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Water Framework Directive Intercalibration Technical Report

Alpine Lake Benthic invertebrate ecological assessment methods

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Introduction

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

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

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

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

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

Contents

Annexes

1. Introduction

In the Alpine Lake Benthic invertebrate GIG:

- Four Member States (Slovenia, France, Germany and Italy) submitted five benthic invertebrates assessment methods (as Germany submitted 2 methods);
- After evaluation of feasibility, only 2 methods were included in the current IC exercise: Slovenian method and German eulittoral method (as both of them address hydromorphological alterations);
- Intercalibration "Option 2" was used - indirect comparison of assessment methods using a common metric;
- IC common metric was developed specifically for this IC exercise comprising 4 metrics, it was benchmark-standardized using "continuous benchmarking" approach;
- 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 German and Slovenian lake benthic invertebrates assessment systems for 2 common types: L-AL3 and L-AL4.

2. Description of national assessment methods

In the Alpine Benthic invertebrates GIG, four countries participated in the intercalibration with 5 finalised benthic invertebrates lake assessment methods (Table 2.1, more details in Annex A).

Table 2.1 Overview of the national lake benthic invertebrates assessment methods in the Alpine GIG.

MS	Method	Status
SI	Slovenian ecological status assessment system for lakes using littoral benthic invertebrates	Finalized agreed national method
DEeul	AESHNA - German lake macroinvertebrate assessment method (part eulittoral of Alpine/Prealpine lakes)	Finalized agreed national method
DEsub	AESHNA - German lake macroinvertebrate assessment method (part sublittoral of Alpine/Prealpine lakes)	Finalized agreed national method
IT	BQI for Italian lakes	Intercalibratable finalized method*
FR	French sublittoral macroinvertebrate assessment method	Intercalibratable finalized method*

Note: *Detailed descriptions of the methods were received early September and, in a revisited form, in early October. Further changes received in Nov2011.

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 benthic invertebrates assessment methods

MS	Full BQE	Taxonomic composition	Abundance	Disturbance sensitive taxa	Diversity
SI	Yes	Taxonomic composition is included in the calculation of fauna index	Relative abundance is included in the calculation of diversity metric	Littoral fauna index	Number of taxa, Margalef diversity index
DE eulitt	Yes	Rel. Abundance of Odonata (% of abundance classes); rel. abundance of feeding type Collectors (% of abundance classes); Reproduction strategy r/k	Rel. abundance included in other metrics	Fauna index	Shannon-Wiener-diversity
DE sublitt	Yes	Insecta% (Individuals %), % Collectors (% abundance classes), Predators (% abundance classes), locomotion type sessiles (% abundance classes), Habitat preference Phytal (% based abundance classes)	Rel. abundance included in other metrics	ETO (% based on taxa number), alpha-Mesosaprobic (% individual number)	Relative taxa richness only for ETO% and Predator% *
FR	Yes	Composition included in other metrics (DTUsS, PtuaS_ol)	DTUsS: Density of Tubificidae without hair setae	PtuaS_ol: Percentage of Tubificidae with hair setae	Es3, Evenness Index (Smith and Wilson, 1996), based on a common form of Simpson index (Es3= (1/D)/S with S the number of taxa and D the Dominance index (Simpson, 1949) (diversity category)
IT	Yes	Taxonomic composition is included in the calculation of the index	Abundance (ind/m2) included in the calculation of the index	Sensitive/tolerant taxa calculated as weight	Diversity included as number of species

Following combination rule of metrics are used :

- SI method - weighted average of all metrics;
- DEeul method - five standardised metrics are averaged using formula: (2*fauna index + Odonata+ Shannon diversity + Collectors gatherer + rk)/6;
- DEsubl method - seven standardised metrics are averaged with equal weighting;
- FR method - average of three metrics EQR (Es3, DTUsS, PtuaS_ol) which has been log10-transformed (in order to be normalised) and again transformed into EQR to obtain values between 0 and 1;
- IT method - weighted average of all metrics .

2.2. Sampling and data processing

German method: Sampling 1/year: February to April (lowland) / May (Alpine) or September to October . Eulittoral: Habitat specific sampling designed for sampling all available habitats at up to 1.2 m depth of water by hand net 500 µm (mesh-size of net). About 0.6 to 1.0 m^2 should be sampled per habitat. The area sampled and the relative presence of each habitat is determined for a later combination to a multi-habitat taxa list. The number of sites depends on the length of the shoreline, according to the formula N=4+shorelength^-2. Sublittoral: Each separate lake basin is sampled at 8 stations (basin < 500ha) or 12 stations (basin > 500ha) distributed in equal sectors of the lake. The sublittoral zone is sampled above the thermocline behind the floating leaf macrophyte zone, outside dense macrophyte beds (depth is usually 4-6 m). Ekman grabs are used – 3 replicates per site.

French method: Sampling period - early spring (March-May). Only sublittoral samples – between 2 and 3 m using Ekman grab in soft sediment and sieved through a 250 µm mesh net. The number of replicates in this sublittoral zone is fixed to 7.

Italian method: Sampling is done biannually: February to April and September to October by Ekman grab. Samples are sieved with sieving net with mesh-size of 250-300 µm. The method follows US-EPA methodologies (http://www.epa.gov/owow/monitoring). A sample consists of 3 grab replicates per site (minimum effort to obtain good data set). Each site is located along a transect in 3 different sampled areas (littoral, sublittoral and profundal). The number of transects depends on lake area and for major lakes (like L. Maggiore, Garda and Como the highest lake areas and maximum depths in Italy, i.e. > 300 m) the number of transects refers to the number of sub-basins.

Slovenian method: One occasion per sampling season (July-August). Hand net with mesh-size of 500 µm, Surber or Hess sampler. Multi-habitat sampling designed for sampling major habitats in proportion to their presence within a sampling reach is carried out. A sample consists of 10 sampling units taken from all habitat types at the sampling site with a share of at least 10 % coverage. A "sampling unit" is a stationary sampling performed by positioning the net and disturbing the substrate in a quadratic area that equals the frame-size upstream of the net (0.25 x 0.25 m). Sediments must be disturbed to a depth of 15-20 cm (where possible) depending on substrate compactness.

2.3. National reference conditions

Slovenian method: Reference conditions were derived using existing near-natural reference sites. A type specific reference value was calculated as a median of reference sites. Reference sites were defined using lake-specific parameters (chemical and physico-chemical parameters) and site-specific parameters (hydromorphological alterations). Reference lakes were derived using pressure data and fulfilling OECD criteria for oligotrophic lakes.

German methods : Reference conditions were derived using a combination of regression line interpolation and percentile approach for all sites, which covered the whole pressure gradient from undisturbed to disturbed.

French methods: Two lakes have been classified as reference at a national scale based on local and catchment scale stressors, Lac du Grand Maclu and Grand lac Etival. These two lakes have population density around 8 habitants per km^2 and agricultural land-cover of 11% in average in the catchment area (while the means for the other lakes are respectively 53 habitants/km^2 and 36% of agricultural land-cover with a maximum value of 243 habitants/km^2 and 74% of agricultural land-cover). These two lakes have also low values of non- natural land-cover %. The average values of the selected metrics of these two lakes will be used as metrics values under reference condition.

Italian methods: T: Reference sites were selected among Austrian lowland lakes; they are in near-natural condition and because in the Alpine GIG biogeographical differences are considered negligible, they were considered representative for the Alpine area. A total of 45 sites (9 sites per 5 lakes) were analyzed in Austrian lakes, but considering reference sites criteria, identified through obligatory variables (O_2% saturation >70%, total phosphorous <6 $\mu g\ l^{-1}$, transparency >8 m, shoreline naturalness >90%), only 36 sites resulted in reference condition. Austrian lakes were chosen as reference sites for the Italian method notwithstanding that difference in type-specific reference conditions could theoretically occur between northern and southern Alps.

Note: This is most problematic aspect of this method as typological and biogeographical differences might influence the anchor points that are derived from the AT lakes. Also, it seems unlikely that reference conditions should be the same for large lakes like Como and Iseo and (relatively) small lakes like the others.

According to authors: "the approach taken to develop an index in this study that overcomes such biogeographic differences is the development of weighted averages which translate the trophic preferences of the species into a common scale to allow comparison across countries."

However, whether this assumption holds or not cannot be checked by this group based solely on the information provided in the method description.

2.4. National boundary setting

Slovenian method: Boundary values between ecological status classes were defined based on the changes in ratio between the number of sensitive and tolerant taxa (Figure 2.1) using following criteria:

Table 2.3 Boundary setting principles used in the Slovenian lake benthic invertebrate assessment method. LBI - Littoral Benthic Invertebrate Index

Boundary	LBI	Boundary setting criteria
High/Good	0.86	Sensitive taxa ≈ Tolerant taxa
Good/Moderate	0.68	Sensitive taxa < Tolerant taxa
Moderate/Poor	0.41	Sensitive taxa << Tolerant taxa
Poor/Bad	0.20	Sensitive taxa = 0

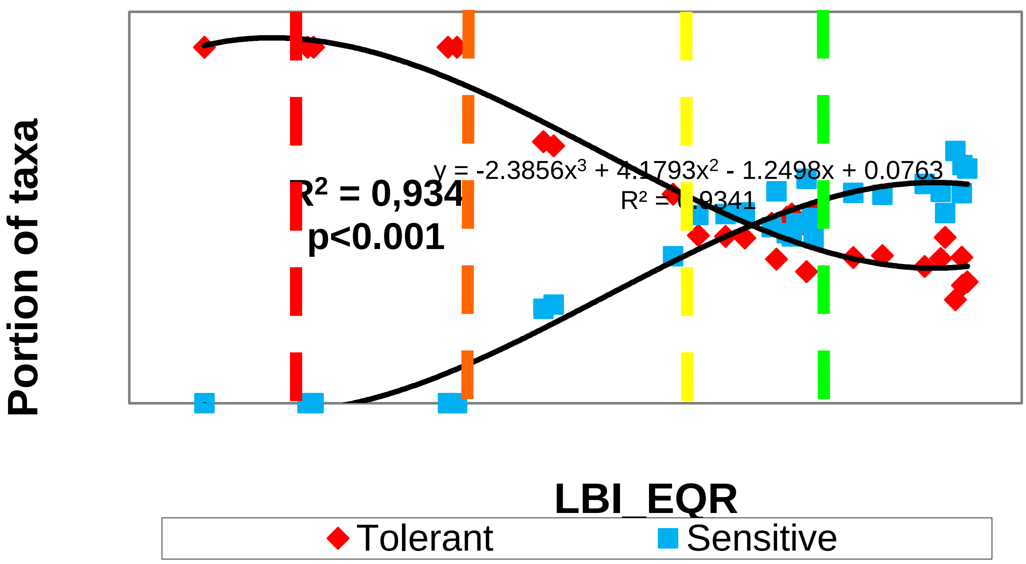

Figure 2.1 Boundary setting between ecological status classes using changes in portion of sensitive and tolerant taxa along the littoral benthic invertebrate index (LBI_EQR).

Biological community at reference sites is high in number of taxa and high diversity (high number of taxa with relatively low abundance) and with high ratio between sensitive and tolerant taxa to hydromorphological degradation.

Reference conditions were derived using existing near-natural reference sites. A type specific reference value was calculated as a median of reference sites. Reference sites were defined using lake-specific parameters (chemical and physico-chemical parameters) and site-specific parameters (hydromorphological alterations). Reference lakes were derived using pressure data and fulfilling OECD criteria for oligotrophic lakes.

German methods: The multimetric index was standardised from 1 (= reference value) to 0 (bad status). This range was equally split up into the 5 quality classes (very good 1-0.8; good 0.8-0.6; moderate 0.6-0.4; poor 0.4-0.2; bad 0.2-0). This approach was chosen, because all other approaches of the boundary setting procedure gave no or highly variable results depending on the metrics used (e.g. paired metrics results depended strongly on the metrics used). Reference conditions were derived using a combination of regression line interpolation and percentile approach for all sites, which covered the whole pressure gradient from undisturbed to disturbed.

French method: Due to low number lake into reference conditions, the class boundaries have been fixed by dividing the EQR range into 5 equal classes. Moreover, it was not possible to ecologically interpret the interaction between paired metrics. However, the High/Good boundary has been controlled in order to contain at least the two reference lakes and biological reference criteria. The values of the metrics have then been examined in high, good and moderate classes (Figure 2.2).

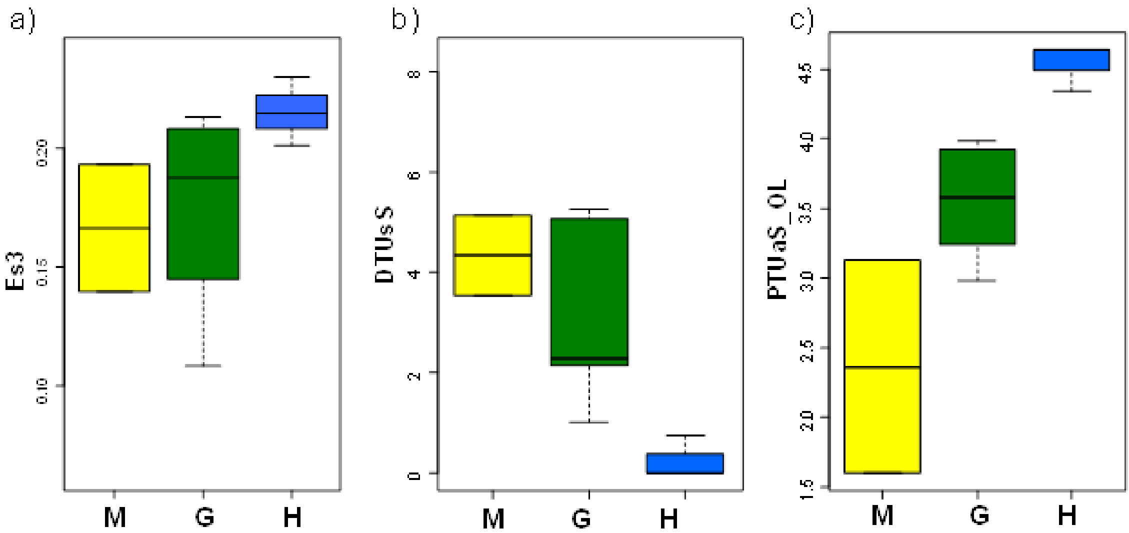

Figure 2.2 Distribution of values of Evenness Index (Es3, a), Density of Tubificidae without hair setae (DTUsS, b) and Percentage of Tubificidae with hair setae (PtuaS_ol, c) in high (H), good (G) and moderate (M) classes.

High values of PTUaS_OL are observed in the High class. Tubificidae with hair setae being composed of sensitive taxa, this result confirmed the High quality status of this class. This metric shows also lower values in the moderate class compared to the Good class. It is clearly the case for Es3 too, as evenness index may decrease with the degradation of the environment.

Italian method:

- The H/G boundary class was established considering biological data alone. All the reference lakes had an EQR>0.8 and all the non reference had a value <0.8;
- The G/M limit boundary established to 0.6 considering the highly significant separation of Como and Iseo lakes by correspondence analysis, both having an EQR comprised between 0.6 and 0.8 (0.8>EQR>0.6);
- The M/P boundary was established at an EQR=0.4 having lakes Viverone, Monate and Levico an EQR<0.6, but greater than 0.4;
- No one of the investigated lakes had an EQR<0.4, so the P/B boundary could not be established. We should guess a 0.2 value;
- The boundary class limits were refined dividing them (0.8, 0.6, 0.4, 0.2) by the median BQIES values of the reference sites, that is 0.9458.

3. Results of WFD compliance checking

All methods WFD-compliant, except IT method (see detailed explanations below). IT method has several characteristics that render compliancy questionable.

1. This is most problematic aspect of this method as typological and biogeographical differences might influence the anchor points that are derived from the AT lakes. Also, it seems unlikely that reference conditions should be the same for large lakes like Como and Iseo and (relatively) small lakes like the others.

   According to authors: "the approach taken to develop an index in this study that overcomes such biogeographic differences is the development of weighted averages which translate the trophic preferences of the species into a common scale to allow comparison across countries."

   However, whether this assumption holds or not cannot be checked by this group based solely on the information provided in the method description.

2. The classification system reported in the y axes seems critically to depend to the weights are introduced to taxa densities based on TSI values (page 4). Therefore, the correlation shown here contains some degree of spuriousness that should be quantified before using the goodness of fit as a quality indicator of a validated relationship.
   According to authors, "to minimize circularity in the reasoning, indicator weights were calculated using all the information available about the sensitivity of species (see http://www.freshwaterecology.info/)". However, how this was done cannot be check by this group based solely on the information provided in the method description.

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).	SI, DEeulittoral, DEsublittoral, FR, IT
2. High, good and moderate ecological status are set in line with the WFD's **normative definitions (Boundary setting procedure)**	SI, DEeu, DEsub, FR, IT
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.	SI, DEeu, DEsub, FR, IT
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	SI, DEeu, DEsub, FR, IT
5. The water body is assessed against **type-specific near-natural reference conditions**	SI, DEeu, DEsub, FR, IT1
6. Assessment results are expressed as **EQRs**	SI, DEeu , DEsub, FR, IT
7. Sampling procedure allows for **representative** information about water body quality/ ecological status in space and time	SI, DEeu, DEsub, FR, IT
8. All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	SI, DEeu, DEsub, FR, IT
9. Selected taxonomic level achieves adequate **confidence and precision** in classification	SI, DEeu, DEsub, FR, IT
10. Validated pressure-response relationship	SI, DEeu, DEsub, FR, IT2

3.1. Detailed description of WFD compliance checking

1. *Ecological status is classified by one of five classes (high, good, moderate, poor and bad).*

All methods comply. See above and in the methods description (Annex A).

2 *High, good and moderate ecological status are set in line with the WFD's normative definitions*

All methods comply. See above and in the methods description (Annex A).

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.*

3.1 SI : Lake littoral benthic invertebrate index (LBI) includes metrics that cover all relevant parameters indicative of the benthic invertebrates (taxonomic composition, abundance, disturbance sensitive taxa and diversity). Three metrics (number of taxa, Margalef diversity and Littoral fauna index) are combined using weighted average approach where LFI is equally weighted as both richness/diversity metrics together.

3.2 DEeul, DEsub: methods cover all relevant parameters indicative of the benthic invertebrates (taxonomic composition, abundance, disturbance sensitive taxa and diversity).

Eulittoral: Five standardised metrics are averaged (rel. abundance of Odonata (% of abundance classes); rel. abundance of feeding type collectors (% of abundance classes; reproduction strategy r/k, Shannon diversity and littoral fauna index) with a double weighting of the fauna index. Formula: (2*fauna index + Odonata+ Shannon diversity + gatherer + rk)/6

Sublittoral: Seven standardised metrics are averaged (ETO-taxa (% based on taxa number), Insecta (% based on individual numbers), habitat preference phythal (% based on abundance classes), feeding type collectors and predators (each in % based on abundance classes), locomotion type sessile (% based on abundance classes), alpha-mesosaprobic (% based on individual numbers) with equal weighting.

3.3 FR: Selected metrics in the multimetric index: DTUsS, PtuaS_ol and Es3:

- PtuaS_ol - Percentage of Tubificidae with hair setae (composition category): Subfamily of Tubificidae with hair setae includes taxa sensitive to organic pollution (e.g. Psammoryctides barbatus, Spirosperma velutinus) (Lafont et al., 2007). Negative correlation with stressors expected.
- DTUsS - Density of Tubificidae without hair setae (abundance catergory). Unlike the Tubificidae with hair setae, these taxa increase with stressors;
- Es3 - Evenness Index (Smith and Wilson, 1996), based on a common form of Simpson index (Es3= (1/D)/S with S the number of taxa and D the Dominance index (Simpson, 1949) (diversity category). Negative correlation with stressors expected.

– These combinations of metrics include also metrics of different type as requested by the normative definition of the WFD.

Finally, the multimetric index selected is then the average of three metrics EQR which has been log10-transformed (in order to be normalised) and again transformed into EQR to obtain values between 0 and 1. This index is correlated to the Pload with a Pearson coefficient of -0.81 (adj R^2 of 0.63, pvalue<0.001) and to the LHMS of -0.63 (adj R^2 of 0.35, pvalue<0.05).

3.4 IT: Benthic Quality Index for Italian Lakes (BQIES) includes all the relevant parameters indicative of the lacustrine macroinvertebrates (taxonomic composition at species level, absolute abundance (ind m^{-2}), sensitive/tolerant taxa ratio calculated as sensitivity value and diversity as number of species and their abundance) with a weighted average approach.

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

4.1 SI: Two national alpine lake types were defined in Slovenia; deep sub-alpine lakes and deep alpine lakes. Both national types belong to intercalibration type L-AL3. However, for the calculation of the national EQR values, national type specific reference values were used.

4.2 DEeul, DEsub: Two national alpine lake types for eulittoral were defined in Germany: major (>5 km2) and minor (<5 km2) which differ in anchor points for metric standardisation.

Both types are treated the same for sublittoral.

4.3 FR: The method was developed based on data from 14 French natural lakes, 6 of AL-3 type and 8 of AL-4

4.4 IT: The index presented here was applied to 5 natural lakes of 2 different types (AL-3, AL-6) located in the Subalpine Region, included in the 18 types described in the national document on lake types. These lakes are located at altitudes below 800 m a.s.l. (AL-3, AL-6). All of them have a mean depth >15 m. The AL-3 type consists of the large Italian lakes with a maximum depth >120 m and a surface area >100 km^2. Italian types AL-3 and AL-6 correspond to IC common type L-AL3.

5 The water body is assessed against type-specific near-natural reference conditions

All methods comply. See above and in the methods description (Annex A).

6 Assessment results are expressed as EQRs

All methods comply. See above and in the methods description (Annex A).

7 Sampling procedure allows for representative information about water body quality/ecological status in space and time

7.1 SI: Benthic invertebrates are sampled once a year in summer (July-August) at six sites along the lakeshore. Sites are chosen based on the hydromorphological pressure. Each water body is sampled every two years.

7.2 DEeul, DEsub: Sampling follows the German standard method for lake eulittoral and sublittoral macroinvertebrates. Benthic invertebrates are sampled once a year in spring (February to May) or fall (September/October) along the lakeshore.

Eulittoral: The number of sites depends on the length of the shoreline, according to the formula:

N = 4 + (shoreline length)$^{-2}$. Sampling stations are selected in a way that all shore types with a portion of more than 10% are covered.

Sublittoral: Each separate lake basin is sampled at 8 stations (basin < 500ha) or 12 stations (basin > 500ha) distributed in equal sectors of the lake. The sublittoral zone is sampled above the thermocline behind the floating leaf macrophyte zone, outside dense macrophyte beds (depth is usually 4-6 m).

7.3 FR: To minimize the effect of seasonal variation in abundance due to emergence of insects, the sampling period has to take place in early spring (between March and May).

The number of replicates in this sublittoral zone is fixed to 7. When the new field protocol was applied (Mazzella et al., 2009), the average abundance value per taxa has been calculated on the seven samples. While the old field sample was applied (Verneaux et al., 2004), more than 7 samples per lake were available (old field protocol). In that case, a random selection of 7 samples repeated several times were applied and the average abundance per taxa was also computed.

7.4 IT: In lakes were obvious depth gradient exists the sampling is performed as stratified sampling, working on transects with sites of sampling arranged to give equal coverage of the different zones.

Sampling period: February to April (Spring) and September to October (Autumn) with a biannual sampling frequency (i.e. during turn over and stratification periods). The number of transects depends on lake area and their position is based on expert knowledge.

8 All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure

8.1 SI: Littoral benthic invertebrates are sampled using the multi microhabitat-type (LMT) sampling procedure. The LMT approach is a proportional stratified sampling where the strata are defined as a combination of four equal depth classes between 0 m and 1 m water depth and inorganic and organic substrate categories. A sample consists of ten sampling units taken from all the microhabitat types at the sampling site with a share of at least 10% coverage. Each sampling unit is 0.0625 m^2, which is equivalent to a total area of 0.625 m^2 of the lake littoral bottom. An actual sampling site is defined as an area covering 10 m of lakeshore either to a distance of 10 m toward open water or to the point at which the water depth exceeds 1 m. Samples are taken using a hand-net.

8.2 DEeul, DEsub

Eulittoral: Habitat specific sampling designed for sampling all available habitats at up to 1.2 m depth of water. About 0.6 to 1.0 m2 should be sampled per habitat with handnets (500 µm mesh-size) or specified other suitable sampling device (e.g. scratcher for flat concrete surfaces). The area sampled and the relative presence of each habitat is determined for a later combination to a multi-habitat taxa list. Samples are sorted out in the field or sieved, fractionated and preserved in ethanol for sorting in the laboratory. The resulting sum of all habitat replicates will be 0.6 to 5 square-meters.

Sublittoral: Sampling is performed with grabs or corers. The number of replicates per station is 3 for standard Birge-Ekman grabs, 2 for Ponar grabs and > 10 for corers covering 35 cm^2. Samples are sieved in the field, conserved and sorted in the laboratory

8.3 FR: Only sublittoral samples are used, the sublittoral zone being between 2 and 3 meters. The sampling has to be done using an Ekman grab in soft sediment. The sediment is sieved through a 250 µm mesh net. Details about the field sampling methodology can be found in (Verneaux et al., 2004) and (Mazzella et al., 2009).

8.4 IT: Three grab replicates per site (minimum effort to obtain good data set) to a maximum of 9 replicates in a transect are taken. Each site is located along transects connecting the shore-line to the max depth, in 3 different sampled areas (littoral, sublittoral and profundal). Sampling is performed through the use of an Ekman grab with an area of 0.0225 m^2 of lake bottom.

9 Selected taxonomic level achieves adequate confidence and precision in classification

9.1 SI: A Slovenian operational taxa (SOT) list with most taxa determined to the species and genus level, and some to the sub-family and family level (e.g. Tubificidae, Chironomidae-subfamilies) is used to calculate metrics. However, Littoral fauna index (LFI) is family based.

9.2 DEeul, DEsub: Organisms of the complete sample are identified. Taxa to be identified are given in a detailed "German lake macroinvertebrate operational taxa list". The level is species or achievable level for all but the following: Family for Oligochaeta and most non-chironomid dipterans, mostly genus for chironomids.

9.3 FR: Determination level is genus level except for Diptera other than Chironomidae (family), Nematoda (phylum) and Oligochaeta for which three groups have been identified (Tubificidae with and without hair setae, other Oligochaeta).

9.4 IT: Specimens are identified to species level in case of Chironomids and Oligochaetes, the genus level is considered acceptable for the other groups or when the material available (only immatures for example) hindered species identification. Numbers are expressed as individual counts abundance and related to area as number of individuals per one m^2 (ind m^{-2}).

10 Validated pressure-response relationship

10.1 SI: Details of pressure-response relationships are given in figure below.

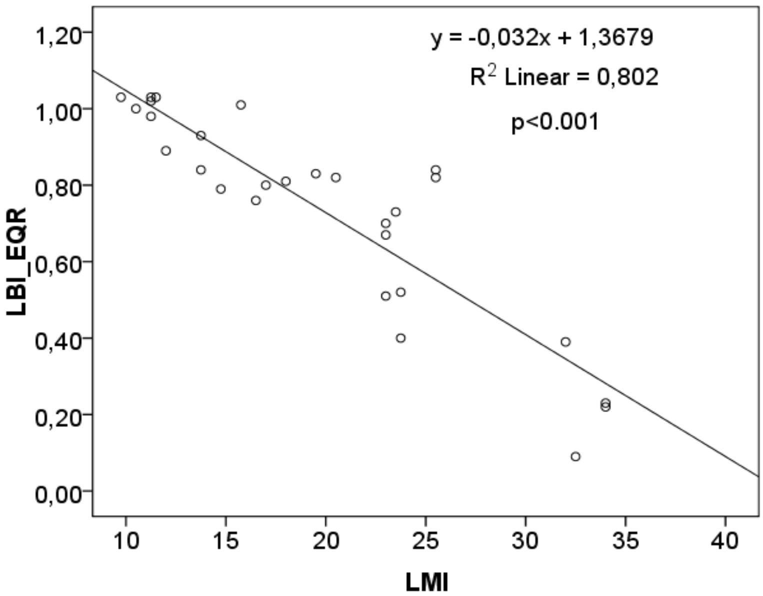

Figure 3.1 Littoral Benthic Invertebrate index (LBI_EQR) in response to lakeshore modification index (LMI).

10.2 DEeul, DEsub: Details of pressure-response relationships are given in figure below.

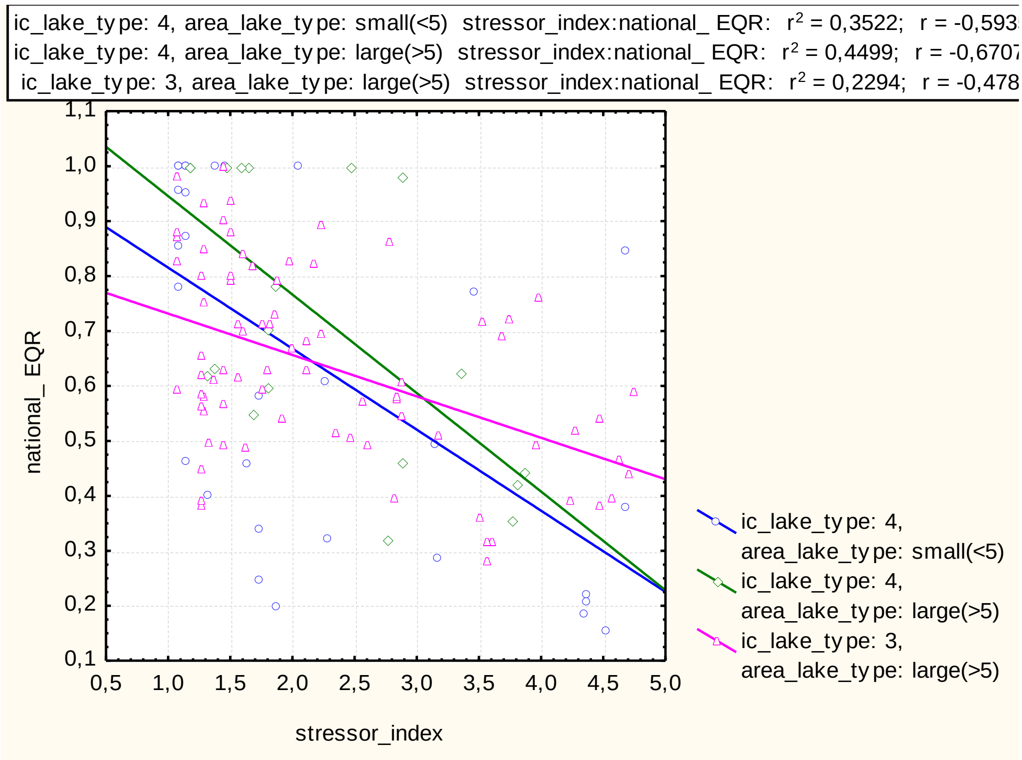

Figure 3.2 EQR of German benthic invertebrate national assessment method (national_EQR) in response to stressor index.

10.3 FR: the multimetric index selected is then the average of three metrics EQR which has been log10-transformed (in order to be normalised) and again transformed into EQR to obtain values between 0 and 1. This index is correlated to the Pload with a Pearson coefficient of -0.81 (adj R^2 of 0.63, p value<0.001) and to the LHMS of -0.63 (adj R^2 of 0.35, pvalue<0.05).

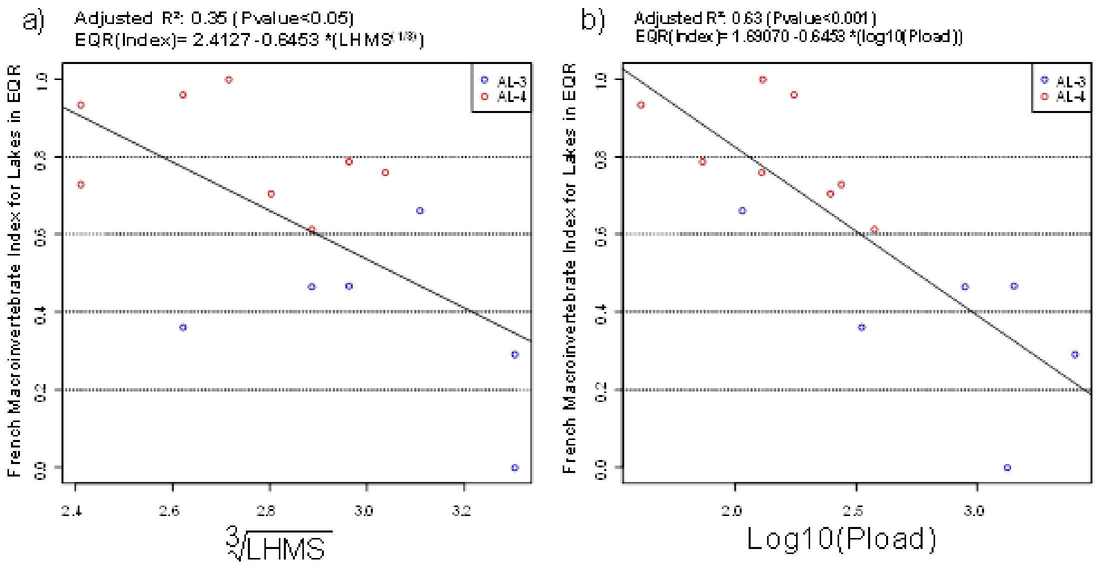

Figure 3.3 EQR of French Macroinvertebrate index in response to (a) Lake Habitat Modification Score (LHMS) and (b) load of phosphorus (Pload).

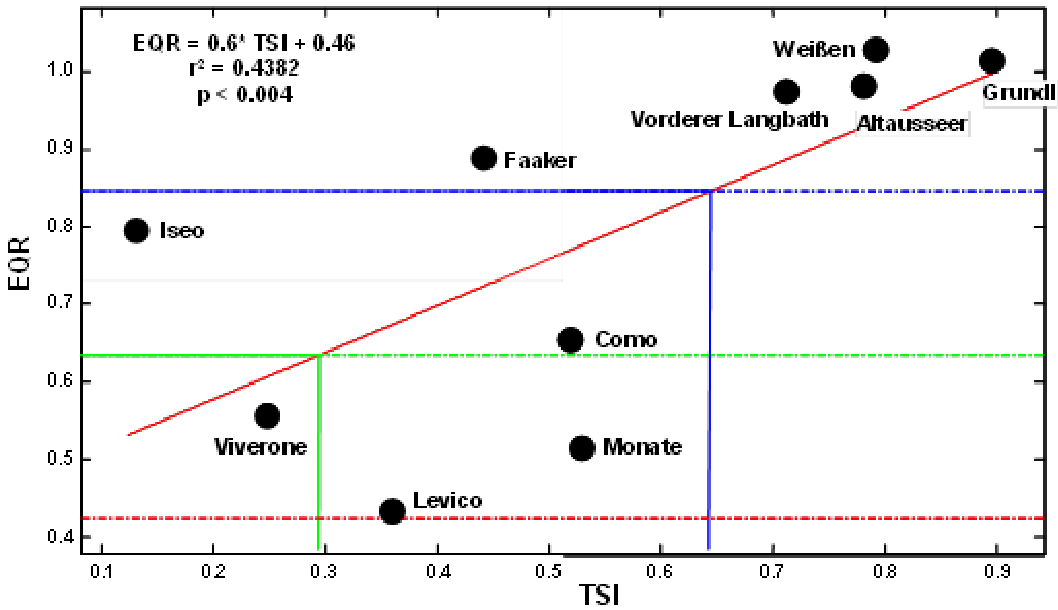

Figure 3.4 EQR of Benthic Quality Index for Italian Lakes (BQIES) in response to TSI (Trophic State index). EQR=BQIES/m_{ref} where m_{ref} is the median of reference sites. Lakes are represented by their mean values calculated on the dataset per each lake.

10.4 IT: the relationship between the macroinvertebrate metric (*BQIES* or EQR) and eutrophication measures *TSI* showed highly significant correlation, r = 0.47, R-squared = 0.22, p<0.001. The correlation was calculated considering all the 106 measures available. Considering the mean values for each lake it was obtained a correlation coefficient r=0.66, with $p<0.04$, an R-square = 0.44, an adjusted R-square = 0.37.

2 The classification system reported in the y axes seems critically to depend to the weights are introduced to taxa densities based on TSI values (see IT method description in Annex A). Therefore, the correlation shown here contains some degree of spuriousness that should be quantified before using the goodness of fit as a quality indicator of a validated relationship.

According to authors, "to minimize circularity in the reasoning, indicator weights were calculated using all the information available about the sensitivity of species (see http://www.freshwaterecology.info/)". However, how this was done cannot be check by this group based solely on the information provided in the method description.

4. IC feasibility check

4.1. Typology

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

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

Common IC type	Type characteristics	MS sharing IC common type
L-AL3	Lowland or mid altitude (50-800 m asl), large (>0.5 km^2), deep (mean depth > 15m), moderate to high alkalinity (>1 meq/ l)	SI, DE, AT, FR, IT
L-AL4	Lowland or mid altitude (50-800 m asl), large (>0.5 km^2), shallow (mean depth 3-15 m), moderate to high alkalinity (>1 meq/ l)	DE, AT, FR

The IC is feasible in terms of typology: All assessment methods are appropriate for the intercalibration water body types

4.2. Pressures

The Intercalibration is feasible in terms of pressures addressed in following combinations:

- IC is feasible for SI method with DEeul method (address morphological alteration);

- IC is feasible for DEsub method with FR method (address morphological alteration and eutrophication);
- Not for IT (address only eutrophication).

Table 4.2 Evaluation if IC feasibility regarding pressures addressed by the methods

Member State	Pressure or combination of pressures	Pressure indicators	Strength of relationship (R^2)	Geographical delineation of the type
SI	Morphological alteration	Lakeshore Modification Index (LMI)	0.8	AL3
DEeul	Morphological alteration	Stressor index*	0.35 0.45 0.23	AL4 small lakes (<5Km2) AL4 large lakes(>5km2) AL3 large lakes(>5Km2)
DEsub	Morphological alteration and eutrophication	Pressure index**	0.62	AL3,AL4
FR	Morphological alteration and eutrophication	Pload LHMS	0.63 0.35	AL3,AL4
IT	Eutrophication	TSI	0.43	AL3,AL4

*Stresor index was used as a pressure gradient, combining six parameters of shoreline alteration, artificial structures and near shore land use up to 100m distance to the water. It is a weighted average according to the following formula:

Stressor index = (landuse_site_15m + naturalness_site + 0,3 * landuse_lake_100m + 0,3 * shore_alteration + 0,5 * hard_shore_alteration + 0,3 * soft_shore_alteration)/3,3

*A pressure index was created based on catchment land use combination with data on sewage treatment plants, lake uses, and expert judgment of impact classes.

4.3. Assessment concept

The Intercalibration is feasible in terms of assessment concepts in following combinations:

- IC is feasible for SI method with DEeul method;
- IC is feasible for DEsub method with FR;
- IC is not feasible No for IT method.

Table 4.3 Evaluation if IC feasibility regarding assessment concepts

Method	Assessment concept
Method SI	Response of eulittoral invertebrates at sampling site level
Method DEeulittoral	Response of eulittoral invertebrates at sampling site level
Method DEsublittoral	Response of invertebrates from sublittoral zone at lake level
Method IT	Response of invertebrates from littoral-sublittoral-profundal zones combined at lake level
Method FRsublittoral	Response of invertebrates from sublittoral zone at lake level

4.4. Conclusion of compliance/feasibility checking:

- Methods included in the current intercalibration: SI method and DEeul method;
- Excluded but compliant methods: DEsub method and FR method. IC was not feasible because final versions of FR method was submitted too late;
- IT method has several characteristics that render compliancy questionable;
- Additionally, IC seems not feasible in term of the pressure addressed (IT is the only method dealing with eutrophication only).

5. Collection of IC dataset

Huge dataset was collected within the Alpine GIG (Table 4.3).

Table 5.1 Overview of the Alpine GIG benthic invertebrates IC dataset

Member State/Type	Number of sites or samples or data values		
	Biological data	Physico- chemical data	Pressure data
SI / LAL-3	28 sitedate/2 lakes	28 sitedate/2 lakes	28 sitedate/2 lakes
DE / LAL-3	77 sitedate /6 lakes	77 sitedate /6 lakes	77 sitedate /6 lakes
IT / LAL-3	55 sitedate/5 lakes	55 sitedate/5 lakes	55 sitedate/5 lakes
DE LAL-4	54 sitedate /6 lakes	54 sitedate /6 lakes	54 sitedate /6 lakes

Note. Additional sublittoral dataset from FR (21 lakes), IT (28 lakes), DE (26 lakes), AT (19 lakes) was collated.

Data acceptance criteria used for the data quality control are described in Table 5.1 (applies to SI and DEeul methods)

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

Data acceptance criteria	Data acceptance checking

Data requirements (obligatory and optional)	We worked out a list of obligatory and optional pressure data to be linked with invertebrate data. For the IC process only the obligatory variables were used (namely: naturalness of shoreline at site level, land use with 15m and 100m at site level, whole lake 100m belt at lake level and % shoreline altered at lake level). Additional obligatory parameters were: depth, area, elevation and invertebrate national assessment results.
The sampling and analytical methodology	Hand net invertebrate samples covering all habitats and individual counts. Samples carried out in the eulittoral with Ekman grab were excluded
Level of taxonomic precision required and taxa lists with codes	Taxonomic levels were family for Oligochaetes and Chironomids, species and genus for the others. Coding was uniformed following Freshwaterecology.info dbase
The minimum number of sites / samples per intercalibration type	Not applicable because no differentiation between IC types was applied
Sufficient covering of all relevant quality classes per type	Both SI and DE provided enough date/sampling site samples to cover the whole range of quality classes

6. Common Benchmarking

Number of **reference sites** is not sufficient to make a statistically reliable estimate.

Benchmark standardization as described in the IC Guidance has not been applied. Instead continuous benchmarking has been applied to standardise all single common metrics (see Central Baltic Benthic invertebrate GIG Annex B for detailed description of the principle). All sites were used for continuous benchmarking.

Pressure criteria for continuous benchmarking:

- Five pressure parameters (naturalness of shoreline at site level, combined land use within 15m and 100m at site level, combined land use within 100m belt at lake level and %shoreline altered at lake level) were first standardizes between 1 to 5 (continuous values);
- Stressor index was calculated for each sampling site by weighted averaging of standardized pressure parameter values following the equation "Stressor index = (2* Naturalness_shoreline + combined Landuse_15m_site + combined Landuse_100m_site + combined Landuse_100m_lake + Shore alteration)/6)".

The offset has been determined using Linear Mixed Models with the biological metrics as dependent variable, the combined pressure variable as covariates and the country as random factor. For this purpose the package 'lme4' of the 'R'-software was used.

To obtain standardised metrics the offsets given by the model were subtracted from the metric values (see Figure 6.1 as example).

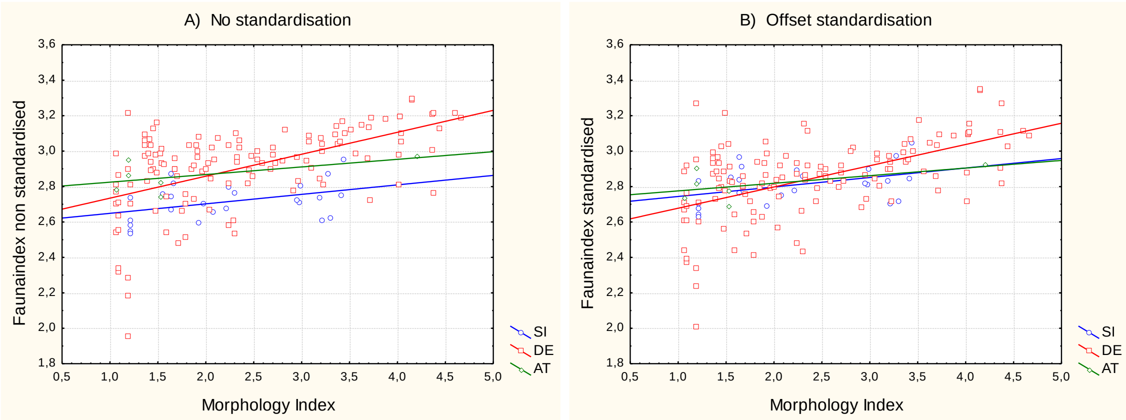

Figure 6.1 Relationships between fauna index and morphology index. A) before and B) after offset standardisation.

The resulting standardisation values (offsets or factors) are given in Table 6.1.

Table 6.1. Offset values for the Alpine lake metric standardisation calculated with linear mixed models. FI_AL - Fauna index; No_Taxa - number of taxa; Gatherers - % feeding type preference gatherer; r/k - reproduction strategy r/k.

Group (country_laketype)	FI_AL	No_Taxa	Gatherers	r/k
AT_small(<5)	0.049	1.19	0.30	-0.0416
DE_large(>5)	0.100	6.47	-4.14	0.0307
DE_small(<5)	-0.054	-6.91	0.91	0.0483
SI_small(<5)	-0.095	-0.75	2.40	-0.0374

After combination of the standardized single metrics into a common multimetric index for boundary comparison all countries followed one common dose response curve (Figure 6.2).

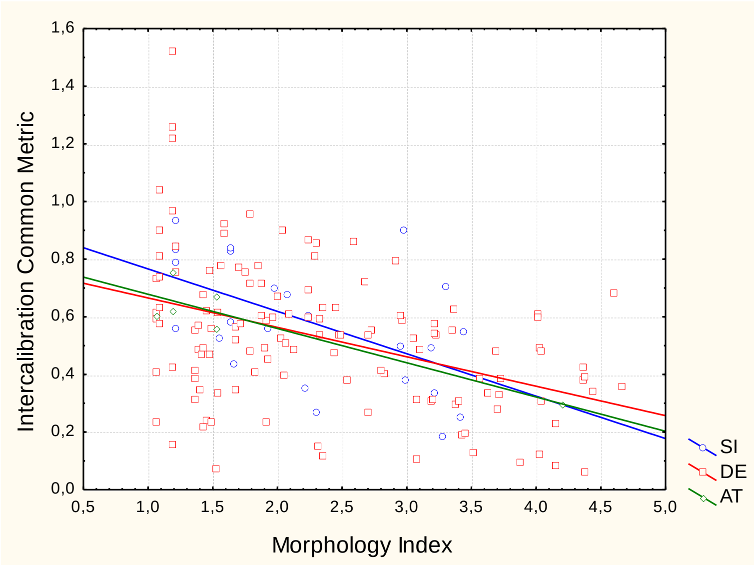

Figure 6.2 Correlation of the final intercalibration common metric (ICM) with the morphology index. The ICM is composed of the standardised single metrics. Therefore all countries follow the same dose response curve.

7. Boundary comparison and harmonisation

Option 2 was used because differences in sampling methods do not allow the application of option 1 and 3. DE sampling method is habitat specific while SI is not. Conversion is not possible because of different sampling.

The Common metric is calculated as the average of 4 normalised metrics (s.

Table 6.1 below for normalization anchors):

Fauna index * 2 + number of taxa + reproduction strategy (r/k) + % feeding type preference gatherer (based abundance classes).

Hence, the common multimetric index addresses all indicative parameters of the WFD: 'Taxonomic diversity', 'ratio of disturbance sensitive to insensitive taxa' as well as 'taxonomic composition and 'abundance'.

Table 7.1 Upper and lower anchors for normalisation of standardised metrics.

	Fauna index	Number of taxa	Gatherers	r/k strategy
Upper anchor	2.61	35	31	0.12
Lower anchor	3.09	14	52	0.3

The outcomes of the regression complied with the following characteristics according to the IC Guidance Table 7.2, Figure 7.1):

- All relationships were highly significant p<=0.001;
- Assumptions of normally distributed error and variance (homoscedasticity) of model residuals must be met;
- Common metric must represent all methods (r>0.5);
- Observed minimum r^2 was > half of the observed maximum r^2;
- Slope of the regression should lie between 0.5 and 1.5 (SI 1.2, DE 1.0, both significantly different from 0).

Table 7.2 Correlation coefficients (R), determination coefficients (R^2) and probabilities (P) for the relationship of each method with the common metric.

Member State/Method	R	R^2	P
SI	0.94	0.88	<0.001
DE	0.76	0.57	<0.001

a) Slovenia **b) Germany**

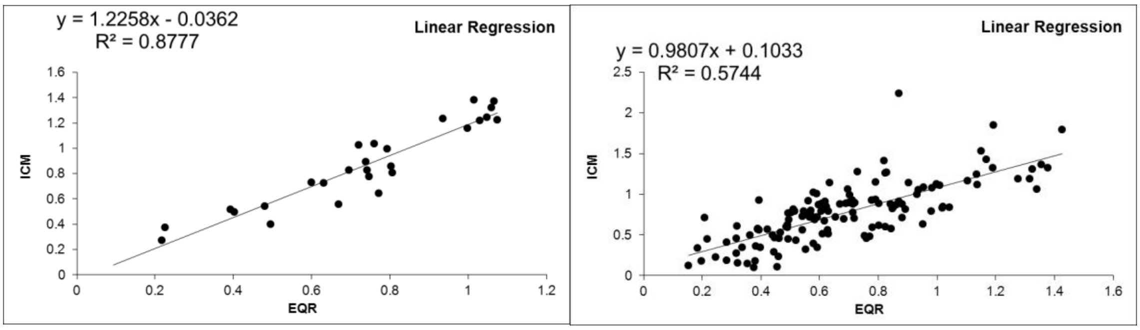

Figure 7.1 Regression between national EQRs (a – Slovenian method, B – German method) and Intercalibration common metrics (ICM).

7.1. Boundary comparison and harmonisation

Boundary comparison was carried out with the Intercalibration Excel Template Sheets (v1.23) for option 2.

Boundaries were compared using IC option 2 with a boundary translation against a common metric scale. Since the common metric was already standardised by continuous benchmarking, the offset was not established using benchmark sites, but was manually set to 0.

Boundary bias was <0.25 class equivalent for high/good (H/G) and good/moderate (G/M) boundary for both eulittoral methods (DE_{HG} - 0.144, DE_{GM} - 0.019, SI_{HG} 0.084 and SI_{GM} 0.015 class equivalent). Class agreement with modelled data was not performed.

Therefore boundaries complied to the comparability criteria, no change was needed (Figure 7.2).

a) High-Good class boundary **b) Good-Moderate boundary**

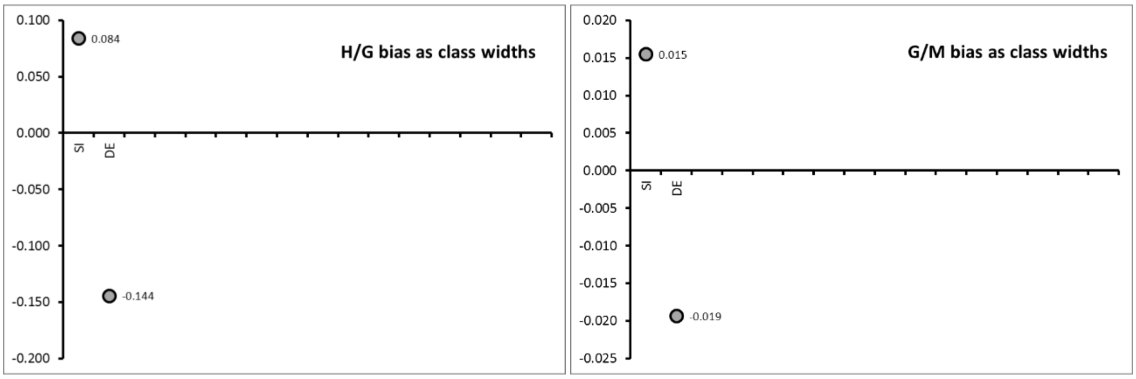

Figure 7.2 Comparison of Alpine GIG benthic invertebrates methods: HG and GM boundary biases (HG – High-Good class boundary, GM- Good-Moderate class boundary).

7.2. IC results

The boundaries for the countries were not adjusted. Therefore the G/M-boundary remained at national EQRs of 0.8 and the H/G-boundary at 0.6 for both countries - DE and SI.

Table 7.3 H/G and G/M boundary EQR values for the national methods for each type.

Member State	Classification	Ecological Quality Ratios	
	Method	High-good boundary	Good-moderate boundary
SI	Metodologija vrednotenja ekološkega stanja jezer z bentoškimi nevretenčarji v Sloveniji (Ecological status assessment system for lakes using benthic invertebrates in Slovenia)	0.80	0.60
DE	AESHNA - Bewertungsverfahren für das eulitorale Makrozoobenthos in Seen zur Umsetzung der EG-Wasserrahmenrichtlinie in Deutschland	0.80	0.60

Gaps of the current Intercalibration:

- to carry out the assessment of the level of class agreement
- to progress in the intercalibration exercise of the sub-littoral methods.

8. Description of biological communities at high and good status

8.1. Description OF the biological communities at reference sites:

Reference status has the same characteristics as good status, but with a gradual difference: high diversity and abundance of sensitive insect taxa (mainly Ephemeroptera, Trichoptera and Odonata), a dominance of sensitive versus tolerant taxa (leading to an increase in Faunaindex, >2.7, for example), low ratios of r-strategists in relation to k-strategists (<0.14) and a low portion of the feeding type gatherers (<35%).

Examples of frequently found taxa with highest abundances at reference status include *Acroloxus lacustris, Cyrnus sp., Dendrocoelum lacteum, Ecnomus tenellus, Sialis lutaria, Oecetis testacea, Athripsodes sp. and Gammarus lacustris*

8.2. Description of the biological communities at good status

Good status is characterised by high diversity and abundance of sensitive insect taxa (mainly Ephemeroptera, Trichoptera and Odonata), a dominance of sensitive versus tolerant taxa (leading to an increase in Faunaindex, for example), low ratios of r-strategists in relation to k-strategists and a low portion of the feeding type gatherers.

About 160 taxa of the IC dataset showed preferences for high to good or moderate to bad status.

Examples of frequently found taxa with higher abundances at high or good status include Odonata *gen. sp.*, Turbellaria *Gen. sp.*, Leptoceridae *gen. sp.*, *Gammarus sp., Athripsodes sp., Ecnomus tenellus, Ecnomus tenellus and Oecetis testacea,*

Moderate or worse status is characterised by high diversity and abundance of insensitive taxa, a dominance of tolerant versus sensitive taxa, higher ratios of r-strategists in relation to k-strategists and a high portion of the feeding type gatherers.

Examples of frequently found taxa with higher abundances at moderate or worse status include *Physa fontinalis., Nebrioporus sp., Glossiphonia sp., Erpobdella sp., Hippeutis complanatus* and *Mystacides sp.*

All in all, this reflects a change from more specialised and sensitive taxa towards generalist and tolerant taxa.

All single metrics as well as the multimetric respond to the pressure in a linear way, without certain changes indicative of class boundaries. Additionally abiotic factor combinations vary leading to specific metric responses. The same holds true for the macroinvertebrate taxa composition. Consequently no borderline communities can be described properly.

Annexes

A. Description of Member states assessment methods

A.1 France - French Macroinvertebrate Index for Lakes - Indice MacroInvertébrés Lacustre (IMAIL)

In France, three assessment index based on benthic invertebrate fauna have been previously developed, however, two of them doesn't fullfil the normative conditions fixed by the WFD:

- The IOBL Oligochaete Index of Lake Bioindication (AFNOR 2005) and the IMOL Indice Mollusques de Bioindication Lacustre (Mouthon 1993) take into account only one group of macroinvertebrates. Moreover, stressor/impact type responses have never been shown with these indicators;
- The third one, LBI Lake Biotic Index (Verneaux et al 2004) needs too heavy field sampling and so cannot be used at a large scale.

The French lake assessment method for macroinvertebrates is multimetrics and follows the requirements of Hering et al. 2006. It is based only on **sublittoral** samples collected according to the field protocol applied for LBI and its simplification (simplified LBI according to Mazzella et al. 2009). This index respond to anthropogenic impacts of two different types: **eutrophication** and **hydromorphological** degradation.

Sampling methodology

To minimize the effect of seasonal variation in abundance due to emergence of insects, the sampling period has to take place in early spring (between March and May). Only sublittoral samples are used, the sublittoral zone being between 2 and 3 meters. Details about the field sampling methodology can be found in Verneaux et al. 2004 and Mazzella et al. 2009.

The number of replicates in this sublittoral zone is fixed to 7. When the new field protocol was applied (Mazzella et al., 2009), the average abundance value per taxa has been calculated on the seven samples. While the old field sample was applied (Verneaux et al., 2004), more than 7 samples per lake were available (old field protocole). In that case, a random selection of 7 samples repeated several times were applied and the average abundance per taxa was also computed.

The sampling has to be done using an Ekman grab in soft sediment. The sediment is sieved through a 250 µm mesh net.

Determination level is genus level except for diptera other than Chironomidae (family), Nematoda (phylum) and Oligochaeta for wich three groups have been identificated (Tubificidae with and without hair setae, other Oligochaeta).

The individuals for the sublittoral samples are counted and the density of each taxa is expressed in number of individuals per m^2.

Dataset

The database we used concerns 14 French natural lakes, 6 of AL-3 type and 8 of AL-4. Samples were collected between 2004 and 2010 according to the field protocol proposed either by the LBI or the simplified LBI.

Reference sites

Two lakes have been classified as reference at a national scale based on local and catchment scale stressors, Lac du Grand Maclu and Grand lac Etival. These two lakes have population density around 8 habitants /km^2 and agricultural land-cover of 11% in average in the catchment area while the means for the other lakes are respectively 53 habitants/km^2 and 36% of agricultural land cover.

Anthropogenic factors

From the stressors available, two have been used in the index development: (i) a local-scale stressor, the **LHMS**, which, according to Rowan et al., (2006) synthesizes a wide array of anthropogenic pressures with a direct impact on the lake hydromorphology. LHMS values ranged from 0 to 42, the latter value corresponding to high levels of anthropogenic pressure. This score then targets the **hydromorphological** stressors; (ii) a catchment scale stressor; the annual Phosphorus load (**Pload**) to target rather the eutrophication. Pload is estimated by:

$$P_{Load} = S \times L_P = \frac{S \times PT \times \frac{Z_{Moy}}{\tau}}{1/\left(1+\sqrt{\tau}\right)}$$

With:

- S the surface of the lake in m^2,
- Lp the annual area load of the total phosphorus (kg/m2/year),
- τ the residence time (year) calculated according to $\tau = \frac{V}{Q}$ with Q which is the mean annual discharge (m3/year) and V the volume of the lake (m3)
- Zmoy the mean depth estimated by $Z_{Moy} = \frac{V}{S}$ and
- PT being the statistic relation developed by (Vollenweider, 1975), is the in-lake phosphorus concentration (e.g. at the spring over-turn) and which can be calculated using : $PT = \frac{L_P}{Z_{Moy}/\tau} \times \left[\frac{1}{1/\left(1+\sqrt{\tau}\right)}\right]$.

Then the equation of Pload can be simplified by :

$$P_{Load} = PT \times Q \times \left(1 + \sqrt{\frac{V}{Q}}\right)$$

Metrics

A metric is defined as a measurable part or process of a biological system empirically shown to change in value along a gradient of human influence (Karr, 1999). For each lake, nearly 100 potential metrics have been calculated. These metrics correspond to different categories: diversity, composition, tolerance and functional. Diversity categories are mainly assemblages based on taxonomic composition or richness. Composition categories are made of percentage of individuals of different taxonomic groups. Tolerance and functional metrics are calculated following the traits value published initially in (Tachet et al., 2000) and updated recently (Usseglio-Polatera, unpublish. data). Each trait is “fuzzy coded” (Chevenet et al., 1994) to account for phenotypic and ecological preference variability among taxa. Thus a score is assigned to each modality from a trait. This score can then been crossed to the fauna list to obtain the percentage of each modality in each lake.

General Index calulation methodology

The development of a mutlimetric index typically involves several steps of analysis leading to metric selections.

Metric selection

- Numerically unsuitable metrics are first excluded from the analysis. Box-whisker plots are drawn in order to detect metrics characterized by a narrow range of values or with many outliers and extreme values (Hering et al., 2006);
- Only metrics with a correct relation with at the stressors are kept in the following analysis process. Metrics are also selected in order to respond to increasing impacts;
- The metric values are transformed into EQR (Ecological Quality Ratio). EQR represents the relationship between the values of the observed metrics and the values for this metric under reference conditions (mean metric values of our reference lakes). The ratio is expressed as a numerical value between zero and one, decreasing with increasing stress.

EQR is calculated as:

$$EQR = \frac{(\mathrm{obs\_metric - ref\_metric}) - \min(\mathrm{obs\_metric - ref\_metric})}{\max(\mathrm{obs\_metric - ref\_metric}) - \min(\mathrm{obs\_metric - ref\_metric})}$$

for metrics that decrease with increasing stress.

For metrics that increase with increasing stress, it is calculated as:

$$EQR = 1 - \frac{(\mathrm{obs\_metric - ref\_metric}) - \min(\mathrm{obs\_metric - ref\_metric})}{\max(\mathrm{obs\_metric - ref\_metric}) - \min(\mathrm{obs\_metric - ref\_metric})}$$

where obs_metric is the value of the observed metric and ref_metric is the average of the reference lakes metric values.

The Pearson correlation between the normalized EQR and the stressor is then calculated. Spearman rank correlation analysis among the remaining metrics is applied and metrics were considered as redundant while Spearman's r > 0.8 (Hering et al.,2010).

The multimetric index should preferably contain at least one metric from each type. A selection of the metrics, which respond to the stressors, is then done when several metrics per type are selected at the previous stage.

Generation of the multimetric index

Several combinations of core metrics EQR are tried using a simple average, and then the final EQR, called here the index is correlated (Pearson) to the stressors used in the model. The comparison of the values obtained for the different combinations as well as the distribution shape of the EQR against the stressors permit the selection of the best index.

The French Lake multimetric index results

From the 100 available metrics, only 9 passed the first selection stages (correct relation with the stressors): piercer, PI, PCR, PCH, PTUaS_OL, DTUsS, Hmax, RS and Es3.

The combination stage of these core metrics to obtain the best multimetric index correctly correlated to the stressors (Table A.1) but also containing metrics not correlated between them (Table A.2) and metrics that will be easily measurable in the future gave the final result of 3 selected metrics in the multimetric index: DTUsS, PtuaS_ol and Es3:

- PtuaS_ol, Percentage of Tubificinae with hair setae (composition category): Subfamily of tubificinae with hair setae includes sensitive taxa to organic pollution (e.g. Psammoryctides barbatus, Spirosperma velutinus) (Lafontetal.,2007). Negative expected correlation with stressors.
- DTUsS, Density of Tubificinae without hair setae (abundance catergory). Unlike the Tubificinae with hair setae, these taxa increase with stressors.
- Es3, Eveness Index (Smith and Wilson, 1996), based on a common form of Simpson index (Es3= (1/D)/S with S the number of taxa and D the Dominance index (Simpson, 1949) (diversity category). Negative expected correlation with stressors.

Table A.1 Spearman correlation between the stressors and the selected metrics.

	LHMS	Pload
Es3	-0.376	-0.539*
DTUsS	0.524*	0.643**
PTUaS_OL	-0.534**	-0.682***

Table A.2 Spearman correlation between core metrics

	Es3	DTUsS	PTUaS_OL
Es3			
DTUsS	-0.44		
PTUaS_OL	0.6 **	-0.49*	

These combinations of metrics gather also metric of different type as requested by the normative definition of the WFD.

Finally, the multimetric index selected is then the average of three metrics EQR which has been log10-transformed (in order to be normalised) and again transformed into EQR to obtain values between 0 and 1 (Figure A.1). This index is correlated to the Pload with a Pearson coefficient of -0.81 (adj R^2 of 0.63, pvalue<0.001) and to the LHMS of -0.63 (adj R^2 of 0.35, pvalue<0.05).

Due to low number lake into reference conditions, the class boundaries have been fixed dividing the EQR range into 5 equal classes. Moreover, it was not possible to ecologically interpret the interaction between paired metrics. However, the High/Good boundary has been controlled in order to contain at least the two reference lakes and biological reference criteria. The values of the metrics have then been examined in the class high, good and moderate (Figure A.2).

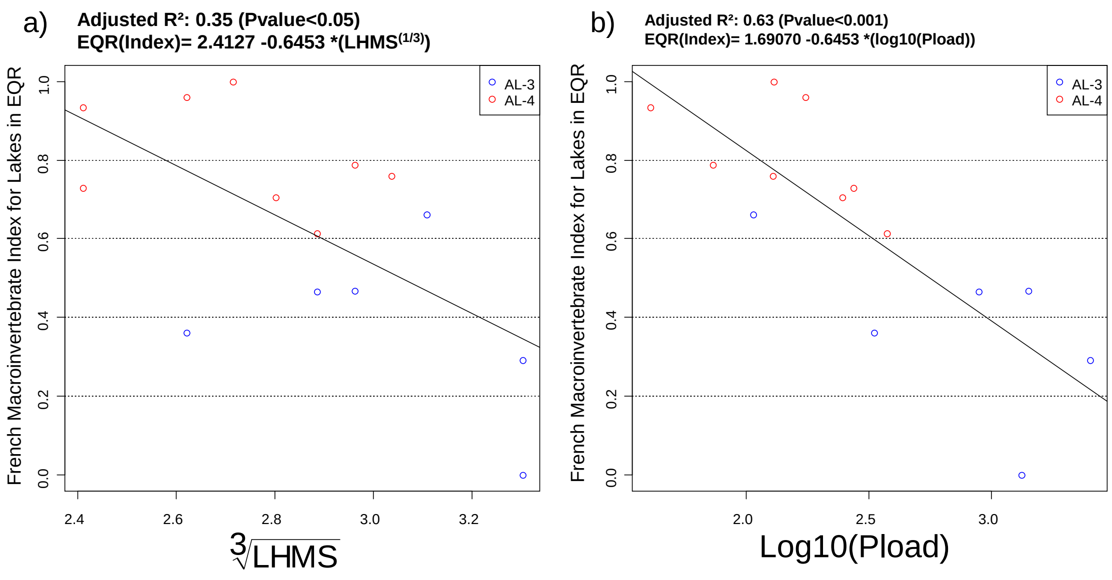

Figure A.1 Relationships between the index and: (a) LHMS; (b) Pload. The model equation, the adjusted r^2 and the significance are noted in the upper part of each figure. The blue dots correspond to the AL3 lakes as the red to the AL4 ones.

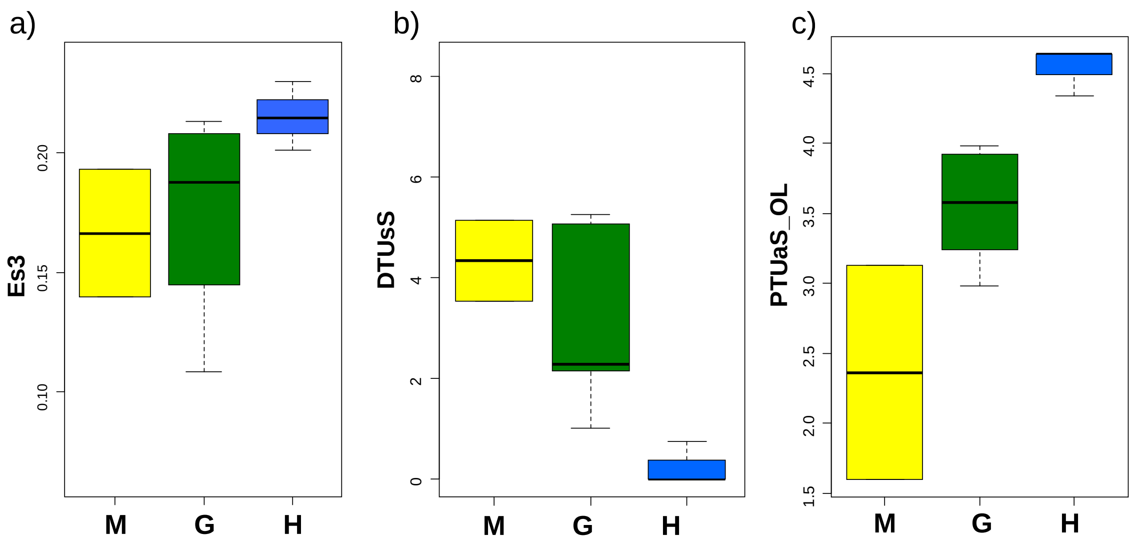

Figure A.2 Distribution value of Es3 (a), DTUsS (b) and PTUaS_OL (c) metrics in the classes High (H), good (G) and moderate (M).

High values of PTUaS_OL are observed in the High class. Tubificinae with hair setae being composed of sensitive taxa, this result confirmed the High quality status of this class. This metric shows also lower values in the moderate class compared to the Good class. It is clearly the case for Es3 too which, as evenness index may decrease with the degradation of the environment.

The final classification of the French lake is then shown in the Table A.3 with three lakes classified in High class, 6 in Good, 2 in Moderate, 2 in Poor and 1 in Bad.

Table A.3Classification of the lakes used in the index development.

Acronym	Lake name	Types	EQR	Class
AIG73	Aiguebelette	AL-3	0.000	B
SPO25	Saint Point	AL-3	0.293	P
PAL38	Paladru	AL-3	0.362	P
NAN01	Nantua (lac de)	AL-3	0.466	M
CHA39	Chalain (lac de)	AL-3	0.469	M
REM25	Remoray (lac de)	AL-4	0.614	G
LAF38	Laffrey (grand lac de)	AL-3	0.662	G
LPC38	Pierre-châtel (lac de)	AL-4	0.704	G
LRO39	Rousses (lac des)	AL-4	0.730	G
GLC39	Clairvaux (Grand lac)	AL-4	0.761	G
PET38	Petitchet	AL-4	0.789	G
ETI39	Etival (grand lac)	AL-4	0.934	H
ILA39	Ilay (lac d')	AL-4	0.960	H
LGM39	Grand maclu (lac du)	AL-4	1.000	H

Summary

This Macroinvertebrates Lake Index is developed on the data collected in the French natural lakes located in the Alpine area. It fulfils the normative definition.

From a statistical point of view, its robustness will have to be checked using other data (collected on new similar lakes). The method will be then improved in the future according to the results of these different validations.

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A.2 Germany: AESHNA - German Lake Macroinvertebrate Assessment System for the Water Framework Directive

This short description of **AESHNA** gives all major information for sampling, calculation of the multimetric assessment index and the classification in ecological status classes according to the Water Framework Directive (Directive 2000/60/ES). The ecological classification system for eulittoral macroinvertebrates will be used in the second RBMP (2015). The sublittoral classification will be used for additional information only.

Lake types

The method is suited to all natural alpine lakes and lowland lakes in Germany. These include all intercalibration lake types occurring in Germany (L-CB1, L-CB2, L-AL3, L-AL4). Within the national typology these comprise the general types 1, 2, 3, 4, 10, 11, 12, 13 and 14, or the benthic fauna lake types small alpine, large alpine, riverine lowland and non-riverine lowland respectively.

AESHNA also includes multimetric assessment indices for other natural and artificial German lake types > 50 ha. But since these have not been officially accepted yet, they were not included here.

Detected pressures

AESHNA was designed for the detection of all kinds of pressures, but the focus was laid on hydromorphological degradation.

Based on **eulittoral** macroinvertebrate samples of 491 central-baltic sampling sites (55 lakes) and 131 alpine sampling sites (12 lakes) pressure-impact and lake morphology data relationships were established for a variety of candidate metrics. Finally multimetric indices were developed consisting of several metrics, which cover all criteria of the WFD. Multimetric indices and lake morphology indices were significantly correlated (Spearman R ranging from 0.5 to 0.8, depending on the pressure index and lake type). Correlations with a combined morphology-TP index were slightly higher, whereas correlations with eutrophication alone or with catchment landuse were significantly lower with Spearman R up to 0.5.

For the **sublittoral** assessment however Spearman R is similar for all landuse in lake surroundings, catchment landuse and eutrophication with values up to 0.6.

Sampling

<u>Short description of eulittoral sampling</u>

A multihabitat sampling procedure is carried out for eulittoral macroinvertebrates in February to April (lowland) / to May (alpine) or September to October.

A minimum of 4 sampling sites per lake (N=4+shorelength^-2) is selected by expert judgement according to the occurrence of shoreline types.

At each sampling site all available habitats have to be covered at up to 1.2 m depth of water. Hand nets (500 µm mesh-size) are used whenever suitable or other devices when more appropriate (e.g. scrapers).

There are two options:

A) The area sampled for each habitat is proportional to the percentage of occurrence at the sampling site. At minimum total of 1 m^2 is sampled.

B) All habitats are sampled with the same intensity, covering 0.6 to 1,0 m^2 per habitat. The area sampled and the relative presence of each habitat is determined for a later combination to a multihabitat taxa list.

<u>Short description of sublittoral sampling</u>

Sublittoral sampling is carried out once in February to April (lowland) / to May (alpine) or September to October.

A minimum of 8 stations per lake (>=12 for lakes >200 ha) is selected by dividing the lake in equal sectors and placing them in the center of the upper sublittoral zone. At each sampling site 3 Ekman grabs are taken.

<u>Sample processing</u>

Samples are sorted out in the field or sieved, fractionated and preserved in Ethanol for sorting in the laboratory. Sublittoral samples may be subsampled.

<u>Level of taxonomical identification</u>

Taxa to be identified are given in a detailed "operational taxa list". The level is mostly species or achievable level for all but the following: Family for oligochaeta and most non-chironomid dipterans, mostly genus for chironomids.

Abundances are recorded as number of individuals per m^2.

Multimetric index / EQR calculation

Multimetric index (MMI) composition and standardisation values differ between benthic fauna lake types. In order to obtain EQR values comparable to other biological quality elements the EQR-values are obtained from the MMI values by linear transformations.

<u>Metric standardisation</u>

Two anchor points for metric standardisation were derived from the data distribution along the pressure gradient using 10%tiles of the whole distribution in combination with extrapolated values (for incomplete pressure gradients): The near reference value and the bad status value. Using the following formula the each metric value (M) is standardised from 0.0 for the bad status value (M0) to 1.0 for the near reference value (M1):

Standardised metric = (M - M0) / (M1 –M0).

The anchor values are specific for each benthic fauna lake type.

Eulittoral alpine MMI/EQR

Five standardised metrics are averaged: relative abundance of Odonata (% of abundance classes), relative abundance of feeding type collectors (% of abundance classes, reproduction strategy r/k, Shannon diversity and littoral faunaindex, with double weighting of the fauna index;

Formula: MMI = (2*fauna index + odonata+ Shannon diversity + gatherer + rk)/6

EQR = MMI*4/3-1.2

Eulittoral non-riverine lowland MMI/EQR

Four standardised metrics are averaged with equal weighting:

Faunaindex, relative abundance of habitat type lithal (% of abundance classes), relative abundance of Odonata (% of abundance classes) and number of ETO-Taxa;

EQR = MMI*4/3-1.2

Eulittoral Riverine lowland MMI/EQR

Three standardised metrics are averaged with equal weighting:

Faunaindex; relative abundance of Chironomidae (% of abundance classes), Margalef-diversity);

EQR = MMI*4/3-1.2

Sublittoral Alpine MMI/EQR

Seven standardised metrics are averaged with equal weighting:

ETO-taxa (% based on taxa number), insecta (% based on individual numbers), habitat preference phythal (% based on abundance classes), feeding type collectors and predators (each in % based on abundance classes), locomotion type sessile (% based on abundance classes), alpha-Mesosaprobic (% based on Individual numbers);

EQR=MMI

Ecological status classification

Ecological status classes are obtained from the EQR values using the following class boundaries:

Boundary	EQR
High/Good	0,8
Good /Moderate	0,6
Moderate/Poor	0,4
Poor /Bad	0,2

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A.3 Italy: Benthic Quality Index for Italian Lakes

Sampling methodologies

The sampling methodologies have been assessed following US-EPA methodologies for soft sediments (http://www.epa.gov/owow/monitoring).

In lakes were obvious depth gradient exists the sampling may be laid out in the form of a stratified sampling, working on transects with sites of sampling arranged to give equal coverage of the different zones.

A sample consists of 3 grab replicates per site (minimum effort to obtain good data set) to a maximum of 9 replicates in a transect. Each site is located along transects connecting the shore-line to the max depth, in 3 different sampled areas (littoral, sublittoral and profundal). Sampling is performed through the use of an Ekman grab with an area of 0.0225 m^2 of lake bottom.

The number of transects depends on lake area (Table A.4) and their position is based on Expert knowledge (e.g. sites most representative of water body, different sediment texture, presence of inflow/outflow rivers and of human impacts, variability of the shore-line habitat, low slope of the banks). For large lakes (like L. Maggiore, Garda and Como the Italian lakes with the highest areas and maximum depths > 300 m) the number of transects refers to the number of sub-basins.

The sampling period is February to April (Spring) and September to October (Autumn) due to Insects life-cycles with a biannual sampling frequency (i.e. during turnover and stratification periods).

Table A.4 Relation between surface lake area and number of transects, of sampling sites and of replicates per site.

Lake area km^2	No transects	No sites	No replicates
<0.6	1	3	9
0.7-2.9	2	6	18

3.0-6.5	3	9	27
>6.6	4	12	36

After taking samples, each of them have to be washed through a sieving net with a mesh size of 250-300 µm to efficiently catch also the smallest organisms, placed in a plastic bottle and preserved with formalin (10%). The sample treatment in the lab includes the sorting of the whole sample under a stereo-microscope, but sub-sampling is allowed when, on the basis of a preliminary survey, the number of specimens is larger than 500. Specimens are identified to species level whenever possible, including Chironomids and Oligochaetes, the genus level is considered when the material available (only immatures for example) hindered species identification. Numbers are expressed as individual counts abundance and related to area as number of individuals per one m^2 (ind m^2).

For a description of the method and sample processing followed see the national guidelines (Bazzanti *et al*., 2007; and for major detail Boggero *et al*., 2011 found on the CNR-ISE Web page http://library.ise.cnr.it/publication.php as *Report CNR-ISE, 02.11*).

Standardisation of methodologies refer to:

ISO 9391.1993. Water quality. Sampling in deep waters for macro-invertebrates. Guidance on the use of colonization, qualitative and quantitative samplers: 13 pp.

ISO/TC 147/SC5. In press. Water Quality. Guidance on the selection of sampling methods and devices for benthic macroinvertebrates in freshwaters: 24 pp.

Italian National Index (Benthic Quality Index for Italian Lakes)

In view of the marked incidence of eutrophication in lake waters, Italy has decided to consider the trophic state as pressure to be addressed. The response of macrobenthic fauna to the hydro-morphological impact will have to be taken into account when data will be available, deriving mostly from WISER and INHABIT European projects.

The index elaborated can be used in all types of lakes in mainland Italy at latitudes > 44°N with conductivity < 2.5 mS cm^{-1}. The use of the index involves different sensitivity values for different species. At present it is not planned to use the index for brackish or mesosaline lakes with conductivity higher than the indicated threshold, as they are probably inhabited by a highly different benthic community.

We decided to focus mainly on Chironomids and Oligochaetes, the main components of the macroinvertebrate lake community, being other taxonomic groups less represented.

The index presented here was applied to 5 natural lakes of 2 different types (AL-3, AL-6) located in the Subalpine Region, included in the 18 types described in the national document on lake types (Buraschi *et al*., 2005). These lakes are located at altitudes below 800 m a.s.l. (AL-3, AL-6). All of them have a mean depth >15 m. The AL-3 type consists of the large Italian lakes with a maximum depth >120 m and a surface area >100 km^2. Italian types AL-3 and AL-6 correspond to IC common type L-AL3. None of the Italian lakes considered in the present report was considered reference for the phytoplankton IC exercise.

For the present method the profundal zone of AL-3 lakes was not included to have an homogeneous dataset including only Italian littoral and sublittoral AL-3 zones, AL-6 lakes, and Austrian reference lakes <800 m a.s.l. with mean depth >15 m, compatible with AL-6 Italian type.

The data used derived from national monitoring sampling campaign. The index described here (Rossaro *et al*., 2011) can be found on the web site of the CNR-ISE (http://library.ise.cnr.it/publication.php as *Report CNR-ISE, 03.11*) and has to be subjected to the intercalibration process.

Reference sites were selected among Austrian lowland lakes; they are in near-natural condition and because in the Alpine GIG biogeographical differences are considered negligible, they were considered representative for the Alpine area. A total of 45 sites (9 sites per 5 lakes) were analyzed in Austrian lakes, but considering reference sites criteria, identified through obligatory variables (O_2% saturation >70%, total phosphorous <6 µg l-1, transparency >8 m, shoreline naturalness >90%), only 36 sites resulted in reference condition. Austrian lakes were chosen as reference sites for the Italian method notwithstanding that differences in type-specific reference conditions could theoretically occur between northern and southern Alps. In fact, according to Free *et al.*, (2006) the Austrian and Italian lakes are characterized by different species: *Evidence was found that several species, either through their differential presence or abundance were indicative of either a country or side of the Alps*. But the the same authors (Free *et al.*, op. cit.) concluded: *The approach taken to develop an index in this study that overcomes such biogeographic differences is the development of weighted averages which translate the trophic preferences of the species into a common scale to allow comparison across countries*. The weighted average method cited by Free *et al.* (2006), just described in Rossaro *et al.* (2006) and reconsidered in the present report, allows to integrate data including species exclusive of a group of sites.

In conclusion, at present, within the Alpine GIG: i) no confounding indication in the scientific literature exists; ii) no sub-types based on biogeographical aspects are possible to describe and iii) no information is available about benthic macroinvertebrates from Italian sites established as reference.

Formulation and use of the *BQIES* index

Data required:

Abundance of each taxon (ind m^{-2}) obtained from an integrated sample of 3 replicates/site taken in the littoral and sub-littoral zones on soft substrate, in at least two field campaigns in the same year (preferably at the overturn and after the summer stratification).

Procedure:

1. insert only the species present in at least 5% of samples in all the lakes considered, taking account of the two samplings required by the protocol; in this way all the species useful for bioindication are included (Table A.5 and Table A.6);
2. the index used is an index of trophy, and as such it has been constructed with the aim of linking the response of macroinvertebrates to physical-chemical factors connected with trophy, i.e. oxygen saturation percentage, transparency (measured by Secchi disc) and total phosphorus.
3. for each species found at a sampling site in a lake, calculate the weighted mean of the three environmental variables. Species abundances are used as weight, following the formula:

   $$\bar{z}_{jk} = \frac{\sum_{i=1}^{n} y_{ij} z_{ik}}{\sum_{i=1}^{n} y_{ij}}$$

   where:

   z_{ik} = value of environmental variable *k* measured at a sampling site *i*,

   y_{ij} = abundance of species *j* at the same sampling site *i*,

   $\bar{z}_{jk}$ = optimum value of the environmental variable *k* for species *j*.

   The weighted means can be interpreted a optimum values for each species (Ter Braak & Prentice, 1988) and used as weights (*BQIW*: Benthic Quality Index Weight) to be assigned to each species in calculating the Benthic Quality Index.

4. The weighted standard deviations for each environmental variable can be interpreted as a tolerance interval; a high value indicates that the species has a lower indicator value than species with a lower tolerance value. However, data available so far are not sufficient to allow us always to interpret the tolerance values in this sense; in fact, some species found with low frequency have a very restricted tolerance value. This does not mean that they are necessarily good indicators, only that if the data are few, estimation of the tolerance value is highly subject to error.
   The formula used for the calculation is:

   $$\bar{s}_{jk} = \sqrt{\frac{\sum_{i=1}^{n} y_{ij} z_{ik}}{\sum_{i=1}^{n} y_{ij}} * (z_{ik} - \bar{z}_{jk})^2}$$

   where the meaning of the symbols is the same as in the previous formula.

5. The weighted means are then rescaled between 1 and 0 using the following formula:

$$\tilde{z}_{jk} = \frac{(\bar{z}_{jk} - z_{\min})}{(z_{\max} - z_{\min})} + 1$$

In this case *k* is one of the q = 3 environmental variables selected (oxygen saturation percentage, transparency and total phosphorus). Total phosphorus is presumed to decrease with water quality, while transparency and oxygen saturation percentage increase, so that $\tilde{z}_{jTP}$ is rescaled as follows:

$$\tilde{z}_{jTP} = 1 - \tilde{z}_{j_{TP}}$$

6. The indicator weight or sensitivity value (BQIWj) is obtained as the mean of the values of $\tilde{z}_{jk}$ rescaled, according to the formula:

$$BQIW_j = \sum_{k=1}^{q} \frac{\tilde{z}_{jk}}{q}$$

where:

q = number of environmental variables used to calculate the BQIW (3 in the present case).

$\tilde{z}_{jk}$ = rescaled mean value of environmental variable *k* weighted for abundance of species *j*.

BQIWj assumes values between 0 and 1.

7. Lastly, the BQIESi for each sampling site i can be calculated using the values of the BQIWj weights using the formula:

$$BQIES_i = \left[\sum_{j=1}^{p} \left(\frac{y_{ij}}{\sum_{j=1}^{p} y_{i_j}} * BQIW_j \right) \right] * \log_{10}(m+1) * \left(\frac{\sum_{j=1}^{m} y_{ij}}{\sum_{j=1}^{m} y_{ij} + 5} \right)$$

where:

p = number of species for which the indicator weight $BQIW_j$ is known

m = total number of species present

BQIWj = indicator weight of species *j*

y_{ij} = $\log_{10}$+1 of the abundance of species *j* at sampling site *i*

$BQIES_i$ = Benthic Quality Index of sampling site *i*

The formula remembers the Benthic Quality Index (*BQI*) proposed by Wiederholm (1976, 1980). The acronym used (*BQIES*) is to distinguish it from the historical *BQI*, *-ES* meaning "expected species number" according to the original Leonardsson *et al.* (2009) formulation, in which *ESW* was used instead of *BQIW* as sensitivity value for each species.

The number of species considered is much higher than the few indicator taxa used by Wiederholm (op. cit.).

The species are not included only as presence/absence, but their abundances are also considered.

In a previously proposed formula of the biotic index (Rossaro *et al.*, 2006, 2007), no account was taken of total fauna abundance. As this is required by the WFD, a suggested change in the index includes abundance measurement (Rosenberg *et al.*, 2004; Leonardsson *et al.*, 2009).

It is also presumed that the indicator weight will be known only for a fraction p out of a total of m species present at a site. To take into account the species whose indicator weight is not known, the $log(m+1)$ and the ratio between the total number of individuals and the total number of individuals $+5$, have been added to the index. This latter ratio is very close to 1 if the number of individuals in the sample is high, but it becomes significantly lower than 1 if the sample comprises few individuals (Leonardsson *et al.*, 2009). So this correction is particularly important for samples containing few individuals, in fact when two samples have the same species composition, the index will be higher in the sample with higher number of individuals. In this way the index includes an estimate of the total abundance of the individuals in each sample, as required by the WFD.

The indicator weights of each species are in Table A.5 and Table A.6. The indicator weights were calculated using a large data set (236 taxa were considered), including all the information available about the sensitivity of species (see http://www.freshwaterecology.info/), in any case different from the one used to calculate the sites index, including 173 species (see Table A.5 and Table A.6) so there is no circularity in the algorithm. In Table A.5 and Table A.6 the species were also separated in tolerant, when exclusive of non-reference sites, and intolerant when exclusive of reference sites, but the algorithm used does not consider this separation.

Table A.5 List of indicator weights for macroinvertebrate taxa used in the index. BQIW: indicator weight of trophic state; T: t=tolerant, i=intolerant.

Order	Family	Species	Author	Code	BQIW	T
Hydrozoa	Hydrozoa	*Hydra* sp.		H.virid	0,3472	i
Turbellaria		*Triclada* sp.		Triclad	0,5208	
		Dugesia tigrina	(Girard, 1850)	D.tigri	0,3299	
		Polycelis nigra	(Müller, 1774)	P.nigra	0,3472	
Nematoidea	Mermitidae	sp.		Mermith	0,3472	t
Nematoda		sp.		Nematod	0,1736	i
Oligochaeta	Naididae	*Amphichaeta* sp.		Amphich	0,3299	i
		Chaetogaster diaphanus	(Gruithuisen, 1828)	C.diaph	0,2951	
		Chaetogaster langi	(Bretscher, 1896)	C.langi	0,4340	i
		Nais barbata	(Müller, 1774)	N.barba	0,3299	
		Nais bretscheri	(Michaelsen, 1899)	N.brets	0,2778	i
		Nais communis	Piguet, 1906	N.commu	0,2257	
		Nais elinguis	Müller, 1774	N.eling	0,2083	i
		Nais pseudobtusa	(Müller, 1774)	N.parda	0,2778	i
		Nais simplex	Piguet, 1906	N.simpl	0,2431	i
		Stylaria lacustris	(Linnæus, 1767)	S.lacus	0,2951	
		Ophidonais serpentina	(Müller, 1774)	O.serpe	0,2083	
		Dero digitata	(Müller, 1774)	D.digit	0,2083	t
		Uncinais uncinata	(Orsted, 184)	U.uncin	0,3993	
		Vejdovskyella intermedia	(Bretscher, 1896)	V.inter	0,3993	i
	Tubificidae	*Psammoryctides albicola*	(Michaelsen, 1901)	P.albic	0,2604	i
		Psammoryctides barbatus	(Grube, 1861)	P.barba	0,3472	
		Spirosperma ferox	(Eisen, 1879)	S.ferox	0,2951	
		Embolocephalus velutinus	(Grube, 1879)	E.velut	0,4167	t
		Aulodrilus pluriseta	(Piguet, 1906)	A.pluri	0,1389	
		Branchiura sowerbyi	Beddard, 1892	B.sower	0,2778	
		Tubifex ignotus	(Stolc, 1886)	T.ignot	0,2778	
		Tubifex tubifex	(Müller, 1774)	T.tubif	0,0694	
		Limnodrilus claparedianus	Ratzel, 1869	L.clapa	0,1910	i
		Limnodrilus hoffmeisteri	Claparède, 1862	L.hoffm	0,0868	
		Limnodrilus profundicola	(Verrill, 1871)	L.profu	0,1563	t
		Limnodrilus udekemianus	Claparède, 1826	L.udeke	0,1215	
		Ilyodrilus templetoni	(Southern, 1909)	i.templ	0,2604	
		Potamothrix bedoti	(Piguet, 1916)	P.bedot	0,0694	t
		Potamothrix hammoniensis	(Michaelsen, 1901)	P.hammo	0,2257	
		Potamothrix heuscheri	(Bretscher, 1900)	P.heusc	0,2083	t
		Potamothrix vejdovskyi	(Hrabe, 1941)	P.vejdo	0,2431	t
	Haplotaxidae	*Haplotaxis gordioides*	(Hartmann, 1821)	H.gordi	0,0833	
	Lumbriculidae	*Stylodrilus heringianus*	Claparède, 1862	S.herin	0,3993	
	Enchytraeidae	sp.		Enchytr	0,5556	
		Cernosvitoviella atrata	(Bretscher, 1903)	C.atrat	0,4861	i
	Lumbricidae	*Eiseniella tetraedra*	(Savigny, 1826)	E.tetra	0,0694	t
Rhyncobdellida	Glossiphoniidae	*Helobdella stagnalis*	(Linnæus, 1758)	H.stagn	0,1389	t
		Glossiphonia complanata	(Linnæus, 1758)	G.compl	0,2604	t
	Hirudinidae	*Limnatis nilotica*	(Savigny, 1822)	Limnati	0,2917	t
	Erpobdellidae	*Erpobdella octoculata*	(Linnæus, 1758)	E.octoc	0,1736	
		Dina lineata	(O.F.Muller, 1774)	D.linea	0,0100	t
Eumalacostraca_Peracarida	Asellidae	*Asellus aquaticus*	(Linnæus, 1758)	A.aquat	0,2083	
	Gammaridae	*Gammarus fossarum*	(Koch, in Panzer, 1835)	G.fossa	0,4167	i
		Gammarus lacustris	Sars, 1836	G.lacus	0,4167	i
		Gammarus roeseli	Gervais, 1835	G.roese	0,2778	i
		Echinogammarus stammeri	(Karaman S., 1931)	E.stamm	0,0694	t

Table A.5 continued

Order	Family	Species	Author	Code	BQIW	T
Plecoptera	Leuctridae	*Leuctra* sp.		Leuctra	0,5382	i
	Nemouridae	*Nemoura* sp.		N.morto	1000	
Ephemeroptera	Baetidae	*Baetis rhodani*	(Pictet, 1843)	B.rhoda	0,3299	
		Centroptilum luteolum	(Muller, 1776)	C.luteo	0,3299	i
	Caenidae	*Caenis horaria*	(Linnæus, 1758)	C.horar	0,3125	
		Caenis luctuosa	(Burmeister, 1839)	C.luctu	0,5903	i
	Ephemeridae	*Ephemera danica*	Müller, 1764	E.danic	0,3819	i
	Leptophlebiidae	*Habrophlebia lauta*	Eaton, 1884	H.lauta	0,4514	i
		Leptophlebiidae sp.		Leptoph	0,3472	i
Odonata	Anisoptera	sp.		Anisopt	0,3472	i
	Corduliidae	*Somatochlora metallica*	(Vander Linden, 1825)	S.metal	0,6111	i
	Gomphidae	*Gomphus vulgatissimus*	(Linnæus, 1758)	G.vulga	0,3472	i
	Libellulidae	Libellulidae sp.		Libellu	0,3472	i
	Platycnemididae	*Platycnemis pennipes*	(Pallas, 1771)	P.penni	0,3472	i
Hemiptera	Corixidae	*Micronecta poweri*	(Douglas & Scott, 1869)	M.power	0,4688	i
	Corixidae	*Micronecta* sp.		Microne	0,4688	i
Neuroptera	Megaloptera	*Sialis lutaria*	(Linnæus, 1758)	S.lutar	0,2951	
Trichoptera	Ecnomidae	Ecnomidae sp.		Ecnomus	0,2778	t
	Hydroptilidae	*Hydroptila* sp.		Hydropt	0,2951	i
		Oxyethira sp.		Oxyethi	0,3819	
	Leptoceridae	*Athripsodes* sp.		Athrips	0,3299	i
		Athripsodes aterrimus	(Stephens, 1836)	A.aterr	0,2778	i
		Athripsodes cinereus	(Curtis, 1834)	A.ciner	0,3299	i
		Leptoceridae sp.		Leptoce	0,2604	i
		Mystacides azureus	(Linnæus, 1761)	M.azure	0,3299	i
	Psychomyiidae	*Tinodes waeneri*	(Linnæus, 1758)	T.waene	0,3125	i
	Polycentropodidae	*Cyrnus trimaculatus*	(Curtis, 1834)	C.trima	0,2778	i
		Polycentropus sp.		Polycen	0,5035	
	Sericostomatidae	Sericostoma flavicorne/personatum		Sericos	0,4688	
	Chaoboridae	*Chaoborus flavicans*	(Meigen, 1830)	C.flavi	0,0500	
	Ceratopogonidae	*Ceratopogonidae* sp.		C.vermi	0,4688	
		Bezzia sp.		Bezzias	0,3472	i
		Dasyhelea sp.		Dasyhel	0,3472	i
Coleoptera	Haliplidae	*Haliplus* sp.		Haliplu	0,3819	i
	Dytiscidae	*Dytiscus marginalis*	Linnæus, 1758	D.margi	0,3299	i
		Graptodytes sp.		Graptod	0,3472	i
		Graptodytes pictus	(Fabricius, 1787)	G.pictu	0,3472	i
		Platambus maculatus	(Linnæus, 1758)	P.macul	0,2951	i
	Hydrophilidae	*Hydrophilidae* sp.		Hydropo	0,3472	i
Hydrachnidia	Hydrachnidia	*Hydracarina* sp.		Hydraca	0,3472	
Bivalvia	Dreissenidae	*Dreissena polymorpha*	(Pallas, 1771)	D.polym	0,3646	
	Pisidiidae	*Pisidium casertanum*	(Poli, 1791)	P.caser	0,2778	
		Pisidium milium	Held, 1836	P.miliu	0,4861	i
		Pisidium subtruncatum	Pfeiffer,	P.subtr	0,3819	i
Prosobranchia	Valvatidae	*Valvata piscinalis*	Müller,	V.pisci	0,3125	
Pulmonata	Bithyniidae	*Bithynia tentaculata*	(Linnæus, 1758)	B.tenta	0,0694	
	Lymnaeidae	*Lymnaea* sp.		Lymnaea	0,3646	t
	Planorbidae	*Gyraulus albus*	(Müller, 1774)	G.albus	0,3819	

Table A.6 List of indicator weights for Chironomid taxa used in the index. BQIW: indicator weight of trophic state; T: t=tolerant, i=intolerant.

Tribe	**Species**	**Author**	**Code**	**BQIW**	**T**
TANYPODINI	*Tanypus punctipennis*	(Meigen, 1818)	T.punct	0,1528	t
PROCLADIINI	*Procladius choreus*	(Meigen, 1804)	P.chore	0,2604	
MACROPELOPIINI	*Macropelopia nebulosa*	(Meigen, 1818)	M.nebul	0,2951	
COELOTANYPODINI	*Apsectrotanypus trifascipennis*	(Zetterstedt, 1838)	A.trifa	0,3125	
PENTANEURINI	*Ablabesmyia longistyla*	Fittkau, 1962	A.longi	0,3299	
	Ablabesmyia monilis	(Linnæus, 1758)	A.monil	0,2951	
	Thienemannimyia carnea	(Fabricius, 1805)	T.carne	0,3819	i
	Zavrelimyia sp.		Zavreli	0,6076	i
	Larsia atrocincta	(Fittkau, 1962)	L.atroc	0,5556	i
	Conchapelopia pallidula	(Meigen, 1818)	C.palli	0,3333	t
PROTANYPINI	*Protanypus morio*	(Zetterstedt, 1838)	Protany	0,4688	i
PRODIAMESINI	*Prodiamesa olivacea*	(Meigen, 1818)	P.oliva	0,2257	
ORTHOCLADIINI	*Orthocladius* sp.		Orthocl	0,6250	i
	Psectrocladius limbatellus	(Holmgren, 1869)	P.P.lim	0,3819	i
	Psectrocladius psilopterus	(Kieffer, 1906)	P.P.psi	0,3819	i
	Synorthocladius semivirens	(Kieffer, 1909)	S.semiv	0,3472	i
	Paracladius conversus	(Walker, 1856)	P.conve	0,3125	i
	Cricotopus albiforceps	(Kieffer In Thienemann E Kieffer, 1916)	C.albif	0,3646	i
	Cricotopus annulator	Goetghebuer, 1927	C.annul	0,3646	i
	Cricotopus fuscus	(Kieffer, 1924)	C.fuscu	0,3646	
	Cricotopus(Isocladius) reversus	Hirvenoja, 1973	i.rever	0,5903	i
METRIOCNEMINI	*Heterotrissocladius marcidus*	(Walker, 1856)	H.marci	0,4688	
	Epoicocladius flavens	(Malloch, 1915)	E.flave	0,3993	i
	Parakiefferiella bathophila	(Kieffer, 1912)	P.batho	0,3472	i
	Parakiefferiella coronata	(Edwards, 1929)	Parakie	0,3819	i
	Parakiefferiella gracillima	(Kieffer, 1924)	P.graci	0,6076	i
CORYNONEURINI	*Corynoneura lacustris*	Edwards, 1924	C.lacus	0,4688	i
TANYTARSINI	*Stempellinella* sp.	Brundin, L., 1947	Stempel	0,5208	i
	Stempellinella minor	(Edwards, 1929)	S.minor	0,5035	i
	Stempellina bausei	(Kieffer, 1911)	S.bause	0,3993	i
	Constempellina brevicosta	(Edwards, 1937)	C.brevi	0,5556	i
	Tanytarsus bathophilus	Kieffer, 1911	T.batho	0,3472	i
	Tanytarsus brundini	Lindeberg, 1963	T.brund	0,3472	i
	Tanytarsus ejuncidus	(Walker, 1856)	T.ejunc	0,3472	i

Table A.6 continued

Tribe	Species	Author	Code	BQIW	T
	Tanytarsus gregarius	Kieffer, 1909	T.grega	0,3472	
	Tanytarsus recurvatus	Brundin, L., 1947	T.recur	0,6076	i
	Cladotanytarsus atridorsum	Kieffer, 1924	C.atrid	0,3299	
	Cladotanytarsus mancus	(Walker, 1856)	C.mancu	0,3299	i
	Paratanytarsus austriacus	(Kieffer In Albrecht, 1924)	P.austr	0,4861	
	Paratanytarsus bituberculatus	(Edwards, 1929)	P.bitub	0,3472	i
	Paratanytarsus laccophilus	(Edwards, 1929)	P.lacco	0,3299	i
	Micropsectra atrofasciata	(Kieffer, 1911)	M.atrof	0,2778	
	Micropsectra contracta	Reiss, F., 1965	M.contr	0,4688	t
	Pagastiella orophila	(Edwards, 1929)	P.oroph	0,5556	
PSEUDOCHIRONOMINI	*Pseudochironomus prasinatus*	(Stäger, 1839)	P.prasi	0,3819	
CHIRONOMINI	sp.		CHiRONO	0,3472	
	Chironomus anthracinus	Zetterstedt, 1860	C.anthr	0,2257	
	Chironomus lacunarius	Wülker, 1973	C.lacun	1000	i
	Chironomus plumosus	(Linnæus, 1758)	C.plumo	0,0694	t
	Cladopelma cladopelma	Kieffer, 1921	Cladope	0,1389	i
	Cladopelma lateralis	(Spies & Sæther, 2004)	C.later	0,3125	i
	Cladopelma viridulum	(Linnæus, 1767)	C.virid	0,3125	t
	Cryptochironomus defectus	(Kieffer, 1913)	C.defec	0,1389	
	Cryptotendipes pseudotener	(Goetghebuer, 1922)	C.pseud	0,5556	
	Demicryptochironomus vulneratus	(Zetterstedt, 1838)	D.vulne	0,2951	
	Dicrotendipes modestus	(Say, 1823)	D.modes	0,1389	
	Einfeldia pagana	(Meigen, 1838)	E.pagan	0,3125	
	Endochironomus tendens	(Fabricius, 1775)	E.tende	0,2604	i
	Glyptotendipes paripes	(Edwards, 1929)	G.parip	0,2604	i
	Microchironomus tener	(Kieffer, 1818)	M.tener	0,4340	t
	Microtendipes chloris	(Meigen, 1818)	M.chlor	0,2951	i
	Microtendipes pedellus	(De Geer, 1776)	M.pedel	0,2951	
	Paracladopelma camptolabis	(Kieffer, 1913)	P.campt	0,3646	
	Paracladopelma nigritulum	(Goetghebuer, 1942)	P.nigri	0,3646	i
	Paralauterborniella nigrohalteralis	(Malloch, 1915)	P.nigro	0,5556	
	Paratendipes albimanus	(Meigen, 1818)	P.albim	0,2951	
	Phaenopsectra flavipes	(Meigen, 1818)	P.flavi	0,2604	
	Polypedilum nubeculosum	(Meigen, 1804)	P.nubec	0,2951	
	Polypedilum(Tripodura) bicrenatum	Kieffer, 1921	P.T.bic	0,2951	
	Polypedilum(Tripodura) pullum	(Zetterstedt, 1838)	P.T.pul	0,2951	i
	Polypedilum(Tripodura) scalaenum	(Schrank, 1803)	P.T.sca	0,2951	i
	Sergentia sp.	Kieffer, 1922	Sergent	0,3819	
	Stictochironomus pictulus	(Meigen, 1830)	S.pictu	0,2083	

Results

Ecological data including 106 samples of macroinvertebrates belongs to 5 Italian and 5 Austrian lakes, the last used as reference sites. The Italian lakes were examined to establish pressure-impact relationship between macroinvertebrates metrics and eutrophication gradient.

TSI (Trophic Status Index) is defined as the mean of three variables (O_2% saturation, total phosphorous and transparency) rescaled between 0 and 1; 1 correspond to the highest value observed for O_2% saturation and transparency and the lowest TP value, 0 corresponds to the lowest values of O_2% saturation and transparency and the highest TP value.

The relationship between the macroinvertebrate metric (*BQIES* or EQR) and eutrophication measures *TSI* showed highly significant correlation, r = 0.4655, R-squared = 0.2167, $p<0.001$, with 104 d.f. The correlation was calculated considering all the 106 measures available. Considering the mean values for each lake it was obtained a correlation coefficient r=0.6619, with p<0.04, an R-square = 0.4382, an adjusted R-square = 0.3679, with 8 d.f., p<0.04.

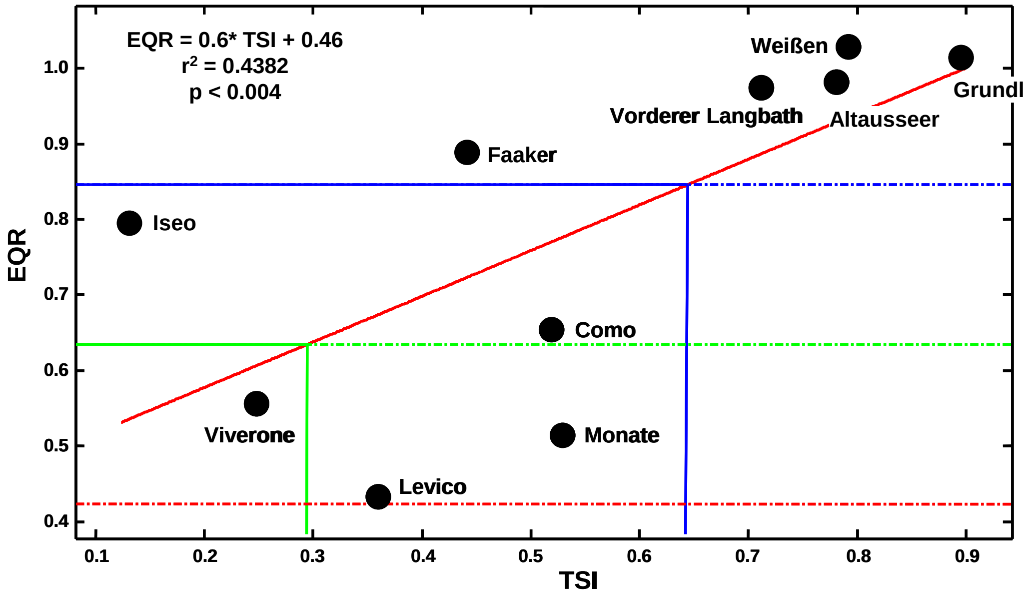

Figure A.3 Relation between TSI and EQR=BQIEJ/mref where mref is the median of reference sites. Lakes are represented by their mean values calculated on the sitedate dataset per each lake.

The most abundant species are given in Figure A.4.

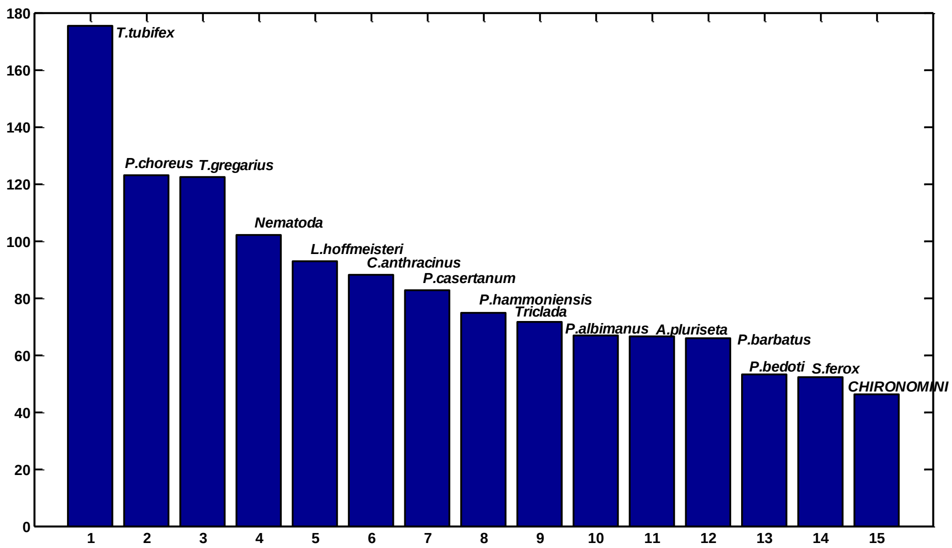

Figure A.4 Most represented specimens in the studied lakes.

The biological community at reference conditions lakes includes 152 taxa, 90 exclusive of reference sites (Table A.5 and Table A.6). In particular, each of the 5 reference lakes have ≥ 20 taxa (Figure A.5), above the number of taxa present in non reference lakes. Reference lakes have communities rich in different insects orders, whereas in non reference lakes Oligochaeta and Chironomids prevail (Table A.5 and Table A.6).

The reference lakes are characterized by the presence of a larger number of species including different orders of aquatic insects (Plecoptera, Ephemeroptera, Trichoptera, Diptera, Coleoptera). Among Chironomids the presence of species (*Parakiefferiella gracillima*, *Pagastiella orophila*, *Tanytarsus recurvatus*, *Orthocladius* spp.) indicators of optimum quality is observed.

In particular, 7 taxa are present in all reference lakes: Chironomidae (*S.bausei, P.P.psilopterus*, *P.austriacus*), Oligochaeta (*N.simplex*), Trichoptera (*M.azurea*), Ephemeropetra (*C.luteolum*), and Ceratopogonidae (*Bezzia* sp.) Eleven in four out of five: Chironomidae (*T.brundini, Parakiefferiella, P.bathophila, C.mancus, C.lacustris*), Trichoptera (Leptoceridae, *C.trimaculatus,* and *Athripsodes*), Hydrozoa (*Hydra* sp.), Oligochaeta (Enchytraeidae), Ceratopogonidae (*Dasyhelea* sp.). Nineteen in three out of five, 27 in two out of five, and 26 in one reference lake only.

In the lakes in good conditions many sensitive species disappear in comparison with reference sites, so that the community is represented by 6 exclusive taxa present in both lakes: Chironomidae (*C.plumosus*), Hirudinea (*D.lineata, H.stagnalis,*), Gammaridae (*E.stammeri*), Oligochaeta (*L.profundicola* and *P.bedoti*), and 8 taxa present in one lake only: Chironomidae (*C.pallidula, M.contracta*), Oligochaeta (*E.velutinus* and *P.vejdovskyi*), Hirudinea (*G.complanata, Limnatis* sp.), Gastropoda (*Lymnaea* sp.), and Nematoda Mermithidae. Finally, six taxa are in common with at least one lake in high conditions Chironomidae (*M.atrofasciata, P.nigrohalteralis*), Gastropoda (*V.piscinalis* and *G.albus*), Trichoptera (*Sericostoma* sp.), Chironomidae (*C.plumosus*), Hirudinea (*D.lineata, H.stagnalis,*), Gammaridae (*E.stammeri*), Oligochaeta (*N.barbata*). Some species with high sensitivity value as *Paralauterborniella nigrohalteralis* are present both in high and good quality lakes (Iseo). In L. Iseo *Micropsectra contracta* and *Embolocephalus velutinus* are easily found, they also indicators of good quality. The Oligochates *Sltylodrilus* heringianus is present in both high and good lakes, but not in moderate ones. Crustacea seem to characterize the good state lakes, whereas avoid the moderate ones.

The lakes in the moderate state have a poorer species composition, including Oligochaeta (Tubifex tubifex and Branchiura sowerbyi), Chironomids (Pseudochironomus prasinatus) and Chaoboridae (Chaoborus flavicans) above all. No one of the taxa present in lakes in moderate conditions are exclusive.

To sum up the communities present in the three classes include different characteristic species. More samples will allow a still better description of the differences and to establish boundaries of the poor and bad classes.

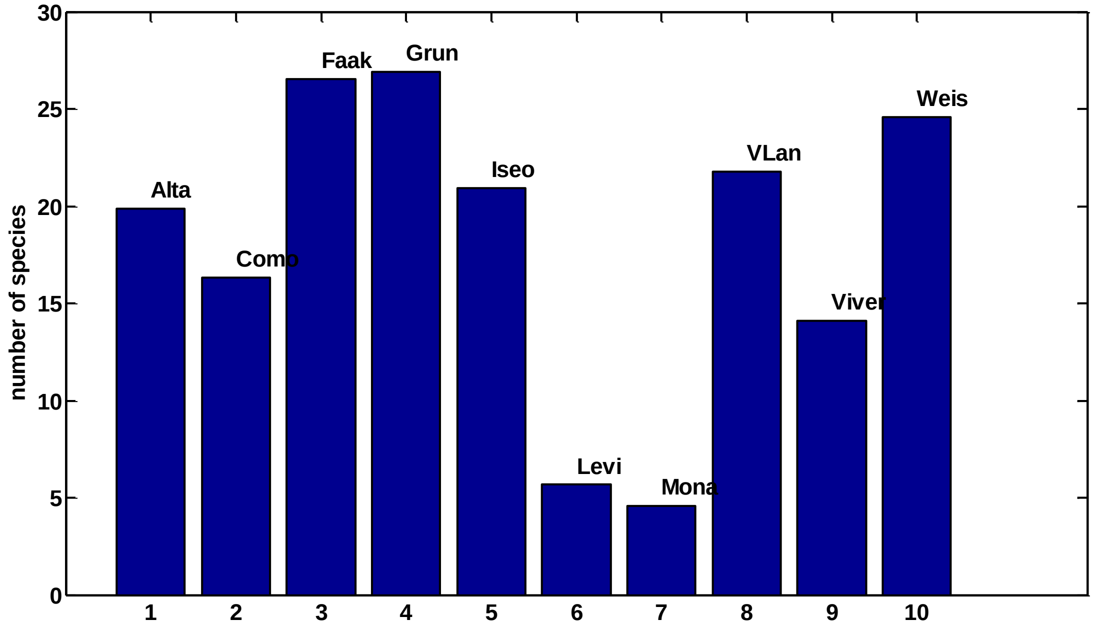

Figure A.5 Mean number of species in each lake.

Figure A.6 Plot of environmental variables in the plan of the first and third coinertia axes.

The conclusion is that benthic macroinvertebrates allow the separation of lakes according to morphometric and not only to trophic factors, but trophic factors are in any case relevant.

The *BQIES* values were transformed in EQR dividing them by the median of *BQIES* values measured in reference lakes. The median value of BQIES for reference lakes was 0.9458 (reference value), so the EQR values were obtained dividing BQIES by 0.9458 (Table A.7).

Table A.7 TSI values calculated by regression between EQR and TSI.

	BQIES	EQR
HG	0.8	0.846
GM	0.6	0.634
MP	0.4	0.423
PB	0.2	0.212

Assuming BQIES equal to 0.8, 0.6, 0.4, 0.2, as suggested by correspondence analysis and calculating EQR dividing BQIES by 0.9458, it was possible to recalculate the expected boundary between classes based on TSI on the basis of the regression line.

The correlation between correspondence analysis scores and *BQIES* (or EQR) values ($r=0.37$, $p<0.01$) allowed to derive the H/G boundary class (Table A.8). This limit was established considering biological data alone. All the reference lakes had and EQR>0.8 and all the non- reference had a value <0.8. The G/M limit was established to 0.6

considering the highly significant separation of Como and Iseo lakes by correspondence analysis, both having an EQR comprised between 0.6 and 0.8 (0.8>EQR>0.6).

Table A.8 TSI and EQR values for the monitored lakes. C: class assigned according to TSI or EQR values; L = boundary between two classes; n = number of samples; std = standard deviation; pw020 = power of classification assuming a cutoff separating 20 % of the area of the central t-student distribution (the power of classification in this case is the probability to assign a lake to a class, when it is effectively a member of this class).

Lake	TSI	C	EQR	C	L	n	std	pw020
Weißensee	0.790	h	1.033	h	0.846	7	0.07	100
Grundlsee	0.892	h	1.012	h	0.846	9	0.211	92.901
Altausseer See	0.784	h	0.979	h	0.846	9	0.107	99.761
Vorderer Langbathsee	0.708	h	0.978	h	0.846	9	0.115	99.405
Faaker See	0.451	m	0.891	h	0.846	9	0.248	38.075
Iseo	0.131	p	0.799	g	0.634	15	0.149	99.963
Como	0.521	m	0.65	g	0.634	12	0.166	30.423
Viverone	0.252	p	0.558	m	0.423	12	0.262	81.977
Monate	0.532	m	0.514	m	0.423	12	0.167	84.182
Levico	0.360	p	0.428	m	0.423	12	0.328	21.485

The M/P boundary was established at an EQR=0.4 having lakes Viverone, Monate and Levico an EQR<0.6, but greater than 0.4. No one of the investigated lakes had an EQR<0.4, so the PB limit could not be established. We should guess a 0.2 value.

The boundary class limits were refined dividing them (0.8, 0.6, 0.4, 0.2) by the median BQIES values of the reference sites, that is 0.9458.

A regression between *TSI* and EQR (Figure A.3) allowed to emphasize the relation between the chemical and biological classification. It is evident that four Italian lakes have an EQR lower than expected by *TSI* values (Monate and Levico, in particular), whereas Iseo has a benthic community in better condition than expected by trophic measures.

The uncertainty of classification was estimated calculating the power of classification (Figure A.7); the power of classification of each lake was obtained considering a non central *t* distribution of the EQR values; the non centrality parameter was estimated on the basis of the mean EQR calculated from each lake, the standard deviation of the mean and the number of samples (Winer, 1962; Carstensen, 2007). For example considering the Grundlsee, its mean EQR value was calculated from 9 sites and was 1.012, well above the HG boundary, which is equal to 0.8458; the EQR standard deviation is 0.153, the non centrality parameter is equal to 4.8019, giving a power of 92.9% to assigne Grundlsee to the high class.

Lakes Faaker, Como and Levico were classified with uncertainty, having a power <80 %

Conclusions

The *BQIES* index is a useful tool for the ecological assessment of the quality of natural lakes in mainland Italy. The index will in future be validated for all types of lakes.

The method will be improved as new information will be available with the prosecution of the monitoring program at regional scale, allowing a refinement of the sensitivity values listed in tables 2 and 3.

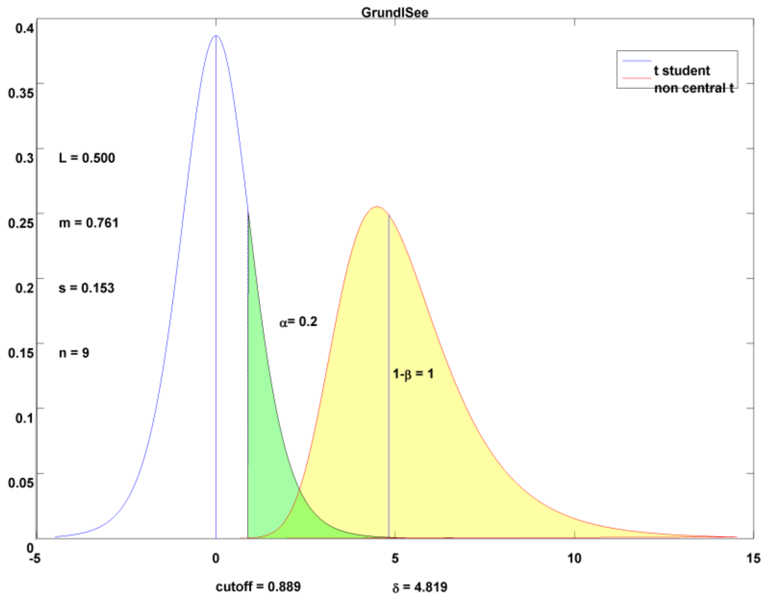

Figure A.7 Power of classification for Grundlsee: central (blue line) and non central (red line) t distribution of the EQR values.

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A.4 Slovenia: Lake littoral benthic invertebrate index (LBI)

Short description of the **Lake littoral benthic invertebrate index (LBI)** given below includes all crucial information for calculation of the LBI and classification of the sampling sites in ecological status classess according to the Water Framework Directive (Directive 2000/60/ES). Littoral benthic invertebrate data were obtained using the multi microhabitat-type sampling (microhabitat types were defined as combination of substrate and depth zones) with a hand-net to the depth of 1 m in two Alpine lakes (Bohinjsko jezero, Blejsko jezero).

Lake types

According to Slovenian national lake typology (Urbanič et al. 2007) two lake-types can be found in the Alps in Slovenia. Lake Bled is described as deep sub-Alpine lake, whereas Lake Bohinj as deep Alpine lake.

Assessment system

<u>Reference sites</u>

Criteria for selection of referene sites followed a national approach (Urbanič & Smolar-Žvanut 2005) where lake-specific and site-specific criteria are used addressing trophic status, pollution sources, lakeshore modifications and water use (Appendix 1).

Using all criteria sites within a lake Bohinj were recognised as reference (Table A.9).

Table A.9 Pressure data of slovenian alpine lakes with data of benthic invertebrates used in the intercalibration process.

Lake	TP (· g/L) ± SD	Chl_a amean (· g/L)±SD	Natural cat (%)	Agricultural cat (%)	Urban cat (%)	Reference lake
Lake Bohinj	4.7 ± 0.6	1.1 ± 0.1	97.1	2.6	0.3	Yes
Lake Bled	15.6 ± 2.6	5.0 ± 2.0	59.6	26.8	13.6	No

Legend: amean – annual mean, TP – total phosphorous, chl – chlorophil *a*, SD – standard deviation, cat – land use in a lake catchment

Benthic invertebrates were sampled at fourteen (14) reference sites. In addition to reference sites in lake Bohinj also partial reference sites in lake Bled were defined using a tiered approach and only criteria related to hydomorphological alterations (first morphological class of the sampling site – an area up to 100 m offshore is considered, the natural vegetation must be preserved, reference sites are not used for mass recreational purposes) were considered. This was possibile as LBI index primarily addresses an impact of lakeshore alterations and in lake Bled natural lakeshore can also be found. Benthic invertebrates were sampled at nine (9) partial reference sites in lake Bled.

<u>Pressure gradient</u>

A Lakeshore modification index (LMI) was used as a pressure gradient (Peterlin & Urbanič 2007). Final score of the LMI included alterations scores and lakeshore use scores in the littoral zone, shoreline zone, riparian zone and lakeshore region up to 100 m offshore. LMI was defined on the scale between 10 and 40. Sampling sites used in the analyses covered almost whole pressure gradient (see Fig. A8).

Metrics, index calculation and classification

LBI is a multimetric index consisting of three metrics;

a. Littoral fauna index (Urbanič et al. 2007) $LFI = \sum_{i=1}^{n} LIVi$

Where LIVi – Lakeshore modification indicative value of the i-th family (Table A.14);

b. Margalef diversity index MDI = (S-1)/ln N

Where S – number of taxa; N – number of individuals;

c. Number of taxa.

Metrics were calculated using Slovenian operational taxa (SOT) list with most taxa determined to the species and genus level, and some to the sub-family and family level (e.g. Tubificidae, Chironomidae-subfamilies). However, Littoral fauna index (LFI) is family based. Reference values of selected metrics were defined as median values calculated using reference sites (Table A.10).

Table A.10 Reference values and lower anchors of three metrics used in Littoral benthic invertebrate index (LBI).

Metric	Metric code	Reference value deep Alpine lake	Reference value - deep sub-Alpine	Lower anchor
Littoral fauna index	LFI	125	110	0
Margalef diversity	MDI	5,3	4,8	0
Number of taxa	S	37	37	0

LBI is calculated according to the equation:

$$LBI = \frac{2 * LFI + MDI + S}{4}$$

Boundary values between ecological status classes were defined based on the ratio of sensitive and tolerant taxa (Figure A.9, Table A.11).

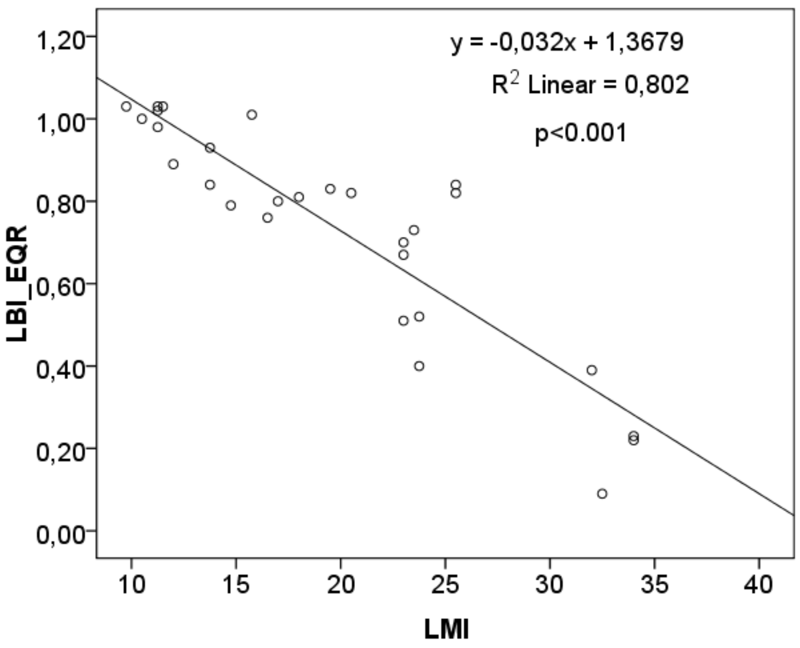

Figure A.8 *Littoral benthic invertebrate index (LBI_EQR) in rensponse to Lakeshore modification index (LMI).*

Table A.11 *Description of Ecological quality classes boundaries based on ratio between sensitive and tolerant taxa.*

Boundary	LBI	Boundary setting
High/Good	0,86	Sensitive ≈ Tolerant
Good	0,68	Sensitive < Tolerant
Moderate/Poo	0,41	Sensitive << Tolerant
Poor /Bad	0,20	Sensitive = 0

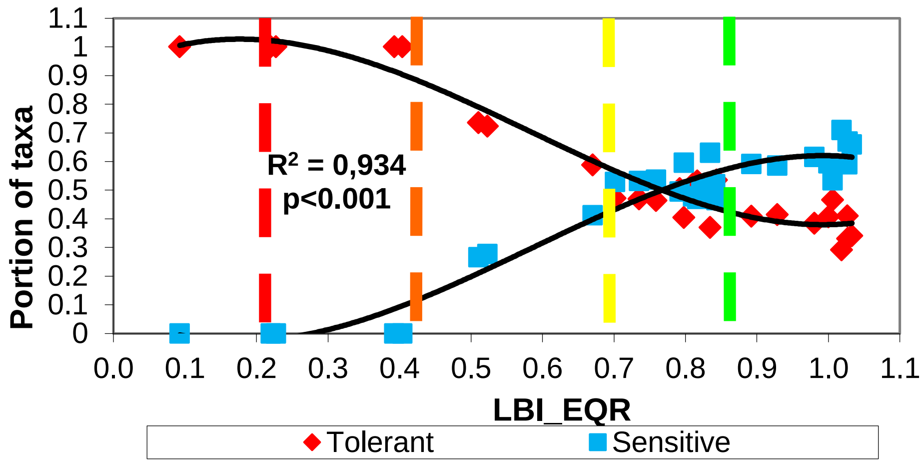

Figure A.9 *Boundary setting between ecological status classes using changes in portion of sensitive and tolerant taxa along the Littoral benthic invertebrate index (LBI_EQR).*

In order to compare EQR values with other Biological quality elements EQR values, all values were pieceweise lineary transformed and five equidistant classes were obtained (Table A.11 and Table A.12). Final classification of the lake is obtained calculating Lake-LBI value averaging six Site-LBI values calculated at six sites in the lake. All lake-LBI values >1 are set to 1.

Table A.12 Piecewise linear transformation equations for Lake littoral benthic invertebrate index (LBI).

Ecological status	LBI	Transformed LBI
High	>0.85	0.8+0.2*(LBI-0.85)/(0.15)
Good	0.68-0.85	0.6+0.2*(LBI -0.68)/(0.18)
Moderate	0.41-0.67	0.4+0.2*(LBI -0.41)/(0.17)
Poor	0.20-0.40	0.2+0.2*(LBI -0.20)/(0.21)
Bad	0.00-0.19	

Table A.13 Transformed boundary values between five ecological status classes using Lake littoral benthic invertebrate index (LBI_EQR_transformed).

Boundary	LBI_EQR_transformed
High/Good	0,8
Good	0,6
Moderate/Poo	0,4
Poor /Bad	0,2

Table A.14 Taxa list with family Lakeshore modification Indicative Value (LIV), used in the Littoral Fauna Index (LFI) calculation

Higher taxon	Family	LIV
Gastropoda	Ancylidae	9
Decapoda	Astacidae	9
Trichoptera	Goeridae	9
Trichoptera	Limnephilidae	9
Gastropoda	Bithyniidae	8
Plecoptera	Leuctridae	8
Heteroptera	Aphelocheiridae	8
Coleoptera	Gyrinidae-Ad.	8
Coleoptera	Haliplidae-Lv.	8
Coleoptera	Hydraenidae-Ad.	8
Coleoptera	Hydraenidae-Lv.	8
Turbellaria	Dendrocoelidae	7
Turbellaria	Dugesiidae	7

Higher taxon	Family	LIV
Turbellaria	Planariidae	7
Oligochaeta	Lumbricidae	7
Gastropoda	Hydrobiidae	7
Gastropoda	Physidae	7
Gastropoda	Planorbidae	7
Ephemeroptera	Ephemeridae	7
Plecoptera	Nemouridae	7
Odonata	Aeshnidae	7
Odonata	Coenagrionidae	7
Odonata	Corduliidae	7
Odonata	Platycnemididae	7
Heteroptera	Corixidae	7
Coleoptera	Dytiscidae-Ad.	7
Trichoptera	Sericostomatidae	7
Oligochaeta	Enchytraeidae	6
Gastropoda	Valvatidae	6
Isopoda	Asellidae	6
Ephemeroptera	Ephemerellidae	6
Megaloptera	Sialidae	6
Coleoptera	Dytiscidae-Lv.	6
Diptera	Tabanidae	6
Diptera	Tipulidae	6
Oligochaeta	Tubificidae	5
Hirudinea	Erpobdellidae	5
Ephemeroptera	Siphlonuridae	5
Odonata	Gomphidae	5
Coleoptera	Hydrophilidae-Ad.	5
Diptera	Chaoboridae	5
Arachnida	Hydrachnidia	4
Trichoptera	Polycentropodidae	4
Oligochaeta	Lumbriculidae	3
Oligochaeta	Naididae	3
Gastropoda	Lymnaeidae	3
Bivalvia	Sphaeriidae	3
Amphipoda	Gammaridae	3
Ephemeroptera	Caenidae	3
Coleoptera	Elmidae-Ad.	3
Trichoptera	Ecnomidae	3
Trichoptera	Leptoceridae	3

Higher taxon	Family	LIV
Diptera	Ceratopogonidae	3
Ephemeroptera	Baetidae	2
Ephemeroptera	Potamanthidae	2
Coleoptera	Elmidae-Lv.	2
Trichoptera	Hydroptilidae	2
Trichoptera	Psychomyiidae	2
Diptera	Psychodidae	2
Diptera	Chironomidae	1

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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 Benthic fauna IC group.

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