

Divergent reaction of fish metrics to human pressures in fish assemblage types in Europe

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Abstract Using a large pan-European dataset, we compared least disturbed sites to sites impacted by human pressures across broad river types to assess which aspects of bio-ecological traits of the fish assemblage are most sensitive to alterations of the river ecosystem. To control for variation across river types and large-scale environmental gradients, we began by clustering the least disturbed sites ($n = 716$) into four homogenous fish assemblage types (FATs) differing by four fish metrics, i.e., lithophilic, rheophilic, omnivorous, and potamodromous fish. We predicted these FATs (headwater streams, medium gradient rivers, lowland rivers, and Mediterranean streams) using environmental variables, i.e., altitude, river slope, temperature, precipitation, latitude, and longitude for impacted sites in our dataset ($n = 2,389$). Using tests of sensitivity and intensity, 17 fish metrics

showed a clear reaction to human pressures. However, 12 metrics responded exclusively within only one of the four FATs. Hence we observed a divergent reaction of fish metrics to human pressures in, e.g., headwater versus lowland rivers. Type-specific reactions are useful in customizing impact assessment for particular river types. It is of primary importance to understand the comparative sensitivity and efficiency of fish-based indicators of water quality for detecting human-induced degradation of river ecosystems.

Keywords Fish · Trait · Assemblage metrics · Human pressure · River type · European running waters · Impact

Abbreviations

| | |
|--------------|---|
| EFT | European fish assemblage type (Melcher et al., 2007) |
| EQR | Ecological quality ratio |
| FAT | Fish assemblage type |
| HWS | Head water stream |
| LLR | Lowland River |
| MES | Mediterranean Stream |
| MGR | Medium Gradient River |
| Trait status | Fish species are assigned to a status that expresses their preferred habitat, diet, spawning habitat, oxygen supply, reproduction strategy, migration behaviour, etc. |
| Trait | Biological and ecological functional trait of fish species |

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Introduction

The development of fish-based methods to assess human pressures on the aquatic ecosystem has already been a long history. Based on the concept of the “index of biotic integrity” (IBI), (Karr, 1981) and under the frame of the “Clean Water Act”, many scientists tried to find appropriate fish metrics and fish indices for the assessment of the ecological status of running waters (Fausch et al., 1984; Lyons et al., 1996; Oberdorff et al., 2002; Hughes et al., 2004; Johnston et al., 2011). Many were successful but the results were restricted to regional extents.

In Europe, the entering into force of the EU Water Framework Directive (EU WFD) in December 2000 played a key role for scientific investigations, as from then, many scientists, environmental agencies and governmental institutions were confronted with the development of standardized fish-based assessment methods for the determination of the ecological status of European rivers. Especially the EU-funded projects FAME (FAME-Consortium, 2004) and (EFI+ Consortium, 2009) developed multi-metric indices based on fish assemblages and analysed relationships between human pressures and fish assemblages. Pont et al. (2007) already found 10 fish metrics (six based on species and four on density data) that showed strong responses to human pressures and therefore were qualified to form the “European fish index (EFI)”. In the FAME project, beside this “site specific approach”, the “spatially based approach” was considered, which classifies rivers into units with homogenous fish assemblages (Melcher et al., 2007). Accordingly, studies in multiple regions in Europe by Ferreira et al. (2007), Grenouillet et al. (2007), Melcher et al. (2007), Noble et al. (2007), Schmutz et al. (2007) and Virbickas & Kesminas (2007) aimed to find appropriate fish metrics that show reactions to human pressures.

Type-specific approach

According to Hughes et al. (1998), Karr & Chu (2000), Hering et al. (2006), Pont et al. (2006), Stoddard et al. (2008) and Logez & Pont (2011), to be applicable for anthropogenic impact assessment, the fish metric should be sensitive only to the variability of human pressure and not the environmental differences among sites. However, any IBI and IBI criteria incorporate natural

regional and local characteristics and therefore these IBIs may be poorly suited for application to areas outside those for which they were developed (Hughes & Oberdorff, 1999; Roset et al., 2007; Pont et al., 2009).

The crucial task is to appropriately delineate regions or river types to develop regional IBIs (Strange, 1998) because of large-scale natural variability in fish communities. Based on these facts, Schmutz et al. (2007) and Melcher et al. (2007) developed the European fish assemblage types (EFT) as an underlying concept for a spatially based method of classification (i.e., a river type-specific approach). They applied discriminant function analysis in two steps of the approach: (1) to predict EFT membership for impacted sites and (2) to find fish metrics that discriminate between the human pressures. However, this statistical method acts like a “black box”, as it tells main effects but not any thresholds. In addition, this method requires all variables to be transformed and it is not based on expected ecological responses, which limits ecological interpretations of the results. Furthermore, this approach was limited by the types of metrics tested (e.g., excluding biomass) and the information on pressure status available at that time at the European scale (mainly expert judgment). Our approach follows the idea of the “typological” method for grouping water bodies but uses biological data from river stretches instead of a priori definition of river types based on environmental characteristics. Our objectives therefore are (1) to describe and model homogenous functional fish assemblage types (FATs) at the European scale by species composition and environmental characteristics to make them predictable everywhere and (2) to identify significance and intensity of changes of fish assemblages upon human pressure within these FATs. We use fish metrics of species presence/absence, density, and biomass to measure characteristics of the fish assemblage.

Methods

A number of 3,105 sampling sites in 16 ecoregions and 14 countries were extracted from an extensive database (EFI+ Consortium, 2009) containing fish surveys conducted by several academic institutions and environmental agencies across Europe. Sites were sampled by electrofishing during low flow periods considering European standards (CEN, 2003). We apply two filters

to select a reliable, plausible and spatially independent dataset: first, a biological filter where only sites with fished areas greater than 100 square metres and more than 50 individuals caught are considered, to minimize the risk of false absences. Second a spatial filter to compensate for possible spatial autocorrelation, because of numerous sampling within many rivers. The spatial filter to obtain dispersed sampling sites was defined in three classes based on upstream catchment size and three thresholds for distance along stream network between sampling sites, respectively. Threshold for (1) small catchments ($<1,000 \text{ km}^2$) was $>5 \text{ km}$ distance, (2) for medium catchments ($1,000\text{--}10,000 \text{ km}^2$) $>10 \text{ km}$, and (3) for large catchments ($\geq 10,000 \text{ km}^2$) $>50 \text{ km}$. The dataset encompassed 2,079 rivers of which 1,553 (74.6%) rivers held only one sample site. Median catchment size was 82 km^2 and 90% of the sites had a catchment size below $1,000 \text{ km}^2$.

Pressure data

Fifteen pressure criteria for sampling sites were stored in the EFI+ database (EFI+ Consortium, 2009). Schinegger et al. (2011) categorized all 15 pressures into four main groups affecting hydrology, morphology, water quality, and connectivity of the river stretch. We categorized sites as impacted if any pressure occurred in one or more of these groups and as minimally disturbed if only slight pressures occurred. Minimally disturbed sites were used for fish assemblage type modelling and impacted sites for testing the fish metric reaction to pressure. In total, 716 sites were classified as minimally disturbed and thus 2,389 sites showed some impact in at least one of the four pressure groups (Fig. 1).

Fish metrics description

European fish species were classified in biological and ecological traits based on some degree of overlap in their ecological niches, regardless of taxonomic relationships (Pont et al., 2006; Noble et al., 2007; EFI+ Consortium, 2009; Logez et al., 2013). For each trait, each species was assigned to one of the different states (Logez et al., 2013), e.g., trait trophic guild of brown trout is assigned to the state insectivorous, trait reproduction of brown trout is assigned to the state lithophilic, and so on. The 116 fish species in the

dataset already were assigned to these states in the EFI+ project, based on published or grey literature and completed by expert judgment when necessary (EFI+ Consortium, 2009). The complete list of classified species can be retrieved from the EFI+ website (<http://efi-plus.boku.ac>). Fish metrics are a quantitative unit-specific measure of the fish assemblage and they were calculated as six selected metrics of the states: absolute number of species, density (ind. ha^{-1}) and biomass (kg ha^{-1}), and their percentages in the total number, density and biomass in the sample. In total, 129 metrics were pre-selected for further analyses (Appendix Table 4 in Supplementary material). According to Noble et al. (2007) and Virbickas & Kesminas (2007), these metrics reflect the most important bio-ecological functional traits of fish assemblages.

Fish metric reaction to pressures

Many fish metrics decrease in response to human pressures (less fish of a trait status leading to diminished density and biomass, disappearance of species) (Pont et al., 2006; Melcher et al., 2007). In contrast, several fish metrics represent generalist and tolerant species and thus tend to increase in response to human pressures (Logez & Pont, 2011) and therefore their testing for sensitivity and intensity is in reverse direction. The direction of a metric's reaction upon human pressures was set from literature and later used for the direction of the statistical tests (Karr, 1981; Verneaux, 1981; Grandmottet, 1983; Noble et al., 2007; Oberdorff et al., 2002). Metrics of the tolerance to pollution trait may increase or decrease under water quality degradation, depending if species do or do not tolerate degradation. Metrics of trophic guilds trait indicate changes in the food web due to human pressure (Karr, 1981; Oberdorff et al., 2002). Metrics of the insectivorous trait status decrease with the degree to what the invertebrate assemblage is degraded, metrics of the piscivorous trait status decrease as surrogate for missing prey fish, and metrics of the trait status omnivorous increase with the degree to what the food base is altered to favour species that can digest both plant and animal foods (generalists). Metrics of the reproduction trait reflect loss or gain of spawning habitats. Metrics of the lithophilic trait status tend to decrease in response to human disturbances such as siltation (Berkman &

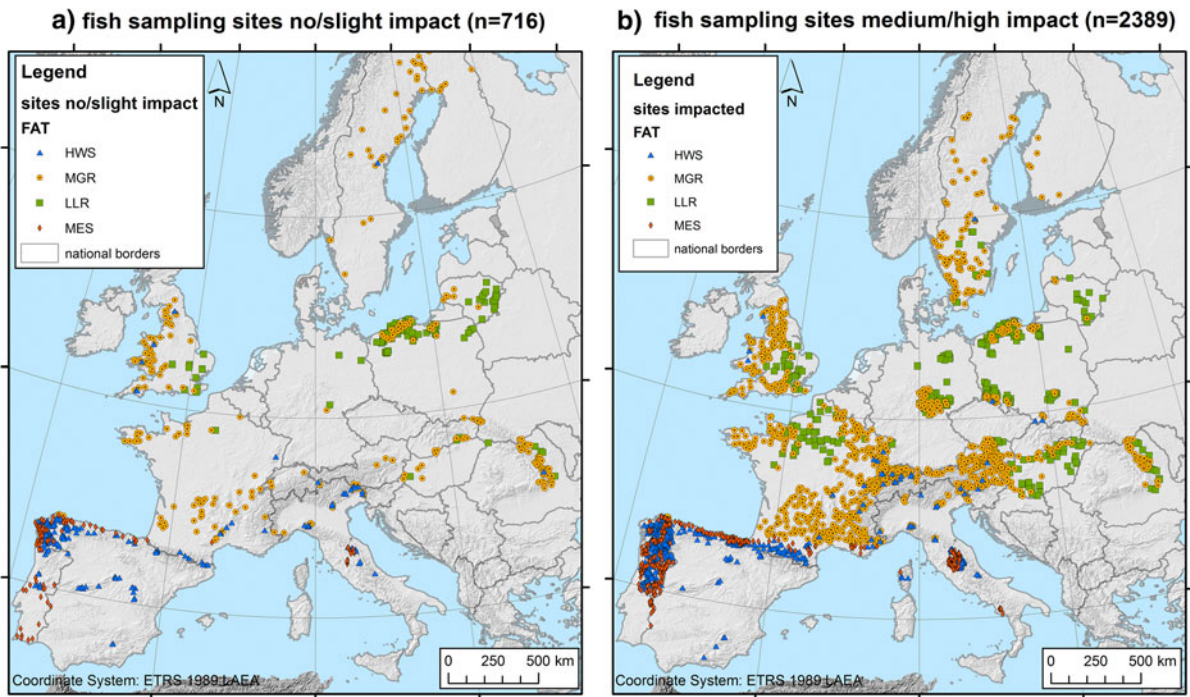


Fig. 1 **a** Assignment of minimally disturbed sites ($n = 716$) to four FATs, **b** geographical distribution of impacted sites ($n = 2,389$); *HWS* headwater streams (high gradient), *MGR* medium gradient rivers, *LLR* flat lowland rivers, *MES* mediterranean streams

Rabeni, 1987), whereas pelago-, phyto-, and polyphilic trait status metrics commonly increase with aquatic vegetation in relation with eutrophication or stagnant water conditions due to impoundments (Pont et al., 2006). Metrics associated with habitat guilds indicate alterations of aquatic habitats where fish prefer to live and feed. Metrics of the trait status intolerance to habitat degradation decrease with degraded habitats from morphological alterations (Karr, 1981; Oberdorff et al., 2002). Metrics of the trait status tolerance to habitat degradation and of the status eurytopic tend to increase with increasing pressure as associated species are more flexible and species of the status limnophilic may find their preferred habitat in impoundments (Noble et al., 2007). The total number of species measures species diversity and declines with environmental degradation (Karr, 1981) but under impacted conditions (e.g., eutrophication), this metric can increase because of higher productivity (Oberdorff et al., 2002). Especially headwaters naturally bear low numbers of species and alterations can lead to an increase of species (Logez & Pont, 2011). Out of a total of 129 metrics, 60 were defined as decreasing with human

pressure, 69 as increasing (see description and abbreviation of functional fish guilds and assumed direction of metric reaction, Appendix Table 4 in Supplementary material). True zero values can occur in metrics of rare species (e.g., status piscivorous) which may be absent in reference conditions and thus cannot decrease under impacted conditions. Hence, a metric response test is unfeasible and we set a criteria to >50% non-zero values in minimally disturbed sites for each metric classified as decreasing (>50% non-zero values in impacted sites for each metric classified as increasing, respectively).

Fish assemblage types (FATs)

To identify homogenous FATs we used fish metrics out of four different functional traits of fish species, i.e., relative species composition of lithophilic, omnivorous, potamodromous and rheophilic (Repro_LITH_%nsp, Atroph_OMNI_%nsp, Mig_POTAD_%nsp, Hab_RH_%nsp) characterizing a wide range of the functional diversity of the European fish fauna. Longitude and latitude were added to account for regionalization, i.e., to identify fish assemblages of

similar structure but disjunctive distribution across Europe. We applied agglomerative hierarchical clustering [R-package ‘cluster’, (Maechler et al., 2005)] with Ward’s method and Euclidean distance as similarity measure using only minimally disturbed sites of the dataset. The threshold for identifying distinct FATs was set by eye in the cluster dendrogram to find a feasible number of strong, well-separated FATs. The final input variables for the cluster algorithm were explored in an iterative process.

To describe the environmental characteristics of FATs, we conducted classification tree analysis [R-package ‘rpart’ (Therneau & Atkinson, 2011)] with FAT as the dependent variable and seven environmental variables as descriptors: altitude, river slope, mean annual precipitation, mean annual air temperature, mean temperature in January, latitude, and longitude. These variables were chosen because they describe both the regional position in the hydrographical network of European rivers and the organization of sites along the longitudinal continuum of rivers. Furthermore, those variables have been tested for their ability to structure fish assemblages across Europe in previous attempts (Hering et al., 2006; Sandin & Verdonshot, 2006; Melcher et al., 2007; Schmutz et al., 2007). The chosen model fitting algorithm ‘rpart’ uses a tenfold cross-validation. The training set is split into 10 roughly equally sized parts and the tree is grown on nine parts while using the tenth for testing (Venables, 2003). The results are averaged and expressed as xerror, which is the cross-validated error estimation of the model as mean square error of the predictions at each split in the tree.

The fitted classification tree model from minimally disturbed sites allows predicting the theoretical FATs for impacted sites by environmental variables. By comparing the mean metric values of minimally disturbed with impacted sites within each FAT we can define the FAT-specific sensitivity and intensity of the alteration of fish assemblages as a reaction to human pressures. To avoid extrapolation in the prediction, impacted sites outside the range of the environmental characteristics of the minimally disturbed sites (between 5% and the 95% percentile) were eliminated from the dataset.

Significance and intensity of fish metrics change

We used a one-sided Welch two sample *t* test with the alternative hypothesis that the true difference in means

is greater than zero (minimally disturbed—impacted) for those metrics supposed to decrease with human pressure. Increasing metrics were tested with the alternative hypothesis that the true difference in means is less than zero.

Furthermore, to identify the intensity of metric reaction between minimally disturbed and impacted condition, the ecological quality ratio (EQR) for each metric was calculated as follows:

$$\text{eqr}_{\text{FAT } i} = \frac{\bar{x}[\text{unimpacted}_{\text{FAT } i}]}{\bar{x}[\text{impacted}_{\text{FAT } i}]}$$

where *i* is one of the four defined FATs and \bar{x} is the arithmetic mean of fish metric values.

EQR is calculated inverse for increasing metrics:

$$\text{eqr}_{\text{FAT } i} = \frac{\bar{x}[\text{unimpacted}_{\text{FAT } i}]}{\bar{x}[\text{impacted}_{\text{FAT } i}]}$$

where *i* is one of the four defined FATs and \bar{x} is the arithmetic mean of fish metric values.

This is to ensure an EQR scale from 0 (bad quality) to 1 (minimally disturbed condition). A metric was selected if it reacted significantly to impacted conditions ($P < 0.05$) and if the EQR was less than 0.7—i.e., the difference between minimally disturbed and impacted condition was greater than 30%.

For the final metric selection, Pearson correlation analysis was conducted for the overall dataset and for each FAT in order to exclude redundant metrics (correlation higher than 0.7). Pairwise box-whisker-plots of minimally disturbed and impacted values of 17 final metrics visualize distribution, level and difference (placed to the Appendix in Supplementary material). Notches in boxes (Chambers et al., 1983) helped for interpretation of the results. The idea is to give roughly a 95% confidence interval for the difference in two medians (R Development Core Team, 2011).

All analyses were performed in R version 2.13.1 (R Development Core Team, 2011). Maps of the FATs were produced with ArcMap10.0 (ESRI, 2011).

Results

Fish assemblage types (FAT)

Cluster analysis resulted in four FATs that could clearly be drawn from the cluster dendrogram (Fig. 2).

Differentiation for the input fish metrics was clear for Head Water Streams (HWS): mean metric values were 100% lithophilic species, 100% potamodromous, 100% rheophilic, and 0% omnivorous species. Low gradient lowland rivers (LLR) bore highest mean of omnivorous (38%) and smallest mean of lithophilic species (37%). Medium gradient rivers (MGR) and Mediterranean streams (MES) showed similar means in lithophilic (67%, 75%), omnivorous (5%, 8%), and potamodromous (36%, 45%) but differed in the mean of rheophilic species (MGR: 91%, MES: 51%).

HWS are small streams in high elevations with high gradient, dominated up to 100% by brown trout (*Salmo trutta f. fario*); MGR represent larger cool rivers with medium gradient dominated by European minnow (*Phoxinus phoxinus*) and brown trout. LLR represent flat lowland rivers dominated by roach (*Rutilus rutilus*) and gudgeon (*Gobio gobio*), and finally MES represent small streams with medium-to-high gradient in Mediterranean climate. One-third of the fish in MES were brown trout, 11.8% European minnow and 7.0% dace (*Telestes souffia*). Figure 3 shows the environmental gradients of FATs, Table 1 their species composition.

The classification tree model could correctly classify 75% of the 716 minimally disturbed sites into the

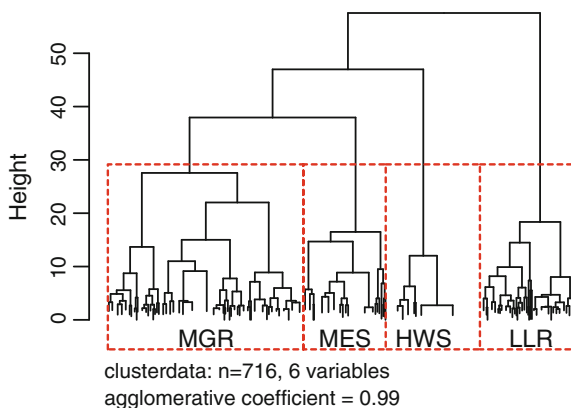


Fig. 2 Dendrogram of agglomerative hierarchical clustering; not all individual 716 minimally disturbed sites are shown because of technical reasons (last 2 branches are cut off); input variables: relative species composition of lithophilic, omnivorous, potamodromous and rheophilic (Repro_LITH_%nsp, Atroph_OMNI_%nsp, Mig_POTAD_%nsp, Hab_RH_%nsp) and geographical latitude and longitude. Dashed rectangles around branches of the dendrogram highlight clusters of fish assemblage types selected by graphical interpretation and later described as HWS headwater streams, MGR medium gradient rivers, LLR large lowland rivers, MES mediterranean streams

four FATs. The classification tree algorithm (rpart) selected only five of the seven environmental variables (altitude, slope, mean annual air temperature, mean annual precipitation and latitude) finally in tree construction. Correct classification ratios were 85% for HWS, 79% for MGR, 69% for LLR, and 61% for MES. The confusion matrix of predicted against original FAT (Table 2) shows that 43 sites (34%) of MES were wrongly predicted as HWS but none as LLR. MGR prediction is confused to 10% with LLR and MGR each. There was no misclassification of predicted LLR as HWS but 24% were misclassified as MGR. The validation of the model supports a quite stable model with an estimated error of 0.43 rising to 0.52 in tenfold cross-validation.

Latitude was the first split variable in the classification tree followed by river slope and altitude (Fig. 4). Further differentiation of the four FATs is achieved by mean annual temperature and annual precipitation in the upstream catchment. HWS sites had steep slopes (≤ 43.98 per mill) and occurred north and south of the latitude threshold. The left branches contain the majority of MGR sites. Their paths indicate that they are northerly of 43.7° latitude and between 3.39 and 43.98 per mill river slope. LLR sites were mainly located in rivers with a slope below 3.39 per mill with mean annual precipitation in the catchment below 727.7 mm. MES sites were in southern latitudes ($< 43.7^\circ$) at low altitudes (< 148 m.a.sl.) and highest mean annual temperatures ($\geq 11.65^\circ\text{C}$).

Finally, prediction of FATs for all sites classified 22% as HWS, 48% as MGR, 15% as LLR, and 15% as MES (Fig. 1).

Metric reaction

Before testing metric reactions to pressures we excluded 63 metrics out of 129 candidate metrics that did not fulfil the zero-value criteria ($> 50\%$ non-zero values). From the remaining 66 metrics, 63 metrics showed significant responses ($P < 0.05$) to pressures in at least one of the four FATs. Six metrics were significant through all FATs and 21 metrics were significant in one FAT exclusively. Thirty-two metrics did not fulfil the EQR criteria for intensity of the reaction (at least 30% difference between means) and thus, 31 metrics were retained (of which 21 metrics were reacting in one FAT exclusively).

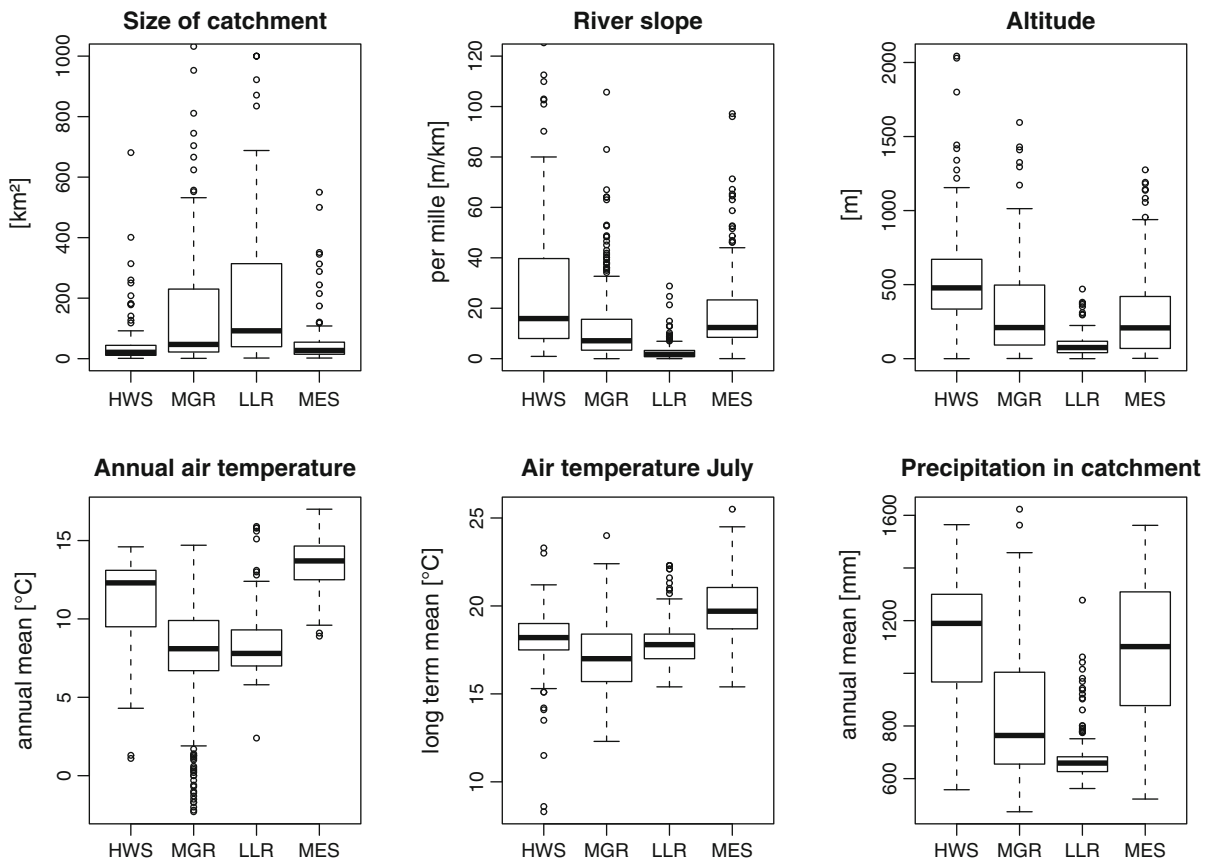


Fig. 3 Box-whisker-plots showing environmental differences across the four fish assemblage types; *HWS* headwater streams, *MGR* medium gradient rivers, *LLR* large lowland rivers, *MES* mediterranean streams

Finally, we excluded 14 out of 31 remaining metrics due to redundancy (based on correlation analysis within each FAT in an iterative process so that 17 unique metrics remained (Table 3). Three of these metrics measured biomass (kg ha^{-1}), three metrics density (ind. ha^{-1}), and two metrics the absolute number of species. The other 9 metrics are “relative” metrics (5 metrics percentage in the total number of species, 2 metrics percentage in density of total and 2 metrics percentage in total biomass of the sample).

Type-specific reaction of fish metrics to pressures

The metric selection procedure filtering for best reacting metrics resulted in different numbers of metrics for the four FATs: 6 metrics reacted in headwater streams (HWS), 2 metrics in MGR, 7 metrics in LLR, and 8 metrics in MES (Table 3).

There were five overlaps of metrics reacting in two or three FATs (Fig. 5) and, hence, 12 metrics were specific to one single FAT. The Venn diagram (Fig. 5) also indicates a separation between the FATs when there are no common metrics between two or more FATs. HWS and MGR did not share a metric, neither did MGR and LLR. In the appendix in Supplementary material we provide 23 pairs of box-whisker-plots of minimally disturbed and impacted sites in the four FATs (Appendix Fig. 7 in Supplementary material).

We found three bio-ecological traits as relevant for HWS, i.e., tolerance to pollution (two metrics of status intolerant), tolerance to low oxygen (two metrics of status intolerant), spawning habitat (one metric), and the metric number of species represented taxonomic composition (Fig. 6). All metrics decreased under impacted conditions expect total number of species. The increase of number of species metric can be considered as an impact as in this river type the fish

Table 1 Percentage of fish species of total catches in four fish assemblage types

| Headwater streams (HWS) | | Medium gradient riv. (MGR) | | Lowland rivers (LLR) | | Mediterranean streams (MES) | |
|-------------------------------|--------|----------------------------|--------|-------------------------------|--------|--------------------------------|--------|
| <i>Salmo trutta fario</i> | 70.5% | <i>Phoxinus phoxinus</i> | 25.6% | <i>Rutilus rutilus</i> | 15.9% | <i>Salmo trutta fario</i> | 33.6% |
| Pseudochondr dr. ^a | 7.6% | <i>Salmo trutta fario</i> | 20.0% | <i>Gobio gobio</i> | 13.7% | <i>Phoxinus phoxinus</i> | 11.8% |
| <i>Squalius pyrenaicus</i> | 5.6% | <i>Cottus gobio</i> | 9.6% | <i>Salmo trutta fario</i> | 12.0% | <i>Telestes souffia</i> | 7.0% |
| Achondr. arc. ^a | 4.2% | <i>Barbatula barbatula</i> | 9.3% | <i>Phoxinus phoxinus</i> | 11.0% | <i>Rutilus rubilio</i> | 6.4% |
| <i>Squalius carolitertii</i> | 3.3% | <i>Salmo salar</i> | 7.5% | Alburnoides bip. ^a | 8.4% | <i>Anguilla anguilla</i> | 6.4% |
| | | <i>Leuciscus cephalus</i> | 4.2% | <i>Cottus gobio</i> | 5.9% | <i>Squalius pyrenaicus</i> | 4.0% |
| | | <i>Gobio gobio</i> | 2.8% | <i>Barbatula barbatula</i> | 4.2% | <i>Leuciscus cephalus</i> | 3.7% |
| | | <i>Barbus petenyi</i> | 2.3% | <i>Leuciscus cephalus</i> | 3.9% | Pseudochondr. dr. ^a | 3.0% |
| | | | | <i>Rhodeus amarus</i> | 3.5% | <i>Squalius alburnoides</i> | 2.9% |
| | | | | Gasterosteus ac. ^a | 2.7% | Achondrostoma og. ^a | 2.8% |
| | | | | <i>Perca fluviatilis</i> | 2.5% | <i>Squalius aradensis</i> | 2.4% |
| Others | 9.0% | Others | 18.6% | Others | 16.1% | Others | 15.9% |
| Total number fish | 21,506 | | 51,503 | | 24,757 | | 12,538 |
| Mean # species | 1.66 | | 4.40 | | 7.23 | | 3.47 |
| SD # species | 0.92 | | 2.60 | | 3.23 | | 1.71 |

^a Alburnoides bip., *Alburnoides bipunctatus*, Gasterosteus ac., *Gasterosteus aculeatus*, Pseudochondr. dr., *Pseudochondrostoma duriense*, Achondr. arc., *Achondrostoma arcasii*, Achondrostoma og., *Achondrostoma oligolepis*

Table 2 Confusion matrix (number of sites) of predicted (rows) against original FATs (columns); prediction from classification tree with 5 environmental variables; original FAT were minimally disturbed sites as input for classification tree model

| | HWS | MGR | LLR | MES | Total |
|-------|------------|------------|-----------|-----------|-------|
| HWS | 123 | 29 | 0 | 43 | 195 |
| MGR | 16 | 239 | 35 | 6 | 296 |
| LLR | 1 | 29 | 98 | 0 | 128 |
| MES | 5 | 4 | 10 | 78 | 97 |
| Total | 145 | 301 | 143 | 127 | 716 |

HWS headwater streams, MGR medium gradient rivers, LLR large lowland rivers, MES mediterranean streams correctly predicted sites in bold

species richness is naturally very low (median number of species = 1). Abundance metrics occurred three times, biomass only once. Abundance of species intolerant to pollution (WQgen_INTOL_dens) had the lowest EQR (0.47) in HWS, which means that the mean value in impacted sites is reduced to 47% of the mean value in minimally disturbed sites. Four metrics were exclusively reacting in HWS and two metrics overlapped with MES and LLR.

Both reacting metrics in MGR represented the trait of tolerance to pollution. Species assigned to the trait status tolerance to pollution were largely not present in

minimally disturbed conditions and the median value of this metric rose to 1 species in impacted conditions. Therewith the relative density of pollution tolerants increased. The same effect was observed in MES.

In LLR four reacting metrics were associated with the tolerance to pollution trait, two with the trophic guild trait, and one with the tolerance to habitat degradation trait. Four metrics increased under impacted conditions. Box-whisker plots indicated that there is a wide range in the metric values for both minimally disturbed and impacted conditions. Variation in relative biomass of habitat degradation tolerants and relative density of pollution intolerants was high in both minimally disturbed and impacted conditions. Density of pollution intolerants also reacted in HWS and MES and relative density of pollution intolerants in MES.

In MES seven out of eight reacting metrics were from the trait tolerance to pollution, one from trophic guild. Metrics of the trait status intolerance to pollution and intolerance to low oxygen showed strong reactions. Relative density of pollution intolerants had a high variation in the data which is similar to its result in the assemblage type LLR. Lowest EQR was found for density of pollution intolerant species (WQgen_INTOL_dens, eqr = 0.38). Three of eight metrics were exclusively selected for MES, the other five overlapped with the selections for either HWS, or MGR, or LLR, respectively (overview in Fig. 5).

Fig. 4 Classification tree model for fish assemblage types as response variable (HWS headwater streams, MGR medium gradient rivers, LLR large lowland rivers, MES mediterranean streams; true split criteria at each node follows left branch; branch end numbers give original classification of response variable: HWS/MGR/LLR/MES; model validation: estimated error 0.43

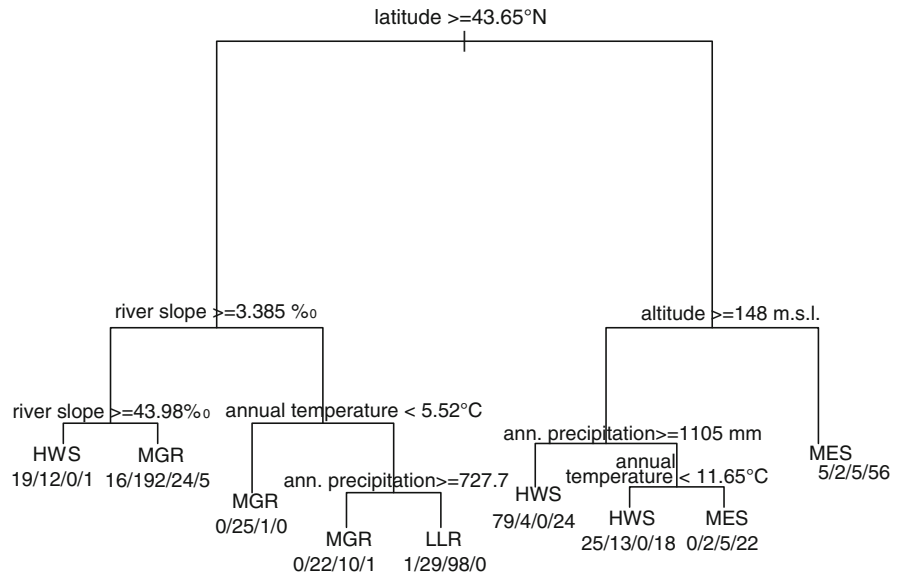


Table 3 Fish metrics reacting to pressures in four fish assemblage types

| Fish metric ^a | Trait ^b | m.react | pHW | qrHW | pMG | qrMG | pLL | qrLL | pME | qrME |
|--------------------------|--------------------|---------|------|------|------|------|------|------|------|------|
| Nsp_all | TX | Incr | 0.00 | 0.59 | 0.00 | 0.79 | 0.21 | 0.96 | 0.00 | 0.73 |
| HabSp_RHPAR_dens | SPH | Decr | 0.00 | 0.66 | 0.77 | 1.06 | 0.67 | 1.09 | 0.05 | 0.81 |
| WQO2_O2INTOL_biom | OXY | Decr | 0.00 | 0.68 | 1.00 | 1.50 | 0.59 | 1.05 | 0.00 | 0.54 |
| WQgen_INTOL_%nsp | POL | Decr | 0.00 | 0.69 | 0.57 | 1.01 | 0.00 | 0.73 | 0.00 | 0.61 |
| WQO2_O2INTOL_dens | OXY | Decr | 0.00 | 0.64 | 0.06 | 0.84 | 0.56 | 1.03 | 0.01 | 0.66 |
| WQgen_INTOL_dens | POL | Decr | 0.00 | 0.47 | 0.12 | 0.91 | 0.03 | 0.66 | 0.00 | 0.38 |
| WQgen_TOL_nsp | POL | Incr | | | 0.00 | 0.46 | 0.02 | 0.85 | 0.00 | 0.61 |
| WQgen_TOL_%dens | POL | Incr | | | 0.00 | 0.59 | 0.02 | 0.80 | 0.00 | 0.66 |
| WQO2_O2TOL_%nsp | OXY | Incr | | | | | 0.00 | 0.70 | 0.98 | 1.25 |
| WQgen_TOL_biom | POL | Incr | | | | | 0.00 | 0.36 | 0.34 | 0.93 |
| HTOL_HTOL_%biom | HD | Incr | | | 0.00 | 0.58 | 0.00 | 0.70 | 0.69 | 1.08 |
| Atroph_OMNI_%biom | TRG | Incr | | | | | 0.00 | 0.61 | | |
| Atroph_PISC_%nsp | TRG | Decr | | | | | 0.00 | 0.69 | | |
| WQgen_INTOL_%dens | POL | Decr | 0.00 | 0.69 | 0.70 | 1.02 | 0.00 | 0.63 | 0.00 | 0.49 |
| WQgen_INTOL_biom | POL | Decr | 0.00 | 0.72 | 1.00 | 1.94 | 0.70 | 1.13 | 0.01 | 0.56 |
| WQO2_O2INTOL_%nsp | OXY | Decr | 0.00 | 0.71 | 0.00 | 0.91 | 0.01 | 0.86 | 0.00 | 0.68 |
| Atroph_OMNI_%nsp | TRG | Incr | | | | | 0.02 | 0.88 | 0.00 | 0.65 |
| Number of metrics | | | HWS | 6 | MGR | 2 | LLR | 7 | MES | 8 |

^a Fish metric abbreviations in (Appendix Table 4 in Supplementary material)

^b Trait: TX taxonomic composition, SPH spawning habitat, OXY tolerance to low oxygen, POL tolerance to pollution, HD habitat degradation, TRG Adult trophic guild, m. react reaction of metric to pressure, incr increasing, decr decreasing; pHW p-value of t test between impacted and minimally disturbed sites in headwater streams (HWS), pMG p-value in medium gradient rivers (MGR), pLL p-value in lowland rivers (LLR), pME p-value in Mediterranean streams (MES), qrHW, qrMG, etc., ecological quality ratio of the metric in the four assemblage types, empty cell means metric did not fulfil zero-value criteria, italicised values shows the final selection in each FAT (correlation |r| < 0.70)

Fig. 5 Venn diagram showing overlap and nesting of relevant fish metrics for four fish assemblage types (FATs)

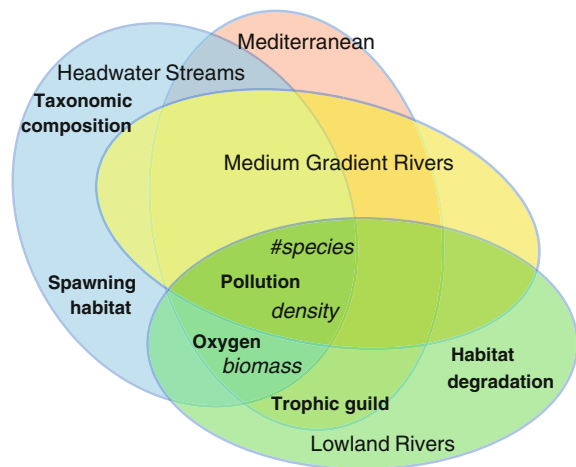
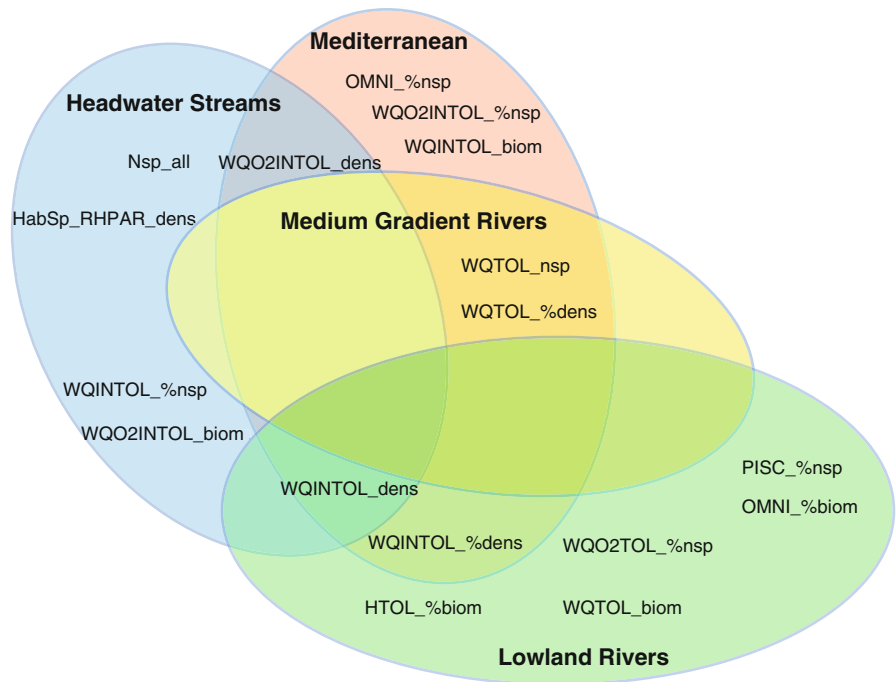


Fig. 6 Venn diagram for traits (in bold letters) and fish metrics (in italic letters) over four fish assemblage types

Crosschecking metric reactions in FATs as shown in Table 3 underlined that some metrics were clearly type specific. Four metrics of HWS were not significant in MGR and LLR and their EQR was bad in MGR and LLR. These metrics indicating intolerance status reacted significantly in MES though but with weaker intensity ($eqr > 0.73$ for two metrics) and so they seem to be very appropriate for small HWS.

Number of species and relative density of pollution tolerants are very general and hence significant in the *t* test in all four types but intensity of reaction was lower in LLR and furthermore they were filtered out by the selection criteria of frequent zero-values in HWS (absence of species assigned to the trait status tolerance to pollution even in impacted conditions). Relative number of species of trait status piscivorous and relative biomass of trait status omnivorous were exclusively selected for LLR because they did not fulfil the zero-value criteria in all other FATs. Piscivorous species are very rare in small streams and therefore are inappropriate to assess a reaction under impacted conditions in other FATs than LLR. Three metrics were exclusively selected for MES mainly because of EQR.

Summarizing, all reacting metrics in HWS were associated with some traits status of in tolerance to certain pressures, whereas metrics in MGR were associated with tolerance. In LLR and MES, both types of metrics (tolerant and intolerant) were reacting. Pollution trait showed a response in all river types in contrast to habitat related traits that only reacted in HWS and LLR. In terms of trophic guild we observed piscivorous species to decrease in LLR and omnivorous to increase in LLR and MES.

Discussion

This study is a river type based attempt to show the reaction of functional FATs to human pressures in European running waters. Pont et al. (2007) and the EFI+ Consortium (2007) already developed fish-based assessment methods using different metrics in salmonid and cyprinid river types. As it is unclear, whether a separation of salmonid and cyprinid river type is adequate, we investigated in a further subdivision into distinct FATs to account for different patterns of human impacts on fish. In the present study, we use two classes of impact (no/slight impact and moderate/strong impact) caused by human pressures. This simplification was necessary to overcome the multitude of combinations from four river types with 129 metrics. Schinegger & Trautwein (2013) focused on metric responses to specific and multiple pressures (hydro-morphological pressures or pollution pressures) and could identify metrics that responded specifically to pollution pressures and hydromorphological pressures.

However, human pressures are widespread and Schinegger et al. (2011) showed that rivers in the alpine region are predominately affected by hydromorphological pressures, whereas pollution pressures mostly in combination with hydromorphological pressures prevail in LLR.

Fish assemblage types

We classified fish assemblages at the European scale based on fish metrics out of four functional fish traits (rheophilic, lithophilic, omnivorous and potamodromous trait status). These metrics were selected based on the assumption that they give a representative overview of the dominating fish assemblages in minimally disturbed sites. To consider various river types and FATs across Europe, Melcher et al. (2007) and Schmutz et al. (2007) already developed fish types. However, they used stepwise discriminant analysis to predict the fish types for impacted conditions and could not analyse type-specific metric reactions because of the high number of FATs that were defined. In contrast, we searched for environmental variables that were able to properly characterize our FATs and used regression trees to predict FATs for impacted conditions.

Hering et al. (2006) analysed two main stream types, namely small mountain and medium-sized

lowland streams and did not find high correlation between fish metrics and human pressures (water quality and hydromorphology). Correlation was weakest for lowland streams although they were subdivided into seven subgroups. The predefined stream type classification may be inappropriate for fish as it was developed for macroinvertebrates (Hering et al., 2006; Verdonschot, 2006). Instead of using a classification system with abiotic variables, we were effective in developing FATs representative for most important river types from empirical data. Sandin and Verdonschot (2006) also analysed biological data in the STAR project and compared biological with environmental based types. They found comparable major types from both points of view: Mountains, Lowlands and Mediterranean. We worked out one additional FAT for MGR and this type was closest related to the Mediterranean stream type in biological terms. MGR and MES differed in the mean of rheophilic species although having similar river slopes. The main environmental difference lies in air temperature and precipitation in the catchment (Fig. 3).

Taxa and guild classification

The classification for species in terms of tolerance to pollution and oxygen depletion is overlapping. This means, a species assigned to status tolerant in the trait tolerance pollution can even be assigned to status intolerant in the trait tolerance to low oxygen. Four species are assigned as both tolerant to pollution and to oxygen depletion. Ten species are classified intolerant to both pollution and oxygen depletion. However, five species were classified as tolerant to pollution but not to oxygen depletion (intermediate). These species make the difference between the trait status tolerant to pollution and tolerant to low oxygen. In contrast, 2 species are intolerant to pollution but they tolerate intermediate oxygen depletion and hence differentiate between trait status intolerant to pollution and intolerant to low oxygen. In our results, the metrics reacting in HWS were of the trait tolerance to pollution and trait tolerance to low oxygen and mainly depend on the dominance of *Salmo trutta* fario but both metrics remained in the selection because correlation was below 0.7. The trait tolerance to pollution and tolerance to low oxygen for LLR and MES were based on higher numbers of classified species and were not dependent on one dominant species. In MES

for example, differentiation between the traits tolerance to pollution and tolerance to low oxygen was determined by *Barbus bocagei*.

In general, the number of piscivorous species is low and that's why only a small number of species/taxa in our samples are classified as piscivorous—mainly of the family Salmonidae but also *Perca fluviatilis* and *Esox lucius*. The other trophic metric, omnivorous species is calculated from more than 30 species, mainly of the family Cyprinidae. Piscivorous and omnivorous species' occurrence is higher in medium and large rivers than in headwaters and the present results showed that these metrics reacted for LLR only.

Metrics

We tested 129 metrics and finally selected 17 that showed a significantly different response under minimally disturbed/impacted conditions and analysed their specific reaction in four FATs. Pont et al. (2007) worked out 10 metrics as responsive to human pressures and implemented them in the EFI for all lotic systems in Europe. Compared to these 10 metrics, we found 5 trait status in common (omnivorous, rheophilic spawning, tolerant to pollution, intolerant to pollution, and migratory guild metrics) but showed that they divergently reacted in the FATs. However, metrics of the insectivorous, benthivorous, and lithophilic status were not represented in the results of the present study. Some metrics of the insectivorous and lithophilic status showed significant differences in the *t* tests for some FATs but reaction intensity was below our EQR criteria.

The EFI plus (EFI+ Consortium, 2007), a revised version of the EFI, consists of 4 final metrics (depending on the fish zone, two of these four metrics are selected): rheophilic reproduction habitat species richness, oxygen depletion intolerant species abundance, lithophilic reproduction habitat species abundance and abundance of individuals <15 cm of habitat intolerant species. In our selection, only the trait status related to oxygen depletion and rheophilic spawning were retained. Instead of habitat intolerants we found habitat tolerants; lithophilic metrics reacted significantly in three FATs but reaction was not strong and therefore this metric was not selected.

Our results confirmed the use of density metrics which are based on fish count data standardized by the

sampling area. In contrast, many other studies in the past used species presence/absence data for impact assessment of running waters (Fausch et al., 1984; Lyons et al., 1996; Oberdorff et al., 2002; Hughes et al., 2004; Johnston et al., 2011) either because of lacking standards in methods of sampling or lack of data due to higher costs in the sampling. In our analyses, metrics based on presence/absence (nsp) responded in all four river types (Fig. 6), as did density metrics. Density metrics are important to reflect degradation before species are extirpated. Biomass metrics reacted in HWS, LLR, and MES. Biomass gives important information about the populations' health and we expected metrics measuring biomass to react more sensible than nsp. The use of quantitative fish metrics based on density and biomass was an important component in our results; otherwise the trait status spawning in running waters or trait status tolerance to habitat degradation, the only two metrics concerning habitat quality, would not have shown up to be responsive in any of the four FATs. Higher biomass indicates larger fish that might be more susceptible to habitat degradation such as channelization.

Species composition, density metrics occurred relevant in all four FATs and biomass metrics in all but MGR (Fig. 6). Schinegger & Trautwein (2013) found that most metrics of any functional trait responded to multiple pressures instead to specific pressures. Further research should investigate whether impact strength (e.g., low, medium, and strong) is better explained by density or biomass metrics.

Metrics of tolerance to pollution trait proved to be significant in all four FATs and tolerance to low oxygen trait were significant in HWS, LLR, and MES (Fig. 6). Trophic guild metrics were significant in LLR and MES. However, we observed a differentiation by bio-ecological functional traits between the four FATs for the traits spawning habitat, habitat degradation, and taxonomic composition.

Weaknesses and uncertainties

In the EFI+ project, 14 countries provided data to assemble a common database and hence the dataset is heterogeneous in terms of geographical and ecological regions covered as well as river types represented. In general, the quality of the dataset is high as it underwent several stages of quality checks during data collection, database management, and the site

selection for the present study. However, the dataset lacks data from large rivers and especially reference data from large rivers. Therefore, our results are focusing on medium sized rivers and streams.

For fish data, the CEN norm (CEN, 2003) for electric fishing was considered for all the samples in the EFI+ database. Pressure data were collected from multiple sources (Schinegger et al., 2011) and the information was harmonized in an ordinal ranking scheme along a gradient ranging from 1 (nearly undisturbed) to 5 (strongly impacted) (EFI+ Consortium, 2007). Nonetheless, multiple sources and harmonization were likely bringing noise in the data that weakened the signal of impact in our results.

Conclusions

The development of FATs was very challenging for a European continental scale. We identified river types along a gradient of catchment size and slope (from headwaters to lowlands) whereas the type MES is mainly a type of headwaters with more Mediterranean climate (higher air temperatures). Later in the interpretation metric reactions it became obvious that response of fish assemblages to human pressure was divergent in the four FATs. Especially MGR appeared as biologically well separated type despite similar catchment size to LLR. We conclude that density and biomass metrics of biological and ecological functional fish traits are useful in impact assessment in distinct river types. The future development of assessment methods should take type-specific response into account. It is of primary importance to gain a comparative idea of the sensitivity and efficiency of these different indicators in detecting river human-induced degradations.

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