Water Framework Directive Intercalibration Technical Report: Lake Phytobenthos ecological assessment methods

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JRC TECHNICAL REPORTS

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Edited by Sandra Poikane

2014

Report EUR 26512 EN

Joint Research Centre

European Commission
Joint Research Centre
Institute for Environment and Sustainability

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This publication is a Technical Report by the Joint Research Centre of the European Commission.

JRC88347

EUR 26512 EN

ISBN 978-92-79-35468-7 (pdf)
ISBN 978-92-79-35469-4 (print)

ISSN 1831-9424 (online)
ISSN 1018-5593 (print)

doi: 10.2788/7466

Cover photo: Sandra Poikane

Luxembourg: Publications Office of the European Union, 2014

Printed in Ispra, Italy

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1. Introduction

In the Phytobenthos cross-Geographical Intercalibration Group (GIG):

- 11 Member States (see Table 2.1) submitted their lake phytobenthos assessment methods;
- Intercalibration (IC) was carried out for three broad common types: high alkalinity (HA), moderate alkalinity (MA) and low alkalinity (LA) lakes;
- Intercalibration “Option 2” was used for HA and MA types - indirect comparison of assessment methods using a common metrics, while direct comparison was carried out for LA type where only 2 assessment systems were compared;
- The Trophic Index (Rott et al. 1999) was used as IC common metric, it was benchmark-standardized using “continuous benchmarking” approach;
- 8 countries participated in HA type intercalibration (HU and PL were excluded from calculation of harmonization ban, but included later), 8 countries participated in MA type intercalibration (FR, IT and DE excluded), 4 countries participated in LA type intercalibration (SE and FI excluded);
- The final results include harmonised EQRs of BE, DE, HU, IE, PL, SE, SI and UK phytobenthos methods for HA type; BE, FI, IE, SE, UK - for MA type, IE and UK – for LA type.

2. Description of national assessment methods

In the Phytobenthos Cross-GIG, eleven MS submitted their phytobenthos assessment methods to the intercalibration (Table 2.1, for detailed description see Annex H.1).

Table 2.1 Phytobenthos lake assessment methods submitted to the IC.

MS	Method	Status
BE-F	Proportions of Impact-Sensitive and Impact-Associated Diatoms (PISIAD)	Finalized formally agreed national method
DE	PHYLIB	Intercalibratable finalized method
FI	IPS	Intercalibratable finalized method
FR	Indice Biologique Diatomées (IBD)	Under development
HU	MIL- Multimetric Index for Lakes	Finalized formally agreed national method
IE	Lake Trophic Diatom Index (LTDI) mark 1	Intercalibratable finalized method
IT	Multimetric method ICM (IPS and TI)	Finalized
PL	PL IOJ (multimetryczny Indeks Okrzemkowy dla Jezior = multimetric Diatom Index for Lakes)	Intercalibratable finalized method
SE	IPS	Intercalibratable finalized method
SI	Trophic index (TI)	Finalized formally agreed national method
UK	DARLEQ mark 2	Finalized formally agreed national method

2.1. Methods and required BQE parameters

“Phytobenthos” is not a BQE: it is one element of “macrophytes and phytobenthos” and compliance checking for phytobenthos alone is inappropriate.

All MS assess the composition and relative abundance of diatoms, assumed to be proxies for the phytobenthos. The opinion of the phytobenthos group is that this alone does not fulfil the obligation to assess "abundance". Some MS, however, include larger algae in their macrophyte methods and include measures of abundance or percent cover. Whilst this should be sufficient to detect "nuisance" growths of algae, we do not believe that such methods alone are adequate to evaluate compositional changes in phytobenthos, or that macrophytes alone are adequate proxies for the entire BQE, e.g. in situations where these are impacted by hydromorphological pressures.

"Undesirable disturbances" are mentioned in the normative definitions but are not an explicit feature of any national assessment methods although it is possible that these were used in the establishment of status classes by some MS. Bacterial tufts are not assessed by any MS but are not generally regarded to be a problem in lakes. BE-F considers visible cyanobacterial films in the littoral equivalent to 'bacterial tufts'; their development is also undesired.

The collective view of the phytobenthos expert group is that an MS cannot be considered to be fully compliant with the normative definitions for macrophytes and phytobenthos if they only possess a macrophyte (or only phytobenthos) method. There are situations (e.g. where the lake is subject to hydromorphological stress, navigation etc.) where macrophytes will not give a reliable indication of the impact of nutrients on littoral flora, and also that the two elements react at different rates to changes in their environment.

It is possible that assessments may be based on either macrophytes or phytobenthos if there is evidence that both elements give similar assessment results within a MS but this assumption should be based on evidence.

For further details on methods, along with scientific literature and computation details see Annex H.1.

2.2. National reference condition and boundary setting

All methods have set reference conditions and boundaries using a method that complies with WFD CIS Guidance (see table 2.2.) except PL G/M boundary and HU H/G boundary (in bold). Therefore these methods were excluded from the calculation of boundary "harmonization band" (see Chapter 6).

Table 2.2 Overview of the methodology used to derive ecological class boundaries.

MS	Methodology used to set class boundaries
BE-F	• Type-specific values for the H/G boundaries were derived from the 90th percentiles of the relative abundance of impact-sensitive diatoms in historical assemblages predating 1940 (best 10%); • G/M boundaries were derived from the 90th percentiles of the relative abundance of impact-associated diatoms in such historical assemblages (best 90%); • G/M boundaries were cross checked against the 75th percentiles for actual assemblages from sites with TP and chl-a below G/M, as inferred from modelling; • For lake types with few historical data, the minimum relative abundance of impact-sensitive diatoms was set to the 90th percentile observed for sites with TP and chl-a below G/M, as inferred from modelling (best 10%), whereas G/M was based on the 75th percentiles of the relative abundance of impact-associated taxa (best 75%);

MS	Methodology used to set class boundaries
	• Lower boundaries were obtained by linear interpolation between the relative abundance of impact-associated diatoms corresponding to the G/M boundary and 100%, assuming equal class intervals. All percentages serving as boundary values were rounded to the nearest 5.
DE	• At first, reference conditions were investigated spatially based on reference sites (littoral sites with no biological and no hydromorphological and no trophic status impacts). It was found, that reference trophic status was somewhat different among national lake types. • Reference conditions were derived for each lake type separately, spatially based and validated by sediment cores, using diatom - TP transfer functions. • Secondly, the class boundaries were assigned for each type equidistantly at trophic index intervals of 0,5, beginning at the H/G boundary. This means, that all class boundaries are type specific, but all classes have the same witdth along the logTP scale (main pressure gradient, explored by CCA).
FI	• High/good boundary is the 25th percentile of EQR reference sites for the medium alkalinity type; • The other boundaries are arithmetical divisions of the remaining EQR scale
FR	• H/G boundary : 25th percentile of reference values for IBD (for every diatom-derived biotype covering all the national lake types) • G/M boundary : statistical division
HU	• **HG boundary is the 25th percentile of alternative benchmark sites;** • GM boundary is the “crossover” between sensitive and tolerant tax a based on indices values; • Other boundaries are arithmetical divisions of the remaining EQR scale.
IE	• H/G: Similar to the UK but calibrated to fit better to Irish reference data; • G/M: The cross over between nutrient sensitive and nutrient tolerant; • M/P/B: Equal divisions of the remaining scale.
IT	• Plans to adopt median boundaries at end of the IC exercise
PL	• H/G: the median value of reference sites; • **G/M: median value of the remaining (non-reference) sites**
SE	• High status: Lakes fulfill the national reference criteria, e.g. TP < 10 µg/l, no acidification, land use: < 20 % farming, < 0,1 % urban area; • The G/M boundary was set to the IPS value where the nutrient tolerant and pollution tolerant species exceed a relative abundance of ca. 30 % (and the amount of sensitive species falls below ca. 30 %).
SI	• High/good boundary is the 25th percentile of EQR reference sites; • Good/moderate, moderate/poor and poor/bad are arithmetical divisions of the remaining EQR scale.
UK	• HG boundary is the 25th percentile of EQR reference sites for the type; • GM boundary is the “crossover” between sensitive and tolerant taxa; • Moderate/poor and poor/bad are arithmetical divisions of the remaining EQR scale.

3. Results of WFD compliance checking

Compliance checking should be performed at the level of the BQE, rather than just the "macrophyte" or "phytobenthos" sub-element. Table 3.1 presents an overview of compliance for the phytobenthos sub-element only.

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

Table 3.1 Outcome of compliance checking of phytobenthos methods.

Compliance criteria	Conclusions
1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes. See Note 1
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions (Boundary setting procedure)**	Yes. See table 2.2. Exceptions are IT (that plans to set boundaries as median of other national boundaries), HU and PL systems (see table 2.2.)
3. **All relevant parameters** indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole.	See Note 2
4. Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the WFD Annex II and approved by WG ECOSTAT	Yes
5. The water body is assessed against **type-specific near-natural reference conditions**	Yes, except HU system (see Table 2.2.)
6. Assessment results are expressed as **EQRs**	Yes
7. Sampling procedure allows for **representative** information about water body quality/ ecological status **in space and time**	Yes. Practices vary from MS to MS: in some cases, a single sample is used to characterize a water body for an assessment period; other MS use multiple samples in either space or time
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	See Note 2
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes

Note 1 IT does not yet fulfil compliance criteria. It will adopt the ICM as national metric, along with median positions of intercalibrated boundaries.

Note 2 This exercise intercalibrates one component of the BQE "Macrophytes and phytobenthos".

Conclusions

- No phytobenthos method submitted to this exercise fulfils all the requirements of the normative definitions; however, in most cases, these methods are used alongside a complementary set of macrophyte metrics;
- Few MS evaluate bacterial tufts in standing waters but this is unlikely to affect classifications as these are rarely a problem in standing waters.

4. Results IC Feasibility checking

4.1. Typology

As this was a cross-GIG exercise, GIG-specific types were amalgamated to form three "supertypes" (Table 4.1).

Table 4.1 Common intercalibration water body types and list of the MS sharing each type

Common type	Common type characteristics, contributing types, region	MS sharing IC common type
HA	High alkalinity lakes CB-GIG: L-CB1, L-CB2 MED-GIG: L-M1 ALP-GIG: L-AL3	BE-F, DE, HU*, IE, IT, PL, SE, SI, UK,
MA	Moderate alkalinity lakes CB-GIG: L-CB3, N-GIG: L-N8 **	BE-F, DE, FR, FI, IE, IT**, SE, UK
LA	Low alkalinity lakes N-GIG: L-N2, L-N3	FI, IE, SE, UK

* HU lakes classified into CB-GIG types ** IT has also submitted some moderate alkalinity lakes from ALP and MED GIGs which do not correspond to any IC types

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

- All methods (with the exception of BE-F, DE and PL) are based on generic weighted average equations (IPS, LTDI, TI) or related concepts (IBD) and are, thus, suitable for all IC types so long as an estimate of the "expected" value of the metric is available;
- BE-F, DE and PL have methods which depend wholly or partly on comparisons with type-specific reference assemblages; however, these methods generally correlate with the ICM, and are appropriate for the common types.

4.2. Pressures addressed

All national methods developed to date are calibrated against eutrophication gradients and this was the focus of the intercalibration exercise (

Table 4.2):

- All MS methods assess trophic status, some metrics were designed for rivers and address "general degradation"; however, there is an assumption that nutrients are the key factor determining outcomes in lakes and that such metrics are therefore usable;
- There is some evidence of a confounding influence of acidity in LA lakes. The implications of this will be discussed later in the report;
- Salinity is a possible confounding factor in a few HA lakes in HU but these are not included in this intercalibration exercise.

*Table 4.2 Pressure response relationships between national metrics and log TP (total phosphorus). R^2 - coefficient of determination. *N.s. - relationship non-significant, p>0.05.*

MS	Site /sample	R^2	Equation	P-value
LA lake type				
FI	Samples	0.11	y = -0.0757x + 0.9915	N.s.*
IE	Samples	0.35	y = -0.4491x + 1.4043	P < 0.05
SE	Samples	0.06	y = -0.0445x + 0.999	N.s.
UK	Site	0.09	y = -0.059x + 1.0163	P <0.05
MA lake type				
BE	site	0.86	y = -0.4145x + 1.3885	P < 0.05
FI	Samples	0.65	y = -0.2987x + 1.2789	P < 0.05
FR	Samples	0.68	y = -0.2791x + 1.4114	P < 0.05
DE	Sample	0.01	y = -0.0208x + 0.8572	N.s.
IE	Samples	0.29	y = -0.2593x + 1.2645	P < 0.05
IT	Sites	0.06	y = -0.0728x + 0.9564	N.s.
SE	Samples	0.19	y = -0.0994x + 1.1102	P < 0.05
UK	Sites	0.29	y = -0.2237x + 1.3081	P < 0.05
HA lake type				
BE	Sites	0.83	y = -0.4259x + 1.416	P < 0.05
DE	Sites	0.20	y = -0.2804x + 1.0416	P < 0.05
HU	Sites	0.14	y = -0.0868x + 0.8362	P < 0.05
IE	Sites	0.48	y = -0.4068x + 1.357	P < 0.05
IT	Sites	0.04	y = 0.1087x + 0.6999	N.s.
PL	Sample	0.15	y = -0.1722x + 1.1044	P < 0.05
SE	Sample	0.31	y = -0.1759x + 1.0045	P = 0.05
SI	Samples	0.38	y = -0.3652x + 1.1695	P < 0.05
UK	Sites	0.63	y = -0.4966x + 1.7748	P < 0.05

4.3. Assessment concept

All national methods follow a similar assessment concept (see table below)

- All assessments focus on the littoral zones of lakes, sampling either stones (usually cobble-sized) or macrophyte stems;
- Two types of assessment are employed:
  - Reference indices (in which the composition is compared with that expected at reference conditions);
  - Pressure metrics – either purpose-designed trophic indices or general pressure metrics.
- As the main gradient in most national datasets is nutrients, there are generally high correlations between these types of metrics. The only confounding pressure is acidity in LA lakes.

Table 4.3 Summary of assessment concepts by Member State

Method	Assessment concept
BE-F	Littoral assemblages are sampled in summer from hard substrates (preferably reed; choice of alternative substrates and sampling procedures are fixed by rules) after a sufficiently prolonged period of submergence at 9 spatially separated sites. The proportions of type-specific impact-sensitive and impact-associated diatoms are estimated from a fixed count of 500 valves in a sample. Identifications are at species or lower taxonomic level. Lake classification is based on results for at least 3 samples from the same season (the number of samples increases with the divergence in assessment results). The presence of cyanobacterial films and abundance of filamentous algae are considered in the macrophyte method.
DE	Each lake is sampled during summer at 5 to 40 fixed sites; the number of sites depends on lake size. Sampling is replicated after 3 years to monitor changes. The sampling sites are distributed more or less equidistantly along the shore line, to support averaging the results of all sites within a water body. Littoral diatom samples are sampled from the natural (type specific) bottom, preferably at 0.3 - 1.5 m depth. Stones are preferred, but sampling on sand, mud or dead stalks of *Phragmites* and *Typha* from the last year is allowed, if stones are absent. The assessment is based on two metrics, one is a trophic index and the second is a ratio, expressing the degree of disturbance of the assemblage at the species level. At least 500 valves are determined at species and variety level to calculate a Trophic Index, especially designed separately for each ecoregion. Slides are screened for another 30 minutes for rare species. The species ratio between sensitive species and indicators of disturbances is used as a second metric.
FI	Littoral diatoms are sampled from five to ten cobbles. Preferred number of littorals is 3 per lake and they are sampled once in a year. Species-level identification is used for calculating IPS index.
FR	Littoral assemblages are sampled from stems of emergent macrophytes, if present, otherwise from rocks; species-level identification of the diatom assemblage is used to calculate a trophic index. Samples are collected on observation units used for macrophytes assessment.
HU	Littoral assemblages are sampled first of all from reed stems, if present (otherwise from any other stems of emergent or submerged macrophytes. In the lack of macrophytes, sampling from rocks is also allowed). Species-level identification of the diatom assemblage is used to calculate a trophic index. A single location per lake is sampled once a year; data from several years are combined to give an integrated assessment.
IE	Littoral diatom assemblages are sampled from natural hard substrate, when present, otherwise rarely from stems of emergent macrophytes; species-level identification of the diatom assemblage is used to calculate a trophic index. Single, or multiple locations (depending on a categorization of lake area) are sampled once in April and in July/August; filamentous algae are also considered in IE's national macrophyte method.
IT	Littoral diatoms are sampled from three to five cobbles or macrophyte stems, preferring *Phragmites* stems. At least one littoral sample per lake is sampled once in a year. Species-level identification is used for calculating the index.

Method	Assessment concept
PL	Littoral assemblages are sampled once a year, in summer, from macrophyte (*Phragmites, Typha, Chara* or others) parts submerged in water at a depth of at least 30 cm; number of sampling sites depends on a lake characteristics. The assessment is based on a multimetric weighted index composed of 2 modules: the trophic index and the reference species index showing deviation when comparing with a reference assemblage. Ca. 500 valves are determined and counted in a sample to calculate the multimetric diatom index. Filamentous algae are considered in PL macrophyte method.
SE	The lake method follows closely the method for running waters. Littoral assemblages are sampled from 5-10 rocks, if present, otherwise from 5-10 stems of emergent macrophytes at a ~10 m reach. Diatom identification to lowest possible level is used to calculate IPS, %PT, TDI and ACID. The assessment is based on a single autumn sampling. Percent cover of other benthic algae than diatoms is noted on the field protocol.
UK	Littoral assemblages are sampled from rocks, if present, otherwise from stems of emergent macrophytes; species-level identification of the diatom assemblage is used to calculate a trophic index. A single location per lake is sampled twice a year; data from several years are combined to give an integrated assessment. Filamentous algae are also considered in UK's macrophyte method.

5. IC dataset collected

Huge dataset was collected within the Phytobenthos cross-GIG (Table 5.1 and Table 5.2)

Table 5.1 Data acceptance criteria used for the data quality control and the data acceptance checking

Data acceptance criteria	Data acceptance checking
Data requirements (obligatory and optional)	Obligatory: littoral diatom samples and TP, collected according to criteria below; Optional: other water chemistry
The sampling and analytical methodology	All: sampling and analysis is based on CEN 13946 and 14407
Level of taxonomic precision required and taxalists with codes	All: Species level identification; data provided with Omnidia (four letter) codes (i.e. *Achnanthidium minutissimum* = ADMI)
The minimum number of sites/samples per intercalibration type	See Note 1
Sufficient covering of all relevant quality classes per type	See Note 1

Note 1: These issues vary from type to type and will be discussed in more detail below.

Table 5.2 Summary of intercalibration dataset

MS	Number of sites/samples/data values		
	Biological samples	Sites	Notes
HA lakes			
BE	68	14	Full gradient but limited coverage at High ecological status
DE	698	119	Full gradient
HU	84		Limited coverage of HES and GES
IE	120	62	Limited coverage of PES and BES.
IT	17	15	
PL	156	134	Full gradient
SE	28	15	Limited coverage of PES and BES.
SI	36		Full gradient
UK	320	66	Full gradient
MA lakes			
BE-F	79	18	Full gradient
FI	25	25	Limited number of poor/bad sites
FR	33	5	29 samples from 4 lakes, if Hourtin is excluded. Mostly H/G status.
DE	14	3	Mostly H/G status
IE	34	14	Mostly H/G status
IT	7	7	Limited number of sites because of the rarity of this type in Italy
SE	21	15	Mostly H/G status
UK	201	40	Limited number of poor/bad sites
LA lakes			
FI	25	21	Limited gradient (mostly H/G)*
IE	45	22	Limited gradient (mostly H/G)
SE	32	21	Limited gradient (mostly H/G)
UK	438	72	Limited gradient (mostly H/G)

*The limited gradient is common to all participating MS and reflects the often remote locations and unsuitability of the catchments for agriculture and settlement.

6. Common benchmarking

Different approaches were adopted for different types:

- Low alkalinity lakes: sufficient **reference sites** were available for all MS;
- Moderate and high alkalinity lakes: some MS lacked reference sites; others lacked a full pressure gradient and **continuous benchmarking** was adopted.

Continuous benchmarking was done using General Linear Model (GLM) in SPSS Statistics version 17.0 (SPSS Inc. 2008). In the model IC common metrics - Trophic Index (Rott et al. 1999) expressed as an EQR value (TI_EQR) was used as a dependent variable, member state as a random variable and the logarithmic value of total phosphorous (log TP) as the covariate. Analyses were conducted separately for high alkalinity (HA) lakes and moderate alkalinity (MA) lakes. Results of the GLM approach are given below (Table 6.2. and Table 6.4).

6.1. Common metrics

The Trophic Index (TI), one of the two component metrics of the pICM (phytobenthos Intercalibration Common Metrics), used for river phytobenthos intercalibration, was used as a common metrics for MA and HA supertypes during this exercise. This is a trophic index based on a weighted average equation: all taxa are given a sensitivity score, depending on the optimum nutrient concentration under which they are found in nature. The TI is the average of the sensitivities of all taxa present, “weighted” by their relative abundance (so a common nutrient-sensitive taxon will have more influence on the final index value than a nutrient-tolerant taxon that is only sparsely represented in the sample).

Additional analysis has been carried out in order to show that (River) Trophic Index (Rott et al. 1998) can provide a reliable assessment of the trophic status of lakes using lake littoral diatoms (see Annex H.2):

- Trophic Index (TI) showed a good relationship with the eutrophication gradient.
- A statistically significant difference in TI was observed between reference and impaired sites and high percentage of recorded littoral diatom taxa was indicative according to TI in all samples.
- Moreover, a new developed littoral diatom-based trophic index (LLTI) was highly correlated with the (River) Trophic Index using all data and alpine data only.
- Thus, diatom-based Trophic Index might considerable well address eutrophication pressure in lakes, although lake littoral diatom specific indices might be more applicable.

6.2. Continuous benchmarking: High alkalinity (HA) lakes

Nine MS participated. Relationships between the common metric (TI-EQR) and TP were significant for all but IT (Table 6.1.)

Table 6.1 Pressure-response relationships between common metric (TI-EQR) and TP in HA lakes.

MS	Site/sample	Metrics tested	R^2	Equation	ANOVA
BE	Site	TI_EQR	0.622	y = -0.4248x + 1.478	P < 0.001
DE	Site	TI_EQR	0.347	y = -0.2413x + 1.1787	P < 0.001
HU	Site	TI_EQR	0.160	y = -0.208x + 1.1592	P = 0.009
IE	Site	TI_EQR	0.564	y = -0.3737x + 1.2958	P < 0.001
IT	Site	TI_EQR	0.029	y = 0.1223x + 0.68	N.s.
PL	Sample	TI_EQR	0.138	y = -0.2065x + 1.1571	P < 0.001
SE	Sample	TI_EQR	0.233	y = -0.3005x + 1.191	P = 0.009
SI	Samples	TI.EQR	0.429	y = -0.3566x + 1.6315	P < 0.001
UK	Site	TI_EQR	0.676	y = -0.375x + 1.4787	P < 0.001

The relationship between TI_EQR and log TP is shown in Figure 6.1. Two groups of outliers above the main trend are apparent: Slovenian sites cluster in the top left corner of the plot whilst a number of Polish samples also lie above the main trend. There is no obvious reason why these behave differently from other Polish samples.

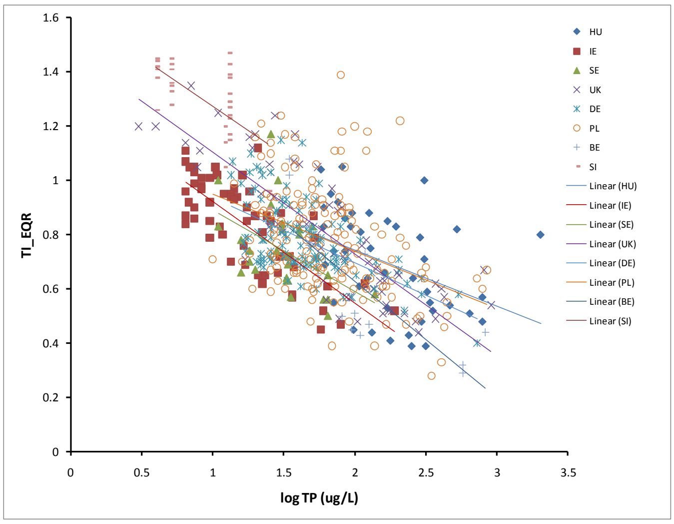

Figure 6.1Relationship between TI_EQR and log TP for high alkalinity lakes. y = -0.3133x + 1.3384; $R^2 = 0.4269$

Continuous benchmarking, using generalised linear models to define Member State-specific offsets (Tbale 6.2.), was adopted. Both subtraction and division methods were then applied. The division approach resulted in a slightly poorer fit than the unadjusted data (r^2 = 0.41, compared to 0.43) whilst subtraction improved the fit slightly (r^2 = 0.45). The SI outliers are now closer to the main trend but the cluster of PL outliers remains (Figure 6.2).

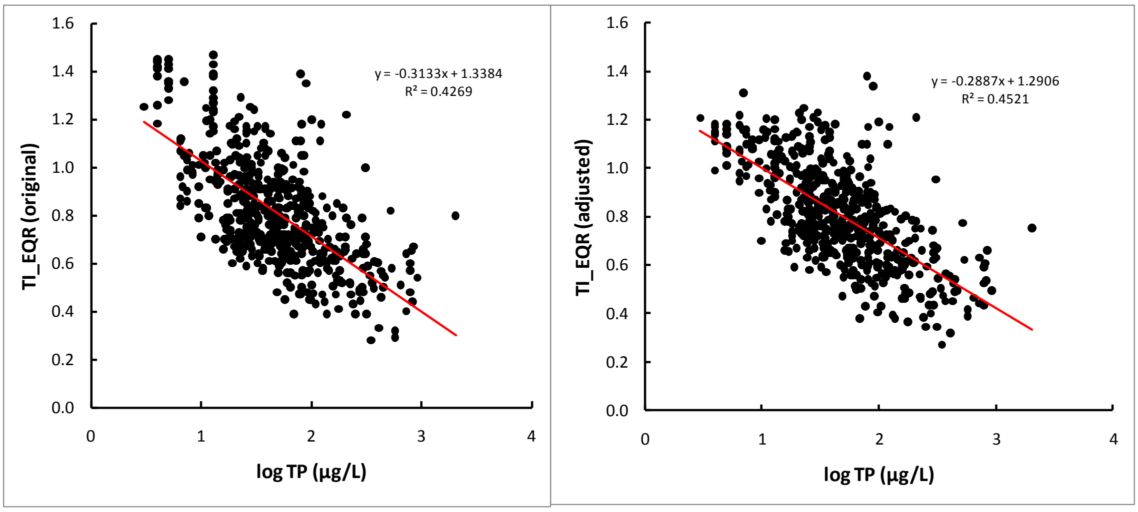

Figure 6.2Comparison between pressure response relationship using metrics before (left) and after (right) national offsets had been subtracted.

National offsets, calculated by GLM, are given in Table below.

Table 6.2 National offsets calculated by GLM for HA intercalibration

Member state	Mean TI_EQR	Std. Error	95% Confidence Interval		Offset	1 - offset
			Lower Bound	Upper Bound		
BE	0.719[a]	0.042	0.637	0.800	-0.097	1.097
DE	0.775[a]	0.014	0.748	0.803	-0.041	1.041
HU	0.862[a]	0.026	0.811	0.913	0.046	0.954
IE	0.708[a]	0.021	0.667	0.749	-0.108	1.108
PL	0.826[a]	0.013	0.801	0.850	0.01	0.99
SE	0.691[a]	0.029	0.634	0.749	-0.125	1.125
SI	1.085[a]	0.029	1.028	1.142	0.269	0.731
UK	0.860[a]	0.018	0.826	0.895	0.044	0.956
Common view	0.816 [a]	0.009	0.799	0.833		

Covariates appearing in the model are evaluated at the log TP = 1.6665

6.3. Continuous benchmarking: MODERATE alkalinity (MA) lakes

Eight MS participated; relationships between national metric were significant for all except DE and IT.

Table 6.3 Pressure-response relationships between common metric (TI-EQR) and TP for MA lakes

MS	Site or sample?	R^2	Equation	
BE	site	0.413	y = -0.0007x + 0.8617	P < 0.05
FI	Samples	0.704	y = -0.4292x + 1.2477	P < 0.05
FR	Samples	0.5943	y = -0.0035x + 1.1232	P < 0.05
DE	Sample	0.0534	y = 0.0903x + 0.8251	n.s
IE	Samples	0.2866	y = -0.2098x + 1.2021	P < 0.05
IT	Sites	0.0019	y = -0.0128x + 0.8294	n.s
SE	Samples	0.3573	y = -0.2447x + 1.2037	P < 0.05
UK	Sites	0.32	y = -0.2949x + 1.2351	P < 0.05

These relationships are plotted in Figure 6.3. The relationship based on all data has r^2 = 0.239.

Lac Hourtin in France is an obvious outlier – having both very high TP and very high TI-EQR. This is a lowland, shallow lake with a high N:P ratio. Excluding Hourtin from this relationship increases this to r^2 = 0.375, and the slope also increases.

Overall, there is some heteroscedasticity in the relationship, with a wide range of values of pICM recorded at low pressure, and a possible response threshold at about 10 µg L^{-1} TP. However, few MS had data that spanned the whole gradient and that there are few sites with >100 µ L^{-1} TP.

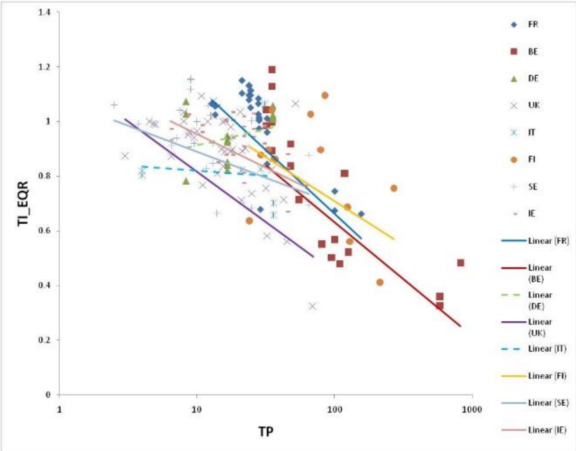

Figure 6.3 Relationship between TI_EQR and log TP for moderate alkalinity lakes.

As not all MS have valid reference sites, continuous benchmarking was adopted, using generalised linear models to define Member State-specific offsets (see Table 6.4).

It was not immediately clear whether to use the "division" or "subtraction" approach. Both were tried, but "subtraction" gave slightly better results, with the relationship between pICM and TP improving from $r^2 = 0.351$ for the uncorrected data to $r^2 = 0.527$ for corrected data (cf 0.512 for the "division" method). Hourtin remains outside the main trend even after the corrections were applied and has been omitted from subsequent analyses. The pressure-response relationship, using corrected pICM values, is shown in Figure 6.4.

Table 6.4 National offsets calculated by GLM for MA lakes

Member state	Mean TI_EQR	Std. Error	95% Confidence Interval		Offset	1 - offset
			Lower Bound	Upper Bound		
BE	0.900[a]	0.037	0.827	0.972	-0.03	1.03
DE	0.914[a]	0.038	0.839	0.989	0.021	0.979
FI	0.662[a]	0.028	0.606	0.718	0.022	0.978
FR	1.076[a]	0.025	1.026	1.126	0.166	0.834
IE	0.911[a]	0.024	0.863	0.959	0.016	0.984
IT	0.774[a]	0.054	0.668	0.880	-0.116	1.116
SE	0.859[a]	0.032	0.796	0.921	-0.024	1.024
UK	0.834[a]	0.024	0.787	0.880	-0.053	1.053
Common view	0.866 [a]	0.012	0.843	0.889		

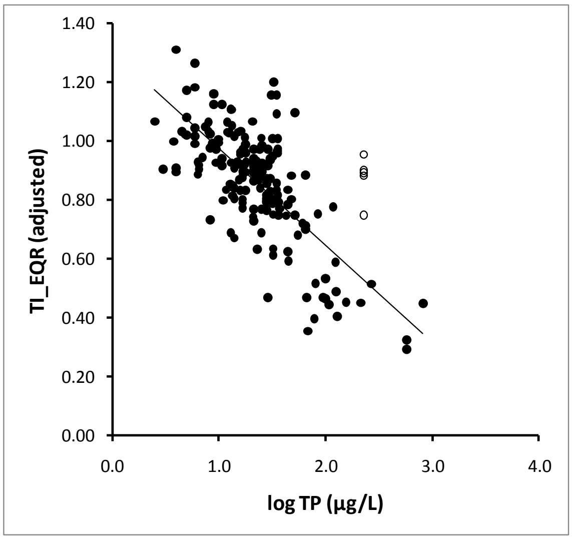

Figure 6.4. Pressure-response for MA lakes, corrected values. Open circles: Lac Hourtin (France).

All relationships are significant except DE and IT. FR relationship excludes Hourtin.

6.4. Common metrics in low alkalinity (LA) lakes

LA lakes

The relationship between TI_EQR and pressure (TP) has a data cloud with a "Y"-shape: the upper branch shows little response to increasing nutrient levels, whist the lower branch shows decreasing TI_EQR values as TP increases (Figure 6.5). Preliminary investigations suggest that this is not easily explainable by typological factors (both branches include strongly humic lakes) but the "upper" group tends to have lower pH (6-6.4) than the "lower" group (pH 6.5-6.9 – based on FI data). We suspect that this reflects an interaction between metrics and the pH gradient but we cannot evaluate this is driven by "natural" acidity or acidification without use of MAGIC or similar models.

In view of the relatively low strength of pressure-response relationships (Table 6.5.) the confounding effect of acidity and the fact that one of the four methods is still under development in time of the Intercalibration (FI), and two methods do not have a significant pressure-response relationship (FI and SE), we will only proceed with formal IC of UK and IE at this stage. These countries have official methods which are almost identical (differing only in the expected value of the national metric).

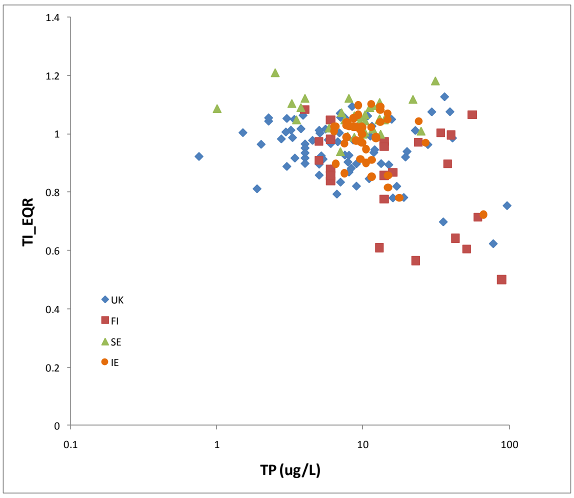

Figure 6.5. Relationship betweeen TI-EQR and TP for low alkalinity lakes

Table 6.5.Pressure-response relationships between common metrics and TP for LA lakes

LA lakes	Site or sample?	R^2	Equation	ANOVA
FI	Samples	0.2202	y = -0.1927x + 1.0951	P = 0.0179
IE	Samples	0.1593	y = -0.185x + 1.1754	P = 0.0066
SE	Samples	0.0241	y = -0.0282x + 1.0418	n.s.
UK	Site	0.0425	y = -0.0472x + 0.9833	P = 0.0224

7. Comparison of methods and boundaries

IC **Option 2** has been adopted for the lake phytobenthos intercalibration for MA and HA lakes:

- Option 3 is not possible for all MS, e.g., DE has particular requirements for counting strategies that were not met by most other MS;
- Methods that are based on type-specific reference assemblages (e.g. BE-FL, DE, PL) are, to some extent, “tuned” to local sub-types, making regional comparisons more difficult;
- Option 2 was used successfully for the river phytobenthos exercise.

For LA lakes, however, only two MS had data that permitted intercalibration: these both used the same assessment method (with very minor differences in reference conditions) and Option 3 was used for these.

7.1. Results of intercalibration FOR High alkalinity (HA) type

Initially 8 countries participated in HA type intercalibration (see table below).

Table 7.1 National boundaries for HA lakes

MS	BE	DE	HU	IE	PL	SE	SI	UK
Ref	1.00	1.00	1.00	1.00	1.00	1.000	1.00	1.00
H/G	0.80	0.78	0.80	0.90	0.80	0.890	0.80	0.92
G/M	0.60	0.55	0.60	0.63	0.60	0.740	0.60	0.70
M/P	0.40	0.33	0.40	0.42	0.40	0.500	0.40	0.46
P/B	0.20	0.10	0.20	0.21	0.20	0.250	0.20	0.23

Boundaries were compared using IC option 2 with a boundary translation to common metrics – TI-EQR (see table below)

Table 7.2 Relationship between national metric and common metric (TI_EQR) for HA lakes.

MS	Intercept (c)	Slope (m)	Pearson's r	R^2	Notes
BE	0.152	1.01	0.88	0.77	
DE	0.529	0.50	0.77	0.60	
IE	0.303	0.75	0.89	0.79	
PL	-0.008	0.96	0.80	0.64	
SE	-0.187	1.25	0.63	0.40	
UK	0.320	0.72	0.94	0.88	
SI	0.320	0.86	0.94	0.88	
HU	-0.576	1.91	0.87	0.76	High slope

The outcomes of the regression complied with the following characteristics according to the IC Guidance

- All relationships were highly significant p<=0.001;
- Assumptions of normally distributed error and variance (homoscedasticity) of model residuals were met;
- Common metric represented all methods (r>0.5);
- Observed minimum r^2 > half of the observed maximum r^2 – this criterion is not fulfilled as min r^2 0.4< max r^2 0.88/2, but maximum r^2 may be artificially high as some MS use the intercalibration metric (TI) as their national metric;
- Slopes of the regression lie between 0.5 and 1.5 (with exception of HU 1.9);

Two countries were excluded from the calculation of boundary bias:

- HU exceeds the requirement for the slope and also set "expected" values by a procedure that did not comply with intercalibration guidelines. As there are no true reference sites in HU this is clearly a challenge and, for this reason, HU was omitted from the boundary setting procedure;
- PL set their good/moderate boundary using a procedure that did not comply with ECOSTAT guidelines, so was also omitted from the boundary setting procedure.

However, once a common view of the boundaries had been established using data from the remaining MS, boundaries for HU and PL were reassessed and, where necessary, adjusted.

Using this as the basis of boundary comparisons, we get the following boundary bias values:

- **High/Good boundary:**
  - Within ± 0.25 class widths of median - BE, IE, SE, SI, UK;
  - Greater than 0.25 deviation -DE (relaxed boundaries);
- **Good/Moderate boundary:**
  - Within ± 0.25 class widths of median - BE, DE, IE, SE, SI, UK;
  - Greater than 0.25 deviation - SI (stringent boundaries).

a) High / Good class biass **b) Good / Moderate class biass**

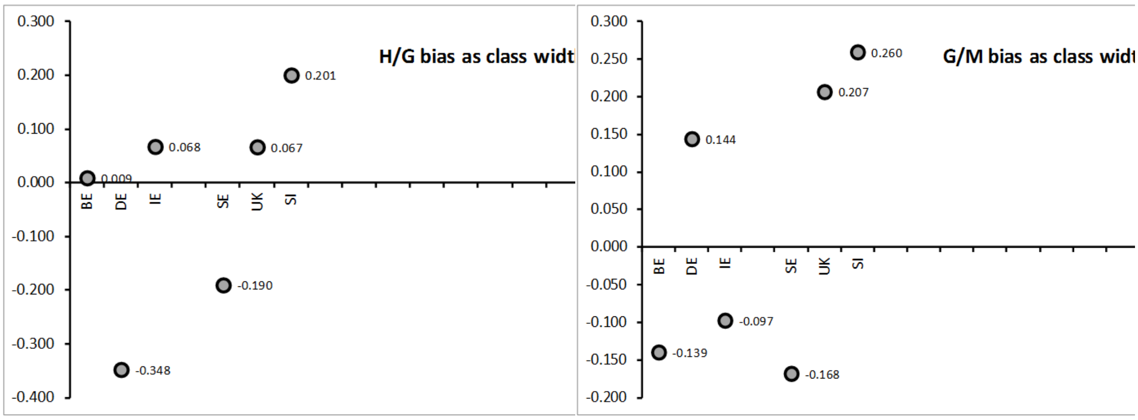

Figure 7.1 Class width bias at High/Good and Good/Moderate for HA lakes

DE has agreed to raise their H/G from 0.78 to 0.80.

The average boundaries, as TI-EQR, are 0.965 (high/good) and 0.790 (good/moderate). The boundaries for HU and PL were then checked manually (see table below):

- HU has agreed to raise their G/M to 0.69, which reduces their bias to within ± 0.25 class widths;
- PL has agreed to raise their H/G to 0.91 and G/M to 0.76, both of which reduces their bias to within ± 0.25 class widths).

Table 7.3 Original and proposed boundaries, and associated bias (as class width) for high/good and good/moderate boundaries for Hungary and Poland.

		Boundaries		Bias, as class width	
MS	**Version**	**H/G**	**G/M**	**H/G**	**G/M**
Hungary	Original	0.80	0.60	0.286	-1.155
	Proposed	0.80	0.69	0.286	-0.233
Poland	Original	0.80	0.60	-0.656	-1.070
	Proposed	0.91	0.76	-0.036	-0.222

The final view of HA boundaries, therefore, is as follows:

Table 7.4 Revised view of national boundaries for HA lakes (in bold: adjustments to original boundary values)

National Method	BE	DE	HU	IE	PL	SE	SI	UK
Ref	1.00	1.00	1.00	1.00	1.00	1.000	1.00	1.00
H/G	0.80	0.80	0.80	0.90	0.91	0.890	0.80	0.92
G/M	0.60	0.55	0.69	0.63	0.76	0.740	0.60	0.70
M/P	0.40	0.33	0.40	0.42	0.40	0.500	0.40	0.46
P/B	0.20	0.10	0.20	0.21	0.20	0.250	0.20	0.23

7.2. Results of intercalibration FOR Moderate alkalinity (MA) type

Initially 7 countries participated in MA type intercalibration (see table below). In addition, IT has some MA lakes but will adopt the ICM as the national metric, and base its boundaries on the results of the intercalibration process.

Table 7.5 National boundaries for MA lakes

National Method	BE	DE	FI	FR	IE	SE	UK
Ref	1.00	1.00	1.00	1.00	1.00	1.00	1.00
H/G	0.80	0.78	0.80	0.94	0.90	0.89	0.92
G/M	0.60	0.55	0.60	0.80	0.63	0.74	0.66
M/P	0.40	0.33	0.40	0.55	0.42	0.50	0.44
P/B	0.20	0.10	0.20	0.30	0.21	0.25	0.22

Boundaries were compared using IC option 2 with a boundary translation to common metrics – TI-EQR (see table below)

Table 7.6 Relationship between national metrics and TI-EQR for MA lakes

	Intercept (c)	Slope (m)	Pearson's r	R^2	Notes
BE	0.007	1.190	0.90	0.80	
DE	0.825	0.090	**0.003**	**0.005**	Small dataset (N = 14 from 3 lakes), weak relationship, low slope
FI	-1.009	**2.315**	0.90	0.80	High slope
FR	0.601	1.593	0.68	0.83	High slope; small dataset (N = 33 samples from 5 lakes, including one (Hourtin) that behaves atypically)
IE	0.302	0.628	0.77	0.59	
IT	0.008	0.948	0.92	0.85	Small dataset (N = 7)
SE	-0.409	1.349	0.74	0.55	
UK	-0.182	1.054	0.87	0.76	

Several MS were excluded from the IC based on the analysis of these relationships:

- DE because of small dataset and non-significant relationship (r=0.003) and low slope;
- FR because of small dataset and high slope of regression, as well as we suspected that their lakes (particularly Lac Hourtin) were responding in a manner that was different to other MA lakes;

IT was excluded due to small dataset (also there was no significant pressure-response relationship between IT metric and TP, see

- Table 4.2);
- FI was retained despite a high slope.

Using this as the basis for boundary comparison yields the following:

- **High/good boundary:**
  - Within 0.25 classes of median: FI, IE;
  - Greater than 0.25 deviation: BE-F (stringent), SE, UK (relaxed);
- **Good/moderate boundary:**
  - Within 0.25 classes of median: SE, UK;
  - Greater than 0.25 deviation: BE-F, IE (stringent), FI (relaxed).

High / Good class biass **Good / Moderate class biass**

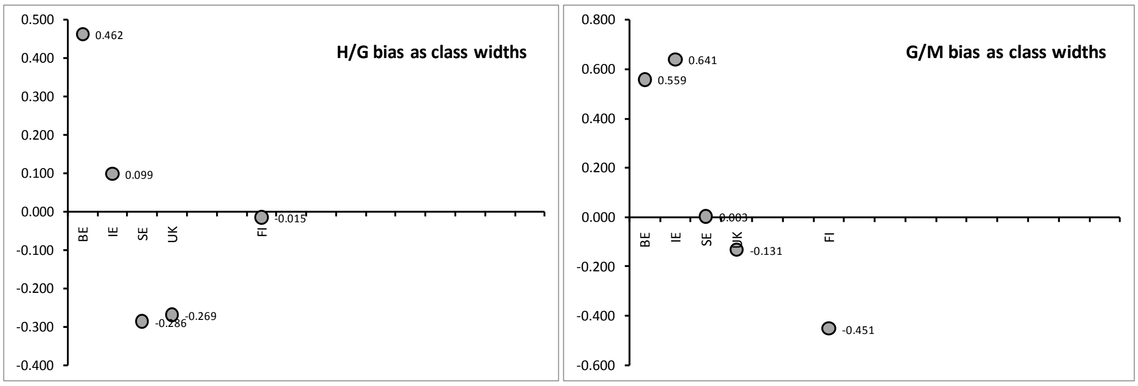

Figure 7.2 Class width bias at H/G anG/M for MA lakes

Note that IT and DE were omitted (small datasets, no pressure-response relationships).

The causes of high bias were investigated. Too stringent (precautionary) boundaries :

- BE: The dataset spans the whole pressure gradient and the relationship with pICM is good. The high bias may reflect genuinely precautionary boundaries. The high analysis threshold for TP may obscure the relation in the lowermost part of the TP gradient, increasing the slope of the relation and the bias relative to other MSs. BE-F is also the only region with sites at TP > 500 µg.L^{-1}, constraining the slope of the regression and thus increasing the relative offset at low values.
- IE: The original dataset spanned a relatively short gradient (mostly HG sites), which may yield an unreliable regression equation with the pICM. The IE dataset was, therefore, supplemented by data from MA lakes in the UK to produce a

larger dataset that spanned a longer gradient before the final calculations. However, the G/M boundary was still precautionary, compared to other MS.

Both BE-F and IE have decided to retain precautionary boundaries.

FI, SE and UK all showed relaxed boundaries for either H/G or G/M. All three have made adjustments to bring their boundaries into line with the common view.

Therefore, the final view of MA boundaries is as follows:

Table 7.7 Revised view of boundaries for MA lakes (in bold: adjustments to original boundary values)

National Method	FI	IE	SE	UK	BE
Ref	1.00	1.00	1.00	1.00	1.00
H/G	0.80	0.90	**0.90**	**0.93**	0.80
G/M	**0.64**	0.63	0.74	0.66	0.60
M/P	0.40	0.42	0.50	0.44	0.40
P/B	0.20	0.21	0.25	0.22	0.20

7.3. Results of intercalibration for Low Alkalinity (LA) supertype

Initially 4 countries participated in LA type intercalibration (see table below) with officially-adopted methods.

Table 7.8 National boundaries for LA lakes

MS	FI	IE	SE	UK
Ref	1.00	1.00	1.00	1.00
H/G	0.80	0.90	0.89	0.92
G/M	0.60	0.66	0.74	0.70
M/P	0.40	0.44	0.50	0.46
P/B	0.20	0.22	0.25	0.23

We will not proceed with formal IC of all MS at this stage because of:

- Relatively low strength of pressure-response relationships with TP and the confounding effect of acidity;
- One of the four methods was still under development at the time key decisions about the strategy for intercalibrating low alkalinity lakes was made on (FI);
- SE and FI methods does not have a significant pressure-response relationship (
-
- Table 4.2);
- However, UK and IE have official methods which are almost identical (differing only in the expected value of the national metric) and an "option 3" intercalibration has been performed for these MS.

The regression equation between UK and IE national metrics is : UK_NM = 0 + 0.975IE_NM (R^2 = 1.0), where UK_NM and IE_NM are the UK and Irish national metrics respectively.

Bias calculations yield the following:

Table 7.9 Outcome of "option 3" intercalibration between UK and IE

	IE	UK
G/M boundary bias	-0.12	0.12
H/G boundary bias	-0.07	0.10

In other words, bias for both H/G and G/M between UK and IE is acceptable and the final view of LA boundaries is as follows:

Table 7.10 Revised view of boundaries for LA lakes

MS	IE	UK
Ref	1.00	1.00
H/G	0.90	0.92
G/M	0.66	0.70
M/P	0.44	0.46
P/B	0.22	0.23

7.4. Remaining tasks:

1. Italian position was to adopt the ICM as national metric, along with median values of H/G and G/M as national boundaries. However, their datasets are small and do not show strong pressure-response relationships (possibly complicated, in some instances, by typological factors). We recommend that IT is not included in the Decision at this stage, and that they collect more data in order to perform a more thorough evaluation of appropriate metrics.
2. Having intercalibrated "macrophytes" and "phytobenthos" separately, it would now be useful to check that the combined "macrophyte and phytobenthos" BQE (IC Guidance sect. 2.1) yields comparable results between MS (e.g. check that differences in combination rules do not increase the amount of class bias, compared to evaluations of the separate components, and to make a more extensive comparison of classifications based on phytobenthos and macrophytes separately, to test the assumptions made by those MS without phytobenthos methods that an adequate classification can be obtained from macrophytes alone..
3. Low alkalinity lakes: the intercalibration needs to be repeated, taking account of acidification as well as nutrients.

8. Description of communities

Method

The lake intercalibration database was used to calculate TI_EQR for all samples, and these values were then adjusted by the national offsets used in the boundary comparison. The relative abundance of common taxa in the moderate and high alkalinity supertypes in all records in the database was then plotted against this EQR scale Low alkalinity lakes were not included in this exercise due to the potentially confounding impact of acidification on the dataset.

Within the context of this report, TI_EQR represents a consensus view of "ecological status" as all national methods have a significant relationship with this metric. The plots here describe taxa changes along this common view of the EQR gradient. These taxa also contribute to the TI calculation, so there is interdependence between "x" and "y" on these plots. This is easily rationalised so long as you remember that "EQR" distils ecological properties of a water body into a single gradient, and that the y axis on these plots simply showing how constituents of this property vary along the gradient.

Note, too, that the limited number of Poor and Bad status sites, particularly for moderate alkalinity, means that the decline in some taxa below Moderate status may be an artefact of the dataset, rather than a genuine biological effect. Also, this analysis considers only the predominant nutrient gradient and other types of pressure (e.g. heavy metals) may exert different responses on some taxa.

Taxa names generally refer to aggregates, following practices in Kahlert *et al*. (2012) and Kelly and Ector (2011).

Results

Most of the abundant taxa were found across the EQR gradient, albeit with some clear patterns in relative abundance emerging between both types and status classes. *Achnanthidium minutissimum* ag., for example, is the most commonly recorded taxon in the database, often forming more than 40% of the total in high and good status sites, but declining in relative abundance as EQR decreased, and there were few sites with >20% *A. minutissimum* at moderate status or below. Other taxa with a predominately high/good distribution included *Brachysira microcephala* (more abundant in MA than in HA lakes), *Gomphonema angustum* ag. and *Tabellaria flocculosa* (the latter, again, more common in MA than in HA lakes).

Amphora pediculus showed almost the opposite pattern to *Achnanthidium minutissimum*, increasing in relative abundance from high to moderate status, particularly in high alkalinity lakes, where it was often abundant (>20% of total), before declining again in poor and bad status.

Other taxa which tended to increase as EQR decreased were *Cocconeis placentula., Nitzschia dissipata* and *N. fonticola*. *C. placentula* can live as both directly on rocks and as an epiphyte, and the increase may, in part, reflect an increase in filamentous green algae as EQR decreases. The two *Nitzschia* species reflects a general pattern of increasing motile diatoms as EQR decreases.

Not all taxa showed such clear patterns: distributions of *Encyonema minutum, Fragilaria capucina* ag., *F. vaucheriae* ag. and *Navicula cryptotenella* ag. are less easy to interpret, and it is possible that these complexes are composed of taxa with different responses along the gradient. However, experience from the river intercalibration exercise showed that there was insufficient consistency in identifications between national datasets to be able to separate these reliably in the multinational datasets used in these exercises.

To provide further insights into the characteristics of assemblages at high, good and moderate status, we used indicator species analysis (ISP: Dufrene & Legendre, 1997) to identify taxa that can be used as indicators for a particular status class or classes. ISP combines measures of faithfulness to a group (always present) and exclusivity (never found in other groups) to derive an indicator value for each taxon in each group which is then tested for significance using a randomization test. As originally described, ISP contrasts the distributions of taxa across individual groups of sites. Samples were allocated to status classes based on their TI-EQR value, adjusted by the national offset, and classified by the median of all national boundary values. Results are shown in Table 8.1 and Table 8.2..

For both HA and MA, *Achnanthidium minutissimum* ag. is a strong indicator of high status, though it is also found at good and moderate status too. Samples with >20% of this taxon are unlikely to be found at moderate status. *Amphora pediculus* is a strong indicator of moderate status for HA lakes but is reported as indicating good status at MA. Examination of scatter plots suggests that it is much less common in MA lakes and the peak in good status should, perhaps, be treated with caution. The "Anomoeoneis vitrea" complex also comes out as a strong indicator of high status for both HA and MA lakes, as do several *Cymbella, Delicata* and *Encyonopsis* spp. Whilst a few *Nitzschia* and *Navicula* species are characteristic of high and good status, there is a general tendency for motile taxa to increase in significance as status decreases.

Table 8.1 Indicator species for high (H), good (G) and moderate (M) status for moderate alkalinity lakes. All taxa which show a significant preference for one of these classes is listed. Note: taxonomy follows conventions in Kelly & Ector (2012); "Anomoeoneis vitrea" represents the complex of Brachysira species including B. vitrea, B. neoexilis and others, but classified as A. vitrea in Krammer & Lange-Bertalot (1986)

Taxon	Status class	Indicator Value	Mean	SDev	p
Achnanthidium minutissimum (Kütz.) Czarnecki and allies	H	53	35.8	1.73	0.0002
"Anomoeoneis vitrea" (Grunow) Ross	H	33.5	22.9	4.46	0.031
Cymbella affinis Kutzing var.affinis	H	18.5	10.8	3.52	0.0376
Denticula tenuis Kutzing	H	23.6	12.2	3.72	0.0174
Encyonema neogracile Krammer	H	23.8	14	3.94	0.0318
Encyonopsis microcephala (Grunow) Krammer	H	38.7	20.6	4.8	0.0072
Eunotia implicata Nörpel. Lange-Bertalot & Alles	H	15.5	8.5	3.25	0.0466
Gomphonema angustum Agardh	H	39.2	26.5	4.97	0.0234
Psammothidium levanderi (Hustedt)Czarnecki in Czarn. et Edlund	H	13.6	7.8	2.86	0.0432
Rossithidium pusillum (Grun.) Round & Bukhtiyarova	H	25	15.5	3.93	0.0302
Tabellaria flocculosa(Roth)Kutzing	H	42.2	29.7	4.74	0.021
Achnanthes clevei Grunow var. clevei	G	21.3	11.1	3.5	0.0186
Achnanthidium subatomus (Hustedt) Lange-Bertalot	G	6.8	2.7	1.61	0.034
Amphora pediculus (Kutzing) Grunow	G	31.1	17.9	4	0.0114
Cymbella amphicephala Naegeli	G	7.3	3	1.75	0.043
Cymbella leptoceros(Ehrenberg)Kutzing	G	5.5	2.2	1.49	0.0298
Encyonema reichardtii (Krammer) D.G. Mann	G	6.8	2.9	1.74	0.03
Encyonopsis minuta Krammer & Reichardt	G	13.4	6.1	2.76	0.0258
Epithemia sorex Kutzing	G	18.7	8.2	3.18	0.0138
Navicula cryptocephala Kutzing	G	30.1	17	4.31	0.0164
Navicula menisculus Schumann	G	8.7	3.9	2.16	0.0336
Nitzschia fonticola Grunow in Cleve et Möller	G	28.5	16.8	4.3	0.0222

Taxon	Status class	Indicator Value	Mean	SDev	p
Nitzschia lacuum Lange-Bertalot	G	22.5	7.5	3.07	0.0036
Planothidium lanceolatum(Brebisson ex Kützing) Lange-Bertalot	G	26.9	16	4.13	0.0242
Reimeria uniseriata Sala Guerrero & Ferrario	G	8.2	2.7	1.6	0.0154
Amphora veneta Kutzing	M	17.5	3.9	2.12	0.001
Cocconeis placentula Ehrenberg	M	55.4	27.6	4.95	0.0006
Denticula kuetzingii Grunow var.kuetzingii	M	17.8	4.1	2.16	0.0036
Diatoma elongatum (Lyngbye) Agardh	M	6.1	1.9	1.37	0.0122
Eolimna minima(Grunow) Lange-Bertalot	M	33.5	17.8	4.53	0.0114
Fragilaria bidens Heiberg	M	11	5.2	2.5	0.0362
Gomphonema angustatum (Kutzing) Rabenhorst and allies	M	17	5.7	2.54	0.0046
Gomphonema parvulum (Kützing) Kützing	M	61.4	21.6	4.71	0.0002
Gomphonema subtile Ehr.	M	7.3	2.4	1.57	0.0276
Mayamaea atomus (Kutzing) Lange-Bertalot	M	13	6.3	2.67	0.0322
Melosira varians Agardh	M	31.3	5.1	2.5	0.0004
Navicula gregaria Donkin	M	65.9	13.7	4.06	0.0002
Navicula lanceolata (Agardh) Ehrenberg	M	26.3	6.9	2.83	0.0006
Navicula seminulum Grunow	M	13.9	5.8	2.59	0.0186
Navicula subminuscula Manguin	M	14.6	2.5	1.59	0.0008
Navicula submuralis Hustedt	M	9.7	4.6	2.36	0.0324
Navicula trivialis Lange-Bertalot	M	8.1	2.5	1.55	0.0262
Nitzschia amphibia Grunow	M	33.2	6.7	2.93	0.0004
Nitzschia capitellata Hustedt in A.Schmidt & al.	M	9.7	2.5	1.62	0.004
Nitzschia gracilis Hantzsch	M	23	11.1	3.55	0.0142
Nitzschia inconspicua Grunow	M	28.1	6.3	2.65	0.0002

Taxon	Status class	Indicator Value	Mean	SDev	p
Nitzschia intermedia Hantzsch ex Cleve & Grunow	M	12.9	2.9	1.7	0.0036
Nitzschia linearis(Agardh) W.M.Smith var.linearis	M	18.4	8.2	3.19	0.0178
Nitzschia palea (Kutzing) W.Smith	M	47.8	22.9	4.35	0.0002
Nitzschia pusilla(Kutzing)Grunow	M	8.5	2.2	1.58	0.0236
Nitzschia tubicola Grunow	M	4.8	1.6	1.3	0.0498
Pinnularia species	M	10.5	4.2	2.19	0.024
Planothidium delicatulum(Kutz.) Round & Bukhtiyarova	M	34.4	5.5	2.48	0.0002
Planothidium frequentissimum(Lange-Bertalot)Lange-Bertalot	M	14.2	7.4	3	0.034
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	M	20.6	10.5	3.65	0.0212
Surirella brebissonii Krammer & Lange-Bertalot	M	14.9	3.5	1.94	0.0014
Surirella minuta Brebisson	M	8.8	2.2	1.48	0.016
Synedra ulna (Nitzsch.)Ehr.	M	37.2	15.6	3.94	0.0012
Ulnaria biceps (Kutzing) Compère	M	4.8	1.5	1.25	0.0448

Table 8.2 Indicator species for high, good and moderate status for high lakes. All taxa which show a significant preference for one of these classes is listed.

Taxon	Status class	IndicatorValue	Mean	SDev	p
Achnanthes inflata (Kutzing) Grunow	H	3.4	1	0.34	0.0002
Achnanthidium minutissimum (Kütz.) Czarnecki and allies	H	61.1	32.6	1.08	0.0002
“Anomoeoneis vitrea” (Grunow) Ross	H	17	4.6	0.85	0.0002
Brachysira brebissonii Ross in Hartley	H	1.5	0.6	0.27	0.0096
Brachysira serians(Breb.)Round et Mann	H	1.3	0.5	0.27	0.025
Brachysira styriaca (Grunow) Ross in Hartley	H	3	0.8	0.32	0.0002
Cavinula cocconeiformis (Gregory ex Greville) Mann & Stickle	H	3.3	1.5	0.4	0.001

Taxon	Status class	IndicatorValue	Mean	SDev	p
Cocconeis neodiminuta Krammer	H	1.4	0.6	0.27	0.0128
Cymbella affinis Kutzing var.affinis	H	38.7	10.6	1.16	0.0002
Cymbella helvetica Kutzing	H	20	5.2	0.83	0.0002
Cymbella lacustris(Agardh)Cleve	H	2.1	1.1	0.4	0.0268
Cymbella leptoceros(Ehrenberg)Kutzing	H	5.7	3.5	0.65	0.0056
Delicata delicatula (Kützing) Krammer	H	15	2.9	0.62	0.0002
Denticula tenuis Kutzing	H	34.9	7.2	0.92	0.0002
Diadesmis gallica var. perpusilla (Grunow) Lange-Bertalot	H	2.2	0.7	0.29	0.0004
Diatoma ehrenbergii Kutzing	H	5.1	1.9	0.61	0.0004
Encyonopsis cesatii (Rabenhorst) Krammer	H	21.8	4.6	0.75	0.0002
Encyonopsis descripta (Hustedt) Krammer	H	2.2	0.6	0.28	0.0008
Encyonopsis microcephala (Grunow) Krammer	H	64.5	15.8	1.18	0.0002
Epithemia smithii Carruthers 1864	H	3.8	2.2	0.61	0.016
Eucocconeis flexella (Kützing) Brun	H	6.1	1.6	0.42	0.0002
Eucocconeis laevis (Oestrup) Lange-Bertalot	H	3.2	1.6	0.46	0.0052
Eunotia arcus Ehrenberg	H	4.3	1.4	0.44	0.0002
Eunotia pectinalis (Dyllwyn) Rabenhorst	H	1.9	0.8	0.34	0.0048
Fragilaria tenera (W.Smith) Lange-Bertalot	H	7.4	3.4	0.7	0.0004
Frustulia krammeri Lange-Bertalot & Metzeltin	H	2.3	0.9	0.35	0.001
Gomphonema angustum Agardh	H	45.4	24.7	1.74	0.0002
Gomphonema clevei Fricke	H	1.3	0.5	0.25	0.019
Gomphonema species	H	15.5	3.1	0.62	0.0002
Martyana martyi (Héribaud) Round in Round Crawford & Mann	H	2.5	1.2	0.38	0.0086
Mastogloia smithii Thwaites	H	5.3	1.6	0.45	0.0002
Navicula arvensis Hustedt	H	2.2	0.7	0.29	0.0008

Taxon	Status class	IndicatorValue	Mean	SDev	p
Navicula digitoradiata (Gregory) Ralfs	H	2.3	1	0.41	0.0102
Navicula ignota Krasske 1932 emend Lund 1948	H	1.6	0.7	0.3	0.0172
Navicula subalpina Reichardt	H	13.4	7.5	0.93	0.0002
Navicula subtilissima Cleve	H	2.6	0.9	0.35	0.0008
Nitzschia angustata Grunow	H	8.6	2.5	0.59	0.0002
Nitzschia lacuum Lange-Bertalot	H	16.2	7.2	0.89	0.0002
Rhopalodia parallela (Grunow) O.Müller	H	3.3	1.4	0.44	0.002
Rossithidium pusillum (Grun.) Round & Bukhtiyarova	H	4.4	1.8	0.44	0.0002
Tabellaria fenestrata(Lyngbye)Kutzing	H	1.2	0.6	0.28	0.0454
Achnanthes clevei Grunow	G	26.3	14.4	1.21	0.0002
Achnanthes holsatica Hustedt in Schmidt et al.	G	7.8	1.9	0.51	0.0002
Achnanthes ziegleri Lange-Bertalot	G	14.5	3.9	0.65	0.0002
Achnanthidium exiguum (Grunow) Czarnecki	G	20.7	6.9	0.88	0.0002
Amphipleura pellucida Kutzing	G	8.7	4	0.77	0.0004
Aneumastus minor (Hustedt) Lange-Bertalot	G	13.4	3.2	0.64	0.0002
Aneumastus stroesei (Ostrup) Mann	G	4.1	1.4	0.46	0.0004
Caloneis bacillum (Grunow) Cleve	G	8.6	6.1	0.83	0.0112
Cavinula scutelloides (W.Smith) Lange-Bertalot	G	15.5	7.3	0.98	0.0002
Cocconeis disculus (Schumann) Cleve in Cleve & Jentzsch	G	4.5	2.2	0.58	0.003
Cocconeis neothumensis Krammer	G	26.3	10.4	1.05	0.0002
Craticula cuspidata (Kutzing) Mann	G	7.1	3.3	0.73	0.0002
Cymbella affiniformis Krammer	G	7.3	3.4	0.75	0.0008
Cymbella cymbiformis Agardh	G	14.4	10.6	1.32	0.013
Cymbella excisa Kutzing	G	15.2	6.8	1.02	0.0002
Cymbella hustedtii Krasske	G	12.9	8.3	1.31	0.003

Taxon	Status class	IndicatorValue	Mean	SDev	p
Cymbella lanceolata (Agardh ?)Agardh	G	8	6.2	0.81	0.0314
Cymbella lange-bertalotii Krammer	G	8.9	4	0.66	0.0002
Cymbella proxima Reimer in Patrick & Reimer	G	10.9	5	0.74	0.0002
Cymbella subhelvetica Krammer	G	7.4	3.9	0.68	0.0002
Cymbella subleptoceros Krammer	G	14.4	5.1	0.74	0.0002
Cymbella vulgata Krammer	G	4.8	2.9	0.67	0.015
Diploneis oblongella (Naegeli) Cleve-Euler	G	2.3	1.2	0.4	0.0196
Encyonema caespitosum Kützing	G	22.5	15.2	1.27	0.0004
Encyonema lacustre (Agardh) F.W.Mills	G	3.1	1.6	0.48	0.0114
Encyonopsis krammeri Reichardt	G	6.5	3.1	0.74	0.0004
Encyonopsis minuta Krammer & Reichardt	G	17.1	7	0.86	0.0002
Encyonopsis subminuta Krammer & Reichardt	G	14.4	6.5	0.88	0.0002
Epithemia adnata (Kutzing) Brebisson	G	36.4	16.3	1.41	0.0002
Epithemia sorex Kutzing	G	27.6	15.3	1.33	0.0002
Epithemia turgida (Ehr.) Kutzing	G	26.1	9.7	1.21	0.0002
Eunotia minor (Kutzing) Grunow in Van Heurck	G	3.7	2.2	0.61	0.026
Fragilaria construens (Ehr.) Grunow	G	35.8	23	1.25	0.0002
Fragilaria lapponica Grunow in van Heurck	G	16	4.7	0.74	0.0002
Fragilaria pinnata Ehrenberg	G	32	20.9	1.35	0.0002
Geissleria cummerowi (L.Kalbe) Lange-Bertalot	G	26.2	7.8	0.91	0.0002
Geissleria schoenfeldii (Hustedt) Lange-Bertalot & Metzeltin	G	12.1	3.2	0.7	0.0002
Gomphonema brebissonii Kützing	G	5.1	3.3	0.7	0.0202
Gomphonema truncatum Ehr.	G	16.1	12.8	1.15	0.0122
Gyrosigma attenuatum (Kützing) Rabenhorst	G	13	7.6	0.93	0.0002
Gyrosigma nodiferum (Grunow) Reimer	G	3.1	2	0.5	0.044

Taxon	Status class	IndicatorValue	Mean	SDev	p
Mastogloia lacustris (Grunow) van Heurck	G	5.3	2.8	0.72	0.0054
Navicula cari Ehrenberg	G	27	10.5	1.13	0.0002
Navicula cryptotenelloides Lange-Bertalot	G	27.7	11.4	1.1	0.0002
Navicula oblonga Kutzing	G	15	6.8	1.32	0.0002
Navicula radiosa Kützing	G	27.5	17.7	1.57	0.0002
Navicula seibigiana Lange-Bertalot	G	21.7	5.9	0.87	0.0002
Navicula subrotundata Hustedt	G	20	6.8	0.84	0.0002
Navicula trophicatrix Lange-Bertalot	G	7.3	3.7	0.7	0.0002
Naviculadicta laterostrata Hustedt	G	3.3	1.5	0.47	0.0056
Naviculadicta pseudoventralis (Hustedt) Lange-Bertalot	G	14.8	3.8	0.78	0.0002
Nitzschia alpina Hustedt	G	1.1	0.4	0.22	0.0238
Nitzschia dissipata(Kutzing)Grunow var.media (Hantzsch.) Grunow	G	2.5	1.5	0.47	0.049
Nitzschia recta Hantzsch in Rabenhorst	G	10.9	7.2	0.95	0.0036
Nitzschia sigmoidea (Nitzsch)W. Smith	G	9.1	4	0.73	0.0002
Planothidium joursacense (Héribaud) Lange-Bertalot	G	17.7	5.9	0.8	0.0002
Planothidium rostratum (Oestrup) Lange-Bertalot	G	16.2	9.4	1.14	0.0002
Platessa conspicua (A.Mayer) Lange-Bertalot	G	17.9	11.3	1.08	0.0004
Pseudostaurosira parasitica var. subconstricta (Grunow) Morales	G	4.1	2	0.47	0.0012
Rhopalodia gibba (Ehr.) O.Muller	G	21.6	11.3	1.23	0.0002
Sellaphora pupula (Kutzing) Mereschkowksy	G	10.7	7.4	0.85	0.0022
Sellaphora verecundiae Lange-Bertalot	G	11.7	3.2	0.65	0.0002
Staurosira construens Ehrenberg	G	8.1	3.9	0.76	0.0002
Tabellaria flocculosa(Roth)Kutzing	G	13	8.2	1.19	0.0022
Achnanthes minuscula Hustedt	M	3.2	1.9	0.58	0.0234
Achnanthes ploenensis Hustedt var.gessneri (Hustedt) Lange-Bertalot	M	2.6	1.3	0.44	0.0146

Taxon	Status class	IndicatorValue	Mean	SDev	p
Amphora libyca Ehr.	M	32.8	14.1	1.16	0.0002
Amphora ovalis (Kutzing) Kutzing	M	15.1	8.6	0.94	0.0002
Amphora pediculus (Kutzing) Grunow	M	44	28.5	1.22	0.0002
Amphora veneta Kutzing	M	10.1	3.9	0.77	0.0002
Caloneis amphisbaena (Bory) Cleve	M	2.6	1	0.37	0.0018
Cocconeis pediculus Ehrenberg	M	29.7	16	1.41	0.0002
Cocconeis placentula Ehrenberg	M	40.6	29.4	1.81	0.0002
Craticula accomoda (Hustedt) Mann	M	1	0.5	0.25	0.043
Craticula molestiformis (Hustedt) Lange-Bertalot	M	2.3	1	0.39	0.0098
Ctenophora pulchella (Ralfs ex Kutz.) Williams et Round	M	4.7	2.5	0.71	0.0088
Cymatopleura solea (Brebisson) W.Smith	M	7.7	5.4	0.92	0.0214
Cymbella tumida (Brebisson)Van Heurck	M	6	2.1	0.52	0.0002
Diadesmis confervacea Kützing	M	1.2	0.4	0.21	0.0098
Diatoma vulgaris Bory	M	9.8	5.9	0.97	0.0026
Diploneis parma Cleve	M	4.1	2.7	0.63	0.034
Ellerbeckia arenaria (Moore) Crawford	M	5.8	3.6	0.79	0.0096
Eolimna minima(Grunow) Lange-Bertalot	M	34	11.4	1.33	0.0002
Eolimna subminuscula (Manguin) Moser Lange-Bertalot & Metzeltin	M	6	2.1	0.55	0.0002
Fragilaria bidens Heiberg	M	3.7	2.1	0.58	0.0176
Fragilaria leptostauron(Ehr.)Hustedt	M	9.1	3.3	0.64	0.0002
Fragilaria nitzschioides Grunow in Van Heurck	M	1.5	0.8	0.3	0.0156
Fragilaria vaucheriae (Kutzing) Petersen	M	27.1	18.3	1.47	0.0002
Gomphonema augur Ehrenberg	M	3.3	1.9	0.56	0.0176
Gomphonema clavatum Ehr.	M	9.9	6.8	1.12	0.0132
Gomphonema insigne Gregory	M	2.6	1.6	0.46	0.0388

Taxon	Status class	IndicatorValue	Mean	SDev	p
Gomphonema micropus Kützing	M	6.7	2.4	0.61	0.0002
Gomphonema parvulum (Kützing) Kützing	M	28.5	15.4	1.44	0.0002
Gomphonema.micropus(Kutzing) Cleve	M	9	3.5	0.69	0.0002
Gyrosigma acuminatum (Kutzing)Rabenhorst	M	2.8	1.3	0.37	0.0034
Hippodonta capitata (Ehr.)Lange-Bert.Metzeltin & Witkowski	M	18.3	6.8	1.14	0.0002
Hippodonta hungarica(Grunow) Lange-Bertalot Metzeltin & Witkowski	M	1.9	0.9	0.35	0.019
Kolbesia ploenensis (Hust.) Kingston	M	13.2	4.3	0.87	0.0002
Lemnicola hungarica (Grunow) Round & Basson	M	5.6	1.7	0.53	0.0002
Mayamaea atomus (Kutzing) Lange-Bertalot	M	16.1	5.3	1.13	0.0002
Melosira varians Agardh	M	24.7	8.1	1.11	0.0002
Meridion circulare (Greville) C.A.Agardh	M	2.6	1.6	0.49	0.0336
Navicula angusta Grunow	M	1.2	0.4	0.22	0.0094
Navicula antonii Lange-Bertalot	M	23.8	8.6	1.4	0.0002
Navicula capitatoradiata Germain	M	23	12.9	1.2	0.0002
Navicula cincta (Ehr.) Ralfs in Pritchard	M	4.9	2.1	0.58	0.0018
Navicula costulata Grunow in Cleve & Grunow	M	2	0.8	0.31	0.007
Navicula cryptocephala Kutzing	M	14.4	9.4	1.24	0.0012
Navicula decussis Oestrup	M	5.8	1.6	0.47	0.0002
Navicula elginensis (Gregory) Ralfs in Pritchard	M	1.4	0.6	0.27	0.0082
Navicula gregaria Donkin	M	18.1	7.3	1.04	0.0002
Navicula ignota Krasske var.palustris (Hustedt) Lund	M	1.2	0.7	0.3	0.045
Navicula jakovljevicii Hustedt	M	2.6	1.2	0.43	0.01
Navicula lanceolata (Agardh) Ehrenberg	M	5.9	3.4	0.61	0.0028
Navicula menisculus Schumann var. menisculus	M	14.9	6	0.85	0.0002
Navicula moskalii Witkowski & Lange-Bertalot	M	4.3	1.9	0.53	0.0014

Taxon	Status class	IndicatorValue	Mean	SDev	p
Navicula oppugnata Hustedt	M	8.8	4.5	0.97	0.0006
Navicula pseudoanglica Cleve-Euler	M	4.6	1.6	0.47	0.0002
Navicula pseudotuscula Hustedt	M	2.6	1.2	0.39	0.0042
Navicula recens (Lange-Bertalot) Lange-Bertalot	M	2.9	1.1	0.4	0.0018
Navicula reichardtiana Lange-Bertalot	M	27.7	13	1.31	0.0002
Navicula reinhardtii (Grunow) Grunow in Cl. & Möller	M	4.7	3	0.64	0.0186
Navicula rhynchocephala Kutzing	M	4.6	1.7	0.47	0.0002
Navicula salinarum Grunow in Cleve et Grunow	M	3	1.3	0.47	0.0018
Navicula schoenfeldii Hustedt	M	3.8	1.7	0.48	0.0034
Navicula schroeteri Meister	M	0.9	0.4	0.2	0.0288
Navicula seminulum Grunow	M	5.7	3.5	0.69	0.01
Navicula slesvicensis Grunow	M	3.7	1.5	0.42	0.0012
Navicula tenelloides Hustedt	M	2.1	1.2	0.44	0.041
Navicula tripunctata (O.F.Müller) Bory	M	37.1	16	1.42	0.0002
Navicula trivialis Lange-Bertalot	M	5	2.6	0.62	0.0036
Navicula veneta Kutzing	M	11.1	5.4	0.81	0.0002
Navicula viridula (Kutzing) Ehrenberg	M	3.5	1.9	0.5	0.0098
Nitzschia acicularis(Kutzing) W.M.Smith	M	5	2.7	0.54	0.002
Nitzschia amphibia Grunow f.amphibia	M	33.3	15.3	1.16	0.0002
Nitzschia capitellata Hustedt in A.Schmidt & al.	M	3.7	1.6	0.46	0.0022
Nitzschia communis Rabenhorst	M	0.9	0.4	0.19	0.028
Nitzschia dissipata(Kutzing)Grunow	M	34.6	17.6	1.82	0.0002
Nitzschia filiformis (W.M.Smith) Van Heurck	M	2.4	0.8	0.36	0.0002
Nitzschia fonticola Grunow in Cleve et Möller	M	25	12.6	1.34	0.0002
Nitzschia frustulum(Kutzing)Grunow	M	17.6	5.5	0.84	0.0002

Taxon	Status class	IndicatorValue	Mean	SDev	p
Nitzschia inconspicua Grunow	M	22.8	6	0.98	0.0002
Nitzschia intermedia Hantzsch ex Cleve & Grunow	M	2.9	1.5	0.42	0.0078
Nitzschia palea (Kutzing) W.Smith	M	24.5	9.7	1.09	0.0002
Nitzschia paleacea (Grunow) Grunow in van Heurck	M	27	9.6	1.24	0.0002
Nitzschia pusilla(Kutzing)Grunow	M	2.9	1.3	0.47	0.0034
Nitzschia sinuata (Thwaites) Grunow var.delognei (Grunow)Lange-Bertalot	M	2.8	1.2	0.44	0.004
Nitzschia sociabilis Hustedt	M	4.9	3.3	0.7	0.0298
Nitzschia supralitorea Lange-Bertalot	M	3.6	1.8	0.59	0.0084
Planothidium delicatulum(Kutz.) Round & Bukhtiyarova	M	7.6	4.1	0.78	0.0008
Planothidium frequentissimum(Lange-Bertalot)Lange-Bertalot	M	16.5	11.1	1.11	0.001
Planothidium lanceolatum(Brebisson ex Kützing) Lange-Bertalot	M	27.1	9.6	1.01	0.0002
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	M	47.3	19	1.47	0.0002
Stephanodiscus species	M	3.8	1.7	0.52	0.0036
Surirella angusta Kutzing	M	3.3	1.3	0.43	0.0016
Surirella brebissonii Krammer & Lange-Bertalot	M	2.1	1.2	0.38	0.026
Synedra ulna (Nitzsch.)Ehr.	M	27.4	15.7	1.43	0.0002
Tabularia fasciculata (Agardh)Williams et Round	M	15.2	5	0.86	0.0002

Annexes

A. Lake Phytobenthos classification systems of Member States

A.1 Belgium – Flanders BE-FL lake phytobenthos method (PISIAD)

Sampling

Littoral diatom assemblages are sampled in summer from hard substrates (preferably reed; choice of alternative substrates and sampling procedures are fixed by protocols) after a sufficiently prolonged period of submergence at 9 spatially separated sites. A peroxide treatment followed by sedimentation is used for cleaning. Cleaned diatom valves are embedded in Naphrax for identification and counting by interference light microscopy at high magnification (EN 14407). Identifications are at species or lower taxonomic level, using up-to-date literature. The proportions of type-specific impact-sensitive and impact-associated diatoms are estimated from a fixed count of 500 randomly selected valves in a sample. Valves from all taxa are considered, except for those which are clearly reworked from coastal deposits. Lake classification is based on averaged results for at least 3 samples. The necessary number of samples increases with the divergence in assessment results up to the number where the standard deviation on the average EQR ≤ 0.2 EQR units, with a maximum of 9 samples. The presence of cyanobacterial films and abundance of filamentous algae are considered in the macrophyte method.

Metric calculation

In PISIAD, the Ecological Quality Ratio (EQR) is obtained from the summed relative abundances of *impact-associated* and impact-sensitive diatoms. The abundance of impact-associated taxa is assumed to remain below a certain treshold at good or high status, increasing progressively up to 100 % with decreasing quality, whereas the proportion of impact-sensitive allows to distinguish high from good status; high status requires a significant percentage abundance of sensitive taxa (Figure A.1). Matching these (not necessarily linear) proportional changes with an EQR scale divided into equal intervals provides a direct and transparent measure of community integrity. Taxa showing no distinct relation to disturbance are not considered, as such, allowing for good status if evenness is very low as long as impact-indicative diatoms remain scarce. Disregarding the percentage of impact-sensitive diatoms in the interval from moderate to bad, minimizes memory and recruitment effects, thereby emphasizing the disturbance signal.

The following formulas are used to calculate the EQR (x = % impact-associated taxa, y = % impact-sensitive taxa, a = lower class limit, b= upper class limit):

If % impact-associated taxa > boundary value good/moderate:

$$EQR = EQRa + \frac{(x-a)}{(b-a)} * 0.2$$

If % impact-associated taxa < boundary value good/moderate:

$$EQR = EQRa + \frac{(y-a)}{(b-a)} * 0.2$$

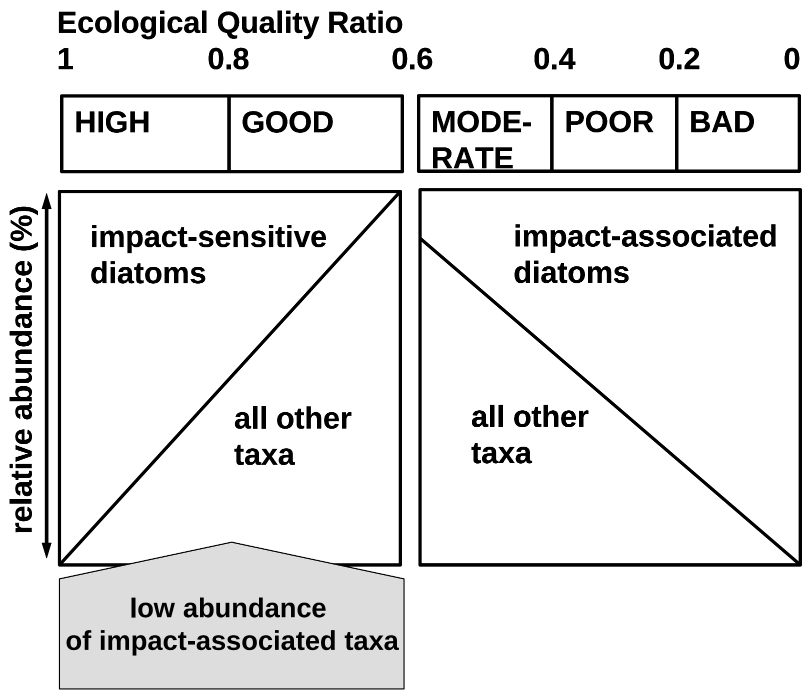

Figure A.1 General principle for the assessment of phytobenthic diatoms in PISIAD.

Identification of impact-sensitive and impact-associated taxa

The procedure for compiling the first version of a list of type-specific indicators is described by Hendrickx & Denys (2005):

- An inventory of all diatom taxa observed in recent and historical epiphyton samples from BE-FL was made (ca. 1080);
- 156 historical epiphyton samples from the period 1852-1945 were attributed to the BE-FL lake types (non-acid waters only) using information on provenance and a comparison of their assemblage composition with 139 recent assemblages of these water types;
- For each water type, an IndVal analysis for 'old' versus 'recent' samples, including only taxa occurring at least once with an abundance of 1%, identified taxa that declined or increased significantly in abundance during the second half of the 20th C;
- A similar analysis was done using similarity in species composition, rather than water type, as a base for comparison;
- Both analyses provided a number of (type-specific) potential indicators;
- DCCA- analyses of the assemblage composition of 137 recent epiphyton samples against gradients of median total inorganic N, median total organic N, maximum TP and median potential gross oxygen production (a measure for phytoplankton productivity; all these variables are proxies for eutrophication and were shown to be significantly related to assemblage composition) were carried out and taxa in the 20th and 80th percentile of the scores on the constrained axis were selected. These taxa were considered also as potential indicators, and increasingly so if they scored as such for a larger number of variables and if they had more effective observations (estimated by Hill's N2);

- A critical evaluation of all potential indicators was made, using literature sources, additional observations from (limited) regional paleolimnological records and their appreciation in NL and DE assessment methods for comparable lake types.

After 2005, some minor revisions of this list were made based on additional observations, mainly to incorporate some taxa that had not been observed previously in BE-FL.

Boundary setting

Type-specific values for the H/G boundaries were derived from the 90th percentiles of the relative abundance of impact-sensitive diatoms in historical assemblages predating 1940 (best 10%) and G/M boundaries from the 90th percentiles of the relative abundance of impact-associated diatoms in such assemblages (best 90%). The latter were cross checked against the 75th percentiles for recent assemblages from sites with TP and chl a below G/M, as inferred from empirical regressions. For lake types with few historical data, the minimum relative abundance of impact-sensitive diatoms was set to the 90th percentile observed for sites with inferred TP and chl a below G/M (best 10%), whereas G/M was based on the 75th percentiles of the relative abundance of impact-associated taxa (best 75%). Lower boundaries were obtained by linear interpolation between the relative abundance of impact-associated diatoms corresponding to the G/M boundary and 100%, assuming equal class intervals. All percentages serving as boundary values were rounded to the nearest 5.

Table A.1 Class Boundaries for different diatom metrics / water body types

BE-FL type	ISD-Ref	ISD-H_G	IAD-G_M	IAD-M_P	IAD-P_B
Cb	0.85	0.7	0.25	0.5	0.75
Ami-e	0.7	0.4	0.25	0.5	0.75
Ami-om	0.8	0.6	0.1	0.4	0.7
Aw-e	0.7	0.4	0.2	0.45	0.75
Aw-om	0.7	0.4	0.2	0.45	0.75
Ai	0.8	0.6	0.25	0.5	0.75

ISD: relative proportion impact-sensitive diatoms; IAD: relative proportion impact-associated diatoms; Ref: reference; H: high; G: good; M: moderate; P: poor; B: bad

EQR boundaries: H/G: 0.8, G/M: 0.6, M/P: 0.4, P/B: 0.2

Pressure addressed

From the above, it follows that the principal pressure addressed by the BE-Fl metric will be eutrophication and impacts that increase the sensitivity of lakes to nutrient-loading (increased stock of zooplanktivorous/benthivorous fish, reduced macrophyte abundance due to pollutants, degradation of riparian habitat,…). The representation of both groups of indicators – impact-sensitive and impact-associated diatoms – in relation to chlorophyll *a* and median TP was examined at water-type level by Hendrickx & Denys (2005). General scatter plots for the combined metric against maximum TP and median chl *a* (202 data points, mostly from smaller WBs) are shown below (see Figure A.2). Although discrimination is rather good for good and high status (EQR ≥ 0.6), there is much scatter at lower values. This is not unexpected considering, among others, the factors influencing chl *a* and TP. Further support for a consistent relation to eutrophication variables and metrics from other MSs is given by the intercalibration results (see final Milestone reports).

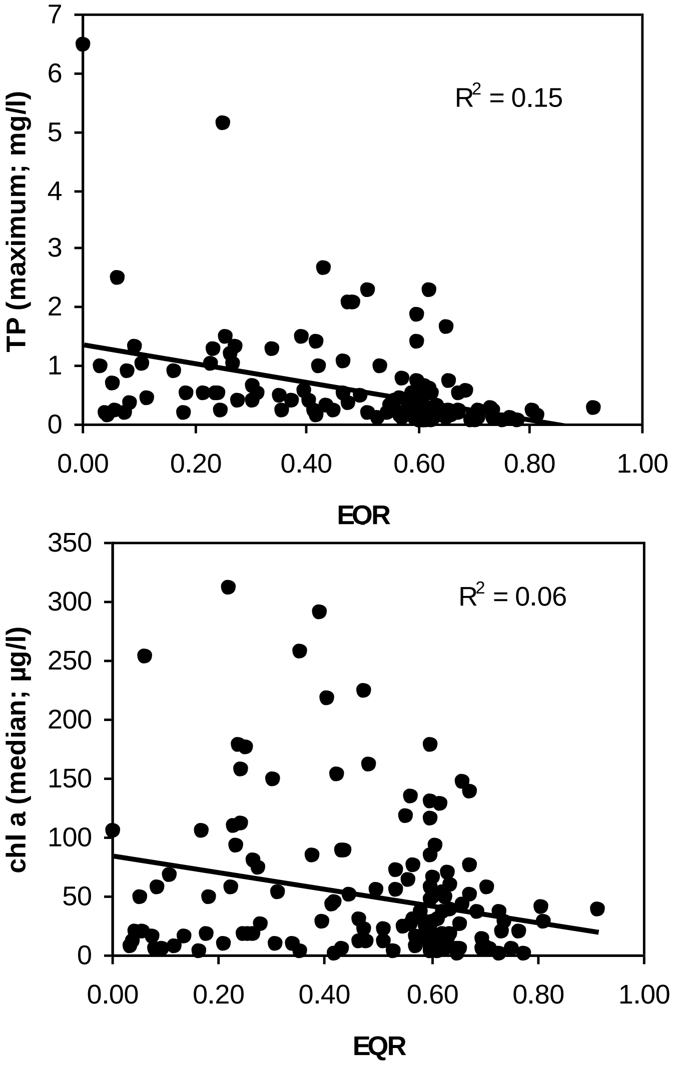

Figure A.2 General scatter plots for the combined metric EQR against maximum TP and median chl a (202 data points, mostly from smaller WBs).

Table A.2 List of indicators – impact sensitive and impact associated taxa for different water body types.

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Achnanthes brevipes									X		X	X
Achnanthes intermedia							X	X	X	X	X	X
Achnanthes lutherii	X	X	X									
Achnanthes microscopica	X	X	X									
Achnanthes nodosa	X	X	X	X	X							
Achnanthes rupestris					X	X						
Achnanthes trinodis		X	X	X	X							
Achnanthidium affine						X		X		X		
Achnanthidium caledonicum	X	X	X	X	X							

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Achnanthidium catenatum			X		X							
Achnanthidium eutrophilum							X	X		X		
Achnanthidium exiguum					X	X	X	X		X		
Achnanthidium gracillimum	X	X	X	X	X							
Achnanthidium jackii						X		X		X		
Achnanthidium linearioide	X	X	X									
Achnanthidium microcephalum						X						
Achnanthidium minutissimum						X						
Achnanthidium minutissimum var. inconspicuum					X	X						
Achnanthidium minutissimum very narrow MT	X	X	X	X	X	X						
Achnanthidium pyrenaicum						X						
Achnanthidium saprophilum							X	X	X	X	X	X
Achnanthidium straubianum						X						
Actinocyclus normanii							X	X	X	X	X	X
Actinocyclus normanii f. subsalsus							X	X	X	X	X	X
Adlafia bryophila			X		X	X						
Adlafia minuscula	X	X	X									
Adlafia minuscula var. muralis							X	X				
Amphora copulata							X	X		X		
Amphora fogediana	X	X	X									
Amphora hemicycla							X	X		X		
Amphora inariensis	X	X	X	X	X							
Amphora lange-bertalotii var. tenuis							X	X		X		
Amphora montana							X	X		X		
Amphora oligotraphenta		X	X	X	X							
Amphora ovalis							X	X		X		
Amphora pediculus							X	X		X		
*Amphora thumensis**		X	X	X	X							
Amphora veneta							X	X	X	X	X	
Aneumastus stroesei		X	X	X	X							
Aneumastus tusculus		X	X	X	X							
Anomoeoneis sphaerophora							X	X	X	X	X	
Astartiella bahusiensis									X		X	X

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Asterionella formosa			X		X	X	X					
*Asterionella ralfsii**	X	X	X									
Aulacoseira alpigena	X	X	X									
Aulacoseira ambigua							X	X	X	X	X	
Aulacoseira crassipunctata	X	X	X	X	X							
Aulacoseira distans	X	X	X									
Aulacoseira granulata							X	X	X	X	X	X
Aulacoseira granulata MT curvata							X	X	X	X	X	X
Aulacoseira granulata var. angustissima							X	X	X	X	X	X
Aulacoseira italica						X						
Aulacoseira muzzazensis							X	X	X	X	X	X
Aulacoseira pusilla							X	X	X	X		
Aulacoseira subarctica	X	X	X									
Aulacoseira subarctica f. recta	X	X	X									
Aulacoseira tenella	X	X	X									
Bacillaria paxillifera							X	X	X	X	X	
Brachysira brebissonii	X	X	X	X	X							
Brachysira calcicola		X	X	X	X							
Brachysira follis	X	X	X	X	X							
Brachysira garrensis	X	X	X									
Brachysira hofmanniae		X	X	X	X							
Brachysira lilianae		X	X	X	X							
Brachysira microcephala	X	X	X	X	X							
Brachysira procera	X	X	X									
Brachysira serians	X	X	X									
Brachysira styriaca	X	X	X	X	X							
Brachysira vitrea		X	X	X	X							
Brachysira wygaschii	X	X	X									
Brachysira zellensis	X	X	X	X	X							
Caloneis alpestris		X	X	X	X							
Caloneis amphisbaena							X	X	X	X	X	
Caloneis bacillum			X		X	X						
Caloneis fontinalis			X		X	X						
Caloneis latiuscula		X	X	X	X							
Caloneis obtusa		X	X	X	X							
Caloneis permagna							X	X	X	X	X	
Caloneis schumanniana						X						
Caloneis tenuis	X	X	X	X	X							
Caloneis undulata	X	X	X									
Cavinula cocconeiformis	X	X	X	X	X							
Cavinula jaernefeltii	X	X	X	X	X							
Cavinula lapidosa	X	X	X									

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Cavinula pseudoscutiformis	X	X	X	X	X							
Cavinula pusio	X	X	X									
Cavinula scutelloides							X	X	X	X		
Cavinula variostriata	X	X	X									
Cocconeis neothumensis			X		X	X	X	X				
Cocconeis pediculus						X	X	X		X		
Cocconeis placentula							X	X		X		
Cocconeis placentula var. euglypta							X	X		X		
Cocconeis placentula var. klinoraphis							X	X		X		
Cocconeis placentula var. lineata							X	X		X		
Cocconeis pseudolineata							X	X		X		
Craticula accomoda							X	X	X	X	X	X
Craticula accomodiformis							X	X	X	X	X	X
Craticula ambigua							X	X	X	X	X	X
Craticula buderi							X	X	X	X	X	X
Craticula citrus							X	X	X	X	X	X
Craticula cuspidata							X	X	X	X	X	X
Craticula halophila							X	X	X	X	X	X
Craticula molestiformis							X	X	X	X	X	X
Craticula riparia							X	X				
Craticula vixnegligenda							X	X		X		
Ctenophora pulchella							X	X	X	X	X	
Cyclostephanos dubius							X	X	X	X	X	X
Cyclostephanos invisitatus							X	X	X	X	X	X
Cyclostephanos tholiformis							X	X	X	X	X	X
Cyclotella atomus							X	X	X	X	X	X
Cyclotella comensis			X									
Cyclotella cyclopuncta			X									
Cyclotella distinguenda			X									
Cyclotella meneghiniana							X	X	X	X	X	X
Cyclotella ocellata			X									
Cyclotella scaldensis							X	X	X	X	X	X
Cyclotella striata							X	X	X	X	X	X
Cymatopleura elliptica			X			X						
Cymatopleura solea							X	X		X		
Cymatopleura solea var. apiculata							X	X		X		
Cymbella affiniformis						X						
Cymbella affinis		X	X	X	X							
Cymbella alpestris	X	X	X	X	X							
Cymbella alpina		X	X	X	X							
Cymbella ancyli		X	X	X	X							

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Cymbella aspera						X		X		X		
Cymbella cistula auct.						X						
Cymbella cymbiformis					X	X						
Cymbella excisa (= C. affinis MT 2)						X	X	X		X		
Cymbella excisa var. angusta		x	x	x	x	x						
Cymbella excisiformis		X	X	X	X							
Cymbella hantzschiana var. borealis		x	x	x	x	x						
Cymbella helmckei												
Cymbella helvetica		X	X	X	X							
Cymbella hustedtii		X	X	X	X							
Cymbella hybrida		X	X	X	X							
Cymbella laevis		X	X	X	X							
Cymbella lanceolata					X	X		X		X		
Cymbella lancettula		X	X	X	X							
Cymbella lange-bertalotii			X		X	x						
*Cymbella lapponica**	X	X	X	X	X							
Cymbella neocistula						X						
Cymbella neoleptoceros						X						
Cymbella neoleptoceros						X						
Cymbella proxima			X		X	X						
Cymbella reinhardtii		X	X	X	X							
Cymbella similis		X	X	X	X							
Cymbella simonsenii		X	X	X	X	X						
Cymbella subcistula						X						
Cymbella subtruncata	X	X	X	X	X	X						
Cymbella tumida							X	X		X		
Cymbella vulgata			X		X	X						
Cymbopleura amphicephala		X	X	X	X	X						
Cymbopleura anglica					X	X						
Cymbopleura angustata		X	X	X	X							
Cymbopleura citrus	X	X	X	X								
Cymbopleura cuspidata					X	X						
Cymbopleura diminuta				X	X							
Cymbopleura frequens		X	X	X	X	X						
Cymbopleura inaequalis						X						
Cymbopleura incerta	X	X	X	X	X							
Cymbopleura naviculacea	X	X	X	X	X							
Cymbopleura subaequalis	X	X	X	X	X							
Cymbopleura subcuspidata	X	X	X									
Delicata delicatula	X	X	X	X	X							
Diatoma moniliformis							X	X		X		
Diatoma problematica							X	X		X		

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Diatoma tenuis							X	X		X	X	
Diatoma vulgaris							X	X		X		
Diatoma vulgaris f. lineare							X	X		X		
Diploneis burgitensis		X	X	X	X							
Diploneis elliptica		X	X	X	X	X						
Diploneis fontanella						X						
Diploneis fontium						X						
Diploneis krammeri						X						
Diploneis marginestriata	X	X	X	X	X	X						
Diploneis modica		X	X	X	X							
Diploneis oblongella auct.						X						
Diploneis parma	X	X	X	X	X							
Diploneis petersenii	X	X	X	X	X							
Diploneis separanda						X						
Discostella pseudostelligera							X	X	X	X	X	X
Distrionella incognita				X	X							
Encyonema brehmi		X	X	X	X							
Encyonema caespitosum							X	X		X		
Encyonema elginense	X	X	X									
Encyonema hebridica	X	X	X									
Encyonema kuelbsii	X	X	X									
Encyonema minutum			X		X	X						
Encyonema neogracile	X	X	X									
Encyonema norvegica	X	X	X									
Encyonema obscurum	X	X	X	X	X							
Encyonema paucistriatum	X	X	X	X	X							
Encyonema perpusillum	X	X	X									
Encyonema prostratum						X	X	X		X		
Encyonema vulgare aggr.	X				X							
Encyonopsis cesatii	X	X	X	X	X							
Encyonopsis descripta	X	X	X	X	X							
Encyonopsis falaisensis	X	X	X	X	X							
Encyonopsis gaeumanii	X	X	X									
Encyonopsis krammeri	X	X	X	X	X	X						
Encyonopsis lanceola	X	X	X									
Encyonopsis microcephala	X	X	X	X	X	X						
Encyonopsis subminuta	X	X	X	X	X	X						
Entomoneis ornata	X	X	X									
Entomoneis paludosa							X	X		X		
Eolimna minima							X	X	X	X	X	X
Eolimna subminuscula							X	X	X	X	X	X
Epithemia adnatum					X	X	X	X		X		

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Epithemia goeppertiana		X	X	X	X							
Epithemia smithii		X	X	X	X							
Epithemia sorex					X	X	X	X		X		
Epithemia turgida					X	X	X	X		X		
Epithemia turgida var. granulata					X	X	X	X		X		
Epithemia turgida var. westermanii						X	X	X		X		
Eucocconeis alpestris	X	X	X	X	X							
Eucocconeis flexella	X	X	X	X	X							
Eucocconeis laevis	X	X	X	X	X							
Eunotia arcubus	X	X	X	X	X							
Eunotia arculus	X											
Eunotia arcus	X	X	X									
Eunotia bidens	X											
Eunotia bilunaris					X	X						
Eunotia bilunaris var. linearis					X	X						
Eunotia botuliformis	X	X	X									
Eunotia circumborealis	X	X	X									
Eunotia compacta	X											
Eunotia diadema	X	X	X									
Eunotia diodon	X	X	X									
Eunotia elegans	X											
Eunotia eurycephaloides	X											
Eunotia exigua							X					
Eunotia exigua var. tridentula	X											
Eunotia faba	X	X	X									
Eunotia fallax	X	X	X									
Eunotia fennica	X											
Eunotia flexuosa	X											
Eunotia formica					X	X						
Eunotia glacialis					X	X						
Eunotia groenlandica	X	X	X									
Eunotia iatriaensis	X	X	X									
Eunotia implicata	X	X	X									
Eunotia incisa	X	X	X									
Eunotia intermedia	X	X	X									
Eunotia jemtlandica	X											
Eunotia meisteri	X	X	X									
Eunotia microcephala	X	X	X									
Eunotia minor						X						
Eunotia monodon	X	X	X									
Eunotia mucophila	X	X	X									
Eunotia naegelii	X											
Eunotia nymanniana	X											
Eunotia parallela	X	X	X									

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Eunotia parallela var. angusta	X	X	X									
Eunotia pectinalis	X	X	X									
Eunotia praerupta	X	X	X									
Eunotia praerupta var. bigibba	X	X	X									
Eunotia rhomboidea	X	X	X									
Eunotia rhynchocephala	X	X	X									
Eunotia serra	X	X	X									
Eunotia soleirolii				X	X							
Eunotia sudetica	X	X	X									
Eunotia tenella	X											
Eunotia tetraodon	X	X	X									
Eunotia ursamaioris	X	X	X									
Eunotia veneris	X	X	X									
Fallacia lenzii		X	X	X	X							
Fallacia monoculata							X	X	X	X		
Fallacia pygmaea							X	X	X	X	X	
Fallacia subhamulata							X	X		X		
Fallacia sublucidula							X	X		X		
Fallacia vitrea	X	X	X									
Fistulifera pelliculosa												
Fistulifera saprophila							X	X	X	X	X	X
Fragilaria bidens							X	X	X	X	X	X
Fragilaria capucina var. distans							X	X		X		
Fragilaria crotonensis						X						
Fragilaria famelica							X	X	X	X		
Fragilaria gracilis					X	X						
Fragilaria mesolepta							X	X		X		
Fragilaria nanana	X	X	X									
Fragilaria perminuta		X	X	X	X							
Fragilaria radians					X	X						
Fragilaria sopotensis							x	x	x	x	x	x
Fragilaria sundayensis									X		X	
Fragilaria tenera	X	X	X	X	X							
Fragilaria tenuistriata							X	X		X		
Fragilaria vaucheriae							X	X	X	X	X	X
Fragilariforma bicapitata							X	X		X		
Fragilariforma constricta	X	X	X									
Fragilariforma exiguiformis	X	X	X									
Fragilariforma virescens	X											
Frustulia erifuga	X	X	X									
Frustulia rhomboides	X	X	X									
Geissleria declivis		X	X									
Geissleria decussis			X				X	X		X		

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Gomphonema acuminatum						X						
Gomphonema acuminatum var. pusillum			X		X	X						
Gomphonema acutiusculum	X	X	X	X	X							
Gomphonema affine							X	X		X		
Gomphonema angustatum						X						
Gomphonema angustum		X	X	X	X							
Gomphonema augur							X	X	X	X	X	X
Gomphonema auritum	X	X	X	X	X							
Gomphonema bavaricum		X	X	X	X							
Gomphonema brebissonii						X						
Gomphonema calcifugum						X	X					
Gomphonema calcifugum						X						
Gomphonema clavatum												
Gomphonema contraturris							X	X	X	X		
Gomphonema coronatum	X	X										
Gomphonema cuneolus	X	X	X		X							
Gomphonema dichotomum		X	X	X	X							
Gomphonema exilissimum	X	X	X	X	X	X						
Gomphonema hebridense	X	X	X	X	X							
*Gomphonema helveticum**		X	X	X	X							
Gomphonema innocens							x	x	x	x	x	x
Gomphonema insigne							X					
Gomphonema insigneforme							X					
Gomphonema lagerheimii	X	X	X									
Gomphonema lateripunctatum		X	X	X	X							
Gomphonema micropumilum			X		X	X						
Gomphonema minusculum			X		X	X						
Gomphonema minutum						X	X					
Gomphonema minutum f. curtum						X						

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Gomphonema minutum f. syriacum						X						
Gomphonema occultum						X						
Gomphonema olivaceum						X	X					
Gomphonema olivaceum var. olivacealacuum						X	X					
Gomphonema parvulius	X	X	X	X	X	X						
Gomphonema parvulum							X	X	X	X	X	X
Gomphonema parvulum f. saprophilum							X	X	X	X	X	X
Gomphonema pratense					X	X						
Gomphonema procerum		X	X	X	X							
Gomphonema productum	X	X	X									
Gomphonema pseudoaugur							X	X	X	X	X	X
Gomphonema pseudobohemicum	X	X	X									
Gomphonema pseudotenellum			X	X	X	X						
Gomphonema pumilum			X		X	X						
Gomphonema pumilum var. 4-9 Reichardt 1997, pl. 12, fig. 4-10			X		X	X						
Gomphonema pumilum var. elegans			X		X	X						
Gomphonema pumilum var. rigidum			X		X	X						
Gomphonema sarcophagus							X	X	X	X	X	X
Gomphonema subclavatum							X	X	X	X	X	X
Gomphonema subtile	X	X	X	X	X							
Gomphonema tergestinum			X		X							
Gomphonema utae							X	X	X	X	X	X
Gomphonema ventricosum		X	X	X	X							
Gomphonema vibrio		X	X	X	X							
Gomphosphenia tackei						X						
Grunowia solgensis							X	X	X	X		
Gyrosigma attenuatum					X	X						
Gyrosigma obtusatum							X	X		X		
Hippodonta capitata							X	X	X	X	X	
Hippodonta hungarica							X	X	X	X	X	
Hippodonta ruthnielseniae							X	X		X		
Karayevia clevei						X	X	X		X		
Karayevia clevei var. rostrata						X	X	X		X		

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Karayevia laterostratra	X	X	X	X	X							
Kobayasiella jaagii				X	X							
Kobayasiella micropunctata	X											
Kobayasiella parasubtilissima	X											
Kobayasiella subtilissima	X											
Kolbesia gessneri			X		X	X	X					
Kolbesia ploenensis			X		X	X	X					
Kolbesia suchlandtii	X	X	X									
Kraskella kriegeriana	X	X	X									
Lemnicola hungarica							X	X	X	X	X	X
Luticola cohnii												
Luticola goeppertiana							X	X	X	X	X	X
Mastogloia grevillei		X	X	X	X	X						
Mastogloia lacustris		X	X	X	X							
Mayamaea atomus							X	X	X	X	X	X
Mayamaea atomus var. alcimona							X	X		X		
Mayamaea atomus var. permitis							X	X	X	X	X	X
Mayamaea lacunolaciniata							X	X	X	X	X	X
Melosira varians							X	X		X		
Meridion circulare							X	X		X		
Microcostatus krasskei	X	X	X									
Microcostatus maceria	X	X	X									
Navicula angusta	X	X	X									
Navicula antonii							X	X		X		
Navicula aquaedurae		X	X	X	X							
Navicula associata							X	X		X		
Navicula capitatoradiata							X	X	X	X		
Navicula concentrica				X	X							
Navicula cryptocephala							X	X	X	X		
Navicula dealpina		X	X	X	X							
Navicula denselineolata				X	X							
Navicula detenta	X											
Navicula digitoconvergens							X	X	X	X		
Navicula digitoradiata							X	X	X	X	X	X
Navicula digitulus	X	X	X									
Navicula diluviana		X	X	X	X							
Navicula erifuga							X	X	X	X	X	
Navicula gottlandica		X	X	X	X							
Navicula gregaria							X	X	X	X	X	
Navicula heimansioides	X	X	X									
Navicula hofmanniae					X	X						
Navicula integra							X	X	X	X	X	X

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Navicula lanceolata							X	X		X		
Navicula laticeps		X	X	X	X							
Navicula leistikowii		X	X	X	X							
Navicula leptostriata	X	X	X									
Navicula libonensis							X	X		X		
Navicula lundii							X	X		X		
Navicula margalitii							X	X		X		
Navicula mediocostata		X	X	X	X							
Navicula menisculus							X	X		X		
Navicula oblonga						X						
Navicula oligotraphenta		X	X	X	X	X						
Navicula oppugnata						X	X	X	X	X		
Navicula perminuta									X		X	X
Navicula praeterita		X	X	X	X							
Navicula pseudosilicula	X											
Navicula pseudoventralis	X	X	X									
Navicula radiosafallax												
Navicula radiosola	x											
Navicula recens							X	X	X	X	X	
Navicula reichardtiana							X	X		X		
Navicula reinhardtii					X	X						
Navicula rhynchotella							X	X	X	X	X	X
Navicula rostellata							X	X	X	X		
Navicula salinarum							X	X	X	X	X	X
Navicula schassmannii	X	X	X									
Navicula schroeteri							X	X	X	X		
Navicula seibigiana							X	X		X		
Navicula slesvicensis							X	X		X		
Navicula stancovicii		X	X	X	X							
Navicula subalpina		X	X	X	X							
Navicula tenelloides							X	X		X		
Navicula tripunctata						X	X	X		X		
Navicula trivialis							X	X	X	X	X	X
Navicula trophicatrix							X	X	X	X		
Navicula vandamii							X	X		X		
Navicula veneta							X	X	X	X	X	X
Navicula viridula							X	X	X	X		
Navicula vulpina		X	X	X	X							
Navicula wiesnerii							X	X	X	X		
Navicula wildii		X	X	X	X							
Naviculadicta cosmopolitana							X	X		X		
Neidiopsis levanderii	X	X	X									
Neidium affine	X	X	X									
Neidium alpinum	X	X	X									
Neidium ampliatum						X						
Neidium binodis		X	X	X	X							

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Neidium bisulcatum	X	X	X									
Neidium carteri	X	X	X									
Neidium densestriatum	X	X	X									
Neidium dubium							X					
Neidium hercynicum	X	X	X									
Neidium iridis						X						
Neidium ladogensis	X	X	X									
Neidium longiceps	X	X	X									
Neidium productum	X	X	X									
Neidium septentrionale	X	X	X									
Nitzschia acicularis							X	X	X	X	X	X
Nitzschia agnewii									X	X	X	X
Nitzschia agnita									X		X	X
Nitzschia alpinobacillum		X	X	X	X							
Nitzschia amphibia							X	X	X	X		
Nitzschia angustatula						X						
Nitzschia angusteforaminata							X	X	X	X	X	X
Nitzschia archibaldii							X	X	X	X	X	X
Nitzschia aurariae							X	X	X	X	X	
Nitzschia bacillum						X						
Nitzschia bulnheimiana							X	X	X	X	X	
Nitzschia capitellata var. tenuirostris							X	X	X	X	X	X
Nitzschia clausii							X	X	X	X		
Nitzschia communis							X	X	X	X	X	
Nitzschia dealpina		X	X	X	X	X						
Nitzschia denticula				X	X	X						
Nitzschia desertorum							X	X	X	X	X	
Nitzschia dissipata							X	X	X	X		
Nitzschia dissipata var. oligotraphenta	X	X	X	X	X							
Nitzschia diversa					X	X						
Nitzschia draveillensis							X	X	X	X		
Nitzschia dubia							X	X	X	X		
Nitzschia filiformis							X	X	X	X	X	X
Nitzschia fonticola							X					
Nitzschia frequens							X	X	X	X	X	X
Nitzschia frustulum							X	X	X	X	X	
Nitzschia gandersheimiensis							X	X	X	X	X	X
Nitzschia graciliformis							X	X	X	X	X	X
Nitzschia gracilis			X		X	X						
Nitzschia inconspicua							X	X	X	X	X	
Nitzschia intermedia							X	X	X	X		
Nitzschia lacuum						X						
Nitzschia liebetrutii							X	X	X	X		
Nitzschia media							X	X	X	X		

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Nitzschia microcephala							X	X	X	X	X	X
Nitzschia palea							X	X	X	X	X	X
Nitzschia palea var. debilis						X						
Nitzschia palea var. minuta							X	X	X	X	X	X
Nitzschia palea var. tenuirostris							X	X	X	X	X	X
Nitzschia paleacea							X	X	X	X	X	X
Nitzschia perminuta	X	X	X			X						
Nitzschia pumila							X	X	X	X	X	X
Nitzschia pussila							X	X	X	X		
Nitzschia radicula					X	X						
Nitzschia sigma							X	X	X	X		
Nitzschia sigmoidea							X					
Nitzschia sociabilis							X	X	X	X		
Nitzschia solita							X	X	X	X	X	X
Nitzschia subacicularis							X					
Nitzschia subtilis							X	X	X	X	X	X
Nitzschia supralitorea							X	X	X	X	X	X
Nitzschia tubicola							X	X	X	X	X	X
Nitzschia umbonata							X	X	X	X	X	X
Nitzschia valdestriata						X						
Nitzschia vermicularis							X	X	X	X		
Nitzschia vitrea							X	X	X	X		
Nupela impexiformis	X	X	X									
Nupela lapidosa	X	X	X									
Nupela silvahercynica	X	X	X									
Parlibellus crucicula												x
Parlibellus protracta							X	X		X		
Peronia fibula	X	X	X									
Pinnularia biceps	X											
Pinnularia brauniana	X											
Pinnularia divergens	X	X	X									
Pinnularia divergentissima var. minor	X	X	X									
Pinnularia gibba	X	X	X									
Pinnularia nobilis	X	X	X									
Pinnularia parvulissima							X	X	X	X		
Pinnularia polyonca	X	X	X									
Pinnularia rhombarea	X	X	X									
Pinnularia rupestris	X	X	X									
Pinnularia stomatophora	X	X	X									
Placoneis clementis							X	X		X		
Placoneis constans					X	X						
Placoneis constans var. symmetrica			X		X	X						

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Placoneis explanata		X	X	X	X	X						
Placoneis gastrum							X	X		X		
Placoneis navicularis	x	x	x	x	x	x						
Placoneis neglecta							X	X		X		
Placoneis placentula							X	X		X		
Placoneis porifera var. opportuna		X	X									
Placoneis pseudanglica							X	X	X	X		
Planothidium biporomum			X									
Planothidium calcar	X	X	X	X	X							
Planothidium daui	X	X	X									
Planothidium delicatulum							X	X	X	X	X	
Planothidium distinctum	X	X	X									
Planothidium dubium							X	X		X		
Planothidium engelbrechtii							X	X	X	X	X	
Planothidium frequentissimum							X	X	X	X	X	X
Planothidium frequentissimum var. rostratiformis							X	X	X	X	X	X
Planothidium granum			X									
Planothidium hauckianum			X									
Planothidium joursacense	X	X	X									
Planothidium lanceolatum							X	X	X	X	X	X
Planothidium lanceolatum var. magna							X	X				
Planothidium oestrupii	X	X	X									
Planothidium peragallii	X	X	X									
Planothidium robustius							X	X				
Planothidium rostratum			X		X	X	X	X				
Planotihidium minusculum							X	X	X	X		
Planotihidium schwabei							X	X	X	X	X	
Planotihidium septentrionalis							X	X	X	X	X	
Platessa conspicua							X	X	X	X	X	X
Platessa hustedtii					X	X						
Pleurosira laevis							X	X	X	X	X	
Pleurosira laevis f. polymorpha							X	X	X	X	X	
Psammothidium altaicum	X	X	X									
Psammothidium bioretii	X	X	X									
Psammothidium chlidanos	X	X	X									
Psammothidium daonense	X	X	X									

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Psammothidium didymum	X	X	X	X	X							
Psammothidium helveticum	X	X	X									
Psammothidium kryophilum	X	X	X	X	X							
Psammothidium kuelbsii	X	X	X									
Psammothidium lacus-vulcani	X	X	X									
Psammothidium lauenburgianum							X					
Psammothidium levanderi	X	X	X									
Psammothidium marginulatum	X	X	X									
Psammothidium oblongellum	X	X	X									
Psammothidium perpusillum	X	X	X									
Psammothidium rechtensis	X	X	X									
Psammothidium rosenstockii		X	X	X	X							
Psammothidium rossii	X	X	X									
Psammothidium scoticum	X	X	X									
Psammothidium subatomoides	X	X	X									
Psammothidium ventralis	X	X	X									
Pseudostaurosira brevistriata						X						
Pseudostaurosira elliptica						X						
Pseudostaurosira perminuta									X		X	X
Rhoicosphenia abbreviata							X	X		X		
Rhopalodia gibba					X	X						
Rhopalodia parallela		X	X	X	X							
Rhopalodia rupestris				X	X							
Rossithidium petersenii	X	X	X	X	X							
Rossithidium pusillum	X	X	X									
Sellaphora americana						X						
Sellaphora bacillum							X	X		X		
Sellaphora disjuncta			X		X							
Sellaphora joubaudii							X	X	X	X	X	X
Sellaphora laevissima	X	X	X									
Sellaphora mutata					X	X						
Sellaphora rectangularis					X	X						
Sellaphora seminulum							X	X	X	X	X	X

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Sellaphora stroemii		X	X	X	X							
Simonsenia delognei							X	X	X	X		
Skeletonema potamos							X	X	X	X	X	X
Skeletonema subsalsum							X	X	X	X	X	X
Stauroneis anceps	X	X	X									
Stauroneis siberica	X	X	X									
Stauroneis smithii							X	X		X		
Staurosira berolinensis							X	X	X	X	X	X
Staurosira oligotraphenta nom.prov.				X	X							
Staurosira subsalina							X	X	X	X	X	X
Staurosirella lapponica				X	X	X						
Staurosirella leptostauron						X						
Staurosirella oldenburgiana		X	X	X	X							
Stenopterobia curvula	X	X	X									
Stenopterobia delicatissima	X	X	X									
Stenopterobia densestriata	X	X	X									
Stephanodiscus binderanus							X	X	X	X	X	X
Stephanodiscus hantzschii							X	X	X	X	X	X
Stephanodiscus medius							X					
Stephanodiscus minutulus							X	X	X	X	X	X
Stephanodiscus neoastraea							X	X		X		
Stephanodiscus parvus							X	X	X	X	X	X
Surirella angusta							X	X	X	X		
Surirella bifrons			X		X	X						
Surirella biseriata			X			X						
Surirella brebissonii							X	X	X	X	X	
Surirella brebissonii var. kuetzingii							X	X	X	X	X	
Surirella capronii			X			X						
Surirella minuta							X	X	X	X	X	
Surirella ovalis							X	X	X	X	X	
Surirella roba	X	X	X									
Surirella robusta			X		X	X						
Surirella splendida			X			X						
Surirella tenera			X		X	X						
Surirella visurgis							X	X	X	X		
Tabellaria binalis	X	X	X									
Tabellaria binalis var. elliptica	X	X	X									
Tabellaria fenestrata	X	X	X		X							

Taxon	Impact-sensitive taxa						Impact-associated taxa					
	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai	Cb	Aw-om	Aw-e	Ami-om	Ami-e	Ai
Tabellaria flocculosa	X	X	X									
Tabularia fasciculata							X	X	X	X		
Thalassiosira lacustris							X	X	X	X	X	
Thalassiosira pseudonana							X	X	X	X	X	X
Thalassiosira weissflogii							X	X	X	X		
Tryblionella levidensis							X	X	X	X	X	X
Tryblionella apiculata							X	X	X	X	X	
Tryblionella calida							X	X	X	X	X	X
Tryblionella debilis							X	X	X	X		
Tryblionella gracilis							X	X	X	X		
Tryblionella hungarica							X	X	X	X	X	X
Tryblionella salinarum							X	X	X	X	X	X
Ulnaria biceps							X	X		X		
Ulnaria capitata					X	X						
Ulnaria danica							X	X		X		
Ulnaria delicatissima	X	X	X	X	X	X						
Ulnaria ulna var. angustissima							X	X		X		

A.2 Finland

FI lake phytobenthos method: IPS – Indice de Polluo-Sensibilité Spécifique in medium alkalinity lakes

Sampling

Three littoral zones are sampled per lake for identification of diatom assemblages. If there are only one or two stony littoral zones, those are sampled. Diatom samples are brushed from littoral zone in autumn (August – October) from randomly sampled 5-10 cobbles with toothbrush. The cobbles should not have filamentous algae on them. Samples are preserved with ethanol and stored in cold and dark. (Meissner et al. 2012)

The samples are cleaned with strong acid method (Eloranta et al. 2007), mounted with Naphrax for identification and counting based on SFS-EN 14407. Approximately 400 valves are counted and indentified to the species level, if possible, from the sample. All species are taken into account.

Used metric

The metric IPS (Indice de Polluo-Sensibilité Spécifique, Coste in Cemagref 1982) is used to estimate the status of lake. The relative abundance of species is applied for lake status assessment. IPS has been used to estimate ecological status of Finnish rivers, and evaluated to work well in Finnish conditions (Eloranta & Soininen 2002). As the metric is based on the indicator values of species, which partly are same in lake littoral zone as in rivers, the IPS was tested on the diatom communities of lake littoral zone. IPS was found to reflect well the eutrophication pressure in medium alkalinity lakes (Figure A. 3). The IPS values are calculated with the latest version of OMNIDIA database modified with the classifications added and changed by Amelie Jarlman (November 2009).

For calculating EQR of a littoral site, the IPS value of that site is divided by the mean of IPS values of reference sites. The mean of EQRs of littoral sites within the lake is calculated for lake phytobenthos EQR value.

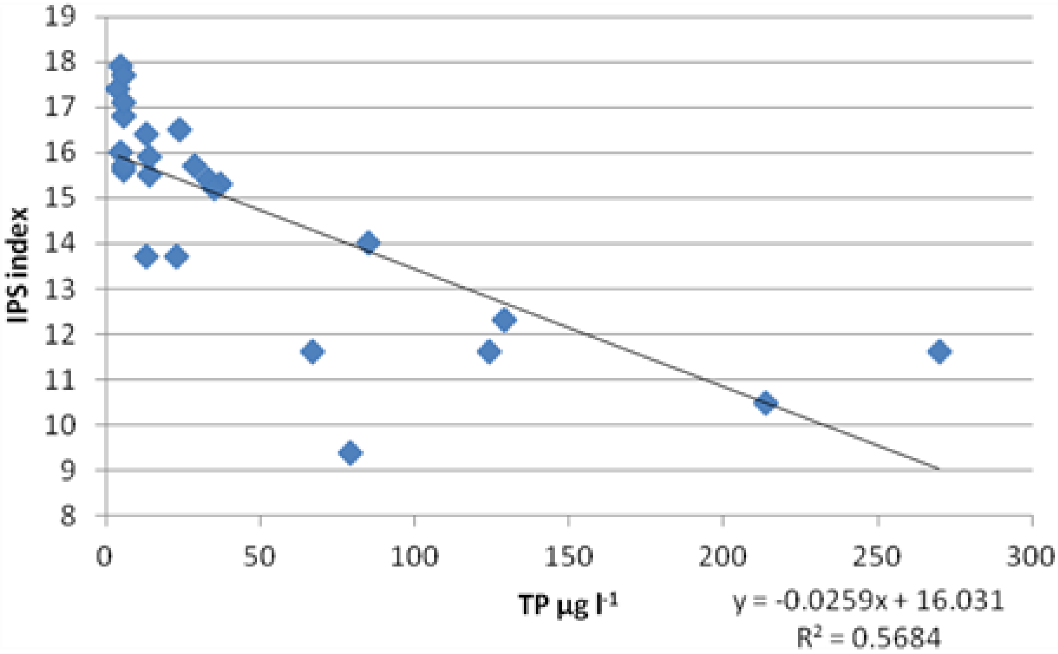

Figure A. 3 The IPS index of lake phytobenthos community vs. median of total phosphorus concentrations of the medium alkalinity lake.

Reference conditions and classification boundary settings

The state of lakes for reference conditions was taken from Finnish water quality register, where regional environment experts have stated the conditions of water body. These conditions follow the criteria set for reference lakes in Vuori et al. (2006), i.e. the reference sites have no point source loading, there is <20% agriculture in the catchment area and no adjacent fields to the lake, and no compact scattered settlement in the catchment area. The reference lake is also not artificially hydromorphologically changed.

High/good boundary is the 25th percentile of EQR reference sites for the medium alkalinity type. The lower limit of Bad is zero, and the boundaries of Good/Moderate, Moderate/Poor and Poor/Bad are arithmetical divisions of the remaining EQR scale.

The Finnish boundary values in IPS are H/G 17, G/M 15, M/P 12, P/B 9.

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A.3 Germany: Status of the German Lake method

Short Description of the method for the entire BQE Macrophytes and Phytobenthos

There are two modules to be monitored and calculated: Module 1 Macrophytes and Module 2 Phytobenthos – diatoms. For Macrophytes one metric is to be calculated: The reference species index. For Diatoms two metrics are to be calculated: The reference species quotient (RAQ) and the trophic index (TI-Nord).

The two diatom indices are to be combined by averaging to the Diatom-Index, which was used and reported for Intercalibration. The Diatom-Index and the Macrophyte-Index are to be combined by averaging to the result for ecological status for macrophytes and phytobenthos for one lake site. All monitored lake sites are combined by averaging to the lake water body result.

Description of the Modul Phytobenthos (in lakes = diatoms)

Which indicators are used?

Composition and abundance of phytobenthos:

Only benthic diatoms (Bacillariophyceae) are used as indicators for Phytobenthos. In order to obtain a representative distribution, about 500 valves are determined in a prepared slide to the species level. The frequencies are presented as percentages.

Summary

For the German method several transects are assessed separately. The lake (waterbody) assessment is calculated as the mean of transect results.

<u>Metrics:</u>

<u>Trophic-Index ($TI_{Nord(North)}$):</u> diatom index related to trophic status according to Schönfelder et al. (unpublished, but complete list of indicator values and formulas cited in Schaumburg et al. (2007)

http://www.lfu.bayern.de/wasser/gewaesserqualitaet_seen/phylib_deutsch/publikationen/doc/bundesweiter_test_mppb_seen.pdf

Quotient of Reference Species (ReferenzArtenQuotient, RAQ): number of the diatom species of two different ecological species groups (reference indicators (A) and degradation indicators (C))

How are these indicators monitored?

Sampling strategy

Type-specific substrate, preferably stones are sampled in their original position and the periphyton (Aufwuchs) or sediment cover is scratched off with a tea spoon, spatula or a similar device and is transferred into a labeled wide neck sampling container. Generally, sampling is carried out in the open water and not amidst dense stands of macrophytes. The sampling depth should always exceed 0.30 m. Fluctuations of the water level must be kept in mind when scheduling sampling dates. If mainly sand or soft sediments are present, the upper millimetres are lifted off with a spoon or sediment tube corer or were exhausted by a pipette.

Numbers of samples per lake

According to lake size and shape, usage of shore and catchment area 4 to 30 transects (=sites) are investigated. Each transect covers a minimum of 20 m of homogeneous shoreline (=width).

At each transect approximately 5 stones or other bottom sediments are sampled.

When is monitored and with which frequency?

Samples are taken once in the middle of growing season i.e. summer.

Use of equipment

Samples are taken with a tea spoon, spatula, pipette, sediment tube corer or a similar device and transferred into a labeled wide neck sampling jar. Diatoms are preserved by adding ethanol.

Analysis of sample and level of determination

Samples are oxidized (KRAMMER & LANGE-BERTALOT (1986)). Determination with microscope (interference/phase contrast) with 1000- to 1200 fold magnification. A minimum number of 500 shells is determined in a prepared slide to the species level. “Diatomeen im Süßwasserbenthos von Mitteleuropa” of Hofmann et al. (2011) is used as standard determination literature. It can be completed by the volumes of the “Diatoms of Europe”, 4 volumes of KRAMMER & LANGE-BERTALOT (1986–1991), supplementary volumes and revisions of individual species published since 1993 by the following authors: KRAMMER (2000, 2002), LANGE-BERTALOT (1993, 2001), LANGE-BERTALOT & MOSER (1994), LANGE-BERTALOT & METZELTIN (1996).

Assessment

Data requirements

The data of a sample should include

- a list of benthic taxa, determined at species and variety level, percentage values of each taxon, based on minimum 500 counted valves (or closed frustules)
- a list of additional rare benthic species in the sample, found during extra checking the slide after counting the mimimum fo 500 valves.

A software tool for the automatically calculation of the German assessment is available. The tool accepts names or numeric codes of the taxa and percentage values. The following information is needed for correct assessment: lake type according to LAWA, German diatom lake subtype (for phytobenthos assessment), natural/ artificial/ HMWB, changes in water level, for each taxon: abundance (percentage).

Methods of calculation

Trophic index

The indicative species of the trophic index (Annex B) which were found at the littoral site to be assessed and their percentages are the basis for calculating the Trophic Index according to Schönfelder et al. (unpublished) (Equation 1).

Table A.3 Value of the $TI_{Nord(North)}$ at the transition „high" – „good" (PHYLIB version 2.6, as intercalibrated)

Diatom lake type	Transition H/G $TI_{Nord(North)}$	Intercalibration lake type
13.1	1.74	L-CB 1
13.2	2.24	L-CB 1
10.1	2.24	L-CB 1
10.2*	2.74*	
14*	2.24*	
11	2.49	L-CB 2
12*	2.99*	

* subtype was not included in IC-Excercise because not fitting to IC-Type

Equation 1: Trophic-Index according to Schönfelder et al. (unpublished) $TI_{Nord(North)}$

$$TI_{Nord} = \frac{\sum_{i=1}^{n} \sqrt{H_i} * T_i}{\sum_{i=1}^{n} \sqrt{H_i}}$$

$TI_{Nord(North)}$	=	Trophic-Index Nord(North)
H_i	=	Percentage of the i-th species
T_i	=	Trophic value of the i-th species

For the combination with the „Quotient of Reference Species (RAQ)" the calculated values of the „Trophic-Index (TI)" are transformed according to the following equation 2.

Equation 2: Transformation of the calculated trophic value $TI_{Nord(North)}$ (modified according to Schönfelder 2006, unpublished)

$$M_{TI_{Nord}} = 0{,}8 - 0{,}8 * ((TI_{Nord} - TI_{Nord_{H/G}}) / 2{,}00)$$

MTI_{Nord}	=	Module Trophic-Index $_{Nord(North)}$
0.8	=	Module value for transition H/G"
TI_{Nord}	=	calculated Trophic-Index$_{Nord(North)}$

TI_{Nord} H/G	=	Value $TI_{Nord(North)}$ of the transition H/G (Table A.7)
2.00	=	Scale width between classes „high“ and „good“and the type specific worst Trophic-IndexNord with the module value 0,00 (at the lower class limit of the ecological status class “poor”)

If module values calculated with Equation 4 are greater than 1, the result is set to be 1. For values smaller than 0, the value is set to be 0.

Phytobenthos: „Quotient of Reference Species“ (ReferenzArtenQuotient, RAQ)

The type specific occurrence in different ecological conditions is used to distinguish two different species groups (compare Annex C).

For assessment the quotient of reference species is determined under consideration of the type specific reference species and their ecological groups. Only the number of species is considered whereas the abundance of the individual species is neglected (compare Equation 3).

Equation 3: Calculation of the Quotient of Reference Species for the lakes of the North German Lowland

$$RAQ = \frac{Number\,of\ taxa\ A - Number\,of\ taxa\ C}{Number\,of\ taxa\ A + Number\,of\ taxa\ C}$$

The RAQ-values are transformed according to equation 6.

Equation 6: Transformation of the type specifically calculated quotient of reference species

$$M_{RAQ} = (RAQ + 1) * 0{,}5$$

M_{RAQ}	=	Module Quotient of Reference Species
RAQ	=	calculated Quotient of Reference Species

The overall assessment of the component Phytobenthos-Diatoms is carried out by a combination of the modules „Trophic-Index (TI)“ and „Quotient of Reference Species (RAQ)“. For this purpose the arithmetic mean of the results is determined to obtain the Diatom- $Index_{Seen}$ ($DI_{Seen(Lakes)}$) following Equation 7.

Equation 7: Calculation of the $DI_{Seen(Lakes)}$

$$DI_{Seen} = \frac{M_{RAQ} + M_{TI}}{2}$$

DI_{Seen}	=	Diatom-$Index_{Seen(Lakes)}$
M_{RAQ}	=	Module Quotient of Reference Species
M_{TI}	=	Module Trophic-Index

Example:

A site within a lake of national type DS 10.1 (L-CB 1) with a calculated $TI_{Nord(North)}$ = 3.00 leads to a transformed $M_{TI\ Nord(North)}$= 0.8 – 0.8 * ((3.00-2.24)/2) = 0.496 in the middle of the range of “moderate” status.

In the same sample from this site were 4 sensitive reference taxa recorded (“taxa A”) and 8 pressure indicative taxa (“taxa C”). The RAQ is determined as RAQ = (4 – 8) / (4 + 8) = -0.33, also in the range of “moderate” status; transformed into M_{RAQ}=(-0.33+1)*0.5 = 0.335.

→ $DI_{Seen(Lakes)}$= (0.496+0.335)/2= 0.4155, in the range of “moderate” status.

According to lake types, the $DI_{Seen(Lakes)}$-values are assigned to ecological quality classes. Table A.4 gives an example for lakes of LCB 2.

The entire lake assessment is derived from the mean of the ecological status classes of the transects.

Table A.4 Index limits for classification of the ecological status: stratified lakes of the North German Lowland, type 10 according to Mathes et al. (2002)

Mathes et al. (2002)	**Typ 10**					
Diatoms	**DS 10.1**			**DS 10.2**		
Ecological status class						
1	1,00	-	0,78	1,00	-	0,78
2	< 0,78	-	0,55	< 0,78	-	0,55
3	< 0,55	-	0,33	< 0,55	-	0,33
4	< 0,33	-	0,10	< 0,33	-	0,10
5	< 0,10	-	0,00	< 0,10	-	0,00

How are reference conditions, H/G and G/M boundaries derived?

The reference of the intercalibrated German lake types and subtypes was based on (few) existing true reference sites, sampled in 2003 - 2005 during collection of the German calibration data set. Additionally the reference conditions of the intercalibrated lake types and subtypes are validated by data from 128 reference sites in 20 reference lakes, sampled in 2007 and 2008 during the first monitoring cycle of the federal states of Mecklenburg-Vorpommern and Brandenburg.

Only sites with no (100% woodland and peatland in the catchment area) or very minor (>90% woodland and extensive used meadows in the catchment area, UNESCO conservation status “National Park”) human impacts were used. Information from historical diatom samples and sediment core investigations was included in the selection of reference sites. Only sites showing nearly undisturbed physico-chemical (e.g. pH, salinity, saprobic and trophic status), hydromorphological and biological conditions were chosen.

How well correlate the indicators with pressure indicators?

The German assessment metrics are correlating quite well with the eutrophication related parameter TP. Figure A.4 show examples for the correlation of the diatom assessment with TP.

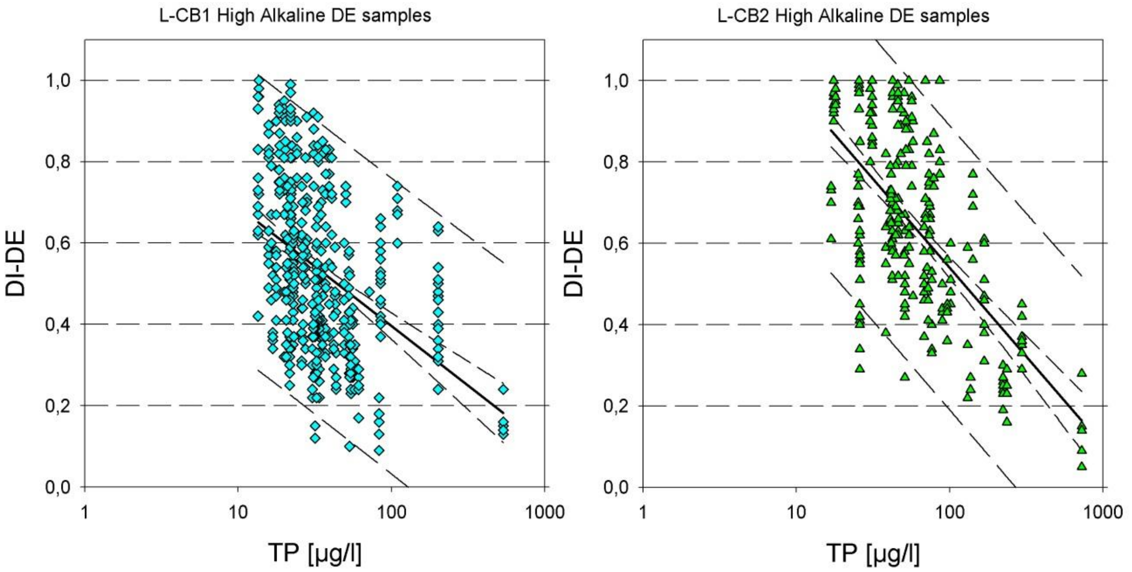

Figure A.4 Correlation between German EQR for diatom assessment and TP concentration in German lakes.

The wider scatter of the German Diatom Index DI-DE plotted against TP for L-CB1 lakes is caused by merging together three national different lake types with three different reference ranges of TP, all showed in one plot.

Type specific plots for the German metrics and the ICM TI developed by Rott al al. (1999) vs. TP are given below for the German types, summarized in L-CB 1 for the purpose of intercalibration

How is dealt with differences between national data and assessment vs. GIG data and assessment?

Completeness of method

The German assessments were slightly more stringent for L-CB1 and slightly more relaxed for L-CB2, but were in the range (band) of accepted deviation. The German diatom method was not completely finished for all the German lake subtypes at the end of collating the intercalibration data set (May 2011). Therefore Germany did not contribute samples and assessments for the national subtype DS 10.2, which can formally be placed in L-CB1. The reasons for leaving data from DS 10.2 out were

- the boundary of residence time or catchment size-volume-quotient for subtype DS 10.2 against DS 10.1 is unclear;
- the indicator species list for the RAQ was not practically tested and should be completed in the next years;
- lakes of DS 10.2 have very small residence times, not comparable with those of the cross-european poulation of L-CB1.

Lake subtype DS 10.2 is assessed one class more relaxed when compared with DS 10.1. So relative (in comparison with other European MS) stringent assessments of Germany for the intercalibrated national types DS 13.1, DS 13.2 and DS 10.1 within L-CB1 was expected by us.

The German assessments for its lakes in L-CB2 appeared relatively relaxed. One reason is, that the PHYLIB method for national type 14, partly mergable into L-CB2, was not finished and Germany was unable to contribute official assessments of samples and sites in lake type 14. Recently PHYLIB 4.1 proposed a method for national type 14. This has to be tested and revised in 2012 and will assess lakes of type 14 half a class more stringent, compared with national type 11 in the same IC type L-CB2. So the results of the intercalibration will influence the finishing of development of the national method, not influencing the results contributed to IC, because only small "extra" groups of lakes in Germany are affected.

Data transformation to GIG data base

Species and environment data are reported in sheets, prepared by the IC leader (Martyn Kelly). Species data have been coded using the OMNIDIA codes. Percentage values were not transformed, only reported. Metrics were scaled to IC scale by the formulas provided by the diatom IC leader.

Assessment transformation to the GIG data base

The assessment results were transformed as in Table A.5.

Table A.5 Assement transformation to the GIG data base

PHYLIB Assessment	Status class	Reported as
1	High	H
2	Good	G
3	Moderate	M
4	Poor	P
5	Bad	B

Effects on final results

Transformations on national methodology

The national method PHYLIB was changed after finishing the report, adopting the final results presented in the report, as follows:

Subtype DS 13.1 (part of L-CB 1). The EQR for the H/G boundary was lowered from 0.83 to 0.78. It was a harmonization within the German method, the EQR for the H/G boundaries are now the same for all German types and subtypes. Lakes of DS 13.1 will be assessed slightly more relaxed, taking into account, that lakes of L-CB1 are reported to be assessed slightly too stringent.

Type DS 14 (part of L-CB 2, but data not provided for IC). The new introduced type DS 14 will be assessed half a class more stringent as type 11, taking into account, that the German assessments of german L-CB2 are reported to be assessed slightly too relaxed.

River Metrics in German Lake Assessment method

In the German Lake method no river metrics are used. The Diatom Indices RAQ, TI-Nord and TI-Süd were especially developed for lakes. The use of Rott-Index as a common IC-metric is a matter of the IC-GIG and should be explained by the GIG lead.

Refrences

Schaumburg, J., Schmedtje, U., Schranz, C., Köpf, B., Schneider, S., Meilinger, P., Stelzer, D., Hofmann, G., Gutowski, A., Foerster, J. (2004) Erarbeitung eines ökologischen Erarbeitung eines ökologischen Bewertungsverfahrens für Fließgewässer und Seen im Teilbereich Makrophyten und Phytobenthos zur Umsetzung der EU-Wasserrahmenrichtlinie. Bayerisches Landesamt für Wasserwirtschaft, Abschlußbericht an das Bundesministerium für Bildung und Forschung (FKZ 0330033) und die Länderarbeitsgemeinschaft Wasser (Projekt Nr. O 11.03), 635 S., München.

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A.4 Hungary

Hungarian phytobenthos methods for lakes

Sampling

When choosing sampling time /sampling site/ substrate, the under mentioned viewpoints should be considered:

- The most appropriate period for sampling diatoms in lakes is the middle of June-early July. In the case of sample series, it is recommended to collect samples in 3 weeks, in order to get comparable results.
- In the case of lakes with outflows, it is recommended to sample a site near to the outflow, where flow rate is near zero, and sunshine is enough for biofilm development (northwest, north, northeast).
- In the case of lakes without outflows, samples should be collected at the site which is the most exposed to sunshine (northwest, north, northeast).
- Due to its frequent occurence, reed (*Phragmites australis*), other macrophytes (*Scirpus lacustris*, *Typha latifolium* and *T. angustifolium, Sparganium)* or the small-leaved *Myriophyllum*, *Ceratophyllum* species are also appropriate substrates. We can get undistorted results if we collect samples at the same site. Regarding literature (King et al 2006), it is suggested to choose the substrate that is the most characteristic for the littoral region of the lake. In Hungary, in the case of numerous lakes, green reed stems worth to be favoured as sampling substrate, as reed is characteristic substrate of the littoral region, and it provides a fresh biofilm which dispense frustules from the previous years, and rarely contains epipelic/planctonic species. It is important to collect mature biofilm (thus reed should be at least 6 weeks old).
- Samples should be collected from 10-30 cm deep, in 5 replicates, from randomly chosen substrates. In the case of reed, samples are collected from the open water-side of the reed.

The best situation is if our sampling site is in connection with the open-water region, thus it is suggested to collect samples from the open-water side of the reed-belt. We should collect those stems that were covered with water permanently (as well in the last months before the sampling)

Evaluation

Investigations of the correlation between diatom indices and chemical properties have pointed out, that we can get better correlations between chemical parameters and diatom indices if we compose multimetric indices. The MIL (Multimetric Index for Lakes) can be calculated from 3 indices: MIL= ($TDIL_{(1-20}$+IBD+EPI-D)/3. The IBD and the EPI-D can be calculated with OMNIDIA, and their value varies between 1-20. The TDIL can be calculated with a special self-developed utility (DILSTORE, Hajnal et al. 2009), and its value varies between 1-5. In order to calculate MIL, TDIL values should be corrected with the following equation:

$TDIL_{(1-20)}$: a = 3,8 * b + 1

We used different index in case of Lake Balaton: MIB (Multimetric Index for Balaton) which is the mean of the indices IBD and $TDIL_{(1-20)}$.

EQR = MIL/MIL max

EQR = MIB/MIB max

A new index was worked out concerning conductivity as the main driver of sodic lakes and we used this index in case of Type 8: SCIL, which can be calculated with a special self-developed utility (DILSTORE, Hajnal et al. 2009).

In the first step, the optimum and tolerance values of algae were calculated by weighted averaging method in terms of conductivity. The results of both analyses were examined: both in which only diatoms were involved and in which all other algae as well that occurred in the epiphyton.

Based on the obtained optimum and tolerance values, the sensitivity of the species (s) were determined on a scale ranging from 1 to 5 (where 1 meant species that preferred waters with low conductivities), and based on the tolerances of the species, the indicative values of the species (v) in terms of conductivity was also given (where 1 meant the sensitive species).

After this, the initial value of the soda index ($SCIL_V$ = initial value of Soda Conductivity Index for Lakes) that can be calculated by the formula of Zelinka & Marvan (1961) is the following:

$$= \frac{\sum_{i=1}^{n} a_i s_i v_i}{\sum_{i=1}^{n} a_i v_i} \qquad \mathbf{SCIL_V}$$

Where:

a_i = relative abundance of the species.

v_i = the indicator value of the given species.

s_i = the sensitivity of the given species to conductivity.

Since the value of SCIL ranges between 1 (the worst) and 5 (the best), so that the value of the index can be comparable with the diatom indices calculated by OMNIDIA, the OMNIDIA water quality rate (Y/20) is calculated by the following equation:

SCIL = 3,8 $SCIL_V$ + 1

In this way, the value of SCIL will be between 1 (the worst) and 20 (the best), in which the boundaries are:

- Excellent 20-17;
- Good 16.9-13;
- Moderate 12.9-9;
- Poor – 8.9-5;
- Bad < 4.9.

By the use of the values of the index, EQR based qualification can be given in the following way:

EQR = SCIL/$SCIL_{max.}$ Therefore, the boundary of the excellent and good ecological condition is

17/20 = 0.85. The boundary of good and medium condition is 13/20 = 0.65

Boundary setting

The highest median values belong to type 12 and type 16 (Figure A.5). The highest class limits are recommended for these groups (Table A.6). The medians of type 7 and type 14 are already lower; the difference between the medians of type 12 and type 16 is one, thus the limit was decreased by one. The same procedure was followed with the 1 and 2 artificial types. The lowest limit was determined at type 13. The limit between tolerable and bad (P/B) was decreased only once from 4, 8 to 3, 8, and this was also applied for all the naturally loaded stagnant water types. In types 6 and 8 the results of the lake Velencei monitoring was considered. The limits of the indices were determined by dividing the values between the potential maxima (20) and minima (1) to five equal portions, and the above mentioned corrections (decreasing the index values) were applied in the given types.

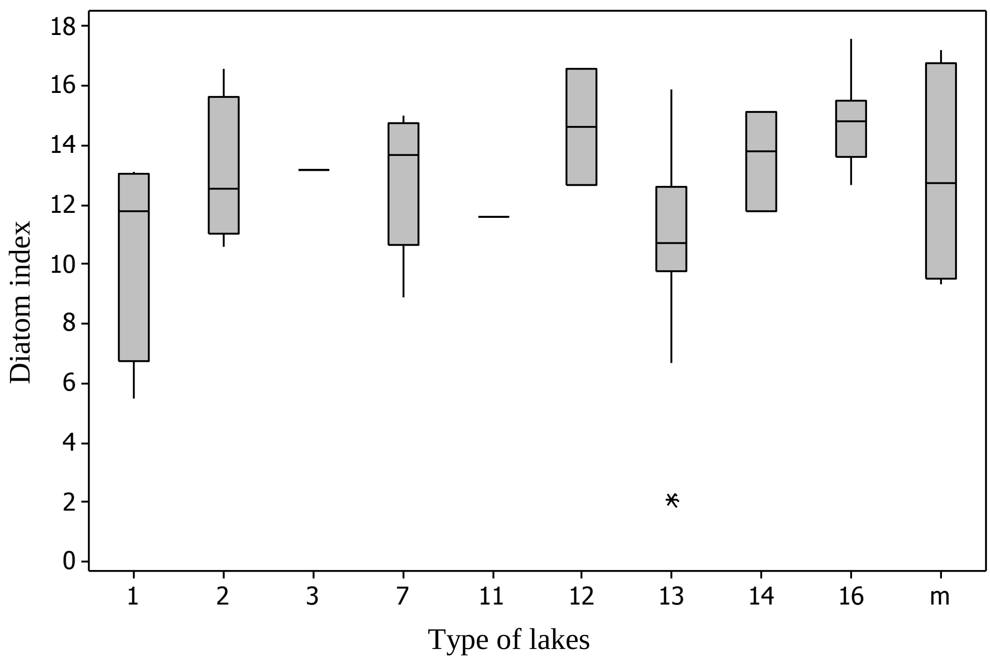

Figure A.5 The box-plot of diatom indices in the different Hungarian lake types (m = artificial).

Table A.6 Boundaries of indices end EQR in different lake types.

Type	Index	index H/G	index G/M	index M/P	index P/B	EQR H/G	EQR G/M	EQR M/P	EQR P/B
1	MIL	14.2	10.4	6.6	3.8	0.71	0.52	0.33	0.19
2	MIL	14.2	10.4	6.6	3.8	0.71	0.52	0.33	0.19
6	IBD	16.2	12.4	8.6	3.8	0.81	0.62	0.43	0.19
7	MIL	15.2	11.4	7.6	3.8	0.76	0.57	0.38	0.19
8	(IBD+ SCIL)/2	16.2	12.4	8.6	3.8	0.81	0.62	0.43	0.19
12	MIL	16.2	12.4	8.6	4.8	0.81	0.62	0.43	0.24
13	MIL	13.2	9.4	5.6	3.8	0.66	0.47	0.28	0.19
14	MIL	15.2	11.4	7.6	3.8	0.76	0.57	0.38	0.19
16	MIB	16.2	12.4	8.6	3.8	0.81	0.62	0.43	0.19
m	MIL	14.2	10.4	6.6	3.8	0.71	0.52	0.33	0.19

Reference conditions

We have not found reference sites.

Literature

Hajnal, É., Stenger–Kovács, Cs., **Ács, É.,** Padisák, J. (2009): DILSTORE software for ecological status assessment of lakes based on benthic diatoms. – Fottea 9(2): 351–354.

A.5 Ireland: Assessment of lakes in Ireland using phytobenthos - Lake Trophic Diatom Index (IE)

Introduction

Phytobenthos is one component of the biological quality element “macrophytes and phytobenthos” to be assessed in lakes in order to comply with the objectives of the WFD. Benthic diatoms and macroscopically visible filamentous algae are two separately monitored components of the phytobenthos, with the latter incorporated into the national lake macrophyte monitoring tool (Free Index) and with diatoms assessed separately using the Lake Trophic Diatom Index (LTDI) developed by agencies in the UK for application in Ecoregion 17 and 18. Sampling, processing and analysis are carried out in conformance with CEN guidance (2003a, 2004) and Kelly et al., 2008. This document provides a summary explanation of the general methodology and application of the method and is based on information given in Kelly et al., 2008 and WFD – UKTAG, 2008.

Sampling

Diatoms are sampled from approximately 0.25 metres to wadeable depth along the lake littoral, from cobble and boulder substrate when present (rarely from large gravel or the stems of emergent macrophytes from the same habitat, when the preferable substrate is lacking). Artificial substrate is not utilised. A phytobenthos sample is obtained from two seasons (April & July/August) every three years, by brushing the epilithon into a tray with a toothbrush and fixing with 0.5 ml of a non-acidified Lugol's iodine solution. The number of individual site samples required per lake is based on a lake area categorisation; < 500 hectares = 1 site required, 500 – 2000 hectares = 2 sites required, or >2000 hectares = 3 sites required. For lakes with multiple sites - the average spring and summer site specific LTDI values are calculated, with the overall lake status being then reported as an average of the individual site values.

Processing & Identification

The sample is digested in strong acids with potassium permanganate and dilute suspensions of the cleaned valves are mounted in Naphrax for identification and counting under x1000 magnification using phase contrast. The standard European diatom floras are used for identification. At least 300 non-planktonic and relatively intact frustules are enumerated.

Lake Trophic Diatom Index Calculation

The method has been designed to detect the impact of nutrient enrichment on the quality element. Each taxon listed in Column 1 of Table 2 and identified as present in the lake sample should be assigned the corresponding nutrient sensitivity score in Column 2 of Table 2.

The observed value of the parameter is then given by the equation:

Observed value of lake trophic diatom index : (W x 25) – 25

Where W is given by equation:

$$W = \frac{\sum_{j=1}^{n} a_j \times s_j}{\sum_{j=1}^{n} a_j}$$

"aj" is the number of valves of taxon j, where "j" represents a taxon listed in Column 1 of Table A.8 and present in the sample;

"j" has a value of 1 to "n" indicating which of the all the taxa (total number = "n") listed in Column 1 and present in the sample it represents;

"sj" is the nutrient sensitivity score in column 2 of Table A.8 corresponding to the taxon in column 1 of that Table represented by j.

Calculation of the EQR

Status is reported on an EQR scale from 0 (bad) to 1 (high) status by the equation:

EQR = (100 – observed value LTDI) / (100 – expected value LTDI).

A reference screening procedure combining information from percentage catchment landuse activity (CORINE), physiochemical data and paleolimnology was carried out for each potential reference lake. After careful screening, no moderate alkalinity lakes considered acceptable as reference status were identifiable. Due to the relatively low number of reference lake examples at high alkalinity, and the concordance of those metric values available with that found at low alkalinity, the expected value for all Irish lake types was combined at LTDI = 20, which is approximately the 90th percentile (0.92) of samples from the reference lake network.

The EQR values of the status class boundaries for the lake types are given in Table A.7. The high/good boundary was placed at the index value of LTDI = 28. A detailed rationale for the location of moderate, poor and bad values is given in Kelly et al. (2008) but in summary; the good/moderate boundary was placed at the “crossover” between sensitive and tolerant taxa, while the moderate/poor and poor/bad are arithmetical divisions of the remaining EQR scale.

Table A.7 EQR values for the status class boundaries utilised in Irish Lakes

	H/G	G/M	M/P	P/B
LA	0.9	0.63	0.44	0.22
MA	0.9	0.63	0.42	0.21
HA	0.9	0.63	0.42	0.21

Application of the method

The index is designed to classify the benthic diatom community into 5 status classes along the trophic gradient. Status is assigned using both the macrophyte and diatom metrics on a one-out-all-out basis, i.e. the EQR for both tools is calculated separately and the lowest value is used to assign status for the entire quality element. The method has been found to have a good relationship along the phosphorus gradient at high alkalinity (see Figure A.7). The relationship with phosphorus at moderate and low alkalinity is linear but has lower regression significance, although the gradient length is also substantially shorter in both instances. There is evidence for a confounding effect of acidification at low alkalinity.

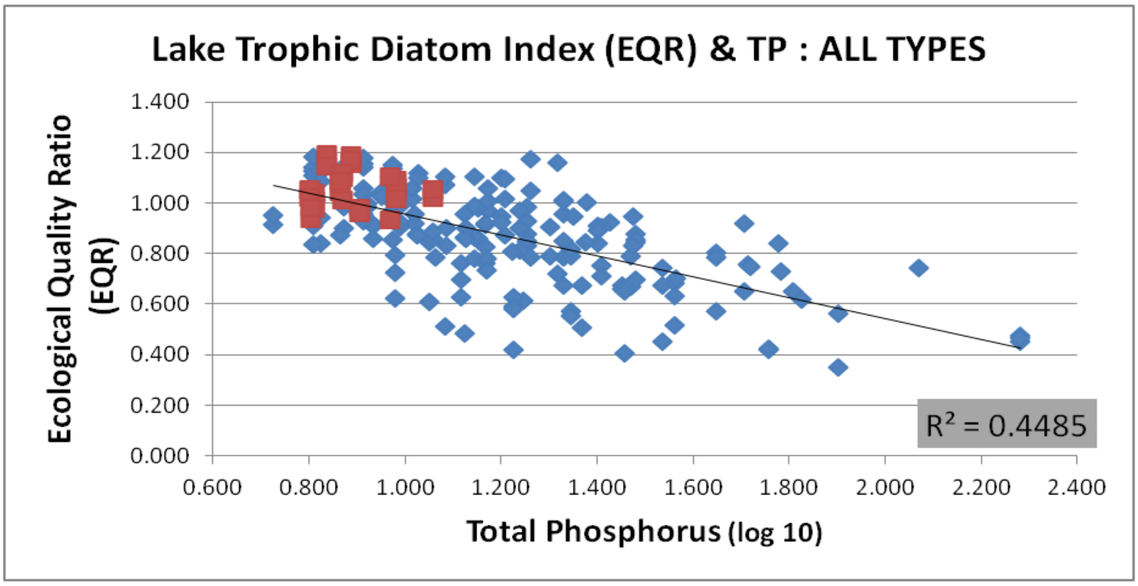

Figure A. 6 The relationship between the LTDI (expressed as an EQR) and total phosphorus, with reference samples indicated in red.

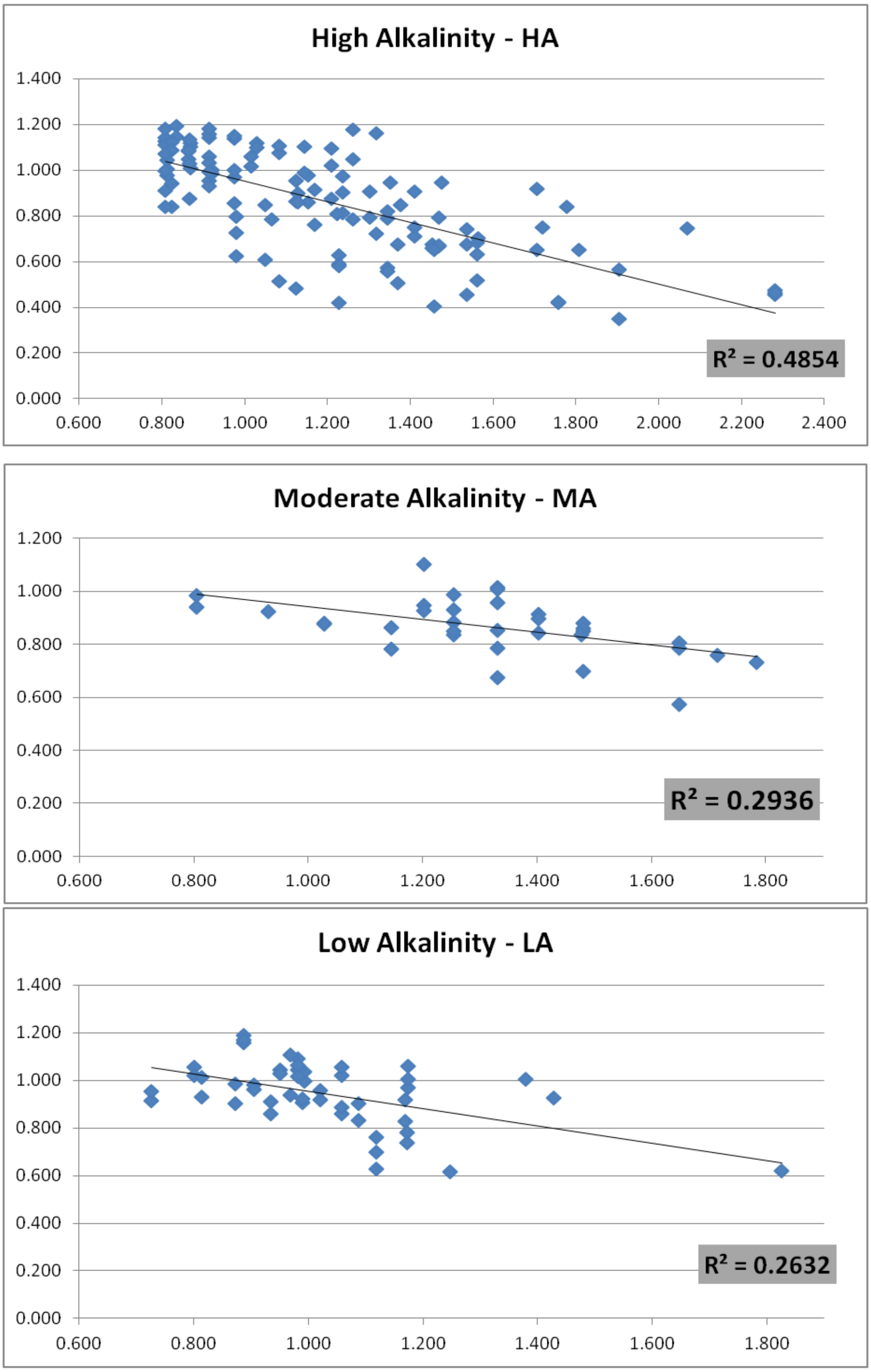

Figure A.7 The relationship between the LTDI (expressed as an EQR) and total phosphorus by lake alkalinity type.

Table A.8 List of diatom taxa and associated nutrient sensitivity scores for the purposes of calculating the value of the parameter, lake trophic diatom index

Column 1	Column 2
Diatom taxa	**Nutrient sensitivity score**
Achnanthes calcar Cleve	3
Achnanthes carissima Lange-Bertalot	5
Achnanthes coarctata (Breb. in W. Sm.) Grun. in Cleve & Grun.	3
Achnanthes conspicua A. Mayer	4
Achnanthes curtissima J.R. Carter	3
Achnanthes exigua Grun. in Cleve & Grun.	4
Achnanthes frigida Hust. in A. Schmidt	3
Achnanthes joursacense Herib.	3
Achnanthes kriegeri Krasske	3
Achnanthes kryophila J.B. Petersen	3
Achnanthes laevis Ostr.	2
Achnanthes minuscula Hust.	5
Achnanthes oblongella Ostr.	2
Achnanthes oestrupii (A. Cleve-Euler) Hust.	3
Achnanthes pseudoswazi J.R. Carter	1
Achnanthes ricula Hohn & Hellerman 1963	5
Achnanthes rosenstockii Lange-Bertalot 1989	5
Achnanthes saccula J.R. Carter in J.R. Carter & Watts	3
Achnanthes silvahercynia Lange-Bertalot 1989	2
Achnanthes sp. Bory	4
Achnanthes straubiana Lamge-Bertalot	1
Achnanthes suchlandtii Hust.	4
Achnanthes ventralis (Krasske) Lange-Bertalot	1
Achnanthes ziegleri Lange-Bertalot 1991	2
Achnanthidium biasolettiana (Grunow) L. Bukhtiyarova	4
Achnanthidium minutissimum (Kütz.) Czarnecki 1994	2
Amphipleura kriegerana (Krasske) Hust.	1
Amphipleura pellucida (Kutz.) Kutz.	1
Amphipleura sp. (Grunow) L. Bukhtiyarova	1
Amphora delicatissima Krasske ex Hust.	5
Amphora dusenii Brun	3
Amphora fogediana Krammer	4
Amphora inariensis Krammer	4
Amphora libyca Ehr.	4
Amphora ovalis (Kutz.) Kutz.	4
Amphora pediculus (Kutz.) Grun.	4
Amphora sp. Ehrenb. ex. Kütz.	5
Amphora veneta Kutz.	5
Aneumastus tuscula (Ehrenb.) Mann & Stickle	1
Anomoeoneis follis (Ehrenb.) Cleve	1
Aulacoseira subarctica (O.Mull.) Haworth	2
Brachysira brebissonii fo. brebissonii R. Ross in Hartley	1
Brachysira neoexilis Lange-Bertalot	1
Brachysira procera L-B & Moser	2
Brachysira serians (Breb. ex Kutz.) Round & Mann	1
Brachysira sp. Kutz.	1
Brachysira styriaca (Grun. in Van Heurck) R. Ross in Hartley	1
Brachysira vitrea (Grun.) R. Ross in Hartley	1
Caloneis bacillum (Grun.) Cleve	4
Caloneis silicula (Ehrenb.) Cleve	2
Caloneis sp. Cleve	2
Cavinula cocconeiformis (Greg. ex Greville) Mann & Stickle	3
Cavinula variostriata (Krasske) Mann	3

Column 1	Column 2
Diatom taxa	**Nutrient sensitivity score**
Cocconeis disculus (Schum.) Cleve	3
Cocconeis neothumensis Krammer	3
Cocconeis pediculus Ehrenb.	4
Cocconeis placentula Ehrenb.	3
Cocconeis pseudothumensis Reichardt 1982	3
Craticula accomoda (Hust) Mann	5
Craticula halophila (Grun. ex Heurck) Mann	4
Ctenophora pulchella (Ralfs ex Kutz.) Williams & Round	3
Cymbella aequalis W. Sm. ex Grev.	1
Cymbella affinis Kutz.	1
Cymbella aspera (Ehrenb.) H. Perag. in Pell.	1
Cymbella brehmii Hust.	3
Cymbella cesatii (Rabenh.) Grun. in A. Schmidt	1
Cymbella cistula (Ehrenb. in Hempr. & Ehrenb.) Kirchner	2
Cymbella cuspidata Kutz.	4
Cymbella cymbiformis Ag.	1
Cymbella delicatula Kutz.	1
Cymbella descripta (Hust.) Krammer & Lange-Bertalot	1
Cymbella gaeumannii Meister	2
Cymbella helvetica Kutz.	2
Cymbella hustedtii Krasske	4
Cymbella incerta Grun. in Cleve & Moller	2
Cymbella lacustris (Ag.) Cleve	3
Cymbella lanceolata (Ag.) Ag.	2
Cymbella lapponica Grun. ex Cleve	1
Cymbella leptoceras (Ehr.) Grun.	2
Cymbella leptoceros var. angusta Grun.	4
Cymbella microcephala fo. microcephala Grun. in Van Heurck	1
Cymbella naviculiformis Auersw. ex Heib.	2
Cymbella perpusilla A. Cleve	2
Cymbella pusilla Grun. ex A. Schmidt	1
Cymbella reinhardtii Grun. ex A. Schmidt	5
Cymbella sp. Ag.	2
Cymbella subaequalis Grun. in Van Heurck	4
Cymbella turgidula Grun.	3
Cymbellonitzschia diluviana Hust.	4
Denticula kuetzingii Grun.	4
Denticula tenuis Kutz.	1
Diadesmis contenta (Grun. ex Van Heurck) Mann	3
Diatoma mesodon (Ehrenber) Kutzing	1
Diatoma moniliformis Kutz	1
Diatoma tenue Ag.	2
Diatoma vulgare Bory	4
Diploneis elliptica (Kutz.) Cleve	3
Diploneis marginestriata Hust.	3
Diploneis oblongella (Naegeli ex Kutz.) R. Ross	3
Diploneis ovalis (Hilse) Cleve	3
Diploneis parma Cleve	4
Diploneis sp. Ehrenberg	1
Ellerbeckia arenaria (Moore) Crawford	5
Encyonema caespitosum Kutz.	3
Encyonema gracile Ehrenberg	2
Encyonema hebridicum Grun. ex Cleve	1

Column 1	Column 2
Diatom taxa	**Nutrient sensitivity score**
Encyonema minutum (Hilse in Rabenhorst) Mann	4
Encyonema reichardtii (Krammer) Mann	4
Encyonema silesiacum (Bleisch in Rabenhorst) Mann	3
Epithemia adnata (Kutz.) Rabenh.	2
Epithemia argus (Ehrenb.) Kutz.	1
Epithemia sorex Kütz.	3
Epithemia sp. Bréb.	3
Eucocconeis flexella Kütz.	2
Eunotia arculus (Grunow) Lange-Bert et Nörpel	1
Eunotia arcus Ehrenb.	1
Eunotia bidentula W. Sm.	1
Eunotia bilunaris (Ehrenb.) F.W. Mills	3
Eunotia diodon Ehrenb.	1
Eunotia elegans Ostr.	1
Eunotia exigua (Breb. ex Kutz.) Rabenh.	1
Eunotia faba (Ehrenb.) Grun. in Van Heurck	1
Eunotia fallax A. Cleve	1
Eunotia flexuosa Kutz.	1
Eunotia formica Ehrenb.	2
Eunotia glacialis Meister	1
Eunotia implicata Norpel, Lange-Bertalot & Alles	1
Eunotia incisa W. Sm. ex Greg.	2
Eunotia intermedia (Hust) Norpel, Lange-Bertalot & Alles	1
Eunotia meisteri Hust.	1
Eunotia microcephala Krasske ex Hust.	1
Eunotia minor (Kutz) Grunow in Van Heurck	4
Eunotia monodon var. bidens (W. Sm.) Hust.	1
Eunotia muscicola Krasske	1
Eunotia muscicola var. tridentula Norpel & Lange-Bertalot 1991	2
Eunotia naegelii Migula	1
Eunotia paludosa Grun.	1
Eunotia paludosa var. trinacria (Krasske) Norpel 1991	4
Eunotia pectinalis (O.F. Mull.) Rabenh.	1
Eunotia pirla Carter et Flower	1
Eunotia praerupta Ehrenb.	2
Eunotia rhomboidea Hust.	1
Eunotia rhyncocephela Hustedt	1
Eunotia serra Ehrenb.	1
Eunotia serra var. diadema (Ehrenb.) Patr.	1
Eunotia soleirolii (Kutz) Rabenhorst	1
Eunotia sp. Ehrenb	2
Eunotia subarcuatoides Alles, Norpel, Lange-Bertalot	2
Eunotia sudetica O. Mull.	1
Eunotia tenella (Grun. in Van Heurck) A. Cleve	2
Fragilaria bidens Heib.	4
Fragilaria capucina Desm.	1
Fragilaria capucina var. amphicephala Grun) Lange-Bert.	1
Fragilaria capucina var. austriaca (Grun) Lange-Bertalot	4
Fragilaria capucina var. distans (Grunow) Lange-Bertalot	3
Fragilaria capucina var. mesolepta (Rabenh.) Rabenh.	3
Fragilaria capucina var. rumpens (Kutz.) Lange-Bertalot	2
Fragilaria construens var. exigua (W. Sm.) Schulz	1
Fragilaria construens var. pumila Grun. in Van Heurck	2

Column 1	Column 2
Diatom taxa	**Nutrient sensitivity score**
Fragilaria incognita Reichardt 1988	1
Fragilaria karelica Molder	2
Fragilaria lapponica Grun. in Van Heurck	2
Fragilaria nitzschioides Grun. in Van Heurck	2
Fragilaria perminuta (Grunow) Lange-Bert.	3
Fragilaria pseudoconstruens Marciniak	3
Fragilaria sp. H.C. Lyngb.	4
Fragilaria vaucheriae (Kutz.) J.B. Petersen	4
Fragilaria vaucheriae var. capitellata (Grun. in Van Heurck) R. Ro	2
Fragilariforma virescens (Ralfs) Williams & Round	3
Fragilariforma virescens var. exigua (Grunow) Poulin	3
Frustulia rhomboides (Ehrenb.) De Toni	1
Gomphonema acuminatum Ehrenb.	3
Gomphonema affine Kutz.	2
Gomphonema angustatum (Kutz.) Rabenh.	4
Gomphonema anoenum Lange-Bertalot	1
Gomphonema augur Ehr.	4
Gomphonema clavatum Ehr.	3
Gomphonema exiguum var. minutissimum Grun in Van Heurck	2
Gomphonema gracile Ehrenb.	2
Gomphonema hebridense Gregory	1
Gomphonema lateripunctatum Reichardt & Lange-Bertalot	1
Gomphonema minutum (Ag.) Ag.	3
Gomphonema olivaceoides Hust.	2
Gomphonema olivaceum (Hornemann) Breb.	5
Gomphonema parvulum (Kutz.) Kutz.	4
Gomphonema parvulum var. exilissimum Grun. in Van Heurck	3
Gomphonema procerum Reichardt & Lange-Bertalot	1
Gomphonema pseudoaugur Lange-Bertalot	1
Gomphonema pseudotenellum Lange Bertalot	3
Gomphonema sp. Ehrenb.	3
Gomphonema subtile Ehrenb.	1
Gomphonema tergestinum (Grun. in Van Heurck) Fricke in A. Sc	3
Gomphonema truncatum Ehrenb.	4
Gomphonema vibrio Ehrenb.	1
Gyrosigma acuminatum (Kutz.) Rabenh.	4
Gyrosigma attenuatum (Kutz.) Rabenh.	4
Hannaea arcus (Ehrenb.) Patr. in Patr. & Reimer	1
Karayevia clevei (Grunow) Round et L. Bukhtiyarova	4
Karayevia laterostrata (Hust.) Round et L. Bukhtiyarova	4
Lemnicola hungarica (Grunow) Round et P.W. Basson	3
Luticola mutica (Kutz.) Mann	5
Mastogloia smithii Thwaites ex W. Sm.	2
Mastogloia smithii var. amphicephala Grun. in Van Heurck	1
Mastogloia sp. Thwaites ex W. Sm.	1
Melosira varians Ag.	5
Meridion circulare (Grev.) Ag.	1
Navicula agrestis Hust.	5
Navicula angusta Grun.	5
Navicula aquaedurae Lange-Bertalot	1
Navicula arcus Ehrenb.	2
Navicula arvensis Hust.	1
Navicula atomus (Kutz.) Grun.	5

Column 1	Column 2
Diatom taxa	**Nutrient sensitivity score**
Navicula bryophila J.B. Petersen	3
Navicula capitata Ehrenb.	5
Navicula capitatoradiata Germain	4
Navicula cari Ehrenb.	4
Navicula caterva Hohn & Hellermann	2
Navicula cincta (Ehrenb.) Ralfs in Pritch.	3
Navicula cryptocephala Kutz.	4
Navicula cryptotenella Lange-Bertalot	5
Navicula cuspidata (Kutz.) Kutz.	4
Navicula decussis Ostr.	5
Navicula dicephala Ehrenb.	4
Navicula difficillima Hust.	3
Navicula digitoradiata var. digito-radiata (Greg.) Ralfs in Pritch.	4
Navicula gallica var. perpusilla (Grun) Lange-Bertalot	2
Navicula gastrum (Ehrenb.) Kutz.	3
Navicula graciloides A. Mayer	3
Navicula gregaria Donk.	5
Navicula hungarica Grun.	5
Navicula ignota var. acceptata (Hustedt) Lange-Bertalot	2
Navicula ignota var. palustris (Hust.) J.W.G. Lund	5
Navicula jaernefeltii Hust.	3
Navicula lanceolata (Agardh) Kutz.	4
Navicula leptostriata Jorgensen	2
Navicula libonensis Schoeman	4
Navicula mediocris Krasske	1
Navicula menisculus Schum.	5
Navicula mimima Grun. In Van Heurck	3
Navicula minuscula Grun. in Van Heurck	5
Navicula phyllepta Kutz.	2
Navicula placenta Ehrenb.	3
Navicula porifera var. opportuna (Hust.) Lange-Bertalot	2
Navicula pseudoanglica Lange-Bertalot	3
Navicula pseudolanceolata Lange-Bertalot	4
Navicula pseudoscutiformis Hust.	2
Navicula pseudotuscula Hust.	3
Navicula pygmaea Kutz.	3
Navicula radiosa Kutz.	2
Navicula radiosafallax Lange-Bertalot	3
Navicula reichardtiana Lange-Bertalot	5
Navicula reinhardtii Grun. in Van Heurck	5
Navicula rhynchocephala Kutz.	4
Navicula rotunda Hust.	5
Navicula salinarum Grun. in Cleve & Grun.	5
Navicula saprophila Lange-Bertalot & Bonik	4
Navicula saxophila Brock ex Hust	5
Navicula schoenfeldii Hust.	2
Navicula scutelloides W. Sm. ex Greg.	4
Navicula seminuloides Hust.	5
Navicula seminulum	4
Navicula slesvicensis Grun. in Van Heurck	5
Navicula soehrensis Krasske	1
Navicula soehrensis var. hassiaca (Krasske)Lange-Bertalot	1
Navicula sp. Bory	4

Column 1	Column 2
Diatom taxa	**Nutrient sensitivity score**
Navicula stroemii Hust.	4
Navicula subatomoides Hust. ex Patr.	5
Navicula subminuscula Manguin	5
Navicula submuralis Hust.	5
Navicula subrotundata Hust.	4
Navicula subtilissima Cleve	1
Navicula tenelloides Hust.	5
Navicula tenuicephala Hust.	1
Navicula tripunctata (O.F. Mull.) Bory	5
Navicula trivialis Lange-Bertalot	3
Navicula veneta Kutz.	5
Navicula vixvisibilis Hust.	3
Neidium affine (Ehrenb.) Pfitz.	1
Neidium ampliatum (Ehren) Krammer	1
Neidium bisulcatum (Lagerst.) Cleve	1
Neidium hercynicum A. Mayer	1
Neidium sp. Pfitzer	2
Nitzschia acicularis (Kutz.) W. Sm.	3
Nitzschia acidoclinata Lange Bertalot	2
Nitzschia amphibia Grun.	5
Nitzschia angustatula Lange-Bertalot	4
Nitzschia angustiforaminata Lange-Bertalot	5
Nitzschia archibaldii Lange-Bertalot	1
Nitzschia bacillum Hustedt in A.Schmidt et al	2
Nitzschia capitellata Hust.	5
Nitzschia commutata Grun. in Cleve & Grun.	4
Nitzschia dissipata (Kutz.) Grun.	5
Nitzschia flexa Schum.	1
Nitzschia fonticola Grun. in Van Heurck	4
Nitzschia frustulum (Kutz.) Grun. in Cleve & Grun.	5
Nitzschia gracilis Hantzsch	3
Nitzschia hantzschiana Rabenh.	3
Nitzschia heufleriana Grun.	2
Nitzschia incognita Legler & Krasske	1
Nitzschia inconspicua Grun.	5
Nitzschia intermedia Hantzsch ex Cleve & Grun.	1
Nitzschia lacuum Lange-Bertalot	3
Nitzschia linearis W. Sm.	3
Nitzschia microcephala Grun. in Cleve & Grun.	3
Nitzschia obtusa var. scalpelliformis Grun. in Van Heurck	3
Nitzschia palea (Kutz.) W. Sm.	4
Nitzschia paleacea (Grun. in Cleve & Grun.) Grun. in Van Heurck	4
Nitzschia paleaeformis Hust.	1
Nitzschia pumila Hust.	3
Nitzschia pura Hustedt	4
Nitzschia pusilla Grun.	4
Nitzschia recta Hantzsch ex Rabenh.	3
Nitzschia sigma (Kutz.) W. Sm.	1
Nitzschia sigmoidea (Nitzsch) W. Sm.	1
Nitzschia sinuata var. delognei (Grun. in Van Heurck) Lange-Bert	5
Nitzschia sinuata var. tabellaria (Grun.) Grun. ex Van Heurck	1
Nitzschia sociabilis Hust.	5
Nitzschia solita Hustedt	5

Column 1	Column 2
Diatom taxa	**Nutrient sensitivity score**
Nitzschia sp. Hassall	4
Nitzschia sublinearis Hust.	2
Nitzschia supralitorea Lange-Bertalot	5
Nitzschia valdestriata Aleem & Hust.	1
Nitzschia vermicularis (Kutz.) Hantzsch. in Rabenh.	2
Opephora sp. Petit	2
Pennate undifferentiated	2
Peronia fibula (Breb. ex Kutz.) R. Ross	2
Pinnularia appendiculata (Ag.) Cleve	1
Pinnularia borealis Ehrenb.	4
Pinnularia brebissonii (Kutz.) Rabenh.	2
Pinnularia gibba (Ehrenb.) Ehrenb.	1
Pinnularia intermedia (Lagerst.) Cleve	2
Pinnularia interrupta W. Smith	1
Pinnularia major (Kutz.) W. Sm.	3
Pinnularia microstauron (Ehrenb.) Cleve	2
Pinnularia rupestris Hantzsch in Rabenh.	2
Pinnularia sp. Ehrenb.	3
Pinnularia subcapitata Greg.	2
Pinnularia viridis (Nitzsch) Ehrenb.	1
Placoneis clementis (Grunow) E.J. Cox	4
Placoneis elginensis (Greg.) E.J. Cox	5
Placoneis placentula (Ehrenb.) Heinzerl.	4
Planothidium daui (Foged) Lange-Bert.	2
Planothidium delicatulum (Kütz.) Round et L. Bukhtiyarova	5
Planothidium granum (Hohn et Hellerman) Lange-Bert.	5
Planothidium haukianum (Grunow) Round et L. Bukhtiyarova	5
Planothidium lanceolatum (Bréb.) Round et L. Bukhtiyarova	4
Planothidium peragalli (Brun et Hérib.) Round et L. Bukhtiyarova	3
Psammothidium bioretii (Germain) L. Bukhtiyarova et Round	2
Psammothidium chlidanos (Hohn et Hellerman) Lange-Bert.	2
Psammothidium grishunun fo. daonensis (Lange-Bert.) L. Bukhti	2
Psammothidium lauenburgianum (Hust.) L. Bukhtiyarova et Rour	5
Psammothidium levanderi (Hust.) L. Bukhtiyarova et Round	2
Psammothidium marginulatum (Grunow) L. Bukhtiyarova et Rour	3
Psammothidium rossii (Hust.) L. Bukhtiyarova et Round	3
Pseudostaurosira brevistriata (Grun. in Van Heurck) Williams & F	4
Pseudostaurosira robusta (Fusey) Williams & Round	3
Rhopalodia brebissonii Krammer	2
Rhopalodia gibba (Ehrenb.) O. Mull.	2
Rhopalodia gibberula var. rupestris (W. Sm.) O. Mull.	1
Rossithidium linearis (W. Sm.) Round et L. Bukhtiyarova	2
Rossithidium petersenii (Hust.) Round et L. Bukhtiyarova	1
Rossithidium pusillum (Grunow) Round et L. Bukhtiyarova	2
Sellaphora bacillum (Ehenb.) Mann	4
Sellaphora pupula (Kutz.) Mereschkowsky	3
Sellaphora seminulum (Grun.) Mann	4
Simonsenia delognei (Grun. in Van Heurck) Lange-Bertalot	5
Skeletonema sp. Grev.	4
Stauroneis kriegeri Patr.	4
Stauroneis palustris Hust.	2
Stauroneis sp. Ehrenb.	4
Staurosira construens Ehrenb.	4

Column 1	Column 2
Diatom taxa	**Nutrient sensitivity score**
Staurosira elliptica (Schumann) Williams & Round	4
Staurosirella pinnata (Ehrenb.) Williams & Round	4
Stenopterobia curvula (W Smith) Krammer	1
Surirella angusta Kutz.	4
Surirella brebissonii Krammer & Lange-Bertalot	5
Surirella elegans Ehrenb.	5
Surirella minuta Breb. ex Kutz.	5
Surirella roba Leclercq	1
Surirella sp. Turpin	1
Synedra acus Kutz.	3
Synedra acus var. delicatissima (W. Sm.) Grun.	1
Synedra delicatissima W. Sm.	2
Synedra fasciculata (Ag.) Kutz.	5
Synedra parasitica (W. Sm.) Hust.	3
Synedra parasitica var. subconstricta (Grun. in Van Heurck) Hust.	4
Synedra sp. Ehrenb.	2
Synedra tenera W. Sm.	1
Tabellaria binalis (Ehrenb.) Grun. in Van Heurck	1
Tabellaria fenestrata (Lyngb.) Kutz.	1
Tabellaria flocculosa (Roth) Kutz.	2
Tabellaria quadriseptata Knudson	1
Tabellaria ventricosa Kütz.	1
Tabularia fasciculata (Ag.) Williams & Round	4
Tetracyclus lacustris Ralfs	1
Thalassiosira pseudonana (Hust.) Hasle & Heimdal	5
Tryblionella acuminata W. Sm.	4
Tryblionella hungarica (Grun) Mann	5
Tryblionella levidensis W. Sm.	4

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A.6 Poland - Multimetric Diatom Index Iojfor lakes in Poland (Multimetryczny Indeks Okrzemkowy Ioj)

Background

In Poland, all lakes with an area $\geq$ 50 ha are located in lowlands. 13 abiotic types of them have been determined. For assessment compliant with the WFD requirements, two groups have been distinguished:

1. softwater lakes with Ca content in water < 25 mg/L;
2. hardwater (alkaline) lakes with Ca content in water > 25 mg/L.

Majority of lakes that should be monitored belong to the alkaline group. They fit to intercalibration types L-CB1 or LC-B2 And the super type HA (high alkalinity).

Sampling

Diatom phytobenthos community recommended for routine monitoring – epiphyton.

Period of sampling – middle summer – middle autumn, once per vegetation season.

Sampling locality – in a littoral zone, in places not impacted by frequent and strong wave action. A sample should be taken from macrophytes adjacent to the open lake waters and submerged at a depth of at least 30 cm distance from a water table surface.

One sample from a monitoring site is composed of 5-6 subsamples collected from different plants, e.g. reed stem pieces of a length 1-2 cm. Collected material is fixed with Lugol's solution.

Laboratory pretreatment and preparing of permanent slides- according to PN-EN 13846. 2006 and Polish manual.

Diatom analysis

300-500 not damaged valves of indicator and reference diatom taxa are counted in a permanent slide from a sample using light microscope and immersion oil objective (100x) (PN–EN 14407:2007).

Metrics

Multimetric diatom index **IOJ** for Polish lakes has been developed specifically for lakes. The IOJ consists of two modules: the trophic **TJ** and a module of reference species **GR_J**.

The trophic **TJ** is calculated as follows:

$$TJ = TJ_i*wTJ_i*L_i)/\square wTJ_i*L_i$$

TJ_i – trophic (sensitivity) value of i-taxon, according to Schaumburg et al. 2007, acc. to Schoenfelder unpbl), range: 0-10, Table A.9;

wTJ_i – weight (tolerance) value of i-taxon, range: 1-3; based on results from Polish lakes, Table A.9

L_i – relative abundance of i-taxon (number of valves of i-taxon per number of all counted valves in a permanent slide, i.e. 300-400).

The TJ values change from theoretical 0 (ultraoligotrophy) to 10 (hypertrophy).

Reference species module **GR_J**: three groups of reference species have been determined: O – general (for all lake types), MW – for softwater lakes and TW – for alkaline lakes. Each reference species is given a value 1, Table A.9.

The **GR_J** module is calculated as follows:

GR_J = □tR_J

tR_i – relative abundance of i-reference talon.

The GR_J values vary from 1 (all taxa in a sample are reference) to 0 (none taxon in a sample is a reference one).

The TJ is converted into the scale identical with the GR_J scale1-0) as follows:

Z_{TJ} = 1–((TJ*0,1)

The **Z_{TJ}** chan ges from 1 (best state) do 0 (worst state).

Finally, the Polish multimetric diatom index IOJ for lakes is calculated according to a weighted formula:

IOJ = 0,6*Z_{TJ} + 0,4*GR_J

The IOJ values vary from 1 (best ecological status) to 0 (worst ecological status).

Table A.9 List of indicator taxa for trop hic index TJ (TJi – trophic value of a taxon, wTJi – weight value) and reference taxa for all Polish Lake types (O), dla for soft water lakes (MW) and for alkaline lakes (TW)

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
AALM	Achnanthes	altaica	0.38	3		1	
ABIA	Achnanthes	biasolettiana	0.52	1	1		
ACLE	Achnanthes	clevei	2.25	2			1
ACON	Achnanthes	conspicua	2.62	1			1
ADAO	Achnanthes	daonensis	0.98	1		1	
ADAU	Achnanthes	daui	0.98	1		1	
ADEL	Achnanthes	delicatula ssp. delicatula	5.43	3			
AEUT	Achnanthes	eutrophila	3.04	1			
AEXG	Achnanthes	exigua	2.41	2			1
AEXI	Achnanthes	exilis	0.52	1			1
AFAL	Achnanthes	flexella var. alpestris	0.54	2	1		
AFLE	Achnanthes	flexella var. flexella	0.02	3	1		
AGRN	Achnanthes	grana	4.23	1			
AHEL	Achnanthes	helvetica	0.48	3		1	
AHUN	Achnanthes	hungarica	6.67	3			
AJOU	Achnanthes	joursacense	1.96	2	1		
AKOL	Achnanthes	kolbei	4.12	2			
ALVS	Achnanthes	laevis	0.52	2	1		
ALBP	Achnanthes	lanceolata ssp. biporoma	2.28	1			1
ALFR	Achnanthes	lanceolata ssp. frequentissima	2.28	2			1
ALAN	Achnanthes	lanceolata ssp. lanceolata	1.15	2			1
ALDU	Achnanthes	lanceolata ssp. robusta	2.28	2			1
ALAR	Achnanthes	lanceolata ssp. rostrata	2.28	2			1

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
ALAT	Achnanthes	laterostrata	0.48	3	1		
ALAU	Achnanthes	lauenburgiana	4.23	2			
ALVD	Achnanthes	levanderi	0.38	3		1	
ALIO	Achnanthes	linearioides	0.38	3		1	
AMAR	Achnanthes	marginulata	0.48	3		1	
AMIS	Achnanthes	minuscula	3.04	2			
AMAF	Achnanthes	minutissima var. affinis	3.38	2			
AMGR	Achnanthes	minutissima var. gracillima	0.38	3			1
AMII	Achnanthes	minutissima var. inconspicua	0.48	2			1
AMJA	Achnanthes	minutissima var. jackii	0.48	2			1
AMIN	Achnanthes	minutissima var. minutissima	0.74	1	1		
AMSC	Achnanthes	minutissima var. scotica	0.14	3	1		
ANOD	Achnanthes	nodosa	0.38	3		1	
AOBG	Achnanthes	oblongella	0.48	3		1	
AOST	Achnanthes	oestrupii	1.55	1			1
APET	Achnanthes	petersenii	0.66	1			1
APLO	Achnanthes	ploenensis var. ploenensis	4.23	3			
APUS	Achnanthes	pusilla	0.75	3	1		
AROK	Achnanthes	rosenstocki	0.09	3			1
ASAT	Achnanthes	subatomoides	0.66	3		1	
ATRI	Achnanthes	trinodis	0.43	3			1
AVTL	Achnanthes	ventralis	0.48	3		1	
AZIE	Achnanthes	ziegleri	1.72	2			1
APEL	Amphipleura	pellucida	1.21	2			1
AMFO	Amphora	fogediana	0.90	3			1
AINA	Amphora	inariensis	0.98	1			1
ALIB	Amphora	libyca	3.96	3			
AOVA	Amphora	ovalis	3.26	1			
APED	Amphora	pediculus	2.89	1			
ATHU	Amphora	thumensis	0.38	3			1
AMVC	Amphora	veneta var. capitata	0.77	3			1
AVEN	Amphora	veneta var. veneta	5.70	2			
ABLT	Aneumastus	balticus					1
ASPH	Anomoeoneis	sphaerophora	5.30	3			
BBRE	Brachysira	brebissonii	0.48	3		1	
BNEO	Brachysira	neoexilis	0.74	2	1		
BPRO	Brachysira	procera	0.38	3	1		
BSER	Brachysira	serians	0.38	3		1	
BSTY	Brachysira	styriaca	0.40	3	1		
BVIT	Brachysira	vitrea	0.48	3			1
CAER	Caloneis	aerophila	0.48	3		1	
CAPS	Caloneis	alpestris	0.40	2			1
CAMP	Caloneis	amphisbanena	4.05	3			
CBAC	Caloneis	bacillum	3.21	2			
CAOB	Caloneis	obtusa	0.38	3	1		

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
CSHU	Caloneis	schumanniana	1.86	3			1
CSIL	Caloneis	silicula	3.25	2			
CNTH	Cocconeis	neothumensis	2.15	2			1
CPED	Cocconeis	pediculus	4.33	3			
CPLE	Cocconeis	placentula var. euglypta	3.45	2			
CPLI	Cocconeis	placentula var. lineata	2.93	2			
CPLA	Cocconeis	placentula var. placentula	3.45	2			
COPL	Cocconeis	placentula var. pseudolineata	3.45	2			
CELL	Cymatopleura	elliptica (wraz z odmianami)	3.33	3			
CSOL	Cymatopleura	solea (wraz z odmianami)	4.08	3			
CAFF	Cymbella	affinis	1.09	3			1
CAFN	Cymbella	affiniformis	1.02	1			1
CAPH	Cymbella	amphicephala var. amphicephala	1.41	3	1		
CASP	Cymbella	aspera	2.58	1			1
CCAE	Cymbella	caespitosa	1.55	3			1
CCES	Cymbella	cesatii	0.45	3	1		
CCIS	Cymbella	cistula	2.56	1			1
CCUS	Cymbella	cuspidata	0.77	1			1
CCYM	Cymbella	cymbiformis	0.71	2			1
CDEL	Cymbella	delicatula	0.48	3			1
CDES	Cymbella	descripta	0.38	3	1		
CEHR	Cymbella	ehrenbergii	2.36	2			1
CELG	Cymbella	elginensis	0.38	3		1	
CAEX	Cymbella	excisa	2.15	2			1
CFAL	Cymbella	falaisensis	0.68	2	1		
CGAE	Cymbella	gaeumannii	0.48	2	1		
CGRA	Cymbella	gracilis	0.97	3		1	
CHEB	Cymbella	hebridica	0.48	3	1		
CHCO	Cymbella	helvetica var. compacta	3.04	2			
CHEL	Cymbella	helvetica var. helvetica	0.50	2			1
CHUS	Cymbella	hustedtii	1.47	3			1
CHYB	Cymbella	hybrida	0.40	3			1
CINC	Cymbella	incerta	0.40	3	1		
CLAC	Cymbella	lacustris	0.04	2			1
CLAE	Cymbella	laevis	0.62	2			1
CLAN	Cymbella	lanceolata	3.60	2			
CLAT	Cymbella	lata	1.51	2			1
CLEP	Cymbella	leptoceros	0.95	3			1
CMIC	Cymbella	microcephala	1.02	3	1		
CMIN	Cymbella	minuta	0.70	3	1		
CPER	Cymbella	perpusilla	0.48	3	1		
CPRO	Cymbella	prostrata	3.39	3			
CPRX	Cymbella	proxima					1
CREI	Cymbella	reichardtii	3.97	3			
CSLE	Cymbella	silesiaca					1

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
CSIN	Cymbella	sinuata	2.79	1			
CSAE	Cymbella	subaequalis	0.83	2	1		
CTUM	Cymbella	tumida	4.49	3			
CTLA	Cymbella	tumidula var. lancettula	0.48	3			1
CTMD	Cymbella	tumidula var. tumidula	0.48	3			1
CVEN	Cymbella	ventricosa					1
CVUL	Cymbella	vulgata					1
DKUE	Denticula	kuetzingii	0.97	2			1
DTEN	Denticula	tenuis	0.80	1			1
DEHR	Diatoma	ehrenbergii	1.44	2			1
DMES	Diatoma	mesodon	0.66	3	1		
DMON	Diatoma	moniliformis	5.74	3			
DPRO	Diatoma	problematica	5.74	3			
DITE	Diatoma	tenuis	4.97	2			
DVUL	Diatoma	vulgaris	5.61	3			
DELL	Diploneis	elliptica	1.44	1			1
DOBL	Diploneis	oblongella	0.30	2			1
DOVA	Diploneis	ovalis	0.44	3			1
DPET	Diploneis	petersenii	0.66	2		1	
EADN	Epithemia	adnata	2.42	2			1
EFRI	Epithemia	frickei					1
ESMI	Epithemia	smithii					1
ESOR	Epithemia	sorex	2.46	2			1
ETUR	Epithemia	turgida	2.95	2			
EARB	Eunotia	arcubus	0.62	3			1
EARC	Eunotia	arcus			1		
EBIL	Eunotia	bilunaris	3.66	3			
EBMU	Eunotia	bilunaris var. mucophila				1	
EBOT	Eunotia	botuliformis	1.61	2		1	
EEXI	Eunotia	exigua	0.64	3		1	
EFAB	Eunotia	faba	0.42	3		1	
EFOR	Eunotia	formica	5.86	1			
EGLA	Eunotia	glacialis	1.81		1		
EGFA	Eunotia	glacilifalsa			1		
EIMP	Eunotia	implicata	1.11	3	1		
EINC	Eunotia	incisa	1.02	3		1	
EMEI	Eunotia	meisteri	0.38	3		1	
EMIN	Eunotia	minor			1		
EMTR	Eunotia	muscicola var. tridentula	0.48	3		1	
ENAE	Eunotia	naegeli	1.07	3		1	
ENYM	Eunotia	nymanniana	0.38	3		1	
EPEC	Eunotia	pectinalis	0.48	3		1	
EPRA	Eunotia	praerupta var. praerupta	0.48	3	1		
ERHO	Eunotia	rhomboidea	0.48	3		1	
ERHY	Eunotia	rhynchocephala				1	

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
ESDI	Eunotia	serra (wraz z odmianami)	0.38	3		1	
FBCP	Fragilaria	biceps	5.27	1			
FBID	Fragilaria	bidens	6.87	1			
FBRE	Fragilaria	brevistriata	2.81	2			
FCPH	Fragilaria	capucina var. amphicephala	0.51	3			1
FCAU	Fragilaria	capucina var. austriaca	0.98	3			1
FCAP	Fragilaria	capucina var. capucina	3.79	3			
FCDI	Fragilaria	capucina var. distans	0.38	3			1
FCGR	Fragilaria	capucina var. gracilis			1		
FCME	Fragilaria	capucina var. mesolepta	3.82	2			
FCPE	Fragilaria	capucina var. perminuta	3.82	2			
FCRP	Fragilaria	capucina var. rumpens	4.12	1			
FCVA	Fragilaria	capucina var. vaucheriae	5.33	3			
FCBI	Fragilaria	construens f. binodis	2.81	2			
FCON	Fragilaria	construens f. construens	2.81	2			
FCVE	Fragilaria	construens f. venter	2.81	2			
FDEL	Fragilaria	delicatissima	0.90	3			1
FEXI	Fragilaria	exigua	0.48	3	1		
FFAM	Fragilaria	famelica	4.23	3			
FFAS	Fragilaria	fasciculata	5.66	3			
FLAP	Fragilaria	laponica	2.50	2			1
FLEP	Fragilaria	leptostauron (wraz z odmianami)	4.00	2			
FNAN	Fragilaria	nanana	1.57	2			1
FNIT	Fragilaria	nitzschioides	5.66	1			
FPAR	Fragilaria	parasitica (wraz z odmianami)	3.28	2			
FPIN	Fragilaria	pinnata	2.57	2			1
FPUL	Fragilaria	pulchella	5.92	3			
FROB	Fragilaria	robusta	1.51	3			1
FTEN	Fragilaria	tenera	1.89	3	1		
FUAC	Fragilaria	ulna var. acus	3.78	2			
FUAN	Fragilaria	ulna var. angustissima	5.74	3			
FULN	Fragilaria	ulna var. ulna	5.27	2			
FVIR	Fragilaria	virescens	0.66	3		1	
FERI	Frustulia	erifuga	0.48	2		1	
FRCR	Frustulia	rhomboides var. crassinervia	0.48	2		1	
FRHO	Frustulia	rhomboides var. rhomboides	1.00	2		1	
FRSA	Frustulia	rhomboides var. saxonica	0.48	2		1	
FVUL	Frustulia	vulgaris	5.71	3			
GACU	Gomphonema	acuminatum	3.31	2			
GANT	Gomphonema	angustum	0.76	2			1
GAUG	Gomphonema	augur	4.99	3			
GAUR	Gomphonema	auritum	0.27	3	1		
GBAV	Gomphonema	bavaricum	0.48	2			1
GBOH	Gomphonema	bohemicum	0.48	2		1	
GBRE	Gomphonema	brebissonii	3.31	2			

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
GCLA	Gomphonema	clavatum	4.00	2			
GDIC	Gomphonema	dichotomum	0.61	2	1		
GGRA	Gomphonema	gracile	1.35	1	1		
GHEB	Gomphonema	hebridense	0.23	3	1		
GHEL	Gomphonema	helveticum	0.40	3			1
GLAT	Gomphonema	lateripunctatum	0.25	3			1
GMIC	Gomphonema	micropus	6.49	3			
GMIS	Gomphonema	minusculum					1
GMIN	Gomphonema	minutum	4.23	2			
GOCU	Gomphonema	occultum	0.57	3			1
GOOL	Gomphonema	olivaceum var. olivaceoides	0.98	3	1		
GOLI	Gomphonema	olivaceum var. olivaceum	4.30	2			
GPXS	Gomphonema	parvulum var. exilissimum	0.98		1		
GPPA	Gomphonema	parvulum var. parvulius	0.48			1	
GPAR	Gomphonema	parvulum var. parvulum	2.95	3			
GPRC	Gomphonema	procerum	0.66	3			1
GPTE	Gomphonema	pseudotenellum	0.66	3	1		
GPUM	Gomphonema	pumilum	2.75	2			
GSUB	Gomphonema	subtile	0.13	1	1		
GTER	Gomphonema	tergestinum	3.04	2			
GTRU	Gomphonema	truncatum	3.25	1			
GVIB	Gomphonema	vibrio	0.77	3			1
GYAC	Gyrosigma	acuminatum	3.62	3			
GYAT	Gyrosigma	attenuatum	3.62	3			
GNOD	Gyrosigma	nodiferum	4.40	3			
MGRE	Mastogloia	grevillei					1
MSLA	Mastogloia	smithii var. lacustris	0.37	3			1
MVAR	Melosira	varians	4.89	3			
MCIR	Meridion	circulare var. circulare	4.92	1			
NABL	Navicula	absoluta	0.60	3	1		
NANT	Navicula	antonii	3.04	2			
NATO	Navicula	atomus var. atomus	4.74	2			
NAPE	Navicula	atomus var. permitis	5.74	2			
NBAC	Navicula	bacillum	2.48	2			1
NBRY	Navicula	bryophila	0.52	2	1		
NCAP	Navicula	capitata var. capitata	5.37	3			
NCHU	Navicula	capitata var. hungarica	5.37	3			
NCLU	Navicula	capitata var. lueneburgensis	4.59	3			
NCPR	Navicula	capitatoradiata	4.20	3			
NCAR	Navicula	cari	3.06	3			
NCIN	Navicula	cincta	2.20	3			
NCIT	Navicula	citrus	5.74	3			
NCLE	Navicula	clementis	2.72	2			1
NCOC	Navicula	cocconeiformis	0.66	2	1		
NCST	Navicula	constans	3.04	2			

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
NCOS	Navicula	costulata	5.86	2			
NCRY	Navicula	cryptocephala	3.00	3			
NCFA	Navicula	cryptofallax	4.23	3			
NCTE	Navicula	cryptotenella	1.37	2			1
NCTO	Navicula	cryptotenelloides	1.37	2			1
NCUS	Navicula	cuspidata	4.85	3			
NDEC	Navicula	decussis	3.02	2			
NDET	Navicula	detenta	0.48	3		1	
NELG	Navicula	elginensis	2.50	2			1
NERI	Navicula	erifuga	5.74	3			
NEXI	Navicula	exilis	0.66	2		1	
NGPE	Navicula	gallica var. perpusilla	0.48	3		1	
NGAS	Navicula	gastrum	3.57	3			
NGOE	Navicula	goeppertiana	5.74	3			
NGOT	Navicula	gottlandica	0.22	2			1
NGRE	Navicula	gregaria	6.76	3			
NHMD	Navicula	heimansioides	0.48	3		1	
NITG	Navicula	integra	4.23	3			
NJOU	Navicula	joubaudii	3.04	2			
NLAE	Navicula	laevissima	2.32	1			1
NLAN	Navicula	lanceolata	7.05	3			
NMED	Navicula	mediocris	0.48	3		1	
NMEN	Navicula	menisculus var. menisculus	4.67	3			
NMUP	Navicula	menisculus var. upsaliensis	4.00	3			
NMIN	Navicula	minima	4.00	1			
NMMU	Navicula	minuscula var. muralis	5.74	3			
NMNO	Navicula	minusculoides	5.74	3			
NMLF	Navicula	molestiformis	5.74	3			
NMOC	Navicula	monoculata	5.74	3			
NMOK	Navicula	moskalii	3.04	2			
NNOT	Navicula	notha	0.66	1		1	
NOBL	Navicula	oblonga	2.02	2			1
NOPU	Navicula	oppugnata	4.62	2			
NPLA	Navicula	placentula	2.64	2			1
NPOR	Navicula	porifea	2.70	2			
NPRA	Navicula	praeterita	0.41	3			1
NPRO	Navicula	protracta	3.23	3			
NAPG	Navicula	pseudoanglica	3.13	2			
NPBY	Navicula	pseudobryophila	0.48	3		1	
NPSC	Navicula	pseudoscutiformis	0.42	3	1		
NPTU	Navicula	pseudotuscula	1.12	1			1
NPVE	Navicula	pseudoventralis	2.63	1			1
NPUP	Navicula	pupula (wraz z odmianami)	3.01	2			
NPYG	Navicula	pygmaea	4.23	3			
NRAD	Navicula	radiosa	1.90	2			1

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
NRCS	Navicula	recens	5.74	3			
NRCH	Navicula	reichardtiana var. reichardtiana	3.51	2			
NREI	Navicula	reinhardtii	3.31	2			
NRHT	Navicula	rhynchotella	5.74	3			
NSAP	Navicula	saprophila	5.74	3			
NSCH	Navicula	schoenfeldii	2.71	3			1
NSHR	Navicula	schroeteri	5.74	3			
NSCD	Navicula	scutelloides	3.91	3			
NSEM	Navicula	seminulum	5.70	3			
NSLE	Navicula	slesvicensis	4.65	3			
NSOR	Navicula	soehrensis (wraz z odmianami)	0.48	3		1	
NSTR	Navicula	stroemii	0.72	2			1
NSBN	Navicula	subalpina	0.54	2			1
NSBU	Navicula	subhamulata	1.17	1			1
NSLU	Navicula	sublucidula	4.23	3			
NSBM	Navicula	subminuscula	5.74	3			
NSBR	Navicula	subrotundata	2.43	1			1
NSUB	Navicula	subtilissima	0.48	3		1	
NSUC	Navicula	suchlandtii	0.48	3		1	
NTPT	Navicula	tripunctata	5.31	3			
NTRV	Navicula	trivialis	4.92	3			
NTCX	Navicula	trophicatrix	2.62	2			1
NTMI	Navicula	tuscula var. minor	1.36	2			1
NTUS	Navicula	tuscula	1.17	2			1
NVEN	Navicula	veneta	5.74	2			
NVTL	Navicula	ventralis	0.48	1			1
NVIR	Navicula	viridula (wraz z odmianami)	5.74	3			
NVUL	Navicula	vulpina	0.71	2			1
NEAF	Neidium	affine var. affine	0.48	3	1		
NEAM	Neidium	ampliatum	0.92	2	1		
NBIS	Neidium	bisulcatum	0.48	3		1	
NEDU	Neidium	dubium	2.20	2			1
NACI	Nitzschia	acicularis	5.83	3			
NACD	Nitzschia	acidoclinata	2.85	1			
NACU	Nitzschia	acula	5.74	3			
NZAL	Nitzschia	alpina	0.48	3	1		
NAMP	Nitzschia	amphibia	4.99	3			
NIAN	Nitzschia	angustata	1.76	2			1
NBCL	Nitzschia	bacillum	1.34	2			1
NICA	Nitzschia	calida	5.74	3			
NCTN	Nitzschia	capitellata	7.29	3			
NCOM	Nitzschia	communis	5.74	3			
NICO	Nitzschia	commutata	9.72	3			
NZCO	Nitzschia	constricta	6.72	3			
NDEB	Nitzschia	debilis	5.74	3			

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
NDIS	Nitzschia	dissipata var. dissipata	3.92	3			
NDME	Nitzschia	dissipata var. media	2.91	3			
NFIL	Nitzschia	filiformis	5.74	3			
NFON	Nitzschia	fonticola	3.72	3			
NIGR	Nitzschia	gracilis	3.72	2			
NHEU	Nitzschia	heufleriana	2.78	3			
NHOM	Nitzschia	homburgiensis	0.98	3		1	
NIHU	Nitzschia	hungarica	5.74	3			
NINC	Nitzschia	inconspicua	5.74	3			
NINT	Nitzschia	intermedia	5.74	3			
NILA	Nitzschia	lacuum	1.27	2			1
NLEV	Nitzschia	levidensis (wraz z odmianami)	8.08	3			
NLIN	Nitzschia	linearis var. linearis	4.77	3			
NLSU	Nitzschia	linearis var. subtilis	5.74	3			
NMIC	Nitzschia	microcephala	5.74	3			
NPAL	Nitzschia	palea var. palea	3.05	2			
NPAE	Nitzschia	paleacea	3.50	3			
NIPM	Nitzschia	perminuta			1		
NIPR	Nitzschia	pura			1		
NIPU	Nitzschia	pusilla	5.74	3			
NZRA	Nitzschia	radicula	0.98	2			1
NREC	Nitzschia	recta	3.72	3			
NIRE	Nitzschia	regula	0.43	3			1
NSIO	Nitzschia	sigmoidea	3.40	3			
NSOC	Nitzschia	sociabilis	4.23	3			
NISO	Nitzschia	solita	5.74	3			
NSUA	Nitzschia	subacicularis	3.49	3			
NSBL	Nitzschia	sublinearis	3.72	2			
NZSU	Nitzschia	supralitorea	5.74	3			
NTRY	Nitzschia	tryblionella	5.74	3			
NUMB	Nitzschia	umbonata	5.74	3			
NWUE	Nitzschia	wuellerstorfii	5.74	3			
PBOR	Pinnularia	borealis	2.95	1			
PMAJ	Pinnularia	major	0.48	1	1		
PMIC	Pinnularia	microstauron	2.41	1			1
PNOB	Pinnularia	nobilis	4.06	1			
PNOD	Pinnularia	nodosa	1.72	1			1
PRUP	Pinnularia	rupestris	2.91	1			
PSIL	Pinnularia	silvatica	0.48	3		1	
PSCA	Pinnularia	subcapitata	0.94	3		1	
PSGI	Pinnularia	subgibba	2.16	1			1
PVIF	Pinnularia	viridiformis	2.91	1			
RABB	Rhoicosphenia	abbreviata	4.35	3			
RGIB	Rhopalodia	gibba var. gibba	2.81	3			1
RGPA	Rhopalodia	gibba var. parallela	0.54	3			1

Code	Genus	Species	TJ_i	wTJ_i	O	MW	TW
STKR	Stauroneis	kriegeri	3.84	2			
SSMI	Stauroneis	smithii	3.04	2			
STCU	Stenopterobia	curvula	0.48	3		1	
STDE	Stenopterobia	delicatissima	0.48	3		1	
SANG	Surirella	angusta	7.05	3			
SBIF	Surirella	bifrons	2.42	3			1
SBRE	Surirella	brebissonii (wraz z odmianami)	6.83	3			
SLCO	Surirella	linearis var. constricta	0.48	3	1		
SLIN	Surirella	linearis var. linearis	1.69	2	1		
SUMI	Surirella	minuta	5.74	3			
SRBA	Surirella	roba	0.66	2		1	
TBEL	Tabellaria	binalis var. elliptica	0.38	3		1	
TFEN	Tabellaria	fenestrata			1		
TFLO	Tabellaria	flocculosa	1,13	3	1		
TVEN	Tabellaria	ventricosa	0.38	3		1	

Reference conditions

Reference sites have been chosen according to REFCOND (Wallin et al. 2003). Basic chemical data and land use in a catchment area are includes in Appendix. Seven lakes for stratified water bodies (LCB! – Borówno, Gostomskie, Krępsko Długie, Maróz, Niegocin,Ostrowite and Sołtmany) and five for non stratified lakes (LCB2 – Białe Sosnowickie, Iławki, Kołowin, Płaskie and Tauty) have been indicated.

Class boubdaries

The IOJ values range from 0 (worst state) to 1 (best state). Basing on the IOJ data from reference sites, following values were calculated: average, median, 75 percentile and 90 percentile (Table A.10).

Class boundaries of ecological status of Polish lakes according to the IOJ values are the same for all lakes. The boundary High/Good has been set between median and 75 percentile values from reference sites of both LCB1 and LCB2. The boundary Good/Moderate is a median value from all data of submitted to IC exercise LCB1 or LCB2 lakes.

Table A.10 The average, median, 75 and 90 percentiles IOJ values from reference sites of Polish alkaline lakes.

	IOJ value	
	Stratified lakes (LCB1)	Non stratified lakes (LCB2)
Average	0.781	0.794
Median	**0.761**	**0.790**
75 percentile	0.839	0.805
90 percentile	0.865	0.860

Table A.11Class boundaries of ecological status of Polish lakes according to the IOJ values

Ecological status	IOJ value
High	> 0.80
Good	0.60
Moderate	0.40
Poor	0.15
Bad	< 0.15

Pressure-response relationship

Diatom phytobenthos in lakes respond to eutrophication, especially in a littoral zone. The relationship between the Polish IOJ and IC metrics, and pressure indicator – total phosphorus (IP and log10 TP) are not very strong but all of them are significant statistically (Table A.11).

Table A.12 Relationship for metrics and TP and log_{10} TP of Polish lakes data set submitted to the IC exercise

Metric	b	a	R^2
PL lake type CB1 HA			
pICM	-0.4288 TP	+0.0976	**0.0976**
EQR_IOJ	-0.3986 TP	+0.6508	0.1052
EQR_IPS	-0.2154 TP	+0.9672	0.0638
EQR_TI	-0.6422 TP	+0.8869	0.1001
pICM	-0.1152 log_{10}TP	+0.7486	0.0822
EQR_IOJ	-0.13 log_{10}TP	+0.4566	0.1308
EQR_IPS	-0.0433 log_{10}TP	+0.8956	0.0301
EQR_TI	-0.1871 log_{10}TP	+0.6016	0.0993
PL lake type CB2 HA			
pICM	-0.3404 TP	+0.4443	0.1432
EQR_IOJ	-0.3714 TP	+0.4413	0.1669
EQR_IPS	-0.2835 TP	+0.4023	0.0677
EQR_TI	-0.2672 TP	+0.3716	0.1611
pICM	-1.0175 log_{10}TP	-0.1612	0.2312
EQR_IOJ	-0.986 log_{10}TP	-0.27	0.2126
EQR_IPS	-0.8908 log_{10}TP	-0.247	0.1207
EQR_TI	-0.7824 log_{10}TP	-0.3925	0.2496

References

Official reference: Multimetryczny Indeks Okrzemkowy IOJ (ROZPORZĄDZENIE MINISTRA ŚRODOWISKA z dnia 9 listopada 2011 r. w sprawie sposobu klasyfikacji stanu jednolitych części wód powierzchniowych oraz środowiskowych norm jakości substancji priorytetowych. Dziennik Ustaw z dnia 29 listopada 2011 – pozycja 1545).

PN–EN 13946. 2006. Jakość wody. Wytyczne do rutynowego pobierania próbek oraz wstępnego przygotowania do analiz okrzemek bentosowych z rzek.

PN–EN 14407:2007 Jakość wody. Wytyczne dotyczące identyfikacji, oznaczania ilościowego i interpretacji wyników badania próbek okrzemek bentosowych z wód płynących.

PN–EN ISO 5667–1:2008. Jakość wody. Pobieranie próbek. Część 1: Wytyczne opracowywania programów pobierania próbek i technik pobierania.

ROZPORZĄDZENIE MINISTRA ŚRODOWISKA z dnia 9 lisyopada 2011 r. w sprawie sposobu klasyfikacji stanu jednolitych części wód powierzchniowych oraz środowiskowych norm jakości substancji priorytetowych. Dziennik Ustaw z dnia 29 listopada 2011 – pozycja 1545.

Schaumburg, J., Schranz, Ch., S., Stelzer, D. & Hofmann, G., 2007. Action Instructions for the ecological Evaluation of Lakes for Implementation of the EU Water Framework Directive: Macrophytes and Phytobenthos. Bavarian Water Management Agency. München. s. 1–69.

A.7 Slovenia: Ecological Quality Assessment of lakes in Slovenia using phytobenthos and macrophytes – Part 1: Phytobenthos

Sampling protocol

Lake sampling site represents a lakeshore section up to 100 m in length. In each lake 3 sapling sites are selected and samples are treated separately. Phytobenthos is sampled in the littoral zone to the depth of 0.6 m. A “multihabitat” sampling approach is used. Thus, phytobenthos is collected from various substrates (stones, sand, macrophytes and wood). Sample is preserved using formaldehyde to the final 1-4% solution. In the laboratory at first phytobenthos of each sample is determined in order to prepare a phytobenthos species list. In the second step, 500 valves of diatoms per sample are counted and identified to the species level under the microscope and used in the index calculation.

Metric description

Phytobenthos and macrophytes are one biological element under the Water Framework Directive (Directive 2000/60/ES). In Slovenian lake ecological classification system both sub-elements are used together as one element. Phytobenthos assessment system consists of one metric - Trophic Index (Rott et al. 1998).

Trophic index respond to eutrophication. In lakes of Slovenia we have found a good relationship between mean annual total phosphorous concentrations in lakes and the Trophic Index (Figure A.8).

Whole lake Trophic Index (Lake – TI) is calculated according to the equation:

$$Lake - TI = \frac{\sum_{j=1}^{n} Site{-}TI_j}{n} \qquad (2)$$

where

Site-TIj is aTrophic index value of the sampling site »j« and »n« is number of sampling sites in a lake.

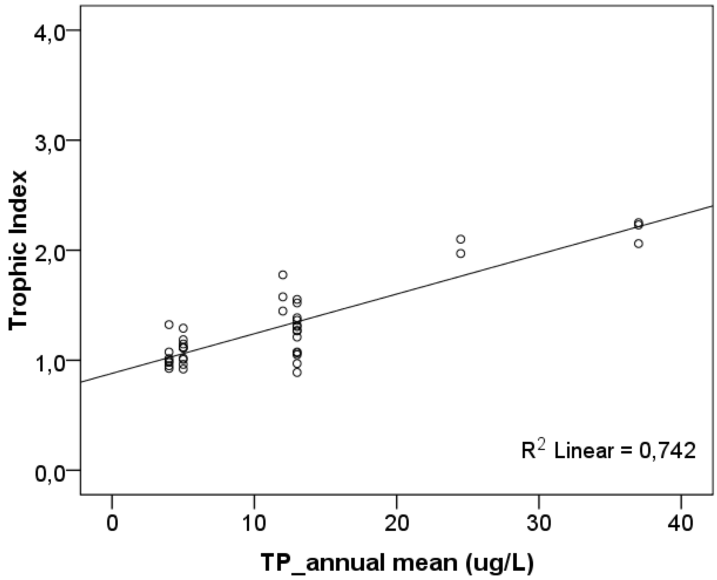

Figure A.8 Trophic Index in rensponse to mean annual total phosphorous (TP) concentration.

Description of reference conditions and boundary setting

In Slovenia, two types of reference site criteria were choosen. First group contains criteria that address the whole lake (lake specific criteria), whereas second group criteria are related to the lakeshore sections (site specific criteria) (Appendix 1). Trophic Index addressess eutrophication pressure and thus lake-specific criteria were used (same criteria were used for phytoplankton). Based on addressed pressure Lake Bohinj is a reference lake. Comparison of reference and non-reference sites revealed significant differences in the Trophic Index (Figure A.9).

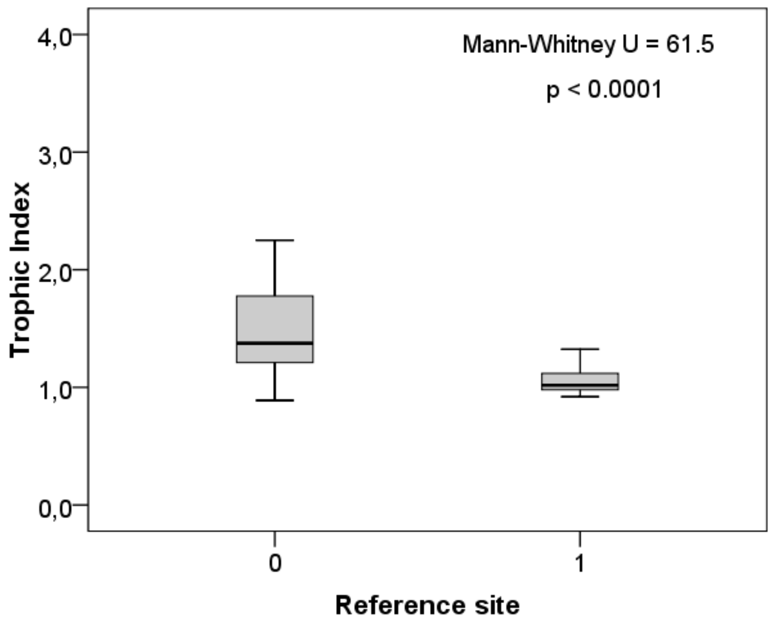

Figure A.9 Distribution of Trophic index values between reference (1) and impaired (0) sites and results of the Mann-Whitney U test.

Due to very low number of moderate sites and absence of poor and bad sites, it was not possible to use any other boundary setting procedure. Boundary values calculated using described approach are given in the Table A.13. In order to combine phytobenthos score with a macrophyte score for the classification of the waterbody all calculated TI_EQR values are piecewise transformed in order to get five equidistant boundary values (Table A.14) which are official Slovenian boundary values.

Table A.13 Piecewise linear transformation equations for normalized Trophic index (TI_EQR).

Ecological status	TI	TI_EQR	Transformed TI_EQR
High	≤1.13	>0.95	0.8+0.2*(TI_EQR-0.96)/(0.04)
Good	1.14-1.82	0.95-0.72	0.6+0.2*(TI_EQR-0.72)/(0.24)
Moderate	1.83-2.52	0.71-0.48	0.4+0.2*(TI_EQR-0.48)/(0.24)
Poor	2.53-3.21	0.47-0.24	0.2+0.2*(TI_EQR-0.24)/(0.24)
Bad	>3.21	<0.24	0.2*(TI_EQR)/(0.24)

Table A.14 Transformed boundary values between five ecological status classes using Trophic index (TI_EQR_transformed).

Boundary	TI_EQR_transformed
High/Good	0.8
Good/Moderat	0.6
Moderate	0.4
Poor /Bad	0.2

References

Kosi G., Bricelj M., (2006). Metodologija vzorčenja in laboratorijske obdelave fitobentosa v jezerih v skladu z zahtevami vodne directive (Direktiva 2000/60/ES). Nacionalni inštitut za biologijo, Ljubljana, 11 pp.

Kosi G., Bricelj M., Eleršek T., Stanič K. (2007). Prilagoditev trofičnega indeksa zahtevam Vodne directive (Direktiva 2000/60/ES) za vrednotenje ekološkega stanja jezer v Sloveniji na podlagi fitobentosa. Nacionalni inštitut za biologijo, Ljubljana, 47 pp.

Rott E., Pipp E., Pfister P., van Dam H., Ortler K., Binder N., Pall K. 1998. Indikationslisten fur Aufwuchsalgen. Teil 2: Trophieindikation. Bundesministerium fur Land-und Forstwirtschaft, Wien.

Urbanič G., Smolar-Žvanut N. (2005). Criteria for selecting river and lake reference sites in Slovenia. Institute for Water of the Republic of Slovenia, Ljubljana, 9pp.

Urbanič G., Kosi G. (2011). Completion of the ecological classification system for Alpine lakes using phytobenthos. Institute for Water of the Republic of Slovenia, Ljubljana, 12 pp.

Appendix 1. Reference condition criteria for selection of lake reference sites in Slovenia (Urbanič & Smolar-Žvanut 2005)

a. The length of the reference site or lake shore

   The reference site or lake shore length is 100 m.

b. Morphological changes

   The reference site must be classified in the first morphological class acording to the classification of the shore and the littoral belt of lakes Bled and Bohinj, with regard to morphological changes (after Peterlin et al., 2005)

c. Residence time of the water

   There is no change in the natural residence time.

d. Shore vegetation

   The natural vegetation must be preserved, corresponding to the type and geographical position of the lake.

e. Land use of the catchment area

   The percent of natural surfaces of the lake catchment area (after Corine Land Cover) is:

   - > 70 % or
   - > 50 %, if at least 50 m from the lake there are no agricultural or urban areas (after Corine Land Cover).

f. Pyhsico-chemical conditions

   - There is no point source of pollution on the reference site (such as industrial waste outflow, communal waste outflow or water treatment plant outflow), that would influence physico-chemical parameters.
   - There are no known sources of pollution or loading with any specific synthetic or non-synthetic pollutants (data from MOP-ARSO 2004).

g. *Trophical status of the lake acording to OECD criteria

h. *Given only for the hydroecoregion Alps, since that is the only region in Slovenia where there are natural lakes that fit the size standards of the Water Framework Directive (Directive 2000/60/EC).

   – Hydroecoregion Alps: oligotrophic

i. Biological pressures

   – There is no impact from non-autochtonous species, which would competitively endanger autochtonous species, disrupt the habitats and genetically weaken the populations.
   – There is very little or no impact from fishery. The reference site is chosen on the section of the river that is either not used for fishing or it is categorised as protected water (after Bertok et al. 2000, 2003).

j. Other pressures

   Reference sites are not used for mass recreational purposes (camping, swimming, rowing).

A.8 Sweden

Introduction

Periphytic algae play an important role as primary producers, in lakes and running water, and diatoms are often the dominant group in the periphyton community. Diatoms are good indicators of water quality and methods of classification and other evaluations of lakes and watercourses based on diatoms are in wide use in Europe and other parts of the world.

The background of the method can be found in the documents (Kahlert et al. 2007, Kelly et al. submitted, Kahlert 2009, Kahlert & Gottschalk 2009). A handbook on how quality requirements in bodies of surface water can be determined and monitored describes several parameters to be used in the lake assessment (see Table below).

Table A.15 Parameters used in the lake assessment by diatoms

Parameter	Primarily shows the effects of	How often measurements need to be taken?	At what times of the year?
IPS	Nutrient impact and organic pollution	Once a year	Late summer/autumn
ACID	Acidity	Once a year	Late summer/autumn
%PT (support parameter)	Organic pollution	Once a year	Late summer/autumn
TDI (support parameter)	Nutrient impact	Once a year	Late summer/autumn

Input parameters

The parameters which must be classified for the diatom quality factor are the two indices IPS (Indice de Polluo-sensibilité Spécifique) and the acidity index ACID.

The support parameters %PT (Pollution Tolerant valves; Kelly 1998) and TDI (Trophic Diatom Index; Kelly 1998) can also be assessed, to obtain better evidence in doubtful cases.

IPS (Cemagref, 1982) shows the impact of nutrients and organic pollution. The support parameters %PT (indicates organic pollution) and TDI (indicates nutrient impact) may be used to obtain a more reliable classification. It is nevertheless IPS which must chiefly be used for the classification.

ACID (Andren & Jarlman 2008) indicates acidity. The acidity index, however, gives no status class but only groups the lake or watercourse respectively in a pH-regime. ACID thus does not distinguish between what is naturally acidic and what is anthropologically acidified. That must be determined by use of physico-chemical assessment criteria for acidification, as described in Chapter 15.

Classifications according to these two indices function throughout Sweden and the reference values and class boundaries are the same for the whole country.

Requirements for supporting data

The classification must be based on sampling and analyses in accordance with SSEN 13946:2003 and SS-EN 14407:2005, or by another method which gives equivalent results. The latest version of the Agency's survey type: 'Periphyton in running water – diatom analysis' is also a good procedure to follow.

One sample per year, preferably taken in the late summer/autumn, is sufficient to classify the water quality, although several samples of course give a more reliable classification. It is important that the diatom analysis is carried out at the species level and also that the person conducting it has good knowledge of the species and makes use of sufficient taxonomic literature (described in the Swedish EPA's survey type: 'Periphyton in running water – diatom analysis'), since the most important source of error lies in the identification of species.

The software program Omnidia, available through CLCI (Catherine Lecointe Conseil Informatique) (http://perso.club-internet.fr/clci/tour_guide.htm) facilitates the calculation of IPS, %PT, TDI and ACID.

IPS index

IPS is calculated as follows: IPS = Σ AjIjVj/ Σ AjVj

Aj = the relative abundance in percentage of taxon j

Vj = the indicator value of taxon j (1-3, where a high value means that a taxon only tolerates limited ecological variations, i.e. it is a strong indicator)

Ij = the pollution sensitivity of taxon j (1-5, where high values show a high pollution sensitivity).

Results obtained according to the above formula are recalculated on a scale of 1-20 according to 4.75 * original index value – 3.75.

The ecological quality ratio (EQR) is calculated as follows:

EQR = calculated IPS / reference value

Reference values and class boundaries are given in Table A.16. As a complement to the IPS index, it is suggested that a computation of TDI and %PT, which show the diatoms' tolerance of nutrient impact and organic pollution respectively, should be carried out. TDI is calculated in the same way as IPS using TDI-specific indicator values and sensitivity values respectively. Results obtained according to the above formula are recalculated on a scale of 1-100 according to 25 * original index value – 25.

%PT is the sum of the relative abundance of all diatom species that are classed as organic pollution tolerant. These parameters are, however, only a support and it is IPS which indicates the status class. Class boundaries for TDI and %PT are given in Table A.17

Calculation of the index and support parameters can be carried out with the aid of the software program Omnidia. Indicator values and pollution sensitivity classifications for common diatoms in Sweden are also shown in the method description in the Agency's survey type: 'Periphyton in running water – diatom analysis'.

Table A.16 Reference values and class boundaries for IPS. Method-bound measure of uncertainty: Margin of error +/- 0.5 unit if IPS > 13, margin of error +/- 1 unit if IPS < 13.

Status	IPS value	EQR value
Ref Value	19.6	
High	≥17.5	≥0.89
Good	≥14.5 and <17.5	≥0.74 and <0.89
Moderate	≥11 and <14.5	≥0.56 and <0.74
Poor	≥8 and <11	≥0.41 and <0.56
Bad	<8	< 0.41

For status classification it is recommended to use the IPS values. Conversion to EQR values and use of these class boundaries gives the same result but can be an unnecessary step in the calculation in normal cases. If the assessment is nonetheless that the lake or watercourse respectively is naturally nutrient-rich, the reference value can be adjusted and in that case the EQR class boundaries are used to obtain the same deviation from the reference value as before.

Table A.17 The class boundaries for the support parameters %PT and TDI may be used to distinguish the classes further in uncertain cases (it is however IPS that gives the main status classification)

Status	%PT	TDI
Reference value	-	-
High	< 10	< 40
Good	< 10	40-80
Moderate	< 20	40-80
Poor	20-40	> 80
Bad	> 40	> 80

ACID index

The acidity index ACID is calculated as follows:

ACID = [log((ADMI/EUNO)+0.003))+2.5] + [log((circumneutral+alkaliphile+alkalibiont)/ /(acidobiont+acidophile))+0.003)+2.5]

A numerator or denominator = 0 is replaced by 1, when the relative abundance is expressed as a percentage. In Omnidia the relative abundance of van Dam groups is given per mille, and 0 is then replaced by 10.

The first part of the index is based on the ratio between the relative abundance of *Achnanthidium minutissimum* (ADMI) and the genus *Eunotia* (EUNO). The second part of the index takes into account all diatoms in the sample and is based on the following classification (van Dam et al. 199410), which is given in the software program Omnidia:

- acidobiont - mainly present at pH <5.5;
- acidophile - mainly present at pH <7;
- circumneutral - mainly present at pH values around 7;
- alkaphile - mainly present at pH >7;
- alkalibiont - only present at pH >7.

Class boundaries between the various acidity classes are given in Table A.18

Table A.18 Assessment of acidity in lakes and watercourses with the aid of diatoms (acidity index ACID). Division into five acidity classes. The classes show different stages of acidity and do not relate to status. Corresponding mean and minimum pH is also given. Method-bound measure of uncertainty: Margin of error ± 10%.

Acidity classes	Acidity index ACID	Corresponds to mean pH (of the 12 months preceding sampling)	Corresponds to minimum pH (during the 12 months preceding sampling)
Alkaline	7.5	7.3	-
Almost neutral	5.8-7.5	6.5-7.3	-
Moderately acidic	4.2-5.8	5.9-6.5	< 6.4
Acidic	2.2-4.2	5.5-5.9	< 5.6
Highly acidic	< 2.2	< 5.5	< 4.8

The acidity classes relate to the reaction of diatoms to pH changes. For the quality factors benthic fauna in lakes and watercourses, and phytoplankton in lakes, there are also acidity classes bearing the same names. Since e.g. benthic fauna do not react as quickly as diatoms to a reduction in pH, their attribution to classes is somewhat different. That is fully in line with the Water Framework Directive. It is the biological response that must be measured. Since different quality factors have different sensitivities to impact they will in certain cases result in different status classes for the same body of water. Because the operating principle is that the worst quality factor determines the classification, this ensures that the most sensitive quality factor is also protected.

Management of uncertainty

To make a good classification, it is appropriate to use data from a number of samplings. Several readings give a more reliable classification and an uncertainty interval in the form of a standard deviant can be calculated for the parameter in the water body in question. In cases where only data from one year is available, the fixed value for method-bound uncertainty for IPS or ACID given in Tables 5.1 and 5.3 may be used. In cases where the uncertainty interval around the calculated value overlaps any of the class boundaries between high and good status, or between good and moderate status, it means that the calculated value lies very close to a class boundary. For this reason, a reasonability assessment should be made, as described in Chapter 4.1.1 of the main handbook. See also Chapter 4.1.2 in the main handbook for more guidance on how to handle uncertainty.

Human impact or natural ?

If the lake or the watercourse is classified in one of the acidity classes ‘moderately acidic’, ‘acidic’ or ‘highly acidic’, an assessment must be made about whether the acidity conditions are anthropogenic in origin or whether the lake or the watercourse is naturally acidic. A more thorough analysis should be made with the aid of the assessment criteria for acidification in accordance with Chapter 15. The analysis can be further improved by making an assessment of the impact or stress caused by the acidification. The impact of forestry, for example, can provide important evidence about this. Furthermore, data on deposits may be used if analyses of large areas are to be made. If the assessment is that the water is naturally acidic, a reference value for pH for the water body should be calculated in accordance with Chapter 15. The pH reference value is compared with the pH values which correspond with the acidity classes for diatoms (Table 5.3). The acidity class for which the interval for mean pH covers the calculated reference value for pH corresponds to high status. The subsequent classes correspond to good, moderate, poor and bad status following the order of descending pH values.

When the status classification results in a ‘moderate’, or worse, status it may be necessary to make an assessment whether that is a result of anthropogenic eutrophication or whether the lake is naturally nutrient-rich. However, it is not particularly common for lakes or watercourses to have naturally high nutrient content. In order to evaluate this, a comparison can be made with results for the assessment criterion for phosphorus. The assessment can further be improved by looking at the impacts/pressures on the water body. Source distribution data, historical data, etc. provide important supporting material, produced in connection with the characterisation. If the evaluation that the lake or watercourse is naturally rich in nutrients is made, on the basis of an expert assessment by the water authority, a revision of the reference value for the specific water body should be made. In this case, the EQR class boundaries in Table 5.1 are used instead of the stated IPS values. The calculated IPS value for the water body is divided by the new reference value, to obtain an EQR that is then compared with the EQR class boundaries.

Verification of Swedish stream method for use in lakes

Why verification of stream index for lakes ?

The Swedish stream method with the main indices used for classification IPS ((Indice de Polluo-sensibilité Spécifique, eutrophicatipo and organic pollution) and ACID (ACidicty Index for Diatoms, used only to assess acidity, not ecological status) and supporting parameters %PT (Pollution Tolerant valves) and TDI (Trophic Diatom Index) (both used to support IPS classification) has been tested for its use in lakes since 2008 because the stream method is very well accepted and several pilot studies have shown that it functions in a similar way for Swedish lakes. If it could be used, Sweden would have the advantage to make use of a readymade method where errors already have been removed to a great deal, and which is accepted among the users.

Reference lakes

Reference condition setting - how we have set RC in our method. 25 lakes with spread over entire Sweden have been selected as reference lakes. They had to pass a national reference filter (Johnson et al 2003, cited in lake background report Kahlert et al. 2007) with chemical and landuse thresholds. In short, lakes had to pass the following:

National reference criteria for lakes

Tot-P < 10 µg/l or no eutrophication (arealspecific loss of Tot-P = class 1; in case of missing data for calculation of arealspecific loss: Tot-P < 20 µg/l AND colour > 100 mg Pt/l), no acidification, land use: < 20 % farming, < 0,1 % urban area.

The same lakes were used in the EU lake intercalibration, where they passed in principle the same reference filter (EU, Kaelly et al. submitted):

- No point sources of pollution
- Population density < 15 people per square kilometre
- <0.4% artificial land use
- < 20% agriculture in the catchment, not adjacent to lake (low intensity stock raising on semi-natural landscapes excluded)*
- <10% of lake shoreline is artificial*
- No alteration of natural lake hydrology (i.e.. no dams or similar structures)
- no introductions of carp or other bottom-feeding fish
- no intensive (commercial) fishing

The indices IPS, TDI and %PT and ACID have been calculated for theses 25 lakes and it has been checked if they were significantly different from the index values for the stream method, which they were not (Kahlert 2009). Therefore, it was concluded that the Swedish stream IPS reference value of 19,6 can be used for lakes as well (Kahlert 2009).

Verifying lakes in classes in classes from “good” to “poor”

First study (included in EU intercalibration as well):

29 lakes in total were used to verify the class boundaries. The lakes were classified into different ecological status classes using only non-diatom Swedish metrics (Tot-P, Secchi, Chl a, *Swedish handbook 2007:4)* to ensure the exclusion of a circle argumentation. Some lakes were sampled several more than once (once a year, several years), and some lakes were sampled horizontally at one occasion to ensure that sampling at one place would be enough. The assessment of ecological status class was done for an entire year because Sweden assumes the diatoms to integrate about 12 months of water chemistry (Kahlert 2007), i.e. a lake in some few occasions could belong to class god in one year but moderate in another. 12 lakes were assessed as good, 14 lakes as moderate, 4 as poor and 2 as bad. Additionally, 5 lakes without sufficient background data to assess an independent ecological status were included in the test-set, as they at least had Tot-P values to compare the stress of nutrients.

First, the repeated horizontal sampling showed that one sample per lake is sufficient to reflect the ecological status of a lake, as it also is for streams (Kahlert & Gottschalk 2009). Then, all Swedish indicators were calculated and compared with a) the range of indices in the different ecological classes derived in the stream study and b) the nutrient and pH background values were compared for the respective stream and lake classes. Both index values and nutrient and pH values were not significantly different from each other

(Kahlert 2009). The diatom taxa were not exactly the same for streams and lakes, but their index values were shown to be similar. Therefore, it was assumed that the same ecological boundaries as for the streams can be used. The good/moderate boundary is then the IPS value where the nutrient tolerant and pollution tolerant diatom taxa exceeded a relative abundance of ca. 30% (and the amount of sensitive taxa falls below ca. 30%). The detailed class table can be found in the method.

Second study (additional sampling of lakes to confirm stress test filling gaps in Tot-P gradient, not included in EU intercalibration):

Additionally to the lake set above, samples were taken in additionally 41 lakes to confirm the nutrient stress test filling gaps in Tot-P gradient. Those lakes were not pre-classified into an ecological status class but are all included in chemical monitoring programs with Tot-P measurements.

All samples from first and second study were used for index calculation. IPS and ACID was plotted against pH respectively Tot-P (mean for 12 months before diatom sampling, see figure). The outcome of these stress tests were the same and not significantly different from each other (Kahlert 2009, and Kahlert & Gottschalk, 2014). IPS had the same strong relationship for with TP when tested in lakes, as well as ACID had with pH (see figure). Regarding Tot-P background values, the good-moderate boundary separates streams and lakes with a Tot-P < 60 µg/l (high and good status) from those with Tot-P higher than 60 µg/l.

Therefore it was concluded that the indices reflect the same ecological background in streams as well as in lakes and it was confirmed that the stream method and class boundaries can be used in the same way.

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A.9 UK: DARLEQ mark 2 (Diatoms for Assessing River and Lake Ecological Quality)

Official references: Biological Method Statement (Lake Phytobenthos): http://www.wfduk.org/resources%20/lake-%E2%80%93-phytobenthos

R&D report: Bennion, H., Burgess, A., Juggins, S., Kelly, M., Reddihough, G., Yallop, M. (2012). Assessment of ecological status in UK lakes using diatoms. Report SC070034/TR3, Environment Agency, Bristol.

Sampling and data analysis:

Samples should be collected by brushing or scraping the upper surface of cobbles or small boulders obtained from the littoral zones of lakes in order to remove the biofilm. Where the bed of the lake is dominated by fine sediments, samples should be collected from submerged stems of emergent macrophytes such as Phragmites australis, Sparganium erectum, Glyceria maxima or Typha species. The sampling method used should follow the general principles set out in the standard method EN 13946 : 2003 Water quality – Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers. Samples should be collected twice a year – in Spring and Autumn.

Samples should be analysed to identify the presence, and number of valves, of all diatom taxa. The analytical method used should conform to EN 14407 : 2004 Water quality – Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters. The Lake Trophic Diatom Index is calculated using a weighted average equation.

Metrics and calculation of final EQR.

Full taxon list and calculation method is given in

http://www.wfduk.org/resources%20/lake-%E2%80%93-phytobenthos

Reference condition setting

Reference conditions for phytobenthos were established alongside reference conditions for other BQEs. For N GIG, this involved screening based on P and chlorophyll concentrations performed as part of the REBECCA project (Carvalho et al., 2008) whilst for Central GIG, lakes were considered to be at reference if they had have no point sources of P, <10% non-natural land use and <10 inhabitants km-2. Evidence from palaeolimnology was also considered (Bennion et al., 2004). For more details see Bennion et al. 2012, Carvalho et al 2008.

Boundary setting:

- High/Good: 25th percentile of EQRs for reference samples
- Good/moderate: “crossover” between “sensitive” and “tolerant” diatom species

Pressures addressed:

The method is calibrated against a eutrophication gradient, expressed as TP (Table A.19).

The regression for LA lakes is signficant; the low R2 for LA lakes is due to the short gradient (lack of nutrient-impacted LA lakes)

Table A.19 Regression characteristics (UK diatom EQR against eutrophication gradient epressed as Total Phosphorus)

Type	Significance of regression (by ANOVA)	R2
LA	P = 0.0116	0.088
MA	P < 0.001	0.375
HA	P < 0.001	0.291

References

Bennion, H., Burgess, A., Juggins, S., Kelly, M., Reddihough, G., Yallop, M. (2012). Assessment of ecological status in UK lakes using diatoms. Report SC070034/TR3, Environment Agency, Bristol.

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B. Using (River) Trophic Index for assessment of the lake trophic status

Introduction

Phytobenthos and macrophytes are one biological element under the Water Framework Directive (Directive 2000/60/ES). In Slovenian lake ecological classification system both sub-elements are used together as one element. Phytobenthos assessment system consists of one metric - Trophic Index (Rott et al. 1998). Trophic Index is calculated as weighted average of the diatom taxa trophic values (TW), where taxa abundance (H), and taxa indicative weights (G) are weighting factors. Individual trophic values (TW) and indicative weights (G) were defined according to the occurrence of the diatom taxa along the eutrophication gradient in rivers (Rott et al. 1998).

The aim of our work is to show that (River) Trophic Index (Rott et al. 1998) can provide a reliable assessment of the trophic status of lakes using lake littoral diatoms.

Study area: Altogether, 13 lakes were investigated and 96 diatom samples were taken between 2005 and 2011 (Table B.1 and Table B.2).

Table B.1 The main characteristics of the studied lakes.

Lake	Ecoregion (Urbanič 2008)	Elevation (m a.s.l.)	Surface area (km^2)	Volume (Mio m^3)	Depth - maximum (m)	Average depth (m)
Blejsko jezero	Alps	475	1.43	26.6	31	19
Bohinjsko jezero	Alps	526	3.28	92.4	45	28
Družmirsko jezero	Alps	340	0.55	>12.0	87	24
Velenjsko jezero	Alps	367	1.35	25.0	55	19
Klivnik	Dinaric western Balkan	460	0.36	4.3	20	9
Mola	Dinaric western Balkan	450	0.68	4.3	12	6
Gajševsko jezero	Pannonian lowland	206	0.77	2.6	10	~3
Ledavsko jezero	Pannonian lowland	225	2.18	5.7	5	~3
Pernica 1	Pannonian lowland	245	0.57	1.2	4	3
Pernica 2	Pannonian lowland	245	0.66	2.1	4	3
Slivniško jezero	Pannonian lowland	292	0.84	4.0	14	5
Šmartinsko jezero	Pannonian lowland	265	1.07	6.5	12	6
Vogeršček	Po lowland	101	0.82	8.5	20	10

Table B.2 Number of sampling sites for each lake and year of sampling.

Lake/year	2005	2006	2007	2008	2009	2010	2011	Sum
Blejsko jezero	3		7		7	3		**20**
Bohinjsko jezero	3		7		7	3		**20**
Družmirsko jezero							3	**3**
Velenjsko jezero					3		3	**6**
Klivnik				3				**3**
Mola				3				**3**
Gajševsko jezero		3					3	**6**
Ledavsko jezero		3					3	**6**
Pernica 1		3						**3**
Pernica 2		3					3	**6**
Slivniško jezero					3		3	**6**
Šmartinsko jezero		5					3	**8**
Vogeršček		3		3				**6**
Sum	**6**	**20**	**14**	**9**	**20**	**6**	**21**	**96**

Physico-chemical parameters and chlorophyll *a* were measured 4 times a year in a vegetation period. Water samples were taken at the deepest part of the lake (Table B.3).

Table B.3 Minimum and maximum values of measured parameters of the whole dataset and used in the development and validation dataset for Lake Littoral Trophic Index.

	Dataset	Whole		Development		Validation	
Parameter	**Code**	**min**	**max**	**min**	**max**	**min**	**max**
Total Phosphorous – mean (µg/L)	TP-log	3,6	224,0	4,0	224,0	3,6	101,0
Total Nitrogen – mean (µg/L)	TN-log	296	1693	299	1693	296	1534
Secchi depth – mean (m)	Secchi depth	0,3	9,7	0,3	9,0	0,3	9,7
Chlorophyll *a* – mean (µg/L)	Chlorophyll	1,0	37,6	1,0	36,4	1,0	37,6

Trophic Index (TI)

Trophic Index (Rott et al. 1998) shows a response to eutrophication. In lakes of Slovenia we have found a good relationship between mean annual total phosphorous concentrations in lakes and the Trophic Index (Figure B.1).

Comparison of reference and non-reference sites in alpine lakes revealed significant differences in the Trophic Index (Figure B.2).

On average slightly more than 33 diatoma taxa were present in lake littoral samples (Figure B.8). The number of taxa ranged from 10 to 56, whereas in alpine lakes from 25 to 49 (Figure B.4 and Figure B.5). Number of indicator taxa used for the calculation of the Trophic Index ranged from 8 to almost 50, whereas in alpine lakes from 20 to 42 Figure B.5 and Figure B.6). Percentage of Trophic Index indicator taxa in the diatom sample was always relatively high and on average exceeds 80 % of the present diatom taxa. Only in one diatom sample indicator taxa represent <60% (Figure B.7). In the alpine lake littoral samples percentage of indicator diatom taxa was never below 70% whereas the mean percentage was even slightly higher in comparison to all considered samples (Figure B.8).

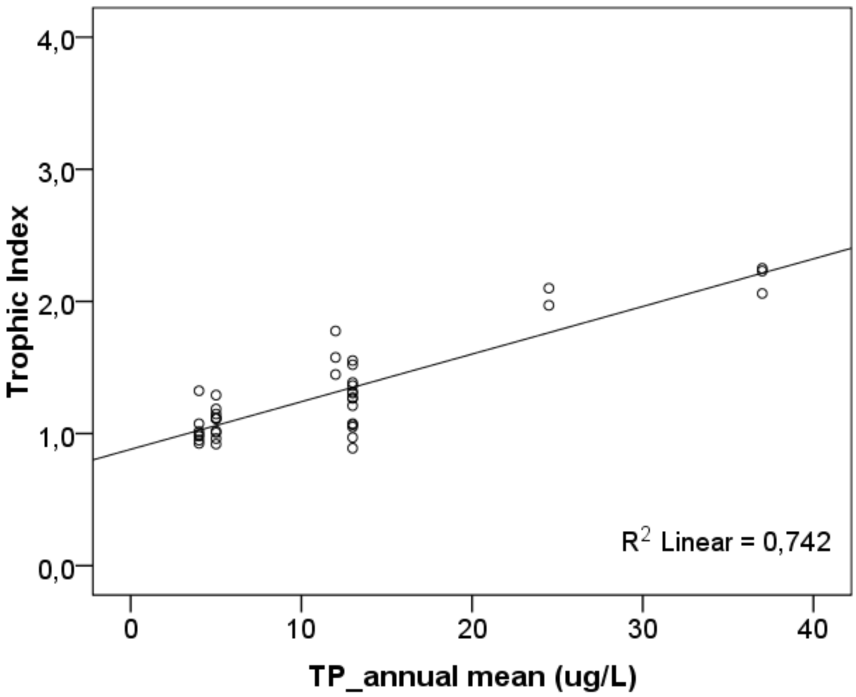

Figure B.1 Regression plots of the mean annual Total phosphorous vs. Trophic Index using diatom data from alpine lakes (Slovenian intercalibration dataset).

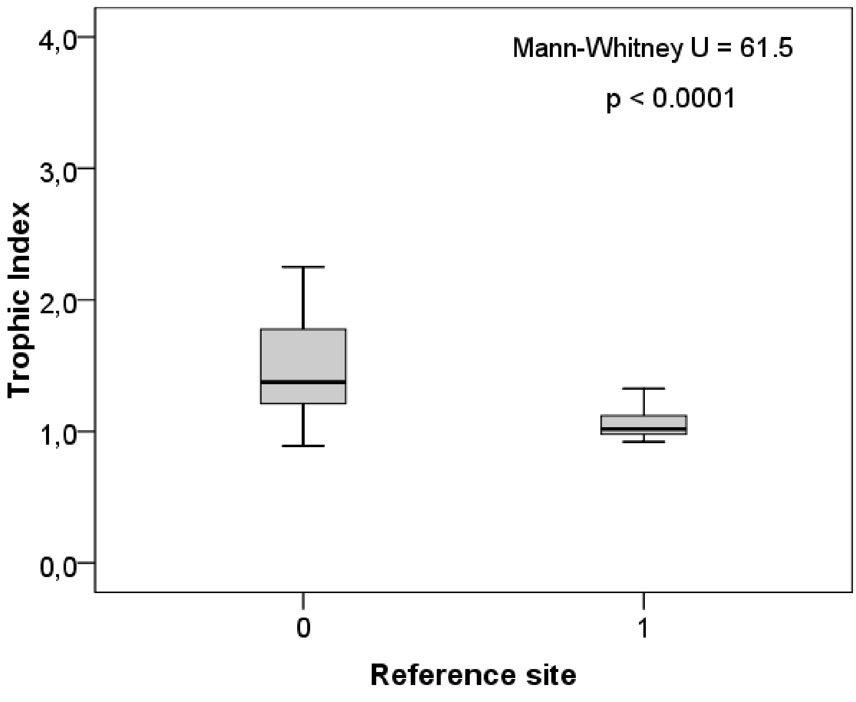

Figure B.2 Distribution of Trophic index values between reference (1) and impaired (0) sites of the alpine lakes (Slovenian intercalibration dataset) with the results of the Mann-Whitney U-test.

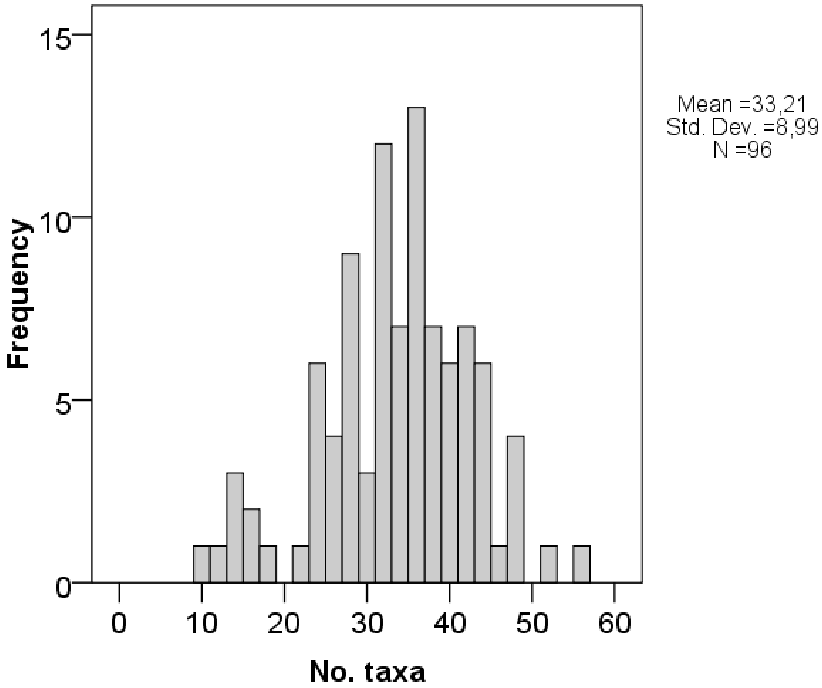

Figure B.3 Frequency distribution of number of taxa in lake littoral diatom samples.

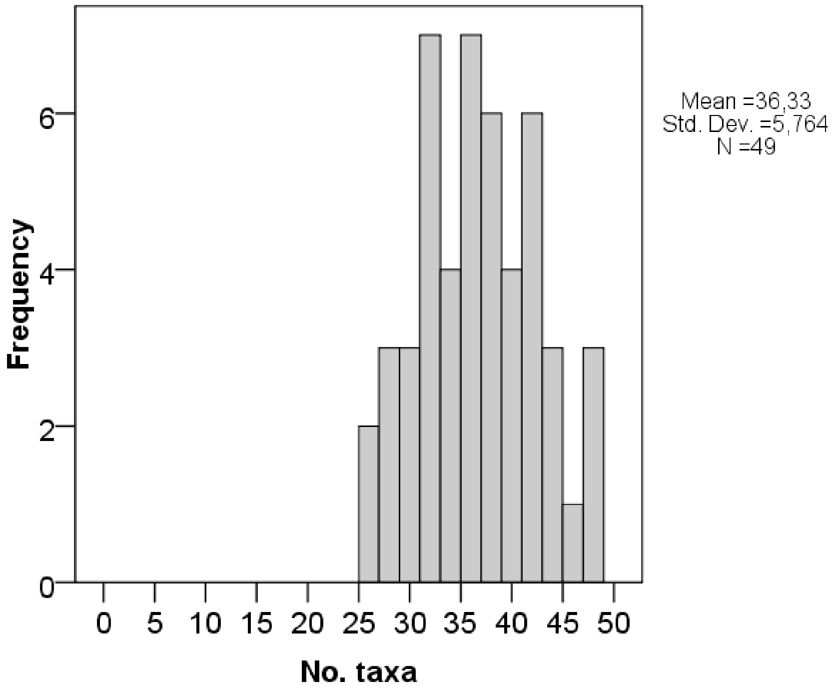

Figure B.4 Frequency distribution of number of taxa in alpine lake littoral diatom samples.

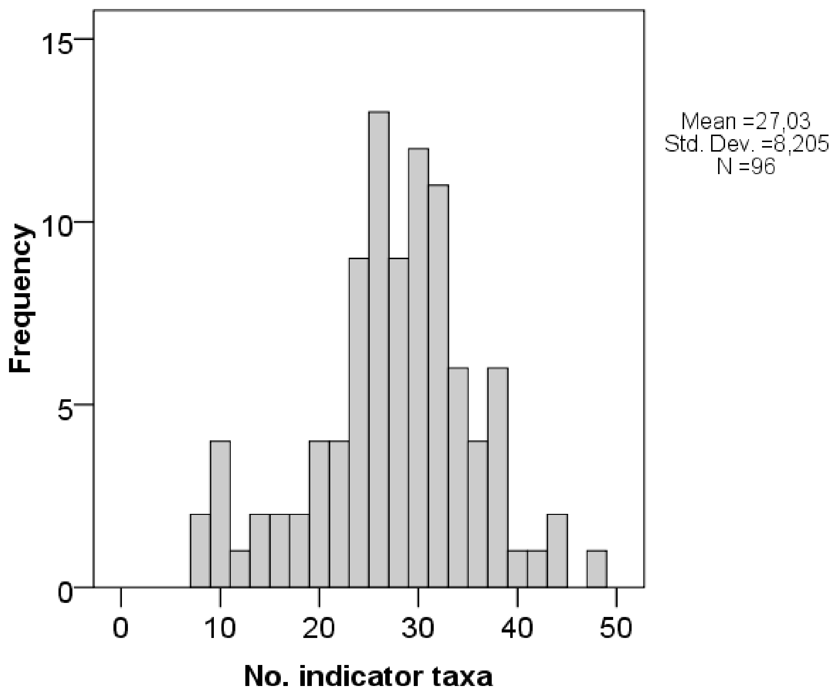

Figure B.5 Frequency distribution of number of Trophic Index indicator taxa in lake littoral diatom samples.

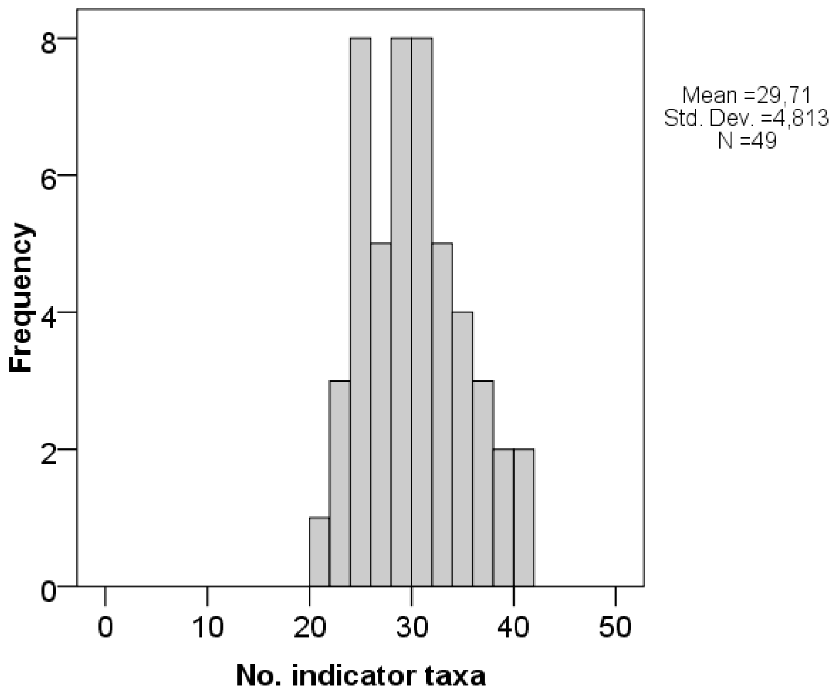

Figure B.6 Frequency distribution of number of Trophic Index indicator taxa in alpine lake littoral diatom samples.

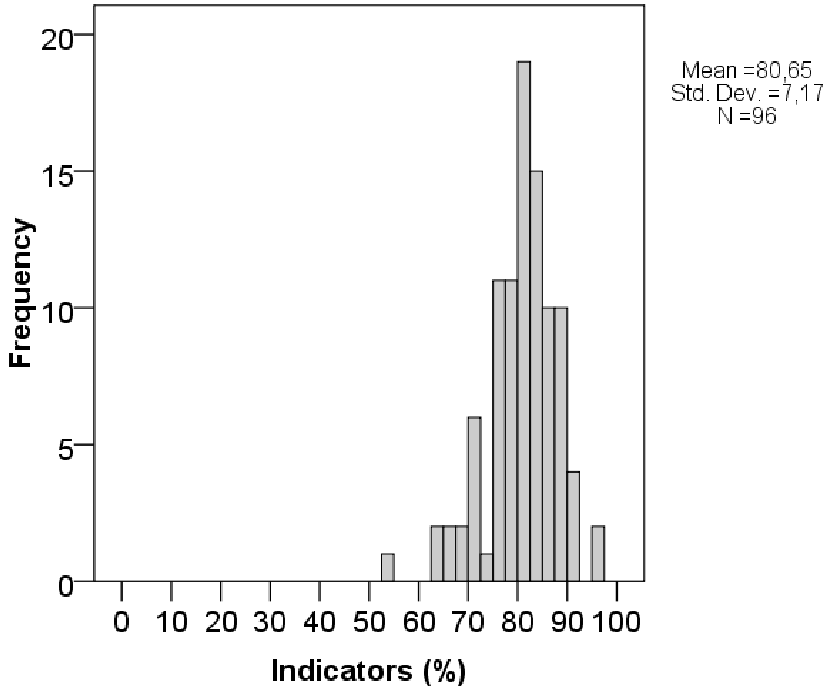

Figure B.7 Frequency distribution of percentage of Trophic Index indicator taxa in lake littoral diatom samples.

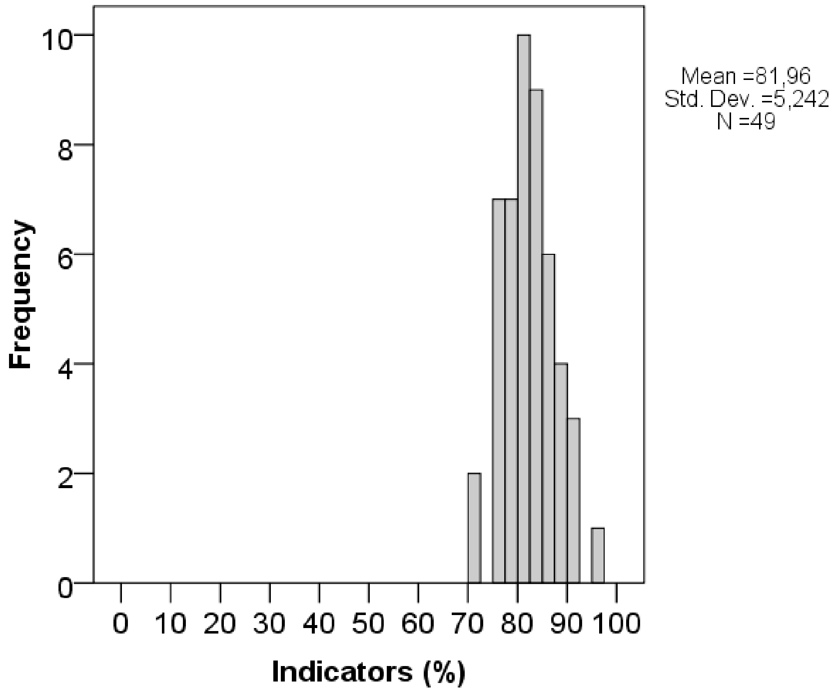

Figure B.8 Frequency distribution of percentage of Trophic Index indicator taxa in alpine lake littoral diatom samples.

Lake Littoral Trophic Index (LLTI)

A whole dataset was divided in a development dataset (62 sites) and a validation dataset (34 sites). A Lake Littoral Trophic Index was developed using a development dataset. A canonical correspondence analysis (CCA) was performed with 185 diatom taxa (Table B.6) and four environmental parameters (Table B.3 and Table B.4, Figure B.9).

Table B.4 Marginal (Lambda 1) and conditional (Lambda A) effects of the environmental parameters, P-value and F-value.

Variable-code	Lambda 1	Lambda A	P	F
Chlorophyll	0.45	0.45	0.001	6.76
TP-log	0.44	0.24	0.001	3.86
Secchi depth	0.37	0.15	0.001	2.45
TN-log	0.29	0.22	0.001	3.45

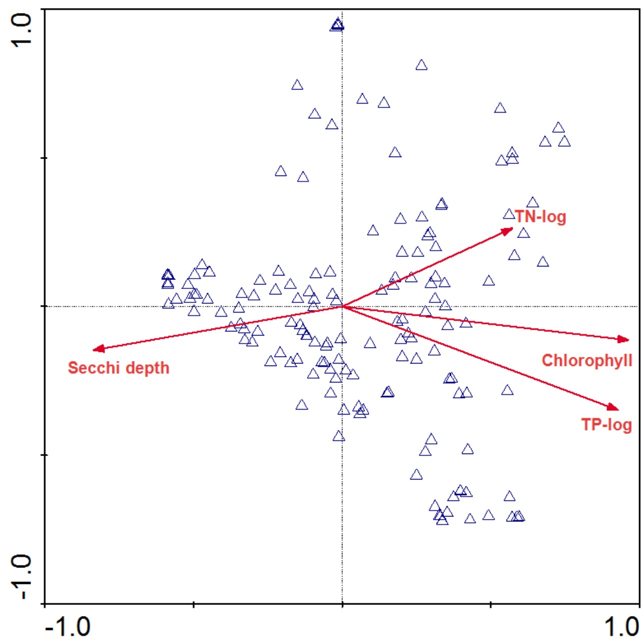

Figure B.9 CCA ordination diagram with 185 diatom taxa (open triangles) and four, environmental variables (arrows)

The LLTI was developed using trophic preferences (diatom trophic values - Dtv) and tolerance (trophic indicative weights - Tiw) of taxa along the first CCA axes. Diatom trophic values (Dtv) were then determined using CCA ordination axis 1 species scores (biplot scaling):

$$Dtv_i = \frac{SC\_CCA1_i}{SC\_CCA1_{max}} \qquad (1)$$

where SC_CCA1i is the CCA ordination axis 1 species score (biplot scaling) of the i-th taxon and SC_CCA1max is the absolute maximum value of the CCA ordination axis 1 species score (biplot scaling). Trophic indicative weights (Tiw) were determined using the CCA ordination axis 1 species tolerance (root mean squared deviation for species) according to Table B.5.

Table B.5 Determination of the trophic indicative weight (Tiw) from the CCA axis 1 species tolerance (root mean squared deviation for species).

Tolerance (t_i)	Tiw
$\mathbf{t_i}$ <0.2	5
0.2< $\mathbf{t_i}$ <0.4	4
0.4< $\mathbf{t_i}$ <0.6	3
0.6< $\mathbf{t_i}$ <0.8	2
$\mathbf{t_i}$ >0.8	1

The LLTI was calculated according to the following equation:

$$LLTI_j = \frac{\sum_{i=1}^{n} a_i * Dtv_i * Tiw_i}{\sum_{i=1}^{n} a_i * Tiw_i} \qquad (2)$$

where a_i is the abundance of the i-th taxon, Dtv_i is the diatom trophic value of the i-th taxon, Tiw_i is the trophic indicative weight of the i-th taxon and n is the number of indicative taxa.

A good relationship was observed between annual mean total phosphorous concentration (log value) and LLTI using development (R^2 = 0.85) and validation dataset (R^2 = 0.70) (Figure B.10 and Figure B.11). Statistically significant differences were observed in LLTI values between reference sites and impaired sites using data from all lakes (Mann-Whitney U = 71, p <0.0001) and from alpine lakes (Mann-Whitney U = 67, p <0.0001) (Figure B.12 and Figure B.13).

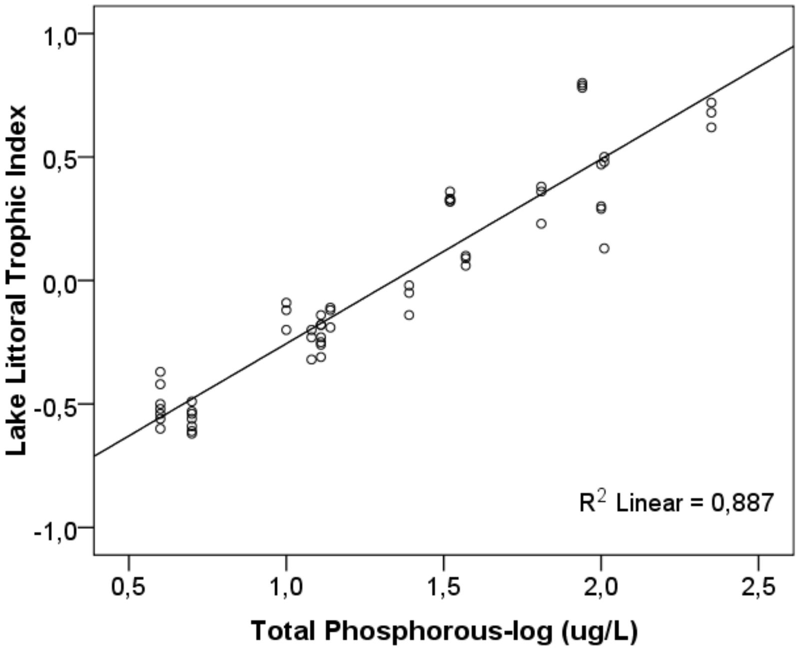

Figure B.10 *Regression plots of of the mean annual Total phosphorous (log value) vs. Lake Littoral Trophic Index using a diatom development dataset*

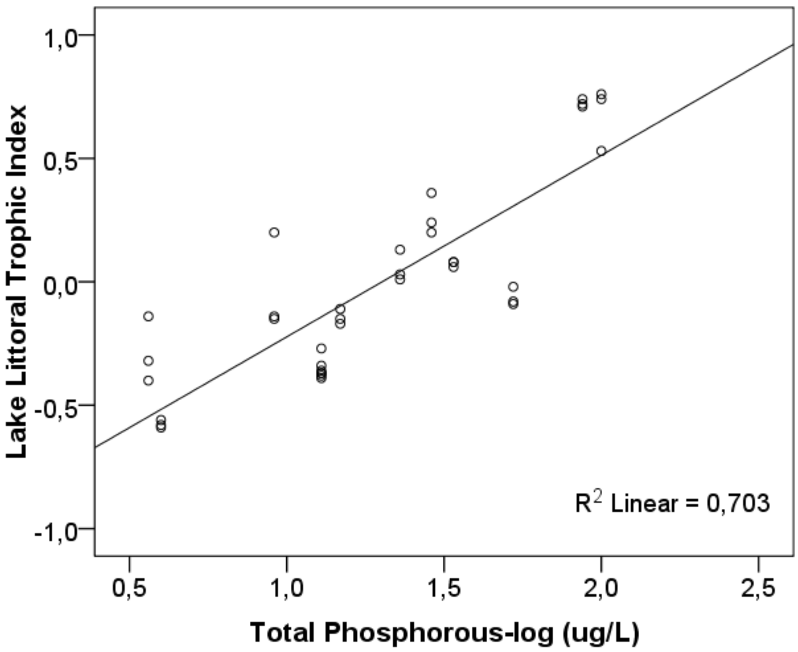

Figure B.11 *Regression plots of the mean annual Total phosphorous (log value) vs. Lake Littoral Trophic Index using a diatom validation dataset*

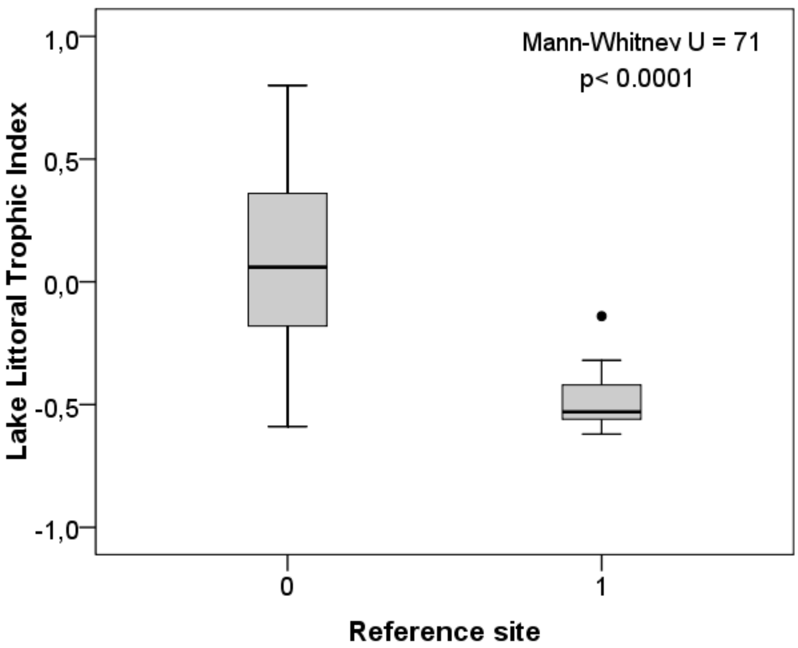

Figure B.12 *Boxplots of the Lake Littoral Trophic Index values recorded at reference (1) and impaired (0) sites with the results of the Mann-Whitney U-test*

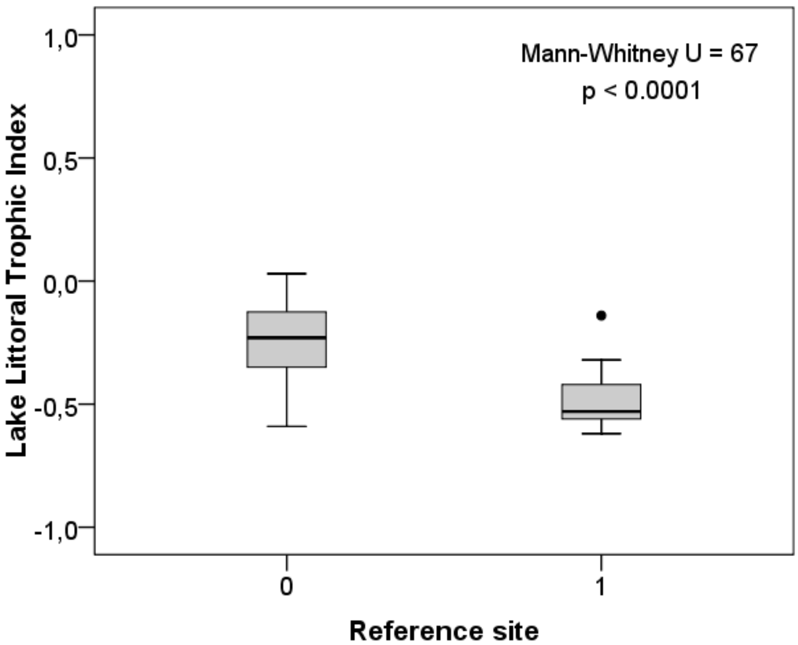

Figure B.13 *Boxplots of the Lake Littoral Trophic Index (LLTI) values recorded at reference (1) and impaired (0) sites of the alpine lakes with the results of the Mann-Whitney U-test*

Trophic Index (TI) vs. Lake Littoral Trophic Index (LLTI)

A good relationship was observed between TI and LLTI using samples from all lakes (R^2 = 0.85) and alpine lakes (R^2 = 0.74) (Figure B.14 and Figure B.15).

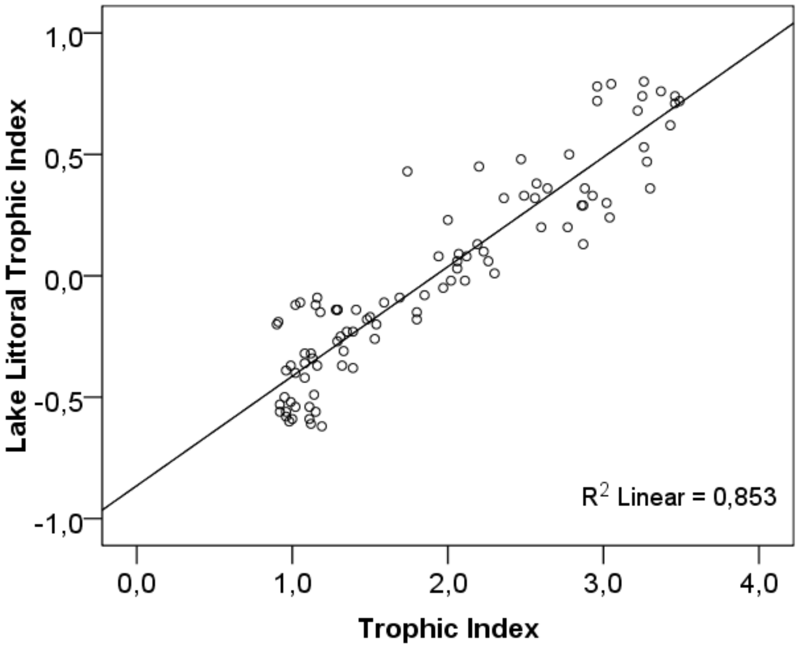

Figure B.14 Regression plots of the Trophic Index vs. Lake Littoral Trophic Index using data from all lakes.

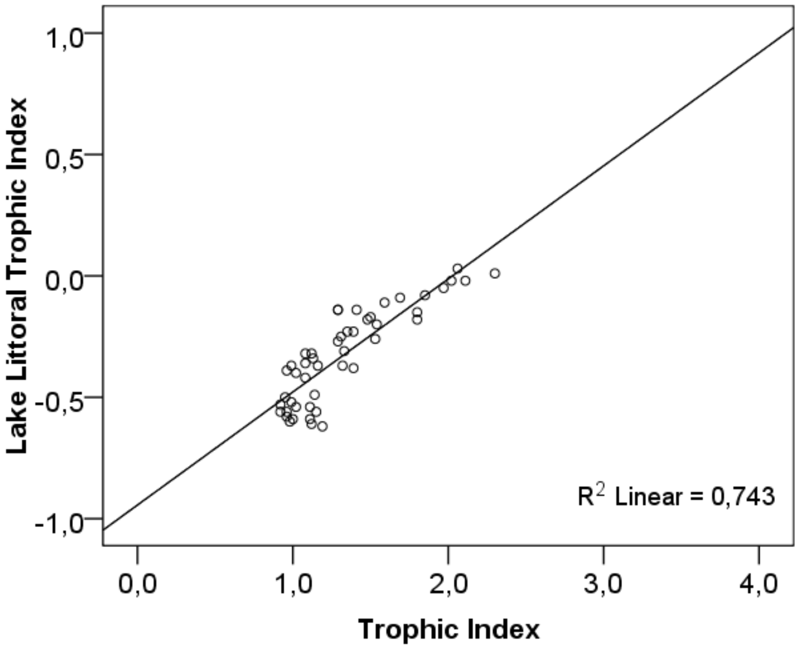

Figure B.15 Regression plots of the Trophic Index vs. Lake Littoral Trophic Index using data from alpine lakes.

Summary

1. (River) Trophic Index (TI) showed good relationship with lake total phosphorous concentrations.
3. Reference sites showed statistically significantly lower TI values than impaired sites.
4. High percentage of diatom taxa occurring in considered littoral samples have assigned trophic values (TW) according to Rott et al. (1998); on average >80% of recorded taxa. In alpine lakes >70% of diatom taxa recorded in each littoral sample were indicator taxa.
5. In the each littoral diatom sample at least eight TI indicator taxa were recorded, whereas on average >25. In samples of alpine lakes at least 20 indicative taxa occurred in the each sample.
6. A relationship between lake littoral diatom taxa and environmental variables representing eutrophication gradient in lakes was tested using Canonical correspondence analysis. Data were collected from varied lake types (lowland and alpine lakes).
7. A new Lake Littoral Trophic Index (LLTI) was developed using littoral diatom data and four environmental variables representing eutrophication gradient.
8. Lake Littoral Trophic Index (LLTI) showed good relationship with mean annual total phosphorous concentration (log data) using development (R^2 = 0.85) and validation dataset (R^2 = 0.70).
9. Reference sites showed statistically significantly lower LLTI values than impaired sites using all data and alpine data.
10. (River) Trophic Index showed a good relationship with new developed Lake Littoral Trophic Index (LLTI) using samples from all lakes (R^2 = 0.85) and alpine lakes (R^2 = 0.74).

Conclusions

(River) Trophic Index (TI) showed a good relationship with the eutrophication gradient. A statistically significant difference in TI was observed between reference and impaired sites and high percentage of recorded littoral diatom taxa was indicative according to TI in all samples. Moreover, a new developed littoral diatom-based trophic index (LLTI) was highly correlated with the (River) Trophic Index using all data and alpine data only. Thus, diatom-based (River) Trophic Index might considerable well address eutrophication pressure in lakes, although lake littoral diatom specific indices might be more applicable.

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Table B.6 List of developed diatom trophic values (Dtv) and trophic indicative weights (Tiw) of diatom taxa for calculation of the Lake Littoral Trophic Index (LLTI).

Taxon	Omnidia code	Dtv	Tiw
Achnanthes helvetica	AHAL	-0,44	5
Achnanthes biasolletiana	ABIA	-0,28	5
Achnanthes bioretii	ABIO	-0,78	5
Achnanthes clevei	ACLE	0,49	1
Achnanthes exiqua	AEXG	0,49	1
Achnanthes flexella	AINF	-0,37	5
Achnanthes hungarica	AHUN	0,80	1
Achnanthes lanceolata	ALAN	0,25	3
Achnanthes minutissima v. gracillima	AMGR	-0,65	5
Achnanthes minutissima	AMIN	-0,04	5
Achnanthes oblongella	AOBG	0,09	5
Achnanthes sp.	ACHS	0,42	2
Achnanthes minutissima v.saprophila	AMSA	0,80	1
Amphora aequalis	AAEQ	-0,02	5
Amphora montana	AMMO	0,39	2
Amphora ovalis	AOVA	0,37	2
Amphora lybica	ALIB	0,34	3
Amphora pediculus	APED	0,13	4
Amphora sp.	AMPS	-0,79	5
Amphipleura pellucida	APEL	-0,12	5
Brachysira vitrea	BVIT	-0,66	5
Anomoeoneis sphaerophora	ASPH	0,45	2
Anomoeoneis vitrea	AVIT	-0,07	5
Asterionella formosa	AFOR	0,19	4
Caloneis alpestris	CAPS	-0,44	5
Caloneis amphisbaena	CAMP	-0,02	5
Caloneis bacillum	CBAC	-0,13	5
Caloneis silicula	CSIL	0,36	2
Fragilaria arcus	FARC	-0,02	5
Cocconeis pediculus	CPED	-0,13	5
Cocconeis placentula	CPLA	0,31	3
Cyclotella meneghiniana	CMEN	0,75	1
Cyclotella ocellata	COCE	0,45	2
Cyclotella sp.	CYLS	0,36	2
Cymatopleura elliptica	CELL	-0,05	5

Taxon	Omnidia code	Dtv	Tiw
Cymatopleura solea	CSOL	-0,20	5
Cymbella affinis	CAFF	-0,30	5
Cymbella amphycephala	CAPH	-0,05	5
Cymbella caespitosa	CCAE	0,02	5
Cymbella cesatii	CCES	-0,69	5
Cymbella cistula	CCIS	0,20	4
Cymbella delicatula	CDEL	-0,75	5
Cymbella ehrenbergii	CEHR	0,07	5
Cymbella falaisensis	CFAL	-0,18	5
Cymbella helvetica	CHEL	-0,60	5
Cymbella incerta	CINC	-0,19	5
Cymbella lanceolata	CLAN	-0,01	5
Cymbella microcephala	CMIC	-0,23	5
Cymbella minuta	CMIN	-0,38	5
Cymbella naviculiformis	CNAV	-0,79	5
Cymbella sp.	CYMS	0,78	1
Cymbella prostrata	CPRO	0,33	3
Cymbella silesiaca	CSLE	-0,29	5
Cymbella sinuata	CSIN	-0,61	5
Cymbella tumida	CTUM	0,53	1
Denticula kuetzingii	DKUE	-0,12	5
Denticula tenuis	DTEN	-0,68	5
Diatoma moniliformis	DMON	-0,08	5
Diatoma vulgaris	DVUL	-0,20	5
Diploneis elliptica	DELL	0,24	3
Diploneis oblongella	DOBL	-0,02	5
Diploneis ovalis	DOVA	-0,79	5
Diploneis subconstricta	DSCO	-0,79	5
Epithemia sorex	ESOR	-0,12	5
Epithemia adnata	EADN	-0,54	5
Eunotia arcus	EARC	-0,63	5
Eunotia bilunaris	EBIL	-0,28	5
Fragilaria capucina	FCAP	-0,05	5
Fragilaria capucina v. austriaca	FCAU	-0,47	5
Fragilaria capucina v. capucina	FCAP	-0,23	5
Fragilaria capucina v. distans	FCDI	-0,02	5
Fragilaria construens	FCON	-0,07	5
Fragilaria crotonensis	FCRO	0,26	3
Fragilaria leptostauron	FLEP	-0,78	5
Fragilaria pinnata	FPIN	-0,16	5
Fragilaria capucina v. vaucheriae	FCVA	-0,05	5
Frustulia vulgaris	FVUL	-0,13	5
Gomphonema angustatum	GANG	-0,79	5
Gomphonema augur	GAUG	0,49	1
Gomphonema clavatum	GCLA	-0,50	5

Taxon	Omnidia code	Dtv	Tiw
Gomphonema gracile	GGRA	0,31	3
Gomphonema micropus	GMIC	-0,32	5
Gomphonema minutum	GMIN	-0,23	5
Gomphonema pumilum	GPUM	-0,40	5
Gomphonema olivaceum	GOLI	0,21	4
Gomphonema parvulum	GPAR	0,46	1
Gomphonema sp.	GOMS	-0,09	5
Gomphonema truncatum	GTRU	0,37	2
Gyrosigma acuminatum	GYAC	0,71	1
Gyrosigma attenuatum	GYAT	0,56	1
Gyrosigma nodiferum	GNOD	0,47	1
Gyrosigma scalproides	GSCA	0,80	1
Gyrosigma spencerii	GSPE	0,77	1
Hantzschia amphioxys	HAMP	0,30	3
Aulacoseira granulata	AUGR	0,77	1
Melosira varians	MVAR	0,10	5
Navicula atomus	NATO	0,74	1
Navicula bacillum	NBAC	-0,79	5
Navicula bryophyla	NBRY	-0,78	5
Navicula cari	NCAR	0,08	5
Navicula capitata	NCAP	0,42	2
Navicula cincta	NCIN	0,46	1
Navicula citrus	NCIT	0,90	1
Navicula clementis	NCLE	0,24	3
Navicula contenta	NCON	-0,78	5
Navicula cryptocephala	NCRY	0,42	2
Navicula capitatoradiata	NCPR	0,26	3
Navicula veneta	NVEN	0,42	2
Navicula cuspidata	NCUS	0,50	1
Navicula elginensis	NELG	0,45	2
Navicula gallica	NGAL	-0,78	5
Navicula gallica v. perpusilla	NGPE	-0,78	5
Navicula halophila	NHAL	-0,13	5
Navicula goeppertiana	NGOE	0,86	1
Navicula gregaria	NGRE	0,53	1
Navicula lanceolata	NLAN	0,56	1
Navicula menisculus	NMEN	0,18	4
Navicula oblonga	NOBL	-0,02	5
Navicula protracta	NPRO	0,14	4
Navicula pupula	NPUP	0,27	3
Navicula pygmaea	NPYG	0,53	1
Navicula placentula	NPLA	-0,78	5
Navicula radiosa	NRAD	-0,15	5
Navicula cryptotenella	NCTE	-0,18	5
Navicula reichardtiana	NRCH	0,01	5

Taxon	Omnidia code	Dtv	Tiw
Navicula reinhardtii	NREI	0,45	2
Navicula rhynchocephala	NRHY	-0,78	5
Navicula schroeteri	NSHR	0,75	1
Navicula sp.	NASP	-0,03	5
Navicula splendicula	NSPD	0,45	2
Navicula subalpina	NSBN	-0,46	5
Navicula trivialis	NTRV	0,39	2
Navicula tripunctata	NTPT	0,56	1
Navicula tuscula	NTUS	-0,66	5
Navicula viridula	NVIR	0,91	1
Navicula viridula v. rostellata	NVRO	0,33	3
Neidium ampliatum	NEAM	0,47	1
Neidium binodis	NBID	-0,02	5
Neidium dubium	NEDU	0,27	3
Nitzschia acicularis	NACI	0,56	1
Nitzschia amphibia	NAMP	0,58	1
Nitzschia angustata	NIAN	-0,67	5
Nitzschia angustatula	NZAG	-0,03	5
Nitzschia constricta	NZCO	0,45	2
Nitzschia capitellata	NCPL	0,05	5
Nitzschia dissipata	NDIS	-0,03	5
Nitzschia dubia	NDUB	-0,13	5
Nitzschia fonticola	NFON	-0,40	5
Nitzschia frustulum	NIFR	0,66	1
Nitzschia gisela	NGIS	-0,78	5
Nitzschia heufleriana	NHEU	0,45	2
Nitzschia incospicua	NINC	0,49	1
Nitzschia levidensis	NLEV	0,79	1
Nitzschia linearis	NLIN	0,42	2
Nitzschia littoralis	NLIT	0,45	2
Nitzschia microcephala	NMIC	-0,20	5
Nitzschia palea	NPAL	0,40	2
Nitzschia paleacea	NPAE	0,76	1
Nitzschia recta	NREC	-0,18	5
Nitzschia sigmoidea	NSIO	0,48	1
Nitzschia sinuata	NSIN	0,27	3
Nitzschia sinuata v. delognei	NSDE	0,49	1
Nitzschia sp.	NZSS	0,23	4
Nitzschia umbonata	NUMB	0,45	2
Nitzschia tryblionella	NTRY	0,82	1
Pinnularia viridis	PVIR	-0,45	5
Rhiocosphenia abbreviata	RABB	0,40	2
Rhopalodia gibba v. minuta	RGMI	-0,79	5
Rhopalodia gibba	RGIB	0,45	2
Stauroneis anceps	STAN	-0,79	5

Taxon	Omnidia code	Dtv	Tiw
Stauroneis smithii	SSMI	-0,02	5
Stephanodiscus sp.	STSP	0,97	1
Surirella angusta	SANG	0,66	1
Surirella bifrons	SBIF	0,44	2
Surirella brebissonii	SBRE	0,45	2
Surirella biseriata	SBIS	-0,02	5
Surirella minuta	SUMI	0,44	2
Surirella ovalis	SOVI	-0,02	5
Surirella tenera	SUTE	1,00	1
Fragilaria ulna v. acus	FUAC	0,72	1
Fragilaria parasitica	FPAR	0,49	1
Fragilaria capucina v.rumpens	FCRP	-0,13	5
Fragilaria ulna	FULN	-0,16	5
Tabellaria flocculosa	TFLO	-0,60	5
Thalassiosira weisflogii	TWEI	0,45	2

European Commission
EUR 26512 EN – Joint Research Centre – Institute for Environment and Sustainability

Title: Water Framework Directive Intercalibration Technical Report: Phytobenthos ecological assessment methods

Authors: Martyn Kelly, Éva Ács, Vincent Bertrin, Helen Bennion, Gábor Borics, Amy Burgess, Luc Denys, Frauke Ecke, Maria Kahlert, Satu Maaria Karjalainen, Bryan Kennedy, Aldo Marchetto, Soizic Morin, Joanna Picinska - Fałtynowicz, Geoff Phillips, Ilka Schönfelder, Jörg Schönfelder, Gorazd Urbanič, Herman van Dam, Tomasz Zalewski

Edited by Sandra Poikane

Luxembourg: Publications Office of the European Union

2014– 121 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424 (online), ISSN 1018-5593 (print)

ISBN 978-92-79-35468-7 (pdf)
ISBN 978-92-79-35469-4 (print)

doi: 10.2788/7466

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 cross-GIG Phytobenthos group.

LB-NA-26512-EN-N

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ISBN 978-92-79-35468-7