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Water Framework Directive Intercalibration Technical Report

Northern Lake Fish fauna ecological assessment methods

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Introduction

The European Water Framework Directive (WFD) requires the national classifications of good ecological status to be harmonised through an intercalibration exercise. In this exercise, significant differences in status classification among Member States are harmonized by comparing and, if necessary, adjusting the good status boundaries of the national assessment methods.

Intercalibration is performed for rivers, lakes, coastal and transitional waters, focusing on selected types of water bodies (intercalibration types), anthropogenic pressures and Biological Quality Elements. Intercalibration exercises were carried out in Geographical Intercalibration Groups - larger geographical units including Member States with similar water body types - and followed the procedure described in the WFD Common Implementation Strategy Guidance document on the intercalibration process (European Commission, 2011).

In a first phase, the intercalibration exercise started in 2003 and extended until 2008. The results from this exercise were agreed on by Member States and then published in a Commission Decision, consequently becoming legally binding (EC, 2008). A second intercalibration phase extended from 2009 to 2012, and the results from this exercise were agreed on by Member States and laid down in a new Commission Decision (EC, 2013) repealing the previous decision. Member States should apply the results of the intercalibration exercise to their national classification systems in order to set the boundaries between high and good status and between good and moderate status for all their national types.

Annex 1 to this Decision sets out the results of the intercalibration exercise for which intercalibration is successfully achieved, within the limits of what is technically feasible at this point in time. The Technical report on the Water Framework Directive intercalibration describes in detail how the intercalibration exercise has been carried out for the water categories and biological quality elements included in that Annex.

The Technical report is organized in volumes according to the water category (rivers, lakes, coastal and transitional waters), Biological Quality Element and Geographical Intercalibration group. This volume addresses the intercalibration of the Lake Northern Fish fauna ecological assessment methods.

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1. Introduction

In the Northern Fish Geographical Intercalibration Group (GIG):

- Four Member States (Ireland, Norway, Sweden and UK) submitted their lake fishbased assessment methods;
- After evaluation of the IC feasibility, 2 methods were included in the current IC exercise: IE and SE (as NO method follows different assessment concept/ assesses different pressures, but Swedish method shows low correlation with the common metric);
- Intercalibration "Option 3" was used direct comparison of assessment methods via regression (as only 2 methods were compared);
- The comparability analysis show that methods give a closely similar assessment (in agreement to comparability criteria defined in the IC Guidance), so no boundary adjustment was not needed;
- The final results include EQRs of Irish and Finnish lake fish-based assessment systems for 2 common types: LNF-1 and LNF-2.

2. Description of national assessment methods

Four fish-based lake assessment methods participate in the Intercalibration:

- Finland Finnish Lake Fish Classification Index EQR4;
- Norway Norwegian Method for Fish in Lakes FCI;
- Ireland and UK (North Ireland) Fish in Irish lakes classification tool FIL2;
- Sweden Assessment criteria for ecological status of fish in Swedish lakes EQR8.

Methods and required BQE parameters

All methods include metrics of taxonomic composition and fish abundance, whereas age structure is included indirectly:

- Finland age structure included indirectly, based on population structure of perch and roach as indicator species;
- Norway age structure included based on age determination;
- Ireland & UK (North Ireland) age structure included indirectly: maximum length of the dominant species is used as a surrogate for age structure in one typology;
- Sweden age structure and sensitive species are only indirectly included.

The Finnish EQR4, Irish FIL2 and Swedish EQR8 are all multimetric fish indices, with no need for any extra combination rule. The Norwegian FCI basically relies on observed changes in the fish community rather than on metrics derived from test fishing according to EN 14757. This method also needs no combination rule. For more information, see Table 2.1 and Annex A to E.

In conclusion, all Northern GIG lake fish assessment methods pass the WFD compliance check regarding included metrics.

Table 2.1	Overview of th	e metrics	included	in the	national	fish-based	lake	assessment
	methods (for m	ore detail	s see Ann	ex A)				

MS	Species composition	Abundance	Age structure	Combination rule of metrics
IE (RoI and NI)	RHEO_BIO %: individuals that are rheophilic SPE_EVEN: Species evenness/dominance (1/D=1/(Nmax/Ntot) BREAM_%_IND: % composition of bream based on CPUE PHYT_%_BIO: % individuals that are phytophilic 2_%_BIO: % biomass of Group 2 species non native species influencing biology CYP_BIO: % biomass of cyprinid species, inc hybrids RUDD_%_IND: % composition of rudd based on CPUE LITH_IND:% individuals (based on CPUE excl. eels and adult salmon) that are lithophilic PERCH_BIO: Mean perch biomass per unit effort	TOT_BPUE: sum of mean biomass per unit effort NAT_BPUE: sum of mean biomass per unit effort of native fish species PERCH_BIO: Mean perch biomass per unit effort	MAX_L_DOM _BIO: Maximun length of dominant species (based on BPUE) (only used for one typology)	Discriminant anlysis and typology specific multivariate regression analysis (using posterior probablilities of the typology specific qualitative classification rules – average of two weighted scores)
FI	Biomass proportion of cyprinid fish; Occurrence of indicator species	Total biomass of fish per gillnet night (BPUE); Total number of fish individuals per gillnet night (NPUE)	Occurrence of indicator species (incl population structure of P. fluviatilis, E. lucius and/or R. rutilus)	Average metric scores
SE	Number of native fish species; Simpson's Diversity Index (based on individuals and biomass); Proportion of piscivorous percids; Ratio perch / cyprinids (based on biomass)	Relative biomass (BPUE) of native fish species; Relative abundance (NPUE) of native fish species	Mean (individual) biomass	Average metric scores

NO	Categorical metrics of	Categorical	Included in	Sums of
	"abundance" and "change" for	metrics of	the categorical	categorical
	all fish species in the original	"abundance" and	metric change	metrics are
	(reference state) fish	"change" for all		used in the
	community	fish species in the		equation for
		original		the fish
		(reference state)		community
		fish community		index

Sampling and data processing

All countries are using benthic survey nets according to EN 14757 standard (Table 2.2.).

Table 2.2	Overview	of	the	sampling	of	national	fish-based	lake	assessment	methods
	(Finland a	nd	Swe	den)						

	Finland	Sweden
How many sampling / survey occasions (in time) are required to allow for ecological quality classification of sampling / survey site or area?	1-8 sampling nights per sampling season (depending on the lake size and depth)	One occasion
How many spatial replicates per sampling / survey occasion are required?	5 to 68 gillnet nights, depending on lake size and depth	8-68 benthic gillnets, depending on lake area and maximum depth
Sampling / survey months	Mid-July to early September	Late summer (usually between July 15 and August 31), when deep lakes are thermally stratified
How the sampling / survey sites or areas were selected?	Expert knowledge, random sampling / surveying, stratified samplings	Lakes in a national network of monitoring sites, including operative monitoring of lakes restored by liming, complemented by lakes monitored by county level administration
What is the total sampled / surveyed area or volume, or total sampling duration to classify site or area?	Lake area 0.01-10 km ² , in larger lakes a representative sub area up to 10 km ² is selected by expert judgment	Standard effort of benthic gillnets set for 12 hours (+/- 1 hour) including dusk and dawn
Sampling / survey device	Gill net (Nordic multimesh surveynets (CEN 14757:2005))	Gill net (Benthic and pelagic gillnets as specified in EN 14757)
Minimum size of organisms sampled and processed	40 mm (0+ fish)	Down to about 3 cm total length

What is the level of	Species / species groups	Species / species groups,
taxonomical identification?		family (if possible hybrids of
What groups to which level?		cyprinids are identified at this
		level)

Table 2.3 Overview of the sampling of the national fish-based lake assessment methods (Ireland and NI, Norway)

	Ireland and UK (North Ireland)	Norway
How many sampling / survey occasions (in time) are required to allow for ecological quality classification of sampling / survey site or area?	One occasion (1-8 sampling nights depending on depth and lake area))	Can be assessed from comparisons of interviews (one occasion) and gill netting data. In the gill netting, mainly one occasion, in some cases 2-3 sampling for larger lakes
How many spatial replicates per sampling / survey occasion are required?	Depends on lake area and maximum depth (4-60 benthic nets) plus other nets	Depends on lake area and depths, 5-45 gillnets nights (benthic nets) + floating nets (2-4)
Sampling / survey months	Mid June to 1 st week in Oct	August and September
How the sampling / survey sites or areas were selected?	Expert opinion, random sampling, stratified sampling	Mainly based on expert knowledge. The lakes are included in a national network of monitoring sites
What is the total sampled / surveyed area or volume, or total sampling duration to classify site or area?	Depends on lake area and maximum depth, lake area varies from 1.4Ha to 11650Ha	Overnight sampling for about 12 hours including dusk and dawn
Sampling / survey device	Gill net (Nordic multimesh survey nets (CEN 14757:2005)) and surface floating nets, fyke nets and additional larger mesh braided survey gill nets in high alkalinity lakes	Gill net (Nordic multimesh survey nets (CEN 14757:2005))
Minimum size of organisms sampled and processed	10mm	40 mm (0+ fish)
What is the level of taxonomical identification? What groups to which level?	Species	Species

National reference conditions

Tables below summarize the methodology used to derive the reference conditions. The GIG conclusion: the methods` reference conditions are in line with the WFD requirements.

Table 2.4 Overview of the methodologies used to derive the reference conditions for the national lake fish assessment methods

Member State	Methodology used to derive the reference conditions
IE (RoI and NI)	Existing near-natural reference sites and least disturbed sites (for high alkalinity lakes), 48 reference lakes (majority in high status, high alkalinity lakes in good status), all sites in RoI
FI	Existing near-natural reference sites, least disturbed conditions, 127 sites
SE	Existing near-natural reference sites and least disturbed conditions (i.e. expected to be in either high or good status), modelling (extrapolating model results), 116 sites
NO	Existing near-natural reference sites, least disturbed conditions, 86 sites

Table 2.5	Description of the methodologies used to derive the reference conditions for the
	national lake fish assessment methods (Finland and Sweden)

	Finland	Sweden
Scope of reference conditions	Surface water type-specific	Site-specific
Key sources to derive reference conditions	Existing near-natural reference sites, least disturbed conditions	Existing near-natural reference sites, least disturbed conditions, modeling (extrapolating model results)
Number of sites, location and geographical coverage of sites used to derive reference conditions	127 sites, located fairly evenly throughout the country; reference sites found in Finnish River Basin Districts 1-7	116 sites (in high and good status); all parts of Sweden, covering the following range of environmental factors: altitude $10 - 894$ m above sea level, lake area $2 - 4236$ ha, maximum depth $1 - 65$ m, annual mean in air temperature $-2 - 8$ °C
<i>Time period of data from sites used to derive reference conditions</i>	1995-2007	Fish data were extracted from the National Register of Survey Test- fishing in 2005, and the latest date of standardized sampling was used for each lake
<i>Reference sites</i> <i>characterization: criteria</i> <i>to select them</i>	Lack or minor presence of human induced environmental pressures, including: main nutrients (Ptot, Ntot), land use information (including Corine land cover) and nutrient load model calculations	Fish metrics at reference sites are expected to have low deviation from site-specific reference values

	Ireland and UK (North Ireland)	Norway
Scope of reference conditions	Fish type and surface water specific	Site-specific
Key sources to derive reference conditions	Existing near-natural reference sites and least disturbed sites (for high alk lakes)	Existing near-natural reference sites, least disturbed conditions
Number of sites, location and geographical coverage of sites used to derive reference conditions	43 reference lakes (majority in high status, high alkalinity lakes in good status), all sites in RoI, majority in west of Ireland	About 40 sites, located in different counties
Time period of data from sites used to derive reference conditions	2005 to 2009 (latest date of sampling was used for each lake)	1995-2010
Reference sites characterization: criteria to select them	Lack or minor presence of anthropogenic pressures. Reference sites chosen based on following parameters in u/s catchment: >80% natural land, <20% agricultural land, <20% urban land, <20% forestry, pH >6 Connectivity (no impassable barriers present d/s, impassable natural barriers present d/s and natural barriers present d/s but passable on some occasions) TP mean <12 and <20 is rejection threshold Chlor a mean <8 and <15 is rejection threshold Fish metrics should be near reference Palaeolimnology also confirmed ref status for some lakes.	Lack or minor presence of anthropogenic pressures. Assessment from water quality and land use

Table 2.6 Description of the methodologies used to derive the reference conditions for thenational lake fish assessment methods (Ireland and NI, Norway)

National boundary setting

The GIG conclusion: the methods` ecological class boundaries are set according to the WFD requirements. **In the Irish method**, the boundary setting is based on the results of the qualitative classification rule and quantitative EQR model which were cross-tabulated at various cut-points in order to quantify class boundaries (see Annex C).

In the Finnish method, the class boundaries are simply equidistant (but different in different lake types) and H/G boundary is based on the 25 percentile in the EQR-distribution of type-specific reference lakes. (Exception is indicator species variable where EQR is based on presence/absence of intolerant species or population structure of core species, see Annex B) We argue based on several studies (Jeppesen et al. 2000, Olin et al.

2002) that (within a lake type) the changes in lake fish communities due to eutrophication are gradual rather than by jumps and thus the equidistant boundary setting is justified.

In the Swedish method, the class boundary setting was based on statistical distribution of reference and impacted lakes (see Annex E).

In the Norwegian method, the starting point in boundary setting was in determining of reference conditions, based on unchanged/healthy populations of dominant, subdominant and rare species (see tables below and Annex D).

Table 2.7 Overview of the methodology used to derive ecological class boundaries

Member State	Methodology used to set class boundaries
IE (RoI and NI)	Discriminant analysis and cross tabulation with pressure gradient
FI	Equidistant division of the EQR gradient, High-good boundary derived from metric variability at near-natural reference sites
SE	Statistical distribution of reference and impacted lakes
NO	Based on expert judgement - lakes with any signs of damaged fish stocks get moderate or worse status
UK	Similarly to Ireland

Table 2.8	Description	of	the	methodology	used	to	derive	ecological	class	boundaries
	(Finland and	1 Sv	vede	n)						

	Finland	Sweden
Setting of the ecological status boundaries: methodology and reasoning to derive and set boundaries	Equidistant division of the EQR gradient (linear response to eutrophication), high-good boundary derived from metric variability at near-natural reference sites	G/M boundary set at the EQR8 value with equal risk of misclassification of high plus good sites versus moderate to bad sites (according to procedures used in the FAME project, for development of a European fish index for rivers)
Boundary setting procedure	High/good class boundary was set to the 25th percentile of the EQR-distribution of fish parameters in reference lakes of each lake type; other class were set by dividing into even distances the EQR values of a lake type from the H/G class boundary to the extreme EQR recorded	The good-moderate boundary was set at the EQR8 value which minimized the risk for type I and type II errors; the high- good boundary was conservatively set at the 95th percentile of EQR values in reference lakes; the poor-bad boundary at the 10th percentile of EQR values in impacted lakes; the moderate-poor boundary was more arbitrarily set at the mean of EQR values at good-moderate and moderate-poor boundaries
Is the description of the communities of reference / high-	Yes: natural fish communities from reference lakes, usually 1 to 10 species per lake;	Fish metrics at high status sites have no or very low deviation from site-specific reference values, inferred by multiple

good-moderate status provided?	good status fish communities in Finnish lakes are close to those in reference conditions including the possible occurrence of sensitive indicator species	regression models with environmental factors

Table 2.9	Description	of	the	methodology	used	to	derive	ecological	class	boundaries
	(Ireland and	N	, No	rway)						

	Ireland (RoI and NI)	Norway
Setting of the ecological status boundaries: methodology and reasoning to derive and set boundaries	Discriminant analysis typology-specific classification rules, relevant to eco-region 17, were derived from the training dataset to describe a lake as High, Good, Moderate or Poor/Bad. Stepwise multiple linear regression analysis and expert opinion were used to develop the EQR models.	The boundaries are based on expert judgement.
Boundary setting procedure	The results of the qualitative classification rule and quantitative EQR model were cross-tabulated at various cut-points in order to quantify class boundaries. A High lake was defined to be [0.76, 1]; Good [0.53, 0.76); Moderate [0.32, 0.53); and, Poor/Bad [0, 0.32).	The H/G and G/M boundaries are the at the 95 and 75 percentile
Is the description of the communities of reference/high- good-moderate status provided?	Yes, high status lakes=reference lakes, natural fish communities, Good status are close to reference with sensitive indicator species present	The reference condition means that the status of each species in a community is unchanged. Score equal 1.

Pressures-response relationships

FI and IE methods show significant correlations with eutrophication indicators (Chl-a. TP – IE methods, TP, land-use – FI method).

SE method assessments show significant difference between reference and impacted lakes (selected using criteria for acidification, eutrophication and general degradation). Lakes with high total P had lower mean EQR8 than reference lakes, but the effect was weaker (P=0.06) than for acidity and general degradation (both P < 0.001).

There are generally a significant correlation between Norwegian FCI and two water chemistry acidified-related variables – pH and inorganic Al (not significant for ANC). The best fit was obtained between FCI and water chemistry in non-limed lakes containing \geq 3 species.

GIG conclusions

We decided to carry out the IC by using **eutrophication** because it is the only relevant common pressure. Lakes impacted by acidification and liming must then be excluded to refine the pressure-response relationships.

The task is challenging as **only the Irish FIL2 and the Finnish EQR4** were originally developed more or less directly to detect the eutrophication pressure.

Instead, the Norwegian FCI is targeted in first hand to acidification and the Swedish EQR8 is most ambitious, covering eutrophication, acidification and common degradation of lakes.

Table 2.10 Pressures addressed by the MS assessment methods. EU – eutrophication, AC – acidification, GD – general degradation. TP – total phosphorus, ANC – anion neutralising capacity.

Member State	Metrics tested	Pressure	Pressure indicators	Strength of relationship
IE (RoI and NI)		EU	TP and chl- a	Pearsons correlation EQR vs TP R=0.598 and EQR vs Chl-a R=0.536
FI	EQR	EU	TP, % cultiv land	Correlation TP R= 0.56, % of cultivated fields 0.42 (p<0.001) ANOVA tests diff. between ref and impacted (p<0.001)
NO	EQR	AC	pH, ANC, inorganic Al	R ² values FCI and pH, Inorganic AI and ANC for all lakes in the data set (both limed and unlimed containing 3 or more fish species was 0.38 (p< 0.005), 0.40 (p < 0.05) and 0.12 (p>0.05) Significant relationships
SE	EQR,	EU, AC, GDR	pH, TP, landuse	t-tests between ref and impacted lakes significant, for acidification and general degradation P<0.001, for eutrophication (expressed as TP) P=0.06
SE	separat e metrics	EU, AC, GDR	pH, TP, landuse	t-tests also sign for separate metrics (different pressures)



Figure 2.1 Original EQRs (y-axes) of the four national methods (Finnish EQR4, Irish FIL2, Norwegian FCI and Swedish EQR8) in relation to total phosphorus concentration (x-axes, μg/L) in the lakes of common IC type (n=169). Determination coefficients (R²) and P-values (<0.05 = *, <0.01 = ** and <0.001 = ***) of the regression analyses are shown

Analysis of the common dataset shows (see Figure 2.1 below):

- Strong significant relationships for Finnish EQR4 (all lakes R²=0.34, P<0.001) and Irish FIL2 (all lakes R²=0.13, P<0.001) with total phosphorus;
- For Swedish EQR8 significant relationship with total phosphorus only for lakes of Sweden (R²=0.08, P<0.05);
- No relationship with total phosphorus for Norwegian FCI.

3. Results of WFD compliance checking

The table below lists the criteria from the IC guidance and compliance checking conclusions

Table 3.1 List of the WFD compliance criteria and the WFD compliance checking process and results

Com	pliance criteria	Compliance checking conclusions
1.	Ecological status is classified by one of five classes (high, good, moderate, poor and bad).	All methods (EQR4, FCI, FIL2 and EQR8) are compliant.
2.	High, good and moderate ecological status are set in line with the WFD's	Finland – Yes

Compliance criteria	Compliance checking conclusions
normative definitions (Boundary setting procedure)	 Norway – More or less, because lakes with any signs of damaged fish stocks get moderate or worse status Rep. of Ireland & NI - Yes Sweden – Yes to minimize type I and II errors when assigning a lake above or below the good-moderate boundary
3. All relevant parameters indicative of biological quality element are covere (see Table 1 in the IC Guidance). A combination rule to combine param assessment into BQE assessment has be defined. If parameters are missing Member States need to demonstrate the method is sufficiently indicative c status of the QE as a whole.	f the YES, SEE TABLE ABOVE d Yes for all countries. For details, see table p. 1-2 neter to , that of the
 Assessment is adapted to intercalibration common types that defined in line with the typological requirements of the WFD Annex II an approved by WG ECOSTAT 	 Finland – No, but to Finnish lake types Norway – No, because the assessment method can be applied to any lake type. Rep. of Ireland & NI – No, but to Irish FIL2 lake types Sweden – No, but using models of lake-specific reference values
 The water body is assessed against ty specific near-natural reference conditions 	ype- YES, SEE TABLE ABOVE
6. Assessment results are expressed as l	EQRs Yes, for all methods (EQR4, FCI, FIL2 and EQR8)
 Sampling procedure allows for representative information about wa body quality/ ecological status in spa and time 	Yes, using benthic survey nets according to EN 14757 standard in all countries (Finland, Norway, Rep. of Ireland & NI and Sweden)
 All data relevant for assessing the biological parameters specified in th WFD's normative definitions are cove by the sampling procedure 	Yes, they are all covered by EN 14757. Sampling for age structure is optional in Finland and Sweden and compulsory in Rep. of Ireland & NI. In Norway, age structure is obligatory for lakes included in the national assessment program.
 Selected taxonomic level achieves adequate confidence and precision classification 	Yes in
10. Other criteria	No

General conclusion of the compliance checking: All L-N-F methods pass the compliance check!

4. Results IC Feasibility checking

Typology

Intercalibration feasible in terms of typology - all assessment methods are appropriate for the common types

Table 4.1 Description of common intercalibration water body types and the MS sharing each type

Common IC type	Type characteristics	MS sharing IC common type
LNF1	Dimictic clear water (colour < 30 mg Hg/l), lakes smaller than 40 km ²	All L-N-F member states
LNF2	Dimictic humic (colour 30-90 mg Hg/l), lakes up to 5 km ² in area	All L-N-F member states

Pressures addressed

Intercalibration is feasible for eutrophication because it is the only relevant common pressure. Lakes impacted by acidification and liming must then be excluded to refine the pressure-response relationships.

- The task is challenging as **only the Irish FIL2 and the Finnish EQR4** were originally developed more or less directly to detect the eutrophication pressure;
- Instead, the **Norwegian FCI** is targeted in first hand to acidification;
- The **Swedish EQR8** is most ambitious, covering eutrophication, acidification and general degradation of lakes.

Table 4.2	Evaluation if IC feasibility regarding pressures addressed by MS fish assessment
	systems

Method	Pressure	Remarks
Finnish EQR4	Eutrophication	May be applied also for acidification, not tested. Acidification is not a relevant pressure in Finland today.
Irish (RoI and NI) FIL2	Eutrophication and general land use pressures	
Norwegian Fish Index	Mixed pressures, mostly acidification	Best suited for effects of acidification (damaged and lost stocks)
Swedish EQR8	Acidification, eutrophication, mixed pressures	

Assessment concept

All national methods follow a similar assessment concept except **Norwegian method**:

- Intercalibration is feasible **between Finnish**, **Irish and Swedish methods** although the response of the Swedish method to eutrophication is weak (Figure 2.1);
- Intercalibration between the Norwegian and the other methods is not feasible as:
 - the Norwegian FCI is not responding to eutrophication pressure;
 - it relies on enquiry data in assessing the magnitude of change over longer time span;
 - it is not possible to provide enquiry or monitoring data on long-term changes of fish stocks from many Finnish, Irish and Swedish lakes, in addition to present state data from gillnet sampling. Obtaining reliable inquiry data from larger lakes with 15-20 fish species proved to be difficult, if not impossible;
 - The Norwegian fish community index (FCI) has been calculated only for 35 of 169 in the IC data set or for 3-13 lakes per country, which appeared to be a too small number.

Method	Assessment concept	Remarks
Finnish EQR4	Benthic and pelagic habitat, 4 structural/functional metrics	IC can be conducted using data from benthic nets only
Irish FIL	Benthic and pelagic habitat, 13 structural/functional metrics	
Norwegian Fish Community Index (FCI)	Fish Index, which is based on inquires is expressed as the deviation from the reference conditions (RC), ranging from 1.0 (no damage) to zero (all stocks lost). Status for all stocks in a community is defined as unchanged, damaged or lost. RC is defined as an unchanged and healthy population. Change in fish status is estimated from number of lost and damaged population in each community. Their evenness is considered and given different weights. Different weights are also given for unchanged, damaged and lost stocks.	IC can be conducted only for lakes with enquiry data in addition to gillnet sampling or for lakes with long time series of fish status data
Swedish EQR8	Benthic habitat, 8 structural/functional metrics	

Table 4.3	Evaluation if IC feasibility regarding pressures addressed by MS fish assessment
	systems

5. IC dataset collected

The IC dataset of L-N-F group is based on the data delivered to the cross-GIG database since 2009. After some additional data deliveries to the cross-GIG database and after receiving some lake fish data from UK (England and Scotland), the L-N-F data set reached a total number of 1577 lakes. As all participating countries use the common sampling method and procedure (EN 14757), the comparability of the data was considered to be sufficient.

In a pilot study 2008-2009, when Finnish and Swedish fish indices were applied to gillnet data from Finnish, Irish, Norwegian and Swedish lakes (Holmgren et al. 2010), a set of 640 lakes was used (89-305 lakes from each country). The results of the pilot study indicated clear differences in the classification output of the Finnish and Swedish tools from the common data set. This was followed by critical examination and refinements of both the data and the methods. Moreover, it was understood that a successful IC exercise would not be possible without a more detailed determination of IC common lake type and without directing the work to one pressure common for all participating countries.

Therefore, a reduced IC dataset was collated. It is a subset of the original common dataset, selected according to the following criteria:

- 1. Only lakes with delivered fish index values using at least the three national methods (Finnish EQR4, Irish FIL2 and Swedish EQR8);
- 2. Only the data of the last test fishing occasion used in calculations for all methods specified in criterion 1;
- Only non-acid (mean pH ≥6) and non-limed lakes included, in order to focus the IC along a eutrophication gradient;
- 4. Only lakes fitting the criteria of one of the common IC types.

The number of lakes in this reduced IC data set summed up to 320 non-acidic and nonlimed lakes. To avoid too broad IC type, further reduction of the IC dataset was still done. For the present IC trial, lakes corresponding LNF1 and LNF2, were included with a total number of 169 lakes.

Table 5.1	Overview of the Northern	GIG fish IC dataset	(common IC types – LNF 1 and
	LNF2)		

Mambar State	Number of sites or samples or data values				
Member State	Biological data	Physico- chemical data	Pressure data		
Finland	62	62	62		
Norway	17	17	17		
Republic of Ireland	41	41	41		
Sweden	48	48	48		
North Ireland	1	1	1		

Table 5.2	List the data acceptance	criteria	used for	the data	quality	control	and	the	data
	acceptance checking proc	ess and	l results						

Data acceptance criteria	Data acceptance checking
Data requirements (obligatory and optional)	Our present Fish data enable calculating Finnish EQR4, Irish FIL2 and Swedish EQR8 using Finnish, Irish, Norwegian and Swedish fish data. The enquiry data required for calculation of the Norwegian Fish Index are not available in the common European database. Therefore, separate calculations of the Norwegian Fish Index were done for a subset of Finnish, Irish, Norwegian and Swedish lakes in the common IC data set (n=35). Physico-chemical data : Missing values sometimes occurred for one or two variables needed for estimating reference values for one or more method-specific fish metric, leading to less than the selected 169 lakes in some calculations.
The sampling and analytical methodology	OK, when accepting minimal differences between Finnish, Irish, Norwegian and Swedish fish data, as revealed in the pilot study (Holmgren et al. 2010).
Level of taxonomic precision required and taxa lists with codes	OK for Finnish, Irish, Norwegian and Swedish fish data
Sufficient covering of all relevant quality classes per type	Yes, in the sense that we have enough lakes close to the most important class boundaries H/G and G/M and also several lakes with increasing pressure and down to bad classification.

6. Common benchmarking

Common approach for setting reference conditions

The L-N-F group followed agreements in the Fish lake cross-GIG group. Summary of Fish lake cross-GIG **reference criteria:**

- at least 81% natural or semi-natural land in the catchment,
- not more than 10 inhabitants / km² catchment,
- annual mean of total phosphorus not more than 12 μg/L
- annual mean pH between 6-9,
- no upstream barriers,
- no lack of connectivity,
- no significant water level fluctuation,
- 0-10% shoreline (bank) modified,
- no urban/industrial discharge,
- no stocking,
- no biological &/or chemical manipulation (e.g. liming to counteract acidification),
- low exploitation of fish population by fishing .

Reference sites

In the dataset of 169 lakes in our common IC type, 106 lakes passed the reference filter. These reference lakes were used as benchmark sites (BM-sites), and they were distributed between member states in the following way: 49 for Finland, 6 for Republic of Ireland, 17 for Norway, and 34 for Sweden.

Two tables below show summary statistics for all fish indices at BM-sites.

Table 6.1	Descriptive statistics of fish indices at reference sites (BM sites) in lakes of the
	common intercalibration type (common lake types LNF1 and LNF2)

Fish index	N	Min	Max	Mean	SD
EQR8	106	0.03	0.85	0.40	0.18
EQR4	106	0.37	0.96	0.79	0.14
FCI	32	0.20	1.00	0.86	0.18
FIL2	106	0	0.89	0.61	0.27

Fish index	Finland	Ireland	Norway	Sweden
EQR8	0.35	0.13	0.23	0.56
EQR4	0.87	0.85	0.83	0.82
FCI	0.96	0.96	0.83	0.85
FIL2	0.71	0.80	0.82	0.57

Table 6.2 Median fish index value at BM sites of the common IC type in different countries

Benchmark standardisation

Original EQR's of national index values (FIL2, EQR8 and EQR4) were **benchmark standardised by subtraction** as the differences between the methods remained along eutrophication gradient (Birk et al. 2011). For the 169 lakes, an offset (deviation of the national median to the average of all benchmark medians) was added to original EQR-values (see Table 6.3).

Method	Country	Lake n	Median EQR	Offset
EQR4	Finland	49	0.87	-0.03
	Ireland	6	0.85	-0.01

	Sweden	34	0.82	0.02
	Norway	17	0.83	0.01
FIL2	Finland	49	0.71	0.02
	Ireland	6	0.80	-0.08
	Sweden	34	0.57	0.16
	Norway	17	0.82	-0.10
EQR8	Finland	49	0.35	-0.03
	Ireland	6	0.13	0.19
	Sweden	34	0.56	-0.24
	Norway	17	0.23	0.09

7. Comparison of methods and boundaries

IC Option and Common Metrics

We chose **IC option 3** for intercalibration as we had similar data acquisition (similar sampling method), common set of lakes, common lake IC types but different numerical evaluation.

We used **pseudo-common metrics (PCM**) i.e. we compared the benchmark standardized EQR values of a one method against the average of the benchmark standardized EQR values of two other methods.

Results of the regression comparison

FI and IE methods have significant regressions to the pseudo-common metrics (see table below), while SE method had non-significant regression.

 Table 7.1 The correlation coefficients (r) and the probability (p) for the correlation of each method with the common metric

Member State/Method	Ν	r	р
Finland	169	0.523	< 0.001
Ireland	169	0.530	< 0.001
Sweden	169	0.036	0.642

The Swedish method was excluded due to its low correlation with the common metric. **Thus we intercalibrated only two methods: the Irish method (FIL2) and the Finnish method (EQR4).** We made the comparison calculations for dataset including only the Finnish and Irish lakes belonging to the lake types LNF1 and LNF2 (total lake n = 104) with benchmark standardization by subtraction.

 Table 7.2 Benchmark standardization offsets (including median of benchmark sites)

Method	Country	Lake n	Median EQR	Offset
EQR4	Finland	49	0.87	-0.01
	Ireland	6	0.85	0.01

FIL2	Finland	49	0.71	0.05
	Ireland	6	0.80	-0.05

Evaluation of comparability criteria

Finland: FIL2 and EQR4 methods gave on average very similar output and were comparable without any harmonization when applied to the common IC lake types. The comparability of these two methods seems to be similar in other Finnish lake types than 1 and 2 as well.



Figure 7.1 Direct comparison of benchmark standardised (subtraction) EQR values by the EQR4 and FIL2 methods obtained from the Finnish and Irish lakes of common IC types (n = 104).

According to the boundary comparison of two methods:

- There is no need for boundary adjustments as boundary biases are below 0.25 class equivalents;
- The absolute average class difference (0.74) was above the limit of 0.5 for two methods but it was not possible to decrease without increasing boundary bias.

The relatively high variation is mainly due to differences in benchmark data (that seems to not be fully corrected by benchmark standardization), biogeographical differences, sampling and different metrics measured:

- the class boundaries of the Finnish method are based on the data from Finnish reference lakes, whereas the boundaries of the Irish method are based on modeling the data from Irish, lakes ranging from oligotrophic reference lakes to highly eutrophicated lakes with very different fish communities compared to the Finnish reference lakes (see the method descriptions in Annexes);
- The Irish assessment method included 13 quite detailed (species level) metrics whereas the Finnish method had only four, more general metrics;
- The Irish sampling included smaller number of gillnets (which increase random variation) supported by fyke netting while the Finnish method was based only on gillnet data;

Low catch or absence of native Irish species (e.g. brown trout, sea trout, salmon, eels, char, pollan and three-spined stickleback) and rheophilic species, and high catches of non-native Irish species (perch and roach that are native in Finland) drop the FIL2 classification in many (mainly shallow, low alkalinity) Finnish reference lakes that had high classification by EQR4.

Mombor	Classification	Ecological Quality Ratios		
State	Method	High-good boundary	Good-moderate boundary	
Finland	EQR4	0.80	0.60	
Ireland	FIL2	0.76	0.53	

Table 7.3 Class boundaries to be included in the IC Decision

Only 2 methods were intercalibrated (the EQR4 and FIL2) as their passed Feasibility check 2.

NO and SE methos did not pass Feasibility check 2 (all method assess the same pressure), and can therefore not be harmonised with the other methods along the eutrophication gradient.

Final conclusions:

- 1. **The biogeographical differences** between N-GIG countries make the lake fish IC very challenging. This is because the fish fauna in Ireland and most parts of Norway is originally very scarce in number of species, mainly dominated by salmonids, whereas in major parts of Finland and Sweden in ecoregion 22, the species richness is higher and fish fauna is mainly dominated by percid and cyprinid species.
- 2. IC work has been **completed for FI and IE methods** and these methods should be included in the EC decision. Apparently this result was because these two methods were originally targeted to detect the fish community responses to eutrophication pressure.
- 3. NO and SE methods were excluded from the intercalibration as they did not pass all steps of feasibility check (address different pressures or no correlation with pseudo-common metrics). These methods are not included in the IC decision. Both methods are still useful for national purposes, NO for acidification, SE for acidification and to some extent eutrophication. In lakes with no acidification pressure (past or present), the Swedish EQR8 method could be complemented with the Finnish EQR4. This could be the case in lowland lakes in the northernmost part of Sweden. For information on fish community responses across the pressure gradient and description of the differences between G and M status lake fish communities, see annexes D and E.
- 4. From the point of IC for NO and SE methods, future development is needed:
 - In Norway. there are plans to carry out further work in order to revise/improve the method. This will involve: (i) increase the data set (limited to < 60 lakes at the present stage), (ii) include different fish communities,

with emphasis on cyprinid species and (iii) testing to what extent different weights used for species richness (dominant, subdominant and rare) and community status (unchanged, changed, i.e. increase or reduction in abundance and lost) will change the values of FCI.

 In Sweden, the EQR8 method will be revised within a new research program, Waterbody Assessment Tools for Ecological Reference conditions and status in Sweden (WATERS), which started in 2011. A new or revised official Swedish method will be expected for practical use until the end of 2016, after completion of the WATERS project in March 2016.

8. Description of biological communities and changes across pressure gradient

Biological communities at reference sites

The most common fish species in the IC reference sites of L-N-F were perch (*Perca fluviatilis*), roach (*Rutilus rutilus*), pike (*Esox lucius*) and ruffe (*Gymnocephalus cernuus*) with occurrence of 57-88 % (See Table below). They are considered to be "core species" in boreal lakes of Sweden and Finland in the ecoregion 22 (Rask et al. 2010). In Norway and Ireland salmonid species brown trout (*Salmo trutta* spp) and arctic char (*Salvelinus* sp) were most common, in Norway also the perch. Salmonid species are more sensitive to eutrophication than the "Finnish and Swedish core species". For these countries, for example, coregonic fishes (*Coregonus albula* and *C. lavaretus*) and the burbot (*Lota lota*) are considered relevant sensitive indicator species to eutrophication.

No single fish species occurred at all BM-sites in each L-N-F country. The main reason for this is biogeographical because – due to differences in post-glacial distribution history – the natural fish fauna in Ireland and most parts of Norway is much poorer as compared to Finland and Sweden. Thus, there is, in addition to decreasing south-north gradient in fish species number, also an increasing one from west (Norway) to east (Finland). Perch occurred in all Swedish lakes, all but one Finnish, in two thirds of Norwegian lakes, but in none of the 6 Irish lakes. The dominant fish species in Irish reference lakes is brown trout (*Salmo trutta fario*, including ferox trout). In deep lakes Arctic char (*Salvelinus alpinus*) are present. Sea trout (*Salmo trutta trutta*), salmon (*Salmo salar*) and 3-spined stickleback (*Gasterosteus aculeatus*) can also be present. Non-native *Phoxinus phoxinus* occurred in a few Irish lakes. All fish species in Finnish, Norwegian and Swedish lakes were considered native at a national basis. Perch and other cyprinid species are non-native in Ireland. Many fish species with native occurrence in Fennoscandia and continental Europe have been spread as non-native species in Ireland, but only in lakes not passing the reference filter.

As the most common species of our lake IC fish data set are quite tolerant along environmental gradients, they are often present both in reference and in impacted lakes. Therefore, attention has been paid to the relative abundance of fish as the increasing productivity of lake ecosystems, including eutrophication effects, usually result in higher fish numbers and biomasses and in increased proportion of cyprinid fishes. Therefore the CPUE data is of importance. And really, in our set of 169 lakes of common IC type, the average total weight and number of fish per unit effort in gillnet sampling was 1193 g (SD=912) and 37 ind. (SD=35) in reference lakes (benchmark sites, n=106) while the corresponding catches in impacted lakes were 1690 g (SD=1104) and 47 ind. (SD=49). The difference between reference and impacted lakes was significant (F-test, P<0.05 in both weight and number data).

Biological communities representing the "borderline" conditions between good and moderate ecological status:

- In Finnish IC common type lakes with moderate status, total abundance and biomass, and cyprinid % are higher and occurrence of indicator species lower than in reference conditions. Mainly normal population structure of core species perch, pike, ruffe and roach is typical whereas symptoms of decrease in indicator species like burbot and vendace that demand oxygen rich hypolimnetic water are common. More information on the responses of fish communities to eutrophication in Finnish lakes is given in Rask et al. (2010, 2011b).
- In Irish lakes of common IC type intolerant fish species (such as brown trout and Arctic char) were dominant above the G/M boundary whereas lakes below the boundary were characterised by a higher biomass of tolerant fish species (roach, perch). In a national analysis it appeared that an equal biomass proportion of tolerant and sensitive fish species is characterising the G/M boundary in Irish lakes.

Table 8.1 Number of BM-sites (Reference_IC = 1) with occurrence of certain fish species. For comparison the total numbers of BM-sites are 49 for Finland, 6 for Republic of Ireland, 17 for Norway, and 34 for Sweden.

Species	Finland	Ireland	Norway	Sweden	Total
Perca fluviatilis	48		11	34	93
Rutilus rutilus	40		4	33	77
Esox lucius	26		4	33	63
Gymnocephalus cernuus	42			18	60
Osmerus eperlanus	20		3	12	35
Coregonus lavaretus	24		2	7	33
Coregonus albula	22			9	31
Alburnus alburnus	18			10	28
Lota lota	12		1	9	22
Abramis brama	13			5	18
Scardinius erythrophthalmus	5			10	15
Salmo trutta	2		12		14
Phoxinus phoxinus	6	3	2	2	13
Salvelinus alpinus	1	5	6	1	13
Tinca tinca	1			7	8
Salmo trutta fario		6			7
Sander lucioperca	6			1	7
Salmo salar	1	4			5
Abramis bjoerkna	3			1	4
Cottus poecilopus	1			3	4
Leuciscus idus	2			2	4
Leuciscus leuciscus	3			1	4
Pungitius pungitius	2			2	4
Salmo trutta trutta		4			4
Anguilla anguilla		2		1	3
Cottus gobio	3				3
Gasterosteus aculeatus		1	1	1	3
Thymallus thymallus	2				2
Carassius carassius			1		1
Cobitis taenia				1	1
Cottus sp				1	1
Cyprinidae unknown				1	1

Comparison with WFD Annex V, normative definitions for each QE/ metrics and type Finnish method:

- In high and good status Finnish lakes of the common IC type, the fish abundance parameters (NPUE and WPUE) were close to natural or undisturbed levels. Also the high occurrence of sensitive indicator species (Table B.1 in Annex B) was in line with the normative definition of high and good ecological status.
- In lakes of moderate status, moderate differences exist in the fish communities reflected as higher NPUE and biomass proportion of Cyprinid species.
- Based on the data from reference and impacted lakes, the G/M boundary is two times higher than the reference value for NPUE, and 1.54 times higher than

reference value for Cyprinid biomass proportion in the lakes of common IC type. The average occurrence of the indicator species in lakes with high or good status is 1.02 species / lake and in lakes with moderate status 0.66 species / lake.

Irish method:

- In high and good status Irish lakes (common IC type and all Irish types) the values of core metrics, Total BPUE, NAT_BPUE and Perch_BIO are at or close to natural or undisturbed levels.
- In lakes of moderate status, moderate differences are observed in these metrics reflected as higher TOT_BPUE and Perch_BIO and lower NAT_BPUE. See Annex C.

Changes across pressure gradient

Finnish EQR4: examples of statistically significant pressure-response relationships of metrics used in the assessment tool EQR4 are given in recent publications (Rask et al 2010, 2011b). In the present common IC data set (n = 169), the relation between EQR4 and total phosphorus was significant (y = $-0.138*\ln(x) + 1.036$, R²=0.344, P<0.001), see also Annex B.

Irish FIL2:

- In general, native BPUE (e.g salmonids) and proportion of lithophilic species were negatively correlated with the pressure
- Instead, total BPUE, roach BPUE, phytophilic species BPUE, and proportion of cyprinid fish in biomass were positively correlated with the pressure. (Kelly et al. 2012)
- These responses differ slightly for each of the 4 typologies.
- In the common IC data set, the relation between FIL2 (with transformed class boundaries of 0.8, 0.6, 0.4 and 0.2) and total phosphorus was significant (y = -0.126*ln(x) + 0.904, R²=0.130, P<0.001), also see Annex C.

EQR values of both methods also correlated significantly with other metrics related to eutrophication, like with the percentage of agricultural land in the catchment area (Finland) and with chlorophyll *a* concentration (Ireland).

Annexes

A. Overview of the Northern GIG Fish fauna assessment methods

		Finland	Sweden	Ireland (RoI and NI)	Norway
1.1	List of methods used	Finnish Lake Fish Classification Index (EQR4)	Assessment Criteria for Ecological Status of Fish in Swedish Lakes (EQR8)	Fish in Irish lakes classification tool (FIL2)	Fish Community Index (FCI)
1.2	What relevant parameters indicative of the BQE are covered?	Biomass, abundance, occurrence of indicator species	Diversity, biomass, abundance, ratio sensitive to insensitive taxa	Biomass, abundance, % composition, indicator species	Dominance category and relative fish status
1.3	Complete list of biological metrics used in the assessment with short description	 Total biomass of fish per gillnet night (BPUE); Total number of fish individuals per gillnet night (NPUE); Biomass proportion of cyprinid fish and Occurrence of indicator species 	 Number of native fish species; Simpson's diversity index (based on number of individuals); Simpson's diversity index (based on biomass); Relative biomass of native fish species; Relative abundance of native fish species; 	 RHEO_BIO %: individuals that are rheophilic SPE_EVEN: Species evenness/dominance (1/D=1/(Nmax/Ntot) BREAM_%_IND: % composition of bream based on CPUE ROACH_BPUE (mean BPUE of roach) PHYT_%_BIO: % individuals that are phytophilic 2_%_BIO: % biomass of Group 2 species non native species influencing biology CYP_BIO: % biomass of cyprinid species, inc hybrids RUDD_%_IND: % composition of rudd based on CPUE LITH_IND:% individuals (based on CPUE excl. eels and adult salmon) that are lithophilic 	 Species list Dominance category: (i) dominance, (ii) subdominant and (iii) rare. These categories are given the weights 1.0, 0.75 and 0.50 Fish status (i) unchanged, (ii) marked change (increased or decreased), (iii) exterminated.

		Finland	Sweden	Ireland (RoI and NI)	Norway
			 Mean mass; Proportion of piscivorous percids; Ratio perch /cyprinids (based on biomass) 	 TOT_BPUE: sum of mean biomass per unit effort NAT_BPUE: sum of mean biomass per unit effort of native fish species PERCH_BIO: Mean perch biomass per unit effort MAX_L_DOM_BIO: Max length of dominant species (based on BPUE) (only used for one typology) 	 Lost stocks for the three categories are given the weights 1.0, 0.75 and 0.50, while changed populations are given the weights 0.75, 0.50 and 0.25.
1.4	From which biological data are the metrics calculated?	Aggregated data from multiple sampling/survey occasions in time, data from single sampling/survey occasion in time	Data from single sampling/survey occasion in time	Data from a single sampling survey	Aggregated data from interviews, test fishing, reports etc
1.5	Combination rule for metrics	Average metric scores	Average metric scores	Discriminant analysis rules were developed for each typology using a stepwise procedure. (Combines metrics using a classification rule - Mahalanobis squared distances between the units and the group means are calculated from the canonical variate scores. Each unit is then allocated to the group for which it has the smallest Mahalanobis squared distance to the group mean. It moves away from scoring individual metrics). Posterior probablilities of the typology-specific qualitative classification rules were used to	Not available

		Finland	Sweden	Ireland (RoI and NI)	Norway
				derive a pseudo EQR, 2 weighted scores were calculated and these were averaged to get an eqr.	
1.6	Is the assessment method applied to water bodies in the whole country?	Applied in Ecoregion 22 (Fennoscandian Shield) covering > 95% of the area	Yes	Applied in Ecoregion 17 (RoI and NI (part of UK) and Scotland	Used in different regions
1.7	Does the selection of metrics differ between types of water bodies?	No	No, all metrics that can be calculated from fish samples from a specific site are used.	Yes, 3 core metrics are used for all lake types and an additional 2 or 3 are also used for each of four lake types.	No
2.1	Scope of detected pressures	Catchment land use, eutrophication, general degradation	Acidification, eutrophication, general degradation	Eutrophication, general degradation	Acidification, eutrophication, general degradation
2.2	Has the pressure- impact relationship of the assessment method been tested?	Yes, with quantitative data (e.g. against range of sites reflecting continuous gradient of pressure)	Yes, with qualitative data (e.g. response at reference against impacted sites).	Yes with quantitative data	Yes, with quantitative data based on test-fishing and water chemistry related to acidification (e.g ANC)
3.1	Scope of reference conditions	Surface water type- specific	Site-specific	Fish type and surface water specific	Site-specific
3.2	Key sources to derive reference conditions	Existing near-natural reference sites, least disturbed conditions	Existing near-natural reference sites, least disturbed conditions, modeling (extrapolating model results)	Existing near-natural reference sites and least disturbed sites (for high alk lakes)	Existing near-natural reference sites, least disturbed conditions

		Finland	Sweden	Ireland (RoI and NI)	Norway
3.3	Number of sites, location and geographical coverage of sites used to derive reference conditions	127 sites, located fairly evenly throughout the country; reference sites found in Finnish River Basin Districts 1-7	116 sites (in high and good status); all parts of Sweden, covering the following range of environmental factors: altitude $10 - 894$ m above sea level, lake area 2 - 4236 ha, maximum depth $1 - 65$ m, annual mean in air temperature -2 - 8 °C	43 reference lakes (majority in high status, high alkalinity lakes in good status), all sites in RoI, majority in west of Ireland	About 40 sites, located in different counties
3.4	Time period of data from sites used to derive reference conditions	1995-2007	Fish data were extracted from the National Register of Survey Test- fishing in 2005, and the latest date of standardized sampling was used for each lake	2005 to 2009 (latest date of sampling was used for each lake)	1995-2010
3.5	Reference sites characterization: criteria to select them	Lack or minor presence of human induced environmental pressures, including: main nutrients (Ptot, Ntot), land use information (including Corine land cover) and nutrient load model calculations	Fish metrics at reference sites are expected to have low deviation from site-specific reference values	Lack or minor presence of anthropogenic pressures. Reference crietria: 1) >80% natural land, <20% agricultural land, <20% urban land, <20% forestry 2) pH >6, 3) Connectivity (no impassable barriers present d/s, impassable natural barriers present d/s and natural barriers present d/s but passable on some occasions)	Lack or minor presence of anthropogenic pressures. Assessement fram water quality and land use

		Finland	Sweden		Ireland (RoI and NI)	Norway
				4) TP n Chlor a 5) Fish 6) Pala status	nean <12 and <20 is rejection threshold, a mean <8 and <15 is rejection threshold metrics should be near reference eolimnology also confirmed reference for some lakes.	
3.6	Are the assessment results expressed as EQRs?	Yes	Yes	Yes		Yes
4.1	How many sampling / survey occasions (in time) are required to allow for ecological quality classification of sampling / survey site or area?	1-8 sampling nights per sampling season (depending on the lake size and depth)	One occasion		One occasion (1-8 sampling nights depending on depth and lake area))	Can be assessed from interviews (one occasion)
4.2	How many spatial replicates per sampling / survey occasion are required?	5 to 68 gillnet nights, depending on lake size and depth	8-68 benthic gillnets, depe on lake area and maximun depth	ending n	Depends on lake area and maximum dept (4-60 benthic nets) plus other nets	h Information may be obtained from different persons or written sources (expert judgement)
4.3	Sampling / survey months	Mid-July to early September	Late summer (usually betw July 15 and August 31), wh deep lakes are thermally stratified	veen hen	Mid June to 1 st week in October	Not dependent of time of year

		Finland	Sweden	Ireland (RoI and NI)	Norway
4.4	How the sampling / survey sites or areas were selected?	Expert knowledge, random sampling / surveying, stratified samplings	Lakes in a national network of monitoring sites, including operative monitoring of lake restored by liming, complemented by lakes monitored by county level administration	of Expert opinion, random sampling, stratified sampling	Data from all lakes in a catchment or random selection of survey sites
4.5	What is the total sampled / surveyed area or volume, or total sampling duration to classify site or area?	Lake area 0.01-10 km ² , in larger lakes a representative sub area up to 10 km ² is selected by expert judgment	Standard effort of benthic gillnets set for 12 hours (+/- hour) including dusk and day	Depends on lake area and maximum 1 depth, lake area varies from 1.4Ha to wn 11650Ha	Not relevant, see 4.1
4.6	Sampling / survey device	Gill net (Nordic multimesh surveynets (CEN 14757:2005))	Gill net (Benthic and pelagic gillnets as specified in EN 14	Gill net (Nordic multimesh survey nets (CEN 14757:2005)) and surface floating nets, and fyke nets and additional larger mesh braided survey gill nets in high alkalinity lakes also used	Standard questionnaire (No CEN standard exists)
4.7	Minimum size of organisms sampled and processed	40 mm (0+ fish)	Down to about 3 cm total le	ngth 10mm	Mainly from fish of a certain size that are caught by fishermen
4.8	What is the level of taxonomical identification? What groups to which level?	Species / species groups	Species / species groups, fan (if possible hybrids of cyprini are identified at this level)	nily Species ids	Species

		Finland	Sweden	Ireland (RoI and NI)	Norway	
5.1	Setting of the ecological status boundaries: methodology and reasoning to derive and set boundaries	Equidistant division of the EQR gradient (linear response to eutrophication), high- good boundary derived from metric variability at near-natural reference sites	G/M boundary set at the EQR8 value with equal risk of misclassification of high plus good sites versus moderate to bad sites (according to procedures used in the FAME project, for development of a European fish index for rivers)	Discriminant analysis typology-specific classification rules, relevant to eco-region 17, were derived from the training dataset to describe a lake as High, Good, Moderate or Poor/Bad. Stepwise multiple linear regression analysis and expert opinion were used to develop the EQR models.	The boundaries are based on expert judgement.	
5.2	Boundary setting procedure	High/good class boundary was set to the 25th percentile of the EQR-distribution of fish parameters in reference lakes of each lake type; other class were set by dividing into even distances the EQR values of a lake type from the H/G class boundary to the extreme EQR recorded	The good-moderate boundary was set at the EQR8 value whic minimized the risk for type I ar type II errors; the high-good boundary was conservatively s at the 95th percentile of EQR values in reference lakes; the poor-bad boundary at the 10th percentile of EQR values in impacted lakes; the moderate- poor boundary was more arbitrarily set at the mean of Ev values at good-moderate and moderate-poor boundaries	 The results of the qualitative classification rule and quantitative EQR model were cross-tabulated at various cut-points in order to quantify class boundaries. A High lake was defined to be [0.76, 1]; Good [0.53, 0.76); Moderate [0.32, 0.53); and, Poor/Bad [0, 0.32). A 	The H/G and G/M boundaries are the at the 95 and 75 percentile	
5.3	Is the description of the communities of reference / high- good-moderate status provided?	Yes: natural fish communities from reference lakes, usually 1 to 10 species per lake;	Fish metrics at high status sites have no or very low deviation from site-specific reference values, inferred by multiple	 Yes, high status lakes=reference lakes, natural fish communities, Good status are close to reference with sensitive indicator species present 	The reference condition means that the status of each species in a community is	

		Finland	Sweden	Ireland (RoI and NI)	Norway
		good status fish communities in Finnish lakes are close to those in reference conditions including the possible occurrence of sensitive indicator species	regression models with environmental factors		unchanged. Score equal 1.
6.1	Has the uncertainty of the method been quantified? Is it regarded in the assessment?	Yes, preliminarily	Yes	Yes, preliminarily	Will be regarded
6.2	Specify how the uncertainty has been quantified and regarded.	Uncertainty was tested by classifying a 320 lake subsample of Nordic lakes from the gross-GIG database. In 16% of reference lakes the classification output was worse than good whereas46% of impacted lakes was classified to status better than moderate.	A general measure of uncertainty is recommended when assessment is based on only one sampling occasion; the general uncertainty measure was set as the median standard deviation of the EQR8 value in a dataset of 113 lakes with at least 3 years of data	Uncertainty was tested by classifying a 320 lake subsample of Nordic lakes from the gross-GIG database. In 37% of reference lakes the classification output was worse than good whereas43% of impacted lakes was classified to status better than moderate.	It will be quantified using different weights of changed and lost populations

B. Finnish lake fish assessment method EQR4

EQR4 is mainly based on data from standard gillnet test fishing with Nordic gillnets. Three of the four metrics in EQR4 are calculated from the gillnet data. All available data (including previous study or restoration projects, catch statistics of local fishermen, fishery inquires) is used for the metrics "indicator species" (however, in many cases gillnet data is the only available data).

Finnish lakes are categorised into 12 lake types based on physical-chemical and geographic properties (Figure B.1). For the metrics calculated from the gillnet data, reference values (RV) and class boundaries (CB) are based on the data of type-specific reference lakes (n = 127). Compared to the reference lakes of the previous version of EQR4, the criteria were tighter and small (<10 ha), previously severely acidified and uncertain cases were dropped.

Preliminary class boundaries have been calculated for 10 lake types (lake types 3 and 5 are combined due to lack of data). For lake type "lakes with low retention time", the existing boundaries are adapted from nearest lake types. Lake type "high altitude lakes" can not yet be classified. Lake type "Naturally eutrophic and high calcium lakes" is lacking reference sites and "best left" sites (n = 10, based on total phosphorus classification, class = good) are used for calculating RVs and CBs.

The ecological classification by the metrics "indicator species" is defined as expert judgement based on presence, absence/extinction of certain indicator species (Table B.1). This metrics is same for all lake types. However, for small lakes(<200 ha) the criteria are less demanding. Previously, we have tested the sensitivity of seven metrics ("number of fish species", "indicator species", "total biomass of fish", "total number of individuals", "species diversity", "biomass proportion of cyprinids" and "biomass proportion of piscivorous percids") against eutrophication pressure, which is the main problem in Finnish lakes. Four metrics responded significantly to eutrophication pressure: "indicator species", "total biomass of fish (totBPUE)", "total number of individuals (totNPUE)" and "biomass proportion of cyprinids". These metrics are used for official classification of Finnish lakes (EQR4). In the updated version of EQR4, only cyprinids that are known to indicate eutrophic conditions are included in the metrics "biomass proportion of cyprinids" (e.g. minnow, dace, asp and ide are excluded).

In the Finnish fish based lake classification system, reference value (RV) is the median value of the gillnet data of the type-specific reference lake group (Table B.2). EQR-value is calculated by dividing the observed value with the reference value if the values of the metrics decrease with human impact. When the values of the metrics increase with human impact, EQR-value is calculated by dividing the reference value with the observed value. "Total biomass of fish" and "total number of individuals" are bidirectional metrics: both exceptionally high and low values decrease the classification. Low values don't affect the classification unless there is environmental pressure that decreases the fish abundance (e.g. severe eutrophication causing anoxia).

In the Finnish assessment method EQR4, the high/good boundary (H/G) is based on the data from the reference lakes which corresponds to the WFD requirements. The high/good boundary (H/G) was set to the 25th percentile from the type-specific EQRdistributions of reference lakes. Other boundaries were set to even distances from H/G boundary to observed type-specific minimum or maximum value from all available gillnet data. Equidistant boundaries were used as the response of total fish biomass and cyprinid biomass to eutrophication has found to be linear in Finnish lakes (Olin et al. 2002). The assessment method results in high and good ecological status can be compared to the normative definitions according to WFD: In high and good status Finnish lakes of the common IC type, the fish abundance parameters (NPUE and WPUE) were close to natural or undisturbed levels. Also the high occurrence of sensitive indicator species, like burbot and vendace that demand oxygen rich hypolimnetic water, was in line with the normative definition of high and good ecological status. In lakes of moderate status, moderate differences exist in the fish communities reflected as higher NPUE, lower occurrence of indicator species and higher biomass proportion of Cyprinid species. Based on the data from reference and impacted lakes, the G/M boundary is two times higher than the reference value for NPUE, and 1.54 times higher than reference value for Cyprinid biomass proportion in the lakes of common IC type. The average occurrence of the indicator species in lakes with high or good status is 1.02 species / lake and in lakes with moderate status 0.66 species / lake.

To calculate the class boundaries, EQR-values were calculated for each reference lake. The EQR-values of each type-specific reference lake group produced the EQRdistribution from where the class boundaries were calculated. The high/good boundary was decided to be the 25th percentile from the EQR-distribution of the reference lakes. Other boundaries were set to equal distances from high/good boundary to observed lowest, type-specific EQR value. There are few exceptions to the above presented boundary setting procedure. In the lake type "Naturally eutrophic and high calcium lakes" the H/G boundary is the median value of the "best left" sites. For small totBPUEs or totNPUEs, the G/M boundary is the observed minimum, type-specific value in the reference data.

EQR-values from different metrics have different ranges thus they are transformed to scale from 0 to 1. This is done by multiplying by certain constant (metric values decreasing with increasing pressure) or by boundary translation by using regression (metric values increasing with increasing pressure, Table B.2). The fish based lake classification i.e. EQR4 value is the average of the EQR-values of the four metrics.

Table B.1 Criteria for EQR according to indicator species. Documented extinction of indicator species decrease classification to next lower class. Stocking of indicators does not increase classification.

EQR	Criteria, >200 ha lakes	Criteria, <200 ha lakes
0.9	Natural population: <i>S. alpinus, C. lavaretus, P. phoxinus, B. barbatula, T. quadricornis</i>	As in >200 ha lakes



Figure B.1 Finnish lake typology.



Figure B.2 Regression between total phosphorus (TP) and the updated EQR4.

In a lake set of 124 Finnish lakes, the updated EQR4 was significantly and negatively correlated with total phosphorus and percentage (Figure B.2) of agricultural land in catchment area (Figure B.3). The difference between impacted and reference lakes in this lake set was also significant (Figure B.4).



Figure B.3 Regression between percentage of agricultural land and the updated EQR4.



- Figure B.4 The updated EQR4 (minimum, lower quartile, median, upper quartile and maximum) in reference and impacted Finnish lakes.
- Table B.2 The reference values (RV) and class boundaries (H/G = high/good, etc.) for 3 metrics expressed as BPUE (biomass per unit effort = g / gillnet night), NPUE (n per unit effort = n / gillnet night) or biomass proportion of cyprinids. Equations to transform EQR-values on the scale from 0 to 1 are also shown.

Metrics	Туре	Lake n	RV	H/G	G/M	M/P	P/B	Scaling to 0-1
Total biomass	1	32	522	178	133	89	44	EQR*2.349
(low BPUEs)	2	17	546	227	170	113	57	EQR*1.926
	3&5	13	466	384	288	192	96	EQR*0.971
g/gillnet night	4	16	425	150	113	75	38	EQR*2.261
	6	14	727	534	401	267	134	EQR*1.089
	7	11	988	829	622	415	207	EQR*0.953
	8	12	1205	337	253	169	84	EQR*2.858
	9	12	1155	699	524	349	175	EQR*1.322
	12	10	2344	1313	985	657	328	EQR*1.428
Total biomass	1	32	522	884	1095	1437	2090	1.759*EQR-0.240
(high BPUEs)	2	17	546	932	1163	1547	2308	1.716*EQR-0.206
	3&5	13	466	813	992	1274	1779	1.927*EQR-0.304
g/gillnet night	4	16	425	828	1011	1297	1811	2.676*EQR-0.486
	6	14	727	885	1048	1284	1659	1.258*EQR-0.305

Metrics	Туре	Lake n	RV	H/G	G/M	M/P	Р/В	Scaling to 0-1
	7	11	988	1895	2105	2367	2704	3.845*EQR-1.205
	8	12	1205	1595	1983	2622	3866	1.352*EQR-0.221
	9	12	1155	1368	1579	1867	2284	1.772*EQR-0.696
	12	10	2344	1895	2338	3052	4394	0.853*EQR-0.255
Total number	1	32	21.0	2.7	2.0	1.4	0.7	EQR*6.185
(low NPUEs)	2	17	23.8	10.6	7.9	5.3	2.6	EQR*1.799
	3&5	13	22.8	11.7	8.8	5.9	2.9	EQR*1.551
ind./gillnet night	4	16	9.9	5.3	4.0	2.7	1.3	EQR*1.492
	6	14	24.3	13.6	10.2	6.8	3.4	EQR*1.428
	7	11	53.4	34.4	25.8	17.2	8.6	EQR*1.242
	8	12	40.8	12.3	9.2	6.1	3.1	EQR*2.658
	9	12	40.3	13.4	10.0	6.7	3.3	EQR*2.411
	12	10	112.1	46.2	34.7	23.1	11.6	EQR*1.940
Total number	1	32	21.0	33.1	41.8	56.9	88.8	1.502*EQR-0.156
(high NPUEs)	2	17	23.8	38.0	47.4	63.1	94.3	1.607*EQR-0.205
	3&5	13	22.8	30.8	37.3	47.4	64.9	1.544*EQR-0.341
ind./gillnet night	4	16	9.9	39.1	47.2	59.4	80.3	4.598*EQR-0.370
	6	14	24.3	32.1	41.0	56.6	91.5	1.223*EQR-0.124
	7	11	53.4	61.5	69.9	81.0	96.3	1.915*EQR-0.862
	8	12	40.8	51.6	64.8	87.0	132.3	1.246*EQR-0.184
	9	12	40.3	50.2	61.3	78.6	109.4	1.384*EQR-0.310
	12	10	112.1	89.4	112.1	150.1	227.4	0.788*EQR-0.189
Cyprinid	1	20	33.4	42.7	48.7	56.6	67.6	2.075*EQR-0.827
biomass	2	17	36.5	55.0	59.1	63.7	69.2	4.416*EQR-2.129
proportion	3&5	13	36.1	38.8	44.2	51.4	61.4	1.750*EQR-0.830
%	4	14	24.7	37.8	39.8	42.1	44.6	6.037*EQR-3.149
	6	14	33.8	48.0	53.5	60.4	69.3	2.776*EQR-1.153
	7	10	38.9	46.9	52.7	60.2	70.1	2.179*EQR-1.010
	8	11	39.7	43.8	49.7	57.4	67.9	1.870*EQR-0.895
	9	10	37.1	57.5	61.9	67.0	73.0	4.384*EQR-2.027
	12	10	62.2	56.5	61.8	68.3	76.2	2.106*EQR-1.519

C. Irish fish assessment system (FIL2)

An ecological classification tool (FIL2) suitable for establishing ecological status of lakes in Ireland based on fish population parameters has been recently developed to comply with the requirements of the Water Framework Directive. Agencies from the Republic of Ireland and Northern Ireland have contributed data from netting surveys and supporting information which was used in model development. A suite of metrics from native and non-native fish species were combined to derive a classification, using nutrients (total phosphorus and chlorophyll a) as the predominant pressure as this is the primary pressure on lakes in Ireland (Tierney *et al*, 2010)

Sampling Method

Fish sampling was conducted using standard Nordic monofilament multi-mesh benthic and surface survey gill nets. The gill netting procedure was in accordance with a modified version of the European standard multi-mesh gillnetting method (CEN, 2005) which was adapted by Inland Fisheries Ireland for WFD fish monitoring in Irish lakes (Kelly *et al.*, 2008b). Fyke nets and surface floating survey gill nets were used to supplement the gill netting effort in all lakes. In some lakes (particularly high alkalinity lakes) the netting effort was supplemented with single panel multifilament survey gillnets (27.5 x 2.0m) of larger mesh sizes (60-70mm knot to knot). Fish data from 137 lakes (151 surveys) in the Republic of Ireland and Northern Ireland were used. 43 reference sites were included in the database.

FIL2 model

A lake typology relevant to fish populations in lakes from Ecoregion 17 was produced as part of the ecological classification tool development. Four lake types were determined based on fish metrics and abiotic variables from 43 "reference" lakes using cluster analysis and stepwise discriminant analysis. The specific lake fish typology categorised lakes into low (\leq 67 CaCO₃ mg L⁻¹) or high (> 67 CaCO₃ mg L⁻¹) alkalinity, and shallow (\leq 17m) or deep (> 17m) maximum depth.

The fish in lakes classification tool (FIL2) follows a multimetric predictive approach and assigns ecological status to a lake using a novel approach of two independent methods. FIL2 qualitatively defines a lake's ecological status based on fish metrics using discriminant classification rules and, using a generalised linear model, quantitatively derives an Ecological Quality Ratio (EQR, 0<EQR<1), along with associated 95% confidence intervals. It is recommended that both methods are used to validate output and cross-check and highlight potential misclassification. The results of the qualitative classification rule and quantitative EQR model were cross-tabulated at various cut-points in order to quantify class boundaries. A High lake was defined to be [0.76, 1]; Good [0.53, 0.76); Moderate [0.32, 0.53); and, Poor/Bad [0, 0.32).

An investigation was also carried out to assess if FIL2 could be used to classify lakes in Scotland. Initial results are positive and the Scottish Environmental Protection Agency is provisionally adopting the tool for use in Scotland.

The relationship between FIL2 and pressure

The mean EQR of lakes classified as 'reference' (0.71) during the tool development was significantly higher than those classified as 'impacted' (0.43) (Independent t-test, P<0.001) (Figure C.1). FIL2 EQR values were negatively correlated with both mean total phosphorus (Pearsons correlation, r=-0.598, P<0.01) and maximum chlorophyll a (Pearsons correlation, r=-0.536, P<0.01) (Figure C.2 and Figure C.3). There was also a significant difference in the EQR between each pressure index class (Independent samples Mann Whitney U test, High vs Good, P<0.05; Good vs Moderate P<0.05, Moderate vs Poor/Bad P<0.05; High vs Moderate P<0.05; High vs Poor/bad P<0.05; Good vs Poor/Bad P<0.05) (Figure C.4).



Figure C.1 Box and whisker plots of FIL2 ecological quality ratio (EQR) scores in reference and impacted lakes (minimum, 1st quartile, median, 3rd quartile and maximum).



Figure C.2 FIL2 ecological quality ratio (EQR) scores versus total phosphorus (mean) in Irish lakes.



Figure C.3 FIL2 ecological quality ratio (EQR) scores versus chlorophyll a (maximum) in Irish lakes.



Figure C.4 Box and whisker plots of FIL2 ecological quality ratio (EQR) scores in relation to the pressure index in Irish lakes.

Boundary setting

The Irish assessment method FIL2 has a multimetric predictive approach and assigns ecological status to a lake using a novel approach of two independent methods. FIL2 qualitatively defines a lake's ecological status based on fish metrics using discriminant classification rules for each of the four typologies using a water quality gradient and, using a generalised linear model, quantitatively derives an Ecological Quality Ratio (EQR, 0<EQR<1), along with associated 95% confidence intervals. Both methods are used to validate output and cross-check and highlight potential misclassification. A range of bounary values were investigated to determine the High/Good, Good/Moderate, Moderate/Poor and Poor/Bad boundaries. The results of the qualitative classification rule and quantitative EQR model were cross-tabulated at various cut-points (boundaries) in order to quantify the class boundaries. Each boundary was determined when the maximum correct classification from the cross tabulation of EQR ecological status class and discriminant analysis ecological status class was achieved for that ecological status class. This resulted in an overall correct classification between the EQR ecological status class and discriminant analysis ecological status class of 56.9%. Expert opinion was then used to verify if the boundaries and ecological status classes could be compared to the normative definitions according to WFD. In high status Irish lakes all type specific intolerant or disturbance sensitive species fish species (e.g. trout and char) are present and dominant. The species composition and abundance of these species corresponds to

undisturbed conditions. There was no observed failure in the reproduction or development of any particular species. In good status Irish lakes only a slight decrease in the type specific communities was observed and there was no observed failure in the reproduction or development of any species. In moderate status Irish lakes there was a moderate decrease in the type specific fish community and a moderate increase in the proportion of tolerant species (e.g. cyprinidae and percidae). Analysis showed that there appears to be an equal proportion of tolerant and sensitive species at the G/M boundary.

Description of the biological community representing the borderline conditions between good and moderate ecological status and between good and high ecological status

Method: Compare the fish community half a class over and half a class below the considered (H/G and G/M)

Ireland has a depauperate and distinctly young freshwater fish fauna compared with the rest of Europe. It is widely believed that Irish freshwaters were frozen to the point where there were no freshwater fish during the last glaciation, ending approximately 11,000 years ago. (Went 1949, 1950). This has resulted in a native fish fauna derived from salt tolerant, often migratory, ancestors that would have been able to colonise Irish freshwaters at the end of the last Ice Age. In addition to this native group there are nonnative species present, very probably introduced by man over the past 1000 years for food, bait, sport or accidentally. The result is a highly patchy and discontinuous fish species distribution in Irish freshwaters, which is further and strongly influenced by a "who put what where when?" effect. A consequence of this history is that not all water bodies have been exposed to colonisation by all fish species present on the island. Rather, fish communities in Irish freshwaters tend to separate into three main groups; the first group contains mainly native species, primarily salmonids and is characteristic of upland or more isolated lakes. The second group contains native species, along with cyprinids, perch and pike. The third group, typical of lowland lakes linked by river and canal systems, contains no (or a limited number of) native species and is dominated by cyprinids, perch and pike (Kelly et al., 2008a). Therefore it is quite difficult to describe the fish communities representing the borderline conditions between high and good and food and moderate status for Iriah lakes.

Mean TOTAL_BPUE, mean TOL_%_BIO (% BPUE tolerant fish species) and mean INTOL_%_BIO (% BPUE of intolerant fish species) were calculated for each EQR half class for each lake (Figure C.5 and Figure C.6). Data analysis shows that there was a continuous increase in TOTAL_BPUE in relation to decreasing ecological status/decreasing water quality (Figure C.4). Statistical analysis revealed that TOTAL_BPUE was significantly different between the high-good boundary and the good-moderate boundary (Independent samples Mann Whitney U test; Hlwr vs Gupr P<0.05; Glwr vs Mupr P<0.05).

Intolerant fish species (such as brown trout and Arctic char) were the dominant fish species in High and Good status lakes (Figure C.6). Nutrient enriched lakes (moderate and poor/bad) were characterised by a higher biomass of tolerant fish species than intolerant fish species. Analysis also showed that in general intolerant fish species

decreased and tolerant fish species increased in relation to in relation to decreasing ecological status (Figure C.6). Although there was no significant difference between the high-good (hlwr/gupr) and good-moderate (glwr/mupr) boundaries for intolerant and tolerant fish species (% bpue), the mean tol_%_BIO at Hlwr was slightly lower than at Gupr and Glwr was also lower than Mupr Figure C.6). For mean intol_%_bio the hlwr was greater than the gupr and glwr was greater than mupr (Figure C.6).



Figure C.5 TOTAL_BPUE (all fish species) vs ecological status (as indicated by half class boundaries) in Irish lakes. N=176).



Figure C.6 Mean percentage BPUE of tolerant and intolerant fish species in Irish lakes in relation to ecological status (as indicated by half class boundaries) N=176.

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D. Norwegian fish community index FCI

The Norwegian FCI was developed in 2009, as part of the national classification work of ecological and water chemical status in different water bodies (Anonymous 2009). This index was addressed mainly to acidification as a pressure. However, it intended to be used for any pressure. Information on the occurrence of different species of fish and their present community status is based on interviews or inquiries with local fishermen, land owners etc (Hesthagen et al. 1993). In addition, all historical sources, as well as data from all kind of test-fishing, will improve the result. Historical information is especially important for lake where fish populations have been lost several decades earlier. First, the FCI requires estimates of the reference condition (RC), defined as an unchanged and healthy population. For each fish community, this value is obtained by grouping the species into three categories; dominant [D], subdominant [S] and rare [R]. The weighted *RC* value for a given fish community is defined as:

$$RC = n_D \times w_{D,RE} + n_S \times w_{S,RE} + n_R \times w_{R,RE} = \sum_{i=(D,S,R)} n_i \times w_{i,RE}$$

where n_i is the number of species in abundance category *i* and the weights $[w_{D,RE}, w_{S,RE}, w_{R,RE}]$ correspond to the importance we assign to each of the three categories, using the weights $[w_{D,RE}, w_{S,RE}, w_{R,RE}] = [1.0, 0.75, 0.50]$.

Secondly, the FCI requires data on population status in terms of changes in abundance relative to the reference condition, grouping the species into three categories; unchanged/no damage [U], marked change (either increased or decreased) [C], or lost [L]. The change in fish status (CS) is then estimated as:

$$CS = \left[n_{D,C} \times w_{D,C} + n_{S,C} \times w_{S,C} + n_{R,C} \times w_{R,C} \right] + \left[n_{D,L} \times w_{D,L} + n_{S,L} \times w_{S,L} + n_{R,L} \times w_{R,L} \right]$$
$$= \sum_{i=(D,S,R)} n_{i,C} \times w_{i,C} + \sum_{i=(D,S,R)} n_{i,L} \times w_{i,L} = \sum_{i=(D,S,R)} \sum_{j=(C,L)} n_{i,j} \times w_{i,j}$$

where $n_{i,j}$ is the number of species from abundance category *i* assigned to change category j, and $w_{i,j}$ the corresponding weights. For changes (e.g. either increased or stocks, we use decreased) and losses of fish the weights $\left[w_{D,L}, w_{S,L}, w_{R,L} \right] = [1.0, 0.75, 0.50]$ $[w_{D,C}, w_{S,C}, w_{R,C}] = [0.75, 0.50, 0.25]$ and respectively. Finally, the fish community index [FCI] is defined as the relative deviation from the reference condition (RC): $FCI = \frac{RC - CS}{RC}$. By diving (RC-CS) with RC, the FCI value for a fish community will range from 1.0 (no change) down to zero (all species lost).

As stated, the FCI was addressed mainly to acidification as a pressure. We have tested the index on a data set from Enningdal watershed, which contained 60 lakes > 1.2 ha (Norwegian part of the watershed). Seven of these lakes have never had fish, while the remaining 53 lakes either still contain fish, or have lost their fish populations. Number of lakes with 1, 2 and \geq 3 species accounts to 23, 11 and 19, respectively. Perch (*Perca*)

fluviatilis) is the dominant species of fish in these lakes (Hesthagen et al. 2007). Twenty five lakes are either limed or affected by liming, having a mean pH of 6.12±0.70 SD. The remaining non-limed lakes, being situated both above and below the formerly marine line, had mean pH values of 4.92±0.24 (n=19) and 5.48±0,66 (n=16), respectively. We related pH, inorganic Al and ANC to FCI, grouped into fish communities with 2 and \geq 3 species, and for \geq 3 species grouped into non-limed and limed lakes (Table D.1, Figure E.1, Figure E.2). There are generally a significant correlation between FCI and these three water chemistry acidified-related variables, except for that of ANC. The best fit was obtained between FCI and water chemistry in non-limed lakes containing \geq 3 species, different from that in limed lakes (Figure E.2). We found relatively low values for FCI in limed lakes in spite of a good water quality. Thus, fish has so far failed to respond positively to this improved water quality because lost fish populations have not yet been re-established. The low rate of fish recovery in limed lakes is considered to be due to physical barriers between the different lakes, preventing them from invading these lakes (cf. Hesthagen et al. 2007). However, we conclude that FCI exhibited to some extent a pressure response to acidification.

Table D.1 P-values for regressions between pH, inorganic Al and ANC vs. lakes with either 2 or \geq 3 species (left), and between pH, inorganic Al and ANC and non-limed and limed lakes with \geq 3 species (right).

Water chemistry	All lakes (non-li with either 2	med and limed) or ≥ 3 species	Lakes with ≥ 3 species			
Variable	2 species	≥ 3 species	Non-limed	Limed		
рН	0.023	< 0.005	< 0.001	>0.05		
Inorganic Al	< 0.05	< 0.05	0.012	>0.05		
ANC	0.017	0.155	>0.05	>0.05		



Figure D.1The relationship between FCI and pH, inorganic Al (μ g/L) and ANC (acid neutralizing capacity, μ ekv/L) for both limed and non-limed lakes in Enningdal watershed containing 2 and \geq 3 fish species, respectively. Data from three lakes with ANC > 200 μ eg/L were excluded.



Figure D.2The relationship between FCI and pH, inorganic Al (μ g/L) and ANC (acid neutralizing capacity, μ ekv/L) for non-limed and limed lakes in Enningdal watershed containing \geq 3 fish species. Data from three lakes with ANC > 200 μ eq/L were excluded.

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E. Swedish fish index EQR8

Fish index development and response to pressures

The Swedish EQR8 was developed in 2005, using data from standardised time series sampling with Nordic multi-mesh gillnets (EN 14757: 2005). A draft report was reviewed by endusers during 2006, before finally published in 2007 (Holmgren et al. 2007, summarised in Holmgren et al. 2010). Index development followed, as closely as possible, procedures described for the European Fish Index for rivers (FAME CONSORTIUM 2004, Pont et al. 2007). Fish monitoring data had been delivered to the official national data host since 1996, and included in the National Register of Survey test-fishing (NORS). A previous Swedish fish index (FIX, Appelberg et al. 2000) measured deviation from typical values, rather than reference conditions, because pressure data were not available in NORS. In 2005, fish samples from 1157 Swedish lakes were available for revision of fish assessment criteria. Pressure data matching lakes with fish data were actively searched for in external data bases, and by a questionnaire sent to the county administration offices. Among requested pressure variables, pH was most often available (995 lakes), followed by total phosphorous concentration (total P, 592 lakes) and land use in the catchment (443 lakes). Reference lakes were selected if they had pH \geq 6, total P < 20 g/L, and/or < 25 % of the catchment covered by agricultural land use and < 1 % by urban land use. Lakes regularly treated with lime (737 lakes) to mitigate acidification effects were also excluded. 116 reference lakes remained for calibration of reference values for 16 candidate metrics using multiple regression models with environmental lake characteristics. Metrics where then expressed as standardised residuals (Z- and P-values). Metrics response to pressures, were evaluated by t-tests of differences between reference lakes and each of two groups of disturbed lakes (40 lakes with acidity stress and 56 lakes with nutrient stress). Ten metrics responded significantly to either acidity or nutrient stress, and eight metrics remained (Table E.1) after exclusion of two redundant metrics (i.e. highly correlated, Pearson's r > 0.8, with other metrics). Metric responses in limed lakes were similar to the responses in acidic lakes (not shown here). The fish index EQR8 was taken as the mean of all P-values that can be calculated, i.e. 3-8 metrics in a given fish sample. EQR8 was lower in disturbed lakes than in reference lakes (Figure E.1a). Two-way ANOVA revealed significant effects of general pressure ($F_{1,504} = 26.5$, P < 0.001) and the interaction between the predefined pressure and liming ($F_{1,504} = 4.13$, P = 0.04). Acidic lakes had lower EQR8 than reference lakes (Figure E.1b), with a significant effect of acidity ($F_{1,372} = 27,2, P < 0.001$), but no effects of liming ($F_{1,372} = 0.07$ och P = 0.79) or interaction between acidity and liming ($F_{1, 372} = 0.04$, P = 0.85). Lakes with high total P had lower mean EQR8 than reference lakes (Figure E.1c), but the effect was weaker than

for acidity. ($F_{1, 414} = 3.66$, P = 0.06). The effect of total P was significant when excluding limed lakes (t-test, t = -2.64, df = 130, P = 0.009).

Table E.1 Retained fish metrics expressed as Z-values. N1 and N2 are number of reference and disturbed lakes, respectively. Diff = difference between group means. Pvalue revealed by t-test. NS = not significant difference, i.e. P > 0.05. The Table is modified from Holmgren et al. 2007.

	Z-value	Acio	lity stre	ess ¹⁾	Nutrient stress ²⁾			
Metric	code	N1 - N2	Diff	Р	N1 - N2	Diff	Р	
1. Number of native fish species	Zniart	124 - 40	-1.09	<0,001	124 - 56	+0.39	0.031	
2. Simpson's D (numbers)	ZS Dn	124 - 36	-1.08	<0,001	124 - 56	+0.11	NS	
3. Simpson's D (biomass)	ZS Dw	124 - 36	-1.36	<0,001	124 - 56	+0.92	0.001	
4. Relative biomass of native species	ZlgWiart	123 - 40	-2.79	<0,001	123 - 56	+0.92	<0.001	
5. Relative abundance of native species	ZlgNiind	123 - 40	-1.36	<0,001	123 - 56	+0.55	0.031	
6. Mean mass (from total catch)	ZlgMeanW	124 - 36	+0.12	NS	124 - 56	+0.51	0.008	
7. Biomass proportion of piscivorous percids	Zandpis	123 - 36	+0.87	0,001	123 - 56	-0.03	NS	
8. Ratio perch / cyprinids (biomass)	ZlgAbCyW	102 - 16	+0.14	NS	102 - 53	-0.76	0.001	

¹⁾ non-limed lakes with total P < 20 μ g/l, disturbed lakes with pH < 6.

 $^{3)}$ non-limed lakes with pH \geq 6, disturbed lakes with total P > 20 µg/l.



Figure E.1 EQR8 (mean <u>+</u> 2 SE) in disturbed ("Påverkade") and reference lakes ("Referenser"), further divided in non-limed (filled symbols) och limed lakes (unfilled symbols). The number of lakes are given at each group. Comparisons between groups refer to a) general pressure, b) acidity stress and c) nutrient stress. From Holmgren et al. (2007)

Fish index implementation and practical evaluation

EQR8 was implemented in Swedish legislation (Naturvårdsverket 2008), i.e. prescribed for assessment of ecological status of the fish fauna in Swedish lakes. The endusers at the county administration have reported ecological status assessments for Swedish lakes in the Water Information System Sweden (VISS, <u>www.viss.lst.se</u>). Most of the lakes were assessed by expert judgement, because only a small proportion of the Swedish lakes have been sampled for fish or any other biological quality element. Some endusers provide new fish data to NORS each year. On request, the national data host delivers calculated fish metrics and EQR8 for all lakes and years in the actual county or one of the five water districts of Sweden. The enduser might then accept the EQR8-based

classification of ecological status, or modify the classification based on expert judgement motivated by other available information. It is not easy to track how many lakes have been officially assessed by EQR8 from one or more years of fish data. Several sources indicate low performance of EQR8 in regions with low representation of reference lakes in the data set to model site-specific reference condition (e.g. Sairanen et al. 2008), as well as in lakes of neighbouring countries (Holmgren et al. 2010).

Comments to intercalibration issues on 'boundary setting description'

Most of the Swedish lakes in the fish database NORS have unknown status of many important pressure variables (especially true for hydromorphological pressures, fish introductions, exotic species and exploitation by fishing). Selection of lakes for the index EQR8 was therefore primarily based on availability on pH and Total P, measured in surface water at least once within less than five years from a date of fish sampling. Because of the generally poor knowledge of pressures except for pH, precence/absence of liming and Total P, the data set of 'reference lakes' was considered to be a mixture of high and good status lakes, rather than lakes of purely high status.

The good-moderate boundary was set at the EQR8-value with equal probability of misclassification of lakes predefined as 'reference' (high + good) or 'disturbed' (moderate or worse). The most important boundary was set therefore set at EQR8 = 0.46 (at cross-section of curves in Figure E.2). Other class boundaries were suggested on more arbitrary grounds (Holmgren et al. 2007). For example, the high-good boundary (at EQR8 = 0.72) corresponds to less than 5 % probability of classifying a 'reference' lake as 'disturbed'.



Figure E.2 Change in proportion of correctly classified lakes when gradually increasing a potential threshold of EQR8 from zero to the maximum value observed. The thick (descending) curve represents 116 non-limed 'reference' lakes and the thin (ascending) curve represents 113 'disturbed' lakes (i.e. the same lakes as for filled symbols in Figure E.1a . The horizontal reference lines are set at 5, 50 respektive 95 % correctly classified lakes. From Holmgren et al. (2007)

Uncertainty in the EQR8-value was estimated using the 3-5 latest fish samples from 113 lakes sampled in three or more different years. For each lake the standard deviation in EQR8 was calculated. The median of SD's was 0.077 (Holmgren et al. 2007). This value was later suggested as a general measure of uncertainty in EQR8 for lakes with only one sample (Naturvårdsverket 2008).

Comments to intercalibration issues on 'high and good status communities'

The calibration data set of 116 'reference' lakes (high + good status) had altitudes of 10-894 m above sea level, lake area of 2- 4236 ha, maximum depth of 1-65 m, and annual mean air temperature of -2 to +8 °C. The variable characteristics of Swedish reference lakes imply a correspondingly high variation in fish communities. The site-specific reference values of metrics are calculated using intercepts and regression coefficients in Table E.2. The reference fish community may be exclusively Arctic charr (Salvelinus alpinus) at high altitude, or only perch (Perca fluviatilis) or pike (Esox lucius) in very small and shallow lakes at lower altitude. The species richness will be higher in larger and deeper lakes at lower altitude. Most often the benthic fish catches are dominated by perch and roach (Rutilus rutilus). Pike is also an expected member of the fish community in most low-land lakes, although it is less efficiently caught with the standard method. The three most frequently occurring species may occur together with other warm water species, and with cold water species if there is a large enough cold and well oxygenated hypolimnion throughout the summer stratification of deep lakes.

Table E.2	ntercept d	and coeffici	ients of r	regressior	n for	calculation of re	efer	ence value.	s, and
	standard	deviation	(SD _{resid})	needed	for	transformation	to	Z-values.	From
	Holmgren	17)							

		Altitude	Lake area	Maximum depth	Annual ait temp.	нс	
Metric	intercept	lg10(x+1)	lg10(x)	lg10(x)	(°C)	(0 or 1)	SD _{resid}
1. Number of native fish species	-0.410		2.534		0.347	-0.916	1.538
2. Simpson's D (numbers)	2.537	-0.460	0.380				0.570
3. Simpson's D (biomass)	1.223		0.345		0.153		0.753
4. Relative biomass of native species	3.666	-0.202	0.121	-0.394			0.202
5. Relativt number of native species	2.171	-0.397	0.081	-0.262	0.044		0.241
6. Mean mass (from total catch)	1.181	0.307			-0.038		0.235
7. Biomass proportion of piscivorous percids	0.057			0.198			0.175
8. Ratio perch / cyprinids (biomass)	1.223				-0.186		0.472

Upcoming index revision

The fish index EQR8, and other Swedish assessment methods, will be revised within a new research program, Waterbody Assessment Tools for Ecological Reference conditions and status in Sweden (WATERS), which started in 2011 and will continue until end of March 2016. For fish in small and medium-sized lakes, site-specific reference values of present and additional metrics will be calibrated in relation to hydromorphological and biogeographical lake characteristics. The amount of data from medium-sized lakes as well as high-altitude lakes will be increased by including new samples as well as sampling occasions with somewhat less than recommended number of gillnets per depth stratum. Metric selection, calculation of EQR and setting class boundaries will follow the most recently recommended procedures, e.g. considering results from the WISER program (www.wiser.eu), the ongoing intercalibration exercise, as well as from WATERS itself. The Swedish Agency for Marine and Water Management (SWAM) have no explicit plans to change the present recommendations on assessment methods (Naturvårdsverket 2008) before the WATERS program is completed.

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Abstract

One of the key actions identified by the Water Framework Directive (WFD; 2000/60/EC) is to develop ecological assessment tools and carry out a European intercalibration (IC) exercise. The aim of the Intercalibration is to ensure that the values assigned by each Member State 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. In total, 83 lake assessment methods were submitted for the 2nd phase of the WFD intercalibration (2008-2012) and 62

intercalibrated and included in the EC Decision on Intercalibration (EC 2013). The intercalibration was carried out in the 13 Lake Geographical Intercalibration Groups according to the ecoregion and biological quality element. In this report we describe how the intercalibration exercise has been carried out in the Northern Lake Fish fauna group.

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