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Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium)

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ABSTRACT

The European Water Framework Directive requires that member states assess all their surface waters based on a number of biological elements, including macroinvertebrates. Since 1989, the Flemish Environment Agency has been using the Belgian Biotic Index for assessing river water quality based on macroinvertebrates. Throughout the years, the Belgian Biotic Index has proven to be a reliable and robust method providing a good indication of general degradation of river water and habitat quality. Since the Belgian Biotic Index does not meet all the requirements of the Water Framework Directive, a new index, the Multimetric Macroinvertebrate Index Flanders (MMIF) for evaluating rivers and lakes was developed and tested. This index was developed in order to provide a general assessment of ecological deterioration caused by any kind of stressor, such as water pollution and habitat quality degradation. The MMIF is based on macroinvertebrate samples that are taken using the same sampling and identification procedure as the Belgian Biotic Index. The index calculation is a type-specific multimetric system based on five equally weighted metrics, which are taxa richness, number of Ephemeroptera, Plecoptera and Trichoptera taxa, number of other sensitive taxa, the Shannon–Wiener diversity index and the mean tolerance score. The final index value is expressed as an Ecological Quality Ratio ranging from zero for very bad ecological quality to one for very good ecological quality. The MMIF correlates positively with dissolved oxygen and negatively with Kjeldahl nitrogen, total nitrogen, ammonium, nitrite, total phosphorous, orthophosphate and biochemical and chemical oxygen demand. This new index is now being used by the Flemish Environment Agency as a standard method to report about the status of macroinvertebrates in rivers and lakes in Flanders within the context of the European Water Framework Directive.

Introduction

According to the European Water Framework Directive (WFD; EU, 2000), a good status should be achieved for all natural surface waters in the European Union by the end of 2015. A good surface water status is more specifically defined as the attainment of both a good ecological and chemical status. The assessment of the ecological status is based on a number of biological quality elements as well as hydromorphological, chemical and physical–chemical elements supporting these biological elements. To assess the status of the biological quality elements, member states must choose or develop a classification method, taking into account a set of parameters depending on the quality element (EU, 2000).

The biological elements that must be taken into account depend upon the category of surface waters. For the categories 'rivers' and 'lakes', one of the relevant elements is the 'benthic invertebrate fauna', commonly referred to as macroinvertebrates. For this quality element, the parameters 'taxonomic composition and abundance' and 'ratio of disturbance sensitive to insensitive taxa' should be taken into account. The quality index must be in agreement with an Ecological Quality Ratio (EQR) showing relative proportion of the index compared to the reference conditions. This EQR has a value between zero and one, where 0 corresponds with a (minimal) bad and 1 with a (maximal) high ecological status. The interval between 0 and 1 is divided into 5 classes reflecting bad, poor, moderate, good and high status, respectively.

Macroinvertebrates have a long history of application in water quality assessment, resulting in a large variety of indices, many of them being country- or region-specific (e.g. Rosenberg and Resh,

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1993; De Pauw et al., 2006). Its application in routine river monitoring schemes by the Flemish Environment Agency (VMM) for more than a decade confirmed the reliability and robustness of the Belgian Biotic Index (BBI; De Pauw and Vanhooren, 1983) as a water quality assessment method. However, a number of difficulties arose with regard to the potential application of the BBI for WFD implementation. A first difficulty in this context was that it is not a type-specific method. All types of rivers are evaluated by means of the same criteria. However, it is known that the composition of the macroinvertebrate communities changes with progression from headwater stream to river (Vannote et al., 1980). Also, the BBI was intended as an assessment system for watercourses (De Pauw and Vanhooren, 1983) and hence an index for stagnant waters was still missing. For the implementation of the WFD, the assessment by means of macroinvertebrates should incorporate the parameters 'taxonomic composition and abundance' as well as 'ratio of disturbance sensitive to insensitive taxa' (EU, 2000). Abundance is not taken into account in the BBI calculation. A minimum abundance of two individuals is required for inclusion of a taxon in the index calculation, but the abundance as such is not incorporated in the index calculation.

To overcome the technical shortcomings of the BBI with regard to the WFD implementation, the development of a new, type-specific multimetric index was envisaged. A multimetric index describes the state of an ecosystem by means of several individual variables (metrics). These metrics each represent a different component of ecosystem quality and are combined into one index value. Multimetric indices were first developed for fish communities (e.g. Karr, 1981; Fausch et al., 1984) and later also for other indicator groups, including macroinvertebrates (e.g. Kerans and Karr, 1994; Thorne and Williams, 1997; Böhmer et al., 2004). An important advantage of multimetric indices is that they are flexible and can easily be adjusted by adding or removing metrics or fine-tuning the metric scoring system. In several European member states, multimetric indices have been developed or are under development for application within the WFD. For example, within the AQEM project, multimetric indices were developed for 28 types of streams throughout Europe (Hering et al., 2004).

The new index, called the Multimetric Macroinvertebrate Index Flanders (MMIF), combines the robustness of the BBI with the versatility of multimetric indices, allowing for an adaptation of scoring criteria for each river or lake type to reflect the relative distance to reference conditions. A preliminary concept of this index was described by Gabriels et al. (2006). The present paper provides an overview of the final version of the MMIF and its development process for all types of rivers and lakes in Flanders.

Development of the Multimetric Macroinvertebrate Index Flanders

Typology

The MMIF is a type-specific index, which means that index calculation depends on the type of river or lake a sampling site belongs to. A typology covering all categories of waterbodies (rivers, lakes, transitional and coastal waters) in Flanders was developed by Jochems et al. (2002) according to the WFD requirements as a framework for the development of assessment methods based on the relevant biological quality elements. For the river category, one adaptation was applied to the typology of Jochems et al. (2002): because the catchment area was considered sufficiently representative for the size of the watercourse, a further correction using the river order according to Strahler (1952) is presently not used as a criterion. For lakes, no adaptations were introduced to the typology, but for the purpose of the MMIF, the ten lake types defined by Jochems et al. (2002) were clustered into four more general types.

An overview of the Flemish types of rivers and lakes as used within the MMIF, including their abbreviations and determining properties are presented in Table 1. The category of lakes includes all stagnant waterbodies with a surface area larger than 0.5 km^2.

Table 2
Taxonomic identification level of macroinvertebrates (De Pauw and Vanhooren 1983; Gabriels et al. 2005).

Taxon	Identification level
Plathelminthes	Genus
Polychaeta	Family
Oligochaeta	Family
Hirudinea	Genus
Mollusca	Genus
Hydracarina s.l.	Presence (i.e. counted as one taxon)
Crustacea	Family
Diptera, Chironomidae	Group (*thummi-plumosus* or non *thummi-plumosus*)
Diptera, other	Family
Megaloptera	Genus
Coleoptera	Family
Hemiptera	Genus
Odonata	Genus
Ephemeroptera	Genus
Trichoptera	Family
Plecoptera	Genus

Table 1
Main characteristics of different types of rivers and lakes in Flanders (Belgium), as defined for application of the Multimetric Macroinvertebrate Index Flanders (based on Jochems et al. 2002).

River types	Abbreviation	Hydro-ecoregion	Catchment area
Small stream	Bk	Sand/sandy loam/loam	< 50 km^2
Small stream Kempen	BkK	Kempen	< 50 km^2
Large stream	Bg	Sand/sandy loam/loam	50–300 km^2
Large stream Kempen	BgK	Kempen region	50–300 km^2
Small river	Rk	Any	300–600 km^2
Large river	Rg	Any	600–10,000 km^2
Very large river	Rzg	Any	> 10,000 km^2
Polder watercourse	P	Polder	Not applicable
Lake types	**Abbreviation**	**Properties**	
Alkaline	A	pH ≥ 7.5	
Circumneutral	C	7.5 > pH ≥ 6.5; no clay	
Acidic	Z	pH < 6.5; only sand/sandy loam/loam	
Very slightly brackish	Bzl	Na > 250 mg/L; no sand/sandy loam/loam	

Table 3
Taxa taken into account for calculating the Multimetric Macroinvertebrate Index Flanders, with their respective tolerance scores (TS), ranging from 10 for very pollution sensitive to 1 for very pollution tolerant taxa.

Taxon	TS
Plathelminthes	
Bdellocephala	5
Crenobia	7
Dendrocoelum	5
Dugesia s.l.	5
Phagocata	5
Planaria	6
Polycelis	6
Polychaeta	
Ampharetidae	3
Oligochaeta	
Aelosomatidae	2
Branchiobdellidae	2
Enchytraeidae	2
Haplotaxidae	4
Lumbricidae	2
Lumbriculidae	2
Naididae s.s.	5
Tubificidae	1
Hirudinea	
Cystobranchus	4
Dina	4
Erpobdella	3
Glossiphonia	4
Haementeria	4
Haemopis	4
Helobdella	4
Hemiclepsis	4
Hirudo	4
Piscicola	5
Theromyzon	4
Trocheta	4
Mollusca	
Acroloxus	6
Ancylus	7
Anisus	5
Anodonta	6
Aplexa	6
Armiger	6
Bathyomphalus	5
Bithynia	5
Bythinella	8
Corbicula	5
Dreissena	5
Ferrissia	7
Gyraulus	6
Hippeutis	6
Lithoglyphus	6
Lymnaea s.l.	5
Margaritifera	10
Marstoniopsis	5
Menetus	5
Myxas	7
Physa s.s.	5
Physella	3
Pisidium	4
Planorbarius	5
Planorbis	6
Potamopyrgus	6
Pseudamnicola s.l.	5
Pseudanodonta	6
Segmentina	6
Sphaerium	4
Theodoxus	7
Unio	6
Valvata	6
Viviparus	6
Acari	
Hydracarina s.l.	5

Table 3 (*continued*)

Taxon	TS
Crustacea	
Argulidae	5
Asellidae	4
Astacidae	8
Atyidae	7
Cambaridae	6
Chirocephalidae	6
Corophiidae	5
Crangonyctidae	4
Gammaridae	5
Janiridae	5
Leptestheriidae	6
Limnadiidae	6
Mysidae	5
Palaemonidae	5
Panopeidae	4
Sphaeromatidae	4
Talitridae	5
Triopsidae	6
Varunidae	4
Diptera	
Athericidae	7
Blephariceridae	7
Ceratopogonidae	3
Chaoboridae	3
Chironomidae	
-non *thummi-plumosus*	3
-*thummi-plumosus*	2
Culicidae	3
Cylindrotomidae	3
Dixidae	6
Dolichopodidae	3
Empididae	3
Ephydridae	3
Limoniidae	4
Muscidae	3
Psychodidae	3
Ptychopteridae	3
Rhagionidae	3
Scatophagidae	3
Sciomyzidae	3
Simuliidae	5
Stratiomyidae	4
Syrphidae	1
Tabanidae	3
Thaumaleidae	3
Tipulidae	3
Megaloptera	
Sialis	5
Coleoptera	
Dryopidae	6
Dytiscidae	5
Elminthidae	7
Gyrinidae	7
Haliplidae	6
Hydraenidae	6
Hydrophilidae	5
Hygrobiidae	5
Noteridae	5
Psephenidae	6
Scirtidae	7
Hemiptera	
Aphelocheirus	8
Arctocorisa	5
Callicorixa	5
Corixa	5
Cymatia	6
Gerris s.l.	6
Glaenocorisa	5
Hebrus	6
Hesperocorixa	5
Hydrometra	6
Ilyocoris	5
Mesovelia	6

Table 3 (*continued*)

Taxon	TS
Micronecta	6
Microvelia	7
Naucoris	6
Nepa	6
Notonecta	5
Paracorixa	5
Plea	6
Ranatra	6
Sigara	5
Velia	7
Odonata	
Aeshna	6
Anax	6
Brachytron	7
Calopteryx	8
Cercion	7
Ceriagrion	7
Coenagrion	6
Cordulegaster	9
Cordulia	7
Crocothemis	7
Enallagma	7
Epitheca	7
Erythromma s.s.	7
Gomphus	7
Ischnura	6
Lestes	7
Leucorrhinia	7
Libellula	7
Nehalennia	7
Onychogomphus	7
Ophiogomphus	7
Orthetrum	7
Oxygastra	7
Platycnemis	7
Pyrrhosoma	7
Somatochlora	7
Sympecma	7
Sympetrum	7
Ephemeroptera	
Baetis	6
Brachycercus	7
Caenis	6
Centroptilum	7
Cloeon	6
Ecdyonurus	9
Epeorus	10
Ephemera	8
Ephemerella s.l.	8
Ephoron	9
Habroleptoides	8
Habrophlebia	8
Heptagenia s.l.	10
Isonychia	7
Leptophlebia s.s.	8
Metreletus	7
Oligoneuriella	7
Paraleptophlebia	8
Potamanthus	8
Procloeon	7
Rhitrogena	10
Siphlonurus	7
Trichoptera	
Beraeidae	9
Brachycentridae	9
Ecnomidae	6
Glossosomatidae	9
Goeridae	9
Hydropsychidae	6
Hydroptilidae	8
Lepidostomatidae	9
Leptoceridae	8
Limnephilidae s.l.	8
Molannidae	9

Table 3 (*continued*)

Taxon	TS
Odontoceridae	9
Philopotamidae	6
Phryganeidae	9
Polycentropodidae	6
Psychomyiidae	7
Rhyacophilidae	8
Sericostomatidae	8
Plecoptera	
Amphinemura	9
Brachyptera	10
Capnia s.l.	10
Chloroperla s.l.	10
Dinocras	10
Isogenus	10
Isoperla	10
Leuctra	9
Marthamea	10
Nemoura	8
Nemurella	8
Perla	10
Perlodes	10
Protonemura	9
Rhabdiopteryx	10
Taeniopteryx	10

Sampling

The samplings should be carried out during spring, summer or autumn. It is recommended to avoid sampling macroinvertebrates during winter in order to avoid extreme conditions, both of hydrological regime and temperature, to ensure a reliable water quality assessment.

Macroinvertebrates are sampled using a standard handnet, as described by De Pauw and Vanhooren (1983) and NBN (1984). This handnet consists of a metal frame of approximately 0.2 m by 0.3 m to which a conical net is attached with a mesh size of minimum 300 and maximum 500 μm. The frame is attached to a 2 m long shaft with two handles enabling it to be handled in a similar way as a scythe. With the handnet, a stretch of approximately 10–20 m is sampled during 3 minutes for water-courses less than 2 m wide or up to 5 minutes for larger rivers. Sampling effort is proportionally distributed over all accessible aquatic habitats. This includes the bed substrate (stones, sand or mud), macrophytes (floating, submerged, emerged), immersed roots of overhanging trees and all other natural or artificial substrates, floating or submerged in the water. Each aquatic habitat is explored, either with the handnet or manually, in order to collect the highest possible diversity of macroinvertebrates. For this purpose, kicksampling is performed by vertically positioning the handnet on the bed and turning over bottom material located immediately upstream by foot or hand. In addition to the handnet sampling, animals are manually picked from stones, leaves or branches along the same stretch (De Pauw and Vanhooren, 1983). For lakes, macroinvertebrates are sampled using the same method, distributing the sampling effort proportionally over all accessible aquatic habitats within a stretch of 10–20 m.

If a site is too deep to be sampled with the handnet method, macroinvertebrates can alternatively be sampled using the so-called Belgian artificial substrates as described by De Pauw et al. (1986) and De Pauw et al. (1994). These substrates are composed of a plastic netting filled with medium-sized (4–8 cm) pieces of brick, with a total volume of approximately 5 L. Per sampling site, three substrates are placed in the water, anchored with a rope to a fixed point located on the bank. The substrates should not be placed in open water but along the banks: in protected sites

Table 4
Overview of metrics taken into account in the Multimetric Macroinvertebrate Index Flanders.

#	Abbreviation	Name	Calculation
1.	TAX	Taxa richness	Total number of present taxa
2.	EPT	Number of EPT taxa	Number of present Ephemeroptera, Plecoptera and/or Trichoptera taxa
3.	NST	Number of sensitive taxa	Number of present taxa with tolerance score >5, not including Ephemeroptera, Plecoptera and Trichoptera
4.	SWD	Shannon–Wiener diversity	$-\sum_{i=1}^{S} p_i \ln p_i$ (Shannon and Weaver 1949) with S=taxa richness, p_i=relative abundance of taxon i
5.	MTS	Mean tolerance score	Mean of the tolerance scores of all present taxa

Table 5
Overview of the expert-based reference values that were used to calculate the type-specific criteria (cf. Tables 1 and 4 for abbreviations).

	Rivers								Lakes			
	Bk	BkK	Bg	BgK	Rk	Rg	Rzg	P	A	C	Z	Bzl
TAX	34	34	38	38	40	42	44	37	33	35	28	30
EPT	7	8	8	9	9	9	10	8	6	8	5	5
NST	9	9	10	10	12	12	12	10	10	10	8	9
SWD	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3	3.2
MTS	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.3	6	6	6	6

among the vegetation near the surface, in unprotected sites, which are exposed to surface turbulence, in deeper water. After an exposure time of at least 3 weeks, the substrates are lifted from the water and transferred into a closed container (De Pauw et al., 1986).

Sorting, identification and counting

All collected material is thoroughly examined for presence of macroinvertebrates. Identification is carried out according to the taxonomic levels defined by De Pauw and Vanhooren (1983). This means family, genus or an intermediate level for all taxa (except for watermites, which are considered as a single taxon). The identification levels are summarised in Table 2.

A list of all taxa taken into consideration for the MMIF is presented in Table 3. This list, consisting of 225 taxa, is based on Gabriels et al. (2005).

After identification, the total number of individuals of each taxon is recorded. If more than ten individuals of the same taxon are encountered, the total abundance can be estimated instead. Estimates of the total abundance of individual taxa, where necessary, can be obtained by homogenising the sample and subsequently counting the number of individuals in a representative subsample.

Metric selection, reference state and index calculation

A preliminary index system was developed, based on an identical set of metrics with type-specific scoring criteria. A preliminary set of metrics was proposed based on a literature review, analysis of existing data and expert judgement. This draft list of metrics, together with a set of proposed reference values per metric for each type of river or lake as well as a set of tolerance scores ranging from 1 to 10 for each taxon, was submitted to a panel of macroinvertebrate experts. After receiving their remarks, a new list of metrics, reference values and tolerance scores was established in order to integrate all assembled expert knowledge. The new values were submitted to the same panel again in order to further refine the developed index (Gabriels et al., 2004). This resulted in a final list of five metrics, a set of type-specific reference values for each metric and a list of tolerance scores.

The tolerance scores, ranging from 10 for very pollution sensitive to 1 for very pollution tolerant taxa, are included in Table 3. The metrics comprised in the MMIF are Taxa Richness (TAX), Number of Ephemeroptera, Plecoptera and Trichoptera Taxa (EPT), Number of other (i.e. non-EPT) Sensitive Taxa (NST), the Shannon–Wiener Diversity (SWD) Index and the Mean Tolerance Score (MTS) (Table 4).

For each type of river and lake, a set of reference values for all five metrics was determined using the previously discussed procedure. An overview of the reference values for all metrics for all types of rivers and lakes is presented in Table 5.

Based on the references, a scoring system was developed for each metric consisting of threshold values needed for assigning a score ranging from zero to four (four being assigned to the metric values that were nearest to the reference value). These criteria were developed by equally dividing the interval between the expert-based target reference value and a value corresponding to bad ecological quality into five smaller intervals. The resulting scoring criteria are summarised in Table 6. These five metric scores are summed and subsequently divided by 20 to obtain the final index, ranging from zero for a very poor ecological quality to one for a very good ecological quality.

When displaying index results for MMIF, the type of river or lake should always be specified because the calculation method is type-specific.

Ecological quality ratio and quality class boundaries

As described above, the MMIF is calculated as the sum of the 5 scores divided by 20, resulting in a final index ranging from 0 to 1. This means that the maximum MMIF value of 1 can only be obtained when all metric values are near the type-specific reference value for that metric. For this reason, the range of the MMIF index can be considered as an EQR scale.

The quality class boundary values were initially constructed by equally dividing the total range of MMIF values into five classes, resulting in class boundaries of 0.80, 0.60, 0.40 and 0.20, respectively. However, subsequent to the European intercalibration exercises (CB-GIG, 2008) the quality class boundaries were modified in order to harmonise quality class evaluation by methods of different member states. As a result, the class boundaries were modified for all Flemish types of rivers that were included in the intercalibration exercise (small stream, small stream Kempen, large stream, large stream Kempen and small river) as well as Flemish river types from similar hydro-ecoregions (large river and very large river) and for lakes. For polder watercourses, the original class boundaries were maintained due to their very specific characteristics. Table 7 provides an overview of the class boundaries used and Table 8 provides an example of MMIF calculations for a river and a lake.

Table 6
Scoring criteria for calculating the Multimetric Macroinvertebrate Index Flanders for all types of rivers and lakes in Flanders. Columns and rows, respectively, correspond to the surface water types and the scores that can be assigned to the respective metrics (abbreviations are explained in Tables 1 and 4).

Type	Rivers								Lakes			
	Bk	BkK	Bg	BgK	Rk	Rg	Rzg	P	A	C	Z	Bzl
Score	**TAX**											
0	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5
1	≤ 12.25	≤ 12.25	≤ 13.25	≤ 13.25	≤ 13.75	≤ 14.25	≤ 14.75	≤ 13	≤ 12	≤ 12.5	≤ 10.75	≤ 11.25
2	≤ 19.5	≤ 19.5	≤ 21.5	≤ 21.5	≤ 22.5	≤ 23.5	≤ 24.5	≤ 21	≤ 19	≤ 20	≤ 16.5	≤ 17.5
3	≤ 26.75	≤ 26.75	≤ 29.75	≤ 29.75	≤ 31.25	≤ 32.75	≤ 34.25	≤ 29	≤ 26	≤ 27.5	≤ 22.25	≤ 23.75
4	> 26.75	> 26.75	> 29.75	> 29.75	> 31.25	> 32.75	> 34.25	> 29	> 26	> 27.5	> 22.25	> 23.75
Score	**EPT**											
0	0	0	0	0	0	0	0	0	0	0	0	0
1	≤ 1.75	≤ 2	≤ 2	≤ 2.25	≤ 2.25	≤ 2.25	≤ 2.5	≤ 2	≤ 1.5	≤ 2	≤ 1.25	≤ 1.25
2	≤ 3.5	≤ 4	≤ 4	≤ 4.5	≤ 4.5	≤ 4.5	≤ 5	≤ 4	≤ 3	≤ 4	≤ 2.5	≤ 2.5
3	≤ 5.25	≤ 6	≤ 6	≤ 6.75	≤ 6.75	≤ 6.75	≤ 7.5	≤ 6	≤ 4.5	≤ 6	≤ 3.75	≤ 3.75
4	> 5.25	> 6	> 6	> 6.75	> 6.75	> 6.75	> 7.5	> 6	> 4.5	> 6	> 3.75	> 3.75
Score	**NST**											
0	0	0	0	0	0	0	0	0	0	0	0	0
1	≤ 2.25	≤ 2.25	≤ 2.5	≤ 2.5	≤ 3	≤ 3	≤ 3	≤ 2.5	≤ 2.5	≤ 2.5	≤ 2	≤ 2.25
2	≤ 4.5	≤ 4.5	≤ 5	≤ 5	≤ 6	≤ 6	≤ 6	≤ 5	≤ 5	≤ 5	≤ 4	≤ 4.5
3	≤ 6.75	≤ 6.75	≤ 7.5	≤ 7.5	≤ 9	≤ 9	≤ 9	≤ 7.5	≤ 7.5	≤ 7.5	≤ 6	≤ 6.75
4	> 6.75	> 6.75	> 7.5	> 7.5	> 9	> 9	> 9	> 7.5	> 7.5	> 7.5	> 6	> 6.75
Score	**SWD**											
0	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2	≤ 0.2
1	≤ 1.025	≤ 1.025	≤ 1.025	≤ 1.025	≤ 1.025	≤ 1.025	≤ 1.025	≤ 1.025	≤ 1.025	≤ 1.025	≤ 0.9	≤ 0.95
2	≤ 1.85	≤ 1.85	≤ 1.85	≤ 1.85	≤ 1.85	≤ 1.85	≤ 1.85	≤ 1.85	≤ 1.85	≤ 1.85	≤ 1.6	≤ 1.7
3	≤ 2.675	≤ 2.675	≤ 2.675	≤ 2.675	≤ 2.675	≤ 2.675	≤ 2.675	≤ 2.675	≤ 2.675	≤ 2.675	≤ 2.3	≤ 2.45
4	> 2.675	> 2.675	> 2.675	> 2.675	> 2.675	> 2.675	> 2.675	> 2.675	> 2.675	> 2.675	> 2.3	> 2.45
Score	**MTS**											
0	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2	≤ 2
1	≤ 3.125	≤ 3.125	≤ 3.125	≤ 3.125	≤ 3.125	≤ 3.125	≤ 3.125	≤ 3.075	≤ 3	≤ 3	≤ 3	≤ 3
2	≤ 4.25	≤ 4.25	≤ 4.25	≤ 4.25	≤ 4.25	≤ 4.25	≤ 4.25	≤ 4.15	≤ 4	≤ 4	≤ 4	≤ 4
3	≤ 5.375	≤ 5.375	≤ 5.375	≤ 5.375	≤ 5.375	≤ 5.375	≤ 5.375	≤ 5.225	≤ 5	≤ 5	≤ 5	≤ 5
4	> 5.375	> 5.375	> 5.375	> 5.375	> 5.375	> 5.375	> 5.375	> 5.225	> 5	> 5	> 5	> 5

Table 7
Relation between Multimetric Macroinvertebrate Index Flanders (MMIF) and quality classes (abbreviations are explained in Table 1).

Types Bk, BkK, Bg, BgK, Rk, Rg, Rzg, A, C, Z, Bzl	Type P	Evaluation of quality	Colour code
0.90–1.00	0.80–1.00	High	Blue
0.70–0.89	0.60–0.79	Good	Green
0.50–0.69	0.40–0.59	Moderate	Yellow
0.30–0.49	0.20–0.39	Poor	Orange
0.00–0.29	0.00–0.19	Bad	Red

Correlation of index values with environmental variables

Based on available sampling data collected by the VMM between 2000 and 2009, the relationship was assessed between the MMIF and a number of environmental variables associated with water pollution. The MMIF was positively correlated with oxygen concentration (Spearman $R=0.45$, $n=304$) and oxygen saturation (Spearman $R=0.46$, $n=304$) and negatively correlated with Kjeldahl nitrogen (Spearman $R=-0.66$, $n=282$), total nitrogen (Spearman $R=-0.43$, $n=301$), ammonium (Spearman $R=-0.69$, $n=297$), nitrite (Spearman $R=-0.41$, $n=301$), total phosphorous (Spearman $R=-0.61$, $n=296$), orthophosphate (Spearman $R=-0.53$, $n=170$), 5 day biochemical oxygen demand (Spearman $R=-0.62$, $n=261$) and chemical oxygen demand (Spearman $R=-0.43$, $n=237$) ($p<0.001$ in all cases) (Fig. 1). However, the MMIF was not significantly correlated with nitrate (Spearman $R=-0.015$, $p=0.80$, $n=301$).

Discussion

Period of sampling

Seasonal variations are important in macroinvertebrate community composition (e.g. Furse et al., 1984; Rosillon, 1989; Linke et al., 1999; Bêche et al., 2006). Consequently, the period of sampling might affect the evaluation of a sampling site. However, not all metrics necessarily differ significantly between seasons. For example, Šporka et al. (2006) found that EPT metric values did not markedly differ between seasons because in any single month a reasonably representative selection of the three EPT orders was always present.

Still, seasonality should not be neglected when developing a monitoring and/or assessment system. Often, this is addressed by constraining the time frame of sampling (Linke et al., 1999). Although this strategy may result in missing information on the overall community at a site (Linke et al., 1999), it can be assumed to be sufficient for water quality assessment purposes. On the other hand, for the purpose of a large-scale monitoring network, it is advisable to choose a timeframe that is sufficiently large to visit all sampling sites in time. Therefore, it is recommended to avoid sampling in winter to avoid extreme hydrological regimes and temperatures and for logistical reasons (e.g. Šporka et al., 2006). Constraining the sampling period to

Table 8
Two random examples of Multimetric Macroinvertebrate Index Flanders calculation for samples taken by the Flemish Environment Agency: one on 10.V.2000 at the river Dijle, a river of the type Rk (small river) and another taken on 30.V.2007 at lake Heerenlaak, a lake of the type A (alkaline lake).

Taxon	TS	EPT	NST	Abundance Dijle	Abundance Heerenlaak
Lumbriculidae	2	–	–	11	
Naididae s.s.	5	–	–	2	1
Tubificidae	1	–	–	11	1
Erpobdella	3	–	–	40	
Glossiphonia	4	–	–	10	
Helobdella	4	–	–	8	8
Theromyzon	4	–	–		2
Trocheta	4	–	–		1
Bithynia	5	–	–		11
Corbicula	5	–	–		25
Dreissena	5	–	–		28
Gyraulus	6	–	1		5
Lymnaea s.l.	5	–	–	8	8
Physella	3	–	–		1
Pisidium	4	–	–		22
Planorbis	6	–	1		1
Potamopyrgus	6	–	1		40
Sphaerium	4	–	–		5
Unio	6	–	1		1
Valvata	6	–	1		30
Hydracarina s.l.	5	–	–	2	1
Asellidae	4	–	–	42	2
Corophiidae	5	–	–		3
Gammaridae	5	–	–		60
Mysidae	5	–	–		3
Chironomidae, non *thummi-plumosus*	3	–	–	6	70
Culicidae	3	–	–	1	
Psychodidae	3	–	–	1	
Simuliidae	5	–	–	1	
Micronecta	6	–	1		30
Sigara	5	–	–		1
Orthetrum	7	–	1		1
Baetis	6	1	–	5	
Caenis	6	1	–		30
Psychomyidae	7	1	–		1
Index calculation	**Dijle**			**Heerenlaak**	
	Value	**Score (Rk)**		**Value**	**Score (A)**
TAX	14	2		28	4
EPT	1	1		2	2
NST	0	0		7	3
SWD	2.06	3		2.59	3
MTS	3.79	2		4.89	3
Sum of scores		8			15
MMIF		0.4			0.75
Quality class		Poor			Good

For each taxon, the tolerance score (TS) is given and in the columns EPT and NST, taxa belonging to these groups are marked '1'.

spring, summer and autumn is therefore a pragmatic and reasonable option.

Taxa list

The MMIF taxa list was based on the list of 221 taxa proposed by Gabriels et al. (2005) for the BBI. Gabriels et al. (2005) pointed out that, in order to ensure comparable calculations over time, taxonomic modifications should not be adopted in existing taxa lists. But since the MMIF is a new index, adaptations to the cited taxa list can be made as long as they are sustained in the future. Both *Physa* s.s. and *Physella*, formerly constituting a single taxon (*Physa* s.l.), can therefore be included in the proposed taxa list. Other genera that were actually split up into two or more genera (e.g. *Lymnaea*) were maintained as a single genus because their separation was not considered to improve the sensitivity of the index system. Such genera are indicated with 's.l.'. Also, three new taxa were added: the crustacean families Sphaeromatidae and Panopeidae and the mollusc genus *Menetus*.

According to Gabriels et al. (2005), the list of taxa used for the BBI calculation should be 'semi-fixed', i.e. all included taxa cannot be altered at a later stage, but the list should be revised on a regular basis to allow for the inclusion of newly encountered (exotic) taxa. This principle should be applied for the MMIF as well, with an appropriate tolerance score assigned to each new included taxon.

Metrics used

The final selection of metrics was based on a number of considerations: they should be useful for all Flemish water body types, they should represent a variety of metric categories, they should all have been successfully used throughout Europe to assess water quality and they should reflect a number of criteria that are required by the WFD.

An identical set of metrics was used for all types, while the scoring thresholds were type-specific. This resulted in a straightforward and transparent index calculation method, while typological differences were still accounted for. A similar approach can be found in Butcher et al. (2003), who differentiated the Benthic Community Index by varying the threshold values of a number of metrics linearly with the natural logarithm of watercourse width.

Multimetric indices combine several metrics into a single evaluation. In this way, it is assumed that several aspects of ecosystem functioning or different measures of ecological integrity are combined into a more holistic evaluation. Also, combining several metrics is generally assumed to enhance reliability and robustness of an index, because accidental outliers of one metric can be smoothed by the other metrics. Metrics can be classified into several categories, each based on different principles of ecological quality assessment (e.g. Resh and Jackson, 1993; Thorne and Williams, 1997; Verdonschot, 2000; De Pauw et al., 2006): richness or diversity metrics; sensitivity metrics; similarity metrics; metrics based on functions, such as feeding groups and metrics that combine two or more of these categories, such as biotic indices.

Considering the metrics included in the MMIF, TAX (Taxa Richness) and SWD (Shannon–Wiener Diversity index) can be classified among the richness or diversity metrics, MTS (Mean tolerance Score) among the sensitivity metrics and EPT (EPT Richness) and NST (Number of Sensitive Taxa) among both of these categories. Similarity metrics are not explicitly included, although each individual metric could alternatively be seen as a measure of similarity to the reference status, expressed as the expert-based reference value from Table 5. Functional feeding group metrics were not used (see further). This examination of metric types illustrates the similarity between the MMIF and the BBI, the index on which the MMIF development was largely based. While the BBI may be seen as a hybrid method using taxa richness on the one hand and sensitivity of the encountered taxa on the other hand, the MMIF uses both properties in a number of metrics.

Metrics of richness or diversity are frequently used as indicators of ecological integrity. Diversity metrics are based on the assumption that disturbance of the water ecosystem or communities under stress leads to a reduction in diversity (De Pauw et al., 2006). Richness is widely used in water quality assessments based on macroinvertebrates because it integrates a

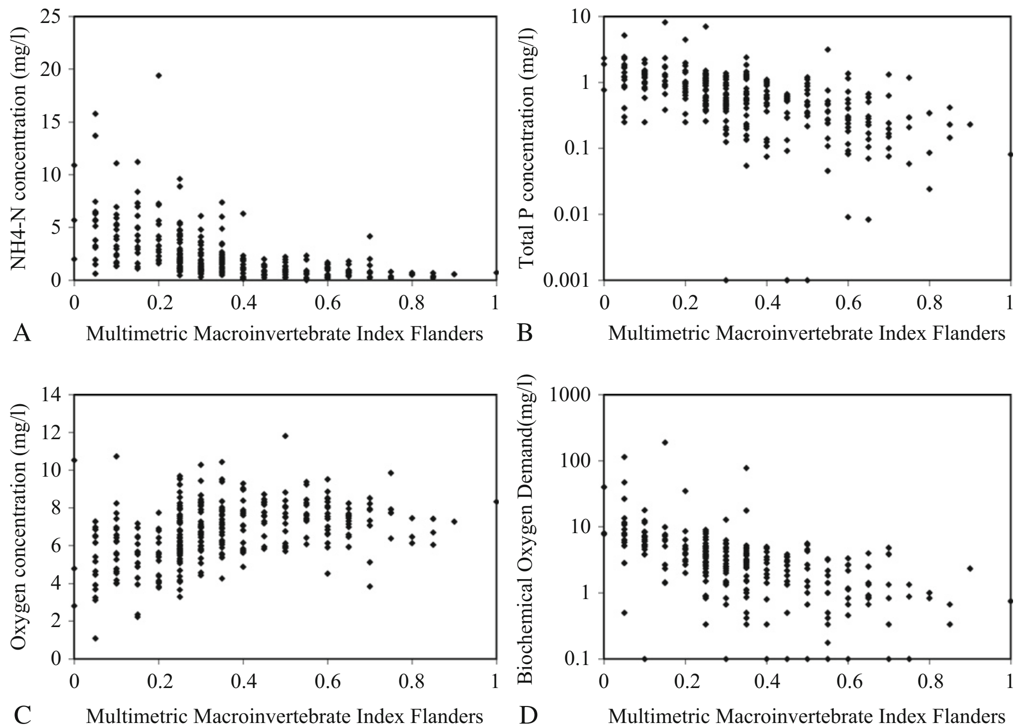

Fig. 1. Relationship between Multimetric Macroinvertebrate Index Flanders and ammonium concentration (A), total phosphorus concentration (B), oxygen concentration (C) and 5 day Biochemical Oxygen Demand (D) based on the samples taken by the Flemish Environment Agency between 2000 and 2009.

wide range of environmental effects. For example, Carlisle and Clements (1999) demonstrated the superiority of taxa richness measures in terms of sensitivity, variability and statistical power when it came to detecting metal-pollution effects. The majority of macroinvertebrate indices that are used for indicating general degradation of aquatic ecosystems include some measure of taxa richness. In the MMIF, included metrics based on richness and diversity are respectively TAX and SWD. The metric SWD is a diversity index that combines diversity and evenness of the encountered community.

Sensitivity metrics are also widely used in water quality assessments based on macroinvertebrates. In comparison to richness or diversity metrics, metrics based on sensitivity offer the advantage that taxon-specific information can be included. These metrics are based on the principle that different taxa respond in various ways to disturbance. This principle has been included in most assessment systems based on macroinvertebrates. The MTS is similar to the British ASPT (Average Score Per Taxon; Armitage et al., 1983), but with the identification levels and tolerance scores defined in Tables 2 and 3, respectively.

The metrics EPT and NST can be both assigned to the category metrics of richness or diversity as well as to the category sensitivity metrics. They are a measure of taxonomic richness within the overall macroinvertebrate richness. Both groups are composed of taxa that are sensitive to various sources of disturbance.

Functional feeding group metrics were not used, because the identification level was considered insufficient to reliably assign each taxon to a functional feeding group. Moreover, Karr (1999) questions the use of functional feeding group metrics for macroinvertebrates. Assigning invertebrates into functional feeding groups is, according to this author, often guesswork. Relative abundance of predators is the only macroinvertebrate functional feeding group that seems moderately reliable (Karr, 1999). Palmer et al. (1996) could not demonstrate a pattern in functional feeding group distribution and water quality in a South African River, although individual species had a strong relationship with water quality variables. Also, Fore et al. (1996) concluded that feeding ecology metrics failed to distinguish the most from the least disturbed sites.

Correlation with environmental variables

Among the environmental variables tested, the values of oxygen (either expressed as concentration or as saturation) typically decrease with increased environmental stress, while the other environmental variables decrease with increased environmental stress. The positive correlation of MMIF with the oxygen values is therefore in agreement with the assumption that the multimetric index negatively responds to environmental stress. Similarly, a negative correlation of MMIF with Kjeldahl nitrogen, total nitrogen, ammonium, nitrite, total phosphorous, orthophosphate and biological and chemical oxygen demand corresponds with what can be expected for stress-related variables. Only for nitrate, no significant correlation with MMIF was found.

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