected metrics, the relative variable importance of SBM ranged from 0.002 to 0.9% while the relative variable importance of total phosphorus ranged from 15.2 to 29.1% and the relative variable importance of non-native species proportion was between 6 and 21.1%. In all cases, eutrophication was the stressor the more strongly related to fish metrics and the Shore Bank Modification index was systematically the least important variable.

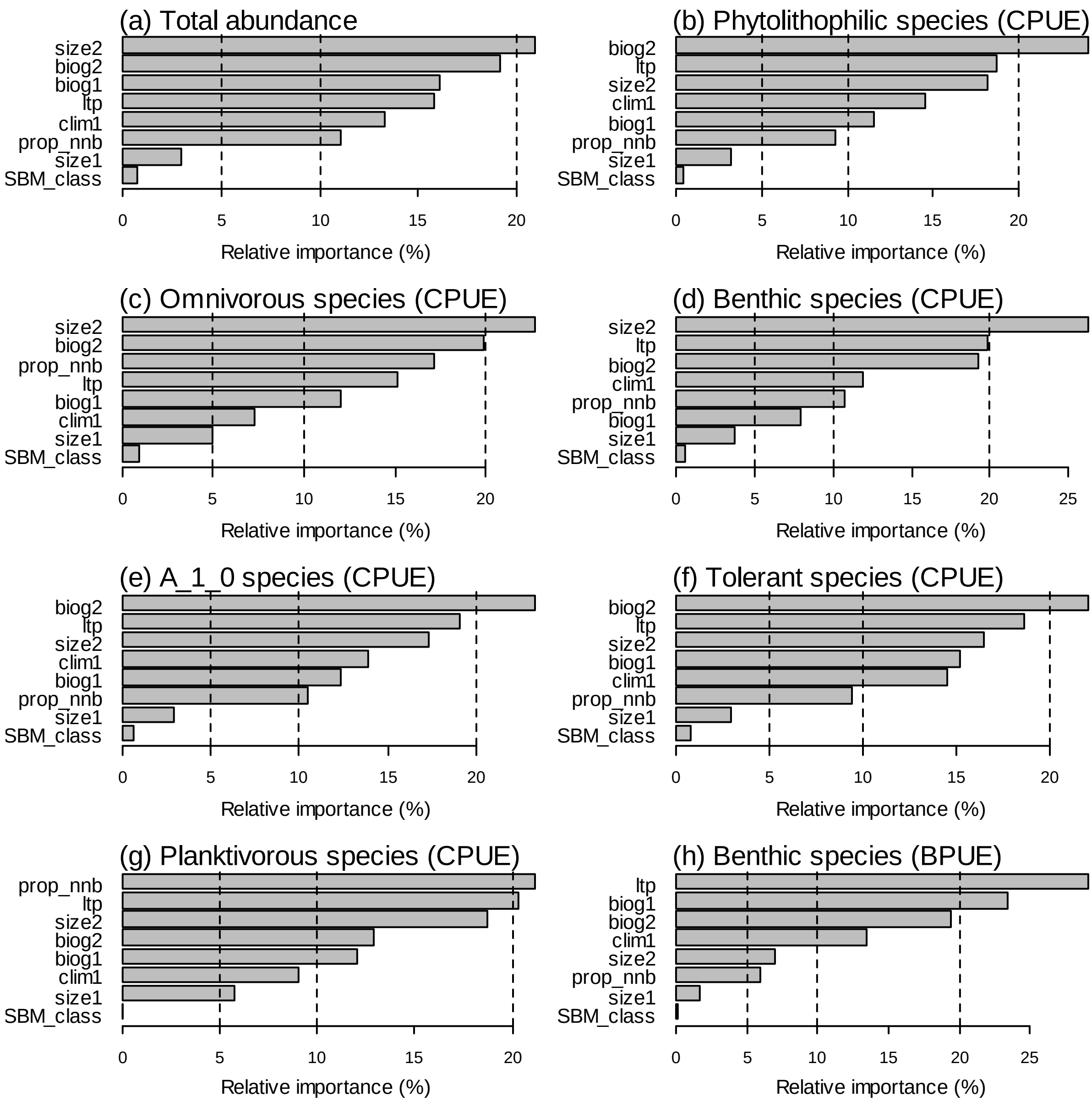

Figure 13 – Relative importance of environmental and stressor variables for the selected fish metrics. Biog1 & 2, size1 & 2 and clim1 are the PCA axes summarizing biogeography, ecosystem size and climate, respectively. Ltp and prop_nnb stand for the concentration of total phosphorus and the proportion of non-native species in biomass, respectively. SBM_class is a measure of Shore Bank Modification.

Interactions between stressors in European natural lakes

Differences and relative differences between Paired and Additive importance of stressor variables were weak (Figure 14). The greatest relative difference was -1.96% which indicates the very weak interactions between stressors studied on the fish European dataset we used. Co-plots indicated strictly additive interactions between eutrophication and the proportion of non-native species (Figure 15). A threshold effect of eutrophication seems to appear between first oligotrophic and then meso- and eutrophic lakes (Figure 15).

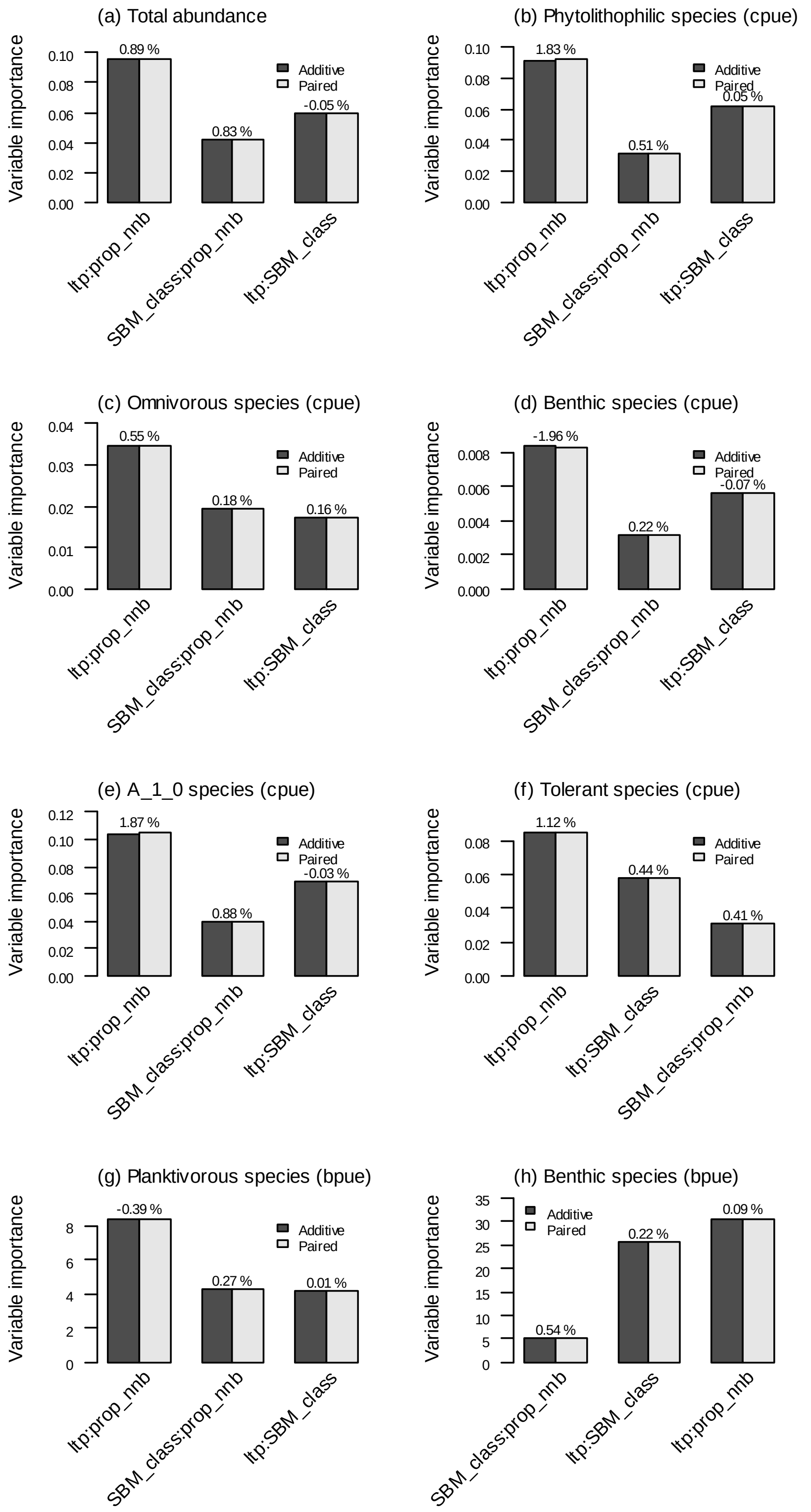

Figure 14 - Paired and additive importance of stressor variables in European natural lakes. The difference between paired and additive values of variable importance measures the interaction between the variables. Interactions are ordered according to the difference value that is written above the bars and expressed relatively to the Additive importance. Positive values indicate synergistic interactions while negative values indicate antagonistic interactions.

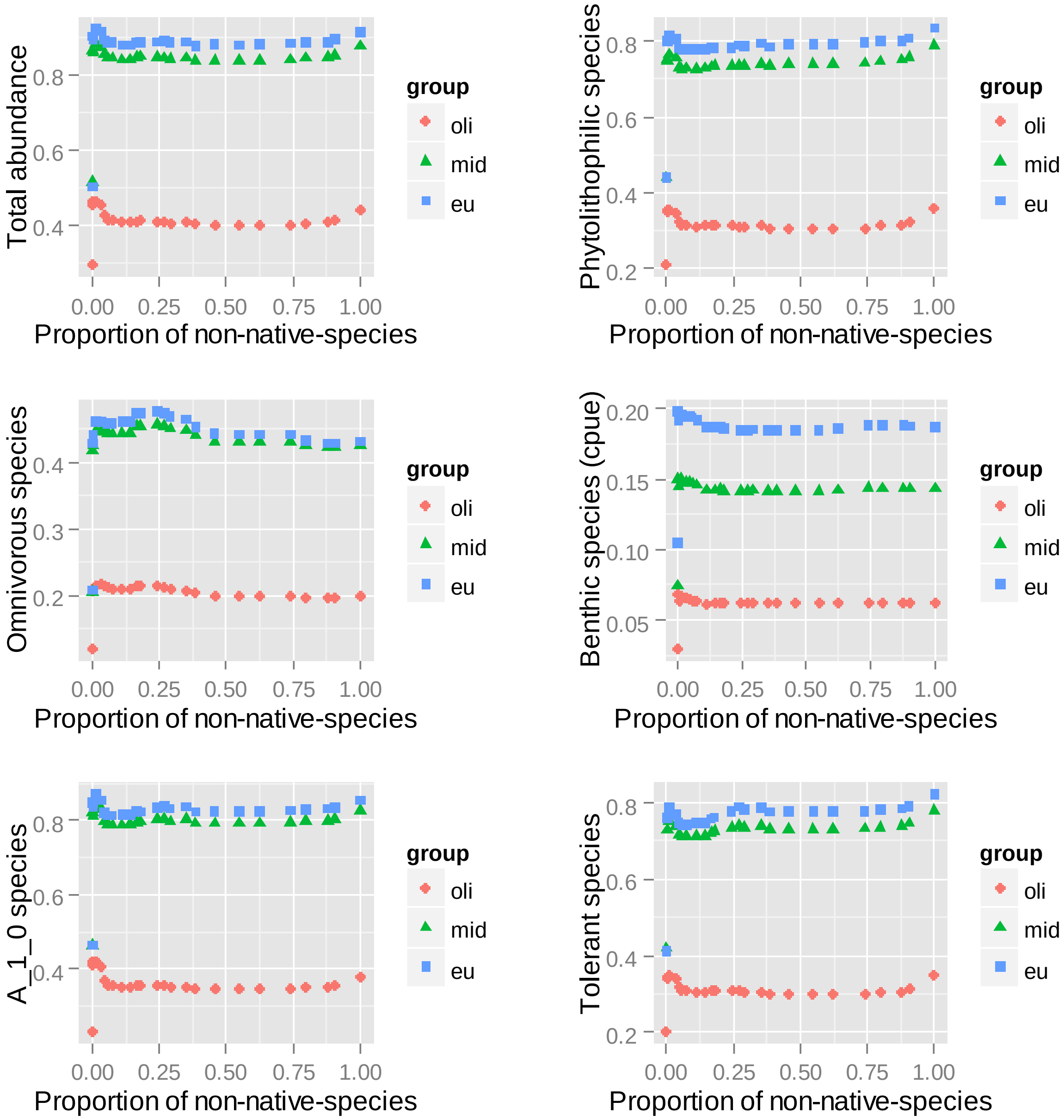

Figure 15 - Partial relations of non-native species with selected fish metrics in different contexts of eutrophication. Thresholds between oligotrophic, meso- and eutrophic groups were 20 and 45 µg L^{-1}.

Effect of stressors of fish metrics in the French and Portuguese reservoirs

Regarding the French and Portuguese reservoirs, random forest models explained up to 55.3% of the variance of all the fish metrics calculated on the dataset, and stressors accounted a maximum of 32.0% of the explaining power (Figure 16). Nine fish metrics in which at least 10% of the variance was explained by stressor variables were selected. The total explained variance of these selected metrics ranged from 27.8 to 55.3% (Figure 16).

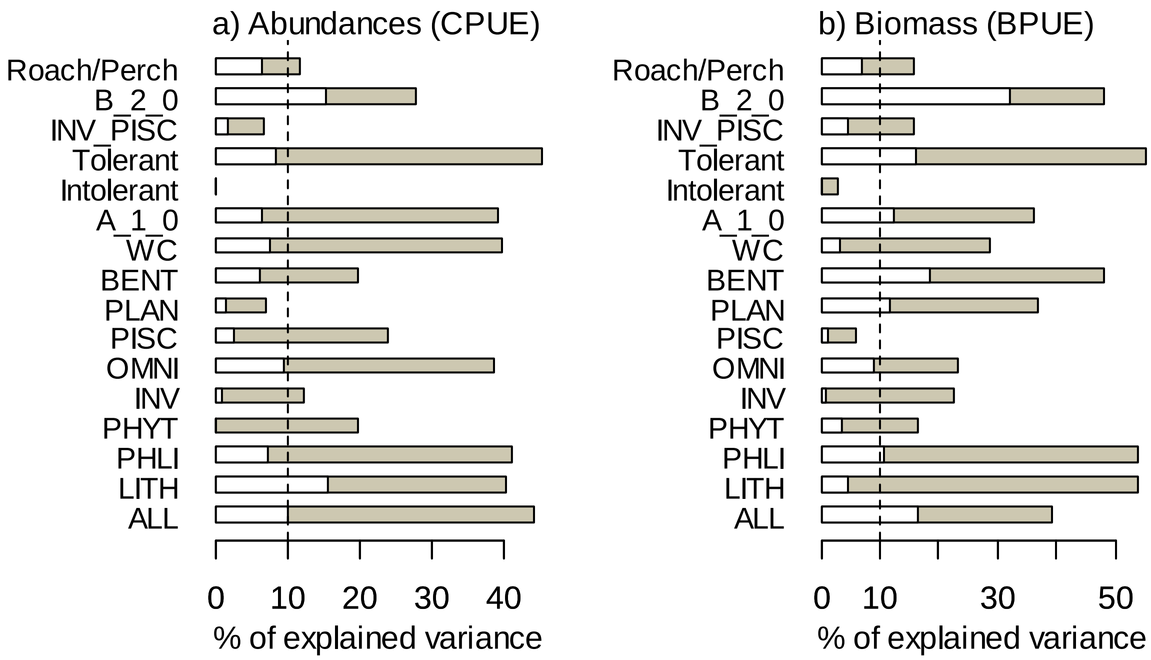

Figure 16 - Total percentage of variance explained by stressor variables (in white) and the environment for each fish metric calculated with the abundance of fish (a) and the biomass of fish (b). The dashed line represents the threshold of variance explained above which the model was selected.

The first result was that hydromorphological impacts were only weakly related to fish metrics compared to eutrophication and non-native species relative importance (Figure 17).

Again, biogeography and ecosystem size variables were important while climate was usually a minor environmental variable (maximum relative importance variable = 13.4% for the abundance of lithophilic species).

B_2_0 species abundance and *biomass*, and *total biomass* were clearly related to stressor variables. The proportion of non-native species and eutrophication explained more variance than environmental variables. *Planktivorous species*, *benthic species*, *tolerant species* and *A_1_0 species* were also strongly related to eutrophication which was systematically the main or one of the main variables and had a relative importance > 20%. *Lithophilic species* were mainly explained by the proportion of non-native species (relative importance = 32.7%).

In the model explaining the *biomass of tolerant species*, the shore use intensity variable had a relative importance of 3% and was ranked 6th out of 11 (Figure 17). Except in that case, hydromorphological variables were systematically the four least important variables.

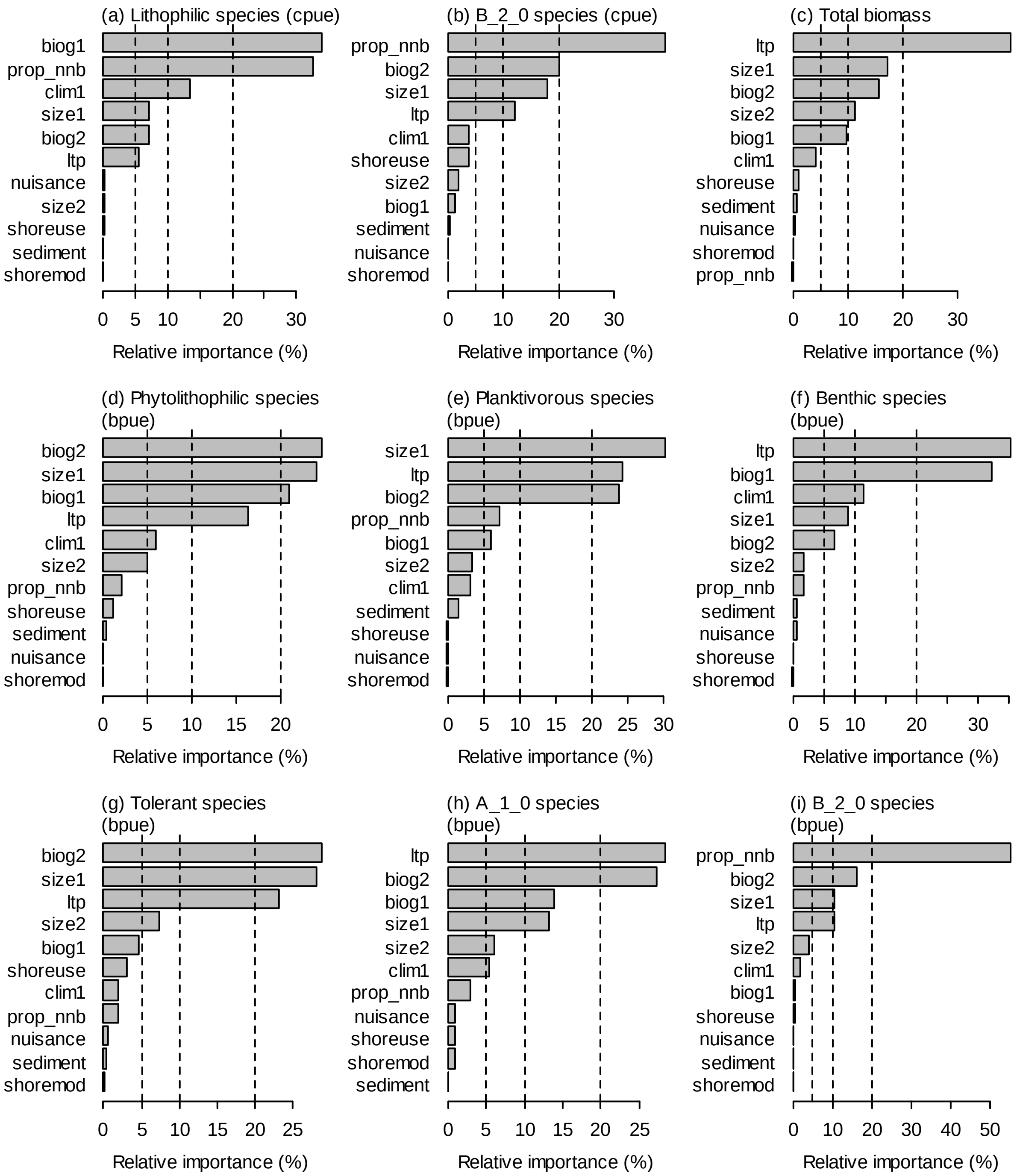

Figure 17 - Relative importance of the environmental and stressor variables for each selected fish metric. Biog1 & 2, size1 & 2 and clim1 are the PCA axes summarizing biogeography, ecosystem size and climate, respectively. Ltp and prop_nnb stand for eutrophication and the proportion of non-native species in biomass, respectively. SBM_class is a measure of Shore Bank Modification.

Interactions between stressors in French and Portuguese reservoirs

The strength of interactions between stressor variables was assessed by computing the difference between additive and paired importance (Figure 18 ;

Figure *19* ; Figure 20) and, similar to natural lakes, most of the time the differences were weak. Although we observed some great relative differences (see Figure 18a shoremod:shoreuse and Figure 18b shoremod:nuisance), the variance explained by these stressors indicated only a poor significance of the interaction.

For metrics based on the fish occurrences (Figure 18), in the models explaining the *abundance of lithophilic species* and of *B_2_0 species* (building a nest), eutrophication and the proportion of non-native species had slightly different importance whether they were separated or paired (Figure 18ab ; Figure 19ab). The co-plot showed synergitic interactions of these stressors on the two metrics. In the model explaining the *abundance of B_2_0 species* (Figure 18b ; Figure 19c), the interaction between eutrophication and the intensity of shore use seems significant with a relative difference of -6.65% but the graphical analyses of the co-plot didn't confirm a synergistic or antagonistic interaction (Figure 20).

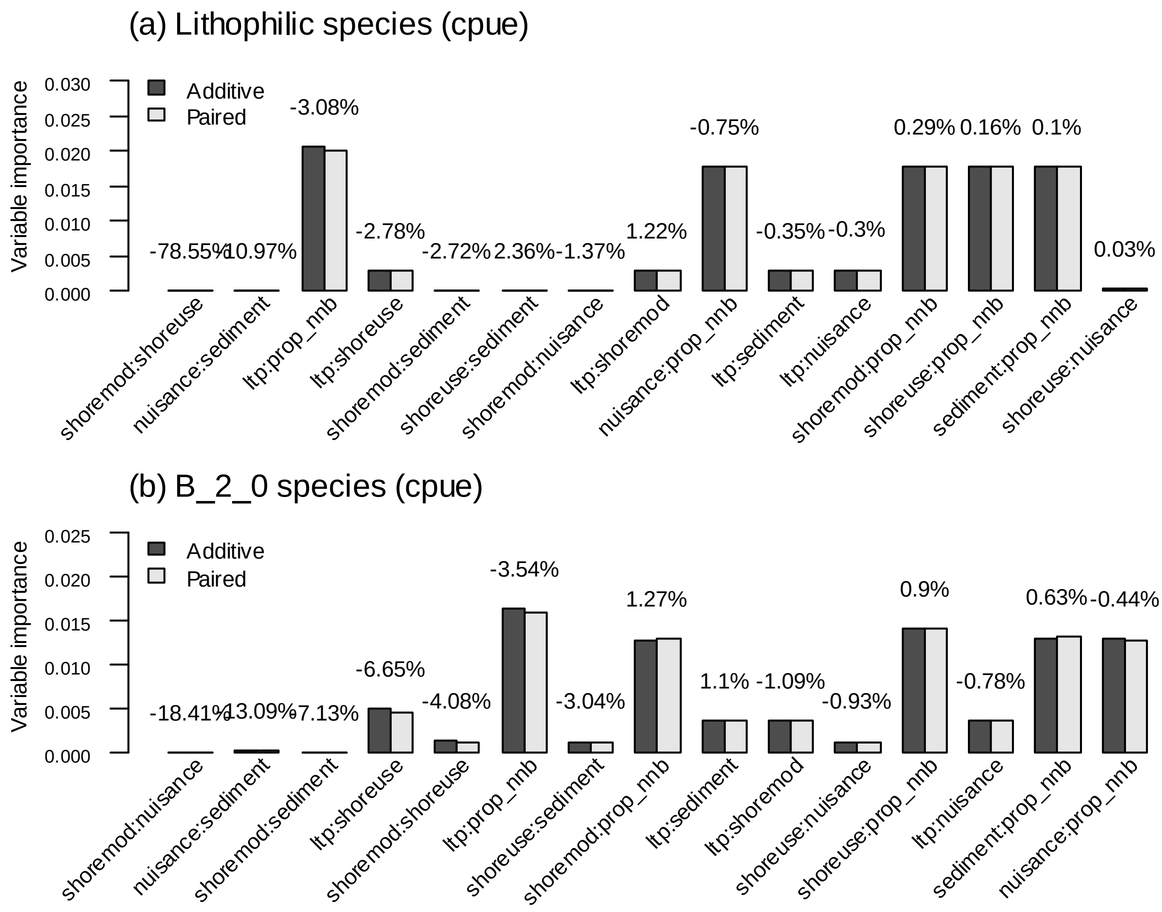

Figure 18 - Paired and additive importance of stressor variables for metrics based on abundances in the French and Portuguese reservoirs. The difference between paired and additive values of variable importance measures the interaction between the variables. Interactions are ordered according to the difference value that is plotted above the bars and expressed relatively to the Additive importance.

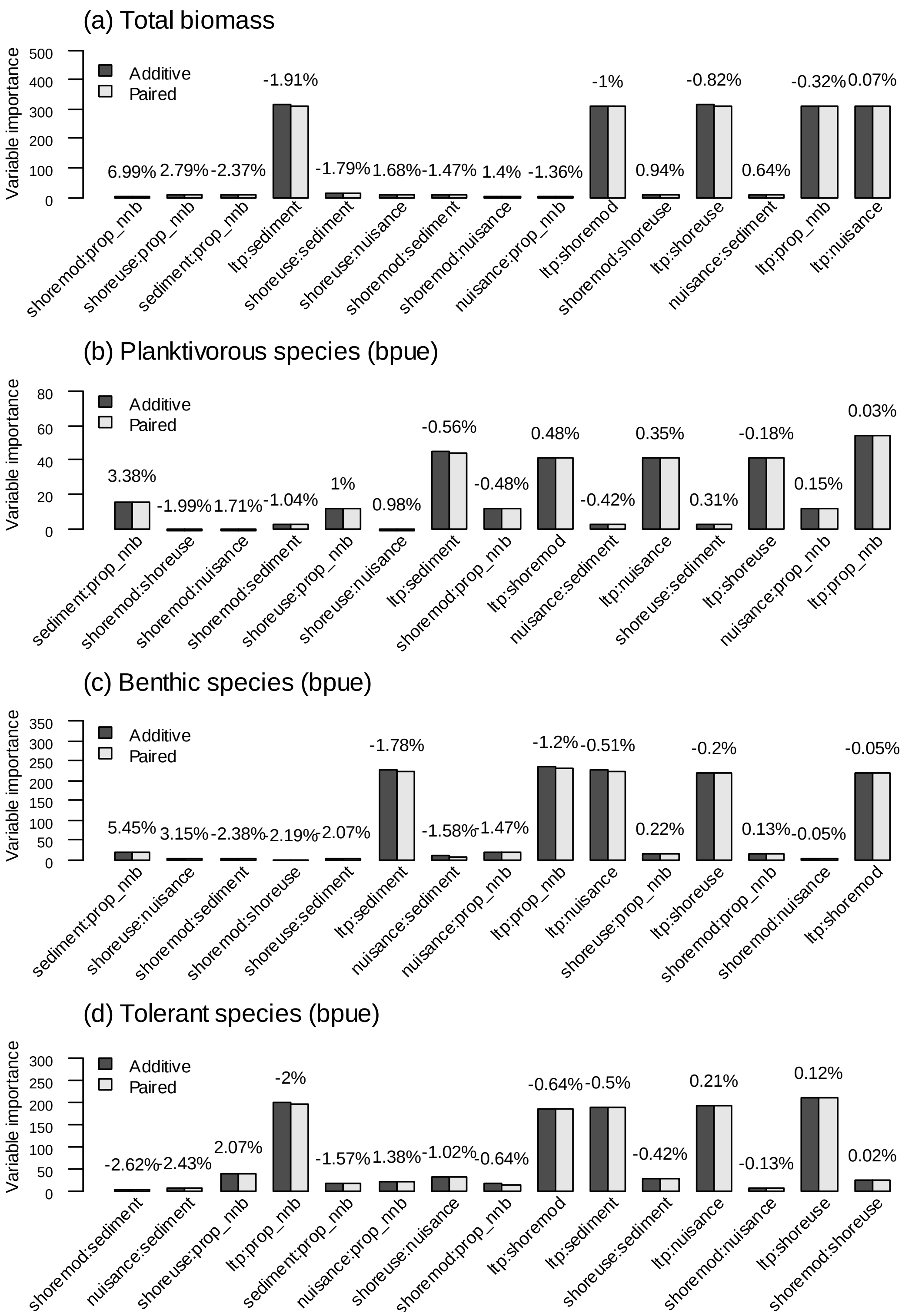

Figure 19 - Paired and additive importance of stressor variables for functional metrics based on biomass in the French and Portuguese reservoirs. The difference between paired and additive values of variable importance measures the interaction between the variables. Interactions are ordered according to the difference value that is plotted above the bars and expressed relatively to the Additive importance.

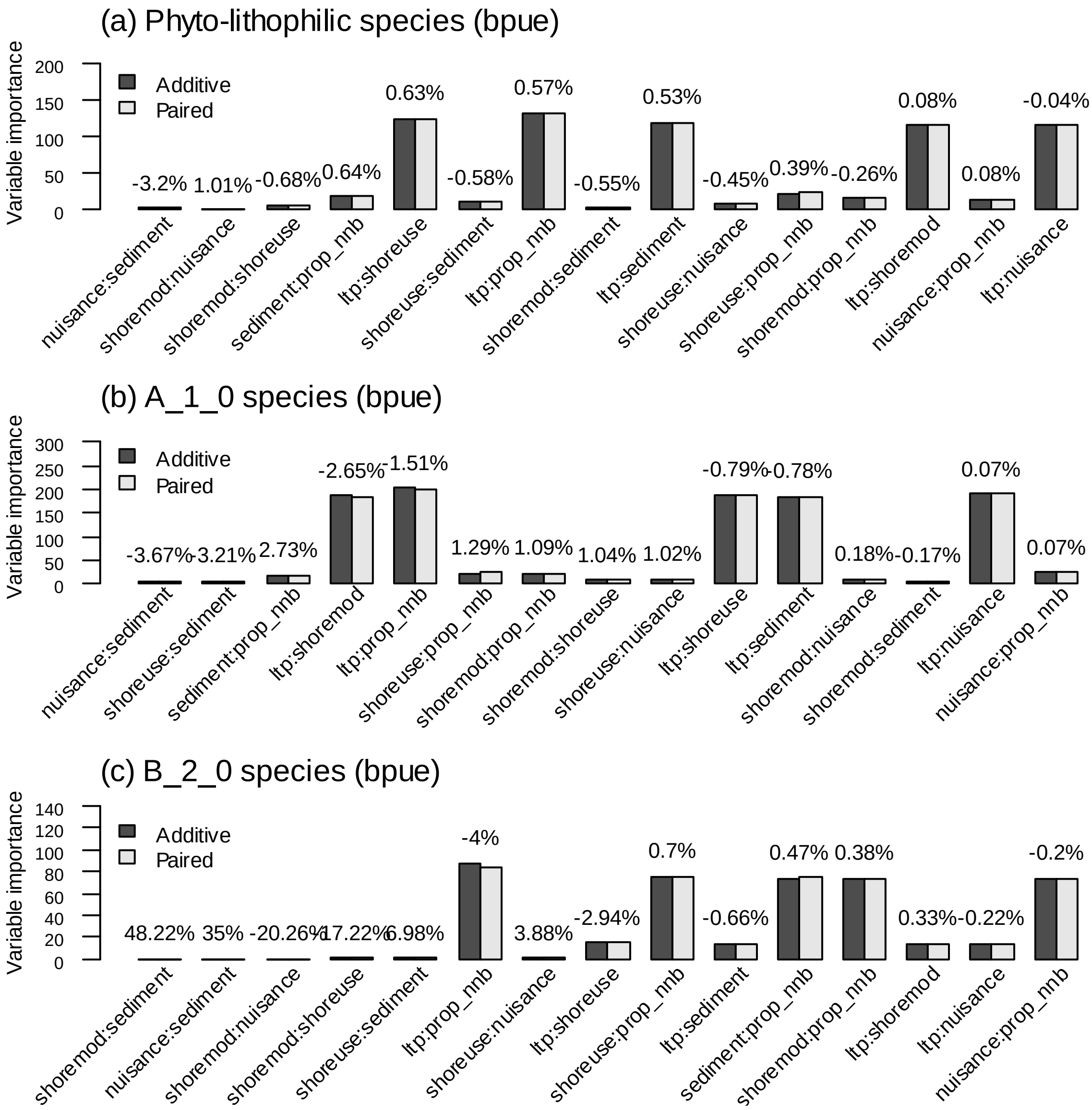

Figure 20 - Paired and additive importance of stressor variables for reproductive trait metrics based on biomass in the French and Portuguese reservoirs. The difference between paired and additive values of variable importance measures the interaction between the variables. Interactions are ordered according to the difference value that is plotted above the bars and expressed relatively to the Additive importance.

In the models testing fish metrics biomass, interactions were globally weaker than for models testing fish metric abundance (Figure 21 and Figure 22). In many cases, interactions between eutrophication and the proportion of non-native species were selected due to their great importance compared to hydromorphological alteration variables (Figure 17). For *total biomass* and *biomass of tolerant species*, the interaction between eutrophication and shore use was selected.

Additive effects of stressors were most frequently observed for the selected metrics (Figure 22). Slight synergistic effects between eutrophication and the abundance of non-native species was observed on the *biomass of tolerant species*. A clear synergetic effect was also observed between these two stressors on the *nest building species* while the interaction was antagonistic for the *planktivorous species* (Figure 22)

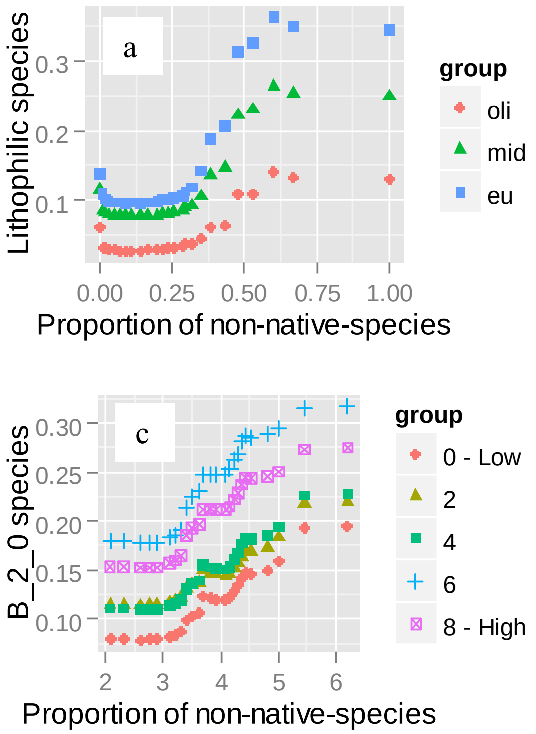

Figure 21 - Partial relations of non-native species with the abundance of lithophilic (a) and the abundance of B_2_0 species (b) in different contexts of eutrophication. (c) Partial relation of eutrophication with the abundance of B_2_0 species in different contexts of shore use intensity. Thresholds between oligotrophic, meso- and eutrophic groups were 20 and 45 µg L^{-1}.

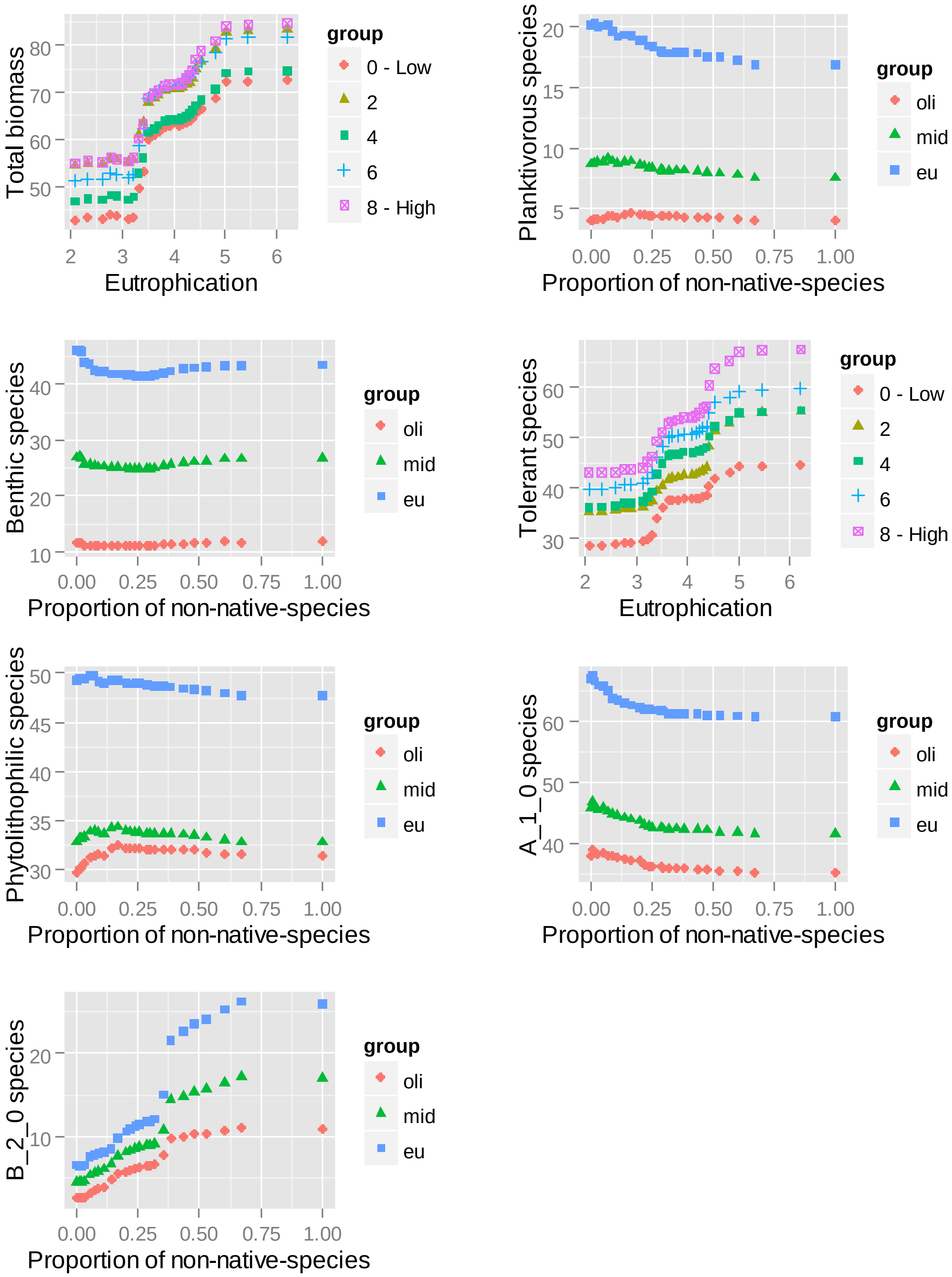

Figure 22 - Partial relations of non-native species with selected fish metrics based on biomass in different contexts of eutrophication. Thresholds between oligotrophic, meso- and eutrophic groups were 20 and 45 µg L^{-1}.

Response of fish communities to multiple stressors in estuaries

The present study investigated the influence of multiple stressors on fish ecological quality and diversity in European estuaries, with the aim of assisting environmental managers to develop efficient strategies of restoration. We used a random forest algorithm to detect the dominant stressors in estuaries and their effects on the EQR (defined by the fish indices in use) and fish diversity in the North-East Atlantic countries. Model simulations were undertaken to evaluate the ecological benefits resulting from lowering the intensity of stressors on EQR, and investigate the type of pairwise interaction between stressors involved in the model (i.e. additive, synergistic and antagonistic). Finally, a step-by-step scheme of restoration was proposed for the studied stressor on the basis of an iterative process maximizing the ecological benefit at each step (i.e. mitigation of one stressor).

Data and methods

The ecological status of 90 European transitional waters, from the North-East Atlantic (NEA) (Figure 23), was assessed according to their EQR calculated based on fish communities (in few methods the demersal assemblages include also crustaceans in the assessment).

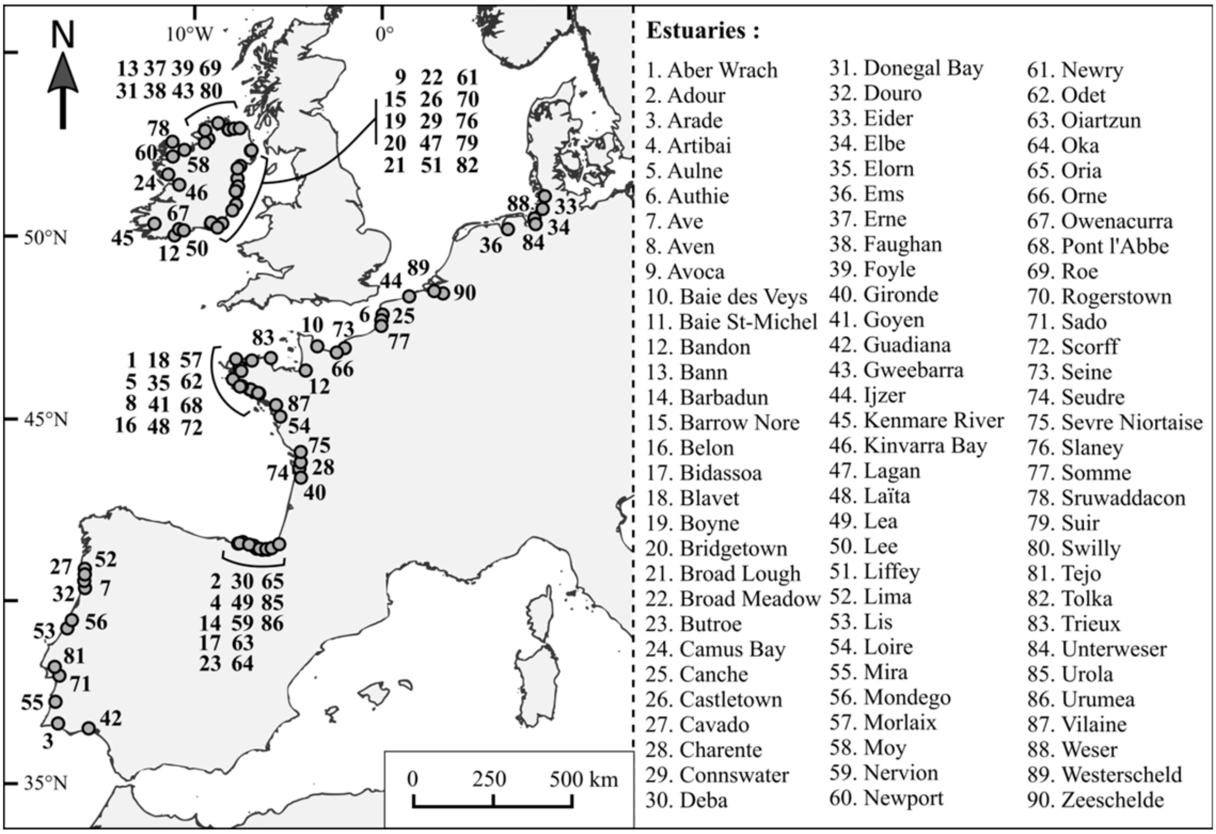

Figure 23 - ***Location of the 90 European estuaries considered for the investigation of combined stressor impacts on fish communities.***

The EQR value ranges between zero and one, with high ecological status represented by values close to one and bad ecological status by values close to zero. Fish-based indices for transitional waters have been developed as part of the WFD, but assessment methods differ across Europe (Perez-Dominguez et al., 2012). A total of seven multimetric indices were commonly used in the area covered by this study (Borja et al., 2004; Breine et al., 2007; Cabral et al., 2012; Coates et al., 2007; Delpech et al., 2010; Harrison and Kelly, 2013; Scholle and Schuchardt, 2012). A great effort of intercalibration has been made to harmonize the results obtained from national assessment methods and to ensure comparability between the different countries within the NEA (Lepage et al., 2012; Poikane et al., 2014). We assumed that EQR values are the most comparable fish-based assessments available at the European scale for transitional waters despite the bias induced by the combination of results obtained from different methods. A total of 272 EQR values were used to evaluate the ecological status of estuaries. EQR data were obtained from the intercalibration exercise (n = 188), plus some other available evaluations for French and Belgian estuaries (n = 84). Assessments were performed for samples taken between 1989 and 2014, allowing to record from one to eleven EQR values per water body.

Stressor evaluation

A total of 18 environmental descriptors merged into ten stressors categories were selected to assess the stressor intensity affecting estuaries (Table 6; Appendix 2).

Some descriptors can be considered as real stressors directly affecting the fish communities in estuaries, while some others are more considered as drivers. Access to direct stressors on fish population and ecosystems is always difficult to get, and using proxies is often necessary. The selected descriptors reflected the three broad categories of disturbance described in estuaries and coasts: “coastline morphological change”, “resource use change”, and “environmental quality and its perception” (Aubry and Elliott, 2006). They were evaluated according to their incidence at the estuary scale because most of stressor effects are acting beyond the salinity zone or water body level.

The stressor evaluation was achieved one time for each estuary, except for those subjected to significant modifications of human stressors between two biological assessments, normally because of restoration works (i.e. Barbadun, Nervion and Zeeschelde estuaries). All the stressor descriptors were classified based on standardized thresholds criteria in six common classes according to their intensity of disturbance (i.e. ‘no disturbance’, ‘very low’, ‘low’, ‘moderate’, ‘high’ and ‘very high’), apart from the eutrophication assessment that was classified in three classes (i.e. ‘problem, ‘potential problem’ and ‘non-problem’ areas).

Table 6 - Anthropogenic stressor categories and their related descriptors used as predictors in the random forest analysis

Stressors categories	Stressor descriptors	Code	Data source
Coastline urbanisation	1. Anthropogenically affected coastline	coast_ant	(1)
	2. Intensity of marina developments	marina	(2)
Dredged sediments	3. Maintenance dredging - disposal area	dre_area	(2)
	4. Maintenance dredging - disposal amount	dre_am	(2)
	5. Capital dredging	dre_cap	(2)
Fisheries and aquaculture	6. Aquaculture	farm	(2)
	7. Fisheries activities	fish	(2)
Flow changes	8. Interference with the hydrographical regime	hydro_dist	(1)
	9. Interference with fish migration routes	mig_dist	(2)
Intertidal lost	10. Intertidal area lost; Realignment schemes; Land claim; Gross change in the bathymetry and topography	tidal_lost	(1)
Eutrophication	11. OSPAR Eutrophication assessment	eutro	(3)
Oxygen depletion	12. Dissolved oxygen (temporal)	DO_time	(1)
	13. Dissolved oxygen (spatial)	DO_space	(1)
Port development	14. Intensity of port developments	port	(2)
Sea bed alteration	15. Benthic ecological status	benth	(1)
Water pollution	16. Water chemical quality	chem_qual	(1)
	17. Water quality biological effects	bio_eff	(1)
	18. Water pollution incidents	pol_inc	(2)

Sources : (1) Lepage et al. 2012, (2) unpublished intercalibration data, (3) OSPAR Comprehensive Procedure

The stressor classification developed by Aubry and Elliott (2006) and revised by Lepage et al. (2012) was used to define the thresholds values for 17 stressor descriptors (Appendix 3). The choice of descriptors is often limited for the estuaries to ensure data availability and similar resolution in all estuaries (Vasconcelos et al., 2007). Data used in the analysis were derived from the intercalibration exercise conducted in 2011 to harmonize the assessment of stressor descriptors between all countries (Lepage et al., 2012). This evaluation was achieved from best data available in literature, WFD monitoring surveys, local maps and Google earth® views. Where such data were not available, the assessments were based on local expert judgment. Eutrophication assessments were derived from the common procedure for the identification of the eutrophication status of the OSPAR Maritime Area (Claussen et al., 2009; OSPAR, 2005). Common criteria were used throughout the OSPAR regions to characterize maritime and estuarine areas with regard to their eutrophication status as, 'problem areas', 'potential problem areas', and 'non-problem areas'.

Random forest model

A forest of 2500 regression trees was built to predict the median EQR value using the 18 stressor descriptors as independent predictors. As the EQR values derived from different assessment methods, we tested if the model residuals varied significantly between methods using a Kruskal-Wallis test, followed by post-hoc pairwise multiple comparisons tests. Significant difference suggests that at least one method deviates from the model predictions. The relationship between the model residuals and three environmental variables (i.e. entrance width, estuary area, latitudinal location) known to affect fish diversity in estuaries (Nicolas et al., 2010a) was assessed using Pearson correlation tests.

Analysis of stressors on fish diversity

In addition, for the identification of stressors with the strongest effect on fish diversity, a random forest method was also undertaken in a subset of the estuarine dataset (35 French estuaries) (see sampling protocol details in Courrat et al. (2009). This was because this subset had sufficient data to undertake such analysis. We derived metrics for each water body, hence differentiating for instance Adour amont from Adour aval when an estuary was split into two or more waterbodies. Each water body has different sampling effort (e.g. Aber Wrach has been sampled once, Adour amont : 11 times, …). Three diversity measures were estimated: 1) Mean Species Richness (*S*, calculated per sample and then averaged per water body); 2) Shannon H' Log Base 2 (*H*, calculated per sample and then averaged per water body); 3) Rarefication (species richness rarefied at the site with the minimum number of individuals, i.e. 43). Subsequently, a random forest model was built to predict diversity measures according to the stressor levels in the same way as for EQR (i.e. a forest of 2500 trees was built to be sure to consider all the possible combinations of predictor). The relative importance of each stressor metric was evaluated and the partial dependence graphs were plotted to investigate the shape of the biological responses along to the gradient of stressor descriptors.

Simulation of EQR restoration benefits

The effects of restoration events on EQR were investigated before and after theoretical stressor restoration using the predictive performances of the random forest. Model simulations were achieved to define the fish ecological response in the estuary after 1) individual actions of stressor categories mitigation, and 2) combined actions of mitigation for each pair of stressor categories. A dataset of 1000 virtual sites was generated with random values for each stressor metric. EQR values were then predicted from the random forest model for each site to produce assumed baseline values (initial conditions before restoration). The benefit of restoration actions was then evaluated by the difference between the baseline values and the predicted EQRs after mitigating the intensity of descriptors related to the target category of stressor. The stressor metric intensities (between 'no disturbance' to 'very high disturbance') were reduced either separately for each stressor (i.e. individual actions of restoration) or simultaneously for each pair of stressors (i.e. combined actions of restoration). The obtained values reflected the expected improvement of the ecological status after the mitigation measures and can be interpreted as an expected restoration benefit. The significance of ecological response difference was tested by comparing predicted EQRs before and after the restoration simulations, using *t*-test for paired samples. The intensity of stressor descriptors used for the simulations was 'very low' for all the mitigation events because substantial decreases of the partial ecological responses often occurred after this level. Additionally, the total removal of anthropogenic stressors in natural conditions could be difficult for some of them and often takes several decades. For the simulations, the descriptors already at 'no' or 'very low' levels of disturbance remained unchanged to avoid a degradation of the predicted ecological status.

Classification of interactions

The inherent ability of random forest to model complex interactions among predictors (Cutler et al., 2007) was used to highlight the interaction effects between pairs of stressor categories in a restoration context. When two stressors are mitigated in combination, the ecological response can result either in additive, antagonistic or synergic effects (Crain et al., 2008). An additive interaction suggests that the cumulative effect of mitigated stressors is equivalent to the sum of effects produced by the mitigation of stressors one by one. By contrast, the interaction effect is synergic or antagonistic whenever the benefit of mitigation is "more-than" or "less-than" the sum of its individual components. In order to investigate the type of interactions involved in the random forest model, the restoration benefits for each combined events of mitigation ('Paired') were compared to the sum of individual restoration benefits predicted separately for two mitigated stressor categories ('Added'), using *t*-test for paired samples. A significant difference between 'Paired' and 'Added' predictions supposed that the combined effect deviated from the additive model (Ishwaran, 2007). The strength of non-additive interactions were expressed as mean percentage difference of combined predictions compared to the additive predictions to facilitate the interpretation. The interaction effects were categorized using the systematic classification proposed by Piggott et al. (2015), which is based on magnitude and response direction of the cumulative effect and the interaction effect. Three non-additive interactions were observed in our analysis. Positive antagonistic interaction (+A) reflects a deviation from additive model less positive than the sum of individual effects, whereas negative antagonistic interaction (-A) is less negative than predicted additively. Positive synergistic interaction (+S) reflects a deviation from additive model more positive than the sum of individual effects.

Scheme of restoration

The random forest model was used to propose an efficient step-by-step restoration scheme for the studied stressor categories, taking into account the interaction effects among stressor descriptors. An iterative procedure was achieved to rank the ten stressor categories by maximizing the fish ecological status at each step (i.e. mitigation of one category). At each step, the stressor categories were sorted according to their expected benefit after restoration compared to the current state of restoration. The stressor category offering the maximum of restoration benefit was recorded, and then restored at 'very low' level of disturbance for the next step. The process was repeated until the classification of all stressors was complete. This procedure allowed accounting for the interactions of the target stressor category with those previously selected in the scheme. The outcomes were thus average expected benefits for each category of stressor without prior knowledge of intensities of both target and co-occurring stressors.

Results

The proportion of variance of EQR explained by the random forest was 47.3%. The model residuals varied weakly according to the evaluation assessment method with a statistical significance set at P = 0.05 (Kruskal-Wallis test, χ^2 = 12.7, df = 6, P = 0.047). Pairwise multiple comparisons between methods were non-significant (Nemenyi tests, all P<0.05) except for one case (P = 0.045), suggesting that the combination of EQR values derived from different assessment methods was acceptable. The model residuals were non-significantly correlated with the estuary entrance width (Pearson correlation test, r = 0.04, t = 0.74, df = 270, P = 0.459), estuary area (r = 0.004, t = 0.06, df = 270, P = 0.951), or latitudinal geographical location (r = 0.01, t = 0.19, df = 270, P = 0.846).

Ecological responses for stressor descriptors

The relative importance of stressor descriptors for predicting the EQR values ranged between 22.8%, for the water quality biological effect, and 0.4%, for the eutrophication status (Figure 24).

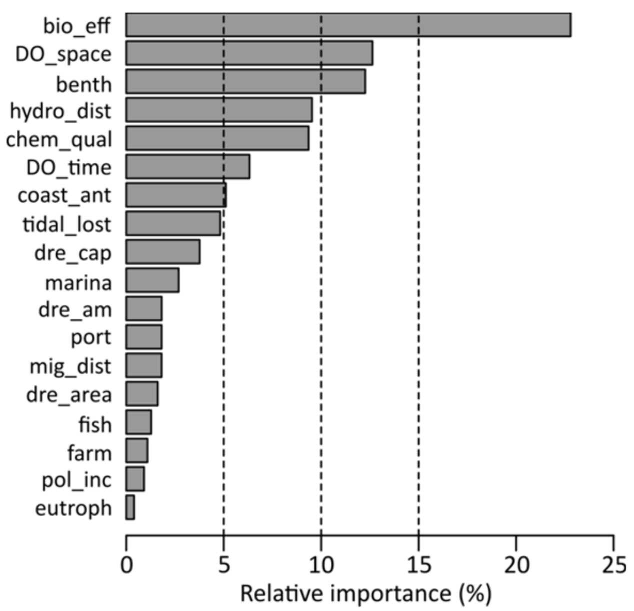

Figure 24 - Relative importance of stressor descriptors (%) for predicting the ecological status of European estuaries. For codes, see Table 6

The partial ecological response of the nine most important stressor descriptors revealed various patterns, including nonlinear relationships and thresholds shifts, whereas the other descriptors did not show obvious response according to the level of disturbance (Figure 25).

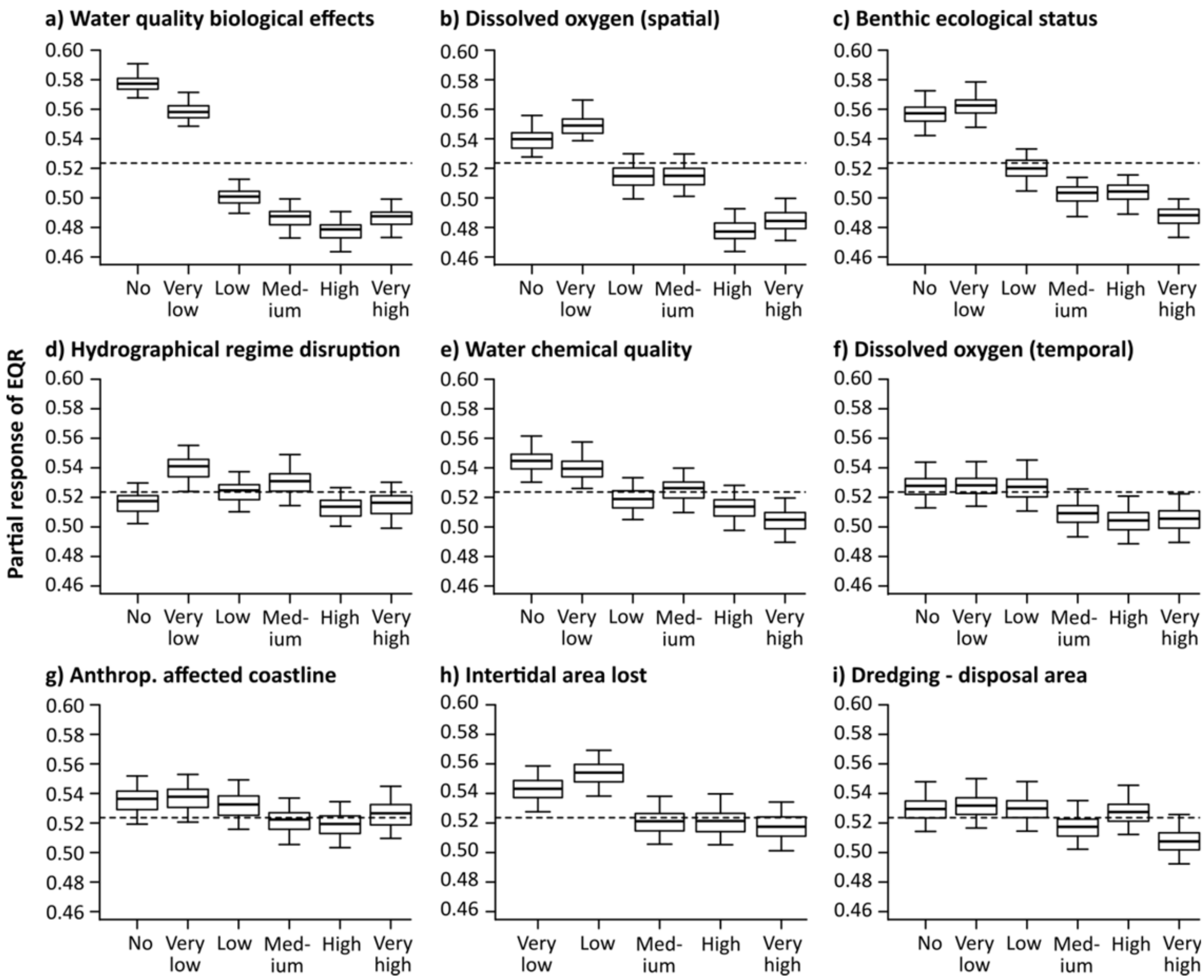

Figure 25 - Partial dependence plots of the nine most important stressor descriptors for predicting the fish ecological status of European estuaries: a) water quality biological effects, b) dissolved oxygen in space, c) benthic ecological status, d) interference with the hydrographical regime, e) water chemical quality, f) dissolved oxygen in time, g) anthropogenically affected coastline, h) intertidal area lost, and i) maintenance dredging - disposal area. The dashed line shows the median value. EQR: ecological quality ratio.

For the water quality biological effect (Figure 25a), the partial response was highest for low concentration of all metals (i.e. 'no' disturbance). A threshold response was observed when the concentration for one or more metals was substantially higher than the national level (i.e. from 'low' disturbance). The same type of pattern was observed for the benthic ecological status with a substantial decrease of the response occurring when the benthic invertebrates of estuaries begin to present deterioration signs (i.e. from 'low' disturbance; Figure 25c). For the dissolved oxygen, the indicator reflecting spatial extent of hypoxia showed a higher relative importance that the one reflecting the duration problem. The EQR response of the spatial component displayed two successive thresholds when hypoxia occurred above 1% (i.e. 'low' disturbance) and above 5% (i.e. 'moderate' disturbance) of the estuary length (Figure 25b). For temporal component (Figure 25f), the decrease of ecological response occurred for dissolved oxygen saturation below 70% for 95% of the time (from 'moderate' disturbance). The ecological response along the gradient of interference with the hydrographic regime showed a decreasing trend with the extension of areas impacted by constructions affecting the prevailing water currents (Figure 25d). Interestingly, the ecological response in estuaries with no reported constructions was lower than those with 5-20% of area affected by construction, suggesting a positive effect of some submerged structures. For the water chemical quality, the partial response decreased progressively with the degree of non-compliance with the Environmental Quality Standards of the EU Dangerous Substances Directive. The ecological response also showed noticeable threshold shifts from 'moderate' level of disturbance for the anthropogenically affected coastline (from 30% of the coastal area impacted; Figure 25g), for the intertidal area lost (from 1% of area lost over the last decade; Figure 25h), and for dredging disposal area (from 10% of area designated for disposal).

Effects of stressors on fish diversity

Capital dredging was the main stressor that explained 1.5% of the variance of the species richness (Figure 26). The percentage of Shannon variance explained by stressors was 13.6% (Figure 26), and the main stressor was also the capital dredging effects. The proportion of variance of rarefaction explained by stressors was 5.6%, and the main stressor was the water quality biological effects (Figure 26), as in the case of EQR. The proportion of variance of EQR for this subset data explained by stressors was 14.0%.

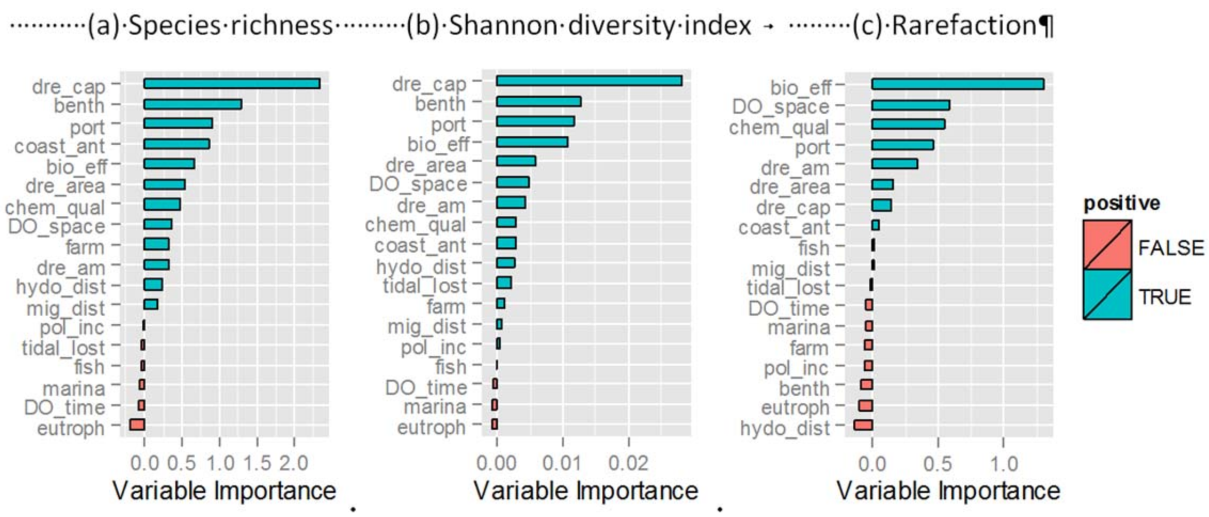

Figure 26 - Relative importance of stressor descriptors for predicting the fish diversity of French estuaries

The species richness and Shannon diversity index decreased with capital dredging stressor, presenting a threshold response from stressor level 'low' to 'medium' (*Figure 27*). The response of rarefaction to the intensity of port developments decreases more gradually and starts decreasing at stressor level 'medium'.

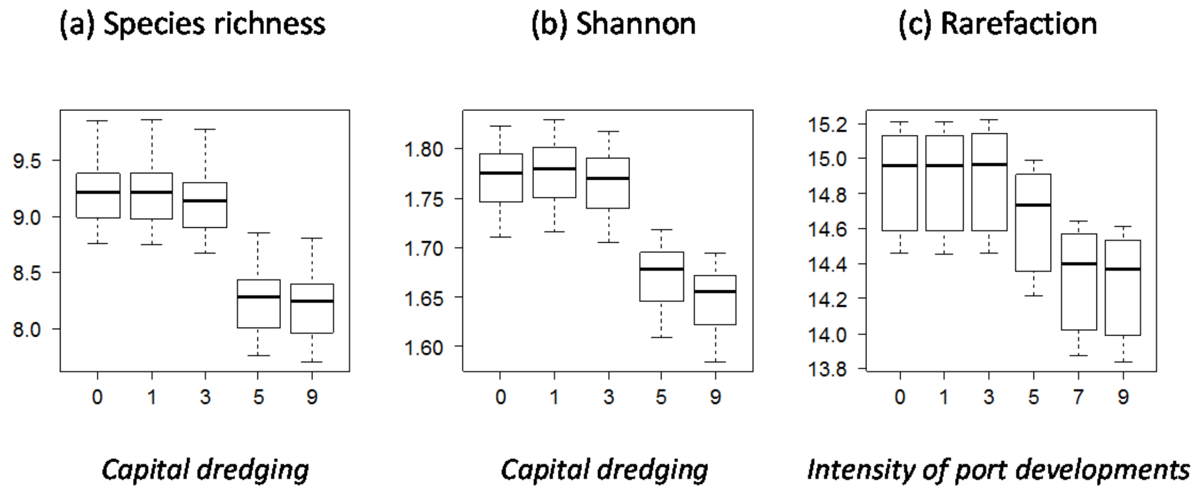

Figure 27 - Partial dependence plots of the most important environmental indicators for predicting the diversity indices of French estuaries: a) capital dredging on species richness, b) capital dredging on Shannon index, c) intensity of ports development on rarefaction

Simulation of EQR restoration benefits

The simulations of stressors mitigation significantly changed the ecological responses (i.e. predicted EQR) when stressor categories were restored separately at 'very low' level of disturbance (*t*-tests for paired samples, n = 1000, all $P < 0.001$). The mitigation effect on the ecological response was positive for all categories except for fisheries and aquaculture for which a slight decrease was observed (negative effect). The mitigation of the water pollution provided the maximum of the restoration benefits, as evaluated by the mean EQR improvement (Figure 28). Substantial benefits were also expected for the mitigation of oxygen depletion and seabed alteration. The expected EQR improvement was lower for the other stressor categories. The combined events of mitigation also significantly changed the biological response for all sequences of simulation (*t*-tests for paired samples, n = 1000, all $P < 0.001$). The pairs of stressor categories showing the highest expected benefits of restoration usually were related to the mitigation of water pollution, oxygen depletion and sediment alteration that is consistent with the individual predictions (Figure 28).

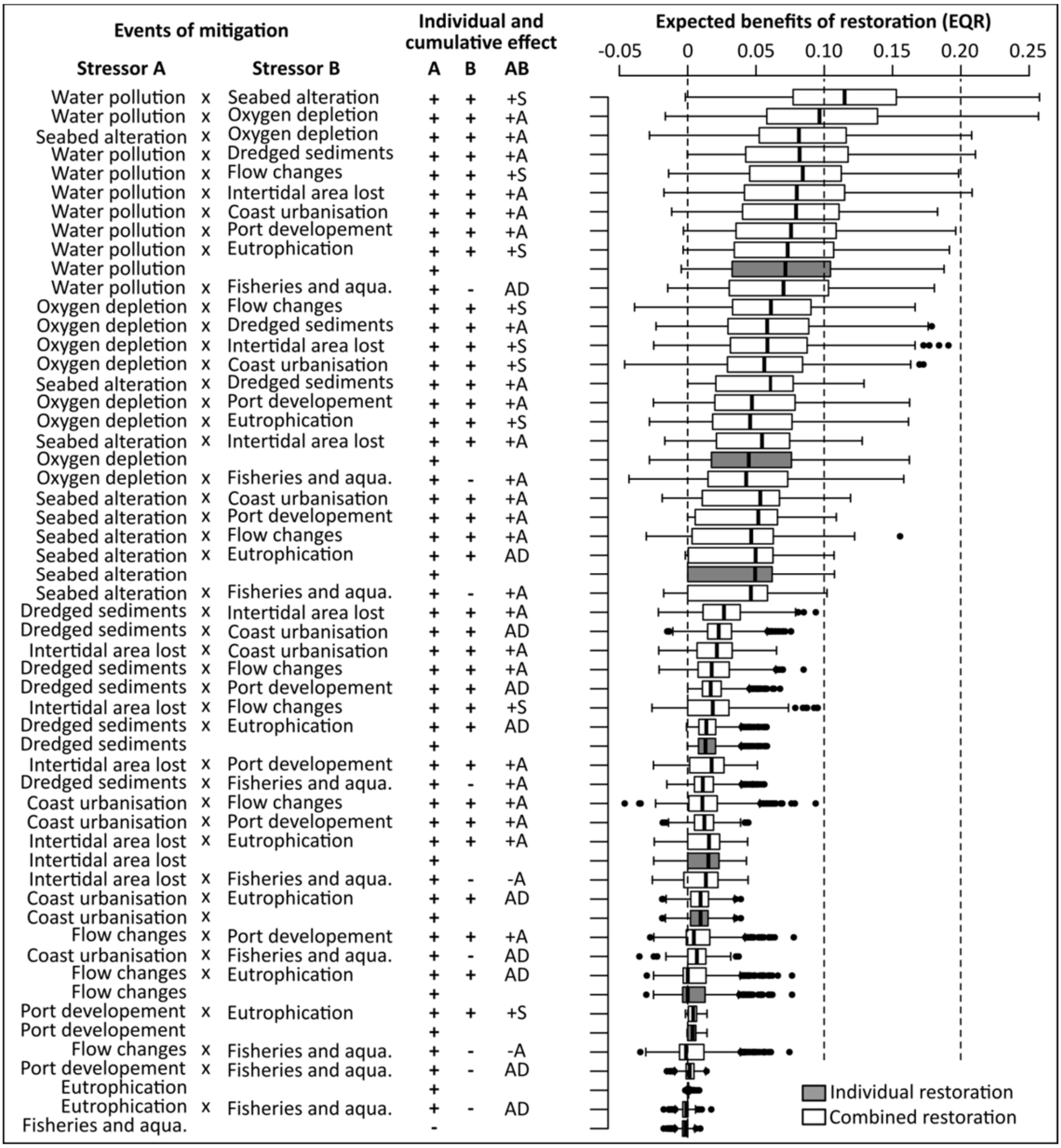

Figure 28 - Boxplots of the restoration benefits predicted for each individual (grey boxes) and combined (white boxes) actions of stressor restoration, as evaluated by the improvement of fish ecological status. The kind of relationship between the combined stressor categories (additive or interactive) and the interaction sign are specified. AD: additive effect; +A: positive antagonism; -A: negative antagonism; +S: positive synergism. EQR: Ecological Quality Ratio

Classification of interactions

Among the 45 possible pairs of assessed stressors, 36 combinations were double positive and nine were opposing (Figure 28). Additive effect was highlighted for ten pairs of stressor categories (*t*-tests for paired samples, n = 1000, $P > 0.05$). The most common interaction classes for the non-additive effects were positive antagonisms (n=24) and positive synergisms (n=9), whereas negative antagonisms were less represented (n=2). Positive antagonisms were the most frequent interactions among the studied stressor categories with the exceptions of ‘fisheries and aquaculture’ and ‘eutrophication’ that mainly acted additively. The strength of non-additive interactions ranged from 0.3 to 16.3%, as measured by the mean percentage difference of combined predictions compared to the additive predictions. The strongest interaction was a positive antagonism observed between water pollution and oxygen depletion (Table 7). Flow changes were involved in 7/10 of the stressors combinations with interaction strength above 5%.

Table 7 - Mean restoration benefits for separated (i.e. sum of individual predictions; ‘Added’) and combined (‘Paired’) events of stressor mitigation. The class (+A positive antagonism, -A negative antagonism, and +S positive synergism) and strength (%) of interactions is presented. P*-values refer to the significance of the* t*-tests for paired samples*

Stressor A	Stressor B	additive	paired	class	strength (%)	*P*-value
Water pollution	Oxygen depletion	0.1191	0.0996	+A	16.31	<0.001
Flow changes	Oxygen depletion	0.0536	0.0619	+S	15.43	<0.001
Flow changes	Fisheries and aqua.	0.0029	0.0033	-A	12.03	<0.001
Flow changes	Coastline urbanisation	0.0139	0.0125	+A	10.13	<0.001
Flow changes	Seabed alteration	0.0434	0.0390	+A	9.96	<0.001
Coastline urbanisation	Seabed alteration	0.0473	0.0429	+A	9.32	<0.001
Flow changes	Intertidal lost	0.0164	0.0178	+S	8.03	<0.001
Flow changes	Water pollution	0.0753	0.0804	+S	6.83	<0.001
Water pollution	Seabed alteration	0.1087	0.1144	+S	5.27	<0.001
Flow changes	Port development	0.0086	0.0081	+A	5.03	<0.001

Scheme of restoration

Each step of the combined stressor restoration scheme significantly changed the ecological response with a positive effect for the eight first steps (Table 8). Restoration benefit was negative for the mitigation of fisheries and aquaculture and coastline urbanization. The most important benefits were expected for water pollution mitigation, seabed alteration, and oxygen depletion, as predicted by the individual and combined approaches. However, the order of priority for stressor restoration differed between the two approaches from step 2, highlighting the influence of interactions with the stressors previously mitigated. For example, the rank of oxygen depletion dropped in the combined approach because of its strong positive antagonistic interaction with water pollution. By contrast, the rank of flow changes was increased because of the positive synergism with water pollution and oxygen depletion.

Table 8 - Summary of the step-by-step restoration scheme for the studied stressors in estuaries. The selected category of stressor for each restoration step was specified for both the individual and combined approach. The number and sign (+ or -) show the changing direction between approaches. The mean Ecological Quality Ratio (EQR) predictions and the mean restoration benefits (%) were provided for the combined scheme of restoration. The significance of the restoration benefit is specified.

Restoration steps	Individual approach	Combined scheme of restoration				
	Selected stressor	Selected stressor		Mean EQR prediction	Mean restoration benefit	*P*-value
Baseline	-	-		0.507	-	-
Step 1	Water pollution	Water pollution		0.578	0.070	< 0.001
Step 2	Oxygen depletion	Seabed alteration	+1	0.622	0.044	< 0.001
Step 3	Seabed alteration	Oxygen depletion	-1	0.650	0.028	< 0.001
Step 4	Dredged sediments	Flow manipulation	+3	0.661	0.011	< 0.001
Step 5	Intertidal area lost	Intertidal area lost		0.671	0.010	< 0.001
Step 6	Coastline urbanisation	Dredged sediments	-2	0.677	0.006	< 0.001
Step 7	Flow manipulation	Eutrophication	+2	0.679	0.001	< 0.001
Step 8	Port development	Port development		0.679	0.001	< 0.001
Step 9	Eutrophication	Fisheries and aquaculture	+1	0.676	-0.003	< 0.001
Step 10	Fisheries and aquaculture	Coastline urbanisation	-1	0.672	-0.004	< 0.001

Discussion

Methodological considerations

In terms of methodological issues, the authors of this report first have to state that fifteen years after the implementation of the Water Framework Directive, there are still a lot of gaps in terms of environmental and biological information on European water bodies. These gaps are mainly related to environmental characteristics of lakes and reservoirs (both regarding natural environmental variability and human induced stressors). One reason of the absence of available information is, for instance, that reservoirs are currently not always included in the reporting of the countries. Another concern is also the absence of standards that make the collection and comparison of data between regions difficult. This is, for example, the case for the alteration of hydrology and morphology of European lakes, just described by some countries by a two-category index, which is probably too rough to allow detecting a response of fish assemblages. For rivers, the available dataset is covering a wide range of different ecoregions (Illies, 1978) across Europe and rivers of various fish assemblage types. Nevertheless, there was some geographical heterogeneity and a paucity of data for some areas (EFI+ Consortium, 2007). For example, Schinegger et al. (2012) and Schinegger et al. (2013) have already shown that there are data gaps for particular regions of Europe (e.g. south-eastern countries) and in certain river types (particularly in large rivers) in the EFI+ dataset. Moreover, the availability of fish sampling sites with frequent single pressures and pressure combinations was limited, as pressure distribution was very dispersed. To achieve a better understanding of the interplay of single pressures on fish assemblages at the European scale, it is crucial to fill data gaps in some key regions. Also, more accurate data on human pressure classification are needed on a single pressure level for future attempts, as only some of the pressure variables in this dataset are based on measured data, others rely on expert judgement. In these regards, the WISE-dataset of EEA including the pressure information gathered by EU member states during the 1st river basin management plans will be ready for analyses soon, however, the quality of these data cannot be evaluated yet.

Some of the problems encountered in this work were also in relation with the non-availability of fish data and/or with the difficulties to collect them in a reasonable time frame (in Mediterranean estuaries and in most of the European reservoirs for example). Regarding these fish data, it also has to be highlighted that differences of fish sampling methods (fishing gears and sampling strategy) prevent consistent comparisons of some fish metrics at a large spatial scale.

From a modelling point of view, despite the unavoidable sources of variability related to the constraints of large-scale analysis, the random forest regression provided a valuable framework to highlight the most important stressors and investigate the effect of interactions using field-monitoring data. Indeed, the models implemented here allowed explaining a large part of the metrics variability (Table 9).

Table 9 – Proportion of variance explained by the models on the different water body types.

	Rivers	Lakes	Reservoirs	Estuaries
Proportion of variance explained by the models	5.3 - 36.9	2.3 - 51.4	0 - 55.3	47.3
Relative proportion of variance explained by eutrophication	28	29.1	39.6	0.4
Relative proportion of variance explained by the main hydromorphology variable	18.5	0.9	3.6	9.5

Fish responses to stressors

In the river and lake analyses the natural environment explains the main part of the metrics' variability. In this way the fish assemblage types that reflect the fish community and environmental characteristics explained a large proportion of the metrics' variability in rivers. Lake size, which relates with habitat diversity, biogeography and – to a lesser extent – the climate variables explain generally the main part of the fish metrics variability. In reservoirs, climate is not so important compared to lakes due to the more limited spatial scale of the reservoir-dataset. As a consequence, residual variability of metrics associated to stressors is often limited at these large scales (Brucet et al., 2013). In estuaries natural environment also explain a large part of the variance of the fish assemblages (Nicolas et al., 2010b) but in this study, the residuals of the model were uncorrelated to the tested environmental features (i.e. entrance width, estuary area and latitudinal location), because EQR values were used instead of fish metrics. The construction of the fish indices and the related EQR takes into account regional environmental particularities in their reference conditions.

In all the analyses, whatever water body type considered, pollution is the dominant stressor. However, because the descriptors of this pressure are not homogenous between the datasets and water body types, it is difficult to discuss further implications. It seems that eutrophication linked to nutrient loading (phosphorus in particular) explains the main part of the residual metric variability in freshwaters whereas in estuaries eutrophication effects measured through nutrients load did not give significant responses of the fish community seen through the EQR. The relatively short residence time of water in estuaries could explain this poor pressure-impact relationship. Because estuaries are often turbid with very low water transparency, the presence of nutrients in high quantity does not automatically lead to phytoplankton bloom or development of macroalgae due to a lack of light availability. The effect of nutrient loads at large scale in estuaries is therefore minimized by general natural physico-chemical characteristics when it does not lead to oxygen depletion.

Effects of frequent stressors on fish in rivers

The main single occurring stressors in rivers were connectivity disruption upstream and downstream, water abstraction, flood protection, morphological alteration, acidification, eutrophication and organic pollution.

As also shown in Table 3, the frequent stressor combinations were connectivity disruption, both up- and downstream and in combination with eutrophication and/or organic pollution. Further, organic pollution and/or eutrophication occurred frequently with flood protection and/or embankment as well as with morphological alteration. Out of these, the EQRs of the five selected fish metrics showed the strongest response to morphological alteration and eutrophication (as single pressures), as well as to pairwise combinations (organic pollution and eutrophication) and triple pressure (eutrophication with organic pollution and morphological alteration). According to EFI+ Consortium (2009), Segurado et al. (2008), Schinegger et al. (2013) and Trautwein et al. (2013), these metrics already showed a significant response to single stressors and multiple stressors and for rivers featuring various fish assemblage types. Three of the five selected metrics were tolerance metrics, all including the presence/abundance of intolerant species (*density of juveniles intolerant to habitat degradation, % of species intolerant to oxygen depletion, % of abundance of intolerant fish to general Water Quality*), showed an overall negative response to stress, while the remaining two (*total richness* and *% of biomass of omnivorous fish*) showed an overall positive response to stress. The inclusion of the metric related with omnivorous species is relevant because this is in fact the only truly functional guild among the selected metrics. This metric reflects the dominance of generalist species regarding feeding habits and there are two processes that may be responsible for its positive response to stress: a decrease in specialist species/individuals at disturbed sites or/and an increase of generalist species/individuals at disturbed sites, especially at nutrient-rich environments.

Finally, total richness of species (Nsp_all) was found in our selection of most reactive metrics (see Table 1). However, this metric has to be interpreted differently than all other metrics, as described by Schinegger et al. (2013). Especially for headwaters and medium gradient rivers, an increasing total richness can often be interpreted as "potamalization effect" (e.g. due to impoundments) etc., where a modified fish fauna occurs. Thus, this metric always has to be interpreted in relation with specific traits of fish assemblages.

Effects of frequent stressors on fish in lakes

In lakes and reservoirs, the selected metrics are related to the different traits, i.e. reproductive guild, trophic guild, tolerance and habitat. In natural lakes, the metrics best explained by stressors are based on the abundance of fish (CPUE) whereas in reservoirs, most of the selected metrics are calculated with biomass.

Since several decades, many authors have demonstrated the importance of chemical stressors, especially nutrient loadings, because they constitute the most common stressors affecting most of the lakes worldwide resulting in eutrophication (Carpenter et al., 1998; Henriksen and Brakke, 1988; Vitousek et al., 1997). Eutrophication may indirectly result in extensive fish kills due to deoxygenation of lakes (Dybas, 2005; Jeppesen et al., 1998). Eutrophication may reduce fish recruitment due to incubating fish eggs suffocation (Wilkonska and Zuromska, 1982), or impair the balance between species due to differences in species condition traits (Persson, 1991; Winfield, 2004). Thus, species composition and biomass of fish communities can be affected by indirect effects of eutrophication. Our results confirm previous results obtained on a wider European dataset on the importance of eutrophication that explains most of the metrics residual variability in shaping fish assemblages (Brucet et al., 2013). Alteration of the morphology (of the littoral zone in particular) and hydrological regime of the lakes are also recognised to affect directly or indirectly fish assemblages of lentic systems. Habitats required for resting, foraging and reproduction of fish are degraded or lost (Baras, 1995; Crowder et al., 1981; Fischer and Eckmann, 1997; Gafny et al., 1992). This situation can lead to a modification of the whole fish assemblage and can disrupt the biological equilibrium (Cohen and Radomski, 1993; Gafny et al., 1992; Johnson, 1957; Michaletz, 1997; Piet, 1998; Rowe et al., 2002). In European natural lakes, we failed in showing a significant effect of these hydromorphological alterations but this result is not surprising because the descriptor used to characterise the hydromorphology alteration is very rough. In reservoirs, shore use is a proxy of morphological pressure that explains part of some metrics but always less than 5% of the variability. One explanation is probably related to the sampling strategy. Indeed, small fish associated to the littoral habitats are generally not caught, whereas metrics using these small fish are expected to be more relevant to assess morphological characteristics of the littoral zone. Finally, the impacts of fish introductions on the trophic equilibrium of lakes have been well documented (e.g. (Allendorf, 1991; Holcik, 1991). By modifying competition and/or predation relationships, these introductions can modify ecosystems functioning and sometimes species richness and biodiversity (Englbrecht et al., 2002; Ostendorp et al., 2004). Local extinctions of native species were also frequently related to introduction of new species (Crivelli, 1995; Holcik, 1991; Olenin et al., 2007; Welcomme, 1974; Winfield, 2004). Our results show that these introductions are able to explain more than 30% of the variability of some metrics.

Effects of frequent stressors on fish in estuaries

In estuaries, it was shown that EQR were more sensitive to stressors than fish diversity indices, especially for species richness and rarefaction. The low number of sites available for the diversity indices analysis limits the statistical power and such preliminary conclusions should be taken with cautious. Note that the low sensitivity of diversity indices was also shown in lakes (Argillier et al, 2013).

The chemical quality of waters is a crucial component for shaping abundance and assemblages in estuaries (Delpech et al., 2010; Le Pape et al., 2007; Whitfield and Elliott, 2002). Chemical contaminants can directly or indirectly impact fish physiology by disturbing fundamental biological functions, such as reproduction or growth, and can induce lethal effects in extreme cases (Fleeger et al., 2003; Johnson et al., 1998; Pankhurst and Van Der Kraak, 1997; Scott and Sloman, 2004). The diversity of habitats in estuaries supports various crucial biological functions for marine, freshwater and estuarine resident species (Beck et al., 2001; Elliott and Hemingway, 2008). The seabed alteration (including capital dredging) was classified at the second rank of priority, as evaluated by the benthic invertebrates' ecological status. Again, a threshold response was observed when benthos was deteriorated with a low level of alteration, suggesting that a slight disturbance of the complex relationships between sediment quality and macrobenthic communities can substantially impact the fish assemblages. In fact, we have demonstrated that fish diversity indices, such as richness, Shannon diversity and rarefaction, decreased with the stressor level of capital dredging (as well as intensity of port development and interference with fish migration routes). Habitat diversity and complexity, affected by dredging, are also essential in subtidal and intertidal areas to ensure their functional roles (Peterson, 2003). The intertidal area lost was classified at the fourth rank of priority in the restoration scheme. The EQR remained high up to 1-4 % of intertidal area lost in estuary over the last decade. Beyond this threshold level, the EQR dropped reflecting a negative impact of the restriction of intertidal area availability on fish communities.

Flow changes were classified at the fifth rank of priority in the combined scheme of restoration. This stressor directly contributes to the disturbance of the natural habitat conditions through the modification of the current patterns, wave regime, sediment transport, and system connectivity (Whitehead et al., 2009). The interference of fish migration routes did not produce an apparent ecological response, but ecological status decreased substantially with an increase of hydrographical regime interference. Immerged constructions (e.g. jetty, bridge supports or wharves) probably act as artificial reefs and contribute to increase the diversity of habitat conditions.

The lack of obvious response for several stressors did not necessarily reflect an absence of biological effect, but could arise from the limitations of indices/metrics for responding to spatially or temporally restricted disturbances and/or to limitation of the descriptors used to characterise the stressors.

Interactions between stressors

A summary of the main results obtained on the interactive effects of stressors studied in each water body type is given in Table 10 where “0” is equal to the sum of two stressors (additive effect) “+“ is an interactive effect superior to the sum of the two stressors (synergetic effect) and “-“ is an interactive effect inferior to the sum of stressors (antagonistic effect)..

The degradation of European rivers is widespread, as almost 60% of fish sampling sites were affected by a combination of stressors and only about 20% by single stressors. The remaining approx. 20% of sites were still in natural condition in terms of the investigated stressors. The maximum stressor combination was six (including organic pollution, eutrophication, morphological alteration, embankment and connectivity disruption, both up- and downstream of the sampling site), but it only occurred in 27 sites that are located in Switzerland, mainly in medium gradient rivers (MGR, see Table 3). For rivers, a consistency in the ranked importance of some pairwise pressure interaction was found among the analysed metrics. This is especially the case of the interaction between eutrophication and organic pollution, which consistently was found to be a major interaction according both to the pairwise and combinatory approaches. In rivers, most of the other important pairwise interactions also involved eutrophication.

In natural lakes, among the selected interactions, we identified only additive effects of the stressors studied. However, this result can be partly explained by the low proportion of variance explained by other stressors than eutrophication. At this large scale a consistent effort on data collection will have to be done in order to improve the quality of the models. In reservoirs, we showed that for half of the selected metrics, interactive effects between eutrophication and non-native species are additive.

Table 10 – Most relevant pairwise interactions of stressors acting on the metrics best explained by the models. (0) additive, (+) synergistic, (-) antagonistic effects.

	Metrics the best explained by the stressors/EQR			
Interactions tested	Rivers	Lakes	Reservoirs	Estuaries
Eutrophication & organic pollution	(+) total abundance of juveniles intolerant to habitat degradation (-) % of species intolerant to oxygen depletion (+) Total number of species (+) % of omnivorous biomass (-) % of abundance of intolerant to general water quality degradation			
Eut & instream habitat alteration	(-) total abundance of juveniles intolerant to habitat degradation (-) % of abundance of intolerant to general water quality degradation			
Eut & hydrograph modification	(+) % of species intolerant to oxygen depletion			
Eut & flood protection :	(+) Total number of species			
hydrograph modification & impoundment	(-) % of omnivorous biomass			
Eutrophication & non native species		(0) Total abundance (0) CPUE Phytophilic (0) CPUE Omnivorous (0) CPUE Benthic (0) CPUE A_1_0 (0) CPUE Tolerant (0) BPUE Planktivorous (0) BPUE Benthic	(+) CPUE Lithophilic (+) CPUE B_2_0 (+) BPUE Tolerant (+) BPUE B_2_0 (-) BPUE Planktivorous (0) Total Biomass (0) BPUE Benthic (0) BPUE Phyto-Lithophilc (0) BPUE A_1_0	
Eutrophication & shore use modification			(0) Total biomass (0) CPUE tolerant species (0) BPUE tolerant species	
Water pollution & oxygen depletion				(-) EQR
Flow changes & oxygen depletion				(+) EQR
Flow changes & Fisheries and aqua				(-) EQR
Flow changes & coastline urbanization				(-) EQR
Flow changes & seabed alteration				(-) EQR
Coastline urbanization & Seabed alteration				(-) EQR
Flow changes & intertidal lost				(+) EQR
Flow changes & water pollution				(+) EQR
Water pollution & seabed alteration				(+) EQR
Flow changes & Port development				(-) EQR

However, antagonistic effect is observed between these two stressors on the biomass of planktivorous species whereas four synergistic interactions were observed. The understanding of these interactions would require a detailed analysis of species manipulations that is not easily feasible at large scale. The composition of some metrics can greatly influence the importance of the non-native species contribution in the explanation of their variance. Indeed, for example, among the eight B_2_0 species encountered in the south-western dataset, only three are native from the area (bullhead *Cottus gobio*, three-spined stickleback *Gasterosteus aculeatus* and freshwater blenny *Salaria fluviatilis*) and they are far less frequent than the five non-native species (Appendix 1). This bias due to the taxonomic composition of European south-western lakes causes a relation between B_2_0 fish metric and the proportion of non-native species. This explains our results showing that the proportion of non-native species is the most important variable explaining both the abundance and the biomass of B_2_0 species. Nevertheless, the interactions between stressors are frequent and this will have to be taken into account in further analyses and in a management perspective. Note that the impact of non-native species is poorly considered in the fish index developed for lakes and this can lead to some bias in the assessment of their ecological status. Indeed, this stressor can explain a large part of some metrics variability and lead to an underestimation of eutrophication effect due to the antagonistic effect of the two stressors.

In estuaries, according to our results, non-additive effects were involved in more than three-quarters of studied stressor combinations, showing that the benefit for mitigating stressors often differed to the expectation. Four types of pairwise combined effects were observed in our analysis (i.e. AD, +S, +A, -A), but antagonism was the most common interaction. This observation reflects a complex scenario for ecosystem management, because the efforts to mitigate stressors often yield fewer benefits than expected (Brown et al., 2013; Folt et al., 1999). The complete recovery for mitigating one stressor is only expected when other antagonist-related stressors have been also removed. In this context, the identification of dominant stressors in estuarine systems should be accomplished by taking into account the direction and strength of interactions to improve the assessment accuracy of the stressors impacts. Furthermore, the direction of combined stressor effects could be changed with the involvement of higher order interactions complicating the predictability of management actions (Piggott et al., 2015). The use of random forest offsets this problem.

To conclude on these comparative analyses of multi-stressors effects on freshwaters and estuaries fish assemblages, in a lot of cases, the stressor descriptors we used lead to non-additive effects on the metrics and indices studied. This means that fish indices will have a better relationship with the stressors if the interacting stressors are taken into account in the development of indices. However, results show that pairs of stressors may act either synergistically or antagonistically depending on the metrics, which hinders general rules for restoration purposes to be established for fish assemblages at the European scale.

Management purposes on estuaries

Water pollution was classified at the first rank of priority in the combined scheme of restoration and showed a threshold shift for the water quality biological effects. The EQR was higher for the lowest levels of pollution-related stressors and abruptly dropped when the contamination of waters and or sediments was substantially elevated compared to the national background level (in the case of heavy metals). Water quality is also impacted by enrichment in nutrients and organic matter, which cause severe impacts on marine ecosystems functioning, especially through problems of oxygen depletion (Diaz and Rosenberg, 1995, 2008). Although direct effects of eutrophication were not highlighted, oxygen depletion was classified at the third rank of priority in the scheme of restoration. The EQR dropped for saturation values of dissolved oxygen below 70% for 95% of the time and when hypoxia spread over 1-5% of the estuary length. Uriarte and Borja (2009) already demonstrated for fish in estuaries that oxygen saturation value below 80% lead to moderate ecological status, whilst 60% saturation lead to poor status, with a threshold effect. These results suggest that a moderate decline of oxygen saturation can produce impacts in the mobile fauna (Breitburg, 2002), resulting in a modification of the structure of fish communities (Pollock et al., 2007).

For management purpose, a parsimonious strategy of stressors investigation involves checking first if stressors yielding the maximum restoration benefit are acting on the target system (Halpern et al., 2007). The outcomes of the step-by-step restoration scheme can be actually useful for environmental managers, because it provide theoretical ranking in which stressors should be considered to establish a restoration program. The most important average benefits were expected for the mitigation of water pollution, seabed alteration, and oxygen depletion, flow changes, intertidal area lost and dredged sediments, listed in descending order of benefit. These stressors reflect a general degradation of water quality, but also hydro-morphological alterations mainly caused by human drivers, such as agriculture, industry and urban development. The provided ordination assumes that the ecosystem recovery was the strict inverse process of deterioration and that degradation was fully reversible. However, these assumptions appear simplistic in coastal and estuarine ecosystems (Duarte et al., 2015). Degradation and recovery typically follow different pathways because ecosystem buffers act to maintain the degraded state (Duarte et al., 2009; Lotze et al., 2011). In many cases, the thresholds separating alternate states often differ between ecosystem degradation and recovery, suggesting that the ecosystem recovery should require much more efforts to reduce the stressor level than to cause the degradation. In the present study, the ecological benefits were obtained after reducing the intensities of stressors at a common level (i.e. “very low”) for simplification purpose, because a large amount of ecosystem recovery is expected from this value. However, accurate restoration criteria should consider the shape of the EQR-stressor relationship to identify the optimal level below which stressors should be mitigated.

This approach developed on estuaries suggests that processes involved in the degradation of the systems are the same than those involved in the restoration process. This assumption is probably less supported in lakes and reservoirs. Indeed, in these types of systems under equilibrium, the local characteristics of the lakes (size in particular) will greatly influence species richness and diversity whereas under conditions of stress leading to a decrease in species abundance or diversity, we can expect a major effect of the properties of the basin (size and regional diversity) on the ability to recover. Sources and sink models could probably help in these cases where connectivity can have a major influence on the recovery potential of the ecosystem.

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Appendix 1

Description of species traits and occurrences. Trait values written in italic were not taken into account because they concern only a few species and rarely occur.

Latin fish name	Spawning substrate	Trophic guild	Feeding habitat	Reproductive guild	Tolerance	Occurrence in EU dataset	Occurrence in PT - FR dataset
Abramis brama	PHLI	PLAN	BENT	A_1_0	Tolerant	32.7	66.4
Alburnus alburnus	PHLI	PLAN	WC	A_1_0	Tolerant	16.4	39.7
Alburnoides bipunctatus	LITH	INV	WC	A_1_0	Intolerant	0.1	0.6
Alosa fallax	LITH	PLAN	BENT	A_1_0		0.1	
Ameiurus melas	LITH	OMNI	BENT	B_2_0	Tolerant	0.5	22
Anguilla anguilla	PELA	INV_PISC	WC	A_1_0	Tolerant	1.6	1.4
Leuciscus aspius	LITH	PISC	BENT	A_1_0		0.4	
Ballerus ballerus	PHYT	PLAN	WC	A_1_0		0.4	
Barbus barbus	LITH	INV	BENT	A_1_0		0.1	3.2
Luciobarbus bocagei	LITH	OMNI	BENT	A_1_0			3.8
Barbatula barbatula	PHLI	INV	BENT	A_1_0		0.1	3.8
Luciobarbus sclateri	LITH	INV	BENT	A_1_0			0.9
Blicca bjoerkna	PHYT	OMNI	BENT	A_1_0	Tolerant	9.8	47.8
Carassius auratus	PHYT	OMNI	BENT	A_1_0	Tolerant	0.1	3.5
Carassius carassius	PHYT	OMNI	BENT	A_1_0	Tolerant	3.2	9.9
Carassius gibelio	PHYT	OMNI	BENT	A_1_0	Tolerant	0.1	1.4
Pseudochondrostoma duriense	LITH	OMNI	BENT	A_1_0			1.7
Pseudochondrostoma polylepis	LITH	OMNI	BENT	A_1_0			2.6
Pseudochondrostoma willkommii	LITH	OMNI	BENT	A_1_0			1.2
Cobitis taenia	PHYT	*BENT*	BENT	A_1_0		2.1	2
Coregonus albula	LITH	PLAN	WC	A_1_0	Intolerant	10	
Coregonus autumnalis	LITH	*INV_PLAN*	WC	A_1_0		0.1	
Coregonus lavaretus	LITH	INV	WC	A_1_0	Intolerant	8.2	0.6
Cottus gobio	LITH	INV	BENT	B_2_0	Intolerant	0.6	2.9
Cottus poecilopus	LITH	OMNI	BENT	B_2_0	Intolerant	1.1	
Cyprinus carpio	PHYT	OMNI	BENT	A_1_0	Tolerant	1.1	32.2
Esox lucius	PHYT	PISC	WC	A_1_0		62.9	60
Gasterosteus aculeatus	*ARIAD*	INV	BENT	B_2_0	Tolerant	1.8	0.6
Gobio gobio	PHLI	INV	BENT	A_1_0		2.6	17.1
Gymnocephalus cernua	PHLI	OMNI	BENT	A_1_0		29.7	50.4
Hypophthalmichthys molitrix	PELA	PLAN	WC	A_1_0	Tolerant	0.1	0.9
Hypophthalmichthys nobilis	PELA	PLAN	BENT	A_1_0		0.1	
Lepomis gibbosus	LITH	INV	WC	B_2_0	Tolerant	0.9	38.8
Leucaspius delineatus	PHYT	OMNI	WC	*B_1_0*		1	3.2
Leuciscus idus	PHLI	INV_PISC	WC	A_1_0		0.6	1.4

Latin fish name	Spawning substrate	Trophic guild	Feeding habitat	Reproductive guild	Tolerance	Occurrence in EU dataset	Occurrence in PT - FR dataset
Leuciscus leuciscus	LITH	OMNI	WC	A_1_0		0.3	4.6
Liza ramada	PELA	*INV_HERB*	BENT	A_1_0	Tolerant	0.1	0.9
Lota lota	LITH	PISC	WC	A_1_0		7.8	2
Micropterus salmoides	*ARIAD*	PISC	WC	B_2_0	Tolerant	0.1	5.8
Misgurnus fossilis	PHYT	*BENT*	BENT	A_1_0		0.1	
Mugil cephalus	PELA	*DETR_HERB*	BENT	A_1_0			0.3
Neogobius melanostomus	*SPEL*	INV	BENT	*A_2_0*		0.1	
Oncorhynchus mykiss	LITH	INV_PISC	WC	*A_2_0*		0.9	7.8
Osmerus eperlanus	LITH	INV_PISC	WC	A_1_0		6.8	
Perca fluviatilis	PHLI	INV_PISC	WC	A_1_0	Tolerant	48.6	86.7
Phoxinus phoxinus	LITH	INV	WC	A_1_0		2.9	4.1
Platichthys flesus	PELA	INV_PISC	BENT	A_1_0		0.2	
Pomatoschistus minutus	*OSTR*	INV_PISC	BENT	B_2_0		0.1	
Pseudorasbora parva	PHLI	OMNI	WC	B_2_0	Tolerant	0.1	1.7
Pungitius pungitius	PHYT	INV	BENT	B_2_0	Tolerant	0.5	
Rhodeus amarus	*OSTR*	OMNI	WC	*A_2_0*	Intolerant	0.2	2.3
Rutilus rutilus	PHLI	OMNI	WC	A_1_0	Tolerant	67.7	87.5
Salaria fluviatilis	LITH	INV	BENT	B_2_0		0.1	0.6
Salmo ferox	LITH	PISC	WC	*A_2_0*	Intolerant	0.1	
Salmo nigripinnis	LITH	PLAN	WC	*A_2_0*		0.1	
Salmo salar	LITH	INV_PISC	WC	*A_2_0*	Intolerant	0.6	
Salmo stomachicus	LITH	INV	BENT	*A_2_0*		0.1	
Salmo trutta fario	LITH	INV_PISC	WC	*A_2_0*	Intolerant	7.7	11.9
Salmo trutta lacustris	LITH	INV	BENT	*A_2_0*	Intolerant	0.1	1.2
Salmo trutta	LITH	INV_PISC	WC	*A_2_0*		6.4	0.9
Salmo trutta trutta	LITH	INV_PISC	WC	*A_2_0*	Intolerant	0.5	
Salvelinus fontinalis	LITH	INV_PISC	WC	*A_2_0*	Intolerant	0.6	0.9
Salvelinus namaycush	LITH	INV_PISC	WC	A_1_0	Intolerant	0.1	0.9
Salvelinus umbla	LITH	INV_PISC	WC	A_2_0	Intolerant	8.2	5.5
Sander lucioperca	PHLI	INV_PISC	WC	B_2_0		9.3	67.8
Scardinius erythrophthalmus	PHYT	OMNI	WC	A_1_0		23.3	69.9
Silurus glanis	PHYT	PISC	WC	*B_1_0*		0.3	16.5
Squalius carolitertii	LITH	INV	BENT	A_1_0			0.6
Squalius cephalus	PHLI	OMNI	WC	A_1_0		1.5	27.8
Squalius pyrenaicus	LITH	INV	WC	A_1_0			0.9
Telestes souffia	LITH	INV	WC	A_1_0	Intolerant	0.1	0.9
Thymallus thymallus	LITH	INV	WC	*A_2_0*	Intolerant	0.5	0.3
Tinca tinca	PHYT	OMNI	BENT	A_1_0	Tolerant	17.7	36.2
Myoxocephalus quadricornis	LITH	INV	BENT	B_2_0		0.1	

Appendix 2

Description of stressors used for estuaries analyses, according to Lepage et al., 2012.

Stressor descriptor	Description
1. Anthropogenically affected coastline	This indicator estimates the percentage land use given over to industrial and urban development, and agriculture within the coastal zone (1 km landward from the MHW). This parameter should reflect the naturalness around the estuary and can be estimated through the use of aerial photography.
2. Intensity of marina developments	This indicator estimate the intensity of marina developments on the basis of the number of berths.
3. Maintenance dredging - disposal area	This indicator is represented by the area designated for disposal as suggested within the Water Framework Directive for the designation of Heavily Modified Water Bodies (HMWB).
4. Maintenance dredging - disposal amount	This indicator estimates the disposal amount derived from the dredging activities, expressed in tons deposited annually in estuaries.
5. Capital dredging	This indicator is represented by the total tonnage disposed during the last 10 years, including beneficial uses, and the number of licences for the last 10 years.
6. Aquaculture	This indicator reflects the extent of fish farming activities, in term of occupied space.
7. Fisheries activities	This indicator reflects the extent of fisheries activities, in term of occupied space.
8. Interference with the hydrographical regime	This indicator measures the percentage area impacted by man-made structures affecting the current patterns, wave regime and sediment transport patterns within a system.
9. Interference with fish migration routes	The number of physical barriers (e.g. tidal water control facilities and drainage facilities) is used as a proportion of natural watercourses affected. Where possible, the significance of the impact of each structure on fish movements should be based on the count of indicative species such as salmon, trout or lamprey.
10. Intertidal area lost topography	This indicator includes both anthropogenically induced changes and natural variations over the last century. Historical maps and/or aerial photography can be used to estimate the area lost.
11. OSPAR Eutrophication assessment	This indicator is based on the identification of the OSPAR eutrophication status.
12. Dissolved oxygen (temporal)	This indicator is based on the percent oxygen saturation within a system over an annual period.
13. Dissolved oxygen (spatial)	This indicator measures the spatial extent of reduced or elevated (supersaturated) dissolved oxygen problems within a system.
14. Intensity of port developments	This indicator measures the intensity of port developments, as evaluated by the length of quays.
15. Benthic ecological status	This indicator is based on WFD intertidal and subtidal benthic invertebrate monitoring. Where such monitoring is not available, assessments can be based on other benthic studies and local expertise.
16. Water chemical quality	Water chemical quality is measured as the degree of compliance with Environmental Quality Standards (EQSs) for List I and List II substances of the EU Dangerous Substances Directive (e.g. metals, organic compounds, pesticides). Where no monitoring data are available, expert judgment is exercised.
17. Water quality biological effects	This indicator is based on heavy metal and biological effects monitoring data (e.g. imposex, oyster embryo bioassays, bioaccumulation studies). Biological effects may not be monitored for water bodies that are classified as 'good status' under the Water Framework Directive. In such instances, the score would be very low (1).
18. Water pollution incidents	This indicator refers to the number of incidents reported in the literature.

Appendix 3

Stressor descriptors of estuaries classified with standardized thresholds criteria according to their intensity of disturbance.

Stressor descriptors	Thresholds values					
	No	V. low	Low	Med.	High	V. high
1. Anthropogenically affected coastline	No development	<5% of the coastal area impacted by industrial or urban activities	≥5% and <30% of the coastal area impacted by industrial or urban activities	≥30% and < 60% of the coastal area impacted by industrial or urban activities	≥60% and < 90% of the coastal area impacted by industrial or urban activities	≥ 90% of the coastal area impacted by industrial or urban activities
2. Intensity of marina developments	No marina	< 100 berths in marina	≥100 & <150 berths in marina	≥150 & <300 berths in marina	≥300 & <500 berths in marina	≥ 500 berths in marina
3. Maintenance dredging - disposal area	No dredging	<1% of the subtidal area dredged	≥1% & <10% of the subtidal area dredged	≥10% & <30% of the subtidal area dredged	≥30% & <50% of the subtidal area dredged	≥ 50% of the subtidal area dredged
4. Maintenance dredging - disposal amount	no disposal	< 5000 tons deposited annually	≥5000 & <100,000 tons deposited annually	≥100,000 & < 1 million tons deposited annually	≥1 & < 4 million tons deposited annually	≥ 4 million tons deposited annually
5. Capital dredging	No disposal	< 5000 tons deposited for the last 10 years	≥5000 & <100,000 tons deposited for the last 10 years	≥100,000 & < 1 million tons deposited for the last 10 years	≥1 & < 4 million tons deposited for the last 10 years	≥ 4 million tons deposited for the last 10 years
6. Aquaculture	No fish farming	<1% of the intertidal and subtidal area covered	≥1% & <10% of the intertidal and subtidal area covered	≥10% & <30% of the intertidal and subtidal area covered	≥30% & <50% of the intertidal and subtidal area covered	≥ 50% of the intertidal and subtidal area covered
7. Fisheries activities	No fishery activities	< 10% of the length of coast (riverbank) affected by fishery	≥10% & <30% of the length of coast affected by fishery	≥30% & <60% of the length of coast affected by fishery	≥60% & <90% of the length of coast affected by fishery	≥ 90% of the length of coast affected by fishery
8. Interference with the hydrographical regime	No construction	<5% of the area affected	≥5% and <10% of the area affected	≥10% and <20% of the area affected	≥20% and <40% of the area affected	≥ 40% of the area affected
9. Interference with fish migration routes	No interference	<5% of natural drains and rivers affected by a physical barrier	≥5% and <30% of natural drains and rivers affected by a physical barrier	≥30% and <60% of natural drains and rivers affected by a physical barrier	≥60% and <90% of natural drains and rivers affected by a physical barrier	≥90% of natural drains and rivers affected by a physical barrier

10. Intertidal area lost; Realignment schemes; Land claim; Gross change in the bathymetry and topography	Increase	No change	<1% lost over the last decade	≥1% and <5% lost over the last decade	≥5% and <10% lost over the last decade	≥ 10% lost over the last decade
11. OSPAR Eutrophication assessment	Non-problem area / Potential problem area / Problem area					
12. Dissolved oxygen (temporal)	No dissolved oxygen problem	DO saturation >80% for 95% of the time	DO saturation ≤80% and >70% for 95% of the time	DO saturation ≤70% and >50% for 95% of the time	DO saturation ≤50% and >20% for 95% of the time	DO saturation ≤20% for 95% of the time
13. Dissolved oxygen (spatial)	No dissolved oxygen problem	Problems may occur in <1% of the estuary length	Problems may occur in ≥1% and <3% of the estuary length	problems may occur in ≥3% and <5% of the estuary length	problems may occur in ≥5% and <7% of the estuary length	problems may occur in >7% of the estuary length
14. Intensity of port developments	No harbour	<500 m of quays	≥500 & <2 km of quays	≥2 & <5 km of quays	≥5 & <10 km of quays	≥ 10 km of quays
15. Benthic ecological status	High status	High status	Normal (Good status)	Recovering or deteriorating (Moderate status)	Modified (Poor status)	Severely modified (Bad status)
16. Water chemical quality	100% compliance of samples with EQSs for all substances	100% compliance of samples with EQSs for all substances	One List II substance fails to comply with EQS AND no significant increase in the concentration of this substance	One List II substance fails to comply with EQS AND significant increase in the concentration of this substance OR (ii) More than one List II substances fail to comply with EQSs AND no significant increase in the concentration of these substances failing the EQS	More than one List II substances fail to comply with EQSs AND significant increase in the concentration of these substances failing the EQS OR (ii) one List I substance fails to comply with EQSs	More than one List I substance fails to comply with EQSs
17. Water quality biological effects	Low concentration for all metals (< 2 x national background level)	Low concentration for all metals (< 2 x national background level)	The concentration for one or more metals is ≥ 2 x national background level and < substantially elevated level	The concentration for one or more metals is ≥ substantially elevated level and < grossly elevated level	The concentration of one metal is ≥ grossly elevated level	The concentration of more than one metal is > grossly elevated level
18. Water pollution incidents	None	No incidents reported	≥1 & <50 incidents reported	≥50 & <100 incidents reported	≥100 & <200 incidents reported	≥ 200 incidents reported

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