A fish-based index of biotic integrity for upstream brooks in Flanders (Belgium)

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Abstract

From 1994 to 2000, 154 sites belonging to the grayling and trout zones were used to develop a fish-based multimetric Index of Biotic Integrity (IBI) as a tool to assess upstream brooks in Flanders (Belgium). All sites had a slope of at least 3‰ and a maximum width of 4.5 m. Fish assemblages were surveyed by electrofishing. From 28 candidate metrics 9 metrics were selected using ecological criteria and statistica! analyses. The IBI was defined as the average of these 9 metrics after scoring them as 1, 3 or 5 by a modified trisection method and five integrity classes were defined to comply with the European Water Framework Directive (WFD). The IBI was tested intemally by comparison with habitat quality scores at sites. A similar comparison was done using an independent set of data from sites of known ecological quality. Our IBI clearly distinguished good sites from disturbed sites and heavily disturbed sites from moderately disturbed sites, thereby meeting the criteria imposed by the WFD.

lntroduction

Fish-based indices are becoming important assessment tools since the European Union (EU) water policy proposed fishes as an element to assess the ecological condition of water bodies (EU Water Framework Directive, WFD, 2000). Certain parameters, such as fish species composition, abundance, and age structure, are mandatory. Numerous studies have demonstrated that fish assemblage attributes accurately reflect the overall biotic integrity of aquatic ecosystems (KaTI" et al., 1986; Lyons et al., 1996; Hughes & Oberdorff 1999). More traditional approaches, based on chemical water quality parameters fail to account for certain types of degradation, such as channelisation, harriers, alien species, and altered flow regimes (Fore et al., 1993). Originally the Index of Biotic Integrity (IBI) was developed for Midwestem USA streams (KaTI", 1981) but it has since been modified for use all over the world (Hughes & Oberdorff, 1999). Multiple IBI metrics provide information about various aspects of fish assemblages and the metric scores quantify deviations from reference conditions. In Europe these references are most often minimally disturbed sites rather than pristine sites. Metric values are affected by multiple environmental characteristics that influence the expected fish assemblage. Gorman & KaTI" (1978) found that habitat complexity (stream depth, bottom type and stream velocity) correlated with species diversity in lndiana and Panama streams. Angermeier & Schlosser (1989) reported that habitat volume and species relative abundance were coTI"elated in two Panama streams that were not subjected to strong seasonal or annual environmental variations. Hugueny (1990) observed positive correlations between species richness and habitat volume, maximum depth, and current speed diversity in the Niadan (Niger) River.

Metrics in the IBI used for Midwestern USA streams vary with stream size and zoogeographic region (Karr et al., 1986; Osborne et al., 1992). Sheldon (1968) showed a strong correlation between some metrics and river depth in Owego Creek. In the Appalachian Highlands of the USA, Leonard & Orth (1986) modified the IBI by calibrating with gradient. However, McCormick et al. (2001) developed an index for wadable streams in the same region by adjusting several metrics to watershed area alone. Therefore, IBI scoring criteria for some metrics may vary with stream size, slope, or elevation.

The biotic integrity in downstream (bream and barbel type, Huet, l 949a) Flanders rivers is assessed with an IBI having 8 metrics, some of which vary with stream width (Belpaire et al., 2000). In wadable streams of the trout and grayling zone in the Walloon part of Belgium, the IBI is calibrated by watershed area (Kestemont et al., 2000). However a specific IBI is needed for upstream areas in Flanders, since substantial differences between geomorphological conditions in Flanders and Walloon are reflected in the fish assemblages. The grayling and trout zone in Walloon differs from those in Flanders with a higher altitude (up to 600 m) and a calcareous formation. Furthermore the fish assemblages of upstream areas differ significantly from downstream sites. Our objective in this paper is to describe the development of an IBI for rivers belonging to the trout and grayling zone in Flanders.

Material and methods

Study area

The study area is within Flanders the northern part of Belgium, belonging to the centra) plains according to the Water Framework Directive (Fig. 1). The studied water bodies are all tributaries belonging to the grayling or trout zone (Huet, l 949a) from three major rivers in Flanders. They are the river Schelde, IJzer and Maas and all flow into the North Sea. In the northeast, streams are fed by rainwater and superficial groundwater. In the south and centre of Flanders they are spring-fed or are fed by deeper groundwater (Wils et al., 1994). The streams in the northeast therefore have a lower alkalinity and calcium concentration than those in the south and centre of Flanders. The average slope is 9.5%o (± 6.4) and wetted width is 1.6 m in average (± 1.0 m). With the exception of some sites, all are located rather close to the source (4 km in average ± 3) with an average altitude of 49 m (ranging from 8.75 to 190 m). Most locations have a high water velocity. Downstream the sites, the studied rivers have suffered from human impacts such as dam construction or canalisation. Agricultural impact on the studied sites is less important than in the downstream areas. The geological formation is siliceous and the average pH is 7.6 (± 0.7).

Data collection

From 1994-2000, 201 sites in upstream waters and belonging to the grayling or trout zone (Huet, 1949a) were surveyed (Fig. 1). Fish assemblage data were obtained by electrofishing using a 5 kW generator with an adjustable output voltage of 300 to 500 V and a pulse frequency of 480 Hz. The number of electrofishing devices and the number of hand-held anodes used was 2 except when the river was smaller than 1 m. Electrofishing was carried out in upstream direction over a distance of 100 m. Fish data included species, densities, and individual total lengths (TL) and weights. Site data included 4 physical, 4 chemica), and 4 geomorphological variables (Belpaire et al., 2000). The geomorphological variables or predictors (distance to source, wetted width, altitude and slope) were measured using overlays in a geographic information system (GIS) and on topographic maps over a distance of 1000 m. Slope was checked in the field using a theodolite and wetted width was measured. In addition, fish data from 346 locations with similar dimensions but belonging to the downstream zone were collected during the same period with an identical protocol.

Data analysis

TabIe 1 gives an overview of the sequence of activities leading to the development of the upstream IBI.

Composition of calibration data set (step1, Table 1)
We needed to know whether we had to develop another index and if yes whether it was necessary to have two separate indices for the grayling and trout zone respectively. To evaluate this problem we applied the index developed for barbel waters (Belpaire et al.,

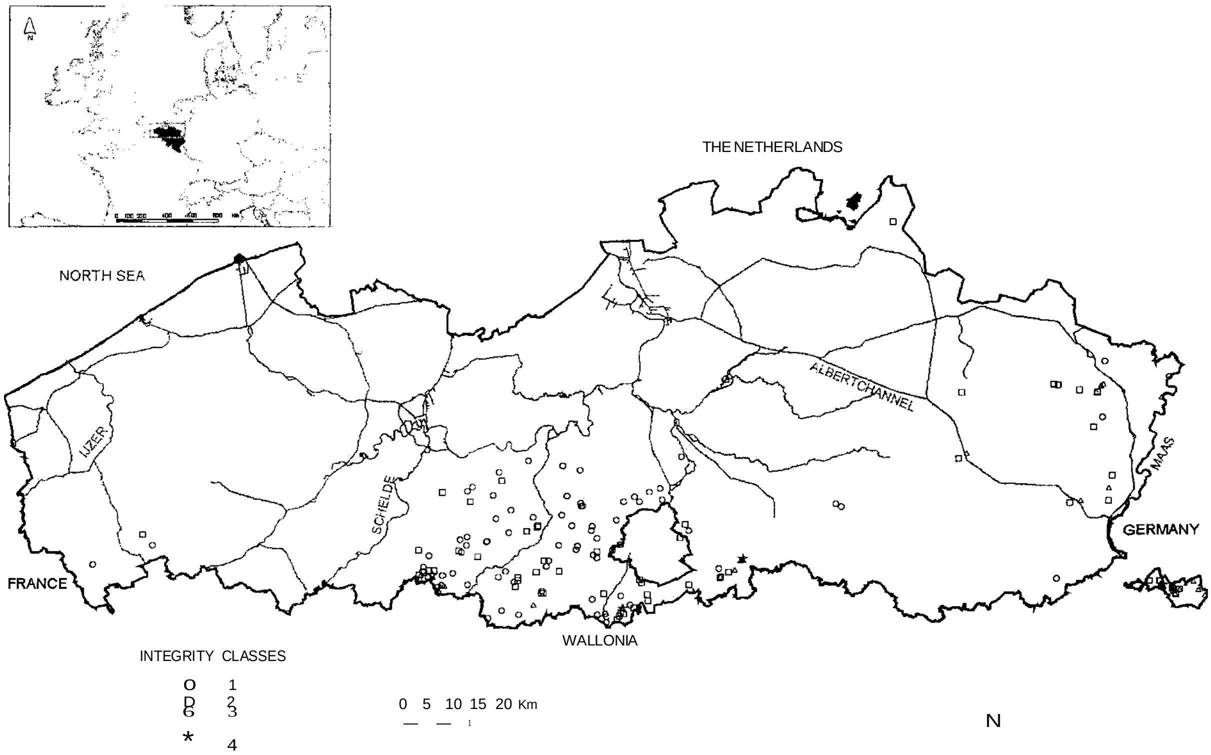

Figure 1. Location of sampling sites and their biotic integrity classes in Flanders.

Table 1. Overview of the activities involved in the development of the upstream !BI

	Step	Description	Input	Methodology	Results
	Composition of calibration data set	Decision: one or more indices?	Biotic and abiotic data from upstream brooks	FA + ANOVA	Pooled data set: one index (for grayling and trout zone)
2	Metric selection	Transformation of data into indicative biologica! variables	Literature and WFD	Guilds PCA + cluster analysis (Fig. 2)	28 candidate metrics (Table 4) Selection of 9 metrics (Table 5)
3	Scoring	Standardisation into a scoring system (1, 3, 5) and aggregation into one biotic index	Descriptors	Trisection (e.g. Fig. 3)	Definition of thresholds (Table 5) Integrity classes (Table 7)
4	Evaluation	Internal and Extemal validation Consistency and redundancy	Ecological quality clas of calibration and independent data	Scoring discrepancy Metric remainder correlation	Discrepancy distribution (Figs. 3 and 4) Consistency evaluation (Table 8)

2000) and the Walloon trout/grayling index (Didier, 1997) to waters of the Flanders grayling and trout zone to check the possible use of those indices. Using a one-way analysis of variance (ANOVA) fish assemblages in downstream sites were compared with those in upstream rivers to assess the dissimilarities between the different types.

Fish assemblages (presence/absence and abundance) in the grayling and trout zones were analysed using multivariate exploratory statistica! techniques (Factor analysis, FA) to define special cases, and ANOVA was used to compare the fish assemblages in the grayling and trout zones.

Metric selection (step2, Table 1)

Based on literature and on the criteria of the WFD a list of candidate biologica] metrics was drawn. For each fish species the ecological demands (reproduction, feeding behaviour, tolerance to oxygen concentration or any relevant special characteristic) were defined from the literature and from our own experiences. Values for the candidate metrics were calculated for each site where fish were caught. A principal component analysis (PCA) on the log transformed metric values ($\log x + 1$) was used to detect low responding metrics and to detect overlapping metrics. An additional cluster analysis was performed. A correlation (Spearman rank) indicated correlated metrics. Metrics composed of few species were rejected to avoid instability in scoring.

Scoring (step 3, Table 1)

After selecting metrics, threshold values for metric scoring classes were determined. The trisection method used was an adaptation of Karr's approach (Karr, 1981) and that developed by Goffaux et al. (2001). We evaluated log values of distance to source, river width, slope, and altitude so that each class had about the same number of data. A Pearson correlation (2-tailed) was used to investigate correlations among the predictor variables. Metric values were plotted against each predictor class to select the predictor most correlated with the metrics. The best-fitted trend line through the highest metric values was drawn and the R^2 values obtained with the different predictors were compared. After comparing the results, we selected one predictor for all parameters based on the correlation. When a specific metric was correlated with the selected predictor, the mean trendline was used to define threshold values and to help estimate reference condition. Scoring criteria were based on the trendline equation of all the values. This mean trendline is a 2nd-order polynomial regression curve between the Log10 of the predictor (X) and all the metric values (Y) and corresponds to the average environmental quality score since a correlation was found between the Jatter and the IBI developed by Belpaire et al. (2000). The average environmental quality score was defined to be 3 or fair. This score was obtained combining different abiotic parameters (see evaluation). The genera] formula of the mean trendline is $y = ax^2 + bx + c$. Lines using $2y/3$ and $4y/3$ were plotted to define the limits of the zone getting a score of 3. Beneath the $2y/3$ line values are scored as 1 or 5 depending on the evolution of the metric value with increasing degradation. Above the $4y/3$ line values are scored as 5 or 1 again depending on the kind of metric considered.

When no correlation was observed, scoring criteria were defined using literature and expert knowledge. The trisection approach defined by Goffaux et al. (2001) was used for the biomass metric.

Evaluation (step 4, Table 1)

Once the threshold values fixed, the index was calculated and different tests were performed to control the robustness and sensitivity of the index. An intemal validation test was performed by comparing the IBI class with the environmental quality class obtained by combining data on habitat structure and chemica) water quality values. We defined the habitat structure quality based on parameters (pools and riffle, meandering and presence of natura! refuges) proposed by Schneiders et al. (1993) in combination with data from Natuur CD, 2000. The chemica! water quality is defined using different parameters: oxygen conccntration (mg 1^{-1}), pH, orthophosphate concentration (PO- µg 1^{-1}), nitrite (N02 µg 1^{-1}) and BOD_0 (mg 1^{-1}). The threshold values for both habitat and chemica] quality used, are those from Goffaux et al. (2001). The final environmental quality score was slightly adapted by giving more weight to oxygen and nitrite concentrations. An independent set of data from locations in the River Maas basin was used for extemal validation of the developed IBI. These Jocations were given an environmental score based on habitat quality (pools and riffle, meandering and presence of natura! refuges) and oxygen concentration (Goffaux et al., 2001).

A Spearman's rank correlation (2-tailed) was applied using metric scores to assess correlations among the selected metrics and between the metrics and the IBI. To assess if a metric was concordant with the others a metric-remainder correlation test with Spearman 's rank correlation coefficient (2-tailed) was dctermined by calculating the IBI without the metric and then calculating the correlation between this changed IBI (r-IBI) and the particular metric value. A positive correlation close to 1 indicates that the given metric is concordant with the other metrics (Hughes et al., 1998).

The statistical packages used were Statistica 5.7, S-PLUS 2000 for Windows and SPSS 9.00 for Windows.

Results

One or two indices?

The ANOVA indicated a significant difference between the fish composition in downstream and upstream waters. The relative number of fish caught, the presence/absence ($p < 0.01$ except for some species) and the trophic composition were significantly higher in the downstream sites ($p < 0.01$). Also we observed a decrease in the percentage of omnivorous and alien individuals between downstream and upstream waters. Applying the barbel IBI to the upstream locations yielded much lower metric scores than we feit were appropriate (average score of 1.66 ± 0.7) out of a possible 5. Walloon index metrics, e.g., bullhead/(bullhead + loach) ratio and percentage of omnivorous individuals were not relevant for Flanders grayling/trout streams in this study. The reason is that in the studied waters nearly no bullhead, loach and omnivorous species are present. Based on these observations it was decided to consider other metrics.

The ANOVA and FA revealed no significant differences in fish assemblages between the trout and grayling zones. The FA reveals one central cloud, and the ANOVA showed that, apart for some species, the between variation is smaller than the within variation ($p > 0.05$) or there is more variation in the different zones than between the two zones. Therefore the option was taken to develop one single index for both zones. Furthermore we reconsidered the composition of our data and decided to exclude brooks wider than 4.5 m due to Jack of data. We therefore developed an index for sites with a maximum width of 4.5 m and a slope of at least 3‰ (grayling and trout typology, Huet, 1949a). In total 154 sites, of which 96 sites had fish, were retained and 30 different species were collected from those 96 sites (Table 3).

Candidatemetrics

From literature (Hughes & Oberdorff, 1999; Belpaire et al., 2000; Goffaux et al., 2001) a list of 28 candidate metrics to assess the upstream waters in Flanders was drawn (Table 2). In addition new variables such as length class value (MANCV) and migrating species

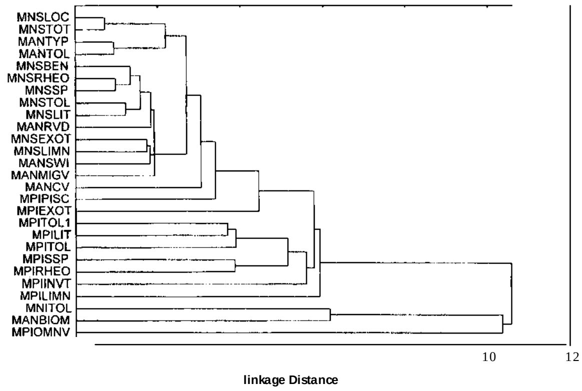

Figure 2. Cluster analysis of log transformed values of candidate metrics (abbreviations see Table 2).

value (MANMIGV) were added, as recommended by the WFD. All 28 metrics are known to respond to human impact and belong to one of the three biological categories proposed by Karr (1981). From this list metrics were screened combining the results of different approaches.

The PCA indicated redundancy and metrics with a low variance. Metrics with a factor loading (1st axis) < 0.7 were rejected (Table 4). However metrics proposed by the WFD were withheld even if the factor loading was low. As such MANCV (length classes value) was selected despite its low contribution to the variance (0.268). The cluster analysis had similar results as the PCA (Fig. 2). It grouped the redundant metrics. Although a cluster analysis is notoriously unpredictable and highly dependent on the algorithm and beta level we decided to keep the results since they corresponded with the PCA results. Among the metrics with a significant contribution to the variance but also strongly correlated, only one (MANMIGW) was selected based on expert judgement and on its applicability to our waters. The combination of the results of the different analyses together with the expert judgement resulted in a selection of 9 metrics (Table 5). The selected metrics contribute each unique ecological information since they represent different ecological aspects of the fish assemblage.

Metric description and defining threshold values for the selected metrics

A significant correlation was found among all predictors except between width and slope and distance and altitude respectively (Table 6). Slope was selected since it can be measured accurately using overlays in a geographical information system (GIS) and since it

Tahle 2. List of candidate metrics (if not genera! in literature the source is added)

Metric	Abbreviation	Source	Predicted response to disturbances
Species richness and composition			
# local species	MNSLOC	Didier (1997)	↓
# species	MNSTOT		↓
% intolerant individuals	MPITOLI	Didier (1997)	↓
Bulhead/loach ratio	MANRVD	Didier (1997)	↓
Typical species value	MANTYP	Belpaire et al. (2000)	↓
% intolerant individuals	MPITOL	Didier modified*	↓
# intolerant specimens	MNSTOL	*	↓
Mean tolerance value	MANTOL	Belpaire et al. (2000)**	↓
Shannon-Wiener diversity index evenness	MANSWI		↓
Migrating species value	MANMJGV	New	↓
Fish condition and abundance			
# individuals	MNITOL		,!,
Biomas (kg/ha)	MANBJOM		,!, and t
% recruitment	MPIRECR	(# species that recruit/# species)* 100	,!,
Length classes value	MANCV***	N=	,!,
# exotic species	MNSEXOT		t
% exotic individuals	MPIEXOT		t
Trophic composition and habitat use			
% omnivorous individuals	MPIOMNV		↑
% invertivorous individuals	MPIINVT		↓
% piscivorous individuals	MPIPISC		↓
# benthic species	MNSBEN		↓
# specialised spawners	MNSSP	****	↓
# lithophilic species	MNSLIT		↓
% lithophih:: individuals	MPILIT		↓
% rheophilic individuals	MPIRHEO		↓
# rheophilic species	MNSRHEO		↓
% limnophilic individuals	MPILIMN		↑
# limnophilic species	MNSLIMN		↑
% specialised spawners (individuals)	MPISSP	Didier (1997)	↓

*Tolerant values used are not those given by Didier (1997) but are values as given in Table 3.
**Tolerant values used are those from EG Life project (Goffaux et al., 2001).
***Only typical species are considered (values 4 and 5 in Table 3) typical is defined by Huet (1949a, 1954).
****See Table 3.

correlates significantly with most of the selected metrics. Wetted width varies according to the season and this creates problems.

As mentioned already, on average our locations obtained an environmental quality score of 3 (ranging from 1 to 5). Considering the correlation between the environmental score and the IBI (unpublished test), we assumed that the mean trendline through all metric values corresponds with a metric score of 3.

Total number of species (MNSTOT)

The number of species decreases with increased human impact (Karr et al., 1986; Miller et al., 1988; Faush et al., 1990; Belpaire et al., 2000). High habitat diversity represents a variety of suitable habitat and food types to support many different species (Wichert & Rapport, 1998). Other authors used the number of native species (Hughes & Gammon, 1987; Steedman, 1988; Crumby et al., 1990; Lyons et al., 1995; Ganasan & Hughes, 1998; Hughes et al., 1998; Kestemont et al., 2000) to avoid positive scoring of alien

Table 3. Guilds for freshwater fishes (species caught in sampling sites are indicated with an *). 1 benthic species, 2 omnivores; 3 exotic species; 4 typical species value; 5 invertivores; 6 limnophilic species; 7 lithophilic species; 8 specialised spawners; 9 length cla5Ses (number of cla5ses = score); 10 migration values (from Breine et al., 2001)

Scientific name		2	3	4	5	6	7	8	9	10
*Abramis brama**	y	y								
Alburnus alburnus				2	y					
Alburnoides bipunctatus				4	y		y	y	1-2=1; 3=3; >3=5	
*Anguilla anguilla**	y	y		3					1=1; 2-6=3; >6=5	2
Barbus barbus	y	y		4			y	y	1=3; > 1=5	
*Barbatula barbatula**	y			5	y			y	1=1; 2-4=3; >4=5	
Blicca bjoerkna		y								
Carassius carassius		y				y				
Chondrvstoma nasus	y	y		4			y	y	l=l ; 2-4=3; >4=5	
Cobitis taenia	y			1	y	y		y		
*Cottus gobio**	y			4	y		y	y	I=I; 2-4=3; >4=5	
*Cyprinus carpio**	y	y		1						
*Esox lucius**				2		y		y		
*Gasterosteus aculeatus**		y		2						
*Gobio gobio**	y			4	y	y		y	1=1; 2-4=3; >4=5	
*Gymnocephalus cernuus**	y				y					
*Lampetra jiuviatilis**	y			2			y	y	1=l; 2-4=3; >4=5	2
*Lampetra planeri**	y			4			y	y	1=3; >1=5	
*Leuciscus cephalus**		y		4			y	y	1-2=1; 3-4=3;>3=5	
*Leucaspius delineatus**				1		y				
*Leuciscus idus**		y		3						
Leuciscus leuciscus		y		4	y		y	y	1 =l ; 2-4=3; >4=5	
Lota lota	y			3			y	y	l=l ; 2-4=3; >4=5	
Misgurnusfossilis	y				y	y		y		
Osmerus eperlanus				1	y					
*Perca jiuviatilis**				2	y					
*Phoxinus phoxinus**		y		4			y	y	1=3; >1=5	
*Pungitius pungitius**		y		1		y				
*Rhodeus sericeus**				1		y		y		
*Rutilus rutilus**		y		2						
*Salmo truttafario**				5	y		y	y	l=l ; 2=3; > 2=5	
Salvelinus fontinalis				4	y		y	y		
Salmo salar				4	y		y	y		2
Salmo trutta trutta				4	y		y	y	1=3; >1=5	2
*Scardinius erythrophthalmus**		y		2		y		y		
Silurus glanis	y			1		y				
Thymallus thymallus				5	y		y	y	1=3; >1=5	
*Tinca tinca**	y	y				y		y		
Vimba vimba			y					y		
*Pimephales promelas**			y							
*Pseudorasbora parva**		y	y							
*Oncorhynchus mykiss**			y		y		y	y		
*Lepomis gibbosus**			y		y	y				
lctaluruspunctatus		y	y			y				
Ameiurus nebulosus	y	y	y			y				
*Ameiurus melas**	y	y	y			y				
*Umbrapygmaea**			y		y	y				
Sander lucioperca			y							
Ctenopharyngodon idella			y							
Aspius aspius			y				y	y		
Chalcalburnus chalcoides			y		y					
*Carassius auratus gibelio**	y		y			y				

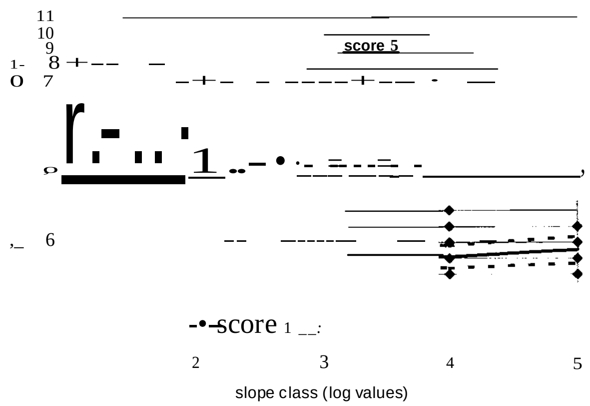

Figure 3. Threshold lines (dotted lines) for MNSTITT (total number of species) versus slope (log slope) based on the medium trendline (full line). Above the upper dotted line score 5 is given, below the lower dotted line score 1. One dot can represent multiple observations.

species. We excluded alien species from certain metrics: average species value, migrating species value and length classes value. An average of 3 species, with a maximum of 10, were collected at the sites. This metric was significantly negatively correlated with slope ($R^2 = 0.81$ and $c = -0.319$, $p < 0.01$). Therefore scoring criteria were based on the trendline equation of all the values. The mean trendline (Fig. 3) was defined as: $y = 0.3744x^2 - 2.9109x + 7.7388$ and as already mentioned corresponds to the environmental quality score 3. The dotted lines in Fig. 3 define the limits of the zone getting a score of 3. Beneath the lower dotted line values are scored as 1, above the top dotted line values are scored as 5. In slope classes 3, 4 and 5 the trendline does not change, therefore, the same scoring criteria are used (Table 5).

Average species value (MANTYP)

This metric consists of the sum of the species value rankings (Table 3, column 4), divided by the total number of species caught. The average species value metric replaced the tolerant and intolerant metrics more commonly used in !Bis. The value attributed to each species is a modification of the typical species value proposed by Belpaire et al. (2000). Species values are based on species distributions and typical habitats (Huet, l 949a, b, 1954; Gerstmeier & Romig, 2000; http://www.fishbase.org). Human impacts increase with distance to source (longitudinal gradient) and with decreasing slope (Pearson correlation coefficient = -0.348 for $p < 0.05$). The average species value is expected to decrease with increasing degradation, since typical species in upstream waters are in genera! intolerant. And as stated by Karr (1981) and Fausch et al. (1990) the number of intolerant species declines with increasing degradation. The metric is strongly correlated to the slope ($R^2 = 0.90$; $c = 0.205$ for $p < 0.05$). Using the same approach as with the metric MNSTOT the trendline obtained with all values was defined, representing the average environmental quality (score 3). The average MANTYP score obtained with our locations was 2.41. Analogous to the method applied with MNSTOT calculating $2y/3$ and $4y/3$ defined the limits for this score (TabIe 5).

Table 4. Factor loadings first two axes (* <0.7) from a PCA with log transformed values of candidate metrics ($\log x + 1$). The first two axes explain 63% (48.7% variance explained in the 1st axis and 14.5% in the second axis). The third and fourth axis explain respectively 8.0 and 5.1%. (abbreviations see Table 2)

metric	Factor 1	Factor 2
MNSLOC	0.791441	-0.50232*
MNSBEN	0.849657	-0.31088*
MNSTOT	0.810347	-0.54320*
MNJTOT	0.633437*	-0.31022*
MPJTOL	0.762783	0.408927*
MANRVD	0.441541*	0.538741*
MPISSP	0.841295	0.334572*
MPIOMNV	-0.35826*	-0.48320*
MANBIOM	0.821412	-0.22405*
MNSEXOT	0.394139*	-0.57224*
MPIEXOT	0.171424*	-0.30268*
MANTYP	0.802053	0.423642*
MPIINVT	0.830077	-0.05397*
MPIPISC	0.425699*	0.202907*
MPIRHEO	0.877021	0.213954*
MNSRHEO	0.947794	-0.04799*
MPILIMN	-0.18387*	-0.52817*
MNSLIMN	0.115673*	-0.78313
MPITOLI	0.726989	0.239801*
MNSTOL	0.914372	-0.01314*
MANTOL	0.689255*	0.394772*
MANSWI	0.750152	-0.476210*
MPILIT	0.757496	0.519996*
MNSLIT	0.872219	0.212232*
MNSSP	0.943323	-0.13203*
MPIRECR	0.087388*	0.145043*
MANCV	0.607856*	-0.26882*
MANMIGV	0.734423	--0.21173*

Shannon-Wiener Diversity Index (MANSWI)

The diversity metric was used because it is a good indicator for sites with many specimens but only a few evenly distributed species. Degradation of the aquatic environment is presumed to reduce evenness (Fausch et al., 1990). The Shannon-Wiener Diversity Index was calculated as $H = -\sum (p_i) \ln(p_i)$ where $p_i = (n_i/n_{tot})$ this is the proportion of individuals that species i contributes to the entire community (Liang & Menzel, 1997). It reflects evenness and species richness, the higher the value the better (Calow, 1998). Though a decreasing tendency with increasing slope was observed, it was not significant ($c = -0.268$, $p = 0.105$). This tendency could be explained by the fact that fewer species can occur in steeper waters. The average value for all locations reflects the average condition of our stations and corresponds with an environmental quality score 3. Here trisection was applied independently from the slope. The average value of the mean trendline in the different classes (ranging from 0.53 to 0.68) received metric score 3. Lower or higher values got a score of 1 or 5 respectively (Table 5).

Migrating pecies value (MANMIGV)

This metric is sensitive to migration barriers. Only fish that migrate over Jonger distances to reproduce or feed are considered (Table 3, column 10). The impact of barriers such as dams on the migration of fish and on fish assemblages has been assessed by different authors (Petts, 1984; Nicola et al., 1996; Knaepkens et al., 2001). Dams not only impede the distribution of material and energy transfer through the drainage basin but also obstruct spawning migrations (Bonetto et al., 1988; Barthem et al., 1991). The metric 'Migrating species value' assesses the impact of dams but also the effect of mitigation processes. This metric is the sum of migration values for each species caught, with a theoretica! maximum score of 14. No significant correlation was found between this metric and river slope ($c = -0.031$, $p = 0.771$). Therefore we used the same approach as for the Shannon-Wiener Diversity Index. Threshold values are given in table 5.

Biomass (MANBIOM kg ha^{-1})

An underlying assumption of the IBI is that fish abundance generally declines with environmental degradation (Fausch et al., 1990). Belpaire et al. (2000) stated that restocking activities might positively affect this metric. However a stocking effect is corrected by the length classes (MANCV) and evenness metrics (MANSWI). Moreover the restocking effect might be temporary since all niches are occupied and restocked fish have little chance to survive. Biomass was initially retained in the Walloon index (Kestemont et al., 2000) as estimated biomass when only one fishing pass was considered. We believe that biomass assessment requires at least two electrofishing passes. We did not attempt to assess true biomass, however our standardised methodology allowed us to compare different sites. Breine et al. (2001) could not find significant biomass differences for these brooks using one or more passes. Although variations (spatial and tempora]) occurred, the integrity class assignment varied in few cases. In Goffaux et al. (2001) biomass was log transformed to obtain a clear trend. For our data this was not necessary. A significant negative correlation for this metric with river slope was observed ($R^2 = 0.72$; $c = -0.263$, $p < 0.05$). This is in accordance with the decreasing number of species and specimens observed with increasing slope. De Maeseneer (1991) stated that on average upstream waters support fish biomass of S 60 kg ha^{-1}. Using the trendline equation for all values brought the threshold score too low (< 20 kg ha^{-1}). Therefore we modified our approach and applied trisection with the trendline through the 95% line as the 5 value score ($y = 27.821x^2 - 241.3x + 613.89$). Calculating the $2y/3$ and $y/3$ values set the limits for scores of 3 and 1 respectively (Table 5). Threshold values for slope classes 4 and 5 are the same since no significant trend difference was observed between those slopes.

Length classes value (MANCV)

This is one of 4 metrics proposed by the WFD (2000). It provides indirect information about natura] recruitment and the ability of the habitat to support long-lived individuals. Natura) recruitment <lid not contribute to the variance and was therefore dropped as a metric. A score was attributed relative to the number of length classes recorded (Table 3, column 9). The length classes for each species were defined from Timmermans (1957), Huet (1961), OVB (1988), De Nie (1996) and Goffaux et al. (2001) and http://www.fishbase.org. The sum of the scores is divided by the sum of the species caught. Only species belonging to the upstream type of water (rheophilic species) were considered. The maximum value that can be obtained is 5. No significant correlation was observed with slope ($p = 0.82$). The average value (1.77) was taken into consideration to define the scoring criteria using the same approach as for Shannon-Wiener Diversity Index (Table 5).

The percentage of invertivorous individuals (M PllNVT)

This metric often is integrated in an IBI (Hughes & Oberdo1ff, 1999) and is correlated with river width

Table 5. Fish-based IBI for upstream waters in Flanders (slope ?:3%o, river width :'.::4.5 m): selected metrics and scoring criteria

Metric	Metric score		
		3	5
Species richness and composition			
Total number of species (MNSTOT)			
Slope class 1 (<4%0)	< 4	4-7	'.:':8
Slope class 2 (4-5%0)	< 3	3-5	?: 6
Slope classes 3, 4 & *5* (>5%o)		2-4	'.:':5
Typical species value (MANTYP)			
Slope class 1	< 1.44	1.44-2.88	> 2.88
Slope class 2	< 1.49	1.49-2.97	> 2.97
Slope class 3 (> 5-8%0)	< 1.57	1.57-3.13	> 3.13
Slope class 4 (>8-12.5%0)	< 1.69	1.69-3.37	> 3.37
Slope class 5 (>12.5%0)	< 1.85	1.85-3.69	> 3.69
Shannon-Wiener diversity index evenness (MANSWI)	< 0.53	0.53--0.68	> 0.68
Migrating species value (MANMIGV)	< 2	2-4	> 4
Fish condition and abundance			
Biomass (kg/ha) (MANBIOM)			
Slope clas 1	:'.:: 130	130.1-250	> 250
Slope class 2	:'.:: 80	80.1-150	> 150
Slope cla5s 3	:'.::46	46.1-100	> 100
Slope cla5ses 4 & 5	:'.:: 30	30.1-60	> 60
Length classes value (MANCV)	< 2	2-3.99	4-5
Trophic composition and habitat use			
% invertivorous individuals (MPIINVT)	< 26	26-45	> 45
Number of benthic species (MNSBEN)	1	2-3	> 3
% specialised spawners (individuals) (MPSSP)			
Slope class 1	< 8	8-15.9	?: 16
Slope cla5s 2	< 10	10-20.9	?: 21
Slope class 3	< 12	12-30.9	?: 31
Slope cla5s 4	< 24	24-47.9	?: 48
Slope class *5*	< 35	35--69.9	?: 70

Table 6. Pearson correlation (2-tailed) between test predictors (raw valucs)

		Width	Slope	Distance to source	Altitude
Correlation	Width	1000			
	Slope	-0.099	1000		
	Distance to source	0.432*	-0.348*	1000	
	Altitude	0.237**	0.392*	-0.160	1000

N = 96.
**Correlation is significant at the 0.05 level (2-tailed).
*Correlation is significant at the 0.01 level (2-tailed).

or stream order (Karr et al., 1986). The percentage of trophic specialists such as invertivores declines with increasing degradation (Fausch et al., 1990). The small increase in the amount of invertivorous fish with increasing slope is natura! since steeper waters are usually less disturbed. The scoring criteria differ slightly from those proposed by Belpaire et al. (2000) for rivers of the barbel and bream zone. A Score of one has a higher threshold value in the upstream zones since it is assumed that the conditions are less disturbed than in the other river type locations. Initially % of omnivorous species was proposed because this metric declines with increasing biotic integrity (Belpaire et al., 2000). However this metric was rejected because few omnivorous species were caught and this metric was negatively correlated with the IBI and the correlation with the r-IBI was low. In our case the small increase in the amount of invertivorous fish with increasing slope observed was not significant ($R^2 = 0.33$, $p = 0.131$). Therefore the average value (ranging from 26-45 over the different slope classes) was considered to define the scoring criteria, using the same approach as for Shannon-Wiener Diversity Index (Table 5).

Number of benthic species (MNSBEN)

The number of benthic species metric measures the habitat quality for bottom dwelling species (McCormick et al., 2001). Benthic species are sensitive to stream siltation, and oxygen deficiency (Oberdorff & Porcher, 1994, Kestemont et al., 2000). Oberdorff & Hughes (1992) stated that benthic species for the most part are sensitive to benthic oxygen depletion since they feed and reproduce in benthic habitats. The attributed threshold values for this metric correspond with those forwarded by Kestemont et al. (2000) where the number of benthic species depends on catchment area and ranges from 3 to 9 species. Scoring criteria proposed by Oberdorff & Porcher (1994) to assess the biologica! impact of fish farms in some Brittany streams were less severe (> 1 benthic species = score 5). An average of 2 benthic species were present at our stations, with a maximum of 6. This metric is negatively correlated with slope ($R^2 = 0.56$, $p = 0.055$). In the absence of a significant correlation, scoring was done without the use of a predictor. Considering the average value for all stations (2), thresholds were fixed using the same approach as for Shannon-Wiener Diversity Index and taking into consideration that the number of benthic species declines in response to degradation of the benthic habitat (Table 5).

Percentage of specialised spawners (MPISSP)

This metric includes gravel spawners, phytophilic and ostracophilic spawners in accordance with Didier (1997) (Table 2, column 8) and it assesses degradation of the spawning habitat. The number of specialised spawners decreases with increasing degradation (Oberdorff & Hughes, 1992; Didier, 1997; Kestemont et al., 2000). Due to degradation fish will not spawn successfully (Belpaire et al., 2000) and this will be reflected by the absence of one or more year-classes or eventually lead to the extinction of one or more species (Nicola et al., 1996). A high correlation was observed between the slope and the metric ($R^2 = 0.90$, $p \ll 0.001$). The mean trendline was expressed by $y = 2.186Ix^2 - 2.824lx + 11.743$. Using this equation the $2y/3$ and $4y/3$ values were calculated to define the threshold values of the score 3 area (Table 5).

Table 7. !BI score ranges and their appreciation, integrity classes and the colour code according to the WFD (EU Water Framework Directive, 2000)

!BI score ranges	!BI appreciation	Integrity class = WFD quality classes	WFD colour code
>4.5-5	Excellent	Very good	Blue
>4-4.5	Very good	Good	Green
>3.5-4	Good		
>3-3.5	Fair	Fair	Yellow
>2.5-3	Critica!		
>2-2.5	Critical-bad	Poor	Orange
>1.5-2	Bad		
1-1.5	Very bad		
0	<lead	Bad	Red

!BI score

The IBI score of a given site is the mean of scores for all metrics, and varies between 0 and 5. An integrity class is assigned to the IBI score, ranging from dead water to a site with excellent quality. Breine et al. (2001) reduced the number of integrity classes to 5 in accordance with other indices and the WFD (2000). The threshold values of the integrity classes described by Belpaire et al. (2000) were adapted to avoid over scoring. Where previously the integrity class intervals were equal, intervals were narrowed for the higher quality classes (Table 7). Internationally the 5 classes system is to be used, but for national purposes the 9 integrity classes can still be used. Figure 1 shows the attributed integrity class for each location.

!BI Internal and external validation

An internal validation of the IBI was executed with data on 29 locations of the design data set. These locations were selected since they represented a range

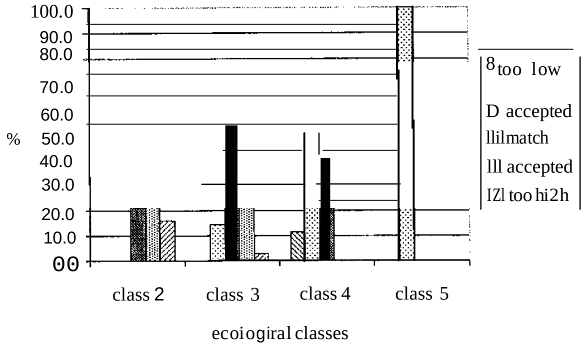

Figure 4. External validation: Distribution of calculated fish-based !BI integrity classes in each attributed environmental quality class ranging from 2 to 5. When the difference between IBJ integrity class and ecological clas is more than one class, the !BI is considered to be a mismatch (too low or too high).

of environmental quality classes from 1 to 4 (bad to good respectively). Comparing the calculated IBI class with the environmental class per location and allowing a class difference of 1 unit, we observed a 17.1% underscoring, 72.4% adequate scoring (41.3% no difference) and 10.3% of overscoring of the IBI classes.

The IBI was also tested using an independent validation data set of 156 locations (river width ::::3 m, slope 2:3%0) in the river Maas basin spread over two countries (France and Belgium, Goffaux et al., 2001). These locations were given an environmental quality score ranging from poor (2) to excellent (5), no site had a bad quality condition (score 1). The IBI scores were compared with the environmental quality score, and 92.7% of the locations were scored appropriately of which 42.6% matched perfectly, while only 7.6% were wrongly scored (difference of two classes, Fig. 4). No class 1 locations were found; for the class 2 locations, 44% were correctly scored, 40 % were within the accepted limit, and 16% of the sites received too high of an IBI score. Class 3 was 53.6% correctly scored with 14.5% and 29% accepted and overscoring, respectively. For class 4, only 11% scored too low. The two locations having an environmental quality score of 5 were attributed to the integrity class 4, which was an acceptable underscoring.

Consistency and redundancy

All metrics were significantly positively correlated with the IBI ($p < 0.01$, Table 8). Most metrics were correlated with each other, but not to a degree making them redundant. The metric-remainder correlation test showed that all metrics had a significant correlation with their respective r-IBI and can therefore be considered concordant but not redundant (Table 8).

Discussion

Composition of calibration data set

The method used to collect the fish is a standardised method used in Flanders (Belpaire et al., 2000). It corresponds also with the suggestions from the CEN document (CEN, 2002) stating that in order to ensure accurate characterisation of a fish community at a given site electric fishing must be conducted over stream (or river) lengths of at least 20 times the stream (or river) width. Although the typology was different, we combined the grayling and trout zone, because of similar fish assemblages and because the threshold values were adjusted for those metrics that were inftuenced by typological conditions (slope). The observed similarity in fish assemblages could have been caused by habitat degradation producing a more homogeneous assemblage.

Metric selection

The list of potential metrics was based on literature but also on knowledge of the aquatic systems. From the evaluation we rejected the low responding metrics. A second criterion was a trade-off between correlation and redundancy. The 9 selected metrics represent diverse aspects of composition, abundance and age structure and cover information of one of the three biologica! categories as proposed by Karr (1981). From the overview presented by Hughes & Oberdorff (1999) we observe that in genera] our metrics correspond well with metrics used in other IBis. The migration species value (MANMIGV) and length class value (MANCV) are new or uncommon but relevant to the biologica! community under study. As already mentioned upstream brooks are important for migrating species. The impact of harriers is therefore important and is assessed by MANMIGV. For MANCV we only considered rheophilic species to avoid overscoring due to the presence of untypical species.

Scoring

Several methodologies can be used to determine metric scoring criteria. In all methods however, reference

Table 8. Spearman's ro correlation between individual metrics and between the metrics and total !BI score (r = correlation coefficient, p = s1gmficance level (2-tailed), and metric remainder coefficient (c = metric remainder coefficient , p^* = level of significance) (abbreviations see Table 5)

		MNSBEN	MNSTOT		MANBIOM	MANTYP	MPIINVT	MANSWI	MANCV	MANMIGV	*c*	*p**
MNSBEN	*r*	1,000									0.729	<0.01
	p											
MNSTOT	*r*	,612**	1,000								0.663	<0.01
	p	,000										
MPSSP	*r*	,313**	,248*	1,000							0.650	<0.01
	p	,002	,015									
MANBIOM	*r*	,278**	,299**	-,032	1,000						0.580	<0.01
	p	,006	,003	,755								
MANTYP	*r*	,298**	,150	,578**	,139	1,000					0.720	<0.01
	p	,003	,143	,000	,176							
MPIINVT	*r*	,271**	,350**	,607**	,096	,472**	1,000				0.720	<0.01
	p	,007	,000	,000	,351	,000						
MANSWI	*r*	,435**	,660**	.305**	,072	,137	,348**	1,000			0.609	<0.01
	p	,000	,000	,003	,488	,182	,OOI					
MANCV	*r*	,194	,186	,131	,348**	,248*	,212*	,015	1,000		0.285	<0.05
	p	,059	,070	,202	,OOI	,015	.038	,886				
MANMIGV	*r*	,466**	,385**	,202*	-,018	,046	.160	,298**	,046	1,000	0.492	<0.01
	p	,000	,000	,048	,861	,660	.120	,003	,658			
!BI	*r*	0.644**	0.672**	0.723**	0.367**	0.643**	0.738**	0.569**	0.408**	0.366**		
	p	,000	,000	,000	,000	,000	,000	,000	,000	,000		

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

sites play a major role. Minimally disturbed reference sites are sometimes used to select optima! metric scores, and score classes are determined by dividing the total metric range in three or five equal portions (assuming a linear behaviour of the metrics). The absence of minimally disturbed reference sites forced us to develop another approach to determine threshold values. Adjusting metric criteria for stream predictors increases our ability to evaluate anthropogenic effects rather than natura) stream characteristic effects. Removing subjectivity when applying the eye-fit method and regression line to define threshold values is not new (Didier, 1997; Liang & Menzel, 1997; Goffaux et al., 2001). However, those approaches used the 95% value, thus omitting the rest of the values. Using the median trend line with all available values gave a more realistic image and provided more robust relationships between metric values and predictors.

Validation

The internal IBI validation should be considered as an initia) test of the overall accuracy and pertinence of the developed index. Fish species diversity is correlated with habitat complexity (Gorman & Karr, 1978). Our unpublished research established a positive correlation between habitat structure and the IBI. Roth et al. (1996) found a correlation between the habitat quality and IBI at the catchment scale. We therefore used the calculated values of habitat structure quality and chemica! quality to evaluate the IBI. Given the u nccrtainty in the environmental quality classification, we feit that one class difference did not reftect a significant divergence and that a two classes deviation or more represented true estimation errors. This validation helped us to define tendencies of under- or overestimation and was also used in Goffaux et al. (2001). From the internal validation we concluded that the IBI discriminated good from bad locations very well, but tended to underscore locations classified as having good environmental quality. From the extemal validation with comparable locations we observed closer agreement. The tendency of underscoring was less pronounced than in the intemal validation. From both validation tests it was clear that the IBI did not give a good score to poor conditions nor a bad or poor score to good or excellent conditions.

We used Spearman's rank correlation to assess the correlation between the metrics since our data did not conform to a bivariate normal distribution. All metrics should provide spccific information concerning the ecological quality of the site. If two or more metrics overlap or are strongly correlated one can be considered as superfluous. Still all parameters should show certain coherence together since they determine the IBI score and their individual contribution should follow an analogous pattem. From the correlation tests (Table 8) it was observed that two metrics (length class value and migrating species value) were less correlated ($r < 0.5$) with the IBI and they were less concordant ($r < 0.5$) with the other parameters. However, both correlations were significant. Furthermore length class value is one of the variables to be assessed by the WFD. From the PCA we observed that all 9 parameters were positively associated with the first axis indicating intemal consistency. The contribution of MANCV and MANMIGV to the first axis was less than the other parameters (0.425 and 0.441 for MANCV and MANMIGV respectively).

Conclusion

Using a standardised sampling methodology a multimetric index to assess upstream water locations was developed. Even though the intrinsic metric variability could not be assessed due to Jack of repeated samples we think that the selected metrics are relevant and allow the appropriate assessment of anthropogenic impacts on fish communities. The IBI includes a balanced set of metrics having a good discriminating power. The fact that they are not redundant is preferable as stated by Whittier & Hughes (2001). The developed IBI responses also to the criteria stipulated in the Water Framework Directive. As mentioned by Triest et al. (2001) the IBI should be considered as a complementary instrument to other ecological indices.

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