

## WP 6

### SPATIALLY-BASED APPROACH ***Western Highlands and Western Plains*** (Ecoregions 8 and 13)

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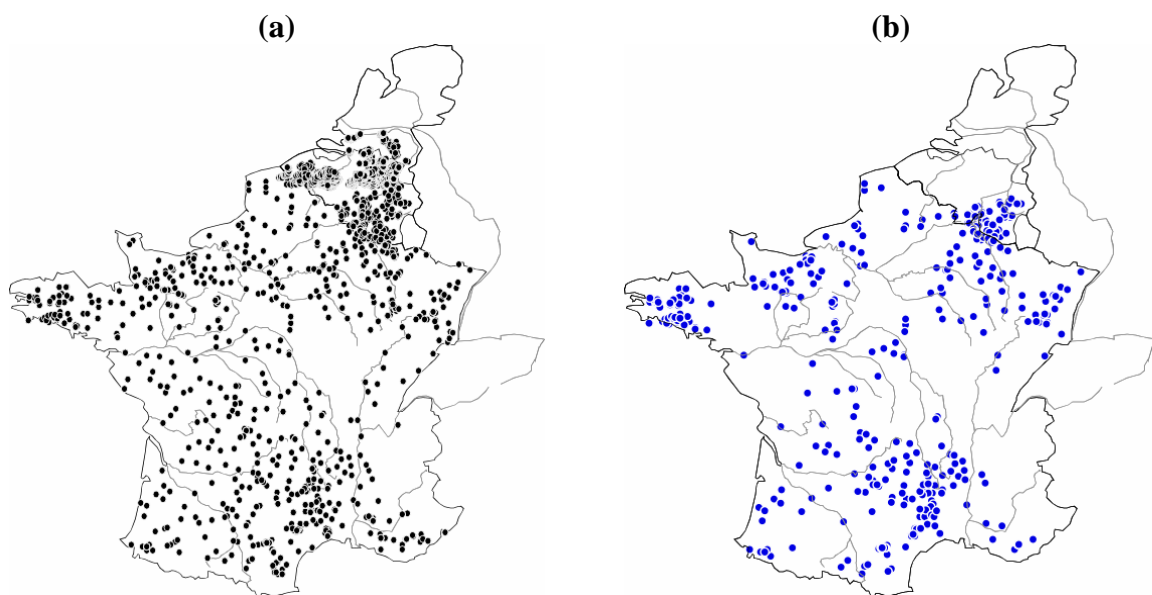
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**SPATIALLY-BASED APPROACH**  
**Western Highlands and Western Plains**  
(Ecoregions 8 and 13)

## 1. Dataset

### 1.1. Fishing occasions

For ecoregions 8 and 13, FIDES database contains 3390 fishing occasions from Belgium-Flanders (BE,  $n=1684$ ), Belgium-Wallonia (BW,  $n=158$ ), France (FR,  $n=1392$ ) and The Netherlands (NL,  $n=156$ ) (Fig. 1a).

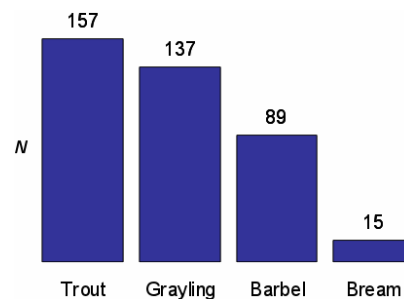


**Fig. 1:** Localisation of the sampling sites. **(a)** Fishing occasions in the FIDES database ( $n=3390$ ), **(b)** reference sites ( $n=398$ )

### 1.2. Reference dataset

In order to avoid the disequilibrium due to temporal repetition in each site, only one sample per site (the most recent one) was selected. Sites with impact classes 1 and 2 were retained to define the reference dataset. No reference conditions were found in Flanders and The Netherlands. Thus, our reference data set contained 398 sites in BW and FR (45 and 353 sites, respectively) (Fig. 1b).

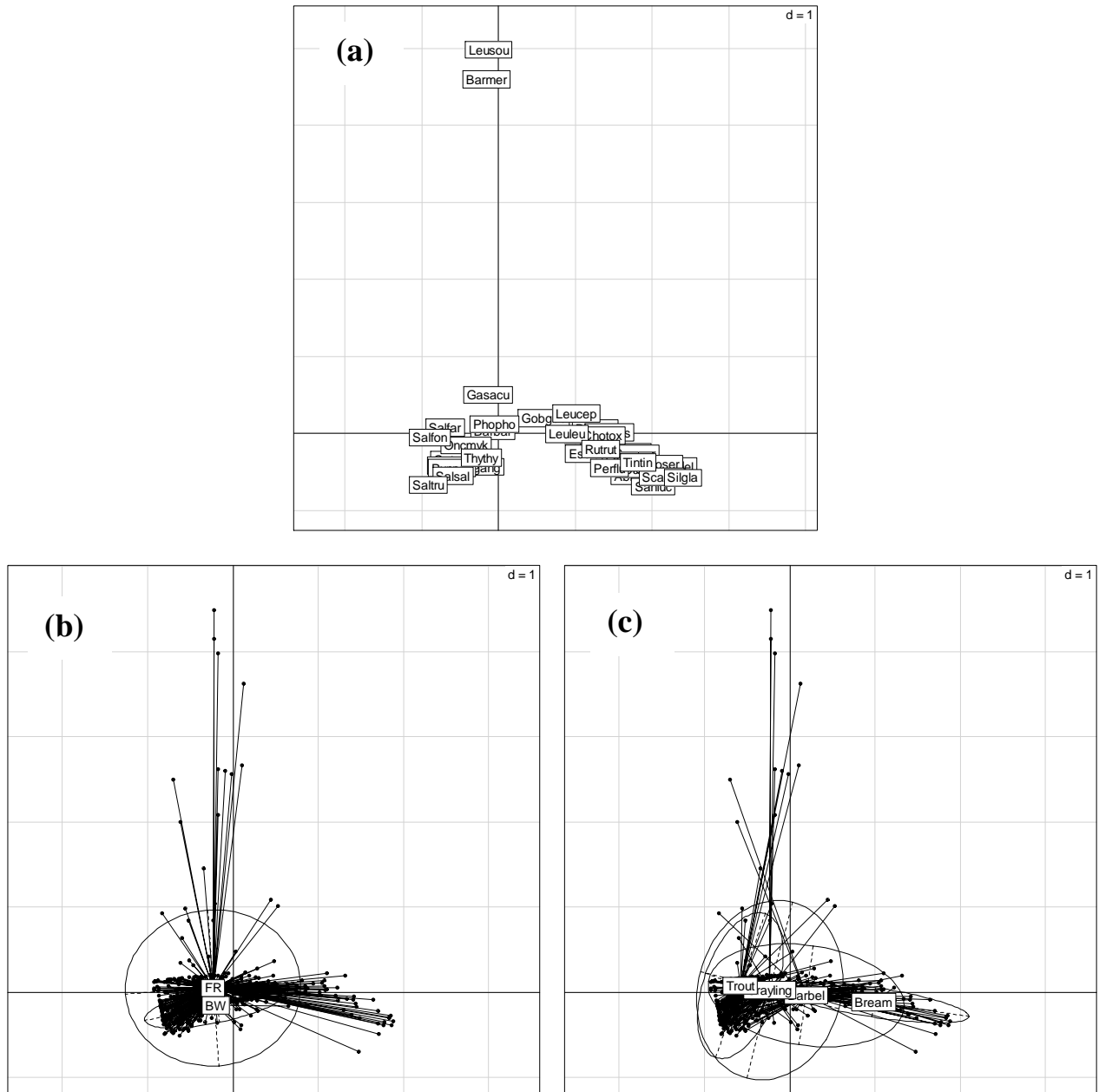
These samples were roughly evenly distributed among the first three Huet river types, with less samples in the Bream zone (Fig. 2).



**Fig. 2:** Distribution of the reference sites among the different Huet river types.

## 2. Preliminary analysis: Correspondence analysis

Correspondence Analysis was performed on the transformed ( $\log(X+1)$ ) matrix of species abundance (number of fish / 100 m<sup>2</sup>) after the rare species, which highly influence the analysis, were excluded. We retained only species with occurrence > 1%.



**Fig. 3:** Correspondence Analysis of species abundance (number of fish / 100 m<sup>2</sup>).  
 (a) factorial map of fish species, (b) grouping of sites according to their country,  
 (c) grouping of sites according to their Huet river type.

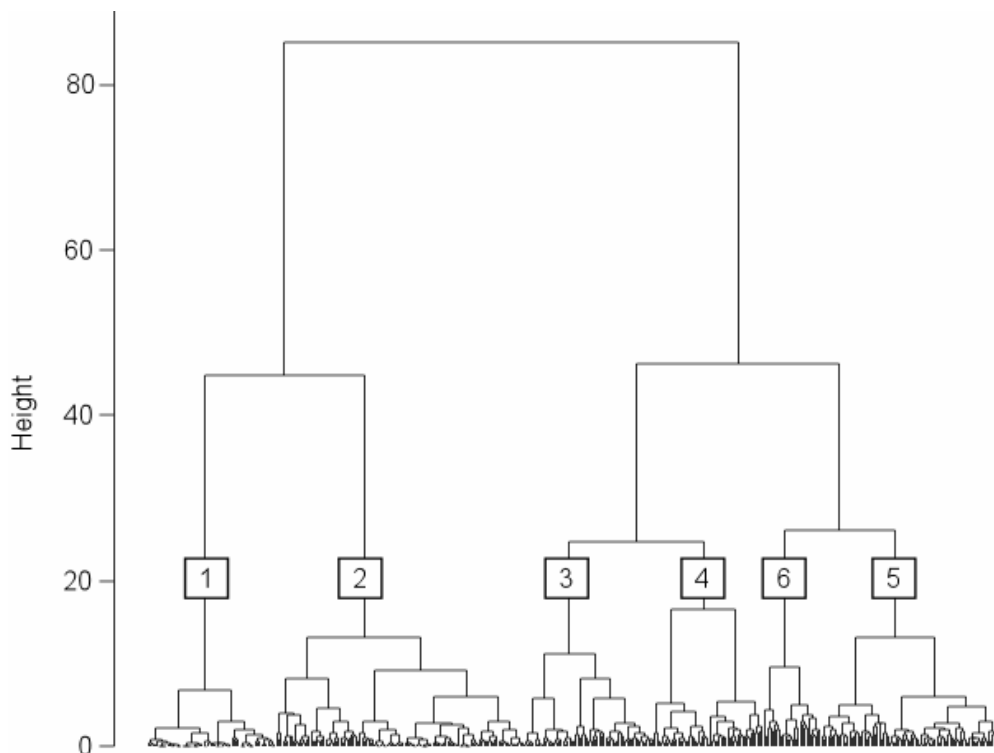
The first axis revealed the longitudinal organisation of species abundance (Fig. 3a), well represented by the Huet zonation (Fig 3c). The second axis separated two particular species (*Leuciscus soufia* and *Barbus meridionalis*), which were exclusively found in South France. Sites could not be separated according to their country (Fig. 3b). The observed longitudinal gradient was thus common for both countries.

### 3. Cluster analysis

#### 3.1. Cluster identification (new variable for FIDES: “*Fish\_type\_ecoregional*”)

We applied a Hierarchical Cluster Analysis (using the Ward’s method) on the transformed ( $\log(X+1)$ ) matrix of species abundance (number of fish / 100 m<sup>2</sup>) (Fig. 4).

We defined 6 clusters. Each cluster has a numerical code (“*Fish\_type\_ecoregional*” in FIDES), which follows the longitudinal gradient (1 is for the upper type and 9 for the lower one).



**Fig. 4:** Dendrogram of sites based on Ward’s method

#### 3.2. Cluster description: physical characteristics

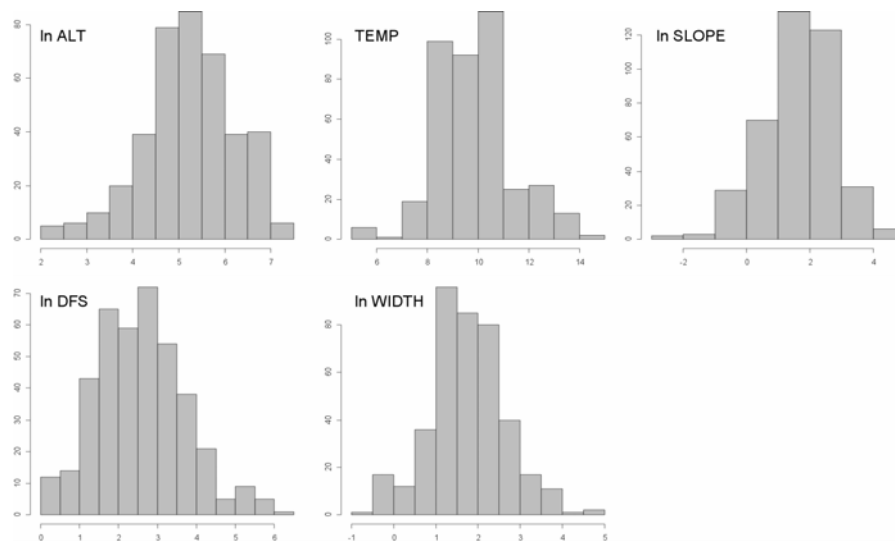
Five environmental parameters were used to describe physical habitat in the reference dataset (no missing value): altitude, mean air temperature, slope, distance from source and wetted width (Table 1).

**Table 1:** Environmental characteristics of the different fish clusters. Given are number of observation (N), minimum, maximum, and mean values, and standard deviation (SD) for altitude (ALT), mean air temperature (TEMP), slope (SLOPE), distance from source (DFS) and wetted width (WIDTH).

| <b>Cluster</b> |            | <b>ALT</b> | <b>TEMP</b> | <b>SLOPE</b> | <b>DFS</b> | <b>WIDTH</b> |
|----------------|------------|------------|-------------|--------------|------------|--------------|
| <b>1</b>       | N of cases | 60         | 60          | 60           | 60         | 60           |
|                | Minimum    | 10.000     | 5.800       | 1.000        | 1.000      | 0.800        |
|                | Maximum    | 1320.000   | 14.000      | 130.000      | 29.000     | 12.000       |
|                | Mean       | 494.133    | 9.608       | 21.103       | 8.100      | 4.635        |
|                | SD         | 320.582    | 1.569       | 23.945       | 7.118      | 2.885        |
| <b>2</b>       | N of cases | 116        | 116         | 116          | 116        | 116          |
|                | Minimum    | 18.000     | 5.800       | 1.100        | 1.000      | 0.500        |
|                | Maximum    | 1190.000   | 12.000      | 60.000       | 149.000    | 20.000       |
|                | Mean       | 251.345    | 9.468       | 11.160       | 12.121     | 4.612        |
|                | SD         | 201.719    | 1.103       | 9.609        | 16.954     | 3.280        |
| <b>3</b>       | N of cases | 61         | 61          | 61           | 61         | 61           |
|                | Minimum    | 14.000     | 5.800       | 1.500        | 1.000      | 0.800        |
|                | Maximum    | 1200.000   | 13.500      | 60.000       | 61.000     | 16.300       |
|                | Mean       | 469.016    | 10.161      | 9.962        | 17.410     | 6.677        |
|                | SD         | 322.814    | 1.834       | 10.038       | 13.045     | 3.696        |
| <b>4</b>       | N of cases | 51         | 51          | 51           | 51         | 51           |
|                | Minimum    | 10.000     | 5.800       | 0.900        | 2.000      | 1.000        |
|                | Maximum    | 1090.000   | 13.500      | 13.200       | 316.000    | 26.000       |
|                | Mean       | 137.373    | 10.244      | 5.153        | 22.216     | 7.560        |
|                | SD         | 174.741    | 1.048       | 3.626        | 43.711     | 5.878        |
| <b>5</b>       | N of cases | 83         | 83          | 83           | 83         | 83           |
|                | Minimum    | 10.000     | 7.500       | 0.400        | 2.000      | 3.000        |
|                | Maximum    | 760.000    | 14.500      | 25.000       | 289.000    | 50.000       |
|                | Mean       | 185.651    | 10.613      | 3.611        | 50.783     | 13.910       |
|                | SD         | 133.935    | 1.764       | 3.689        | 54.730     | 10.713       |
| <b>6</b>       | N of cases | 27         | 27          | 27           | 27         | 27           |
|                | Minimum    | 31.000     | 9.000       | 0.100        | 4.000      | 4.000        |
|                | Maximum    | 315.000    | 14.500      | 3.000        | 420.000    | 120.000      |
|                | Mean       | 129.481    | 10.963      | 0.989        | 96.222     | 29.326       |
|                | SD         | 82.011     | 1.658       | 0.748        | 112.131    | 29.120       |

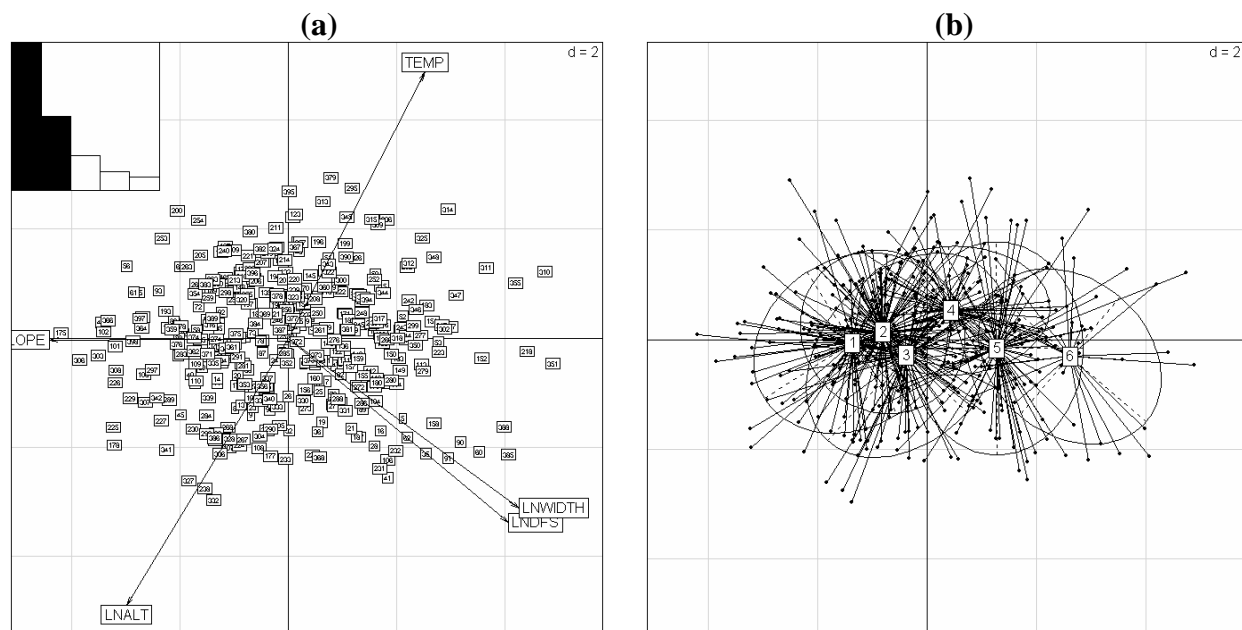
### 3.3. Principal Component Analysis

Before performing the PCA, altitude (ALT), slope (SLOPE), distance from source (DFS) and wetted width (WIDTH) were log-transformed to achieve normality (Fig. 5).



**Fig. 5:** Frequency distributions for environmental variables used in the Principal Component Analysis

The five environmental variables were then used to describe physiographic characteristics in the reference dataset (Fig. 6).



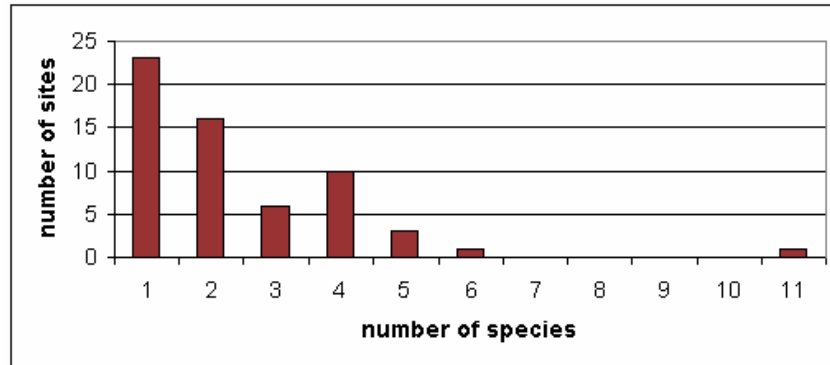
**Fig. 6:** Correspondence Analysis of species abundance (number of fish / 100 m<sup>2</sup>).  
 (a) factorial map (PC1 and PC2) showing sites position and environmental parameters,  
 (b) grouping of sites according to their cluster number

The first axis reflected the longitudinal gradient (Slope, Width and DFS) whereas the second axis separated sites according to their altitude and temperature characteristics (Fig. 6a). The fish clusters were ordered along the first axis, but were highly overlapping (Fig. 6b).

### 3.4. Fish composition of the different clusters (based on species with occurrence >1%)

#### Cluster 1

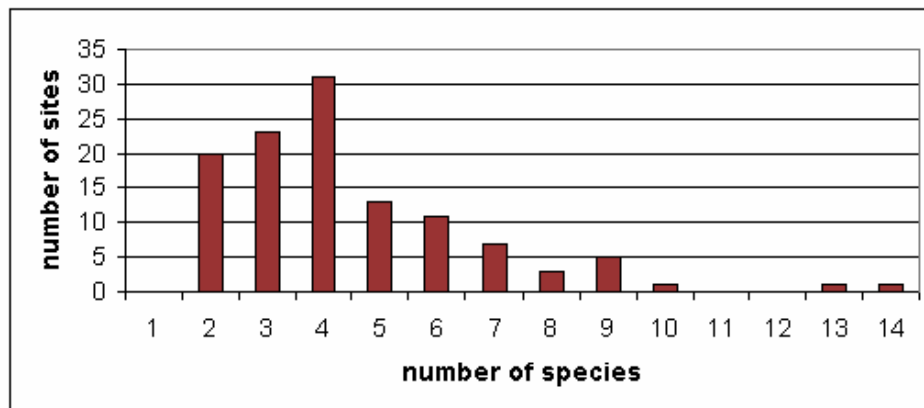
There are 60 sites and 23 different species in this type. In average sites contain 2.6 species.



Dominant species are *Salmo trutta fario* (brown trout, 100 %) and *Poxinus phoxinus* (minnow, 30.0 %). *Cottus gobio* (bullhead (21.6%) and *Barbatulla barbatulla* (stone loach, 16.6 %) are less frequent. The presence of the remaining species is quite low ( $\leq 10$  %)

#### Cluster 2

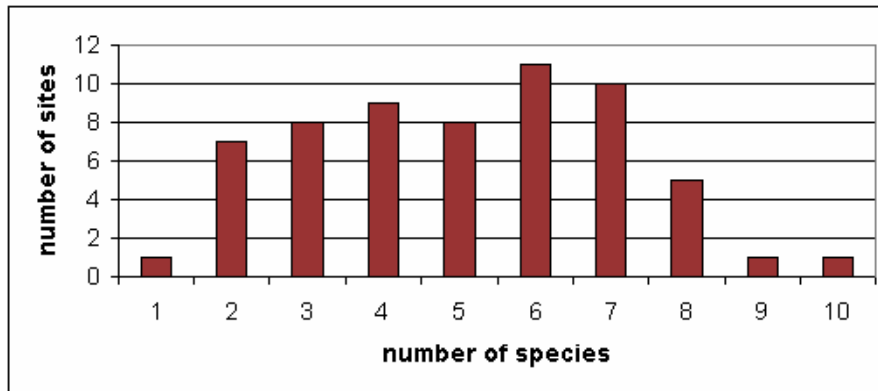
This cluster contains 28 species spread over 116 sites. Each site contains in average 4.47 species.



All sites contain brown trout and bullhead. *Lampetra planeri* (brook lamprey, 46.5 %), stone loach (41.3 %), minnow (31.8 %) and eel (*Anguilla anguilla*, 31.0 %) are relatively frequent too.

### Cluster 3

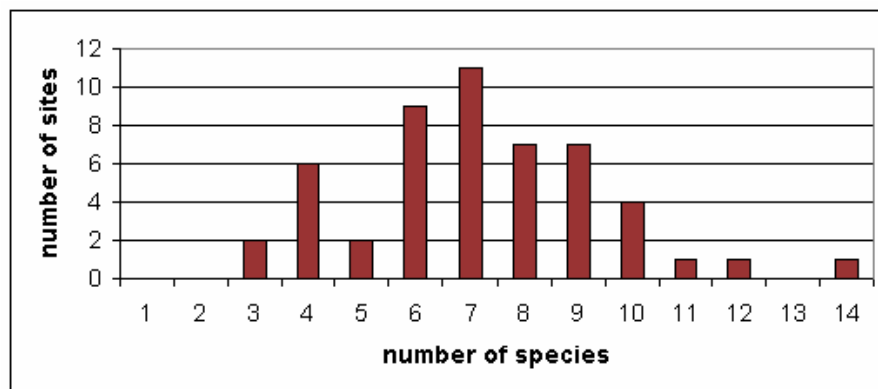
This cluster groups 61 sites having 23 different fish species in total. In average, the sites contain 5.0 species.



All sites have brown trout and in 91.8 % of them minnow is present. Stone loach (72.2 %) and *Gobio gobio* or gudgeon (67.2 %) are also frequently found. Chub (*Leuciscus cephalus*) is more abundant in this type (36.0 %) compared to the previous clusters. Newcomers are soufie (*Leuciscus soufia*) and Mediterranean barbel (*Barbus meridionalis*) with a frequency of 13.1 and 21.3% respectively. Brook lamprey has a frequency of 21.3 %.

### Cluster 4

This type contains 51 sites having a total of 21 different fish species. In average sites contain 7.1 species.

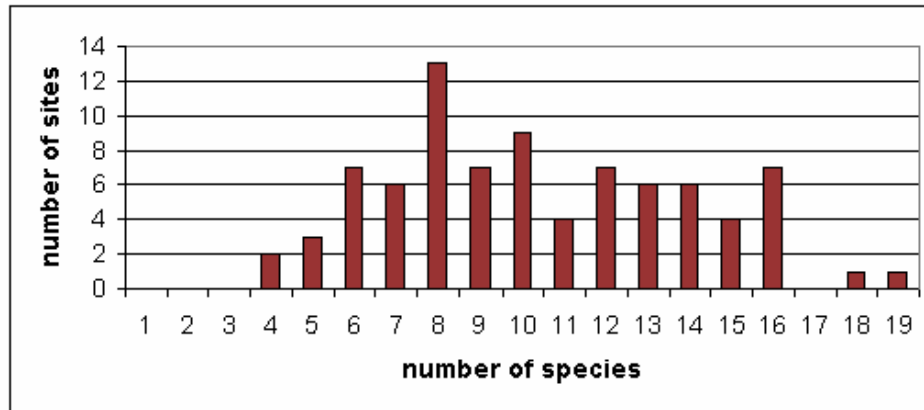


Again, brown trout is present in all sites and together with bullhead (96.2 %) and minnow (92.1 %). Stone loach and eel are also quite frequent (88.2 and 74.5 % respectively). Brook lamprey (56.8 %) and salmon (*Salmo salar*, 52.9 %) are fairly present.



### Cluster 5

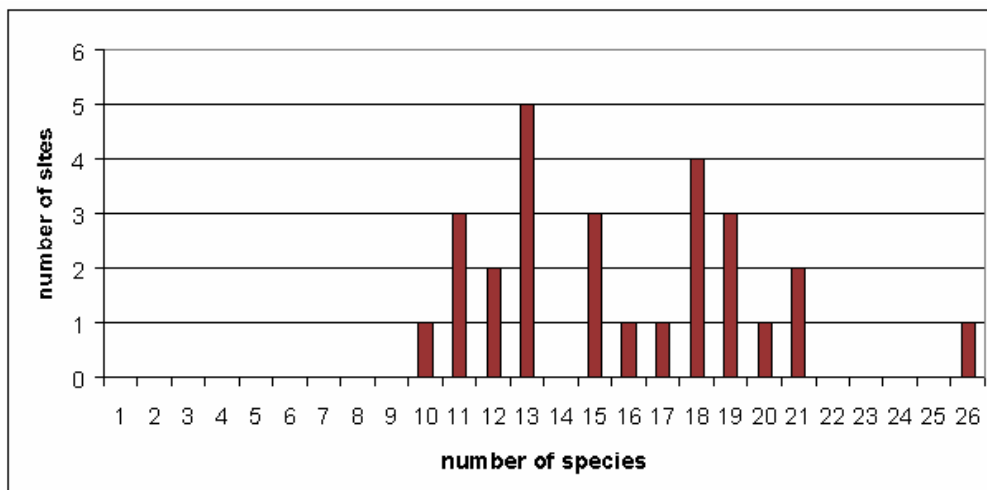
Here we have 83 sites with in total 36 different fish species. In average, one site contains 10.3 species.



The most abundant species in this type are minnow (96.3 %), gudgeon (89.1 %), stone loach (87.9 %), brown trout (81.9 %) and chub (80.7 %). In more than 50 % of the sites, we find bullhead (74.6 %), dace (*Leuciscus leuciscus*, 66.2 %), roach (*Rutilus rutilus*, 57.8 %), eel (54.2 %) and barbel (*Barbus barbus*, 53.0 %).

### Cluster 6

This is a cluster with only 27 sites and 34 different species. Sites in average have 15.8 species.



This cluster is defined by the presence of roach, chub and gudgeon that are present in all sites. Bleak (*Alburnus alburnus*) is found in 96.3 % of the sites. Perch (*Perca fluviatilis*) and barbel are also important (85.2 %) as well as dace present in 81.5 % of the sites. Stone loach and eel are present in 74.1 and 70.4 % of the sites respectively. Pike (*Esox lucius*) is found in 66.7 % of the sites. Minnow and bitterling (*Rhodeus sericeus*) are present in 63 % of the sites. Bullhead is present in 51.9 % of the sites. Brown trout is less important here (22.2 %) but species like rudd (*Scardinius erythrophthalmus*, 48.1 %), tench (*Tinca tinca*, 44.4 %), Silver bream (*Blicca bjoerkna*, 48.1 %) and Schneider (*Alburnoides bipunctatus*, 44.4 %) are more important than in the other clusters.

Tables 2 and 3 summarise the fish characteristics of each fish cluster.

**Table 2:** Overview per cluster of the species and their percentage of occurrence.

| <i>Fish species</i>                | <i>Cluster</i> |            |             |             |             |             |
|------------------------------------|----------------|------------|-------------|-------------|-------------|-------------|
|                                    | 1              | 2          | 3           | 4           | 5           | 6           |
| <i>Salmo trutta fario</i>          | <b>100</b>     | <b>100</b> | <b>100</b>  | <b>100</b>  | <b>81.9</b> | 22.2        |
| <i>Phoxinus phoxinus</i>           | 30.0           | 31.8       | <b>91.8</b> | <b>92.1</b> | <b>96.3</b> | 63          |
| <i>Leuciscus cephalus</i>          | 5.0            | 7.7        | 36.0        | 21.5        | <b>80.7</b> | <b>100</b>  |
| <i>Gobio gobio</i>                 | 10.0           | 11.2       | 67.2        | 37.2        | <b>89.1</b> | <b>100</b>  |
| <i>Barbatula barbatula</i>         | 16.6           | 41.3       | 72.1        | <b>88.2</b> | <b>87.9</b> | 74.1        |
| <i>Leuciscus soufia</i>            | 1              | 0.8        | 13.1        |             | 1.2         | 3.7         |
| <i>Barbus meridionalis</i>         | 21.3           |            | 21.3        |             | 7.2         | 0           |
| <i>Cottus gobio</i>                | 21.7           | <b>100</b> | 29.5        | <b>96.0</b> | 74.6        | 51.9        |
| <i>Lampetra planeri</i>            |                | 46.5       | 21.3        | 56.8        | 28.9        | 11.1        |
| <i>Anguilla anguilla</i>           | 10             | 31.0       | 13.1        | 74.5        | 54.2        | 70.4        |
| <i>Rutilus rutilus</i>             |                | 8.6        | 4.9         | 13.7        | 57.8        | <b>100</b>  |
| <i>Perca fluviatilis</i>           |                | 5.1        | 1.6         | 9.8         | 37.3        | 85.2        |
| <i>Leuciscus leuciscus</i>         |                | 4.3        | 3.2         | 13.7        | 66.2        | 81.5        |
| <i>Salmo salar</i>                 |                |            | 1.6         | 52.9        | 3.6         |             |
| <i>Barbus barbus</i>               |                | 3.4        | 4.9         | 1.9         | 53.0        | 85.2        |
| <i>Alburnus alburnus</i>           |                | 0.8        | 3.2         |             | 21.6        | <b>96.3</b> |
| <i>Thymallus thymallus</i>         | 1.6            | 14.6       | 3.2         | 9.8         | 24.0        |             |
| <i>Scardinius erythrophthalmus</i> |                | 0.8        |             |             | 4.8         | 48.1        |
| <i>Esox lucius</i>                 |                | 3.4        | 3.2         | 7.8         | 43.3        |             |

**Table 3:** Fish characteristics of each fish cluster (Fish\_type\_ecoregional). Given are total number of species (>1%), mean number of species per type and the dominant fish species.

| <i>Fish_type_ecoregional</i> | <i>Total number of species per type &gt; 1 %</i> | <i>Mean number of species per type</i> | <i>Fish species (dominant)</i> |
|------------------------------|--|--|--------------------------------|
| 1                            | 23   | 2.6                                    | <i>Salmo trutta fario</i>      |
| 2                            | 28   | 4.5                                    | <i>Salmo trutta fario</i>      |
| 3                            | 25   | 5.0                                    | <i>Salmo trutta fario</i>      |
| 4                            | 21   | 7.1                                    | <i>Phoxinus phoxinus</i>       |
| 5                            | 36   | 10.3                                   | <i>Phoxinus phoxinus</i>       |
| 6                            | 34   | 15.8                                   | <i>Rutilus rutilus</i>         |

### 3.5. Conclusions

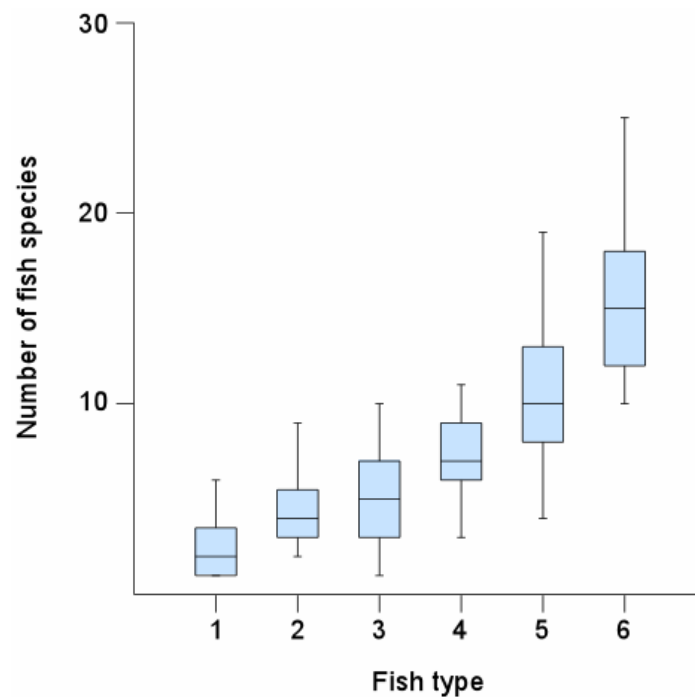
Cluster 1 is characterised by brown trout. It is distinguished from the other types through its low number of species per site.

Clusters 2, 3 and 4 are firstly characterised by brown trout. In addition type 2 is further characterised by bullhead, type 3 by minnow and type 4 by minnow and bullhead. In average their sites have less species than clusters 5 and 6.

Cluster 5 is different from the other clusters since it is defined by minnow, bullhead, barbel, brown trout and chub.

Cluster 6 is characterised by gudgeon, chub, roach and bleak. In average it contains most species per site.

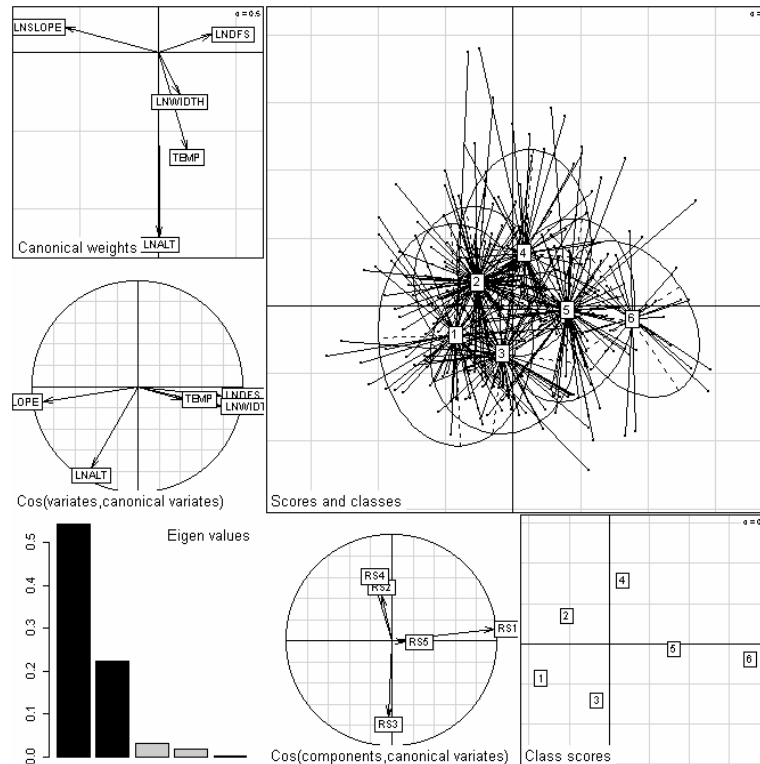
These fish clusters, ordered along the longitudinal gradient, reflect an increase in fish species richness with increasing stream size (Fig.16).



**Fig. 16:** 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.

#### 4. Linking fish clusters and abiotic variables: Discriminant Analysis

We performed a discriminant analysis (DA) to predict clusters belonging to the five environment parameters (ln-transformed for all except Mean Air Temperature).



**Fig. 17:** Results of the Discriminant Analysis

The first two axes (LD1 and LD2) represented 77.7 and 18.8 % of the total inertia, respectively.

The factorial maps (Fig. 17) show that slope and altitude were the main factors involved in the prediction of the fish-clusters. Slope was negatively related to the first axis, whereas the second axis was strongly related to altitude (Table 4).

**Table 4:** Coefficients of linear discriminants.

|         | LD1             | LD2           | LD3     | LD4     | LD5      |
|---------|-----------------|---------------|---------|---------|----------|
| LNALT   | 0.01522         | <b>1.3438</b> | -0.2653 | 0.4212  | -0.20564 |
| TEMP    | 0.17736         | 0.4470        | 0.1990  | -0.2709 | -0.50425 |
| LNSLOPE | <b>-0.77352</b> | -0.1620       | 0.8438  | -0.6433 | 0.48147  |
| LNDFS   | 0.43522         | -0.1138       | 1.0590  | 0.7306  | 0.02152  |
| LNWIDTH | 0.22827         | 0.3382        | -0.5061 | -1.3999 | 1.02050  |

To validate this analysis, we examined how the linear discriminants classify the calibration data set. We compared the fish type predicted by the discriminant analysis to the fish type resulting from the cluster analysis (Table 5).

**Table 5:** Validation of the Discriminant Analysis. For each fish type defined by the cluster analysis, given are the number of sites assigned to the different fish types, and the percentage of sites well classified

|                               |   | Fish type |           |           |           |           |           |
|-------------------------------|---|-----------|-----------|-----------|-----------|-----------|-----------|
|                               |   | 1         | 2         | 3         | 4         | 5         | 6         |
| Predicted<br>fish type        | 1 | <b>22</b> | 5         | 11        | 1         | 1         | 0         |
|                               | 2 | 22        | <b>91</b> | 14        | 25        | 10        | 0         |
|                               | 3 | 10        | 8         | <b>21</b> | 0         | 4         | 0         |
|                               | 4 | 4         | 3         | 1         | <b>12</b> | 5         | 1         |
|                               | 5 | 2         | 9         | 14        | 13        | <b>60</b> | 10        |
|                               | 6 | 0         | 0         | 0         | 0         | 3         | <b>16</b> |
| % of sites well<br>classified |   | 0.37      | 0.78      | 0.34      | 0.24      | 0.72      | 0.59      |

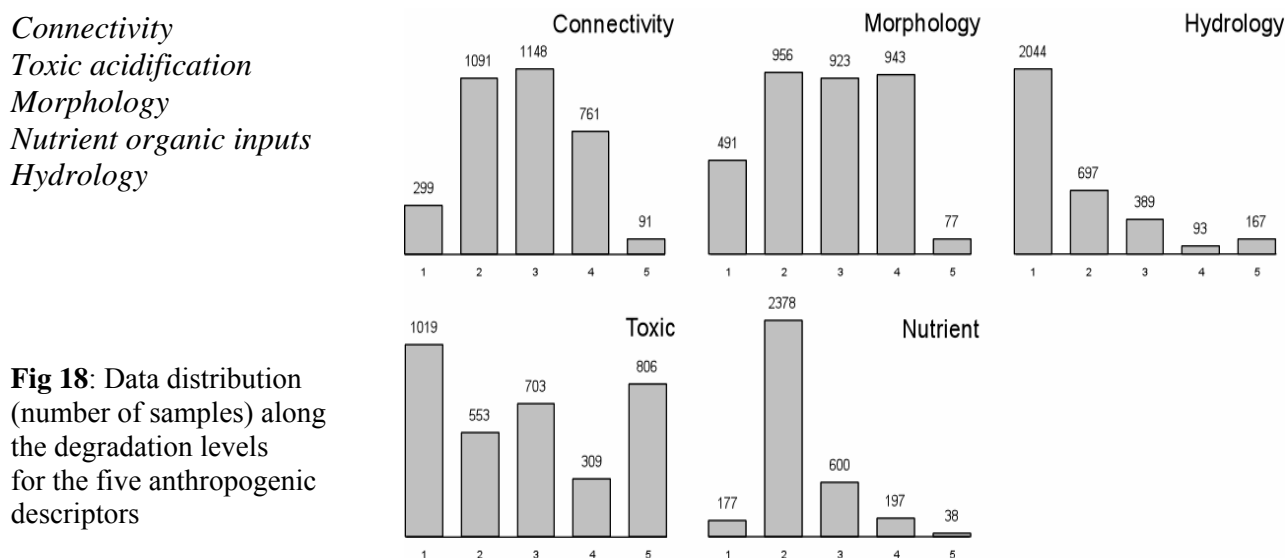
*Note:* One site was well classified when the Discriminant Analysis assigned it to the right fish type (defined by to the cluster analysis). For each fish type, the numbers of sites well classified are in bold.

Globally, this analysis allowed to classify **55.8 %** of sites to the right fish type. We well classified only 24 % of sites from fish type 4, whereas sites from fish types 2 and 5 were the best classified (with 78 and 72 % of site well classified, respectively).

The results of the DA can then be used to assign clusters to all the samples of the ecoregions 8 and 13 based on a linear combination of these abiotic parameters. Thus, we assigned each site to a particular cluster whatever its degree of ecological integrity (i.e. reference and every levels of degradation).

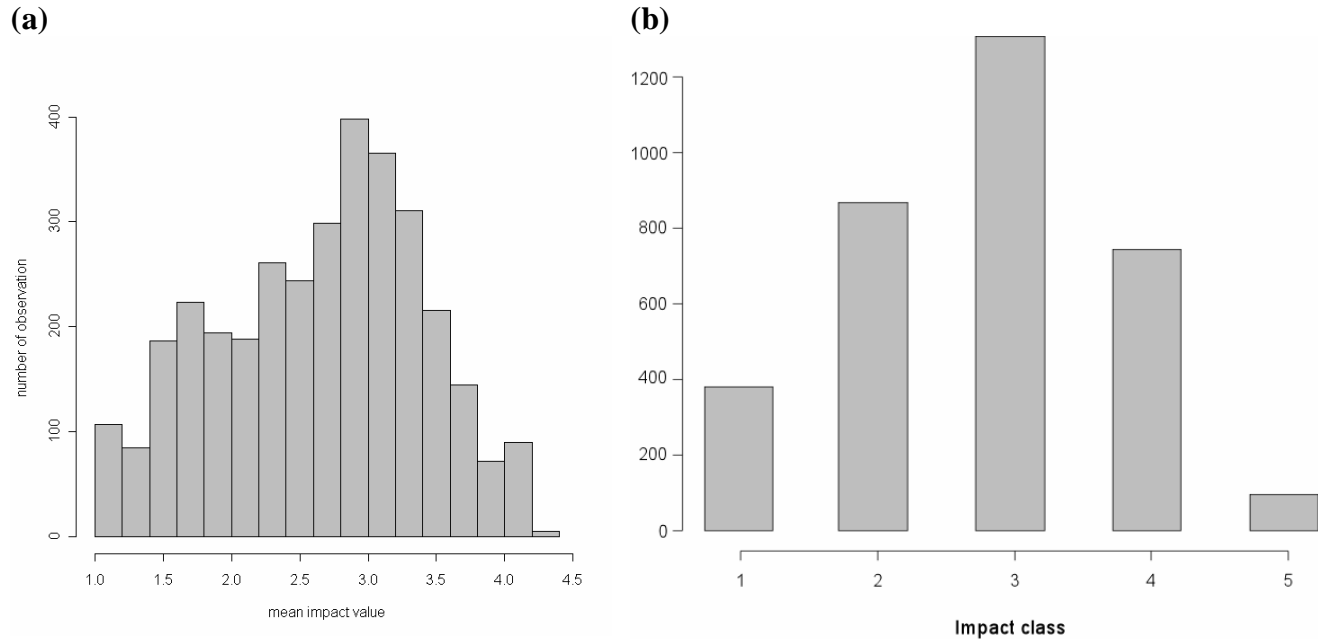
## 5. Impact characteristics in the degraded data set.

Five anthropogenic variables were used to describe impact level of each degraded site:



**Fig 18:** Data distribution (number of samples) along the degradation levels for the five anthropogenic descriptors

We defined a global level of anthropogenic impact as the mean of the five anthropogenic criteria, coded in five classes afterwards (Fig. 19). The class limits were as follows: [1.0, 1.8] [1.8, 2.6] [2.6, 3.4] [3.4, 4.2] [4.2, 5.0].



**Fig. 19:** Data distribution (number of samples) along the degradation levels expressed by (a) the mean value of five degradation criteria and (b) five impact classes.

High impact levels (class 5) were absent from fish types 1, 3 and 5 (Table 6).

**Table 6:** Distribution of samples in the degraded data set according to their fish type belonging and the global impact level (impact class).

|                       | <i>Fish type</i> |          |          |          |          |          |
|-----------------------|------------------|----------|----------|----------|----------|----------|
|                       | <i>1</i>         | <i>2</i> | <i>3</i> | <i>4</i> | <i>5</i> | <i>6</i> |
| <i>Impact class 1</i> | 59               | 169      | 46       | 38       | 60       | 7        |
| <i>Impact class 2</i> | 28               | 204      | 88       | 92       | 358      | 96       |
| <i>Impact class 3</i> | 5                | 332      | 11       | 543      | 159      | 255      |
| <i>Impact class 4</i> | 1                | 162      | 1        | 414      | 34       | 131      |
| <i>Impact class 5</i> | 0                | 27       | 0        | 33       | 0        | 35       |

## 6. Selection of fish metrics

Fish metrics have been calculated using (1) all species, or (2) sentinel species. Thus, two metric tables were available in FIDES and referenced as “all species metrics” and “sentinel species metrics”. We did not use “Historical metrics” because of the lack of information for ecoregion 8 and 13 (this information was available only for Flanders sites). Here we focused on metrics based on species composition (number, %), density and biomass.

### 6.1. First selection of metrics: Spearman correlations between metrics and impact class

For each fish type, we used Spearman rank correlation to screen the response of each metric to degradation. The number of metrics significantly responding to degradation varied from 61 (fish type 1) to 138 (fish type 6) (Table 7).

**Table 7:** Number of metrics significantly correlated with impact class for each fish type.

|                  | <i>Fish type</i> |            |            |            |            |            |
|------------------|------------------|------------|------------|------------|------------|------------|
|                  | 1                | 2          | 3          | 4          | 5          | 6          |
| all species      | 58               | 117        | 99         | 97         | 79         | 112        |
| sentinel species | 3                | 12         | 4          | 14         | 22         | 26         |
| <b>Total</b>     | <b>61</b>        | <b>129</b> | <b>103</b> | <b>111</b> | <b>101</b> | <b>138</b> |

Few metrics based on sentinel species were selected, except downstream (fish types 5 and 6).

When examining in more details which metrics responded to degradation (see *Annex 1* and *Annex 2*), then it appeared that some metrics responded whatever the fish type (i.e., perc\_sp\_Intol or n\_ha\_Hab\_li), whereas some others showed a response to degradation only for some fish types (i.e., perc\_kgha\_Hab\_b in fish types 1-2-3)

### 6.2. Selection of metrics: Stepwise Discriminant Analysis

Stepwise Discriminant Analysis was performed to avoid redundancy between metrics and to select metrics discriminating the classification variable (impact class). We used a forward stepwise procedure, so that only metrics which significantly ( $P < 0.05$ ) discriminated among the different impact classes were added to the model.

This was done for each fish type and the number of selected metrics per fish type varied from 13 (fish type 1) to 19 (fish type 4) (Table 8).

**Table 8:** Selected metrics from Stepwise Discriminant Analysis for each fish type.

| Fish type    | Metrics             | F             | Fish type          | Metrics              | F     |
|--------------|---------------------|---------------|--------------------|----------------------|-------|
| 1            | <b>NHAHABB</b>      | 54.00         | 4                  | BSPNATIVE            | 3.85  |
|              | KGHAHABB            | 32.73         |                    | NSPINTOL             | 7.35  |
|              | NHAHABLI            | 11.78         |                    | PNHAINOL             | 5.18  |
|              | PERCNHAHABLI        | 22.21         |                    | PNHAHABRH            | 8.85  |
|              | <b>PERCNHARELIT</b> | 42.69         |                    | PNHAHABEURY          | 9.14  |
|              | PERCSPREPHYT        | 29.53         |                    | NSPREPHYT            | 4.53  |
|              | PERCKGHAREPH        | 13.85         |                    | <b>PNHAMILONG</b>    | 21.92 |
|              | PERCNHALONSL        | 32.01         |                    | KHAMIPOTAD           | 9.98  |
|              | PERCNHAFEINS        | 29.49         |                    | DSPSENTINELC         | 13.36 |
|              | PERCNHAFEOMN        | 26.34         |                    | DSPSENTINELE         | 6.23  |
|              | <b>KGHAFEOMNI</b>   | 43.53         |                    | DSPSENTINELL         | 7.56  |
|              | PERCKGHAFEOM        | 9.39          |                    | D0COTGOB             | 5.35  |
|              | PRESENCE0PLU        | 19.62         |                    | <b>POSALFAR</b>      | 38.48 |
| 2            | NSPNATIVE           | 15.44         | BSPSENTINELC       | 5.02                 |       |
|              | NSPINTOL            | 6.87          | BSPSENTINELE       | 7.49                 |       |
|              | PSPINTOL            | 5.34          | BSPSENTINELL       | 6.96                 |       |
|              | PKHAINOL            | 6.21          | BSPSENTINELS       | 5.69                 |       |
|              | PNHAHABB            | 15.11         | PRES0COTGOB        | 7.15                 |       |
|              | PSPHABRH            | 13.28         | <b>PRES0SALFAR</b> | 80.61                |       |
|              | <b>NSPRELITH</b>    | 17.68         | 5                  | PSPTOL               | 7.42  |
|              | PNHARELITH          | 8.42          |                    | NHAHABLI             | 15.23 |
|              | PKHARELITH          | 7.99          |                    | <b>PNHAHABLI</b>     | 55.28 |
|              | NSPREPHYT           | 4.57          |                    | PKHAHABLI            | 39.84 |
|              | KHAREPHYT           | 4.39          |                    | <b>PSPHABEURY</b>    | 59.52 |
|              | PNHAFEINSEV         | 6.12          |                    | NSPRELITH            | 28.08 |
|              | <b>PSPMILONG</b>    | 21.75         |                    | <b>PSPRELITH</b>     | 75.31 |
|              | NHAMIPOTAD          | 4.09          |                    | PSPREPHYT            | 23.64 |
|              | KHARUN1HABB         | 7.69          |                    | PKHAREPHYT           | 30.42 |
|              | D0PLUSCOTGOB        | 4.59          |                    | PSPFEINSEV           | 8.53  |
|              | BSPSENTINELC        | 6.79          |                    | PNHAFEINSEV          | 6.66  |
| PRES0PLUSSAL | 4.51                | PNHAFEOMNI    |                    | 6.44                 |       |
| 3            | NHAHABB             | 4.27          |                    | NHAMILONG            | 6.92  |
|              | NSPHABLI            | 14.31         | D0PLUSBARMER       | 9.02                 |       |
|              | <b>PKHAHABEURY</b>  | 39.68         | 6                  | NSPINTOL             | 7.73  |
|              | NSPREPHYT           | 7.10          |                    | NHAINOL              | 3.90  |
|              | PSPREPHYT           | 8.20          |                    | <b>PSPHABB</b>       | 12.32 |
|              | NHAREPHYT           | 27.68         |                    | PNHAHABRH            | 4.56  |
|              | KHAREPHYT           | 27.45         |                    | NSPRELITH            | 8.43  |
|              | <b>NSPFEPISC</b>    | 228.58        |                    | PNHALONLL            | 7.31  |
|              | <b>PSPFEPIESC</b>   | 769.70        |                    | <b>NSPMILONG</b>     | 11.93 |
|              | NHAFEPIESC          | 52.49         |                    | PSPMIPOTAD           | 8.40  |
|              | PKHAFEPIESC         | 14.07         |                    | DSPSENCOTGOB         | 8.83  |
|              | PNHAFEINSEV         | 15.00         |                    | <b>DSPSENE SOLUC</b> | 13.90 |
|              | <b>KHARUN1HABEU</b> | 181.88        |                    | DSPSENSALFAR         | 7.20  |
| KHARUN1REPHY | 22.04               | BSPSENBARBAB  |                    | 5.43                 |       |
|              |                     | BSPSENCHONAS  |                    | 5.33                 |       |
|              |                     | BSPSENE SOLUC | 9.54               |                      |       |
|              |                     | PRES0PLUSLEU  | 6.64               |                      |       |

Note: The most discriminant metrics are given in bold.

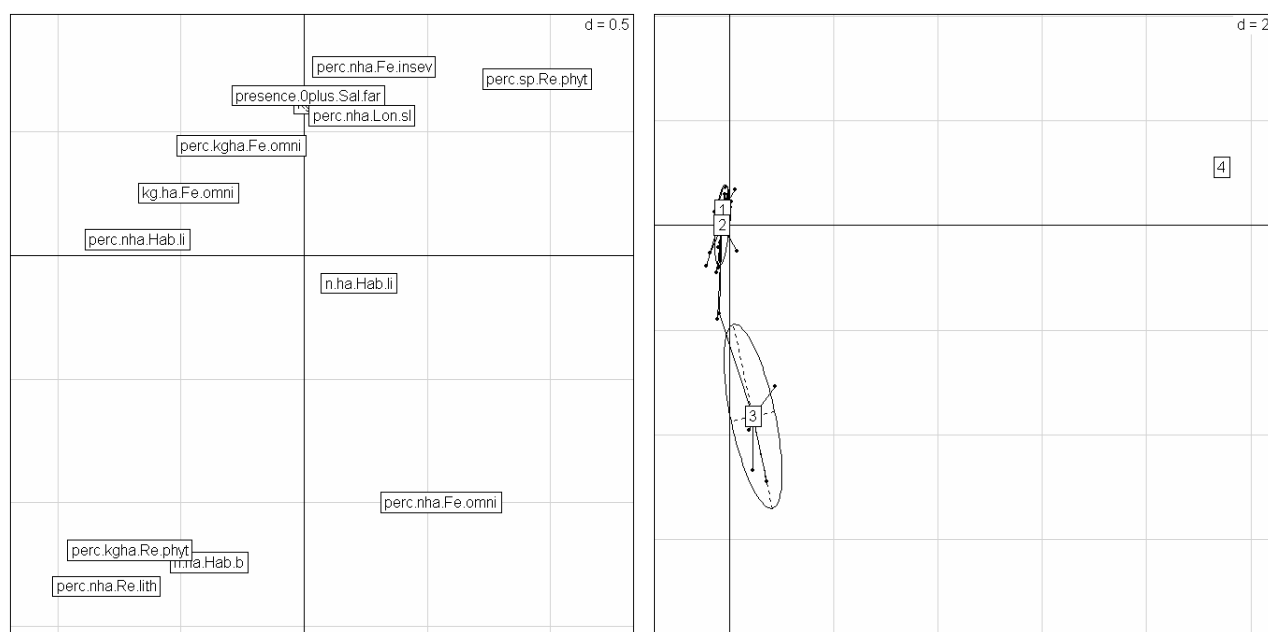


## 7. Linking metrics and impact class: Discriminant Analysis

For each fish type, we performed Discriminant Analysis to examine how selected metrics discriminated impact classes.

Below is given, for each fish type, the coefficients of linear discriminants and the proportion of trace (the part of total inertia represented by each axes).

### Fish type 1



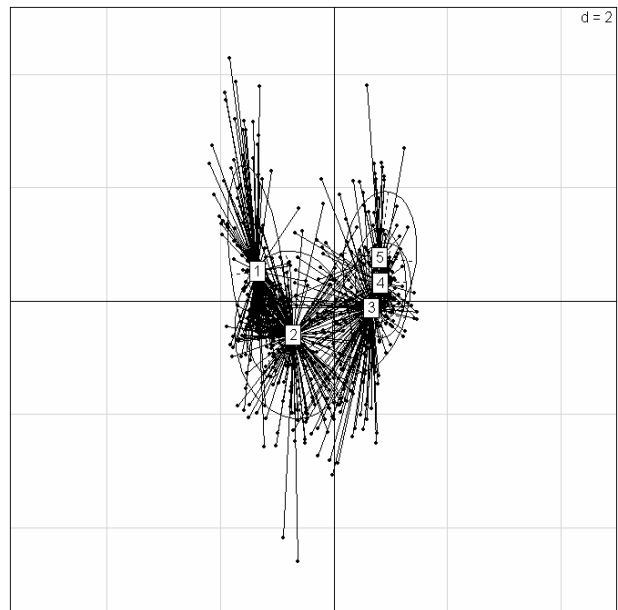
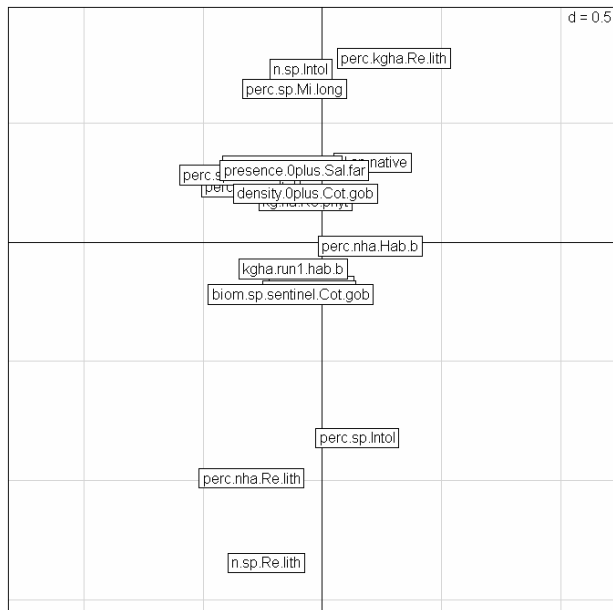
#### Coefficients of linear discriminants:

|                        | LD1        | LD2        | LD3        |
|------------------------|------------|------------|------------|
| n.ha.Hab.b             | -0.0016604 | -0.0013407 | -8.807e-05 |
| kg.ha.Hab.b            | 0.0002984  | 0.0003447  | 6.391e-05  |
| n.ha.Hab.li            | 0.0234324  | -0.0029637 | 3.915e-03  |
| perc.nha.Hab.li        | -1.5702601 | 0.0372589  | -4.700e-02 |
| perc.nha.Re.lith       | -0.3513003 | -0.1471455 | 2.377e-02  |
| perc.sp.Re.phyt        | 0.7019682  | 0.1333477  | -1.852e-01 |
| perc.kgha.Re.phyt      | -0.5638523 | -0.2390132 | 1.255e-01  |
| perc.nha.Lon.sl        | 0.0706469  | 0.0429786  | 4.788e-02  |
| perc.nha.Fe.insev      | 0.0685035  | 0.0464706  | 1.289e-04  |
| perc.nha.Fe.omni       | 0.2825933  | -0.1278371 | 3.073e-02  |
| kg.ha.Fe.omni          | -0.0821636 | 0.0111300  | -4.211e-03 |
| perc.kgha.Fe.omni      | -0.1336273 | 0.0585813  | 5.883e-02  |
| presence.0plus.Sal.far | 0.4143835  | 4.2080316  | -7.610e-01 |

#### Proportion of trace:

| LD1    | LD2    | LD3    |
|--------|--------|--------|
| 0.9397 | 0.0471 | 0.0131 |

## Fish type 2



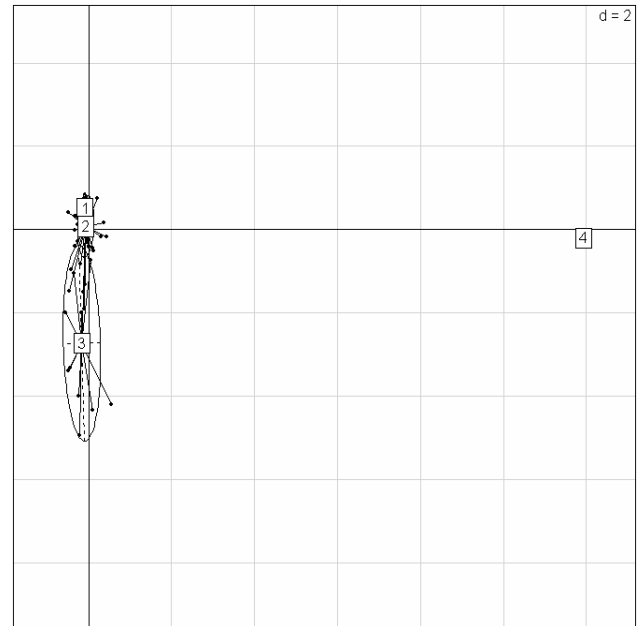
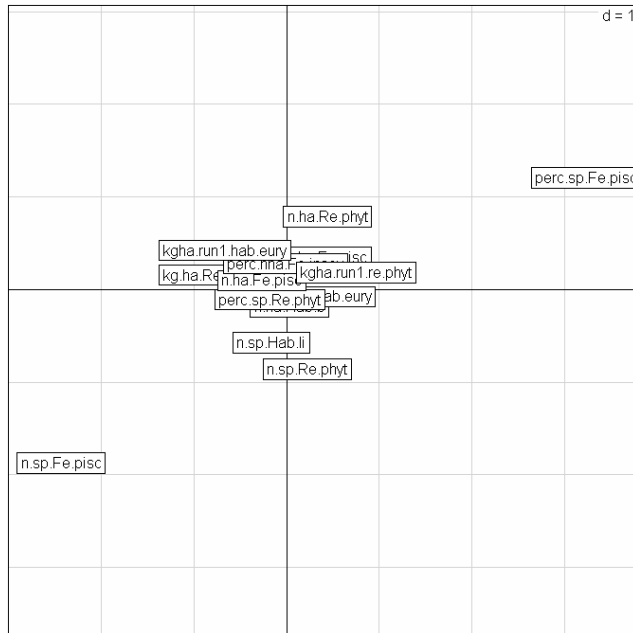
### Coefficients of linear discriminants:

|                          | LD1        | LD2        | LD3        | LD4        |
|--------------------------|------------|------------|------------|------------|
| N.sp.native              | 1.337e-01  | 1.125e-01  | 6.001e-01  | 2.764e-02  |
| n.sp.Intol               | -1.429e-01 | 6.037e-01  | 1.335e+00  | 3.390e-01  |
| perc.sp.Intol            | 9.377e-03  | -2.794e-02 | -1.871e-02 | 1.848e-02  |
| perc.kgha.Intol          | -1.572e-02 | 6.414e-03  | 1.752e-03  | 2.392e-02  |
| perc.nha.Hab.b           | 1.333e-02  | -5.933e-04 | -1.298e-02 | -8.114e-03 |
| perc.sp.Hab.rh           | -2.014e-02 | 7.795e-03  | 2.044e-02  | -5.819e-02 |
| n.sp.Re.lith             | -2.157e-01 | -6.499e-01 | -1.357e+00 | 4.575e-02  |
| perc.nha.Re.lith         | -1.371e-02 | -2.426e-02 | -2.143e-02 | 2.467e-02  |
| perc.kgha.Re.lith        | 1.448e-02  | 1.981e-02  | 2.636e-02  | 6.634e-03  |
| n.sp.Re.phyt             | -1.189e-01 | -2.527e-01 | -6.202e-01 | 4.833e-01  |
| kg.ha.Re.phyt            | -7.362e-05 | 9.754e-05  | -4.700e-05 | 5.623e-05  |
| perc.nha.Fe.insev        | -8.728e-03 | 8.844e-03  | -4.647e-03 | -2.286e-02 |
| perc.sp.Mi.long          | -2.657e-02 | 7.720e-02  | -3.893e-02 | -4.330e-03 |
| n.ha.Mi.potad            | -6.208e-05 | -1.245e-04 | 8.420e-05  | -1.237e-04 |
| kgha.run1.hab.b          | -4.446e-05 | -2.144e-05 | 1.097e-05  | -4.299e-05 |
| density.0plus.Cot.gob    | -1.790e-04 | 2.670e-04  | -3.058e-05 | 2.125e-04  |
| biom.sp.sentinel.Cot.gob | -1.316e-03 | -1.166e-03 | 3.234e-04  | -5.112e-04 |
| presence.0plus.Sal.far   | -5.265e-01 | 7.152e-01  | -2.707e-01 | -3.574e-02 |

### Proportion of trace:

| LD1    | LD2    | LD3    | LD4    |
|--------|--------|--------|--------|
| 0.9016 | 0.0575 | 0.0330 | 0.0078 |

### Fish type 3



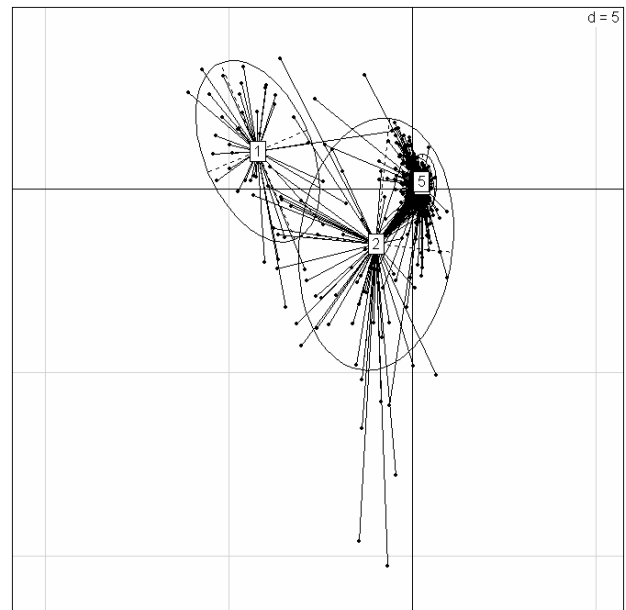
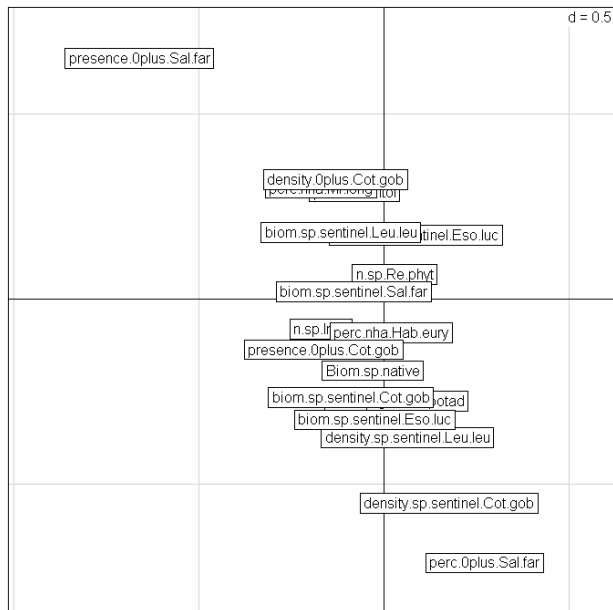
#### Coefficients of linear discriminants:

|                    | LD1        | LD2        | LD3        |
|--------------------|------------|------------|------------|
| n.ha.Hab.b         | 4.879e-05  | -6.847e-05 | -6.397e-05 |
| n.sp.Hab.li        | -1.310e+00 | -1.028e+00 | -2.015e-01 |
| perc.kgha.Hab.eury | 1.207e-01  | -9.357e-03 | 8.507e-03  |
| n.sp.Re.phyt       | 1.864e+00  | -1.647e+00 | -1.452e+00 |
| perc.sp.Re.phyt    | -1.871e-01 | -2.601e-02 | 4.626e-02  |
| n.ha.Re.phyt       | 5.237e-02  | 2.116e-02  | 1.109e-02  |
| kg.ha.Re.phyt      | -8.829e-01 | 3.685e-02  | 4.269e-02  |
| n.sp.Fe.pisc       | -8.314e+01 | -1.452e+01 | -6.733e+00 |
| perc.sp.Fe.pisc    | 1.049e+01  | 8.857e-01  | 3.099e-01  |
| n.ha.Fe.pisc       | -2.876e-01 | 2.331e-02  | 1.444e-02  |
| perc.kgha.Fe.pisc  | 2.246e+00  | 7.550e-01  | 6.072e-01  |
| perc.nha.Fe.insev  | -2.396e-03 | 1.316e-02  | -3.165e-02 |
| kgha.run1.hab.eury | -1.322e-01 | 1.895e-02  | 6.513e-03  |
| kgha.run1.re.phyt  | 8.492e-01  | 4.640e-02  | -2.976e-03 |

#### Proportion of trace:

| LD1    | LD2    | LD3    |
|--------|--------|--------|
| 0.9605 | 0.0326 | 0.0069 |

## Fish type 4



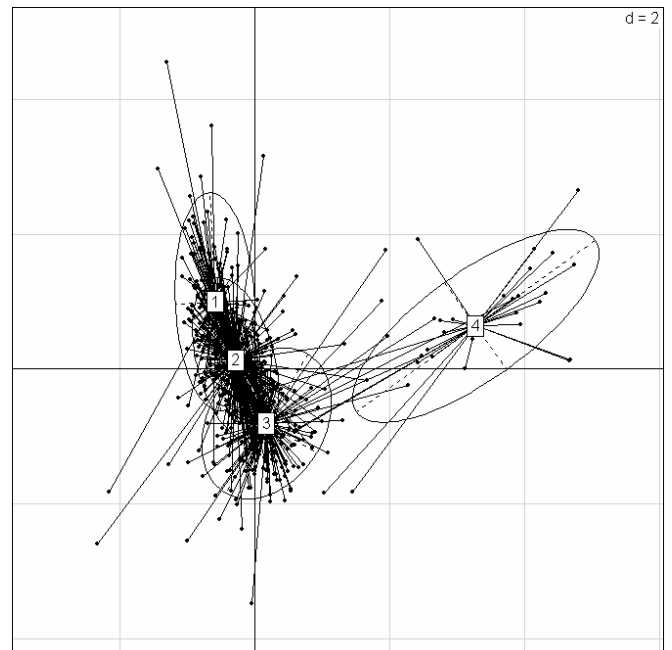
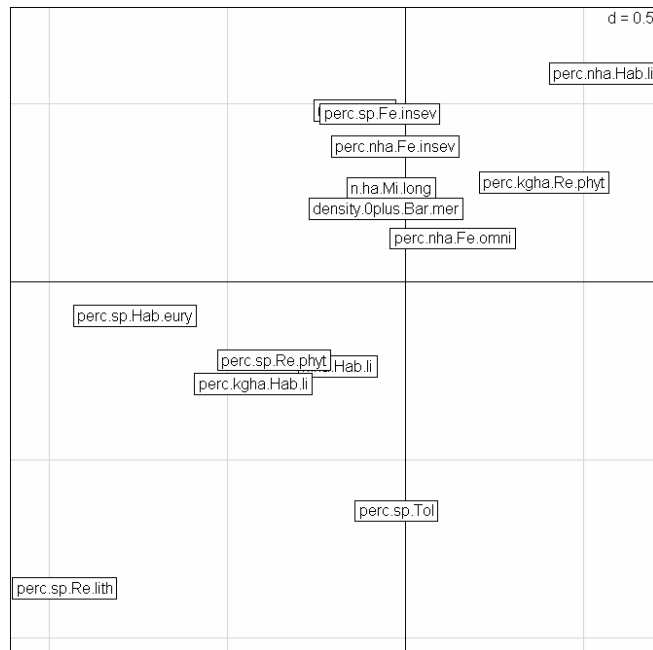
### Coefficients of linear discriminants:

|                             | LD1        | LD2        | LD3        | LD4        |
|-----------------------------|------------|------------|------------|------------|
| Biom.sp.native              | -2.051e-05 | -7.456e-05 | -1.561e-05 | -2.797e-05 |
| n.sp.Intol                  | -3.558e-01 | -9.847e-02 | -1.213e-01 | 9.725e-01  |
| perc.nha.Intol              | -1.007e-02 | 2.110e-02  | 6.469e-03  | -1.980e-02 |
| perc.nha.Hab.rh             | -9.412e-04 | -1.051e-02 | 1.988e-03  | -2.525e-02 |
| perc.nha.Hab.eury           | 1.131e-03  | -2.999e-03 | 2.082e-02  | -1.045e-02 |
| n.sp.Re.phyt                | 3.994e-02  | 5.478e-02  | 3.427e-01  | 8.786e-02  |
| perc.nha.Mi.long            | -3.276e-02 | 3.378e-02  | -1.615e-02 | -3.972e-02 |
| kg.ha.Mi.potad              | 3.925e-03  | -7.008e-03 | 1.166e-03  | -5.221e-03 |
| density.sp.sentinel.Cot.gob | 8.757e-04  | -1.617e-03 | 9.279e-06  | 9.708e-05  |
| density.sp.sentinel.Eso.luc | 1.699e-02  | 2.018e-02  | -2.133e-02 | 7.763e-03  |
| density.sp.sentinel.Leu.leu | 2.448e-03  | -8.507e-03 | -2.198e-04 | -2.343e-03 |
| density.0plus.Cot.gob       | -2.827e-03 | 4.040e-03  | 9.909e-05  | -3.609e-04 |
| perc.0plus.Sal.far          | 7.443e-02  | -1.145e-01 | 9.461e-03  | -1.297e-02 |
| biom.sp.sentinel.Cot.gob    | -1.620e-03 | -2.815e-03 | -1.409e-03 | -2.303e-04 |
| biom.sp.sentinel.Eso.luc    | -1.690e-03 | -1.283e-02 | -7.279e-03 | 6.465e-03  |
| biom.sp.sentinel.Leu.leu    | -6.023e-03 | 5.379e-03  | -1.988e-03 | 6.892e-03  |
| biom.sp.sentinel.Sal.far    | -7.229e-04 | 9.824e-05  | 2.144e-03  | 2.353e-03  |
| presence.0plus.Cot.gob      | -1.389e+00 | -6.780e-01 | 2.599e-01  | -9.468e-01 |
| presence.0plus.Sal.far      | -5.431e+00 | 3.124e+00  | 2.930e-02  | -4.181e-01 |

### Proportion of trace:

| LD1    | LD2    | LD3    | LD4    |
|--------|--------|--------|--------|
| 0.8743 | 0.0942 | 0.0236 | 0.0079 |

## Fish type 5



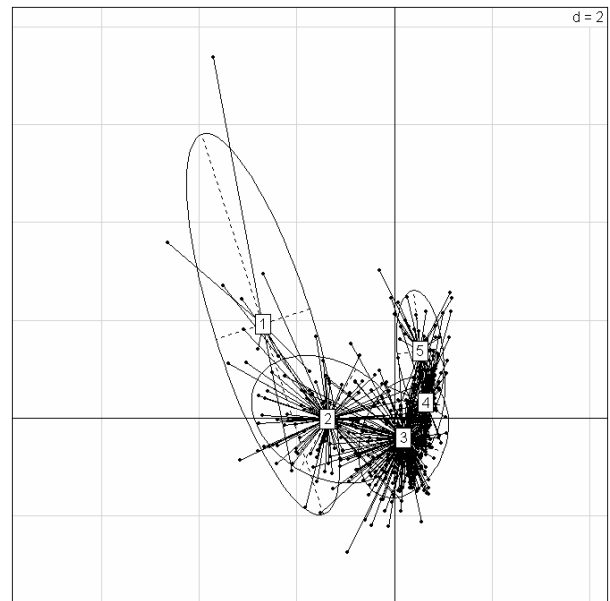
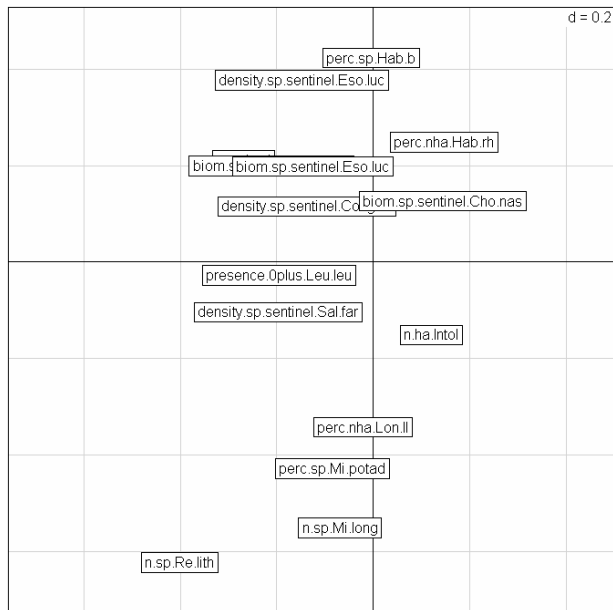
### Coefficients of linear discriminants:

|                       | LD1        | LD2        | LD3        |
|-----------------------|------------|------------|------------|
| perc.sp.Tol           | -0.0023548 | -0.0389801 | 3.876e-03  |
| n.ha.Hab.li           | -0.0003384 | -0.0002813 | -5.785e-05 |
| perc.nha.Hab.li       | 0.0757376  | 0.0530421  | 3.362e-02  |
| perc.kgha.Hab.li      | -0.0662492 | -0.0297214 | -5.026e-02 |
| perc.sp.Hab.eury      | -0.0774781 | -0.0065644 | -1.690e-02 |
| n.sp.Re.lith          | -0.0977501 | 0.2207798  | 3.671e-01  |
| perc.sp.Re.lith       | -0.0714979 | -0.0429555 | -2.996e-02 |
| perc.sp.Re.phyt       | -0.0599017 | -0.0238814 | 2.115e-03  |
| perc.kgha.Re.phyt     | 0.0452748  | 0.0216637  | -1.528e-02 |
| perc.sp.Fe.insev      | -0.0092923 | 0.0405919  | 2.624e-02  |
| perc.nha.Fe.insev     | -0.0025211 | 0.0208973  | -1.451e-02 |
| perc.nha.Fe.omni      | 0.0082335  | 0.0050213  | 1.582e-02  |
| n.ha.Mi.long          | -0.0002055 | 0.0008602  | -4.409e-04 |
| density.0plus.Bar.mer | -0.0068021 | 0.0164348  | -2.823e-02 |

### Proportion of trace:

| LD1    | LD2    | LD3    |
|--------|--------|--------|
| 0.7929 | 0.1539 | 0.0532 |

## Fish type 6



### Coefficients of linear discriminants:

|                             | LD1        | LD2        | LD3        | LD4        |
|-----------------------------|------------|------------|------------|------------|
| n.sp.Intol                  | -4.355e-01 | 2.607e-01  | 4.731e-01  | -8.222e-01 |
| n.ha.Intol                  | 8.673e-05  | -8.196e-05 | -4.386e-05 | 7.672e-05  |
| perc.sp.Hab.b               | -2.559e-04 | 2.547e-02  | 1.072e-02  | 4.100e-02  |
| perc.nha.Hab.rh             | 8.845e-03  | 1.132e-02  | 3.006e-03  | -9.219e-03 |
| n.sp.Re.lith                | -2.499e-01 | -2.951e-01 | -1.027e-02 | 3.547e-01  |
| perc.nha.Lon.ll             | -1.595e-03 | -2.056e-02 | -1.068e-02 | 2.112e-02  |
| n.sp.Mi.long                | -2.033e-01 | -1.240e+00 | -2.366e-01 | -6.873e-01 |
| perc.sp.Mi.potad            | -9.698e-03 | -3.719e-02 | -2.155e-02 | -2.048e-03 |
| density.sp.sentinel.Cot.gob | -5.367e-03 | 3.408e-03  | -1.466e-02 | -2.343e-03 |
| density.sp.sentinel.Eso.luc | -4.002e-03 | 7.754e-03  | -1.156e-02 | -7.629e-04 |
| density.sp.sentinel.Sal.far | -7.690e-03 | -3.077e-03 | 2.347e-03  | -2.214e-03 |
| biom.sp.sentinel.Bar.bab    | -2.881e-03 | 2.081e-03  | -3.043e-04 | -2.791e-03 |
| biom.sp.sentinel.Cho.nas    | 6.118e-03  | 4.072e-03  | -1.132e-02 | -2.219e-03 |
| biom.sp.sentinel.Eso.luc    | -1.997e-03 | 2.396e-03  | 5.295e-03  | 4.008e-03  |
| presence.0plus.Leu.leu      | -9.494e-01 | -1.013e-01 | 1.118e+00  | -7.134e-01 |

### Proportion of trace:

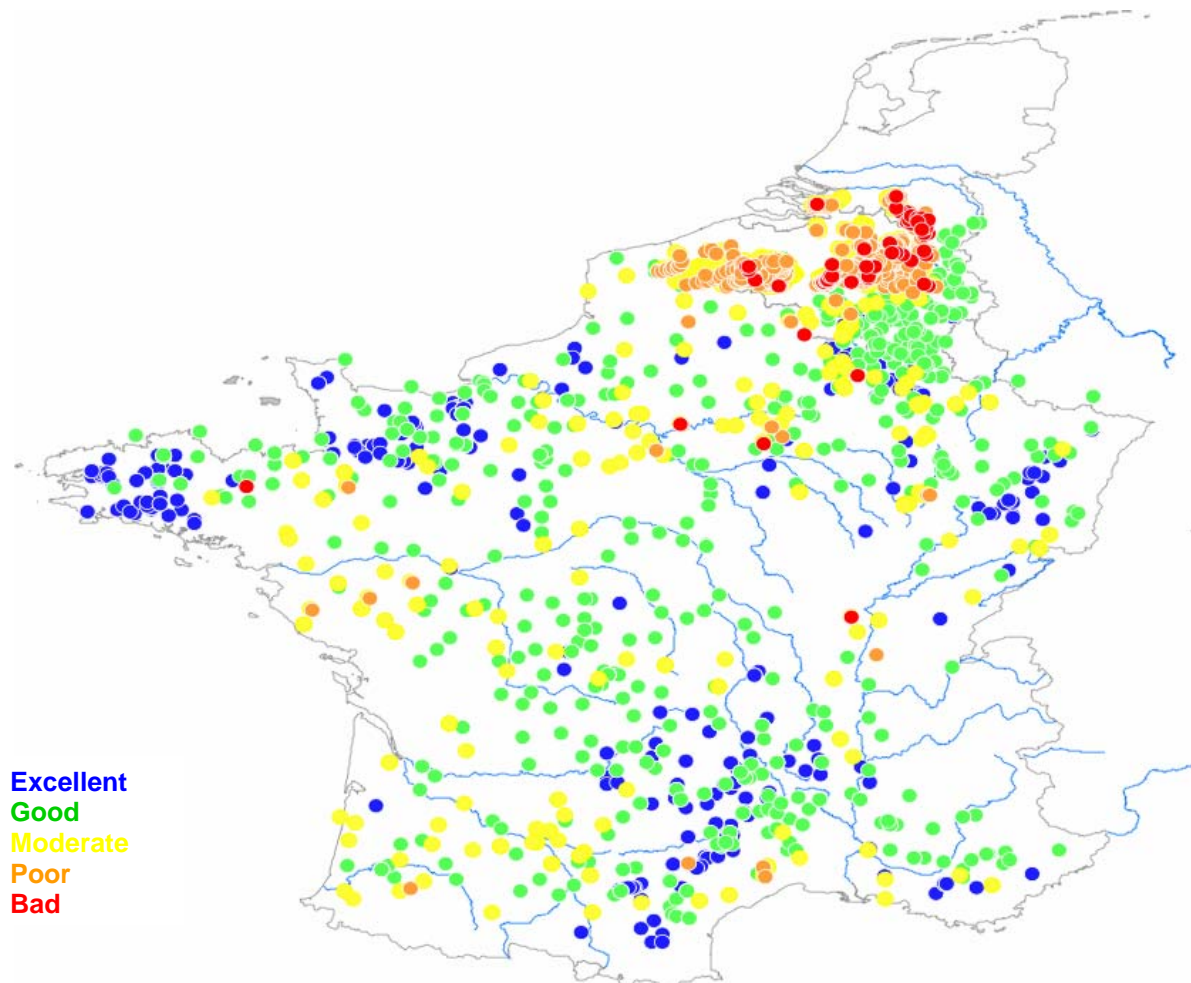
| LD1    | LD2    | LD3    | LD4    |
|--------|--------|--------|--------|
| 0.6574 | 0.1815 | 0.0935 | 0.0677 |

## 8. Prediction of impact class for each site and validation

### 8.1. Prediction

Each site was assigned to a fish type (see 4. *Linking fish clusters and abiotic variables*), so that we used the coefficients of corresponding linear discriminants (from Discriminant Analysis with metrics) to predict ecological impact.

This produced a new variable in FIDES: *Status\_ecoregional*, ranging from 1 (excellent) to 5 (bad) (Fig. 20).



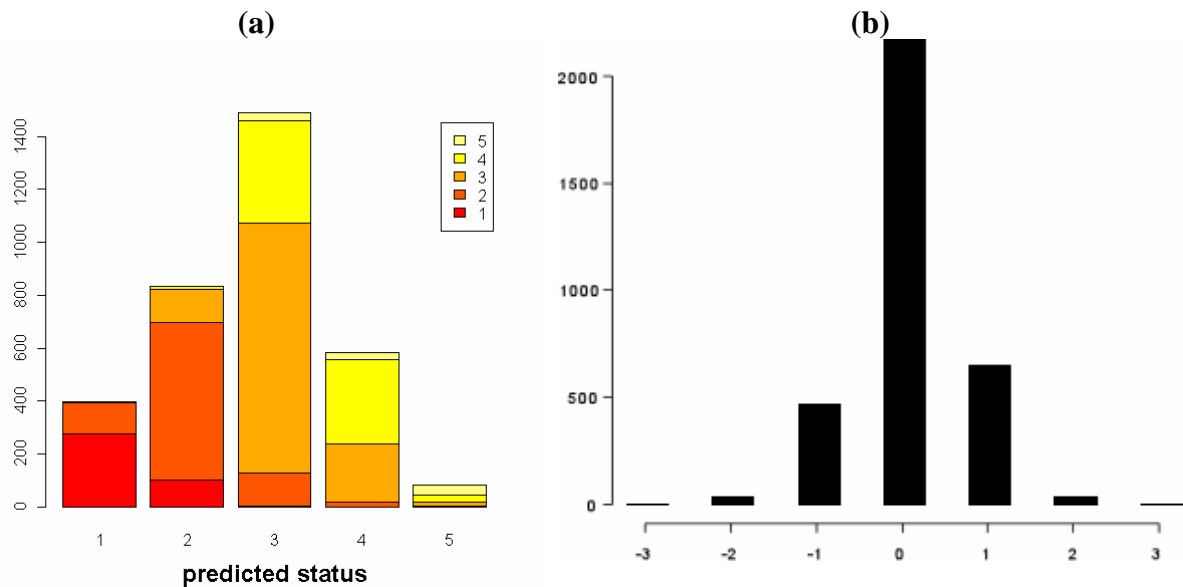
**Fig. 20:** Predicted values of impact class (*Status\_ecoregional*).

## 8.2. Validation

We examined the ability of our method to classify site status by comparing predictions to observed impact status at two levels: the “ecoregional” and the “fish type” level.

### 8.2.1 At the ecoregion level (for the whole data set)

First, we examined the efficiency to classify sites into five impact classes (Fig. 21).

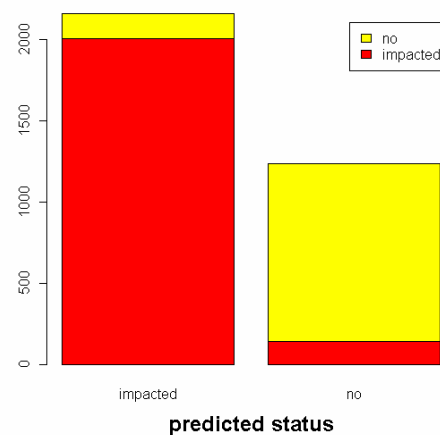


**Fig. 21:** (a) observed impact class for each predicted class and (b) distribution of differences between observed and predicted impact class.

Globally, **64.1%** of the sites were well classified into five impact classes. When examining the discrepancy between observed and predicted impacts, only **2.5%** of sites had a difference  $>1$  (in absolute value)

Then we compared predictions for ecological status, this is after grouping impacts 1 and 2 in a “not impacted” status, whereas impacts 3, 4 and 5 are defined as “impacted” status (Fig. 22).

**91.3%** of the sites were well classified into “impacted” or “not impacted” status.

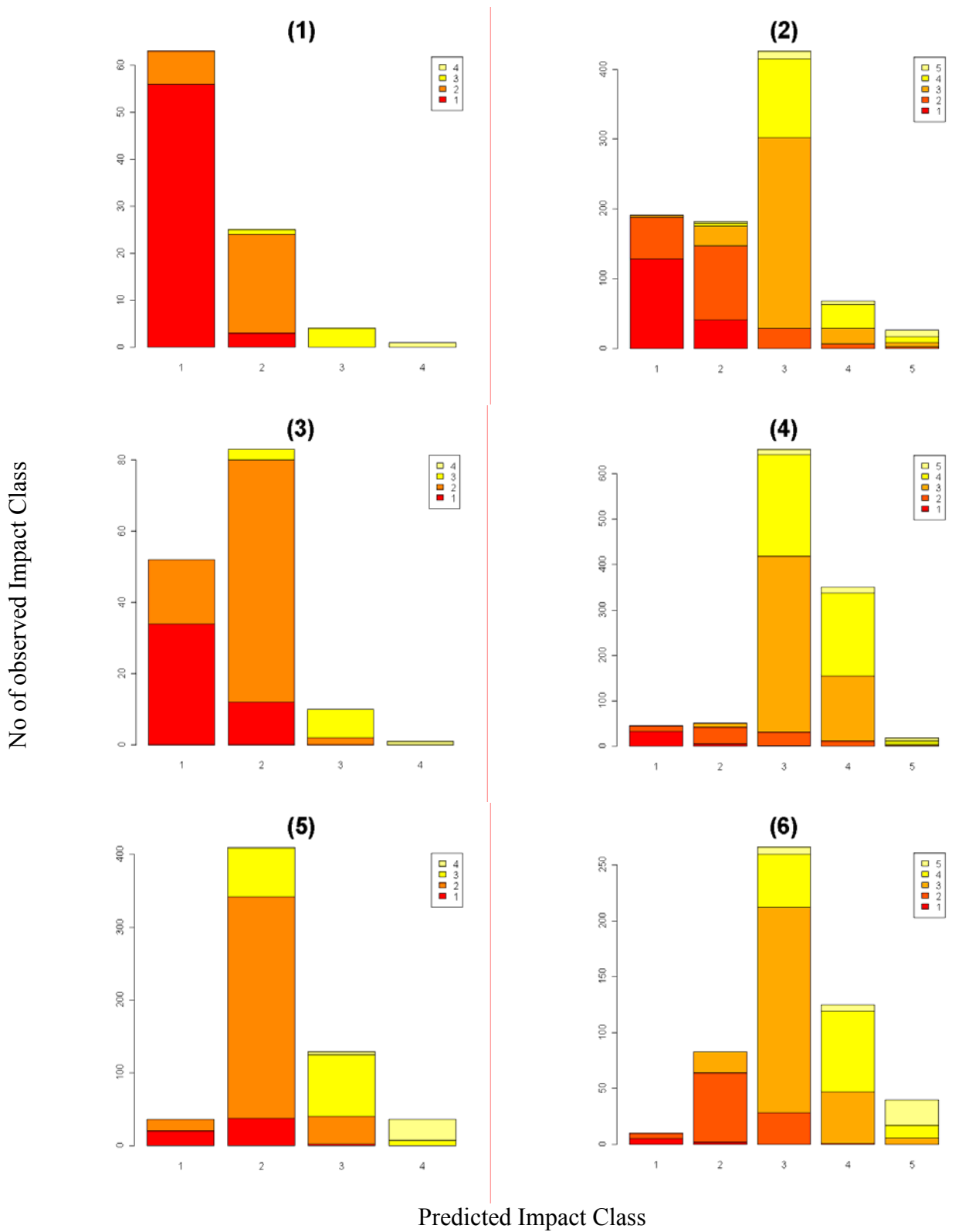


**Fig. 22:** observed status for each predicted status



### 8.2.2 At the fish-type level

The efficiency of our method to classify site varied among fish types (Fig. 23 and Table 9).

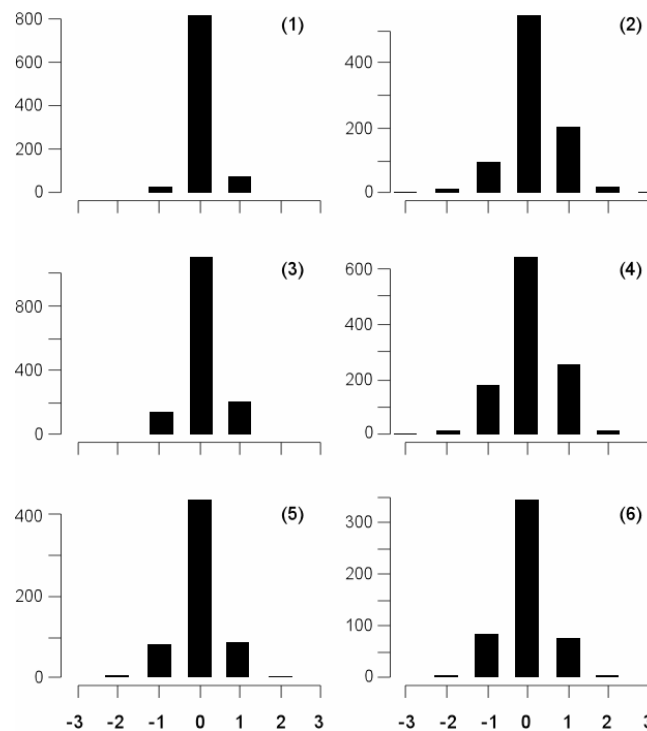


**Fig. 23:** observed impact class versus predicted impact for each fish type (in brackets)

**Table 9:** Percentage of well-classified sites according to two classifications:  
 (1) Impact Class ranging from 1 to 5, and (2) Impact Status (impacted or not)

| <i>Fish type</i> | <i>Impact class</i> | <i>Impact status</i> |
|------------------|---------------------|----------------------|
| 1                | 0.880               | 0.989                |
| 2                | 0.615               | 0.914                |
| 3                | 0.760               | 0.966                |
| 4                | 0.575               | 0.951                |
| 5                | 0.716               | 0.823                |
| 6                | 0.660               | 0.908                |

The percentage of well-classified sites varied from 57.5% (fish type 4) to 88% (fish type 1) for a classification into five classes, and from 82.8 % (fish type 5) to 98.9 % (fish type 1) for a classification into two ecological statuses.



**Fig. 24:** Distribution of differences between observed and predicted impact class for each fish type (in brackets).

The percentage of sites for which the discrepancy between observed and predicted impact class was more than one class ranged from 0% (fish types 1 and 3) to 4.1% (fish type 2) (Fig. 24).

## 9. Conclusions

- Based on fish species composition, 6 main fish types were revealed by cluster analysis. These types could be separated along the longitudinal (upstream-downstream) gradient.
- The separation of the clusters, based on physiographic characteristics, was significant, but these clusters were highly overlapping.
- We measured impact level by the mean of five anthropogenic criteria, coded in five classes afterwards.
- For each fish types, we screened the response to degradation for all fish metrics, retaining only those exhibiting highly significant responses. Then we eliminated redundancy among metrics by performing discriminant analysis with a stepwise procedure. Only metrics that significantly discriminated among the different impact classes were retained. The number of selected metrics varied from 13 to 19 according to the fish type.
- We used discriminant analysis to predict an expected impact level and we compared this prediction to the observed impact.
- Globally, this approach well predicted impact class for 64% of the sites. When discrepancy was observed, the difference between observed and predicted impact was more than one class only for 2.5% of sites. This percentage ranged from 0 to 4.1% among fish types.
- When grouping impact classes into two ecological statuses, 91.3% of the sites were well classified into “impacted” or “not impacted” status. This percentage ranged from 82.8 % to 98.9 % among fish types.

**Annex 1** : List of “*all species metrics*” correlated with impact class (Spearman correlation,  $P < 0.0003$  after Bonferroni correction).

| metric            | Fish type |   |   |   |   |   | metric               | Fish type |   |   |   |   |   |
|-------------------|-----------|---|---|---|---|---|----------------------|-----------|---|---|---|---|---|
|                   | 1         | 2 | 3 | 4 | 5 | 6 |                      | 1         | 2 | 3 | 4 | 5 | 6 |
| N_sp_all          | x         | x | x |   |   | x | n_sp_Fe_pisc         |           |   | x |   |   | x |
| N_sp_native       | x         | x | x |   | x | x | perc_sp_Fe_pisc      |           |   | x |   |   |   |
| Perc_sp_native    |           | x | x | x | x |   | n_ha_Fe_pisc         |           |   | x |   |   | x |
| Density_sp_all    |           | x |   |   |   | x | perc_nha_Fe_pisc     |           |   |   |   |   |   |
| Biom_sp_all       |           | x |   |   |   | x | kg_ha_Fe_pisc        |           |   | x |   |   | x |
| Density_sp_native |           | x |   |   |   | x | perc_kgha_Fe_pisc    |           |   | x |   |   |   |
| Biom_sp_native    |           | x |   | x | x | x | n_sp_Fe_insev        |           | x |   | x | x | x |
| Density_sp_alien  |           | x | x | x | x | x | perc_sp_Fe_insev     | x         | x | x | x | x | x |
| Biom_sp_alien     |           | x | x | x |   | x | n_ha_Fe_insev        | x         | x | x | x | x | x |
| n_sp_Intol        |           | x |   | x | x | x | perc_nha_Fe_insev    | x         | x | x | x | x | x |
| perc_sp_Intol     | x         | x | x | x | x | x | kg_ha_Fe_insev       | x         | x | x | x | x | x |
| n_ha_Intol        | x         | x | x | x | x | x | perc_kgha_Fe_insev   | x         | x | x | x | x | x |
| perc_nha_Intol    | x         | x | x | x | x | x | n_sp_Fe_omni         | x         | x | x | x | x | x |
| kg_ha_Intol       | x         | x | x | x | x | x | perc_sp_Fe_omni      | x         | x | x | x | x |   |
| perc_kgha_Intol   | x         | x | x | x | x | x | n_ha_Fe_omni         | x         | x | x | x | x | x |
| n_sp_Tol          |           | x | x | x | x | x | perc_nha_Fe_omni     | x         | x | x | x | x |   |
| perc_sp_Tol       |           | x | x | x | x | x | kg_ha_Fe_omni        | x         | x | x | x |   | x |
| n_ha_Tol          | x         | x | x | x | x | x | perc_kgha_Fe_omni    | x         | x | x | x | x |   |
| perc_nha_Tol      |           | x | x | x | x |   | n_sp_Mi_long         |           | x |   | x | x | x |
| kg_ha_Tol         |           |   | x |   |   | x | perc_sp_Mi_long      |           | x |   | x |   |   |
| perc_kgha_Tol     |           |   | x | x | x | x | n_ha_Mi_long         |           | x |   | x | x | x |
| n_sp_Hab_wc       | x         | x | x |   |   | x | perc_nha_Mi_long     |           | x |   | x | x |   |
| perc_sp_Hab_wc    |           |   |   |   |   |   | kg_ha_Mi_long        |           | x |   | x |   | x |
| n_ha_Hab_wc       |           | x |   |   |   | x | perc_kgha_Mi_long    |           | x |   | x |   | x |
| perc_nha_Hab_wc   | x         |   | x |   |   |   | n_sp_Mi_potad        |           | x | x | x | x | x |
| kg_ha_Hab_wc      |           | x |   |   | x | x | perc_sp_Mi_potad     |           | x | x | x | x | x |
| perc_kgha_Hab_wc  | x         | x | x |   | x |   | n_ha_Mi_potad        |           | x | x | x |   | x |
| n_sp_Hab_b        |           | x | x |   | x | x | perc_nha_Mi_potad    |           | x | x | x |   | x |
| perc_sp_Hab_b     |           | x |   |   |   | x | kg_ha_Mi_potad       |           | x | x | x |   | x |
| n_ha_Hab_b        | x         | x | x |   |   | x | perc_kgha_Mi_potad   |           | x | x | x |   | x |
| perc_nha_Hab_b    | x         | x | x |   |   |   | n_sp_hist            |           | x |   | x | x | x |
| kg_ha_Hab_b       | x         | x | x |   |   | x | perc_sp_hist         |           | x |   | x | x | x |
| perc_kgha_Hab_b   | x         | x | x |   |   |   | perc_histsp_intol    |           | x |   | x | x | x |
| n_sp_Hab_rh       |           | x | x | x | x | x | perc_histsp_tol      |           |   | x |   |   | x |
| perc_sp_Hab_rh    |           | x | x | x | x | x | perc_histsp_hab_wc   |           | x |   | x | x | x |
| n_ha_Hab_rh       |           | x |   | x |   | x | perc_histsp_hab_b    |           | x |   | x | x | x |
| perc_nha_Hab_rh   |           | x | x | x | x | x | perc_histsp_hab_rh   |           | x |   | x | x | x |
| kg_ha_Hab_rh      |           | x |   | x | x | x | perc_histsp_hab_li   | x         | x | x | x | x | x |
| perc_kgha_Hab_rh  |           | x | x | x | x | x | perc_histsp_hab_eury |           |   | x |   |   | x |
| n_sp_Hab_li       | x         | x | x | x | x |   | perc_histsp_re_lith  |           | x |   | x | x | x |
| perc_sp_Hab_li    | x         | x | x | x | x |   | perc_histsp_re_phyt  | x         | x | x | x |   | x |
| n_ha_Hab_li       | x         | x | x | x | x | x | perc_histsp_lon_ll   |           |   | x |   |   | x |
| perc_nha_Hab_li   | x         | x | x | x | x |   | perc_histsp_lon_sl   |           | x |   | x | x | x |
| kg_ha_Hab_li      | x         | x | x | x | x | x | perc_histsp_fe_pisc  |           |   |   |   |   | x |
| perc_kgha_Hab_li  | x         | x | x | x | x |   | perc_histsp_fe_insev | x         | x |   | x | x | x |
| n_sp_Hab_eury     |           | x | x | x |   | x | perc_histsp_fe_omni  |           | x | x |   |   | x |

|                    |   |   |   |   |   |
|--------------------|---|---|---|---|---|
| perc_sp_Hab_eury   | x | x | x | x |   |
| n_ha_Hab_eury      | x | x | x |   | x |
| perc_nha_Hab_eury  | x | x | x | x |   |
| kg_ha_Hab_eury     |   | x |   |   | x |
| perc_kgha_Hab_eury | x | x | x |   |   |
| n_sp_Re_lith       | x | x | x | x | x |
| perc_sp_Re_lith    | x | x | x | x | x |
| n_ha_Re_lith       | x |   | x | x | x |
| perc_nha_Re_lith   | x | x | x | x | x |
| kg_ha_Re_lith      | x |   | x | x | x |
| perc_kgha_Re_lith  | x | x | x | x | x |
| n_sp_Re_phyt       | x | x | x | x | x |
| perc_sp_Re_phyt    | x | x | x | x | x |
| n_ha_Re_phyt       | x | x | x | x | x |
| perc_nha_Re_phyt   | x | x | x | x |   |
| kg_ha_Re_phyt      | x | x | x | x |   |
| perc_kgha_Re_phyt  | x | x | x | x | x |
| n_sp_Lon_ll        | x |   | x | x | x |
| perc_sp_Lon_ll     | x |   | x | x | x |
| n_ha_Lon_ll        | x |   | x | x | x |
| perc_nha_Lon_ll    |   |   | x |   | x |
| kg_ha_Lon_ll       | x |   | x |   | x |
| perc_kgha_Lon_ll   | x |   | x | x | x |
| n_sp_Lon_sl        | x | x | x |   | x |
| perc_sp_Lon_sl     |   |   |   |   |   |
| n_ha_Lon_sl        | x | x |   |   | x |
| perc_nha_Lon_sl    | x |   |   |   |   |
| kg_ha_Lon_sl       | x | x | x |   | x |
| perc_kgha_Lon_sl   | x |   |   |   | x |

|                      |   |   |   |   |   |
|----------------------|---|---|---|---|---|
| perc_histsp_mi_long  | x |   | x | x | x |
| perc_histsp_mi_potad | x |   | x | x | x |
| kgha_run1_intol      | x | x | x | x | x |
| kgha_run1_tol        |   | x |   |   | x |
| kgha_run1_hab_wc     | x |   |   | x | x |
| kgha_run1_hab_b      | x | x | x |   | x |
| kgha_run1_hab_rh     | x |   | x | x | x |
| kgha_run1_hab_li     | x | x | x | x | x |
| kgha_run1_hab_eury   |   | x |   |   | x |
| kgha_run1_re_lith    | x |   | x | x | x |
| kgha_run1_re_phyt    | x | x | x | x |   |
| kgha_run1_lon_ll     | x |   | x |   | x |
| kgha_run1_lon_sl     | x | x | x |   | x |
| kgha_run1_fe_pisc    |   | x |   |   | x |
| kgha_run1_fe_insev   | x | x | x | x | x |
| kgha_run1_fe_omni    | x | x | x | x | x |
| kgha_run1_mi_long    | x |   | x |   | x |
| kgha_run1_mi_potad   | x | x | x |   | x |
| biom_run1_all        | x |   |   |   | x |
| biom_run1_native     | x |   |   | x | x |
| biom_run1_alien      | x | x | x |   | x |

**Annex 2** : List of “*sentinel species metrics*” correlated with impact class (Spearman correlation,  $P < 0.0002$  after Bonferroni correction).

| metric                       | Fish type |   |   |   |   |   |
|------------------------------|-----------|---|---|---|---|---|
|                              | 1         | 2 | 3 | 4 | 5 | 6 |
| density_sp_sentinel_Abr_bra  |           |   |   |   |   |   |
| density_sp_sentinel_Alb_bip  |           |   |   |   |   |   |
| density_sp_sentinel_Asp_asp  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_bar  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_bab  |           |   |   | x | x |   |
| density_sp_sentinel_Bar_boc  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_com  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_cyc  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_gra  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_gui  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_haa  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_mer  |           |   |   | x |   |   |
| density_sp_sentinel_Bar_pel  |           |   |   |   |   |   |
| density_sp_sentinel_Bar_scl  |           |   |   |   |   |   |
| density_sp_sentinel_Cho_arr  |           |   |   |   |   |   |
| density_sp_sentinel_Cho_lem  |           |   |   |   |   |   |
| density_sp_sentinel_Cho_mie  |           |   |   |   |   |   |
| density_sp_sentinel_Cho_nas  |           |   |   |   | x |   |
| density_sp_sentinel_Cho_pol  |           |   |   |   |   |   |
| density_sp_sentinel_Cho_tox  |           |   |   |   | x |   |
| density_sp_sentinel_Cho_will |           |   |   |   |   |   |
| density_sp_sentinel_Cot_gob  | x         |   | x | x | x |   |
| density_sp_sentinel_Cot_poe  |           |   |   |   |   |   |
| density_sp_sentinel_Eco_pyg  |           |   |   |   |   |   |
| density_sp_sentinel_Eso_luc  |           |   | x |   | x |   |
| density_sp_sentinel_Huc_huc  |           |   |   |   |   |   |
| density_sp_sentinel_Leu_car  |           |   |   |   |   |   |
| density_sp_sentinel_Leu_cep  |           |   |   |   |   |   |
| density_sp_sentinel_Leu_idu  |           |   |   |   |   |   |
| density_sp_sentinel_Leu_leu  |           |   | x | x | x |   |
| density_sp_sentinel_Leu_ple  |           |   |   |   |   |   |
| density_sp_sentinel_Leu_pyr  |           |   |   |   |   |   |
| density_sp_sentinel_Leu_sou  |           |   |   |   |   |   |
| density_sp_sentinel_Lot_lot  |           |   |   |   |   |   |
| density_sp_sentinel_Pho_pho  |           |   |   |   |   |   |
| density_sp_sentinel_Pse_sty  |           |   |   |   |   |   |
| density_sp_sentinel_Rut_rut  |           |   |   |   |   |   |
| density_sp_sentinel_Sal_flu  |           |   |   |   |   |   |
| density_sp_sentinel_Sal_sal  |           |   |   |   |   |   |
| density_sp_sentinel_Sal_far  | x         | x | x | x | x | x |
| density_sp_sentinel_Sal_tru  |           |   |   |   |   |   |
| density_sp_sentinel_Sal_lac  |           |   |   |   |   |   |
| density_sp_sentinel_Sal_alp  |           |   |   |   |   |   |
| density_sp_sentinel_San_luc  |           |   |   |   |   |   |
| density_sp_sentinel_Sil_gla  |           |   |   |   |   |   |
| density_sp_sentinel_Squ_alb  |           |   |   |   |   |   |

| metric                    | Fish type |   |   |   |   |   |
|---------------------------|-----------|---|---|---|---|---|
|                           | 1         | 2 | 3 | 4 | 5 | 6 |
| perc_0plus_Huc_huc        |           |   |   |   |   |   |
| perc_0plus_Leu_car        |           |   |   |   |   |   |
| perc_0plus_Leu_cep        |           |   |   |   |   |   |
| perc_0plus_Leu_idu        |           |   |   |   |   |   |
| perc_0plus_Leu_leu        |           |   |   |   |   | x |
| perc_0plus_Leu_ple        |           |   |   |   |   |   |
| perc_0plus_Leu_pyr        |           |   |   |   |   |   |
| perc_0plus_Leu_sou        |           |   |   |   |   |   |
| perc_0plus_Lot_lot        |           |   |   |   |   |   |
| perc_0plus_Pho_pho        |           |   |   |   |   |   |
| perc_0plus_Pse_sty        |           |   |   |   |   |   |
| perc_0plus_Rut_rut        |           |   |   |   |   |   |
| perc_0plus_Sal_flu        |           |   |   |   |   |   |
| perc_0plus_Sal_sal        |           |   |   |   |   |   |
| perc_0plus_Sal_far        |           | x | x | x | x |   |
| perc_0plus_Sal_tru        |           |   |   |   |   |   |
| perc_0plus_Sal_lac        |           |   |   |   |   |   |
| perc_0plus_Sal_alp        |           |   |   |   |   |   |
| perc_0plus_San_luc        |           |   |   |   |   |   |
| perc_0plus_Sil_gla        |           |   |   |   |   |   |
| perc_0plus_Squ_alb        |           |   |   |   |   |   |
| perc_0plus_Thy_thy        |           |   |   |   |   |   |
| perc_0plus_Vim_vim        |           |   |   |   |   |   |
| biom_sp_sentinel_Abr_bra  |           |   |   |   |   |   |
| biom_sp_sentinel_Alb_bip  |           |   |   |   |   |   |
| biom_sp_sentinel_Asp_asp  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_bar  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_bab  |           |   |   |   | x | x |
| biom_sp_sentinel_Bar_boc  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_com  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_cyc  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_gra  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_gui  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_haa  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_mer  |           |   |   |   | x |   |
| biom_sp_sentinel_Bar_pel  |           |   |   |   |   |   |
| biom_sp_sentinel_Bar_scl  |           |   |   |   |   |   |
| biom_sp_sentinel_Cho_arr  |           |   |   |   |   |   |
| biom_sp_sentinel_Cho_lem  |           |   |   |   |   |   |
| biom_sp_sentinel_Cho_mie  |           |   |   |   |   |   |
| biom_sp_sentinel_Cho_pol  |           |   |   |   |   |   |
| biom_sp_sentinel_Cho_nas  |           |   |   |   |   | x |
| biom_sp_sentinel_Cho_tox  |           |   |   |   |   | x |
| biom_sp_sentinel_Cho_will |           |   |   |   |   |   |
| biom_sp_sentinel_Cot_gob  | x         |   | x | x | x |   |
| biom_sp_sentinel_Cot_poe  |           |   |   |   |   |   |

|                             |   |   |   |   |   |
|-----------------------------|---|---|---|---|---|
| density_sp_sentinel_Thy_thy | x |   |   | x |   |
| density_sp_sentinel_Vim_vim |   |   |   |   |   |
| density_0plus_Abr_bra       |   |   |   |   |   |
| density_0plus_Alb_bip       |   |   |   |   |   |
| density_0plus_Asp_asp       |   |   |   |   |   |
| density_0plus_Bar_bar       |   |   |   |   |   |
| density_0plus_Bar_bab       |   |   |   |   | x |
| density_0plus_Bar_boc       |   |   |   |   |   |
| density_0plus_Bar_com       |   |   |   |   |   |
| density_0plus_Bar_cyc       |   |   |   |   |   |
| density_0plus_Bar_gra       |   |   |   |   |   |
| density_0plus_Bar_gui       |   |   |   |   |   |
| density_0plus_Bar_haa       |   |   |   |   |   |
| density_0plus_Bar_mer       |   |   |   | x |   |
| density_0plus_Bar_pel       |   |   |   |   |   |
| density_0plus_Bar_scl       |   |   |   |   |   |
| density_0plus_Cho_arr       |   |   |   |   |   |
| density_0plus_Cho_lem       |   |   |   |   |   |
| density_0plus_Cho_mie       |   |   |   |   |   |
| density_0plus_Cho_nas       |   |   |   |   | x |
| density_0plus_Cho_pol       |   |   |   |   |   |
| density_0plus_Cho_tox       |   |   |   |   |   |
| density_0plus_Cho_will      |   |   |   |   |   |
| density_0plus_Cot_gob       | x |   | x | x | x |
| density_0plus_Cot_poe       |   |   |   |   |   |
| density_0plus_Eco_pyg       |   |   |   |   |   |
| density_0plus_Eso_luc       |   |   |   |   |   |
| density_0plus_Huc_huc       |   |   |   |   |   |
| density_0plus_Leu_car       |   |   |   |   |   |
| density_0plus_Leu_cep       |   |   |   |   |   |
| density_0plus_Leu_idu       |   |   |   |   |   |
| density_0plus_Leu_leu       |   |   |   |   | x |
| density_0plus_Leu_ple       |   |   |   |   |   |
| density_0plus_Leu_pyr       |   |   |   |   |   |
| density_0plus_Leu_sou       |   |   |   |   |   |
| density_0plus_Lot_lot       |   |   |   |   |   |
| density_0plus_Pho_pho       |   |   |   |   |   |
| density_0plus_Pse_sty       |   |   |   |   |   |
| density_0plus_Rut_rut       |   |   |   |   |   |
| density_0plus_Sal_flu       |   |   |   |   |   |
| density_0plus_Sal_sal       |   |   |   |   |   |
| density_0plus_Sal_far       | x | x | x | x | x |
| density_0plus_Sal_tru       |   |   |   |   |   |
| density_0plus_Sal_lac       |   |   |   |   |   |
| density_0plus_Sal_alp       |   |   |   |   |   |
| density_0plus_San_luc       |   |   |   |   |   |
| density_0plus_Sil_gla       |   |   |   |   |   |
| density_0plus_Squ_alb       |   |   |   |   |   |
| density_0plus_Thy_thy       |   |   |   |   |   |
| density_0plus_Vim_vim       |   |   |   |   |   |
| perc_0plus_Abr_bra          |   |   |   |   |   |

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| biom_sp_sentinel_Eco_pyg |   |  |   |   |   |   |   |
| biom_sp_sentinel_Eso_luc |   |  | x | x | x |   |   |
| biom_sp_sentinel_Huc_huc |   |  |   |   |   |   |   |
| biom_sp_sentinel_Leu_car |   |  |   |   |   |   |   |
| biom_sp_sentinel_Leu_cep |   |  |   |   |   |   |   |
| biom_sp_sentinel_Leu_idu |   |  |   |   |   |   |   |
| biom_sp_sentinel_Leu_leu |   |  |   | x | x | x |   |
| biom_sp_sentinel_Leu_ple |   |  |   |   |   |   |   |
| biom_sp_sentinel_Leu_pyr |   |  |   |   |   |   |   |
| biom_sp_sentinel_Leu_sou |   |  |   |   |   |   |   |
| biom_sp_sentinel_Lot_lot |   |  |   |   |   |   |   |
| biom_sp_sentinel_Pho_pho |   |  |   |   |   |   |   |
| biom_sp_sentinel_Pse_sty |   |  |   |   |   |   |   |
| biom_sp_sentinel_Rut_rut |   |  |   |   |   |   |   |
| biom_sp_sentinel_Sal_flu |   |  |   |   |   |   |   |
| biom_sp_sentinel_Sal_sal |   |  |   |   |   |   |   |
| biom_sp_sentinel_Sal_far | x |  | x | x | x | x |   |
| biom_sp_sentinel_Sal_tru |   |  |   |   |   |   |   |
| biom_sp_sentinel_Sal_lac |   |  |   |   |   |   |   |
| biom_sp_sentinel_Sal_alp |   |  |   |   |   |   |   |
| biom_sp_sentinel_San_luc |   |  |   |   |   |   |   |
| biom_sp_sentinel_Sil_gla |   |  |   |   |   |   |   |
| biom_sp_sentinel_Squ_alb |   |  |   |   |   |   |   |
| biom_sp_sentinel_Thy_thy | x |  |   |   | x |   |   |
| biom_sp_sentinel_Vim_vim |   |  |   |   |   |   |   |
| presence_0plus_Abr_bra   |   |  |   |   |   |   |   |
| presence_0plus_Alb_bip   |   |  |   |   |   |   |   |
| presence_0plus_Asp_asp   |   |  |   |   |   |   |   |
| presence_0plus_Bar_bar   |   |  |   |   |   |   |   |
| presence_0plus_Bar_bab   |   |  |   |   |   | x |   |
| presence_0plus_Bar_boc   |   |  |   |   |   |   |   |
| presence_0plus_Bar_com   |   |  |   |   |   |   |   |
| presence_0plus_Bar_cyc   |   |  |   |   |   |   |   |
| presence_0plus_Bar_gra   |   |  |   |   |   |   |   |
| presence_0plus_Bar_gui   |   |  |   |   |   |   |   |
| presence_0plus_Bar_haa   |   |  |   |   |   |   |   |
| presence_0plus_Bar_mer   |   |  |   |   |   | x |   |
| presence_0plus_Bar_pel   | x |  |   |   |   | x |   |
| presence_0plus_Bar_scl   |   |  |   |   |   |   |   |
| presence_0plus_Cho_arr   |   |  |   |   |   |   |   |
| presence_0plus_Cho_lem   |   |  |   |   |   |   |   |
| presence_0plus_Cho_mie   |   |  |   |   |   |   |   |
| presence_0plus_Cho_nas   |   |  |   |   |   | x |   |
| presence_0plus_Cho_pol   |   |  |   |   |   |   |   |
| presence_0plus_Cho_tox   |   |  |   |   |   |   |   |
| presence_0plus_Cho_will  |   |  |   |   |   |   |   |
| presence_0plus_Cot_gob   | x |  |   |   | x | x | x |
| presence_0plus_Cot_poe   |   |  |   |   |   |   |   |
| presence_0plus_Eco_pyg   |   |  |   |   |   |   |   |
| presence_0plus_Eso_luc   |   |  |   |   |   |   |   |
| presence_0plus_Huc_huc   |   |  |   |   |   |   |   |

perc\_Oplus\_Alb\_bip  
 perc\_Oplus\_Asp\_asp  
 perc\_Oplus\_Bar\_bar  
 perc\_Oplus\_Bar\_bab  
 perc\_Oplus\_Bar\_boc  
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 perc\_Oplus\_Bar\_gui  
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 perc\_Oplus\_Cot\_gob  
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presence\_Oplus\_Leu\_cep  
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 presence\_Oplus\_Leu\_pyr  
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 presence\_Oplus\_Lot\_lot  
 presence\_Oplus\_Pho\_pho  
 presence\_Oplus\_Pse\_sty  
 presence\_Oplus\_Rut\_rut  
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 presence\_Oplus\_Sal\_lac  
 presence\_Oplus\_Sal\_alp  
 presence\_Oplus\_San\_luc  
 presence\_Oplus\_Sil\_gla  
 presence\_Oplus\_Squ\_alb  
 presence\_Oplus\_Thy\_thy  
 presence\_Oplus\_Vim\_vim

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