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6 FISH ZONATION AND INDICATOR SPECIES FOR THE EVALUATION
7 OF THE ECOLOGICAL STATUS OF RIVERS: EXAMPLE
8 OF THE LOIRE BASIN (FRANCE)
9

10 EMILIEN LASNE,^{a*} BENJAMIN BERGEROT,^a SOVAN LEK^b and PASCAL LAFFAILLE^a

11 ^a *Université de Rennes 1, ERT 52 – Bâtiment 25, 1er étage, Campus Beaulieu – 35 042 Rennes, Cedex, France*

12 ^b *Université de Toulouse 3, Lab. Evolution & Diversité Biologique, UMR 5174, CNRS – 118, route de Narbonne – 31062 Toulouse, Cedex, France*
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17 ABSTRACT

18 In the context of river alteration, ecologists are asked to develop tools for the assessment of river integrity. Fish are known to be
19 good bioindicators of the ecological condition of rivers. The Loire basin, (France) is often considered as relatively little impacted
20 compared to most other large European systems. But curiously, no study clearly addressed the question of fish assemblages
21 patterns in this system in order to assess this status. Thus, we studied fish assemblages along the river network in the Loire basin
22 using self-organizing maps (SOMs) and we built a fish typology. Four basic assemblages were described and indicator species
23 were identified. These assemblages varied in terms of individual species patterns as well as in terms of flow preference guilds and
24 species richness. A discriminant analysis carried out on environmental variables revealed that they could be mainly determined
25 by the slope, temperature and depth. Finally, fish assemblages were arrayed along a longitudinal gradient and roughly fitted the
26 theoretical zonation expected in European rivers with the succession of brown trout (*Salmo trutta fario*), grayling (*Thymallus*
thymallus), barbel (*Barbus barbus*) and bream (*Abramis brama*) zones in a downstream direction. Such patterns are still rarely
27 observed in large European systems. However, the fish assemblage characteristic of the bream zone occurred more frequently
28 than predicted on the basis of environmental variables. Such deviations between field data and theory suggest lotic-to-lentic
29 shifts probably due to anthropogenic disturbances, especially in the grayling and barbel zones. In these river sectors, eurytopic
30 and limnophilic species tend to replace rheophilic ones. Finally, the method used in this study to investigate fish patterns may be
31 helpful to detect disturbances and may serve as a tool for the establishment of management plans. Copyright © 2007 John Wiley
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32 KEY WORDS: fish assemblage; zonation; indicator species; disturbance; ecological status; fish guilds

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37 INTRODUCTION

38 Biodiversity loss is a global phenomenon particularly striking in freshwater systems (Ricciardi and Rasmussen,
39 1999; Gibbs, 2000; Saunders *et al.*, 2002; Dudgeon *et al.*, 2006), and it is well recognized that it is mainly due to
40 human activities (Abell, 2002; Saunders *et al.*, 2002). The conservation of natural resources and biodiversity,
41 especially in river systems, is a great challenge for the coming century (Ormerod, 1999). Environmental policies are
42 being developed throughout the world. In order to identify priority areas for restoration or conservation, scientists
43 are asked to propose simple, synthetic and—if possible—cheap tools for the evaluation of the ecological status of
44 rivers (Wessel *et al.*, 1998; European Union, 2000; Darwall and Vié, 2005).
45

46 In this context, the construction of classifications of sites (or areas) based on the species assemblages is a very
47 up-to-date exercise in running water conservation ecology (Aarst and Nienhuis, 2003; Aarts *et al.*, 2004; Tison
48 *et al.*, 2005; Fieseler and Wolter, 2006). For instance, the Water Directive Framework of the European Union
49 (European Union, 2000) demands that member countries classify their surface water bodies on the basis of species
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51 *Correspondence to: Emilien Lasne, Université de Rennes 1, ERT 52 – Bâtiment 25, 1er étage, Campus Beaulieu – 35 042 Rennes, Cedex,
52 France. E-mail: emilien.lasne@univ-rennes1.fr
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1
2 assemblages. Generally, the deviation between the observed assemblage type and the one expected in undisturbed
3 (theoretical) conditions provides an assessment of their ecological status. Because of their longevity, their mobility
4 and their sensitivity to habitat modification, fish are good bioindicators and they are often used for the assessment of
5 the ecological integrity of rivers (e.g. Karr, 1981; Verneaux, 1981; Schiemer, 2000; Oberdorff *et al.*, 2002; Aarst
6 and Nienhuis, 2003; Chovanec *et al.*, 2003; Pont *et al.*, 2006; Welcomme *et al.*, 2006).

7 Many theoretical classifications of running waters, notably fish-based classifications, have been proposed since
8 the end of the 19th century (Miranda and Raborn, 2000). Among them, one of the most famous is the longitudinal
9 fish zonation proposed by Huet (1959) in Western Europe. Huet distinguished four main fish zones from
10 upstream to downstream reaches of natural river systems. These zones are determined by environmental factors
11 (e.g. the slope and the width of the river section) and may be characterized by the dominant species: the brown trout
12 (*Salmo trutta fario*), the grayling (*Thymallus thymallus*), the barbel (*Barbus barbus*) and the bream (*Abramis*
13 *brama*) zones.

14 While the zonation concept, notably Huet's zonation (1959), is still highly cited in the literature and still serves as
15 a baseline for many studies (e.g. Lorenz *et al.*, 1997; Aarst and Nienhuis, 2003; Fieseler and Wolter, 2006; Petry and
16 Schulz, 2006), some limits that may be problematic in conservation as well as in conceptual perspectives have been
17 pointed out (Matthews, 1998; Miranda and Raborn, 2000; Aarts and Nienhuis, 2003). Three main criticisms are
18 made. Firstly, the zonation concept suggests the existence of discrete entities (zones), whereas the response of a
19 species to environmental gradients is usually continuous and variable depending on species (Pont *et al.*, 2005). It is
20 more likely that the community shifts occur by 'transition' rather than by 'zonation' (Matthew, 1998; Verneaux
21 *et al.*, 2003). Second, the identification of zones is based on the occurrence of indicator species. However, such
22 indicator species may be absent from regions and streams so that the zonation concept cannot be applied (for
23 instance, in France the grayling is absent from the Garonne basin; Park *et al.*, 2006). Third, most European systems,
24 notably where the zonation has been developed, are now heavily altered, so that the zonation does not fit the
25 observed patterns. Thus, the zonation concept is sometimes considered to be an old-fashioned paradigm for most
26 river ecologists notably because it cannot provide solutions for actual challenges (Miranda and Raborn, 2000).
27 Now, it has been largely ousted by the continuum concept (Vannote *et al.*, 1980).

28 However, some authors have shown that the relevance of the zonation concept and its usefulness for the
29 assessment of the ecological integrity of rivers can be enhanced. Indeed, instead of just considering characteristic
30 species, it is possible to also consider environmental fish guilds (Aarst and Nienhuis, 2003; Welcomme *et al.*, 2006).
31 In a similar way, it is also likely that the use of modern methodological approaches to investigating spatial
32 assemblage patterns could provide more flexibility to the zonation concept. Indeed, scientists who studied zonation
33 in the last century often used very basic—and therefore not very powerful—analytical methods (e.g. graphical one;
34 Miranda and Raborn, 2000). Some recent studies (Manel *et al.*, 1999; Olden and Jackson, 2002; Olden *et al.*, 2006;
35 Park *et al.*, 2006), using methods such as artificial neural networks, have shown that it was possible to successfully
36 investigate the assemblage patterns as well as the individual species patterns along complex gradients. In addition,
37 while the zonation concept largely relies on the occurrence of characteristic species, their indicator ability has been
38 rarely evaluated, though useful tools are available (e.g. the IndVal method of Dufrêne and Legendre, 1997).

39 In this study, we test whether such methods can help to provide a further refinement of the zonation concept and
40 to improve its usefulness in the conservation context, for instance, to evaluate the ecological status of rivers. We
41 chose to carry out our study in the Loire basin, because in a recent study based on the analysis of the distribution
42 patterns of individual species in the four largest French basins, Pont *et al.* (2005) showed that the Loire basin was
43 less impacted than the others. Assessing fish assemblages patterns in this system was therefore interesting, notably
44 to precise if it could serve as a reference system for calibrating a fish classification. Moreover, the Loire basin is
45 quite large and hosts various riverine landscapes—ranging from mountains, foothills and floodplain rivers—and a
46 relatively rich fish fauna, including the four characteristic species of the Huet zonation (1959). We firstly therefore
47 classified the fish assemblages using self-organizing maps (SOMs; Kohonen, 2001). Secondly, we analyzed
48 individual species patterns and we identified indicator species that could be used to summarize the assemblages
49 using the IndVal method (Dufrêne and Legendre, 1997). Thirdly, we modelled these assemblages according
50 to environmental variables using stepwise discriminant analysis. Finally, divergences between the predictions
51 of the theoretical zonation and field reality are discussed in the framework of the ecological status of river
52 assessment.

MATERIALS AND METHODS

Study area

The Loire River is the largest French river (1012 km) and drains a basin of 117 000 km². (Figure 1). The Loire basin presents a wide array of habitat conditions, ranging from mountain streams to foothills and large lowland rivers. The total length of the river network is estimated to be 107 000 km. The main axis of the basin (i.e. the Loire River and its main tributary, the Allier River) is less regulated and presents a good longitudinal connectivity, compared to other tributaries (including large rivers) that may be substantially impounded. However, the Loire basin is often considered as less impacted than other large systems in Europe (Pont *et al.*, 2005).

Data set

We used long-term data of the French Superior Council of Fisheries. This data set consists of a network of sampling sites distributed throughout the Loire basin and sampled each year between 1995 and 2003. Sampling was carried out in late summer during low water levels. Sampling could be by electrofishing by wading in shallow waters or electrofishing from a boat in deeper areas. In each case, all available habitats were investigated to obtain the most reliable picture of the fish community present in a given site (see Oberdorff *et al.*, 2001 for more details). In order to standardize data, we selected sites that have been sampled in each of the nine sampling years. Finally, our data set was composed of 108 sites (Figure 1).

Since abundance data are likely to be substantially influenced by the fishing technique or habitat characteristics (that in return may influence fishing efficiency), we used presence–absence data at each site and for a given sampling occasion (Oberdorff *et al.* 2002; Pont *et al.* 2005). Despite the simplicity of such input data, they may provide reliable information for analysing fish assemblage patterns (Ibarra *et al.*, 2005; Park *et al.*, 2006). Nevertheless, under the assumption that fish assemblages did not significantly change in the course of the study and in order to include more information, we used the mean occurrence along the study period (i.e. the frequency of years in which the species was detected) as an index of local species abundance.

Sampling sites were described by measuring the distance to the source (km), the upstream drainage area (km²), the altitude (m), the mean depth (m), the mean width (m), the slope (‰) and the mean air temperature of January and July (°C). According to Pont *et al.* (2005), the air temperature may be used as a surrogate for water temperature.

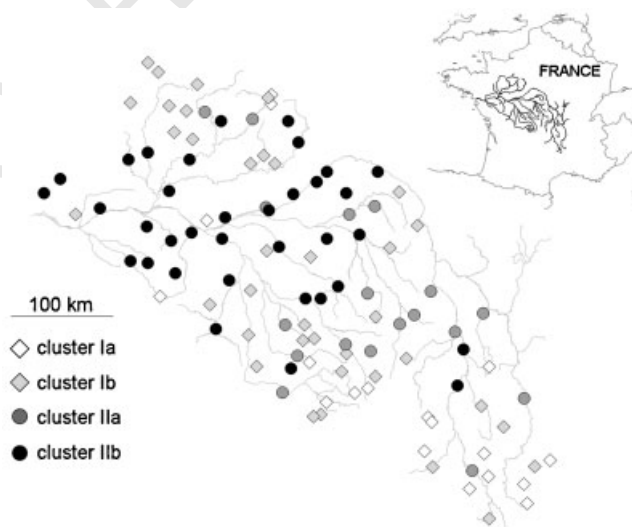


Figure 1. Map of the Loire basin river network and sampling sites. Clusters of sites identified by the SOM procedure based on assemblage similarities are indicated by various symbols and colours. NB: only the main tributaries of the basin are shown on the map for a better legibility

Statistical analysis

Fish assemblage patterns. A hierarchical classification of sites based on fish assemblages was performed using a SOM procedure. This non-supervised artificial neural network method can be used to analyze complex data sets and for the analysis of non-linear relationships (Kohonen, 2001). It has been recognized as a powerful tool for describing species distributions and assemblages (Lek *et al.*, 2005), notably in fish (Ibarra *et al.*, 2005; Park *et al.*, 2006; Konan *et al.*, 2007) where more details on the method are available. Samples with similar species composition and structure were classified in the same cell or in the neighbour cells. However, using weighed vectors of a trained SOM, a clustering technique (Ward's method) can subdivide the SOM cells into several clusters, i.e. subgroups of species assemblages.

The abundances of each species, the number of species according to guilds of flow preferences: rheophilic, limnophilic or eurytopic (according to Aarts *et al.*, 2003), the number of native and exotic species (according to Keith and Allardi, 2001 and Copp *et al.*, 2005) and species richness (the number of species per sampling site throughout the study) in the different clusters identified were compared using Kruskal–Wallis tests and Dunn's post test.

Indicator species. The comparison of mean occurrences between clusters of sites provides indications on individual patterns among clusters. However, to identify indicator species, account has to be taken of two important aspects of a species distribution. The first is the fidelity of the species to a given cluster. The fidelity is highest when the species is present in all the sites of a cluster. The second is the specificity to a given cluster. The specificity is highest when all the individuals of a species are found in the same cluster. We therefore used the indicator value (IndVal) according to the method developed by Dufrêne and Legendre (1997). IndVal is based on both the fidelity and the specificity of species for each cluster of sites. The IndVal of species *i* in cluster type *j* is expressed as a % and is calculated as follows: $\text{IndVal}_{ij} = A_{ij} \times B_{ij} \times 100$, where A_{ij} (Abundance *ij*/Abundance *i*) is a measure of the specificity of species *i* to the type *j*, and $B_{ij} (= \frac{N_{sites\ ij}}{N_{sites\ i}})$ is a measure of the fidelity of species *i* to type *j*. A randomization procedure is used to test the difference ($\alpha=0.05$) of the IndVal of each species in the different clusters of a hierarchy level. Only significant $\text{IndVal} > 25$ have been taken into account, because an $\text{IndVal} > 25$ implies that the species is present in at least 50% of the sites of the cluster, and that this cluster contains at least 50% of the total abundance of the species. As suggested by Dufrêne and Legendre (1997), the level for which a species has its highest IndVal value should be considered as the best classification level for that indicator species. However, lower IndVal values may provide supplementary information on the distribution pattern trends of the species especially at lower hierarchical levels.

Environmental factors. A backward stepwise discriminant analysis was used to determine whether clusters of sites derived from the SOM procedure and based on mean species occurrence could be discriminated based on a set of selected environmental variables. A random Monte Carlo permutation test and a leave-one-out cross validation were used to assess the ability of these variables to predict the clusters of fish communities.

Analyzes of indicator values were performed using INDVAL 2.0. The SOM and cluster analysis were computed with the SOM toolbox[©] (Alhoniemi *et al.*, 2000, <http://www.cis.hut.fi/projects/somtoolbox/>) under the Matlab environment (The Mathworks, Inc., Natick, MA, USA) and other statistical analyzes were conducted with SYSTAT 8.0 (SPSS, Inc., Chicago, IL, USA) and R^{Q2} (Ihaka and Gentleman, 1996).

RESULTS

Fish assemblage patternizing

A total of 32 species were sampled in the 108 sites in the course of this 9-year study (Table I). Among them, 14 species were rheophilic, 14 were eurytopic, and only four were limnophilic. Only five species were non-native of the Loire basin but they were all well acclimated. Among them, the Gibel carp *Carassius auratus gibelio*, the black bullhead *Ameiurus melas* and the pumpkinseed *Lepomis gibbosus* are 'undesirable' species suspected of competing with native species and disturbing natural communities, whereas the pikeperch *Sander lucioperca* and common carp *Cyprinus carpio* have a fishery value and are stocked in some areas. The mean occurrence (i.e. the frequency of years in which the species was detected) was highly variable depending on species. It was on an average 32.6% (Table I). Three species were very common (mean occurrence >75%): the gudgeon *Gobio gobio*, the stone loach

Table I. List of species classified according to flow preferences: eurytopic, limnophilic and rheophilic according to Aarts and Nienhuis (2003)

Scientific name	Common name	Abbreviation	Order	Origine	Mean occurrence (SD)	Mean occurrence (SD) in clusters				
						KW	Ia	Ib	Ia Ib	
Eurytopic										
<i>Abramis brama</i>	Common bream	A.br	Cypr	N	28.1 (39.7)	***	0 (0) a	8.4 (20.2) a	16.7 (27.5) a	69.2 (38.6) b
<i>Alburnus alburnus</i>	Bleak	A.al	Cypr	N	41.8 (44.4)	***	0 (0) a	8.7 (18.9) a	80.2 (28.1) b	78.4 (33.8) b
<i>Anguilla anguilla</i>	Eel	A.an	Angu	N	44.1 (42.2)	***	2.5 (10.5) a	25.2 (34.2) ab	48.1 (36.8) b	83.5 (26.9) c
<i>Carassius auratus gibelio</i>	Gibel carp	C.au	Cypr	E	9.4 (16.5)	***	1.2 (3.6) a	4.2 (11.8) a	9.3 (10.3) ab	19 (22.2) b
<i>Cottus gobio</i>	Common sculpin	C.go	Scor	N	49.7 (44.9)	**	40.7 (47.9) ab	71.5 (42) a	52.5 (42.6) ab	29.8 (38) b
<i>Cyprinus carpio</i>	Carp	C.ca	Cypr	E	19.5 (27)	***	5.6 (2.1) a	14.7 (28.1) a	15.4 (20.6) ab	34 (26.1) b
<i>Exocoetis lucius</i>	Pike	E.lu	Salm	N	26 (35.1)	***	0.6 (2.6) a	9.9 (22.9) a	16.7 (22.6) a	61 (33.8) b
<i>Gasterosteus aculeatus</i>	Three-spined stickleback	G.ac	Gast	N	2.2 (12)	ns	0 (0) a	1.8 (11) a	1.2 (3.6) b	4.1 (17.7) b
<i>Gymnocephalus cernuus</i>	Ruffe	G.ce	Perc	N	14.9 (28.3)	***	0 (0)	1.2 (7.3)	21.6 (29.4)	33.7 (36.4)
<i>Lepomis gibbosus</i>	Pumpkinseed	L.gi	Perc	E	47.3 (40.9)	***	11.1 (25.3) a	25.2 (30.4) ab	56.8 (38.5) bc	84.4 (22.9) c
<i>Percia fluviatilis</i>	Perch	P.fl	Perc	N	53 (39.4)	***	13 (21.3) a	37.8 (32.5) ab	48.1 (36) b	92.1 (14.4) c
<i>Pungitius pungitius</i>	Ten-spined stickleback	P.pu	Gast	N	15.2 (30.6)	ns	6.2 (23.6)	23.1 (37.6)	4.9 (12.8)	16.8 (30.4)
<i>Rutilus rutilus</i>	Roach	R.ru	Cypr	N	69.9 (39.7)	***	10.5 (16.8) a	61.6 (37.3) b	91.4 (20.7) c	98.1 (11.3)c
<i>Sander lucioperca</i>	Pikeperch	S.lu	Perc	E	11 (21.7)	***	0 (0) ab	0.9 (4) a	10.5 (14.5) bc	27.6 (30.1) c
Limnophilic										
<i>Ameiurus melas</i>	Black bullhead	I.me	Silu	E	21.8 (34)	***	1.9 (7.9) a	8.7 (21.3) a	30.9 (34.2) b	41.3 (41.4) b
<i>Rhodeus sericeus amarus</i>	Bitterling	R.se	Cypr	N	26.3 (38.1)	***	0 (0) a	5.4 (19.2) a	48.8 (36.6) b	50.5 (42.5) b
<i>Scardinius erythrophthalmus</i>	Rudd	S.er	Cypr	N	22.9 (27.4)	***	0 (0) a	13.2 (18.5) ab	17.3 (15.3) b	47.9 (29.1) c
<i>Tinca tinca</i>	Tench	T.ti	Cypr	N	27.3 (31.6)	***	2.5 (8.1) a	16.5 (24.7) a	17.9 (19.1) a	56.2 (30.5) b
Rheophilic										
<i>Alburnoides bipunctatus</i>	Stream bleak	A.bi	Cypr	N	29.2 (41.8)	***	0 (0) a	17.4 (34.1) ab	88.9 (22.9) b	26 (38.9) a
<i>Barbatula barbatula</i>	Stone loach	B.bt	Cypr	N	77.9 (34.7)	***	60.5 (47.5) a	96.4 (10.8) b	92 (11.3) ab	60 (38.8) a
<i>Barbus barbus</i>	Barbel	B.ba	Cypr	N	31.2 (42.7)	***	0 (0) a	6.6 (14.2) a	96.3 (8.5) b	39.7 (44.8) c
<i>Chondrostoma nasus</i>	Nase	C.na	Cypr	N	14.9 (31)	***	0 (0) a	0.3 (1.8) a	51.9 (43.3) b	19 (31.5)c
<i>Chondrostoma toxostoma</i>	Toxostome	C.to	Cypr	N	1 (4.7)	***	0 (0) a	0 (0) a	4.9 (9.5) b	0.6 (3.8) a
<i>Gobio gobio</i>	Gudgeon	G.go	Cypr	N	78.8 (36.6)	***	8 (16.1) a	91.6 (20.4) b	99.4 (2.6) b	91.1 (20.7) b
<i>Lampetra planeri</i>	Brook lamprey	L.pl	Petr	N	26.9 (36.1)	***	14.2 (29.7) a	54.4 (37.6) b	19.8 (32.7) a	7.9 (18) a
<i>Leuciscus cephalus</i>	Chub	L.ce	Cypr	N	75.7 (38.8)	***	2.5 (8.1) a	78.1 (30.7) b	99.4 (2.6) c	98.7 (4.5) c
<i>Leuciscus leuciscus</i>	Dace	L.le	Cypr	N	33.4 (37.1)	***	0 (0) a	18 (29.1) a	80.2 (18.1) b	42.9 (34.1) b
<i>Lota lota</i>	Burbot	L.lo	Gadi	N	2 (11.8)	***	0 (0) a	0 (0) a	11.7 (27.6) b	0 (0) a
<i>Phoxinus phoxinus</i>	Minnow	P.ph	Cypr	N	72.9 (40.7)	***	61.7 (44.6) ab	99.4 (2.5) c	85.2 (29.5) ac	44.4 (45) b
<i>Salmo salar</i>	Salmon	S.sa	Salm	N	4.4 (19.7)	ns	0 (0)	7.5 (24.4)	11.1 (32.3)	0 (0)
<i>Salmo trutta fario</i>	Brown trout	S.tr	Salm	N	60.4 (44.4)	***	100 (0) a	94.9 (11.3) a	59.9 (33.9) b	3.8 (8.9) c
<i>Thymallus thymallus</i>	Grayling	T.th	Salm	N	4.7 (17.4)	*	0 (0) a	9.6 (25.5) a	8 (19.7) a	0.3 (1.9) a

Order: Angu = anguilliforme, Cypr = cypriniforme, Gadi = Gadiforme, Gast = Gasteroforme, Perc = Perciforme, Petr = Petromyzoniforme, Salm = Salmoniforme, Scop = Scorpaeniforme, Silu = Siluriforme; origin: N = native, E = exotic according to Keith and Allardi, 2001 and Copp *et al.*, 2005). Mean occurrence (SD in parentheses) in overall sites and by cluster are presented. In the latter case, mean values followed by the same letter are not statistically different ($\alpha = 0.05$) according to Dunn's post test when Kruskal-Wallis tests are significant ($\alpha = 0.05$; ** = < 0.01, *** = < 0.001, ns = not significant).

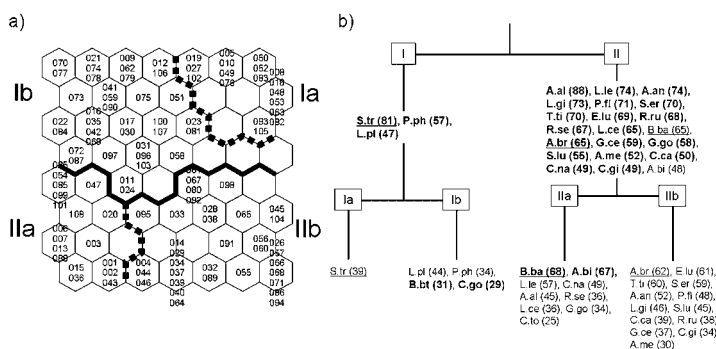


Figure 2. (a) Distribution of the sampled sites on the SOM. Clusters of sites identified by the Ward's Euclidean method are indicated by a full black and bold line (higher hierarchical level) and dotted black and bold lines (lower hierarchical level). (b) Indicator species of the clusters of sites at the two levels. Indicator values (%) are given in parentheses, and bold characters indicate the highest indicator value for a given species. Characteristic species of the Huet zonation are underlined

Barbatula barbatula and the chub *Leuciscus cephalus*. Three others were common (mean occurrence between 50 and 75%): the minnow *Phoxinus phoxinus*, the roach *Rutilus rutilus* and the brown trout. In contrast, five species were scarce (mean occurrence <25%), and among them, some were very scarce (<5%): the toxostome *Chondrostoma toxostoma*, the burbot *Lota lota*, the Atlantic salmon *Salmo salar*, the grayling and the three-spined stickleback *Gasterosteus aculeatus*.

The SOM procedure ordinated the samples in a two-dimensional map (8×7 cells), in which the sites were quite equally distributed (Figure 2(a)). Then, on the basis of the similarity of the cells on the SOM, the clustering procedure identified two main clusters I and II that were each subdivided into two smaller clusters named Ia and Ib on one side, and IIa and IIb on the other side, that were composed of 18, 37, 18 and 35 sites respectively (Figure 2b). We did not consider any further subdivisions to allow a sufficient number of sites per cluster for statistical investigations. Each cluster of sites may be represented on the map of the Loire basin (Figure 1). Cluster Ia was mainly located in upstream reaches, and cluster IIb in the most downstream reach, except for particular sites. However, for the two clusters, Ib and IIa, the patterns of distribution within the basin were less evident. They appeared to be located between clusters Ia and IIb, with cluster Ib being located upstream of IIa.

The mean occurrence in the four basic clusters varied significantly for most species (Table I), except for very scarce species for which the data are so scarce that it was not possible to clearly determine the patterns (e.g. the three-spined stickleback, the burbot, the Atlantic salmon, the toxostome). The four species characteristic of the Huet zonation presented very contrasting patterns between the four clusters (Table I). The mean occurrence of brown trout was highest in cluster Ia where it was present in all sites and all the sampling years. It was also high in cluster Ib, but decreased strongly in clusters IIa and IIb where it was very scarce. The grayling was on average very scarce and the mean occurrence differences between clusters were not statistically significant, though the highest mean occurrence was observed in cluster Ib. The barbel was totally absent from type Ia. It was scarce in Ib, and the highest occurrence was observed in cluster IIa, though it is also represented in cluster IIb. Finally, the Bream was totally absent from cluster Ia, its mean occurrence increased in clusters Ib and IIa, and reached its maximum in IIb.

The relative proportion of the three flow preference guilds (Figure 3(a), (b) and (c)) varied greatly between clusters at both the hierarchical levels. The rheophilic dominated in cluster Ia and their number decreased in clusters Ib, IIa and then IIb. Eurytopic species followed the opposite pattern, as did limnophilic species. The proportions of native and exotic species did not differ significantly between clusters (Figure 3(d) and (e)), probably due to the low number of exotic species in the data set. Finally, the mean species richness (Figure 3(f)) increased from cluster Ia to cluster IIa. Though the difference was not significant, it tended to decrease in cluster IIb.

Indicator species

The IndVal method enabled species to be identified that were characteristic of the clusters at both the levels of the classification (Figure 2(b)). The number of species with significant indicator values varied according to clusters. It

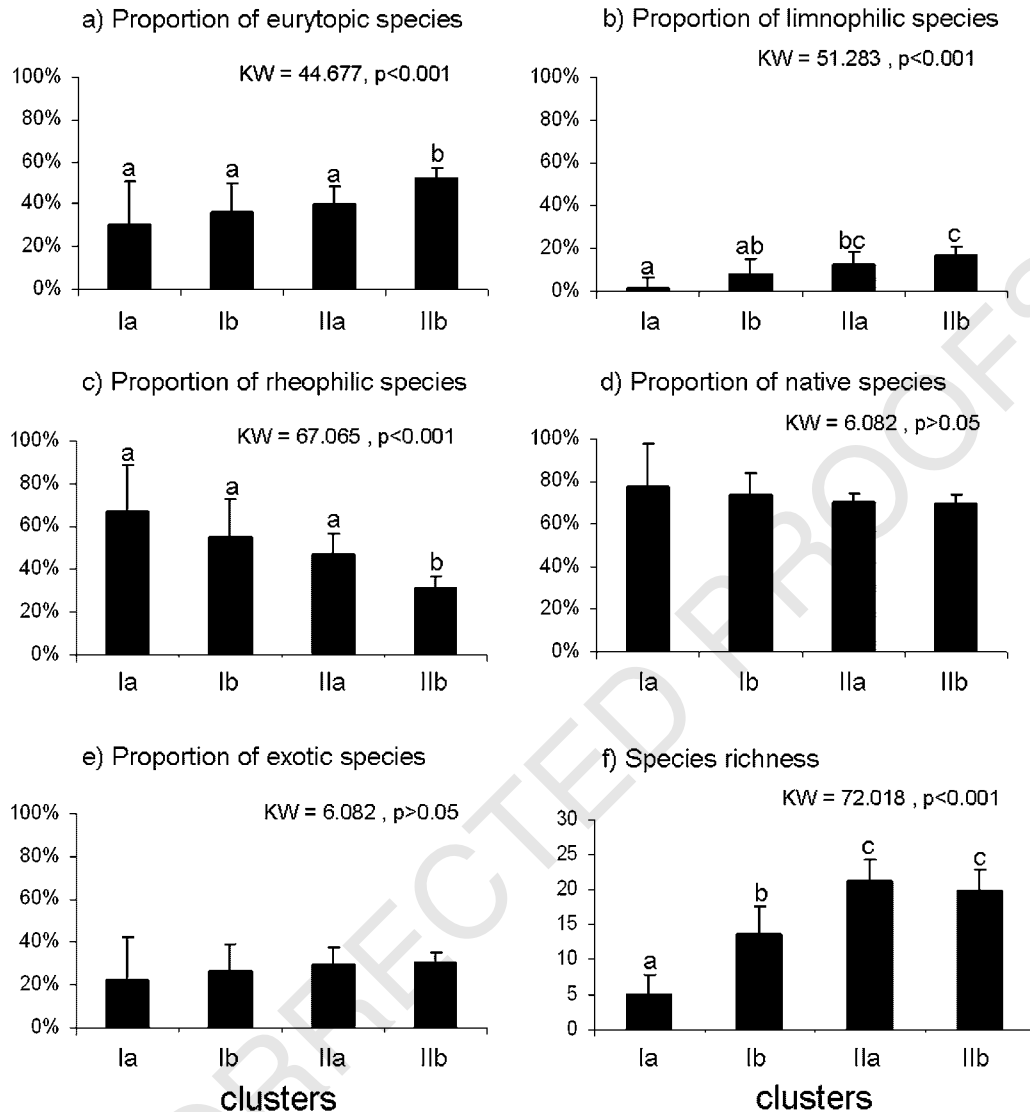


Figure 3. Comparison of the proportion of (a) eurytopic, (b) limnophilic, (c) rheophilic, (d) native and (e) exotic species, and (f) species richness between clusters. In each graph, bars represent standard deviation. The same letter above bars shows that values are not statistically different (Dunn's post tests; $\alpha=0.05$) when Kruskal–Wallis (KW) p -value < 0.05

was lower in cluster I than in cluster II (3 vs. 21), and increased from clusters Ia, Ib, IIa and IIb (1, 4, 9 and 13, respectively). Most of the indicator species (22 out of a total of 26 different species) had their highest IndVal at the first classification level, and only a few (4) at the second one. This suggests that the first dichotomy (cluster I vs. cluster II) had a strong ecological significance.

Most of the indicator species in cluster I were rheophilic species (brown trout, minnow, brook lamprey *Lampetra planeri*), whereas in cluster II, they were more varied with eurytopic (bleak *Alburnus alburnus*, European eel *Anguilla anguilla*, pumpkinseed, perch *Perca fluviatilis*, pike *Esox lucius*, roach, common bream, ruffe *Gymnocephalus cernuus*, pikeperch, carp, Gibel carp), limnophilic (rudd *Scardinius erythrophthalmus*, tench *Tinca tinca*, bitterling *Rhodeus sericeus amarus*, black bullhead) and rheophilic (nase *Leuciscus leuciscus*, chub, barbel, gudgeon, toxostome, stream bleak *Alburnoides bipunctatus*) species. At the lower level, only the brown trout was

Table II. Mean values (SD in parentheses) of environmental variables in the four clusters of sites

Variable	KW	p	Ia	Ib	IIa	IIb
Upstream drainage area (km ²)	63.026	***	30.2 (27.1) a	129.1 (181.3) a	927.8 (896.6) b	3099.9 (7258.4) b
Distance to source (km)	58.376	***	8.1 (5.6) a	18 (16.1) a	72.3 (41.7) b	103.8 (136.5) b
Width (m)	50.296	***	3.5 (1.9) a	6 (4.7) a	18.1 (13.1) b	28.9 (53) b
Slope (%)	60.670	***	14.7 (7.5) a	4.7 (3.4) b	2.7 (3.5) bc	1.3 (1.6) c
Depth (m)	41.590	***	0.3 (0.1) a	0.4 (0.1) a	0.6 (0.2) b	0.9 (0.5) b
Altitude (m)	45.112	***	661.6 (383) a	282.7 (255.1) b	210.2 (113.6) ab	90.1 (74.7) c
Mean air temperature of July (°C)	40.749	***	16.1 (2.2) a	17.9 (1.3) a	19 (0.9) b	18.9 (0.5) b
Mean air temperature of January (°C)	35.079	***	1 (2) a	3.1 (1.3) b	3.2 (0.6)b	3.8 (0.5)c

In each cluster column, when Kruskal–Wallis (KW) values are significant ($\alpha = 0.05$; *** = <0.0001), mean values followed by the same letter are not statistically different (Dunn's post tests; $\alpha = 0.05$).

an indicator of cluster Ia, whereas cluster Ib was characterized by four species; three were small rheophilic (brook lamprey, minnow and stone loach) species and the common sculpin *Cottus gobio*. The indicator species of cluster IIa were mainly characteristic rheophilic species (stream bleak, nase, chub, gudgeon, toxostome) and the barbel was the most significant indicator species. In contrast, species indicative of cluster IIb were mainly eurytopic (pike, European eel, perch, pumpkinseed, pikeperch, carp, roach, ruffe, Gibel carp) and limnophilic (tench, rudd, black bullhead) species and the common bream was the most significant indicator species.

Environmental factors

The environmental variables varied greatly between clusters (Table II). In fact, the upstream drainage area, distance to source, width, depth and mean air temperature increased from assemblage Ia to IIb. However, the slope and the altitude decreased significantly from cluster Ia to IIb. All the environmental variables were more or less correlated and responded to an upstream–downstream gradient.

The backward stepwise discriminant analysis identified the most influential factors that separated the four clusters Ia, Ib, IIa, IIb (Figure 4).^{Q3} Among all the input variables, the slope, the upstream drainage area, the mean air temperature in July, the distance to the source, the mean width and the mean depth were selected. Three discriminant functions were generated, and the random Monte Carlo permutation test showed that they were highly significant ($p < 0.001$). These functions (F1, F2 and F3) accounted for 77, 13 and 9% of the between–clusters variability, respectively. F1 separated clusters Ia, Ib and II (i.e. IIa and IIb). It was mainly determined by the slope (cosine = 0.93) and second by the mean air temperature in July (cosine = -0.78). Sites in cluster Ia were mainly located in the more upstream reaches with strong slopes and cold temperatures, cluster Ib in the middle reaches, and clusters IIa and IIb in downstream reaches. F2 was mainly determined by the mean depth (cosine = -0.63) and separated assemblages IIa and IIb. Sites in cluster IIb were located in wider and deeper areas typical of the most downstream reaches of river systems. Finally, the four clusters were roughly arrayed along the longitudinal gradient.

The six selected variables predicted the assignment of each site to the right cluster with an average success of 66% (Table III). In other words, based on environmental variables it was possible to predict the clusters and consequently, the type of fish assemblage occurring. The prediction success was relatively good for clusters Ia and IIb (76.5% and 75%, respectively), but it was lower for clusters Ib (62%) and IIa (53%). Moreover, about 40% of the sites that had been classified in cluster IIa (characterized by the barbel) on the basis of environmental variables in reality hosted a type IIb assemblage (characterized by the bream). Similarly, about 20% of the sites predicted as belonging to cluster Ib (characterized by the brook lamprey) hosted an assemblage of cluster IIb. Finally, 46% of total errors in prediction based on habitat features were due to the presence of a type IIb assemblage when it was not expected. Thus, the type IIb was more frequently observed than expected. Only 14, 16 and 24% of bad predictions were due to the presence of type Ia, Ib and IIa assemblages respectively when others were expected.

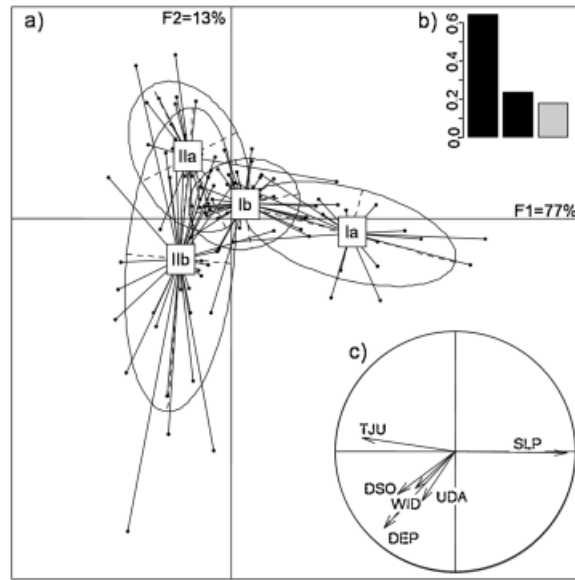


Figure 4. Results of the backward stepwise discriminant analysis using six environmental variables to predict the four clusters of sites (identified from SOM model). (a) Axis 1 accounts for 77% and axis 2 for 13% of between-group variability, respectively. Each group of clusters is presented as ellipsoid with the cluster name in the centre. (b) Histogram showing eigenvalues of the discriminant analysis. (c) Circle showing the contribution of the six selected variables to F1 and F2. TJU = mean air temperature in July, SLP = slope, DSO = distance to source, WID = width, UDA = upstream drainage area, and DEP = depth

DISCUSSION

Fish patterns in the Loire catchment

In our classification, the first hierarchical level contrasts upstream sites characterized by rheophilic against downstream sites, with mainly eurytopic species. This supports the distinction between the rhithron and the potamon as proposed by Illiès and Botosaneanu (1963). It is also consistent with the zonation of Jens (1980), who distinguished upstream reaches where salmonids dominate, and the downstream reaches where the cyprinids dominate. More precisely, at the first hierarchical level, upstream sites were characterized by the brown trout and a set of accompanying species, and downstream sites were characterized by numerous indicator species (and among them many cyprinids) such as the bleak that was a strong indicator. Finally, Hawkes (1975) considered that European systems were divided into two distinct fish zones based on thermal criteria. They proposed that the brown trout and grayling zones correspond in reality to a cold water zone (in upstream reaches) and that the barbel and bream zones correspond to a warm water zone (in downstream reaches). In our study, the temperature was one of

Table III. Confusing matrix showing the leave-one-out cross validation of the discriminant model using the six variables selected by the backward stepwise procedure. The overall percentage of successful prediction is 66%

		predicted				%success
		Ia	Ib	IIa	IIb	
Observed	Ia	13	5	0	0	76.5
	Ib	3	31	1	2	62
	IIa	1	4	9	4	53
	IIb	0	10	7	18	75

1
2 the main factors affecting assemblage composition and confirms that this dichotomy based on thermal
3 consideration was relevant in the Loire basin.

4 Nevertheless, at the lower hierarchical level it was possible to refine this classification. In the most upstream
5 reaches of the rhithron, a first assemblage was characterized by the brown trout. Few species accompanied the
6 brown trout in this sector. The relatively cold temperatures and the strong slopes probably constitute constraints that
7 filter out other species, as shown elsewhere (Jackson *et al.*, 2001; Quist *et al.*, 2004; Pont *et al.*, 2005). In the lower
8 parts of the rhithron, the slope decreases and the temperature increases, allowing the co-occurrence of the brown
9 trout (that is still largely present), small-sized rheophilic species and the common sculpin. Though the grayling was
10 very scarce in the Loire basin, many of the sites where it was detected were located in this sector. In addition, most
11 of its accompanying species described in the Huet classification (1959) are present and indicative of the lower
12 rhithron. This suggests that this river sector may be compared to the grayling zone of the Huet zonation (1959). In
13 the potamal reaches of the basin, the barbel was indicative of the most upstream (e.g. lotic) reaches, whereas the
14 bream was indicative of lower (e.g. lentic) areas. Finally, the longitudinal fish patterns in the Loire basin were quite
15 similar to those expected in the Huet zonation (1959) and the assemblages identified in the four clusters of sites Ia,
16 Ib, IIa and IIb could be more or less compared to the 'trout', 'grayling', 'barbel' and 'bream' zones.

17 However, it was evident that the boundaries between fish zones were diffuse in the Loire basin and that
18 assemblage shifts occurred by transition rather than by strict zonation, as supported by other studies on fish or for
19 instance aquatic invertebrates (e.g. Vannote *et al.*, 1980; Matthews, 1998; Verneaux *et al.*, 2003). Moreover, the
20 IndVal method is able to mitigate the notion of characteristic species in the zonation framework by quantifying the
21 indicator power of species, taking both the fidelity and the specificity of species into account (Dufrêne and
22 Legendre, 1997). It showed that, even for the most characteristic species, the boundaries between zones are not as
23 sharp as postulated in the original zonation concept (Miranda and Raborn, 2000). Indeed, indicator species showed
24 variable indicator values (i.e. variable fidelity and/or specificity) toward the assemblage they characterized, and no
25 species was strictly related to a given type. Such analysis of the indicator power of characteristic species has rarely
26 been achieved in past studies, whereas the zonation concept largely relies on this notion (Petry and Schulz, 2006).
27 Moreover, for most species, the mean occurrence patterns were gradual along the longitudinal gradient. Our results
28 were very similar to those of Pont *et al.* (2005). In a species-based approach, these authors studied the individual
29 responses of 13 species to environmental gradients in four French basins. They notably showed the role of the slope,
30 the temperature, the width and the upstream drainage area. For instance, they showed that the common sculpin and
31 the brown trout had similar requirements in terms of slope. However, the common sculpin's distribution was limited
32 by cold temperatures, whereas brown trout's distribution was not. In our study, the wider temperature tolerance of
33 the brown trout may explain its presence in headwaters, where other species such as the common sculpin are absent.
34 Thus, while the species-based approach may provide precise details on species-habitat relationships, the method
35 used in this study to investigate community assemblage patterns is also suitable for the investigation of species
36 patterns. Species patterns in our classification were quite continuous, reflecting the non-discrete nature of fish
37 assemblages, and supporting the hypothesis of a fish community continuum as proposed by Zalewski and Naiman
38 (1985).

39 40 *Mechanisms of the shifts in assemblage patterns*

41
42 Another open question concerns the mechanisms behind the shifts in species composition along the longitudinal
43 gradient (Rahel and Hubert, 1991; Matthews, 1998; Petry and Schulz, 2006). Some authors advocate the
44 mechanism of species replacement downstream (Huet, 1959), whereas others advocate the mechanism of species
45 addition (Sheldon, 1968), and finally some authors suggest that these two mechanisms coexist (Rahel and Hubert,
46 1991; Petry and Schulz, 2006). The mechanism of species addition suggests that an increase in habitat diversity
47 downstream (Gorman and Karr, 1978; Angermeier and Schlosser, 1989; Rahel and Hubert, 1991) allows the
48 co-occurrence of species with various life-history strategies, leading to maximum species richness in the most
49 downstream areas (Oberdorff *et al.*, 1993; Mahon, 1984; Mastroiello *et al.*, 1998). The mechanism of species
50 replacement suggests that species are replaced by others in the next zone because of change in environmental
51 constraints. Under the assumption of species replacement, species diversity should be highest in the middle reaches
52 where species of adjacent zones may co-occur. In the Loire basin, the addition of species occurred first between the
53

1 trout zone and the grayling zone. While the addition of species was mainly predominant between the grayling zone
2 and the barbel zone, this transition may also be characterized by the replacement of the brown trout and the brook
3 lamprey probably because of physico-chemical constraints such as higher temperatures and lower dissolved
4 oxygen concentrations. Downstream, the transition between the barbel and the bream zone was mainly
5 characterized by species replacement, more precisely by the decrease of rheophilic cyprinids, probably because
6 conditions become lentic in the most upstream reaches of the basin. This replacement seems to reflect a shift from
7 gravel-spawning to vegetation-spawning species related to a change in dominant bottom substrate that was not
8 measured. Many species of the bream zone were also well represented in the barbel zone probably because of the
9 high habitat heterogeneity in the middle reaches where both lotic and lentic areas may be found. Thus, species
10 addition and replacement occurred in the Loire basin and confirmed that these two mechanisms may coexist (Rahel
11 and Hubert, 1991; Petry and Schulz, 2006). Finally, in the Loire basin, the species richness was highest in the
12 potamon and tended to be higher in the barbel zone. Such a pattern was probably due to a conjunction of both
13 species' addition and replacement. Indeed, in the middle reaches (i.e. in the barbel zone), species characteristic of
14 upstream and downstream areas can co-occur at the limit of their distribution, which might result in a higher
15 richness.
16

17 *Contribution to the assessment of the ecological status of rivers*

18 Most of the temperate rivers are today heavily altered by human activities such as pollution, flow regulation,
19 water abstraction and species introductions (Dudgeon *et al.*, 2006). Particularly, the alteration of the natural flow
20 regime, and the subsequent lotic-to-lentic shift of hydrological conditions, is suspected to play a major role (Poff
21 *et al.*, 1997). As a general consequence, most of the threatened fish species in temperate systems are rheophilic
22 species (Galat and Zweimüller, 2001; Aarts and Nienhuis, 2003), whereas in contrast, eurytopic species, including
23 exotic species, tend to develop in habitats where the natural flow regime has been altered, providing new conditions
24 (Schiemer, 2000; Bunn and Arthington, 2002; Aarts *et al.*, 2004; Lytle and Poff, 2004; Kennard *et al.*, 2005). Thus,
25 habitat alteration may create disturbance in zonation patterns (Vila-Gispert, 2002). Aarts and Nienhuis (2003)
26 suggested that most river systems in Europe are so much altered that fish assemblages no longer fit the zonation. In
27 US rivers, Scott and Helfman (2001) showed that habitat alteration (e.g. deforestation that in turn lead to physical
28 modifications of the habitat) could lead to assemblages homogenization. More precisely, they found that eurytopic
29 species (both native and exotic ones) typical of the downstream reaches may invade disturbed upstream habitats. In
30 the Loire basin, the natural and gradual shift from rheophilic dominant to eurytopic dominant fish along the
31 upstream-downstream gradient was evident, and only five species were exotic. Besides, fish patterns in the Loire
32 basin were roughly consistent with those expected in natural systems (i.e. Huet's zonation, 1959). However, the
33 frequency of the assemblages characterized by the barbel and other associated rheophilic cyprinids was very low
34 and their geographical patterns remain somewhat unclear (Figure 1). Conversely, the bream assemblage was widely
35 and very regularly represented in a large downstream part of the Loire, even in areas where habitat features were
36 favourable for the barbel or grayling assemblages. This deviation between expected assemblages based on
37 environmental variables and observed ones was consistent with the lotic-to-lentic shift of aquatic habitats trends
38 observed in other systems (Aarst and Nienhuis, 2003). The barbel and grayling zones are transition zones in middle
39 reaches often considered to be the most sensitive ones to human impacts (Aarts and Nienhuis, 2003). Our results
40 suggest that such a phenomenon also occurs in the Loire basin. The sites where the typical bream assemblage was
41 found whereas more rheophilic ones should occur could be considered as disturbed. Further field investigation
42 could identify more precisely the perturbation and serve to establish management (e.g. restoration) planning. On
43 the other hand, sites where the expected assemblage occurred could be considered as reference sites. Yet references
44 sites should not be viewed as pristine, but as less altered (Oberdorff *et al.*, 2002).
45

46 Some authors have suggested that management of freshwater resources should be undertaken at a comprehensive
47 (i.e. whole catchment) scale (Saunders *et al.*, 2002; Collares-Pereira and Cowx, 2004; Darwall and Vié, 2005). The
48 method used in this study was able to detect local (i.e. at the individual site scale) and more widespread problems
49 and seemed quite suitable in such a perspective. Although it does not provide a clear measurement of disturbance, it
50 provides cues for managers to identify dysfunction (e.g. hydraulic ones). Furthermore, it is complementary with the
51 fish based index (FBI) proposed by Oberdorff *et al.* (2002) in France or that proposed by Pont *et al.* (2006) at the
52

1
2 European scale. Indeed, these authors built their index based on the calibration of a model in reference sites, and
3 tested it on disturbed sites. In such an *a priori* method, the selection of reference sites is a key issue (Pont *et al.*,
4 2006). However, this selection is mainly based on expert opinion, and by definition, they may be considered to be
5 somewhat subjective. In our study, we proposed an *a posteriori* approach with few prerequisites. It could be easily
6 adapted to other systems, with other faunas. Moreover, most input variables in our predictive model are often
7 already available.

8 9 10 *Conclusions*

11 Our main objective was to investigate fish assemblage patterns in the Loire basin. We showed that the zonation
12 concept, e.g. the Huet zonation (1959), was still useful in conceptual and conservation perspectives, if it is refined
13 by including additional information, e.g. on individual species patterns. Indeed, we provided new information on
14 the mechanisms of the shifts from one type to another to make the classification concept more manageable and
15 pragmatic. We also confirmed the potential use of fish guilds as a tool for the assessment of the ecological integrity
16 of rivers (according to Aarts and Nienhuis, 2003 and Welcomme *et al.*, 2006). More precisely, the methods used
17 reconciled the zonation and the continuum paradigms (Miranda and Raborn, 2000), by specifying the nature and the
18 sharpness of the boundaries between biotic zones. The original combination of different analytical methods
19 established a compromise between, on the one hand, the need to simplify the complex ecological information for
20 policy and management purposes, and on the other hand, the need to conserve relevant ecological information.
21 Furthermore, the method mitigated the concept of characteristic species and suggested that the presence of the
22 characteristic species of the fish zonation was not a prerequisite for the application of the concept. Conversely, it is
23 possible to identify other characteristic species that are more relevant in a particular context. Finally, while fish
24 patterns are highly disturbed in most temperate systems, in the Loire basin they are roughly coherent with the
25 original longitudinal fish zonation.

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Q5

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