Water Framework Directive Intercalibration Technical Report: Alpine Lake Phytoplankton ecological assessment methods

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Georg Wolfram, Fabio Buzzi, Martin Dokulil, Maria Friedl, Eberhard Hoehn, Christophe Laplace-Treyture, Maud Menay, Aldo Marchetto, Giuseppe Morabito, Markus Reichmann, Špela Remec-Rekar, Ursula Riedmüller, Gorazd Urbanič

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Introduction

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

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

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

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

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

Contents

Annexes

1. Introduction

In the Alpine Lake Phytoplankton Geographical Intercalibration Group:

- Four Member States (Austria, Germany, Italy and Slovenia) compared and harmonised their national lake phytoplankton assessment systems (France withdrew their method in the final stage of the Intercalibration);
- All methods address eutrophication pressure and follow a similar assessment principle (including biomass metrics and trophic index based on indicator taxa);
- Intercalibration "Option 3" was used - direct comparison of assessment methods using a common dataset via application of all assessment methods to all data available;
- The comparability analysis show that methods give a closely similar assessment (in agreement to comparability criteria defined in the IC Guidance), so no boundary adjustment was needed;
- The final results include EQRs of Austrian, German, Italian and Slovenian lake phytoplankton assessment systems for 2 common intercalibration lake types: LAL-3 and L-AL4.

2. Description of national assessment methods

In the Alpine Phytoplankton GIG, four countries participated in the intercalibration with finalised phytoplankton assessment methods (Table 2.1, for detailed descriptions see Annex A).

Table 2.1 Overview of the national phytoplankton assessment methods

MS	Method	Status
AT	Evaluation of the biological quality elements, Part B2 – phytoplankton	Finalized formally agreed national method
DE	PSI (Phyto-Seen-Index) - Bewertungsverfahren für Seen mittels Phytoplankton zur Umsetzung der EG-Wasserrahmenrichtlinie in Deutschland	Finalized formally agreed national method
IT	Italian Phytoplankton Assessment Method (IPAM)	Finalized formally agreed national method
SI	Metodologija vrednotenja ekološkega stanja jezer s fitoplanktonom v Sloveniji (Ecological status assessment system for lakes using phytoplankton in Slovenia)	Finalized formally agreed national method

2.1. Methods and required BQE parameters

All MS have developed full BQE methods (see Table 2.2).

Table 2.2 Overview of the metrics included in the national phytoplankton assessment methods

MS	Biomass	Taxonomic composition and abundance	Algal blooms	Combination rule of metrics
AT	Average of the nEQR of annual mean total biovolume and chlorophyll-a	Brettum index (calculated from annual mean relative biovolume, weighted average of the trophic scores for the indicator taxa)	Metric not considered	Arithmetic mean of nEQRs
DE	Total biovolume (seasonal mean) and chlorophyll-a concentration (seasonal mean and maximum)	Algae groups/classes or combination of groups. Evaluation related to type specific decision tables. PTSI (Phytoplankton Taxa Seen Index) - Evaluation related to log transformed biomass and trophic weighting factors of indicator species	Metric not considered	Weighted average metric scores
IT	Average of the nEQR of annual mean total biovolume and chlorophyll-a	PTIot Phytoplankton Trophic Index. Log transformed biovolume (annual mean), weighted average of the trophic scores and the indicator values for all indicator taxa	Metric not considered	Arithmetic mean of nEQRs
SI	Average of the nEQR of annual mean total biovolume and chlorophyll-a	Brettum index (calculated from annual mean relative biovolume, weighted average of the trophic scores for the indicator taxa)	Metric not considered	Arithmetic mean of nEQRs

There are many questions regarding the use of blooms in the assessment of lakes using phytoplankton. It is still unclear:

- Whether only Cyanobacteria or also other algal taxa should be regarded;
- How to deal with surface scums in routine sampling of the epilimnion or euphotic zone;
- How to deal with blooms occurring in sheltered bays while the sampling point is situated in the centre of the lake etc.;
- Besides, sampling frequency is most critical when blooms occur only for a short period – which is more interesting than persistent blooms in lakes, which will then probably be classified as moderate, poor or bad anyway.

At present it seems that blooms (significant peaks of blue-greens at the surface or within the whole epilimnion) do hardly occur in Alpine lakes under high and good status. As stated by Carvalho et al. (unpubl. WISER deliverable), blooms are rare at TP concentrations of less than 20–25 µg L^{-1}. The risk of missing a bloom is thus very high, causing a high uncertainty and stochasticity when using a bloom metric. Even under moderate status, many lakes do not have "persistent blooms during summer months" (cf Annex V of WFD). Figure 2.1 shows that an algal bloom is an unlikely phenomenon under high and good status in Alpine lakes.
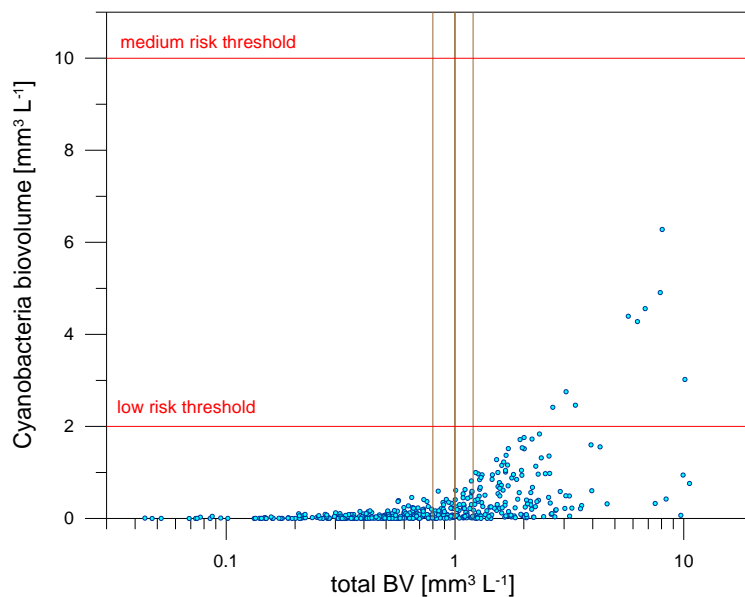
Figure 2.1. Relationship between total phytoplankton biovolume (total BV) and Cyanobacteria biovolume in Alpine lakes. Low risk (2 mm3/l) and risk threshold (10 mm3/l) of Cyanobacteria blooms shown as horizontal lines. Good - moderate class boundary for biovolume (1 mm3/l, range 0.8-1.2 mm3/l) in L-AL3 shown as a vertical line.

In order not to add a metric with high uncertainty and little relevance to the existing, well working assessment methods, **the Alpine GIG has agreed not to include blooms in their classification systems**. This approach may be revised as soon as positive experience with the use of the WISER blooms metric becomes available.

As stated by the representative of Germany, the maximum chlorophyll-a concentration as used as a metric in the German method cannot be considered as a bloom metric.

2.2.   Sampling and data processing

All countries use similar sampling strategies / data processing techniques (Table 2.3).

Table 2.3 Overview of the sampling and data processing of the national phytoplankton assessment methods

MS	Sampling strategy	Data processing
AT/SI	Integrated sample over the euphotic zone or epilimnion or fixed depth range at the lake's deepest point at least 4 times a year	Utermöhl technique
DE	Integrated sample over the euphotic zone at the lake's deepest point at least 6 times during vegetation period.	Utermöhl technique
IT	Integrated sample over the euphotic zone at the lake's deepest point at least 6 times a year	Utermöhl technique

2.3. National reference conditions

All countries have set national reference conditions based on near-natural reference sites in combination with other approaches (Table 2.4).

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

Member State	Methodology used to derive the reference conditions
AT	Existing, Expert knowledge, Historical data, Modelling (extrapolating model results)
DE	Existing near-natural reference sites and palaeo-limnological studies
IT	Existing near-natural reference sites, Expert knowledge, Historical data, Modelling
SI	Existing near-natural reference sites, Expert knowledge, Historical data, Modelling (extrapolating model results)

2.4. National boundary setting

<u>AT/SI and IT:</u> Reference values and class boundaries of total biovolume BV and chlorophyll-a Chl-a were set using the selected population of reference sites. The median was defined as reference value, the 90%percentile as H/G boundary – both supported by expert judgment.

An alternative approach for setting the class boundaries of Chl-a (regression with total biovolume, as performed in phase 1 of the IC exercise) did not give reliable results and was dismissed.

The other class boundaries of BV and Chl-a were derived using equidistant class widths on a log-scale (as described in phase 1).

<u>AT:</u> The class boundaries for the Brettum index BI were derived in the same way as for BV and Chl-a, supported by expert judgment.

<u>IT:</u> The class boundaries for the Italian PTI were derived using the following criteria: For H/G 10th percentile of reference sites. G/M boundaries calculated for those lakes classified as good by Brettum index. The distance between H/G – G/M was used to define the position of the other boundaries.

<u>GE:</u> The class boundaries for the German metrics were set using a combination of methods: Reference values and high-good-boundary e.g. by modelling, following historical data, reference site data (international data set). The ongoing boundaries were set using regression with TP or the LAWA index and were harmonized with the LAWA-Index classes and intercalibration values. BV-boundaries are derived by regression to the Chl-a-boundaries.

2.5. Pressures-response relationships

All MS assessment methods address eutrophication pressure (Table 2.5, Figure 2.2.)

Table 2.5 Pressures addressed by the MS assessment methods (EU – eutrophication, TP- total phosphorus concentration, nEQR – normalised Ecological Quality Ration of national assessment methods)

Member State	Metrics tested	Pressure	Pressure indicators	Strength of relationship (determination coefficient R2)
AT/SI	nEQR	EU	TP	L-AL3 0.62; L-AL4 0.62
GE	nEQR	EU	TP	L-AL3 0.57; L-AL4 0.70
IT	nEQR	EU	TP	L-AL3 0.52; L-AL4 0.52

AT/SI: Y = -0.1781 * ln(X) - 0.1618 (N = 236, R² = 0.62)
GE: Y = -0.1762 * ln(X) - 0.1415 (N = 410, R² = 0.57)
IT: Y = -0.1755 * ln(X) - 0.1199 (N = 264, R² = 0.52)

L-AL3

AT: Y = -0.2296 * ln(X) - 0.2523 (N = 132, R² = 0.62)
GE: Y = -0.2559 * ln(X) - 0.3173 (N = 299, R² = 0.70)
IT: Y = -0.2034 * ln(X) - 0.1023 (N = 133, R² = 0.52)

L-AL4
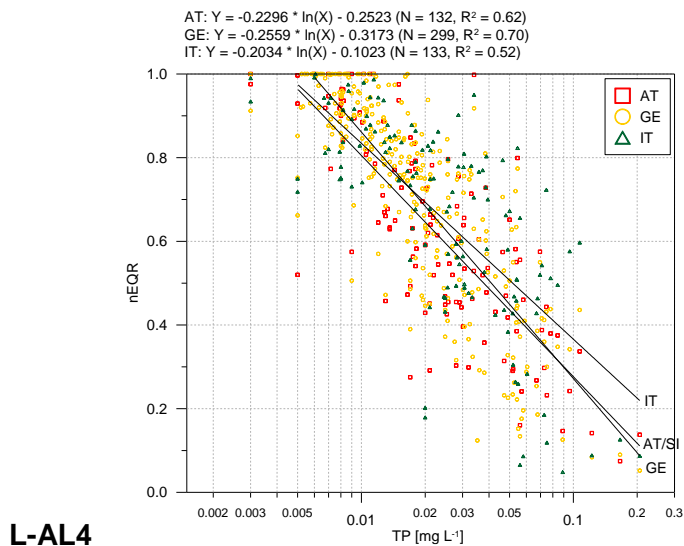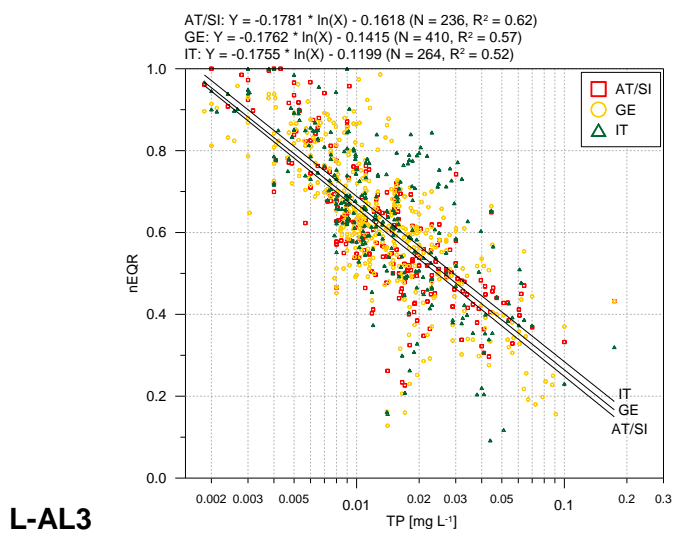
Figure 2.2. Regressions between pressure (TP) and response (normalised EQRs of national methods)

3. Results of WFD compliance checking

All MS methods are considered WFD compliant (Table 3.1). FR will submit an updated version of its assessment method later.

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

Compliance criteria	Compliance checking conclusions
1. Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	All MS: yes
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions (Boundary setting procedure)**	All MS: yes
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.	All MS: all parameters except blooms are included. For abundance/biomass, both chlorophyll-a and biovolume (GE, IT, AT/SI) are used.
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	All MS: yes
5. The water body is assessed against **type-specific near-natural reference conditions**	All MS: yes
6. Assessment results are expressed as **EQRs**	All MS: yes, on the level of single metrics; these are converted to normalized EQR (nEQR; class width = 0.2) and combined to final nEQR. This procedure complies with the approach of other GIGs in phase 1 as well as with the current phase 2 approach of the Alpine macrophyte group.
7. Sampling procedure allows for **representative** information about water body quality/ ecological status in **space and time**	All MS: yes, min 4 samples per year in data set. Future monitoring: GE & IT 6 times, AT, FR & SI 4 times per year (in AT the 4 sampling dates may distribute over the whole year and hence with only 3 dates during the vegetation season March – October) Samplings of epilimnion or euphotic zone.
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	All MS: yes (except blooms)
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	All MS: yes

4. Results IC Feasibility checking

4.1. Typology

Two common intercalibration types were define din the Alpine GIG – L-AL3 and L-AL4 (Table 4.1).

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

Common IC type	Type characteristics	MS sharing IC common type
L-AL3	Deep (mean depth usually >15 m), true Alpine catchment	All MS
L-AL4	Moderately deep – shallow (mean depth usually 3–15 m), usually pre-Alpine catchment	All MS but SI (where both lakes >0.5 km2 belong to L-AL3)

There are several lakes in most MS of the Alpine GIG which do not fall into the range of criteria of the two IC lake types. As decided already in phase 1 of the IC process, these lakes were excluded from the intercalibration, since the criteria for performing the IC (at least 2 lakes in 2 MS each) were not fulfilled. During phase 2, the Alpine GIG could extend the data set, but it was still not possible or justified to create new IC types or to define clear sub-types of L-AL3 or L-AL4 using additional criteria such as geology or biogeography.

Intercalibration is feasible in terms of typology (Table 4.2.).

Table 4.2 Evaluation if IC feasibility regarding common IC types

Method	Appropriate for IC types/subtypes	Remarks
AT method	L-AL3 L-AL4	several national types; ranges are used to cover differences between national types
GE method	L-AL3 L-AL4	4 types are defined for German lakes, 3 of them fit to the IC types, some shallow lakes were included to L-AL4.
IT method	L-AL3 L-AL4	For biomass metrics, ranges are used to cover variability within the types.
SI method	L-AL3	Ranges are used to cover variability within the IC types (i.e. difference between the two lakes > 0.5 km2, both L-AL3)

4.2. Pressures

Intercalibration is feasible in terms of **pressures** addressed by the methods: all methods address eutrophication

4.3. Assessment concept

Intercalibration is feasible in terms of **assessment concepts** as all MS follow the same approach: a combination of a quantitative metric (chlorophyll-a and/or biovolume) and a trophic index.

5. Collection of IC dataset

Huge dataset was collected within the Alpine Phytoplankton GIG (Table 5.1. Only lake-years with at least 4 sampling dates per year, where the dates are more or less evenly distributed over the year or the vegetation season, are taking into account (for description of data acceptance criteria, see Table 5.2). Some shallow (Z_{mean} <3 m) and small (<50 ha) lakes are included under L-AL4.

Table 5.1 Overview of the Alpine GIG phytoplankton IC dataset

Member State	Number of lake years		
	Biovolume data	Chlorophyll-a data	Total phosphorus data
L-AL3			
AT	240	65	196
FR	24	24	24
DE	148	121	136
IT	42	39	42
SI	26	24	24
L-AL4			
AT	160	12	158
FR	4	4	4
DE	75	62	64
IT	44	40	44

Table 5.2 Overview of the data acceptance criteria used for the data quality control

Data acceptance criteria	Data acceptance checking
Data requirements (obligatory for all MS)	1. Both total biovolume and chlorophyll-a data must be available. 2. Sampling frequency per year must be four at minimum. 3. Sampling dates must be more or less evenly distributed over the year or the vegetation season (e.g. Seehamer See 1996 with 5 sampling dates was excluded, since sampling started in September) 4. AT method: only lake-years are included where centric diatoms are identified to species level
The sampling and analytical methodology (obligatory for all MS)	1. Sampling of phytoplankton (and chlorophyll-a) must cover the whole epilimnion or euphotic zone. Samples are taken using an integrating water sampler or as a mixed samples of several sampling depths. 2. Total phosphorus is calculated as volume weighted annual average. If this is not available, the TP

Data acceptance criteria	Data acceptance checking
	concentration during spring circulation is used as alternative. 3. Data stemming from surface sampling only were excluded from the dataset.
Level of taxonomic precision required and taxa lists with codes	Taxa are identified at highest level possible, viz. species, species-group or genus. The Rebecca-Wiser codes are used.
The minimum number of sites/samples per intercalibration type	This criteria is easily met
Sufficient covering of all relevant quality classes per type	This is no criteria for the data selection, but an outcome of the IC exercise. However, considering the range of TP concentration (0.002–0.2 mg L–1), it can be assumed that the data cover all quality classes.

6. Common benchmarking

A tiered approach to define reference sites of Alpine lakes has been prepared by the Invertebrates and Phytoplankton group of the Alpine GIG and proposed to the Macrophytes and Fish groups (see Annex B).

6.1. Reference conditions

Reference sites for phytoplankton total biovolume:

- L-AL3: 71 lake-years from 24 sampling sites (AT, DE, SI);
- L-AL4: 59 lake-years from 14 sampling sites (AT, DE).

Reference sites for phytoplankton Chlorophyll-a:

- L-AL3: 29 lake-years from 15 sampling sites (AT, DE, SI);
- L-AL4: 29 lake-years from 13 sampling sites (AT, DE).

The number is considered sufficient to make a statistically reliable estimate, with support from modelling and expert judgment.

Pre-selected 'reference condition sites' for L-AL3 lakes (for some lake-years chl-a was missing or one of the national method could not be applied. Only lake-years underlined were included in the final comparability checking): Achensee 2008, Alpsee bei Füssen 2001, 2004, Altausseer See 2002, Attersee 1997-1998, 2002, 2003-2004, 2007-2009, Bohinjsko jezero 2005-2007, Eibsee 2005, Fuschlsee 1997-1999, 2007-2008, Grundlsee 2002, Heiterwanger See 2007-2008, Königssee 2000, 2008, Millstätter See 1932-1937, Obersee 2000, 2007, Ossiacher See 1934-1938, Plansee 2008, Starnberger See 2007-2008, Sylvensteinsee 2007, Traunsee 2007, 2009, Walchensee 1995, 1997, 2008, Weißensee 1933-1935, 1987, 1989, 1993, 1997, 2003, 2006, Wolfgangsee/Gilgen 2007-2008, Wolfgangsee/St. Wolfgang 2007-2008, Wörthersee 1931-1938, Zeller See 2000, 2008.

Pre-selected 'reference condition sites' for L-AL4 lakes: <u>Faaker See</u> 1937, 1987, 1990-2003, <u>2007</u>, <u>Großer Ostersee 2004, 2008</u>, <u>Irrsee 2002-2009</u>, <u>Keutschacher See</u> 2000-2003, 2006, <u>2007-2008</u>, Kirchsee 2001, <u>Lago di Ganna 2007</u>, <u>Lago di Segrino 2008</u>, Längsee 1999-2000, 2002, <u>Lustsee 1996-1998</u>, 1999, <u>2000</u>, Mattsee 1997-1999, 2007-2008, Pressegger See 2002, Staffelsee 2007, Weissensee (GE) 1997-1998, 2001, <u>Wörthsee</u> 1993-1994, 2002, 2005, <u>2008.</u>

Screening of the biological data:

- Candidate reference sites were checked for possible impacts from hydro-morphological pressures (water level fluctuations), but no significant deviations were found (cf the German report on HMWB & AWB lakes, HOEHN et al. 2009);
- Lakes that have undergone a eutrophication – re-oligotrophication process, but have not yet reached stable trophic conditions were excluded, even if they already met the reference criteria (e.g. TP already ≤8 µg L^{-1}, but still decreasing). This was done in order to exclude lakes where the phytoplankton might react with some delay to the improvement;
- One lake, which has been affected by mining activities, was excluded from the candidate list of reference sites for safety reasons, although no impact on phytoplankton could be detected;
- Very small lakes outside the typology criteria were excluded.

Setting reference conditions (summary statistics used):

1. The arithmetic mean of total biovolume and chlorophyll-a from different years was calculated for each reference site in order to give equal weight to each site;
2. Both parameters, the median of all reference sites was calculated and rounded to 1 digit;
3. Where the reference values for L-AL3 and L-AL4 only slightly deviated from the values already derived in phase 1 of the IC exercise, the old values were accepted also for IC phase 2.

6.2. Benchmark standardisation

Benchmark standardization serves to homogenize the EQR results of common datasets *where needed*, minimising typological and methodological differences between the Member states which may otherwise influence the comparability of their classifications.

Biogeographical differences within the Alpine GIG are considered negligible. Differences in type-specific reference conditions could theoretically occur between northern and southern Alps, but there is no confounding indication in the scientific literature nor was it possible to describe sub-types within the Alpine GIG based on biogeographical aspects. According to the internal guidance benchmark standardization is *not needed* when there are no significant subtypological differences between Member states. This is the case in the Alpine GIG. Some MS use, however, a kind of fine-tuning of reference values via ranges, in order to cope for variability within the two types L-AL3 and L-AL4, which cannot be used to define separate IC types. The ranges take into account altitude, depth, mixing type and other characteristics (cf Wolfram et al. 2009) and are included in the calculation of the EQR and nEQR.

As required by the JRC guidance mentioned above, we explored if there might exist subtypes in the common type between or within countries. This was done by comparing if the EQR values of a given national method for the benchmark sites (in our case: reference sites) do not differ between Member states.

The following box-whisker-plots (Figure 6.1) show the nEQR values of reference lake-years from L-AL3 and L-AL4 in different MS as calculated from the three methods available. There is no clear trend of deviation in the boxplots.

In consequence, the checking of comparability was carried out ***without the standardization step*, but starts with the normalization.** This was done by manually setting the offset to zero (cells B34:E37 in sheet calc in <IC_Opt3_sub.xlsx>).
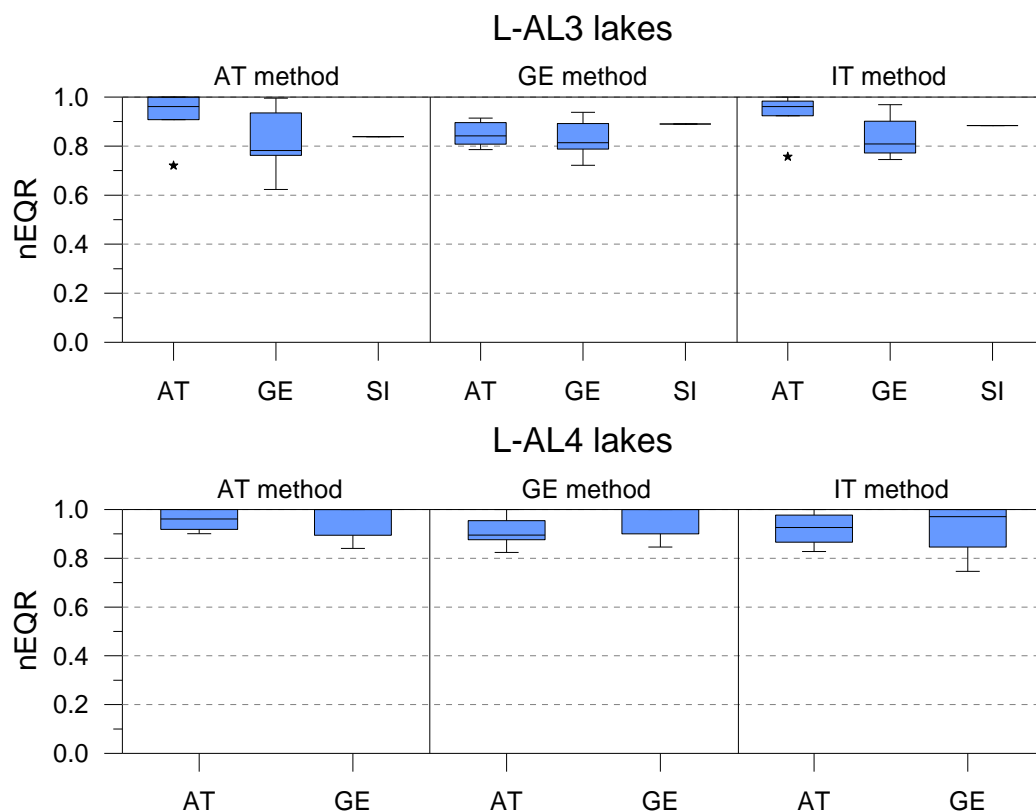
Figure 6.1. Comparison on nEQR values of reference lake-years from L-AL3 and L-AL4 in different MS as calculated from the three methods available. Number of reference lake-years in L-AL3 type: AT 7, GE 13, SI 1. Number of reference lake-years in L-AL4 type: AT 10, GE 7 (IT 2, not included)

7. Comparison of methods and boundaries

7.1. IC Option and Common Metrics

Option 1 was followed between AT and SI, since both countries use the same method. The reference values and class boundaries of total biovolume and of chlorophyll-a are fully identical in AT, SI and IT. Hence, the methods from AT/SI and IT differ only in the trophic index.

For the whole GIG, **option 3a** is followed, since data acquisition was very similar in all MS of the Alpine GIG. The arithmetic mean of the normalized EQR values was used as pseudo-common metric (PCM). No additional common metric was selected.

Results of the regression comparison (National EQRs vs PCM)

All methods have significant correlations with common metrics (Table 7.1):

- The **Pearson correlation coefficient** ranges from 0.928 to 0.959 in L-AL3 and from 0.944 to 0.955 in L-AL4. The requirement that r ≥ 0.5 is fulfilled in both IC types.
- The **ratio min R^2 : max R^2** in the regression models between the national methods and the PCM is 0.94 for L-AL3 lakes and 0.97 for L-AL4 lakes. The requirement of a ratio >0.5 is fulfilled in both IC types.

Table 7.1   Regression characteristics (National EQRs vs PCM)

MS/IC type	Intercept (c)		Slope (m)	
	L-AL3	**L-AL4**	**L-AL3**	**L-AL4**
AT/SI	0.117	0.136	0.916	0.828
GE	−0.038	0.022	0.936	0.959
IT	0.034	−0.043	0.932	1.022
	Regressions with PCM R2		**Pearson R**	
AT/SI	0.919	0.884	0.959	0.940
GE	0.860	0.913	0.928	0.955
IT	0.899	0.899	0.948	0.948
	Ratio min R^2 : max R^2			
	0.94	0.97		

7.2.   Boundary comparison and harmonisation

All national methods comply with comparability criteria (Table 7.2, Figure 7.1):

- The **boundary bias** in L-AL3 type ranges between −0.12 and +0.18 for the H/G boundary and −0.14 and +0.18 for the G/M boundary;
- In L-AL4 type, the **boundary bias** ranges between −0.11   and 0.09 for the H/G boundary and −0.24 and +0.24 for the G/M boundary. The boundary bias never exceeds −0.25, which would indicate that a method was too relaxed;
- **Absolute class differences** range from 0.10 to 0.20 in L-AL3 and from 0.19 to 0.22 in L-AL4. They are clearly less than 1 class for all methods.

Table 7.2 Overview of the IC comparability criteria

| | Class difference (boundary bias) | | | | Absolute Class Difference | |
| | H/G boundary bias | | G/M boundary bias | | | |
MS/IC type	**L-AL3**	**L-AL4**	**L-AL3**	**L-AL4**	**L-AL3**	**L-AL4**
AT/SI	0.18	−0.09	0.18	0.24	0.17	0.22
GE	−0.06	0.05	−0.04	0.07	0.18	0.19
IT	−0.12	−0.11	−0.14	−0.24	0.20	0.22

a) LAL-3 type – HG boundary bias
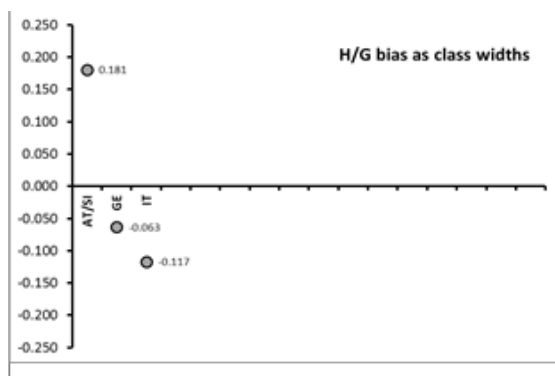
b) LAL-3 type – GM boundary bias
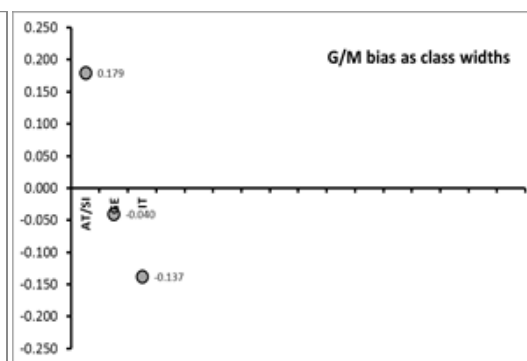
c) LAL-4 type – HG boundary bias
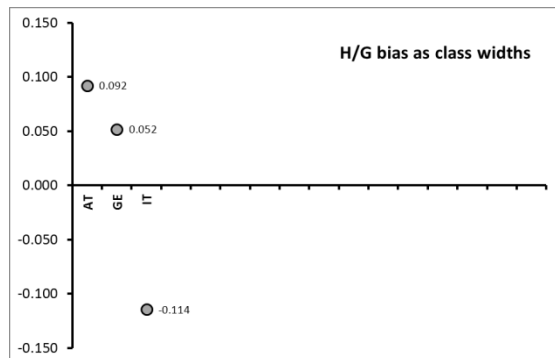
d) LAL-4 type – GM boundary bias
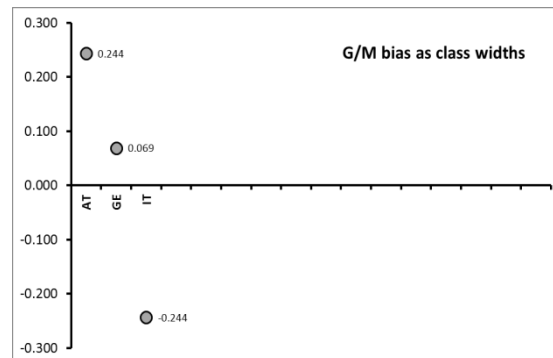
Figure 7.1. Comparison of Alpine GIG phytoplankton methods: HG and GM boundary biases (HG – High-Good class boundary, GM- Good-Moderate class boundary)

Final results of the IC - EQRs of the Alpine GIG phytoplankton assessment methods are given in Table 7.3.

Table 7.3 Overview of the IC results: EQRs of the Alpine GIG phytoplankton assessment methods

MS	Classification	Ecological Quality Ratios	
	Method	High-good boundary	Good-moderate boundary
AT	Evaluation of the biological quality elements, Part B2 – phytoplankton	0.8	0.6
DE	PSI (Phyto-Seen-Index) - Bewertungsverfahren für Seen mittels Phytoplankton zur Umsetzung der EG-Wasserrahmenrichtlinie in Deutschland	0.8	0.6
IT	Italian Phytoplankton Assessment Method (IPAM)	0.8	0.6
SI	Metodologija vrednotenja ekološkega stanja jezer s fitoplanktonom v Sloveniji (Ecological status assessment system for lakes using phytoplankton in Slovenia)	0.8	0.6

7.3. Correspondence between common intercalibration types and national typologies/assessment systems

In some MS, the national types can be directly related to common IC types (e.g. GE type 4 = IC type L-AL3, AT type B2 = IC type L-AL4). If ranges are used for fine-tuning of typology, the criteria as described in Wolfram *et al.* (2009) can be used. However, all classification results will be expressed as nEQR, having the same boundaries for all national and IC types: 0.8 for H/G, 0.6 for G/M.

7.4. Gaps of the current Intercalibration

- All members of the Alpine GIG recognize **standardization of methods** as an important issue, although there is no common and agreed view in various methodological questions. Critical issues are: sampling depth and sampling frequency, and the identification of centric diatoms.
- The existing CEN standard of Utermöhl counting is overloaded with statistics and thus not practical. It should be revised in near future!
- More effort should be undertaken to quantify measurement of uncertainty (sampling, frequency, counting, ...), although the Alpine GIG members are aware that the quantification of analytical results in chemistry (*cf* EN 17025) and biology are different issues and cannot be performed with the same level of precision.
- France has to submit an agreed national assessment method

8. Description of biological communities and changes across pressure gradient

8.1. Biological communities at reference sites

In general, the algal community under reference conditions is comparatively poor in taxa richness. A characteristic feature in the phytoplankton community of many deep Alpine

lakes (L-AL3) is a strong dominance of *Cyclotella* species. This fact is proved by monitoring data from reference sites (also historical data), but also from palaeo-reconstruction. Typical accompanying taxa besides *Cyclotella* are *Ceratium hirundinella*, *Asterionella formosa*, various chrysoflagellates, cryptoflagellates and Chroococcales. Some of these taxa may also occur at higher trophic levels, but form a significant part of the community at oligotrophic conditions.

The annual mean biomass under reference conditions is within the same range as it was prior to major urbanisation, industrialisation and agriculture, which can be proved by historical data available from the 1930s. Planktonic blooms do not occur at high status.

In moderately deep lakes (IC type L-AL4), variability and biovolume is slightly higher than in deep lakes (reference conditions = oligo-mesotrophic). The trophic gradient spanned by L-AL4 lakes is however higher than in deep lakes, which makes this group more heterogeneous than the L-AL3 lake group. At the lower trophic end of L-AL4 lakes, biovolume and taxonomic composition is similar to the situation in deep lakes. At the upper trophic end, species richness may be significantly higher than in oligotrophic lakes. Also the proportion of nutrient tolerant taxa such as *Fragilaria crotonensis*, *Stephanodiscus* spp., *Tabellaria fenestrata* or various filamentous blue-green algae (such as *Planktothrix rubescens*) may be slightly higher than in typical high status lakes of type L-AL3.

Like in L-AL3 lakes, the annual mean biomass under reference conditions in L-AL4 lakes is within the same range as it was prior to major urbanisation, industrialisation and agriculture, which can be proved by historical data available from the 1930s. Planktonic blooms do not occur at high status, but potentially bloom forming taxa such as *Planktothrix rubescens* may occur in low density (also proven by historical data).

8.2. Biological changes across pressure gradient

In most Alpine lakes, the total biovolume of phytoplankton is directly related with nutrient supply. An increase of total phosphorus concentration (which is the limiting nutrient in most cases) will lead to an increase of biomass. The significant positive correlation of TP and BV and Chl-a, resp., has been proved already in phase 1 of the IC exercise.

Each of the four trophic indices used in the Alpine GIG (Brettum index, PTI, PTSI, MCS) is based on list of indicator taxa with different trophic optima. A change in trophic state – that means, a shift along the pressure gradient – will thus cause a change in the trophic index. Like for BV and Chl-a, the significant correlation between TP and the trophic indices was demonstrated already in phase 1 for the first three indices (Brettum Index, PTI and PTSI). As an example, the relation between TP and the Italian PTI is given below (Figure 8.1).
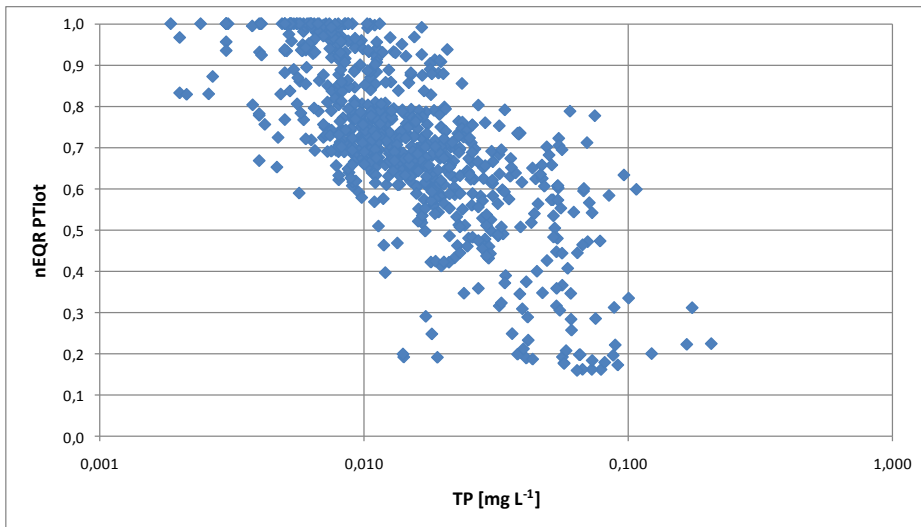
Figure 8.1. Relationship between total phosphorus concentration (TP) and normalised EQR (nEQR) of Italian phytoplankton trophic index (PTlot).

8.3.   Comparison with WFD Annex V normative definitions

Since L-AL3 (deep) and L-AL4 (moderately deep – shallow) just represent two expressions along a continuum of lakes with decreasing depth, the metrics used in the national methods are expected to react in similar way in both lake types. They will thus be discussed together.

Biomass (BV, Chl-a):

NormDef High: The average phytoplankton biomass is consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type-specific transparency conditions.

NormDef Good: There are slight changes in the composition and abundance of planktonic taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbance to the balance of organisms present in the water body or to the physico-chemical quality of the water or sediment.

NormDef Moderate: Biomass is moderately disturbed and may be such as to produce a significant undesirable disturbance in the condition of other biological quality elements and the physico-chemical quality of the water or sediment.

EQR for H/G is 0.60–0.64 (BV) and 0.70–0.75 (Chl-a), EQR for G/M is 0.25–0.26 (BV) and 0.40–0.41 (Chl-a). Hence, phytoplankton biomass under good status is about 1.5–4 times the biomass under reference conditions. This is considered as 'slight' and will not result in disturbance to the balance of organisms present in the water body or to the physico-chemical quality of the water or sediment. Annual mean Secchi depth at good status is 3 to 10.5 m (median 5.7 m) in L-AL4 and 1.3–5 m (median 3.2 m) in L-AL3 lakes (Figure 8.2). In addition to organic turbidity from phytoplankton, biogenic calcification or inorganic turbidity from inflows can reduce Secchi depth.
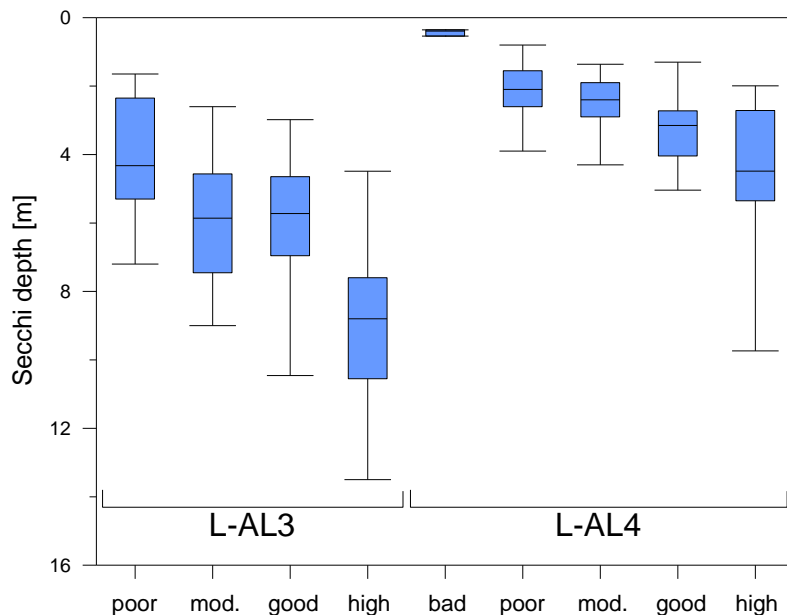
Figure 8.2. Secchi depth (m) distribution at different ecological status classes in L-AL3 and L-AL4 lakes.

Taxonomic composition (trophic indices):

NormDef High: The taxonomic composition and abundance of phytoplankton correspond totally or nearly totally to undisturbed conditions.

NormDef Good: There are slight changes in the composition and abundance of planktonic taxa compared to the type-specific communities. Such changes do not indicate any accelerated growth of algae resulting in undesirable disturbance to the balance of organisms present in the water body or to the physico-chemical quality of the water or sediment.

NormDef Moderate: The composition and abundance of planktonic taxa differ moderately from the type-specific communities.

Taxa dominant at high status still play a significant role at good status, but their relative proportion of total biovolume rapidly diminishes under moderate status. This is reflected by the three trophic indices, which are calculated from taxon-specific relative proportions of total biovolume. Nutrient tolerant taxa such as *Fragilaria crotonensis*, *Stephanodiscus* spp., *Tabellaria fenestrata* or various filamentous blue-green algae (such as *Planktothrix rubescens*) increase in relative proportion.

Blooms:

NormDef High: Planktonic blooms occur at a frequency and intensity which is consistent with the type specific physicochemical conditions.

NormDef Good: A slight increase in the frequency and intensity of the type specific planktonic blooms may occur.

NormDef Moderate: A moderate increase in the frequency and intensity of planktonic blooms may occur. Persistent blooms may occur during summer months.

There are many questions regarding the use of blooms in the assessment of lakes using phytoplankton. It is still unclear whether only Cyanobacteria or also other algal taxa

should be regarded, how to deal with surface scums in routine sampling of the epilimnion or euphotic zone, how to deal with blooms occurring in sheltered bays while the sampling point is situated in the centre of the lake etc. Besides, sampling frequency is most critical when blooms occur only for a short period – which is more interesting than persistent blooms in lakes, which will then probably be classified as moderate, poor or bad anyway.

At present it seems that blooms (significant peaks of blue-greens at the surface or within the whole epilimnion) do hardly occur in Alpine lakes under high and good status. As stated by Carvalho et al. (unpubl. WISER deliverable), blooms are rare at TP concentrations of less than 20–25 µg L^{-1}. The risk of missing a bloom is thus very high, causing a high uncertainty and stochasticity when using a bloom metric. Even under moderate status, many lakes do not have "persistent blooms during summer months" (cf Annex V of WFD).

In order not to add a metric with high uncertainty and little relevance to the existing, well working assessment methods, the Alpine GIG has agreed not to include blooms in their classification systems. This approach may be revised as soon as positive experience with the use of the WISER blooms metric becomes available.

(As stated by the representative of GE, the maximum chlorophyll-a concentration as used as a metric in the GE method cannot be considered as a blooms metric.)

8.4.   Description of IC type-specific biological communities representing the "borderline" conditions between good and moderate ecological status

Biomass: Algal biomass *(BV, Chl-a)* is about 2.5–4 times the values that can be expected under reference conditions (EQR = 0.25–0.40). Subtypological variability and hydro-morphological differences (mixing type, very deep/large lakes) are not reflected in this *ratio*, but in differences of the *absolute reference values* for *BV* and *Chl-a*.

Taxonomic composition: Taxa dominating under reference conditions are still present at G/M, but clearly differ in their relative proportion of total biovolume. This can be demonstrated by using the Brettum scores for selected trophic classes (Figure 8.3, example only for L-AL3). At the "borderline" G/M, which is defined here by using the Austrian method, the scores for the "best" trophic class (TP <5 µg L^{-1}) are <1 in most cases, while the scores for the "lowest" class (TP >60 µg L^{-1}) increase. The scores of the third trophic class (8–15 µg L^{-1}) have their optimum slightly above the G/M boundary.

This is in line with the normative definitions, which require moderate difference in the composition and abundance of planktonic taxa from the type-specific communities.
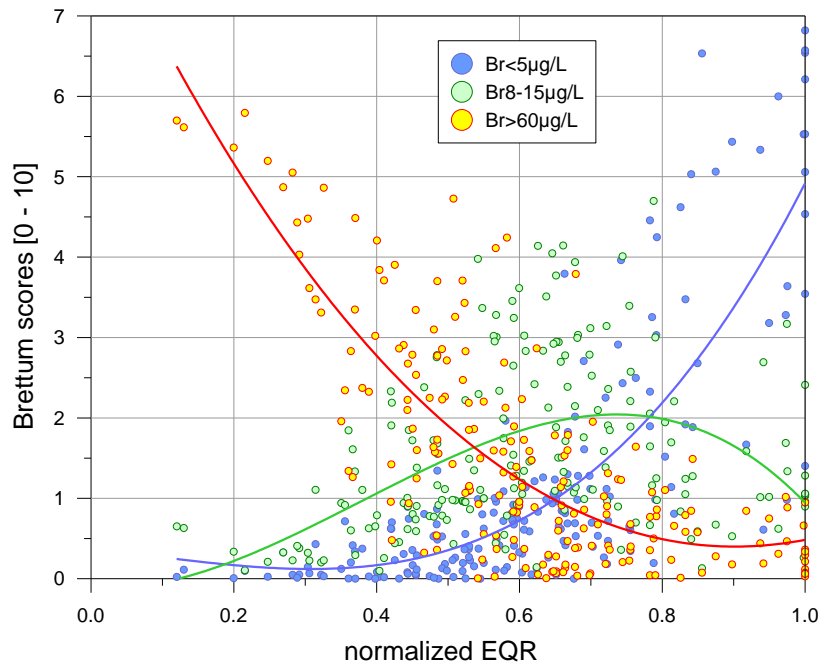
Figure 8.3. Brettum scores for selected trophic classes in relation to normalized EQR (L-AL3 type)

Annexes

A.   Description of Member states assessment methods

A.1   Austria: Evaluation of the biological quality elements

Overview

<u>Application of the method</u>

An assessment of the ecological status of lakes using phytoplankton is mainly a classification of their nutrient and productivity levels. The assessment method presented in this manual was developed for Austrian lakes with a surface area >50 ha. The assessment method can be applied for lakes <50 ha, but in every single case it must be determined whether the same trophic reference states developed for larger lakes can be reasonably applied to smaller ones. This is especially true for small lakes in the lowlands. If necessary, the reference state has to be refined.

The majority of Austrian lakes >50 ha lie in the Alpine and pre-Alpine region. In addition to these lakes, there are a few others in Eastern Austria that also have to be considered: the special lake type represented by the Old Danube and Neusiedler See, and certain large salt (soda) pans in the so-called Seewinkel. The general description of sampling and sample processing, as provided in this manual, is valid also for these lakes; however, an official WFD-compliant assessment method is thus far not available. The assessment methods recently developed for the Neusiedler See and Old Danube still need to be validated and therefore are not considered in this manual.

Principles of the Method

1. According to this manual, assessment of the ecological status of a lake is based on several phytoplankton samples collected from the epilimnion or the euphotic zone at different sampling dates. The chlorophyll-a concentration is determined from an additional sample taken from the same water layer and following the same technique (as a mixed or integrated sample) as used for the phytoplankton sample.
2. The **phytoplankton samples** are analysed in the laboratory with respect to taxonomy (qualitative analysis), with the abundance and total biovolume of the planktonic algae determined from a subsample observed using an **inverted microscope** (quantitative analysis after UTERMÖHL 1958, DIN EN 15204/2006, CEN TC 230/WG 2/TG 3/2007). Taxonomic analyses are carried out at the species level, as far as possible with reasonable effort. If the relative proportion of centric diatoms exceeds 10% of the total biovolume per sample, an additional detailed analysis of diatoms is required in order to enhance the degree of confidence in the taxonomic analyses (burn mount, after EN 14407:2004). **Qualitative sampling** with a plankton-net after DIN EN 15204/2006 also should be carried out. In the monitoring programme of Austrian lakes, a (formaldehyde-preserved) qualitative sample should be taken, storing it for later analysis. The qualitative analysis can be omitted only if the species composition is very well known and the ecological status can be assessed with a high degree of confidence solely on the basis of the quantitative analysis.
3. For the analysis of **chlorophyll-a**, the respective standards should be consulted. In principle, chlorophyll-a can be determined spectrophotometrically (reference standard DIN 38412 part 16) or using HPLC. See also GZÜV (BGBl. II 479 from 14 Dec 2006).

4. For each year, the mean chlorophyll-a concentration and, for each taxon, the mean biovolume are determined as the arithmetic means of four or more sampling dates. The mean total biovolume of a lake is calculated as the sum of the mean biovolumes of the single taxa. The relative proportions of the mean biovolumes of these single taxa and the taxon-specific trophic scores are used to calculate the Brettum index.
The **final classification** of the lake using phytoplankton is based on the mean chlorophyll-a concentration, the mean total biovolume and the Brettum index.

The full manual is available on the homepage of the Federal Ministry of Agriculture and Forestry, Environment and Water Management (Ministry of Life) under www.lebensministerium.at/section „Wasser/Wasserrahmenrichtlinie".

Sampling Frequency and Sampling Dates

Sampling Frequency

The assessment of phytoplankton is based on the annual mean of data acquired from several sampling dates. For large Austrian lakes, at least four sampling dates per year are required to reliably calculate the mean. A higher sampling frequency will improve the confidence in the calculation and avoid biases of the annual means due to outliers. The assessment is carried out on the basis of a running average of three subsequent years.

Selection of the Sampling Date

The minimum requirement for the classification is sampling at four different, limnologically important dates: spring circulation, beginning of the summer stagnation, peak of the summer stagnation, beginning of the autumn circulation (often at the end of autumn).

Selection of the Sampling Site

The morphology of the lake basin of most standing waters in Austria is relatively simple. Hence, most lakes represent a water body as defined by the WFD and are sampled at one sampling site only. Examples of lakes with a more complex basin morphology are Wolfgangsee in Salzburg and the Old Danube in Vienna, both of which are sampled at two sites in accordance with the GZÜV. Several sampling sites are defined for Lake Constance and Neusiedler See.

In general, sampling is carried out from a boat positioned above the deepest point of the lake (or the lake basin). The four sampling sites of Neusiedler See are spread along an imaginary line from north to south (see Annex 9 of the GZÜV).

Sampling Depth

Quantitative samples of phytoplankton (total biovolume and chlorophyll-a) are taken from the epilimnion or, as in some neighbouring countries, from the euphotic zone. To ensure data harmonisation, it is recommended to sample the epilimnion according to the monitoring programmes under the GZÜV. If the euphotic zone is smaller than the epilimnion, the latter must be sampled.

Calculations and Assessment of the Ecological Status

General

The assessment of the ecological status of a lake is a classification of its nutrient and productivity levels. The parameters used in the assessment are: the chlorophyll-a

concentration (annual mean), the total biovolume (annual mean) and the Brettum index (calculated from the taxa list and the corresponding annual mean biovolumes). Derivation of the taxon-specific trophic scores, calculation of the Brettum index, calculation of the EQR and normalized EQR values as well as the final assessment are presented in the following sections.

Brettum Index: Basis of the Calculation

In its general outline, calculation of the Brettum index is comparable to that of the saprobic index since it is based on taxon-specific trophic scores, currently for 80 taxa (species and genera). Following the approach of BRETTUM (1989) as modified by DOKULIL et al. (2005) and WOLFRAM et al. (2007), the trophic scores are calculated on the basis of occurrence and on the relative proportion of biovolume within six total phosphorus (TP) concentration levels.

Depending on the occurrence of the phytoplankton taxa within each of the six trophic ranges, the trophic scores are calculated as follows. The first three steps follow the approach described by DOKULIL et al. (2005).

1. The probability p_{ij} to find a taxon i within the trophic range j at a certain relative proportion of the total biovolume of the phytoplankton is calculated as:

$$p_{ij} = \frac{n_{ij}}{N_j} b_i \qquad (2)$$

n_{ij} Number of findings of a taxon i within the trophic range j (presence/absence)

N_j Total number of all samples within the trophic range j

b_i Mean relative proportion of the taxon i of the total phytoplankton biovolume within the trophic range j („dominance")

and where

$\frac{n_{ij}}{N_j}$ is a measure of the occurrence of a taxon

2. After the probability pij for each trophic range has been calculated, the TP range with the highest relative proportion of biovolume is given the index value xi = 100. The index values of the other TP ranges are calculated relative to it, in order to numerically describe the distribution of the taxon along the TP gradient. The more a taxon is confined to one or a few TP ranges, the higher its indicator value.

3. Based on the index values x_{ij} for all indicator taxa and on their biovolumes v_i (as annual means) a total index I_j is calculated for each of the six trophic level as follows:

$$I_j = \frac{\sum_{i=1}^{n} v_i x_{ij}}{\sum_{i=1}^{n} v_i} \qquad (3)$$

The trophic assessment now results in six indices I_j.

4. In their modification of the original Brettum approach, DOKULIL et al. (2005) and WOLFRAM et al. (2007) did not use a maximum value of 100 but instead distributed 10 points along the trophic gradient with its six different ranges, weighted by the probabilities p_{ij}, (analogous to the saprobic index in MOOG 1995). This leads to a higher weight of stenoecious species (refined to a few TP ranges) and to the reduced weight of indifferent species (similar probability along a wider TP gradient).

   The following example illustrate this step, but also show the database used, which comprises data from lakes in Austria, Slovenia, Italy, Germany and France.

Bitrichia chodatii

Rebecca ID	R1155
Order	Stylococcales
Class	Chrysophyceae

Distribution along TP gradient

lake years	234
occurrence	32,7%
max. %biovol.	1,12%

	AT	FR	GE	IT	SI
lakes / country	15	6	30	3	2

weighted avg of TP			TP classes					
avg	**0,011**	**mg L^{-1}**	TP class	lakes	years	occurr	avg	scores	10 pts

TP (cumul. %biovol.)			TP class	lakes	years	occurr	avg	scores	10 pts
min	0,002	mg L^{-1}	<=5 µg L^{-1}	14	15	42%	0,11%	4,4	4
25perc.	0,006	mg L^{-1}	5-8 µg L^{-1}	15	39	42%	0,08%	3,5	4
median	**0,009**	**mg L^{-1}**	8-15 µg L^{-1}	28	107	40%	0,04%	1,6	2
75perc.	0,011	mg L^{-1}	15-30 µg L^{-1}	19	61	31%	0,01%	0,4	0
max	0,100	mg L^{-1}	30-60 µg L^{-1}	8,0	11	13%	0,01%	0,1	0
			>60 µg L^{-1}	1	1	3%	0,01%	0,0	0
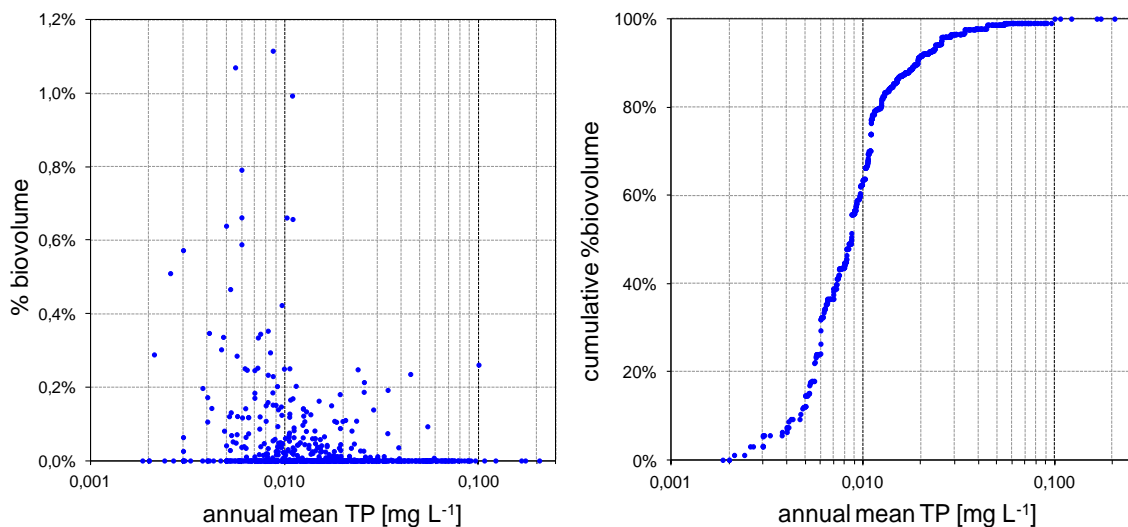
Figure A.1   Distribution along total phosphorus gradient of Bitrichia chodatii (% of total phytoplankton biovolume)

Calculation of the Brettum Index

To calculate the Brettum index BI, a weighted average is calculated from the six indices I_j, where the TP ranges j have the values $T_j = 6$ (<5 µg L^{-1}) to 1 (>60 µg L^{-1}) in descending order:

$$BI = \frac{\sum_{j=1}^{5} I_j T_j}{\sum_{j=1}^{5} I_j} \qquad (4)$$

The values of the Brettum index range between 1 (very nutrient-poor) and 6 (nutrient-rich).

Note: The extension and re-calculation of the taxon-specific trophic scores made it necessary to recalculate also the reference values and class boundaries for the Brettum index. Consequently, the index values calculated from the new taxa list in this manual cannot be compared with those calculated following the previous version of the manual. However, the results can be compared at the level of the normalized EQR values.

Taxon-Specific Trophic Scores

The calculation described above was carried out for a large number of phytoplankton taxa (from species to class level). The finally selected indicator taxa are listed in Table A.1. The selection was done during the intercalibration process on the basis of the dominance and occurrence of the taxa in the whole Alpine region (with application of the method potentially in other countries as well) and after plausibility checking of the distribution of the single scores x_{ij}.

Table A.1 Taxon-specific trophic scores. The scores given for genera are valid for all species belonging to it unless the latter are listed separately.

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
A. Ordered by trophic indication		7		0	0	0	0
R0040	*Cyclotella bodanica*	7	3	0	0	0	0
R2195	*Cyclotella cyclopuncta*	7	3	0	0	0	0
R2196	*Cyclotella distinguenda*	8	1	1	0	0	0
R0733	*Pseudoquadrigula* sp.	8	1	1	0	0	0
R0042	*Cyclotella comensis*	7	2	1	0	0	0
R1070	*Dinobryon cylindricum*	7	2	1	0	0	0
R2058	*Discostella glomerata*	6	3	1	0	0	0
R1903	*Peridinium umbonatum* - complex	7	2	0	1	0	0
R1166	*Chrysolykos planctonicus*	5	4	1	0	0	0
R1446	*Chroococcus turgidus*	5	3	2	0	0	0
R1167	*Chrysolykos skujae*	2	8	0	0	0	0
R1155	*Bitrichia chodatii*	4	4	2	0	0	0

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
R0493	*Botryococcus braunii*	5	2	2	1		0
R1037	*Kephyrion* sp.	6	1	1	1	1	0
R0191	*Diatoma vulgaris*	5	2	1	1	1	0
R1697	*Peridinium pusillum*		9	1	0	0	0
R1066	*Dinobryon bavaricum*	3	3	2	2		0
R1438	*Chroococcus limneticus*	4	2	2	1	1	0
R1660	*Gymnodinium uberrimum*	1	6	2	1		0
R0442	*Tabellaria flocculosa*	1	4	5	0	0	0
R2174	*Ulnaria delicatissima* var. *angustissima*	2	3	3	2	0	0
R1654	*Gymnodinium* sp.	1	5	2	1	1	0
R1691	*Peridinium inconspicuum*	1	4	3	2	0	0
R1069	*Dinobryon crenulatum*	2	2	3	2	1	0
R1443	*Chroococcus minutus*	1	3	4	1	1	0
R0033	*Aulacoseira subarctica*		1	8	1	0	0
R1209	*Cosmarium depressum*	2	2	3	1	1	1
R1704	*Peridinium willei*	1	4	2	1	1	1
R0440	*Tabellaria fenestrata*	1	1	4	4	0	0
R1642	*Glenodinium* sp.		2	5	3	0	0
R1151	*Uroglena* sp.		3	3	3	1	0
R0606	*Coenococcus planctonicus*		1	5	4	0	0
R1413	*Aphanocapsa delicatissima*		3	3	2	2	0
R1617	*Planktothrix rubescens*	1	1	3	4	1	0
R0582	*Didymocystis* sp.		1	4	4	1	0
R1510	*Snowella lacustris*		1	4	4	1	0
R1549	*Anabaena spiroides*		1	6	1	1	1
R1282	*Staurastrum chaetoceras*			3	7	0	0
R2549	*Urosolenia longiseta*		1	3	3	3	0
R2556	*Crucigeniella irregularis*	0	0	4	4	2	0
R0025	*Aulacoseira islandica*	0	1	3	3	2	1
R0083	*Stephanodiscus neoastraea*		1	2	4	3	0
R0533	*Coenochloris fottii*	0	1	3	3	2	1
R1074	*Dinobryon divergens* var. *schauinslandii*	0	0	1	9		
R2503	*Achnanthidium catenatum*	0	0	1	8	1	
R1081	*Dinobryon sertularia*	0	1	1	5	3	
R1096	*Mallomonas acaroides*	0	1	2	4	2	1
R1342	*Sphaerozosma* sp.	0	0	1	8	1	
R1687	*Peridinium cinctum*	0	1	2	4	2	1
R0649	*Lagerheimia genevensis*	0	0	3	3	4	
R1303	*Staurastrum pingue*	0	0	2	5	3	
R1375	*Chroomonas* sp.	0	1	2	2	5	
R0048	*Cyclotella ocellata*	0	1	1	4	3	1
R0848	*Tetraedron minimum*	0	1	1	4	3	1
R0736	*Pseudosphaerocystis lacustris*	0		2	5	2	1
R1414	*Aphanocapsa elachista*	0	1	2	2	4	1

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
R0571	*Dictyosphaerium pulchellum*	0	0	1	5	4	
R1097	*Mallomonas akrokomos*	0	0	2	4	3	1
R2169	*Staurosira construens*	0	0	2	2	6	0
R1100	*Mallomonas caudata*	0	0	1	4	5	0
R1427	*Aphanothece clathrata*	0	0	1	4	5	0
R1776	*Trachelomonas volvocina*	0	0	1	4	5	0
R2520	*Fragilaria capucina* ssp. *rumpens*	0	0	2	3	3	2
R0555	*Crucigeniella rectangularis*	0	0	1	5	2	2
R0690	*Nephrocytium agardhianum*	0	0	0	5	5	0
R0782	*Scenedesmus ellipticus*	0	0	1	5	2	2
R0935	*Chlamydomonas globosa*	0	0	1	3	6	0
R0051	*Cyclotella radiosa*	0	0	1	3	5	1
R0682	*Monoraphidium* sp.	0	0	1	2	7	0
R0971	*Pandorina morum*	0	0	2	2	4	2
R1377	*Cryptomonas curvata*	0	0	1	3	5	1
R1536	*Anabaena flos-aquae*	0	1	1	2	3	3
R1620	*Pseudanabaena catenata*	0	1	1	2	3	3
R1205	*Cosmarium bioculatum*	0	0	1	1	8	0
R1506	*Rhabdogloea* sp.	0	0	1	1	8	0
R0490	*Ankyra lanceolata*	0	0	1	3	4	2
R0762	*Scenedesmus armatus*	0	0	1	3	4	2
R0975	*Phacotus lenticularis*	0	0	1	3	4	2
R1818	*Chrysochromulina parva*	0	0	1	3	4	2
R1004	*Mougeotia thylespora*				3	7	0
R0184	*Diatoma ehrenbergii*	0	0	0	3	7	0
R1141	*Synura* sp.	0	0	1	3	3	3
R0697	*Oocystis lacustris*	0	0	1	2	5	2
R0743	*Quadrigula lacustris*	0	0	1	1	7	1
R1288	*Staurastrum gracile*	0	0	0	3	6	1
R1487	*Microcystis flos-aquae*	0	1	1	1	3	4
R0701	*Oocystis parva*	0	0	1	1	6	2
R0760	*Scenedesmus obtusus*	0	0	0	1	9	0
R0966	*Gonium pectorale*	0	0	0	1	9	0
R0996	*Tetraselmis cordiformis*	0	0	0	2	7	1
R0998	*Volvox aureus*	0	0	0	1	9	0
R1181	*Closterium acutum* var. *variabile*	0	0	0	2	7	1
R1300	*Staurastrum paradoxum*	0	0	0	2	7	1
R1519	*Synechocystis aquatilis*	0	0	0	2	7	1
R1560	*Aphanizomenon gracile*	0	0	1	2	4	3
R1613	*Planktothrix agardhii*	0	0	1	3	2	4
R0082	*Stephanodiscus minutulus*	0	0	0	3	4	3
R0489	*Ankyra judayi*	0	0	0	1	8	1
R0633	*Kirchneriella* sp.	0	0	0	2	6	2
R0654	*Lagerheimia subsalsa*	0	0	0	1	8	1

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
R0923	*Carteria* sp.	0	0	1	1	5	3
R1095	*Erkenia subaequiciliata*	0	0	1	2	3	4
R1386	*Cryptomonas ovata*	0	0	1	2	3	4
R1199	*Closterium pronum*	0	0	0	1	8	1
R1283	*Staurastrum cingulum*	0	0	0	1	8	1
R1621	*Pseudanabaena limnetica*	0	0	0	3	4	3
R0189	*Diatoma tenuis*	0	0	1	1	4	4
R0529	*Coelastrum pseudomicroporum*	0	0	0	1	7	2
R0530	*Coelastrum reticulatum*	0	0	1	2	2	5
R1726	*Euglena* sp.	0	0	1	2	2	5
R0993	*Sphaerocystis schroeteri*	0	0	0	2	5	3
R1191	*Closterium limneticum*	0	0	0	1	7	2
R1525	*Woronichinia naegeliana*	0	0	0	3	3	4
R0891	*Gloeocystis* sp.	0	0	0	1	6	3
R0660	*Micractinium pusillum*	0	0	0	1	6	3
R0820	*Schroederia setigera*	0	0	0	1	6	3
R1482	*Microcystis aeruginosa*	0	0	1	1	3	5
R0016	*Acanthoceras zachariasii*	0	0	0	2	3	5
R0024	*Aulacoseira granulata* var. *angustissima*	0	0	0	2	3	5
R0343	*Nitzschia acicularis*	0	0	1	1	2	6
R0527	*Coelastrum microporum*	0	0	1	1	2	6
R1178	*Closterium acutum*	0	0	1	1	2	6
R0704	*Oocystis solitaria*	0	0	0	2	3	5
R1003	*Mougeotia* sp.	0	0	0	1	5	4
R0806	*Scenedesmus quadricauda*	0	0	0	1	4	5
R0940	*Chlamydomonas reinhardtii*	0	0	0	1	4	5
R0047	*Cyclotella meneghiniana*	0	0	0	1	4	5
R0963	*Eudorina elegans*	0	0	0	2	2	6
R1176	*Closterium aciculare*	0	0	0	0	6	4
R1311	*Staurastrum tetracerum*	0	0	0	0	6	4
R1153	*Pseudopedinella erkensis*	0	0	0	2	2	6
R0023	*Aulacoseira granulata*	0	0	0	1	3	6
R0506	*Chlorococcum* sp.	0	0	0	0	5	5
R0698	*Oocystis marssonii*	0	0	0	1	3	6
R1518	*Synechococcus* sp.	0	0	0	0	5	5
R1558	*Aphanizomenon flos-aquae*	0	0	0	1	3	6
R0713	*Pediastrum boryanum*	0	0	0	0	4	6
R0722	*Pediastrum simplex*	0	0	0	1	2	7
R0725	*Pediastrum tetras*	0	0	0	1	2	7
R0754	*Scenedesmus acuminatus*	0	0	0	0	4	6
R1499	*Microcystis wesenbergii*	0	0	0	1	2	7
R1582	*Limnothrix redekei*	0	0	0	1	2	7
R0488	*Ankyra ancora*	0	0	0	1	1	8
R0523	*Coelastrum astroideum*	0	0	0	0	3	7

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
R0616	*Golenkinia radiata*	0	0	0	1	1	8
R0716	*Pediastrum duplex*	0	0	0	0	3	7
R0777	*Scenedesmus dimorphus*	0	0	0	1	1	8
R1531	*Anabaena circinalis*	0	0	0	1	1	8
R1544	*Anabaena planctonica*	0	0	0	0	3	7
R1748	*Phacus longicauda*	0	0	0	1	1	8
R0078	*Stephanodiscus binderanus*	0	0	0	0	2	8
R0079	*Stephanodiscus hantzschii*	0	0	0	0	2	8
R0484	*Ankistrodesmus* sp.	0	0	0	0	2	8
R0781	*Scenedesmus ecornis*	0	0	0	0	2	8
R0999	*Volvox globator*	0	0	0	0	2	8
R1622	*Pseudanabaena mucicola*	0	0	0	0	2	8
R0503	*Chlorella* sp.	0	0	0	0	2	8
R0020	*Aulacoseira ambigua*	0	0	0	0	1	9
R0500	*Characium* sp.	0	0	0	0	1	9
R1610	*Planktolyngbya limnetica*	0	0	0	0	1	9
R0028	*Aulacoseira italica*	0	0	0	0	0	10
R0930	*Chlamydocapsa planctonica*	0	0	0	0	0	10
A. In alphabetic order		7	0	0	0	0	0
R0016	*Acanthoceras zachariasii*	0	0	0	2	3	5
R2503	*Achnanthidium catenatum*	0	0	1	8	1	0
R1531	*Anabaena circinalis*	0	0	0	1	1	8
R1536	*Anabaena flos-aquae*	0	1	1	2	3	3
R1544	*Anabaena planctonica*	0	0	0	0	3	7
R1549	*Anabaena spiroides*	0	1	6	1	1	1
R0484	*Ankistrodesmus* sp.	0	0	0	0	2	8
R0488	*Ankyra ancora*	0	0	0	1	1	8
R0489	*Ankyra judayi*	0	0	0	1	8	1
R0490	*Ankyra lanceolata*	0	0	1	3	4	2
R1558	*Aphanizomenon flos-aquae*	0	0	0	1	3	6
R1560	*Aphanizomenon gracile*	0	0	1	2	4	3
R1413	*Aphanocapsa delicatissima*	0	3	3	2	2	0
R1414	*Aphanocapsa elachista*	0	1	2	2	4	1
R1427	*Aphanothece clathrata*	0	0	1	4	5	0
R0020	*Aulacoseira ambigua*	0	0	0	0	1	9
R0023	*Aulacoseira granulata*	0	0	0	1	3	6
R0024	*Aulacoseira granulata* var. *angustissima*	0	0	0	2	3	5
R0025	*Aulacoseira islandica*	0	1	3	3	2	1
R0028	*Aulacoseira italica*	0	0	0	0	0	10
R0033	*Aulacoseira subarctica*	0	1	8	1	0	0
R1155	*Bitrichia chodatii*	4	4	2	0	0	0
R0493	*Botryococcus braunii*	5	2	2	1	0	0
R0923	*Carteria* sp.	0	0	1	1	5	3
R0500	*Characium* sp.	0	0	0	0	1	9

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
R0930	*Chlamydocapsa planctonica*	0	0	0	0	0	10
R0935	*Chlamydomonas globosa*	0	0	1	3	6	0
R0940	*Chlamydomonas reinhardtii*	0	0	0	1	4	5
R0503	*Chlorella* sp.	0	0	0	0	2	8
R0506	Chlorococcum sp.	0	0	0	0	5	5
R1438	Chroococcus limneticus	4	2	2	1	1	0
R1443	Chroococcus minutus	1	3	4	1	1	0
R1446	Chroococcus turgidus	5	3	2			
R1375	Chroomonas sp.	0	1	2	2	5	0
R1818	Chrysochromulina parva	0	0	1	3	4	2
R1166	Chrysolykos planctonicus	5	4	1			
R1167	Chrysolykos skujae	2	8				
R1176	Closterium aciculare	0	0	0	0	6	4
R1178	Closterium acutum	0	0	1	1	2	6
R1181	Closterium acutum var. variabile	0	0	0	2	7	1
R1191	Closterium limneticum	0	0	0	1	7	2
R1199	Closterium pronum	0	0	0	1	8	1
R0523	Coelastrum astroideum	0	0	0	0	3	7
R0527	Coelastrum microporum	0	0	1	1	2	6
R0529	Coelastrum pseudomicroporum	0	0	0	1	7	2
R0530	Coelastrum reticulatum	0	0	1	2	2	5
R0533	Coenochloris fottii	0	1	3	3	2	1
R0606	Coenococcus planctonicus	0	1	5	4	0	0
R1205	Cosmarium bioculatum	0	0	1	1	8	0
R1209	Cosmarium depressum	2	2	3	1	1	1
R2556	Crucigeniella irregularis	0	0	4	4	2	0
R0555	Crucigeniella rectangularis	0	0	1	5	2	2
R1377	Cryptomonas curvata	0	0	1	3	5	1
R1386	Cryptomonas ovata	0	0	1	2	3	4
R0040	Cyclotella bodanica	7	3	0	0	0	0
R0042	Cyclotella comensis	7	2	1	0	0	0
R2195	Cyclotella cyclopuncta	7	3	0	0	0	0
R2196	Cyclotella distinguenda	8	1	1	0	0	0
R0047	Cyclotella meneghiniana	0	0	0	1	4	5
R0048	Cyclotella ocellata	0	1	1	4	3	1
R0051	Cyclotella radiosa	0	0	1	3	5	1
R0184	Diatoma ehrenbergii	0	0	0	3	7	0
R0189	Diatoma tenuis	0	0	1	1	4	4
R0191	Diatoma vulgaris	5	2	1	1	1	0
R0571	Dictyosphaerium pulchellum	0	0	1	5	4	0
R0582	Didymocystis sp.	0	1	4	4	1	0
R1066	Dinobryon bavaricum	3	3	2	2	0	0
R1069	Dinobryon crenulatum	2	2	3	2	1	0
R1070	Dinobryon cylindricum	7	2	1	0	0	0

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
R1074	Dinobryon divergens var. schauinslandii	0	0	1	9	0	0
R1081	Dinobryon sertularia	0	1	1	5	3	0
R2058	Discostella glomerata	6	3	1			
R1095	Erkenia subaequiciliata	0	0	1	2	3	4
R0963	Eudorina elegans	0	0	0	2	2	6
R1726	Euglena sp.	0	0	1	2	2	5
R2520	Fragilaria capucina ssp. rumpens	0	0	2	3	3	2
R1642	Glenodinium sp.	0	2	5	3		
R0891	Gloeocystis sp.	0	0	0	1	6	3
R0616	Golenkinia radiata	0	0	0	1	1	8
R0966	Gonium pectorale	0	0	0	1	9	0
R1654	Gymnodinium sp.	1	5	2	1	1	0
R1660	Gymnodinium uberrimum	1	6	2	1		
R1037	Kephyrion sp.	6	1	1	1	1	0
R0633	Kirchneriella sp.	0	0	0	2	6	2
R0649	Lagerheimia genevensis	0	0	3	3	4	0
R0654	Lagerheimia subsalsa	0	0	0	1	8	1
R1582	Limnothrix redekei	0	0	0	1	2	7
R1096	Mallomonas acaroides	0	1	2	4	2	1
R1097	Mallomonas akrokomos	0	0	2	4	3	1
R1100	Mallomonas caudata	0	0	1	4	5	0
R0660	Micractinium pusillum	0	0	0	1	6	3
R1482	Microcystis aeruginosa	0	0	1	1	3	5
R1487	Microcystis flos-aquae	0	1	1	1	3	4
R1499	Microcystis wesenbergii	0	0	0	1	2	7
R0682	Monoraphidium sp.	0	0	1	2	7	0
R1003	Mougeotia sp.	0	0	0	1	5	4
R1004	Mougeotia thylespora				3	7	
R0690	Nephrocytium agardhianum	0	0	0	5	5	
R0343	Nitzschia acicularis	0	0	1	1	2	6
R0697	Oocystis lacustris	0	0	1	2	5	2
R0698	Oocystis marssonii	0	0	0	1	3	6
R0701	Oocystis parva	0	0	1	1	6	2
R0704	Oocystis solitaria	0	0	0	2	3	5
R0971	Pandorina morum	0	0	2	2	4	2
R0713	Pediastrum boryanum	0	0	0	0	4	6
R0716	Pediastrum duplex	0	0	0	0	3	7
R0722	Pediastrum simplex	0	0	0	1	2	7
R0725	Pediastrum tetras	0	0	0	1	2	7
R1687	Peridinium cinctum	0	1	2	4	2	1
R1691	Peridinium inconspicuum	1	4	3	2		
R1697	Peridinium pusillum	0	9	1	0	0	0
R1903	Peridinium umbonatum - complex	7	2		1		
R1704	Peridinium willei	1	4	2	1	1	1

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
R0975	Phacotus lenticularis	0	0	1	3	4	2
R1748	Phacus longicauda	0	0	0	1	1	8
R1610	Planktolyngbya limnetica	0	0	0	0	1	9
R1613	Planktothrix agardhii	0	0	1	3	2	4
R1617	Planktothrix rubescens	1	1	3	4	1	0
R1620	Pseudanabaena catenata	0	1	1	2	3	3
R1621	Pseudanabaena limnetica	0	0	0	3	4	3
R1622	Pseudanabaena mucicola	0	0	0	0	2	8
R1153	Pseudopedinella erkensis	0	0	0	2	2	6
R0733	Pseudoquadrigula sp.	8	1	1	0	0	0
R0736	Pseudosphaerocystis lacustris	0	0	2	5	2	1
R0743	Quadrigula lacustris	0	0	1	1	7	1
R1506	Rhabdogloea sp.	0	0	1	1	8	0
R0754	Scenedesmus acuminatus	0	0	0	0	4	6
R0762	Scenedesmus armatus	0	0	1	3	4	2
R0777	Scenedesmus dimorphus	0	0	0	1	1	8
R0781	Scenedesmus ecornis	0	0	0	0	2	8
R0782	Scenedesmus ellipticus	0	0	1	5	2	2
R0760	Scenedesmus obtusus	0	0	0	1	9	
R0806	Scenedesmus quadricauda	0	0	0	1	4	5
R0820	Schroederia setigera	0	0	0	1	6	3
R1510	Snowella lacustris	0	1	4	4	1	0
R0993	Sphaerocystis schroeteri	0	0	0	2	5	3
R1342	Sphaerozosma sp.	0	0	1	8	1	0
R1282	Staurastrum chaetoceras	0	0	3	7		
R1283	Staurastrum cingulum	0	0	0	1	8	1
R1288	Staurastrum gracile	0	0	0	3	6	1
R1300	Staurastrum paradoxum	0	0	0	2	7	1
R1303	Staurastrum pingue	0	0	2	5	3	0
R1311	Staurastrum tetracerum	0	0	0	0	6	4
R2169	Staurosira construens	0	0	2	2	6	
R0078	Stephanodiscus binderanus	0	0	0	0	2	8
R0079	Stephanodiscus hantzschii	0	0	0	0	2	8
R0082	Stephanodiscus minutulus	0	0	0	3	4	3
R0083	Stephanodiscus neoastraea		1	2	4	3	0
R1518	Synechococcus sp.	0	0	0	0	5	5
R1519	Synechocystis aquatilis	0	0	0	2	7	1
R1141	Synura sp.	0	0	1	3	3	3
R0440	Tabellaria fenestrata	1	1	4	4		
R0442	Tabellaria flocculosa	1	4	5			
R0848	Tetraedron minimum	0	1	1	4	3	1
R0996	Tetraselmis cordiformis			0	2	7	1
R1776	Trachelomonas volvocina			1	4	5	0
R2174	Ulnaria delicatissima var. angustissima	2	3	3	2		

Code	Taxon	Trophic range (as TP in µg L–1)					
		≤5	5-8	8-15	15-30	30-60	>60
R1151	*Uroglena* sp.	0	3	3	3	1	0
R2549	*Urosolenia longiseta*	0	1	3	3	3	0
R0998	*Volvox aureus*	0	0	0	1	9	0
R0999	*Volvox globator*	0	0	0	0	2	8
R1525	*Woronichinia naegeliana*	0	0	0	3	3	4

Reference Conditions and Class Boundaries

The reference conditions and class boundaries of the three parameters chlorophyll-a, total biovolume and Brettum index were developed during the intercalibration process and are now harmonised between Slovenia, Italy, France, Germany and Austria. For Austrian lakes, ranges rather than fixed values were defined for the three parameters and the two IC lake types. Table A.3 lists the ranges of the original values for total biovolume, chlorophyll-a concentration and Brettum index, the EQR values and the normalised EQR values. Following the proposal on the position of the Austrian lakes within these ranges, concrete reference values and class boundaries are now available for each of the 38 Alpine lakes >50 ha listed in Table A.4.

Ecological Quality Ratio and the Classification of the Ecological Status

Any classification following the principles of the WFD is based on a comparison of the status quo with the reference state. The deviation is calculated as the ecological quality ratio for the chlorophyll-a concentration (EQR_{Chl}), the total biovolume (EQR_{BV}) and the Brettum index (EQR_{Bi}):

EQR_{Chl} and EQR_{BV} = reference value/measured value   (5)

EQR_{Bi} = measured value/reference value   (6)

In order to enable a combination of EQR values, they are transformed ("normalised") such that the class boundaries are equidistant. This allows the ecological status class to be directly identified from the "normalised" EQR value (nEQR 0.8 = class boundary high / good, 0.6 = good / moderate etc.).

Transformation of EQR to nEQR is done using the following algorithms:

EQR_i	$nEQR_i$
≥ 1	1
≥ $EQR_{H/G}$	$(EQR_i - EQR_{H/G}) / (1 - EQR_{H/G}) * 0.2 + 0.8$
$EQR_{H/G} > EQR_i ≥ EQR_{G/M}$	$(EQR_i - EQR_{G/M}) / (EQR_{H/G} - EQR_{G/M}) * 0.2 + 0.6$
$EQR_{G/M} > EQR_i ≥ EQR_{M/P}$	$(EQR_i - EQR_{M/P}) / (EQR_{G/M} - EQR_{M/P}) * 0.2 + 0.4$
$EQR_{M/P} > EQR_i ≥ EQR_{P/B}$	$(EQR_i - EQR_{P/B}) / (EQR_{M/P} - EQR_{P/B}) * 0.2 + 0.2$
< $EQR_{P/B}$	$EQR_i / EQR_{P/B} * 0.2$

The **assessment** of **single years** is based on the arithmetic means of the normalised EQR values for chlorohyll-a, total biovolume and the Brettum index:

$$nEQR_{gesamt} = \frac{(nEQR_{BV} + nEQR_{Chl})/2 + nEQR_{BI}}{2}$$ (7)

The **final assessment** of the ecological status is based on the **average** of the final normalised EQR values **of three subsequent years**. The status classes include the lower class boundary, whereas the upper class boundary is assigned to the higher class (Table A.2).

The starting point of the assessment is the reference values and EQR class boundaries given in Table A.3and Table A.4. All calculations based on these values (e.g. class boundaries for chlorophyll-a, total biovolume or the Brettum index, normalised EQR values, combination of nEQR values and final calculation) are carried out without rounding off the values.

Table A.2 Assessment of the ecological status using phytoplankton. Values lying exactly on the class boundaries are classified in the higher class (0.8 = high, 0.6 = good etc.).

Ecological status	$nEQR_{total}$
High	≥0,80
Good	0,60 – 0,80
Moderate	0,40 – 0,60
Poor	0,20 – 0,40
Bad	<0,20

At present, it is not possible to confidently assign reference values to the Neusiedler See, the salt pans in the Seewinkel region and the Old Danube. The assessment methods recently developed by WOLFRAM et al. (2008, 2011) remain to be validated. There are also uncertainties in the definition of the reference state for some Alpine lakes, where either no or limited data on the phytoplankton are available. For instance, there are currently no reference values for the very shallow Almsee (mean depth <3 m) (Table A.4).

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Table A.3 *Ranges, EQR values and normalised EQR values of the reference conditions and class boundaries of total biovolume (mm3 L–1), chlorophyll-a concentration (µg L–1) and the Brettum index in the two IC lake types, L-AL3 and L-AL4. Within the ranges of the two types, the reference conditions may vary depending on the hydromorphology. For Austrian lakes, the positions within the ranges are given in Table A.4 (1 = minimum value, 2 = median value, 3 = maximum value).*

	Total biovolume L-AL3							Total biovolume L-AL4					
	biovol.	ranges			EQR			biovol.	ranges			EQR	
	mm^3 L^{-1}	1	2	3	EQR	nEQR		mm^3 L^{-1}	1	2	3	EQR	nEQR
Ref	0.2-0.3	0.20	0.25	0.30	1.00	1.0	Ref	0.5-0.7	0.50	0.60	0.70	1.00	1.0
H/G	0.3-0.5	0.33	0.42	0.50	0.60	0.8	H/G	0.8-1.1	0.78	0.94	1.09	0.64	0.8
G/M	0.8-1.2	0.80	1.00	1.20	0.25	0.6	G/M	1.9-2.7	1.92	2.31	2.69	0.26	0.6
M/P	2.1-3.1	2.00	2.50	3.10	0.10	0.4	M/P	5.0-7.0	5.00	6.00	7.00	0.10	0.4
P/B	5.3-7.5	5.00	6.25	7.50	0.04	0.2	P/B	12.5-17.5	12.50	15.00	17.50	0.04	0.2
	Chlorophyll-a L-AL3							Chlorophyll-a L-AL4					
	conc.	ranges			EQR			conc.	ranges			EQR	
	µg L^{-1}	1	2	3	EQR	nEQR		µg L^{-1}	1	2	3	EQR	nEQR
Ref	1.5-1.9	1.50	1.70	1.90	1.00	1.0	Ref	2.7-3.3	2.70	3.00	3.30	1.00	1.0
H/G	2.1-2.7	2.14	2.43	2.71	0.70	0.8	H/G	3.6-4.4	3.60	4.00	4.40	0.75	0.8
G/M	3.8-4.8	3.75	4.25	4.75	0.40	0.6	G/M	6.6-8.0	6.59	7.32	8.05	0.41	0.6
M/P	6.8-8.6	6.82	7.73	8.64	0.22	0.4	M/P	11.7-14.3	11.74	13.04	14.35	0.23	0.4
P/B	12.5-15.8	12.50	14.17	15.83	0.12	0.2	P/B	22.5-27.5	22.50	25.00	27.50	0.12	0.2
	Brettum-Index L-AL3							Brettum-Index L-AL4					
	index	ranges			EQR			index	ranges			EQR	
		1	2	3	EQR	nEQR			1	2	3	EQR	nEQR
Ref	5.09-5.29	5.29	5.19	5.09	1.000	1.0	Ref	3.97-4.17	4.17	4.07	3.97	1.000	1.0
H/G	4.21-4.37	4.37	4.29	4.21	0.827	0.8	H/G	3.45-3.62	3.62	3.54	3.45	0.869	0.8
G/M	3.33-3.46	3.46	3.39	3.33	0.654	0.6	G/M	2.93-3.08	3.08	3.00	2.93	0.738	0.6
M/P	2.45-2.54	2.54	2.50	2.45	0.481	0.4	M/P	2.41-2.53	2.53	2.47	2.41	0.607	0.4
P/B	1.57-1.63	1.63	1.60	1.57	0.308	0.2	P/B	1.89-1.98	1.98	1.94	1.89	0.476	0.2

Table A.4 *Reference values, class boundaries (H = high, G = good, M = moderate, P = poor, B = bad) and EQR values for the Brettum index and the total biovolume (mm^3 L^{-1}). No values are available thus far for the Neusiedler See, the Seewinkel salt pans and the Old Danube.*

Lake		Natural lakes	IC	Range	Chlorophyll-a			Biovolume			Brettum index		
Type			type		Ref	H/G	G/M	Ref	H/G	G/M	Ref	H/G	G/M
B	B1	Bodensee	3	1	1.5	0.70	0.40	0.20	0.60	0.25	5.29	0.827	0,654
	B2	Obertrumer See	4	2	3.0	0.75	0.41	0.60	0.64	0.26	4.07	0.869	0,738
		Mattsee	4	1	2.7	0.75	0.41	0.50	0.64	0.26	4.17	0.869	0,738
		Irrsee	4	2	3.0	0.75	0.41	0.60	0.64	0.26	4.07	0.869	0,738
		Grabensee	4	3	3.3	0.75	0.41	0.70	0.64	0.26	3.97	0.869	0,738
		Wallersee	4	2	3.0	0.75	0.41	0.60	0.64	0.26	4.07	0.869	0,738
C	C1a	Ossiacher See	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Wörthersee	3	3	1.9	0.70	0.40	0.30	0.60	0.25	5.09	0.827	0,654
		Klopeiner See	3	3	1.9	0.70	0.40	0.30	0.60	0.25	5.09	0.827	0,654
	C1b	Faaker See	4	1	2.7	0.75	0.41	0.50	0.64	0.26	4.17	0.869	0,738
		Pressegger See	4	1	2.7	0.75	0.41	0.50	0.64	0.26	4.17	0.869	0,738
		Keutschacher See	4	2	3.0	0.75	0.41	0.60	0.64	0.26	4.27	0.869	0,738
		Längsee	4	3	3.3	0.75	0.41	0.70	0.64	0.26	3.97	0.869	0,738
D	D1	Hallstätter See	3	1	1.5	0.70	0.40	0.20	0.60	0.25	5.29	0.827	0,654
		Traunsee	3	1	1.5	0.70	0.40	0.20	0.60	0.25	5.29	0.827	0,654
		Mondsee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Attersee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Fuschlsee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Wolfgangsee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
	D2a	Lunzer See	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Erlaufsee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Offensee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
	D2b	Almsee	–	–	–	–	–	–	–	–	–	–	–
		Hintersee	(3)	(3)	(1.9)	0.70	0.40	(0.30)	0.60	0.25	(5.09)	0.827	0,654
		Walchsee	(3)	(3)	(1.9)	0.70	0.40	(0.30)	0.60	0.25	(5.09)	0.827	0,654
	D3	Millstätter See	3	3	1.9	0.70	0.40	0.30	0.60	0.25	5.09	0.827	0,654

Lake		Natural lakes	IC	Range	Chlorophyll-a			Biovolume			Brettum index		
Type			type		Ref	H/G	G/M	Ref	H/G	G/M	Ref	H/G	G/M
		Zeller See	3	3	1.9	0.70	0.40	0.30	0.60	0.25	5.09	0.827	0,654
E	E1	Vorderer Gosausee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Altausseer See	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Grundlsee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Toplitzsee	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Hintersteiner See	3	2	1.7	0.70	0.40	0.25	0.60	0.25	5.19	0.827	0,654
		Plansee	3	1	1.5	0.70	0.40	0.20	0.60	0.25	5.29	0.827	0,654
		Haldensee	(3)	(2)	(1.7)	0.70	0.40	(0.25)	0.60	0.25	(5.09)	0.827	0,654
		Heiterwanger See	3	1	1.5	0.70	0.40	0.20	0.60	0.25	5.29	0.827	0,654
		Vilsalpsee	(3)	(2)	(1.7)	0.70	0.40	(0.25)	0.60	0.25	(5.09)	0.827	0,654
		Achensee	3	1	1.5	0.70	0.40	0.20	0.60	0.25	5.29	0.827	0,654
	E2	Weißensee	3	3	1.9	0.70	0.40	0.30	0.60	0.25	5.29	0.827	0,654

A.2 Germany: PSI (Phyto-Seen-Index) - Bewertungsverfahren für Seen mittels Phytoplankton zur Umsetzung der EG-Wasserrahmenrichtlinie in Deutschland

Sampling and analyses

The German assessment procedure includes and requires a fixing of standardized methods for sampling, preservation and storage, and microscopic analysis (Nixdorf et al. 2010).

For the assessment six samples per year are needed from epilimnion or euphotic zone (clear water lakes, of which four samples must be taken in the period May-September. The taxa are counted according the Utermöhl technique and coded by the operational phytoplankton taxa list. To determine indicator species additional diatom preparation is recommended.

Assessment

The German phytoplankton-based assessment system for lakes (Mischke et al. 2008) yields a multi-metric index value, the Phyto-See-Index (PSI), and differentiates between different lake types. It classifies water bodies into one of five status classes in accordance with the Water Framework Directive (WFD). The PSI consists of three mandatory metrics: "biomass", "algal classes" and the "Phytoplankton-Taxa-Seen-Index" (PTSI).

The three compulsory metrics along the stressor "eutrophication" are calibrated and adjusted in accordance with reference sites and trophic reference conditions. Total phosphorus and the actual assessment value of the German Trophic Index (LAWA 1999) served as the stressor scale. The German Trophic Index is based on the combined classification of the common trophic parameters "chlorophyll-a", "total phosphorus" and "secchi depth" as a measure of lake transparency..

The PSI is composed of three mandatory metrics and an optional fourth metric, DI-PROF, latter not included into intercalibration. Some of these metrics are multi-parameter variables.

1. Biomass metric: this is composed of:
   a. The total biovolume of phytoplankton in the epilimnic or euphotic zone of the lake (arithmetic mean in the vegetation period from April to October of six samples);
   b. Chlorophyll-a concentration (arithmetic mean in the vegetation period from April to October;
   c. Maximum Chlorophyll-a value, if it deviates from the mean more than 25%.

2. Algal class metric: the biovolume or its percentage of total biovolume in specific annual periods (e.g. mean values of cyanophytes, dinophytes and of chlorophytes from July to October; mean value from chrysophytes from April to October);

3. PTSI (Phytoplankton Taxa Lake Index): this index evaluates the species composition based on lake-type specific lists of indicator species (332 different species) and their special trophic scores and weighting factors. The method works in two steps:
   a. trophic assignment results in a PTSI index per sample or lake year;

b. assessment by comparing current trophic state with the lake type specific trophic reference status

The results of all components and of the final index are an index value between 0.5 and 5.5 which can be easily transformed to a normalized EQR ($y = -0.2x + 1.1$).

Table A.5 Transformation of the metric index value to normalized EQR.

German metric index value	Normalized EQR
0.5 – 1.5	0.8 – 1
1.51 – 2.5	0.6 – 0.8
2.51 – 3.5	0.4 – 0.6
3.51 – 4.5	0.2 – 0.4
4.51 – 5.5	0.0 – 0.2

The final score is summarized using weighting factors of used components before averaging the metric results (details in Mischke et al. 2008).

Reference and boundary setting

The class boundaries for the total biovolume and the metric algal classes are derived by using a pre-assignment of ecological quality of the lakes. The assignment was based on a trophic score, the German LAWA-index, the estimation of local experts and in consideration of the lake type specific trophic reference state. The trophic reference status of lake types are defined (in first draft) with a view to palaeolimnological investigations, true reference sites without anthropogenic impact and ideas about background concentrations of total phosphorus and morphometric conditions in lakes. Trophic reference status is given as a trophic class according to the German LAWA-approach for assessing lakes (LAWA 1999), which combines criteria for chlorophyll a, total phosphorous and transparency (SD). During the intercalibration exercise the German reference boundaries for chlorophyll a were adjusted to intercalibration results. The trophic scores of indicator species for the PTSI were developed along the trophic gradient, German LAWA index and total phosphorus concentrations.

References

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Download link for taxa list codes and for calculation tool PhytoSee: http://www.igb-berlin.de/staff_.html?per_page=0&search=lastname&for=mischke&show=117#ankerartikel0

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A.3   Italy: Italian classification method for phytoplankton in lakes

Summary

This document outlines how status is assigned for the biological quality element phytoplankton and how boundaries have been assigned in Italy. The metrics included in the Italian phytoplankton assessment method developed for Alpine lakes are the biomass metrics chlorophyll a and total biovolume and the taxonomic composition metric PTI_{ot}. The reference value and HG boundary for each metric and each type were set from the median and the 90th %ile of the AlpGIG reference sites (lake-years) respectively. The GM, MP and PB metric boundaries were set using equidistant class widths on a log scale.

Introduction

In the Alpine GIG a phytoplankton assessment method based on three parameters was intercalibrated: chlorophyll, biomass and a taxonomic composition metric (PTI_{ot}).

Yearly average of chlorophyll a concentration, biovolume and PTIot are combined in order to calculate the overall index for phytoplankton (ICF), giving the final classification score:

* normalized EQRs of average chlorophyll and biomass metrics are averaged to give the Biomass Index;
* the normalized EQR of average PTI_{ot} metric is the Composition Index;
* Arithmetic mean of Biomass and Composition Indexes gives the final ICF value.

The Italian assessment phytoplankton method was developed from a dataset including natural lakes of the Alpine ecoregion, belonging to L-AL3 and L-AL4 types:

* L-AL3 are Lowland or mid-altitude (50-800 m a.s.l.), deep, moderate to high alkalinity (alpine influence), large;
* L-AL4 are medium or low altitude lakes (200-800 m a.s.l.), calcareous (alk > 1meq/l), with a surface area higher than 0.5 km^2 and an average depth lower than 15 m.

Metric description: sampling, analyses, principles for setting reference value and boundaries

Sampling strategies

Phytoplankton samples are taken from mid-lake stations, as integrated samples of euphotic water column. Sampling frequency is 6 times per year, according to the seasonal development of phytoplankton succession, as follows:

* Sample 1: period January to mid-March for winter assemblages;

- Sample 2: period April to mid-May for spring assemblages;
- Sample 3: period mid-May – mid June for transition between spring and summer assemblages;
- Sample 4: period July – August for summer assemblages;
- Sample 5: September for transition between summer and autumn assemblages;
- Sample 6: period mid-October – November for autumn assemblages.

All the metrics were calculated using data obtained with the same sampling strategy.

Chlorophyll a

Chlorophyll a is determined following extraction using spectrophotometric analysis. Reference values were decided at GIG level and are detailed in the Water Framework Directive Intercalibration Technical Report - Part 2: Lakes (Poikane, 2009) and in the Intercalibration decision (EC, 2008). Reference values and class boundaries of and chlorophyll-a Chl-a were set using the selected population of reference sites. The median was defined as reference value, the 90%percentile as H/G boundary – both supported by expert judgment. The GM, MP and PB metric boundaries were set using equidistant class widths on a log scale. The chlorophyll a EQR is calculated using Equation 1 below where the $Chla_{ref}$ is the GIG chlorophyll reference value in µg/l and the $Chla_{obs}$ is the observed growing season mean chlorophyll value in µg/l.

$$Chla_{EQR} = Chla_{ref}/Chla_{obs} \qquad (1)$$

Total biovolume (mg/l)

Phytoplankton samples are counted using the Utermöhl technique and total biovolume is calculated from the sum of the biovolumes of each taxon in the sample (cell number x specific cell volume). Reference values and class boundaries of total biovolume BV were set using the selected population of reference sites. The median was defined as reference value, the 90%percentile as H/G boundary – both supported by expert judgment. The GM, MP and PB metric boundaries were set using equidistant class widths on a log scale. The biovolue EQR is calculated using Equation 2 below where the BV_{ref} is the GIG biovolume reference value in mg/l and the BV_{obs} is the observed growing season mean biovolume value in mg/l.

$$BV_{EQR} = BV_{ref}/BV_{obs} \qquad (2)$$

Phytoplankton composition metric (PTI$_{ot}$)

The phytoplankton composition metric provides an indication of the state of community composition and relative abundance in relation to the eutrophication pressure gradient. Assessment is based on 6 yearly samples, collected as explained above.

Phytoplankton samples are counted following the Utermöhl technique (CEN standard). Assessment is based on an index called PTIot (Phytoplankton Trophic Index).

The metric has been implemented using the entire dataset of Alpine GIG. A comparison of results obtained with the use of datasets showed that the use of the entire pool of data has allowed us to obtain a better correlation between total phosphorus and PTIot in Lakes / year.

The criteria used to select the species for which to calculate the index were:

- Species present in at least 3 lakes;
- Species with a percentage of biovolume higher than 1%.

The necessary condition for the application of the index is that at least 70% of the total biovolume of the species for the water body is used to calculate the index.

The index is based on the calculation of the weighted average (niche centroid, ter Braak & Verdonshot, 1995) of the species k with respect to the gradient of total phosphorus for all the lakes (Equation 3). These values represent the **trophic index** for each species (TI_k). The higher this index, the higher the quality of the trophic species. Prior to the calculation of TI_k, the concentrations of total phosphorus have been transformed into logarithmic values and scaled from 1 to 5.

$$TI_k = \sum_{i=1}^{n} \frac{Y_{ik}}{Y_{+k}} TPi \qquad (3)$$

Where

Y_{ik} = biomass of species k in site i as annual average,

Y_{+k} = biomass of species k in all sites,

TP_i= Total phosphorus concentration in site i.

In a second step **tolerance** is calculated, representing the goodness of species as trophic indicator: the higher the tolerance, the worse the indicator quality of a species.

Tolerance was calculated as follows:

$$t_k = \sqrt{\sum_{i=1}^{n} \frac{Y_{ik}}{Y_{+k}} \left(TPi - TI_k\right)^2} \qquad (4)$$

From the ratio between tolerance (t_k) and trophic index (TI_k) were obtained the **indicator values** (v_i), ranging from 1 to 4. When the ratio is higher than 0.8, then $v_i = 1$; when lies between 0.8 and 0.6, $v_i = 2$; when lies between 0.4 and 0.6 $v_i = 3$; when is lower than 0.4, $v_i = 4$.

Trophic indices and indicator values for each species are reported in Table A.5.

Finally, the PTI_{ot} value for the lake is obtained from:

$$PTI_{ot} = \frac{\sum a_i TI_k v_i}{\sum a_i v_i} \qquad (5)$$

Where:

a_i = annual average of biovolume of species I,

TI_k = trophic index of species I,

v_i = indicator value of species i.

Reference values and class boundaries for PTI_{ot} was done by using the values calculated over the whole AlpGIG dataset. Reference values correspond to the median value of the reference lakes. As H/G boundary the 10th %ile of the values of the reference lakes was taken. G/M boundary was set as the 10th %ile of the index values calculated in those lakes classified as Good using the Austrian Brettum Index (Dokulil & Teubner, 2006). We chose this approach because we didn't identified any discontinuity in the relationship between eutrophication gradient and PTiot values, therefore we compared the results

obtained by the use of percentile with those obtained using lakes classified as Good by Austrian Brettum index, the only index developed at the time of the first boundary setting procedure. We also analysed the species composition of these lakes and, by an experts judgement, we agreed that the phytoplankton communities would fit with the classification of Good Quality reported by WFD. The results of the 2 intercalibration exercises confirmed the goodness of our choice.

Table A.6 reports reference values and class boundaries for the two intercalibrated lake types.

Table A.6 EQRs, reference values and quality class boundaries for PTI$_{ot}$ metric.

Type	Ecological Quality Ratio (EQR)		Index values		
	H/G boundary	G/M boundary	Reference	H/G boundary	G/M boundary
L-AL3	0.95	0.89	3.62	3.43	3.22
L-AL4	0.95	0.85	3.54	3.37	3.01

In Figure A.2 the relationship between Log TP and PTI$_{ot}$ is reported. The regression model is based on each lake/year for the whole Alpine GIG data set.

$$y = -0.655x + 4.0628$$
$$r^2 = 0.6043$$
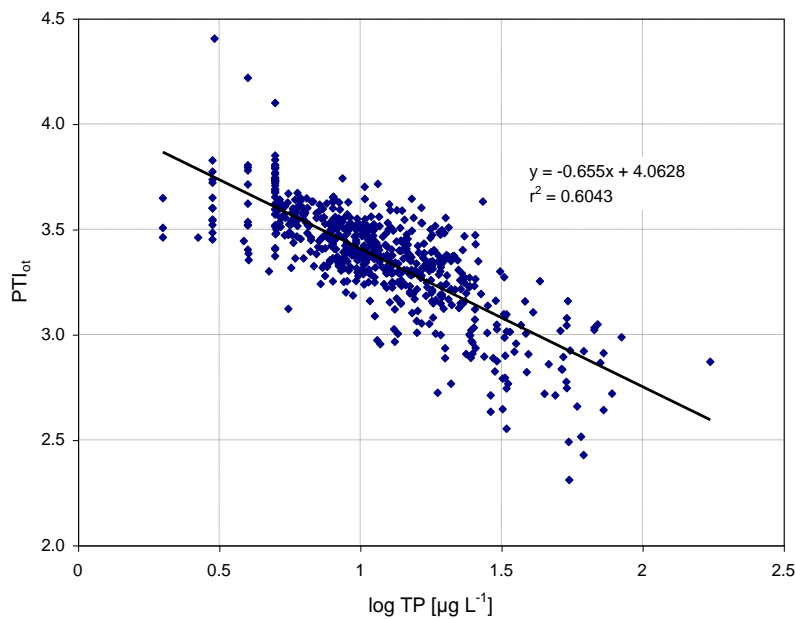
Figure A.2   Regression model between PTIot and Log TP for the Alpine GIG data set.

EQR for PTI$_{ot}$ are calculated as in Equation 6:

  EQR = PTIot$_{EQR}$ = PTIot$_{obs}$ / PTIot$_{ref}$ (6)

Calculation of EQR and normalised EQR for all metrics

EQR values for the metrics used in the Italian phytoplankton assessment method are calculated as reported in Equation 1, 2 and 6.

When EQR is higher than 1, EQR value must be set to 1.

Calculation of normalised EQR

In order to allow combination of all metrics to a whole BQE assessment, each metric EQR has to be converted to the normalised scale with equal class widths and standardised class boundaries, where the HG, GM, MP, and PB boundaries are 0.8, 0.6, 0.4, 0.2 respectively. When using indices with classes ranges different from each other, the normalization equation is based on a linear interpolation between classes boundaries. Moreover, the interpolation process takes into account the reference value as upper limit of the High-Quality class (EQR and normalised EQR equal to 1) as well as the minimum EQR value as the lower threshold of the Bad-Quality class (normalised EQR equal to 0).

Therefore, the normalization equations will be different for each quality class, as below.

$EQR_{norm} = 1 - 0.2*(1-EQR)/(1-EQR_{HG})$	(7)	Class "High"
$EQR_{norm} = 0,8 - 0,2*(EQR_{HG} - EQR)/(EQR_{HG} - EQR_{GM})$	(8)	Class "Good"
$EQR_{norm} = 0,6 - 0,2*(EQR_{MP} - EQR)/(EQR_{GM} - EQR_{MP})$	(9)	Class "Moderate"
$EQR_{norm} = 0,4 - 0,2*(EQR_{MP} - EQR)/(EQR_{MP} - EQR_{PB})$	(10)	Class "Poor"
$EQR_{norm} = 0,2 - 0,2*(EQR_{PB} - EQR)/(EQR_{PB} - EQR_{min})$	(11)	Class "Bad"

Where: EQR are the measured values, EQR_{HG}, EQR_{GM}, EQR_{MP}, EQR_{PB} are the EQR values (not normalized) at the boundary between two quality classes and EQR_{min} is the minimum EQR of each metric.

EQR_{min} is set to 0 for PTI_{ot} and to 20 times the value of the P/B threshold for biovolume and chlorophyll.

Combination of metrics to whole quality element result

The following process is used to combine single metrics to a whole quality element results for lake phytoplankton (to be done for a whole growing season only, not for single samples):

- Average the normalised EQRs of chlorophyll a and total biovolume (two biomass metrics). This is important to avoid too heavy weight on the biomass metric relative to the other metrics.
- Average the normalised EQRs for the biomass metrics from point 1 with the normalised EQRs of the PTI_{ot}.

Reference values and class boundaries for each type

Table A.7, Table A.8, and Table A.9 gives all the reference values and class boundaries for the Italian classification system for each metric both as absolute values and as EQRs (non-normalised). The final whole BQE class boundaries are simply the normalised boundaries: 0.8, 0.6, 0.4, and 0.2 for the HG, GM, MP, PB boundaries respectively. Ranges were used instead of fixed values, for biovolume and chlorophyll-a, because IC lake types are rather broad and do not reflect geographical or other typological differences. Fixed values may cause problems when there is the need to transpose the values of the common IC type to their more detailed national typology.

Table A.7 Reference values, class boundaries and EQR for the total biovolume (BV) for the IC lake types L-AL3 and L-AL4 (GIG agreement).

	L-AL3	L-AL4

	BV [mm³ L⁻¹]	EQR	BV [mm³ L⁻¹]	EQR
Ref	0.2–0.3	1.00	0.5–0.7	1.00
H/G	0.3–0.5	0.60	0.8–1.1	0.64
G/M	0.8–1.2	0.25	1.9–2.7	0.26
M/P	2.1–3.1	0.10	5.0–6.9	0.10
P/B	5.3–7.8	0.04	12.5–17.4	0.04

Up to now in Italy we do not have enough data to calculate the boundaries for all the national typologies. Use of the ranges will allow us to discriminate, for example, large deep subalpine lakes, characterized by lower algal production and, therefore, also lower reference values, from the other L-AL3 with a mean depth close to 15 metres. Within L-AL4 lakes, we can separate very shallow polymictic lakes, with higher algal production and reference values, from the other small lakes. In the Italian official phytoplankton assessment method, lakes of the Alpine Eco Region are subdivided in 4 types, coincident with the ranges showed before. Ranges are going to be officially adopted, after the applicability to all the lakes of Italian alpine eco region will be verified.

*Table A.8 Reference values, class boundaries and EQR for the **chlorophyll-a** concentration (chl-a) for the IC lake types L-AL3 and L-AL4 (GIG agreement).*

	L-AL3		L-AL4	
	chl-a [µg L⁻¹]	EQR	chl-a [µg L⁻¹]	EQR
Ref	1.5–1.9	1.00	2.7–3.3	1.00
H/G	2.1–2.7	0.70	3.6–4.4	0.75
G/M	3.8–4.7	0.40	6.6–8.0	0.41
M/P	6.8–8.7	0.22	11.7–14.6	0.23
P/B	12.5–15.4	0.12	22.5–26.7	0.12

*Table A.9 Reference values, class boundaries and EQR for and **PTI$_{ot}$***

	L-AL3		L-AL4	
	PTI$_{ot}$	EQR	PTI$_{ot}$	EQR
Ref	3.62	1.00	3.54	1.00
H/G	3.43	0.95	3.37	0.95
G/M	3.22	0.89	3.01	0.85
M/P	3.01	0.83	2.64	0.75
P/B	2.80	0.77	2.28	0.64

Correlation of Italian combined whole BQE phytoplankton method against pressure (total-P)

The Italian Phytoplankton Assessment Method (IPAM) is well correlated with pressure (Total-P) for both lakes typologies . The r^2 vary from 0.56, for L-AL3, to 0.57 for L-AL4 as reported in Figure A.3 and Figure A.4.
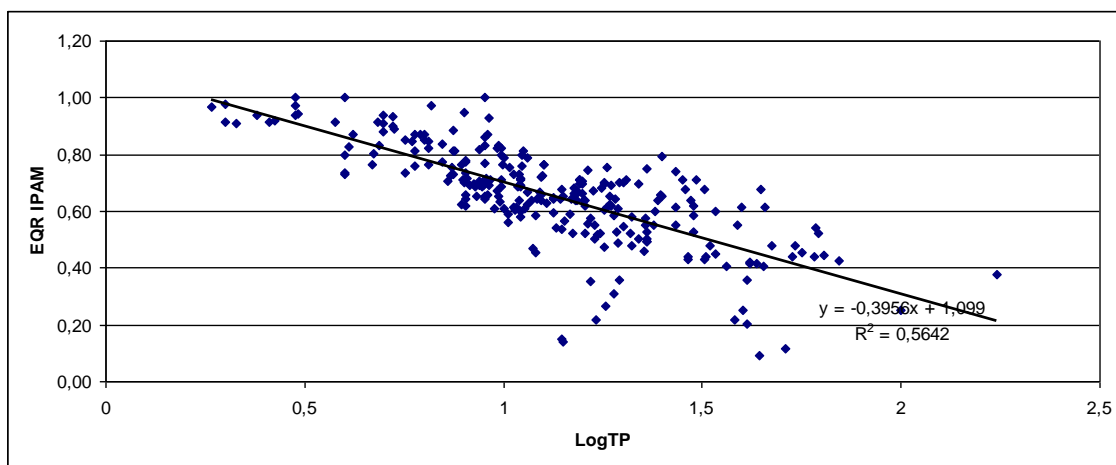
Figure A.3   Correlation between LogTP and EQR's IPAM for 132 L-AL3 lakes-year of Alpine GIG.
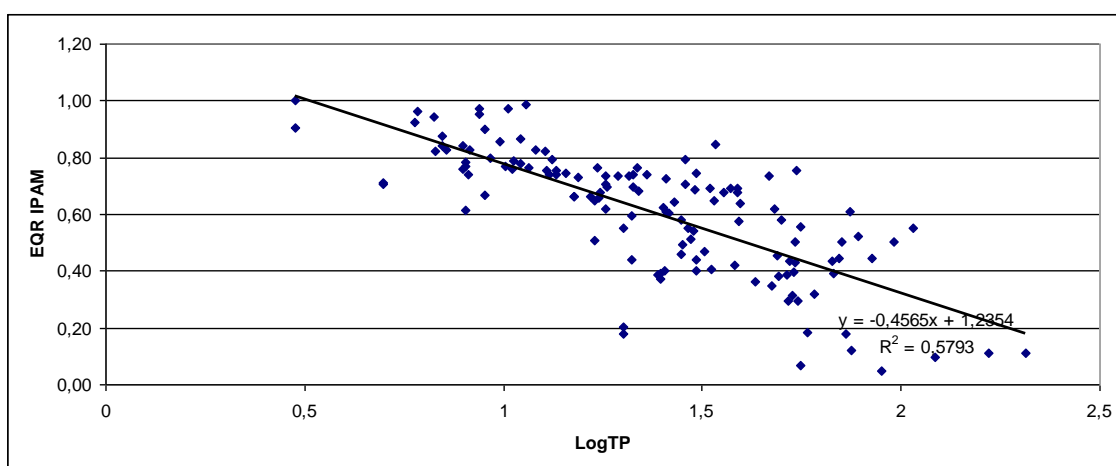
Figure A.4   Correlation between LogTP and EQR's IPAM for 165 L-AL4 lakes-year of Alpine GIG

Table A.10   Phytoplankton taxa used in the implementation of PTI_{ot} Index. The table reports frequency of occurrence in the dataset, trophic index and indicator values for each species.

Taxon	Frequency	TI_k	v_i
Amphora	11	3.14	2
Amphora ovalis	25	3.33	4
Anabaena	73	3.27	1
Anabaena circinalis	18	2.87	1
Anabaena flos-aquae	221	3.15	1
Anabaena planctonica	46	2.19	4
Anabaena solitaria	5	1.85	4
Anabaena sphaerica	3	3.29	4
Anabaena spiroides	75	3.47	3
Anabaena viguieri	5	2.30	4
Ankistrodesmus	67	3.00	1

Taxon	Frequency	TI_k	v_i
Ankyra ancora	44	2.55	4
Ankyra judayi	56	2.53	4
Ankyra lanceolata	60	2.83	2
Aphanizomenon	68	3.38	1
Aphanizomenon flos-aquae	204	2.57	2
Aphanizomenon gracile	16	2.97	3
Aphanocapsa	58	3.20	2
Aphanocapsa delicatissima	143	3.35	2
Aphanocapsa elachista	97	3.00	2
Aphanocapsa holsatica	4	2.55	4
Aphanothece	90	3.45	3
Aphanothece clathrata	87	3.02	2
Asterionella formosa	659	3.29	1
Aulacoseira	38	3.36	3
Aulacoseira ambigua	13	2.80	4
Aulacoseira granulata	98	2.59	3
Aulacoseira granulata v. angustissima	74	2.64	3
Aulacoseira islandica	116	3.16	2
Aulacoseira islandica v. helvetica	5	2.29	4
Aulacoseira italica	11	1.97	3
Aulacoseira subarctica	19	3.55	3
Bitrichia chodatii	184	3.69	3
Botryococcus	7	2.52	4
Botryococcus braunii	180	3.39	1
Carteria	147	2.86	2
Ceratium	47	3.89	2
Ceratium cornutum	33	3.95	3
Ceratium furcoides	5	2.10	4
Ceratium hirundinella	657	3.44	1
Chamaesiphon	4	2.41	4
Chlamydocapsa planktonica	19	1.98	4
Chlamydomonas	384	3.41	1
Chlamydomonas globosa	23	2.71	4
Chlamydomonas reinhardtii	37	2.22	3
Chlorella	68	2.39	3
Chlorella vulgaris	62	2.60	3
Chlorolobion	62	3.49	4
Choricystis	14	3.15	4
Choricystis chodatii	12	3.38	1
Chromulina	151	3.19	2
Chroococcus	118	3.46	4
Chroococcus limneticus	122	3.46	1
Chroococcus minimus	6	3.41	2
Chroococcus minutus	82	3.54	2
Chroococcus turgidus	19	3.32	4

Taxon	Frequency	TI_k	v_i
Chroomonas	86	3.34	1
Chrysidiastrum catenatum	51	3.44	4
Chrysochromulina	3	3.20	3
Chrysochromulina parva	150	2.92	2
Chrysococcus	29	3.32	2
Chrysococcus minutus	5	3.14	4
Chrysococcus rufescens	21	2.97	4
Chrysolykos	69	3.55	2
Closterium aciculare	61	3.22	1
Closterium acutum	107	2.85	2
Closterium acutum v. variabile	96	2.58	3
Cocconeis placentula	31	2.78	4
Coelastrum	62	2.92	4
Coelastrum astroideum	28	2.39	3
Coelastrum microporum	117	2.82	1
Coelastrum pseudomicroporum	20	2.43	4
Coelastrum reticulatum	74	3.21	2
Coelosphaerium	33	3.05	3
Coelosphaerium kuetzingianum	53	2.72	2
Coenochloris	22	3.53	4
Coenocystis	4	2.58	4
Cosmarium	191	3.33	1
Cosmarium bioculatum	30	3.15	3
Cosmarium depressum	145	3.38	1
Crucigenia	36	2.67	4
Crucigenia quadrata	20	2.91	3
Crucigenia tetrapedia	103	2.65	4
Crucigeniella	21	3.40	2
Crucigeniella rectangularis	49	3.04	2
Cryptomonas	606	3.35	2
Cryptomonas	3	2.66	4
Cryptomonas curvata	38	2.98	4
Cryptomonas erosa	201	2.98	1
Cryptomonas erosa var. reflexa	8	2.59	3
Cryptomonas marssonii	339	3.18	1
Cryptomonas obovata	63	3.85	3
Cryptomonas ovata	263	2.81	2
Cryptomonas reflexa	42	2.52	3
Cryptomonas rostratiformis	127	2.72	3
Cryptomonas tetrapyrenoidosa	3	2.27	4
Cyanodictyon planktonicum	11	3.45	4
Cyclotella	467	3.69	2
Cyclotella bodanica	92	3.81	1
Cyclotella comensis	197	3.87	2
Cyclotella comta	3	2.33	3

Taxon	Frequency	TI_k	v_i
Cyclotella glomerata	27	3.87	2
Cyclotella meneghiniana	16	3.34	2
Cyclotella ocellata	40	2.85	2
Cyclotella pseudostelligera	19	3.38	4
Cyclotella radiosa	192	3.06	2
Cyclotella stelligera	8	3.68	3
Cymatopleura elliptica	11	4.19	1
Cymatopleura solea	23	4.05	3
Cymbella	54	3.56	1
Cymbella prostrata	32	3.68	4
Diatoma	43	2.36	3
Diatoma ehrenbergii	9	2.85	4
Diatoma tenuis	68	3.17	2
Diatoma vulgaris	69	3.09	2
Dictyosphaerium	45	2.71	2
Dictyosphaerium pulchellum	42	3.19	2
Didymocystis	77	2.66	3
Dinobryon	324	3.55	3
Dinobryon bavaricum	107	3.42	3
Dinobryon crenulatum	109	3.38	3
Dinobryon cylindricum	62	3.50	2
Dinobryon divergens	417	3.31	2
Dinobryon divergens v. schauinslandii	13	3.26	4
Dinobryon sertularia	107	3.02	3
Dinobryon sociale	244	3.17	2
Dinobryon sociale v. americanum	15	2.76	4
Dinobryon sociale v. stipitatum	52	3.50	4
Elakatothrix	150	3.36	1
Elakatothrix gelatinosa	132	2.88	2
Elakatothrix genevensis	33	3.60	2
Erkenia subaequiciliata	117	3.05	1
Eudorina	14	2.15	3
Eudorina elegans	77	2.47	2
Euglena	76	3.32	1
Euglena acus	24	2.47	4
Eunotia	20	2.88	2
Eutetramorus	31	3.10	2
Eutetramorus fottii	133	3.01	2
Eutetramorus planktonicus	22	3.54	4
Fragilaria	132	3.49	2
Fragilaria berolinensis	6	3.63	4
Fragilaria capucina	81	3.08	2
Fragilaria construens	43	3.29	1
Fragilaria crotonensis	538	3.39	1
Fragilaria ulna	134	3.33	1

Taxon	Frequency	TI_k	v_i
Fragilaria ulna v. acus	501	3.42	2
Fragilaria ulna v. angustissima	432	3.37	2
Fragilaria virescens	11	2.11	4
Glenodinium	122	3.59	3
Gloeocapsa	5	4.61	4
Gloeococcus	4	4.36	4
Gloeocystis	21	2.29	4
Golenkinia radiata	10	2.75	3
Gomphonema	26	3.25	4
Gomphosphaeria	46	4.05	1
Gomphosphaeria aponina	39	2.81	3
Gomphosphaeria lacustris	5	3.25	4
Gymnodinium	428	3.51	1
Gymnodinium fuscum	12	3.43	4
Gymnodinium helveticum	576	3.32	1
Gymnodinium lantzschii	113	3.08	2
Gymnodinium ordinatum	5	3.49	3
Gymnodinium uberrimum	276	3.54	3
Gyrosigma acuminatum	3	3.35	4
Gyrosigma attenuatum	13	3.28	4
Katablepharis	29	3.29	3
Kephyrion	158	3.43	1
Kirchneriella	51	2.78	3
Korshikoviella	22	4.17	1
Lagerheimia	42	3.75	1
Lagerheimia subsalsa	14	2.42	4
Limnothrix	14	2.76	4
Limnothrix redekei	10	3.13	4
Lyngbya	17	3.82	2
Lyngbya limnetica	18	3.53	1
Mallomonas	344	3.27	1
Mallomonas acaroides	80	3.12	2
Mallomonas akrokomos	102	3.12	3
Mallomonas caudata	116	2.80	3
Mallomonas crassisquama	4	3.49	1
Mallomonas elongata	71	3.24	1
Mallomonas tonsurata	13	3.30	4
Melosira varians	80	2.76	3
Merismopedia	45	3.19	1
Merismopedia glauca	8	3.13	3
Merismopedia tenuissima	60	2.34	3
Merispomedia tenuissima	4	3.03	4
Micractinium pusillum	21	2.38	3
Microcystis	157	3.45	2
Microcystis aeruginosa	173	3.17	2

Taxon	Frequency	TI_k	v_i
Microcystis firma	5	3.37	2
Microcystis flos-aquae	47	3.11	2
Microcystis incerta	10	2.90	4
Microcystis viridis	3	2.89	4
Microcystis wesenbergii	23	2.93	1
Monoraphidium arcuatum	43	2.87	4
Monoraphidium contortum	63	3.49	1
Monoraphidium griffithii	22	2.76	2
Monoraphidium komarkovae	42	3.41	2
Monoraphidium minutum	29	2.19	4
Mougeotia	63	2.40	1
Mougeotia thylespora	29	2.66	4
Navicula	118	3.22	3
Navicula cryptocephala	14	3.13	4
Navicula radiosa	17	3.13	3
Nephrocytium	56	3.18	2
Nephrocytium agardhianum	56	2.68	3
Nephrocytium lunatum	5	3.51	4
Nitzschia	61	2.64	3
Nitzschia acicularis	110	2.97	1
Nitzschia fruticosa	22	2.91	4
Ochromonas	213	3.39	1
Oedogonium	11	2.52	4
Oocystis	191	3.42	1
Oocystis borgei	3	2.20	4
Oocystis lacustris	163	3.09	2
Oocystis marssonii	78	2.29	4
Oocystis parva	21	2.92	2
Oocystis solitaria	7	2.85	4
Oscillatoria	104	3.26	1
Oscillatoria limosa	22	3.28	1
Pandorina	15	1.81	4
Pandorina morum	116	2.76	3
Pediastrum	20	2.78	4
Pediastrum boryanum	221	2.55	3
Pediastrum duplex	142	2.69	2
Pediastrum simplex	23	3.72	1
Peridiniopsis	14	3.21	4
Peridinium	471	3.53	2
Peridinium aciculiferum	61	3.05	1
Peridinium bipes	7	3.15	4
Peridinium cinctum	80	3.13	3
Peridinium inconspicuum	139	3.39	3
Peridinium palatinum	5	2.73	4
Peridinium pusillum	55	3.80	3

Taxon	Frequency	TI_k	v_i
Peridinium willei	187	3.37	1
Phacotus	46	2.64	3
Phacotus lendneri	70	2.93	2
Phacotus lenticularis	43	2.74	3
Phacus	6	2.81	4
Phacus tortus	25	3.11	1
Phytodinium globosum	7	2.38	4
Pinnularia	8	3.33	4
Planktonema	13	3.97	3
Planktonema lauterbornii	7	3.05	4
Planktosphaeria gelatinosa	103	3.27	1
Planktothrix agardhii	18	3.34	4
Planktothrix prolifica	8	2.01	4
Planktothrix rubescens	424	3.29	2
Pseudanabaena catenata	58	3.14	1
Pseudanabaena limnetica	18	2.87	4
Pseudoanabaena	6	2.92	4
Pseudokephyrion	24	3.38	2
Pseudosphaerocystis lacustris	128	3.16	3
Quadrigula lacustris	35	2.88	2
Quadrigula pfitzeri	18	2.48	4
Radiocystis geminata	14	3.49	4
Rhabdogloea	35	3.30	2
Rhizosolenia	5	2.08	4
Rhizosolenia longiseta	16	2.67	4
Rhodomonas	301	3.69	1
Rhodomonas lacustris	413	3.37	1
Rhodomonas lens	200	3.00	2
Rhodomonas minuta	22	2.82	3
Scenedesmus	193	2.62	3
Scenedesmus acutus	12	2.54	4
Scenedesmus costato-granulatus	3	2.56	4
Scenedesmus ecornis	37	2.42	3
Scenedesmus linearis	45	2.99	2
Scenedesmus obtusus	42	2.37	4
Scenedesmus quadricauda	127	2.87	2
Schroederia setigera	47	2.54	2
Snowella	3	3.47	4
Snowella lacustris	216	3.80	2
Sphaerocystis	19	2.53	4
Sphaerocystis schroeteri	125	2.74	3
Sphaerozosma	28	3.13	4
Spirulina	8	3.22	4
Staurastrum	196	3.02	2
Staurastrum chaetoceras	26	3.32	4

Taxon	Frequency	TI_k	v_i
Staurastrum cingulum	23	2.39	4
Staurastrum gracile	50	2.61	4
Staurastrum paradoxum	59	2.62	4
Staurastrum tetracerum	20	2.52	3
Stephanodiscus	38	3.04	2
Stephanodiscus alpinus	74	3.64	1
Stephanodiscus binderanus	39	2.20	4
Stephanodiscus hantzschii	57	2.05	4
Stephanodiscus minutulus	99	2.87	2
Stephanodiscus neoastraea	205	3.20	2
Stephanodiscus parvus	9	2.89	2
Synechococcus	42	2.51	3
Synedra acus	7	2.52	4
Synedra ulna	6	2.76	4
Synura	49	2.90	2
Synura uvella	22	2.73	4
Tabellaria	11	2.43	3
Tabellaria fenestrata	282	3.28	2
Tabellaria flocculosa	56	3.52	4
Tabellaria flocculosa v.asterionelloides	5	3.12	4
Tetrachlorella	16	2.45	3
Tetraedron	70	3.38	4
Tetraedron caudatum	43	2.99	3
Tetraedron incus	4	3.76	3
Tetraedron minimum	221	2.85	3
Tetraselmis cordiformis	33	2.85	3
Tetrastrum triangulare	26	2.92	4
Trachelomonas	125	3.18	2
Trachelomonas oblonga	3	2.85	4
Trachelomonas volvocina	95	2.79	3
Tribonema	4	2.36	4
Ulothrix	21	1.94	4
Ulothrix subconstricta	16	2.66	4
Uroglena	293	3.40	3
Uroglena americana	51	2.69	3
Uroglena volvox	22	3.19	3
Volvox aureus	17	2.15	4
Willea irregularis	56	3.32	3
Woronichinia naegeliana	33	2.97	2

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A.4 Slovenia: Metodologija vrednotenja ekološkega stanja jezer s fitoplanktonom v Sloveniji (Ecological status assessment system for lakes using phytoplankton in Slovenia)

Overview

There are only two natural lakes >50 ha in Slovenia. Both are in the Alpine region and the same IC L-AL3 type. According to the national tipology, Lake Bled belongs to the deep prealpine type and Lake Bohinj to the deep alpine type of lakes. Base for differentiation is different biogeographical region.

Slovenia adopted AT phytoplankton method during the process of intercalibration. The only adaptation of AT method for Slovenian lake conditions relate to the sampling depth, all other important things i.e. reference conditions and class boundaries are the same as in AT methodology.

Sampling and data analysis (sampling frequency, depth etc)

Sampling frequency: At the defined sampling points minimal 4-times per year; 1 sampling during spring homothermic period is obligatory.

Sampling depth: integrated water samples from the euphotic zone = 2,5 X Secchi depth; in cases when the euphotic zone exceed 20 m, sampling is carried out from the surface to the depth of 20 m.

Metrics and calculation of final EQRs

Table A.11   Metrics, standard nethods, recalculation to EQR values

Metric	Unit	Standard methodology	EQR calculation
Biovolume (BV)	mm³/l	UTERMÖHL SIST EN 15204:2007 CEN TC 230/WG 2/TG 3/2007	BV_{ref} / BV_i
Chlorophyll a(Chl-a)	µg/l	SIST ISO 10260:2001	$Chl\text{-}a_{ref} / Chl\text{-}a_i$
Brettum index (BI)	-	Indicator species list with trophic scores	$BI_i / BI_{ref.}$

Final result is multimetric phytoplankton index **(MMI_ FPL)** the combination of nEQR values of a single metric .

$$MMI\_FPL = \frac{(nEQR_{BV} + nEQR_{Chl})/2 + nEQR_{BI}}{2}$$

Table A.12   Indicator species list with trophic scores, ordered on rising trophy

Code	Taxon	Trophic class (as TP in µg L^{-1})					
		<=5	5-8	8-15	15-30	30-60	>60
R0040	*Cyclotella bodanica*	7	3	0	0	0	0
R2195	*Cyclotella cyclopuncta*	7	3	0	0	0	0
R2196	*Cyclotella distinguenda*	8	1	1	0	0	0
R0733	*Pseudoquadrigula* sp.	8	1	1	0	0	0
R0042	*Cyclotella comensis*	7	2	1	0	0	0
R1070	*Dinobryon cylindricum*	7	2	1	0	0	0
R2058	*Discostella glomerata*	6	3	1	0	0	0
R1903	*Peridinium umbonatum* - complex	7	2	0	1	0	0
R1166	*Chrysolykos planctonicus*	5	4	1	0	0	0
R1446	*Chroococcus turgidus*	5	3	2	0	0	0
R1167	*Chrysolykos skujae*	2	8	0	0	0	0
R1155	*Bitrichia chodatii*	4	4	2	0	0	0
R0493	*Botryococcus braunii*	5	2	2	1	0	0
R1037	*Kephyrion* sp.	6	1	1	1	1	0
R0191	*Diatoma vulgaris*	5	2	1	1	1	0
R1697	*Peridinium pusillum*	0	9	1	0	0	0
R1066	*Dinobryon bavaricum*	3	3	2	2	0	0
R1438	*Chroococcus limneticus*	4	2	2	1	1	0
R1660	*Gymnodinium uberrimum*	1	6	2	1	0	0
R0442	*Tabellaria flocculosa*	1	4	5	0	0	0
R2174	*Ulnaria delicatissima* var. *angustissima*	2	3	3	2	0	0
R1654	*Gymnodinium* sp.	1	5	2	1	1	0
R1691	*Peridinium inconspicuum*	1	4	3	2	0	0
R1069	*Dinobryon crenulatum*	2	2	3	2	1	0
R1443	*Chroococcus minutus*	1	3	4	1	1	0
R0033	*Aulacoseira subarctica*	0	1	8	1	0	0
R1209	*Cosmarium depressum*	2	2	3	1	1	1
R1704	*Peridinium willei*	1	4	2	1	1	1
R0440	*Tabellaria fenestrata*	1	1	4	4	0	0
R1642	*Glenodinium* sp.	0	2	5	3	0	0
R1151	*Uroglena* sp.	0	3	3	3	1	0
R0606	*Coenococcus planctonicus*	0	1	5	4	0	0
R1413	*Aphanocapsa delicatissima*	0	3	3	2	2	0
R1617	*Planktothrix rubescens*	1	1	3	4	1	0
R0582	*Didymocystis* sp.	0	1	4	4	1	0
R1510	*Snowella lacustris*	0	1	4	4	1	0
R1549	*Anabaena spiroides*	0	1	6	1	1	1
R1282	*Staurastrum chaetoceras*	0	0	3	7	0	0
R2549	*Urosolenia longiseta*	0	1	3	3	3	0
R2556	*Crucigeniella irregularis*	0	0	4	4	2	0
R0025	*Aulacoseira islandica*	0	1	3	3	2	1
R0083	*Stephanodiscus neoastraea*	0	1	2	4	3	0
R0533	*Coenochloris fottii*	0	1	3	3	2	1
R1074	*Dinobryon divergens* var. *schauinslandii*	0	0	1	9	0	0

Code	Taxon	Trophic class (as TP in µg L^{-1})					
		<=5	5-8	8-15	15-30	30-60	>60
R2503	*Achnanthidium catenatum*	0	0	1	8	1	0
R1081	*Dinobryon sertularia*	0	1	1	5	3	0
R1096	*Mallomonas acaroides*	0	1	2	4	2	1
R1342	*Sphaerozosma* sp.	0	0	1	8	1	0
R1687	*Peridinium cinctum*	0	1	2	4	2	1
R0649	*Lagerheimia genevensis*	0	0	3	3	4	0
R1303	*Staurastrum pingue*	0	0	2	5	3	0
R1375	*Chroomonas* sp.	0	1	2	2	5	0
R0048	*Cyclotella ocellata*	0	1	1	4	3	1
R0848	*Tetraedron minimum*	0	1	1	4	3	1
R0736	*Pseudosphaerocystis lacustris*	0	0	2	5	2	1
R1414	*Aphanocapsa elachista*	0	1	2	2	4	1
R0571	*Dictyosphaerium pulchellum*	0	0	1	5	4	0
R1097	*Mallomonas akrokomos*	0	0	2	4	3	1
R2169	*Staurosira construens*	0	0	2	2	6	0
R1100	*Mallomonas caudata*	0	0	1	4	5	0
R1427	*Aphanothece clathrata*	0	0	1	4	5	0
R1776	*Trachelomonas volvocina*	0	0	1	4	5	0
R2520	*Fragilaria capucina* ssp. *rumpens*	0	0	2	3	3	2
R0555	*Crucigeniella rectangularis*	0	0	1	5	2	2
R0690	*Nephrocytium agardhianum*	0	0	0	5	5	0
R0782	*Scenedesmus ellipticus*	0	0	1	5	2	2
R0935	*Chlamydomonas globosa*	0	0	1	3	6	0
R0051	*Cyclotella radiosa*	0	0	1	3	5	1
R0682	*Monoraphidium* sp.	0	0	1	2	7	0
R0971	*Pandorina morum*	0	0	2	2	4	2
R1377	*Cryptomonas curvata*	0	0	1	3	5	1
R1536	*Anabaena flos-aquae*	0	1	1	2	3	3
R1620	*Pseudanabaena catenata*	0	1	1	2	3	3
R1205	*Cosmarium bioculatum*	0	0	1	1	8	0
R1506	*Rhabdogloea* sp.	0	0	1	1	8	0
R0490	*Ankyra lanceolata*	0	0	1	3	4	2
R0762	*Scenedesmus armatus*	0	0	1	3	4	2
R0975	*Phacotus lenticularis*	0	0	1	3	4	2
R1818	*Chrysochromulina parva*	0	0	1	3	4	2
R1004	*Mougeotia thylespora*	0	0	0	3	7	0
R0184	*Diatoma ehrenbergii*	0	0	0	3	7	0
R1141	*Synura* sp.	0	0	1	3	3	3
R0697	*Oocystis lacustris*	0	0	1	2	5	2
R0743	*Quadrigula lacustris*	0	0	1	1	7	1
R1288	*Staurastrum gracile*	0	0	0	3	6	1
R1487	*Microcystis flos-aquae*	0	1	1	1	3	4
R0701	*Oocystis parva*	0	0	1	1	6	2
R0760	*Scenedesmus obtusus*	0	0	0	1	9	0

Code	Taxon	Trophic class (as TP in µg L^{-1})					
		<=5	5-8	8-15	15-30	30-60	>60
R0966	*Gonium pectorale*	0	0	0	1	9	0
R0996	*Tetraselmis cordiformis*	0	0	0	2	7	1
R0998	*Volvox aureus*	0	0	0	1	9	0
R1181	*Closterium acutum* var. *variabile*	0	0	0	2	7	1
R1300	*Staurastrum paradoxum*	0	0	0	2	7	1
R1519	*Synechocystis aquatilis*	0	0	0	2	7	1
R1560	*Aphanizomenon gracile*	0	0	1	2	4	3
R1613	*Planktothrix agardhii*	0	0	1	3	2	4
R0082	*Stephanodiscus minutulus*	0	0	0	3	4	3
R0489	*Ankyra judayi*	0	0	0	1	8	1
R0633	*Kirchneriella* sp.	0	0	0	2	6	2
R0654	*Lagerheimia subsalsa*	0	0	0	1	8	1
R0923	*Carteria* sp.	0	0	1	1	5	3
R1095	*Erkenia subaequiciliata*	0	0	1	2	3	4
R1386	*Cryptomonas ovata*	0	0	1	2	3	4
R1199	*Closterium pronum*	0	0	0	1	8	1
R1283	*Staurastrum cingulum*	0	0	0	1	8	1
R1621	*Pseudanabaena limnetica*	0	0	0	3	4	3
R0189	*Diatoma tenuis*	0	0	1	1	4	4
R0529	*Coelastrum pseudomicroporum*	0	0	0	1	7	2
R0530	*Coelastrum reticulatum*	0	0	1	2	2	5
R1726	*Euglena* sp.	0	0	1	2	2	5
R0993	*Sphaerocystis schroeteri*	0	0	0	2	5	3
R1191	*Closterium limneticum*	0	0	0	1	7	2
R1525	*Woronichinia naegeliana*	0	0	0	3	3	4
R0891	*Gloeocystis* sp.	0	0	0	1	6	3
R0660	*Micractinium pusillum*	0	0	0	1	6	3
R0820	*Schroederia setigera*	0	0	0	1	6	3
R1482	*Microcystis aeruginosa*	0	0	1	1	3	5
R0016	*Acanthoceras zachariasii*	0	0	0	2	3	5
R0024	*Aulacoseira granulata* var. *angustissima*	0	0	0	2	3	5
R0343	*Nitzschia acicularis*	0	0	1	1	2	6
R0527	*Coelastrum microporum*	0	0	1	1	2	6
R1178	*Closterium acutum*	0	0	1	1	2	6
R0704	*Oocystis solitaria*	0	0	0	2	3	5
R1003	*Mougeotia* sp.	0	0	0	1	5	4
R0806	*Scenedesmus quadricauda*	0	0	0	1	4	5
R0940	*Chlamydomonas reinhardtii*	0	0	0	1	4	5
R0047	*Cyclotella meneghiniana*	0	0	0	1	4	5
R0963	*Eudorina elegans*	0	0	0	2	2	6
R1176	*Closterium aciculare*	0	0	0	0	6	4
R1311	*Staurastrum tetracerum*	0	0	0	0	6	4
R1153	*Pseudopedinella erkensis*	0	0	0	2	2	6
R0023	*Aulacoseira granulata*	0	0	0	1	3	6

Code	Taxon	Trophic class (as TP in µg L^{-1})					
		<=5	5-8	8-15	15-30	30-60	>60
R0506	*Chlorococcum* sp.	0	0	0	0	5	5
R0698	*Oocystis marssonii*	0	0	0	1	3	6
R1518	*Synechococcus* sp.	0	0	0	0	5	5
R1558	*Aphanizomenon flos-aquae*	0	0	0	1	3	6
R0713	*Pediastrum boryanum*	0	0	0	0	4	6
R0722	*Pediastrum simplex*	0	0	0	1	2	7
R0725	*Pediastrum tetras*	0	0	0	1	2	7
R0754	*Scenedesmus acuminatus*	0	0	0	0	4	6
R1499	*Microcystis wesenbergii*	0	0	0	1	2	7
R1582	*Limnothrix redekei*	0	0	0	1	2	7
R0488	*Ankyra ancora*	0	0	0	1	1	8
R0523	*Coelastrum astroideum*	0	0	0	0	3	7
R0616	*Golenkinia radiata*	0	0	0	1	1	8
R0716	*Pediastrum duplex*	0	0	0	0	3	7
R0777	*Scenedesmus dimorphus*	0	0	0	1	1	8
R1531	*Anabaena circinalis*	0	0	0	1	1	8
R1544	*Anabaena planctonica*	0	0	0	0	3	7
R1748	*Phacus longicauda*	0	0	0	1	1	8
R0078	*Stephanodiscus binderanus*	0	0	0	0	2	8
R0079	*Stephanodiscus hantzschii*	0	0	0	0	2	8
R0484	*Ankistrodesmus* sp.	0	0	0	0	2	8
R0781	*Scenedesmus ecornis*	0	0	0	0	2	8
R0999	*Volvox globator*	0	0	0	0	2	8
R1622	*Pseudanabaena mucicola*	0	0	0	0	2	8
R0503	*Chlorella* sp.	0	0	0	0	2	8
R0020	*Aulacoseira ambigua*	0	0	0	0	1	9
R0500	*Characium* sp.	0	0	0	0	1	9
R1610	*Planktolyngbya limnetica*	0	0	0	0	1	9
R0028	*Aulacoseira italica*	0	0	0	0	0	10
R0930	*Chlamydocapsa planctonica*	0	0	0	0	0	10

Table A.13   Reference interval, ranges and class boundaries for the Biovolume, Chlorophyll-a and Brettum-Index

	Biovolume mm³ L⁻¹	range type				Chlorophyll-a µg L⁻¹	range type				Brettum Index	range type		
		1	2	3			1	2	3			1	2	3
Ref	0,2-0,3	0,20	0,25	0,30	Ref	1,50 –1,90	1,50	1,70	1,90	Ref	5,09–5,29	5,29	5,19	5,09
H/G	0,3-0,5	0,33	0,42	0,50	H/G	2,10– 2,70	2,10	2,40	2,70	H/G	4,21–4,37	4,37	4,29	4,21
G/M	0,8-1,2	0,80	1,00	1,20	G/M	3,80 - 4,70	3,80	4,25	4,70	G/M	3,33–3,46	3,46	3,39	3,33
M/P	2,1-3,1	2,00	2,50	3,10	M/P	6,80 -8,70	6,80	7,75	8,70	M/P	2,45–2,54	2,54	2,5	2,45
P/B	5,3-7,5	5,00	6,25	7,50	P/B	12,5 -15,8	12,50	14,15	15,80	P/B	1,57–1,63	1,63	1,6	1,57

Table A.14   Reference value and EQR value for class boundaries high/good (H/G) and good/moderate (G/M) for Biovolume, Chlorophyll-a and Brettum index for both Slovenian Lakes

Lake	National Type	IC Type	Range	Chlorophyll-a			Biovolumen			Brettum-Index		
				Ref	H/G	G/M	Ref	H/G	G/M	Ref	H/G	G/M
Lake Bled	Prealpine	L-VL3	3	1,50	0,7	0,4	0,20	0,6	0,25	5,29	0,827	0,654
Lake Bohinj	Alpine	L-VL3	1	1,90	0,7	0,4	0,30	0,6	0,25	5,09	0,827	0,654

Reference condition setting

Reference condition and class boundaries for the total biovolume, Chlorophyll-a and Brettum index were set during the IC process and harmonized among AT,DE, FR, IT, SI. The base were existing near-natural reference sites, epert knowledge, historical data, modelling (extrapolating model results).

Reference values and class boundaries of the total biovolume BV and chlorophyll-a Chl-a were set using **the selected population of reference sites. The median** was defined as reference value, the **95% percentile as H/G boundary** – both supported by **expert judgment.**

The other class boundaries of BV and Chl-a were derived using equidistant class widths on a log-scale. The class boundaries for the new version of the Brettum index BInew were derived in the same way as for BV and Chl-a, supported by expert judgment.

Reference values for Biovolume, Chlorophyll-a and Brettum-Index were not set as fixed values but interval value.

According to the different type conditions specific »rang« value for each Lake type was selected.

Class Boundary setting

HG boundary derived from metric variability at near-natural reference sites (95th percentile).

The class boundaries of BV and Chl-a were derived using equidistant class widths on a log-scale. The class boundaries for the Brettum index were derived in the same way as for BV and Chl-a.

Table A.15   Recalculation EQRi to normalised nEQR values

EQRi	nEQRi
≥ 1	1
$\geq EQR_{H/G}$	$(EQR_i - EQR_{H/G}) / (1 - EQR_{H/G}) * 0.2 + 0.8$
$EQR_{H/G} > EQR_i \geq EQR_{G/M}$	$(EQR_i - EQR_{G/M}) / (EQR_{H/G} - EQR_{G/M}) * 0.2 + 0.6$
$EQR_{G/M} > EQR_i \geq EQR_{M/P}$	$(EQR_i - EQR_{M/P}) / (EQR_{G/M} - EQR_{M/P}) * 0.2 + 0.4$
$EQR_{M/P} > EQR_i \geq EQR_{P/B}$	$(EQR_i - EQR_{P/B}) / (EQR_{M/P} - EQR_{P/B}) * 0.2 + 0.2$
$< EQR_{P/B}$	$EQR_i / EQR_{P/B} * 0.2$

Table A.16   The final boundary values

Ecological status	MM_FPL
Very good	≥0,80
Good	0,60 – 0,80
Moderate	0,40 – 0,60
Poor	0,20 – 0,40
Bad	<0,20

B. Tiered approach to define harmonized reference criteria for the Alpine GIG

Introduction

In phase 1 of the intercalibration exercise, the phytoplankton and the macrophyte Alpine GIG defined criteria for selecting reference lakes, which, however, differed in some respect. In 2008, the invertebrate Alpine GIG started its work also with defining reference criteria, which again partly complied, partly differed from what proposed by the other BQE groups. The lack of harmonized criteria for defining reference conditions for the same IC types is criticized in the consistency check report of Pardo *et al.* (2010), who strongly recommended to work on agreed criteria for the whole GIG.

The different approaches for defining reference conditions both between and within GIGs were also criticized by the Reference Conditions Working Group, which contributed to Annex III of the new IC guidance document.

In this document we propose a way to harmonize the criteria for defining reference conditions for Alpine lakes by using a tiered approach. It is based on the Refcond guidance and takes into account the recommendations of Pardo *et al.* (2010) and of the Reference Conditions Working Group proposed for Annex III of the new IC guidance document (Table B.1).

The criteria proposed in this document are used to select:

1. **Reference lakes (RL)**, i.e. lakes that are in reference conditions for all BQE as well as for hydro-morphological and chemical conditions.
2. **Reference conditions lakes (RCL)**, i.e. lakes where at least one BQE is in high status. A can be considered as RCL for phytoplankton, macrophytes, invertebrates or fish – or for various combinations of 2 to 4 BQE.
3. **Reference conditions sampling sites (RCSS)**, i.e. sampling sites for invertebrates in the littoral or sublittoral zone, or transects for macrophyte sampling, where both reference criteria valid for the whole lake (such as trophic state, water level conditions) and criteria valid at local scale (such as morphological condition of the shore within a certain area) meet the criteria for reference conditions.

Criteria which are valid one (or at least less then all four) BQE and which are used to define RCL are defined as **'specific criteria'**. The sum of the specific criteria for the four BQE give the **'general criteria'** that are required to be met in RL.

Table B.1 List of important REFCOND pressures and potential pressure indicators for each type of pressure per water category.

Rivers		Lakes		Transitional		Coastal	
Type of pressure	**Pressure indicators**	**Type of pressure**	**Pressure indicators**	**Type of pressure**	**Pressure indicators**	**Type of pressure**	**Pressure indicators**
1. Point source pollution	population density, oxygen, phosphate, nitrogen	1. Point source pollution	population density, oxygen, phosphate, nitrogen	1. Point source pollution	population density, oxygen, phosphate, nitrogen	1. Point source pollution (from rivers + coastline)	population density, oxygen, phosphate, nitrogen
2. Diffuse source pollution	Agriculture land use, phosphate, nitrogen	2. Diffuse source pollution	Agriculture land use, phosphate, nitrogen	2. Diffuse source pollution	Agriculture land use, phosphate, nitrogen	2. Diffuse source pollution (from rivers	Agriculture land use, phosphate, nitrogen
3. Riparian zone vegetation	Riparian use, riparian composition, riparian longitudinal and lateral connectivity	3. Riparian zone vegetation	Riparian use, riparian composition, riparian longitudinal and lateral connectivity	3. Riparian zone vegetation	Riparian use, riparian composition, riparian longitudinal and lateral connectivity	3. Shoreline modification/harbours in supralittoral/ terrestrial	Shoreline ocupation, continuity between coastal perimeter and natural settings
4. Morphological alterations	Sediment transport, river continuity, channelisation, bank stabilisation, siltation, river profile, absence of weirs & dams	4. Hydromorphological alterations	Quantity and dynamics of flow, water level, residence time, goundwater connection, depth variation, substrate and structure of shore zone	4. Hydromorphological alterations	Quantity and dynamics of flow, water level, residence time, goundwater connection, depth variation, substrate and structure of shore zone	4. Hydromorphological alterations in littoral and sublittoral	Changes in deposition/erosional areas, groyns
5. Water abstraction	Abstraction below a threshold	5. Water abstraction	Abstraction below a threshold				
6. River flow regulation	No dams influencing natural flow regime, storage and seasonal patterns not influenced						
7. Biological pressures	No invasive species, no biomanipulation, no intensive fishery /aquaculture	7. Biological pressures	No invasive species, no biomanipulation, no intensive fishery /aquaculture	7. Biological pressures	No invasive species, no biomanipulation, no intensive fishery /aquaculture	7. Biological pressures	No invasive species, no biomanipulation, no intensive fishery /aquaculture
8. Other pressures	No intensive recreational use	8. Other pressures	No intensive recreational use	8. Other pressures, recreational	No intensive recreational use	8. Other pressures	No intensive recreational use

Tiered approach

<u>Tier 1</u>

Reference criteria for the pressure 'eutrophication' are defined according to the proposal made by the **phytoplankton** group in phase 1 of the IC exercise (Wolfram *et al.* 2009). The other BQE will adopt these criteria.

The criteria focus on TP as proxy for *land use* as driving force and for *eutrophication* or *nutrient load* as pressure (Table B.2). The approach to use TP as proxy rather than the driving force 'land use in the catchment area' is justified by the insufficient correlation between land use and the trophic state of Alpine lake (Figure B.1 and Figure B.2). This owes to strong model uncertainties, a lack of additional data such as buffer stripes around the lake, the degree of construction of WWTP or the presence of waste water ring channels (Figure B.3 and Figure B.4). In spite of good availability of land use data, the overall model between land use and the biological response is subject to very large uncertainty (Table B.4). Hence, land use data from the catchment area will not be used as threshold criteria for selecting RCL, but as <u>supporting criteria</u> (Table B.3). In case of conflicts between the strict criteria listed in Table B.2 and the supporting criteria listed thereafter, it has to be proved in each single case whether or not the lake can be treated as RCL. Up to then, the lake is considered as candidate RCL only.

For assessing local trophic impacts, however, land use in the near surrounding will be used (see below).
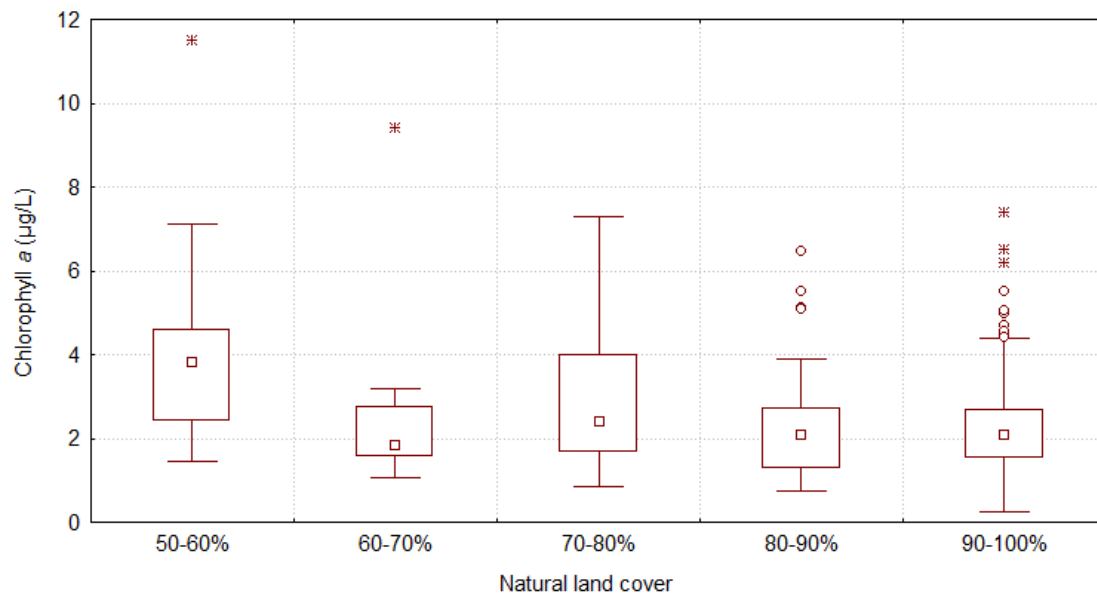
Figure B.1   Comparison of chlorophyll-a values in reference lakes with different natural land cover in catchments (all GIGs and all lake types) (from: Pardo et al. 2010).
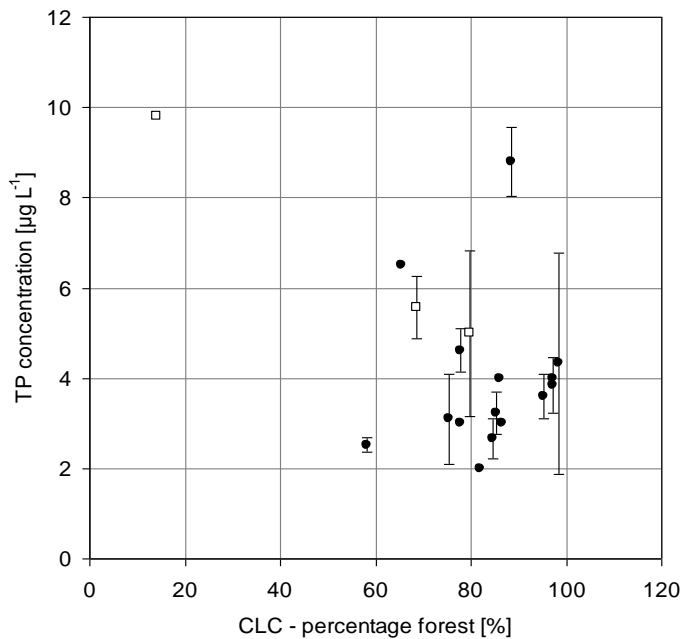
Figure B.2   Correlation of the CORINE category 'forest' and the TP concentration in oligotrophic Alpine lakes (from Wolfram et al. 2006). The different symbols refer to L-AL3 (filled) and L-AL4 (open) lakes, the bars give the 95% confidence limits (based on time series of monitoring).
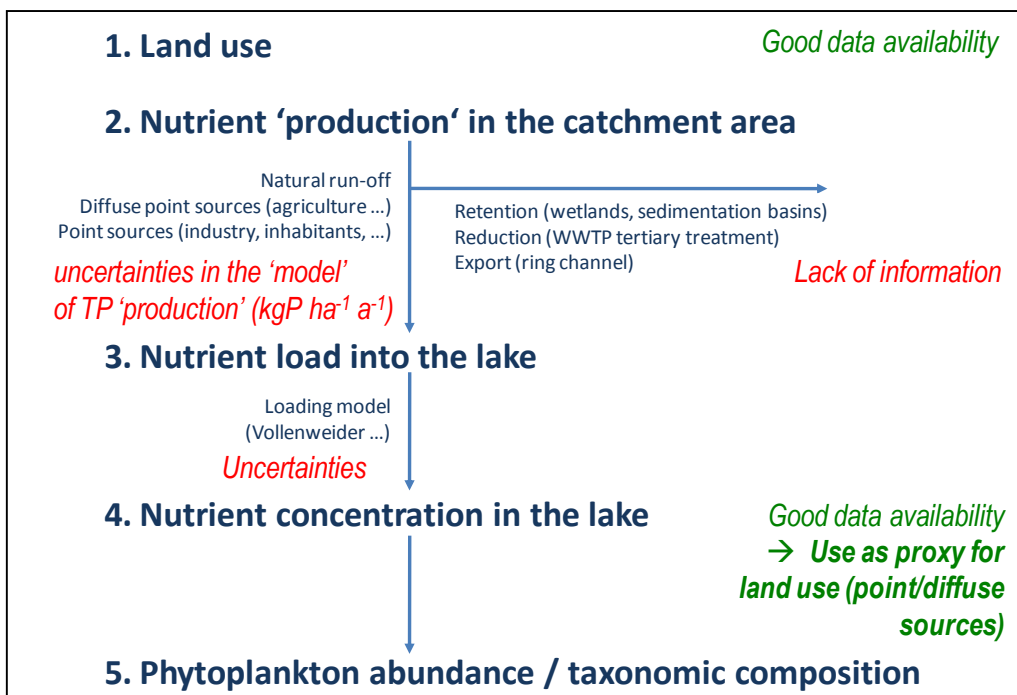
Figure B.3   Driving force (land use → nutrient production/export), pressure (nutrient load/ eutrophication → nutrient concentration) and state (phytoplankton abundance and taxonomic composition).
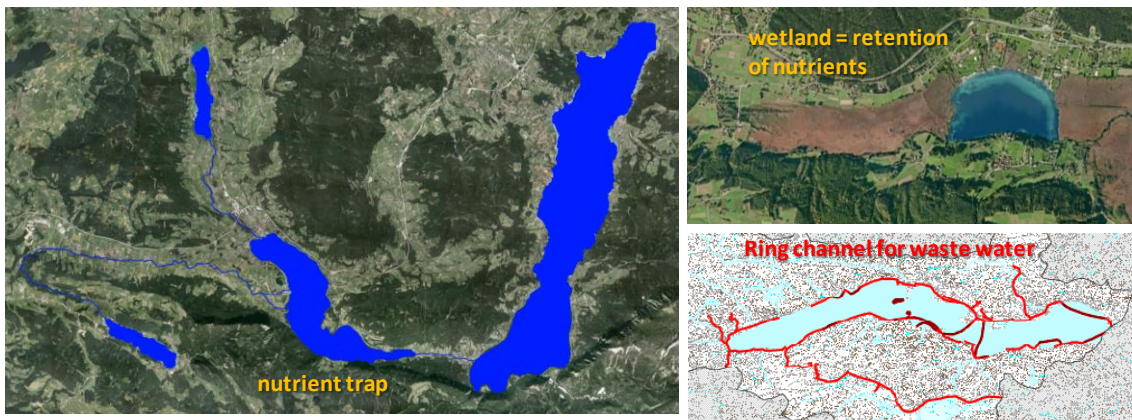
Figure B.4   Example 1: Lake chain in the Northern Limestone Alps with upstream lakes serving as sediment and nutrient trap for the downstream Lake Attersee, hence biasing possible effects of land use on the nutrient load and the trophic state. Example 2: Wetlands as buffer zones which reduce the inflow of nutrients into Lake Pressegger See. Example 3: Export of waste water from the catchment area in a ring channel around Lake Wörthersee.

Table B.2 Specific reference criteria for the selection of RCL for the BQE phytoplankton in Alpine lakes.

Pressure	Pressure indicators	Criteria
Eutrophication	Trophic state (TP, chl-a, loading models *etc.*)	No deviation of the actual from the natural trophic state
		Insignificant contribution of anthropogenic to total nutrient load (historical data prior to major industrialization, urbanization and intensification of agriculture; calculations on nutrient loading)
	Total phosphorus concentration	L-AL3: TP ≤8 µg L^{-1} L-AL4: TP ≤12 µg L^{-1}

Table B.3 Supporting specific reference criteria for the selection of RCL for the BQE phytoplankton in Alpine lakes.

Pressure	Pressure indicators	Criteria
Eutrophication	Land use in the whole catchment area	>80–90% natural forest, wasteland, moors, meadows, pasture (CLC classes 3.1.1, 3.1.2, 3.1.3, 3.2, 3.3, 4 and 5)
		No (or insignificant) intensive crops, vines (CLC classes 2.1, 2.2, 2.4.1, 2.4.2)
		No (or insignificant) artificial areas (CLC class 1)
		No deterioration of associated wetland areas

<u>Tier 2</u>

Reference criteria for the pressure 'hydro-morphology' <u>at whole lake level</u> as well as <u>at sampling site (transect) level</u> are defined as proposed by the **macrophyte** group in phase 1 of the IC exercise. They take into account water level fluctuations, eutrophication effects of local point sources and local shore line modifications. The other BQE will adopt these criteria.

A 'conversion' of criteria valid for sampling sites to criteria valid for the whole lake is proposed in this document.

The near surrounding is defined as a stripe around the lake between the shore line (0 m) and a distance of 100 to 300 m. Analyses carried out by J. Böhmer (unpubl.) on lakes in the Central Baltic revealed little difference in the general pattern of land use when using a small (0-100 m) or a broad (0-300 m) stripe. The conditions at local scale are derived for a zone of of 50-100 m shore length.

Table B.4 Specific reference criteria for the selection of RCL for the BQE macrophytes in Alpine lakes.

Pressure	Pressure indicators	Criteria
At the whole lake level		
Eutrophication	Adopted from phytoplankton (Table B.2)	Adopted from phytoplankton (Table B.2)
Hydrological alterations	Water level fluctuations	Artificial water level fluctuations not larger than the range between the natural mean low water level (MLW) and the natural mean high water level (MHW)
At sampling site (transect) level		
Eutrophication	Land use in the near surrounding	No intensive agriculture
		No artificial areas
	'Conversion' to whole lake level	<10% of total shore length with intensive agriculture or artificial areas
		No (or insignificant) direct local nutrient input near the sampling site
(+ habitat destruction)	Recreational use of the water body	No recreation area (beaches etc.) near the sampling site
Morphological alterations	Shore line modifications	No (or insignificant) artificial modifications of the shore line at the sampling site
	'Conversion' to whole lake level	<10% of total shore length with artificial modifications

A 'conversion' of the criteria 'direct local nutrient input near the sampling site' and 'modifications of the shore line at the sampling site' to whole lake level is not necessary, since possible effects on the trophic state are covered already by the criteria listed in Table B.2. The same is true for the criterion 'recreational use of the water body'.

Additional to the criteria listed in Table B.4, reference condition sites (RCS) for macrophytes should not be situated near tributaries, which may change the typical pattern of the macrophyte community structure.

The reference criteria for the pressure 'eutrophication' and 'hydro-morphological alterations' as defined by the phytoplankton and the macrophyte group Table B.2 and Table B.4) are adopted as specific reference criteria for selecting RCL for **benthic invertebrates** in the littoral and sublittoral zone. The criteria aiming at the pressure 'hydro-morphological alterations' are not relevant for profundal invertebrates, hence, for this group only the criteria listed in Table B.2 (pressure 'eutrophication') are adopted as specific criteria.

However, additional specific criteria are required for the profundal fauna. They take into consideration the fact that the profundal benthic fauna often shows a delay in recovery from eutrophication. During re-oligotrophication processes, the epilimnetic flora and fauna may show near-natural conditions already, whereas the profundal fauna lags behind and mirrors higher trophic conditions (e.g. Lang 1991, Lang & Lods-Crozet 1997, Wolfram *et al.* 2002).

Another criterion concerns a change on the mixing behavior, which may cause oxygen depletion in the hypolimnion (e.g. artificial meromixis and development of an anoxic monimolimnion). This can be a result from eutrophication (which then is covered by other criteria already) or by mining activities (e.g. salt intrusion in Lake Hallstätter See). A deviation from natural oxygen conditions is also used as criterion in spite of matching the eutrophication criteria in Table B.2, since it may be caused by other insufficiently known pressures, such as organic pollution. It seems, however, difficult to set a threshold for O_2 concentration (above ground vs hypolimnion, extent vs duration of O_2 deficiency *etc.*). Hence, this criterion is used by expert judgment.

Table B.5 Specific reference criteria for the selection of RCL for the BQE littoral and sublittoral invertebrates in Alpine lakes.

Pressure	Pressure indicators	Criteria
Eutrophication	*Adopted from phytoplankton (*Table B.2*)*	*Adopted from phytoplankton (*Table B.2*)*
Hydrological alterations	*Adopted from macrophytes (*Table B.4*)*	*Adopted from macrophytes (*Table B.4*)*
Eutrophication and morpho-logical alterations at local scale	*Adopted from macrophytes (*Table B.4*)*	*Adopted from macrophytes (*Table B.4*)*

Table B.6 Specific reference criteria for the selection of RCL for the BQE profundal invertebrates in Alpine lakes.

Pressure	Pressure indicators	Criteria
Eutrophication	*Adopted from phytoplankton (*Table B.2*)*	*Adopted from phytoplankton (*Table B.2*)*
	Trophic state	No significant eutrophication phase in the past, *i.e.* no mesotrophic conditions in L-AL3, no meso-eutrophic conditions in L-AL4
	Oxygen conditions	Unnatural O_2 conditions in late summer (expert judgment)

Hydrological alterations	Mixing behavior, e.g. artificial (facultative) meromixis	No change of natural mixing behavior

Tier 4

The reference criteria for the pressure 'eutrophication' and 'hydro-morphological alterations' as defined by the phytoplankton and the macrophyte group (Table B.2 and Table B.4) as well as the additional specific criteria for the profundal benthic fauna (Table B.6) are adopted as specific reference criteria for selecting RCL for fish, since this BQE inhabits all lake zones and is thus prone to all anthropogenic alterations described above.

Two additional criteria have to be added specifically for fish: the connectivity of tributaries and the outflow, and effects from intensive fisheries and aquaculture (including intensive stocking of indigenous and/or non-indigenous species).

Table B.7 Specific reference criteria for the selection of RCL for the BQE fish in Alpine lakes.

Pressure	Pressure indicators	Criteria
Eutrophication	*Adopted from phytoplankton (*Table B.2*)*	*Adopted from phytoplankton (*Table B.2*)*
Hydrological alterations	*Adopted from macrophytes (*Table B.4*)*	*Adopted from macrophytes (*Table B.4*)*
	Connectivity to tributaries and outflow	No interruption of the continuum to major tributaries and the outflow
Eutrophication and morpho-logical alter-ations at local scale	*Adopted from macrophytes (*Table B.4*)*	*Adopted from macrophytes (*Table B.4*)*
Biological pressures	Intensive fishery/aquaculture	No intensive fishery/aquaculture (including stocking)

Tier 5

Some pressures may affect several or all BQE. Their relevance and impact on the ecological status is, however, often little known. They are listed in Table B.8 and have to be regarded as specific criteria for defining reference conditions for all four BQE, if there are data or indication that they play a significant role.

Table B.8 Specific reference criteria for the selection of RCL for all BQE in Alpine lakes, if there are data or hints that the pressures may significantly affect the ecological status.

Pressure	Pressure indicators	Criteria
Toxicity	Substances listed in the EU Decision 2455/2001/EC	EQS values as defined in the EU Directive 2008/105/EC are not exceeded
Biological pressures	Invasive (proliferating) species	No significant impact from invasive species

Overview on reference criteria

The BQE specific criteria for selecting reference condition sampling sites (macrophytes and littoral/sublittoral invertebrates) and reference condition lakes are summed up to give the general criteria for selecting reference lakes (Table B.9). The scheme in

Figure B.5 illustrates the tiered approach to develop a common set of reference criteria in the Alpine GIG.

Table B.9 General reference criteria for the selection of RL in Alpine lakes. Supporting criteria in grey

| Pressure	Pressure indicators	Criteria
Eutrophication	Trophic state (TP, chl-a, loading models *etc.*)	No deviation of the actual from the natural trophic state
		Insignificant contribution of anthropogenic to total nutrient load (historical data prior to major industrialization, urbanization and intensification of agriculture; calculations on nutrient loading)
		No significant eutrophication phase in the past, *i.e.* no mesotrophic conditions in L-AL3, no meso-eutrophic conditions in L-AL4
	Total phosphorus concentration	L-AL3: TP ≤8 µg L^{-1} L-AL4: TP ≤12 µg L^{-1}
	Oxygen conditions	Unnatural O_2 conditions in late summer (expert judgment)
	Land use in the whole catchment area	>80–90% natural forest, wasteland, moors, meadows, pasture (CLC classes 3.1.1, 3.1.2, 3.1.3, 3.2, 3.3, 4 and 5)
		No (or insignificant) intensive crops, vines (CLC classes 2.1, 2.2, 2.4.1, 2.4.2)
		No (or insignificant) artificial areas (CLC class 1)
		No deterioration of associated wetland areas
	Land use in the near surrounding	<10% of total shore length with intensive agriculture or artificial areas
Hydrological alterations	Water level fluctuations	Artificial water level fluctuations not larger than the range between the natural mean low water level (MLW) and the natural mean high water level (MHW)
	Mixing behavior, e.g. artificial (facultative) meromixis	No change of natural mixing behavior
	Connectivity to tributaries and outflow	No interruption of the continuum to major tributaries and the outflow
Morphological alterations	Shore line modifications	<10% of total shore length with artificial modifications
Biological pressures	Intensive fishery/aquaculture	No intensive fishery/aquaculture (including stocking)
	Invasive (proliferating) species	No significant impact from invasive species
Toxicity	Substances listed in the EU Decision 2455/2001/EC	EQS values as defined in the EU Directive 2008/105/EC are not exceeded
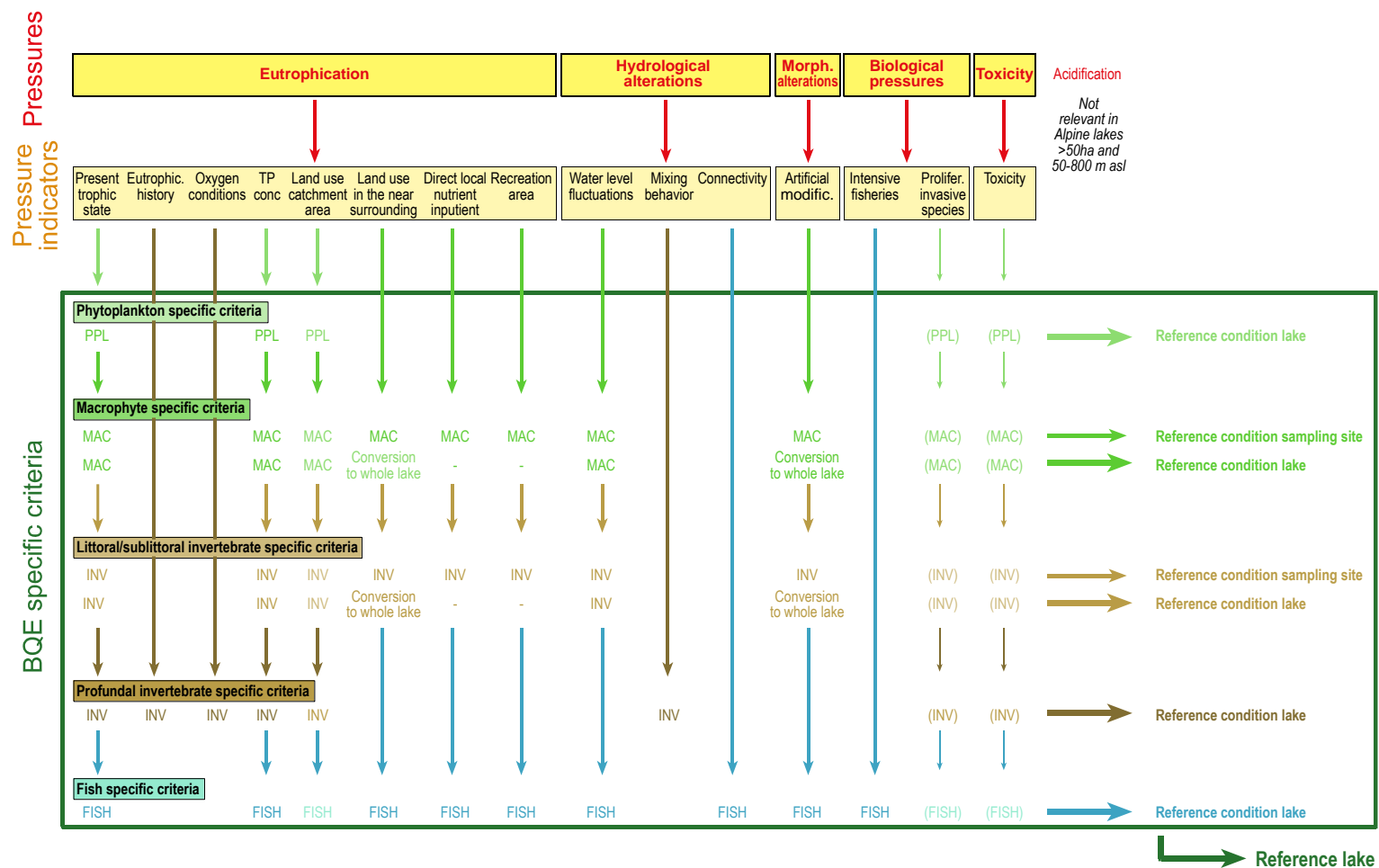
Figure B.5   Scheme on the tiered approach to develop a common set of reference criteria in the Alpine GIG.

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Abstract

One of the key actions identified by the Water Framework Directive (WFD; 2000/60/EC) is to develop ecological assessment tools and carry out a European intercalibration (IC) exercise. The aim of the Intercalibration is to ensure that the values assigned by each Member State to the good ecological class boundaries are consistent with the Directive's generic description of these boundaries and comparable to the boundaries proposed by other MS.
In total, 83 lake assessment methods were submitted for the 2nd phase of the WFD intercalibration (2008-2012) and 62 intercalibrated and included in the EC Decision on Intercalibration (EC 2013). The intercalibration was carried out in the 13 Lake Geographical Intercalibration Groups according to the ecoregion and biological quality element. In this report we describe how the intercalibration exercise has been carried out in the Alpine Lake Phytoplankton group.

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