The Dutch assessment of macroinvertebrates in international comparison

Analysis of the Dutch WFDi assessment method and comparison of ICM-metric scores of Dutch references with references from other member states

Rijkswaterstaat RIZA

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SUMMARY

The Dutch national assessment index (WFDi) is developed to assess water bodies in the Netherlands, mainly characterized as lowland streams. During the intercalibration pilot, the Dutch WFDi appeared to correlate weakly to the international Intercalibration Common Metric index (ICMi). Moreover, during the meeting in Lithuania the steering committee decided that the Dutch method may be not compliant with the WFDi, because no reference sites are available. This report explains, underpinned by several studies, why the weak correlation with ICMi and the lack of reference sites do not limit the possibilities of including Dutch values in proper harmonization.

The weak correlation between ICMi and WFDi can be explained by the following facts:
- The Dutch metric is sensitive to hydromorphological pressure, whereas the ICMi is not. At this point, WFDi assesses Dutch waters more accurately.
- EPT taxa, in particular Plectoptera, which are determinant for ICMi-scores, are relatively rare and are not characteristic for Dutch reference sites.
- The Dutch low quality sites contain relatively many families, whereas species indicating high quality are divided among a few different families only. Therefore, the metrics 'number of families' and 'ASPT' are not accurate indicators. The Dutch metric can account for this, because indicators are considered at species level.

As a consequence, the band of quality expressed on the ICM scale is relatively small for the Netherlands and expresses a weak correlation. The method for harmonization as it is presented, however, does not limit the participation of the Netherlands because:
- The test whether a member state reference value is within the band of confidence is practiced on the median value. Statistical noise is thus not taken into account.
- Comparison on the level of classes shows that the good status of ICMi is similar to the mean good status calculated by the Dutch metric.
- The figures showing the 95% confidence limits (e.g. option 4) show nearly no differences in the sizes of the intervals in comparison with other member states. The fact that the Dutch dataset is relatively large, probably results in reduced confidence limits.

Because the range of confidence limits of the estimations of the class boundaries (H/G, G/M) is not different from those of other member states, the requirements for harmonization are met. The values of the ICMi metric, derived from the Dutch 'high' status sites, are statistically comparable with the reference values of one or more other Member States. We therefore conclude that the use of the 75th percentile of the Dutch high sites is justified for use in the harmonization.

CONTENTS

1 INTRODUCTION

The Dutch national assessment index (WFDi) is developed to assess water bodies in the Netherlands, which mainly consist of lowland streams. During the intercalibration pilot, the Dutch WFDi appeared to correlate weakly to the International common metric index (ICMi). Moreover, during the meeting in Lithuania the steering committee decided that the Dutch method may be not compliant with the WFDi, because no reference sites are available. This report explains why the lack of reference sites and the weak correlation with ICMi are not a problem for the quality of the intercalibration exercise: chapter 2 explains the development of the Dutch metric in order to clarify its working principle as well as its response to pressures. The system is developed based on all information available, and is validated on the judgement of independent experts. Chapter 3 describes the search for reference sites in the Netherlands according to internationally established criteria. Only one site could meet these requirements. Chapter 4 explores the possibilities for using high classified sites as Dutch references in the international intercalibration process. The potential Dutch references are therefore compared to references of other member states. Furthermore, the ICMi score ranges of several member states that have lowland streams are compared in chapter 5. The report presents recommendations based on scientific arguments for fulfilling successfully the Dutch participation in the intercalibration exercise.

2 DEVELOPMENT AND WORKING OF THE DUTCH WFD-INDEX

2.1 Summary of development of WFDi ('KRW-maatlat')

A multi-metric WFD-index for Dutch running waters has been developed based on species composition and abundances of macroinvertebrates. Expert judgement based on all available information is used to determine which macroinvertebrate species characterize the different classes of ecological quality (from 'bad' to 'high'). From these characteristic communities, macroinvertebrate species are listed and used for ecological assessment. Three lists of indicator species were developed per water type and consist of: critical species, dominant positive species, and dominant negative species. The relative contribution of these groups is determined and the calculation of the EQR is integrated in one formula:

EQR = {200*(%KM/KMmax) + 2*(100-%DN) + %(DP+KM)}/500

% KM	= relative number of critical species in a sample
KMmax	= maximum achievable number of critical species under reference conditions
% DN	= relative abundance of dominant negative species
% (DP+KM)	= sum of relative abundances of dominant positive species and critical species

Attachment I gives a more detailed explanation of the development of WFDi, whereas Attachment II shows an example of calculating EQR using WFDi.

2.2 Brief explanation of working of WFDi

Figure 1a shows the divergent influences of the three WDFi-parameters. The parameter %KM is most determining in distinguishing EQR-classes. Lower EQR-classes are characterized by high values of %DN and low values of %KM. The increase of %KM and the decrease of %DN result in higher quality classes. From the class 'good', the role of the parameter %(DP+KM) becomes more important and determines whether the class 'high' can be achieved. Figure 1b presents the number of critical, dominant negative and dominant positive taxa, characteristic for different WFD-classes. Dominant negative species indicate low-quality sites, whereas critical species mostly determine high quality sites. The low quality classes are characterized by a low number of macroinvertebrates. Taxa of critical species lack and dominant negative species are mostly abundant. The 'moderate/good' boundary is characterized by a low number of dominant negative taxa, a fairly high occurrence of KM taxa and an increase of dominant positive species, and is mostly influenced by the increase of abundance of dominant positive and critical species.

The boundaries for the different EQR-classes (bad, poor, moderate, good and high) are set, based on expert judgement and follow a more or less equal division of quality. The WFDi and its class-boundaries were validated by experts judging species lists from anonymous sites, using normative definitions. A more detailed description can be found in Attachment I of this document.

Figure 1a: The influence of WFD-parameters %KM (taxa), %DN (abundance) and %(DP+KM) (abundance) on EQR

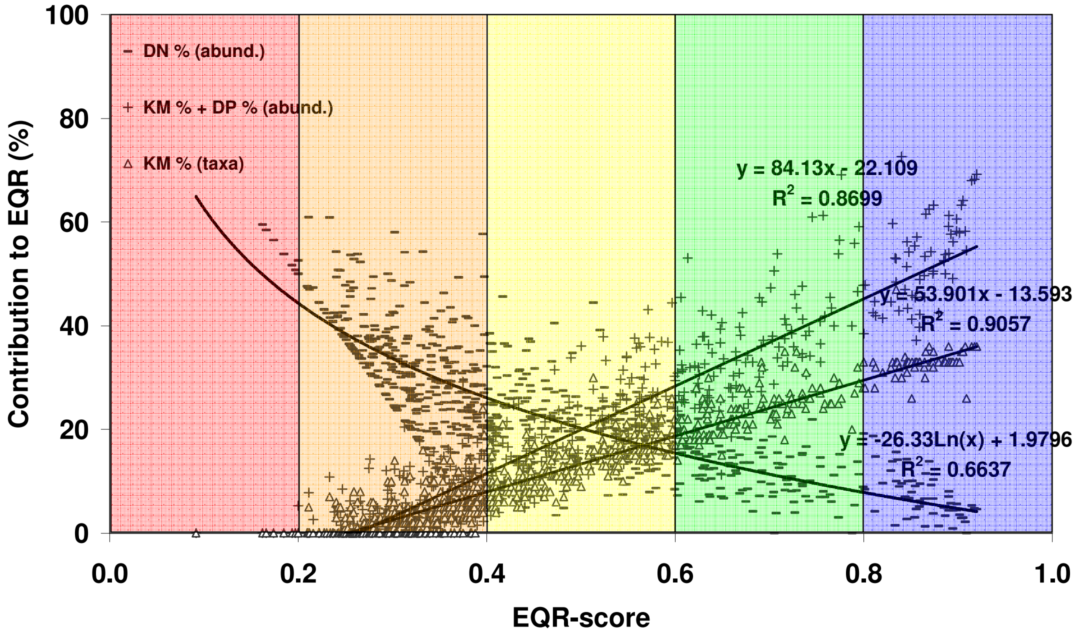
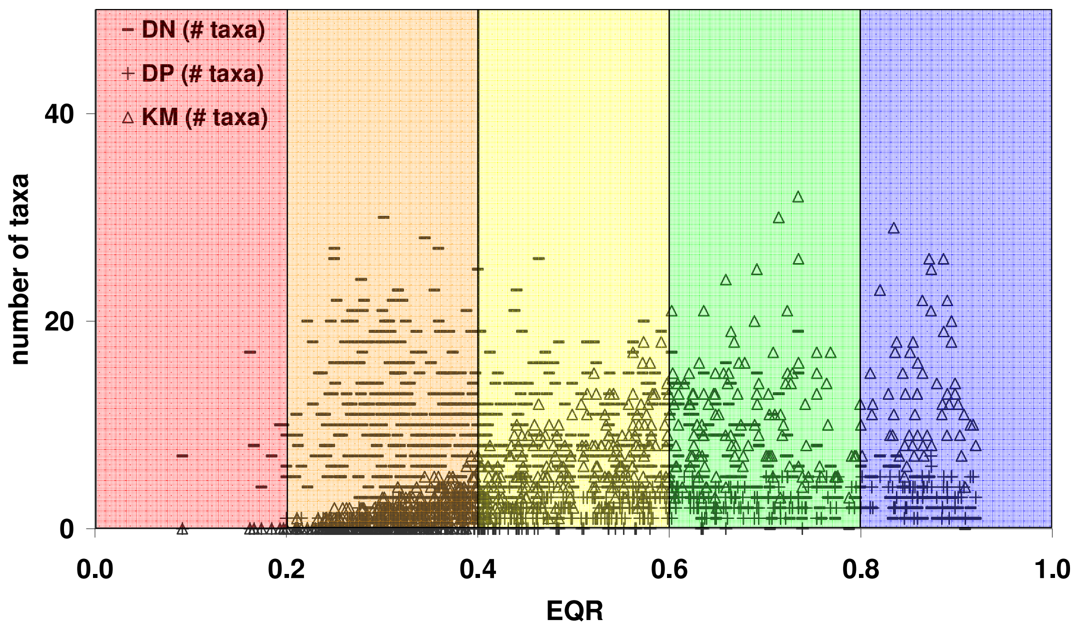


Figure 1b: The influence of dominant negative, dominant positive and critical taxa (# taxa) on EQR



2.3 Setting EQR-class boundaries

A pool of macroinvertebrate samples was pre-classified by expert judgement in combination with multivariate gradient analysis.

The combination of metrics that fitted the pre-classification best were selected and transformed in a formula that calculates the EQR. The selection of these metrics and development of the formula are described in attachment I. The formula calculates EQR from 0 to 1. The class boundaries values are:

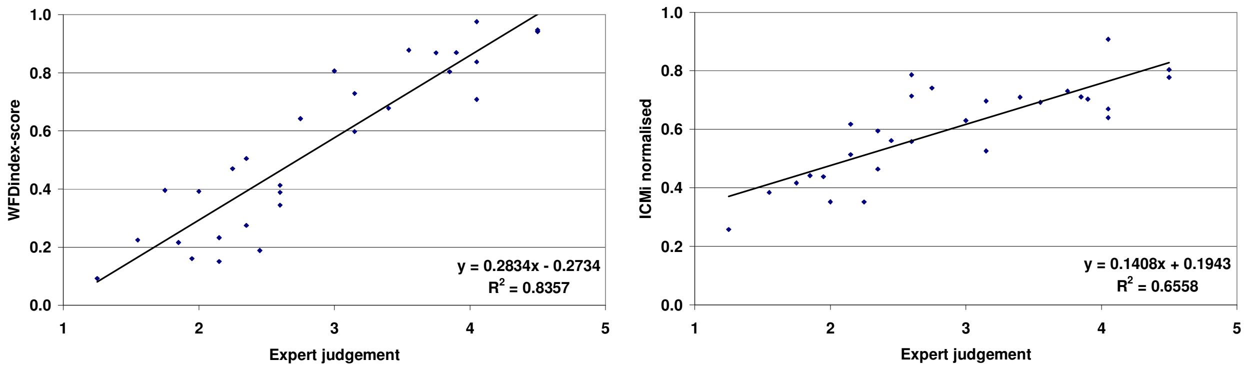
- boundary bad-poor = 0.2;
- boundary poor-moderate = 0.4;
- boundary moderate-good = 0.6;
- boundary good-high = 0.8.

The G/M boundary is the just before the moment where dominant negative taxa are getting more important contribution to EQR as compared to the dominant positive taxa. Additionally, we tried to set boundaries using to the protocol for setting boundaries (Pollard and van de Bund, 2006). Unfortunately, our data appeared not suitable to complete the process. The results are shown in attachment III.

2.4 Validation of the Dutch WFD-index

Validation of the Dutch WFD-index and the normalized ICMi to expert judgement showed equal EQR-classification for 81% of the samples tested. Expert judgement was performed according to the 'Delphi-method' by sending thirty species list of anonymised sampling sites to ten macroinvertebrate experts distributed over the country. These experts had not been involved in the development of the WDF-index before, to avoid circularity. Expert judgement correlated well with both the WFD-index and the normalized ICMi (Figure 2).

Figure 2: Correlation of mean WFDi-scores and mean ICMi-scores with expert judgement (n = 29)



The validation shows clearly that the Dutch WFD-index correlates much stronger to physical environmental pressures, described by hydromorphological pressure, than to chemical pressures. The ICMi on the other hand, showed poor correlations with hydromorphological pressure (Figure 3). Water bodies in the Netherlands are hydromorphologically altered, making physical pressure an important factor in assessment of Dutch water bodies. At this point, the WFDi describes the Dutch situation better than the ICMi does and is thus more accurate for Dutch waters. Despite of the correlation between ICMi and WFDi, classification into WFD-classes is highly comparable between ICMi and the Dutch WFDi (Figure 4). This comparison on the level of classes shows that the good status of ICMi is similar to the mean good status calculated by the Dutch metric.

Figure 3: Correlation of mean WFDi-scores and mean ICMi-scores with the hydromorphologic pressure gradient (n = 279)

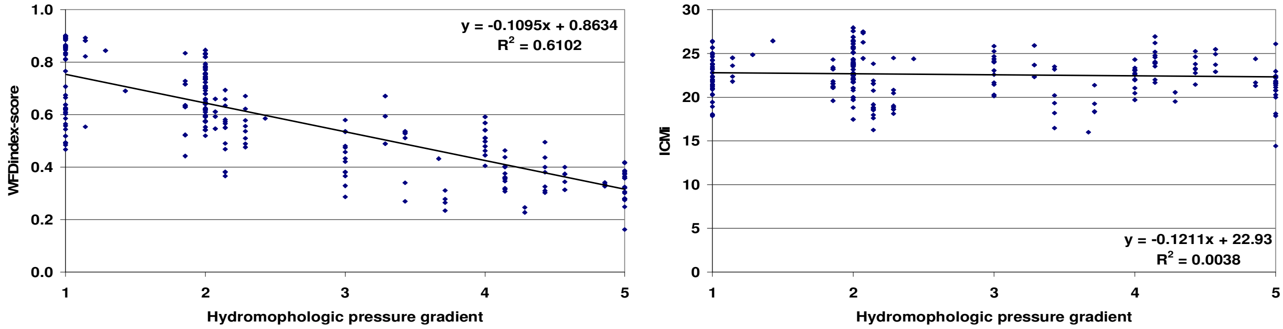
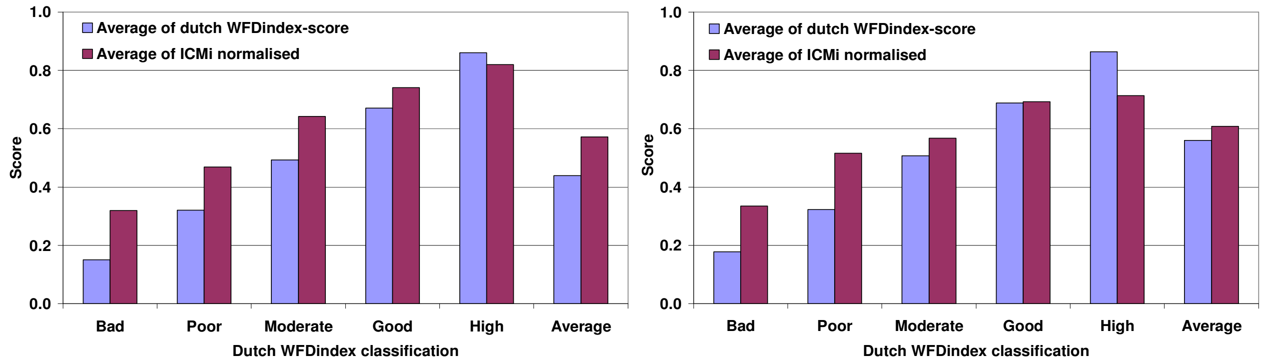


Figure 4: Correlation of mean class scores of WFDi and ICMi for RC-1 (n = 459) and RC-4 (n=436) waters



3 SELECTING TYPE-SPECIFIC REFERENCE SITES

3.1 From sites with high ecological quality that meet the criteria of Wasson

Reference sites have been identified from sites with high ecological quality (WFDi-class ‘high’) according to the criteria defined by Wasson (April, 2006). Most of the Dutch waters could not meet these requirements as most of them have been hydromorphologically altered and do not correspond with the conditions set for nitrogen and phosphate. The selection criteria for the Dutch reference waters implied that the land use of the drainage basin meets: nature for at least 50% of the catchment, less than 4% urban area, less than 15 kilogram nitrogen per hectare, nor 1 kilogram phosphate per hectare in the catchment. The criteria for water chemistry are summarized in table 2. Furthermore, a reference site may not contain point sources and may not be hydromorphologically altered. Recreation or bio-manipulation must be restricted to a minimum. The stream Hierdensche beek is the only stream that meets all criteria set for a reference site.

Table 2: Reference criteria

Parameter	RC-1	RC-4	Remark
BOD5	2.4 mg/l	2.4 mg/l	Yearly average
BOD5	3.6 mg/l	3.6 mg/l	90 percentile
O2 saturation	95-105	95-105	Yearly average
O2 saturation	85-115	85-115	10-90 percentile
N-NH4	0.1 mg N/l	0.1 mg N/l	Yearly average
N-NH4	0.25 mg N/l	0.25 mg N/l	90 percentile
P-PO4	0.04 mg P/l	0.04 mg P/l	Yearly average
N-NO3	6 mg N/l	6 mg N/l	90 percentile
N-NO3	2-4 mg N/l	2-4 mg N/l	Yearly average

3.2 Using criteria for chemical pressures only

Potential reference sites have been identified from all data available according to the criteria for water chemistry only, defined by Wasson (April, 2006). The analysis is based on summer averages (from April to September) of the various parameters. Ecological quality and land-use have not been considered in this analysis, as most of the Dutch waters could not meet these requirements due to hydromorphological alterations. The criteria for water chemistry are summarized in table 2.

A few sites seem to fit to the chemical requirements for reference sites, but lack information on two or more chemical parameters. BOD for instance was not available for any of the potential reference sites (table 3). These sites and their chemical characteristics are shown in table 3. All sites scored high on EQR of macrofauna (from 0.83-0.91). From these sites, Steenhaarswatergang and Eerbeekse beek are the least disturbed streams. These streams run through a forest, are not pressed by pollution, and their hydrology is not seriously altered. Additional measurements can be taken to see if these streams may consider for reference sites. The ICMi metric scores of these sites are shown in Attachment IV.

The median values of metric scores of these potential reference sites are, however, lower than the 75-percentiles of the scores of all high quality sites. Using 75-percentiles of high scoring sites for intercalibration will thus be preferable.

Table 3: Potential Dutch reference sites, if only chemical parameters are considered and land use criteria are not applied (n.a.= not available)

Location	O_2 (%)	P-total (mg P/l)	NH_4(mg N/l)	NO_3(mg N/l)	EQR
Kroonbeek Ivo	95.67	n.a.	n.a.	n.a.	0.89
Swalm Hoosterhof	103.11	n.a.	n.a.	n.a.	0.87
Tielebeek Vagevuur	101.40	n.a.	n.a.	n.a.	0.89
Tungelroysebeek Baanbrug	103.00	n.a.	n.a.	n.a.	0.86
Vissesteert instroom Neerpeelbeek	101.50	n.a.	n.a.	n.a.	0.90
Vliet Baldersstraat	96.00	n.a.	n.a.	n.a.	0.85
Steenhaarswatergang	n.a.	0.02	0.10	0.10	0.91
B18 middelste horthoekerbeek Epe	n.a.	0.02	0.10	2.00	0.89
C24 Eerbeekse beek Eerbeek	n.a.	0.03	0.05	0.89	0.83
C210 Loenense beek: bij sportterrein	n.a.	0.02	0.10	6.47	0.90

3.3 Using sites from neighboring countries

We tried to find reference sites close to the German-Dutch border and the Belgian-Dutch border. However, none of the Belgian sites could meet the requirements either, and we did not get permission within the available timeframe from the German nature conservation authority for sampling the German sites. A sampling campaign is planned for September 2006.

4 COMPARING DUTCH HIGH STATUS SITES WITH REFERENCE SITES OF OTHER EU-MEMBER STATES

General
For normalizing ICMi-metric scores, a correction factor (representing the upper end of the EQR scale from 0 to 1) derived from metric scores of reference sites is needed. Unfortunately, only one Dutch site can be considered as reference site (see paragraph 2), which is too few for setting reliable correction factors. As explained in chapter 2, the Dutch assessment system is WFDi compliant and is validated on independent judgment of experts. Therefore the Dutch ‘high’ status sites can be considered suitable for conversion to ICMi reference values. To test whether this consideration is valid, we compared the ICMi values of the Dutch ‘high’ sites with the reference sites of some other member states within the Central/Baltic GIG.

4.1 Comparing ICMi-metric scores of international references

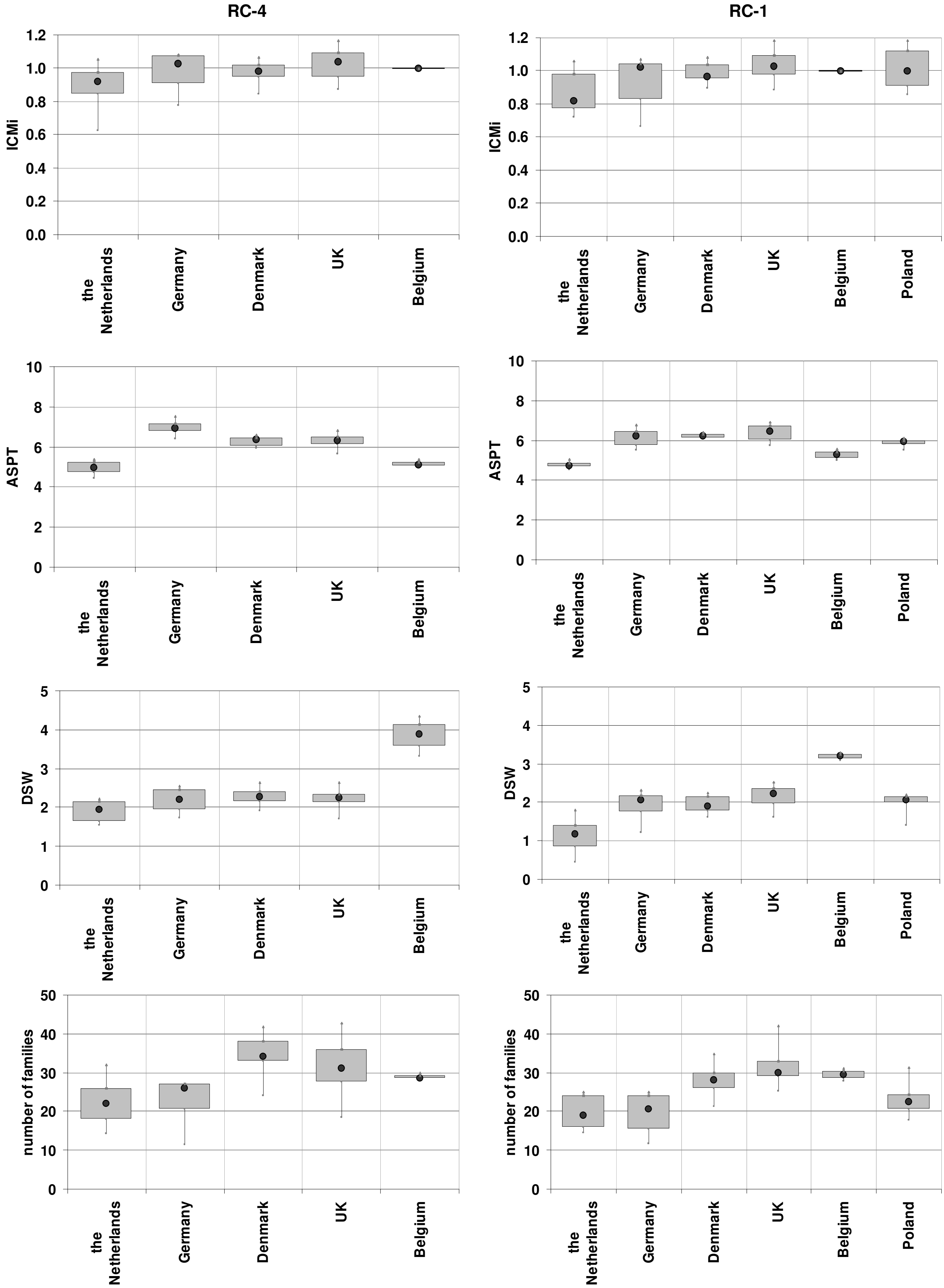
Every member state attending the intercalibration process selected reference sites for the water types RC-1 and RC-4, if available and applicable. The Dutch high status sites were compared to reference sites from other lowland member states. The ICMi-metrics of these references were calculated by Asterics (Aqem 2.5) from macroinvertebrate abundances at the level of family.

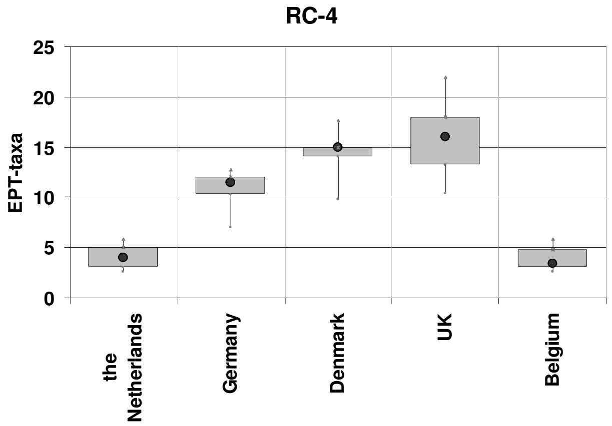
Figure 4 shows the 5-95 percentile ranges of absolute metric scores for reference sites of Germany, Denmark and Great Britain, the 75-percentile references of the Netherlands and the theoretical references of Belgium. The boxes in the figure refer to the 25 to 75-percentile range. Black dots in the boxes indicate median values. Most of the metric scores of the Dutch high status sites are within the same range as the scores of reference sites from other European countries (Figure 5). The Dutch 75-percentiles are in far most cases within the range of the 25-75 percentiles of other references. Statistical analysis, performed on mean values, show statistical differences between the absolute values of ICMi metrics of reference sites of different European countries (Appendix V). The Dutch references differed most from those of the UK and were most similar to references from Belgium and Germany. Additionally, the Dutch 75-percentiles were compared to Dutch potential references, discussed in Chapter 3. This comparison is described in Attachment IV. The 75-percentiles were within the score range of these potential references.

Overall, scores of Dutch references on metrics related to ETP-taxa remain low, as a logical consequence of natural low abundances of EPT-taxa. EPT-taxa are rare in the area chosen as a natural reference for the Netherlands as well (i.e. the river Pripjat, Appendix VI), indicating that these groups may not be essential for biotopes similar to the Dutch waters. The Netherlands therefore rather validates its samples to European waters that are situated within the same ecological region and that are hydromorphologically pressured as well and, such as Germany or Belgium. Remarkably, Dutch sites with high EQR-scores contain less different families than low quality sites, explaining the low scores on the metric for ASPT and low number of families. The metric score ranges of the references from the other member states show more similarities to one another than to Dutch references.

Many explanations can account for this, the most important being the fact that member states used different sampling techniques for collecting macrofauna, resulting in catching a different, extended or limited part of the macroinvertebrate population.
In conclusion, the reference values of the ICMi of Dutch ‘high’ status sites are statistically comparable with those of one or more other member states. The Dutch values are in some cases lower, which is probably due to the fact that Dutch rivers can be characterized as extremes within the lowland member states.

Figure 5: Absolute metric scores of reference sites from European countries

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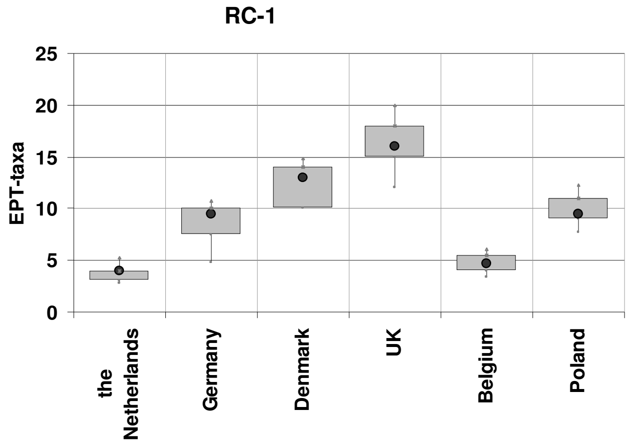
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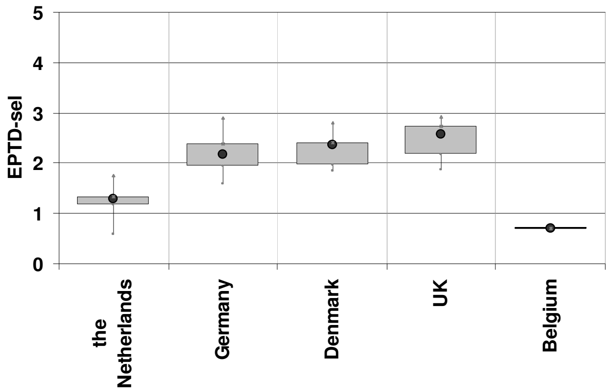
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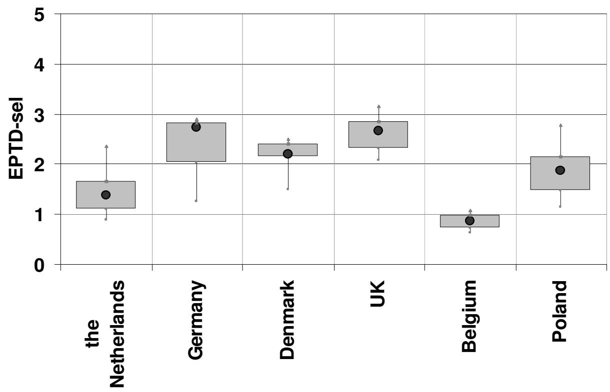
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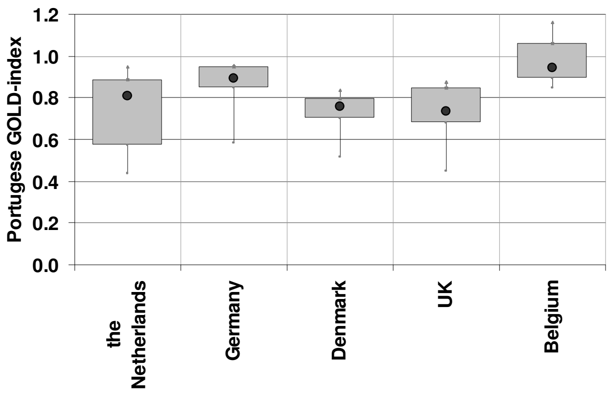
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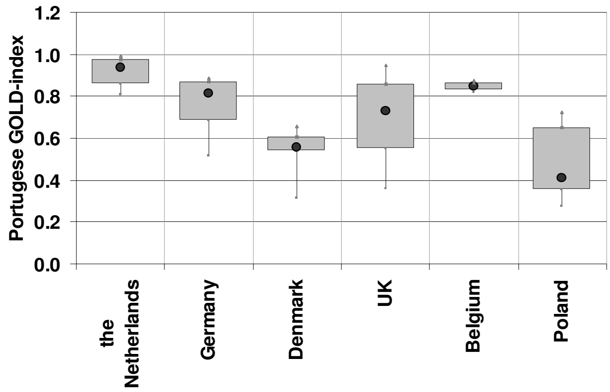
Portugese GOLD-index
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5 COMPARING METRIC SCORES OF FOREIGN NON-REFERENCE SITES

A range of equally many good (highest national class) and bad (lowest national class) samples of each country was analyzed using the Dutch ICMi-tool, as comparing the scores of total ranges can lead to deviating ranges due to differences in water body quality. The ICMi-score ranges of these high and low end ecological status are compared (Figure 6). The Dutch worst quality samples score remarkably high compared to those of the other member states, whereas the Dutch best quality samples score may be equal or very slightly lower. Apparently, the Dutch samples show a more narrow range in quality as compared to other member states. This is an additional reason for the weak correlation between ICMi and the Dutch metric.

The Dutch samples scored especially low at metrics related to the occurrence of Ephemeroptera, Plecoptera and Trichoptera (i.e. EPT-taxa and EPTDsel, Figure 7), as these families (in particular Plecoptera) are rare or even absent in Dutch macroinvertebrate communities and are not considered as very characteristic for Dutch reference waters.
Unfortunately, information on abiotic conditions of the different sampling sites from different countries was not available. We therefore could not test if these directly affect the EQR and are comparable within member states.

Figure 6: Absolute ICMi scores of the best (open symbols) and worst (closed symbols) quality samples from European countries

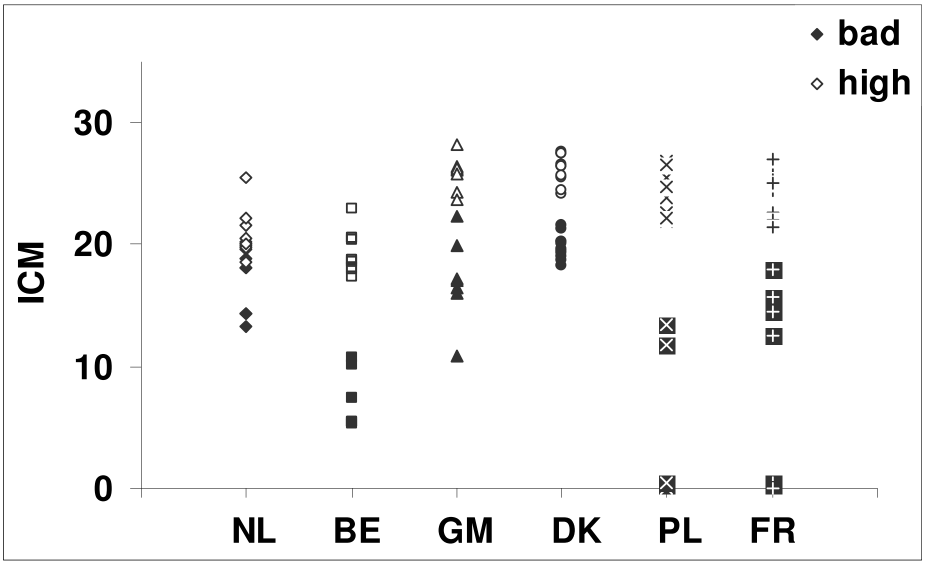
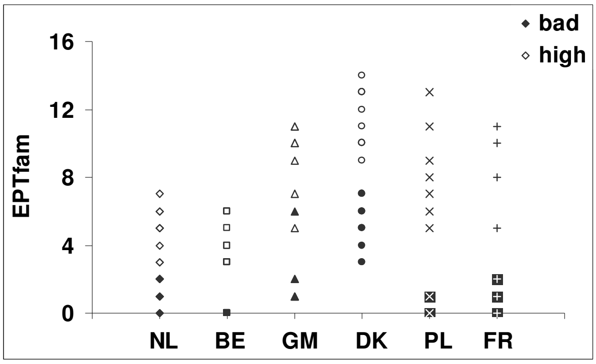
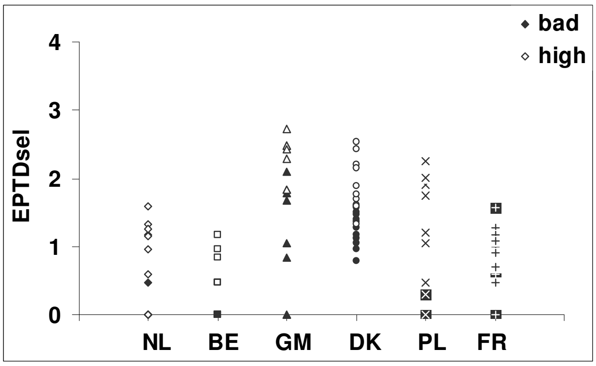


Figure 7: EPTfam and EPTDsel scores of the best (open symbols) and worst (closed symbols) quality samples from other member states of the Central-Baltic GIG





6 CONCLUSION

The Dutch metric shows a weak correlation with the ICMi, which may be caused by a different response of ICMi and the Dutch metric to pressures. The Dutch metric is sensitive to hydromorphological pressure, but the ICMi appaers not to respond to this type of pressure. This is a problem because the quality of Dutch streams is strongly affected by hydromorphological changes. Furthermore, the ICMi is less suitable for the assessment of macroinvertebrates because:

- EPT taxa (in particular Plecoptera) are relatively rare and are not characteristic for Dutch reference sites;
- The Dutch low quality sites contain relatively many families, whereas species indicating high quality are divided among a few different families only. Therefore, the metrics ‘number of families’ and ‘ASPT’ are not accurate indicators. The Dutch metric can account for this, because indicators are considered at species level.

As a consequence, the band of quality expressed on the ICM scale is relatively small for the Netherlands and expresses a weak correlation.

The question arises whether the weak correlation between ICMi and Dutch metric limits the possibilities of proper harmonization. The method for harmonization as it is presented now may not limit the participation of the Netherlands because:

- The test whether a member state reference value is within the band of confidence is practiced on the median value. Statistical noise is thus not taken into account;
- Comparison on the level of classes shows that the good status of ICMi is similar to the mean good status calculated by the Dutch metric;
- The figures showing the 95% confidence limits (e.g. option 4) show nearly no differences in the sizes of the intervals in comparison with other member states. The fact that the Dutch dataset is relatively large, probably results in reduced confidence limits.

Because the range of confidence limits of the estimations of the class boundaries (H/G, G/M) is not different from those of other member states, the requirements for harmonization are met.

The values of the ICMi metric, derived from the Dutch ‘high’ status sites, are statistically comparable with the reference values of one or more other Member States. The Dutch values are in some cases lower than values of other member states, which may be due to the extremely lowland character of Dutch rivers. We therefore conclude that the use of the 75th percentile of the Dutch high sites is justified for use in the harmonization.

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Annex 1
Development of the Dutch WFD-index

1. Dutch WFDindex ('KRW-maatlat') in a nutshell

A multi-metric WFD-index for Dutch running waters has been developed based on species composition and abundances of macroinvertebrates. Expert judgement determined based on all available information is used to determine which macroinvertebrate species characterize the different classes of ecological quality (from 'bad' to 'high'). From these characteristic communities, macroinvertebrate species are listed and used for ecological assessment. Three lists of indicator species were developed per water type and consist of: critical species, dominant positive species, and dominant negative species. The relative contribution of these groups is determined and the calculation of the EQR is integrated in one formula:

EQR = {200*(%KM/KMmax) + 2*(100-%DN) + %(DP+KM)}/500

% KM	= relative number of critical species in a sample
KMmax	= maximum achievable number of critical species under reference conditions
% DN	= relative abundance of dominant negative species
% (DP+KM)	= sum of relative abundances of dominant positive species and critical species

1.1 Selecting indicator species

Indicator species have been selected from indicator species lists (Verdonschot et al. 1992; Verdonschot 2000; Verdonschot & Janssen 2000; Verdonschot, 1990; Verdonschot et al., 2000; Verdonschot et al., 1999; Janssen et al., 1998; Verdonschot & Nijboer, 2004). Ecological quality is indicated by the presence of indicator species as well as their abundances. Species indicative for ecological quality have been added to the species list. The critical abundance classes are based on abundances of indicator species in natural stream types. Indicator species with abundance classes greater than 6 (i.e. 91-244 individuals in a sample taken with a standard net over a distance of 5m) are referred to as dominant positive species, whereas species with lower abundances are referred to as critical species. Species that occur in high densities in impacted or polluted water systems are referred to as dominant negative species. The list containing dominant positive species was extended with species that are commonly present (i.e. >90 individuals) in natural reference waters situated in Poland, Germany, Denmark and Ukraine. Species from reference waters abroad that had already been incorporated in the list of critical species or have never been found in Dutch waters were not included in the list of dominant positive species. The lists composed were lastly judged by experts and were adjusted accordingly.

1.2 Development of the Dutch WFD-metrics (%KM, %DN and %(DP+KM))

WFD-parameters were developed on the basis of medium sized lowland rivers, corresponding with intercalibration water type RC-1. This water type is well documented and biological as well as chemical variables are monitored frequently.
WFD-metrics were developed from RC-1 macroinvertebrate samples, which had been classified to EQR by expert judgement supported by multivariate data analysis (CANOCO 4.0). According to expert judgement, sufficient samples represented the classes 'bad', 'poor', 'moderate' and 'good', assuming that no or very minor number of Dutch streams could be qualified as 'high' due to human pressure.

Subsequently, the EQR from the classified macroinvertebrate samples was plotted to the abundances of dominant negative species, dominant positive species, critical species and rare species. Pearson coefficient analysis calculated which groups of species determined EQR and therefore had to be implemented in the WFD-index parameters. Using relative abundances instead of absolute abundances of the selected species groups improved correlation with EQR. Using relative instead of absolute abundances furthermore diminishes side effects from using different sample methods on the calculation of EQR. Critical species (KM), dominant negative species (DN) and dominant positive species (DP) determined EQR most and were therefore selected as parameters determining the WFD-index. These parameters were selected using a score system for analyzing the role of the selected species groups (KM, DN and DP) on the EQR. The abundance of dominant positive species appeared to be related to the abundance of critical species as the number of critical species and the number of positive dominant species cannot be high at the same time. Positive dominant species en critical species have therefore been combined to one parameter**:** %(DP+KM). Subsequently, the scores of the parameters %KM, %DN and %(DP+KM) were translated to a normalized EQR-qualification, ranging from 0 to 1. Within this range, the class boundaries were equally distributed, with 0.6 being the most important boundary as it distinguishes between the classes ‘moderate’ and ‘good’. As macroinvertebrate composition highly depends on water type, translations have been made according to relative abundances of DP, DN and KM in reference communities for each water type individually. Table A1.1 shows an example of this translation for the water type RC-1. The exact values for the KRW-parameters at the boundary ‘moderate/good’ (EQR = 0.6) can not be calculated, but it can roughly be indicated by %DN (abundance) values of more than 41, %KM (number of taxa) values of more than 33 and %(DP+KM) (abundance) values of more than 25.

Table A1.1: Translation from parameter scores to EQR for water type RC-1

	Abundance score	EQR-score
%DN (abundance)	≥ 41	0.1
	< 41	0.2
%KM (number of taxa)	≤ 7	0.1
	7 < %KM < 18	0.2
	18 ≤ %KM < 33	0.3
	≥ 33	0.5
%(DP+KM) (abundance)	< 5	0.1
	5 ≤ %KM < 25	0.2
	≥ 25	0.3

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Annex 2
Example of determining EQR using the Dutch WFDi

In order to illustrate the working of the Dutch WFDi, we have included an example of ecological assessment determined by using WFDi. The assessment is done on a Dutch macroinvertebrate sample from a type R05 (RC-1) stream.

Step 1: *List the occurring macroinvertebrate at species level.*
Species occurring in the test sample are listed in table A2.1.

Step 2: H*ighligh indicative taxa.*
Taxa indicative for ecological quality are listed for R05 in table A2.2 and A2.3.
Determine which indicator species occur in the sample.

In this example: Positive dominant indicators are highlighted green in table A2.1, whereas negative dominant indicators are highlighted red and critical species are highlighted yellow.

Step 3: *Determine the values of the three WFDi-parameters.*

- DN% (abundance class); percentage (based on abundance classes) of individuals belonging to negative dominant taxa;
- KM% (number of taxa); percentage critical taxa;
- DP% + KM% (abundance class); percentage (based on abundance classes) of individuals belonging to critical or positive dominant taxa.

Abundances classes are determined using table A2.1 (van der Hammen, 1992; Evers *et al*, 2005). These classes are based on real abundances.

Table A2.1: Abundance classes, corresponding with absolute abundances (van der Hammen, 1992; Evers *et al*, 2005)

Absolute number of individuals	1	2-4	5-12	13-33	34-90	91-244	245-665	666-1808	>1808
Abundance class	1	2	3	4	5	6	7	8	9

In this example:

$$DN\% = \frac{\text{total abundance DN (based on classes)}}{\text{Total abundance all taxa (based on classes)}} *100 = 9/99*100 = \mathbf{9.09}$$

$$DP\% + KM\% = \frac{\text{total abundance DP and KM (based on classes)}}{\text{Total abundance all taxa (based on classes)}} *100 = 46/99*100 = \mathbf{46.46}$$

$$KM\% \text{ (taxa)} = \frac{\text{number of critical taxa}}{\text{total number of taxa}} *100 = 19/54*100 = \mathbf{35.19}$$

Step 5: *Fill in the formula.*
Search for KMmax corresponding to the watertype of the sample in table A2.2.

Table B2.2: KMmax per WFD-watertype

KRW-type	R01	R02	R03	R04	R05	R06	R09	R10	R11	R12	R13	R14	R15	R17	R18
KMmax	56	63	56	26	33	36	26	33	26	33	36	51	51	36	51

KMmax for R05 = 33 (table A2.2)
DN% = 9.09
DP% + KM% = 46.46
KM% (taxa) = 35.19

Calculate EQR:

EQR = {200*(%KM/KMmax) + 2*(100-%DN) + %(DP+KM)}/500

The value for parameter KM/KMmax may not exceed 1. In this example, KM/KMmax (35.19/33) >1. Therefore, the value of this parameter is set at 1.

In this example:

EQR = (200* 1 +2* (100-9.09) + 46.46)/500 = 0.86

Step 6: *Determine WFDi-class.*
Look for the WFDi-class corresponding to the calculated EQR in table A2.3. In this example: EQR-value 0.88 corresponds to WFDi-class 'high'.

Table A2.3: EQR and corresponding WFD-classes

WFDmetric score (EQR)	**Ecological status**
0.8-1.0	High
0.6-0.8	Good
0.4-0.6	Moderate
0.2-0.4	Poor
0-0.2	Bad

Table A2.4: Species lis of macroinvertabrates present in sample

code	date	Taxon	number of individuals	abundance classes*
R5-01	05/01/2006	Stagnicola palustris	1	1
R5-01	05/01/2006	Anisus vortex	1	1
R5-01	05/01/2006	Glossiphonia complanata	1	1
R5-01	05/01/2006	Erpobdella octoculata	1	1
R5-01	05/01/2006	Lumbriculus variegatus	2	2
R5-01	05/01/2006	Asellus aquaticus	1	1
R5-01	05/01/2006	Proasellus meridianus	3	2
R5-01	05/01/2006	Gammarus pulex	30	4
R5-01	05/01/2006	Hydrodroma torrenticola	1	1
R5-01	05/01/2006	Lebertia inaequalis	29	4
R5-01	05/01/2006	Lebertia insignis	4	2
R5-01	05/01/2006	Hygrobates fluviatilis	2	2
R5-01	05/01/2006	Hygrobates longipalpis	3	2
R5-01	05/01/2006	Hygrobates nigromaculatus	11	3
R5-01	05/01/2006	Hygrobates trigonicus	2	2
R5-01	05/01/2006	Forelia variegator	1	1
R5-01	05/01/2006	Mideopsis crassipes	1	1
R5-01	05/01/2006	Mideopsis orbicularis	6	3
R5-01	05/01/2006	Calopteryx splendens	2	2
R5-01	05/01/2006	Caenis horaria	9	3
R5-01	05/01/2006	Nemoura cinerea	13	4
R5-01	05/01/2006	Sialis lutaria	1	1
R5-01	05/01/2006	Haliplus	2	2
R5-01	05/01/2006	Laccophilus hyalinus	1	1
R5-01	05/01/2006	Laccophilus minutus	1	1
R5-01	05/01/2006	Graptodytes pictus	1	1
R5-01	05/01/2006	Nebrioporus depressus	1	1
R5-01	05/01/2006	Agabus didymus	1	1
R5-01	05/01/2006	Orectochilus villosus	1	1
R5-01	05/01/2006	Hydraena testacea	1	1
R5-01	05/01/2006	Limnebius nitidus	5	3
R5-01	05/01/2006	Helophorus arvernicus	1	1
R5-01	05/01/2006	Anacaena lutescens	2	2
R5-01	05/01/2006	Oulimnius tuberculatus	40	5
R5-01	05/01/2006	Odagmia ornata	2	2
R5-01	05/01/2006	Orthocladius	109	6
R5-01	05/01/2006	Clinotanypus nervosus	1	1
R5-01	05/01/2006	Odontomesa fulva	1	1
R5-01	05/01/2006	Potthastia longimana	1	1
R5-01	05/01/2006	Prodiamesa olivacea	1	1
R5-01	05/01/2006	Cricotopus bicinctus	9	3
R5-01	05/01/2006	Nanocladius rectinervis	1	1
R5-01	05/01/2006	Orthocladius oblidens	4	2
R5-01	05/01/2006	Paratrichocladius rufiventris	8	3
R5-01	05/01/2006	Thienemanniella flaviforceps	9	3
R5-01	05/01/2006	Microtendipes pedellus agg	1	1
R5-01	05/01/2006	Parachironomus gr arcuatus	1	1
R5-01	05/01/2006	Paracladopelma nigritula	1	1
R5-01	05/01/2006	Paratendipes gr albimanus	1	1
R5-01	05/01/2006	Polypedilum scalaenum	3	2
R5-01	05/01/2006	Athripsodes cinereus	1	1
R5-01	05/01/2006	Mystacides azurea	1	1
R5-01	05/01/2006	Triaenodes bicolor	1	1
R5-01	05/01/2006	Anabolia nervosa	2	2
		TOTAL (sum of abundance classes)		99
		total DN (abundance based on abundance class)		9
		total DP+KM (abundance based on abundance class)		46
		total KM (taxa)		19

* Transformed according to (van der Hammen, 1992; Evers *et al*, 2005).

Table A2.5: Positive and negative indicator taxa for watertype R5

Positive dominant taxa	Negative dominant taxa	
Gammarus fossarum	*Anisus vortex*	*Lumbriculus variegatus*
Gammarus pulex	*Arrenurus globator*	*Lymnaea stagnalis*
Gammarus roeseli	*Asellus aquaticus*	*Musculium lacustre*
Glyphotaelius pellucidus	*Bathyomphalus contortus*	*Nais elinguis*
Hydroptila	*Bithynia leachi*	*Ophidonais serpentina*
Hygrobates nigromaculatus	*Bithynia tentaculata*	*Piona pusilla pusilla*
Limnephilius bipunctatus	*Caenis horaria*	*Planorbis planorbis*
Micropsectra	*Chironomus gr annularis*	*Polypedilium gr nubeculosum s.l.*
Nais barbata	*Chironomus gr thummi*	*Polypedilium gr sordens*
Odagmia ornata	*Clinotanypus nervosus*	*Potamopyrgus antipodarum*
Pisidium supinum	*Cloeon dipterum*	*Potamothrix hammoniensis*
Polypedilum scalaenum	*Crangonyx pseudogracilis*	*Proassellus coxalis*
Simulium lineatum	*Cricotopus gr sylvestris*	*Psectrotanypus varius*
	Cryptochironomus	*Radix ovata*
	Endochironomus albipennis	*Radix peregra*
	Erpobdella octoculata	*Radix peregra/ovata soortsgroep*
	Glyptotendipes	*Sigara falleni*
	Gyraulus albus	*Sigara striata*
	Helobdella stagnalis	*Sphaerium corneum*
	Limnesia maculata	*Stylaria lacustris*
	Limnesia undulata	*Tubifex tubifex*
	Limnodrilus claparedeanus	*Valvata piscinalis*
	Limnodrilus hoffmeisteri	

Table A2.6: Critical taxa for watertype R5

Hydropsyche contubernalis	*Nautarachna crassa*	*Rhithrogena semicolorata*
Hydropsyche exocellata	*Nebrioporus depressus elegans*	*Scarodytes halensis*
Hydropsyche pellucidula	*Nemoura avicularis*	*Sericostoma personatum*
Hydropsyche saxonica	*Nemoura cinerea*	*Sialis fuliginosa*
Hydropsyche siltalai	*Nemoura dubitans*	*Sigara hellensii*
Hydroptila cornuta	*Nemurella pictetii*	*Silo nigricornis*
Hydroptila sparsa	*Neureclipsis bimaculata*	*Simulium equinum*
Hygrobates fluviatilis	*Notidobia ciliaris*	*Simulium lundstromi*
Hygrobates longiporus	*Ochthebius metallescens*	*Simulium morsitans*
Ironoquia dubia	*Odontomesa fulva*	*Simulium vernum*
Ithytrichia lamellaris	*Orectochilus villosus*	*Siphlonurus aestivalis*
Kongsbergia materna	*Orthocladius oblidens*	*Siphlonurus armatus*
Laccobius obscuratus	*Orthocladius thienemanni agg*	*Siphlonurus lacustris*
Laccobius sinuatus	*Osmylus fulvicephalus*	*Specaria josinae*
Laccobius striatulus	*Oulimnius major*	*Sperchon*
Lasiocephala basalis	*Oulimnius tuberculatus*	*Sperchon clupeifer*
Lebertia fimbriata	*Oxus setosus*	*Sperchon compactilis*
Lebertia insignis	*Paracladopelma laminata agg*	*Sperchon setiger*
Lebertia porosa	*Paracladopelma nigritula*	*Sperchon turgidus*
Lebertia rivulorum	*Paraleptophlebia cincta*	*Sperchonopsis verrucosa*
Lepidostoma hirtum	*Paraleptophlebia submarginata*	*Sphaerium rivicola*
Leptophlebia marginata	*Paratrichocladius rufiventris*	*Stempellina*
Leuctra fusca	*Pedicia rivosa*	*Stempellinella*
Leuctra nigra	*Platambus maculatus*	*Stictotarsus duodecimpustulatus*
Limnebius crinifer	*Platycnemis pennipes*	*Stylodrilus heringianus*
Limnebius nitidus	*Plectrocnemia conspersa*	*Synorthocladius semivirens*
Limnebius truncatellus	*Polycentropus flavomaculatus*	*Taeniopteryx nebulosa*
Limnephilus centralis	*Polycentropus irroratus*	*Thienemanniella clavicornis*
Limnephilus elegans	*Polypedilum convictum*	*Thienemanniella flaviforceps agg*
Limnephilus extricatus	*Polypedilum gr bicrenatum*	*Thyas palustris*
Limnephilus fuscicornis	*Polypedilum laetum agg*	*Tinodes assimilis*
Limnius volckmari	*Polypedilum pedestre agg*	*Tinodes unicolor*
Lype phaeopa	*Potamophylax cingulatus*	*Tinodes waeneri*
Micronecta poweri	*Potamophylax rotundipennis*	*Torrenticola amplexa*
Micropsectra atrofasciata	*Potthastia longimana*	*Ylodes simulans*
Micropsectra notescens	*Procloeon bifidum*	*Tvetenia calvescens agg*
Micropterna sequax	*Prodiamesa rufovittata*	*Tvetenia discoloripes agg*
Microtendipes pedellus	*Protonemura meyeri*	*Unio crassus*
Mideopsis crassipes	*Protzia eximia*	*Unio tumidus*
Molanna angustata	*Pseudanodonta complanata*	*Velia caprai caprai*
Monodiamesa bathyphila	*Psychomyia pusilla*	*Velia saulii*
Mundamella germanica	*Rheocricotopus chalybeatus*	*Wettina podagrica*
Mystacides azureus	*Rheocricotopus fuscipes*	*Yola bicarinata*
Nanocladius bicolor	*Rheopelopia ornata*	*Zavrelimyia barbatipes*
Nanocladius rectinervis	*Rheotanytarsus*	*Zavrelimyia nubila agg*

Annex 3
Setting boundaries using the EEWAI-protocol

Step 1: Identify qualifying criteria for type-specific reference conditions

Describe the criteria used to identify reference sites for the biological quality element:Identify the specific values or criteria for the relevant hydromorphological and physico-chemical conditions considered to correspond to no, or only very minor, anthropogenic alteration.

Reference sites were selected according to the criteria of Wasson (April, 2006). The Dutch WFD-index does not define exact hydromorphological and physico-chemical conditions which a high status site should meet. However, theoretical values concerning chemical conditions of high-status sites can be distracted from former studies and are described in the table below.

	Dutch WFD-index (KRW-maatlatten	**GIG interpretation**
General statement	High status or reference conditions is a state corresponding to very low pressure, without the effects of major industrialisation, urbanisation and intensified agriculture, and with very minor modification of physico-chemistry, hydromorphology and biology.	*High status or reference conditions is a state corresponding to very low pressure, without the effects of major industrialisation, urbanisation and intensified agriculture, and with very minor modification of physico-chemistry, hydromorphology and biology.*
Diffuse source pollution		
Land-use intensification: Agriculture, forestry	The percentage land-use intensification was calculated for high status sites using GIS. Subsequently, phosphate and nitrogen delivery was analysed. Sites consisting for more than 50% of nature and for less than 4% of urban area, and are loaded with less than 1 kg/ha phosphate and less than 15 kg/ha nitrogen (CBS-data, 2000) have been selected. Only one Dutch site (R-C1) met these requirements: Hierdensche beek, which is loaded with 0.49 kg phosphate and 14 kg nitrogen per hectare per year.	*At least 50% of a reference site can be qualified as nature, the site does not contain more than 4% of urban area, does not exceed 15 kilogram nitrogen per hectare, nor 1 kilogram phosphate per hectare.*
Point source pollution		
Synthetic and non-synthetic pollutants	No point source pollution occurs at the reference site.	*A reference site does not contain point sources.*
Other effluents/ discharges	No discharges are present at the reference site	*No ecological effects by discharges or effluents.*
Morphological alterations		
River morphology	The upperstream of the Hierdensche Beek is only slightly altered by hydromorphological alterations. More alterations are present downstream. This is exceptional for Dutch standards, as almost every Dutch stream has been altered hydromorphologically.	*A reference site may not contain hydromorphological alterations.*
Water abstraction		
	?	?
Flow regulation		
River flow regulation	No flow regulation occurs upstream. Downstream, the flow is regulated due to hydromorphological alterations.	*Absence of significant flow regulation upstream.*

Riparian zone vegetation		
	Qualification of macroinvertebrate reference sites by the Dutch WFD-index (KRW-maatlat) does not consider riparian zone vegetation.	*Riparian vegetation is appropriate to the type and geographical location of the river.*
Biological pressure		
Introduction of alien species	Qualification of macroinvertebrate reference sites by the Dutch WFD-index (KRW-maatlat) does not consider alien species.	*Alien species are not considered.*
Fisheries and aquaculture	No major fisheries or aquaculture are practised at the reference site.	*Fisheries and aquaculture do not influence the structure and functioning of the system.*
Biomanipulation	Biomanipulation does not occur at the reference site.	*Biomanipulation does not occur at the reference site.*
Other pressures		
Recreation uses	The reference site is not intensively used for recreation purposes.	*The reference site is not affected by recreation.*

State whether it was possible to identify reference values for the biological quality element using data from reference sites:

> Only one high state site met the requirements set for reference sites. Biological data was sufficient available for this site; macroinvertebrate samples were taken during 1995-2002 and were mostly qualified as high by the Dutch WFD-index (KRW-Maatlatten).

If it was possible to use reference sites:

Specify which summary statistic (e.g. median value or arithmetic mean) of the values for the biological quality elements at reference conditions were used to quantify reference conditions for the purpose of calculating EQRs.
Specify which summary statistic (e.g. 95 percentile) of the values for the biological quality elements at reference were used to identify the high-good boundary.

> As only one Dutch site could be selected as reference site, no reliable quantitative reference conditions could be defined based on reference sites.

If it was not possible to use reference sites:

Specify the relevant criteria used to define reference values and the high-good boundary (e.g. when using modelling methods; paleolimnological methods; expert judgement; etc.).

> Reference values and the high-good boundary was defined by multivariate analysis (CANOCO 4.0) combined with expert judgement. The gradient obtained from expert judgement was used for analysis of determining hydromorphological and physico-chemical variables. Analysis of these variables resulted in definitions of reference values and the high-good boundary.

Step 2:
Describe how the biological quality element is expected to change as the impact of the pressure or pressures on supporting elements increases[1]; and
Relate this description to the normative definitions.

Specify the relevant pressure or combination of pressures and the associated impacts on the supporting elements that are being considered.

High concentrations of phosphate, and nitrogen to a lesser extend, result in eutrofication. Hydromorphological pressure alters the rate of flow and decreases heterogeneity of river habitats. Rate of flow influences oxygen availability and nutrient concentrations. Low oxygen availability will decrease survival of macroinvertebrates. Pressure from any of these variables will result in a decrease of critical macroinvertebrate species, a decrease in abundance of dominant positive species, and a dominance of tolerant species that influence the EQR negatively.

Specify the quality element(s) being considered.

The quality elements considered are benthic and lothic macroinvertebrates at the level of species. These macroinvertebrate species have been classified into either critical species, dominant positive species, or dominant negative species, all contributing to the EQR-score.

In the form of a conceptual model, describe how the biological quality element(s) is expected to respond as the impact (or impacts) on the supporting elements increases[2]. The conceptual model should be designed to highlight key changes to ecosystem structure and function as anthropogenic disturbance increases.

With increasing pressure there is a gradual decrease of ecological quality:
- decreasing diversity;
- decrease of the ratio sensitive (critical) versus tolerant (dominant negative) species;
- decreasing abundance of positive species and increasing abundance of negative species.

Based on the normative definitions and the conceptual model, provide an ecological description of the condition of the biological quality element at high, good and moderate status.

[1] The direct effects of most pressures are on the supporting elements (i.e. physico-chemical conditions and hydromorphological conditions). The changes in these supporting elements lead to impacts on biological quality elements. Relatively few pressures act directly on the biological quality elements (e.g. fishing). If relevant, the effects of such pressures should be taken into account when using the protocol

[2] An example of a general, rather than a type-specific, conceptual model is set out in Annex B.

High status is characterized by a high abundance of dominant positive species and a high diversity and abundance of critical species. Dominant negative species are nearly absent.
Good status is characterized by a high diversity and abundance of critical species and an increasing abundance of dominant positive species. The abundance of dominant negative species is low.
Moderate status is characterized by a lower number of critical taxa and a dominance of negative species.
Poor status is characterized by dominance of negative (tolerant) species, and absence of critical species or dominant positive species.
Bad status has very low diversity and abundance of macroinvertebrates. The macroinvertebrates that are present are tolerant species.

Step 3: Select suitable metric(s) of the quality element; assess whether the metric(s) responds to the gradient of impact contained in the data set; and quantify the reference conditions for the metric

This purpose of this step is to organise the data in the biological data set so that they describe the way in which the biological quality element responds to increasing impacts (i.e. they describe the degradation curve for the biological quality element)
Select a metric (or metrics) of the quality element that is representative of the effects on the quality element predicted in the Step 2 analysis of the normative definitions.

EQR = {200*(%KM/KMmax) + 2*(100-%DN) + %(DP+KM)}/500

In which:
% KM = relative number of critical species in a sample
KMmax = maximum achievable number of critical species under reference conditions
% DN = relative abundance of dominant negative species
% (DP+KM) = sum of relative abundances of dominant positive species and critical species

Identify a descriptor, or composite descriptor, of the degree of impact on the relevant supporting element or elements, noting that the biological metric(s) may be affected by a combination of impacts on the supporting elements.

The multimetric index is considered to determine impacts on macroinvertebrates sufficiently.

Identify whether the biological metric being considered responds over the whole potential gradient of impact on the supporting element(s). If not, try to find a combination of metrics for the quality element that will together cover the whole spectrum[3]

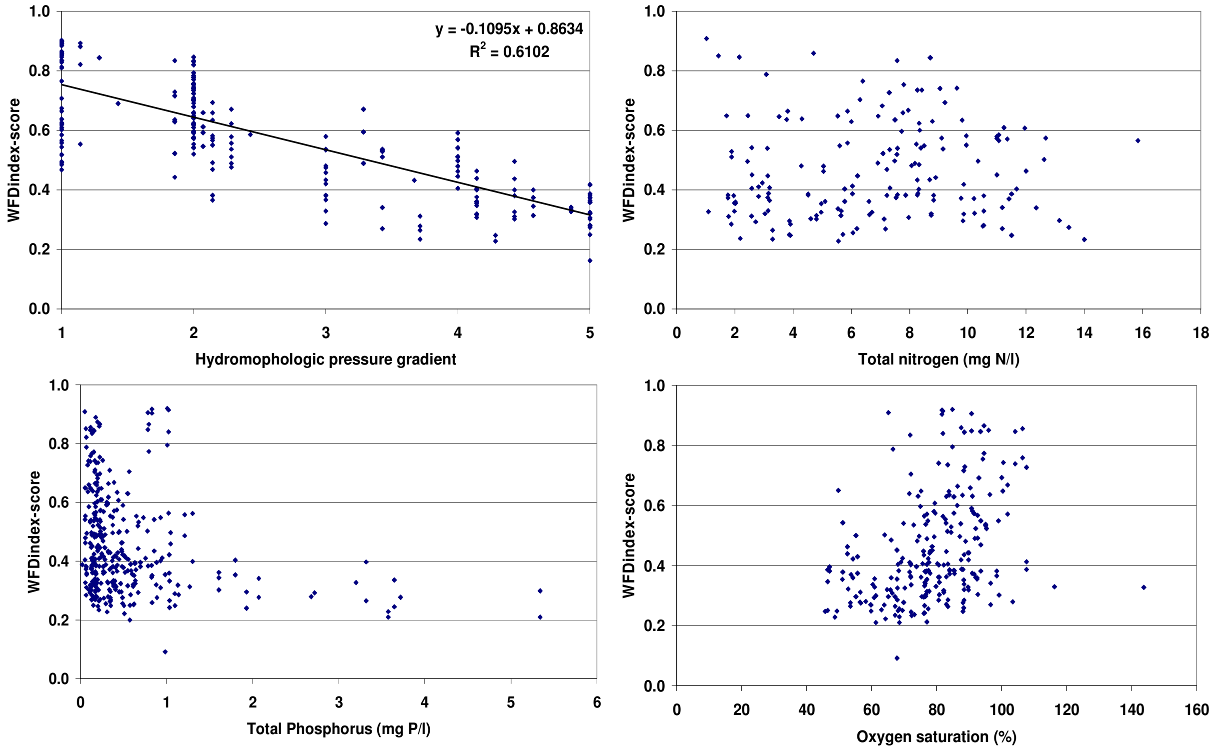
The multimetric index is expected to respond over the whole pressure gradient.

[3] If it is not possible to calculate metrics responding over the whole spectrum of the impact gradient, ensure a metric is selected that shows a response likely to span at least high, good and moderate status.

Collate comparable data on the selected biological metric or metrics from a range of sites subject to varying degrees of anthropogenic impact, including reference sites if possible.

Data from 177 sites, RC-1 and RC-4 water types that are situated in the Netherlands, have been compiled. The estimated ecological status ranges from high to the poor-bad class boundary. The dataset contains 1 site that is classified as reference site following the criteria specified by Wasson (April, 2006). From these sites, 24 sites were classified by the Dutch WFD-multimetric as high status sites, but did not meet the requirements for reference site due to hydromorphologic alterations.

Figure B2.1: The relation of the Dutch WFD-multimetric index with hydromorphologic and chemical pressure gradients

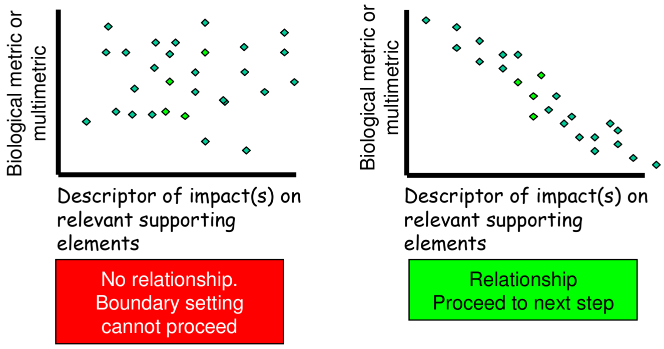


If the metric shows relationships with the impact gradient:
Quantify the reference conditions and the high-good boundary following the procedure outlined in step 1 > Continue with step 4.

The multimetric index showed a fuzzy relationship with the pressure gradients. Quantitative values of pressures, determining the high-good boundary could not be calculated from the gradient shown.

If the metric shows no relationship with the impact gradient represented in the dataset, the boundary setting process for this metric cannot proceed. In such cases:

- the use of another metric of the quality element should be considered;
- the collection of better data on the original metric of the quality element should be considered; and The appropriateness of the way in which the impact gradient has been defined should be considered (e.g. Are other pressures acting? Is the definition of the impact gradient sufficiently type-specific?).



According to the figures above, we unfortunately cannot precede the boundary setting process due to the lack of sufficient linear correlations of the Dutch WFD-multimetric with pressures.

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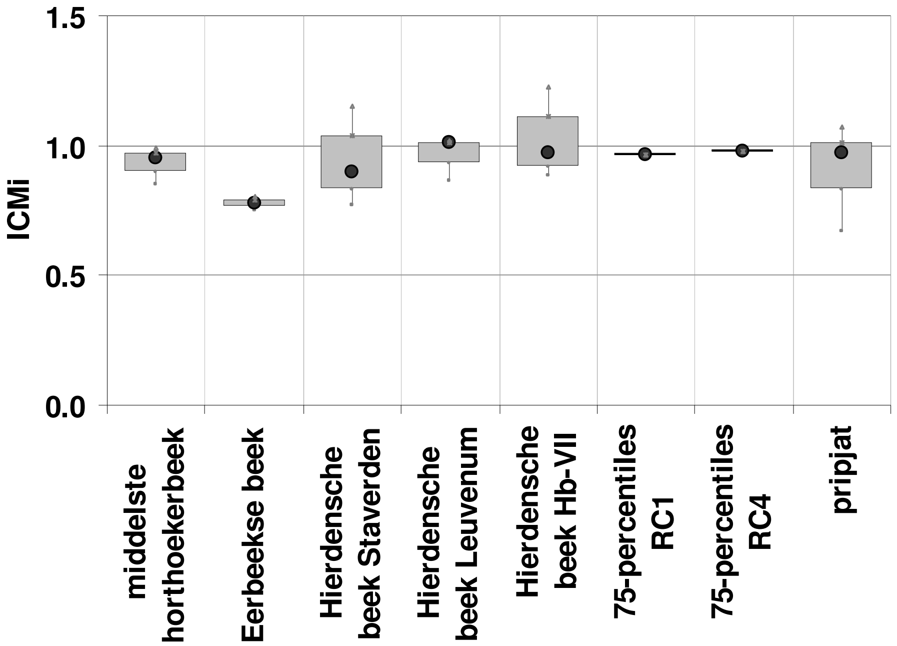
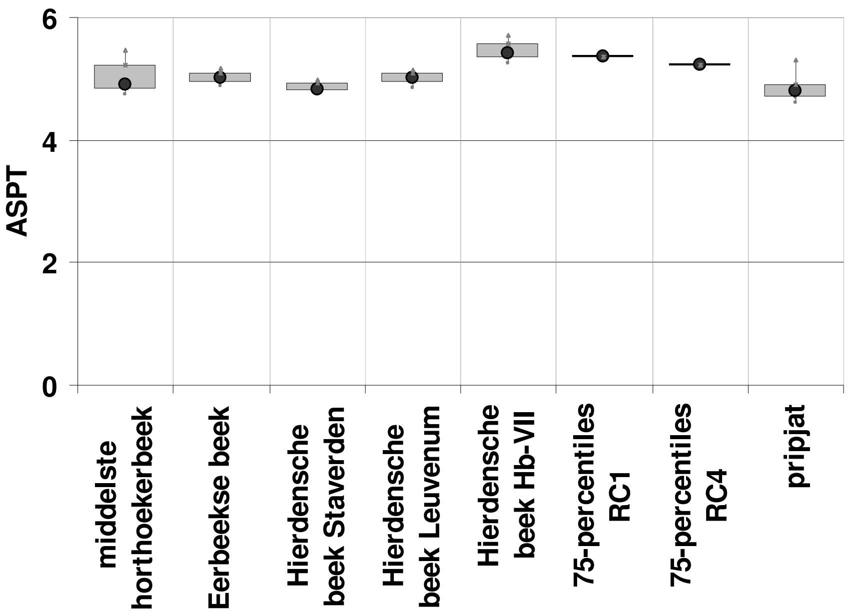
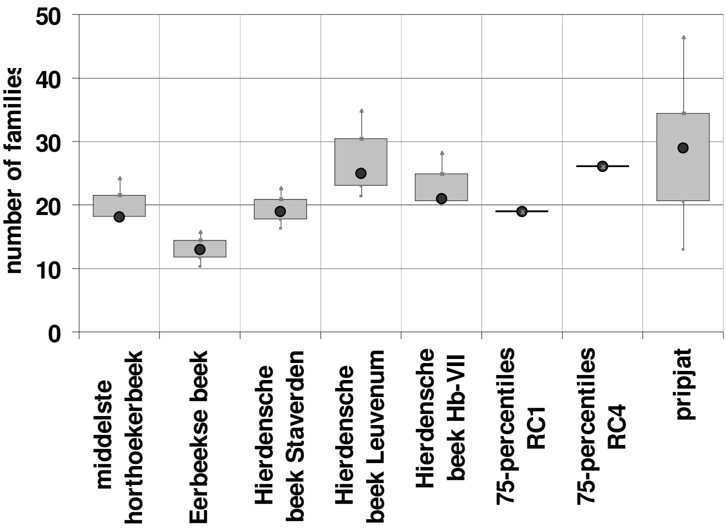
Annex 4
ICMi metric scores of potential Dutch reference sites

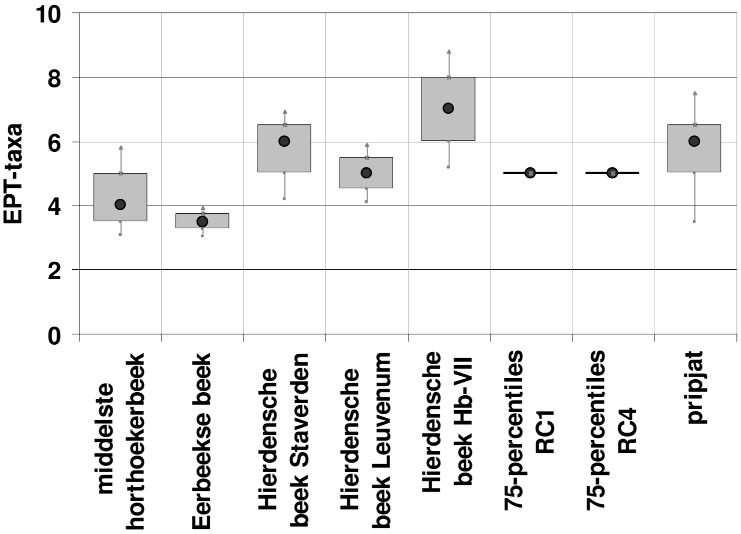
Following figures show ICMi metric scores for potential Dutch reference sites, for 75-percentiles and for the natural reference site Pripjat (Poland). ICMi scores of the potential references are high (ICMi almost equals 1). Scores on the other ICMi metrics are diverse, and poorly comparable. Eerbeekse beek scores most divergent from those of the other sites, as ICMi, number of families and EPT-taxa score low, whereas GOLD-index scores high. Metric scores of 75-percentiles are within the range of the metric scores of potential reference sites. The median values of metric scores of these potential reference sites are lower than the 75-percentiles of the scores of all high quality sites. Using 75-percentiles of high scoring sites for intercalibration will thus be preferable.



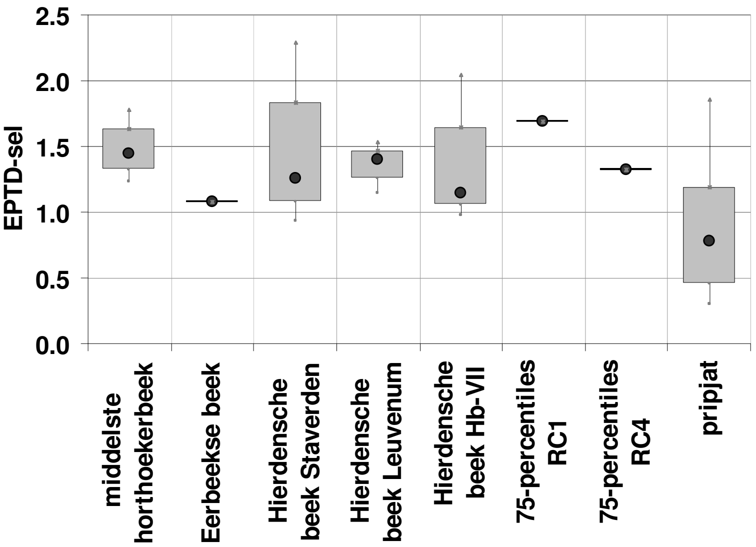




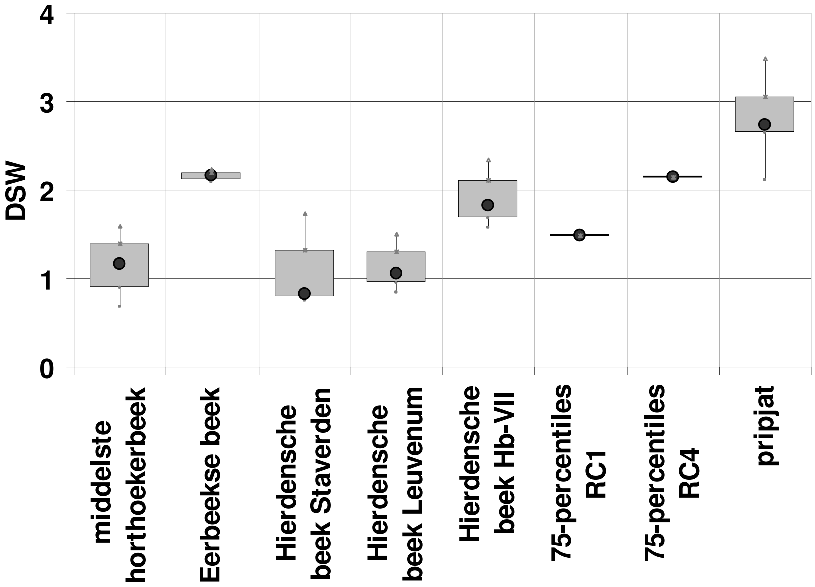
number of families
0
10
20
30
40
50
middelste horthoekerbeek
Eerbeekse beek
Hierdensche beek Staverden
Hierdensche beek Leuvenum
Hierdensche beek Hb-VII
75-percentiles RC1
75-percentiles RC4
pripjat

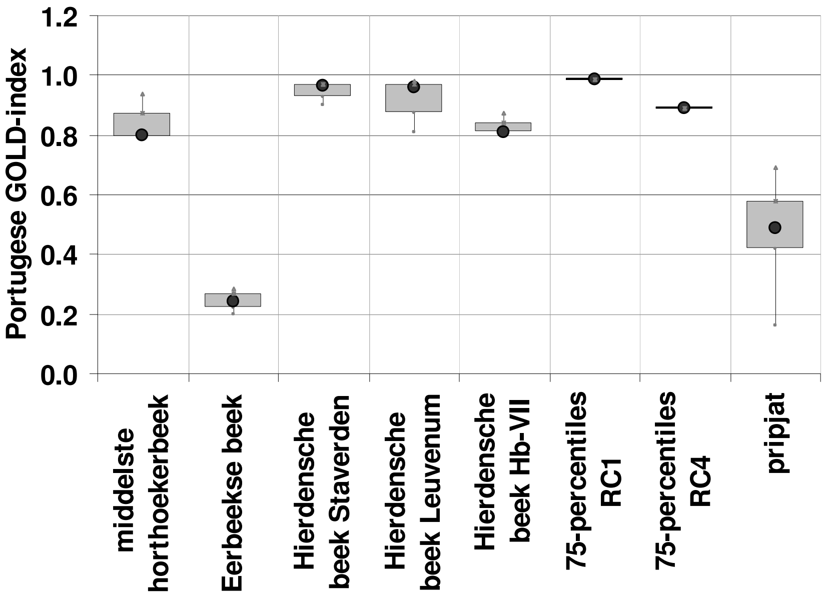


EPT-taxa
0
2
4
6
8
10
middelste horthoekerbeek
Eerbeekse beek
Hierdensche beek Staverden
Hierdensche beek Leuvenum
Hierdensche beek Hb-VII
75-percentiles RC1
75-percentiles RC4
pripjat



EPTD-sel
0.0
0.5
1.0
1.5
2.0
2.5
middelste horthoekerbeek
Eerbeekse beek
Hierdensche beek Staverden
Hierdensche beek Leuvenum
Hierdensche beek Hb-VII
75-percentiles RC1
75-percentiles RC4
pripjat


4
3
2
1
0
DSW
middelste horthoekerbeek
Eerbeekse beek
Hierdensche beek Staverden
Hierdensche beek Leuvenum
Hierdensche beek Hb-VII
75-percentiles RC1
75-percentiles RC4
pripjat


1.2
1.0
0.8
0.6
0.4
0.2
0.0
Portugese GOLD-index
middelste horthoekerbeek
Eerbeekse beek
Hierdensche beek Staverden
Hierdensche beek Leuvenum
Hierdensche beek Hb-VII
75-percentiles RC1
75-percentiles RC4
pripjat

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Annex 5
Statistical comparison between mean metric scores of reference sites of European countries and Dutch '75-percentile reference sites'

Statistical analysis was performed on ICMi (non-normalized) scores and absolute metric scores, calculated from RC-1 and RC-4 reference sites of different European countries. Most of the European countries have selected reference sites to the criteria of Wasson (2006). The Netherlands could not meet these requirements for reference sites due to hydromorphological adjustments. Belgium could neither meet the requirements for reference sites, a problem which they solved by developing theoretical values for reference metric scores. The Netherlands developed metric reference values from Dutch sites with high EQR-scores. In this way, reference values derived from actual metric scores of sites that could be considered as reference sites in view of macroinvertebrate quality.
The metric scores of these '75-percentile reference sites' were compared to metric scores of reference sites of other European countries, using an oneway ANOVA. If variances were not homogene, differences were tested using a Kruskal-Wallis test. Games-Howell post hoc test indicated between which countries the differences in metric scores occurred.

1. Results from RC-1 reference sites analysis

Table A5.1: Results (p-values) of statistical analysis of European RC-1 reference sites scores, indicating if reference site scores differ between EU-countries

Metric	Levene's test	ANOVA	Kruskal-Wallis
ICMi	p=0.008	n.a.	p=0.011
ASPT	p=0.000	n.a.	p=0.000
DSW	p=0.461	p=0.000	
Number of Families	p=0.918	p=0.000	
EPT-taxa	p=0.036	n.a.	p=0.000
EPTDsel	p=0.136	p=0.000	
Portuguese GOLD-index	p=0.001	n.a.	p=0.000

n.a.=not allowed
$p<0.05$ indicates a significant difference

Table A5.2: Results of Games-Howell post hoc test, indicating which countries differ from which considering RC-1 reference site scores. Different marks point out significant differences

	Netherlands	Germany	Denmark	United Kingdom	Belgium	Poland
ICMi	A	AB	AB	B	B	AB
ASPT	A	BC	BC	B	AC	C
DSW	A	AC	C	BC	B	C
Number of Families	B	B	AB	A	AB	B
EPT-taxa	A	B	BC	C	AC	B
EPTDsel	A	AB	AB	B	AB	AB
Portuguese GOLD-index	A	ABC	BC	BC	AB	C

Significant differences between metric values of different EU-countries were found for every metrics (table A5.1). The metric scores of Dutch samples differ significantly from those of the United Kingdom, but are comparable to those of Belgium and Germany for most metrics (table A5.2). Dutch 75-percentile reference sites differ from:

- Belgian and English samples for ICMi;
- all countries except for Belgium for ASPT;
- all countries except for Germany for DSW;
- the United Kingdom for number of families;
- all countries except for Belgium for EPT-taxa;
- the United Kingdom for EPTDsel;
- Denmark, the United Kingdom and Poland for the Portuguese GOLD-index.

2. Results from RC-4 reference sites analysis

Table A5.3: Results (p-values) of statistical analysis of European RC-4 reference sites scores, indicating if RC-4 reference site scores differ between EU-countries

Metric	Levene's test	ANOVA	Kruskal-Wallis
ICMi	p=0.084	p=0.021	
ASPT	p=0.805	p=0.000	
DSW	p=0.560	p=0.000	
Number of Families	p=0.329	p=0.001	
EPT-taxa	p=0.034	n.a.	p=0.000
EPTDsel	p=0.213	p=0.000	
Portuguese GOLD-index	p=0.362	p=0.066	

n.a.=not allowed

p<0.05 indicates a significant difference

Table A5.4: Results of Games-Howell post hoc test, indicating which countries differ from which considering RC-4 reference site scores. Different marks point out significant differences

	Netherlands	Germany	Denmark	United Kingdom	Belgium
ICMi	A	A	A	A	A
ASPT	A	B	B	B	A
DSW	A	AB	B	B	AB
Number of Families	A	AB	B	B	B
EPT-taxa	A	B	BC	C	A
EPTDsel	A	B	B	B	C
Portuguese GOLD-index	A	A	A	A	A

Significant differences between metric values of different EU-countries were found for every metric, except for the Portuguese GOLD-index (table A5.3). The metric scores of Dutch samples differ significantly from those of the United Kingdom and Denmark, but are more comparable to those of Belgium and Germany (table A5.4).

Dutch 75-percentile reference sites differ from:
- non for ICMi;
- all countries except for Belgium for ASPT;
- Denmark and the United Kingdom for DSW;
- All countries except for Germany for number of families;
- all countries except for Belgium for EPT-taxa;
- all countries for EPTDsel;
- non for the Portuguese GOLD-index.

Annex 6
Occurrence of critical taxa in the river Pripjat

The occurrence of critical species at reference sites in the river Pripjat was studied. Ten samples from reference sites in the Pripjat were analysed for Plecoptera, Thrichoptera and Ephemeroptera taxa. Only four of these 22 critical families were present (table A6.1). The abundances of these critical taxa were also low (table A6.2). From these data we conclude that critical species, especially Plecoptera, are even low abundant at lowland reference sites. Especially streams with low velocity and sand substrate often lack these critical EPT-species.

Table A6.1: Most critical families (ASPT-score = 10). The families in white boxes were found in Pripjat samples

Family	Orde
Aphelocheiridae	Heteroptera
Beraeidae	Trichoptera
Brachycentridae	Trichoptera
Capniidae	Plecoptera
Chloroperlidae	Plecoptera
Ephemerellidae	Ephemeroptera
Ephemeridae	Ephemeroptera
Goeridae	Trichoptera
Heptageniidae	Ephemeroptera
Lepidostomatidae	Trichoptera
Leptoceridae	Trichoptera
Leptophlebiidae	Ephemeroptera
Leuctridae	Plecoptera
Molannidae	Trichoptera
Odontoceridae	Trichoptera
Perlidae	Plecoptera
Perlodidae	Plecoptera
Phryganeidae	Trichoptera
Potamanthidae	Ephemeroptera
Sericostomatidae	Trichoptera
Siphlonuridae	Ephemeroptera
Taeniopterygidae	Plecoptera

Table A6.2: Mean number of critical species individuals per Pripjat sample

Family	Total per sample
Brachycentridae	5.64
Heptageniidae	1.79
Leptoceridae	1.98
Phryganeidae	0.1
Total	**9.51**