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Report on Harmonisation of freshwater biological methods

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CONTENTS

Background and purpose of the document

1. The Water Framework Directive (WFD) requires (Annex V 1.3.6) that standard~~s~~ methods are used for the monitoring of water quality elements: '*Methods used for the monitoring of type parameters shall conform to the international standards listed below or such other national or international standards which will ensure the provision of data of an equivalent scientific quality and comparability*'.

2. In the context of the WFD Common Implementation Strategy (CIS) Intercalibration Working Group (WG), a common issue raised by lake and river experts was the lack of comparability of national biological assessment systems due to different sampling and analytical procedures, and use of different metrics for the assessment of the degree of impact due to the same pressure. This resulted in a strong recommendation from these networks of experts for the harmonisation of these methodologies and the possible identification of common metrics.

3. Thus, in the workshop of the WFD-CIS Intercalibration WG, in March 2003 at the JRC, Ispra, it was decided to initiate a task on the review of the needs for harmonisation of the freshwater biological methods, under the WFD-CIS WG 2A ECOSTAT. The first contacts and presentation of this activity was at the meeting of the WG 2A in October 2003 at the JRC, Ispra.

4. The report from this task will support the Geographic Intercalibration Groups (GIGs) in the intercalibration exercise by providing a concise overview of the available methods to be considered as potential candidates for common metrics. This can be applied for the harmonised assessment of the water bodies belonging to the intercalibration network. Furthermore, it identifies the need for the development of new methods or the further harmonisation and standardisation of existing methods and proposes a procedure to link WFD relevant groups with Comité Européen de Normalisation (CEN) working group elaborating biological and ecological assessment methods.

5. The overall objectives of this task are 1) to make an overview and comparison of the national biological methods currently in use in EU countries; 2) to evaluate their applicability in the assessment of the ecological quality of inland surface waters (lakes and rivers) as required by the WFD; 3) to evaluate their suitability for use as common

metrics for the purpose of the intercalibration exercise, and 4) to identify a way of interaction amongst CEN, ECOSTAT WG2A and the WFD Committee allowing for prioritization of WFD relevant methods for standardization.

Sources of information

6. The following information was collected (Table 1) and analysed:

- Reports of the methods currently in use, and in development, in the Member States and Candidate Countries (requested from the ECOSTAT WG 2A);
- WFD-CIS Monitoring guidance fact-sheets on biological assessment methods;
- WFD-Intercalibration Metadata base (January 2004) containing information on the biological methods applied in the assessment of the intercalibration sites;
- STAR, WATERVIEW database of river methods.

Table 1. Information gathered on the biological national methods: sources of information and countries represented.

Source	Lakes	Rivers
ECOSTAT WG2A	14	25
WFD-CIS Monitoring WG	4	7
Intercalibration metadata (January 2004)*	21	27
STAR WATERVIEW Database	NA	All MS but Fin, CY and Malta, also countries in negotiation

NA Non applicable
* In Annex I is the distribution of countries by GIG for lakes and rivers.

7. Altogether, the different sources of data provided a good geographic coverage of the biological monitoring systems used in assessing the quality of lakes and rivers across Europe. However, some methods are poorly described invalidating the use of the information for a comprehensive comparison, which thus in some cases can only be done in general terms.

A. Lake

8. The information on the lake biological methods reported in the fact sheets prepared within the WFD-CIS Monitoring WG and information of the national methods sent by the ECOSTAT WG2A is summarized in Annex II. Altogether there is information from 14 countries, thus, information on the biological methods in use in Europe is incomplete.

9. Also, it is worth noting that the monitoring fact sheets were compiled during 2002 and therefore may soon not be representative of the methods available in the Member

States and candidate countries. Information from ECOSTAT WG 2A members clearly indicates that Member States and candidate countries are working on the development of WFD compatible biological methods.

10. To establish the register of sites for the intercalibration exercise, Member States and Candidate Countries were asked to fill in a metadata questionnaire along with the sites submitted for the exercise. The returned answers give important information in terms of compliance with the ecological assessment requirements of the WFD and comparability of the national methods.

11. In our evaluation, we have used data stored in the intercalibration metadata base of January 2004[1]. At this date, the database contained the data used for assessment of the ecological quality of 314 lakes submitted by 21 countries. However, the lakes were mostly assessed making use of physico-chemical paremeters (all countries, for most lakes) and phytoplankton (18 countries), followed by benthic invertebrates and Macrophytes (Fig. 1).

B. River

12. The overview of the river phytoplankton and phytobenthos monitoring systems includes the approaches of 18 countries: 4 methods for phytoplankton and 15 for phytobenthos. Only 4 countries (Estonia, Latvia, Romania and Hungary) use phytoplankton in their monitoring programs, and in one of these, Latvia, it is still under development. For phytobenthos, only a few countries have current monitoring programmes, i.e. Austria, France, Slovenia and Romania.

[1] At the time the Harmonisation Task started and until the end of 2004 the metadata of the final register was not available. This contains information for one more country and more lakes than the previous draft register. However, we considered that for the purpose of comparison of methods these are not so important changes and have decided to use the metadata of the intercalibration register as of January 2004.

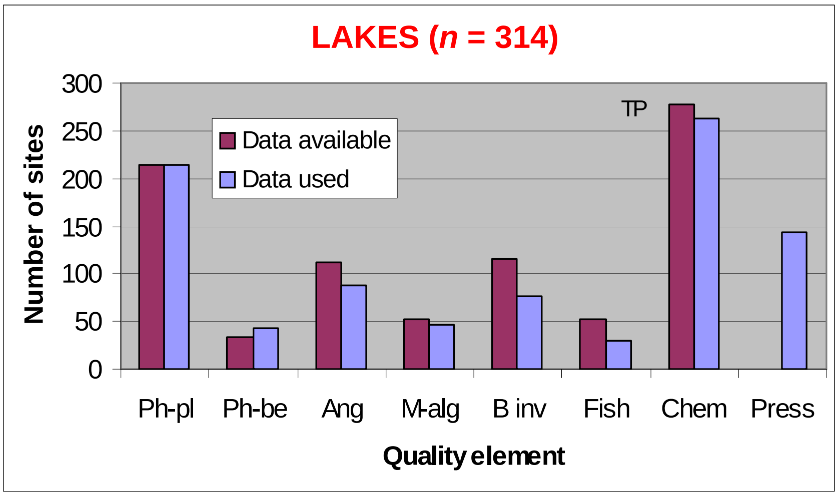

Figure 1. Elements (biological, chemical and pressure) used for ecological quality assessment of the lakes submitted to the intercalibration metadata (January 2004). Ph-pl= Phytoplankton, Ph-be= Phytobenthos, Ang= Angiosperms, M-alg= Macroalgae, Binv= Benthic invertebrates, Chem= Chemical Parameter (Total phosphorus), Press= Pressures. Note that angiosperms and macroalgae together form the group of the macrophytes.

13. The overview of biological monitoring systems using benthic invertebrates comprises 44 systems applied in 32 European countries. All methods discussed are listed in the Annex III comprising data on status and literature references. Detailed descriptions of most methods comprising the complete set of acquired data are available at http://starwp3.eu-star.at (Waterview Database).

14. The overview of the river macrophytes monitoring systems includes the approaches of 10 countries Austria, Denmark, Estonia, France, Germany, Latvia, The Netherlands, Norway, Sweden and UK.

15. The overview of fish monitoring systems focus on methods developed within the European research project FAME that is designed to provide direct support to the WFD. This Project, included the participation of twelve countries, Austria, Belgium, France, Greece, Germany, Latvia, Poland, Portugal, Spain, Sweden, The Netherlands and UK.

Overview and Comparison of the national methods

16. The comparison of the national biological monitoring systems is complete, wherever information is available, by considering three different steps in the monitoring process: sampling and laboratory processing, estimation of metric and

classification, each of these being the source of an independent variation to the final result of the assessment contributing the uncertainty of the final classification (Fig. 2).

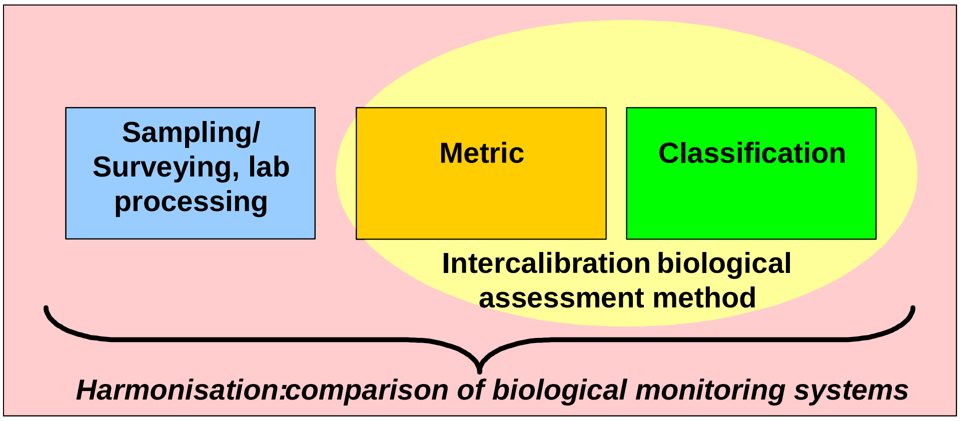

Figure 2. The three steps in the biological monitoring systems considered for comparison in the harmonisation task.

A. Lake

17. The comparison of the biological monitoring systems for lakes uses the intercalibration metadata and compares approaches in the GIGs (see composition of the lake GIGs in Annex I).

18. The intercalibration of lake biological assessment methods is confined to the effects of eutrophication and acidification, focusing on the quality elements considered most relevant for the selected pressures:

- Nutrient loading - Eutrophication:

  Phytoplankton (including. Chlorophyll-a): necessary for all lake types and widely used in Member States

- Acidification:

  Macroinvertebrates: necessary for all lake types, widely used in Member States

19. Thus, the information analysed may be biased towards methods respecting the above combinations of pressures and quality elements, even if the intercalibration metadata questionnaire gather information on the other biological elements.

20. In general terms, every country has different sampling procedures for each of the biological elements. Even within a country there may be different sampling procedures adopted for different lakes or lake areas monitored. Most sampling

procedures, for any of the biological elements, have some degree of standardization at a national level, and when international standards are available these are often followed. The biological material collected have generally a good taxonomic resolution with identification of many organisms to species. For the majority of the countries, the metrics estimated for the biological elements do not respect the WFD requirements. Type specific reference conditions, are only known for some benthic invertebrate metrics in two countries, i.e. Sweden and UK. Also, there are some preliminary reference conditions (mostly lake specific) for fish metrics also for Swedish lakes. The UK and Germany have developed multimetric indexes based on macroinvertebrates and aquatic plants in small lakes, for which reference conditions are derived from a wide range of environmental conditions and geology. It is as appears to have good applicability to the WFD. However, only 2 national assessment methods based on benthic invertebrates are considered to be WFD compatible (see Annex VII).

21. A more detailed comparison of the lake methods can be found in Annex IV.

BIOLOGICAL QUALITY ELEMENT: CHLOROPHYLL

22. In the GIGs the percentage of lakes for which chlorophyll *a* is measured varies between 40% (Eastern Continental, EC) and 100% (Mediterranean, ME), with an overall average of 82.5%. However, there is great heterogeneity both within GIG and between GIGs in terms of the sampling and analytical methods.

23. The most frequent sampling method in all the GIGs involves the collection of surface samples between 2 and 12 times per year (whole year (monthly) or concentrated during spring, summer, or vegetation periods) at only one station.

24. The extraction methods vary widely within GIGs, among GIGs and even within a single country. These can explain considerable variance in the measurement of chlorophyll *a* concentration.

25. Only 4 countries have informed to employ international standard methods for the determination of chlorophyll *a*, and 2 other countries make use of national standardized methods.

BIOLOGICAL QUALITY ELEMENT: PHYTOPLANKTON

26. Most countries include in their biological monitoring systems some metric for phytoplankton. The lowest number of lakes for which phytoplankton is monitored is in the NO GIG (54%), in all other GIGs at least 60% of the lakes are monitored for phytoplankton. However, there is great heterogeneity both within GIG and between GIGs in terms of the sampling and metrics estimated.

27. The most frequent sampling method in all the GIGs involves the collection of surface samples between 2 and 12 times per year (spring and summer) at one station.

28. The metrics used in the assessment differ both within and among GIGs, but most countries measure abundance and biomass and successively less countries record also bloom occurrence, size composition and primary productivity. There are no countries that completely fulfill the WFD metric requirements.

BIOLOGICAL QUALITY ELEMENT: PHYTOBENTHOS

29. Most countries do not use phytobenthos in their biological monitoring. The highest number of lakes monitored for phytobenthos is found in the EC GIG followed closely by CE and AL GIGs, while in the NO GIG countries this biological element is not used at all at intercalibration sites. Like for other elements, there is great heterogeneity both within GIG and between GIGs in terms of the sampling and metrics estimated.

30. In cases where phytobenthos is sampled, the most frequent sampling frequency is between 2 and 6 times per year, and the number of sampling stations in each lake vary in the GIGs from one lake station (all lakes in the AT and EC GIGs), 2-10 stations (most lakes in the BA and CE GIGs), or more than 10 stations (all lakes in the ME GIG and most in the AL GIG).

31. As with chlorophyll *a* and phytoplankton, sampling is held during the vegetation growth period, spring and summer. The habitats sampled differ, some countries sample epilithic and others epiphytic phytobenthos. The composition metrics are species composition, presence or absence of species and indicator taxa. The quantity metrics are the relative abundance of species, indicator taxa and excessive growth of nuisance species such as *Cladophora*. Most countries include in their assessment both

an estimate of phytobbenthos community composition and an estimate of their abundance.

BIOLOGICAL QUALITY ELEMENT: MACROPHYTES

32. The aquatic Macrophytes are widely used in the biological monitoring systems of the AT, BA and CE GIGs and less in all other GIGs.

33. Sampling occurs mostly between 2 and 6 times per year, during the summer or other vegetation period, in a variable number of sampling stations (1 to 20) in each lake. In the AL, AT, BA and ME GIGs all emergent, floating and submerged plants are sampled. In contrast in the CE and NO GIGs not all countries sample the three groups of plants.

34. Macrophytes communities' composition and abundance are recorded from visual inspections in the field from the shore. In cases when the whole lake is monitored this is done by boat. In most countries the plants are identified to species and abundance and/or diversity determined.

BIOLOGICAL QUALITY ELEMENT: BENTHIC INVERTEBRATES

35. The percentage of lakes for which benthic invertebrates are sampled within the GIGs is for part most below 40%, with exception of AT and CE.

36. Sampling frequency is variable between GIGs, the most common frequencies are 1 or 2-6 times per year, and occurs mostly in spring and summer but in the AL and NO GIGs it is performed all year round.

37. A wide number of sampling approaches and metrics are used. The collection of samples is performed by several different devices: grab, Eckman skip, sticking cylinder, triangle bottom scraper and hand net. The mesh size varies widely from 100µm to 670µm. When kick sampling is used time (1-3 minutes), mesh size (100-670µm) and habitat sampled are different. (littoral in general or stones only). Some countries sampled in lake littoral, other the profundal and some in both lake zones. Only 2 countries have informed to make use of international sampling standard methods. These standards are currently under revision.

38. Every country uses a different combination of metrics, the following metrics are either used alone or in combination: abundance and relative abundance, diversity

indicators, species lists, frequency of occurrence of individual taxa, number of taxa, group ratios, average score per taxa, biotic score, biotic integrity index, saprobic index, average score per taxon and ratio of littoral to profundal taxa.

BIOLOGICAL QUALITY ELEMENT: FISH

39. Most countries do not use fish in their biological monitoring and assessment programmes. The higher number of lakes sampled for fish is found in the BA, CE and NO GIGs.

40. The sampling frequency is variable generally 2-12 times per year with summer and autumn as the most common sampling seasons.

41. A total of 13 countries are sampling fishes in lakes using a total of 7 different approaches, either alone or in combination, for gathering information: net fishing (gillnets and trammel net), electrofishing, hydroacoustics, catch statistics, information from anglers and historic data. The majority of the countries determine species composition, some the native species and or functional group ratios. Fish abundance is determined as total biomass, relative biomass (CPUE), either for the whole fish community per species, and as density. The age structure or size structure is determined in only two countries.

B. River

42. A more detailed comparison of river methods can be found in Annex V.

BIOLOGICAL QUALITY ELEMENT: PHYTOPLANKTON

43. The overview of the river phytoplankton monitoring systems includes the approaches of 4 national methods. There is great heterogeneity in terms of the sampling and metrics estimated. Sampling frequency vary from weekly at some sites to every five years.

44. The level of taxonomic resolution used in all the assessment methodologies based on river phytoplankton is the species or genus. Absolute or relative abundance is determined in all 4 methods, while biomass is determined by two countries.

45. Three different categories of assessment methods were identified in this review: biotic indices, assemblage/community assessment and multimetric indices.

RIVER BIOLOGICAL QUALITY ELEMENT: PHYTOBENTHOS

46. The overview of the river phytobenthos monitoring systems includes the approaches of 15 countries. The phytobenthos community includes diatom and non-diatom groups. However, most of the developed methods, especially in monitoring programs are based only on the diatom group. Only one country was found to have a monitoring programme covering both diatom and non-diatom groups, while another has a new assessment method under development that includes both groups.

47. The sampling procedure is based in the European Norms EN 13946 (2003) Water quality: *Guidance standard for the routine sampling and pretreatment of benthic diatoms for rivers for water quality assessment* and/or in national methodological guidelines. Some countries, for example Germany and the United Kingdom use the recommendations of Kelly *et al.* (1998).

48. Both documents only concern diatoms, but a working document for a proposed new European Standard is in preparation which includes algal groups other than diatoms (cyanobacteria, green algae, red algae, etc.).

49. A recent draft summarising the methods using benthic algae to assess water quality in running water concluded that the main processes in use for routine sampling are similar, only details that depend on current velocity and dominating type of available substratum may vary (CEN TC230 N68).

50. The available information shows that scraping with a brush from the natural substrates (stones) is the most common method. Quantitative sampling is performed in some cases, using a fixed surface area for sampling that varies between 9cm^2 and 100cm^2. Artificial subtracts are only used by 2 countries.

51. Concerning the sampling habitat, 6 methodologies are multihabitat, 6 are single habitat related with hard subtracts (cobbles or stones) and 3 did not answer. These different procedures do not allow comparability between the results as they reflect different ecological situations.

52. The sampling frequency for phytobenthos monitoring varies from annually to every 3 or 4 years. The level of taxonomic resolution used is the species or species groups. Only one country uses higher taxonomic levels, which varies from species to family or higher.

53. The identification and enumeration of relative proportions of diatom *taxa* is based on a European Standard EN 14407 (2004) Water quality: *Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running water*. Some countries use the OMNIDIA software for taxonomic identification (Lecointe *et al.*, 1993), which allows the calculation of a great number of diatom indices and contains a systematic and an ecological database. Consultation of other literature is also recommended for the algal identification, like floras, identification guides and iconographs appropriated to the habitats and geographic region under consideration.

54. The recording of abundance is usually expressed as the number of cells per *taxon* per sample on occasions using abundance classes. Relative coverage of the river bed is also mentioned by one country.

55. Three different categories of assessment methods were identified in this review: biotic indices, assemblage/community assessment and multimetric indices.Biotic indices are the most common assessment method including 5 different approaches all based in diatom assemblages, applied to 9 countries. Only one country is using assemblage/community assessment (Austria) and two are using multimetric indices Estonia and Germany). The Biological Diatom Index' (IBD- Indice Biologique Diatomées; AFNOR, 2000) is the only standardized method applied on a national level.

RIVER BIOLOGICAL QUALITY ELEMENT: MACROPHYTES

56. Macrophytes are monitored in ten countries of the European Union also including Norway. All methods comprise the investigation of watercourse vegetation by means of regular visual from the banks or by wading in the stream to record the taxonomic composition and abundance of water plants. Assessment of the ecological quality of watercourses is done by five different schemes. Recently 3 countries have developed macrophyte methods to meet the requirements of the WFD.

57. Sampling area differs between individual methods: separate transects of 0.25 x 0.25 m^2 are sampled, survey an area of at least 100 m^2, other schemes prescribe the investigation of stream reaches of 100 m or 500 m. Only two countries follow the standard EN 14184:2003 Water quality: *Guidance standard for the surveying of aquatic macrophytes in running waters*.

58. Most commonly macrophytes are identified to species level, which is usually done in the field. Uncertain identifications are verified in the laboratory by referring to herbarium species. Abundance is recorded in most countries as the relative plant coverage or specifies the ‘plant mass estimate’ accounting for the three-dimensional extension of the plant stand. In both options abundance is expressed in classes, but for most methods the number of classes and the defined ranges deviate.

59. The numerical evaluation of macrophyte composition and abundance is based on a single biotic index integrating indicator species and their abundance, and is included in assessment methods of 5 countries.

60. A multimetric assessment based on macrophytes is done by 2 countries. One assessessment method is dependent on the stream type, is based on a separately evaluation of mosses and phanerogams to which further metrics (e.g. evenness, percentage of *Sparganium emersum* etc.) can be added to the results of the main index. Besides the assessment of general degradation, the method comprises modules for the detection of acidification in base-poor mountain streams. The other method considers the percent coverage of plant growth forms and the abundance of stream type-specific indicator species to assess general stream degradation. In both methods the overall quality of the watercourse is derived on the basis of the analysis of the entire aquatic flora including phytobenthos.

61. The number of quality classes ranges from four to seven.

RIVER BIOLOGICAL QUALITY ELEMENT: BENTHIC INVERTEBRATES

62. The biodegradable organic pollution represents the main focus of the biological assessment in European rivers.

63. The sampling procedure of a large number of methods applied in monitoring programmes using benthic invertebrates is based on the International Standard ISO 7828 (1985) or the adopted European Norm EN 27828 (1994) Water quality. Methods of biological sampling: *Guidance on handnet sampling of aquatic benthic macro-invertebrates*. Several methods which do not directly referring to these international standards carry out ‘kick and sweep’ sampling that is regulated by national norms/ methods.

64. In general, this technique is the most common sampling procedure and applied in 30 methods using a hand-net. The nets used differ in size of the opening and mesh. Net-openings specified by the assessment methods vary between approximately 600 and 900 square centimetres. In half of the schemes, animals are retained by mesh-sizes of about 500 µm.

65. The procedure of quantitative sampling is standardised by the guidance ISO 8265 (1988) or EN 28265 (1994) Methods of biological sampling: *Guidance on the design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters*. Surber samplers are most commonly used for quantitative, area-related sampling. The recommendations of this standard for maximum aperture size of the net range between 250 to 750 µm. The models currently applied in various national watercourse monitoring programmes differ in sampled area (0.01 to 0.12 m^2) and mesh-size (100 to 500 µm).

66. In deeper streams benthic macroinvertebrates are taken using of grabs, dredges and artificial substrates. The application of these devices is standardised in ISO 9391 (1993) or EN ISO 9391 (1995). These are the same standards as the previous ones. The difference in dates is caused by the different dates of publication by the two organisations.

67. ISO and CEN standards for sampling invertebrates are currently under revision to make them more WFD compliant, but the timescale for publication will be several years.

68. Sampling varies from seasonal collections to procedures conducted every five years. Annual sampling is the most common interval applied in river monitoring. In some monitoring programs observing the saprobiological water quality sampling is seasonal. This inevitably influences the degree of uncertainty of the resulting ecological classifications, i.e. the likelihood of the banding allocated

69. Nearly 60 percent of the methods applied determine at least selected orders of benthic invertebrates to species- or species groups-level. The remaining approximately 40 percent of methods, identify organisms to genus or family. It must be noted that in several assessment methods taxa are identified to lower levels than required to adequately compute the respective quality index.

70. Nearly 50 percent of macroinvertebrate methods record the abundance in number of individuals per area. In fact, purely quantitative data require area-related sampling procedures by means of quadrate samplers, grabs or similar devices. Since these requirements are only met by a few schemes, abundance statements based on semi-quantitative hand-net sampling are in most cases of restricted reliability but they are cheap, practical and effective.

71. There are different abundance classification schemes in use, the most common are: i) 3-class scheme, derived from Pantle and Buck (1955) applying the Saprobic Index; ii) 5-class scheme, there two systems in Europe: one based on a logarithmic scale of organisms' abundance (Murray-Bligh, 1999) and the *Quality Rating System*; iii) 7-classes scheme, established by Knöpp (1955) this classification has been included in the German standard DIN 38 410 (1990, 2003): *Determination of Saprobic Index of Running Waters*.

72. Assessment methods operating on the basis of presence/absence data of macroinvertebrate taxa do not necessarily need to record taxa abundance. Many biotic indices like *I.B.G.N.*, *IBE*, *BMWP-ASPT* or *BBI* are not designed to include abundance information. Their outputs may be biased by single organisms drifting into the sample from upstream reaches. Therefore, the individual systems prescribe to include only taxa that exceed a certain threshold of abundance to avoid false results.

73. Seven different categories of assessment methods can be distinguished among the methods currently used according to the type and scope of measured parameters ("metrics") (Knoben *et al.*, 1995; Verdonschot, 2000):

- Saprobic Indices (represent specific modes of biotic scores)
- Diversity Indices
- Predictive Assessment
- Process Assessment
- Rapid Bioassessment
- Multimetric Assessment
- Ecosystem Components Assessment

RIVER BIOLOGICAL QUALITY ELEMENT: FISH

74. In November 2004 the European –FAME (Fish-based assessment method for the ecological status) project was completed– This was aimed at developing, evaluating and implementing a new fish based assessment method for the ecological status of European rivers in direct support to the WFD. This project, included the participation of twelve European countries, and has delivered recommendations concerning fish sampling and data interpretation.The results of the project are being considered for CEN standardization and has potential to be included in the future WFD monitoring plans.

75. Currently, different fish based methods are used throughout Europe to assess the ecological status of rivers.

76. A short description of the methods currently in use and those developed within the FAME project is given in Annex V.

77. In Europe, fish sampling methods differ greatly between countries, and even between regions or states belonging to the same country. As a corollary, national sampling methods rarely exist. However, in some European countries, fish monitoring programmes have been designed to assess the ecological quality of rivers based on fish assemblages. These monitoring programmes have, to some extent, led to the standardisation of sampling procedures, at least at the regional level. At the national or international levels, the development of fish based methods of river quality assessment are limited by the diversity of fish sampling procedures, and consequently the fish databases were restricted to sampling sites for which similar fish sampling methods were applied.

78. The majority of the procedures and devices described in the WFD intercalibration metadata base consist of electric fishing and occasionally hand nets.

ISO and CEN Methods

79. In Annex VI is described the CEN standardisation process and are listed the published standards of relevance in relation to the WFD.

A. International Organisation for Standardization (ISO) and Comité Européen de Normalisation (CEN): General background

80. The standardization of biological methods is undertaken in ISO Technical Committee (TC) 147 subcommittee (SC) 5 – Biological methods and CEN/TC 230 working group (WG) 2 – Biological and Ecological Assessment Methods. These committees and their parent bodies work closely together to ensure that there is no overlap in their respective work programmes.

81. The work programme of ISO/TC 147/SC 5 is primarily to develop toxicity and biodegradability methods including the supporting statistical methodologies and hence are not relevant to the intercalibration exercise and will not be considered in detail here.

82. The development of biological and ecological assessment methods is now solely within CEN/TC 230/WG 2, which is comprised of 6 task groups (TGs) and an *ad hoc* strategy group (Fig. 3).

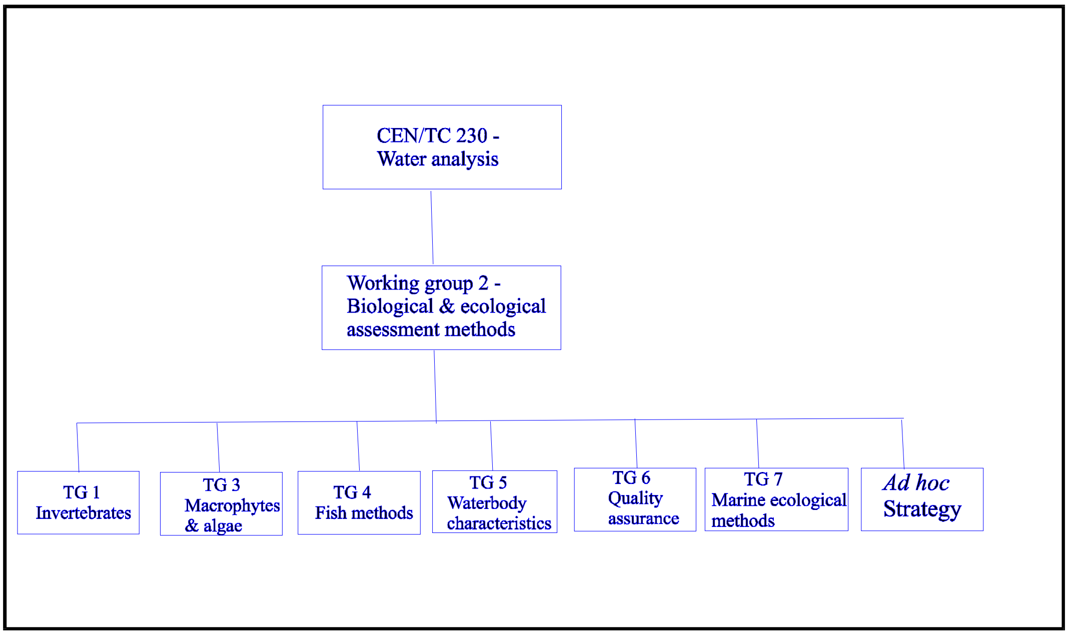

Figure 3. Structure of WG 2 biological and ecological assessment methods within the context of the technical committee, CEN/TC 230.

83. The work programme of WG 2 is in support of all legislation where there is an inherent or specific requirement under existing EU legislation for biological and ecological assessment methods. However, it is obvious from the titles of the Task groups that WFD has a large influence on the standards being developed.

84. The development of specific standards is very much the domain of the individual task groups. WG 2 manages the work programme of its task groups advised by its *ad hoc* strategy group in liaisons with expert groups, DG Environment, Joint Research Centre, ECOSTAT (**Eco**logical **Sta**tus) etc, in prioritising future work items for the task groups.

B. Current work programme

85. The CEN/TC 230/WG 2 work programme will change over time as standards are completed and as new work items are initiated. Table 2 provides a summary of active formal work items up to July 2005.

Table 2. CEN/TC 230/WG 2 formal work programme (July 2005).

CEN Reference	Work item	Comment
CEN230217 prENXXX	Water Quality – Guidance standard for the surveying of macrophytes in lakes	NWIP approved
CEN 230175	Water Quality – Guidance standard on the routine sampling of benthic algae in fast flowing, shallow waters to include laboratory procedures	Original WI deleted but NWIP will be requested to include expanded scope
CEN 230171 PrEN14962	Water Quality – Guidance on the scope and selection of fish sampling methods.	
CEN 230169 prEN/ISO16665	Water Quality – Guidelines for quantitative investigations of marine soft-bottom benthic fauna in the marine environment	ISO lead but proposed for parallel adoption
CEN 230216 prEN/ISO 19493	Water Quality – Guidance on marine biological surveys of littoral and sublittoral hard bottom	CEN lead
CEN 230207 prEN15204	Water Quality – Guidance standard for routine analysis of phytoplankton abundance and composition using inverted microscopy (Utermöhl technique)	
CEN230209 prEN 14996	Water Quality – Guidance on assuring the quality of biological and ecological assessments in the aquatic environment	
CEN 230208 prEN15110	Water Quality – Guidance standard for the routine sampling of zooplankton from standing waters	
CEN230171 prEN14962	Water Quality – Guidance on the scope and selection of fish sampling methods	
CEN 230213 prEN/ISO15196	Water Quality – Guidance on the sampling and processing of the pupal exuviae of Chironomidae (Order Diptera) for ecological assessment	Include in work programme in June 2004
WI 230165	Water quality – Guidance on data collation, interpretation and classification of running waters based on aquatic macrophytes	
CEN230172 prEN 14757	Water quality – Sampling of fish with multi-mesh gillnets	
WIXX N72 E	Water quality – Guidance standard for surveying of benthic macro-invertebrates in lentic waters	
CEN230118 prEN 14614	Water quality – Guidance standard for assessing the hydromorphological features of rivers	
prEN 14393	Water quality – Guidance on quality assurance aspects of aquatic macrophytes surveying and analysis in running waters	
prEN 8692	Water quality – Fresh water algal growth inhibition test	

	with unicellular green algae (ISO/DIS 8692)	
CEN230211 prEN/ISO 20079	Water quality – Determination of the toxic effects of water constituents and waste water to duckweed (*Lemna minor*) – Duckweed growth inhibition test	UAP-vote, ISO/CEN
CEN230210 prEN/ISO 16712	Water quality – Determination of acute toxicity of marine or estuarine sediments to amphipods	UAP-vote, ISO/CEN
CEN230XXX prENXXX	Water quality - Guidance on *pro-rata* multi-habitat-sampling of benthic invertebrates from wadeable rivers	
CEN230XXX prENXXX	Water quality – Guidance standard on the design of multimetric indices	

86. In WFD terms the current programme contributes to but does not completely meet the more immediate needs for intercalibration or subsequent monitoring. This reflects the lack of resources available to WG 2 in supporting the relevant specialist workshops, encouraging experts to devote time to standardization especially those from the new EU countries, pre- and co-normative research, translation services *etc*.

C. Proposals for the formulation of future work programme items in WG 2

87. Under advice from the expert (TG) groups and the *ad hoc* group WG 2 has developed a strategy document designed to prioritise future work programme activities, which has been widely distributed. ECOSTAT is invited to confirm whether or not this prioritisation meets there needs and timescales.

88. The work programme changes in response to the perceived needs of the EU and is constrained by the absence of financial support and the identification of methodologies that are suitable for standardization. In this respect the EU sponsored R&D has a critical role in assisting the standardization process. For example, in the May 2004 meeting the EU STAR (**Sta**ndardization of **R**iver Classification) project submitted two proposals for standardization in relation to multihabitat sampling of invertebrates in wadeable streams and the selection of multimetrics. Both were likely to be proposed as new work items in CEN and will shortly be added to the WG2A workprogramme. Similarly the EU FAME (**F**ish **A**ssessment **M**ethod for **E**urope) project has developed a European classification system based on fish and WG 2 is liaising with the project leaders on the possibility of standardizing this work and a nominated FAME expert will attend the next CEN/ TC 230 meeting.

89. However, numerous other working documents are under consideration including such diverse items as laboratory intercalibration for ecological assessment, hydroacoustics for non-destructive fishery assessment, sampling of marine algae, best

practice guides for identification keys, lake hydromorphological assessment, the development of a scoring system for assessing the quality of physical features in rivers and the quantification and use of performance characteristics in ecological assessment methods, amongst many others that are, at the current time, given lower priority.

D. Current contribution to WFD intercalibration

90. All of the recently published standards contribute to the intercalibration process, where the GIGs choose to use the same method of ecological assessment.

91. Additionally, the advanced drafts available for several biological and ecological assessment methods could also support the process, particularly where these are pending formal vote for adoption as EU standards. In formulating their intercalibration strategies, the GIGs and ECOSTAT should consider not only the use of these standards for future data collection, but also advise WG 2 of priority gaps that need to be addressed in method standardization.

E. Future liaison and involvement with ECOSTAT WG 2A

92. CEN/TC 230/WG 2 states in its mission statement that "it is committed to the delivery of scientifically robust methods for biological and ecological assessment and classification in support of European Union legislation and sound science".

93. The priority objectives of WG 2 are hence to:

(i) examine the present state of standardization of biological and ecological assessment methods;

(ii) advise CEN/TC 230, the Commission, its expert groups and its delegated agencies of progress in, and barriers to, the standardization of biological and ecological assessment methods;

(iii) determine, evaluate and advise CEN and others of gaps in the available research that need to be addressed prior to normalization;

(iv) identify needs in the knowledge-base supporting European standardization and identify and promote appropriate project proposals;

(v) provide standards in a timely manner for EU needs and CEN priorities.

94. Within this context there is considerable scope for ECOSTAT working group 2A to identify future ecological standardization priorities specifically in mandated areas such as the EU Eutrophication strategy.

95. ECOSTAT supports the better resourcing of the standardization work in CEN in order to deliver the outputs required to meet their specific needs and against timescales they foresee as being timely to the delivery of WFD.

96. Following a meeting with DG Environment preliminary discussions have taken place in relation to the mandating of specific standardisation projects. ECOSTAT is invited to contribute to this debate. Ultimately the standards produced should be referred to the Article 21 committee for inclusion in the WFD.

Further information CEN can be obtained through the WEB site www.cenorm.be/boss or through the Secretariat of Working Group 2 at peter.hale @doeni.gov.uk

Evaluation of the usefulness of existing methods in relation to the WFD

97. In Annex VII is summarised the WFD ecological quality classification requirements, in particular for lakes and rivers.

A. Lakes

98. The intercalibration metadata questionnaire directly asked whether the site selected was classified in compliance with the WFD, and most countries, 65% of those that submitted lakes, stated that the assessment was based on a non-WFD-compatible classification system. The countries that considered having classification methods that were at least partially WFD compatible (e.g. not implemented nation wide) are Estonia, Germany, The Netherlands, Spain, Sweden and UK.

99. However, the judgments of compatibility with the ecological classification requirements of the WFD were subjective and generally based on the national interpretations of the Directive; the numbers of non-compatible classifications would probably increase, if there was an agreement on the minimum requirements for a WFD compatible classification.

100. The information received from the national representatives in the ECOSTAT WG2A on the state of compatibility of their national classification methods with the WFD requirements can be summarized as follows (see also table in Annex VIII):

- *aquatic flora*, Lithuania and Sweden are in the process of developing WFD compatible classifications;
- *benthic invertebrates*, UK method is declared to be WFD compatible;
- *phytoplankton*, Austria and Sweden have developed WFD compatible classifications but only for some lake types, while Latvia has now estimated preliminary reference conditions for lakes in the country;
- *Fish*, UK, as well as other countries, is on the way of developing a WFD compatible classification method;
- *Macrophytes and phytobenthos*, Germany has developed a classification method with these two elements that is considered WFD compatible;
- Aquatic plants and benthic invertebrates, UK has developed a classification method with these two elements that is considered WFD compatible.

B. Rivers

101. In terms of metric requirements by the WFD, these are met for *phytobenthos* as all countries include in their methods a measurement of taxonomic composition and abundance. The overview shows, that at the moment, some countries are modifying their own assessment methods for phytobenthos in order to fill the gaps (Austria, Germany and Spain) or have new methods under development that will include these requirements (Portugal). Norway and the Netherlands have assessment methods that respect the parameters to be measured for the WFD, but these are not related with reference conditions.

102. This is not the case for *phytoplankton*, methods include taxonomic composition and abundance, but no reference to recording of the planktonic blooms that is required by the Directive for this biological element. For phytoplankton, none of the assessment methods are WFD compatible.

103. For *macrophytes* only a few methods presented are WFD compliant. This is because the methods developed prior to the implementation of the WFD are generally lacking in the definition of stream type-specific reference conditions. This does not hold for schemes specifically designed for WFD monitoring purposes (Austria, Germany, The Netherlands). Sweden and UK intend to implement a predictive habitat approach (a RIVPACS-type system for aquatic plants) in the near future to model macrophyte reference communities.

104. For *benthic invertebrates*, only a small number of methods included in this overview fulfill the WFD classification demands at least partially. In particular, recently developed methods of the categories predictive and multimetric assessment methods incude stream type-specific evaluation based on reference conditions. Some countries have modified their traditional methods (e.g. Saprobic System) and/ or combined them with new assessment methods (e.g. for morphological degradation) to meet the requirements of the WFD (Rolauffs *et al.*, 2004). Furthermore, it is planned to adjust the site-specific reference of the UK RIVPACS system by consideration of stream morphology (Nixon *et al.*, 1997).

105. Integrated assessment of all indicative parameters of macrozoobenthos communities is done by multimetric indices. Besides the overall appraisal of ecological quality, their modular structure enables indication of the cause of degradation by providing individual metric values. Full ecological evaluation accounting for several biota is carried out by the ecosystem component’s assessment systems described above.

106. More than 50 percent of the methods present results in five classes of quality. Some of these banding schemes represent recent amendments with respect to the demands of the WFD. In this context it has to be noted that the numbering of quality classes is performed conversely. Some schemes label the highest class with ‘1’ and increment the number according to increasing deterioration. Other classifications denote high quality levels with ‘5’ and count down. This anomaly has to be considered and resolved in connection with the exchange and comparison of quality results derived by different methods.

107. Another 12 percent of the methods use seven classes of water quality originating from the classification of saprobity introduced by Liebmann (1962).

Evaluation of the suitability of current metrics as 'common metrics'

108. In Annex IX is summarised the common understanding of the WFD intercalibration process and the significance of common metric in this context.

A. Lakes

109. On the basis of the overview above of the assessment methods we conclude that it is currently not possible to identify a common metric satisfying the requirements for an intercalibration common metric. Independent of the biological element measured, sampling and assessment methods (metrics and classification are not shared by the countries in a GIG. Also, there are few methods that are in compliance with the WFD. This information would have to be collated in order to possible identify Intercalibration common metrics.

B. Rivers

110. The situation that applies to lakes also holds for the river phytoplankton, phytobenthos and macrophytes. At the time the information for this report was gathered Member States were developing WFD compatible methods and thus with the current information it is not possible to identify common metrics for this elements.

111. The overview above of the benthic invertebrate assessment methods can show which metrics are most commonly used to evaluate the quality of running waters in Europe. These metrics are likely to meet the above specified requirements since equal premises have to be fulfilled in different countries (e.g. level of determination, record of abundance etc.) to calculate the metric results.

112. In 15 countries saprobic indices for water quality classification are in usage. Despite of many country-specific modifications the efforts made towards harmonised application of the saprobic system in the Danube River Basin (Knoben *et al.*, 1999; Sommerhäuser *et al.*, 2004) are promising. Cyprus, Estonia, Hungary, Poland, Portugal, Sweden, Spain and United Kingdom use the BMWP score or BMWP-ASPT Index in water quality assessment. The indicator list of this metric operating at family-level has been modified by Hungary, Poland, Portugal and Spain. Based on the original table BMWP scores are part of multimetric indices of the AQEM systems in the Czech Republic, Germany, Greece, Italy and Sweden.

113. Further analysis of the metrics or metric groups integrated in the various AQEM multimetric indices reveals common use of metrics listed in Table 3.

Table 3. Metrics/metric groups most commonly used by the different AQEM systems (AQEM Consortium, 2002).

metric/metric groups	**number of stream types where metric/metric group is applied**
feeding types (*scrapers*, *shredders*, *predators*, …)	11
zonation preference (*crenal*, *rhithral*, *potamal*, …)	10
number of Ephemeroptera, Plecoptera, Trichoptera taxa	10
individuals of certain taxonomic groups	10
number of taxa in individual taxonomic groups	6
microhabitat preference (*pelal*, *argyllal*, *psammal*, *akal*, …)	6
saprobic index (Zelinka and Marvan)	6

114. About Fish metrics, an extensive list of metrics from IBI index, was presented to be tested on the FAME project. (Kestemont, P. and Goffaux, D., 2002). The metrics are not distributed among the four categories of the original IBI, but classified within the 3 major categories of the WFD, *i.e.* species composition (including metrics related to trophic composition, reproduction and condition), fish abundance and age-length structure.

Concluding remarks and recommendations

115. This review of the harmonization of biological monitoring systems is based on data gathered and collated over the drafting period. Many of the outcomes reported continue to be valid but others have been and will continue to be influenced by scientific developments supporting the implementation of the WFD. These include the outcomes of national and European research programmes, the finalisation of relevant CEN standards and the trialing of recently developed or modified ecological assessment methodologies at the national level. Further, as the intercalibration process progresses within the GIGs, new approaches and the possible need for method standardization will be identified.

116. In order to ensure that these rapid developments are taken into account, which will aid the WFD implementation process it is recommended that the harmonization

group should update its primary findings on an annual basis. The outcome would inform ECOSTAT, the Commission and other partners as to the state-of-the-art, as well as demonstrating progress and identifying gaps that need to be filled by targeted research at a European level. (RECOMMENDATION 1)

117. This review clearly demonstrates that at present, lakes and rivers biological monitoring systems in Europe differ widely in terms of the biological elements sampled, sampling methods, metrics and classification schemes adopted. These differences probably reflect varying monitoring objectives, the pressures impacting on the water bodies and technical, economic and cultural features of each country. They are not only obvious at the national level, but also within some countries.

118. It is, however, possible to find European wide a common pattern. For most lakes, quality assessment includes chlorophyll *a* and phytoplankton monitoring and for most rivers, quality assessment includes benthic invertebrates monitoring. This is not to say that the methods are identical, as in many instances this is clearly not the case, and direct comparisons of data using differing sampling and analytical regimes may be problematical. The work within the GIGs will inform this process

119. It is also clear from this review of harmonization that many countries have yet to develop monitoring and classification programmes that are WFD compliant, although it is clear that progress continues to be made through national and European research and development programmes. In order to manage the implementation process it is essential to collate this information and make it widely available. The Harmonisation Group could be central to this process (RECOMMENDATION 2)

120. The report show that the development many methods are still need or are underway at the moment. For this reason it is too early to demand the standardization of special metrics. In this respect ECOSTAT should liaise closely with the work of CEN, with the Harmonisation Group representing the obvious focal point (RECOMMENDATION 3).

121. The previous recommendation should be facilitated through a workshop involving the CEN task group convenors, the Harmonisation Group and other identified ECOSTAT members. This could be facilitated by JRC.

122. It is acknowledged that standardisation of WFD methods within CEN is resource limited. Where ECOSTAT and the GIGs identify priority work for CEN, it would seek a mandate for this work through the representatives of DG Environment (RECOMMENDATION 4)

123. Proposed and completed research in support of WFD has the strong potential now, and in the future, to encourage a wider level of method development and standardisation than currently exists. ECOSTAT needs to take a lead in value adding to the outputs in terms of finalization of ecological methods. The Harmonisation Group could lead in this area (RECOMMENDATION 5)

RECOMMENDATIONS

I. The harmonization group should continue to monitor the development of WFD compliant methodologies for ecological assessment and classification and report to ECOSTAT regularly.

II. The harmonization group should assume responsibility for collating scientific developments relevant to the WFD and ensuring that these are made widely available through the internet.

III. Methods and metrics which are used in a wide geographical scale and have the potential for standardization should be identified by ECOSTAT and the GIGs as a basis for priority areas for technical standardization. This could be managed by the ECOSTAT WG in contact with CEN. A start-up workshop could be facilitated by the JRC.

IV. The harmonisation group would take the lead in establishing priority areas for standardization within CEN and through ECOSTAT recommend areas of work requiring a mandate from DG Environment.

V. The Harmonisation Group on behalf of ECOSTAT could identify areas of ecological research relevant to the implementation of the WFD and ensure dissemination.

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EN ISO 8689-2 Water quality – Biological classification of rivers – Part 2: Guidance on the presentation of biological quality data from surveys of benthic macro-invertebrates (ISO 8689-1:2000)

EN 14184 Water quality – Guidance standard for the surveying of aquatic macrophytes in running waters

EN 13946 Water Quality – Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers

EN 14011 Water analysis – Sampling of fish with electricity

EN 14407 Water quality – Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters

EN 14614 Water quality – Guidace standard for assessing the hydromorlogical features of rivers

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Annex I: Composition of the Geographic Intercalibration Groups

Table 1. Distribution of countries by Geographic Intercalibration Groups (GIGs) and water categories, with numbers of sites in the draft register for each country (25 May 2005).

	Rivers					Lakes				
GIG / Countries	RAL	RCE	REC	RME	RNO	LAL	LAT	LCE	LME	LNO
Austria	20	10	4			15				
Belgium		21						2		
ulgaria			5							
Cyprus				2					2	
Czech Republic		22								
Germany	9	61				13		12		
Denmark		14						21		
Estonia		10						13		
Spain	11	35		55					18	
Finland					8					12
France	22	127		11		8		3	2	
UK		59			36			11		35
Greece				15					2	
Hungary			16					5		
Ireland		15			8		19			9
Italy	18	6		55		9			4	
Lithuania		21						6		
Luxemburg		4								
Latvia		8						6		
Malta				1						
Netherlands		18						21		
Norway					55					49
Poland		13						21		
Portugal				32					8	
Romania			14						8	
Sweden		2			13					25
Slovenia	4					2				
Slovakia			12							
TOTAL	84	446	51	171	120	47	41	121	44	130

Annex II: Summary of the information gathered from ECOSTAT and Monitoring WG on the national lake biological methods

Table 1. Summary of general characteristics of the lake biological methods employed in different countries (The information in this table is not complete but gather only the information received from the ECOSTAT WG 2A during the first half of 2004 and information reported in the WFD CIS Monitoring WG fact sheets).

Biological element	Pressure	Country	Sampling	Metric	Assessment
Phytoplankton	Eutrophication	AT, EE, FIN, IE, IT, LV, LT, NO, PT, SE, SL, SP, UK	Interests differ Lake areas, frequencies, qualitative or quantitative samples. Using national Std or published methods	Chl as biomass, taxonomic composition, abundance and biovolume	Ref. Cond not considered or only for limited No of lakes. None WFD compatible
Aquatic plants	Eutrophication	EE, DE, IE, FIN, LV, LT, SE, SL, UK	Different strategies, influence on plants in transect from shore to deep water	ID to species, abundance as % coverage	One considers ref cond. but for limited No of lakes, mostly used to monitor changes. Not WFD compatible
Benthic Macro-invertebrates	Eutrophication,, acidification	IE, SE, NO UK,	Different sampling according to lake area of interest International or National Std	Species abundance, presence or absence of sensitive species	Species Applicable to different areas (littoral or profundal) ref. cond. or need to develop type specific ref. cond., 5 quality classes. 2 WFD compatible
	Eutrophication	CY, LV, SE	Hand net or beaker, Ekman grab National Std (LT, SE)	Family, genus, species	Applicable to profundal Type specific ref. cond., 5 quality classes, WFD Compatible
Fish	Eutrophication, acidification	SE, SP, UK	5 different methods	Applicable to different lakes. Most ID to species and abundance	One with type species ref. cond. Good basis for developing WFD compatible methods
Macro-inv. aquatic plants	Eutrophication, acidification	UK	National Std for both biol.elements	Multimetric index PSYM	Ref. Cond. for range of env. cond. and geology. Good applicability to WFD. Small standing water only

AT= Austria, CY= Cyprus, DE= Germany, EE= Estonia, FIN= Finland, IE= Ireland, IT= Italy, LV= Latvia, LT= Lithuania, NO= Norway, PT= Portugal, SE= Sweden, SL= Slovenia, SP= Spain, UK= United Kingdom

Annex III: River biological assessment methods from Waterview Database (2004)

Table 1. River biological assessment methods from Waterview Database included in this review.

method	country	status	reference
Assessment of saprobiological quality of rivers	Austria	u	Moog *et al.* (1999)
WFD method for macroinvertebrates (Multimetric Index)	Austria	d	no publication
Belgian Biotic Index (BBI)	Belgium	u	De Pauw and Vanhooren (1983)
Biotic Sediment Index (BSI)	Belgium	n	De Pauw and Heylen (2001)
Biotic Index based on 'Quality Rating System'	Bulgaria	u	MCgarrigle *et al.* (1992)
Determination of Saprobic Index according to Pantle and Buck and Rothschein	Bulgaria	n	Pantle and Buck (1955), Rothschein (1962)
Evaluation of BSI according to BMWP and quantification of BDI according to SCI methodology	Cyprus	u	Cairns *et al.*, 1968
PERLA	Czech Republic	?	Kokes *et al.* (2003)
Saprobiological Monitoring	Czech Republic	?	CSN 757716 (1998)
DSFI (Danish Stream Fauna Index)	Denmark	u	Skriver *et al.* (2000)
Quality Assessment of Estonian Watercourses using Benthic Macroinvertebrates	Estonia	u	SEPA (2000)
I.B.G.N. - Indice Biologique Global Normalisé	France	u	AFNOR (1992)
I.O.B.S. - Indice Oligochètes de Bioindication des Sédiments	France	n	AFNOR (2002)
Ecological classification of benthic fauna in rivers	Germany	u	Meier *et al.* (in print)
Potamon-Characterisation-Index (PTI)	Germany	u*	Schöll and Haybach (2001)
AQEM Greece	Greece	n	Skoulikidis *et al.* (2004)
Hellenic Evaluation System (HES)	Greece	n	Lazaridou-Dimitriadou *et al.* (2004)
BMWP - HY (adapted to Hungarian conditions)	Hungary	?	Just *et al.* (1998)
Quality Rating System	Ireland	u	MCgarrigle *et al.* (1992)
Extended Biotic Index - IBE ('Indice Biotico Esteso') modified according to Ghetti	Italy	u	Ghetti (1997)
Operative Evaluation of the Biological Quality of Small Streams by Saprobic Index of Macrozoobenthos Communities	Latvia	u	Latvian Standard LVS 240 (1999)
Water Quality Assessment	Liechtenstein	n	Moog *et al.* (1999)
Operative Evaluation of the Biological Quality of Small Streams by Biotic Integrity Index of Macrozoobenthos Communities	Lithuania	u	no information
I.B.G.N. - Indice Biologique Global Normalisé	Luxembourg	u	no publication
AMOEBE - general method for ecosystem description and assessment	Netherlands	n	Ten Brink *et al.* (1991)
Ecological classification system for rivers	Netherlands	u	Molen *et al.* (2004)
Acidification Index	Norway	n	Raddum (1999)
Invertebrate Monitoring	Norway	n	no information
BMWP - PL (Biological Monitoring Working Party score adapted to Polish conditions)	Poland	d	WATERVIEW DATABASE (2004)
BMWP' (Biological Monitoring Working Party score adopted to Iberian conditions)	Portugal	u	Alba-Tercedor and Pujante (2000)
Determination of Saprobic Index according to Pantle and	Romania	n	Marcoci (1984)

method	country	status	reference
Buck			
Saprobiological Analysis	Slovenia	u	Grbovic (1999)
ECOSTRIMED – Ecological Status of Streams and Rivers in the Spanish Mediterranean Area	Spain	d	Prat *et al.* (2000)
Benthic Fauna in Lake Littorals and Running Water - Time Series	Sweden	u	SEPA (2000)
Acidification Index	United Kingdom	?	Rutt *et al.* (1990)
Biological GQA (General Quality Assessment) classification	United Kingdom	u	ENVIRONMENT AGENCY (1996)
Lotic-invertebrate Index for Flow Evaluation (LIFE) Index	United Kingdom	u*	Extence *et al.* (1999)
System for Evaluating Rivers for Conservation (SERCON)	United Kingdom	n	Boon *et al.* (1997)

u - to be used in WFD-compliant monitoring (only Member States)
u* - part of method to be used in WFD-compliant monitoring
n - not to be used in WFD-compliant monitoring
d - under development to be used in WFD-compliant monitoring (only Member States)
c - monitoring discontinued
? - usage in WFD-compliant monitoring uncertain

Annex IV: Analysis of lake biological monitoring methods (Intercalibration metadata at 14/01/2004)

CHLOROPHYLL

1. The WFD allows for the use of chlorophyll *a* as surrogate for phytoplankton biomass and, thus, is considered a biological parameter, and the most frequently measured in lakes. In the GIGs the percentage of lakes for which chlorophyll *a* is measured varies between 40% (Eastern Continental, EC) and 100% (Mediterranean, ME), with an overall average of 82.5% (Fig. 1). A possible explanation for a smaller percentage of lakes with chlorophyll *a* data in the Eastern Continental and Northern GIGs could be a monitoring strategy targeted to identify acidification impacts and for which chlorophyll *a* is less used.

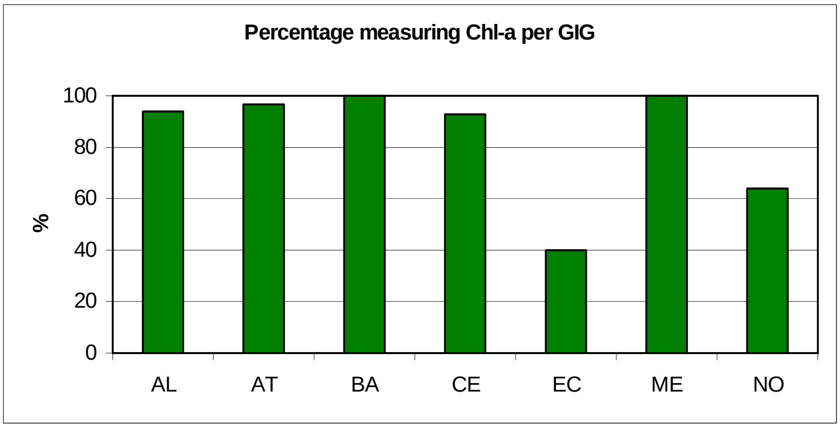

Figure 1. Percentage of lakes for which chlorophyll *a* is measured in the GIG (Al= Alpine; AT= Atlantic, BA= Baltic; CE= Central; EC= Eastern continental, ME= Mediterranean, NO= Northern).

2. However, there is great heterogeneity both within GIG and between GIGs in terms of the sampling methods. Most lakes in the GIGs are sampled for chlorophyll *a* between 2 and 12 times per year (Fig. 2), and samples are taken over the whole year (monthly) or concentrated during spring, summer, or vegetation periods (Fig. 3).

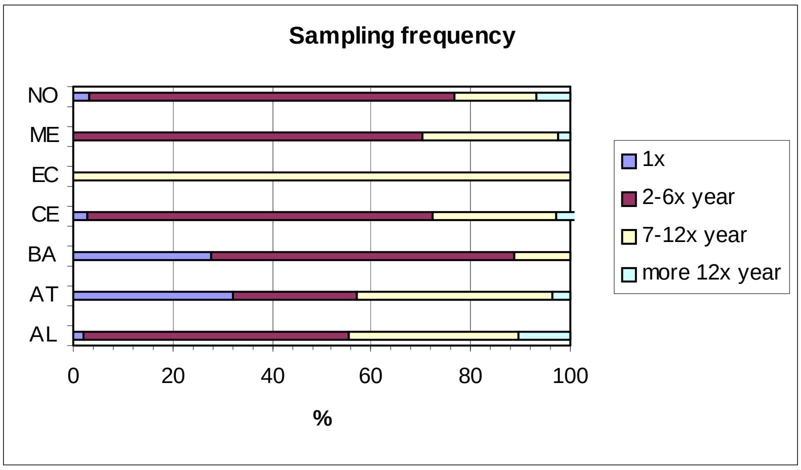

Figure 2. Sampling frequencies for chlorophyll *a* in times (x) per year in the GIG.

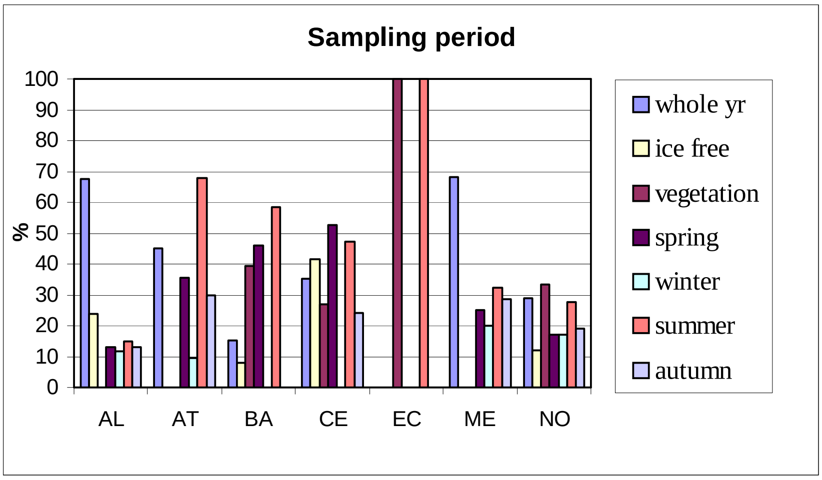

Figure 3. Chlorophyll *a* sampling periods in the GIGs.

3. Most lakes, are monitored for chlorophyll *a* at only one station, a smaller percentage is sampled at two stations, particularly in the EC and ME GIGs (Fig. 4.).

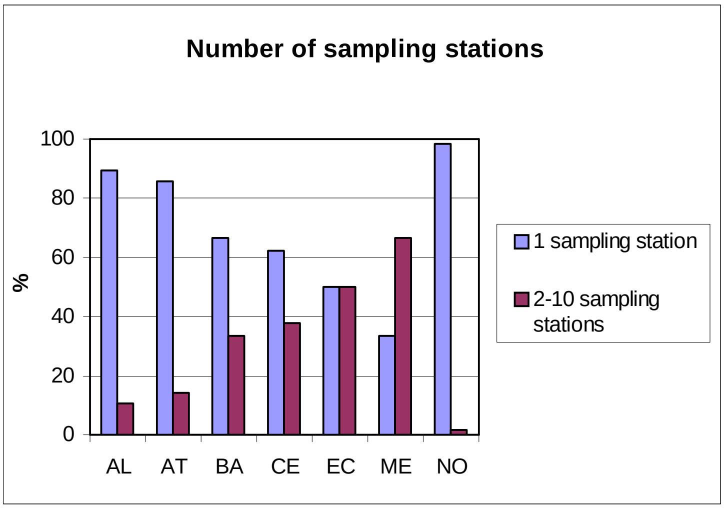

Figure 4. Number of sampling stations for chlorophyll *a* in the GIGs.

4. The sampling depth and volume sampled may contribute to large differences in the determination of chlorophyll *a* concentrations in a lake. The most frequent method in all the GIGs involves the collection of surface samples. In some GIGs (most countries in the AL, ME and NO GIGs), for a few lakes chlorophyll *a* is estimated from integrated samples (Fig. 5). In the AL and BA GIGs the collection of more than one discrete sample at different depths is regularly practiced.

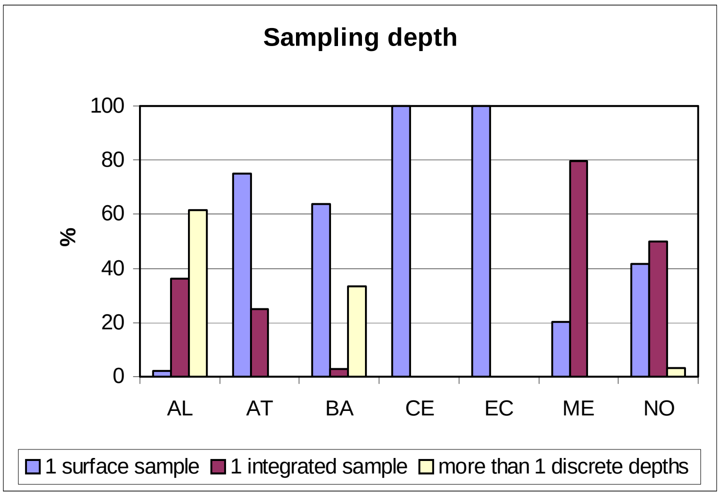

Figure 5. Sampling depth for chlorophyll *a* in the GIGs.

5. Among the most heterogeneous steps in the assessment of chlorophyll *a* are, however, the extraction methods (e.g. Marker, 1972; Pepe et al, 2001), which vary widely within GIGs, among GIGs and even within a single country (Table 1). These can explain considerable variance in the measurement of chlorophyll *a* concentration.

6. Some countries (Cyprus, Latvia, Finland and Slovenia) employ international standard methods for the determination of chlorophyll, a number of other countries make use of national standardized methods (Germany and France), and the rest refer to several different published methods. Some countries gave no reference to the method employed.

Table 1. Chlorophyll determination methods in the different countries within GIGs.

GIG/ Country	Extraction	Measurement	References
ALPINE			
AT		fluorimetric	
DE	ethanol[2] or ethanol and acetone[3]	spectrophotometric or HPLC (since 1999)	DIN 384 12-L 16
ES	methanol (100 %)[4]	spectrophotometric	Parsons and Strickland (1965)
FR			AFNOR
IT	acetone (90%)	spectrophotometric	
SL	methanol	spectrophotometric	SIST ISO 10260:2001 - modified
ATLANTIC			
IE	boiling methanol	spectrophotometric	
UK	cold acetone (100%) or hot methanol (90%)	spectrophotometric	
BALTIC			
EE	ethanol (96%)	spectrophotometric	Jeffrey and Humphrey (1975)
PL	acetone	spectrophotometric	
LV	ethanol	spectrophotometric	ISO 10260:1992
LT		spectrophotometric	
CENTRAL			
BE	acetone (90%)	spectrophotometric	Golterman *et al.*(1978), Strickland and Parsons (1968)
DE	ethanol (90%)		DIN 38 412, modified by Nusch (1980); or Strickland and Parsons (1972)
NL	ethanol (80 %) at 75oC	spectrophotometric	
PL	acetone	spectrophotometric	
UK	acetone (90%)[5] or cold acetone (100%) or hot methanol (90%)	spectrophotometric	Golterman *et al.*(1978), Strickland and Parsons (1968); or Phillips and Kerrison (1991)
EASTERN CONTINENTAL			
HU	methanol		Wolfe-Murphy et al (1991)
MEDITERRANEAN			
CY	acetone (90%) buffered	spectrophotometric	Standard Methods for the examination of Water and Waste water
ES	methanol [6]or acetone (90%)[7] or metanol (100 %)[8]	spectrophotometric	Strickland and Parsons (1968) or Talling and Driver (1963)

[2] With spectrophotometric determination.
[3] With HPLC determination.
[4] Golterman *et al.* (1978)
[5] Strickland and Parsons (1968) or Phillips and Kerrison (1991)
[6] Strickland and Parsons (1968)

IT	90% acetone	spectrophotometric	
PT	acetone	spectrophotometric	Lorenzen (1967)
NORTHERN			
NO	100% methanol	spectrophotometric	
IE	boiling methanol		
UK	cold acetone (100% or hot methanol (90% methanol); acetone)	spectrophotometric	HMSO (1980)
FIN		spectrophotometric	ISO 10260:1992
SE		spectrophotometric	

PHYTOPLANKTON

7. Most countries include in their biological monitoring systems some metric for phytoplankton, as shown in Figure 6. The lowest number of lakes for which phytoplankton is monitored is in the NO GIG (54%), in all other GIGs at least 60% of the lakes are monitored for phytoplankton. A possible explanation for a smaller percentage of lakes with phytoplankton data in the Northern GIG could be a monitoring strategy targeted to identify acidification impacts and for which chlorophyll *a* is less used.

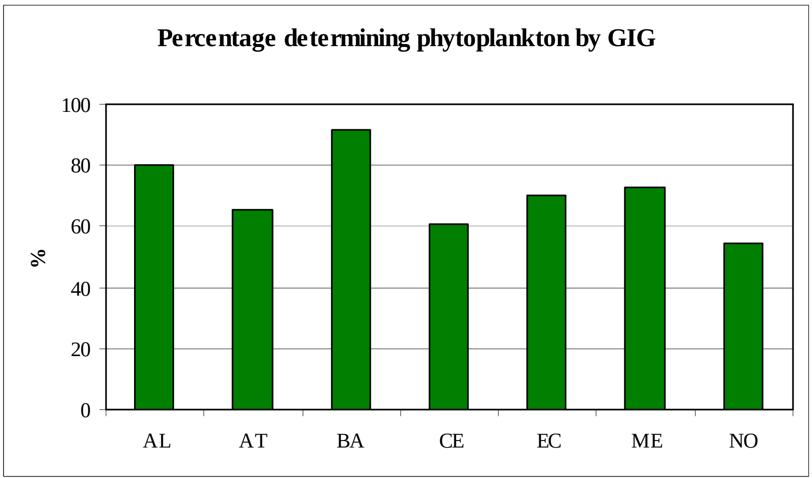

Figure 6. Percentage measuring phytoplankton within the GIGs.

8. Lake sampling frequency is variable but in most GIGs, and for most lakes is 2-12 times per year (Fig.7), and spring and summer as the most common sampling seasons (Fig. 8).

[7] Spectrophotometric determination
[8] Talling and Driver (1963)

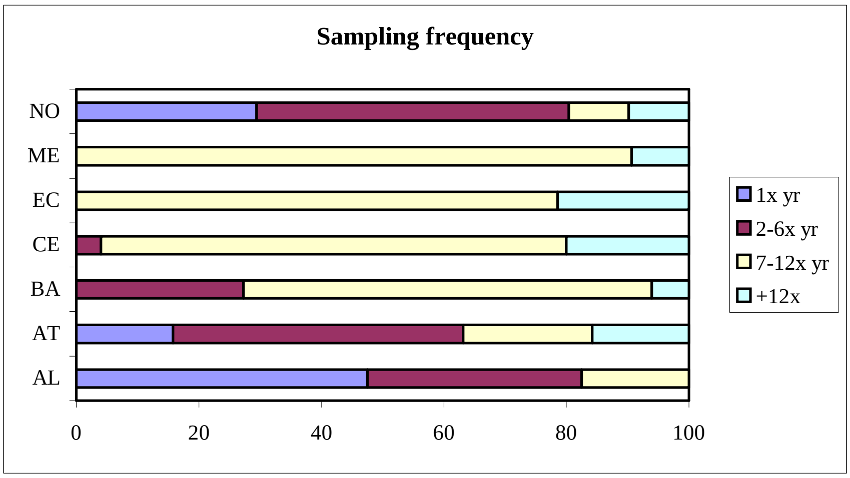

Figure 7. Phytoplankton sampling frequency in the GIGs.

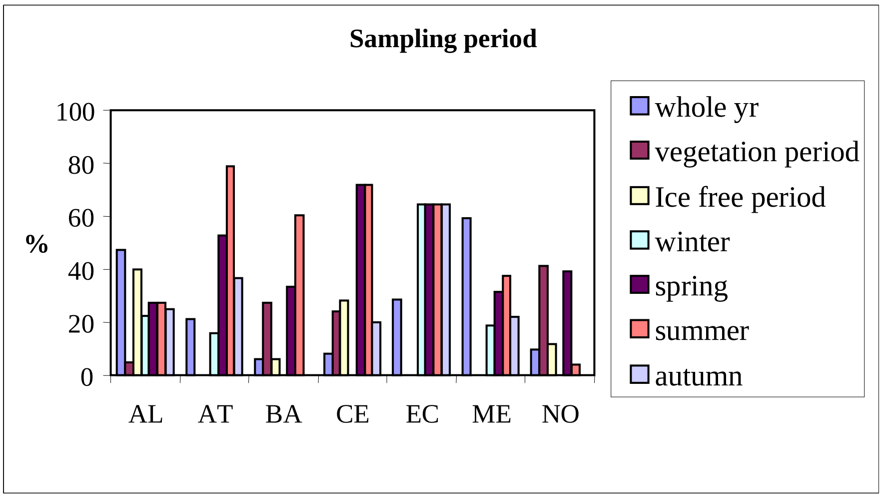

Figure 8. Phytoplankton sampling period in the GIGs.

9. Most lakes are monitored for phytoplankton at only one sampling station, a smaller percentage is sampled in two stations, as is the case in the EC, ME and CE GIGs (Fig. 9).

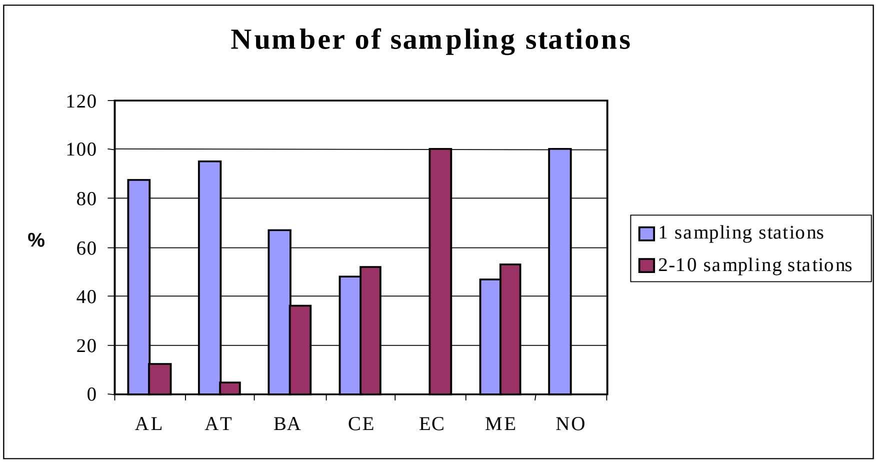

Figure 9. Number of stations sampled per lake per GIG.

10. The sampling depth and volume sampled may contribute to large differences in the measured phytoplankton biomass and taxonomic composition. The most frequent method involves the collection of surface samples or other discrete samples although some countries take integrated samples (a few lakes by most countries in the NO, some in the AL, AT and ME GIGs) (Fig. 10).

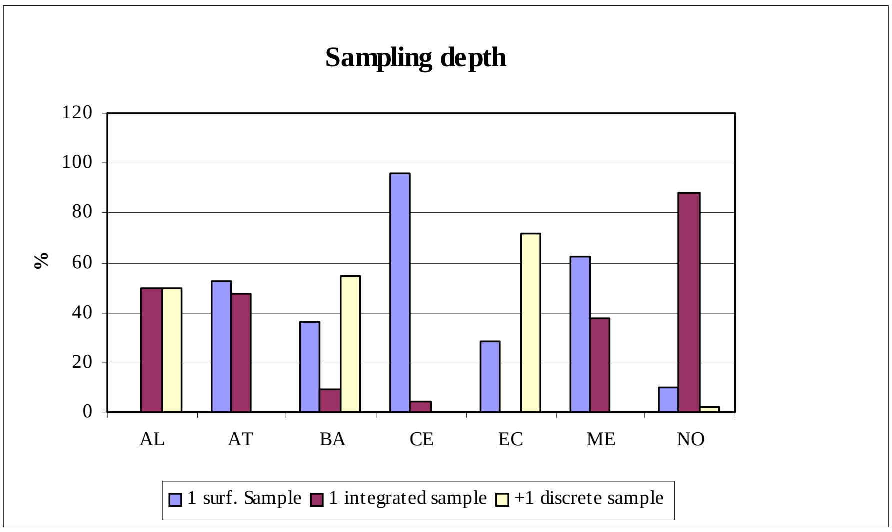

Figure 10. Phytoplankton sampling depth in the GIGs.

11. Also, the metrics used in the assessment differ both within and among GIGs, but most countries measure abundance and biomass and successively less countries record bloom occurrence, size composition and primary productivity (Fig. 11).

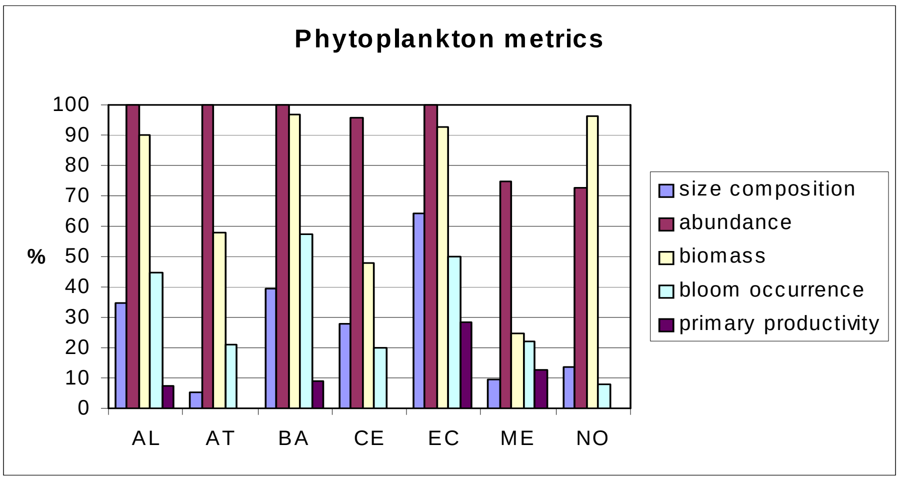

Figure 11. Phytoplankton metrics in the GIGs.

12. Table 2 shows that sampling methods and metrics often differ between countries at GIG level, and that apart from biomass as indicated from chlorophyll concentrations, for a specific lake or lake type each country uses different metrics. Also, there are no countries that completely fulfill the WFD metric requirements.

Table 2. Phytoplankton sampling device and metrics used in different countries within the GIGs.

GIG/ Country	Sampling method	Metric
ALPINE		
AT		abundance, biomass, group ratios, indicator taxa
IT		abundance
AT	point samples	abundance, biomass, indicator taxa, group ratios
SI	single depth samples	temporal and spatial distribution of different species
DE	integrated samples	species composition and abundance or abundance and biomass of single species
FR	net on secchi depth or on integrated sampling from 0 to 50m	abundance
ATLANTIC		
IE	standard net or aliquot of integrated sample	relative abundance, indicator taxa and group ratios, annual cycle, abundance estimated by analysis of the pigment chlorophyll
GB	Rutner water sampler every metre from surface composited, supplemented by phytoplankton net tow over lake surface. Composite tube sampling.	abundance and identification of algal species
BALTIC		
PL	15 l of water filtered by plankton net No25 from the depth of 1m and 5m.	dominance structure, indicator taxa.
LV	with Ruttner bathometer, from 0,5 m depth other from 0.2-0.3 m depth and 5-10m	biomass, indicator taxa, relative contribution of Cyanophyta, Chrysophyta, Bacillariophyta etc, saprobic index, number of species
EE	Ruttner sampler or Van Dorn sampler Utermöhl's (1958) technique	abundance, frequency of occurrence, indicator taxa, group ratios, percentages of larger taxonomic groups, community structure, chlorophyll a concentration
CENTRAL		
PL	1l and qualitative drawn sample from photic zone. plankton-net no 25.	dominance structure, indicator taxa.
NL		cell counting (from colonies cells are estimated), identification to species or to genera level, share of groups (diatoms-blue – green-other algae)
DE	surface sample or standard water sampler in a depth of 1 m, or 5-liter integral sampler, sampling the epilimnetic layer	No information available
RO	samples takes with bucket, Knopp method SR ISO	species density, abundance, wet biomass, biomass on systematical gr, probic index

	5667-2/98 Romanian standardisated method (STAS-ICIM)	
MEDITERRANEAN		
PT	bottle sampler and Utermohl method - qualitative analysis	species abundance classes (present, frequent, abundant and bloom)
ES		abundance and species composition
NORTHERN		
FI	One composite sample from the epilimnion, usually 0 - 2 m. Sampling, preservation and analysis described in detail in Swedish EPA reports 4860 and 4861.	biomass, species number, relative abundance.
IE	Standard net, haul from 6m for quantitative data and sub-surface water	relative abundance, indicator taxa and group ratios
GB	5m integrated tube,	species list
NO	2m tube sampler taken at site of max.depth. Separate samples are mixed from different depth intervals: 0-2, 2-4, 4-6 m etc. down to 2x secchi depth	biomass per species for each species present
SE	depth- and surface integrated pelagial samples collected with a plexiglass tube. Sampling, preservation, and analyses described in detail in Swedish EPA reports 4860 and 4861	total biovolume

PHYTOBENTHOS

13. Most countries do not use phytobenthos in their biological monitoring (Fig. 12), The highest number of lakes monitored for phytobenthos is found in the EC GIG followed closely by CE and AL GIGs, while in the NO GIG countries this biological element is not used at all.

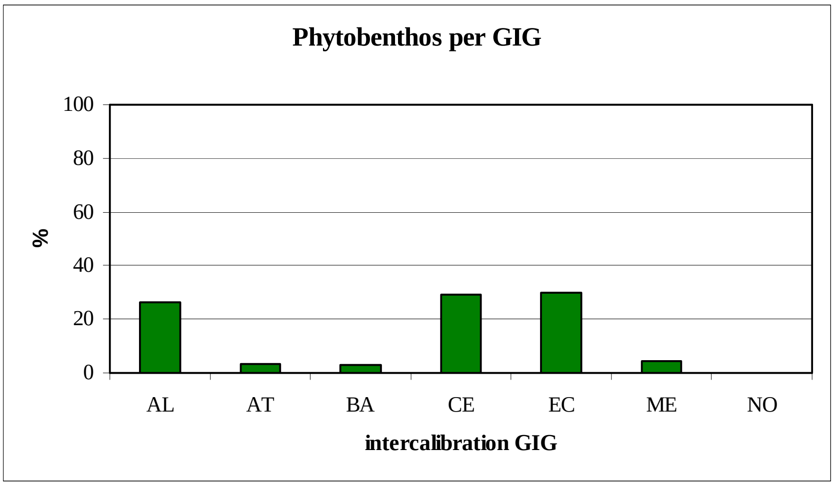

Figure 12. Percentage measuring phytobenthos in the GIGs.

14. In cases where phytobenthos is sampled, the most frequent sampling frequency is 2-6 times per year, but in Mediterranean countries it is only sampled once a year (Fig. 13). The samples are taken in either one lake station (all AT and EC), or 2-10 stations (most BA and CE), or more than 10 stations (all ME and most AL) (Fig. 14).

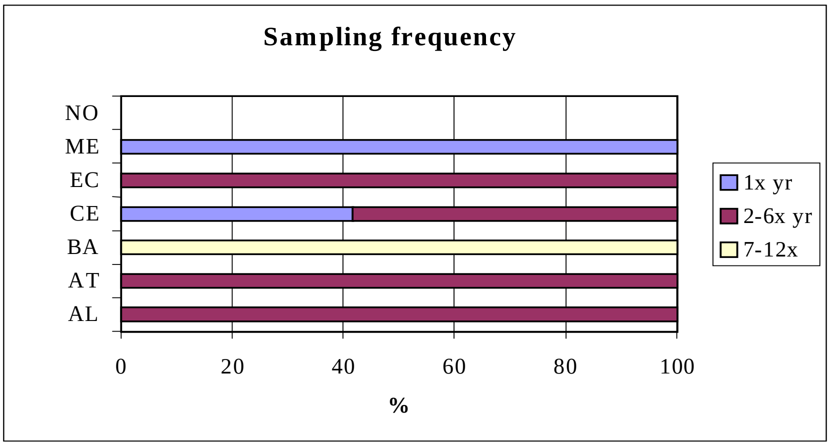

Figure 13. Phytobenthos sampling frequency in the GIGs.

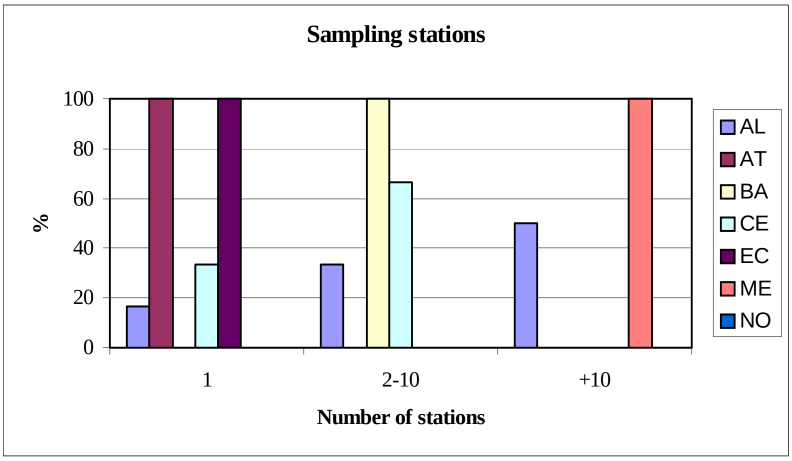

Figure 14. Phytobenthos sampling stations in the GIGs.

15. As with chlorophyll *a* and phytoplankton, sampling is held during the vegetation growth period, spring and summer (Fig. 15). Sampling and metrics vary (Table 3). The habitats sampled differ, some countries sample epilithic and others epiphytic phytobenthos. The composition metrics are species composition, presence or absence of species and indicator taxa. The quantity metrics are the relative abundance of species, indicator taxa and excessive growth of nuisance species such as *Cladophora*.

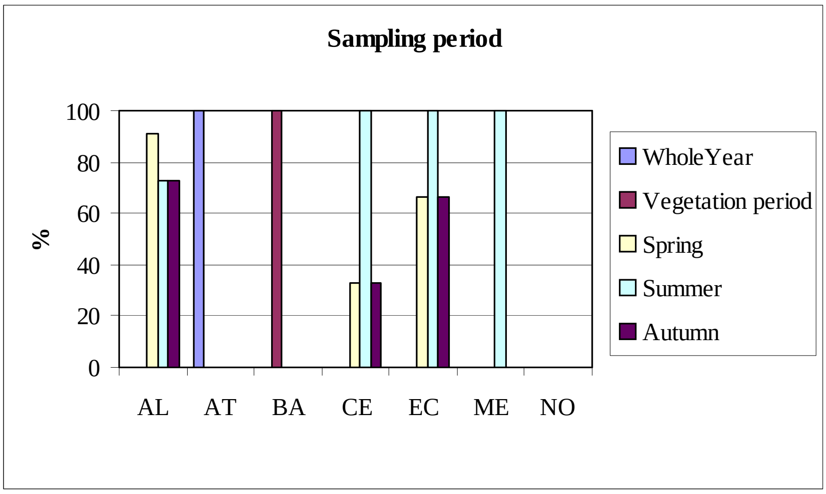

Figure 15. Phytobenthos sampling period in the GIG's.

Table 3. Phytobenthos sampling and metrics used in different countries within the GIGs..

GIG/ Country	Sampling Method	Composition Metric	Quantity Metric
ALPINE			
DE	epilithic diatoms	species composition	relative abundance according to 400 counted valves
ATLANTIC			
GB	direct observation in the littoral zone	presence/absence by direct observation.	excessive growths of nuisance species such as *Cladophora* agg.
BALTIC			
EE		species composition	Biomass as chlorophyll *a*
CENTRAL			
BE	Diatoms relative abundance; sediment assemblage, epiphytes	relative abundance of indicator taxa (species)	relative abundance (counts of 500 valves)
DE	epilithic diatoms	species composition and relative abundance	relative abundance
DE	(1) benthic diatoms attached on reed and submerged macrophytes (2) diatom remains from deepest point surface sediment (Niederreiter gravity corer)	Trophic state index (Hofmann 1994); Index BRB (phosphorus availability, Schönfelder 1997)	relative abundances (percentages) of each species
DE	epilithic diatoms	species composition and relative abundance	relative abundance
EASTERN CONTINENTAL			
RO	Knopp method Pantle - Buck method SR ISO 5667-2/98	species list, indicator taxa; annual medium number of species; annual medium density of species	number of ind/m2
MEDITERRANEAN			
ES	submerged substrates	species determination	

MACROPHYTES

16. The aquatic Macrophytes are widely used in the biological monitoring systems of the AT, BA and CE GIGs and less in all other GIGs (Fig. 16).

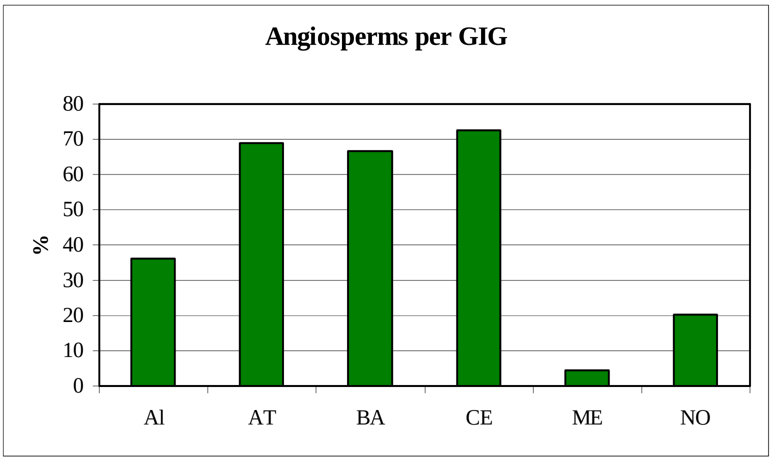

Figure 16. Percentage sampling Macrophytes in the GIGs.

17. Sampling occurs mostly between 2 and 6 times per year, in some GIGs a smaller number of lakes are sampled from 7 to 12 times per year (in the AT, BA, CE and NO GIGs) (Fig. 17), all during the summer or vegetation period (Fig. 18).

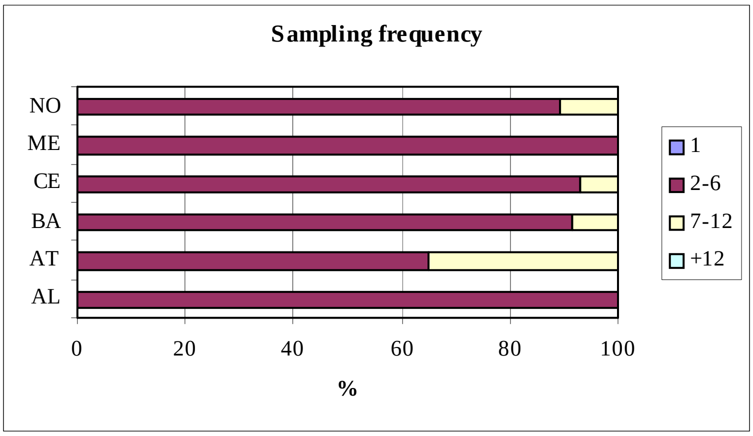

Figure 17. Macrophytes sampling frequency in the GIGs.

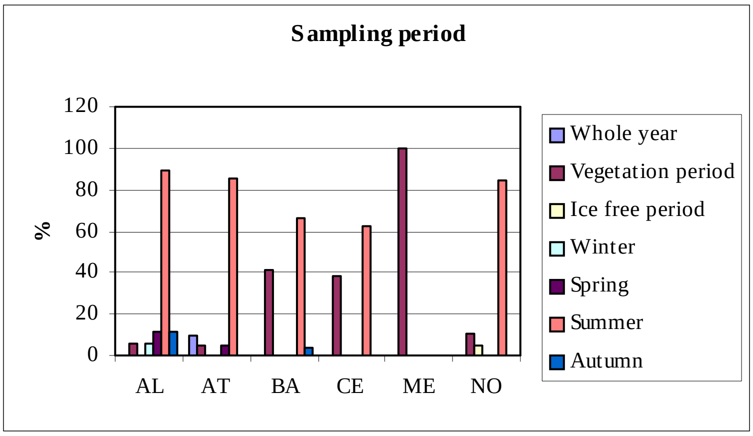

Figure 18. Macrophytes sampling period in the GIGs.

18. The number of sampling stations per lake is widely variable (Fig. 19), in a single GIG the number can be of 1, 2-10 or more than 10, excepting for the ME were the number is 2-20.

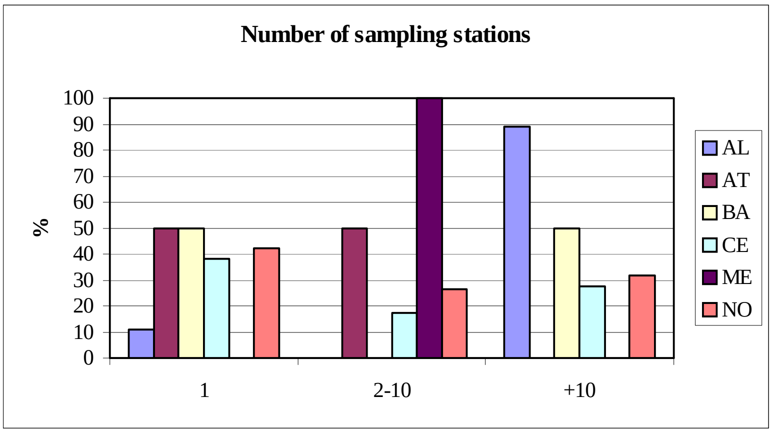

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Figure 19. Macrophytes number of stations in the GIGS.

19. In the AL, AT, BA and ME GIGs all emergent, floating and submerged plants are sampled. In contrast in the CE and NO GIGs not all countries sample the three groups of plants (Fig. 20).

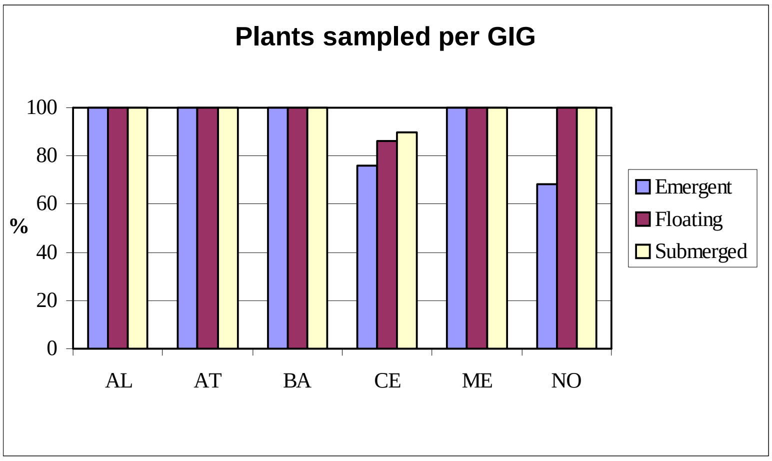

Figure 20. Groups of Macrophytes sampled in the GIGs.

20. In Table 4 the sampling methods and metrics for Macrophytes are presented. Composition and abundance are recorded from visual inspections in the field from the shore. In cases when the whole lake is monitored this is done by boat. One country uses an aqua-scope to monitor plant growth underneath the water surface. Rakes and grapnels are used to remove the plants, along transects, for estimation of abundance and taxonomic identification. In most countries the plants are identified to species and abundance and/or diversity determined.

Table 4. Macrophytes sampling and metrics used in different countries within the GIGs.

GIG/ Country	Sampling Method	Composition Metric	Quantity Metric
ALPINE			
IT	rake	species list	
AT	Jager *et al.* (2002, 2004) Melzer *et al.* (1986).		plant mass
SI	combined geobotanical quadrate	species diversity	relative abundance, estimation by degrees 1-5
DE	transect method with boat and rake/grab; mapping of ecologically homogenous sections of the whole littoral zone	mapping species composition	abundance (semiquantitative), 5 point scale according to Kohler (1978)
ATLANTIC			
IE	shoreline examinations and sampling at 6m intervals along transects from shoreline using rake and underwater cameras Recording of abundance and species in littoral zone of the lake augmented with emergents readily identifiable at distance.	species, frequency of occurrence, indicator taxa, group ratios	abundance, weight of discrete samples DAFOR
GB	inshore transects using rake; shoreline examinations and sampling at 6m intervals along transects from shoreline using rake and underwater	species composition, frequency of occurrence, indicator taxa, group ratios	relative abundance, weight of discrete samples, DAFOR

	cameras; recording of abundance and species in littoral zone of the lake augmented with emergents readily identifiable at distance; believed to be transect using grapnel from boat		
	BALTIC		
PL	fields observations: occurrence of submerged and emergent sensitive angiosperm taxa; plant survey (Braun-Blanquet, 1964)	plant composition, presence of indicator species, diversity of plant communities	colonisation index, index of the age of ecosystem, synanthropisation index, area covered by particular plant communities
EE	mapping of the species of the whole lake, rake method, distribution depth.	species composition, species diversity, coverage and distribution depth PVI (percent volume infested)	relative abundance, depth distribution, density (shoots per m2*, PVI)
	CENTRAL		
NL	vegetation is sampled with a rake, 1000 sample points, cover estimated by eye in eight classes per species, Charophytes and mosses are also sampled, but determined at genus level	species composition , Tensley scale per species available	total covered area, submerged macrophytes, Tansley scale
GB	transect survey with grapnel; transects using a double headed rake; believed to be transect with grapnel	species list, number of species	relative abundance, ranked 1-4, abundance using DAFOR scale
BE	shore-based, rake (littoral); grid, boat, grapnel (whole lake)	type specific species (abundance weighted), disturbance indicators, growth forms	cover/ frequency (Tansley); aquatic vegetation, shore vegetation
PL	Plant survey (Braun-Blanquet, 1964).	presence of indicator species, diversity of plant communities.	colonisation index, index of the age of ecosystem, synanthropisation index, area covered by particular plant community, abundance.
NL	Tansley scale sampling on 50 meter transects, including charophytes	species, tansley scale	abundance, tansley scale
DE	transect method with boat and rake/grab; mapping of ecologically homogenous sections of the whole littoral zone New method for WFD, method of mapping (Kohler, 1978); aquatic spermatophyta, pteridophyta, characeae, bryophyta, rake (Jensen, 1977, Engel and Nichols, 1994, Karttunen and Toivonen, 1995)	mapping specific composition	abundance (semiquantative), abundance in different soundings (Kohler, 1978) 5 point scale according to Braun-Blanquet
NL	Tansley, Charophytes are also sampled, but determined at genus level	Tansley scale	cover of growth forms are estimated
	MEDITERRANEAN		
ES	manual sample collection and grabber samplers	species abundance	% cover
	NORDIC		
IE	shoreline examinations and sampling at 6m intervals along transects from shoreline using rake and underwater cameras.	frequency of occurrence, indicator taxa, group ratios	abundance, weight of discrete samples

NO	qualitativ method	species number, ALS	relative abundance, area coverage
NO	plants recorded using aqua scope and collected by dredging from boat,		relative abundance abundance of the species is scored by a scale(1=rare,2=scattered,3= common, 4=locally dominant and 5=dominant) area coverage - expert opinion
SE	whole-lake investigation with respect to species richness, quantitative estimate of the percentage coverage for randomly selected squares along one transect.	species richness, indicator scores	percentage cover
IE	inshore transects using rake	frequency of occurrence, indicator taxa, group ratios	relative abundance

BENTHIC INVERTEBRATES

21. The percentage of lakes for which benthic invertebrates are sampled within the GIGs is for part most below 40%, with exception of AT and CE (Fig. 21).

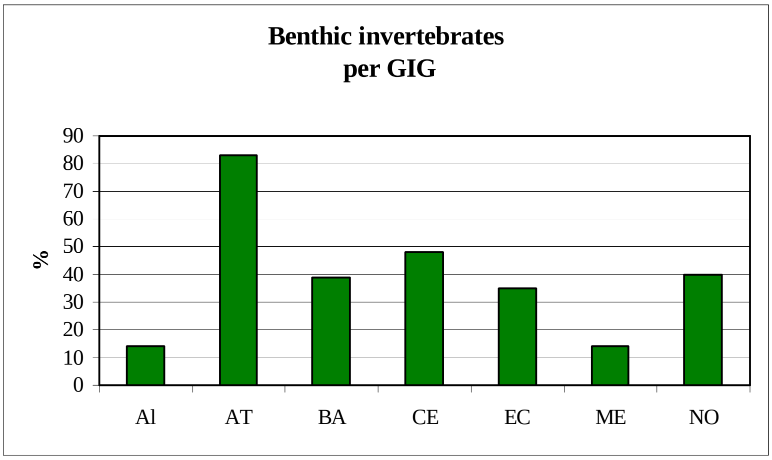

Figure 21. Percentage of lakes sampled for benthic invertebrates in the GIGs.

22. Sampling frequency is variable between GIGs, the most common frequencies are 1 or 2-6 times per year, but EC countries sample a few lakes 7-12 times per year, and the BA, AT and AL include a combination of different sampling frequencies (Fig. 22). Sampling occurs mostly in spring and summer but in the AL and NO GIGs it is performed most often in the Autumn (Fig. 23).

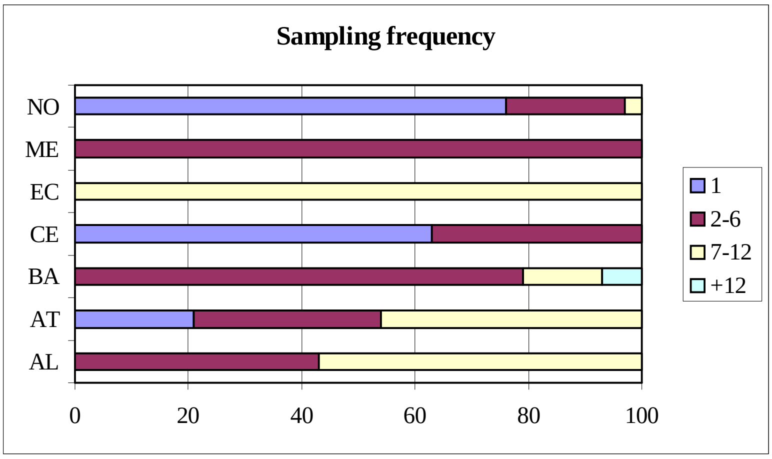

Figure 22. Benthic invertebrates sampling frequency in the GIGs.

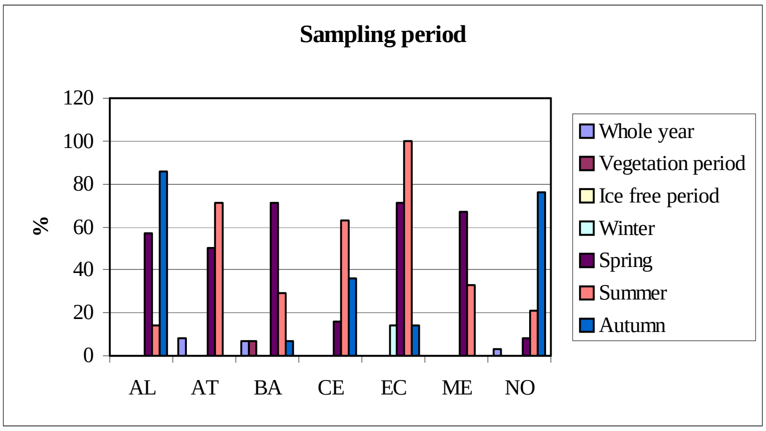

Figure 23 Benthic invertebrates sampling period in the GIGs.

23. In Table 5 the sampling methods and metrics for benthic invertebrates are presented. It shows that a wide number of sampling approaches and metrics are used. The collection of samples is performed by several different devices: grab, Eckman skip, sticking cylinder, triangle bottom scraper and hand net. The mesh size varies widely from 100µm to 670µm. When kick sampling is used time (1-3 minutes), mesh size (100-670µm) and habitat sampled (littoral in general or stones) are different. Some countries sampled in lake littoral, other the profundal and some in both lake zones (IE). Two countries make use of international sampling standard methods, Estonia and Romania. These standards are currently under revision.

24. Every country uses a different combination of metrics, the following metrics are either used alone or in combination: abundance and relative abundance, diversity

indicators, species lists, frequency of occurrence of individual taxa, number of taxa, group ratios, average score per taxa, biotic score, biotic integrity index, saprobic index, average score per taxon and ratio of littoral to profundal taxa.

Table 5. Benthic invertebrates sampling and metrics used in different countries within the GIGs..

GIG/ Country	Sampling Method	Metric
ALPINE		
DE	sampling with grab or sticking cylinder; mesh size for sieving 0.2 mm	
FR	Eckman skip + strainer	
IE	littoral samples taken by kick sampling on stony substrate for 2 minutes using 670µm mesh size; pond net bag with rectangular frame (260mm wide, 200mm high); profundal samples taken by Eckman Grab (5 replicates) at deepest point of lake	abundance, frequency of occurrence, presence of indicator taxa, group ratios and diversity
GB	3 minute kick sample with Freshwater Biological Association pond net mesh 100um	biotic score, number of taxa and average score per taxon, log abundance estimates, indicator taxa.
BALTIC		
PL	Ekmann and Birdge sampler, samples from the depth of 20 - 50 m	dominance structure
LT	5 samples taken in 1/40 m2 area by special device for quantitative measurements and 1 sample by triangle bottom scraper, which is dragged for 2-5 metres	biotic integrity index
EE	guidance of sampling of aquatic benthic macro-invertebrates (ISO 7828:1985),(ISO 8265:1988),(ISO 9391:1993),(ISO 8689-1:2000),(ISO 8689-1:2000).	species composition, number of species
EE	Borutsky or Zabolotsky bottom sampler; international standards: (ISO 7828:1985). (ISO 8689-1:2000)(ISO 8689-2:2000).	percentages of larger taxonomic groups, ASPT, EPT indexes
CENTRAL		
NL	depending on substrate macroinvertebrates are sampled from stones (5 stones), or the substrate itself is sampled with a grab (Ekman 0.15x0.15m), or tube (diameter ca. 0.1m) or hand net (mesh 0.5 mm).	no metric used for classification; Zebra mussels: biovolume and number of samples sites
GB	1 minute kick/sweep	species list
BE	littoral, standard net	
DE	sampling stations in different depths (2-3, 5, 7, 10, 15, 24 m) along one transect, mesh size: 405 µm; or with grab or sticking cylinder; mesh size for sieving 0.2 mm and 0,5 mm; or Ekman-Birge sampler, dredge	indicator taxa, abundance, group ratio: littoral/profundal fauna
EASTERN CONTINENTAL		
RO	Knopp method; Pantle - Buck method; SR ISO 5667-2/98	density, indicator taxa, abundance, number of species, saprobic index
ES	grabber samplers	species determination

CY	replicate samples with hand net or beaker in spring and autumn from 6 points at depth of 1m.	evaluation based on BSI (Benthic Saprobity index) and BDI (Biological diversity index). Manual on Water Quality Evaluation (BDI:quantified according to Sequential Comparison index. BSI: determinations up to family level)
NORDIC		
FI	Littoral: recently some separate preparatory surveys, using various sampling methods. Sampling in Autumn (or also spring). Kick sampling 20s x 1m. Modified ntional standard (SFS 5077). Profundal: sampling most usually at point of maximum depth (but other depths may be sampled) Ekman sampler but also tube sampler used. Sieving mesh size 0.5mm. (SFS-EN-ISO 9391:1995, SFS 5076 and SFS 5730).	Metrics to be further developed
IE	littoral samples taken by kick sampling on stony substrate for 2 minutes using 670µm mesh size; pond net bag with rectangular frame (260mm wide, 200mm high). Profundal samples taken by Eckman Grab (5 replicates) at deepest point of lake.	abundance or relative abundance, frequency of occurrence, presence of indicator taxa, group ratios and diversity
NO	kick-sampling with net in littoral zone: min 120 seek per substrate type, mesh size: 0.25 mm (0.5 mm)	Raddum acidity index (acid sensitive taxa)
SE	kick samples, 20s x 1m, mesh size= 0.5mm	Average Score Per Taxon, Danish Fauna Index, acidity index, Shannon diversity

FISH

25. Most countries do not use fish in their biological monitoring and assessment programmes (Fig 24). The highest number of lakes sampled for fish is found in the BA GIG followed closely by CE and NO GIGs, while in the AT GIG the countries this biological element is not used at all.

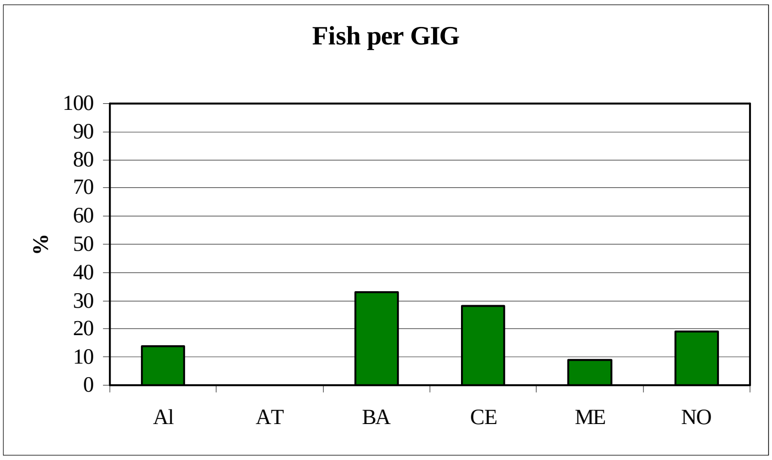

Figure 24. Percentage sampling fish in the GIGs.

26. The sampling frequency is variable generally 2-12 times per year (Fig. 25), with summer and autumn as the most common sampling seasons (Fig. 26).

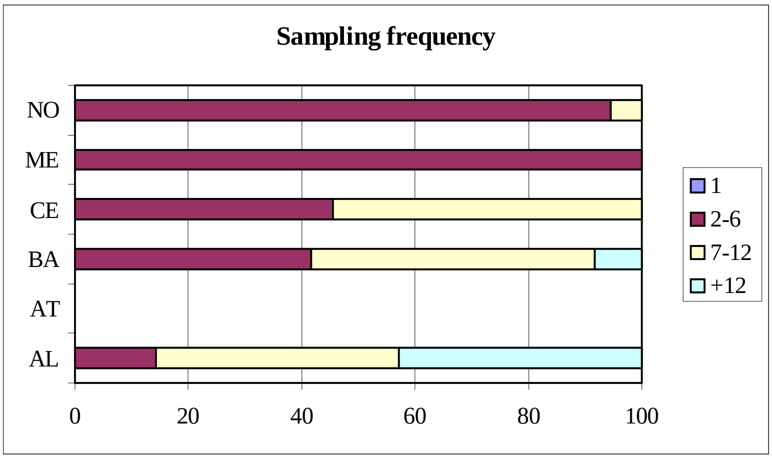

Figure 25. Fish sampling frequency in the GIGs.

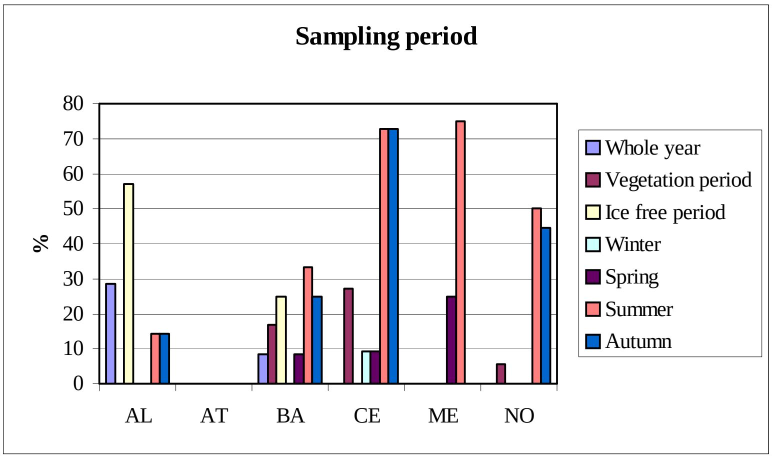

Figure 26. Fish sampling frequency in the GIGs.

27. Table 6 describes the sampling methods and metrics for fishes. It shows a wide number of sampling approaches and metrics. A total of 13 countries are sampling fishes in lakes using a total of 7 different approaches, either alone or in combination, that are used for gathering the information: net fishing (gillnets and trammel net), electrofishing, hydroacoustics, catch statistics, information from anglers and historic data. The majority of the countries determine species composition, some the native species and or functional group ratios. Fish abundance is determined as total biomass, relative biomass (CPUE), either for the whole fish community per species, and as

density. The age structure or size structure is determined in all but three countries: Poland, Finland and UK.

Table 6. Fish sampling and metrics used in different countries within the GIGs..

GIG/ Country	Sampling method	Composition metric	Quantity metric	Age/ size structure
ALPINE				
IT	net fishing	species composition		Y
AT	gillnets, electrofishing, hydroacoustics (Gassner, H. and J. Wanzenböck, 1999)	native species		Y
DE	catch statistic of professional fishermen and sport fishing; test-fishing	caught species	metric tons per year	Y
BALTIC				
PL	information from anglers; historical data	occurrence of the fish taxa on the basis of anglers' information or catches; indicator taxa		N
LT	selective fish-net, length - 5m, porosity - 14, 18, 22, 25, 36, 40, 50, 60 mm	number of species	abundance in units/ha and biomass in kg/ha	Y
EE	selectiv fish-net, 5m length, porosity - 14, 18, 22, 25, 36, 40, 50, 60 mm; Lundgren multi-mesh nylon monofilament gillnets	captured fishes are sorted by mesh size and species, measured by the total length, number of species piscivor./planktivor and piscivor./omnivor. ratios	abundance in units/ha, biomass in kg/ha, CPUE	Y
CENTRAL				
NL	Lundgren type gillnets; trawls (10x1,5 m), mesh 12 mm.	species lists	total biomass and species share of total biomass, CPUE	age distribution, size structure
GB	hydroacoustics - survey carried out using a portable echosounder operating at 200kHz. Central arm had 10 transects with a total length of 1935m	no data available for individual species	fish density at -50db and fish density at -56 dB	N
DE	Point electro fishing according to EN 14011,along transect lines; multimesh gillnets according to Swedish standard	species composition	relative abundance and estimate of biomass, CPUE	Y
MEDITERRANEAN				
ES	Trammel net, RFAI Method	species determination		size composition
NORDIC				
FI	prEN 14757 Water quality – sampling of fish with gillnets; Nordic gillnets in littoral, profundal and pelagic.	native species, species composition	CPUE, number and weight	Y

	CEN/TC 230/WG 2/ TG 4 N 28, 2nd working draft			
NO	prEN 14757 Water quality - Sampling of fish with gillnets; Nordic gillnets in littoral, profundal and pelagial, CEN/TC 230/WG 2/TG 4 N 28, 2nd Working draft	species number	unit catches, relative abundance, CPUE for each species (mainly trout)	Y
SE	Nordic multiple mesh size gillnetting. Both pelagic and bottom nets are used, see Swedish EPA report 4921 and Appelberg, 2000	species number, diversity	abundance, biomass	Y

Annex V: Analysis of river biological monitoring methods.

PHYTOPLANKTON

1. The overview of the river phytoplankton monitoring systems includes the approaches of 4 national methods: Estonia, Hungary, Latvia and Romania.

2. The phytoplankton sampling includes quantitative and qualitative methods: Estonia uses a bottle sampler, Latvia uses the Rutther bathometer (APHA 10200), Romania uses a handnet and Hungary a scoop-sampler.

3. With respect to the sampling frequency, the programmes vary from weekly sampling at some sites (Hungary) to every five years (Estonia).

4. The level of taxonomic resolution used in all the assessment methodologies based on river phytoplankton is the species or genus.

5. Abundance is expressed as number of cells (Estonia and Romania) while in Hungary is expressed in 7 abundance classes. Both Latvia and Romania calculate biomass.

6. Three different categories of assessment methods were identified in this review: biotic indices, assemblage/community assessment and multimetric indices.

7. For phytoplankton, two countries use biotic indices (Romania and Hungary), one a biomass, assemblage/community assessment (Latvia) and another a multimetric index (Estonia).

8. Romania samples phytoplankton in addition to phytobenthos, benthic invertebrates and zooplankton while Hungary samples phytoplankton in addition to phytobenthos and benthic invertebrates. Both countries use the Saprobic Index (Pantle and Buck, 1955) and classify the biological quality of the watercourses into one of five quality classes.

PHYTOBENTHOS

9. The overview of the river phytobenthos monitoring systems includes the approaches of 15 countries. The phytobenthos community includes diatom and non-diatom groups. However, most of the developed methods, especially in monitoring

programs are based only on the diatom group. This is because diatoms are found in abundance in most lotic ecosystems and are differentially adapted to a wide range of ecological conditions (Barbour *et al*, 1999). The identification of the non-diatom groups requires a considerable effort, expertise and expense. In this report only Romania has a monitoring programme covering both diatom and non-diatom groups, while DE (Germany) has a new assessment method under development that includes both groups.

10. The sampling procedure is based in the European Norms EN 13946 (2003) Water quality: *Guidance standard for the routine sampling and pretreatment of benthic diatoms for rivers for water quality assessment* and/or in national methodological guidelines. Some countries, for example Germany and the United Kingdom use the recommendations of Kelly *et al.* (1998).

11. Both documents only concern diatoms and are related to the choice of substratum and sample site selection, field sampling procedure and sample pre-treatment prior to microscope identification and lists sampling equipment and reagents. A working document for a proposed new European Standard is in preparation which includes algal groups other than diatoms (cyanobacteria, green algae, red algae, etc.).

12. A recent draft summarising the methods using benthic algae to assess water quality in running water concluded that the main processes in use for routine sampling are similar, only details that depend on current velocity and dominating type of available substratum may vary (CEN TC230 N68).

13. The available information shows that scraping with a brush from the natural substrates (stones) is the most common method. In France and in Spain a brush or a net-scrapper with 0,3mm mesh-size is used in stream sections 10 times long as wide. Quantitative sampling is performed in some cases, using a fixed surface area for sampling that varies between $9cm^2$ and $100cm^2$.

14. Artificial subtracts are only used by France and the United Kingdom.

15. Concerning the sampling habitat, 6 methodologies are multihabitat, 6 are single habitat related with hard subtracts (cobbles or stones) and 3 did not answer. All these different procedures do not allow comparability between the results as they reflect different ecological situations. The multihabitat sampling best characterizes the

benthic algae in the reach, but results may not be sensitive to subtle water quality changes because of habitat variability between reaches. Species composition of assemblages from a single habitat potentially reflect water quality differences between streams more precisely than multihabitat sampling, but impacts in other habitats in the reach may be missed (Barbour *et al*, 1999).

16. The sampling frequency for phytobenthos monitoring varies from annually in France, every 3 years in Austria and 4 years in Romania.

17. Barbour *et al.* (1999) recommended that single habitat sampling should be used when biomass of periphyton is assessed. In other circumstances the use of multihabitat sampling may be more appropriated. The only references for biomass quantification relate to the assessment method developed by Norway, which is performed in a single substratum, in riffle conditions.

18. The level of taxonomic resolution used in the assessment methodologies based in phytobenthos is the species or species groups. Only Slovenia uses a higher taxonomic level, which varies from species to family or higher taxonomic level. For phytobenthos most of the indices require species-level identification, although some can be used with genera, or a mixture of genera and species. Some *taxa* are difficult to identify and in these cases the *taxa* have been pooled into "paired taxa".

19. Despite the complexity of diatoms identification, this is costly and time consuming process and hence, the lowest taxonomic level is often preferable, providing a better ecological resolution, especially for definition of reference conditions. This problem will be enhanced if the monitoring programmes include diatoms and non-diatoms groups.

20. The identification process is based on a European Standard EN 14407 (2004) Water quality: *Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running water*. This standard establishes methods for identification and enumeration of relative proportions of diatom *taxa* on prepared slides and of data interpretation relevant to assessment of water quality in rivers and streams. It is suitable for use with indices and assessment methods based on the relative abundance of *taxa*.

21. Some countries like France, Estonia and Sweden use the OMNIDIA software for taxonomic identification (Lecointe *et al.*, 1993), which allows the calculation of a great number of diatom indices and contains a systematic and an ecological database. Consultation of other literature is also recommended for the algal identification, like floras, identification guides and iconographs appropriated to the habitats and geographic region under consideration.

22. The recording of abundance is usually expressed as the number of cells per *taxon* per sample on occasions using abundance classes. Relative coverage of the river bed is also mentioned by Norway.

23. With respect to the non-diatom group, Austria estimates abundance in microscope preparations and estimates coverage of channel bed by macroalgae. Germany is also developing a method which includes an abundance specification in 5 classes based in a verbal descriptions.

24. Three different categories of assessment methods were identified in this review: biotic indices, assemblage/community assessment and multimetric indices.

25. For phytobenthos, biotic indices are the most common assessment method including 5 different approaches all based in diatom assemblages, applied to 9 countries. Only one country is using assemblage/community assessment (Austria) and two are using multimetric indices (Estonia and Germany).

26. The Biological Diatom Index' (IBD- Indice Biologique Diatomées; AFNOR, 2000) is the only standardized method applied on a national level. This has been used in France as part of a monitoring program since 1999. Other countries, like Spain and Estonia are also using IBD as their assessment method, while Sweden use "The Generic Diatom Index" (Bourrelly, 1981) and the "Specific Pollution Sensitivity Index" (Cemagref, 1982). Both are diatom indices of French origin, and assess the general quality of the watercourses. The IBD is used to assess different pressures like organic pollution, eutrophication, acidification and toxic substances.

27. Another index commonly used is the British Trophic Diatom Index (TDI - Kelly and Whitton, 1995; Kelly *et al.*, 1998). This index is based on diatoms and provides a monitoring tool that separates the influence of nutrients from that of other constituents of sewage discharges. Its application comprises both an indication of eutrophication

by filtered reactive phosphorus and determination of the percentage pollution tolerant valves (%PTV) to estimate the influence of organic pollution (Birk, 2003). This index is used in United Kingdom and in Estonia and has been applied in Ireland.

28. As stated above, besides Romania and Hungary, Slovenia also uses a saprobiotic analysis in its monitoring programme, including phytobenthos samples in addition to the benthic invertebrates samples. This method is based in the Saprobic Index (Pantle and Buck, 1955) modified by Zelinka and Marvan (1961), in a seven scheme quality classification from unpolluted/very slightly polluted to excessively polluted. The same index is used in Czech Republic but is presented in five quality classes.

29. In Austria, a single metric is used in a diatom based trophic state indicator, which allows a classification of sites according to total phosphorous and nitrogen compounds.

30. Concerning multimetric indices, only Estonia and Germany have a multimetric assessment method under development. Estonia uses both IBD and TDI indices to evaluate the biological quality of the watercourses, while Germany is still developing a method based in non-diatom and diatom groups.

MACROPHYTES

31. Macrophytes are monitored in ten countries of the European Union also including Norway (Table 1). All methods comprise the investigation of watercourse vegetation by means of regular visual surveys to record the taxonomic composition and abundance of water plants. Assessment of the ecological quality of watercourses is done by five schemes using numerical evaluation on the basis of metrics. Austria, Germany and The Netherlands have recently developed macrophyte methods to meet the requirements of the WFD.

Table 1. European methods for monitoring and assessment of macrophytes in watercourses.

Method	Country	Reference
WFD method for macrophytes	Austria	no reference
Macrophyte monitoring	Denmark	Skriver 1999
Macrophyte monitoring	Estonia	no reference
Indice Biologique Macrophytes en Rivière (I.B.M.R.)	France	AFNOR (Association Française de Normalisation) 2002
PHYLIB	Germany	Schaumburg *et al.* 2004
Method for the survey of aquatic macrophytes in running waters	Latvia	Cimdins *et al.* 1995
KRW-Maatlatten for Aquatic Flora	The Netherlands	Molen *et al.* 2004
Macrophyte assessment	Norway	no reference
Macrophyte monitoring	Sweden	Swedish Environmental Protection Agency 2000
Mean Trophic Ranking (MTR)	United Kingdom	Holmes *et al.* 1999

32. Composition and abundance of in-stream macrophytes are recorded in the field by visual inspection from the banks or by wading in the stream. Larger watercourses are investigated by boat or diving. In some countries aqua-scopes are employed, which are specifically designed tools to survey the plant growth underneath the water surface. Rakes or grapnels are used to remove macrophytes for abundance estimation or further taxonomic identification.

33. Sampling area differs between individual methods. In the Nordic countries separate transects of 0.25 * 0.25 m^2 are sampled. The French and Latvian methods specify to survey an area of at least 100 m^2. Other schemes prescribe the investigation of stream reaches of 100 m or 500 m.

34. Latvia and Norway follow the standard EN 14184:2003 Water quality: *Guidance standard for the surveying of aquatic macrophytes in running waters*.

35. Most commonly macrophytes are identified to species level, which is usually done in the field. Uncertain identifications are verified in the laboratory by referring to herbarium species.

36. Two different schemes to record macrophyte abundance are in use. In most countries the relative coverage of the macrophyte community is measured. Methods applied in Austria and Germany specify the 'plant mass estimate' which accounts for the three-dimensional extension of the plant stand. In both options abundance is

expressed in classes. For most methods the number of classes and the defined ranges deviate.

37. The numerical evaluation of macrophyte composition and abundance is done in assessment methods of France, Germany, Latvia, The Netherlands and United Kingdom. Quality appraisal is mainly based on a single biotic index integrating indicator species and their abundance. Most indicator species included in the indices of the French and British schemes are sensitive to the trophic state of the water and the sediment. In Latvia macrophytes are part of the Saprobic System to detect organic pollution.

38. Multimetric assessment based on macrophytes is done by Germany and The Netherlands. Dependent on the stream type assessed by the German method, mosses and phanerogams are separately evaluated, or further metrics (e.g. evenness, percentage of *Sparganium emersum* etc.) are added to the results of the main index. Besides the assessment of general degradation, the method comprises modules for the detection of acidification in base-poor mountain streams. The Dutch method considers the percent coverage of plant growth forms and the abundance of stream type-specific indicator species to assess general stream degradation. In both methods the overall quality of the watercourse is derived on the basis of the analysis of the entire aquatic flora including phytobenthos.

39. The number of quality classes, in the methods discussed ranges from four to seven. The quality classification for sites analysed by the British method is indicated but a three class system has been proposed based on MTR. Here, only recommendations for the interpretation of the index value are given.

BENTHIC INVERTEBRATES

A. Summary of the national assessment methods for rivers using benthic invertebrates

40. The overview of biological assessment methods using benthic invertebrates comprises 44 schemes applied in 32 European countries. Besides methods which will be used in WFD-compliant monitoring programmes by the EU Member States additional assessment methods of specific interest have been included. The range of

countries also covers Non-EU Member States. All discussed methods are listed in the Annex III comprising data on status and literature references. Detailed descriptions of most methods comprising the complete set of acquired data are available at http://starwp3.eu-star.at (Waterview Database).

41. The following section provides a short summary of the state-of-the-art of biological methods and watercourse monitoring using benthic macroinvertebrates in each country. Information has mainly been taken from Waterview Database (2004) and Birk and Schmedtje (in print).

Austria

42. For WFD implementation a multimetric method for assessing the ecological status of rivers is in preparation. This benthic invertebrate based stressor-specific (organic pollution and habitat alteration) multimetric index for monitoring running waters in Austria has been elaborated (Ofenböck *et al.* 2004) and will be finalised by the end of 2004 covering all Austrian stream types. In this context it is planned to extend the national monitoring network to a maximum of 900 sites and to all biological quality elements (benthic invertebrates, macrophytes and phytobenthos, and fish) according to their relevance for the surface water type.

Belgium

43. Applied in Belgium since 1978 (standardised since 1984) the *Belgian Biotic Index* (BBI) represents a simple, rapid, reliable, low cost and practical assessment-tool for watercourses using benthic invertebrates The calculation of the *BBI* is performed by using a table with indicating faunistic groups and number of systematic units. A systematic group involves mostly taxonomical groups at genus or family level. The resulting value of the *BBI* is classified on a five-class quality scale ranging from lightly polluted or unpolluted to very heavily polluted (cf Republic of Ireland system).

44. The *Biotic Sediment Index* (BSI) is an adapted version of the *Belgian Biotic Index* based on the taxonomic diversity of benthic macroinvertebrate community and the presence or absence of indicator taxa in a given sediment sample. The degree of pollution sensitivity of a taxon is related to 13 contaminants (heavy metals, organic toxicants) resulting in five pollution tolerance ranks. *BSI* score can vary between 10 and 0, corresponding with four sediment quality classes.

Bulgaria

44. In Bulgaria, the Danube and its tributaries have been studied on the basis of benthic invertebrate data for more than twenty years. The national method for biological water quality assessment of running waters is a biotic index based on the Irish *Quality Rating System*. The *Saprobic Index* was formerly prescribed in the COMECON[9] agreement, but today it is only used in the frame of scientific analyses (Peev and Gerassimov, 1999).

Cyprus

45. In Cyprus the biodiversity of the macroinvertebrate fauna is being quantified according to the Sequential Comparison Index (SCI) methodology and an estimate of a Species Deficit Score (SDS), comparing the local number of species with the number of species at a reference or upstream area. Organic pollution is being evaluated according to the method prescribed by the Biological Monitoring Working Party (BMWP).

Czech Republic

46. *Saprobiological monitoring* based on investigations of the benthic invertebrate fauna is used for the standardised assessment of organic pollution in Czech rivers. The degree of pollution is evaluated according to the technical norm ČSN 75 7716 (1998) and is applied in a large monitoring network (approx. 1450 sites).

47. Since 2002 small watercourses have been monitored by using the *PERLA* prediction system. Here, the observed fauna is compared with an expected stream type-specific reference fauna in order to assess biological quality.

48. Besides the *PERLA* system, a multimetric approach has been elaborated, which is a Czech modification of the AQEM system (Brabec *et al.*, 2004). In this study organic pollution and morphological degradation have been assessed on three small and mid-sized stream types. For WFD implementation the application of both predictive and multimetric assessment are under discussion.

Denmark

[9] Council for Mutual Economic Assistance

49. The *Danish Stream Fauna Index* is determined on the basis of indicator taxa and the number of diversity groups in the total fauna sample (kick samples and hand-picked samples). The first step of assessing the quality class is done by assigning the sampled fauna to one of six indicator groups based on at least two specimens of selected taxonomic groups found in the kick samples or one specimen found in the hand-picked sample, respectively. Additionally, selected species belong to positive (e.g. Baetidae, *Nemoura*) or negative (e.g. *Erpobdella*, *Sialis*) diversity groups, which are added up and classified (four ranges in total) within the second step of assessment. Carrying-out both steps leads directly to one of seven quality classes, ranging from 'unimpacted' to 'very strongly impacted'.

Estonia

50. The *Estonian quality assessment of watercourses* is based on the Swedish *Benthic Fauna in Lake Littorals and Running Water - Time Series* method. It uses four selected indicator metrics of macroinvertebrate diversity and distribution: Shannon's Diversity Index, ASPT Index, Danish Stream Fauna Index and Acidity Index. This multimetric approach leads to an assessment of both watercourses (riffle areas) and littoral zone of lakes, resulting in one of five quality classes. It is planned to use the ratio between observed index value and expected reference value to indicate the extent to which bottom fauna conditions deviate from an undisturbed (natural) state.

Finland

51. Assessment is based on riffle/ rapid kick net samples with three replicates (Finish satandard SFS – 5077). A multimetric method for assessing the status of macroinvertebrates has been tested. The system under preparation consists of several metrics, e.g. number of type-specific species, EPT taxons and abundance of indicator species.

France

52. The *Standardised Biological Global Index* (I.B.G.N.) is widely used in monitoring programmes in France since 1992. Benthic invertebrates are usually identified to family-level, some groups to higher taxonomical level. The method embraces 138 different taxa to determine the ‘total variety of the sample (Σt)’ split up into 14 variety classes. 38 taxa constitute nine ‘faunal indicator groups (GI)’, which are selected if

three (or ten, respectively) individuals belonging to an indicator taxon are found in the sample. On the basis of an index value-chart the *I.B.G.N.* is calculated directly - dependent on variety class (table rows) and indicator group (table columns) and assigned to 5 quality classes.

53. The *Oligochaeta Index for Sediment Bioindication* (I.O.B.S.) assesses the general quality of stable and permanent fine sediments of natural and artificial watercourses and indicates their susceptibility to gross organic stress and micropollutants such as metals and PCBs. To calculate the index 100 individuals have to be identified. The total number of taxa (only Oligochaeta) and the dominant percentage of Tubificidae with or without hair-chaetae are determined. The latter group indicates the effect of micropollutants.

<u>Germany</u>

54. For WFD implementation a multimetric approach for biological classification based on benthic invertebrates was developed for selected stream types across Europe (AQEM-Project, Hering *et al.*, 2004). In Germany, this approach is now being expanded to cover all stream types that have been identified for WFD implementation. The method includes modules for the detection of organic pollution, acidification and general degradation. The method will undergo testing regarding its practicability in 2005 and 2006.

55. Part of the multimetric scheme is the *Potamon-Characterisation-Index*, which is specifically designed for the ecological status assessment of large rivers and is based on the presence of typical species of the potamon (lower course of the river).

<u>Greece</u>

56. In Greece, no generally applied assessment method exists. The high proportion of endemic species necessitates thorough autecological studies prior to the development of evaluation methodologies. Additionally, a sampling network has to be established.

57. The European AQEM project generated a multimetric classification scheme for three Greek stream types based on macroinvertebrate sampling. Its application is under development. The *Hellenic Evaluation System* is an adaptation of the BMWP-ASPT score to Greece conditions.

Hungary

58. Since 2002 a modification of the British *BMWP-ASPT score* system is applied featuring newly included taxa and modified scores. Combination of total score and average score per taxon results in an index value, which is assigned to one of five classes of watercourse quality. The method is in preliminary phase and practical experience and taxonomic expertise are advancing.

Ireland

59. The *Quality Rating System* used in Ireland relates the relative abundance of five key groups of benthic invertebrates to water quality. In each group taxa of similar sensitivity (mainly to organic pollution) are integrated. The index is based primarily on individuals identified to family level. Additionally, the British *BMWP* system are applied in Northern Ireland. BMWP includes ASPT.

Italy

60. The *IBE* is based on the *Extended Biotic Index* according to (Woodiwiss, 1978) and is a standard method for assessing Italian watercourses. For determination of the *IBE* value two factors are considered: taxa richness (diversity) and presence of sensitive taxa (indicator groups). Calculation is performed with a cross-table. The resulting index reaches values between 0 and 14, the highest number indicating the best water quality.

Latvia

61. The standardised *Operative Evaluation of the Biological Quality of Small Streams* is used for biological quality control of small streams with a flow velocity more than 0,1 m/s. The Saprobic Index is calculated according to Zelinka and Marvan (1961) and the results are assigned to one of five quality classes. Water quality evaluation of larger streams in Latvia is done by the Saprobic *Assessment of River Water Quality based on hydrobiological indicators*.

Liechtenstein

62. The Austrian method *Assessment of saprobiological quality of rivers* is used in Liechtenstein to assess the impact of organic pollution on streams. The modular structure of this method allows different procedures to be chosen for specific purposes

based on increasing temporal (sampling and determination) effort and the level of precision.

Lithuania

63. No information

Luxembourg

64. The official method used by the Environment Administration to assess biological water quality in Luxembourg is the French *I.B.G.N.* Few sites per year are studied and no general and recent maps of biological water quality for the whole country are available at the moment.

65. At the 'Public Research Centre - Gabriel Lippmann' a biocoenotic study on aquatic benthic invertebrates has been conducted since 1994, which primarily aims to study biodiversity. In this context the dataset is used to test several indices (among them BMWP, ASPT and DIN 38 410). Within the next three years it is intended to generate a predictive system (similar to RIVPACS or PERLA) out of which a system for ecological water quality assessment will be developed.

Netherlands

66. In the Netherlands a new method to assess the ecological quality of running waters has recently been developed to fulfill the requirements of WFD-compliant monitoring. The scheme enables stream type-specific evaluation based on reference conditions and comprises composition and abundance metrics.

67. General methods for ecosystem description and assessment that are already applied in the Netherlands are called *AMOEBA.* In principle an *AMOEBA* can be developed for all water types. An important part of the approach is the description of the reference situation. The reference system is quantified by means of target variables, i.e. organisms, which are representative of the different parts of the aquatic ecosystem. By expressing the present-day values of the target variables as a percentage of reference values ecological assessment is possible.

Norway

68. Macroinvertebrates from rivers are used in the assessment of organic pollution, acidification, heavy metal pollution and effects of hydropower regulations. An

Acidification Index, based on the sensitivity of the various taxa, is used to assess the effects of acidification. The classification system is modified to cover acidification of organic rivers. In eutrophication studies, different indices such as the Modified Trent Index, BMWP, ASTP, Shannon-Weaver are used.

69. Knowledge on Norwegian river macroinvertebrates is based on national monitoring programs on acidification and liming (22 rivers including only non-limed sites) and various surveys related to effects of local pollution and river regulations (approx. 900 sites).

Poland

70. The *BMWP* system provides a score for each macroinvertebrate family that is primarily dependant on its sensitivity to organic pollution. In use in the United Kingdom since the late 1970s, this method is intended to be applied in Poland, operating with a modified list of indicator taxa. It is recently elaborated and will soon be utilised within a pilot project. To verify the results obtained by calculating the BMWP score, a diversity index expressed as the ratio of number of families to macroinvertebrate fauna abundance is determined.

Portugal

71. For WFD-compliant monitoring the *BMWP'* will be used which is an adaptation of the British Biological Monitoring Working Party (BMWP) score system. The modifications include the addition of new families, changes in some scores and division of scores into five classes, representing various degrees of organic pollution.

Romania

72. Monitoring of aquatic biota throughout Romania is conducted four times per year within the framework of the National Water Monitoring System, which started in 1978. In the past, biological quality assessment was based on the *Relative Load Method* (Knöpp, 1955). Now, biological quality assessment of running waters is based on the determination of the *Saprobic Index* according to Pantle and Buck (1955). Since the beginning of 2004 the index scores have been classified in a five-band scheme following the recommendations of Knoben *et al.* (1999).

73. In preparation of WFD implementation a national stream typology has been developed. In addition, a multimetric assessment method was completed, which determines the biological water quality based on the effects of organic pollution, toxic substances and morphological degradation.

Spain

74. Within the Spanish project GUADALMED a method to assess the ecological status of streams and rivers in the Spanish Mediterranean area has been developed (ECOSTRIMED). The main objective of *ECOSTRIMED* is to provide an integrated quality assessment system for fluvial ecosystems. It includes the riparian habitat and the biological quality of the water as the main parameters. Designed as a Rapid Bioassessment Protocol (RBP) it is performed directly in the field with a short sampling time schedule.

75. The *ECOSTRIMED* index is calculated by combining the values of two different quality indices: 1) a biological index based on macroinvertebrates (FBILL or IBMWP), 2) a Riparian Habitat Quality Index (QBR). In accordance with the requirements of the WFD the result is presented in one of five classes of ecological status.

Sweden

76. The Time Series as a component of the Swedish method *Benthic Fauna in Lake Littorals and Running Water* uses four selected indicator metrics of macroinvertebrate diversity and distribution: Shannon’s Diversity Index, ASPT Index, Danish Stream Fauna Index and Acidity Index according to Hendikson and Medin. This approach leads to an assessment of watercourses (riffle areas), resulting in one of five condition classes for each metric. The ratio between observed index value and expected reference value is used to indicate the extent to which bottom fauna conditions deviate from an undisturbed (natural) state.

Switzerland

77. Switzerland uses the component *Benthos biology* of the *Methods for Investigation and Assessment of running waters* to evaluate biological watercourse quality. The method‘s objectives are to gain information on the occurrence of common macroinvertebrate taxa and to appraise and assess impacts on its composition.

Identification is predominantly to family level to meet the demands of a rapid, low expenditure system. The two parts of the assessment are the standardised calculation of two quality indices (1. calculation either according to BBI or I.B.G.N., 2. number of scoring taxa) and a verbal characterisation of the river status (discussion of special aspects concerning the invertebrate-community). The values of both quality indices are used to determine the quality class (5-class scheme).

<u>United Kingdom</u>

78. Widely used by the Environment Agency for England and Wales for reporting National River Quality surveys since 1996, the *Biological GQA* method is applied in combination with chemical examinations (Chemical GQA). It is based on the presence of major groups of invertebrates (mostly families). The two indices used for the classification are the average BMWP-score per taxon (ASPT) and the number of scoring taxa (N-taxa, i.e. the number of major groups used in the scheme). Having calculated these indices, they are compared to those expected in an unpolluted river by means of RIVPACS (River Invertebrate Prediction and Classification System - computer-based, mathematical model which predicts the river fauna using physical stream data). This comparison is expressed in the Ecological Quality Index (observed: predicted ratio) for both the number of taxa and the ASPT. The resulting EQI-values are assigned to one of six quality classes. It is planned to include the *Lotic-invertebrate Index for Flow Evaluation* (LIFE) Index into the GQA scheme.

79. In addition, the *Acidification Index* is a biological classification scheme for sensitivity to acidification (index of acid conditions). The *System for Evaluating Rivers for Conservation* (SERCON) is used to identify important rivers for conservation and to monitor river rehabilitation schemes.

B. Comparative analysis

<u>Pressures targeted</u>

80. Table 2 lists the percentage of assessment methods using benthic invertebrates to detect certain anthropogenic pressures. Within the range of particular stressors biodegradable organic pollution represents the main focus of bioassessment in European rivers. All other specific pressures are less prevalent. With nearly 30 percent the detection of general degradation (stressor not specified) holds the second largest portion.

Table 2. Percentage of assessment methods detecting specific pressures.

pressure	percentage
Organic Pollution	48
General Degradation	29
Morphological Degradation	9
Acidification	9
Toxic Substances	5

<u>Sampling techniques</u>

81. The sampling procedure of a large number of methods applied in monitoring programmes using benthic invertebrates is based on the International Standard ISO 7828 (1985) or the adopted European Norm EN 27828 (1994): *Water quality – Methods of biological sampling - Guidance on handnet sampling of aquatic benthic macro-invertebrates*. Several methods which do not directly refer to these international standards carry out 'kick and sweep' sampling that is regulated by national norms/methods.

82. In general, this technique is the most common sampling procedure and applied in 30 methods using a hand-net. The nets used differ in size of the opening and mesh. Net-openings specified by the assessment methods vary between approximately 600 and 900 square centimetres. In half of the schemes, animals are retained by mesh-sizes of about 500 µm. Mesh-sizes of 1 mm are particularly used in British schemes operating at family level (*Biological GQA*, Hungarian adaptation of the *BMWP score*). Hand-nets of less than 350 µm mesh-size are used in methods focussing on selected orders (e.g. Oligochaetes: *Oligochaeta Index for Sediment Bioindication*). In addition, small mesh-sizes are in use in some Mediterranean countries (Croatia, Italy, Spain). To sample sandy or silty substrates or sites with large amount of detritus hydrobiological sieves are used in Bulgaria with a mesh-size of 1.5 mm.

83. The procedure of quantitative sampling is standardised by the guidance ISO 8265 (1988) or EN 28265 (1994): *Methods of biological sampling - Guidance on the design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters*. Surber samplers are most commonly used for quantitative, area-related sampling. The recommendations of this standard for maximum aperture

size of the net range between 250 to 750 µm. The models currently applied in various national watercourse monitoring programmes differ in sampled area (0.01 to 0.12 m^2) and mesh-size (100 to 500 µm).

84. In deeper streams benthic macroinvertebrates are taken using grabs, dredges and artificial substrates. The application of these devices is standardised in ISO 9391 (1993) or EN ISO 9391 (1995). These are the same standards, as are the previous ones, as a result of adoption by ISO and CEN. The difference in dates is caused by the different dates of publication by the two organisations:

85. In many European countries large rivers are monitored by utilisation of quantitative bottom samplers: Different types such as Ponar Grab, Van Veen Grab, Petersen Grab, Birge-Ekman Grab and core samplers are used. All devices sample defined areas of the river bottom ranging from 100 to 500 cm^2.

86. To obtain a qualitative sample of the river bed community, dredges represent adequate tools in deeper watercourses. Dredges are particularly applied in several countries in the Danube catchment (Austria, Bulgaria, Hungary, Romania, Serbia-Montenegro) using mesh-sizes of 225 and 500 µm.

87. The use of artificial substrates allows comparability of different sites by providing similar habitats is not widely used for monitoring purposes. In monitoring programs only Moldova (*Saprobiological assessment based on various metrics*), Austria (*Assessment of saprobiological quality of rivers*) and France (*I.B.G.N.*) employ these colonisation devices.

88. Note that the ISO and CEN standards for sampling invertebrates are currently under revision. This should make them more WFD compliant, but the timescale for publication will inevitable be several years.

Sampling frequency

89. Sampling frequency in biomonitoring programmes using benthic invertebrates as indicators of watercourse quality varies from seasonal collections to procedures conducted every five years. Annual sampling is the most common interval applied in river monitoring. In particular, programs observing the saprobiological water quality in Latvia, Moldova, Romania and Serbia-Montenegro as well as the national Italian monitoring programme based on *IBE* take seasonal samples of macroinvertebrates.

This inevitably influences the degree of uncertainty of the resulting ecological classifications i.e. the likelihood of the banding allocated being accurate.

Metrics

Level of taxonomical resolution

90. The level of taxonomical resolution used in watercourse assessment methods based on macroinvertebrates differs.

91. Nearly 60 percent of the bioassessment methods applied in Europe determine at least selected orders of benthic invertebrates to species- or species groups-level. The latter intends to preserve some of the species-level information without the necessity to identify to species (Buffagni, 1997). The remaining approximately 40 percent of methods, identify organisms to genus or family. Table 3 provides an overview of taxonomical resolution required in various quality assessment methods applied in Europe.

Table 3. Taxonomical resolution required by various watercourse assessment methods applied in Europe.

species-/species groups-level	genus- and higher level
Saprobic Indices (applied in Austria, Bosnia-Herzegovina, Croatia, Czech Republic, Estonia, Germany, Latvia, Lithuania, Moldova, Romania, Serbia-Montenegro, Slovak Republic, Slovenia) ***Multimetric Indices*** (e.g. Austria, Germany and Netherlands) ***Functional Indices*** (PTI – Schöll and Haybach, 2001; LIFE Index – Extence *et al.*, 1999) ***Acidification Indices*** (Germany - Braukmann and Biss, in print; Norway – Raddum, 1999) ***IOBS*** (AFNOR, 2002) ***PERLA*** (Kokeš *et al.*, 2003) ***Swedish Benthic Fauna in Lake Littorals and Running Water*** (SEPA, 2000)	***Acidification Index*** (Rutt *et al.*, 1990) ***Belgian Biotic Index*** (De Pauw and Vanhooren, 1983) ***BMWP-ASPT Index*** (Armitage *et al.*, 1983; Alba-Tercedor and Pujante, 2000) applied in Cyprus, Hungary, Spain, Portugal, Poland, United Kingdom ***Danish Stream Fauna Index*** (Skriver *et al.*, 2000) ***Extended Biotic Index*** (Ghetti, 1997) ***IGBN*** (AFNOR, 1992) ***Quality Rating Scheme*** (McGarrigle *et al.*, 1992) ***Hellenic Evaluation System*** (Lazaridou-Dimitriadou *et al.*, 2004)

92. Several methods demand the identification of genera only for particular groups of organisms (Table 4). The Italian as well as the Belgian index includes all genera of the orders Plecoptera, Ephemeroptera, Odonata and the class Hirudinea amongst others. The identification of selected genera of stoneflies, gammarids and chironomids is required by the Danish and Irish schemes primarily mayflies to genera in the latter. In addition, the *DSFI* comprises indicators of the orders megaloptera and coleoptera, and the genus *Ancylus*. The Irish *Quality Rating Scheme* considers individuals of a

water bug genus (*Aphelocheirus*) and species of *Baetis rhodani*. The British method is based on identification of all genera of the family Chironomidae (Ruse, 2000).

Table 4. Selected methods determining particular organism groups to genus level.

Method	Determined genera
Belgian Biotic Index (De Pauw and Vanhooren, 1983) and ***Biotic Sediment Index*** (De Pauw and Heylen, 2001)	all genera of the phylum/class/order Plathelminthes, Hirudinea, Mollusca, Plecoptera, Ephemeroptera, Odonata, Megaloptera, Hemiptera
Danish Stream Fauna Index (Skriver *et al.*, 2000)	*Brachyptera, Capnia, Leuctra, Isogenus, Isoperla, Isoptena, Perlodes, Protonemura, Siphonoperla, Limnius, Amphinemura, Taeniopteryx, Elmis, Elodes, Ancylus, Asellus, Chironomus, Gammarus, Sialis*
Extended Biotic Index (Ghetti, 1997) and ***Indice a Rapporto*** (Stoch, 1986)	all genera of the class/order Tricladia, Hirudinea, Plecoptera, Ephemeroptera, Odonata
Quality Rating Scheme (McGarrigle *et al.*, 1992)	*Leuctra, Aphelocheirus, Rheotanytarsus, Gammarus, Baetis rhodani, Asellus, Chironomus*
Chironomid Pupal Exuviae Technique (Ruse, 2000)	all genera of Chironomidae

93. The French *Oligochaeta Index for Sediment Bioindication* focuses on selected orders to indicate the quality of watercourses combining achievement of precise ecological information through identification to species or species group level with low-cost sample processing. In general, this approach allows application of specific sampling techniques, ensuring maximum coverage of the group studied.

94. It must be noted that in several assessment methods taxa are identified to lower levels than required to adequately compute the respective quality index.

<u>Record of abundance</u>

95. In general, there are two alternatives to indicate the abundance of particular taxa of benthic invertebrates found in the sample: (1) number of individuals per area, (2) abundance stated in ranges e.g. logarithmic, (3) abundance not recorded - not recording is not an estimate of abundance.

96. The option with the most substantial information content is the specification of the number of individuals per area. Nearly 50 percent of macroinvertebrate methods record the abundance of individuals that way. In fact, purely quantitative data require area-related sampling procedures by means of quadrate samplers, grabs or similar devices. Since these requirements are only met by a few schemes, abundance statements based on semi-quantitative hand-net sampling are in most cases of restricted reliability but they are cheap, practical and.effective.

97. The use of range values to record individual occurrence allows the estimation of taxa abundance. Compared to the first option, this alternative is time saving because an exact count of individual organisms is not necessary. Different abundance classification schemes are in use, the most common are listed in Table 5.

98. The 3-class scheme derives from the well-known publication of Pantle and Buck (1955) and is mostly used in eastern European countries applying the Saprobic Index (Bosnia-Herzegovina, Croatia, Slovenia).

99. Two systems to classify abundance in a five-fold scheme exist in Europe: The classes of the British system are based on a logarithmic scale of organisms' abundance (Murray-Bligh, 1999). Different from this is the allocation of classes in the *Quality Rating System* as applied by Bulgaria. Here, the presence of more than 100 individuals of a certain taxon is assigned to the highest class. Hungary specifies relative taxa abundance of a sample (five metres section kicked for 15 minutes). Other schemes do not specify numerical boundary values but provide only verbal descriptions of abundance classes (e.g. McGarrigle *et al.*, 1992).

100. In German watercourse bioassessment abundance is stated in seven classes. Established by Knöpp (1955) this classification has been included in the German standard DIN 38 410 (1990, 2003): *Determination of Saprobic Index of Running Waters*. Beyond its broad application in Germany, methods in Serbia-Montenegro, Slovakia and Switzerland operate on the basis of a seven-class abundance scheme.

101. Assessment methods operating on the basis of presence/absence data of macroinvertebrate taxa do not necessarily need to record taxa abundance. Many biotic indices like *I.B.G.N.* (France, Luxembourg), *IBE* (Italy), *BMWP-ASPT* (e.g. Cyprus, Portugal, Spain, United Kingdom) or *BBI* (Belgium) are not designed to include abundance information. Their outputs may be biased by single organisms drifting into the sample from upstream reaches. Therefore, the individual systems prescribe to include only taxa that exceed a certain threshold of abundance to avoid false results.

Table 5. Common abundance classification schemes used in European watercourse assessment methods based on benthic invertebrates

<table>
<tr><th></th><th>class</th><th colspan="2">number of organisms</th><th>description</th></tr>
<tr><td rowspan="3">3-class scheme (Pantle and Buck, 1955)</td><td>1</td><td colspan="2">1 – 10</td><td>sporadic</td></tr>
<tr><td>3</td><td colspan="2">10 – 100</td><td>frequent</td></tr>
<tr><td>5</td><td colspan="2">> 100</td><td>in masses</td></tr>
<tr><td rowspan="6">5-class scheme</td><td></td><td>Bulgarian Standard</td><td>Murray-Bligh (1999)</td><td>McGarrigle (1992)</td></tr>
<tr><td>1</td><td>1 – 5</td><td>1 – 9</td><td>usually absent</td></tr>
<tr><td>2</td><td>6 – 20</td><td>10 – 99</td><td>sparse or absent</td></tr>
<tr><td>3</td><td>21 – 50</td><td>100 – 999</td><td>present in small numbers</td></tr>
<tr><td>4</td><td>51 – 100</td><td>1,000 – 9,999</td><td>common</td></tr>
<tr><td>5</td><td>> 100</td><td>10,000 +</td><td>well represented or dominant</td></tr>
<tr><td rowspan="7">7-class scheme Knöpp (1955)</td><td>1</td><td colspan="2">1</td><td>single</td></tr>
<tr><td>2</td><td colspan="2">2 – 20</td><td>sparse</td></tr>
<tr><td>3</td><td colspan="2">21 – 40</td><td>sparse to medium</td></tr>
<tr><td>4</td><td colspan="2">41 – 80</td><td>medium</td></tr>
<tr><td>5</td><td colspan="2">81 – 160</td><td>medium to abundant</td></tr>
<tr><td>6</td><td colspan="2">161 – 320</td><td>abundant</td></tr>
<tr><td>7</td><td colspan="2">> 320</td><td>in masses</td></tr>
</table>

<u>Assessment</u>

102. The assessment of the biological quality of running waters is based on the analysis of measurable attributes of the biotic environment. According to the type and scope of measured parameters (“metrics“) different categories of assessment methods can be distinguished (Knoben *et al.*, 1995; Verdonschot, 2000):

103. Biotic Indices integrate taxa richness and pollution tolerance metrics. The basic principle of this approach is the assumption that taxa showing different sensitivity to disturbance disappear in a certain order as the pressure increases. In addition, the number of taxonomic groups is reduced.

104. Extended by abundance information Saprobic Indices represent specific modes of biotic scores. Based on the work of Kolkwitz and Marsson (1902; 1908; 1909) saprobic systems have been revised with regard to quality classification and

presentation (Liebmann, 1962), calculation (Pantle and Buck, 1955), indication (Zelinka and Marvan, 1961) and general scientific framework (Sládeček, 1973). Due to these modifications different specifications of the system exist. In Europe 15 countries apply Saprobic Indices using different quality elements, class boundaries, indicator lists and calculation formulas. The Saprobic System has been a standard method in former COMECON countries. Thus, its application is especially widespread in the Danube River Basin.

105. Apart from detection of organic pollution biotic indices are used to assess acidification of watercourses. The indices applied in Germany (Braukmann and Biss, in print), Norway (Raddum, 1999), Estonia and Sweden (SEPA, 2000) differentiate diverse macroinvertebrate groups of gradual sensitivity to acidification. Organisms belonging to the same indicator group are summed to determine the acidity-class by exceeding a specific frequency-threshold.

106. Diversity Indices combine measurement of taxa richness and abundance. In non-multimetric assessment they are exclusively applied in connection with scientific monitoring programmes to obtain an integrative picture of community structure. Especially, Shannon and Weaver (1949) and Simpson (1949) diversity are calculated. Results are not directly included in quality classification.

107. Compared to Biotic Indices, which are usually stressor-specific and useful in monitoring water quality changes when the cause of the disturbance is known (Johnson 1995), Predictive Assessment offers an evaluation focusing on shifts in the composition of entire species communities. Hence, it allows the detection of impacts on stream biota caused by any kind of pressure, but provides no information on the type of pressure acting. The British *RIVPACS System* (Wright *et al.*, 2000) is a Predictive Assessment scheme based on multivariate analysis techniques. To implement the scheme, biological data of different reference sites have been classified according to their species assemblage. These classes have been related to a series of environmental watercourse attributes. Based on these variables the undisturbed species community can be predicted at any site and compared to the community observed at this site. Results are presented as Ecological Quality Ratios (EQRs). The Czech *PERLA* system (Kokeš *et al.*, 2003) represents a modification of *RIVPACS* adapted to Czech conditions.

108. Process Assessment focuses on evaluation of taxon characteristics such as functional groups and species traits. The *Lotic-invertebrate Index for Flow Evaluation* (LIFE) assesses the impact of variable flows due to regulation or augmentation on benthic populations. Every regularly encountered invertebrate species and family of Britain has been assigned to one of six groups which are related to certain flow conditions.

109. The definition of distinct reference communities as basis of assessment for large watercourses is difficult due to substantial anthropogenic influence and the occurrence of newcomer species occupying ecological niches. Here, the concept of Process Assessment is suitable as it appraises the performance of ecological functions rather than the presence of individual species. The *Potamon-Characterisation-Index* (PTI) thus operates on the basis of an "open" taxon list in which all species showing preference to potamal habitats are indicators of high quality. By definition newcomers have low ecological values.

110. Rapid Bioassessment represents a combination of biological and habitat quality assessment emphasising a low-cost approach through reduced sampling and efficient data analysis. Investigation of both biotic and habitat features aims at obtaining an integrated ecological watercourse assessment. In Europe the only Rapid Bioassessment Protocol in current usage is the Spanish *ECOSTRIMED* method, which comprises the Spanish modification of the *BMWP score*.

111. A fundamental concept of Multimetric Assessment is to analyse community health composed of community structure, community balance and functional feeding groups (Barbour *et al.*, 1992). In this context it represents an integrative approach to water quality assessment combining various metrics like Biotic, Saprobic and Diversity Indices, and Process Assessment measures.

112. Within the European research project AQEM ("The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates") multimetric assessment systems using benthic invertebrates have been developed for a limited number of stream types in Austria, Czech Republic, Germany, Greece, Italy, Netherlands, Portugal and Sweden (AQEM Consortium, 2002). Based on this experience, Germany (Meier *et al.*, in print) and Austria are implementing multimetric assessment at a

national level in 2005. In Luxemburg and Serbia (Tripković, 2003) studies to test the AQEM approach have been undertaken or are planned, respectively.

113. Comprehension of all essential abiotic and biotic components constituting the stream ecosystem is aim of Ecosystem Components Assessment. In this approach various factors affecting the characteristics of running waters are involved. Additionally, the outputs of assessment directly support water managers in decision making.

114. In the Netherlands the *General method for ecosystem description and assessment* (AMOEBE) relates physical, chemical and biological variables of large water bodies to reference conditions to derive politically passable target values. A special feature is the integrative visualisation of the different parameters.

115. The *System for Evaluating Rivers for Conservation* (SERCON) is used to identify important rivers for conservation and to monitor river rehabilitation schemes. *SERCON* focuses on the physical, chemical and biological features of river channels and banks, riparian zones and associated floodplains, and includes catchment characteristics like land-use and human population density. The system evaluates data of 35 attributes, grouped within six conservation criteria: Physical Density, Naturalness, Representativeness, Rarity, Species Richness and Special Features.

FISH

116. A short description of the methods currently in use and those developed within the FAME project is given below.

<u>'Index of Biotic Integrity' (IBI</u>)

117. First developed in North America by Karr (1981) the concept has been adapted to regional conditions in Europe (Belpaire *et al.*, 2000; Kesminas (2000) Appelberg *et al.*, 2002; Oberdorff *et al.*, 2002; Schager and Peter, 2002).

118. Most IBI developed world-wide and applied in European countries have preserved the four original categories: 1, species composition and diversity, 2, trophic composition, 3, fish abundance and 4, reproduction and condition.

119. The methods below are IBI fish based methods from the FAME project:

Austria

120. MuLFA: Ecological Integrity Assessment Method of Melcher and Schmutz Method proposed by Schmutz *et al.* (2000) as a multilevel concept for fish based, river-type-specific assessment of ecological integrity (EI) in Austria. This method targets the assessment of general degradation, through the following metrics: species diversity (type-specific species, species with self-sustaining populations), species composition (fish region, number of guilds, guild composition), population size (density and biomass), reproductive success and recruitment (population age structure). Reference conditions are estimated from historical abiotic and fish data, data of reference sites and models. Five levels of ecological integrity (EI) and their degradation correspond to the normative definitions of ecological status developed within the WFD.

Belgium

121. IBIP (Wallonia): A fish based index developed for assessment of lotic ecosystem in Wallonia by Kestemont *et al.* (2000). This method retained the metrics of the original IBI (12 metrics) but modifying it to be applicable to the Meuse basin. The metrics were classified into 4 broad categories: species richness and composition, trophic composition, fish abundance, and reproductive function. For a given sampling site, each metric received a score ranging from one to five points, according to the level of similarity (low = 1, high = 5) of its value to that expected for a fish assemblage experiencing little human influence. The total IBI score was the sum of the 12 metric scores and ranged from 12 (worst) to 60 (best). Rationale for the selection of the 12 metrics has been described in Didier (1997) and Kestemont *et al.* (2000).

122. IBI (for upstream brooks used in Flanders): a method developed by Breine and Belpaire (submited) using a fish dataset from electric fishing surveys in Flanders during the period 1994-2000. A total of 154 sites belonging to the grayling and trout zone were used to develop a multimetric index to assess upstream brooks in Flanders. All sites had a slope of at least 3‰ and a maximum width of 4.5 m. The developed IBI consists of a set of metrics scored from 0 to 5. These metrics were selected using univariate and multivariate analyses, taking into account ecological criteria. Some of the selected metrics correlate significantly with the slope of the river. Threshold

values for correlated metrics were defined using the mean trend line through all metric values. If no significant correlation existed scoring criteria were defined using the literature. Five integrity classes were defined. The IBI was tested internally by comparing the IBI scores and the attributed habitat quality scores. A similar comparison was done using an independent set of data of known habitat quality. The individual contribution of the selected metrics was assessed. The developed IBI clearly distinguishes good sites from impacted sites and can separate the heavily impacted sites from the fairly impacted. The IBI meets the criteria imposed by the European Water Framework Directive.

France

123. FBI : Oberdorff *et al.* (2001) developed a 7 metrics index that can be applied in different regions and river types of France despite the complex and heterogeneous geology and climate of this country.

Germany

124. FiBS: A multimetric method for the assessment of the ecological status of rivers using fish assemblages (Dussling *et al.* 2004). The method is based on the comparison of recent fish samples with reconstructed type specific reference fish communities. For that purpose in total 18 metrics were selected, which can be categorized into six ecological quality features as follows: inventory of species and guilds; abundance of species and guilds; age structure; migration (index-based); fish region (index-based); dominant species (index based).

125. The assessment of the ecological river-status with FiBS comprises three steps. In a first step, all metrics are scored according to defined criteria following the approach of the IBI (Karr, 1981). In a second step, an assessment of each of the six ecological quality features is carried out. Finally, in a third step, the overall classification of the referring sampling site is performed by an algorithm calculating a weighted average from the six quality features assessed. Since 2004, FiBS is been tested nation-wide.

Sweden

126. Swedish fish Index: Appelberg *et al* (2000) proposed a set of metrics based on standardised fish sampling, for assessing environmental disturbances in fish communities in Swedish lakes and streams. Reference values for the fish community metrics and scoring criteria in relation to regional and local environments were estimated, using two comprehensive national databases comprising fish community data from lakes and streams. In concordance with Minns *et al.* (1994), the databases were assumed to comprise both degraded and reference habitats.

English

127. IBI: Rahman *et al.* (2002) developed an index to assess ecological integrity in lowland rivers. The same IBI is also used in other countries as Finland, Italy, Lithuania, Netherlands, Spain and Switzerland.

Other Fish Methods

128. Czech Republic **-** Fish Stock assessment CEN/TC 230/WG 2/TG 4N (2001) Water analysis – Sampling of Fish with electricity. Revision of Pr EN 14011. 25. October 2001

129. Denmark - Udvidet biologisk program. Skriver, J *et al* (1999)

130. Finland - A fish based index for classification of the ecological status of rivers is under development. In fish sampling of rivers, electrofishing according to SFS – EN 14011 (Water analysis – sampling of fish with electricity is applied.

131. France - Indice Poisson en Riviere (FBI). Oberdorff, T et al (1992)

132. Italy – Ichthyological Index (I.I. “Indice Ittico”). Lodi, E. Badino, G. (1993)

Austria

133. Several fish sampling methods are currently used in Austria. The main methods are electric fishing (in wadable streams or rivers), electric fishing from a boat, seining, gillnetting and long-lines (in large rivers, such as The Danube). In wadable rivers, the number of electric fishing devices and the number of anodes are dependent on river width. Autumn is the preferred sampling season in wadable rivers.

In non-wadable or large rivers, the main sampling season is also in autumn, but also in the summer in the Danube.

Belgium – Flanders

134. The fish population surveys are standardised both for wadable and non-wadable rivers. The principal method of fish sampling is electric fishing, but other methods such as gillnets, seine netting or fyke nets are also used in large rivers, and in some specific water systems (e.g. in Polder drainage systems).

135. In wadable rivers, the number of electric fishing devices and the number of anodes are dependent on river width, from 1 anode for river width smaller than 1.5 m to 4 anodes for rivers of 6-8 m wide. Electric fishing is performed in an upstream direction. At each station (sampled area = 100 m long), a maximum of 200 specimens of each species are individually weighed and measured). Captures by fyke nets, seine netting and/or gill nets are sometimes used to complement the electric fishing captures, principally in standing waters (channels).

Belgium – Wallonia

136. Despite the absence of a regular fish-monitoring programme defined at the regional level, several institutions (Universities, public administration responsible of natural resource management) perform frequent fish sampling operations in Wallonia. Electric fishing is used by all institutions, with relatively similar, but not standardised, sampling procedure. In addition, fish capture data obtained with gill nets, seine netting, fake nets or fish pass control are also compiled in the regional fish database.

137. In rivers, electric fishing is performed over a distance of 150-350 m, regardless of river width, with 1-3 passages depending on sampling objectives and institutions. Stop nets are used as far as possible, but if the water flow is too high or the river too wide, sampling areas are selected in such a way that natural barriers (small weirs or very shallow riffles) delimit the prospected zones. The preferred sampling seasons are summer and autumn.

138. In non-wadable rivers, electric fishing and horizontal bottom gill net techniques are combined. Electric fishing is performed from a boat, in an upstream or downstream direction along both banks, by a staff of 4 persons including the anode operator. Both electric fishing and gill netting are usually performed during daylight,

but comparisons have been made with sampling performed during the night, indicating that the abundance, frequency and size of species caught vary greatly between day and night. Additional techniques of fish sampling in non-wadable rivers include seining (used in straight canals with width less than 30 m), fyke nets (in connection between main channel and backwaters), and control of fish pass and sport angler catches.

France

139. Electric fishing is the usual method of fish sampling employed nation-wide. The preferred season for sampling is autumn, but sampling in small and medium-sized wadable rivers is also performed in late spring and summer.

140. In streams, fishing is conducted over the whole river width, moving from downstream to upstream. The sampling area is delimited by two stop nets. During one passage (or more), several anodes depending on the river width) are moved in the water and followed by hand-nets (4mm-mesh size) for collecting fishes. Each anode is generally followed by 2 hand with suitable vessels for transporting fish.

141. In wadable rivers, several sampling protocols have been successively tested: the first one was implemented in 1981. The procedure consisted of “continuous bank sampling”, the second protocol used is “point abundance sampling” (Persat and Copp, 1990) and the third protocol was introduced in 1995 and derives from Pouilly (1994) and Capra (1995) and is called “ambience sampling”.

Germany

142. A standardised German river monitoring system has never been established due to the special situation in Germany where inland fisheries is under the responsibility of Federal States. Each Federal State has its own river monitoring programmes and maintains their own database. Only for some large rivers as Rhine or Elbe are fish monitoring systems coordinated by the affected Federal States or countries. For example, the Federal State of Baden-Wuerttemberg use the follow sampling procedures:

143. In wadable rivers, electric fishing is performed using a DC 1,5-7 KW electric generator. For a given site, the sampling area is at least 100 m in small headwater streams and increases with increasing size and habitat complexity of the sampled

riverin order to obtain a representative sample. One passage is performed. Stop nets are not usually used, but are used in some specific cases. The preferred season for electric fishing extends from late summer to early autumn.

144. In non-wadable rivers, electric fishing is performed from a boat, usually by 4 persons, with a 7 KW DC generator. The representative method is used.

Greece

145. In Greece, there is no nation-wide system of fish data collection, and, therefore, there are no nationally standardised sampling methods. The collection of fish data allowing the evaluation of the type and magnitude of impacts on the aquatic environment has never been a sampling objective. As a consequence, sampling techniques have not been appropriately standardised for purposes of water quality assessments.

146. Although samples have been collected with a variety of fishing techniques (gill nets, seine netting, fyke nets, fry nets, fish pass control, etc.), electric fishing has always been a basic component of all riverine investigations. Data exclusively from wadable rivers, collected in spring if the investigation is targeted to aspects of reproduction and early life stages and in late summer or autumn if the target is the study of human impacts and threats to endangered species. DC electric generators (300-600 V) are used. The usual sampling practice is one person operating the anode proceeds upstream (sampling stretches of 40-100 m) and one or two other persons with hand-nets follow behind. One passage is performed, and no stop nets are used.

Lithuania

147. Several sampling gears are currently used in Lithuania. The most extensively used method is electric fishing, representing 88% of the sampling sites, 54% by wading and 34% from a boat. Other methods include stationary gill netting, and the use of drift and dragnets.

148. In wadable rivers, electric fishing is performed with a 600 V PDC battery, usually involving 3 persons of whom one operates the anode. The sampling intensity depends on the river size. Usually, one site per 10 km length river segment is investigated, with the sampling site covering 100-200 m. For each site, 1-3 passages are performed (in most cases 2 passages) and no stop nets are used. The preferred

seasons for sampling are summer and autumn. In non-wadable rivers, electric fishing, gill nets, drift nets and dragnets are used. Electric fishing is performed from a boat, and sections of about 3 m wide and 100-200 m long along the banks are sampled. When floating nets are used, about 2 km length segments of the main river channel are fished. Bottom gill nets (35-40 m long for each mesh size, ranging from 14-60 mm) are set overnight, for about 10 h.

<u>Poland</u>

149. There is no systematic monitoring programme for fish fauna in Polish rivers. There are some proposals as to how to conduct such a monitoring programme and which institution can be mainly involved in it. Up to now the Polish Anglers Association is the main institution that collects fish fauna data from the whole Poland. But not all of them can be easily used used as a basis for monitoring according to purposes of WFD framework.

150. Electric fishing is the main sampling method used in Poland for assessing fish community composition in rivers. Sampling intensity and sampling area are dependent on river size. Sampling is usually performed seasonally, from spring to autumn.

<u>Portugal</u>

151. All data are obtained by electric fishing, 90% from wadable rivers and 10% from a boat, in large rivers.

152. In wadable rivers, each site is selected in order to sample a representative sequence of habitats (riffle, run and pool). A single passage is performed in upstream direction, without stop nets.

<u>Sweden</u>

153. Electric fishing is the main sampling method used in Sweden, only by wading. Stationary gill nets are occasionally used in wide river bays.

154. The sampling strategy is a standardised, representative one, based on the successive removal of fish, with 3 passages per sampling site (length of the sampling stretch = 30-70 m). The preferred season is late summer-early autumn (Aug-Sept).

<u>The Netherlands</u>

155. Fish sampling is performed mainly in large lowland rivers (100-300 m wide). Electric fishing (PDC generator, 300 V, 5A) is usually performed from a boat, along the banks, by a staff of 3 persons. The prospected area averages 1000 m^2, *e.g.* 500 m long, 2 m wide along the banks, where the mean water depth is 0.8 m. The preferred season for electric fishing is from late autumn to early spring. Other techniques, including trawling, fyke and frame nets, are also used in large lowland rivers. The preferred season for trawling extends from April to October. On the other hand, fyke and frame nets are preferably used from spring (April) to autumn (November). Nets of 5-20 m long are set on the bottom of lowland rivers, at a depth of 3 m for periods ranging from 12 to 154 h.

United Kingdom

156. The specific equipement and strategies used are generally region and site dependent. Electric fishing is the most common sampling method used although the gear specifications, technique of application and man-power used has been variable between Environmental Agency (EA) area teams. The review of the EA Monitoring Programme and recent R&D programmes have attempted to standardise the sampling strategy and methods used for each element of the new monitoring programme whilst retaining some flexibility in approach to allow for evolution. The review is also taking full consideration of CEN requirements.

157. Electric fishing surveys are usually carried out in summer months, post spawning season (June - October). However, some regional and site-specific variation occurs depending upon the purpose of the surveys, the accessibility of the site and the conditions for efficient sampling, e.g. weed growth. The new sampling programme has proposed methods to suit different sampling data requirements, different habitat conditions and the manpower required to undertake them.Agencies in Scotland and Northern Ireland are working closely with the EA in developing standardize fish assessment methods.

Annex VI: Standardization process and CEN standards relevant to the WFD

A. THE STANDARDIZATION PROCESS

1. The process of elaborating CEN standards is shown schematically in Figure 1. Essentially this can be broken down into two distinct phases:

   i. The elaboration of an advanced working draft. At this stage the comments of interested experts, including those working with ECOSTAT, the relevant commissions and the broader scientific community, are welcomed directly. This broad peer review with external experts continues through to the submission of the working document as a draft prEN (project European Norm).

   ii. When the working draft is submitted to the CEN/TC 230 Secretariat as a draft prEN it becomes an official document covered by CEN copyright. Subsequent circulations are through the national member bodies and all comments from national experts are fed back officially through the same route and in an official format.

2. The direct involvement of ECOSTAT and other experts is restricted to the first stage. Subsequent involvement of ECOSTAT experts can be most successfully managed through the national member (standards) bodies in individual countries.

3. Voting on a new work item is by weighted majority and at least 5 member bodies must agree to have a practical involvement through nominated national experts. WG 2 now requires the completion of (1.) before requesting a new work item on the assumption that the working document can be circulated with the new work item proposal (NWIP). The text can then be submitted for CEN official procedure as soon as the NWIP is approved, such are the time constraints imposed by the CEN Central Secretariat.

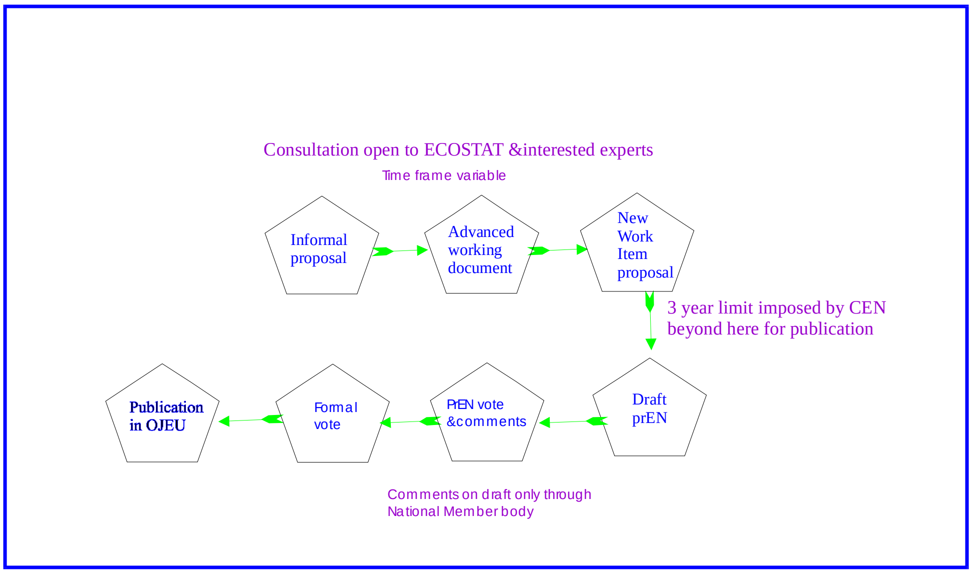

Figure 1. Stages involved in developing a European Norm in CEN.

4. At the draft prEN stage CEN/TC 230 including member (standardization) bodies are asked to approve the document for prEN voting within 6 weeks. At this stage the vote is either yes or no, but inevitably some comments are received but usually these are considered following the subsequent prEN voting stage.

5. At the prEN voting stage, member bodies consult national experts and will vote positively or negatively and provide comments. Where votes are negative explanations for the vote are given.

6. All comments received are collated by the relevant task group convenor and an official response to each and every comment must be given. Comments may be general, editorial or technical. These may be accepted or rejected but each and every comment must be responded to giving a reason, where necessary, for rejection. These comments and the responses are circulated with a revised text following the prEN stage. Every effort is made to reach consensus including delaying further procedure until outstanding issues can be discussed and resolved at a task group meeting, bearing in mind that meetings are usually only held annually.

7. At the formal vote stage voting is either positive or negative. No further comments are acceptable with the possible exception of minor editorial changes. Voting at the prEN and formal vote stage are by weighted majority.

8. Standards, which receive positive support at the formal vote stage, are published in the Official Journal of the European Union. At this stage they are automatically the EU reference method and must be published by the national member bodies, unlike ISO standards where publication is discretionary. CEN does not publish its own standards. This is done through the national member bodies.

9. The whole process between the acceptance of the new work item (NWI) and the formal vote must be completed within 3 years.

B. PUBLISHED CEN STANDARDS RELEVANT TO THE IMPLEMENTATION OF THE WFD AND THE INTERCALIBRATION PROCESS

10. A number of EN standards exist that are directly relevant to the implementation of the WFD and these are tabulated in Table 1.

11. Several features of this tabulation should be noted. Firstly, not all of the standards listed were elaborated in CEN/TC 230.

12. Several of the standards are referred to as ISO EN. These standards may have been elaborated under a CEN or ISO lead but were subsequently co-adopted by the two standards bodies under the Vienna Agreement. The date of publication for the ISO and EN may not be the same as processing and publication of the final drafts may not be identical.

13. In common with ISO, CEN has now adopted a 5 yearly review of standards to ensure that they remain relevant and fit for purpose. The review encourages member bodies to give an opinion as to whether the standards are still representative of the state of the art (confirmation), whether they need to be revised to meet current needs or if they are no longer necessary in which case they recommend withdrawal.

14. An obvious example of this process is with the invertebrate sampling standards cited in the WFD. EN 27828 - handnet sampling, EN 28265- quantitative samplers and EN ISO 9391 - deep water sampling -were published under an ISO lead but adopted in CEN. These are under revision in order to ensure their relevance to WFD. The drafts are being revised in CEN but will be published under an ISO lead, as these were originally ISO documents.

Table 1. List of European standards that are relevant to the implementation of the WFD and the intercalibration process.

Reference	Title	Published	Comment
EN 25667-1	Water quality –Sampling- Part 1: Guidance on the design of sampling programmes (ISO 5667-1:1980)	1993	
EN 25667-2	Water quality – Sampling – Part 2: Guidance on sampling techniques (ISO 5667-2:1991)	1993	
EN 5667-3	Water quality – Sampling – Part 3: Guidance on the preservation and handling of water samples	2003	
EN 27828	Water quality – Methods of biological sampling - Guidance on handnet sampling of aquatic benthic macro-invertebrates (ISO 7828:1985)	1994	
EN 28265	Water quality – Methods of biological sampling – Guidance on the design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters (ISO 8265:1988)	1994	Under revision
EN ISO 5667-3	Water quality – Sampling guidance on the preservation and handling of samples (ISO 5667-3:1994)	1995	Under revision
EN ISO 9391	Water Quality – Sampling in deep waters for macro-invertebrates – Guidance on the use of colonization, qualitative and quantitative samples (ISO 9391:1993)	1995	Under review
EN ISO 5667-16	Water quality – Sampling – Part 16: Guidance on biotesting of samples (ISO 5667-16:1998)	1998	
EN ISO 8689-1	Water quality – Biological classification of rivers – Part 1: Guidance on the interpretation of biological quality data from surveys of benthic macro-invertebrates in running waters (ISO 8689-1:2000)	2000	
EN ISO 8689-2	Water quality – Biological classification of rivers – Part 2: Guidance on the presentation of biological quality data from surveys of benthic macro-invertebrates (ISO 8689-1:2000)	2000	
EN 14184	Water quality – Guidance standard for the surveying of aquatic macrophytes in running waters	2003	
EN 13946	Water Quality – Guidance standard for the routine sampling and pre-treatment of benthic diatoms from rivers	2003	
EN 14011	Water analysis – Sampling of fish with electricity	2003	
EN 14407	Water quality – Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters	2004	
EN 14614	Water quality – Guidace standard for assessing the hydromorlogical features of rivers	2004	

Annex VII: WFD classification and intercalibration requirements

1. In order to evaluate the biological methods in relation to the WFD requirements, we recall the relevant sections of the Directive and the common agreed interpretations of these sections.

2. In the WFD ecological status of surface waters is defined as "…*an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters, classified in accordance with Annex V*" (Article 2.21). This implies that the classification systems should reflect changes taking place in the structure of the biological communities and in the overall ecosystem functioning as response to anthropogenic pressures (e.g. nutrient loading, acidification).

3. The WFD, also, stipulates that the ecological quality of water bodies should be classified into five quality classes (high, good, moderate, poor, and bad) using an Ecological Quality Ratio (EQR), defined as the ratio between type specific reference conditions and observed values of the relevant biological quality elements (Table 1). Guidance on how to establish reference conditions, and on classification of ecological status was prepared within WFD-CIS working groups REFCOND (Anonymous, 2003a) and ECOSTAT (Anonymous, 2004).

Table 1. Biological quality elements and metrics required for the classification of the high, good, and moderate ecological quality status of different surface waters according to the normative definitions described in the Annex V of the WFD. 1 = Taxonomic composition, 2 = Abundance, 3= Biomass, 4 = Plankton blooms, 5= diversity, 6= sensitive taxa (e.g. sensitive vs. insensitive species of organisms), 7 = age structure (from Heiskanen *et al.*, 2004).

Quality element	**Rivers**	**Lakes**
Phytoplankton	1, 2, 3*, 4	1, 2, 3, 4
Aquatic flora	1, 2	1, 2
Benthic invertebrates	1, 2, 5, 6	1, 2, 5, 6
Fish	1, 2, 6, 7	1, 2, 6, 7

*transparency as a proxy of phytoplankton biomass

Annex VIII: Compatibility of the national classification methods with WFD requirements

Table 1. Summary of the information on the compatibility of the national classification methods with the WFD requirements obtained by the Ecostat group WG 2A.

Country	Criteria for WFD compatibility			Method applicability
	5 quality classes	Reference Conditions	Reference conditions WFD compatible	
Aquatic flora				
Estonia	no inf.	no inf.	no inf.	no inf.
Finland	no inf.	no inf.	no inf.	no inf.
Ireland	no inf.	no inf.	no inf.	no inf.
Ireland	no inf.	no inf.	no inf.	no inf.
Latvia	no inf.	no inf.	no inf.	no inf.
Latvia	no inf.	no inf.	no inf.	no inf.
Lithuania	no inf.	no inf.	no inf.	no inf.
Lithuania	no	not yet, depending on development of typology	not yet	needs further elaboration of reference conditions
Sweden	no inf.	tests of monitoring carried out in national reference lakes	no	consistent with WFD when a database of lake vegetation is completed
UK	no inf.	no	no	basis for generating the required measures for the WFD, not related to reference conditions and there is no classification scheme relating to quality status
Slovenia	no inf.	no	no inf.	no
Benthic invertebrate fauna				
CY	no inf.	no	no	no
Lithuania	no	not yet, depending on development of typology	not yet	needs further elaboration of reference conditions
Latvia	no inf.	no inf.	no inf.	no inf.
Sweden	yes	spatially based, best available sites for six ecoregions (types)	no	yes
Ireland*	no inf.	no inf.	no inf.	no inf.
Norway	yes	no	no	Needs further elaboration of reference conditions
UK*	no inf.	temporal referencing, reconstructing cond., pH, NO3 and TP for larval remains in sediment core samples; spatial referencing	yes, indicator assemblages identified for different lake types based on their cond., can be done for pH and nutrients	yes
Phytoplankton				
Latvia	no inf.	draft version	yes	
Austria	yes	monitoring on lakes including reference lakes	for selected lakes in Carinthia only	for selected lakes in Carinthia only
Estonia	no inf.	no inf.	no inf.	no inf.
Finland	no inf.	no	no inf.	no inf.
Ireland	no inf.	no inf.	no inf.	no inf.
Lithuania	no inf.	no inf.	no inf.	no inf.
Norway	yes	not yet, depending on	no inf.	no inf.

		development of typology		
Sweden		Yes, for 3 types of lakes	no, need a more detailed typology	
UK	no inf.	no	no	
Slovenia	no inf.	no	no	
Portugal	no	no	no	no
Spain	no inf.	no	no	
Fish				
Spain	no inf.	no inf.	no inf.	no inf.
Sweden		yes	not exactly, metrics and their reference values are derived using National database	needs to be revised in terms of both metrics and type-specific reference values
UK	no	no	no	basis for generating the required measures for the WFD. Not related to reference conditions and there is no classification scheme relating to quality status. Used in isolation will not provide all data required by WFD
Nordic countries	no inf.	no inf.	no inf.	no inf.
Macrophytes and phytobenthos				
Germany	yes	yes	yes	Good
Aquatic plants and benthic invertebrates				
UK**		Yes	Yes	Good

* Eutrophication and acidification
** Multistressors
Note all other methods target the classification of eutrophication impacts

Annex IX: WFD Common Metric

1. In order to ensure comparability of the Ecological Quality Ratio (EQR) scales between the different EU countries and to obtain a common understanding of the good ecological status of surface waters all over EU, the WFD requires for the intercalibration of the classification results of the biological monitoring systems. In practice, the intercalibration exercise must establish the values for the boundary between the classes of high and good status, and for the boundary between good and moderate status, which shall be consistent with the normative definitions of those class boundaries given in Annex V of the WFD.

2. As in the WFD the intercalibration is a two-phase process, which starts with the establishment of an intercalibration network consisting of sites representing the boundaries between the quality classes ‘High-Good’ and ‘Good-Moderate’, as based on the WFD normative definitions. In a second phase each Member State’s assessment method must be calibrated both in the ecoregion and for the surface water type to which the system is applicable. The results of the second phase must be used to set (EQR) values for relevant class boundaries for each Member States biological assessment system.

3. The intercalibration exercise will focus on specific type/biological quality element/ pressure combinations, which were selected based in data availability within the time constrains of the exercise. For rivers, the intercalibration will focus on sites impacted by organic and/or nutrient loading, river modification and acidification as assessed making use of macroinvertebrates, fish and benthic algae. For lakes, the focus will be on eutrophication and acidification as assessed using, respectively, of phytoplankton and macrophytes, and macroinvertebrates and fish respectively. The different combinations of pressures/ biological quality elements are, both for river and lakes, dependant of the GIG, the pressure and the type.

4. Guidance on the intercalibration process was developed within a drafting group that is part of the ECOSTAT WG 2A. The guidance foresees an intercalibration process based in the selection from three different options, and several hybrids of these options, all firmly based on the definition of a protocol for deriving good ecological status class boundary values from the normative definitions. The choice of

one or other option depends on the degree of compliance of the national assessment methods with the WFD requirements, the nature of the methods for determining reference conditions, and the possibility for identifying a common metric.

5. Given the state of development of the national methods and their possible divergences it is expected that the most viable option involves the use of a common metric identified specifically for the purposes of the intercalibration exercise and to which the national methods will be compared with and then adjusted.

What is a metric and what is a intercalibration 'common metric'

6. A metric is defined in Karr and Chu (1999) as a measurable part or process of a biological system empirically shown to change in value along a gradient of human influence. It reflects specific and predictable responses of the community to human activities, either to a single factor or to the cumulative effects of several or all activities within a watershed.

7. Several metrics types can be distinguished by definition addressing comparable aspects of a community, regardless of the stressor to which the metrics are responding. The following metric types are distinguished:

- ***Composition / abundance metrics***: all metrics giving the share of a taxon or taxonomic group in relation to the total number of individuals counted; all metrics giving the abundance of a taxon or taxonomic group.
- ***Richness / diversity metrics***: all metrics giving the number of taxa within a certain taxon (including the total number of taxa), all diversity indices.
- ***Sensitivity / tolerance metrics***: all metrics giving the ratio of taxa sensitive and insensitive to stress in general or to a certain stress-type, either using presence/absence or abundance information.
- ***Functional metrics***: all metrics addressing the characteristics of taxa other than their sensitivity to stress (species traits, taxa traits, ecological guilds): feeding types, habitat preferences, ecosystem type preferences, current preferences, life-history parameters, body-size parameters. These can be based on taxa abundance.

8. For the purpose of the WFD intercalibration exercise it may be necessary to identify common metrics (see option 2 of the Guidance on the Intercalibration Process[10]). This is the case when Member State's assessment methods are not directly comparable, and involves the agreement on a common WFD method by the Member States in a GIG.

9. The common metrics should be indicative of the relevant biological quality element and sensitive to the pressure that is assessed. These may be selected from one of the Member State's existing assessment methods, if acceptable for the other Member States in the GIG, but also can be specifically developed in the GIGs. For the common metric, type-specific good status boundary values need to be established in the GIGs following the application of an agreed boundary setting procedure using a data set assembled for the purposes of the intercalibration exercise. The results of the common assessment method will be used as the basis for adjusting the boundary EQR values of the national assessment methods.

10. The applicability of a common metric is dependent of the availability of a suitable data set from which the common metric(s) can be calculated to enable reliable application of the agreed boundary setting procedure, and the availability of a means of estimating and taking into account differences in the bias of the methods when applied to the data set referred to above.

11. In general, common metrics are biological metrics widely applicable within a larger geographical region, which can be used to derive comparable information among different countries and waterbody types. Basic features are the ability in discriminating different quality classes and the possibility of calculating them from a wide range of geographical contexts, i.e. where different effort is placed on the monitoring exercise and different expertise is available for taxonomic identification (Buffagni and Erba, 2004).

[10] 'Guidance on the Intercalibration Process' available at http://

Annex X: Summary of the national biological assessment methods for lakes (WFD Intercalibration metadata January 2004)

Austria

- **WFD compatible? No**
- Classification of ecological quality status based on the comparison of reference with present-day trophic state.
- The classification of the trophic state is based on physico-chemical and phytoplankton parameters. The ecological quality status was classified as 'high' or as 'boundary high/good' with moderate to high certainty.

- **Phytoplankton: Yes;** abundance, biomass, indicator taxa, group ratios, chlorophyll-a
- **Phytobenthos: No**
- **Macrophytes: Yes; 4 out of 15;** indicator taxa, limit of vegetation
- **Macroalgae: No**
- **Benthic invertebrates: No**
- **Fish: No**
- **Physicochemical quality: Yes**; Total phosphorus, nitrate, ammonium, oxygen, secchi depth
- **Pressure criteria: Yes;** Nutrient loading

Belgium

- **WFD compatible? No**
- Expert consultation, occasional research of diatoms, macro invertebrates, macrophytes and site specific physico-chemical parameters
- Official methods are not yet operational. Expert agreement is the method, and is a subjective method.

- **Phytoplankton: No**
- **Phytobenthos: Yes;** relative abundance of indicator species; relative abundance (counts of 500 valves)
- **Macrophytes: Yes;** type specific species (abundance weighted), disturbance indicators, growth forms, cover-frequency (Tansley), aquatic vegetation, shore vegetation
- **Macroalgae: No**
- **Benthic invertebrates: Yes;** Taxonomic composition; numbers (Preston scores)
- **Fish: No**
- **Physicochemical quality: Yes**; temperature, salinity, pH, alkalinity, oxygen, absorbance (254 nm, 420 nm), DIC, N-t, P-t, nitrate-N, ammonium-N, phosphate-P, Kj-N, sodium, potassium, calcium, magnesium, iron, sulphate, chloride, silica, COD
- **Pressure criteria: No**

Cyprus

- **WFD compatible? No**

- An integrated holistic evaluation of data and information guided by expert judgment was applied. The sites were classified in two categories representing ecological conditions in the range of high-good; and good-moderate. Formal methods are not yet operational. Expert agreement made, a subjective method.
- The classification was mainly based on synthetic evaluation of a) 226 physicochemical /biological parameters integrated into 9 indices b) on hydrological regime and c) the existing pressures (mainly based on existing register and Nitrate level)

- **Phytoplankton: No**
- **Phytobenthos: No**
- **Macrophytes: No**
- **Macroalgae: No**
- **Benthic invertebrates: Yes;** The Benthic Saprobity index (BSI) .It is evaluated according to the method of the Biological Monitoring Working party (BMWP). The Biological Diversity index (BDI). It is quantified according to the Sequential Comparison index (SCI) methodology.
- **Fish: No**
- **Physicochemical quality: Yes**; All mentioned in paragraph 5.3 except for the Salinity, Secchi depth, Total Nitrogen and Suspended Matter. Within the scheme of an Integrated Pollution Evaluation Organic and inorganic pollutants and toxicity were investigated.
- **Pressure criteria: Yes;** data from the register of population and the activities in catchments zones (1996) and to partially amended in 2003 the results of the Indices. Nutrient loading and organic loading.

<u>Germany</u>

WFD compatible? Yes 19 out of 24

- Trophic method according to LAWA-AK 'Gewaesserbewertung - stehende Gewaesser' (1999). Assessment system according to 'PHYLIB' (Bayerisches Landesamt fuer Wasserwirtschaft, Schaumburg *et al.*, 2003. Macrophytes and diatom Index, Schaumburg *et al.*, 2003).
- Trophic and biological criteria (total P, chlorophyll a, Secchi depth) expressed as Trophic Index. Trophic and biological criteria (phytoplankton, macrophytes , phytobenthos), pressure criteria. Results from macrophytes Index, diatom index; chloroph,yll a, phytoplankton biovolume, secchi depth, total phosphorus, total nitrogen and chloride differ only slightly from those values in reference lakes. One site unknown.

- **Phytoplankton: Yes;** Species composition and abundance (quantitative), chlorophyll a. Or Secchi depth, chlorophyll *a*. Or epilimnetic chlorophyll *a* concentration/, phytoplankton biovolume; diatom remains in the surface sediment from the deepest point.
- **Phytobenthos: Yes; 14 out of 24;** Benthic diatoms: species composition and abundance, diverse indices. 3 lakes: littoral diatoms
- **Macrophytes: Yes; 19 out of 24;** Species composition and abundance, macrophyte index according to MELZER, reference index (see Schaumburg *et al.*, 2003).Or ecological groups of macrophytes indicating the trophic level of the lake; abundance of the macrophytes (Kohler, 1978); structure of the lake

shore (map); aquatic spermatophyta, pteridophyta, characeae, Bryophyta. 3 lakes no information on method.

- **Macroalgae: Yes; 4 out of 24;** Characeae only: same as in Macrophytes. 3 lakes no information on method.
- **Benthic invertebrates: Yes; 8 out of 24;** Characeae only: same as in Macrophytes. Abundance of the profundal fauna and boundary littoral/profundal-fauna (FITTKAU). Species composition. 1 lake with no information on method.
- **Fish: No**
- **Physicochemical quality: Yes**; Temperature, conductivity, secchi depth, TP, Ca. Or Secchi depth, temperature, oxygen, pH, conductivity, total phosphorus, soluble phosphorus, nitrate-N, ammonium-N, silicic acid. Total phosphorus (TP), total nitrogen (TN), chloride (Cl) (2 lakes)
- **Pressure criteria: Yes**; 16 out of 24; Waste water load, tourism, diffuse loads. Or landuse, waste water load, structure of lake shore. In one 1 site no information on method.

Estonia

- **WFD compatible? Yes**
- Classification based on TP as main pressure and biol. and physical-chemical parameters correlating with it. RC for TP are estimated basing on morpho-edaphic index (Vighi and Chiaudani, 1985). Long-term changes in biota were estimated using similarity with 1911-
- Depite of very poor surrounding sand soils, diminished water transparency and opulent macrovegetation refers to human impact. Estonian team worked out quality limits for different quality classes; species composition of charophytes, relative abundance, coverage, distribution depth

- **Phytoplankton: Yes;** community structure, chl-a concentration
- **Phytobenthos: No**
- **Macrophytes: No**
- **Macroalgae: Yes 9out of 12;** similarity coefficient with 1911-13 data; or species composition, relative abundance, coverage, distribution depth
- **Benthic invertebrates: Only for 1 site**
- **Fish: Yes;Only for 1 site**
- **Physicochemical quality: Yes;** pH, total nitrogen, total phosphorus, Secchi transparency; in one site also BOD7
- **Pressure criteria: No**

Spain

- **WFD compatible? Yes**
- OCDE mean Chlorophyll (µg/l) boundary values for trophic classification (OECD, 1982).
- Annual mean chlorophyll values between 3,5 and 7 were selected for the good/moderate class boundary sites (19 sites); annual mean chlorophyll values between 1 and 3 (µg/l) were selected for the high/good class boundary sites (Secchi disk, TP around 10 µg/l) (2 sites); annual mean chlorophyll values in the oligotrophic level selected for the high/good class boundary site. (1site)

- **Phytoplankton: Yes;** Clorophyll as alga biomass measure.
- **Phytobenthos: No**
- **Macrophytes: No**
- **Macroalgae: No**
- **Benthic invertebrates: No**
- **Fish: No**
- **Physicochemical quality: No**
- **Pressure criteria: No**

<u>France</u>

- **WFD compatible? No**
- Based on trophic level.

- **Phytoplankton: No**
- **Phytobenthos: No**
- **Macrophytes: No**
- **Macroalgae: No**
- **Benthic invertebrates: No**
- **Fish: No**
- **Physicochemical quality: Yes;** nutrients, chlorophyll, transparency.
- **Pressure criteria: No**

<u>United Kingdom</u>

- **WFD compatible? Yes** (13 sites); NO (19 sites)
- The 13 compatible sites: SEPA's Standing Water Classification Scheme (1994, SEPA Internal Report). Sediment diatoms to assess the degree of floristic change (diatom species turnover) between core bottom and surface sediment sample using chord distance dissimilarity measure.
- The non-compatible sites: mostly, based on definitions given in Annex V for the biological elements - expert opinion (9 sites). Several different combinations of these definitions, expert judgement, pressure information, ratio of reference to current lake total phosphorus concentration and sediment diatoms used to assess the degree of floristic change (diatom species turnover) between core bottom and a surface sediment sample.
- Boundary criteria have been based on an interpretation of the normative definitions, which have been related to monitoring methodologies used in the UK.

- **Phytoplankton: Yes21 out of 32;** fossil diatom assemblages (14 sites) and Occasional blooms (7 sites).
- **Phytobenthos: Yes 22 out of 32 sites;** fossil Diatom record (14 sites) and absence of bacterial tufts, but occasional algal mats of Cladophora agg (8 sites).
- **Macrophytes: Yes 11 out of 32 sites;** absence of indicator species typical of type and abundances lower than expected for a good quality lake of this type, species presence and absence and relative abundance DAFOR, species composition**.**

- **Macroalgae; 8 out of 32 sites;** periodic growths of Cladophora mats, presence of nuisance growths of indicators such as Cladophora agg.(2 sites).
- **Benthic invertebrates: 8 out of 32 sites**; family level invertebrates and log abundance - presence and absence of pollution sensitive/tolerant taxa.
- **Fish: No**
- **Physicochemical quality: 28 out of 32 sites;** current total phosphorus, hindcast total phosphorus, current acid neutralising capacity and hindcast acid neutralising capacity (13 sites), OECD classification based on total phosphorus (8 sites), Total Phosphorus (7 sites).
- **Pressure criteria: 25 out of 32 sites;** presence / absence / significance of fish farms, other point sources, hydromorphological, recreational and land use pressures (16 sites), Catchment land-use, improved grassland/evergreen forestry (8 sites), expert opinion based on knowledge of lake (1 site).

Ireland

- **WFD compatible? No**
- Ireland's interpretation of normative definitions in Annex V of Water framework Directive.
- Several combinations of the elements macrophytes, macroinvertebrates, phytoplankton species composition and quantity, total phosphorus and other physico-chemical data are used for classification,

- **Phytoplankton: Yes; 19 out of 24 sites;** species composition, biomass and abundance (11 sites), species composition and abundance (4 sites), Phytoplankton abundance as measured by chlorophyll (3 sites).
- **Phytobenthos: No**
- **Macrophytes: Yes; 14 out of 24;** species composition and abundance.
- **Macroalgae: Yes; 11 out of 24 sites;** species composition (6 sites) and abundance (4 sites), Presence or absence (1 site).
- **Benthic invertebrates: Yes; 10 out of 24 sites;** species composition and abundance.
- **Fish: No**
- **Physicochemical quality: Yes; 22 out 24sites**; total Phosphorus only (8 sites), H+, alkalinity, non-marine sulphate, nitrate, cations and heavy metals concentrations (4 sites), several combinations of nutrients, pH, H+, alkalinity, non-marine sulphate, nitrate, cations and heavy metals concentrations, chlorophyll (9 sites).
- **Pressure criteria: No**

Lithuania (4expert knowledge on level of acid deposition in the catchment/region sites)

- **WFD compatible? No**
- Pressure screening, historical and existing monitoring data, expert judgement
- Maximum allowable concentrations, existing biological classification system for macroinvertebrates (biotic integrity index)

- **Phytoplankton: No**
- **Phytobenthos: No**
- **Macrophytes: No**

- **Macroalgae: No**
- **Benthic invertebrates: Yes;** biotic integrity index
- **Fish: No**
- **Physicochemical quality: Yes**; BOD7, total N, total P
- **Pressure criteria: Yes**; Land use (%), wastewater discharges (approximate amount of discharges), recreational use

<u>**Latvia**</u>

- **WFD compatible? No**
- Use of reference physico-chemical conditions (? But reference condition questions were all answered with no), statistical analyses of physico-chemical values.
- Criteria (P total, N total, BOD5, N/NO3, N/NO2, N/NH4, P/PO4, Chlorophyll *a*) the lowest and the highest quartiles were used for approximate defining high/good or the good/moderate class boundaries + expert judgement

- **Phytoplankton: No 5 out of 6 sites**
- **Phytobenthos: No**
- **Macrophytes: No**
- **Macroalgae: No**
- **Benthic invertebrates: No**
- **Fish: No**
- **Physicochemical quality: Yes**; P total, N total, BOD5, N/NO3, N/NO2, N/NH4, P/PO4, Chlorophyll *a*
- **Pressure criteria: No**

<u>**Netherlands**</u>

- **WFD compatible? Yes**
- Modification of method presented in Van den Berg *et al.*, (2002). Boundary high/good: statistical variation in data, 90% percentile of reference values
- good/moderated: class width of good is assumed to be similar as class width for high.

- **Phytoplankton: Yes;** mean chlorophyll *a* concentration (summer period).
- **Phytobenthos: No**
- **Macrophytes: Yes; 3 out of 3 sites;** area covered by submerged macrophytes, 1 site including charophytes.
- **Macroalgae: only 1 site;** Charophytes were included in area covered by submerged macrophytes.
- **Benthic invertebrates: No**
- **Fish: No**
- **Physicochemical quality: Yes;** total P
- **Pressure criteria: No**

<u>**Norway**</u>

- **WFD compatible? No**
- National classification system (SFT-guidance 1997:04) combined with expert judgement

- Total phosphorus and chlorophyll *a* combined with expert judgement of reference conditions for these parameters for the type relevant for the site

- **Phytoplankton: Yes; 21 out of 46;** mean chlorophyll *a* concentration (summer period)
- **Phytobenthos: No**
- **Macrophytes: Yes; only 1 lake;**
- **Macroalgae: only 1 site**
- **Benthic invertebrates: yes; 9 out of 46;** species composition, acidification index based upon indicator taxa sensitive to acidification
- **Fish: Yes; 8 out of 46;** species composition, CPUE and age structure (primarily trout populations)
- **Physicochemical quality: Yes**; 44 out of 46; **pH, ANC. Total phosphorus**
- **Pressure criteria: 24 out of 46;** expert knowledge on level of acid deposition in the catchment/region

Poland

- **WFD compatible? No**
- Lake Quality Evaluation System - method used in routine monitoring of Polish lakes Kudelska *et al.* (1997). Two types of criteria: water quality criteria (mainly eutrophication parameters), morphometric, hydrographic and watershead criteria.

- **Phytoplankton: Yes; 22 out of 25 sites;** quantitative and qualitative composition, indicator taxa (in 10 sites) + biomass (chlorophyll *a* content) (in 11 sites) + number of taxons, indicator taxa, dominance structure (1 site).
- **Phytobenthos: No**
- **Macrophytes: Yes; 17 out of 25 sites;** taxonomic composition, area covered by particular plant community, max. depth of plant growth (15 sites), quantitative and qualitative composition, indicator taxa, biomass (chlorophyll *a* content) (1site), indicator taxa only (1 site).
- **Macroalgae: Yes; 13 out of 25 sites;** presence of Characeae, area covered by *Chara* community, max. depth of plant growth.
- **Benthic invertebrates: 0nly 1;** composition, indicator taxa**.**
- **Fish: No; 20 out of 25 sites;** qualitative composition and 1 site also with indicator taxa**.**
- **Physicochemical quality: Yes**; mainly eutrophication parameters (oxygen conditions, N and P compounds, Secchi disc reading, chlorophyll *a*) and COD-Cr, conductivity.
- **Pressure criteria: Yes**; land use in catchment area, presence of human settlements, presence of sources of pollution, tourism.

Portugal

- **WFD compatible? No**
- Concentration of chlorophyll a.
- Statistical approach based on historical data of chlorophyll *a*.

- **Phytoplankton: Yes;** Chlorophyll *a*.
- **Phytobenthos: No**

- **Macrophytes: No**
- **Macroalgae: No**
- **Benthic invertebrates: No**
- **Fish: No**
- **Physicochemical quality: No**
- **Pressure criteria: No**

<u>**Sweden**</u>

- **WFD compatible? Yes 2 out of 9**
- Swedish Environmental Quality Criteria (Report 4913, Swedish EPA).
- Border class 1 and 2, as deviations from reference values. Not eutrophied/acidified according to pressure criteria. Border class 2 and 3, as deviations from reference values

- **Phytoplankton: Yes 4 out of 9;** total biovolume (values for August)
- **Phytobenthos: No**
- **Macrophytes: No**
- **Macroalgae: No**
- **Benthic invertebrates: Yes; 5out of 9;** Average Score Per Taxon, acidity index
- **Fish: only one site**; species number, diversity (Shannon-Wieners H), biomass, relative abundance of native species, proportion of cyprinids, proportion of pisc. percids, number of acidific.-sensitve species, proportion of species tolerant to low oxygen levels, proportion of introduced species.
- **Physicochemical quality: Yes;** pH, total phosphorus, water colour or only pH
- **Pressure criteria: Yes;** F-factor, percentage agricultural land, percentage clear-cuttings

<u>**Slovenia**</u>

WFD compatible? No

- OECD classification; Eutrophication of waters, Monitoring, Assessment and Control Anon., OECD Paris, (1982)
- N, P annual conc., Ptot.- (SIST EN 1189:1997); N anorg.= Ammonium (SIST EN ISO 11732:1999)+ Nitrate (SIST EN ISO 10304-1:1998) +Nitrite (SIST EN 26777:1996); transparency (Secchi depth), chlorophyll *a* concentration (SIST ISO 10260:2001- modif.)

- **Phytoplankton: Yes;** chlorophyll-a concentration, phytoplankton biomass, bloom frequency.
- **Phytobenthos: No**
- **Macrophytes: Yes;** taxonomic composition, relative abundance.
- **Macroalgae: Yes;** taxonomic composition, relative abundance.
- **Benthic invertebrates: No**
- **Fish: No**
- **Physicochemical quality: Yes;** Secchi depth, Total P, Total inorg. N (Nitrate, Nitrite, Ammonium).
- **Pressure criteria: No**

Annex XI: Harmonisation drafting group members

This task team will be co-ordinated by JRC Ana Cristina Cardoso, Angelo Solimini and Guido Premazzi (JRC/ EEWAI). The task members are Fabrice Martinet (FR)**,** Maria Luisa Serrano (ES), Teresa Rafael (PT), and Sebastian Birk (STAR project), Peter Hale (CEN).

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