

Research article

<https://doi.org/10.26496/bjz.2021.89>

A new fish-based index of biotic integrity for lowland rivers in Flanders (Belgium)

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Abstract. The first fish-based index to assess the ecological quality of lowland rivers in Flanders (Belgium) is based on data obtained from different fishing techniques without considering the gear specificity. As a consequence, this index could not be intercalibrated with other European indices which concentrate on one gear type only. In order to comply with the European Water Framework Directive, we developed a new fish-based index using data obtained from surveys in rivers with electric gear only. All 293 selected rivers belonged to the bream or barbel zone. An updated reference list of fish species was compiled based on previous work and recent data. Abiotic data were collected according to standard methods and habitat quality of all surveyed sites was pre-classified using pressure indicators. To develop the new index candidate metrics were selected from the literature and metric values were calculated. Linear mixed regression models selected metrics based on their response to the pre-classified habitat status. Correlation tests were performed to avoid redundancy among responsive metrics. Boundaries for metric scores were defined based on the calculated metric values. The new index of biotic integrity (IBI) was calculated by summation of the metric scores, and transformed to an ecological quality ratio (EQR), ranging between 0 and 1. Five integrity classes, ranging from bad to maximal ecological potential, were attributed and compared to the pre-classified habitat status of the site. In addition, the new index was also validated with an independent set of data. The new IBI proved to successfully assess the ecological status of the rivers.

Keywords. Multimetric fish-based index, fish reference list, functional guilds, lowland rivers, European Water Framework Directive.

BREINE J., VAN DEN BERGH E., VAN THUYNE G. & BELPAIRE C. (2021). A new fish-based index of biotic integrity for lowland rivers in Flanders (Belgium). *Belgian Journal of Zoology* 151: 107–137.
<https://doi.org/10.26496/bjz.2021.89>

Introduction

Fish are sensitive to changes in habitat quality in rivers (KARR 1981; FAUSCH *et al.* 1990; MINIER *et al.* 2015). Spatial and temporal variability of the environment influences physiological and morphological characteristics of fish and affects their behaviour (TOWNSEND & HILDREW 1994). Fish populations in many European rivers are impacted by deteriorated habitat and water quality. With the European Water

Framework Directive (WFD) the European Union established a framework for the protection, recovery, and rehabilitation of all EU waters. The common goal of the WFD is to achieve and maintain “good ecological status” for all aquatic habitats. The WFD instructs using phytoplankton, macrophytes, benthic invertebrates and fish as biological quality elements to assess the ecological status of rivers (EU WATER FRAMEWORK DIRECTIVE 2000). As a consequence, fish-based indices were developed throughout Europe to assess the ecological quality of rivers (e.g., BELPAIRE *et al.* 2000; KESMINAS & VIRBICKAS 2000; KESTEMONT *et al.* 2000; ANGERMEIER & DAVIDEANU 2004; PONT *et al.* 2006). Fish-based indices are calculated using multiple metrics (KARR 1981). Each of these metrics is a variable assessing an ecological attribute of a fish community that is sensitive to human impact and reacts unambiguously to impact changes (BREINE *et al.* 2015).

In Flanders (Belgium) a fish-based index for rivers was developed by BELPAIRE *et al.* (2000) and subsequently used to report river habitat quality following the WFD requirements. Intensive application of this index has revealed some shortcomings and the need for improvement. First the robustness of this index is questioned as it is based on data obtained using electric fishing, seine netting, fyke nets and gill nets without considering gear specificity. Different sampling methods give selective or biased estimates of fish species abundance, distribution and size structure (LAPONTE *et al.* 2006; JURVELIUS *et al.* 2011; HAN *et al.* 2019). PASQUAUD *et al.* (2012) evaluated monitoring results from four different fishing techniques in the Gironde River, demonstrating that sampling gear had a significant effect on ecological assessments. Moreover, fish responses to particular anthropogenic pressures are gear type specific, hence indices should be tailored to the gear used in the surveys (CHOW-FRASER *et al.* 2006). Second, new robust statistical approaches are now available and have been demonstrated useful for the development of new fish-based indices (e.g., MOSTAFAVI *et al.* 2019; DÉZERALD *et al.* 2020). Third, the European fish index, which was developed as an alternative for existing local indices (PONT *et al.* 2006), is not a valid alternative because it does not assess Flandrian rivers correctly as it is based on references that do not occur in Flanders (QUATAERT *et al.* 2007).

We hypothesise that a more robust fish-based index for rivers in Flanders (the northern part of Belgium) can be designed and validated, based on single fish gear data and a reliable statistical approach that allows to reduce the use of expert judgement for assessment. Only fish data from lowland rivers belonging to the bream and barbel zones (HUET 1949), obtained with electric gear in accordance with the CEN standards (CEN 2003) were used. Our study took advantage of an extensive set of data collected over more than 20 years during 1545 fish stock surveys in 675 sites in Flanders. The different steps in the index development are those described by HERING *et al.* (2006).

Material and methods

Fish data and sampling method

Two data sets were established: one to develop the index (development data set) and one for external validation of the index (validation data set). For the development data set surveys between 1993 and 2015 were used: 722 surveys from 337 sampling sites in bream type rivers and 823 surveys from 348 sampling sites of barbel type rivers. These sites are spread over 281 rivers (Fig. 1). Surveys between 2015 and 2018 were used for the validation data set: 62 surveys in bream type rivers and 108 in barbel type rivers. This data set comprises 86 rivers (validation data set). All rivers were characterised as bream or barbel zone based on riverbed slope (measured on maps) and cross section (measured in the field) according to the HUET (1949, 1962) typology. River width varies between 0.25 and 30 m and the slope is below 3%. All rivers have suffered from human impacts such as dam construction, canalisation, agricultural activities or urbanisation.

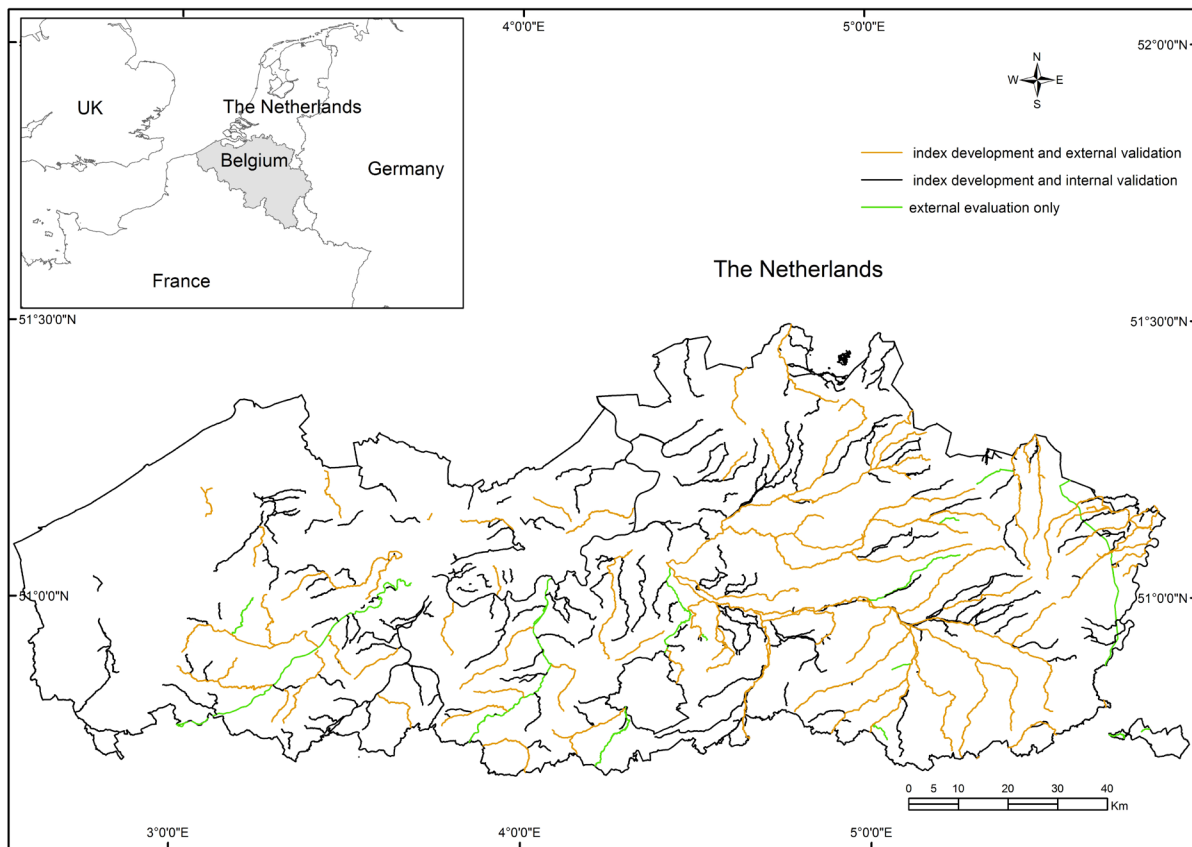


Figure 1 – Overview of assessed rivers (1993–2015) and rivers used for external validation (2015–2018).

Sampling was done year round except for winter. Season was defined according to the meteorological definition: spring includes March, April, and May; summer includes June, July, and August; fall includes September, October, and November. Fish assemblages' data was collected by electric fishing. Trained fish biologists and technicians assessed the rivers either by wading or from a boat. In rivers less than 1 meter wide only one anode was used, for larger rivers two hand-held anodes were used. A 5kW generator with adjustable output voltage generated between 300 and 500V and a pulse frequency of 480Hz. When wading, electric fishing was carried out over a standard distance of 100 m in upstream direction. In rivers less than 8 m wide the total width was surveyed, in larger rivers we monitored 2 m along both banks. From a boat a section of 250 m (2.5 m along both banks) was surveyed. The surveyed river bank length occasionally varied due to obstructions or other disturbances. Each captured individual fish was identified, measured (total length (TL), nearest 0.1 cm) and weighed (nearest 0.1g). To correct for differences in sampling effort, survey results were expressed as catch per unit effort (CPUE), i.e., survey data (numbers of individuals and biomass) were standardised to catch results per m². Data are available from the Fish Information Database (VIS databank: <http://vis.inbo.be>) and in BROSENS *et al.* (2015) and VAN THUYNE *et al.* (2020a).

To assess the relevance of separate indices for the bream and barbel zones we analysed differences between the fish assemblages in these two river types. A Detrended Correspondence Analysis (DCA) using the relative percentage of the 20 most abundant species was performed.

Habitat characteristic

Water quality data recorded in the field with digital multimeters were dissolved oxygen (mg/l), pH, water temperature (°C), conductivity ($\mu\text{S}/\text{cm}$) (Hach HQ40d) and turbidity (NTU, Hach 2100Q). The river width (m) was calculated as the average of three measurements with a tape measure above the water surface at the sampling site. To assess the level of anthropogenic disturbance several variables were recorded and (semi)quantified in an area up to 50 m inland and over a stretch of 250 m up- and downstream along each sampling site (see Pre-classification of habitat quality status).

Index development

Index development was stepwise (Fig. 2), starting with pre-classification of the sites based on a habitat quality assessment. A reference list of fish species attributed to ecological guilds was set up and used for the calculation of candidate metric values. Application of several statistical methods enabled the final selection of metrics. Selected metrics are scored using the development data set, and the Ecological Quality Ratio (EQR) is calculated defining integrity classes. Finally, the validation data set enabled us to evaluate the new index on its performance.

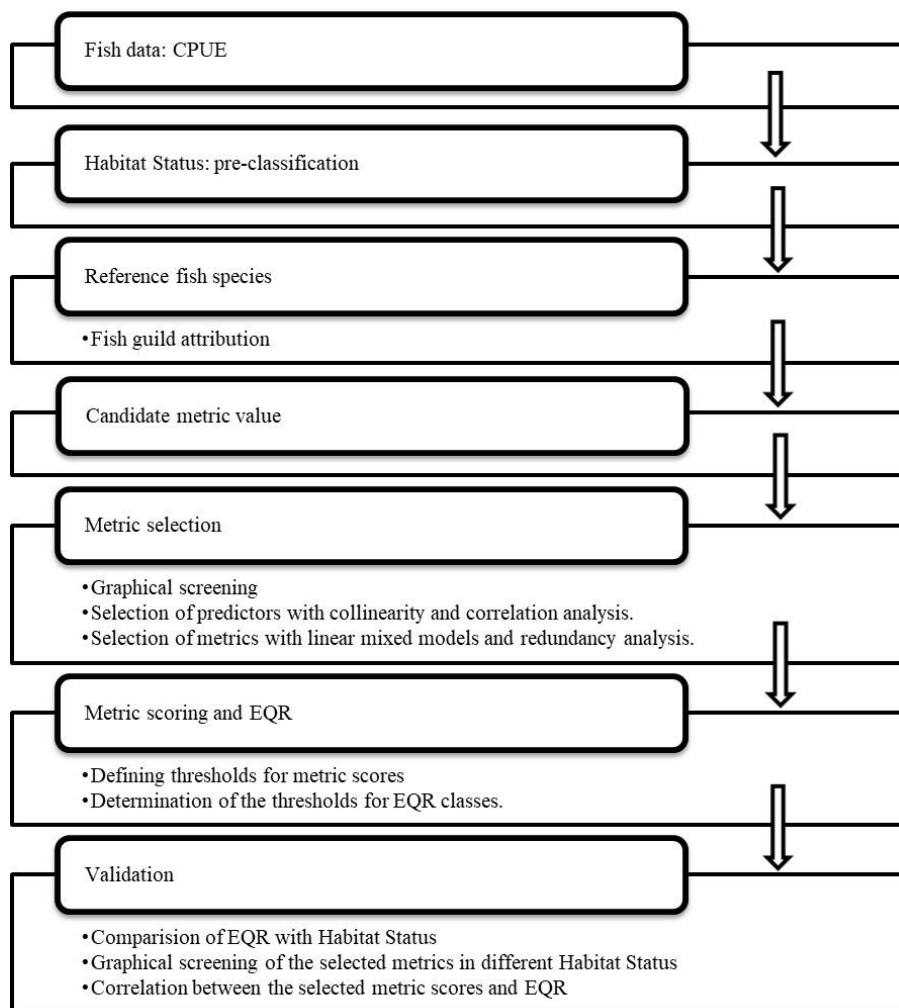


Figure 2 – Flowchart presenting the different steps of the development of the new fish-based multimetric index for lowland rivers in Flanders.

Pre-classification of habitat quality status

The Habitat Status (HabStat) was defined using the habitat characteristics recorded in the field or via Google Earth in those cases where data were incomplete (ERGÖNÜL *et al.* 2019). The variables included in the pre-classification are: **shelter for fish** (e.g., tree trunks, roots, plants), **natural banks** or reinforced (visual observation of concrete, stones, etc.), **industry** (industrial activities, e.g., factories), **agriculture activity** (e.g., crop culture), **urbanisation** (constructions or buildings) and % **trees** obtained by visual estimation. **Meadows** as a proxy of cattle, manure etc., **river course** alteration (e.g., canalization) and **presence of barriers** for fish passage upstream and downstream. Threshold and scores are given in Table 1. The sum of the score of variables (SSV) ranged between 0 and 20 and was transformed for standardisation into a habitat status score (HabStat) ranging between 0 and 4 as follow: HabStat=0 for SSV=0; HabStat=1 for SSV=1-5; HabStat=2 for SVV=6-10; HabStat=3 for SSV=11-15 and: HabStat=4 for SSV=16-20.

TABLE 1

Thresholds and scores for variables to define the pre-classified habitat status.

variable/score	0	1	2	3	maximum value
shelter for fish	none	few patches	on one bank	on both banks	3
banks	>50% reinforced	<50% reinforced	natural		2
industry	on both banks	on one bank	absent		2
agriculture activity	on both banks	on one bank	absent		2
urbanisation	>10 buildings	5<>0 buildings	<5 buildings	absent	3
trees	absent	<10% of area	10<>50% of area	>50% of area	3
meadows	on both banks	on one bank	absent		2
river course	altered	slight change	no change		2
barriers	present	absent			1

Reference fish species and attribution to ecological guilds

BELPAIRE *et al.* (2000) provided a list of 40 fish species occurring in lowland rivers in Flanders. We updated this list and differed between Good and Maximal Ecological Potential (GEP and MEP) status. Fish species were omitted 1) that were locally or regionally extirpated (see VERREYCKEN *et al.* 2014), 2) for which the bream or barbel zone as described by HUET (1950, 1962) is not the preferred habitat (see also BREINE *et al.* 2015) and, 3) species defined as non-indigenous according to VERREYCKEN *et al.* (2007) based on historical and archaeological records. Exceptions in our list are pike-perch (*Sander lucioperca* Linnaeus, 1758) and common carp (*Cyprinus carpio* Linnaeus, 1758) as we consider them as naturalised. We added species that were frequently caught and not yet included. Species that are rarely caught (i.e., <5% catch frequency), are retained in the list representing the MEP, but not in the GEP list (BREINE *et al.* 2011).

All species on the final GEP and MEP lists were attributed to metrics referring to ecological guilds based on a literature review (BREINE *et al.* 2004, 2005). The guilds refer to habitat specificity, reproduction, tolerance to oxygen deficiency and habitat degradation, migration and feeding strategies.

Candidate metrics

Ecologically-relevant candidate metrics (e.g., number of species, percentage of specialised spawners) were selected from literature (see BELPAIRE *et al.* 2000; BREINE *et al.* 2015). These candidate metrics reflect major fish community attributes such as species richness, trophic function etc. Metric values were calculated using reference species only (BREINE *et al.* 2010) except for the Shannon-Wiener index which was calculated using all species present at the site and the metric assessing pioneers including three non-native species.

Metric selection

To explore the response of the candidate metrics to environmental pressures graphically boxplots were used to show how the metric distribution changes along the HabStat score (BREINE *et al.* 2007). Then the response of candidate metrics to predictors (i.e., pH, water temperature, dissolved oxygen, turbidity, river width, season and HabStat) was analysed with linear mixed regression models as described in ERGÖNÜL *et al.* (2018). To retrieve less-skewed distribution, percentage metrics were square-root transformed and count metrics were log-transformed ($\log(x+1)$) (LAUNOIS *et al.* 2011). Diversity metrics (e.g., Shannon-Wiener Index) were not transformed. Only uncorrelated predictors were used in the model. Correlation analysis was done with bivariate Pearson correlation tests (ZUUR *et al.* 2009). In addition, collinearity analyses were performed with a Variance Inflation Factor (VIF) analysis as follows: $VIF(\ln(\log(\text{variable}+1)) \sim \log(\text{variable}_1+1) + \log(\text{variable}_2+1) + \dots + \text{season})$. The predictors with a VIF value higher than 3 were excluded from modelling (ZUUR *et al.* 2009). Secondly, the remaining predictors were rejected if they were highly intercorrelated ($c \geq 0.7$; $p < 0.001$) (TABACHNICK & FIDELL 1996; MOSTAFAVI *et al.* 2019). Sampling site was added as a crossed random effect and sampling date (year) was added as nested random effect. Season (spring, summer or autumn) was added as a factor. Normality assumptions were assessed with the residual plots (Q-Q plots).

To define the goodness-of-fit, the marginal and conditional R^2 values for each fitted model were calculated as described by NAKAGAWA & SCHIELZETH (2013). Only the metric response to pressures was decisive for the selection (R^2 conditional $> 35\%$). Four classes of river width were defined by graphical analyses with smoother of number of species in function of river width. The ascending part was divided in three equal parts and the threshold for the next part is defined when the smoother reaches its plateau. Boxplots visualised patterns of the variation with river width classes of the value of metrics fitted with R^2 conditional $> 35\%$. Redundancy of responsive metrics was analysed with a Pearson correlation. Among the correlated metrics ($c \geq 0.7$; $p \leq 0.001$), the one with the best fitted model was chosen. For the less correlated metrics that were fitted by the model ($c < 0.7$ and ≥ 0.5 ; $p \leq 0.05$), the one that least correlated with other metrics was selected. The fact that a certain metric contains valuable ecological information required by the WFD can overrule the decision when selection criteria mentioned above were not fulfilled.

The statistical software used was R.4.0.2. with packages ImerTest, lme4, nlme and MuMIn (R CORE TEAM 2020).

Scoring selected metrics and EQR

Fish samples should contain a minimum number of individuals for a consistent estimation of the assemblage structure (LOGEZ & PONT 2011). We based the definition of this minimum on the average number of individuals caught in sites with HabStat 1. To correct for catch inefficiency 90% of this average value is used as a benchmark (BREINE *et al.* 2005; VAN LIEFFERINGHE *et al.* 2010). Depending on the selected metric, river width or season is taken into account for scoring. Based on the reference list, a number of species is defined for each selected metric counting species, representing the values

expected in the GEP status. To correct for catch inefficiency we take arbitrarily 70% of the calculated GEP value as the minimum threshold value for metric score 4. Threshold value determination for the selected metrics in other classes (bad, poor or moderate) was based on a combination of its average value in a particular river width class or season and trisection of this value (KARR 1981). This average reflects the lower boundary score for the moderate status (score 3) as most of the sites of this type of rivers in Flanders are considered to be in the moderate status (VAN THUYNE *et al.* 2020b). MEP thresholds (score 5) could be attributed by using the calculated GEP value as the lower score. When the GEP threshold value for a particular metric could not be calculated from the reference (e.g., percentage metrics), it was defined by trisection of the average value in a particular river width class or season. If a percentage metric increases in value with increasing pressure, a minimum value is defined by trisection percentage below which again a low score is given. If a percentage metric decreases in value with increasing pressure, a maximum value is defined by trisection percentage except for metrics assessing recruitment. The detailed scoring procedure of the selected metrics is given in appendix (scoring selected metrics).

For each sampling site total IBI was calculated as the sum of the scores of the selected metrics. To be compatible with the WFD, the IBI values are then transformed to EQR values. The EQR is a numerical value ranging between 0 (bad status) and 1 (MEP) and is calculated with the following formulae: $EQR = (IBI - \text{lowest IBI possible}) / (\text{maximum IBI possible} - \text{lowest IBI possible})$ (HERING *et al.* 2006). Integrity classes (e.g., bad, poor, moderate, GEP and MEP) were defined as described in BREINE *et al.* (2015).

Validation

Allowing a class difference of one unit (small Type I or II error see BREINE *et al.* 2007, 2010), the index was validated by comparing the integrity class obtained per site with its HabStat status. We assessed data of sites used for the index development and an independent set of data. EQR scores were graphically screened with HabStat scores using both data sets. In addition a Spearman's rank test (2-tailed) assessed the correlations among the selected metric-scores and the EQR.

Results

Fish assemblage

A DCA with the relative numbers of the 20 most abundant species shows a big overlap between the barbel and bream zone assemblages (Fig. 3). Because there is no clear difference, we group all data together for further analyses and only one common index for both river types will be developed.

Between 1993 and 2015 45 fish species were caught 10 of which are non-indigenous. In total 216 062 individuals were caught or 0.3 individuals per surveyed m². In both bream and barbel zones three-spined stickleback (*Gasterosteus aculeatus* Linnaeus, 1758) is the most common species followed by gudgeon (*Gobio gobio* Linnaeus, 1758), roach (*Rutilus rutilus* Linnaeus, 1758) and ninespine stickleback (*Pungitius pungitius* Linnaeus, 1758). Only 14 native species have a catch frequency > 10% (Table 2). The maximum number of native species encountered in a single survey is 17 which occurred in 2 sites only (0.13%). Barely 9.7% of the surveyed sites had 10 or more species and in more than half of the stations (54.2%) 4 or less species were caught.

Water quality and HabStat

Water quality parameters in the bream and barbel type of sites show a higher within than between variation (Table 3).

TABLE 2 (continued on next page)

Fish species, catch frequency (CF %) guilds and metric attribution. Ref. = reference species (GEP or MEP); x = fish belongs to metric. Abbreviations: MnsBen = benthic; MnsDia = diadromic; MnsInv = intolerant; MnsPhy = invertivorous; MnsPio = pioneer; MnsPhy = phytophilic; MnsPis = piscivorous; MnsRhe = rheophilic; MnsSpa = specialised spawner; MnsTol = tolerant species.

Guild	Scientific name	common name	origin	catch frequency	Ref sp.	Habitat use			Reproduction			Tolerance			Feeding			Migration	
						MnsRhe	MnsBen	MnsPhy	MnsSpa	MnsPio	MnsTol	MnsInt	MnsPhy	MnsPis	MnsDia	MnsInv	MnsPis	MnsDia	
	<i>Abramis brama</i> (Linnaeus, 1758)	breem	native	6.6	GEP						x								
	<i>Alburnoides bipunctatus</i> (Bloch, 1782)	schneider	native	0.3	MEP	x			x							x			
	<i>Alburnus alburnus</i> (Linnaeus, 1758)	bleak	native	1.3	MEP						x					x			
	<i>Anguilla anguilla</i> (Linnaeus, 1758)	eel	native	36.7	GEP		x												x
	<i>Barbatula barbatula</i> (Linnaeus, 1758)	stone loach	native	41.9	GEP	x	x		x							x			
	<i>Barbus barbus</i> (Linnaeus, 1758)	barbel	native	0.3	MEP	x	x							x					
	<i>Blicca bjoerkna</i> (Linnaeus, 1758)	white bream	native	7.3	GEP								x						
	<i>Carassius carassius</i> (Linnaeus, 1758)	crucian carp	native	0.8	MEP					x									
	<i>Chondrostoma nasus</i> (Linnaeus, 1758)	common nase	native	0.1	MEP	x	x		x										
	<i>Cobitis taenia</i> (Linnaeus, 1758)	spined loach	native	5.4	GEP		x		x							x			
	<i>Cottus gobio</i> (Linnaeus, 1758)	bullhead	native	5.3	GEP	x	x		x							x			
	<i>Cyprinus carpio</i> (Linnaeus, 1758)	common carp	native	11.7	GEP					x									
	<i>Esox lucius</i> (Linnaeus, 1758)	pike	native	14.6	GEP				x						x				
	<i>Gasterosteus aculeatus</i> (Linnaeus, 1758)	three-spined stickleback	native	71.1	GEP				x				x						
	<i>Gobio gobio</i> (Linnaeus, 1758)	gudgeon	native	50.3	GEP	x			x						x				
	<i>Gymnocephalus cernua</i> (Linnaeus, 1758)	ruffe	native	3.5	MEP		x								x				
	<i>Lampetra fluviatilis</i> (Linnaeus, 1758)	river lamprey	native	0	MEP					x									x
	<i>Lampetra planeri</i> (Bloch, 1784)	European brook lamprey	native	4.3	MEP	x	x		x										x
	<i>Leucaspis delineatus</i> (Heckel, 1843)	belica	native	8.7	GEP					x									
	<i>Leuciscus idus</i> (Linnaeus, 1758)	ide	native	10.3	GEP	x										x			
	<i>Leuciscus leuciscus</i> (Linnaeus, 1758)	common dace	native	3.4	MEP	x										x			
	<i>Lota lota</i> (Linnaeus, 1758)	burbot	native	1.2	MEP		x		x										x

Guild	Scientific name	common name	origin	catch frequency	Ref sp.	Habitat use			Tolerance			Feeding			Migration	
						MnsRhe	MnsBen	MnsPhy	MnsSpa	MnsPio	MnsTol	MnsInt	MnsLnv	MnsPis	MnsDia	
	<i>Perca fluviatilis</i> (Linnaeus, 1758)	perch	native	31.9	GEP					x					x	
	<i>Phoxinus phoxinus</i> (Linnaeus, 1758)	Eurasian minnow	native	0.1	MEP	x										
	<i>Platichthys flesus</i> (Linnaeus, 1758)	flounder	native	0.9	MEP		x						x			x
	<i>Pungitius pungitius</i> (Linnaeus, 1758)	ninespine stickleback	native	46.1	GEP					x						
	<i>Rhodeus sericeus</i> (Pallas, 1776)	bitterling	native	11.9	GEP				x							
	<i>Rutilus rutilus</i> (Linnaeus, 1758)	roach	native	42.6	GEP					x						
	<i>Salmo salar</i> (Linnaeus, 1758)	Atlantic salmon	native	0	MEP	x					x					x
	<i>Salmo trutta</i> (Linnaeus, 1758)	sea trout	native	0	MEP	x										x
	<i>Salmo trutta fario</i> (Linnaeus, 1758)	brown trout	native	4.2	MEP	x								x		x
	<i>Sander lucioperca</i> (Linnaeus, 1758)	pike-perch	native	0.7	MEP										x	
	<i>Scardinius erythrophthalmus</i> (Linnaeus, 1758)	rudd	native	27.2	GEP			x								
	<i>Silurus glanis</i> (Linnaeus, 1758)	Wels catfish	native	0.4	MEP		x									x
	<i>Squalius cephalus</i> (Linnaeus, 1758)	chub	native	11.5	GEP	x										
	<i>Tinca tinca</i> (Linnaeus, 1758)	tench	native	14.2	GEP		x								x	
	<i>Ameiurus nebulosus</i> (Lesueur, 1819)	brown bullhead	non-indigenous	7.3												
	<i>Pimephales promelas</i> (Rafinesque, 1820)	fathead minnow	non-indigenous	1.3												
	<i>Carassius auratus</i> (Linnaeus, 1758)	goldfish	non-indigenous	0												
	<i>Umbra pygmaea</i> (DeKay, 1842)	eastern mudminnow	non-indigenous	7.6												
	<i>Ponticola kessleri</i> (Günther, 1861)	bighead goby	non-indigenous	0.1												
	<i>Proterorhinus semilunaris</i> (Heckel, 1837)	western tubenose goby	non-indigenous	0.3												
	<i>Lepomis gibbosus</i> (Linnaeus, 1758)	pumpkinseed	non-indigenous	18											x	
	<i>Pseudorasbora parva</i> (Temminck & Schlegel, 1846)	stone moroko	non-indigenous	33.5											x	
	<i>Carassius gibelio</i> (Bloch, 1782)	Prussian carp	non-indigenous	26.8												x
	<i>Ctenopharyngodon idella</i> (Valenciennes, 1844)	grass carp	non-indigenous	0.1												
	<i>Oncorhynchus mykiss</i> (Walbaum, 1792)	rainbow trout	non-indigenous	0												
	<i>Pomatoschistus microps</i> (Krøyer, 1838)	common goby	estuarine	0.1												

TABLE 3

The range (min-max) of the abiotic data collected during each survey including water quality parameters, river width and HabStat (including mean \pm standard deviation) in rivers of bream and barbel zone (n=number of sites).

	bream zone (n=772)	barbel zone (n=1100)
conductivity (μ S/cm)	114-6415	72-2260
water temperature ($^{\circ}$ C)	1-25.8	1.7-26.7
dissolved oxygen (mg/l)	0.28-21.2	0.1-22.7
turbidity (NTU)	1.5-263	2.3-524
pH	4.0-9.5	3.3-10.1
river width (m)	0.3-25.5	0.6-30
HabStat	1-4 (2.2 \pm 0.7)	1-4 (2.1 \pm 0.7)

In 21.9% surveys of the bream sites and 10.5% surveys of the barbel sites dissolved oxygen was lower than 6 mg/l at the time of the survey. This is the minimum oxygen concentration required for sustainable fish life in rivers as stipulated by Belgian law (VLAREM II 2010). In the sites belonging to the bream zone 18.3% have a HabStat of 1, 41.3% score 2, 38.5% score 3 and only 1.9% get a 4. In the barbel zone 15.3% score 1, 34.9% scores 2, 24.5% score 3 and 25.3% score 4.

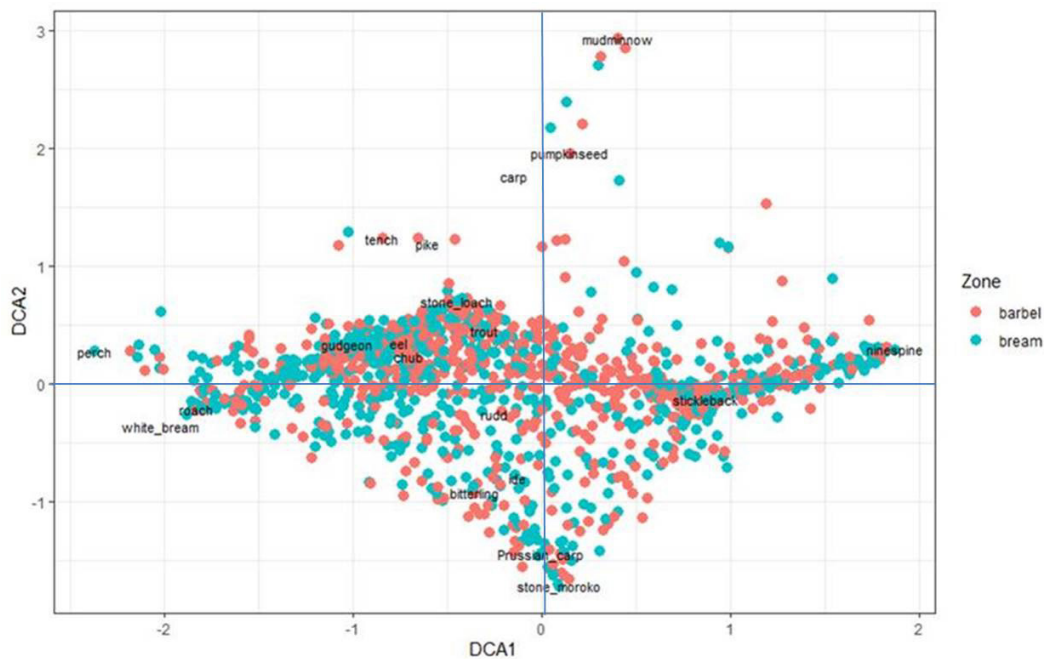


Figure 3 – Biplot based on DCA of relative percentage of the 20 most abundant fish species caught in 772 surveys in bream and 823 in surveys in barbel sites over a period from 1993 to 2015. Eigenvalues first and second axes are 0.66 and 0.51.

Reference fish species and attribution to ecological guilds

The updated reference fish species list for rivers in Flanders includes 37 species (Table 2). Non-indigenous species and the estuarine common goby (*Pomatoschistus microps*, Krøyer, 1838) are not considered as MEP or GEP species for rivers. Eighteen species from the list were rarely caught (<5%) and are considered as MEP species or GEP can still be reached when they are absent. The remaining 19 species contribute to the GEP status. The metric attribution is also presented in Table 2. The metrics belong to habitat use guild (diadromic and benthic species), trophic guilds (piscivores and invertivores); reproductive guilds, e.g., specialised spawners (MnsSpa) including gravel spawners, phytophilic and ostracophilic spawners; habitat use guilds, e.g., benthic and phytophilic species and tolerance guilds, e.g., tolerant and intolerant species and pioneers species which are defined as opportunistic resistant species that are first to be found once a habitat is restored (MnsPio). Pioneers include three non-native species: Prussian carp (*Carassius gibelio* Bloch 1782), stone moroko (*Pseudorasbora parva* Temminck & Schlegel, 1846) and pumpkinseed (*Lepomis gibbosus* Linnaeus 1758).

Selection of metrics

The metric screening from literature resulted in a total of 44 candidate metrics (Table 4).

The measure of association analyses allowed the selection of uncorrelated variables to be used in the model. The correlations among the abiotic variables are small (Table A1, Appendix). From the variance inflating factor analyses we only drop dissolved oxygen (VIF»3 in Table A2 in Appendix). Water temperature is kept as its VIF is just slightly above 3 and is known as an important variable for fish (e.g., WEBB & WALSH 2004; WALBERG 2011).

Except for MniInd/m² (number of individuals per m²), data variation in metrics based on number of individuals did not show a clear pattern in the different HabStat classes and were therefore omitted. This reduced the number of candidate metrics to be tested with linear mixed models to 30 (Table A3, Appendix).

Normality analyses excluded following fitted metrics: invertivorous species (MnsInv), relative percentage piscivorous individuals (MpiPis), number of diadromic and rheophilic species (MnsDia, MnsRhe), biomass non-indigenous fish (BioExo), number of benthic species (MnsBen), total biomass (ManBio), number of phytophilic species (MnsPhy), relative percentage pioneers (MpiPio) and number of tolerant species (MnsTol). The metrics assessing the number of non-indigenous and pioneer species (MnsExo, MnsPio) are removed as they show an opposite reaction to habitat quality improvement as expected. The correlation between the fitted metrics is given in Table A4 (Appendix).

We categorised river width as follows: class 1: ≤ 5m (n=1057); class 2: >5 and ≤10m (n=350); class 3: >10 and ≤ 15m (n=108) and class 4 >15 and ≤30m (n=82). Graphical analyses of the fitted metric values with HabStat and with river width (Figs 4–5) and the correlation analyses resulted into the selection of 5 metrics. These are: **number of species** (MnsTot), **Shannon-Wiener Index** (ManSha), **relative percentage of tolerant individuals** (MpiTol), **relative percentage of invertivorous individuals** (MpiInv) and the **percentage of recruiting species** (MpsRec).

The selected metrics show a gradient with HabStat classes (Fig. 4). Other fitted metrics also showed a gradient but were omitted as they were highly correlated with other metrics (Table A4) or had a small range of variation (number of invertivorous species MnsInv 0-4). The less fitted MpsRec is kept as it is indirectly a metric required by the WFD. It shows a relation with HabStat but is independent from river width (Figs 4–5) and is not correlated with any selected metric. The model shows a seasonal effect on recruitment (Table A3). In a next step the 5 selected metrics needed scoring.

TABLE 4

Candidate metrics with their predicted response to increasing disturbance.

Abbreviation	Explanation	Expected reaction to degradation
MniInd	total number of individuals	↓
MnsTot	total number of native species	↓
MniExo	number of non-indigenous individuals	↑
MnsExo	number of non-indigenous species	↑
MpiExo	relative percentage of non-indigenous individuals	↑
MpsRec	percentage recruitment (species)	↓
MniIndE	total number of native individuals	↓
ManSha	Shannon-Wiener index	↓
MnsSpa	special spawners	↓
MniSpa	number of special spawners individuals	↓
MpiSpa	relative percentage special spawner individuals	↓
MnsInv	number of invertivorous species	↓
MniInv	number of invertivorous individuals	↓
MpiInv	relative percentage of invertivorous individuals	↓
MnsPis	number of piscivorous species	↓
MniPis	number of piscivorous individuals	↓
MpiPis	relative percentage of piscivorous individuals	↓
MnsDia	number of diadromic species	↓
MniDia	number of diadromic individuals	↓
MpiDia	relative percentage of diadromic individuals	↓
MnsRhe	number of rheophilic species	↓
MniRhe	number of rheophilic individuals	↓
MpiRhe	relative percentage of rheophilic individuals	↓
MnsPio	number of pioneer species	↑
MniPio	number of pioneer individuals	↑
MpiPio	relative percentage of pioneer individuals	↑
MniInd/m ²	number of individuals per m ²	↓
MniIndE/m ²	number of native individuals per m ²	↓
BioExo	biomass non-indigenous species	↑
MapExo	relative percentage biomass non-indigenous species	↑
MnsBen	number of benthic species	↓
MniBen	number of benthic individuals	↓
MpiBen	relative percentage of benthic individuals	↓
MnsInt	number of intolerant species	↓
MniInt	number of intolerant individuals	↓
MpiInt	relative percentage of intolerant individuals	↓
ManBio	biomass	↓
ManBio/m ²	biomass per m ²	↓
MnsPhy	number of phytophilic species	↓
MniPhy	number of phytophilic individuals	↓
MpiPhy	relative percentage of phytophilic individuals	↓
MnsTol	number of tolerant species	↑
MniTol	number of tolerant individuals	↑
MpiTol	relative percentage of tolerant individuals	↑

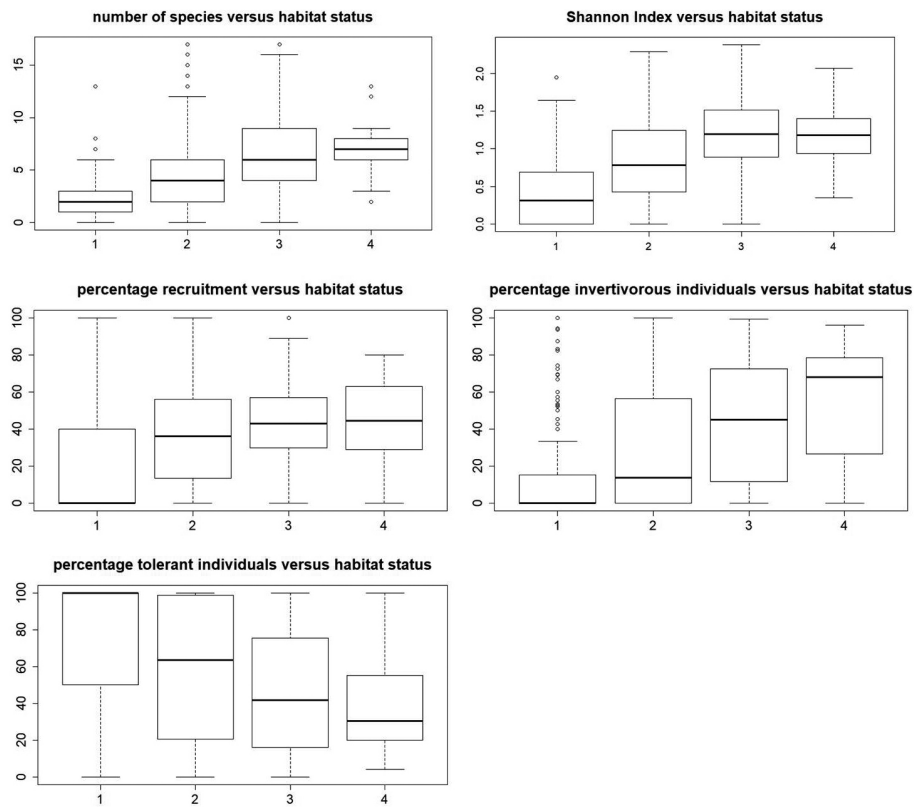


Figure 4 – Boxplots of values of selected metrics as a function of pre-classification of the sites (n = 963), bold line=median, hinges=25th and 75th percentiles, whiskers=range.

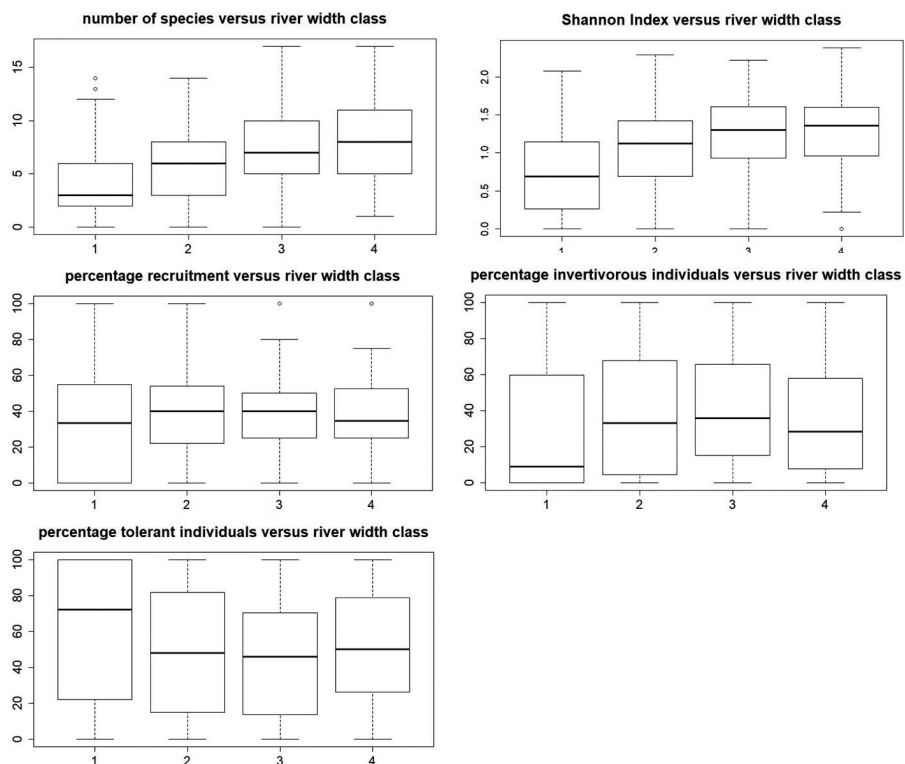


Figure 5 – Boxplots of values of selected metrics as a function of river width classes (n=963), bold line = median, hinges=25th and 75th percentiles, whiskers=range.

Metric scoring

The average number of individuals caught in sites with HabStat 1 was 34. Hence the required number of individuals in a sample for a metric calculation was minimum 30. The rationale of metric scoring is explained for metric MnsTot. The scoring procedure for the other metrics is similar and explained in appendix ‘Scoring selected metric’. Values of the metric percentage of recruiting species (MpsRec) are season dependent (Fig. 6) and scoring criteria are therefore related to season.

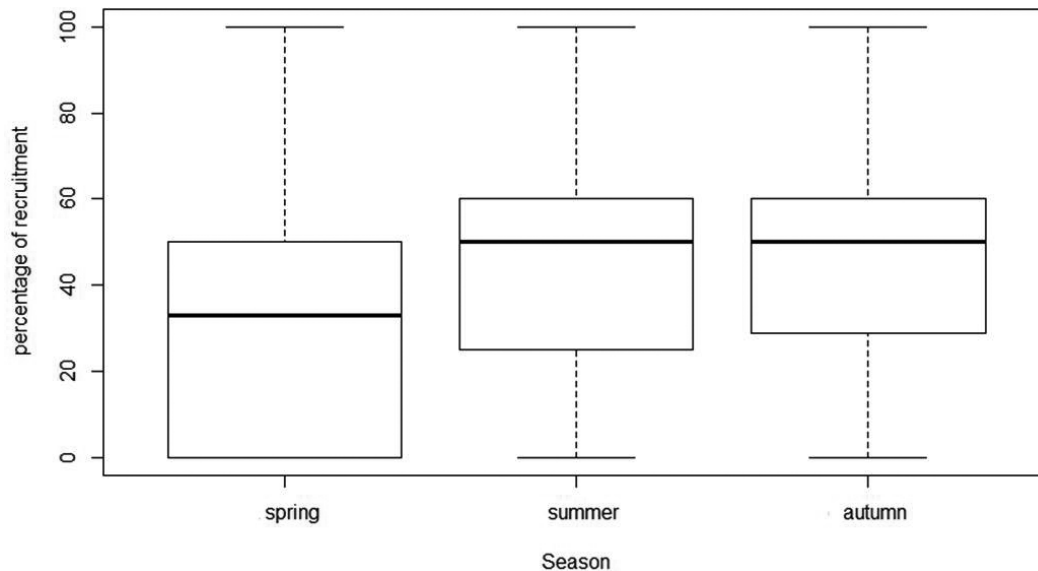


Figure 6 – Boxplots of values of the percentage of recruitment metric as a function of season (n=963), bold line=median, hinges=25th and 75th percentiles, whiskers=range.

Total number of species (MnsTot)

Seventeen was the maximum number of reference species caught at one site in the studied period, while 19 is the number of species in GEP (Table 2). The lower limit of score 4 (GEP) is determined by 70% of 19 species (i.e., 13). Scoring depends on the river width as this metric is affected by it (Fig. 5). The average metric value of MnsTot in each river width class (see table A5 in Appendix) is the lower boundary for the moderate metric score which is 3. The lower score of GEP determines automatically the upper boundary for the moderate score. Trisection gives the lower boundaries for the metric scores 2 and 1. For example in the river width class $\leq 5\text{m}$ the average is 4 species. Therefore, 4 is the lower boundary for score 3, i.e., the moderate metric score. The upper boundary for this score is 12 as 13 is the lower boundary for score 4 (GEP). To obtain the lower boundary for the poor class (score 2) we apply the trisection as follows: $4/3 \times 2 = 3$; for the bad class (score 1): $4/3 = 1$. We apply the same approach for the other river width classes to obtain the upper boundaries of the metric scores 1 and 2. Values lower than the lower value of score 1 are scored as 0. Boundary values for score classes are given in Table 5.

IBI scoring thresholds and EQR

The maximum IBI score with selected metrics is 25 and the minimum is 1. This minimum value is possible when MpiTot scores 1 and the other metrics score zero. The index score was transformed to an EQR calculated as a value between 0 and 1. The appreciation of the status is defined by the EQR value (Table 6).

TABLE 5

Selected metrics for rivers of bream and barbel type and their threshold values for the metric scores (abbreviations, see Table 4).

Scores	0	1	2	3	4	5
River width	MnsTot threshold values					
≤5m	0	1 - 2	3	4 - 12	13 - 19	>19
>5m ≤10m	1	2 - 3	4 - 5	6 - 12	13 - 19	>19
>10m ≤15m	1	2 - 4	5 - 6	7 - 12	13 - 19	>19
>15m ≤30 m	2	3 - 4	5 - 7	8 - 12	13 - 19	>19
River width	ManSha threshold values					
≤5m	<0.245	0.245 - <0.490	0.490 - <0.736	0.736 - <0.981	0.981 - <1.226	≥1.226
>5m	<0.397	0.397 - <0.794	0.794 - <1.191	1.191 - <1.588	1.588 - <1.985	≥1.985
River width	MpiTol (%) threshold values					
≤5m		≤24.4 - >73.1	73.1 - >60.9	60.9 - >48.7	48.7 - >36.5	36.5 - >24.4
>5m		≤19.3 - >57.8	57.8 - >48.2	48.2 - >38.6	38.6 - >28.9	28.9 - >19.3
River width	MpiInv (%) threshold values					
≤5m	<9.7	9.7 - <19.3 & ≥ 58	19.3 - <29	29 - <38.6	38.6 - <48.3	48.3 - <58
>5m	<12.8	12.8 - <25.5 & ≥76.6	25.5 - <38.2	38.2 - <51.5	51.1 - <63.8	63.8 - <76.6
Season	MpsRec (%) threshold values					
spring	<11.3	11.3 - <22.5	22.5 - <33.8	33.8 - <45.1	45.1 - <56.3	≥56.3
summer/autumn	<14.9	14.9 - <29.8	29.8 - <44.7	44.7 - <59.6	59.6 - <74.5	≥74.5

TABLE 6

Threshold values for EQR-scores and appreciation (integrity class).

Integrity class	Threshold EQR-score
MEP	1
GEP	<1 ≥ 0.75
Moderate	<0.75 ≥ 0.50
Poor	<0.50 ≥ 0.25
Bad	< 0.25

Validation

The internal validation with development data set was performed using data of 963 surveys in the period 1993-2015 as in 582 surveys less than 30 fish individuals were caught. Comparing the EQR appreciation with the HabStat status showed that there is a 44.8 % match and a 48.2% one-class difference. Out of 963 surveys 64 scored GEP, 341 moderate, 457 poor and 101 bad. For the external validation with the validation data 39 surveys out of 170 had less than 30 individuals in the sample. 95.2% correspondence was found between the EQR appreciation and the attributed HabStat status of which 42.3% had the same class. Only 4 sites scored GEP, 42 scored moderate, 73 poor and 12 bad. The external validation revealed

that the good HabStat (n=2) was underscored and the moderate HabStat (n=65) was in 100% of the cases scored correctly. The Pearson correlation between EQR values obtained with the initial index (BELPAIRE *et al.* 2000) and the new EQR values showed a low but significant correlation ($c=0.562$; $p<0.001$). In the external validation 6 sites out of 131 are scored too high (Type II error) with the old index while with the new index one site was scored too low (Type I error). Setting out the variation of EQR scores in function of HabStat scores shows a very good gradient with the internal data but with the external data the good HabStat (score 4) is not distinguished from the moderate sites (Fig. 7). All metric scores, except MpsRec with the internal validation data, were significantly positively correlated with the EQR (Table 7).

TABLE 7

Spearman’s rho correlation between the metrics and EQR, ** correlation is significant at the 0.001 level (2-tailed) (For abbreviations, see Table 4).

	MnsTot	ManSha	MpiTol	MpiInv	MpsRec
EQR (internal)	0.655**	0.714**	0.693**	0.409**	0.156
EQR (external)	0.609**	0.731**	0.531**	0.633**	0.343**

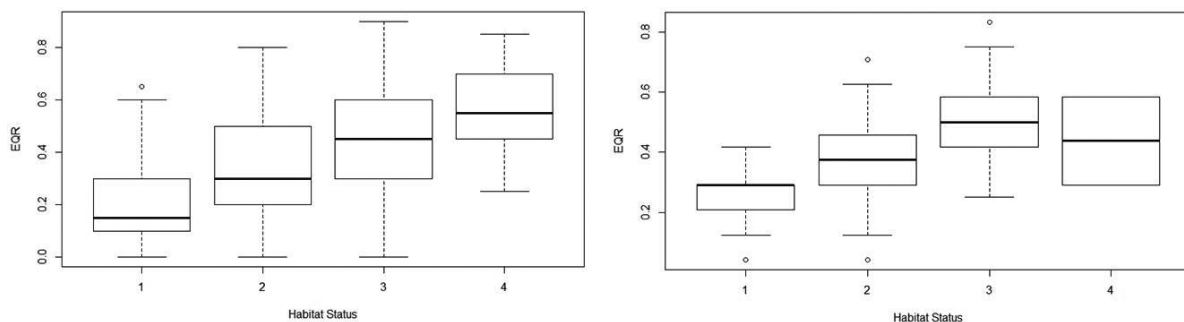


Figure 7 – Boxplots of EQR scores fitted as a function of pre-classification of the sites: bold line = median, hinges = 25th and 75th percentiles, whiskers = range. Left development data (n=963), right validation data (n=131).

Discussion

In our study all necessary steps to design a new fish-based index of biotic integrity (Fig. 2) were successfully taken.

Fish data were obtained with a single standardised fishing methodology according to CEN standards (CEN 2003), reducing the effect of the fish community’s gear specific reactions to disturbances.

Pre-classification of habitat status was used as a technique to rank sampling sites in a reasonable way with respect to anthropogenic pressures, enabling to establish the fish-based metrics’ reaction to multiple stressors (see also in BREINE *et al.* 2007, 2010; QUATAERT *et al.* 2011; WU *et al.* 2014; BREINE *et al.* 2015; ERGÖNÜL *et al.* 2018; SCHINEGGER *et al.* 2018; CONINO *et al.* 2020). Different variables were chosen for their known effects on the fish community at different spatio-temporal scales (e.g., ZIMMERMAN *et al.* 2003; ANGERMEIER & DAVIDEANU 2004; KRUK 2007; FALCONE *et al.* 2010; YAN *et al.* 2013; RADINGER *et al.* 2018; SAÇ *et al.* 2021). These were combined into a single HabStat variable following DEGERMAN *et al.* (2007) and MURPHY *et al.* (2013), integrating local site condition (e.g., presence of shelter) with pressures on a larger scale (e.g., presence of migration barriers, DROUINEAU *et al.* 2018).

Pre-classification threshold values were attributed with expert judgement. The rationale was to obtain a good ranking of habitats with respect to human impact and not necessarily an absolute expression of the habitat quality. The ranking was needed for the first metric selection and for validation. Sometimes HabStat classes 3 and 4 are not clearly distinguished by fitted metrics, probably because there is a disproportion of HabStat class 4 (17) and HabStat 3 (566) sites in our data set.

A reference list of fish species for pristine rivers could not be established because all lowland rivers in Flanders are heavily modified. Instead, we developed a list representing the GEP status and one for the MEP status in accordance with the WFD guidelines. These differ slightly from the list presented by BELPAIRE *et al.* (2000) which was based on HUET (1949) and VANDELANNOOTE *et al.* (1998). We added flounder (*Platichthys flesus* Linnaeus, 1758), river lamprey (*Lampetra fluviatilis* Linnaeus, 1758), Atlantic salmon (*Salmo salar* Linnaeus, 1758) and Wels catfish (*Silurus glanis* Linnaeus, 1758). These species were frequently caught except for salmon. But the latter species has the potential to re-enter the rivers as it is found in the Belgian part of the River Maas. All non-indigenous species were omitted from the list because they are considered as indicators of disturbance (e.g., KARR 1981; BELPAIRE *et al.* 2000). DÉZERARD *et al.* (2020) could detect impairment of river fish communities induced by non-indigenous species in 1527 sites belonging to the French Water Framework Directive's surveillance monitoring. However, their effects on the native communities could not be defined and SCHLAEFFER (2018) argued that biodiversity indices should include non-indigenous species. Thus, we excluded non-indigenous species from all metrics except Shannon-Wiener Index (ManSha) and metrics assessing alien species. Species residing for decades in our waters, e.g., pike-perch, are considered as naturalised and are included in the reference list. We consider the reference list as the pool containing possible fish species in the rivers of bream and barbel type in Flanders.

For the *metric selection* we applied a repeatedly used methodology (OBERDORFF *et al.* 2002; PONT *et al.* 2007; FREUND & PETTY 2007; BREINE *et al.* 2004, 2015; MOSTAFAVI *et al.* 2015; ERGÖNÜL *et al.* 2019), but with minor modifications. Dissolved oxygen, albeit an important water quality parameter (e.g., AZIMI & ROCHER 2016), was omitted as predictor from the model using the Akaike information criterion (AIC). Linear mixed modelling is considered as a reliable tool for ecological studies (EINHEUSER *et al.* 2013) and allowed for a first metric selection based on the sensitivity for one or more predictors. The new index includes three metrics associated with guilds (tolerance, recruitment and a trophic guild) and two associated with diversity (the total number of native species (MnsTot) and Shannon-Wiener index (ManSha)) all of which have already been used in different other fish-based indices. The number of native species decreases with increased human impact and is a widely used metric in fish-based indices (e.g., BREINE *et al.* 2004; MOSTAFAVI *et al.* 2019). Non-indigenous species could indeed mask the response to habitat degradation as they are mostly tolerant (CASATTI *et al.* 2009; CASATTI & TERESA 2012).

ManSha includes all species but integrates their evenness and is often used in fish-based indices (WU *et al.* 2014; YERLI *et al.* 2016; ERGÖNÜL *et al.* 2019). The value of this index usually ranges between 1.5 and 3.5 and rarely exceeds 4.5 (MAGURRAN 2004) and its highest value in our data set was 2.38.

The relative percentage of tolerant individuals (MpiTol) is the only selected metric increasing in value with decreasing habitat quality. It is logical to define a minimum percentage below which again a low score is obtained as a total absence of tolerant species is not natural. Their observed trend in our study is in accordance with observations made in other studies (OBERDORFF *et al.* 2002; VEHANEN *et al.* 2010; CASATTI & TERESA 2012; LI *et al.* 2018).

The relative percentage of invertivorous individuals (MpiInv) is a metric related to a trophic guild. Aquatic habitat degradation may have an adverse effect on fish with specific feeding requirements such

as invertivorous fish (NOBLE *et al.* 2007). A decrease in this metric also indicates the alterations in the aquatic food chain (ERGÖNÜL *et al.* 2019).

Percentage recruitment (MpsRec) contains information about the fish condition and habitat use. Fish should be able to recruit naturally. The metric ‘percentage recruitment’ was retained because we consider that even a less optimal metric sometimes can provide valuable information in combination with other metrics (see also BREINE *et al.* 2007). In addition, this metric is based on length classes present per species and therefore complies with one of the WFD requirements. The scoring of this metric, which is also used in the initial index, is now taking into account seasonal effects.

Metric scoring is often based on comparison with reference sites (e.g., KARR 1981; OBERDORFF *et al.* 2002; CASATTI *et al.* 2009; VEHANEN *et al.* 2011; ADAMCZYK *et al.* 2017). However, the use of reference conditions as a benchmark for metric scoring has a number of limitations. It could be an unrealistic target for management (TWEEDLEY *et al.* 2017). Moreover, global warming can change the environmental conditions in an unpredictable way and can modify the structure of pristine aquatic communities (LATLI *et al.* 2017). During the European fish-based intercalibration exercise it became clear that many countries lack pristine sites and had to use the best available sites as reference (JEPSEN & PONT 2007). PARDO *et al.* (2012) developed a protocol to identify near pristine sites in the Central and Baltic region of Europe. In Flanders no pristine sites or even natural rivers are present. In the current study a data driven methodology was used to score the selected metrics. Different authors applied similar techniques to create a reference based on data (BOZZETTI & SCHULZ 2004; PAGANELLI *et al.* 2011; BORJA *et al.* 2012; SAPOUNIDIS *et al.* 2019). Values for the selected metrics changed with river width or season. Therefore, scoring was adjusted to these variables but always the same rationale was used to define scoring criteria. Using the average metric value within a particular river width class or season assures a realistic and robust approach. Attempts to estimate the slope of metric values, under which trisection is performed, by applying trendline regression (e.g., BELPAIRE *et al.* 2000) suffer from the potential inadequacy of overestimating the expected condition for metrics that vary along natural environmental gradients (KENNARD *et al.* 2006).

For *validation* we allowed a one-class difference between the habitat status (HabStat pre-classification) and the EQR to define tendencies, of under- or over-estimation, as was done by other authors (GOFFAUX *et al.* 2001; BREINE *et al.* 2015; ERGÖNÜL *et al.* 2019). Internal IBI validation with boxplots as an initial pertinence test for the developed index revealed a good correlation for EQR scores HabStat. With the validation dataset good HabStat was not distinguished from moderate but only two good HabStat sites (score 4) were represented. One metric (MpsRec) was not correlated ($r < 0.5$, $p > 0.01$, Table 7) with the EQR for internal data. However, with the external data the correlation was small but still significant. Nevertheless, this metric was retained because it is required by the WFD as a proxy of age structure. Overall, the newly developed index was able to distinguish different degrees of degradation within the pre-classified sites.

Conclusion

The result of our study is a validated method to assess the ecological status of Flandrian lowland rivers, using a single standardised fishing methodology according to CEN standards (CEN, 2003) and with limited reliance on expert judgement compared to BELPAIRE *et al.* (2000). The developed IBI complies adequately to the criteria stipulated in the Water Framework Directive. We ensured that the selected metrics are not redundant and are relevant allowing for appropriate assessment of anthropogenic impacts on the fish communities. Moreover, we demonstrated that fish communities in barbel and in bream type of rivers can be assessed using the same IBI.

Acknowledgements

We are grateful to our technical staff for their enthusiasm and perseverance. Fishing was not always easy. We are also grateful for all the people assisting us in the field. Thanks to all colleagues who helped us with data quests and databank issues. We thank the two anonymous reviewers whose comments and suggestions improved this manuscript.

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Manuscript received: 5 November 2020

Manuscript accepted: 23 November 2021

Published on: 25 November 2021

Branch editor: Merlijn Jocqué

Appendix scoring selected metrics

Shannon-Wiener Index (ManSha)

The model fitted ManSha with river width and HabStat significantly (Table A3). However, the boxplot shows a strong overlap of the variations between the different river width classes (Fig. 5). Only the first river width class is separated from the three other river width classes. Here, the GEP and MEP scores are defined not from the reference but from the average value in the river width class. For rivers ≤ 5 m the average value corresponding to score 3 is 0.736. Here we reason that this lower score is obtained from trisection of the lower MEP value: or $0.736 * 5/3$ equals 1.226 which is the calculated lower boundary value for score 5. Trisection of the MEP boundary gives the lower boundaries for the other metric scores. The average value in other river width classes (>5 m) corresponding to score 3 is 1.191. This value is therefore obtained from $1.191 * 5/3$ or 1.985 is the metric value for the threshold between score 4 and 5. The thresholds for the other scores are determined using the same approach as described above. Values lower than the lower threshold of score 1 receive score zero. Boundary values for score classes are given in Table 5.

Relative percentage tolerant species (MpiTol)

Values of percentage metrics range between 0 and 100. The value of MpiTol increases with decreasing habitat quality. The average value for MpiTol in rivers with river width ≤ 5 m is 60.9% being the upper limit for score 3 or the lower limit for score 2. The lower limit for score 4 equals four fifth of 60.9 and for score 5 three fifth of 60.9. The lower boundary threshold for score 2 is obtained by calculating six fifth of 60.9%. The highest boundary value in score 2 and lowest value in score 5 gives the thresholds for score 1. For the wider rivers we start from 48.2% as being the lower boundary for score 4 and continue defining thresholds as just described. Boundary values for score classes are given in Table 5.

Relative percentage invertivorous species (MpiInv)

The value of MpiInv increases with increasing habitat quality. However, a site is not considered in a good status if only invertivorous individuals are present and therefore a threshold is set beyond which a moderate score is given. The average value for MpiInv in rivers with river width ≤ 5 m is 29%. We use the same rationale as for ManSha whereby this lower threshold for score 3 is obtained from trisection of the lower MEP value: $29 * 5/3 = 48.3\%$. Trisection gives the lower thresholds for the other scores. The upper limit for score 5 is obtained by trisection beyond which the metric value scores 1. Metric values lower than the lower threshold value for score 1 receive 0 as score. For the rivers wider than 5m we start from 38.2% as the lower value for score 3 and apply the rationale as explained with ManSha. Boundary values for score classes are given in Table 5.

Percentage recruiting species (MpsRec)

This metric is seasonally dependent. The variation of MpsRec in summer and autumn is similar (Fig. 6) but the average percentage recruiting species in spring (33.8%) is lower than that in summer (45%) and autumn (44.5%). The average percentage for the latter seasons is 44.7%. The lower threshold for score 3 is obtained from $x/5 * 3$, where x is the lower threshold for score 5. These lower thresholds are 56.3% in spring and 74.5% in the other seasons. The rationale to obtain thresholds for other scores follows that of ManSha and MpiInv. Again metric values lower than the lower threshold for score 1 receive 0 as a score. Boundary values for score classes are given in Table 5.

TABLE A1

Pearson correlation coefficients between the abiotic variables from 1545 surveyed sites between 1993 and 2015 (** $p \leq 0.001$; * $p \leq 0.05$).

variables	pH	temperature	dissolved oxygen	turbidity	river width	HabStat
conductivity	0.2427**	0.1698**	-0.105**	0.000	0.2311**	-0.1013**
pH		0.1219**	0.0417	-0.0926	0.0978**	-0.0516
temperature			-0.2635**	0.0358	0.1183**	-0.0413
dissolved oxygen				-0.2447**	-0.1229**	0.2239**
turbidity					-0.0411	-0.0753
river width						0.1949**

TABLE A2

Variance inflation factor (VIF) values among abiotic variables from 1545 surveyed sites between 1993 and 2015.

	GVIF ^{(1/(2*Df))}	VIF
temperature	1.7372	3.018
conductivity	1.558	2.427
pH	1.2036	1.449
turbidity	1.0795	1.165
river width	1.0729	1.151
HabStat	1.088	1.184
dissolved oxygen	6.292	39.589
season	1.0472	1.097

TABLE A3 (continued on next page)

The linear mixed model (lmer) assessed how far uncorrelated variables describe metrics log (L) or square root (S) transformed (metric abbreviations are explained in Table 4). Season is a factor: spring, summer and autumn.

metric	full model	fitted model	lmer(metric~Lconductivity+LpH+Lturbidity+Lwater temperature+Lriver width+LHabStat+season+(1 location))	p	p	p	p	R ² mar	R ² con
LMnsTot	0.0215-0.02 LCon+0.251 RiverW+1.065LHabStat			0.001	0.001	0.001	0.001	0.3307	0.6655
LMnsExo	0.058-0.01 LCon+0.186 RiverW+0.179 LHabStat-0.03 Season			0.01	0.001	0.001	0.05	0.0659	0.4445
SMpiExo	3.006-0.38 LTemp+1.251 LRiverW-2.904 LHabStat			0.001	0.001	0.001	0.001	0.0243	0.5077
SMpsRec	0.0523+0.346 LTemp+10.63 LHabStat-0.63 Season			0.01	0.001	0.001	0.001	0.1559	0.2926
ManSha	-0.422+0.478 LRiverW+1.895 LHabStat			0.001	0.001	0.001	0.001	0.2337	0.5694
LMnsSpa	-0.055-0.015 LCon+0.186 LRiverW+0.913 LHabStat			0.01	0.001	0.001	0.001	0.2655	0.6293
SMpiSpa	4.935+0.199 LCon-1.453 LpH+0.497 LTur+6.2 LHabStat			0.01	0.001	0.001	0.001	0.0558	0.5097
LMnsInv	-0.259+0.193 LRiverW+0.854 LHabStat			0.001	0.001	0.001	0.001	0.2188	0.6986
SMpiInv	-2.153+2.199 LRiverW+9.393 LHabStat			0.001	0.001	0.001	0.001	0.1204	0.6190
LMnsPis	-0.125-0.009 LCon+0.184 LRiverW+0.364 LHabStat-0.022 Season			0.05	0.001	0.001	0.01	0.1514	0.4733
SMpiPis	-0.367+0.642LpH-0.225 LTur-0.289 LTemp+1.785 LRiverW			0.05	0.05	0.001	0.001	0.0596	0.4155
LMnsDia	-0.115-0.014 LCon+0.130 LRiverW+0.362 LHabStat			0.001	0.001	0.001	0.001	0.1274	0.4715
SMpiDia	-0.654-0.123 LCon+1.545 LRiverW+1.877 LHabStat+0.252 Season			0.05	0.001	0.001	0.01	0.0723	0.4516
LMnsRhe	-0.191-0.012 LCon+0.131 LRiverW+0.811 LHabStat			0.05	0.001	0.001	0.001	0.1723	0.6998
SMpiRhe	-1.452-0.846 LpH+1.331 LRiverW+10.098 LHabStat			0.1	0.001	0.001	0.001	0.1071	0.6567
LMnsPio	0.268+0.040 LRiverW+0.312 LHabStat-0.036 Season			0.1	0.001	0.05	0.001	0.0446	0.3617
SMpiPio	10.645+0.246 LCon+0.265 LTemp-2.875 LRiverW-5.282 LHabStat-0.684 Season			0.05	0.1	0.001	0.001	0.0981	0.6506
LMnInnd	0.047-0.126 LRiverW+0.415 LHabStat-0.045 Season			0.001	0.001	0.001	0.001	0.1248	0.3127
LMnInndE	-0.122-0.249 LRiverW-0.665 LHabStat-0.436 Season			1	0.001	0.001	0.001	0.1201	0.2997
LBtoExo	-0.110-0.088 LCon+0.977 LRiverW+1.261 LHabStat			0.01	0.001	0.001	0.001	0.0783	0.4078

full model	lmer(metric~Lconductivity+LpH+Lturbidity+Lwater temperature+Lriver width+LHabStat+season+(1 year)+(1 location))										
metric	fitted model	p	p	p	p	p	p	p	p	R ² mar	R ² con
LMnsBen	-0.180-0.034 LCond+0.023 LTemp+0.164 LRiverW+0.768 LHabStat	0.001	0.05	0.001	0.001	0.001	0.001	0.001	0.001	0.2035	0.6293
SMpiBen	-0.002-0.234 LCond-1.360 LpH+0.490 LTemp+0.976 LRiverW+6.862 LHabStat	0.05	0.01	0.01	0.01	0.05	0.001	0.001	0.001	0.0923	0.5637
LMnsInt	-0.104-0.008 LCond+0.040 LpH+0.107 LRiverW+0.213 LHabStat-0.023 Season	0.05	0.1	0.001	0.001	0.001	0.001	0.001	0.1	0.0876	0.3381
SMpiInt	-0.352+0.654 LRiverW+0.862 LHabStat	0.001	0.001							0.0230	0.3065
LManBio	-0.208-0.038 LCond-0.046 LTur+ 0.078 LRiverW+1.608 LHabStat	0.001	0.1	1		0.01				0.1670	0.4480
LMnsPhy	-0.175-0.012 LCond-0.026 LTur+0.252 LRiverW+0.443 LHabStat	0.05	0.05	0.001	0.001	0.001	0.001	0.001	0.001	0.1670	0.4305
SMpiPhy	0.3-0.216 LCond-0.363 LTur+1.510 LRiverW+1.2333 LHabStat	0.001	0.01	0.001	0.001	0.05				0.0622	0.4025
LMnsTol	0.110-0.009 LCond+0.127 LRiverW+0.583 LHabStat	0.05	0.001	0.001						0.1714	0.4086
SMpiTol	8.999+1.162 LpH-0.291 LTemp-1.612 LRiverW-3.270 LHabStat	0.05	0.05	0.001	0.001	0.001	0.001	0.001	0.001	0.0422	0.5253

TABLE A4

Pearson coefficient (c) and significance (**p ≤ 0.001; * p ≤ 0.05) for correlation analysis fitted metrics log (L) or square root (S) transformed (abbreviations, see Table 4).

	SMpiExo	SMpsRec	ManSha	LMnsSpa	SMpiSpa	LMnsInv	SMpiInv	LMnsPis	SMpiDia	SMpiRhe	SMapExo	SMpiBen	SMpiPhy	SMpiTot
LMnsTot	0.197**	0.366**	0.843**	0.838**	0.172**	0.772**	0.519**	0.639**	0.245**	0.501**	0.168**	0.359**	0.337**	-0.237**
SMpiExo		-0.056*	0.095**	-0.095**	-0.249**	-0.064*	-0.113**	0.024	-0.049*	-0.096*	0.858**	-0.146**	0.302**	-0.142**
SMpsRec			0.310**	0.363**	0.145**	0.325**	0.239**	0.219**	0.009	0.248**	-0.047	0.099**	0.050*	-0.060*
ManSha				0.757**	0.139**	0.717**	0.503**	0.562**	0.334**	0.470**	0.023	0.436**	0.356**	-0.210**
LMnsSpa					0.515**	0.808**	0.604**	0.552**	0.191**	0.639**	-0.104**	0.459**	0.215**	-0.302**
SMpiSpa						0.248**	0.316**	-0.006	-0.053*	0.433**	-0.226**	0.231**	-0.035	-0.178**
LMnsInv							0.817**	0.616**	0.269**	0.746**	-0.088**	0.585**	0.075*	-0.447**
SMpiInv								0.393**	0.209**	0.882**	-0.153**	0.636**	-0.011	-0.619**
LMnsPis									0.263**	0.273**	-0.005	0.228**	0.294**	-0.170**
SMpiDia										0.217**	-0.116**	0.546**	-0.012	-0.349**
SMpiRhe											-0.145**	0.620**	-0.098**	-0.692**
SMMapExo												-0.187**	0.217**	-0.043
SMpiBen													-0.012	-0.550**
SMpiPhy														-0.116**

TABLE A5

Average and maximum values of selected metrics in function of river width classes (abbreviations, see Table 4).

Average values	MnsTot	ManSha	MpiTol (%)	MpiInv (%)	MpsRec (%)
rivers ≤ 5 m	4	0.74	60.9	29.0	28.3
rivers >5 m ≤ 10 m	6	1.03	48.5	38.5	37.1
rivers >10 m ≤ 15 m	7	1.25	45.4	41.1	36.2
rivers >15 m ≤ 30 m	8	1.29	50.6	35.0	26.5
Maximum values	MnsTot	ManSha	MpiTol (%)	MpiInv (%)	MpsRec (%)
rivers ≤ 5 m	14	2.08	100	100	100
rivers >5 m ≤ 10 m	14	2.29	100	100	100
rivers >10 m ≤ 15 m	17	2.22	100	100	97.5
rivers >15 m ≤ 30 m	17	2.39	100	100	100