VLAAMSE MILIEUMAATSCHAPPIJ

Biological assessment of the natural, heavily modified and artificial surface water bodies in Flanders according to the European Water Framework Directive

September 2009

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FIGURES AND TABLES

ABBREVIATIONS USED

Ad	Alkalic dune lake
Ai	Ion-rich, alkalic lake
Ami	Moderately ion-rich, alkalic lake
Awe	Large, deep, eutrophic, alkalic lake
Awom	Large, deep, oligotrophic to mesotrophic, alkalic lake
BEQI	Benthic Ecology Quality Index
Bg	Large stream
BgK	Large stream Kempen
Bk	Small stream
BkK	Small stream Kempen
Bs	Strongly brackish lake
Bzl	Very slightly brackish lake
Cb	Circumneutral, strongly buffered lake
CFe	Circumneutral, iron-rich lake
Czb	Circumneutral, weakly buffered lake
DIWB	Flemish Decree Integrated Water Policy
EBI	Estuarine Biotic Integrity Index
EFI	European Fish Index
EQR	Ecological Quality Ratio
FQI	Floristic Quality Index
GEP	Good Ecological Potential
GES	Good Ecological Status
GV	Growth forms
HD	Habitat Directive
IBI	Index for Biotic Integrity
INBO	Research Institute for Nature and Forest
IOBS	Indice Oligochètes de Bioindication des Sédiments
KFK	Square Kilometer Frequency Class
K1	Mesotidal tidal inlet or sea arm (Zwin)
MEP	Maximum Ecological Potential
MLz	Freshwater, mesotidal lowland estuary
MMIF	Multimetric Macroinvertebrate Index Flanders
O1b	Brackish macrotidal lowland estuary
O1o	Weakly brackish (oligohaline), macrotidal lowland estuary
O2zout	Saline mesotidal lowland estuary
PAR	Photosynthetically Active Radiation
Pb	Brackish polder watercourse
PISIAD	Proportions of Impact-Sensitive and Impact-Associated Diatoms

Pz	Freshwater polder watercourse
Rk	Small river
Rg	Large river
Rzg	Very large river
TIP	Typspezifischen Indexwertes Potamoplankton
TS	Type Specificity
V	Disturbance
VMM	Flemish Environment Agency
VO	Vegetation Development
WFD	European Water Framework Directive
Zm	Moderately acidic lake
Zs	Strongly acidic lake

1 Introduction

This document provides an overview of the relevant biological assessment methods for Flemish water bodies for the European Water Framework Directive (WFD; EU, 2000).

For most water types and quality elements methods have already been proposed, which were developed in the context of a number of studies conducted by various research institutions. In some cases, later amendments or additions were applied to the methodology, e.g. as a result of the European intercalibration exercises (see section 2.4). In this document, the current state of affairs is given for all available methods.

Because most proposed biological systems are multimetric and the various reports use in some cases different terminologies, for reasons of clarity, the term "metric" is used throughout this text for a variable that is part of an overall assessment system and the term "total index" for the overall multi-metric assessment (not necessarily equalling the Ecological Quality Ratio (EQR) at the water body level). This terminology may in some cases be different from the names used in the original texts.

Chapter 2 briefly outlines the general requirements of the WFD for the biological assessment of water bodies. In chapter 3, the typology used in Flanders is addressed. The subsequent chapters (4-8) deal with the assessment methods for different biological quality elements for natural water bodies belonging to the types of rivers, lakes and transitional waters. The following aspects of the assessment methods are addressed:

-the method of sampling;

-the conservation method (if applicable);

-the level of identification used (if applicable) and, if applicable, identification keys and standard lists used;

-assessment method (index) and calculation of the EQR (if it differs from the index) and its associated division into quality classes.

In chapter 9, the assessment of coastal waters is dealth with. This category includes only one water body.

Water bodies that are designated as artificial or heavily modified, are evaluated by means of the maximum ecological potential (MEP). The objective for these water bodies is achieving a good ecological potential (GEP). The establishment of these objectives for each water body is discussed in chapter 10.

2 Requirements of the Water Framework Directive

The European Water Framework Directive (EU, 2000), in force since December 22, 2000, aims to achieve good ecological status (GES) for natural water bodies by the end of 2015. To assess this, a number of biological quality elements are evaluated. For heavily modified and artificial water bodies an adapted evaluation is used.

2.1 Relevant quality elements for natural water bodies

Which biological quality elements should be evaluated in a natural water body, depends on the category to which the water body belongs, as shown in Table 2.1.

Table 2.1: Biological quality elements to be evaluated per category

Category	River	Lake	Transitional water	Coastal water
Phytoplankton	+**	+	+	+
Phytobenthos*	+	+	-	-
Macrophytes*	+	+	+***	+***
Macroinvertebrates	+	+	+	+
Fish	+	+	+	-

*: phytobenthos and macrophytes are one quality element: “other aquatic flora”
**: phytoplankton is not mentioned separately as a quality element for rivers but is included with phytobenthos and macrophytes under “aquatic flora”
***: macroalgae and angiosperms

2.2 Ecological quality ratio (EQR)

The assessment of a biological quality element is expressed in the form of an Ecological Quality Ratio (EQR), which can take values between zero and one, with one representing a very good ecological status and zero represents a very poor environmental status. This interval is further divided into five classes, high, good, moderate, poor and bad with the associated colour codes blue, green, yellow, orange and red, respectively. For obtaining the GES at least the quality class good must be reached. The EQR and the corresponding class boundaries vary by type of water body and biological quality element.

2.3 Integration of the partial assessments

The integration of the assessments of the various biological quality elements into an overall assessment for a water body is carried out according to the one out, all out principle. This means that the final assessment is the worst figure obtained for any quality element.

2.4 European intercalibration exercises

For each biological quality element and for each water body category, the Member States choose which assessment system they use. To ensure that the various assessment systems are comparable, intercalibration exercises are organized, coordinated by the Joint Research Centre of the European Commission in Ispra. A first series of intercalibration exercises have already been completed. The results were published in the form of a decision of the Commission (EU, 2008). These results have already been incorporated in the present document.

2.5 Assessment of heavily modified and artificial water bodies

According to the framework directive, water bodies designated as heavily modified or artificial should be assessed based on the Maximum Ecological Potential (MEP) and the Good Ecological Potential (GEP). The GEP is the objective that should be achieved by 2015. The starting point for developing the GEP for an artificial or heavily modified water body is always the objective for the most closely resembling type of natural water body. This objective is then adjusted depending on the specific pressures on the water body that can not be removed because of the associated water uses. Such changes are also dependent on the biological quality element. The integration of the assessments per

quality element is done in the same way as for the natural water bodies. A European intercalibration for the assessment of heavily modified and artificial water bodies is not foreseen.

The quality classes for these water bodies are assigned in a different way as for the natural water bodies. When at least the GEP is reached, the class "good and above" is granted, the lower classes are divided into "moderate", "inadequate" and "poor", as for natural water bodies. These four classes are represented by the colour green, yellow, orange and red, respectively, with equal light gray stripes for artificial water bodies and with equal dark gray stripes for heavily modified water bodies.

3 Water body typology

The natural water bodies are classified according to the WFD in four categories, namely rivers, lakes, transitional waters and coastal waters. These categories are then further divided into types (Table 3.1). This classification is based on the classification established by Jochems et al (2002), with a number of adaptations.

Table 3.1: Classification into categories and types of water bodies in Flanders

Category	**Type**	**Code**
Rivers	Small stream	Bk
	Small stream Kempen	BkK
	Large stream	Bg
	Large stream Kempen	BgK
	Small river	Rk
	Large river	Rg
	Very large river	Rzg
	Brackish polder watercourse	Pb
	Freshwater polder watercourse	Pz
	Freshwater, mesotidal lowland estuary	MLz
Lakes	Moderately ion-rich, alkalic lake	Ami
	Large, deep, eutrophic, alkalic lake	Awe
	Large, deep, oligotrophic to mesotrophic, alkalic lake	Awom
	Ion-rich, alkalic lake	Ai
	Alkalic dune lake	Ad
	Circumneutral, strongly buffered lake	Cb
	Circumneutral, weakly buffered lake	Czb
	Circumneutral, iron-rich lake	CFe
	Strongly acidic lake	Zs
	Moderately acidic lake	Zm
	Very slightly brackish lake	Bzl
	Strongly brackish lake	Bs
Transitional waters	Weakly brackish (oligohaline), macrotidal lowland estuary	O1o
	Brackish macrotidal lowland estuary	O1b
	Saline mesotidal lowland estuary	O2zout
Coastal waters	Mesotidal tidal inlet or sea arm (Zwin)	K1

Only features that are digitally available on the scale of Flanders, are included in the current typology. A customized typology map is currently only available for the Flemish surface water bodies.

For a number of biotic assessment systems also other characteristics such as slope, source characteristics or acidity determine the presence of the community. These characteristics can be included in the assessment systems in the form of subtypes. When these characteristics will also be digitally available on the scale of Flanders, the typology map can be further refined.

4 Phytoplankton

4.1 Rivers

For sampling, conservation and identification of phytoplankton in rivers, the method proposed by Van Wichelen et al (2005) for transitional waters is used. Taking subsamples, fixing and counting is identical as for lakes.

For the assessment, Van Wichelen et al. (2008a) propose to apply the German method (Mischke and Behrendt, 2007; Mischke, 2008). The river type MLz, formerly belonging to the transitional waters, is covered in chapter 4.3, with the exception of the water bodies “Getijdenetes” and “Getijdedijle en - Zenne”. For these two water bodies, Speybroeck et al (2008b) also propose the German method (Mischke and Behrendt, 2007; Mischke, 2008) (see below).

For the river types Bk, BkK, Bg, BgK and Rk, there is in principle no assessment based on phytoplankton because phytoplankton is not relevant for these types as a quality element. These types are however included in the description below.

4.1.1 Sampling

Water, ideally from the middle of the stream, is collected in a large container using a large plastic bucket and a rope.

After the sample is taken, subsamples are taken from the large container for microscopic and pigment analysis. The water should be thoroughly stirred in advance in order to homogenize floating organisms.

A 2-liter container is filled with water and stored in a cool box with cooling elements. When a lot of large zooplankton is present in the sample (especially in clear water with a dense macrophyte vegetation), the sample should be filtered in advance over a mesh size of 200 µm. This reduces the grazing of phytoplankton during the transport to the laboratory. If large colonies of blue-green algae are also present (*Microcystis*, *Aphanizomenon*, ...) this filtering should not be done.

The rest is filtered using a phytoplankton net of 10 µm mesh size and the concentrate is also stored cool in a 100 ml jar.

4.1.2 Conservation

A subsample of 250 ml for microscopic analysis is fixed with 125 µl alkaline lugol (dissolve 10 g of potassium iodide in 20 ml distilled water and add 5 g of iodide (solution A), dissolve 50 g of sodium acetate in 50 ml distilled water (solution B), bring the two solutions together), 6.25 ml formaldehyde buffered with borax (35 %) and 250 µl of sodium thiosulphate (5 %) (Sherr & Sherr, 1993). These samples are stored in a cool, dark location.

For the analysis of chlorophyll a, a known volume of sample (depending on the amount of suspended material) is filtered under vacuum on a Whatman GF/F glass fiber filter (diameter 47 mm, pore size 0.7 µm) until the filter clogs. This filter is stored at – 80 °C until the analysis of chlorophyll a.

4.1.3 Identification

Microscopic analysis are conducted according to the European standard EN 15204:2006 (CEN, 2006). This is based on the classical Utermöhl method and counts of at least 400 units. This is carried out with sedimentation cuvettes and a reversed microscope after staining with Bengal Rose B to facilitate the detection of cells in detritus-rich samples (Utermöhl, 1958). The sample is moved up and down about twenty times to obtain a good homogenization before a subsample is placed in the sedimentation chamber using a (pipe-)pipette. For clear water, between 10 and 50 ml of sample is usually taken, for turbid water between 2 and 10 ml. The sedimentation time varies, depending on the amount of sample, between 6 hours (5 ml) and 24 hours (50 ml). The organisms are counted at different magnifications. Per sample, at least 400 individuals are identified, to the species level where possible (otherwise to genus level) in which colonies are regarded as an individual. The most common taxa are counted under a magnification of 400x by 1 or more longitudinal transects or in 50 to 100 random fields. For the dominant phytoplankton taxa at least 100 individuals should be counted and for less common taxa about 25 individuals. Larger organisms are counted at a lower magnification (100 - 200x) along longitudinal transects or in a half or an entire cuvette. For colonies, individual cells are

counted or estimated or when this is not possible (colonial cyanobacteria) a density factor is determined. This shows how densely cells are aggregated within a colony (Table 4.1), which is important for the conversion to biomass. If the cells are far apart and hence much open space is present within the colony, the density factor is low. Filaments are counted as individuals. Of each taxon a number of individuals is measured (length-width). Filaments are fully measured.

Table 4.1: Target values for density estimation of colonial cyanobacteria

	Open colony	**Dense colony**
Chroococcales	0.05 – 0.07	0.07 – 0.1
Microcystis	0.3 – 0.6	0.6 – 0.8
Gomphosphaeroideae	0.3 – 0.6	0.6 – 0.8

Separate counts are also made for picocyanobacteria and floating cyanobacteria, which are largely missed using the ordinary Utermöhl technique. These data are added to the densities determined with the Utermöhl technique.

The quantification of picocyanobacteria (size: 0.2 – 2 µm) is done using epifluorescence microscopy. For this purpose, 5 ml of water per sample is concentrated on a black polycarbonate filter (Isopore GTBP, 0.22 m pore). These filters are placed on a microscope slide and embedded in fluorescent oil (Cargile A), and subsequently stored in the freezer before the microscopic analysis. The counting is carried out with an epifluorescence microscope at a magnification of 1000x under green light, which makes the characteristic organisms strongly illuminate (autofluorescence). Complete fields are counted until 400 units are reached, or a total of 20 complete fields.

For quantification of floating cyanobacteria 1 ml of well homogenized sample is placed in a Sedgewick-Rafter counting chamber and after five minutes all floating organisms (just under the cover slip) in the counting chamber are quantified. In case of a too high density, only a few transects are counted.

With the average size for each species a biovolume is determined using geometric formulas (Tikkanen & Willen, 1992, Hillebrand et al, 1999) of the corresponding best fit forms (sphere, cylinder, ...). Biovolume is converted into C-biomass for each species using the following formulas (Menden-Deuer & Lessard, 2000):

$$\text{pg C (diatoms)} = 0.288 * (\text{biovolume } (\mu m^3))^{0.811}$$

$$\text{pg C (other phytoplankton)} = 0.216 * (\text{biovolume } (\mu m^3))^{0.939}$$

The density is obtained with the following formula:

$$\text{Density (N/ml)} = [(D / C) * B] / A$$

With: A: volume of the subsample

B: surface area of the cuvette

C: the surface area that was counted

D: the number of individuals that was counted for each species

The biomass is calculated as follows:

$$\text{Biomass (µg C/l)} = (\text{pg C/cell} * \text{density}) / 1000$$

The chlorophyll a is extracted from the filter with acetone (90 %). Subsequently, the concentration of chlorophyll a determined by spectrophotometer or HPLC.

4.1.4 Index calculation

For the index calculation five metrics are used.

For this assessment, the types are assigned to those used for the German method (Mischke and Behrendt, 2007; Mischke, 2008). The types Bk, BkK, Bg, BgK and Rk are assessed according to the type "15.1 +17.1", the types Rg, Rzg, Pz according to the type "20.2" and the type Pb according to the type "23" (Table 4.2). In what follows, these assessment types will always be mentioned instead of the

actual types. For some assessment types, not all metrics are taken into account. The metrics used are indicated per type in Table 4.2. Two water bodies of the river type MLz are also assessed using the method for rivers, in contrast to other water bodies of this type. The water body “Getijdenetes” is assessed according to the assessment type "15.1 +17.1" and the water body “Getijdedijle en -Zenne” according to the assessment type "20.2".

All metrics are always based on the seasonal average. This is obtained by taking, for each month in which measurements are performed, the average of all obtained values (if it concerns more than one measurement) and subsequently calculating the average of these values over all months in the growing season (April until September).

Table 4.2: Metrics used for phytoplankton in rivers per type

Types	**Bk, BkK, Bg, BgK, Rk**	**Rg, Rzg, Pz**	**Pb**
Assessment types	**15.1+17.1**	**20.2**	**23**
Biomass	X	X	X
Relative proportion pennate diatoms	X	X	X
Relative proportion green algae			X
Relative proportion cyanobacteria	X		X
Potamoplankton	X	X	X

4.1.4.1 Metric biomass

For the metric biomass, the seasonal average chlorophyll a content is used. Table 4.3 gives the upper boundaries of the different classes, expressed in µg per liter and the calculation of the metric that is used to calculate the total index. If the result of this calculation is less than 0.5 it will be set equal to 0.5 and if it is greater than 5.5 it is set equal to 5.5.

Table 4.3: Upper boundaries for the metric biomass expressed as seasonal average non-corrected chlorophyll a content (µg/l) and calculation formula for the metric score for the different types of rivers

Type	**High**	**Good**	**Moderate**	**Poor**	**Metric score calculation**
15.1+17.1	≤20.0	≤33.0	≤55.0	≤90.0	1.9907 * ln(Chl a) – 4.4749
20.2	≤30.0	≤52.0	≤90.0	≤155.0	1.8168 * ln(Chl a) – 4.6772
23	≤30.0	≤52.0	≤90.0	≤155.0	1.8168 * ln(Chl a) – 4.6772

4.1.4.2 Metric relative proportion pennate diatoms

This metric uses the seasonal average relative proportion of pennate diatom biovolume compared to the total phytoplanktonbiovolume. The delineation of quality classes and corresponding scores for this metric are shown in Table 4.4. The classes moderate, poor and bad are not separated and are all assigned a score of 3.

Table 4.4: Delineation of quality classes for the metric relative proportion pennate diatoms expressed as seasonal average and corresponding score

Type	**High**	**Good**	**Moderate**	**Poor**	**Bad**
15.1+17.1	≥20	≥15 ... <20	<15	-	-
23	≥20	≥15 ... <20	<15	-	-
Score	**1**	**2**	**3**	**-**	**-**

4.1.4.3 Metric relative proportion green algae

This metric is based on the seasonal average relative proportion of green algae biovolume compared to the total phytoplanktonbiovolume. The delineation of quality classes and corresponding scores for this metric are shown in Table 4.5. If the percentage is smaller than or equal to 5, the score of this metric is set equal to the score obtained for the metric biomass.

Table 4.5: Delination of quality classes for the metric relative proportion green algae expressed as seasonal average and corresponding score

Type	High	Good	Moderate	Poor	Bad
20.2	-	-	≤5 see 4.1.4.1	>5 ... ≤15	>15
23	-	-	≤5 see 4.1.4.1	>5 ... ≤15	>15
Score	**-**	**-**	**See 4.1.4.1**	**4**	**5**

4.1.4.4 Metric relative proportion cyanobacteria

This metric uses the seasonal average relative proportion of cyanobacteria compared to the total phytoplanktonbiovolume. However, when the absolute cyanobacterial biovolume is less than or equal to 0.5 mm³/l, a rating of "2" is always assigned with the associated class "good" (Mischke, 2008). When the absolute biovolume is larger, the rating is assigned based on Table 4.6 using the average relative proportion of biovolume. For the types 15.1+17.1 and 20.2 the score of this metric is set equal to that for the metric biomass (see section 4.1.4.1) provided that at least the class 'moderate' is reached.

Table 4.6: Delineation of the quality classes for the metric relative proportion cyoanobacteria expressed as seasonal average and corresponding score

Type	High	Good	Moderate	Poor	Bad
15.1+17.1	-	-	<10 see 4.1.4.1	>10 ... ≤20	>20
20.2	-	-	<2 see 4.1.4.1	>2 ... ≤5	>5
23	≤0.001	>0.001 ... ≤5	>5 ... ≤10	>10 ... ≤20	>20
Score	**1**	**2**	**3**	**4**	**5**

4.1.4.5 Metric potamoplankton

For this metric the Typspezifischen Indexwertes Potamoplankton (TIP) is used. This is a type-specific potamoplankton index based on the weighted average of idicatortaxa. It is calculated as follows:

$$TIP = \Sigma^{n}_{i=1} [TI_i * GW_i * DW_i] / \Sigma^{n}_{i=1} [GW_i * DW_i]$$

With: TI_i: the type-specific indicator value of taxon i;

GW_i: the weight factor of taxon i;

DW_i: the dominance value (seasonal average percentage proportion of biovolume) of taxon i;

n: the total number of taxa encountered.

The type-specific indicator values and weight factors used for TIP calculation can be found in Mischke and Behrendt (2007) for all indicator taxa.

This metric is used only if at least six different indicator taxa are found. Otherwise, this metric is not included in the calculation of the total index.

4.1.4.6 Total index calculation

The final assessment according to the German method (Mischke and Behrendt, 2007; Mischke, 2008) is equal to the average of the scores obtained for all relevant metrics. This is a value that can vary between 0.5 and 5.5, with class boundaries high/good, good/moderate, moderate/poor and poor/bad equalling 1.5, 2.5, 3.5 and 4.5, respectively.

To obtain an EQR Van Wichelen et al (2008a) propose to divide a reference, which is set equal to 1, by this average value. This results in a provisional EQR with the corresponding class limits 0.667, 0.4, 0.286 and 0.222, respectively. This figure is further transformed to a new scale (EQR_T) with class limits 0.9, 0.75, 0.5 and 0.25. This rescaling is done by linearly transforming the provisional EQR value of the corresponding class limits expressed as EQR to the new class boundaries. This transformation is done using the following formula:

$$EQR_T = OG_T + (BG_T - OG_T) * (EQR_{NT} - OG_{NT}) / (BG_{NT} - OG_{NT})$$

Met: BG: upper boundary of the relevant status class

OG: lower boundary of the relevant status class

T: transformed (linear)

NT: non-transformed (original)

For the class "bad", an EQR of 0 is used as a lower boundary. A preliminary EQR of 0.3 (class moderate) will be rescaled to a final assessment of:

$$EKC_T = 0,5 + (0,75 - 0,5) * (0,3 - 0,286) / (0,4 - 0,286) = 0,53$$

4.2 Lakes

4.2.1 Sampling

In small lakes (<5 ha) water is collected in a large container from 8 random locations scattered across the lake using a boat. In large lakes (> 5 ha) 16 random sites are sampled.

In shallow lakes, it is sufficient to take each time a sample of the entire water with a tube sampler (a plastic 2-meter-long tube), ensuring that the soil and submerged vegetation is not touched to avoid contamination. One should also remain at a sufficient distance from the bank in order to avoid contamination with typical littoral species.

In deep lakes, at each point the entire circulating upper layer (epilimnion) is sampled. From the surface to the metalimnion, every meter, or every two meters in case of a very extensive epilimnion, a sample is taken using a Niskin bottle. The depth to which sampling should be done, is determined by the measurement of a vertical temperature and/or oxygen profile. When no data on the average depth of the lake is available, as many depth measurements as possible can be made during the transportation between two points. At a central point (or where the lake is at its deepest) using a multimeter the temperature, oxygen content, conductivity, acidity, the Secchi depth and ideally also the depth (in deep lakes) of the entire water column is measured with an interval of 50 cm. On the basis of the depth profile of the temperature, the thermocline to be determined up to where the biota should be sampled.

During transport between two points, the container should always be closed with a lid. After water is collected at all locations, subsamples are taken from the large container for microscopic and pigment analysis. The water should be thoroughly stirred in advance in order to homogenize floating organisms.

A 2-liter container is filled with water and stored in a cool box with cooling elements. When a lot of large zooplankton is present in the sample (especially in clear water with a dense macrophyte vegetation), the sample should be filtered in advance over a mesh size of 200 µm. This reduces the grazing of phytoplankton during the transport to the laboratory. If large colonies of blue-green algae are also present (*Microcystis*, *Aphanizomenon*, ...) this filtering should not be done.

The rest is filtered using a phytoplankton net of 10 µm mesh size and the concentrate is also stored cool in a 100 ml jar.

4.2.2 Conservation

A subsample of 250 ml for microscopic analysis is fixed with 125 µl alkaline lugol (dissolve 10 g of potassium iodide in 20 ml distilled water and add 5 g of iodide (solution A), dissolve 50 g of sodium acetate in 50 ml distilled water (solution B), bring the two solutions together), 6.25 ml formaldehyde buffered with borax (35 %) and 250 µl of sodium thiosulphate (5 %) (Sherr & Sherr, 1993). These samples are stored in a cool, dark location.

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Table 4.7: Target values for density estimation of colonial cyanobacteria

	Open colony	**Dense colony**
Chroococcales	0.05 – 0.07	0.07 – 0.1
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Separate counts are also made for picocyanobacteria and floating cyanobacteria, which are largely missed using the ordinary Utermöhl technique. These data are added to the densities determined with the Utermöhl technique.

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$$\text{pg C (other phytoplankton)} = 0.216 * (\text{biovolume } (\mu m^3))^{0.939}$$

The density is obtained with the following formula:

$$\text{Density (N/ml)} = [(D / C) * B] / A$$

With: A: volume of the subsample

B: surface area of the cuvette

C: the surface area that was counted

D: the number of individuals that was counted for each species

The biomass is calculated as follows:

Biomass (µg C/l) = (pg C/cell * density) / 1000

The chlorophyll a is extracted from the filter with acetone (90 %). Subsequently, the concentration of chlorophyll a determined by spectrophotometer or HPLC.

4.2.4 Index calculation

The status determination for phytoplankton in lakes is done using the metrics biomass and species composition.

4.2.4.1 Metric biomass

The metric biomass is based on the chlorophyll a content. The measured value for this metric is converted into an EQR by dividing the reference value by the measured value. When the measured chlorophyll content is lower than the reference value, the EQR is set equal to 1.

The class limits high/good and good/moderate for chlorophyll a are determined in the framework of the intercalibration exercises (EU, 2008) for all types except Bzl. For this type Bzl values are provisionally taken from the Dutch type M30 (Van der Molen & Pot, 2007). The values for the lower class boundaries are provisionally derived using the proposed doubling per class. All these class boundaries are shown in Table 4.8.

Table 4.8: Chlorophyll-a criteria for a number of lake types. The high/good and good/moderate boundary values result from the European intercalibration (EU, 2008) with the exception of Bzl.

Type	**Awe, Awom**	**Ai, Ad, Ami**	**Bzl**	**Cb, CFe, Czb**	**Zs, Zm**
Class boundary	**Chl_a (µg/L) – summer average**				
Reference	3.2	7.4	30	3.1	3.1
Boundary high / good	5.8	11.7	40	5.4	5.4
Boundary good / moderate	10	25	60	10	10
Boundary moderate / poor	20	50	120	20	20
Boundary poor / bad	40	100	240	40	40

4.2.4.2 Metric species composition

For the metric species composition, the relative proportion of cyanobacteria expressed as biomass (%) is used. The division into status classes is applied according to Table 4.9.

Table 4.9: Delineation of the various status classes for the metric species composition of phytoplankton in lakes based on the relative proportion of cyanobacteria expressed as biomass (%)

Class boundary	**Relative proportion of cyanobacteria (%) – summer average**	**EQR**
Reference	2,5	1
Boundary high / good	5	0.5
Boundary good / moderate	10	0.25
Boundary moderate / poor	25	0.1
Boundary poor / bad	50	0.05

The average relative proportion of cyanobacteria is adjusted for those lakes that are in high or good status for the chlorofyll a content and are characterised by the presence of picocyanobacteria and/of *Gomphosphaeria*-species. These taxa should not be taken into account.

The measures value for this metric is transformed into an EQR. This is obtained by dividing the reference (2,5 %) by the measured value. When the relative proportion of cyanobacteria is below this reference value, then the EQR is set equal to 1.

4.2.4.3 Total index calculation

The obtained EQR for the species composition is rescaled to a new scale (EQR_T), of which the class boundaries correspond to those for the metric biomass (calculated from the values in Table 4.8). This rescaling is done by linearly transforming the obtained EQR value between the original class boundaries, expressed as EQR, to the new class limits, expressed as EQR. This transformation is done using the following formula:

$$EQR_T = OG_T + (BG_T - OG_T) * (EQR_{NT} - OG_{NT}) / (BG_{NT} - OG_{NT})$$

Met: BG: upper boundary of the relevant status class

OG: lower boundary of the relevant status class

T: transformed (linear)

NT: non-transformed (original)

As lower boundary for the class "bad" (not mentioned in Table 4.8 and Table 4.9), an EQR of 0 is used.

An original EQR for the metric species composition for the type Awe of 0.4, for example, will be transformed as follows:

$$EQR_T = 3.2/10 + (3.2/5.8 – 3.2/10) * (0.4 – 0.25) / (0.5 – 0.25) = 0.46$$

In the original system described by Van Wichelen et al. (2005), the final score is determined by taking the average of both EQR_T's, except when the difference between both metrics is more than 2 classes, in which case, the worst score is decisive. Lock et al. (2007) changed this by introducing the 'one out, all out' principle to this index. Hence the final score is always equal to the worst score of both metrics.

4.3 Transitional waters

The river type MLz, which was formerly assigned to the transitional waters, is also addressed in this chapter, together with the “actual” transitional waters, except for the water bodies “Getijdenetes” and “Getijdedijle en -Zenne”. The water body “Getijdenetes” is assessed according to the river type “15.1+17.1 and the water body “Getijdedijle en -Zenne” is assessed according to the river type “20.2”, as described in section 4.1. The brackish zone (type O1b) is considered a natural mortality zone for the freshwater as well as the saline phytoplankton community. For all water bodies belonging to the type O2, phytoplankton is not considered relevant either. As a consequence, the assessment method described below applies only for the water body “Zeeschelde III + Rupel” (type O1o) and for the water bodies “Zeeschelde I”, “Zeeschelde II” and “Getijdedurme” (type MLz).

For transitional waters, Brys et al. (2005) have proposed a method, largely based on an earlier proposal by Van Damme et al. (2003). The method of Brys et al. (2005) was later further elaborated by Speybroeck et al. (2008b). The sections sampling, conservation and identification are taken from Van Damme et al. (2003).

Because all Flemish transitional waters and water bodies of the type MLz are heavily modified or artificial, the proposed method is an assessment of the ecological potential. In what follows the relevant quality classes for artificial and heavily modified water bodies will therefore be used (see section 2.5).

4.3.1 Sampling

The phytoplankton is sampled with so-called non-concentrated, hence non-filtered samples. A surface sample is taken in a large container from which the necessary subsamples can be taken.

It is important that the phytoplankton monitoring completely covers the occurring periods of blooms. During the winter months, phytoplankton monitoring is not essential, but in the period from March to September, a measuring frequency of at least twice a month is recommended. Monthly samplings during this period are an absolute minimum.

4.3.2 Conservation

A subsample of 250 ml for microscopic analysis is fixed with 125 µl alkaline lugol (dissolve 10 g of potassium iodide in 20 ml distilled water and add 5 g of iodide (solution A), dissolve 50 g of sodium acetate in 50 ml distilled water (solution B), bring the two solutions together), 6.25 ml formaldehyde buffered with borax (35 %) and 250 µl of sodium thiosulphate (5 %) (Sherr & Sherr, 1993). These samples are stored in a cool, dark location.

For the analysis of chlorophyll a, a known volume of sample (depending on the amount of suspended material) is filtered under vacuum on a Whatman GF/F glass fiber filter (diameter 47 mm, pore size 0.7 µm) until the filter clogs. This filter is stored at – 80 °C until the analysis of chlorophyll a.

4.3.3 Identification

Microscopic analysis are conducted according to the European standard EN 15204:2006 (CEN, 2006). This is based on the classical Utermöhl method and counts of at least 400 units. This is carried out with sedimentation cuvettes and a reversed microscope after staining with Bengal Rose B to facilitate the detection of cells in detritus-rich samples (Utermöhl, 1958). The sample is moved up and down about twenty times to obtain a good homogenization before a subsample is placed in the sedimentation chamber using a (pipe-)pipette. For clear water, between 10 and 50 ml of sample is usually taken, for turbid water between 2 and 10 ml. The sedimentation time varies, depending on the amount of sample, between 6 hours (5 ml) and 24 hours (50 ml). The organisms are counted at different magnifications. Per sample, at least 400 individuals are identified, to the species level where possible (otherwise to genus level) in which colonies are regarded as an individual. The most common taxa are counted under a magnification of 400x by 1 or more longitudinal transects or in 50 to 100 random fields. For the dominant phytoplankton taxa at least 100 individuals should be counted and for less common taxa about 25 individuals. Larger organisms are counted at a lower magnification (100 - 200x) along longitudinal transects or in a half or an entire cuvette. For colonies, individual cells are counted or estimated or when this is not possible (colonial cyanobacteria) a density factor is determined. This shows how densely cells are aggregated within a colony (Table 4.10), which is important for the conversion to biomass. If the cells are far apart and hence much open space is present within the colony, the density factor is low. Filaments are counted as individuals. Of each taxon a number of individuals is measured (length-width). Filaments are fully measured.

Table 4.10: Target values for density estimation of colonial cyanobacteria

	Open colony	**Dense colony**
Chroococcales	0.05 – 0.07	0.07 – 0.1
Microcystis	0.3 – 0.6	0.6 – 0.8
Gomphosphaeroideae	0.3 – 0.6	0.6 – 0.8

Separate counts are also made for picocyanobacteria and floating cyanobacteria, which are largely missed using the ordinary Utermöhl technique. These data are added to the densities determined with the Utermöhl technique.

The quantification of picocyanobacteria (size: 0.2 – 2 µm) is done using epifluorescence microscopy. For this purpose, 5 ml of water per sample is concentrated on a black polycarbonate filter (Isopore GTBP, 0.22 m pore). These filters are placed on a microscope slide and embedded in fluorescent oil (Cargile A), and subsequently stored in the freezer before the microscopic analysis. The counting is carried out with an epifluorescence microscope at a magnification of 1000x under green light, which makes the characteristic organisms strongly illuminate (autofluorescence). Complete fields are counted until 400 units are reached, or a total of 20 complete fields.

For quantification of floating cyanobacteria 1 ml of well homogenized sample is placed in a Sedgewick-Rafter counting chamber and after five minutes all floating organisms (just under the cover

slip) in the counting chamber are quantified. In case of a too high density, only a few transects are counted.

With the average size for each species a biovolume is determined using geometric formulas (Tikkanen & Willen, 1992, Hillebrand et al, 1999) of the corresponding best fit forms (sphere, cylinder, ...). Biovolume is converted into C-biomass for each species using the following formulas (Menden-Deuer & Lessard, 2000):

$$\text{pg C (diatoms)} = 0.288 * (\text{biovolume } (\mu m^3))^{0.811}$$

$$\text{pg C (other phytoplankton)} = 0.216 * (\text{biovolume } (\mu m^3))^{0.939}$$

The density is obtained with the following formula:

$$\text{Density (N/ml)} = [(D / C) * B] / A$$

With: A: volume of the subsample

B: surface area of the cuvette

C: the surface area that was counted

D: the number of individuals that was counted for each species

The biomass is calculated as follows:

$$\text{Biomass } (\mu g\ C/l) = (\text{pg C/cell} * \text{density}) / 1000$$

The chlorophyll a is extracted from the filter with acetone (90 %). Subsequently, the concentration of chlorophyll a determined by spectrophotometer or HPLC.

For phytoplankton dominance, the following criterion is used: non-diatoms are dominant over diatoms when the biomass of all algal species together is larger than the biomass of all diatom species together, and vice versa. For this, this biomass is determined as above.

The light regime is described by the ratio of photic depth relative to the mixing depth. For fully mixed estuaries such as the Scheldt, it is assumed that the mixing depth equals the depth of the estuary at an average tide. By convention, the photic depth is to the depth where the incoming light is still only 1% of the light intensity at the surface. The light intensity is hereby further specified as the Photosynthetically Active Radiation (PAR). Measurement of PAR can be done by means of a quantum sensor.

4.3.4 Index calculation

The status determination for phytoplankton in transitional waters is carried out using the metrics biomass and species composition.

4.3.4.1 Metric biomass

The status classes for the metric biomass are based on the chlorophyll a content. These are summarized in Table 4.11. The boundaries depend on the conditions of the environmental variables. When these are all good, a tolerance window is observed for chlorophyll a, the criteria are in that case less strict. The requirements for these good conditions of the environmental variables are shown in Table 4.12. If all conditions in Table 4.12 are met, the tolerance window is used and hence the criteria in column 4 and 5 of Table 4.11 apply. Otherwise, the criteria in column 2 and 3 apply. Intermediate values between the class boundaries have an EQR that is interpolated between the corresponding boundary values for the relevant class. In this way, a chlorophyll a content of 200, in the case that the tolerance window is applied, will result in an EQR of 0.80.

Table 4.11: Boundaries of the various status classes for the metric species composition of phytoplankton in transitional waters based on the relative contribution of cyanobacteria

	Not all environmental variables are good (no tolerance window)		**All environmental variables are good (tolerance window)**	
Chlorophyll a (µg/L)	**Class boundaries**	**EQR**	**Class boundaries**	**EQR**
MEP	<100	1.00	<100	1.00
Good and above	100 - 200	1.00 - 0.60	100 - 300	1.00 - 0.60

Moderate	200 - 250	0.60 - 0.40	300 - 350	0.60 - 0.40
Poor	250 - 300	0.40 - 0.20	350 - 400	0.40 - 0.20
Bad	>300 of <100	0.00	>400 of <100	0.00

Table 4.12: Criteria for the environmental variables for the application of the tolerance window for assessment of the chlorophyll a-content in transitional waters (TN = total nitrogen load; TP = total phosphorus load; DSi = dissolved silicon; Zm = mixing depth; Zp = photic depth)

Environmental variable	**Requirements for good conditions**
Nutrients	TN/DSi $\leq$ 1 TP/DSi $\leq$ 1/16
Light regime	Zm/Zp < 4
Dissolution time	$t_{1/2}$ > 1 d

4.3.4.2 Metric species composition

Speybroeck et al. (2008b) use, for the metric species composition, the dominance of diatoms compared to other phytoplankton taxa as proposed by Van Damme et al (2003). For the class boundaries, they take the values of Van Wichelen et al. (2005) as shown in Table 4.13.

Table 4.13: Status classes for the metric species composition of the phytoplankton in transitional waters based on the proportion of diatoms in the total biomass

Class	**Diatom (%) in total biomass**	**EQR**
MEP	>75	1,00
Good and above	55 - 75	0,60 - 1,00
Moderate	45 - 55	0,40 - 0,60
Poor	25 - 45	0,20 - 0,40
Bad	0 - 25	0,00 - 0,20

4.3.4.3 Total index calculation

For the overall assessment the 'one out, all out' principle is applied. The worst score of both metrics is always decisive. To discern the noise caused by climate variations from the actual long-term evolution, moving averages over the last five growing seasons are used.

5 Phytobenthos

Fytobenthos must be assessed in water bodies belonging to the categories rivers and lakes. In practice, diatoms are used for the quality assessment based on fytobenthos. The diatoms often constitute the most abundant and diverse group within the fytobenthos (Kelly et al, 2008), they are known as a good indicator of water quality and diatoms are also used in most other Member States for this quality element. Kelly et al. (2008) already demonstrated that at least for lakes diatoms are, as an indicator, a reliable representative for the whole fytobenthos community. For rivers and lakes in Flanders the PISIAD index (Proportions of Impact-Sensitive and Impact-Associated Diatoms) was developed by Denys and Hendrickx (2005) and later further completed for a number of types.

5.1 Rivers

For watercourses the PISIAD index (Denys & Hendrickx, 2005) was developed with indicator values (see below) specified for the types Bk, BkK and BgK. For the types Bg, Rk, Rg and Rzg this specification has presently also been carried out. To be able to apply this system for the types Pb and Pz, this specification must still be carried out. The selection of measurement points and the sampling method has been further elaborated by Leyssen et al. (2007). For the river type MLz, formerly belonging to the transitional waters, no assessment based on fytobenthos is planned.

5.1.1 Selection of stretches for phytobenthos and macrophytes in rivers

For fytobenthos and macrophytes three sampling points or stretches are selected for each water body according to the decision tree shown in Figure 5.1.

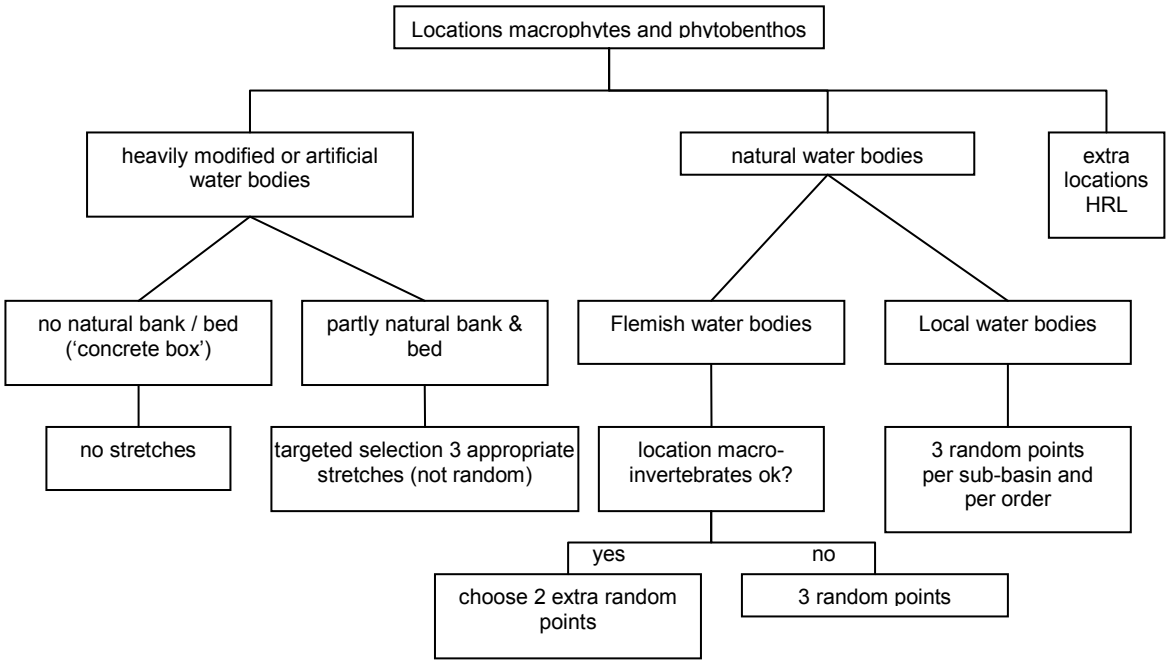

Figure 5.1: Decision tree for selection of stretches or sampling points (Leyssen et al., 2007). HD = Habitat Directive, which is not further discussed here

When field conditions are such that the stretch is too heavily shaded, the bed is recently cleared or ... an alternative stretch is chosen. In Flemish water bodies this is chosen up to one kilometer upstream or downstream, in local water bodies up to 500 meters upstream or downstream.

Bank consolidation is not a valid criterion to choose another stretch, its presence will be indicated in the field form.

For macrophytes stretches are used, for phytobenthos sampling points. The start and/or end of a stretch is preferably not located in the immediate vicinity of a bridge (due to flow effects), but at least 10 meters away from it. For phytobenthos, sampling of stones or bridge pillars is recommended (see

below). If possible, the phytobenthos point is always chosen within the stretch where macrophytes are inventoried.

5.1.2 Sampling

The order of preference for the substrate to be sampled is:

1) stones;

2) non-wooden artificial structures (e.g. bridge pillars);

3) living reed;

4) other similar, living helophytes (monocotyls such as cattail (*Typha*), rushes (*Scirpus*, *Juncus*),…);

5) artificial substrates.

Five different stones that were found spread throughout the location are sampled. These stones are lifted from the water and are sampled to the flow side (upstream side). With a (pocket) knife or sharpened spoon the epilithon is removed from the stones and stored in a container (60 – 100 ml) with a wide screw cap and extra closing lid. The sample is fixed with a small quantity of formaldehyde (<5%). Record code and date on lid and on container (Leyssen et al., 2007) and also an indication of the type of substrate used.

It is important that during sampling no epilithon with sludge is sampled. Stones that are almost completely in the sediment are not eligible. The sampling of rocks to the non-flow side (downstream side) should be avoided. The sampled stones should be permanently submerged. Ensure that the container is not completely filled with water and formol. After the sampling the epilithon should preferably be allowed to settle. Then the excess water can be drained. In most cases one centimeter of water/formaldehyde is already sufficient (Leyssen et al., 2007).

In some cases no (suitable) stones will be present within the stretch. In this case the bridge pillar can alternatively be sampled. This is done in the same way as with stones, only this will be carried out under water. Sample only the outer vertical sections of the pillar because they are most exposed to light. Make sure you sample an area of which one is certain that it is permenantly submerged. Only areas that have not dried the last 1.5 months, are eligible for sampling. In case of doubt, this should be recorded on the field form (Leyssen et al., 2007).

If no stones or bridge pillars are present, one can sample living reeds. These are cut with scissors. Collect only the zone about 10 cm below the water surface. Take 5 different stems to be sampled. They are kept in a similar container and fixed in formaldehyde (<5%) (Leyssen et al., 2007).

In case of artificial substrates, preferably permanent, vandal-resistant constructions are chosen of inert material on which a biofilm can develop undisturbed during the whole year. In the vast majority of cases, however, useful structures will already be present in the water body. Substrates on which 'younger' communities are present, may be more sensitive to disturbances in the catchment that promotes an increase of 'drift' (erosion and suspended solids load, Kutka & Richards, 1996) (Denys & Hendrickx, 2005).

In no case constructions should be sampled that are in wood (Leyssen et al., 2007) or metal.

5.1.3 Conservation

The organic material and the cell content are eliminated by treating the samples with hydrogen peroxide (30%) at a temperature of 70 °C. After cooling, the final reaction is catalyzed by adding a few drops of a saturated, aqueous $KMnO_4$ solution. Manganese compounds and any carbonates are solved by adding a few drops of HCl. Then the sample is centrifuged for 10 minutes at 3000 rpm. The supernatant is decanted and the precipitate is rinsed with distilled water. The last two steps are repeated three times and the cleaned material is kept in distilled water. As an alternative to the rapid centrifugation, which may damage the valves, repeated settling in test tubes for at least 48 hours can be applied. After stirring of the residue several drops of suspended material are pipetted onto a degreased coverslip (24 x 40 mm), evenly distributed and slowly dried up by evaporation. Subsequently, permanent preparations are created using the mounting medium Naphrax.

5.1.4 Identification

The preparations are examined at a magnification of about 1000x with a microscope with interference optics. Exactly 500 valves are counted along randomly chosen transects. Denys & Hendrickx (2005) cite the following identification works: Cleve-Euler (1968), Germain (1981), Hustedt (1961-1966), Krammer & Lange-Bertalot (1986-1991), Krammer (1992, 1997a, 1997b, 2000 , 2002), Lange-Bertalot (1993, 2001), Lange-Bertalot & Moser (1994), Reichardt (1997, 1999), Lange-Bertalot & Metzeltin (1996). Customary procedures are described more in detail in EN 14407 (CEN, 2004) and in the Enumeration protocol of the British DARES project (DARES Consortium, 2004).

A basic list is given in Annex 2 of Denys & Hendrickx (2005) for the water types Bk, BkK and BgK.

5.1.5 Index calculation

First, the total percentage abundance of impact-sensitive and of impact-associated indicators are calculated. Annex 2 of Hendrickx & Denys (2005) indicates for each species whether or not it is an impact-sensitive or impact associated indicator for the water types Bk, BkK and BgK. For the types headwaters - acid, headwaters - calcium-rich, Bg, Rk, Rg and Rzg, these indications have now also been developed by Luc Denys of INBO (Research Institute for Nature and Forest) and made available to the VMM (Flemish Environment Agency). For the types Pzoet, Pzilt and Pzout, a concept for the positive indicators is given in Annex 1 of Leyssen et al. (2006). A list of negative indicators for these types of polder watercourses is still missing.

The calculation of the EQR is done with the following equations:

If the relative abundance of impact associated indicators > threshold good/moderate:

$$EQR = EQR_a + 0.2 * [(x-a)/(b-a)]$$

If the relative abundance of impact associated indicators < threshold good/moderate:

$$EQR = EQR_a - 0.2 * [(y-a)/(b-a)]$$

With: x: percentage abundance impact-associated indicators;

y: percentage abundance impact-sensitive indicators;

a: lower class boundary;

b: upper class boundary;

0.2: The width of the EQR class.

For the water types Bk, BkK, Bg, BgK, Rk, Rg and Rzg the class boundaries and their associated EQR values shown in Table 5.1 (Hendrickx and Denys, 2005; Denys, 2006).

Table 5.1: Class boundaries for the river types BkK, BgK, Bk, Bg, Rk, Rg and Rzg with corresponding EQR values. ISD: Impact-sensitive diatoms; IAD: Impact-associated diatoms

EQR	Class boundary	BkK, BgK	Bk	Bg	Rk	Rg, Rzg
	Reference	75 % ISD	50 % ISD	55 % ISD	65 % ISD	70 % ISD
0,8	High / good	50 % ISD	30 % ISD	25 % ISD	40 % ISD	45 % ISD
0,6	Good / moderate	20 % IAD	20 % IAD	20 % IAD	20 % IAD	20 % IAD
0,4	Moderate / poor	45 % IAD	45 % IAD	45 % IAD	45 % IAD	45 % IAD
0,2	Poor / bad	75 % IAD	75 % IAD	75 % IAD	75 % IAD	75 % IAD

5.1.5.1 Determination of the total index per traject

The index for one stretch is determined based on one sample and calculated as described above.

5.1.5.2 Determination of the total index per water body

In a whole water body, three stretches are sampled. Per stretch, the EQR is calculated as described above. Then the average of these quality indices is used as EQR for the whole water body.

5.2 Lakes

For the lakes, the PISIAD index (Hendrickx & Denys, 2005) was specifically developed with indicator values (see below) for the types Cb, Awom, Awe, Ami and Ai. To be able to apply this system in all types of Flemish lakes, this specification must also be done for the remaining types, which are Ad, Czb, CFe, Zs, Zm, Bzl and Bs. As for the sampling, a number of changes were made by Lock et al. (2007).

5.2.1 Sampling

The order of preference for the substrate to be sampled is:

1) living reed;

2) other similar, living helophytes (monocotyls such as cattail (*Typha*), rushes (*Scirpus*, *Juncus*),…);

3) stones;

4) artificial substrates.

Depending on the differences in vegetation, water bed and structure, reed or morphologically similar plant species, or stone or similar hard substrates, are sampled at the side of the open water at 9 locations along the shore throughout the entire lake. At each site a composite sample of the epifyton is collected based on about 10 stems or stones, which are sampled at a depth of 20-30 cm (Denys & Hendrickx, 2005, Lock et al., 2007).

5.2.2 Conservation

The organic material and the cell content are eliminated by treating the samples with hydrogen peroxide (30%) at a temperature of 70 °C. After cooling, the final reaction is catalyzed by adding a few drops of a saturated, aqueous $KMnO_4$ solution. Manganese compounds and any carbonates are solved by adding a few drops of HCl. Then the sample is centrifuged for 10 minutes at 3000 rpm. The supernatant is decanted and the precipitate is rinsed with distilled water. The last two steps are repeated three times and the cleaned material is kept in distilled water. As an alternative to the rapid centrifugation, which may damage the valves, repeated settling in test tubes for at least 48 hours can be applied. After stirring of the residue several drops of suspended material are pipetted onto a degreased coverslip (24 x 40 mm), evenly distributed and slowly dried up by evaporation. Subsequently, permanent preparations are created using the mounting medium Naphrax.

5.2.3 Identification

The preparations are examined at a magnification of about 1000x with a microscope with interference optics. Exactly 500 valves are counted along randomly chosen transects. Hendrickx & Denys (2005) cite the following identification works: Cleve-Euler (1968), Germain (1981), Hustedt (1961-1966), Krammer & Lange-Bertalot (1986-1991), Krammer (1992, 1997a, 1997b, 2000 , 2002), Lange-Bertalot (1993, 2001), Lange-Bertalot & Moser (1994), Reichardt (1997, 1999), Lange-Bertalot & Metzeltin (1996). Customary procedures are described more in detail in EN 14407 (CEN, 2004) and in the Enumeration protocol of the British DARES project (DARES Consortium, 2004).

A basic list is given in Annex 1 of Hendrickx & Denys (2005) for the lake types Cb, Awom, Awe, Ami and Ai.

5.2.4 Index calculation

First, the total percentage abundance of impact-sensitive and of impact-associated indicators are calculated. Annex 1 of Hendrickx & Denys (2005) indicates for each species whether or not it is an impact-sensitive or impact associated indicator for the lake types Cb, Awom, Awe, Ami and Ai.

The calculation of the EQR is done with the following equations:

If the relative abundance of impact associated indicators > threshold good/moderate:

$$EQR = EQR_a + 0.2 * [(x-a)/(b-a)]$$

If the relative abundance of impact associated indicators < threshold good/moderate:

$EQR = EQR_a - 0.2 * [(y-a)/(b-a)]$

With: x: percentage abundance impact-associated indicators;

y: percentage abundance impact-sensitive indicators;

a: lower class boundary;

b: upper class boundary;

0.2: The width of the EQR class.

For the lake types circumneutral, strongly buffered lake (Cb), large, deep, eutrophic, alkalic lake (Awe), large, deep, oligotrophic to mesotrophic, alkalic lake (Awom), moderately ion-rich, alkalic lake (Ami, further differentiated into an oligotrophic to mesotrophic subtype Ami-om and a eutrophic subtype Ami-e), and ion-rich, alkalic lake (Ai) the class boundaries and their associated EQR values are shown in Table 5.2 (Hendrickx and Denys, 2005).

Table 5.2: Class boundaries for the lake types Cb, Aw, Ami-om Ami-e and Ai with corresponding EQR values. ISD: Impact-sensitive diatoms; IAD: Impact-associated diatoms

EQR	Class boundary	Cb	Awe, Awom	Ami-om	Ami-e	Ai
	Reference	80 % ISD	70 % ISD	80 % ISD	70 % ISD	80 % ISD
0,8	High / good	70 % ISD	40 % ISD	60 % ISD	40 % ISD	60 % ISD
0,6	Good / moderate	10 of 25 % IAD	20 % IAD	10 % IAD	25 % IAD	25 % IAD
0,4	Moderate / poor	40 of 50 % IAD	45 % IAD	40 % IAD	50 % IAD	50 % IAD
0,2	Poor / bad	70 of 75 % IAD	75 % IAD	70 % IAD	75 % IAD	75 % IAD

5.2.4.1 Determination of total index per sample

The index for a sample is determined based on one sample and calculated as described above.

5.2.4.2 Determination of total index per water body

Initially 3 samples are selected based on spatial distribution and any quality differences suggested by vegetation and/or morphology. If the standard deviation of the EQR between these samples is more than 0.2 EQR units, additional samples, randomly chosen from the other samples, are analyzed until a standard deviation of less than 0.2 on the average EQR value is obtained or until all 9 samples are counted. The average value is the EQR for the water body (Hendrickx & Denys, 2005, Lock et al., 2007).

5.3 Transitional waters

For transitional waters, the quality element phytobenthos is not applicable (see section 2.1).

6 Macrophytes

6.1 Rivers

For the river type MLz, formerly beloning to the transitional waters, see chapter 6.3.

6.1.1 Selection of stretches for phytobenthos and macrophytes in rivers

For the selection of stretches, see section 5.1.1.

6.1.2 Survey

The field form for this method can be found in annex in Leyssen et al. (2007). For each location a form with 'environmental features' is completed, preferably also some photos of the stretch are taken. The original field sheet on which the species are recorded can be found in Leyssen et al. (2007). This field sheet is regularly updated by VMM to better adapt it to the needs of the monitoring network.

The fieldwork consists of a survey of a stretch of 100 m. For the survey only the water vegetation is fully recorded; for the riparian vegetation, only the characteristic species are indicated. The water vegetation includes those plants that root in the water or the water bed.

For the survey of the water vegetation the present species are listed for the entire stretch, along with their abundance according to a modified Tansley-scale (Table 6.1).

Table 6.1: Modified Tansley-scale for watercourses

Code	Modified Tansley-scale	Description
1	Rare	Rare; less than 3 individuals
2	Occasional	Occasional; more individuals; never covering
3	Frequent	Frequent; many individuals and <5 % covering
4	Low-abundant	5-25 % covering
5	Abundant	25-50 % covering
6	Co-dominant	50-75 % covering
7	Dominant	75-100 % covering

The survey is done, if possible, from the water. Wading is always done against the flow in order to avoid that the loosened sediment layer would impede the view on the vegetation. If the field conditions do not allow this, the survey is carried out from the bank. In this situation, the macrophytes are sampled by means of a rake, mounted on a telescopic pole. In this case, raking is done three times for each 10 m-strip.

It is important that the survey is done on 'representative' locations. By considering a sufficient number of sites, a good picture of the situation can be obtained.

Some macrophytes can occur as different growth forms (Table 6.2). For these species the growth form is recorded in a separate column.

Table 6.2: Species with several growth forms

Scientific name	English name	Growth form
Apium inundatum	Lesser marshwort	batrachids / riparian/marsh
Hippuris vulgaris	Mare's-tail	elodeids / riparian/marsh
Luronium natans	Floating water-plantain	isoetids / nymphaeids / vallisnerids
Eleocharis acicularis	Needle spike-rush	isoetids / riparian/marsh
Juncus bulbosus	Bulbous rush	Isoetids / riparian/marsh / parvopotamids
Hydrocotyle ranunculoides	Floating marsh-pennywort	nymphaeids / riparian/marsh
Polygonum amphibium	Amphibious bistort	nymphaeids / riparian/marsh

Scirpus fluitans	Floating club-rush	riparian/marsh / parvopotamids
Glyceria fluitans	Floating sweet-grass	riparian/marsh / vallisnerids
Sagittaria sagittifolia	Arrowhead	riparian/marsh / vallisnerids
Sparganium emersum	Unbranched bur-reed	riparian/marsh / vallisnerids
Sparganium natans	Least bur-reed	riparian/marsh / vallisnerids

The submerse vegetation development is taken into account for the types BkK, BgK, Bk and Bg; this is recorded separately during the field survey per 10 m strip. By analogy with the method used for the lakes, the cover of the submerse vegetation is estimated from the cover scale given in Table 6.3. For each 10 m strip, the submerse vegetation development is estimated.

Table 6.3: Cover scale to estimate the submerse vegetation development (Moss et al., 2003)

Code	Covering of submerse vegetation
0	No submerse vegetation
1	Plants scarce, some plants on rake
2	Many rake samples convey plants and the submerse vegetation rarely if ever impedes the passage of a rowing boat
3	Nearly all rake samples convey plants, plants grow up to the surface in most parts of the stretch or filamentous algae cover most part of bottom or surface

For the survey of the riparian vegetation a distinction is made between the vegetation of the 'wet' bank and the 'dry' bank. The 'wet' bank signifies the part of the bank that is regularly submerged; for this part the most dominant species are recorded. The 'dry' side means the upper part of the bank; for this the aspect determining vegetation is described. Alien species located on the bank, should certainly be mentioned: New Zealand pigmyweed, floating marsh-pennywort, *Ludwigia grandiflora*, parrot feather, Japanese knotweed, ...

6.1.3 Conservation

The species are identified in situ and therefore do not need to be taken away. Plants that can not be identified in the field, can be taken away for viewing with a binocular in the laboratory, or if necessary with a microscope. The material can be stored up to a week in a refrigerator.

6.1.4 Identification

A standard list for macrophytes in rivers is given in Leyssen et al. (2005), Annex 4. In Leyssen et al. (2005) an additional list can also be found of species for the type Rzg, because for the Grensmaas the typical gravel bank vegetations are included in the assessment. This standard list is however not static, it must be supplemented when necessary, e.g. when new alien plant species are encountered. Updating of the list is carried out by VMM and INBO. It is important that for assessment always the most recent list is taken into acccount. A list of recommended identification works is given in Leyssen et al. (2007). The basic identification work is Lambinon et al. (1998).

6.1.5 Index calculation

The assessment method for watercourses consists of three metrics: the metric type specificity, the metric disturbance and the metric growth forms. For some types there is an additional metric vegetation development. For the quality assessment of watercourses at the water body level, a sample of at least three stretches of 100 m is considered.

6.1.5.1 Metric type specificity (TS)

For each type a type-specificity value is assigned for each species. This metric is calculated for the water vegetation.

For watercourses two type-specificity values are distinguished:

type specificity value 0: species not belonging in the type;

type specificity value 1: species belonging in the type but that are also found in other types. These are often species with a very broad range;

The type-specificity values for each species can be found in the above-mentioned standard lists. To the type-specificity and disturbance values some changes have been made since the publication of the report.

Because no international consensus has been reached regarding alien plant species, it has provisionally been decided to consider the alien plant species (indicated by 'N' in the standard list) as non-specific for all types.

Taking into account the coverings, the type specificity score for the 100 m stretch is calculated as follows for watercouses:

$$TS = \Sigma^{n}_{i=1} (Ab_i . ts_i) / \Sigma^{n}_{i=1} Ab_i$$

With: Ab_i : the abundance of species i;

ts_i : the type specificity value of species i (0 or 1) according to the standard list;

n: the number of observed species included in the standard list;

TS : the type specificity score.

It should be noted that ΣAb_i is the sum of the abundances of the species that are included in the standard list. If species are observed that are not in the list, they will not be taken into account in the calculations.

The result of TS reflects an EQR scale. The boundaries of the TS score for rivers are given in Table 6.4.

Table 6.4: Class boundaries for assessment of the type specificity (TS) and disturbance (V) for water vegetation in watercourses

TS or V	EQR class
0,80 - 1	High
0,60 - < 0,80	Good
0,40 - < 0,60	Moderate
0,20 - < 0,40	Poor
0 - < 0,20	Bad

6.1.5.2 Metric disturbance (V)

This metric is calculated for the water vegetation. For each type a disturbance value is assigned per species. There is a distinction between:

disturbance value 1: species that indicate disturbance of environmental quality;

disturbance value 0: species that do not indicate evident disturbance of environmental quality.

The disturbance values are given in the standard list. Taking into account the abundances the V-score for the 100 m stretch in the watercourses is calculated as follows:

$$V = 1 - [\Sigma^{n}_{i=1} (Ab_i . v_i) / \Sigma^{n}_{i=1} Ab_i]$$

With: Ab_i: the abundance of species i;

v_i: the disturbance value of species i (0 or 1) according to the standard list;

n: the number of observed species from the standard list;

V: disturbance score.

The score is between 0 and 1, which can be divided into 5 equal classes as for the type specificity in in Table 6.4.

6.1.5.3 Metric growth forms (GV)

For the metric growth forms only species actually present in the water are taken into account. To each species a growth form is assigned (Leyssen et al. (2005), Annex 6).

The species present in the entire 100 m stretch are used as the basis for this calculation. For the watercourses, it is checked which species, and consequently which growth forms are present for each location. In order to be included in the number of growth forms observed, at least one representative of the growth form has to be present.

If a certain growth form is represented by one or more species that indicate an exceptional ecological quality level (marked with a 'B' in the standard list), the number of points scored for that growth form is increased with the number of species that indicate an exceptional quality.

For each water type, it is specified how many and what combination of growth forms must be present to obtain a bad, poor, moderate, good or high assessment (Table 6.5). If the obtained score is more than the maximum score (due to the presence of 'B'-species), this is reduced to the maximum score. Using linear interpolation between the class boundaries, the EQR is obtained. For this, the boundary between two categories is determined by the lowest number of growth forms of the highest of both categories.

Table 6.5: Scoring system for the metric growth forms in running water. The distribution into quality classes is done based on the observed number of growth forms

Growth forms	BB_K	BB_Z	BkK	BgK	Bk	Bg	Rk	Rg	Rzg	Pzoet	Pzilt	Pbr/z
Enteromorpha	-	-	-	-	-	-	-	-	-	-	-	1
Lemnids	-	-	-	1	-	1	1	1	1	1	1	-
Ricciellids	-	-	-	1	-	1	1	1	-	1	1	-
Hydrocharids	-	-	1	1	-	-	-	-	-	1	-	-
Stratiotids												
Ceratophyllids	-	-	-	1	-	1	1	1	1	1	1	-
Charids	1	1	1	1	1	1	-	-	1	1	1	-
Parvopotamids	-	1	1	1	1	1	1	1	1	1	1	1
Myriophyllids												
Elodeids												
Magnopotamids	1	1	1	1	1	1	1	1	1	1	-	-
Nymphaeids	-	-	1	1	1	1	1	1	1	1	-	-
Batrachids	1	1	1	1	1	1	-	-	1	1	1	1
Peplids												
Vallisnerids	-	-	1	1	1	1	1	1	1	1	-	-
Isoetids	-	1	-	-	-	-	-	-	-	-	-	-
Aquatic moss (incl. *Sphagnum*)	1	1	1	-	1	-	-	-	-	-	-	-
riparian/marsh plants (small)	1	1	1	1	1	1	1	1	1	1	1	1
riparian/marsh plants (large) large monocotyledons	-	-	1	1	1	1	1	1	1	1	1	1
Max. score	**5**	**7**	**10**	**12**	**9**	**11**	**9**	**9**	**10**	**12**	**8**	**5**
High	4	4	$\geq$ 7	$\geq$ 9	$\geq$ 7	$\geq$ 6	$\geq$ 7	$\geq$ 7	$\geq$ 7	$\geq$ 9	$\geq$ 6	3

Good	3	3	5-6	6-8	5-6	4-5	5-6	5-6	5-6	7-8	4-5	2
Moderate	2	2	3-4	4-5	3-4	2-3	4	4	3-4	5-6	3	1
Poor	1	1	2	2-3	2	1	2-3	2-3	2	3-4	2	0
Bad	0	0	0-1	0-1	0-1	0	0-1	0-1	0-1	0-2	0-1	0

6.1.5.4 Metric vegetation development (VO)

For the types ‘small stream Kempen‘, ‘large stream Kempen‘, ‘small stream‘, ‘large stream‘ and ‘polderwatercouses‘, a metric for submerse vegetation development is added. Per strip, the cover scale of submerse vegetation (Table 6.3) is converted into a score (Table 6.6). The average score of the entire stretch is calculated. By halving this number, the EQR is obtained (Table 6.7).

Table 6.6: Conversion of the abundance into a score for submerse vegetation development

Abundance	**Score**
0	0
1	1
2	2
3	1

Table 6.7: Conversion of the score for vegetation development into an EQR

Average score	**EQR**
1,6 - 2	0,8 - 1
1,2 - < 1,6	0,6 - < 0,8
0,8 - < 1,2	0,4 - < 0,6
0,4 - < 0,8	0,2 - < 0,4
0 - < 0,4	0 - < 0,2

To avoid locations where the submerse vegetation is well developed, but not type specific, to be assessed more negatively than sites where submerse vegetation is very scarce, an exception rule is introduced. If the difference between VO and TS is greater than 0.4, and TS is bad or poor, a value of 0.2 is added to TS. However, if the VO is the lowest of the two values, nothing changes, and VO continues to strongly affect the final assessment. Or otherwise expressed:

if VO - TS ≥ 0,4 and TS < 0,4 then TS’ = TS + 0,2

6.1.5.5 Determination of the total index per stretch

To determine the final score from the different metrics for watercourses, the principle 'one out - all out' is used. This means the worst score of the different metrics will be the final score.

6.1.5.6 Determination of the total index per water body

Throughout a whole water body at least three stretches are sampled, unless this is impossible, especially for very short water bodies, in which case the number of stretches can be reduced. Per stretch the relevant metrics are assessed. There has not been a decision by INBO yet on the method for taking these results together. Therefore it was decided by VMM to calculate the average result per metric for the sampled stretches, and subsequently use the worst score among these averages as final score for the entire water body.

6.2 Lakes

6.2.1 Survey

For the fieldwork the method described by Schneiders et al. (2004) is used. The pond is divided into several, more or less homogeneous, segments according to adjacent vegetation and land use, morphological structure and vegetation. This partitioning into water and riparian segments is graphically presented in the sheets of Leyssen et al. (2005), Annex 2.

The species occurring in the water zone and in the riparian zone are listed separately. As upper limit of the riparian zone the level is chosen that is reached at normal maximum water level and that is distinguished from the non-flooded areas by vegetation composition. The water level is the lower limit. The vegetation survey consists of a recording made at the bank (riparian and water survey) and, if possible, by additional transect surveys. The survey is based on observations along the bank, as well as wading through the water, while submerged vegetation is collected with a rake. For each species observed per segment the covering is recorded based on a simplified Tansley-scale (Table 6.8).

Table 6.8: Simplified Tansley scale for lakes (Schaminée et al., 1995)

Code	Simplified Tansley scale
R	Rare
1	Occasional
2	Frequent
3	Abundant
4	Co-dominant
5	Dominant

Also, the percentage covering is estimated for the following vegetation components: submerse and non-submerse vegetation and covering of the riparian vegetation (classified as herbaceous, arboreal and shrub layer). Where possible transect surveys are made. The transect surveys are carried out over the entire width of the pond, selected in order to obtain an impression of the vegetation that is as complete and representative as possible. From a motorboat or wading through the water, at regular intervals (transect points) estimates (Table 6.8) are made based on raked or dredged material. Also the depth is recorded for each transect point and an estimate of the total submerse covering is made using the scale given in Table 6.9.

Table 6.9: Cover scale to estimate the submerse vegetation development (Moss et al., 2003)

Code	Covering of submerse vegetation
0	No submerse vegetation
1	Plants scarce, some plants on rake
2	Many rake samples convey plants and the submerse vegetation rarely if ever impedes the passage of a rowing boat
3	Nearly all rake samples convey plants, plants grow up to the surface in most parts of the stretch or filamentous algae cover most part of bottom or surface

The depth to assess the vegetation is adapted. Vegetation surveys are limited to a depth of 4 m for lakes belonging to the types Awom or Awe and a depth of 2 m for lakes belonging to another type. However, the maximum depth up to where vegetation is present and its position is recorded.

6.2.2 Conservation

The species are identified in situ and therefore do not need to be taken away. Plants that can not be identified in the field, can be taken away for viewing with a binocular in the laboratory, or if necessary with a microscope. The material can be stored up to a week in a refrigerator.

6.2.3 Identification

A standard list for macrophytes in lakes is given in Leyssen et al. (2005), Annex 5. This standard list is however not static, it must be supplemented when necessary, e.g. when new alien plant species are encountered. Updating of the list is carried out by VMM and INBO. It is important that for assessment always the most recent list is taken into acccount. A list of recommended identification works is given in Leyssen et al. (2007). The basic identification work is Lambinon et al. (1998).

6.2.4 Index calculation

For the assessment of lakes four metrics are taken into account: the metric type specificity, the metric disturbance, the metric growth forms and the metric vegetation development. For this the entire water body is inventoried.

6.2.4.1 Metric type specificity (TS)

For each type a type-specificity value is assigned for each species. This metric is calculated for the lakes for bank vegetation as well as for water vegetation (TS_o and TS_w).

For lakes two type-specificity values are distinguished:

type specificity value 0: species does not belong in the type;

type specificity value 1: species does belong in the type;

The type-specificity values for each species can be found in the above-mentioned standard lists.

Because no international consensus has been reached regarding alien plant species, it has provisionally been decided to consider the alien plant species (indicated by 'N' in the standard list) as non-specific for all types.

With the following formula the ecological quality ratio is calculated for the total surface area of the lake:

$$TS = \Sigma_{\text{all segments}} [[\Sigma^{n}_{i=1} (Ab_i . ts_i) / \Sigma^{n}_{i=1} Ab_i] . [Opp_a / \Sigma^{z}_{a=1} opp_a]]$$

With: Ab_i: the abundance of species i in the surface segment a;

ts_i : the type specificity value of species i (0 or 1) according to the standard list;

n: the number of observed species in surface segment a that are included in the standard list;

Opp_a: the surface of segment a;

z: the total number of segments;

Σopp_a: the overall surface area of all segments combined;

TS: the type specificity score.

It should be noted that ΣAb_i is the sum of the abundances of the species that are included in the standard list. If species are observed that are not in the list, they will not be taken into account in the calculations.

The result of TS reflects an EQR scale. The boundaries of the TS score for lakes are given in Table 6.10.

Table 6.10: Class boundaries for the assessment of the type specificity (TS) and disturbance (V) for riparian and water vegetation in lakes

TS or V	EQR class
0,80 - 1	High
0,60 - < 0,80	Good
0,40 - < 0,60	Moderate
0,20 - < 0,40	Poor
0 - < 0,20	Bad

6.2.4.2 Metric disturbance (V)

This metric is calculated for the riparian as well as for the water vegetation (V_o en V_w). For each type a disturbance value is assigned per species. There is a distinction between:

disturbance value 1: species that indicate disturbance of environmental quality;

disturbance value 0: species that do not indicate evident disturbance of environmental quality.

The disturbance values are given in the standard list.

The formula for disturbance for the total surface area of lakes is as follows:

$$V = 1 - [\Sigma_{\text{all segments}} [[\Sigma^{n}_{i=1} (Ab_i . v_i) / \Sigma^{n}_{i=1} Ab_i] . [Opp_a / \Sigma^{z}_{a=1} opp_a]]]$$

With: Ab_i: the abundance of species i;

v_i: the disturbance value of species i (0 or 1) according to the standard list;

n: the number of observed species from the standard list;

Opp_a: the surface of segment a;

z: the total number of segments;

Σopp_a: the overall surface area of all segments combined;

V: disturbance score.

The score is between 0 and 1, which can be divided into 5 equal classes as for the type specificity in in Table 6.10.

6.2.4.3 Metric growth forms (GV)

For the metric growth forms only species actually present in the water are taken into account. To each species a growth form is assigned (Leyssen et al. (2005), Annex 6).

The species present in the entire pond are used as the basis for this calculation. So for lakes this score is not determined per segment. In order to be included in the number of growth forms observed, at least one representative of the growth form has to be present. Each expected growth form is assigned a number of score points (Table 6.11). Hereby the growth forms that are most sensitive to a decline in water quality are more strongly weighted. The sum of the score points of a type provides the basic sum.

If a certain growth form is represented by one or more species that indicate an exceptional ecological quality level (marked with a 'B' in the standard list), the number of points scored for that growth form is increased with the number of species that indicate an exceptional quality.

A score is obtained by dividing the resulting points by the basic sum (Table 6.11); if this is more than 1 (due to the presence of 'B'-species), this is rounded to 1. The obtained score is thus between 0 and 1, so it can be divided into five equal quality classes as in Table 6.10.

Table 6.11: Scoring system for diversity of growth forms in different types of lakes

Growth forms	Zs	Zm	Czb	CFe	Cb	Aom	Ae	Ai	Awom	Awe	Ad	Bzl
lemnids	-	-	1	1	1	1	1	1	1	1	1	1
Large pleustophytes	-	-	1	1	1	1	1	1	-	-	-	-
Submerged, in suspension	1	1	1	1	1	1	1	1	-	1	1	1
Charids	-	1	1	1	2	2	2	2	2	2	2	1
Magnopotamids	-	1	1	1	2	1	1	1	2	2	1	1
Other rooting caulescent hydrophytes	1	1	1	1	1	1	1	1	1	1	1	1

Nymphaeids	-	1	1	1	1	1	1	1	1	1	-	-
Vallisnerids	1	1	1	1	1	-	-	-	-	-	-	-
Isoetids	1	2	2	1	2	-	-	-	2	-	2	-
Small and mid-sized riparian and marsh plants	1	1	1	1	1	1	1	1	1	1	1	1
Large monocotyledons	-	-	1	1	1	1	1	1	1	1	1	1
Peat moss	1	1	1	-	-	-	-	-	1	-	-	-
Basic sum	**6**	**10**	**13**	**11**	**14**	**10**	**10**	**10**	**12**	**10**	**10**	**7**
Cyanobacterial film	-	-	-1	-1	-1	-1	-1	-1	-1	-1	-1	-

The large pleustophytes include the hydrocharids and stratiotids. The category 'submerged and in suspension' contains the groups ceratophyllids, ricciellids and water mosses (excluding peat moss). Among the ‘other rooting caulescent hydrophytes’ the parvopotamids, the myriophyllids, the elodeids, the batrachids and the peplids are included.

6.2.4.4 Metric vegetation development (VO)

In Schneiders et al. (2004), the combined abundance of submerged vegetation is taken into account in the assessment of lakes (Table 6.9). For this metric the surface area where growth is possible must be taken into account. Per water segment, the abundance of submerse vegetation is converted to a score (Table 6.12). The average score of the entire pond is calculated, taking into account the relative segment surface areas. By halving this number, the EQR is obtained (Table 6.13).

Table 6.12: Conversion of abundance to a score for the submerse vegetation development

Abundance	Score
0	0
1	1
2	2
3	1

Table 6.13: Conversion of the score for the vegetation abundance to an EQR

Average score	EQR
1,6 - 2	0,8 - 1
1,2 - < 1,6	0,6 - < 0,8
0,8 - < 1,2	0,4 - < 0,6
0,4 - < 0,8	0,2 - < 0,4
0 - < 0,4	0 - < 0,2

6.2.4.5 Calculation of the total index

To determine the final score for lakes from the different metrics, the principle 'one out - all out' is used. This means the worst score of the different metrics will be the final score.

6.3 Transitional waters

The quality element macrophytes for transitional waters includes macroalgae, submerged angiosperms and tidal marsh vegetations. Brys et al. (2005) argue that the first two groups do not or hardly thrive in the Flemish transitional waters and that there is no evidence that the situation was much different in the past. Therefore, they only take tidal marsh vegetations into account for evaluation of the ecological status of the Flemish transitional waters for the quality element macrophytes. The method of Brys et al. (2005) was later further elaborated by Speybroeck et al. (2008a, 2008b).

The river type MLz, formerly belonging to the transitional waters, is also addressed in this chapter, along with the "true" transitional waters.

As all Flemish transitional waters and water bodies of the type MLz are heavily modified or artificial, the proposed method is an assessment of the ecological potential. Consequently, the relevant quality classes for artificial and heavily modified water bodies will be used in what follows (see section 2.5).

6.3.1 Survey

The vegetation diversity of a tidal marsh is determined by means of a vegetation map. Based on recent orthophotos vegetation units can be distinguished to which a vegetation type can be associated in the field. From the vegetation map the vegetation diversity can then be calculated (see below).

At least five vegetation surveys per vegetation type are needed per water body to determine the quality of the tidal marshes. In doing so, it is ensured that these are made throughout the various tidal marshes that are present within the water body (Leyssen et al. 2006).

The size of the vegetation survey varies depending on the fysionomy, which means that the vegetation surveys are larger in the forests than e.g. in the grasslands. Per distinguished stratum a species list is made with a covering code assigned to each species according to the Londo scale (Londo, 1976). The species richness corresponds to the total number of plant species that are encountered in the vegetation surveys, from which then the floristic quality can be determined (see below).

6.3.2 Conservation

Not applicable (identification in situ).

6.3.3 Identification

Identification of higher plants is done using Lambinon et al. (1998).

For the calculation of the Floristic Quality Index (FQI; see below) Brys et al. (2005) refer to Biesbrouck et al. (2001) who give an indication of rareness for each species. However, Van Landuyt et al. (2006) provide a more recent and therefore better indication of the rareness on which the standard list can be based.

6.3.4 Index calculation

The assessment at the body water level is based on the total area of tidal marshes on the one hand and the average quality of all individual tidal marshes within the water body on the other hand. The quality index for each individual tidal marsh is determined based on the shape and on the vegetation quality. The latter is in turn based on vegetation diversity, species richness and floristic quality.

6.3.4.1 Metric tidal marsh surface area

Table 6.14 shows the demarcation of the class boundaries of the maximum ecological potential (MEP) and for the classes good and above, moderate, poor and bad based on the tidal marsh surface area per individual water body and the demarcation at the ecosystem level for Zeeschelde and adjacent tidal rivers combined (the seven water bodies at the top of the table). For the Yser, the assessments at the ecosystem and the water body level are identical, because this tidal zone includes only one water body, Yser Harbour Passage.

Table 6.14: Demarcation of the maximum ecological potential (MEP) and the classes good and above, moderate, poor and bad based on the tidal marsh surface area in hectares per individual water body and the demarcation at the ecosystem level for Zeeschelde and adjacent tidal rivers

Water body	MEP	Goed and above	Moderate	Poor	Bad
Zeeschelde IV	1570	> 500	> 333	> 167	< 167
Zeeschelde III + Rupel	1382	> 440	> 293	> 147	< 147
Zeeschelde II	901	> 287	> 191	> 96	< 96
Zeeschelde I	1439	> 458	> 305	> 153	< 153
Tidal Durme	581	> 185	> 123	> 62	< 62
Tidal Dijle and –Zenne	647	> 206	> 137	> 62	< 62
Tidal Netes	992	> 316	> 210	> 105	< 105
Ecosystem (Scheldt and adjacent tidal rivers)	**7512**	**> 2390**	**> 1593**	**> 797**	**< 797**
Yser Harbour Passage	31,1	> 28,8	> 19,2	> 9,6	< 9,6

This score is transformed into an EQR with class boundaries as shown in Table 6.15. This is done by a linear transformation between the upper and lower limits of the absolute scores.

Table 6.15: Demarcation of the classes good and above, moderate, poor and bad for the tidal marsh surface area

Class	EQR
Good and above	0,75 - 1,00
Moderate	0,50 - 0,75
Poor	0,25 - 0,50
Bad	0,00 - 0,25

6.3.4.2 Metric vegetation index per individual tidal marsh

The metric vegetation is calculated per individual tidal marsh based on the parameters vegetation diversity, species richness and Floristic Quality Index (FQI). This metric is calculated only for the water bodies belonging to the Zeeschelde and adjacent tidal rivers. For the Yser no criteria were derived by Speybroeck et al. (2008a) for this metric due to lack of data and hence this metric is not included.

The vegetation diversity per tidal marsh is calculated using the Shannon-Wiener diversity index (H') (Shannon & Weaver, 1949). This index typically varies from 1 (low diversity) to 5 (high diversity) and uses the proportional abundance p_i of all vegetation types per tidal marsh:

$$H' = - \Sigma^{S}_{i=1} [p_i . \ln p_i]$$

With: S = the total number of vegetation types per tidal marsh;

p_i = the relative abundance of the i-th vegetation type.

The class boundaries for the GEP, the moderate, poor and bad status for the parameter vegetation diversity for freshwater and brackish tidal marshes in the Zeeschelde are summarized in Table 6.16.

Table 6.16: Demarcation of the classes good and above, moderate, poor and bad for the parameter vegetation diversity for freshwater and brackish tidal marshes in the Zeeschelde and adjacent tidal rivers

Water bodies	Tidal Netes and Tidal Dijle and Zenne	Zeeschelde I, Zeeschelde II, Zeeschelde III + Rupel, Tidal Durme	Zeeschelde IV
Good and above	1,10 - 1,40	1,20 - 1,40	0,90 - 1,20
Moderate	0,80 - 1,10	1,05 - 1,20	0,60 - 0,90

Poor	0,40 - 0,80	0,80 - 1,05	0,30 - 0,60
Bad	0,00 - 0,40	0,00 - 0,80	0,00 - 0,30

The parameter species richness equals the total number of observed species. In Table 6.17, the class boundaries are given for the GEP, the moderate, poor and bad status for the parameter species richness for freshwater and brackish tidal marshes in the Zeeschelde.

Table 6.17: Demarcation of the classes good and higher, moderate, poor and bad for the parameter species richness for freshwater and brackish tidal marshes in the Zeeschelde and adjacent tidal rivers

Water bodies	**Tidal Netes and Tidal Dijle and Zenne**	**Zeeschelde I, Zeeschelde II, Zeeschelde III + Rupel, Tidal Durme**	**Zeeschelde IV**
Good and above	89 - 119	93 - 124	54 - 72
Moderate	60 - 89	62 - 93	36 - 54
Poor	30 - 60	31 - 62	18 - 36
Bad	0 - 30	0 - 31	0 - 18

The parameter FQI uses the rareness of each species in function of the total floristic composition of a given area or sample. This index is calculated by the following formula (Lopez & Fennessy, 2002, Cohen et al., 2004):

$$FQI = [\Sigma_{i,j} ZC_{ij}] / \sqrt{(N_j)}$$

With: ZC_{ij}: the rareness coefficient for species i on location j;

N_j: the total number of species on location j.

The rareness coefficient of each species concerned is based on the global indication measure of rareness, the Square Kilometer Frequency Class (KFK), which was defined for the Flemish higher plants in Van Landuyt et al. (2006). This gives for each species a certain score that ranges from 10 (very common) to 1 (very rare). For this analysis, the scores are reversed, hence a rareness coefficient of 10 means very rare and of 1 very common.

In Table 6.18, the class boundaries are presented for the classes good and above, moderate, poor and bad for the FQI for freshwater and brackish tidal marshes in the Zeeschelde and adjacent tidal rivers.

Table 6.18: Demarcation of the classes good and above, moderate, poor and bad for the parameter FQI for freshwater and brackish tidal marshes in the Zeeschelde and adjacent tidal rivers

Water bodies	**Tidal Netes and Tidal Dijle and Zenne**	**Zeeschelde I, Zeeschelde II, Zeeschelde III + Rupel, Tidal Durme**	**Zeeschelde IV**
Good and above	22,6 – 30,16	20,5 - 21,4	27,4 - 25,6
Moderate	15,1 – 22,6	19,6 - 20,5	23,8 - 25,6
Poor	7,5 – 15,1	17,7 - 19,6	22,0 - 23,8
Bad	0,0 - 7,5	0,0 - 17,7	0,0 - 22,0

The scores of each of these three parameters are transformed into an EQR with class boundaries as shown in Table 6.19. This is done by a linear transformation between the upper and lower limits of the absolute scores.

Table 6.19: Demarcation of the classes good and above, moderate, poor and bad for the parameters vegetation diversity, species richness and FQI for assessment of individual tidal marshes

Class	**EQR**

Good and above	0,75 - 1,00
Moderate	0,50 - 0,75
Poor	0,25 - 0,50
Bad	0,00 - 0,25

Once the EQR has been calculated in this way for each of these three parameters, the $EQR_{vegetation}$ is determined with the following formula:

$$EQR_{vegetation} = (2 * EQR_{vegetation\ diversity} + EQR_{species\ richness} + EQR_{FQI}) / 4$$

6.3.4.3 Metric morphology per individual tidal marsh

The metric morphology per individual tidal marsh is calculated by means of the surface of the tidal marsh in relation to the length of the tidal marsh area along the river axis and the 'threshold width' at that location along the estuary. This width is necessary to ensure that the tidal marsh has a favourable profile to develop fully and sustainably.

In Table 6.20, the criteria are listed for assigning a quality class based on the percentage of current surface area relative to the GEP surface area. Through a linear rescaling between the relevant class boundaries this percentage can be converted into an EQR according to the class boundaries from the table.

Table 6.20: Demarcation of the classes good and above, moderate, poor and bad for the metric morphology per individual tidal marsh

Class	**Current surface area / GEP suface area (%)**	**EQR**
MEP	>133	1,00
Good and above	100 - 133	0,75 – 1,00
Moderate	66 - 100	0,50 - 0,75
Poor	33 - 66	0,25 - 0,50
Bad	0 - 33	0,00 - 0,25

6.3.4.4 Calculation of the total index

The macrophytes for transitional waters can be assessed at three different hierarchical levels: the level of the individual tidal marsh, the water body level and the ecosystem level.

The EQR of an individual tidal marsh is based on two metrics (morphology and vegetation index) and the final score is calculated as follows:

$$EQR_{tidal\ marsh} = (2 * EQR_{morphology} + EQR_{vegetation}) / 3$$

For the water body "Yser Harbour Passage" $EQR_{tidal\ marsh}$ is set equal to $EQR_{morphology}$ (see above).

The assessment at the water body level is based on two metrics, the metric surface area tidal marshes within the water body and the average of the EQRs for all individual tidal marshes within that water body. The total score for a water body is determined by the lowest scoring parameter. If both parameters are in the same quality class, the average of both EQRs is calculated. The class boundaries for the overall index are the same as those applicable for the metrics.

At the ecosystem level, the total surface area of tidal marshes is considered. This is assessed in the same way as the surface area of tidal marshes of individual water bodies, but with the class boundaries for the total surface area as indicated in Table 6.14, for "ecosystem (Scheldt and adjacent tidal rivers)". For the Yser this assessment at the ecosystem level is identical to that at the water body level because this tidal zone only comprises one water body. This overall assessment at the ecosystem level is however not necessary for WFD reporting.

7 Macroinvertebrates

7.1 Rivers

A first version of the Multimetric Macroinvertebrate Index Flanders (MMIF) for rivers and lakes was described by Gabriels et al. (2004). The final version of the MMIF for rivers, after applying a number of modifications, is described in Gabriels (2008).

For the river type MLz, formerly belonging to the transitional waters, see chapter 7.3.

7.1.1 Selection of sampling sites

Per water body usually one representative measurement point was assigned for the assessment of macroinvertebrates. In most cases this corresponds to the site where also the physical-chemical quality is determined. Some water bodies have more than one representative measurement point, usually because their quality is not uniform.

7.1.2 Sampling

For sampling, kick-sampling with handnet is applied as described in De Pauw and Vanhooren (1983). For places where this method is not applicable due to a large depth, artificial substrates will be used as described in De Pauw et al. (1986, 1994).

7.1.3 Conservation

For storage of the sampled macroinvertebrates, the same method can be applied as for the Belgian Biotic Index.

7.1.4 Identification

The used identification levels are:

Hydracarina: presence;

Oligochaeta, Crustacea, Coleoptera, Trichoptera, Diptera (except Chironomidae): family;

Chironomidae: groups thummi-plumosus and non thummi-plumosus;

Plathelminthes, Hirudinea, Mollusca, Ephemeroptera, Odonata, Plecoptera, Hemiptera, Megaloptera: genus.

A standard list with all taxa included in index calculation can be found in Gabriels (2008).

As identification key De Pauw and Vannevel (1991) can be used, except for Ampharetidae, Janiridae, Sphaeromatidae, *Corbicula*, which are not included in the cited work, and *Physa* and *Physella*, which are not distinguished in this work.

For all taxa abundances are recorded. For taxa that occur in larger abundances (>10) the abundance may be estimated.

7.1.5 Index calculation

7.1.5.1 Metric taxa richness

The metric taxa richness is calculated as the total number of taxa (according to the specified levels of identification) of which one or more individuals were found in the sample.

7.1.5.2 Metric number of EPT taxa

The metric number of EPT taxa is calculated as the total number of taxa (according to the specified levels of identification) belonging to Ephemeroptera, Plecoptera and/or Trichoptera of which one or more individuals were found in the sample.

7.1.5.3 Metric number of other sensitive taxa

The metric number of other sensitive taxa is calculated as the total number of taxa (according to the specified levels of identification), other than the EPT taxa, with a tolerance score of six or more. The list of tolerance scores (ranging from 10 for very intolerant to 1 for very tolerant) for all taxa is given in Gabriels (2008).

7.1.5.4 Metric Shannon-Wiener Index

The metric Shannon-Wiener Index is calculated using the following formula (Shannon & Weaver, 1949):

$$H' = - \Sigma^{S}_{i=1} [p_i . \ln p_i]$$

With: S = the taxa richness;

p_i = the relative abundance of the i-th taxon.

When no taxa are encountered in a sample at all, this metric is set equal to zero.

7.1.5.5 Metric Mean Tolerance Score

The metric mean tolerance score is calculated as the sum of the tolerance scores of taxa of which one or more individuals were found in the sample, divided by the total number of taxa. The list of tolerance scores (ranging from 10 for very sensitive to 1 for very tolerant) for all taxa can be found in Gabriels (2008). When no taxa are encountered in a sample at all, this metric is set equal to zero.

7.1.5.6 Total index calculation

In order to integrate the values of the five metrics into one index, first they have to be converted into scores of 0 to 4. Per water type criteria are set for each metric by which the value can be converted into the corresponding score. These criteria are summarized in Gabriels (2008) per water type.

The overall index for a sampling point is equal to the sum of the five metric scores, which is a number between 0 and 20, divided by 20. This results in an EQR value that is comprised within the interval 0-1.

7.1.5.7 Determination of the index for the whole water body

When several points were sampled per water body, the reporting is based on the outcome of the most recently taken sample within the water body.

7.1.5.8 Determination of the quality class

The criteria used for determining the quality classes are summarized in Table 7.1 (Gabriels et al., 2004; Gabriels, 2008). These criteria depend on the river type to which the water body belongs.

Table 7.1: Class boundaries for the macroinvertebrate index for rivers

MMIF types Bk, BkK, Bg, BgK, Rk, Rg, Rzg	**MMIF types Pz, Pb**	**Class**	**Colour code**
$\geq$ 0,90	$\geq$ 0,80	High	Blue
< 0,90 and $\geq$ 0,70	< 0,80 and $\geq$ 0,60	Good	Green
< 0,70 and $\geq$ 0,50	< 0,60 and $\geq$ 0,40	Moderate	Yellow
< 0,50 and $\geq$ 0,30	< 0,40 and $\geq$ 0,20	Poor	Orange
< 0,30	< 0,20	Bad	Red

7.2 Lakes

A first version of the Multimetric Macroinvertebrate Index Flanders (MMIF) for rivers and lakes was described by Gabriels et al. (2004). The final version of the MMIF, after applying a number of modifications, is described in Gabriels et al. (2009).

7.2.1 Selection of sampling sites

At least three measurement points are selected throughout the water body. The selection of representative measurement points is preferably based on the partitioning into water and riparian segments as elaborated for the quality element macrophytes (see section 6.2.1).

7.2.2 Sampling

For sampling, kick-sampling with handnet is applied as described in De Pauw and Vanhooren (1983). For places where this method is not applicable due to a large depth, artificial substrates will be used as described in De Pauw et al. (1986, 1994).

7.2.3 Conservation

For storage of the sampled macroinvertebrates, the same method can be applied as for the Belgian Biotic Index.

7.2.4 Identification

The used identification levels are:

Hydracarina: presence;

Oligochaeta, Crustacea, Coleoptera, Trichoptera, Diptera (except Chironomidae): family;

Chironomidae: groups thummi-plumosus and non thummi-plumosus;

Plathelminthes, Hirudinea, Mollusca, Ephemeroptera, Odonata, Plecoptera, Hemiptera, Megaloptera: genus.

A standard list with all taxa included in index calculation can be found in Gabriels et al. (2009).

As identification key De Pauw and Vannevel (1991) can be used, except for Ampharetidae, Janiridae, Sphaeromatidae, *Corbicula*, which are not included in the cited work, and *Physa* and *Physella*, which are not distinguished in this work.

For all taxa abundances are recorded. For taxa that occur in larger abundances (>10) the abundance may be estimated.

7.2.5 Index calculation

7.2.5.1 Metric taxa richness

The metric taxa richness is calculated as the total number of taxa (according to the specified levels of identification) of which one or more individuals were found in the sample.

7.2.5.2 Metric number of EPT taxa

The metric number of EPT taxa is calculated as the total number of taxa (according to the specified levels of identification) belonging to Ephemeroptera, Plecoptera and/or Trichoptera of which one or more individuals were found in the sample.

7.2.5.3 Metric number of other sensitive taxa

The metric number of other sensitive taxa is calculated as the total number of taxa (according to the specified levels of identification), other than the EPT taxa, with a tolerance score of six or more. The list of tolerance scores (ranging from 10 for very intolerant to 1 for very tolerant) for all taxa is given in Gabriels et al. (2009).

7.2.5.4 Metric Shannon-Wiener Index

The metric Shannon-Wiener Index is calculated using the following formula (Shannon & Weaver, 1949):

$$H' = - \Sigma^{s}_{i=1} [p_i . \ln p_i]$$

With: S = the taxa richness;

p_i = the relative abundance of the i-th taxon.

When no taxa are encountered in a sample at all, this metric is set equal to zero.

7.2.5.5 Metric Mean Tolerance Score

The metric mean tolerance score is calculated as the sum of the tolerance scores of taxa of which one or more individuals were found in the sample, divided by the total number of taxa. The list of tolerance scores (ranging from 10 for very sensitive to 1 for very tolerant) for all taxa can be found in Gabriels et al. (2009). When no taxa are encountered in a sample at all, this metric is set equal to zero.

7.2.5.6 Total index calculation

In order to integrate the values of the five metrics into one index, first they have to be converted into scores of 0 to 4. Per water type criteria are set for each metric by which the value can be converted into the corresponding score. These criteria are summarized in Gabriels et al. (2009) per water type.

The overall index for a sampling point is equal to the sum of the five metric scores, which is a number between 0 and 20, divided by 20. This results in an EQR value that is comprised within the interval 0-1.

7.2.5.7 Determination of the index for the whole water body

The index value for the whole lake is the average of the index values of the different representative sampling points.

7.2.5.8 Determination of the quality class

The criteria used for determining the quality classes are summarized in Table 7.2 (Gabriels et al., 2004, 2009).

Table 7.2: Class boundaries for the macroinvertebrate index for lakes

MMIF	Class	Colour code
$\geq$ 0,90	High	Blue
< 0,90 and $\geq$ 0,70	Good	Green
< 0,70 and $\geq$ 0,50	Moderate	Yellow
< 0,50 and $\geq$ 0,30	Poor	Orange
< 0,30	Bad	Red

7.3 Transitional waters

For the macroinvertebrates a concept of assessment system is proposed by Brys et al. (2005). For sampling and determination, Brys et al. (2005) have made recommendations, but no actual method is proposed. What follows is therefore based on the proposal of Van Damme et al. (2003) for these aspects. The assessment method of Brys et al. (2005) was later supplemented by Speybroeck et al. (2008a, 2008b).

The river type MLz, formerly belonging to the transitional waters, is also addressed in this chapter, along with the "true" transitional waters.

As all Flemish transitional waters and water bodies of the type MLz are heavily modified or artificial, the proposed method is an assessment of the ecological potential. Consequently, the relevant quality classes for artificial and heavily modified water bodies will be used in what follows (see section 2.5).

7.3.1 Sampling

The number of samples should be distributed stratified over the relevant habitats, at least including intertidal mud, sandbank, shallow subtidal and deep subtidal.

The sampling of intertidal zones is done by means of the multiple core-tube technique, and in the subtidal by means of a Van Veen grab or a Reinecke “box corer” (Ysebaert & Meire, 1999). Each sample is sieved through a sieve with mesh size of 1 mm.

7.3.2 Conservation

For storage of the sampled organisms, no method is proposed by Van Damme et al. (2003). Provisionally it can be assumed that the same method can be applied as for the Belgian Biotic Index.

7.3.3 Identification

The sampled organisms are identified to the species level. As a basic list for the water bodies of the Zeeschelde and adjacent tidal rivers, one may refer to the list given in Annex C of the method description of the "Indice Oligochètes de Bioindication des Sédiments" (IOBS, AFNOR, 2002). That list however does not include all species that can be found in these water bodies. For the water body Yser Harbour Passage one can provisionally refer to Degraer et al. (2006) but also here, probably not all species that may be found in that water body are listed.

7.3.4 Index calculation

The index consists of two metrics: a metric at the habitat level and a metric at the community level. An additional metric at the ecosystem level (Brys et al., 2005; Speybroeck, 2008a, 2008b) is not further discussed here because one the one hand it is not necessary for the assessment at the water body level and on the other hand it has only been elaborated for a minority of the water bodies.

7.3.4.1 Metric at the habitat level

For the habitat metric, the surface area of shallow zones and of intertidal mud within the water body is used. The class boundaries of the assessment scale for the surface area of shallow zones and intertidal mud for the water bodies of the Zeeschelde and adjacent tidal rivers are listed in Table 7.3.

Table 7.3: Class boundaries for the surface area of shallow subtidal and intertidal mud for the water bodies belonging to the category of transitional waters or the river type MLz

Surface area (ha)	Water bodies	Good and above	Moderate	Poor	Bad
Shallow zone	Zeeschelde IV	388 - 518	258 - 388	128 - 258	0 - 128
	Zeeschelde III + Rupel	225 - 272	150 - 225	75 - 150	0 - 75
	Zeeschelde II	145 - 175	97 - 145	48 - 97	0 - 48
	Zeeschelde I	195 - 235	130 - 195	65 - 130	0 - 65
	Tidal Durme	48 - 58	32 - 48	16 - 32	0 - 16
	Tidal Dijle and –Zenne	53 - 64	35 - 53	18 - 35	0 - 18
	Tidal Netes	96 - 116	64 - 96	32 - 64	0 - 32
	Yser Harbour Passage	22 - 27	15 - 22	7,5 - 15	0 - 7,5
Intertidal mud	Zeeschelde IV	456 - 550	304 - 456	152 - 304	0 - 152
	Zeeschelde III + Rupel	398 - 479	256 - 398	133 - 265	0 - 133
	Zeeschelde II	230 - 277	153 - 230	77 - 153	0 - 77
	Zeeschelde I	195 - 235	130 - 195	65 - 130	0 - 65
	Tidal Durme	113 - 136	75 - 113	38 - 75	0 - 38
	Tidal Dijle and –Zenne	80 - 96	53 - 80	27 - 53	0 - 27
	Tidal Netes	141 - 170	94 - 141	47 - 94	0 - 47
	Yser Harbour Passage	20 - 24	13 - 20	6,6 - 13	0 - 6,6

For both shallow zone and intertidal mud, the surface area is converted into an EQR. This is done by a linear transformation between the upper and lower limits of the absolute scores to an EQR with class boundaires as shown in Table 7.4.

Table 7.4: Demarcation of the classes good and above, moderate, poor and bad for the surface area shallow subtidal and intertidal mud for the water bodies belonging to the category transitional waters or the river type MLz

Class	EQR
Good and above	0,75 - 1,00
Moderate	0,50 - 0,75
Poor	0,25 - 0,50
Bad	0,00 - 0,25

To obtain the total EQR at the habitat level the average is calculated of the EQRs for intertidal mud and shallow zone.

7.3.4.2 Metric at the community level

For the Zeeschelde and adjacent tidal waters, this metric is assessed by Speybroeck et al. (2008b) by means of the "Indice Oligochètes de Bioindication des Sédiments" (IOBS, AFNOR, 2002). This index is calculated as follows:

IOBS = 10 * S / T

with: S = total number of taxa identified within 100 oligochaetes (if less than 100 individuals present: IOBS can not be calculated)

T = percentage that constitutes the dominant group within the family Tubificidae (groups: Tubificidae with capillary hairs and Tubificidae without capillary hairs)

If T = 0 then IOBS is calculated as 10 * S.

The IOBS is zero if no oligochaetes are found.

The calculated value is converted into an EQR through a linear transformation between the upper and lower limits of the absolute scores to the upper and lower boundaries of five classes on the EQR scale as indicated in Table 7.5. An IOBS of e.g. 4.50 will thus be converted into an EQR of 0.70.

Table 7.5: Class boundaries used for a linear transformation of the calculated IOBS and the EQR for the metric at the community level for macroinvertebrates in transitional waters

IOBS	EQR
6,00 - 10,00	0,80 - 1,00
3,00 - 6,00	0,60 - 0,80
2,00 - 3,00	0,40 - 0,60
1,00 - 2,00	0,20 - 0,40
0,00 - 1,00	0,00 - 0,20

For the water body "Yser Harbour Passage" by Speybroeck et al. (2008a) the approach of the BEQI index (Benthic Ecology Quality Index; Van Hoey et al., 2007) is used for the metric at the community level. This assessment is based on four sub-indicators: biomass, density, species richness and species composition.

From the resulting EQR of each of these four sub-indicators, the average is taken to obtain a total EQR.

This total EQR is calculated in three different ways: for the sediment-rich litoral, the sandy litoral and the sub-litoral. For the sediment-rich litoral, this calculation is done with omission of the sub-indicator biomass and with omission of the samples from the high or the low intertidal. For the sandy litoral the sub-indicator biomass is also omitted but samples from the high and the low intertidal are merged with

those from the mid-litoral. For the sub-litoral, the sub-indicator biomass is included and samples from the high and low intertidal are merged with those from the mid-litoral.

The final assessment for the metric at the community level for the water body "Yser Harbour Passage" is equal to the average of the three total EQRs.

7.3.4.3 Total index calculation

Both for Zeeschelde and adjacent tidal rivers and for Yser Harbour Passage the total index is calculated as the average of the scores of the two metrics:

Total index = 0.5 * $EQR_{habitat\ level}$ + 0,5 * $EQR_{community\ level}$

7.3.4.4 Determination of the quality class

Assigning a quality class to the total index is done based on criteria which are summarized in Table 7.6.

Table 7.6: Class boundaries for macroinvertebrates in transitional waters

Index	**Assessment**
1,00	Maximum Ecological Potential
$\leq$ 1,00 and > 0,75	Good and above
< 0,75 and > 0,50	Moderate
< 0,50 and > 0,30	Poor
< 0,30	Bad

8 Fish

8.1 Rivers

Goethals et al. (2006) proposed an assessment method for rivers in Flanders based on fish communities. This proposal is further modified by INBO because for rivers the European Fish Index (EFI) was adopted (Breine et al., 2005). This index however does not fully comply with the requirements of the WFD. For this reason, the cited proposal is not further explained below. However, indices currently exist that are calibrated to the Huet zonation (Huet, 1949) and published in scientific journals. There is one for watercourses of the bream and barbel type (Belpaire et al., 2000), for watercourses of the trout and grayling zone (Breine et al., 2004). These indices are currently used for reporting.

8.1.1 Sampling

For sampling of rivers, two methods are used depending on the size of the location for fishing. At each location fishing is done electrically (wading or with boat) and this according to the CEN. In rivers with larger dimensions also two traps (double fyke nets) are placed in each location. The traps are placed for a period of 24 hours. The captured fish are individually measured (total length) and weighed (g) and subsequently released again.

8.1.2 Conservation

Not applicable.

8.1.3 Identification

The captured fish are identified to the species level. When necessary, the work of Nijssen & De Groot (1987) is used.

8.1.4 Index calculation

Currently four zones are distinguished which can evidently also be inserted into the new typology proposed by Jochems et al. (2002). The indices used correspond to the requirements of the Water Framework Directive.

For the watercourses of the bream and barbeeltype common metrics are used but the score limits are adjusted.

Table 8.1 gives the overview of the selected metrics and their limits for calculating the Index of Biotic Integrity (IBI).

Table 8.1: Determination of metrics and boundaries for calculating the IBI for watercourses of the type bream and barbel

Metric	**Score**				
Type bream	**5**	**4**	**3**	**2**	**1**
Total number of species					
River width < 3m	*≥7*	*6*	*5-4*	*3-2*	*1*
River width 3-6.4m	*≥12*	*11-9*	*8-6*	*5-3*	*≤2*
River width 6.5-8.9m	*≥13*	*12-10*	*9-7*	*6-4*	*≤3*
River width ≥ 9m	*≥14*	*13-10*	*9-7*	*6-4*	*≤3*
Mean tolerance	≥2.4	2.39-2	1.99-1.6	1.59-1.2	<1.2
Mean typical species value	≥3.3	3.29-3	2.99-2.7	2.69-2.4	<2.4
Type species*	≥4.5	4.49-3.5	3.49-2.5	2.49-1.5	<1.5
% *Rutilus rutilus*	*10-25*	*25.1-35*	*35.1-45*	*45.1-55*	*>55*

		7.5-9.9	*5-7.4*	*2.5-4.9*	*<2.5*
% *Scardinius erythrophthalmus*	*≥10*	*5-9.9*	*2-4.9*	*1-1.9*	*<1*
% *Tinca tinca***	*≥15 (+ rekr.)*	*10-14.9 (+rekr.)*	*<10 (+ rekr.)*	*≥15 (- rekr.)*	*<15 (- rekr.)*
Total biomass (kg/ha)	100-349	350-499 75-99	500-649 50-74	650-799 25-49	≥800 <25
Weight % of non-native species	<1	1-3.99	4-6.99	7-9.99	≥10
Trophic composition	5-4.3	4.29-3.5	3.49-2.5	2.49-1.7	<1.7
% omnivorous species	*<1*		*1-5*		*>5*
% invertivorous species	*>45*		*45-20*		*<20*
% piscivorous species	*3-5*		*2.9-1* *5.1-7*		*<1* *>7*
Natural recruitment (%)	≥85	84.9-70	69.9-55	54.9-40	<40
Type barbel					
Total number of species					
River width < 3m	*≥5*	*4*	*3*	*2*	*1*
River width 3-6.4m	*≥7*	*6*	*5-4*	*3-2*	*1*
River width 6.5-8.9m	*≥10*	*9-8*	*7-6*	*5-4*	*≤3*
River width ≥ 9m	*≥12*	*11-9*	*8-6*	*5-4*	*≤3*
Mean tolerance	≥2.4	2.39-2	1.99-1.6	1.59-1.2	<1.2
Mean typical species value	≥3.1	3.09-2.8	2.79-2.5	2.49-2.2	<2.2
Type species*	≥4.5	4.49-3.5	3.49-2.5	2.49-1.5	<1.5
% *Gasterosteus aculeatus*	*<3*	*3-4.9*	*5-6.9*	*7-8.9*	*≥9*
% *Barbatula barbatula*	*≥11*	*10.9-9*	*8.9-7*	*6.9-5*	*<5*
% *Leuciscus cephalus***	*>20 (+ rekr.)*	*20-5 (+ rekr.)*	*<5 (+ rekr.)*	*≥25 (- rekr.)*	*<25 (- rekr.)*
Total biomass (kg/ha)	250-349	350-449 100-249	450-549 60-99	550-649 20-59	≥650 <20
Weight % of non-native species	<1	1-3.99	4-6.99	7-9.99	≥10
Trophic composition	5-4.3	4.29-3.5	3.49-2.5	2.49-1.7	<1.7
% omnivorous species	*<1*		*1-5*		*>5*
% invertivorous species	*>45*		*45-20*		*<20*
% piscivorous species	*3-5*		*2.9-1* *5.1-7*		*<1* *>7*
Natural recruitment (%)	≥85	84.9-70	69.9-55	54.9-40	<40

*: score is obtained by taking the average of the scores obtained for the species printed in italics
**: "+ rekr." and "- rekr." means presence and absence of recruitment

The resulting index score is converted into an EQR which enables to determine a quality class (integrity class) as shown in Table 8.3.

For the trout and grayling zone other metrics are used (see Table 8.2).

Table 8.2: Metrics and boundaries for watercourses of the type trout and/or grayling (slope ≥3‰, river width ≤4.5 m)

Metric	**Score**		
	1	**3**	**5**
Species richness and composition			
Total number of species			
Slope class 1 (<4‰)	<4	4-7	≥8
Slope class 2 (4-5‰)	<3	3-5	≥6
Slope classes 3, 4 & 5 (>5‰)	1	2-4	≥5
Typical species value			
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‰)	<1.57	1.57-3.13	>3.13
Slope class 4 (>8-12.5‰)	<1.69	1.69-3.37	>3.37
Slope class 5 (>12.5‰)	<1.85	1.85-3.69	>3.69
Shannon-Wiener diversity index (evenness)	<0.53	0.53-0.68	>0.68
Migrating species value	<2	2-4	>4
Fish condition and abundance			
Biomass (kg/ha)			
Slope class 1	≤130	130.1-250	>250
Slope class 2	≤80	80.1-150	>150
Slope class 3	≤46	46.1-100	>100
Slope classes 4 & 5	≤30	30.1-60	>60
Length classes value	<2	2-3.99	4-5
Trophic composition and habitat use			
% invertivorous individuals	<26	26-45	>45
Number of benthic species	1	2-3	>3
% specialised spawners			
Slope class 1	<8	8-15.9	≥16
Slope class 2	<10	10-20.9	≥21
Slope class 3	<12	12-30.9	≥31
Slope class 4	<24	24-47.9	≥48
Slope class 5	<35	35-69.9	≥70

Table 8.3: Value assessment of the different IBI scores and EQR values

IBI score	**EQR**	**WFD classification**	**Colour code**	**Situation description**
>4.5-5	>0.9	High	Blue	Natural situation without anthropogenic disturbance. All expected species are

				present, including the most sensitive ones. Balanced trophic structure.
>3.5-4.5	>0.7-0.9	Good	Green	Species richness lower than expected. Less fish and less sensitive species are present. The trophic structure shows signs of stress.
>2.5-3.5	>0.5-0.7	Moderate	Yellow	Only a few or no sensitive species still occur. The trophic structure is broken.
≥1-2.5	≥0.2-0.5	Poor	Orange	Few fish is present. Mainly introduced and tolerant fish species occur.
<1	<0.2	Bad	Red	No or almost no fish are encountered.

8.2 Lakes

Goethals et al. (2006) proposed an assessment method for lakes in Flanders based on fish communities. This proposal is further modified by INBO. For this reason, the cited proposal is not further explained below. For reporting INBO currently uses the index for standing waters (Belpaire et al., 2000) because this index is entirely consistent with the requirements of the water framework directive.

8.2.1 Sampling

The fishing techniques used are: electrical fishing (riparian zones), gill nets and fykes (pelagic) and when possible, seine netting is used. The standing waters are classified in zones based on habitat characteristics.

8.2.2 Conservation

Not applicable.

8.2.3 Identification

The captured fish are identified to the species level. When necessary, the work of Nijssen & De Groot (1987) is used.

8.2.4 Index calculation

The selected metrics and their boundary values are presented in Table 8.4.

Table 8.4: determination of metrics and boundary values for calculating the IBI for standing waters

Metric	**Score**				
	5	**4**	**3**	**2**	**1**
Total number of species	>15	15-12	11-8	7-3	<3
Mean tolerance value	≥2.4	2.39-2	1.99-1.6	1.59-1.2	<1.2
Type species*	≥4.5	4.49-3.5	3.49-2.5	2.49-1.5	<1.5
% *Rutilus rutilus*	*10-25*	*25.1-35*	*35.1-45*	*45.1-55*	*>55*
		9.9-7.5	*7.4-5*	*2.5-4.9*	*<2.5*
% *Scardinius erythrophthalmus*	*≥10*	*9.9-5*	*4.9-2*	*1.9-1*	*<1*
% *Abramis brama*	*0.1-10*	*10.1-20*	*20.1-30*	*30.1-40*	*>40*
					0
Pike recruitment and biomass	≥20	10-19.9	<10	≥20	<20

(kg/ha)**	(+ rekr.)	(+ rekr.)	(+ rekr.)	(- rekr.)	(- rekr.)
Tench recruitment and biomass (kg/ha)**	≥15	10-14.9	<10	≥15	<15
	(+ rekr.)	(+ rekr.)	(+ rekr.)	(- rekr.)	(- rekr.)
Total biomass (kg/ha)	100-349	350-499	500-649	650-799	≥800
		75-99	50-74	25-49	<25
Weight % of non-native species	<1	1-3.99	4-6.99	7-9.99	≥10
Weight ratio piscivores/non-piscivores	0.2-0.14	0.139-0.1	0.09-0.067	0.066-0.05	<0.05
		0.201-0.25	0.251-0.33	0.331-0.5	>0.5

*: score is obtained by taking the mean of the species scores in italics
**: "+ rekr." and "- rekr." means presence and absence of recruitment

The obtained index score is converted into an Ecological Quality ratio (EQR) which enables to determine a quality class (integrity class) as shown in Table 8.5.

Table 8.5: Value assessment for the different IBI scores and EQR values

IBI score	EQR	WFD classification	Colour code	Situation description
>4.5-5	>0.9	High	Blue	Natural situation without anthropogenic disturbance. All expected species are present, including the most sensitive ones. Balanced trophic structure.
>3.5-4.5	>0.7-0.9	Good	Green	Species richness lower than expected. Less fish and less sensitive species are present. The trophic structure shows signs of stress.
>2.5-3.5	>0.5-0.7	Moderate	Yellow	Only a few or no sensitive species still occur. The trophic structure is broken.
≥1-2.5	≥0.2-0.5	Poor	Orange	Few fish is present. Mainly introduced and tolerant fish species occur.
<1	<0.2	Bad	Red	No or almost no fish are encountered.

8.3 Transitional waters

Goethals et al. (2006) proposed an assessment method for transitional waters in Flanders based on fish communities. This proposal is further modified by INBO for the brackish part of the transitional water (Breine et al., 2007). The adaptation for the freshwater transitional water is under development. For this reason, the cited proposal is not further explained below. For reporting the index developed for the brackish transitional waters is used.

8.3.1 Sampling

For transitional waters sampling is done with double fyke nets. These fykes are placed during low tide. After 24 hours the nets are emptied and put back for a period of 24 hours. In the freshwater part the same method is applied, sometimes supplemented with electric fishing (as determined in CEN). INBO reports only on the brackish part because the index for the freshwater tidal water is not yet developed.

8.3.2 Conservation

Not applicable.

8.3.3 Identification

The captured fish are identified to the species level. When necessary, the work of Nijssen & De Groot (1987) is used.

8.3.4 Index calculation

The developed index does not define good or high quality. Indeed we have no references (historical or actual) that are useful to determine the boundaries. Table 8.6 shows the selected metrics and their boundaries and Table 8.7 shows the score boundaries for the integrity classes. If values are obtained that exceed the upper boundary value of the moderate status it can be assumed that the situation is good but we were not able to statistically support this.

Table 8.6: Selected metrics and boundary values (calculated as average value per fyke day)

Metric	**Score**				
	0	**0.25**	**0.5**	**0.75**	**1**
Species richness and composition					
Total number of species	≤7	>7	>9	>10	>11
% Smelt individuals	≤0.33		>0.33	>1.12	>2.68
% Marine juvenile migrating individuals	≤33.0	>33.0	>54.2	>73.1	>82.0
Trophic composition and habitat use					
% Omnivorous individuals	≥16.44	<16.44	<7.90	<3.37	<1.17
% Piscivorous individuals	≤12.84	>12.84	>19.44	>27.23	>41.19

Table 8.7: Estuarine Biotic Integrity Index (EBI) score boundaries expressed as Ecological Quality Ratio (EQR), the associated integrity class and the colour code

EBI boundary values	**Integrity class**	**Colour code**
	High	Blue
	Good	Green
>0.3	Moderate	Yellow
>0.15	Poor	Orange
≤0.15	Bad	Red

9 Assessment of water bodies belonging to the category coastal waters

Only one Flemish water body has been delineated that belongs to the category of coastal waters, the Zwin. It belongs to the only type, K1, 'mesotidal tidal inlet or sea arm' and is designated as a natural water body. As shown in Table 2.1, according to WFD there are three relevant biological quality elements for coastal waters: phytoplankton, macrophytes and macroinvertebrates.

As an indirect result of a decision of the Flemish Government with regard to the extension of the Zwin, this water body will change into a transitional water. The CIW-sub-working group "objectives and monitoring surface water" proposed to examine the applicability of the assessment methods for transitional waters once this change is achieved in practice. The quality elements phytoplankton, macrophytes and macroinvertebrates are therefore provisionally (river basin management plan 2009) assigned as VNB (provisionally not to be assessed) for the Zwin in anticipation of the change into transitional water.

10 Assessment of artificial and heavily modified water bodies

The artificial and heavily modified water bodies are designated according to the approach of the Decree on Integrated Water Policy (DIWB, Ministry of the Flemish Community, 2003). For each Flemish water body the state (natural, heavily modified or artificial) is specified in the draft River Basin Management Plans (CIW, 2008a, 2008b). Natural water bodies are assessed according to the methods discussed in chapters 4 through 8. The artificial and heavily modified water bodies are assessed according to adjusted methods.

Each heavily modified or artificial Flemish water body can receive an adjusted assessment method, either by adjusting class boundaries, or by adjusting the method itself. In specific cases it may also be decided to designate one or more biological quality elements as not relevant for a water body. For all artificial and heavily modified water bodies, the class boundaries, either adjusted or not, are given per quality element in the draft river basin management plans (CIW, 2008a, 2008b). The approach adopted for defining the class boundaries and the possible adaptation of the methods is explained in the following paragraphs. A distinction is made here between the water bodies most closely resembling the category rivers (except the type MLz), the water bodies most closely resembling the category lakes and the water bodies subject to tidal influence (these are the transitional waters and rivers of the type MLz).

10.1 Artificial and heavily modified water bodies most closely resembling the category rivers (except type MLz)

For artificial and heavily modified water bodies most closely resembling the category rivers (excluding the type MLz) a generic method was developed which is briefly outlined in the INBO report INBO.R.2008.12 (Van Looy et al., 2008).

10.1.1 Identification of pressures

First, the water uses have to be identified for each water body. In a study of Soresma (2006), it was indicated for each water body which water uses are relevant. In a next step water managers were asked which pressures are present in the water body that are strictly necessary for a water use. In addition, a criterion was established with the critical load for these pressures, this is the minimum percentage affected proportion of a water body that is necessary to consider it as significant. In this way a list of significant present and permanent pressures is obtained per water body.

10.1.2 Pressures resulting in a change of type

Some of these pressures may lead to a change in the corresponding natural type. In the context of the study on reference conditions and evaluation systems for macrophytes and phytobenthos this change of types is listed for a number of types (Leyssen et al., 2005). These changes have already been taken into account when allocating the most closely resembling types to the artificial and heavily modified water bodies as mentioned in the draft river basin management plans (CIW, 2008a, 2008b).

For phytoplankton, an additional condition is introduced which may give rise to an amended assessment type. For artificial and heavily modified water bodies most closely resembling to the category rivers, the quality assessment for the parameter chloride is checked. If the water body has been given an evaluation "poor" or "bad", then the assessment of the quality element phytoplankton is based on the assessment type 23 (see section 4.1.4). Otherwise, the evaluation type for the natural types is used.

10.1.3 Pressures resulting in a modified assessment

Then the influence of permanent hydromorphological pressures on the different biological quality elements is assessed. Important here is that for a certain number of pressures a mitigation is already assumed.

This adjusted assessment is introduced specifically for the quality element macrophytes. For this, a so-called "pressure-effect" table (Annex 2 in Leyssen et al., 2006) is used to decide which pressures present on the water body give rise to the omission of metrics in the overall assessment. Because the assessment for macrophytes uses a "one out, all out" system, the omission of a metric leads in fact to

a less strict standard, although the figure of the lower boundary (GEP) has not changed compared to the GET (in particular 0,60). Therefore, in the GEP-table in the draft river basin management plans (CIW, 2008a, 2008b) this is indicated by an asterisk (0.60*).

Omission of metrics is evaluated based on field observations in the water body. The "pressure-effect" table indicates which metrics may be omitted in case a given water use is present, but this omission is carried out only on the condition that the pressure is considered neccesary for a present water use. Tabel 10.1 shows for each pressure from the pressure-effect table with what water uses these pressures are permitted (i.e. allow for the omission of the mentioned metrics). So if a certain pressure from Tabel 10.1 is recorded for a water body, and this water body is designated for at least one water use that are associated with it in the table, the metrics indicated in the pressure-effect table for this pressure may be omitted.

Tabel 10.1: Pressures from the pressure-effect table and the water uses for which these pressures allow for the omission of metrics

Pressure	**Water use**	
	Navigation + port facilities	**Flood protection**
Bank protection – impervious	X	
Bank protection – lower part of slope enforced, impervious	X	
Bed – deepened (too deep for macrophytes)	X	X
Bed – sediment agitation by intensive river bed fishery / navigation	X	
Bed – bed enhancement / enforcement (impervious)	X	X
Maintenance – bed (ordinary vegetation removal) – frequent	X	X
Maintenance – bed (intensive vegetation removal) – frequent	X	X
Maintenance – bed (intensive vegetation removal) – occasional	X	X

For artificial water bodies this system is applied as for heavily modified water bodies, but for these water bodies the water uses were not designated. For these water bodies, the presence of the water uses navigation + port facilities and flood protection is therefore assessed by means of the information available at VMM and MOW.

Metrics that have a better or equally good score at the stretch level compared to the EQR which is based on the worst scoring metric that can not be removed from the stretch, are not removed for calculation at the water body level. These metrics do no affect the EQR at the stretch level, but may do so at the water body level.

When calculating the average result for a metric over the different stretches within a water body, only non-omitted results are taken into account, unless this would result in an average that is lower compared to when all stretches are taken into account. In that case, all results for this metric are taken into account. In this way, the possibility that the total EQR for a water body would decrease when the GEP is applied, is excluded.

When the metric vegetation development is omitted and the metric type specificity is not, the exception rule (in case the difference between vegetation development and type specificity is greater than 0.4), as mentioned in paragraph 6.1.5.4, is still applied. In the final assessment, TS' is used as metric result, not TS.

10.1.4 Pressures resulting in a spatially split assessment

The permanent pressures that do not give rise to an adjustment of the corresponding natural type, are hydromorphological pressures that produce a predominantly local effect. Pressures of this type occur scattered throughout the entire length of the water body, making it virtually impossible to split a water body into affected and unaffected zones. Therefore a generic approach was chosen: the percentage

of impervious surface near the banks of the water body was mapped through a GIS-exercise and used as a proxy for the degree of morphological degradation of the entire water body.

This spatially split assessment is applied for the quality elements fish and macroinvertebrates. They also only apply if this rate is between 10% and 90%. If the percentage is lower for the water body in question the quality elements macro-invertebrates and fish are assessed using the GET (0.70 resp. 0.60). There is only one water body to which the percentage is higher, i.e. VL05_79, Dijle III (100%). For this water body, all biological quality elements are designated as "not relevant".

For the types Pz and Pb, this adjusted assessment is also not applied for the macroinvertebrates, and the lower boundaries for GEP, moderate ecological potential and poor ecological potential is maintained at 0.60, 0.40 and 0.20, respectively.

For water bodies where this adjusted method is used, first the zone near the banks that is used for the calculation of the GEP (see below), is delineated depending on the type of water body:

-for Bg and BgK: The area within a distance of 0-25 meters to the middle of the watercourse

-for Rk, Rg and Rzg: The area within a distance of 0-50 meters to the middle of the watercourse

An exception is the Canal Ghent-Terneuzen (most closely resembling to type Rg) where the 50-100 meters buffer was used instead of that of 0-50 meters.

The GEP is then calculated as a weighted average between the standard for good status in the non-affected parts (GET, being 0.70 for macroinvertebrates and 0.60 for fish) and a standard "current status" elsewhere. For the macroinvertebrates, the figure for "current status" is based on the three most recent EQR-assessments carried out within the water body for that quality element, with the lowest value chosen as representative for the part that is affected. This "worst score" should however amount to at least 0.35, otherwise 0.35 is used for the calculation. For the fish, the most recently obtained EQR-value is used, with a lower limit of 0.25 being applied. This lower limit is the value of the EQR that is assumed to be attainable at all times, even if the structural characteristics are very bad (e.g. concrete bank). The values used are determined by "expert judgment". The difference between the two figures reflects differences between the two quality elements (fish and macroinvertebrates) at the level of the EQR (including the extent to which the score responds to changes in structural quality, and related to that, the class boundaries established for these quality elements).

The GEP for a heavily modified water body most closely resembling to the category of rivers is:

GEP = GET x (1 – A) / 100 + HT x A / 100

with: A: percentage of impervious surface within the demarcated area

HT (current status): for macroinvertebrates the lowest of the 3 most recent EQR-determinations within the water body with minimum value 0.35; for fish the most recent EQR-determination with minimum value 0.25.

The thus obtained GEP for macroinvertebrates is rounded to 0.05.

For a limited number of cases insufficient data were available. For the macroinvertebrates the figure for these water bodies is determined based on the available expertise. For the fish the GEP is determined for these water bodies to be 0.60 and INBO will make additional adjustments if necessary. This is indicated in the GEP-table with an asterisk (0.60*).

The other class boundaries of the ecological potential, those between moderate and poor ecological potential, and between poor and bad ecological potential, are not defined by simply dividing the range of the potential-index between GEP and 0 in three parts, but by dividing the interval widths proportionally to the classification of the corresponding index for the quality element in question for the 'natural' type.

The following formulas are therefore applied:

-boundary moderate/poor potential = GEP * (lower boundary moderate for natural water bodies / lower boundary good for natural water bodies)

-boundary poor/bad potential = GEP * (lower boundary poor for natural water bodies / lower boundary good for natural water bodies)

For the macroinvertebrates the thus obtained boundaries are rounded to 0.05.

For fish, these formulas equal 2/3 respectively 1/3 of the GEP because the relevant limits for the natural water bodies are 0.60, 0.40 and 0.20, respectively. For the macroinvertebrates, this is not the case because the class boundaries are different (0.70, 050 and 0.30, respectively).

10.2 Artificial and heavily modified water bodies most closely resembling the category lakes

The assessment schemes for these water bodies were established individually for each water body during a specific study. The table with draft standards for the GEP for artificial and heavily modified water bodies was part of the draft river basin management plan which was approved by the Co-ordination Committee Integrated Water Policy on October 14, 2008.

For Vinne (VL05_119) draft standards were proposed by Louette et al. (2008d).

For Brugse Reien (VL05_155) no specific study has been carried out. For this water body it was decided that macrophytes are not relevant as a biological quality element and these are therefore not assessed. For the quality element phytobenthos the same criteria apply as for the natural water bodies of the type Ami, for the other quality elements (for which the draft river basin management plan mentions "ntb" or "still to be determined" for this water body) provisionally also the criteria for Ami apply.

For Antwerp Harbour Docks + Scheldt-Rhine Connection (VL05_187) draft standards were proposed by Pals & Vercouteren (2008). The quality element macrophytes was indicated as not relevant indicated.

For Blankaart Reservoir (VL05_188) and Kluizen I + II Reservoirs (VL05_199), no studies have been conducted. For these water bodies, the biological quality elements macrophytes, macroinvertebrates and fish are indicated as not relevant. The biological quality elements phytoplankton and phytobenthos (for which the draft river basin management plan mentions "ntb" or "still to be determined" for this water body) are provisionally assessed for these water bodies according to the criteria for Ami.

For Blokkersdijk (VL05_189) draft standards were proposed by Louette et al. (2008a).

For Boudewijn Canal + Achterhaven Zeebrugge (VL05_190) draft standards for phytoplankton were proposed by Van Wichelen et al. (2008b). The quality elements phytobenthos, macrophytes and macroinvertebrates were indicated as not relevant for this water body as mentioned in the draft river basin management plan which was approved by the Co-ordination Committee Integrated Water Policy on October 14, 2008.

For Desselse Zandputten (VL05_191) (most closely resembling the type Awom) and Donkmeer (VL05_192) (most closely resembling the type Ami) no specific studies were conducted yet. These water bodies (for which the draft river basin management plan mentions "ntb" or "still to be determined") are provisionally assessed according to the criteria for natural water bodies for the corresponding types.

For Eisden Mijn (VL05_193), Gavers Harelbeke (VL05_195) and Hazewinkel (VL05_198) (all most closely resembling the type Awe), no specific studies were conducted but for these water bodies the draft standards were determined according to the method proposed by Lock et al. (2007) for the type Awe.

For Galgenweel (VL05_194) draft standards were proposed by Van Ballaer et al. (2008). The quality element phytobenthos was identified as not relevant.

For Grindplas Kessenich (VL05_196) and Spaanjerd + Heerenlaak (VL05_201) draft standards were proposed by Lock et al. (2007).

For Grote Vijver Mechelen (VL05_197) draft standards were proposed by Louette et al. (2008b).

For Schulensmeer (VL05_200) draft standards were proposed by Louette et al. (2008c).

For Spuikom Ostend (VL05_202) draft standards for phytoplankton were proposed by Van Wichelen et al. (2008c). The quality elements phytobenthos, macrophytes, macroinvertebrates and fish were designated as not relevant for this water body.

10.3 Artificial and heavily modified water bodies most closely resembling the category transitional waters or the type MLz

For these water bodies MEP-GEP was already taken into account during the development of the assessment method because all these water bodies are heavily modified or artificial. For these, one can refer to the relevant sections in chapters 4 through 8.

For the artificial transitional waters Blankenberge Harbour Passage + Marinas (VL05_184), Ostend Harbour Passage + Docks (VL05_185) and Zeebrugge Buitenhaven (VL05_186), the quality elements phytoplankton, macrophytes, macroinvertebrates and fish are indicated as not relevant as mentioned in the draft river basin management plan which was approved by the Co-ordination Committee Integrated Water Policy on October 14, 2008. For these water bodies no biological assessments are carried out.

REFERENCES

- AFNOR (2002). Qualité de l'eau. Détermination de l'indice oligochètes de bioindication des sédiments (IOBS). Norme AFNOR T 90-390. Avril 2002. Association Française de Normalisation, Saint-Denis La Plaine, Frankrijk.

- Belpaire, C., Smolders, R., Vanden Auweele, I., Ercken, D., Breine, J., Van Thuyne, G. & Ollevier, F. (2000). An Index of Biotic Integrity characterizing fish populations and the ecological quality of Flandrian waterbodies. Hydrobiologia 434(1-3): 17-33.

- Biesbrouck, B., Es, K., Van Landuyt, W., Vanhecke, L., Hermy, M. & Van den Brempt, P. (2001). Een ecologisch register voor hogere planten als instrument voor het natuurbehoud in Vlaanderen. Eindrapport VLINA 00/01, Brussel. 49 p.

- Breine, J., Simoens, I., Goethals, P., Quataert, P., Ercken, D., Van Liefferinghe, C. & Belpaire, C., (2004). A fish-based index of biotic integrity for upstream brooks in Flanders (Belgium). Hydrobiologia 522(1-3): 133-148.

- Breine, J., Maes, J., Quataert, P., Van den Bergh, E., Simoens, I., Van Thuyne, G. & Belpaire, C. (2007). A fish-based assessment tool for the ecological quality of the brackish Schelde estuary in Flanders (Belgium). Hydrobiologia 575(1): 141-159.

- Breine, J., Simoens, I., Haidvogl, G., Melcher, A., Pont, D., Schmutz, S. & the FAME CONSORTIUM, (2005). Manual for the application of the European Fish Index - EFI. A fish-based method to assess the ecological status of European rivers in support of the Water Framework Directive. Version 1.1, January 2005. 81 p.

- Brys, R., Ysebaert, T., Escaravage, V., Van Damme, S., Van Braeckel, A., Vandevoorde, B. & Van den Bergh, E. (2005). Afstemmen van referentiecondities en evaluatiesystemen in functie van de KRW: afleiden en beschrijven van typespecifieke referentieomstandigheden en/of MEP in elk Vlaams overgangswatertype vanuit de – overeenkomstig de KRW – ontwikkelde beoordelingssystemen voor biologische kwaliteitselementen. Eindrapport. VMM.AMO.KRW.REFCOND.OW. Instituut voor natuurbehoud IN.O. 2005.7. Instituut voor Natuurbehoud, Brussel. 137 p. + bijlagen.

- CEN (2004). EN 14407:2004. Water quality – Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. European Committee for Standardization, Brussel. 16 p.

- CEN (2006). EN 15204:2006. Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique). European Committee for Standardization, Brussel. 46 p.

- CIW (2008a). Ontwerp stroomgebiedbeheerplan voor de Maas. Openbaar onderzoek 16 december 2008 – 15 juni 2009. Coördinatiecommissie Integraal Waterbeleid, Erembodegem.

- CIW (2008b). Ontwerp stroomgebiedbeheerplan voor de Schelde. Openbaar onderzoek 16 december 2008 – 15 juni 2009. Coördinatiecommissie Integraal Waterbeleid, Erembodegem.

- Cleve-Euler, A. (1968). Die Diatomeen von Schweden und Finnland. Verlag J. Cramer, Lehre, Duitsland. 1202 p.

- Cohen, M.J., Carstenn, S. & Lane, C.R. (2004). Evaluation of Floristic Quality Indices for biotic assessment of depressional marsh condition in Florida. Ecological Applications 14(3): 784-794.

- DARES Consortium (2004). Diatom Assessment of River Ecological Status. http://craticula.ncl.ac.uk/DARES/dares_project.htm.

- Degraer, S., Wittoeck, J., Appeltans, W., Cooreman, K., Deprez, T., Hillewaert, H., Hostens, K., Mees, J., Vanden Berghe, W. & Vincx, M. (2006). De macrobenthosatlas van het Belgisch deel van de Noordzee. Federaal Wetenschapsbeleid, Brussel. Wettelijk depot D/2005/1191/5. ISBN 90-810081-5-3. 164 p.

- Denys, L. (2006). Validation and revision of the reference concept for river phytobenthos in Belgium - Flanders proposed for the European Water Framework Directive, based on diatom assemblages from reference sites in the Central-Baltic GIG region. Advies van het Instituut voor Natuur- en Bosonderzoek INBO.A.2006.163. Instituut voor Natuur- en Bosonderzoek, Brussel. 7 p. + appendices.

- De Pauw, N., Lambert, V., Van Kenhove, A. & Bij de Vaate, A. (1994). Performance of two artificial substrate samplers for macroinvertebrates in biological monitoring of large and deep rivers and canals in Belgium and The Netherlands. Environmental Monitoring and Assessment 30(1): 25-47.

- De Pauw, N., Roels, D. & Fontoura, P. (1986). Use of artificial substrates for standardized sampling of macroinvertebrates in the assessment of water quality by means of the Belgian Biotic Index. Hydrobiologia 133(3): 237-258.

- De Pauw, N. & Vanhooren, G. (1983). Method for biological quality assessment of watercourses in Belgium. Hydrobiologia 100(1): 153-168.

- De Pauw, N. & Vannevel, R. (eds.) (1991). Macro-invertebraten en waterkwaliteit. Stichting Leefmilieu, Antwerpen. 316 p.

- EU (2000). Richtlijn 2000/60/EG van het Europees Parlement en de Raad van 23 oktober 2000 tot vaststelling van een kader voor communautaire maatregelen betreffende het waterbeleid. Publicatieblad van de Europese Gemeenschappen L327: 1-72.

- EU (2008). Beschikking van de commissie van 30 oktober 2008 tot vaststelling van de indelingswaarden voor de monitoringsystemen van de lidstaten die het resultaat zijn van de intercalibratie, overeenkomstig Richtlijn 2000/60/EG van het Europees Parlement en de Raad. Publicatieblad van de Europese Unie L332: 20-44.

- Gabriels, W., Goethals, P., Adriaenssens, V. & De Pauw, N. (2004). Toepassing van verschillende biologische beoordelingssystemen op Vlaamse potentiële interkalibratielocaties overeenkomstig de Europese Kaderrichtlijn Water, partim benthische ongewervelden. Eindrapport. Laboratorium voor Milieutoxicologie en Aquatische Ecologie, Universiteit Gent, Gent. 59 p. + bijlagen.

- Gabriels, W. (2008). Annex 2.1.4.2. Proposal for adjusting the Flemish class boundaries according to the intercalibration exercise for river macroinvertebrates. Technical note for the Steering Group of the Central/Baltic Rivers Geographical Intercalibration Group. In: van de Bund, W. (ed.). Water Framework Directive intercalibration technical report. Part 1: Rivers. European Commission, Joint Research Centre, Ispra, Italië.

- Gabriels, W., Lock, K., De Pauw, N. & Goethals, P.L.M. (2009). The Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). Limnologica: in press.

- Germain, H. (1981). Flore des diatomées eaux douces et saumâtres. Société Nouvelle des Éditions Boubée, Parijs, Frankrijk. 444 p.

- Goethals, P.L.M., Breine, J., Simoens, I. & Belpaire, C. (2006). Ontwikkelen van een index voor het biologisch kwaliteitselement vis voor de Kaderrichtlijn Water - Uittesten van de Europese ontwerphandleiding 'REFCOND' voor het kwaliteitselement vis in Vlaanderen. Eindverslag. Project nr. VMM.AMO.KRW.ECO1.2002. Instituut voor Bosbouw en Wildbeheer, Groenendaal. 79 p.

- Hendrickx, A. & Denys, L. (2005). Toepassing van verschillende biologische beoordelingssystemen op Vlaamse potentiële interkalibratielocaties overeenkomstig de Europese Kaderrichtlijn Water. Partim “Fytobenthos”. Rapport IN.R.2005.06, Instituut voor Natuurbehoud, Brussel. 107 p. + bijlagen.

- Hillebrand, H., Durselen, C.D., Kirschtel, D., Pollingher, U. & Zohary, T. (1999). Biovolume calculation for pelagic and benthic macroalgae. Journal of Phycology 35(2): 403-424.

- Huet, M. (1949). Aperçu des relations entre la pente et les populations piscicoles des eaux courantes. Revue Suisse d’ Hydrologie 11(3-4): 332-351.

- Hustedt, F. (1961-1966). Die Kieselalgen Deutschlands, Österreichs und der Schweiz unter Berücksichtigung der übrigen Länder Europas sowie der angrenzenden Meeresgebiete. In: Rabenhorst, L. (ed.). Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Akademische Verlagsgesellschaft, Leipzig, Duitsland. p. 1-816.

- Jochems, H., Schneiders, A., Denys, L. & Van den Bergh, E. (2002). Typologie van de oppervlaktewateren in Vlaanderen. Eindverslag van het project VMM.KRLW-typologie.2001. Instituut voor Natuurbehoud, Brussel. 68 p. + CD-ROM.

- Kelly, M.G., King, L., Jones, R.I., Barker, P.A. & Jamieson, B.J. (2008). Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes. Hydrobiologia 610(1): 125-129.

- Krammer, K. (1992). *Pinnularia*. Eine Monographie der europäischen Taxa. Bibliotheca Diatomologica 26: 1-353.

- Krammer, K. (1997a). Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 1. Allgemeines und *Encyonema* part. Bibliotheca Diatomologica 36: 1-382.

- Krammer, K. (1997b). Die cymbelloiden Diatomeen. Eine Monographie der weltweit bekannten Taxa. Teil 2. *Encyonema* part., *Encyonopsis* und *Cymbellopsis*. Bibliotheca Diatomologica 37: 1-469.

- Krammer, K. (2000). Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 1. The Genus *Pinnularia*. A.R.G. Gantner Verlag K.G, Ruggell, Liechtenstein. 703 p.

- Krammer, K. (2002). Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 3. *Cymbella*. A.R.G. Gantner Verlag K.G, Ruggell, Liechtenstein. 584 p.

- Krammer, K. & Lange-Bertalot, H. (1986). Bacillariophyceae. 1. Teil: Naviculaceae. In: Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (eds). Süsswasserflora von Mitteleuropa. Gustav Fischer Verlag, Stuttgart, Duitsland. 876 p.

- Krammer, K. & Lange-Bertalot, H. (1991). Bacillariophyceae. 3 Teil: Centrales, Fragilariaceae, Eunotiaceae. In: Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (eds). Süsswasserflora von Mitteleuropa. Gustav Fischer Verlag, Stuttgart, Duitsland. 576 p.

- Kutka, F.J. & Richards, C. (1996). Relating diatom assemblage structure to stream habitat quality. Journal of the North American Benthological Society 15(4): 469-480.

- Lambinon, J., De Langhe, J.E., Delvosalle, L. & Duvigneaud, J. (1998). Flora van België, het Groothertogdom Luxemburg, Noord-Frankrijk en de aangrenzende gebieden (Pteridofyten en Spermatofyten). 3e druk. ISBN 9072619234. Nationale Plantentuin van België, Meise. 1091 p.

- Lange-Bertalot, H. (1993). 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süßwasserflora von Mitteleuropa Vol. 2/1-4. Bibliotheca Diatomologica 27: 1-454.

- Lange-Bertalot, H. (2001). Diatoms of Europe. Diatoms of the European Inland Waters and Comparable Habitats. Vol. 2. *Navicula* sensu stricto. 10 Genera Separated from *Navicula* sensu lato. *Frustulia*. A.R.G. Gantner Verlag K.G, Ruggell, Liechtenstein. 526 p.

- Lange-Bertalot, H. & Metzeltin, D. (1996). Oligotrophie-Indikatoren, 800 Taxa repräsentativ für drei diverse Seen-Typen, kalkreich - oligodystroph - schwach gepuffertes Weichwasser. Iconographia Diatomologica 2: 1-390.

- Lange-Bertalot, H. & Moser, G. (1994). *Brachysira*. Monographie der Gattung. Wichtige Indikator-Spezies für das Gewässer-Monitoring und *Naviculadicta* nov. gen. Ein Lösungsvorschlag zu dem Problem *Navicula* sensu lato ohne *Navicula* sensu stricto. Bibliotheca Diatomologica 29: 1-212.

- Leyssen, A., Adriaens, P., Denys, L., Packet, J., Schneiders, A., Van Looy, K. & Vanhecke, L. (2005). Toepassing van verschillende biologische beoordelingssystemen op Vlaamse potentiële interkalibratielocaties overeenkomstig de Europese Kaderrichtlijn Water - partim “Macrofyten”. Instituut voor Natuurbehoud in opdracht van VMM, Brussel. 98 p. + bijlagen.

- Leyssen, A., Denys, L., Schneiders, A., Van Looy, K., Packet, J. & Vanhecke, L. (2006). Afstemmen van referentiecondities en evaluatiesystemen voor de biologische kwaliteitselementen macrofyten en fytobenthos en uitwerken van een meetstrategie in functie van de Kaderrichtlijn Water. Rapport van het Instituut voor Natuurbehoud IN.R.2006.09 in opdracht van VMM, Brussel. 92 p. + bijlagen.

- Leyssen, A., Denys, L., Schneiders, A., Van Looy, K., Packet, J. & Vanhecke, L. (2007). Handleiding macrofyteninventarisatie en fythobenthosstaalname in waterlopen. Instituut voor Natuurbehoud, Brussel. 9 p. + bijlagen.

- Lock, K., Van Wichelen, J., Packet, J., Simoens, I., Van Looy, K., Louette, G., Warmoes, T., Denys, L. & Leyssen, A. (2007). Bepalen van het maximaal en het goed ecologisch potentieel, alsook de huidige toestand, voor een aantal Vlaamse (gewestelijke) waterlichamen die vergelijkbaar zijn met de categorie meren. Deel I. Rapport INBO.R.2007.51. Instituut voor Natuur- en Bosonderzoek, Brussel. 192 p.

- Londo, G. (1976). The decimal scale for releves of permanent quadrats. Vegetatio 33(1): 61-64.

- Lopez, R.D. & Fennessy, M.S. (2002). Testing the floristic quality assessment index as an indicator of wetland condition. Ecological Applications 12(2): 487-497.

- Louette, G., Van Wichelen, J., Packet, J., De Smedt, S. & Denys, L. (2008a). Bepalen van het maximaal en het goed ecologisch potentieel, alsook de huidige toestand voor de zeventien Vlaamse (gewestelijke) waterlichamen die vergelijkbaar zijn met de categorie meren – tweede deel, partim Blokkersdijk. D/2008/3241/376. INBO.R.2008.48. Instituut voor Natuur- en Bosonderzoek, Brussel.

- Louette, G., Van Wichelen, J., Packet, J., Warmoes, T. & Denys, L. (2008b). Bepalen van het maximaal en het goed ecologisch potentieel, alsook de huidige toestand voor de zeventien Vlaamse (gewestelijke) waterlichamen die vergelijkbaar zijn met de categorie meren – tweede deel, partim Grote Vijver Mechelen. D/2008/3241/377. INBO.R.2008.47. Instituut voor Natuur- en Bosonderzoek, Brussel.

- Louette, G., Van Wichelen, J., Packet, J., De Smedt, S. & Denys, L. (2008c). Bepalen van het maximaal en het goed ecologisch potentieel, alsook de huidige toestand voor de zeventien Vlaamse (gewestelijke) waterlichamen die vergelijkbaar zijn met de categorie meren – tweede deel, partim Schulensmeer. D/2008/3241/378. INBO.R.2008.49. Instituut voor Natuur- en Bosonderzoek, Brussel.

- Louette, G., Van Wichelen, J., Packet, J., Warmoes, T. & Denys, L. (2008d). Bepalen van het maximaal en het goed ecologisch potentieel, alsook de huidige toestand voor de zeventien Vlaamse (gewestelijke) waterlichamen die vergelijkbaar zijn met de categorie meren – tweede deel, partim Vinne. D/2008/3241/379. INBO.R.2008.50. Instituut voor Natuur- en Bosonderzoek, Brussel.

- Menden-Deuer, S. & Lessard, E.J. (2000). Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography 45(3): 569-579.

- Meire, P., Rossaert, G., De Regge, N., Ysebaert, T. & Kuijken, E. (1992). Het Schelde-estuarium: ecologische beschrijving en een visie op de toekomst. Adviezen van het Instituut voor Natuurbehoud 1992(57). Insitituut voor Natuurbehoud, Hasselt. 150 p.

- Ministerie van de Vlaamse Gemeenschap (2003). 18 juli 2003 - Decreet betreffende het integraal waterbeleid. Belgisch Staatsblad 14 november 2003: 55038-55058.

- Mischke, U. & Behrendt, H. (2007). Handbuch zum Bewertungsverfahren von Fließgewässern mittels Phytoplankton zur Umsetzung der EU-WRRL in Deutschland. ISBN 978-3-89998-105-6. Weissensee Verlag, Berlijn, Duitsland. 88 p.

- Mischke, U. (2008). Begleitbrief zu Neue PhytoFluss Version 2.0 (Mrz08) und Effekt auf die Bewertung von Fließgewässern mittels Phytoplankton. http://unio.igb-berlin.de/abt2/mitarbeiter/mischke/PhytoFluss_Vers2_0_download.zip Leibniz-Institut für Gewässerökologie und Binnenfischerei, Abteilung Limnologie von Flussseen, Berlijn, Duitsland. 7 p.

- Moss, B., Stephen, D., Alvarez, C., Becares, E., van de Bund, W., Collings, S.E., van Donk, E., De Eyto, E., Feldmann, T., Fernández-Aláez, C., Fernández-Aláez, M., Franken, R.J.M., García-Criado, F., Gross, E., Gyllström, M., Hansson, L.-A., Irvine, K., Järvalt, A., Jenssen, J.P., Jeppesen, E., Kairesalo, T., Kornijów, R., Krause, T., Künnap, H., Laas, A., Lill, E., Lorens, B., Luup, H., Miracle, M.R., Nöges, P., Nöges, T., Nykänen, M., Ott, I., Peczula, W., Peeters, E.T.H.M., Phillips, G., Romo, S., Russel, V., Salujöe, J., Scheffer, M., Siewertsen, K., Smal, H., Tesch, C., Timm, H., Tuvikene, L., Tonno, I., Virro, T. & Wilson, D. (2003). The determination of ecological quality in shallow lakes - a tested system (ECOFRAME) for implementation of the European Water Framework Directive Aquatic Conservation: Marine and Freshwater Ecosystems 13(6): 507-549.

- Nijssen, H. & De Groot, S.J. (1987). De Vissen van Nederland. Stichting KNNV-Uitgeverij, Utrecht. 224 p.

- Pals, A. & Vercoutere, B. (2008). Bepalen van het Maximaal Ecologisch Potentieel en het Goed Ecologisch Potentieel voor het waterlichaam Antwerpse Havendokken en Schelde-Rijn verbinding. Opdrachtgever Gemeentelijk Havenbedrijf Antwerpen. Referentie 817965/R/873173/Mech. Haskoning Belgium, Mechelen.

- Reichardt, E. (1997). Taxonomische revision des artenkomplexes um *Gomphonema pumilum* (Bacillariophyceae). Nova Hedwigia 65(1-4): 99-129.

- Reichardt, E. (1999). Zur Revision der Gattung *Gomphonema*. Die Arten um *G. affine*/*insigne*, *G. angustatum*/*micropus*, *G. acuminatum* sowie gomphonemoide Diatomeen aus dem Oberologozän in Böhmen. Iconographia Diatomologica 8: 1-203.

- Schaminée, J.H.J., Stortelder, A.H.F. & Westhoff, V. (1995). De vegetatie van Nederland: deel 1. Inleiding tot de plantensociologie - grondslagen, methoden en toepassingen. Opulus Press, Leiden, Nederland. 296 p.

- Schneiders, A., Denys, L., Jochems, H., Vanhecke, L., Triest, L., Es, K., Packet, J., Knuysen, K. & Meire, P. (2004). Ontwikkelen van een monitoringsysteem en een beoordelingssysteem voor macrofyten in oppervlaktewateren in Vlaanderen overeenkomstig de Europese Kaderrichtlijn Water. Instituut voor Natuurbehoud, Nationale Plantentuin van België, UA en VUB in opdracht van VMM, Brussel. 153 p.

- Shannon, C.E. & Weaver, W. (1949). The mathematical theory of communication. University of Illinois Press, Urbana, Illinois, USA. 117 p.

- Sherr, E.B. & Sherr, B.F. (1993). Preservation and storage of samples for enumeration of heterotrophic protists. In: Kemp, P.F., Sherr, B.F., Sherr, E.B. & Cole, J.J. (eds.). Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, Florida, USA. p. 207-212.

- Soresma (2006). Aanduiding van sterk veranderde waterlichamen voor de Vlaamse rivieren en overgangswateren overeenkomstig de Europese Kaderrichtlijn Water. Soresma, Antwerpen.

- Speybroeck, J., Breine, J., Vandevoorde, B., Van Braeckel, A., Van den Bergh, E. & Van Thuyne, G. (2008a). KRW doelstellingen in de IJzermonding. Afleiden en beschrijven van typespecifiek maximaal ecologisch potentieel en goed ecologisch potentieel in het Vlaams waterlichaam “Havengeul IJzer” vanuit de – overeenkomstig de Kaderrichtlijn Water – ontwikkelde relevante beoordelingssystemen voor een aantal biologische kwaliteitselementen. Eindrapport 2008. Instituut voor Natuur- en Bosonderzoek, Brussel. INBO.R.2008.55. 77 p.

- Speybroeck, J., Breine, J., Vandevoorde, B., Van Wichelen, J., Van Braeckel, A., Van Burm, E., Van den Bergh, E., Van Thuyne, G. & Vyverman, W. (2008b). KRW doelstellingen in Vlaamse getijrivieren. Afleiden en beschrijven van typespecifiek maximaal ecologisch potentieel en goed ecologisch potentieel in een aantal Vlaamse getijrivier-waterlichamen vanuit de – overeenkomstig de Kaderrichtlijn Water – ontwikkelde relevante beoordelingssystemen voor een aantal biologische kwaliteitselementen. Eindrapport 2008. Instituut voor Natuur- en Bosonderzoek, Brussel & Universiteit Gent. INBO.R.2008.56. 154 p.

- Tikkanen, T. & Willén, T. (1992). Växtplanktonflora. Naturvårdsverket, Solna, Zweden. 280 p.

- Utermöhl, H. (1958). Zur Vervolkommung der quantitativen phytoplankton-Methodik. Mitteilungen Internationale Vereiningung für Theoretische und Angewandte Limnologie 9: 1-38.

- Van Ballaer, B., De Deckere, E., Muylaert, K. & Meire, P. (2008). Bepalen van het maximaal en het goed ecologisch potentieel, alsook de huidige toestand voor de zeventien Vlaamse (gewestelijke) waterlichamen die vergelijkbaar zijn met de categorie meren - partim Galgenweel. Eindrapport. Rapportnummer: ECOBE 08-R118. Universiteit Antwerpen, Onderzoeksgroep Ecosysteembeheer i.s.m. K.U. Leuven, Biologie Campus Kortrijk.

- Van Damme, S., Van Hove, D., Ysebaert, T., de Deckere, E., Van den Bergh, E. & Meire, P. (2003). Ontwikkelen van een score of index voor fytoplankton, macrozoöbenthos, macro-algen en angiospermen voor de Vlaamse overgangswateren volgens de Europese Kaderrichtlijn Water. Universiteit Antwerpen, Onderzoeksgroep Ecosysteembeheer. 69 p. + bijlagen.

- van der Molen, D.T. & Pot, R. (red.) (2007). Referenties en maatlatten voor natuurlijke watertypen voor de Kaderrichtlijn Water. December 2007. Rapport STOWA 2007-32. ISBN 978.90.5773.383.3. 362 p.

- Van Hoey, G., Drent, J., Ysebaert, T. & Herman, P. (2007). The Benthic Ecosystem Quality Index (BEQI), intercalibration and assessment of Dutch Coastal and Transitional Waters for the Water Framework Directive. NIOO rapport 2007-02. Nederlands Instituut voor Ecologie, Yerseke, Nederland. 244 p.

- Van Landuyt, W., Vanhecke, L., & Hoste, I. (2006). Rode Lijst van de vaatplanten van Vlaanderen en het Brussels Hoofdstedelijk Gewest. In: Van Landuyt, W., Hoste, I., Vanhecke, L., Van Den Bremt, P., Vercruysse, W. & De Beer, D. (eds.). Atlas van de flora van Vlaanderen en het Brussel Gewest. Instituut voor Natuur- en Bosonderzoek, Brussel en Nationale Plantentuin van België, Meise i.s.m. Flo.Wer vzw, Brussel.

- Van Looy, K., Denys, L. & Schneiders, A. (2008). Methodiek vaststelling Maximaal en Goed Ecologisch Potentieel (MEP-GEP) voor sterk veranderde waterlopen. Rapporten van het Instituut voor Natuur- en Bosonderzoek 2008 (INBO.R.2008.06). Instituut voor Natuur- en Bosonderzoek, Brussel. 20 p.

- Van Wichelen, J., Denys, L., Lionard, M., Dasseville, R. & Vyverman, W. (2005). Ontwikkelen van scores of indices voor het biologische kwaliteitselement fytoplankton voor de Vlaamse rivieren, meren en overgangswateren in overeenstemming met de Europese Kaderrichtlijn Water. Eindrapport VMM.AMO.SCALDIT.fytoplanktonstudie. Universiteit Gent, Vakgroep Biologie, Onderzoeksgroep Protistologie en Aquatische Ecologie, Gent. 105 p.

- Van Wichelen, J., Fagot, M., Lavens, F. & Vyverman, W. (2008a). Fytoplanktonanalyses van een aantal Vlaamse waterlopen ten behoeve van de Europese Kaderrichtlijn Water. Eindrapportage van de onderhandse overeenkomst (VMM.ARW.001.2008) in opdracht van de Vlaamse Milieumaatschappij. Universiteit Gent, Vakgroep Biologie, Onderzoeksgroep Protistologie en Aquatische Ecologie, Gent.

- Van Wichelen, J., De Bock, D. & Vyverman, W. (2008b). Bepalen van het maximaal en het goed ecologisch potentieel, alsook de huidige toestand voor een aantal Vlaamse (gewestelijke) waterlichamen die vergelijkbaar zijn met de categorie meren – Deel II Partim 'Boudewijnkanaal en Achterhaven Zeebrugge'. Rapport studieopdracht VMM.AMO.KRW.ECOPOT-Meren 01/12/2008. Universiteit Gent, Laboratorium voor Protistologie & Aquatische Ecologie.

- Van Wichelen, J., De Bock, D. & Vyverman, W. (2008c). Bepalen van het maximaal en het goed ecologisch potentieel, alsook de huidige toestand voor een aantal Vlaamse (gewestelijke) waterlichamen die vergelijkbaar zijn met de categorie meren – Deel II Partim 'Spuikom'. Rapport studieopdracht VMM.AMO.KRW.ECOPOT-Meren 01/12/2008. Universiteit Gent, Laboratorium voor Protistologie & Aquatische Ecologie.

- Ysebaert, T. & Meire, P. (1999). Macrobenthos of the Schelde estuary: predicting macrobenthic species responses in the estuarine environment: a statistical analysis of the Schelde estuary macrobenthos within the ECOFLAT project. IN 99/19. Instituut voor Natuurbehoud, Brussel. 178 p.