Northern Rivers GIG Phytobenthos Intercalibration Exercise

Authors:

Martyn Kelly, Amelie Jarlman, Bryan Kennedy, Cathy Bennett, Heikki Mykrä, Juha Miettinen, Kari-Matti Vuori, Maria Kahlert, Mikaela Gonczi.

Table of Contents

Contact details for national representatives/phytobenthos experts:

Amelie Jarlman
Jarlman HB
Stora Tvärgatan 33
22352 Lund, Sweden.
jarlman@mac.com

Bryan Kennedy
Environmental Protection Agency
John Moore Road
Castlebar
County Mayo
Ireland
b.kennedy@epa.ie

Heikki Mykrä
Finnish Environment Institute
Research Programme for Integrated River Basin Management
P.O. Box 413
FI-90014 University of Oulu, Finland
heikki.mykra@ymparisto.fi

Juha Miettinen
juhami@gmail.com
ph. +358400831194

Kari-Matti Vuori
Finnish Environment Institute
Research Programme for Integrated River Basin Management
P.O. Box 413
FI-90014 University of Oulu, Finland
kari-matti.vuori@ymparisto.fi

Maria Kahlert
Dep. of Environmental Assessment
Swedish University of Agricultural Sciences
POBox 7050
SE 75007 Uppsala, Sweden.
maria.kahlert@ma.slu.se

Martyn Kelly
Bowburn Consultancy, 11 Monteigne Drive,
Bowburn, Durham DH6 5QB, UK.
MGKelly@bowburn-consultancy.co.uk

Mikaela Gonczi
Swedish Environmental Protection Agency
(Naturvårdsverket)
SE-106 48 Stockholm, Sweden.
Mikaela.Gonczi@naturvardsverket.se

1. Introduction

1.1. *Objectives*

The **Water Framework Directive** (WFD) establishes a framework for the protection of all waters (including inland surface waters, transitional waters, coastal waters and groundwater). The environmental objectives of the WFD set out that good ecological status[*] of natural water bodies and good ecological potential[†] of heavily modified and artificial water bodies should be reached by 2015.

One of the key actions identified by the WFD is to carry out a European benchmarking or intercalibration (IC) exercise to ensure that good ecological status represents the same level of ecological quality everywhere in Europe (Annex V WFD). It is designed to ensure that the values assigned by each Member State (MS) to the good ecological class boundaries are consistent with the Directive's generic description of these boundaries and comparable to the boundaries proposed by other MS. The intercalibration of surface water ecological quality status assessment systems is a legal obligation, the results of which will be published by the Commission in 2007.

Intercalibration is carried out under the umbrella of Common Implementation Strategy (CIS) Working Group A - Ecological Status (ECOSTAT), which is responsible for evaluating the results of the IC exercise and making recommendations to the Strategic Co-ordination Group or WFD Committee. The IC exercise aims at consistency and comparability in the classification results of the monitoring systems operated by each MS for biological quality elements (CIS WFD Guidance Document No. 14). In order to achieve this, each MS is required to establish Ecological Quality Ratios (EQRs) for the boundaries between high (H) and good (G) status and for the boundary between good (G) and moderate (M) status, which are consistent with the WFD normative definitions of those class boundaries given in Annex V of the WFD.

All 27 MS of the European Union are involved in this process, along with Norway, who has joined the process on a voluntary basis. Expert groups have been established for lakes, rivers and coastal/transitional waters, subdivided into 14 Geographical Intercalibration Groups (GIGs -groups of MSs that share the same water body types in different sub-regions or ecoregions).

The IC exercise aims to ensure that the H/G and the G/M boundaries in all MS's assessment methods for biological quality elements correspond to comparable levels of ecosystem alteration (CIS WFD Guidance Document No. 14). Intercalibration guidance produced by CIS (WFD Guidance Document No. 14) warns that the process will only work if common EQR boundary values are agreed for very similar assessment methods or where the results for different assessment methods are normalised using appropriate transformation factors. Different assessment methods

[*] 'Ecological status' is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V WFD; 'Good ecological status' is the status of a body of surface water so classified in accordance with Annex V.
[†] 'Good ecological potential' is the status of a heavily modified or artificial body of water, so classified in accordance with the relevant provision of Annex V.

(e.g. using different parameters indicative of a biological element) may show different response curves to pressures and therefore produce different EQRs when measuring the same degree of impact (CIS WFD Guidance Document No. 14).

In each GIG, the IC exercise will be completed for those MS that already have data and (WFD compliant) assessment methods to set boundary EQR values for some of the biological quality elements. Countries that do not have data or assessment methods already available, or do not actively participate in the current IC exercise, need to agree with the outcome of the IC exercise and harmonise their assessment methods, taking into account the results of the current exercise, when their data/methods becomes available.

The WFD refers to an 'intercalibration network', comprising sites selected from a range of surface water body types present within each ecoregion, as the basis for intercalibration (Annex V; 1.4.1). For each surface water body type selected, the WFD specifies that at least two sites corresponding to the boundary between high and good status, and between good and moderate status should be submitted by each Member State for intercalibration. However, as the IC exercise evolved, this network has become redundant, as these datasets were too small to permit robust intercalibration.

1.1. *Northern GIG*

The Northern GIG (N GIG) includes (parts of) Finland, Ireland, Norway, Sweden, and UK. Four of these MS are taking part in the phytobenthos IC exercise: Finland (FI), Ireland (IE), Sweden (SE) and the United Kingdom (UK).

Seven[‡] common IC river types were identified for N GIG (**Table 1.1**) and are characterised by the following descriptors:

- catchment area, following System A typology.
- Altitude and geomorphology - three classes: lowland (altitude <200m or below highest coastline), mid-altitude (between lowland and highland), and high (above treeline).
- Alkalinity was used as a proxy for siliceous/calcareous geology, with two classes: low alkalinity (< 0.2 meq/l) and medium alkalinity (0.2-1 meq/l).
- Organic/peat content – two water colour classes: low level (< 30 mg Pt/l) and high level (> 30 mg Pt/l).

However, this river typology was derived primarily for the macro-invertebrate intercalibration. The CB GIG phytobenthos group carried out an evaluation of the CB GIG common IC typology using reference data from eleven participating countries. Their results suggested that the common IC river types for CB GIG did not distinguish between diatom assemblages and consequently the CB-GIG exercise did not use common IC river types. Due to time constraints and the experience of the CB GIG process, the N GIG working group agreed that the "no types approach"

[‡] Nine common river types were initially identified in the N GIG but two types (R-N6 and R-N8 were subsequently deleted because only Norway could assign sites to those river types.

was fit for purpose, providing the data submitted to the exercise fitted one of the N GIG common IC river types.

Table 1.1: Northern GIG common intercalibration river types.

Type	River characterisation	Catchment area (of stretch)	Altitude & geomorphology	Alkalinity (meq/l)	Organic material (mg Pt/l)
R-N1	*Small lowland siliceous moderate alkalinity*	*10-100 km2*	*< 200 m and HC**	*0.2 - 1*	*< 30***
R-N2	*Small-medium lowland siliceous low alkalinity, clear*	*10-1000 km2*	*< 200 m and HC**	*< 0.2*	*< 30*
R-N3	*Small lowland organic*	*10-100 km2*	*< 200 m and HC**	*< 0.2*	*> 30*
R-N4	*Medium/large lowland siliceous moderate alkalinity*	*100-10000 km2*	*< 200 m and HC**	*0.2 - 1*	*< 30*
R-N5	*Small mid-altitude siliceous*	*10-100 km2*	*Between lowland and highland*	*< 0.2*	*< 30*
R-N7	*Small highland siliceous low alkalinity, clear*	*10-100 km2*	*Above treeline*	*< 0.2*	*< 30*
R-N9	*Small – medium mid-altitude siliceous low alkalinity organic (humic)*	*10-1000 km2*	*Between lowland and highland*	*< 0.2*	*> 30*

* - highest coastline
** - Ireland has indicated that they need a higher threshold of 150 mg Pt/l

2. National approaches to assessing ecological status using phytobenthos

2.1. Compliance with normative definitions

Annex V of the WFD treats 'macrophytes and phytobenthos' as a single biological element for the purpose of ecological status assessment and identifies four characteristics of this biological element (taxonomic composition, abundance, likelihood of undesirable disturbances and presence of bacterial tufts) that need to be considered when setting status class boundaries. All MS taking part in the N GIG intercalibration exercise have chosen to develop separate methods for macrophytes and phytobenthos and, in addition, to use diatoms as proxies for phytobenthos. There are, however, differences in national concepts of 'macrophytes' with some MS including larger algae such as *Cladophora* in macrophyte methods whilst others treat these as part of the phytobenthos.

All MS participating in phytobenthos IC were asked to justify their methods in terms of the normative definitions (NDs) and their responses will be considered below. It should be borne in mind that a phytobenthos assessment method does not necessarily need to consider all properties defined in the NDs either because these are considered in a macrophyte method that will be used in parallel with the phytobenthos method or because the MS can demonstrate a relationship between properties defined in the NDs which means that measurement of one property provides an indication of the state of another. In such cases, MS can use a cost-effective method for routine estimation of ecological status whilst, at the same time, demonstrating *de facto* compliance with the NDs.

Table 2.1 shows the extent to which the four properties listed in the NDs are incorporated into the national assessment methods. All methods assess taxonomic composition of diatoms alone, however, Ireland and UK have also evaluated the potential for using non-diatoms (Kelly *et al*., 2006a; Kelly, 2006).

Abundance is problematic. Finland and Sweden report that abundance is assessed, but both measure relative, rather than absolute abundance of diatom taxa. Relative abundance is assessed by Ireland and the UK but neither regard this as an assessment of abundance within the meaning of the NDs. The relationship between taxonomic composition, abundance and ecological status was assessed by Ireland and the UK as part of a joint project. The results of this project revealed a relationship between EQR and the upper 90th percentile of biomass measurements, suggesting that the trophic gradient determined the upper limit of biomass at a site but that other factors acted locally to reduce this (Kelly *et al*., 2006a). These findings are broadly in line with those found in other studies (Bernhardt and Likens 2004, Pan *et al.,* 1999, Biggs & Close, 1989; Biggs, 1996) and suggest that routine evaluation of absolute abundance may not yield significant extra information about ecological status.

This suggests that the requirement for assessment of abundance as outlined in the NDs might be better served by macrophyte survey methods, particularly where these include macroalgae. Phytobenthos biomass is very spatially and temporally heterogeneous and therefore quantitative assessment is unlikely to yield detailed insights about ecological status at low or moderate pressure levels. However, at higher pressure levels, visually-obvious growths of macroalgae such as *Cladophora* are likely to be conspicuous, often at the expense of macrophyte diversity more generally, and routine assessment of such growths using straightforward survey techniques may well yield more useful information than quantitative assessment of phytobenthos abundance.

'Undesirable disturbances' are not defined any further in the WFD itself, but ECOSTAT (2005) defines an undesirable disturbance as: 'a direct or indirect anthropogenic impact on an aquatic ecosystem that appreciably degrades the health or threatens the sustainable human use of that ecosystem.' None of the participants in N GIG phytobenthos consider this to be assessed as part of their national methods. Several of the examples of 'undesirable disturbances' listed in ECOSTAT (2005) relate to the effects of macrophytes and phytobenthos on other biological elements, however, it is difficult to differentiate between direct effects of the pressure gradient on these biological elements and interactions with other biological elements.

Similarly, assessment of 'bacterial tufts' are not included directly in any of the assessment systems evaluated here although Sweden includes these growths in other parts of their overall assessment method. Again, a precautionary approach to boundary setting should ensure that the probability of such growths should be minimal when ecological status is good or better.

The view of the phytobenthos expert groups both in N GIG (like Central Baltic GIG) is that if a precautionary approach to boundary setting is taken using other properties (e.g. taxonomic composition), then the probability of undesirable disturbances and bacterial tufts should be minimal when ecological status is good or better.

Table 2.1: Northern GIG phytobenthos methods: compliance with WFD normative definitions. ✓ = assessed as part of national metric; X = not included in national metric; 0 = assessed but not included in national metric.

MS	Taxonomic composition	Abundance	Undesirable disturbances	Bacterial tufts
FI	✓	✓	**X**	**X**
Comment	Diatoms only.	Relative abundance of diatom taxa.		
IE	✓	**X**	**X**	**X**
Comment	See comments for UK.	See comments for UK.	See comments for UK.	See comments for UK.
SE	✓	✓	**X**	**0**
Comment	Diatoms only.	Relative abundance of diatom taxa. Percent cover of all benthic algae noted on field protocol, and used in expert assessment of status class.		Noted in field protocol, used in expert assessment of status class.
UK	✓	**X**	**X**	**X**
Comment	Diatoms only. The relationship between diatoms and other algae has been tested (Kelly et al., 2006b; Kelly, 2006). Macroalgae are included in the UK macrophyte method.	There is a negative relationship between EQR and abundance (as chlorophyll a concentration) but abundance is not measured routinely and was not used to set status class boundaries – see Kelly et al. (2006b).	Undesirable disturbances have not been considered.	Bacterial tufts have not been considered.

2.2. Evaluation of taxonomic composition

Only two national metrics are currently being used in N GIG by the four participating MS (**Table 2.2**), both of which use existing metrics based on weighted averaging to relate taxonomic composition to ecological status (**Table 2.1**).

Table 2.2: National metric/assessment methods for Northern GIG phytobenthos intercalibration.

MS	National metric
FI/SE	Indice de Polluosensibilité (IPS) (Coste, in CEMAGREF, 1982).
IE/UK	Revised form of Trophic Diatom Index (TDI) (Kelly et al., 2006b)

2.3. Placement of status class boundaries

The metrics used by MS convert the response to a pressure gradient into a continuous variable which then has to be converted into an EQR, computed from Observed (O) and Expected (E) values. MS adopted a variety of approaches to split this EQR scale into separate status classes. **Table 2.3** summarises these approaches.

The NDs define high, good and moderate status in terms of their deviation from the biota expected at the reference state and, therefore, a national method, if it is to be compliant with the NDs, has to be able to express each status class in terms of change from the reference state.

Table 2.3: Rationales for defining phytobenthos high/good and good/moderate class boundaries in Northern GIG.

	High / Good Boundary	Good / Moderate Boundary
FI	High/good boundary: IPS=17	Good/moderate boundary: IPS=15
	Preliminary national boundaries for IPS are based on the study of Eloranta & Soininen (2002). The study was based on data of 56 streams with varying degree of alteration of water chemistry. Streams were first classified into five classes according to land use and alteration of water chemistry: 1) near pristine streams with only minor degree of human activities in drainage area, 2) good quality streams with some forestry activities and low degree of agriculture, but with low load of nutrients or suspended materials, 3) moderate quality streams with moderate degree of agriculture and forestry or/and more dense populated areas, 4) poor quality streams with more intense agriculture and forestry, fish farming or small waste water plants, 5) bad quality streams loaded with effluents from different sources. However, none of the studied sites were heavily polluted. Boundaries for ecological quality classes for IPS were then derived from this classification.	
IE	The high/good boundary is set at the 75th percentile of EQR values for reference sites within a particular type. See comments for UK	'Crossover' between nutrient-sensitive and nutrient-tolerant species (Pollard and van de Bund, 2005).
SE	High/good boundary: IPS=17,5 High status: River/stream fulfils the national reference criteria, e.g. Tot-P < 10 µg/l or no eutrophication (area-specific loss of Tot-P = class 1); no acidification, < 20 % agriculture and < 0,1 % urban area. If water colour > 100 mg Pt/l: Tot-P < 20 µg/l.	Good/moderate boundary: IPS=14,5 The G/M boundary was set to the IPS value where the nutrient tolerant and pollution tolerant species exceed a relative abundance of ca. 30 % (and the amount of sensitive species falls below ca. 30 %).
UK	The high/good boundary is set at the 75th percentile of EQR values for reference sites within a particular type.	'Crossover' between nutrient-sensitive and nutrient-tolerant species (Pollard and van de Bund, 2005).
	Biological metrics tend to show gradual change as the level of nutrient/organic pressure increases, with no distinct discontinuities that could act as criteria for setting class boundaries. An alternative approach – based on the proportions of nutrient-tolerant, nutrient-sensitive and indifferent taxa within samples – was used to define status class boundaries in the UK, with the good/moderate boundary set at	

	High / Good Boundary	Good / Moderate Boundary
	the point where the proportion of sensitive taxa falls below that of tolerant taxa. In ecological terms, the diatom flora at high and good status is characterised by a number of taxa, often with relatively broad niches (e.g. *Achnanthidium minutissimum, Fragilaria capucina*) which occur at different phases of a microsucession from colonisation of bare rock up to a mature biofilm (see Biggs *et al*., 1989). At high status, these are accompanied by other nutrient-sensitive taxa but as nutrient concentrations increase, the most sensitive of these taxa disappear whilst the numbers of nutrient tolerant taxa increases. The ‘crossover’ is, therefore, the point at which the taxa which form the ‘association’ characteristic of a site in the absence of pressure become subordinate to taxa which are favoured by a pressure (nutrients, in this case). The EQR gradient below the good/moderate boundary is then divided into three equally-spaced portions from which the moderate/poor and poor/bad boundaries are derived.	

3. Test datasets

A summary of the number of sites available in each quality class (including reference sites) from each MS is presented in **Table 3.1**. In the N GIG, seven common IC river types were defined (**Table 1.1**). The data submitted for the IC exercise was required to fit into one of these seven IC common river types defined by N GIG even though the expert group also agreed to consider intercalibrating using a common river types approach (as described in **Section 6.1**). Those parts of UK which met criteria for N GIG tended to occur in regions well away from large towns and, consequently, the datasets had relatively few sites with status classes that were moderate or lower. The UK dataset used for intercalibration is, therefore, composed of sites that fulfil criteria for either N GIG or CB GIG in order to cover the entire status gradient. The national assessment systems use a site-specific prediction of expected values which compensates for any typological differences between N GIG and CB GIG sites.

Also the SE dataset is composed like the one from UK. The national approach for SE includes only one type, as there were no significant differences between reference values.

Table 3.1: Number of reference sites and phytobenthos samples available in each quality class from each Member State in the Northern GIG.

	Reference	H	G	M	P	B	Total
FI	66	79	23	10	4		116
IE	36	139	33	18	6	1	197
SE	61	82	16	24	4	1	127
UK	69	454	394	438	124	6	1,416
Total	232	718	466	490	138	8	1,856

4. Standardisation of reference conditions

4.1. Introduction to Reference Conditions

The concept of 'type-specific reference conditions' is central to the WFD as ecological status is defined in terms of deviation from the biota expected under such conditions. Different interpretations of 'reference conditions' may lead to different values being used as the denominator in EQR calculations leading, in turn, to the same 'observed' biota having different ecological status assessments. On the other hand, the WFD also recognises that the 'expected' biota will vary from place to place depending on local factors such as climate, underlying geology and stream order and this too will have an effect on ecological status class boundaries. The challenge facing the IC exercise is to differentiate between those differences in national reference states that reflect genuine biogeographical variability across the GIG and those that reflect differences in approach by those responsible for implementation.

Evaluation of reference conditions and principles of setting classification boundaries within the GIGs assumes a cascade of effects, with alterations to catchments (removal of natural vegetation, replacement by agriculture or urban development) leading to increases in pressure variables in surface water which, in turn, affect the biota. Ideally, evaluation of reference conditions focuses on changes to the catchment, and incorporates data on land use and supports this with data on pressure variables (nutrients, BOD etc). The final approach – use of the biota to define reference conditions – is not encouraged as the NDs define ecological status in relation to the biota expected under undisturbed conditions (Annex V, article 1.2) and the use of land-use and pressure data to define 'undisturbed conditions' ensures rigour and objectivity in the definition of the 'expected' value.

In common with most members of CB-GIG, N-GIG participants used the median metric values of reference samples as the 'expected' value. This is a more stable property than alternatives (e.g. use of 95^{th} percentile values), especially when the population of reference sites is small; however, one consequence is that a number of high status sites will have EQR >1. In such cases, EQR values >1 can be automatically set to 1 for reporting.

4.2. Reference screening procedures

The phytobenthos expert group adopted an approach that is consistent with other Intercalibration working groups (Central Baltic (CB) GIG phytobenthos and N GIG/CB GIG macro-invertebrate groups) to define what is meant by reference conditions. Member States followed REFCOND guidance (Working Group 2.3 - REFCOND Guidance Document No 10.) when initially choosing reference sites. A list of the more detailed criteria and type-specific concentrations ("reference thresholds") of key chemical parameters were developed by the N GIG macro-invertebrate working group for rivers. The thresholds aim to interpret the WFD requirement of "very minor anthropogenic impact".

Representatives from each MS were asked to screen reference sites, chosen using REFCOND guidance, against agreed catchment land use and chemical reference thresholds. The thresholds (**Table 4.2)** were principally derived from datasets linking invertebrates to general chemical elements, but other values taken from national water quality classifications, diatoms datasets (in the case of nutrients), specific studies and expert opinions were also considered. The proposed reference thresholds allow the same criteria to be applied to the selection of all reference samples used in the IC exercise in N GIG rivers and were intended for use in conjunction with other general pressure criteria. Both mean values and 90- or 95-percentile values were proposed for some parameters. The mean is the most robust statistic when few data are available, as is frequently the case for new reference sites. The 90^{th} or 95^{th} percentile should be used only when sufficient data are available (at least 12 monthly chemical samples).

Table 4.2: Northern GIG guidelines for physico-chemical characteristics and general characteristics of reference river sites. Physico-chemical values to be regarded as maximum threshold values for screening reference sites. Values may vary according to national typologies. *Cf.* Appendix Table A1 for guidance from REFCOND and N GIG on reference sites.

Quality Element of Characteristic	Concentration or Descriptor at Reference Condition	Countries Using this Criterion
Pollution Status	Pristine, Unpolluted	ALL
Organic Waste Load	No Observed Effect	ALL
Nutrient Loads	Background	ALL
90%ile B.O.D.	< 2.7 mg/l	IE
Mean BOD	<1.6	IE
Dissolved Oxygen	Close to 100% (>80% and < 120% saturation at all times)	IE, FI
95%ile Non-ionised Ammonia (mg/l N)	Compliant with the Freshwater Fish Directive National Regulations	IE, FI
Annual Mean total Ammonium (mg/l N)	Compliant with the Freshwater Fish Directive National Regulations for total ammonium	IE, FI
95%ile Total ammonium (mg N/l)	<0.04 mg/l	IE, FI, SE
Annual Median ortho-Phosphate	<0.015 mg P/l	IE, UK, SE
Annual Mean ortho-Phosphate	<0.03 mg P/l	IE, UK, SE
Annual mean total P	R-N1 < 20 ug/l R-N3 < 30ug/l R-N4 < 18 ug/l R-N5 <18 ug/l	SE, FI,
Annual Mean Nitrate (mg N/l)	< 1.6 mg N/l	SE, IE, UK, FI
Annual Mean Total N (mgN/l)	<1.8 mg N/l	FI, SE

Table 4.3 indicates which of the N GIG defined reference criteria were used for the screening exercise and what sources of information were available to each MS for this purpose. Member States were also asked to indicate if they used more stringent criteria (or different but equivalent ones).

Table 4.3: Criteria used by Member States for phytobenthos reference site selection in the Northern GIG. Key: 0: missing info; 1: not used; 2, Yes, Measured; 3, Yes, Estimated; 4, Yes, Field inspection; 5, Yes, Expert judgement.

	Landuse	BOD_5	O_2	$N\text{-}NH_4$	P- fraction	$N\text{-}NO_3$	Comments
FI	5	0	0	2	2	2	water chemistry not available for all sites
IE	2	2	2	2	2	2	See paragraph below
SE	2	1	1	1	2	1	
UK	2	0	0	2	2	2	

The following paragraphs give a more detailed description of the screening exercise for reference sites as undertaken by each MS:

Finland:
The main pressure criteria are: no major point sources, agriculture and forestry in catchment upstream of reference sites of low intensity (< 10% agriculture in total catchment area, no large clear cuts, mainly judged from visual observation of GIS land-use), Total P median concentration < 20 µg l-1. Experts from the regional environmental centres were used in the final determination.

Ireland:

Reference screening in Ireland was carried out by selecting reference sites for which maximum catchment land cover limits were below an agreed percentage, as carried out for the NGIG invertebrate intercalibration exercise. The CORINE Land Cover dataset was used to provide an estimate of the upstream land cover using ESRI's Arc View 3.2a GIS software. Water chemistry results for these selected sites were extracted from the Agency's water quality database, for sites where suitable water chemistry existed. Sites that did not meet the criteria for reference site water quality set out in **Table 4.2** were removed from the list. Potential reference sites were also compared against their rTDI score (national metric for phytobenthos) and Q-Value (national metric for invertebrates). The final selection was found to have an rTDI score indicative of high status and a Q-Value of 4.5 – 5, also indicative of high status.

Sweden:

For the N-GIG, we used the following screening factors for a reference stream:
1a) < 10 µg/l Tot-P
1b) IF colour was high (> 100 mg Pt/l), then < 20 µg/l Tot-P
2) pH > 6

United Kingdom:

A database of SEPA-monitored diatom sites (which comprise the majority of N GIG sites in the UK) was used as the basis for reference site selection in the N GIG phytobenthos Intercalibration exercise. Sites were initially assigned to N GIG river types following the descriptors outlined in **Table 1.1**. Expert judgement was used in a minority of situations to make allowances for sites that were marginally outside the upper and lower threshold limits for N GIG river type descriptors. Colour data was not available to distinguish between the two water colour classes.

Screening for physico-chemical and landuse characteristics was carried out for all sites in the SEPA database in the initial stages of the selection process. The full process of reference site selection and validation is described as follows:

1. *Landcover 2000* data obtained for the SEPA database of sites was used as the basis for the landuse screening exercise. With the exception of forestry, the maximum landuse threshold limits used followed the guideline threshold limits for N GIG defined in **Table 4.1**; these were as follows:
   - Arable: 10%
   - Permanent crops: 15%
   - Pasture: 30%
   - Forestry*: 30% (Central Baltic GIG threshold substituted)
   - Urban fabric: <0.8% of catchment

   **Landcover 2000* does not distinguish between (semi-)natural woodland and plantations. A threshold value of 30% forestry was used as a proxy for the N GIG guideline of <5% clear-felled/planted forest.
2. The maximum chemical threshold values for screening of reference sites were as follows (*cf*. **Table 4.2**):
   - Soluble Reactive Phosphorus: 30 ug l^{-1}
   - Nitrate-N: 1.6 mg l^{-1}
3. Following the landuse/physio-chemical screening, expert judgement was used to review the list of proposed reference sites. In addition, the characteristics of each site was validated using the *SEPA GIS interactive Map* to check the proximity of potential sources of point/diffuse inputs, morphological alterations and biological/recreational pressures; any additional information logged against site locations was also taken into account.
4. Sites known to be influenced by acidification and with pH<6 were also eliminated from the selection.

5. The final step in the validation of the N GIG reference sites was on the basis of the revised TDI calculation. Any potential reference sites with revised TDI scores > 50 were removed to ensure that the final selection of sites did not include those influenced by elevated nutrient concentrations.

5. Development of Common Metric

In order to compare status class boundaries developed in each MS, national metrics first had to be converted to a common scale. The mechanism for doing this was to develop an 'intercalibration common metric' (ICM) (corresponding to Option 2 outlined in the Boundary Setting Protocol) similar to that developed for the CB GIG invertebrate IC exercise (Buffagini *et al*., 2005). This ICM should have a statistically-significant relationship with each national metric so that EQR values computed using national metrics can be quoted as the corresponding value of the ICM. In the case of N GIG phytobenthos, there was a high degree of congruence between national methods with common sampling and analysis methods (CEN, 2003, 2004; Kelly *et al*., 1998), and relying on the fact that both metrics used for the exercise are based on the weighted average (WA) equation of Zelinka and Marvan (1961).

5.1. Evaluation of Candidate Metrics

N GIG used a slightly different ICM to that used in CB GIG, although it is based on identical principles. The N GIG ICM is composed of two metrics developed in Austria: Trophien Index (TI) and Saprobien Index (SI). The N GIG ICM had two advantages over the CB GIG ICM:

1. Neither component metric is used by any participant in N GIG, so the ICM is independent of national methods (something that CB GIG were unable to achieve).

2. When tested against the national metrics, the N GIG ICM also had a better relationship with the IE and UK national metrics than the CB GIG ICM (composed of the TI and IPS).

Two variants of the N GIG ICM were tested – one based on the mean of the two component metrics (TISI-mean) and the other based on the minimum (TISI-min). Relationships between national metrics and the ICMs (TISI-mean and TISI-min) were evaluated using identical criteria to those used in CB GIG. These were as follows:

a. Nationally agreed assessment system and boundary values;

b. At least six reference samples (representing at least four sites);

c. A statistically-significant linear relationship with the ICM. More particularly:

- Root mean square error (RMSE) ≤ 0.15
- Coefficient of determination (r^2) ≥ 0.5; and,
- Slope ≥ 0.5 and ≤ 1.5.

The coefficient of determination (r^2) measures association between two variables and gives little indication of the predictive power of that relationship. It is also dependent, to some extent, on the length of the gradient over which the coefficient is applied (see **Fig. 5.3**). RMSE, on the other hand, gives a better indication of the predictive power of the relationship, regardless of gradient length Using both, along with visual examination and slope, provides a robust basis for evaluating relationships between national metrics and the ICMs.

The properties of the relationships are shown in **Table 5.1**. FI and SE metrics showed a stronger relationship with TISI-mean whilst UK and IE had a stronger relationship with TISI-min.

Table 5.1: Regression properties for national metrics versus ICMs for the four national datasets used in the N GIG phytobenthos intercalibration exercise.

		TISI-mean			TISI-min		
	n	**r^2**	**RMSE**	**slope**	**r^2**	**RMSE**	**slope**
FI	112	0.601	0.0945	1.31	0.6292	0.115	1.692
IE	197	0.3716	0.129	0.4865	0.4063	0.157	0.64
SE	122	0.84	0.053	0.7	0.846	0.052	0.54
UK	920	0.562	0.141	0.72	0.612	0.133	0.834

5.2. Evaluation of the Intercalibration Common Metric

Table 5.2 shows the relationship between ICM-min and ICM-mean and nitrogen and phosphorus fractions. Note that the primary purpose of an ICM is to allow values of national metrics to be compared, so the performance characteristics in **Table 5.1** are more instructive for the purposes of selecting an ICM but **Table 5.2** helps to illustrate the relationship between the ICMs and the underlying nutrient / organic gradient.

Table 5.2: Correlation coefficients between nutrients and the minimum (‘min’) and mean (‘mean’) intercalibration metric (TISI) in the Northern GIG phytobenthos Intercalibration exercise. ‘SRP’ = soluble reactive phosphorus (≈ PO_4-P); ‘NOx’ = nitrogen oxides (≈ NO_3-N + NO_2-N).

Member State	Determinand	Data Type	TISI-min	TISI-mean
FI	Log Total N	Median	-0.466***	-0.505***
FI	Log Total P		-0.466***	-0.505***
IE	Log NOx	Spot	-0.5405*	-0.5211**
IE	Log PO_4-P		-0.3597*	-0.3391
SE	Log NH_4-N	Mean	-0.43***	-0.47***
SE	Log Total N		-0.75***	-0.76***
SE	Log NOx		-0.74***	-0.75***
SE	Log Total P		-0.81***	-0.83***
SE	Log PO_4-P		-0.81***	-0.83***
UK	Log NO_3-N	Mean	-0.604 ***	-0.610 ***
UK	Log NOx		-0.515 ***	-0.508 ***
UK	Log SRP		-0.659 ***	-0.648 ***

Significance level: P < 0.05: *; P < 0.01: **; P < 0.001: ***

5.3. Conversion of national metrics to the ICM

For each MS, the N GIG ICM was calculated as follows:

a. EQR values based on SI and TI values were calculated using MS data.

b. The expected value for each EQR value is the median of reference values for the MS.

c. Two ICMs were calculated: one as minimum of TI and SI (TISI-min) and one as the mean of TI and SI (TISI-mean)

d. The regression between the ICMs and the national metric was plotted – based on all sites in H, G and M only (some national datasets had non-linear relationships with the dataset and using just H, G and M confined the relationship to the linear portion). The regression equation and associated statistics (r^2, root mean square error, slope) were calculated (**Table 5.1**).

e. Once the linear relationship was confirmed, values of the national metric representing the High / Good and Good / Moderate boundaries were converted to corresponding values of the ICM for both ICMs. The procedure for doing this is identical to that used in the CB GIG invertebrate IC exercise and is based on a linear regression equation:

   ICM = a + b(national metric as EQR)

   Where: a = constant; b = slope.

   Figure 5.1 shows a regression between the EQR values of a national metric and the ICM for a hypothetical national dataset and illustrates the process of converting the national value of the Good/Moderate boundary to the ICM.

   A single relationship was computed for each national dataset and this relationship was used to convert boundary values for each national type to the ICM.

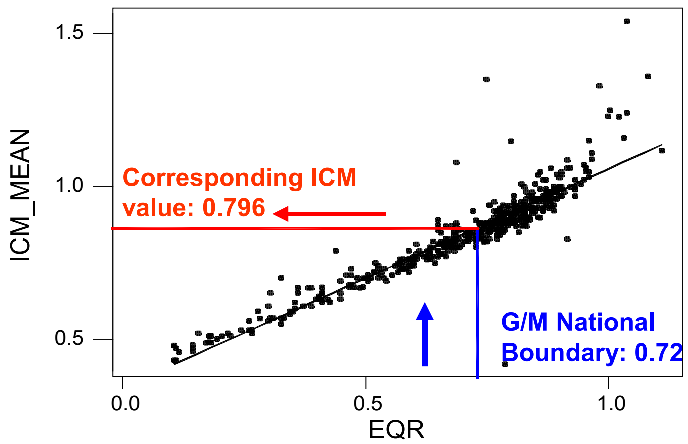

Figure 5.1: Conversion of the Good/Moderate national boundary value for a hypothetical national dataset into an ICM value using the regression formula: ICM = a + b(national metric as EQR).

6. Comparison of boundaries and harmonisation

6.1. Overview of results

The acceptable range of boundary values was calculated by identical criteria to those used in CB GIG, as the median boundary value ± 0.05 EQR units for all MS who fulfilled the statistical criteria described in **Section 5.1**. However, as only four countries are included in the exercise, the statistical power of the exercise is relatively low, and results are presented with an acceptable band based on boundary values for all four MS as well as with an acceptable band based on just those that fulfil the statistical criteria.

Table 6.1 shows a detailed breakdown of results for the high/good and good/moderate boundary for both ICMs. **Table 6.2** presents the results of the intercalibration in terms of the relationship between national boundaries and the limits of the 'acceptable band'. SE boundaries are high for all tests performed using TISI-min (but, as these lie above the 'acceptable band' there are no implications for harmonisation). FI and IE were both marginally below the acceptable band for one of the comparisons. Experience from CB GIG suggests that both differences lie within the statistical limits of the exercise; again, there are no implications for harmonisation. Each of these cases is considered in more detail in **Section 6.2**.

Table 6.1: Boundary values for national methods involved in the N GIG phytobenthos intercalibration exercise.

	H/G			G/M		
	National metric	**TISI-mean**	**TISI-min**	**National metric**	**TISI-mean**	**TISI-min**
FI	0.912	0.892	0.804	0.804	0.751	0.622
IE	0.93	0.846	0.762	0.78	0.773	0.666
SE	0.89	0.905	0.930	0.74	0.800	0.850
UK	0.93	0.898	0.804	0.78	0.790	0.679
Acceptable bands						
	All MS			**All MS**		
	Median	0.895	0.804		0.782	0.673
	Upper limit	0.945	0.854		0.832	0.723
	lower limit	0.845	0.754		0.732	0.623
	Excluding IE			**Excluding IE and FI**		
	Median	0.898	0.804		0.795	0.679
	Upper limit	0.948	0.854		0.845	0.729
	lower limit	0.848	0.754		0.745	0.629

Table 6.2: Implications for harmonisation in the N GIG phytobenthos intercalibration exercise.

	H/G		G/M	
	TISI-mean	**TISI-min**	**TISI-mean**	**TISI-min**
Acceptable band based on all MS				
Inside acceptable band	All	FI, IE, UK	All	FI, IE, UK
Above		SE		SE
Acceptable band based on those MS that fulfill statistical criteria				
Inside acceptable band	FI, SE, UK	FI, IE, UK	All	IE, UK
Above		SE		SE
Below	IE			FI

6.2. Detailed comments

FI: Finland

The national assessment methods for diatoms are under development. The Finnish classification is, therefore, only preliminary and IPS values for class boundaries will be re-evaluated. There was also a wide variation in IPS values among reference sites, indicating that stratification for natural background variability might also be needed. Preliminary results have shown that the stream typology used for macroinvertebrates may not be useful for diatoms. Alternative typologies should thus be considered. Also metrics other than IPS should be tested in near future.

IE: Ireland

Ireland has a low coefficient of determination in the regression between the national EQR and ICM; with the ICM based on TISI-min ($r^2 = 0.4063$) being slightly better than that observed for the TISI-mean ($r^2 = 0.3716$). These regression statistics are lower than that obtained for other member states, including the UK, with whom IE shares a common national metric. These lower regression statistics are probably influenced strongly by several aspects inherent in the IE dataset. The IE dataset is heavily weighted towards the higher quality classes (see **Table 3.1**) with approximately 84% of the sites in high and good status. The number of alkalinity values necessary for the calculation of the national EQR was limited, and when estimated from conductivity for lower alkalinity sites some error in the EQR would be expected. Default rather than measured alkalinity values were also used in the EQR calculation for a large proportion of the sites.

Low correlation coefficients between the ICMs and nutrients were also observed, again with TISI-min giving a slightly better relationship. The relatively small number of sites used in this analysis, coupled with the chemistry results for some of these sites being from different years to that of the biological samples, and again the lack of dynamic range because most are of high or good status explains the low correlation coefficients in this instance.

When the acceptable bands are calculated (excluding IE due to poor regression statistics), Ireland is inside the acceptable band for the H/G and G/M boundary for the ICM based on TISI-min, and the G/M boundary for the TI/SI-mean. Ireland falls just outside the lower boundary of the TISI-mean for the H/G boundary, but only at the third decimal place.

SE: Sweden

The position of the SE boundaries is consistent with the results of the CB-GIG exercise, with both high/good and good/moderate boundaries falling within the ‘acceptable band’, when using the TISI mean.

UK: United Kingdom

The position of the UK boundaries is consistent with the results of the CB-GIG exercise, with both high/good and good/moderate boundaries falling within the ‘acceptable band’.

7. Conclusions/Recommendations

General issues associated with phytobenthos intercalibration exercises are addressed in the report on the CB GIG intercalibration exercise. The conclusions and recommendations listed in that report are all equally valid for the N GIG exercise. This section highlights a few points that are unique to the N GIG exercise.

The CB GIG exercise involved 12 Member States; whilst the N GIG exercise is much smaller, with just four participants. An important implication is that the exercise has lower statistical power and it is not always clear if those MS that fall outside the ‘acceptable band’ do so because there are issues that those MS need to address or because the ‘acceptable band’ is itself based on a small (and potentially atypical sample). On the other hand, however, the ‘acceptable band’ should not be equated with ‘best practice’. MS that comply with the minimum requirements of the exercise are included in the acceptable band and the position of this band, therefore, reflects the consensus of those.

This must affect how results from N GIG and other smaller intercalibration exercises are judged. In particular, a ‘Type 1 error’ (i.e. erroneous rejection of the [null] hypothesis that boundaries are the same) may lead to the conclusion that a MS needs to adjust boundaries when, in fact, the median value of the ICM (which anchors the acceptable band) is unlikely to be stable with such a small sample size.

The approach adopted here was, therefore, to perform a suite of tests using different permutations of the statistical criteria and to make final judgements about the need (or otherwise) to adjust boundaries based on the weight of evidence. Whilst the CB GIG exercise evaluated two versions of the ICM (one based on the mean of component metrics, the other based on the minimum), the N GIG exercise used both versions. TISI-min favoured IE and UK, both of whose national metric was the TDI, which correlates more strongly with the nutrient-sensitive TI, whilst TISI-mean favoured FI and SE whose national metric was the IPS, which correlated more strongly with the SI. Whilst TISI-mean is not biased by a low value of one or other metric, TISI-min better embodies the ‘one out, all out’ principle used when comparing biological elements as part of status assessments.

Three of the four MS taking part in this exercise were also involved in the CB GIG exercise. Boundaries calculated in this exercise are broadly consistent between the two exercises. For H/G, IE, SE and UK were all inside the acceptable band for the CB GIG exercise whilst, for N GIG, UK were inside whilst SE was above the acceptable band for TISI-min but inside for TISI-mean and IE was marginally below for TISI-mean. For G/M, UK and SE were inside the acceptable band whilst IE was above. For the N GIG exercise, IE and UK were inside the acceptable band on all occasions whilst SE was again above the acceptable band when TISI-min was used. In the case of IE, the relatively small size of the dataset plus the low number of poor quality sites may be responsible for the differences in regression equations.

Whilst SE were above the acceptable band on two out of four occasions for each of H/G and G/M comparisons, it is only those MS that fall below the acceptable band that need to consider harmonisation. In this exercise, both IE and FI fell below the acceptable band on one out of four occasions, both were only marginally below the acceptable band on these occasions and we believe that there is no case for either MS to adjust their boundaries.

8. References

Bernhardt, E.S. and Likens, G.E. (2004). Controls on periphyton biomass in heterotrophic streams. Freshwater Biology, 49, 14-27.

Biggs, B.J.F. (1996): Patterns in Benthic Algae of Streams in Stevenson, R.J., Bothwell, M.L. and Lowe, R.L. (eds). Algal Ecology: Freshwater Benthic Ecosystems, Academic Press, San Diego.

Biggs, B.J.F. & Close, M.E. (1989). Periphyton biomass dynamics in gravel bed rivers – the relative effects of flows and nutrients. Freshwater Biology, 22, 209-231.

Common Implementation Strategy (CIS) for the Water Framework Directive (2000/60/EC) Guidance Document No. 14, Guidance on the Intercalibration Process 2004-2006.

ECOSTAT [Working Group A] (2005). Activity on eutrophication. Eutrophication assessment in the context of European water policies. Version 10, 25 October 2005. Draft Guidance.

Eloranta, P. & Soininen, J. 2002. Ecological status of some Finnish rivers evaluated using benthic diatom communities. Journal of Applied Phycology 14: 1-7.

Kelly, M.G., Cazaubon, A., Coring, E., Dell'Uomo, A., Ector, L., Goldsmith, B., Guasch, H., Hürlimann, J., Jarlman, A., Kawecka, B., Kwandrans, J., Laugaste, R., Lindstrøm, E.-A., Leitao, M., Marvan, P., Padisák, J., Pipp, E., Prygiel, J., Rott, E., Sabater, S., van Dam, H., Vizinet, J., 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *Journal of Applied Phycology,* 10, 215-224.

Kelly, M.G. (2006). A comparison of diatoms with other phytobenthos as indicators of ecological status in streams in northern England. Pp. 139-151. In: Proceedings of the 18th International Diatom Symposium (edited by A. Witkowski). Biopress, Bristol.

Kelly, M.G., Rippey, B., King, L., Ní Chatháin, B, McQuillan, C. & Poole, M. (2006a). *Use of phytobenthos for evaluating ecological status in Ireland*. Report to North-South Shared Aquatic Resource (NSShARe) project.

Kelly, M.G., Juggins, S., Bennion, H., Burgess, A., Yallop, M., Hirst, H., King, L., Jamieson, J., Guthrie, R., Rippey, B. (2006b*). Use of diatoms for evaluating ecological status in UK freshwaters*. 160pp: Draft final report to Environment Agency.

Pan, Y., Stevenson, R.J., Hill, B.H., Kaufmann, P.R. and Herlihy, A.T. (1999). Spatial patterns and ecolocical determinants of benthic algal assemblages in mid-atlantic streams, USA. Journal of Phycology, 35, 460-468.

Working Group 2.3 - REFCOND Guidance Document No 10. Rivers and Lakes – Typology, Reference Conditions and Classification Systems. Common Implementation Strategy For The Water Framework Directive (2000/60/EC).

Zelinka, M. & Marvan, P (1961). Zur Präzisierung der biologischen Klassifikation des Reinheit fliessender Gewässer. Archiv für Hydrobiologie 57: 389-407.

9. Glossary

Term	Explanation
Biological metric	A calculated value representing some aspect of the biological population's structure, function or other measurable characteristic that changes in a predictable way with increased human influence.
BQE	Biological quality element.
CEN	Comité European de Normalisation.
CIS	Common Implementation Strategy.
Class boundary	The EQR value representing the threshold between two quality classes.
Ecological status	One of two components of surface water status, the other being chemical status. There are five classes of ecological status of surface waters (high, good, moderate, poor and bad).
ECOSTAT CIS	Common Implementation Strategy (CIS) Working Group A Ecological Status.
EQR	Ecological Quality Ratio.
GIG	Geographic Intercalibration Group i.e. a geographical area assumed to have comparable ecological boundaries conditions.
Good ecological status	Status of a body of surface water, classified in accordance with WFD standards (cf. annex V of the WFD).
Harmonisation	The process by which class boundaries should be adjusted to be consistent (with a common European defined GIG boundary). It must be performed for HG and GM boundaries.
ICM	Intercalibration Common Metric.
Intercalibration	Benchmarking exercise to ensure that good ecological status represents the same level of ecological quality everywhere in Europe.
JRC	Joint Research Council of the EU Commission. Information on the Joint Research Centre, which provides research support for EU policy-making.

MS	Member State (of the European Union)
Pressures	Physical expression of human activities that changes the status of the environment (discharge, abstraction, environmental changes, etc...).
REFCOND	Development of a protocol for identification of reference conditions, and boundaries between high, good and moderate status in lakes and watercourses. EU Water Framework Directive project funded by the European Commission Environment Directorate-General.
Reference conditions	The benchmark against which the effects on surface water ecosystems of human activities can be measured and reported in the relevant classification scheme.
Water body	Distinct and significant volume of water. For example, for surface water: a lake, a reservoir, a river or part of a river, a stream or part of a stream.
WFD	Water Framework Directive.

10. Appendix

Table A.1: REFCOND and N GIG guidance with regard to the description of reference sites to be included in the rivers intercalibration exercise. See Table 4.2 for physico-chemical thresholds.

	REFCOND	N GIG Definition
General statement	High status or reference conditions is a state in the present or in the past corresponding to very low pressure, without the effects of major industrialisation, urbanisation and intensification of agriculture, and with only very minor modification of physico-chemistry, hydromorpology and biology.	High status or reference conditions is a state in the present or in the past corresponding to very low pressure, without the effects of major industrialisation, urbanisation and intensification of agriculture, forestry, aquaculture and with only very minor modification of physico-chemistry, hydromorpology and biology.
Diffuse source pollution	**REFCOND**	**NGIG Definition**
Land-use intensification: Agriculture, forestry	Pre-intensive agriculture or impacts compatible with pressures pre-dating any recent land-use intensification. Pressures pre-dating any recent intensification in airborne inputs that could lead to water acidification.	**Agriculture and Forestry:** Agriculture and forestry in catchment upstream of reference sites of low intensity. Maximum percentage area for screening sites with respect to land cover in catchment upstream of a point at which reference conditions are believed to exist is as follows using CORINE terminology: (Figures are tentative and may vary from region to region. In larger reference catchments proximity of pressure to the proposed reference site may be taken into account. Where CORINE datasets are not available similar land use cover data may be used.) **Agriculture:** Arable land – less than 2 – 10 % Pastures- less than 30% Permanent crops– less than 15% **Forestry:** Forests - clear-felled area/planted area within last 5 years - < 5% **Diffuse Urban Pressures:** Urban fabric – <0.8% of catchment (close to zero)
Point source pollution	**REFCOND**	**NGIG Definition**
Specific synthetic pollutants	Pressures resulting in concentrations close to zero or at least below the limits of detection of the most advanced analytical techniques in general use (A Selection process for relevant pollutants in a river basin is presented as an example of best practice in section 6 of the guidance document from Working Group 2.1, IMPRESS).	• Pressures resulting in concentrations close to zero or below the limits of detection in water of the analytical techniques in general use. Concentrations should be below the NEC level or established national EQS values where available. • No significant point sources. • Airborne pollutants in water at background concentration.
Spec. non-synthetic pollutants	Natural background level/load (see reference above)	• At natural background concentrations or below EQS where available.
Other effluents/discharges	No or very local discharges with only very minor ecological effects.	• No or very local discharges with only very minor ecological effects. • No effects from IPPC controlled industrial plants

		• No other major discharges controlled by other statutory pollution control licences
Morphological alterations	**REFCOND**	**NGIG Definition**
River morphology	Level of direct morphological alteration, e.g. artificial instream and bank structures, river profiles, and lateral connectivity compatible with ecosystem adaptation and recovery to a level of biodiversity and ecological functioning equivalent to unmodified, natural water bodies	Level of direct morphological alteration, e.g. artificial instream and bank structures, river profiles, and lateral connectivity compatible with ecosystem adaptation and recovery to a level of biodiversity and ecological functioning equivalent to unmodified, natural water bodies. No major dams or control structures upstream of reference condition site. The river should not have been subject to any arterial drainage schemes that affect lateral connectivity or cause changes in the natural time of residence. River substratum should be appropriate to the catchment geology and river slope at the point of substratum assessment.
Water abstraction	**REFCOND**	**NGIG Definition**
water abstraction	Levels of abstraction resulting in only very minor reductions in flow levels or lake level changes having no more than very minor effects on the quality elements.	• Abstraction of water from the river upstream of a site regarded as being at reference condition should not **reduce** the 95 percentile discharge flow (m^3/s) by more than 10%. (The 95 percentile flow or discharge is that which is exceeded 95% of the time over the hydrological year).
Flow regulation	**REFCOND**	**NGIG Definition**
River flow regulation	Levels of regulation resulting in only very minor reductions in flow levels or lake level changes having no more than very minor effects on the quality elements.	• Levels of regulation resulting in only very minor reductions in flow levels having no more than very minor effects on the quality elements. As a guideline low flow alteration should be less than 20% of monthly minimum flow. • There should be no major dams or control structures upstream of the reference condition site. Dams located downstream should not affect the flow regime at the reference site and should not impede the passage of migratory fish.
Riparian zone vegetation	**REFCOND**	**NGIG Definition**
	Having adjacent natural vegetation appropriate to the type and geographical location of the river.	• Having adjacent natural vegetation appropriate to the type and geographical location of the river.
Biological pressures	**REFCOND**	**NGIG Definition**
Introductions of alien species	Introductions compatible with very minor impairment of the indigenous biota by introduction of fish, crustacea, mussels or any other kind of plants and animals. No impairment by invasive plant or animal species.	• Introductions compatible with very minor impairment of the indigenous biota by introduction of fish, crustacea, mussels or any other kind of plants and animals. • No impairment by invasive plant or animal species. • No recent introductions (<15 years) that are still causing major ecological changes within a river ecosystem.
Fisheries and aquaculture	Fishing operations should allow for the maintenance of the structure, productivity, function and diversity of the ecosystem (including habitat and associated dependent and ecologically related species) on which the fishery depends Stocking of non indigenous fish should not significantly affect the structure and	• There should be no commercial fishing operations or fish farming which affects the biological quality elements or water quality of the river system. No significant stocking of non-native species or stocking of 'put and take' fish for angling purposes.

	functioning of the ecosystem.. No impact from fish farming.	
Biomanipul-ation	No biomanipulation.	• No biomanipulation or liming of the system in response to acidity pressures.
Other pressures	**REFCOND**	**NGIG Definition**
Recreation uses	No intensive use of reference sites for recreation purposes (no intensive camping, swimming, boating, etc.)	• No intensive use of reference sites for recreation purposes (camping, swimming, boating, etc.) causing physical, chemical or biological disturbance