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Fish assemblages in European Western Highlands and Western Plains: a type-specific approach to assess ecological quality of running waters

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Abstract After typological pre-classification of 398 calibration sites, fish-based metric models were used to predict the impact of human activities on river quality in European Western Highlands and Western Plains ecoregions. Calibration sites were grouped into six assemblage types and according to their geomorphology; test sites were assigned to their corresponding assemblage type. Five anthropogenic variables were used to describe the impact level of each site and stepwise discriminant analysis was performed to: (i) avoid redundancy between metrics; (ii) examine how selected metrics discriminated impact classes and (iii) predict ecological status for each site of the given fish type. Globally, this approach predicted the impact class correctly for 64% of sites. The difference between observed and predicted impact was more than one class for only 2.5% of the sites. When validating this approach with an independent data set, differences between observed and predicted impact values never exceeded 2 impact classes, but these differences varied in size among countries.

KEYWORDS: bioassessment, fish assemblages, predictive models, type-specific approach.

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Introduction

Biological indicators have been widely used to assess human pressure in running waters (Karr 1981; Karr, Fausch, Angermeier, Yant & Schlosser 1986; Karr & Chu 1999). The first index of biotic integrity (IBI) was developed for streams in the mid-western USA (Karr 1981). Subsequently, the IBI has been modified for many regions (reviewed in Karr & Chu 1999; Roset, Grenouillet, Goffaux, Pont & Kestemont 2007), and in particular in Europe (e.g. Belpaire, Smolders, Vanden Auweele, Ercken, Breine, Van Thuyne & Ollevier 2000; Kestemont, Didier, Depiereux & Micha 2000; Breine, Simoens, Goethals, Quataert, Ercken, Van Liefferinghe & Belpaire 2004). Oberdorff, Pont, Hugueny & Chessel (2001, 2002) developed multivariate models to assess biotic integrity of French rivers over large geographical areas.

Most of the predictive models developed, assess ecological status by comparing the biotic condition at sites with the biota expected in the absence of human pressure (Wright 1995). Of the numerous techniques used, two general approaches emerge. First, the type-specific approach that relies on clustering techniques, so that the reference sites are classified into groups based on the homogeneity of their fauna. Secondly, the modelling approach that directly predicts expected fauna according to environmental features of the evaluated site, and does not require a prior development of a classification system (e.g. Oberdorff *et al.* 2001).

The aim of this study was to develop a spatially-based method (SBM) using fish assemblages to assess ecological integrity of Western European rivers from three countries (France, Belgium and the Netherlands), including the rivers Garonne, Loire, Meuse, Rhône, Seine and Scheldt. According to Illies (1978), the study area covered two ecoregions (e.g. Western Highlands and Western Plains). An examination of the Illies' classification highlighted the maladjustment of ecoregions to account for similarities between the fish assemblages at the European scale (Reyjol, Aeconomo, Beier, Caiola, Cowx, Ferreira, Haidvogl, Hugueny, Noble, Pont, Vigneron & Virbickas 2007). In the present study, based on species abundance, similar fish assemblages (e.g. same dominant species and similar assemblage structures) were observed in similar-sized streams in both ecoregions. Moreover, one of the river catchments (e.g. Meuse catchment) covered both ecoregions. For these reasons, it was decided to focus on the two ecoregions jointly.

Material and methods

Abiotic variables and pressure

Data relating to 3388 fishing occasions, all sampled by electric fishing, were selected from the Belgium–Flanders (BE), Belgium–Wallonia (BW), France (FR) and the Netherlands (NL) (1682, 158, 1392 and 156 sites, respectively) national databases.

Five environmental variables were used to describe physiographical characteristics of sites: altitude (ALT, m), wetted width (WID, m), gradient slope (SLO, ‰), mean air temperature (TEM, °C) and distance from source (DFS, km) (Beier *et al.* 2007). These variables were chosen because they describe the spatial position in the hydrographic network (i.e. along the upstream–downstream longitudinal gradient).

Physical and chemical pressures were defined according to five criteria (hydrological regime, river connectivity, morphological conditions, toxic acidification and nutrient organic inputs) (Degerman, Beier, Breine, Melcher, Quataert, Rogers, Roset & Simoens 2007). These criteria, dictated by the European Union Water Framework Directive (WFD), were coded from 1 (no pressure) to 5 (high pressure) based on knowledge of local experts. Only sites with pressure statuses 1 and 2 for all criteria were retained to define the calibration data set (CD). No such conditions were found in Flanders and the Netherlands. As a result the CD contained 398 sites from BW and FR (45 and 353 sites, respectively) (Fig. 1a). The five pressure criteria dictated by the WFD were combined into one variable (Degerman *et al.* 2007). This global level of human pressure was defined as the mean of the five criteria, and coded into five status categories ('pressure', ranging from 1 to 5).

Calibration of models and assessment procedure

Step 1: Classification of calibration sites (cluster analysis) Calibration sites were classified into groups with similar fish assemblages and densities. A hierarchical cluster analysis (using the Ward's method and Euclidean distances) was applied on the transformed [$\log(x + 1)$] matrix of species abundance (number of fish $\times$ 100 m^{-2}). Dissimilarities among fish samples were calculated using various Euclidean and non-Euclidean distances and that other measures of assemblage dissimilarity (e.g. Bray–Curtis distance) were verified to be highly correlated with the Euclidean distance values. The dendrogram produced from this cluster analysis was examined to separate clusters that represent the main biological groups or fish types.

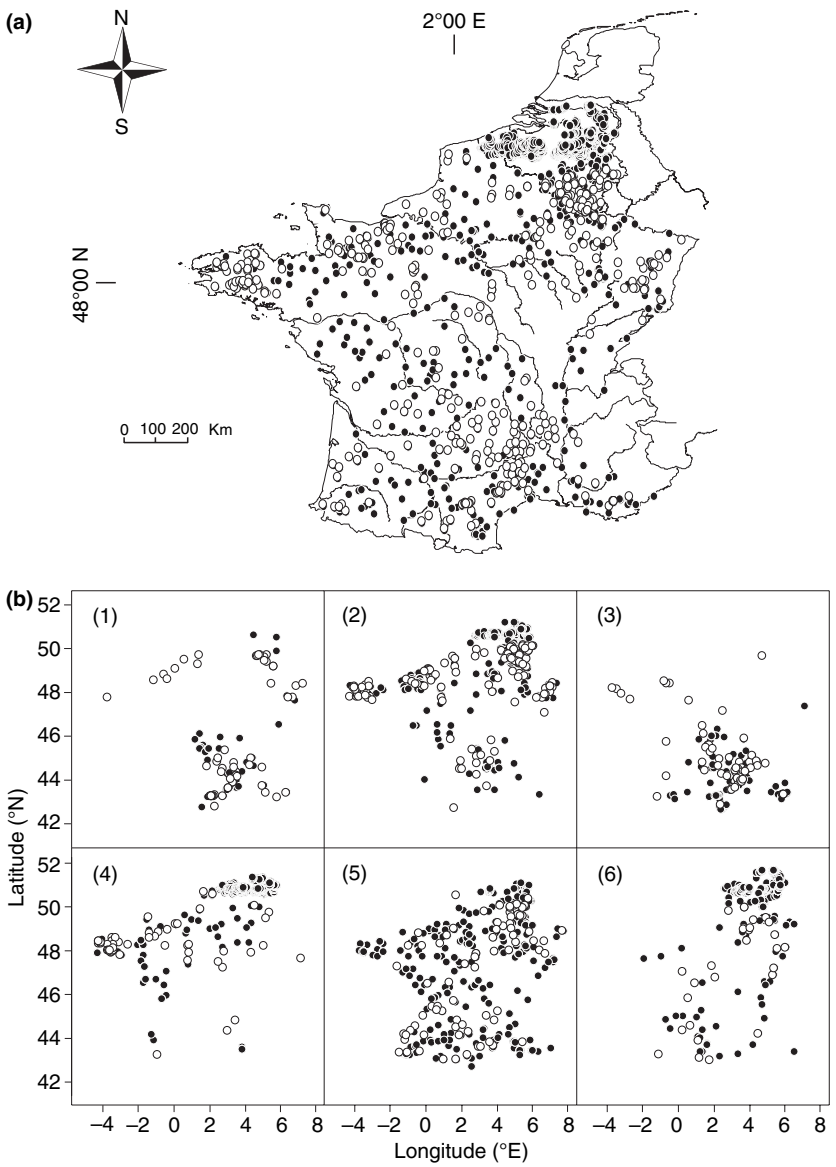

Figure 1. Geographical position of sampling sites (a) within the whole data set, and (b) within each fish type, for calibration (open circles) and test (solid circles) sites. Numbers (1–6) indicate the fish type in which each site belongs.

Step 2: Linking fish types with physiographic variables (discriminant analysis) Altitude, WID, SLO, TEM and DFS were used to describe the mean physiographical characteristics of the habitats of the different fish types. These variables were checked for normality, and all except TEM were log-transformed. Discriminant analysis was performed on these five variables to identify those that best discriminated among the fish types. Cross validation (re-classification of samples) was used to estimate the error rates (i.e. the percentage of incorrectly classified samples), and provide a robust measure of the predictive ability of the discriminant analysis within the CD (Joy & Death 2002). Different classifications (varying 4–9 fish types) were examined and the cross validation error rate was used to identify the best classification.

Step 3: Selecting metrics (Spearman's correlation and stepwise discriminant analysis) All sites were allocated to their corresponding fish types using relationships between physiographical parameters and fish types. For each fish type, correlations between fish metrics and pressure status were

investigated (Spearman's rank correlation), and a first list of potential metrics (i.e. only those responding to pressure) was obtained for each fish type. Stepwise discriminant analysis was then performed to avoid redundancy between these metrics and to select metrics discriminating among pressure status categories.

Step 4: Assessing new sites After allocating new sites to their expected fish types on the basis of their physiographical characteristics (discriminant analysis, Step 2), relationships between fish metrics (discriminant analysis, Step 3) and pressure status categories can be used for predicting pressure status of these new monitoring sites.

Validating the approach on independent data set Finally, the validity of the SBM was checked using an independent dataset. Fifty-five sites were sampled by electric fishing during late summer 2003 in the different countries (9, 6, 26 and 14 sites in BE, BW, FR and NL, respectively). These sites were selected to cover the whole pressure gradient, and to reflect the different fish types. For the discrepancy between observed and predicted pressure status (i.e. model error), differences among fish types and countries were examined using ANOVAS.

Results

Species composition

Forty nine species occurred in the CD, but those found in $<1\%$ of sites were excluded, leaving 40 species (representing 13 families) to be retained for classification of calibration sites. Five species made up to 49.8% of fish occurrences. Brown trout *Salmo trutta* L., was the most widely distributed species (91% of sites). Bullhead, *Cottus gobio* L., minnow *Phoxinus phoxinus* (L.) and stone loach, *Barbatula barbatula* (L.), occurred at 68.3%, 64.1% and 60.3% of calibration sites respectively.

According to the Huet (1959) fish zonation, 157, 137, 89 and 15 calibration sites belonged to the trout, grayling, barbel and bream zones respectively. This highlights that the CD mainly focused on trout and grayling streams (almost 74% of calibration sites), and was thus not representative of lowland rivers ($<4\%$ of sites).

Classifying calibration sites

Six fish types were visually identified from the classification results of calibration data (Fig. 2a). They differed in their fish species composition (Table 1). Fish type 1 was characterised by brown trout and was distinguished from other types by low number of species per site (mean value = 2.6). Fish types 2, 3 and 4 (mean species richness = 4.5, 5.0 and 7.1, respectively) were first characterised by brown trout. Fish type 2 was further characterised by bullhead, type 3 by minnow and type 4 by minnow and bullhead. Fish type 5 (mean species richness = 10.3) was mainly defined by minnow, gudgeon *Gobio gobio* (L.), stone loach, brown trout, chub *Leuciscus cephalus* (L.) and bullhead. Finally, fish type 6 (mean species richness = 15.8) was characterised by gudgeon, chub, roach *Rutilus rutilus* (L.) and bleak *Alburnus alburnus* (L.).

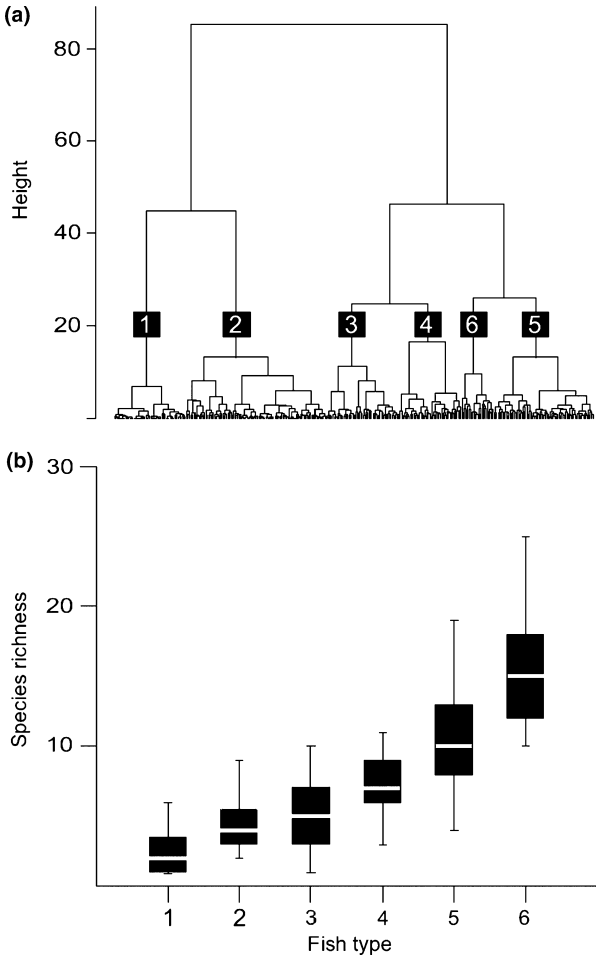

Figure 2. (a) Dendrogram of calibration sites based on Ward's method. (b) Box plots of fish species richness for each fish type. In a box plot, the centre vertical line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third quartiles.

Table 1. Fish frequency and physiographic characteristics among fish types in the calibration data set

Fish species	Code	Fish type						*P*-value
		1 (n = 60)	2 (n = 116)	3 (n = 61)	4 (n = 51)	5 (n = 83)	6 (n = 27)	
Salmo trutta fario	Salfar	1.00[a]	1.00[a]	1.00[a]	1.00[a]	0.82[b]	0.22[c]	< 0.0001
Phoxinus phoxinus	Phopho	0.30[a]	0.32[a]	0.92[b]	0.92[b]	0.96[b]	0.63[c]	< 0.0001
Leuciscus cephalus	Leucep	0.05[a]	0.08[a]	0.36[b]	0.22[a,b]	0.81[c]	1.00[c]	< 0.0001
Gobio gobio	Gobgob	0.10[a]	0.11[a]	0.67[b]	0.37[c]	0.89[d]	1.00[d]	< 0.0001
Barbatula barbatula	Barbar	0.17[a]	0.41[b]	0.72[c]	0.88[c]	0.88[c]	0.74[c]	< 0.0001
Leuciscus souphia	Leusou	0.05[a,b]	0.01[b]	0.13[a]	0.00[b]	0.01[a]	0.04[a]	0.0018
Barbus meridionalis	Barmer	0.07[a]	0.00[a]	0.21[b]	0.00[a]	0.07[a]	0.00[a]	< 0.0001
Cottus gobio	Cotgob	0.22[a]	1.00[b]	0.30[a]	0.96[b]	0.75[c]	0.52[c]	< 0.0001
Lampetra lampetra	Lampla	0.07	0.47[b]	0.21[a]	0.57[b]	0.29[c]	0.11[a,c]	< 0.0001
Anguilla anguilla	Angang	0.10[a]	0.31[b]	0.13[a,b]	0.75[c]	0.54[c]	0.70	< 0.0001
Rutilus rutilus	Rutrut	0.03[a]	0.09[a]	0.05[a]	0.14[a]	0.58[b]	1.00[c]	< 0.0001
Perca fluviatilis	Perflu	0.02[a]	0.05[a]	0.02[a]	0.10[a]	0.37[b]	0.85[c]	< 0.0001
Leuciscus leuciscus	Leuleu	0.02[a]	0.04[a]	0.03[a]	0.14[a]	0.66[b]	0.81[b]	< 0.0001
Salmo salar	Salsal	0.00[a]	0.06[a]	0.02[a]	0.53	0.04[a]	0.00[a]	< 0.0001
Barbus barbus	Barbus	0.02[a]	0.03[a]	0.05[a]	0.02[a]	0.53[b]	0.85[c]	< 0.0001
Alburnus alburnus	Albalb	0.00[a]	0.01[a]	0.00[a]	0.00[a]	0.22[b]	0.96[c]	< 0.0001
Thymallus thymallus	Thythy	0.02[a]	0.15[a,b]	0.03[a]	0.10[a]	0.24[b]	0.04[a]	< 0.0001
Scardinius erythrophtalmus	Scaery	0.02[a]	0.01[a]	0.00[a]	0.00[a]	0.05[a]	0.48[b]	< 0.0001
Esox lucius	Esoluc	0.02[a]	0.03[a]	0.03[a]	0.08[a]	0.43[b]	0.67[c]	< 0.0001
Physiographical characteristics	ALT	494.13[a] (320.58)	251.34[b] (201.72)	469.02[a] (322.81)	137.37[c] (174.74)	185.65[b,c] (133.93)	129.48[c] (82.01)	< 0.0001
	TEM	9.61[a,b] (1.57)	9.47[a] (1.10)	10.16[b,c] (1.83)	10.24[b,c] (1.05)	10.61[c] (1.76)	10.96[c] (1.66)	< 0.0001
	SLO	21.10[a] (23.94)	11.16[b] (9.61)	9.96[b] (10.04)	5.15[c] (3.63)	3.61[d] (3.69)	0.99[e] (0.75)	< 0.0001
	DFS	8.10[a] (7.12)	12.12[a] (16.95)	17.41[b] (13.04)	22.22[b] (43.71)	50.78[c] (54.73)	96.22[c] (112.13)	< 0.0001
	WID	4.63[a] (2.88)	4.61[a] (3.28)	6.68[b] (3.70)	7.56[b] (5.88)	13.91[c] (10.71)	29.33[d] (29.12)	< 0.0001

n, number of observations; percentage of the different fish species, mean values (with SD in brackets) for altitude (ALT), mean air temperature (TEM), slope (SLO), distance from source (DFS) and wetted width (WID). For each variable, values among fish type with a common letter are not significantly different at $P = 0.05$ (pairwise logistic comparison and Tukey–Kramer tests for occurrence and quantitative data, respectively). Probabilities (P) are given for testing the fish type effect on each variable.

Linking fish types with physiographic variables

All physiographical variables differed significantly (ANOVA tests, $P < 0.0001$, Table 1) among fish types. According to SLO, DFS and WID, fish types appeared to be mainly ordered (fish type 1–6) along a longitudinal (upstream–downstream) gradient. These fish types reflected an increase in fish species richness with increasing stream size (Fig. 2b). Slope and ALT were the two main physiographical variables involved in the separation of fish types (Table 2). Slope was negatively related with the first axis (LD1) of the discriminant analysis, whereas the second axis (LD2) was strongly related with ALT. These first two axes represented 77.7% and 18.8% of the total inertia respectively.

Using cross-validation, 222 of the 398 calibration sites (55.8%) were assigned correctly to their predetermined fish type. Only 24% of sites from fish type 4 were well classified, whereas sites from fish types 2 and 5 were correctly classified on 78% and 72% of occasions respectively.

Table 2. Linear discriminant coefficients (with percentage of total inertia explained in parentheses) for the five physiographical variables (code as in Table 1) used in the discriminant analysis

Physiographic variables	Linear discriminant coefficients				
	LD1 (0.777)	LD2 (0.188)	LD3 (0.021)	LD4 (0.012)	LD5 (0.002)
ALT	0.015	1.344	−0.265	0.421	−0.206
TEM	0.177	0.447	0.199	−0.271	−0.504
SLO	−0.774	−0.162	0.844	−0.643	0.481
DFS	0.435	−0.114	1.059	0.731	0.022
WID	0.228	0.338	−0.506	−1.400	1.021

Patterns in fish type spatial distribution were examined by plotting geographical coordinates of sites (Fig. 1b). According to calibration sites, all fish types were more or less spread across the study area, and no mismatch occurred between spatial distribution of calibration and test sites. Fish type 5 was the most evenly distributed across the study area.

Selecting metrics

The number of metrics responding significantly ($P < 0.05$, Spearman's rank correlation) to pressure varied from 61 (fish type 1) to 138 (fish type 6). After this pre-selection, the number of metrics retained by stepwise discriminant analysis varied from 13 (fish type 1) to 19 (fish type 4). In total, 67 metrics were used for model construction, of which 48 (71.6%) were specific to one single fish type. 'Percentage of invertivorous individuals' was the most common metric across the models (i.e. retained for fish types 1, 2, 3 and 5). Five metrics (i.e. 'number of intolerant species', 'number of lithophilic species', 'number of phytophilic species', 'percentage of phytophilic species' and 'presence of juvenile brown trout') were used in three of the six models.

Predicting pressure

Cross-validations showed that 64.1% of the sites were correctly classified into five pressure status, while 91.3% of the sites were correctly classified into 'impacted' (i.e. status >2) or 'not impacted' (i.e. status 1 and 2) sites (Table 3).

Table 3. Predicted pressure status (mean values with SD in parentheses) and percentage of well-classified sites according to two classifications: Pressure status (1–5) and ecological status ('impacted' or 'not impacted')

		Pressure status		Ecological status
Data	*n*	Predicted pressure status	% of well-classified sites	error	% of well-classified sites
All	3388	2.74 (0.96)	0.641	0.056	0.913
Fish type					
1	93	1.39^{a} (0.62)	0.880	0.054^{a,b}	0.989
2	894	2.50^{b} (1.00)	0.615	0.134^{b}	0.914
3	146	1.73^{c} (0.61)	0.760	0.048^{a,b}	0.966
4	1120	3.22^{d} (0.73)	0.575	0.061^{a,b}	0.951
5	611	2.27^{c} (0.66)	0.716	0.003^{a}	0.823
6	524	3.19^{d} (0.86)	0.660	−0.021^{a}	0.908
Country					
BE	1682	3.35^{a} (0.58)	0.586	0.101^{a}	0.957
BW	158	2.36^{b} (0.70)	0.728	−0.095^{b}	0.949
FR	1392	1.96^{c} (0.75)	0.699	0.014^{b}	0.855
NL	156	3.47^{a} (0.84)	0.641	0.100^{a}	0.923

BE, Belgium–Flanders; BW, Belgium–Wallonia; FR, France; NL, the Netherlands.

Probabilities (*P*) from ANOVA models are given for testing the effect of fish type and country on predicted status and the errors (discrepancy between observed and predicted pressure status). Values among fish types and countries with a common letter are not significantly different at $P = 0.05$ (Tukey–Kramer tests).

At the fish type level, predicted status significantly (ANOVA, $P < 0.0001$, Table 3) differed among types. Mean predicted values were highest in fish types 4 and 6, and lowest in fish type 1. The percentage of well-classified sites for each of the five classes varied from 57.5% (fish type 4) to 88% (fish type 1), and for a classification into two ecological statuses from 82.8% (fish type 5) to 98.9% (fish type 1). There was no clear pattern in the relationships between the percentage of well-classified sites in a given fish type and its position along the longitudinal gradient. Model errors (i.e. the differences between observed and predicted pressure status) showed that the efficiency to classify sites differed significantly ($P < 0.0001$) among types (ANOVA tests, Table 3). Mean errors were highest in fish type 2 and lowest in fish type 6. Only 2.5% of sites had a difference >1 (in absolute value), and this percentage ranged from 0 (fish types 1 and 3) to 4.1% (fish type 2) of sites (Fig. 3). Among countries, predicted pressure status was highest in BE and NL, and lowest in FR sites (Table 3). The percentage of well-classified sites varied from 58.6% (BE) to 72.8% (BW) for a classification into five ecological status classes, and from 85.5% (FR) to 95.7% (BE) for a classification into two ecological status classes.

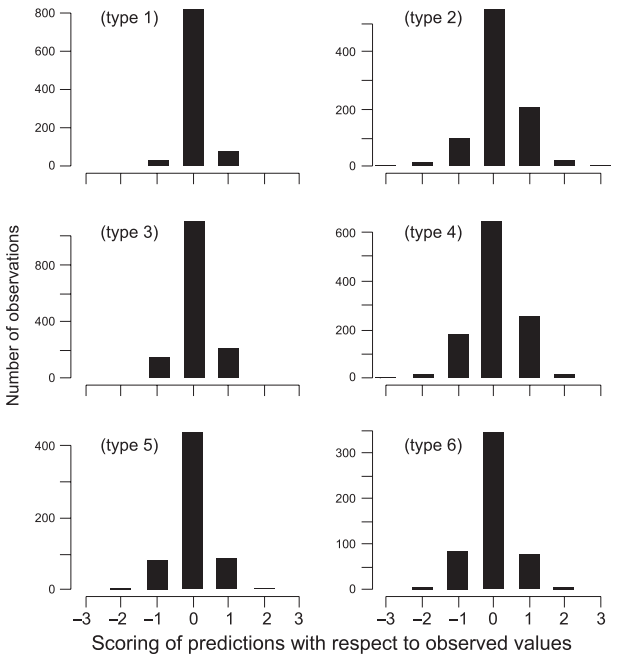

Figure 3. Distribution of differences between observed and predicted pressure status within each fish type.

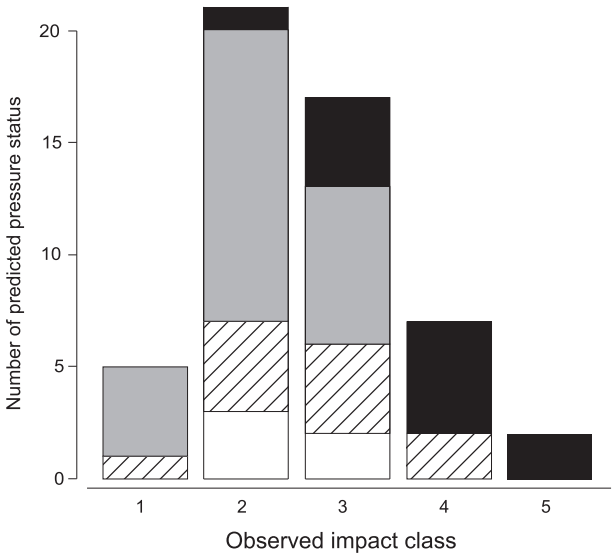

Figure 4. Number of predicted pressure status (1 in white, 2 in cross hatching, 3 in grey and 4 in black) for independent sites (n = 60).

Validating the approach on independent sites

The monitoring data set included sites from the full range of fish types (n = 2, 13, 4, 9, 7 and 20 in fish types 1–6, respectively). Regionally, pressure predictions status varied from 1 to 4, whereas observed status varied from 1 to 5. Differences between observed and predicted pressure status never exceeded two classes (Fig. 4). Note that model predictions were more severe (i.e. predictions higher than pressure observations) for less impacted sites (i.e. observed pressure status 1), whereas predictions were less severe for highly impacted sites (i.e. observed pressure status 5). Differences between observed and predicted pressure status varied among countries (ANOVA test, $P < 0.001$). Predictions tended to under score the ecological quality of BE and NL sites more frequently, but systematically over scored BW sites.

Discussion

Pre-classification of the CD defined groups based on the homogeneity of their fauna, and the large-scale geographical pattern in fish assemblage revealed that no group was restricted to a single catchment. This suggests that similar fish assemblage structures were encountered in the different river catchments, and that differences among sites within catchments were more important than differences among catchments.

Most of the variation in physiographical variables used to explain site classification (e.g. mainly SLO and ALT) could be accounted for by longitudinal (upstream–downstream) gradient. This gradient, reflecting an increase in fish species richness with increasing stream size, is probably the most well-known, worldwide, large-scale pattern in stream fish assemblages (reviewed in Matthews 1986). Joy & Death (2002) tested various environmental variables in discriminating the different fish types and found that ALT and distance from the sea were the most important factors associated with fish assemblage structure, while local-scale variables were less important. In essence, catchment-scale variables are the most important variables driving fish assemblage structure (Jowett & Richardson 1996).

In this study, 55.8% of calibration sites were assigned correctly to their predetermined fish types. This cross-validation error rate (44%) is noticeably higher than those reported in the literature, commonly around 30% for both fish (Joy & Death 2002) and macroinvertebrates (Simpson & Norris 2000). Nevertheless, meaningful output was obtained even in the more misclassified fish type (fish type 4, with only 24% of correct classifications), where the model correctly predicted 57.5% of the pressure status, and 95.1% of the classification into 'impacted' or 'not impacted' sites. Although misclassification for a monitoring site appeared to have little impact on the ability of the method to detect an impaired fish assemblage, this highlights the limits of the current method. Much of the debate on the effect of the longitudinal gradient on fish assemblages has focused on the concepts of 'addition' vs 'replacement' of species from headwaters to large rivers, and most available studies have provided evidence in support of addition as a prevailing pattern, at least in rivers lacking geographical discontinuities (reviewed in Matthews 1986). Overlapping classification, resulting from similarity between some groups, can thus be viewed as a consequence of the requirement to impose groups upon a community continuum (Reynoldson, Rosenberg & Resh 2001).

Previous authors (e.g. Schmutz, Kaufmann, Vogel, Jungwirth & Muhar 2000) called for a fish-based river classification as a prerequisite for the development of large-scale assessment methods. Moreover, defining reference fish types as a starting point could facilitate the identification of metrics responding to pressure in only a restricted stretch of the upstream–downstream gradient. In this study, selected metrics differed among fish types, illustrating that metrics responsive to physical and chemical pressure vary along the longitudinal gradient. As a result, this highlights one of the weaknesses of the method, as numerous selected metrics make the interpretation of the current model

more difficult. From 13 to 19 metrics were retained in the current model, while most IBI adaptations were based on more or less 12 metrics, and probabilistic approaches (e.g. Oberdorff *et al.* 2002) used < 10 metrics.

The proposed approach provided the opportunity to examine the relative importance of both assemblage (e.g. species richness) and species-specific (e.g. abundance, percentage or biomass of 'sentinel' species) metrics. All but one model (e.g. fish type 3) incorporated both assemblage- and species-specific metrics. Regionally, assemblage metrics were more efficient to discriminate among pressure status, except in fish type 4, where presence of trout was the most important to predict pressure status.

Because the European fish fauna mainly consists of generalist feeders (Oberdorff & Hughes 1992), Aarts & Nienhuis (2003) suggested that the feeding guild classification should have a low discriminative capacity and would be poorly suited for ecological integrity assessment. In this study, metrics based on feeding guild showed significant capacity to discriminate among pressure statuses. Four of the six models included feeding metrics, but the capacity of feeding guild metrics to discriminate among pressure statuses was higher in upstream fish types (e.g. fish types 1 and 3) and decreased downstream; these metrics being totally absent in models for fish types 4 and 6.

For the habitat guild, rheophilic and limnophilic metrics were the ones mainly included in the models, while eurytopic metric had very poor discriminative capacity. This confirms the assumption that rheophilic and limnophilic fish are generally sensitive to perturbations of their environment and can be characterised as habitat specialists (Aarts & Nienhuis 2003). For reproductive guild, all models contained metrics belonging to this guild and both phytophilic and lithophilic metrics were included, without clear pattern among fish types.

Goffaux, Roset, Breine, de Leeuw, Oberdorff, Gérard, Micha & Kestemont (2003) compared two assessment approaches on the River Meuse (i.e. IBI and fish-based index, FBI) and found no significant differences among countries using FBI, while predictions from IBI tended to give a systematically worse score (according to observations) to Flemish sites. This study was consistent with former results as model predictions over scored rivers in Flanders and the Netherlands. Three explanations could be put forward: (i) the lack of good reference sites for this region did not take unique regional fish assemblage patterns into account; (ii) the FBI did not reflect the presumed ecological quality or (iii) the initial pressure classification was not accurate or sensitive in these two countries.

Finally, it is worth noting that previous fish-based studies in this area were all performed without pre-classification of calibration sites (e.g. Oberdorff *et al.* 2001; Goffaux *et al.* 2003). Therefore, this study is the first to combine a pre-classification approach and a fish metric-based assessment method. Although results emphasise that the proposed approach can be used to assess ecological quality of running waters over large geographical areas, some drawbacks are evident. These drawbacks can result from the requirement to impose groups upon an assemblage continuum, for which site pre-classification should not be adapted. The choice of a given assessment procedure has strong implications for examining patterns of spatial variability. Although comparisons among numerous approaches to prediction have been conducted using macroinvertebrate fauna (e.g. Moss, Wright, Furse & Clarke 1999), such a comparison using fish assemblages remains to be performed.

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