Eco-AlpsWater

Innovative Ecological Assessment and Water Management Strategy for the Protection of Ecosystem Services in Alpine Lakes and Rivers

Priority 3: Liveable Alpine Space. SO3.2 - Enhance the protection, the conservation and the ecological connectivity of Alpine Space

Deliverable D.T2.1.1

Mutual awareness, learning and exchange of experiences and approaches in water quality assessment among PPs

Project Eco-AlpsWater
Work Package WPT2
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Abstract

The output of the Deliverable D.T2.1.1 consisted of the detailed description of methods adopted in the key lakes and rivers for the assessment of the ecological status of waters, using biological quality elements selected in the Eco-AlpsWater project (phytoplankton, benthic diatoms, and fish). The method description includes sampling procedures, sampling period and frequency, representative sampling site requirements, data processing, and rules to define ecological classes and reference conditions. Project Partners (PPs) responsible for the monitoring of key sites provided the methods for the three biological quality elements (BQE). These methods were shared among PPs and used as base on which the innovative eDNA protocols were developed (WP1).

In some key sites not all the BQE are monitored for the water quality assessment. Below a summary of BQE methods applied in key lakes and rivers of the Eco-AlpsWater project.

BQE		Phytoplankton	Diatoms (Phytobenthos)	Fish
KEY LAKES	Mondsee (A)	✓		✓
	Bourget (F)	✓	Method under development	✓
	Starnberger (D)	✓	✓	Method just approved but not yet adopted
	Garda (I)	✓	✓	Method pending approval
	Bled (SI)	✓	✓	✓
	Lugano (CH-I)	✓		
KEY RIVERS	Steyr (A)		✓	✓
	Drome (F)		✓	✓
	Wertach (D)		✓	✓
	Adige (I)		✓	✓
	Soča (SI-I)		✓	✓

For each key lakes and rivers a list of taxa (BQE) observed in the last 10 years was filled in by PPs, including alloctonous species (see Annex 1). This dataset comes from monitoring programmes and research projects.

Besides the methods adopted for the evaluation of BQE selected in the Eco-AlpsWater project, some PPs provided methods on additional BQE monitoring applied in key sites, like phytoplankton and phytobenthos in rivers (see Annex 2).

The survey showed differences and similarity among the methods used in key lakes and rivers, which are summarised in the table below.

		Similarity	**Differences**
Phytoplankton	Lakes	Sampling point (max. depth) Sampling depth (epilimnion/euphotic depth) Biovolume (Utermöhl) and Chlorophyll-a (ISO) Species level identification Reference conditions and ecological classes (IC) Last sampling (2016-2018)	Sampling frequences (4-16 per year/cycle) INDEX
Diatoms (Phytobenthos)	Lakes	Sampling period (summer) Substrate types Habitat Species/genera level identification Ecological classes	Biological community (phythobenthos other than diatoms) Number of sampling stations (3-50) INDEX and reference conditions Last sampling (2014-2017)
	Rivers	Sampling period (May-October) Sampling site size Substrate types Habitat Species/genera level identification Reference conditions and ecological classes (IC)	Biological community (phythobenthos other than diatoms) Number of sampling stations Sampling frequences (1-6 years) INDEX Last sampling (2015-2018)
Fish	Lakes	Sampling period (July-October) Sampling strategies Species level identification Ecological classes	Number of sampling stations (surface/depth) INDEX and reference conditions Last sampling (2010-2018)

	Rivers	Sampling period Sampling strategies Habitat Species level identification Reference conditions and ecological classes	Number of sampling stations Sampling frequences (1-6 years) INDEX Last sampling (2015-2018)

Water quality assessment-National methods

Austria

Country: Austria
Category: Lake
Biological Quality Element: Phytoplankton

Short description of sampling procedure

An assessment of the ecological status of lakes using phytoplankton is mainly a classification of their nutrient and productivity levels. The assessment method presented in this manual was developed for Austrian lakes with a surface > 50 ha. The sampling methods presented in this method survey are based on "Guidance on the monitoring of the biological quality elements part B2 – Phytoplankton" (Wolfram et al. 2015). The assessment of the ecological status of a lake is based on several phytoplankton samples collected from the epilimnion at different sampling dates. The chlorophyll-a concentration is determined from an additional sample taken from the same water layer and following the same technique (as a mixed or integrated sample) as used for the phytoplankton sample. In general, sampling is carried out from a boat positioned above the deepest point of the lake (or the lake basin). The phytoplankton samples are analyzed in the laboratory with respect to taxonomy (qualitative analysis), with the abundance and total biovolume of the planktonic algae determined from a subsample observed using an inverted microscope.

Sampling:

According to European lake typology only two types are considered: L-AL3: Low to medium altitude (50–800 m above sea level), deep (mean depth usually >15 m), moderate to high alkalinity (usually >1 mmol L-1) surface area >50 ha, Alpine catchment areas and L-AL4: Low to medium altitude (50–800 m above sea level), shallow (mean depth usually 3–15 m), moderate to high alkalinity (usually >1 mmol L–1), surface area >50 ha, usually pre-Alpine catchment areas or within inner-Alpine basins.

Quantitative sampling: A single sample per sampling date is sufficient. It can be taken by using an integrating sampler or by collecting a series of samples from different depths (e.g. 0 m, 3 m, 6 m etc.). In the latter case, the samples from separate depths are pooled in a large container. After careful mixing, one subsample is transferred to a 100- or 200-ml bottle (quantitative sample). Alternatively, the bottle is directly filled from the integrating sampler. For ease of later homogenization, the bottle should only be 80%, rather than completely filled. The diatom sample and the chlorophyll-a sample must be taken from the same mixed sample (container) as the quantitative sample. In meso- to eutrophic lakes, at least 1 liter should be collected for the diatom and chlorophyll-a samples. In samples of oligotrophic lakes at least 2 liters are recommended for the chlorophyll-a sample. In nutrient-poor lakes, the diatom sample can be taken from the qualitative plankton-net sample.

Qualitative sampling: The qualitative sample of fresh material is obtained using a plankton-net with a mesh size of 30–40 µm. The plankton haul should cover the whole epilimnion.

Preservation:

The living (**qualitative**) samples can be stored up to 36 h at low temperatures (4–10 °C) in the dark (DIN EN 15204/2006) before microscopic analysis. Samples taken from waters with a higher temperature should be cooled slowly and to only slightly below the temperature of the epilimnion, in order to prevent fragile algal cells from bursting as well as algal colonies from disintegrating. Samples from warm waters (>20 °C) should be analyzed as soon as possible, without any cooling at all. For high densities of zooplankton, the time between sampling and microscopic analysis should be kept very short, otherwise a few drops of formaldehyde should be added.

An alkaline iodide solution (Lugol's solution, modified after UTERMÖHL 1958) is used to preserve the **quantitative** samples, adding about 8 drops per 100-ml sample, i.e. until the samples becomes light brown / orange (cognac coloured). The Lugol-fixed sample should be stored in the dark (according to DIN EN 15204/2006 also at low temperature), but not for longer than one year. If the sample will be stored for longer than 1 year, a few drops of 37% formaldehyde should be added.

If a separate diatom sample is taken, it must be filtered in the field with a manual filtering apparatus using a cellulose acetate filter (<4-µm pore size). The filter is kept air-dried in a Petridish and can be stored at room temperature. If a manual filter device is not available, filtration should be carried out in the laboratory on the same day. In case of filtration at a later date, preservation of the sample with formaldehyde is required.

Sampling period/frequency

The assessment of phytoplankton is based on the annual mean of data acquired from several sampling dates. For large Austrian lakes, at least four sampling dates per year are required to reliably calculate the mean. A higher sampling frequency will improve the confidence in the calculation and avoid biases of the annual means due to outliers. The assessment is carried out on the basis of a running average of three subsequent years. The minimum requirement for the classification is sampling at four different, limnologically important dates: Spring circulation, beginning of the summer stagnation, peak of the summer stagnation, beginning of the autumn circulation.

Characterization of representative sampling site

The morphology of the lake basin of most standing waters in Austria is relatively simple. Hence, most lakes represent a water body as defined by the WFD and are sampled at one sampling site only. However, there are lakes with a more complex basin morphology (e.g. Wolfgangsee) which are sampled at two sites in accordance with the GZÜV. Quantitative samples of phytoplankton (total biovolume and chlorophyll-a) are taken from the epilimnion or, as in some neighbouring countries, from the euphotic zone. To ensure data harmonization, it is recommended to sample the epilimnion according to the monitoring programs under the GZÜV. If the euphotic zone is smaller than the epilimnion, the latter must be sampled. The same is true it the euphotic zone extends into an anoxic deep water zone.

Short description of processing method and evaluation (e.g. metrics, level of identification) Taxonomic analyses are carried out at the species level, as far as possible with reasonable effort. If the relative proportion of centric diatoms exceeds 10% of the total biovolume per sample, an additional detailed analysis of diatoms is required in order to enhance the degree of confidence in the taxonomic analyses (burn mount, after EN 14407:2004). For each year, the mean chlorophyll-a concentration and, for each taxon, the mean biovolume are determined as the arithmetic means on four or more sampling dates. The mean total biovolume of a lake is calculated as the sum of the mean biovolumes of the single taxa. The relative proportions of the mean biovolumes of these single taxa and the taxon-specific trophic scores are used to calculate the Brettum index. The final classification of the lake using phytoplankton is based on the mean chlorophyll-a concentration, the mean total biovolume and the Brettum index. The different weights of these three metrics are described in detail in "Guidance on the monitoring of the biological quality elements part B2 – Phytoplankton" (Wolfram et al. 2015)
Additional abiotic data recorded The reliability of the trophic assessment usually increases if a larger number of parameters is taken into account (oxygen and potential depletion/hypersaturation in the water column. In particular: Temperature profile, depth of thermocline and thickness of the epilimnion, Secchi depth and thickness of euphotic zone, sampling depth, atmospheric conditions, discharge of the main tributaries, colour , turbidity and type of turbidity (mineral, organic, calcite precipitation).
Method features compliant with WFD The classification of the ecological status is based on a comparison between the reference status and the actual status of a waterbody. Austrian BQE assessment for Phytoplankton in lakes is described in "Guidance on the monitoring of the biological quality elements part B2 – Phytoplankton" (Wolfram et al. 2015). All requirements for the WFD are covered (species composition, abundance and biomass. Furthermore, the ecological status is defined by 5 classes (high, good, moderate, poor and bad).

Rules to define ecological classes and reference conditions

The reference conditions and class boundaries of the three parameters chlorophyll-a, total biovolume and Brettum index were developed during the intercalibration (IC) process and are now harmonized between Slovenia, Italy, France, Germany and Austria. For Austrian lakes, ranges rather than fixed values were defined for the three parameters and the two IC lake types. Any classification following the principles of the WFD is based on a comparison of the status quo with the reference state. The deviation is calculated as the ecological quality ratio for the chlorophyll-a concentration (EQR_{Chl}), the total biovolume (EQR_{BV}) and the Brettum index (EQ_{RBI}). In order to enable a combination of EQR values, they are transformed ("normalized") such that the class boundaries are equidistant. This allows the ecological status class to be directly identified from the "normalized" EQR value. The final assessment of the ecological status is based on the average of the final normalized EQR values of three subsequent years.

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

Table 1 Shows the boundaries to define the ecological status class.

Literature Reference

DIN EN 15204 (2006): Wasserbeschaffenheit – Anleitung für die Zählung von Phytoplankton mittels der Umkehrmikroskopie (Utermöhl-Technik).

EN 14407:2004. Water quality - Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters.

GZÜV: Verordnung des Bundesministeriums für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft über die Überwachung des Zustandes von Gewässern; Gewässerzustandsüberwachungsverordnung samt Anhängen; BGBl. II Nr. 479/2006.

Wolfram et al. (2015) GUIDANCE ON THE MONITORING OF THE BIOLOGICAL QUALITY ELEMENTS PART B2 – PHYTOPLANKTON. B2-01i_PHP_EN (non-binding work translation for information purposes only), Federal Ministry of Agriculture and Forestry,

UTERMÖHL H. (1958): Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. int. Ver. theor. angew. Limnol. 9: 1–38.

Comments
A thorough knowledge of the studied lake is crucial in order to choose the appropriate sampling dates. If the second sampling date is set during the clear-water phase and the third sampling date is set too late in autumn, the annual mean of either the total biovolume or the chlorophyll-a concentration may be significantly underestimated.

Method reported by:
Name: Hans Rund, Rainer Kurmayer
Institute: University Innsbruck

Country: Austria
Category: River
Biological Quality Element: Phytobenthos

Short description of sampling procedure

This Method survey is based on "Guidance on the monitoring of the biological quality elements part A3 - Phytobenthos" (Pfister et al. 2015). Sampling and sample processing is done according to CEN 2003 and CEN 2004. The procedure is based on the recording of all benthic algal communities of natural substrates found in a section of running water. Accordingly, planktic and also metaphytic forms, which are found time and again in benthos samples (these include e.g. almost all centric diatoms or actively-movable, flagellate groups such as Euglenophycea), must generally not be considered for the method described here.

Macro algae / Growth-habit types (mixed populations of micro algae):

Sampling is carried out as a result of macroscopic algae recording. In any event, all proven or logged macro-algae species and discernible growth-habit types (mixed populations of macro algae) are to be extracted. As a rule, the samples are rocks/hard substrates. Also in water bodies primarily exhibiting fine substrates, rocks are, in any case, to be preferred. The number of rocks to be extracted depends on the number and heterogeneity of the existing algal beds. Periphytic forms which are only to be found on large boulders must be extracted separately and directly filled in well-lockable sampling vessels. In water bodies exclusively exhibiting fine/soft sediment, at least three surface-sediment samples must be taken (lifting or suction of top 2 mm with a spoon or a mini cylindrical finger – e.g. PVC core cutter made of disposable syringes). The samples can either be transported to the laboratory as entire rocks (in a bucket and wetted with water; according to experience, a 5-litre bucket per survey site will be sufficient), or all macro algae as well as the different macroscopic growth-habit types are gently stripped off/scraped off/brushed off the rocks on-site and each filled into separate sampling vessels (do not forget to affix exact labelling!). To the extent possible, the samples must be swiftly transported to the lab in a cool and dark state for further treatment (Pfister et al. 2015).

Diatoms:

The base material for the analysis and counting of Diatomophycea must be primarily extracted from rocks (or hard substrates). Immediately after sampling, these must be brushed off on-site with a toothbrush (after the brush has been thoroughly cleaned!) and poured into a well-lockable vessel together with a small amount of site water. Partial samples consisting of at least 5 rocks and extracted from the dominant choriotopes should be processed into a composite sample. From water bodies exclusively exhibiting fine/soft sediment, 2-3 surface-sediment samples should be extracted (lifting or suction of top 2 mm with a spoon or a mini cylindrical finger – e.g. PVC core cutter made of disposable syringes) and joined into a composite sample. The unit containing the diatom sampling material must be labelled accurately and transported - as cool and dark as possible - to the lab for the purpose of further treatment (Pfister et al. 2015).

Sampling period/frequency

Sampling should be carried out when the conditions are favorable (low water level and low turbidity).Therefore sampling should happen during winter (January – March). In general, possible flood events must be taken into consideration prior to the survey. For the current phytobenthos survey, they must date back at least 3 - 4 weeks. The decisive factor for periphytic algae in this

respect is, if the flood has cause major bed-load discharge. At least in our gravel-dominated running waters, such is the case approximately from HQ1 (annual flood).

Characterisation of representative sampling site

The extension of the area which has to be recorded in order to capture, if possible, all species of phytobenthos occurring in a running-water section considerably and generally depends on the range of species and on the heterogeneity of distribution of the individual species/growth forms. In order to meet these requirements, a stretch which is at least appr. 4-5 times the width of the water body, but at least 20 m in length in brooks or 40 m in length in rivers, must be recorded in any event for periphytic algae. The sampled area should be large enough to capture all existing algal habitats (Pfister et al. 2015).

Short description of processing method and evaluation (e.g. metrics, level of identification)

In the overall phytobenthos assessment, "diatoms" and "all other groups of algae" (referred to as "non-diatoms" in the following) are represented at a ratio of 1:1, respectively. Recordings for which the relative share of abundance of the taxa which cannot be determined at species level does not exceed 33% overall (or 15% for mere diatom assessments) can only be assessed to a limited extent. Taxa which cannot be determined at species level, are generally included in the list of species and form part of overall abundance, also with regard to their respective relative frequencies. For the determination of non-diatoms, working on live samples is the premium choice. To this end, however, treatment must be carried out no later than 4 – 5 days after sampling (in the meantime, samples should be stored at 5°C in the refrigerator. For diatom analysis, the treatment of live material is not required. If swift further treatment is not possible (within 1 – 2 days), the material must be fixated until it is cleaned/prepared (e.g. 1 – 2% final formaldehyde concentration). The determination of relative frequencies is the basis for further calculations. In the overall phytobenthos assessment, diatoms and non-diatoms are included at a ratio of 1:1, respectively.

The phytobenthos-based assessment of the ecological status is based on a multimetric approach and includes three modules. For each module, a separate assessment (allocation of the recording to an ecological status class) is carried out:

• The Trophic Status Module assesses nutrient load and is based on the trophic index according to ROTT et al. (1999). The scale for assessment is the deviation of the determined trophic condition from the respective bioregion-specific reference condition.

• The Saprobic Status Module assesses organic load and is based on the saprobic index according to ROTT et al. (1997). The scale for assessment is the deviation of the determined saprobic condition from the respective bioregion-specific reference condition.

• The reference-species Module assesses the deviation of the found biotic community from the reference biocoenosis expected in the respective bioregion and altitudinal belt and indicates synergy effects found in the interaction between nutrient load and organic load as well as other changes in environmental conditions which have, as of yet, not been covered by the two mentioned indication systems. The scale for assessment is the share of reference species in the total abundance or in the total number of species of periphytic algae respectively determined.

Each of the three modules relies on the prepared list of species as well as on the determined bioregion and altitudinal belt of the survey site as their base data. In a first step, the module-specific indices (trophic index, saprobic index and/or referencespecies index) are calculated. Further on, these indices have to be, respectively, converted into a specific value, i.e. the "Ecological Quality Ratio" (EQR), to ensure EU-wide comparability of different national assessment procedures. The

Ecological Quality Ratio indicates the ratio between the index determined for the respective survey and the index value expected for the respective bioregion and altitudinal belt.
For the overall assessment of phytobenthos, the 3 individual results gained from the mentioned modules are intersected with one another according to the "worst-case principle" (i.e. the worst individual assessment which has been determined is decisive). Detailed information on the index calculation is provided in "Guidance on the monitoring of the biological quality elements part A3 – Phytobenthos" (Pfister et al. 2015).

Additional abiotic data recorded

From Pfister et al. (2015):
Bioregion, altitudinal belt, relevant bioregions (corresponding to module "reference species"), Trophic reference condition class, Saprobic reference condition class, current discharge, discharge forecast (to the extent identifiable), references to past flood events, mean flow velocity (estimate), flow-off amount (estimate), choriotope distribution [%] estimate, water turbidity level, shading of survey site [%], survey section/area (length, width, share in running-water cross-section) [m]

Method features compliant with WFD

This operating instruction for the phytobenthos-based ecological assessment of Austrian running waters according to the EU Water Framework Directive 2000/60/EC (European Commission) describes the methods to be applied for all steps required in the process, from field survey/sampling over further sample treatment in the lab all the way to result evaluation and finally the assessment of the ecological status (Pfister et al. 2015). According to the requirements of the Water Framework Directive, the scale to be used for the assessment of the ecological status is the deviation of an existent coenosis from the expected reference coenosis (or the deviation of an existent status from the respective reference status). The overall assessment (as well as the individual assessments) is/are expressed as ecological status class. There are 5 ecological status classes (1 = high, 2 = good, 3 = moderate, 4 = poor, 5 = bad).

Rules to define ecological classes and reference conditions

Since 2001, Austria has been subdivided into 15 bioregions. These bioregions are also suitable for the phytobenthos-based typification of running waters and are, accordingly, also used as a basis for defining the reference/basic conditions which are relevant for algae. Sites surveyed in large rivers must hence be allocated to the respectively corresponding water section (there are 18 water sections). For the phytobenthos assessment modules, there are three altitudinal belts: altitudinal belt 1 (= < 500 m), altitudinal belt 2 (= 501 - 800 m) and altitudinal belt 3 (= > 800 m).

Literature Reference

(CEN) ÖNORM EN 14407 (2004): Wasserbeschaffenheit – Anleitung zur Bestimmung. Zählung und Interpretation von benthischen Kieselalgen in Fließgewässern/ Water condition – Guide for the determination, counting and interpretation of benthic diatoms in running waters

(CEN) ÖNORM EN 13946 (2003): Wasserbeschaffenheit - Leitfaden zur Probenahme und Probenaufbereitung von benthischen Kieselalgen in Fließgewässern/ Water condition - Guidance on sampling and sample treatment of benthic diatoms in running waters.

MOOG O.; SCHMIDT-KLOIBER A.; OFENBÖCK T. & GERRITSEN T. (2001): Aquatische Ökoregionen und Fließgewässer-Bioregionen Österreichs – eine Gliederung nach geoökologischen Milieufaktoren und Makrozoobenthos-Zönosen. Publ. Wasserwirtschaftskataster. BMFLFUW. 1-106.

PFISTER P.; PIPP E. (2015): GUIDANCE ON THE MONITORING OF THE BIOLOGICAL QUALITY ELEMENTS PART A3 - PHYTOBENTHOS

ROTT. E.; HOFMANN. G.; PALL. K.; PFISTER. P. & PIPP E. (1997): Indikationslisten für Periphyticalgen. Teil 1: Saprobielle Indikation. Publ. Wasserwirtschaftskataster. BMLF. 1-73.

ROTT. E.; Van DAM. H.; PFISTER. P.; PIPP. E.; PALL. K.; BINDER. N. & ORTLER K. (1999): Indikationslisten für Periphyticalgen. Teil 2: Trophieindikation. geochemische Reaktion. toxikologische und taxonomische Anmerkungen. Publ. Wasserwirtschaftskataster. BMLF. 1-248.

Comments

The scope of application of the method presented here generally includes all types and sizes of running waters occurring in Austria. The method is certainly best suitable for use in completely accessible-by-foot, more or less clear brooks with rock substrates. In larger rivers, assessments naturally only refer to the river-bank area respectively visible and suitable for collecting samples. The least-well-founded statements on the ecological status with regard to phytobenthos are certainly made for slowly-flowing, frequently-turbid brooks dominated by soft/fine substrates.

Method reported by:
Name Hans Rund, Rainer Kurmayer
Institute University Innsbruck

Country: Austria
Category: Lake
Biological Quality Element: Fish

Short description of sampling procedure

The Austrian BQE (fish) assessment for lakes consists of a combination of 4 standardized methods: Electrofishing along the shoreline, benthic and pelagic gillnet fishing and hydroacoustics surveys.

Electrofishing: Electrofishing in the littoral zone is used to detect fish inhabiting the lakeshore zone and to detect fish that have a low probability to get caught in the gill-nets. The number of sites to be fished has to be chosen depending on the lake surface area (see section on characterization of representative sampling site). Sampling is done by electrofishing using a boat. From the slow-driving boat (along the depth contour of approximately 1.5m), the anode is immersed in the water, and all fish which have been stunned by electricity must be scooped as soon as possible and put into the oxygen-supplied fish barrel. Fishing shall be performed at each site, if possible over a period of 15 min; yet this period may also be shorter depending on habitat size. The length of the section is, in addition, measured via GPS, and the site is photographed (Gassner et al. 2015). Fish are measured (total length), counted and determined to species level. If necessary, fish may be sedated to enable quicker processing of the samples. Fish larvae and 0+ fish may be preserved in alcohol for the purpose of later laboratory species determination. The remaining fish should be released at the capture site, after complete recovery from sedation.

Benthic gillnet fishing: Sampling in the benthic zone is used to detect fish species inhabiting the littoral and the benthic zone. The number of gillnet nights used for sampling depends on the maximum depth and the surface area of the lake (Table 1, European standard EN 14757:2015). Detailed information on the sampling effort in relation to surface area and depth can be found in the appendix. Nets placed at a depth of 0 to 3 m, should be attached to several orange and well-visible buoys fastened in order to prevent danger to swimmers. In addition, designated bathing sites, diving sites and landing sites must be avoided with these nets.

Pelagic gillnet fishing: Sampling in the pelagic zone is used to detect fish species inhabiting the pelagic zone and their relative abundances and biomass. In deviation to existing standards, the Austrian assessment approach uses more sampling sites than required in the European standard. According to CEN (2005, 2015) only the deepest location of the lake should be sampled with pelagic gillnets. However, in Austria the following rules are applied: For lakes with a surface area of ≤ 5km² one sampling site (deepest point) should be used, for lakes between 5 - 10 km² the deepest point and an additional random site should be sampled. For lakes > 10km² the deepest point as well as two random sites should be sampled. As given in EN 14757:2005 the sampling is done covering the full depth range at the deepest point starting at the 0-6 m depth and lowering the 6x30 m (height x length) nets in 6 m intervals down to the bottom. This standard was later modified for deep lakes to cover a depth range of 0 - 70 m only.

Gillnet (benthic and pelagic) sample handling**:** On the shore, the bins containing the fish are cooled with ice. Sample processing is performed per net. Net sheet by net sheet, the fish are removed from the net, sorted according to mesh widths, and then provided in labelled bowls (mesh width, site, net number, net depth, date, time) for further treatment. Individuals are numbered, the species is determined, and total length and full fresh weight are measured. Furthermore, scale or otolith samples are drawn from sentinel fish species for the purpose of age determination. All these data are immediately entered in a data sheet and stored in the PC. After removing the fish, the nets are cleaned from branches, leaves etc. and rinsed in soapy water. After that, they are taken up in order to make them ready-to-use again (Gassner et al. 2016).

Hydroacoustic surveys: Hydroacoustic surveys are used to estimate the overall fish biomass, to survey the spatial-temporal fish distribution and to validate gill-net catches. Hydroacoustic surveying has to be performed with a scientific split-beam echosounder. Surveys must be conducted exclusively during night hours along zigzag transects. Because of the patchy distribution of fish in lakes the quality of a biomass estimation increases with accumulating number of transects and the degree of coverage has to be >5. Position is continuously controlled and logged using a GPS system. Boat speed should be maintained at 5–7 km per hour. The spherical transducer is mounted 0.2 – 0.5 m below the water surface and oriented vertically. Ping rate has to be as fast as possible depending on the water depth, and pulse duration has to be set to 0.064 ms. The threshold for volume backscattering strength (Sv) has to be set at -70 dB, and the single echo detector of the echosounder should accept echoes with minimum target strength (TS) of -61 dB. Minimum and maximum echo lengths are 0.5 and 1.9 of the transmitted pulse length. Before each survey, a standard target test should be performed, and if necessary, the hydroacoustic system must be calibrated using a standard copper sphere (diameter 23 mm). Raw data and positioning data (GPS device) are continuously recorded and stored on the operating computer (Gassner et al. 2015).

Sampling period/frequency

Electrofishing: Sampling should be done between July and mid October.

Benthic & Pelagic gillnet fishing: In order to be able to comply with a catch duration of appr. 12 hours according to CEN (2005), the nets are set in the evening (between 5 p.m. and 8 p.m.) and lifted in the morning (between 5 a.m. and 8 a.m.). Sampling should take place between July and mid October, when the surface water temperature is still > 15° (CEN 2005).

Hydroacoustics: The optimal season for hydroacoustic survey is between September and January. A minimum of 3 surveys should be carried out per lake (monthly interval) to guarantee a good estimation of the total fish biomass.

Characterization of representative sampling site

Electrofishing: Lakes < 4km² surface area need at least 4 lakeshore sites, lakes > 4km² surface area need 1 site for each km². The sampling sites should represent all the different lakeshore habitats (depending on sediment composition, submerged and shoreline vegetation, entrance of

tributaries and natural vs. artificial shorelines) that can be found in the waterbody of interest. According to EN 14011:2003, a minimum of 50 meters needs to be sampled per site.

Benthic gillnet fishing: Number of sampling sites and nets used depends on surface area and depth. Detailed information on the sampling effort in relation of this factors can be found in the appendix.

Pelagic gillnet fishing: For lakes with a surface area of ≤ 5km² one sampling site (deepest point) should be used, for lakes between 5 - 10 km² the deepest point and an additional random site should be sampled. For lakes > 10km² the deepest point as well as two random sites should be sampled.

Hydroacoustic surveys: All offshore areas >15 m depth (CEN 2014) of the investigated lake should be covered.

Short description of processing method and evaluation (e.g. metrics, level of identification)

To determine the ecological status of lakes based on the biological quality element fish, the the Austrian Lake Fish Index (ALFI) is used (Gassner et al. 2013). The Austrian assessment method addresses a set of different pressures (Water level fluctuation, shore line degradation, connectivity, recreation, fisheries intensity, alien (translocated) fish species and eutrophication) by combination of several parameters of the fish community. ALFI is defined by 8 metrics: abundance index (AI) for type-specific species, proportion of AI of alien species, AI of small-bodied species AI of sensitive species, AI of migrating spawners, AI of spawning guilds, Length frequency of sentinel species and the fish biomass. The individual metrics and, consequentially, also the total EQR) can constitute a value between 0 and 1, with 1 representing the reference status and each smaller value expressing the respective deviation from the reference status (Gassner et al. 2015).

Additional abiotic data recorded

Temperature, conductivity, surface area, turbidity, depth, latitude, pH, dissolved oxygen

Method features compliant with WFD

Austrian BQE assessment for lakes is described in" Guidance on surveying the biological quality elements part B1 – fish" (Gassner at al. 2015). All requirements for the WFD are covered (species composition, abundance and age structure approximated by length frequency) and the ecological status is defined by 5 classes (high, good, moderate, poor, bad). Partially the Austrian assessment methods exceeds the WFD requirements e.g. number of pelagic sampling sites.

Rules to define ecological classes and reference conditions

The reference condition is based on the reconstruction of historical fish-species communities in lakes > 50 ha (Gassner et al. 2003b; Gassner et al. 2005). The assessment method starts from the assumption that in the unimpaired historical reference status each fish species belonging to the native fish community would be detectable in the course of standardized fishing with the highest abundance index. Biomass (hydroacoustically measured) is evaluated against the empirical mean

fish biomass hydroacoustically measured in recent decades in Austrian lakes for each lake type defined by sentinel fish species (arctic char, minnow, bleak, pikeperch). The length frequency of sentinel species is evaluated against length frequency data from previous standardized catches. Depending on the sentinel species defined lake type, different length classes and intervals have been established (Arctic char: 18 classes of 12-29 cm with class interval of 1 cm; minnow: 13 classes of 3-9 cm with class interval of 0.5 cm; bleak: 14 classes of 8-14.5 cm with class interval of 0.5 cm; pike-perch: 16 classes of 10-40 cm with class interval of 2 cm). The number of currently detected length classes is divided by the number of definitely available length classes in the reference status. This way, the assessment method measures the current status of each lake on the basis of its individual historical reference and performs an assessment which is not based on a general and type-specific reference status.

Rules to define ecological status classes of lakes:

Ecological status class	EQR (Austrian Lake Fish Index)
high	> 0,8
good	0.60-0.79
moderate	0.40-0.59
poor	0.20-0.39
bad	< 0.20

References

CEN (2003): EN 14011 Water quality — Sampling of fish with electricity.

CEN (2005): EN 14757 Water quality — Sampling of fish with multi-mesh gillnets

CEN (2014): EN 15910 Water quality — Guidance on the estimation of fish abundance with mobile hydroacoustic methods

CEN (2015): EN 14757 Water quality — Sampling of fish with multi-mesh gillnets

GASSNER, H., D. ACHLEITNER, M. LUGER (2015) Guidance on surveying the biological quality elements. Part B1 – Fish. Published by: Austrian Federal Ministry of Agriculture and Forestry, Environment and Water Management (ISBN: 978-3-85174-063-9).

Method reported by:
Name: Hans Rund, Josef Wanzenböck
Institute: University Innsbruck

Appendix

	Depth stratum m	Maximum depth m						
		< 6	6 to 11,9	12 to 19,9	20 to 34,9	35 to 49,9	50 to 75	> 75
Lake area < 20 ha	< 3	4	3	4	4	3		
	3 to 5,9	4	3	4	3	3		
	6 to 11,9		2	4	3	3		
	12 to 19,9			4	3	3		
	20 to 34,9				3	2		
	35 to 49,9					2		
Total number of gillnet-nights		8	8	16	16	16		
Lake area 21 ha to 50 ha	<3	4	5	5	5	5		
	3 to 5,9	4	6	5	5	5		
	6 to 11,9		5	3	5	6		
	12 to 19,9			3	5	6		
	20 to 34,9				4	6		
	35 to 49,9					4		
Total number of gillnet-nights		8	16	16	24	32		
Lake area 51 ha to 100 ha	< 3	8	8	7	7	7	7	
	3 to 5,9	8	8	7	7	7	7	
	6 to 11,9		8	5	9	7	10	
	12 to 19,9			5	6	4	4	
	20 to 34,9				3	4	4	
	35 to 49,9					3	4	
	50 to 75						4	
Total number of gillnet-nights		16	24	24	32	32	40	
Lake area 101 ha to 250 ha	< 3	8	8	8	7	7	7	
	3 to 5,9	8	8	8	7	7	7	
	6 to 11,9		8	8	10	10	6	
	12 to 19,9			8	8	6	6	
	20 to 34,9				8	6	6	
	35 to 49,9					4	4	
	50 to 75						4	
Total number of gillnet-nights		16	24	32	40	40	40	

	Depth stratum m	Maximum depth m						
		<6	6 to 11,9	12 to 19,9	20 to 34,9	35 to 49,9	50 to 75	>75
Lake area 251 ha to 1 000 ha	< 3	12	11	10	10	10	10	10
	3 to 5,9	12	11	10	10	10	10	10
	6 to 11,9		10	10	10	10	10	10
	12 to 19,9			10	10	8	8	8
	20 to 34,9				8	6	8	5
	35 to 49,9					4	6	5
	50 to 75						4	4
Optional	>75							0 or 4
Total number of gillnet-nights		24	32	40	48	48	56	52 to 56
Lake area 1 001 ha to 5 000 ha	<3	12	11	10	10	10	10	10
	3 to 5,9	12	11	10	10	10	10	10
	6 to 11,9		10	10	12	12	10	10
	12 to 19,9			10	12	9	10	10
	20 to 34,9				12	9	10	10
	35 to 49,9					6	10	6
	50 to 75						4	4
Optional	>75							0 or 4
Total number of gillnet-nights		24	32	40	56	56	64	60 to 64

Table 1 Detailed information on number of benthic gillnet nights used within each depth stratum depending on the surface area of the lake

Country: Austria
Category: River
Biological Quality Element: Fish

Short description of sampling procedure

The Austrian Water quality assessment method for rivers, based on the biological quality element fish is based on "Leitfaden zur Erhebung der biologischen Qualitätselemente Teil A1" (Haunschmid et al. 2006). In preparation of sampling, the watershed is analyzed concerning the geographical, morphological and hydrological characteristics to determine appropriate sampling strategies. The Fishing procedures and equipment differ depending on water depth and river width.

Wadable rivers:

Small rivers are electrofished from the bank or by wading if the water depth does not exceed 0.7m. One pole operator and at least one dip-net operator is needed per 5 m river width. The operators fish upstream so that turbidity, caused by wading does not affect efficiency. For absolute population estimates, stop nets are placed at the end of each sampling stretch which prevent the fish from escaping. Each sampling stretch is fished at least 2 times, using identical fishing effort and can be extended to 3 times if the individual number of dominant species is low. Equipment (power source and control box) is best located on the bank with access to the stream section, achieved by fitting long cables to the anode. Another possibility is to use backpack- mounted machines.

River width	Minimum total length	Minimum length per sub-sampling stretch
< 5 m	100 m	35 m
5 m – 15 m	100 m – 150 m	50 m
> 15 m	At least 10 x river width	80 m

Non-wadable rivers:

Whenever water deeper than 0.7m is sampled, a boat is used since wading beyond this depth can be dangerous (anyhow, shallow parts may be sampled by wading). Sampling is done by using the "strip-fishing method" according to Schmutz et al. (2001). The concept is to quantify stocks by fishing a considerable amount of distinct, habitat-specific "strips" with electrofishing-boats and to extrapolate these samples according to a standardized procedure to a whole river reach. This method consists of stratified sampling of all typical habitats in combination of fishing along the shore and the middle of the river on longer sections of water. In preparation of sampling, the proportion of different habitat types is assessed. A representative sample should consist of several sub-samples proportional to the distribution of the existing habitats. If possible, any type of habitat must be sampled at least 3 times. However, a specific stretch should be sampled only once. A "strip" is defined as a certain area that is representative for different habitat types, which can be found in the waterbody of interest. The area of the "strips" vary with the location of the habitat types within the river (shore or middle strips). The length of the individual strips is chosen depending on the structure

of the strips: shore strips should be 50 - 100 m and river strips 100 - 300 m long. If the investigated river reach is < 2.5 km the length of all strips combined need to be at least 2.5 km. For longer reaches, the sum of the lengths of all fished strips must be 30% of the section length and consist of at least 25 strips (50 m -300 m). The width of the strips depend on the boat used for the sampling and can vary between 3.6 m and 5.1 m (Schmutz et al. 2001). For sampling the middle of the river, a specially equipped boat is used, several anodes are mounted on a boom in front of the boat to increases the size of the electric field relative to the fished area. For each type of habitat an average value, weighted according to the strip length, can then be calculated from the individual strips. Thus, inventory values can be specified for each individual habitat type. The total stock is calculated as the average of average habitat stock values, weighted according to their representativeness.

Sample processing for both methods:

Fish affected by the electric current are removed from the electric field as quickly as possible and transferred to suitable holding containers. Due to the increased oxygen demand, a sufficient supply of oxygen is necessary until release. Fish are measured (total length), counted and determined to species level. If necessary, fish may be sedated to enable quicker processing of the samples. Fish larvae and 0+ fish may be preserved in alcohol for the purpose of later laboratory species determination. The remaining fish should be released at the capture site, after complete recovery from sedation.

Sampling period/frequency

Sampling period: The timing of sampling is linked to the life history strategies of target species. In most circumstances sampling is carried out towards the end of the growing season when juveniles are of a sufficient large size to be caught by electro fishing. The sampling in the epi- and meta-rhithral is appropriate from June on, sampling in the hyporhithral and epipotamal is appropriate from March on. Due to reduced fish activity and therefore reduced sampling efficiency, sampling shouldn`t be done at water-temperatures < 5° (epi-, meta- and hyporhithral) respectively < 8° (epipotamal). Furthermore, fishing shouldn´t be done if the water temperature exceeds 20° or if the air temperature is above 30°.

Characterization of representative sampling site

The sampling stretches should represent all existing habitats in the given waterbody. The length of all sub-sampling stretches with the same characteristics should be the same.

Short description of processing method and evaluation (e.g. metrics, level of identification)

To determine the ecological status of rivers based on the biological quality element fish, the Fish Index Austria (FIA) is used (Haunschmid et al. 2006). The FIA is defined by 9 metrics: biomass, percentage of dominant species, percentage of subdominant species, percentage of rare species, flow velocity guilds, spawning guilds, deviations to the fish region index, population-structure of dominant species and the population-structure of subdominant species. The individual metrics and, consequentially, also the total EQR can constitute a value between 1 and 5, with 1 representing the

reference status and higher values expressing the respective deviation from the reference status (Haunschmid et al. 2006).

Additional abiotic data recorded

River bed width, wetted width, discharge, water temperature, mean water depth, maximum water depth, conductivity, turbidity, altitude, (pH)

Method features compliant with WFD

Austrian BQE assessment for rivers is described in "Leitfaden zur Erhebung der biologischen Qualitätselemente Teil A1- Fische (Haunschmid et al. 2006). All requirements for the WFD are covered (species composition, abundance, age structure) and the ecological status is defined by 5 classes (high, good, moderate, poor, bad).

Rules to define ecological classes and reference conditions

Type-specific reference conditions are based on the stratification of the monitored area using zoogeographic features and abiotic variables, with the objective of deriving strata of the highest possible ecological homogeneity. Biological attributes of ecological quality (metrics) are selected with respect to their sensitivity to particular types of environmental stress. The Austrian concept follows an integrated approach using the following types of information: reference sites, historical fish data, historical maps, reference models and expert judgement. Most of the data sources are integrated into a countrywide habitat and fish database (HaFiDat) comprising 2760 fish samples of 281 rivers. Austria follows a biocoenotic river type classification and is extended by fish regions and their subtypes (Economou et al. 2002).

Rules to define ecological status classes of rivers:

Ecological status class	EQR (Fish Index Austria)
high	1,00 - <1,50
good	1,50 - <2,50
moderate	2,50 - <3,50
poor	3,50 - <4,50
bad	4,50 – 5,00

Literature Reference

Economou A., Schmutz, S., Melcher, A., Haidvogl, G., Breine, J., Simoens, I., Kestemont, P., Goffaux, D., Ponz, D., Böhmer, J., Kesminas, V., Virbickas, T., Zalewski, M., Lapinska, M., Backx, J., de Leeuw, J.J., Ferreira, T., Beier, U., Degerman, E., Cowx, I.G., Noble, R.A.A., Starkie, A. (2002) Development, Evaluation & Implementation of a Standardised Fish-based Assessment Method for the Ecological Status of European Rivers - A Contribution to the Water Framework Directive - Defining Reference Conditions

Haunschmid, R., Schotzko, N., Petz-Glechner R., Honsig-Erlenburg W., Schmutz S., Spindler T., Unfer G., Wolfram G., Bammer V., Hundritsch L., Prinz H., Sasano B. (2006) Leitfaden zur Erhebung der biologischen Qualitätselemente Teil A1

Schmutz, S., Zauner, G., Eberstaller, J. & Jungwirth M. (2001): Die „Streifenbefischungsmethode“: eine Methode zur Quantifizierung von Fischbeständen mittelgroßer Fließgewässer. Österreichs Fischerei Jg. 54, Heft 1/2001: 14–27.

CEN (2003): EN 14011 Water quality – Sampling of fish with electricity.

Method reported by:
Name: Hans Rund, Josef Wanzenböck
Institute: Research Department for Limnology, Mondsee

Water quality assessment-National methods
France

Country: France **Category: Lake** **Biological Quality Element: Phytoplankton**

Short description of sampling procedure One sampling station per lake, located at the maximum depth. For artificial lakes the station must be located outside of the influence area of the dam. Sampling is performed in the eutrophic zone with an integrative bottle, from the surface to 2.5 Secchi depth. If the euphotic zone is deeper than the Secchi depth then the sample is taken from the surface to 1m above the bottom of the lake. Two subsampled are taken after water homogenization. The first one (500 mL) is fixed with Lugol to assess the phytoplankton composition, and the second one (1L) is filtered to assess the concentration in chlorophyll-a. At least 400 individuals must be counted and identified at the lowest taxonomic unit. Results are expressed in biovolume ($mm^3.L^{-1}$).
Sampling period/frequency 4 campaigns per year and at least 3 campaigns during the growing period (between May and October). The 1st campaign should take place between mid-February and March, the 2nd between mid-May and June, the 3rd between July and August and the last one between September and mid-October. This could be adapted for lakes freezing in winter. At least three weeks must separate two sampling occasions.
Characterisation of representative sampling site Must be located on the deepest zone of the lake.
Short description of processing method and evaluation (e.g. metrics, level of identification) IPLAC is the French national index to assess lakes with the phytoplankton. It is composed of two metrics: MBA which is the algal biomass metric and MCS which is the specific composition qualifies the trophic status of the lake. Taxa are identified at the species level and results are expressed in biovolume. MBA value is obtained by dividing the expected value of the chlorophyll a concentration by the observed value (mean of the growing period, 3 values). The reference value is computed from the mean depth of the lake through a power function. To compute the value for the metric MCS, for each taxa '*i*'it is necessary to have its biovolume B_i, its stenoecy coefficient S_i and it specific score CS_i.MCS is obtained with the formula: $$MSC_c = \frac{\sum_{i=1}^{n} CS_i \times S_i \times B_i}{\sum_{i=1}^{n} S_i \times B_i}$$

The MSC value it the average of the 3 MSCs computed for the 3 surveys realized during the growing period. The observed MSC value is then traduced in Ecological Quality Ratio (EQR) by dividing the observed value by the reference value. This latest if predicted from a multiple linear regression based on the mean depth and the altitude of the lake.
The index value is obtained by a weighted mean of the two EQRs: IPLAC = $0.7 \times MCS_{EQR} + 0.3 \times MBA_{EQR}$
This value can be traduced in ecological class H/G/M/P/B

Additional abiotic data recorded

Water transparency, and a vertical profile of temperature, pH, conductivity and dissolved oxygen.
These abiotic variables are necessary for the IPLAC computation:
Mean depth
Altitude

Method features compliant with WFD

Yes

Rules to define ecological classes and reference conditions

Ecological classes are defined along a linear scale from 0 to 1.
H: $0.8 < IPLAC \leq 1$
G: $0.6 < IPLAC \leq 0.8$
M : $0.4 < IPLAC \leq 0.6$
P: $0.2 < IPLAC \leq 0.8$
B: $0 \leq IPLAC \leq 0.2$
Reference conditions are defined from linear and non-linear functions that were established on a reference data set (47 'lake-years').

Literature Reference

Laplace-Treyture, C. and T. Feret. 2016. Performance of the Phytoplankton Index for Lakes (IPLAC): A multimetric phytoplankton index to assess the ecological status of water bodies in France. Ecological Indicators 69:686-698.

Laplace-Treyture, C., Barbe, J., Dutartre, A., Druart, J.C., Rimet, F., Anneville, O.,2009. Protocole standardisé d'échantillonnage, de conservation, d'observationet de dénombrement du phytoplancton en plan d'eau pour la mise en oeuvrede la DCE: version 3.3.1, pp. 44.

Method reported by:
Name Maxime Logez
Institute AFB/Irstea

Country: France
Category: Lake
Biological Quality Element: Phytobenthos (diatoms)

Short description of sampling procedure

The diatoms are sampled simultaneously with macrophytes. Only natural or artificial lakes with an annual water level fluctuation lower than 2m are concerned. Several stations (unit of observations) are sampled along the lake shore. The number of potential stations is computed from the Jensen's protocol, which is a geometric positioning. Perpendicular transects are regularly drawn from the baseline, and the contact between these transects and the shoreline defines a potential station. The number of recommended station depends of the lake area (3 for lakes < 2.5km², 6 for lakes between 2.5 and 10 km² and 8 for larger lakes). A station is a portion of bank of 100m long. Stations should be selected to cover the different bank types.

At each station two types of substrates has to be sampled with a toothbrush or knife: large hard substrates (pebble to rocks) and submerged macrophytes. 5 samples must be taken on each substrate. As far as possible, the same species (taxon) of macrophyte must be used as sampling substrate for all stations all over the lake. Helophytes should be preferred.

For the hard substrates, at least 5 random samples must be performed representing at least 100cm². Samples must be located in the euphotic area, in a depth lower than 50cm. Areas influenced by water level fluctuations must be avoided, such as the samples are realised on substrates that are always submerged.

For the macrophytes, 5 random samples are taken on submersed stems of helophytes of 10 cm long. Stems must be sampled in the first 20cm of the water columns on plants located on the boundary of the macrophyte patch.

For each substrate the 5 samples are mixed, and samples are fixed with Lugol.

The sampling must be performed during one occasion, on consecutive days if necessary. Interruptions should be avoided.

Sampling period/frequency

Once per year during the growing season, between July and September.

Characterisation of representative sampling site

A sampling site is a portion of 100m of bank. It should encompass both hard substrates and patch of macrophytes. It must representative of one of the four bank types: typical wetland riparian type, riparian zone colonised by dry-land schrubs and brushes, riparian zone not colonised by dry-land schrubs and bushes and artificial areas.

Short description of processing method and evaluation (e.g. metrics, level of identification)

Individuals are identified at the lowest possible taxonomic level (e.g. genera, species) and counted. At least 400 diatom units must have been identified. Results are expressed in biovolume.

Additional abiotic data recorded None
Method features compliant with WFD The protocol was designed to fulfil WFD requirements.
Rules to define ecological classes and reference conditions No method is available yet.

Literature Reference AFNOR NF XP T 90-238. 2010. Qualité de l'eau – Echantillonnage des communautés de macrophytes en plan d'eau. Irstea Bordeaux. Echantillonnage des communautés de phytobenthos en plans d'eau. 8pp.

Method reported by: **Name: Maxime Logez** **Institute: AFB/Irstea**

Country: France
Category: River
Biological Quality Element: Phytobenthos (diatoms)

Short description of sampling procedure

If hard natural substrates are available, epilithic diatoms are sampled by randomly selecting 5 cobbles (at least 100 cm^2) within riffles that have to be scraped by a toothbrush or knife. Shaded areas have to be avoided and samples have to be taken far from the shores.
If natural hard substrates are not available, five samples have to be realised on 5 plants (macrophytes).
Samples could be fixed with Lugol.

Sampling period/frequency

The sampling must take place after a period hydrologically stable. It is recommended to sample at least 1 week after a low flood and to wait 4 weeks if the flood was high.
Sampling is realized once per year, between May and October, but summertime period should be favorited (growing season).

Characterisation of representative sampling site

Sampling site should be wadeable and located in fast flowing conditions (riffle), in the middle of the stream channel to avoid shaded areas. If possible the sample should be realised at a bridge.

Short description of processing method and evaluation (e.g. metrics, level of identification)

Individuals are identified at the lowest possible taxonomic level (e.g. genera, species) and counted. At least 400 diatom units must have been identified to compute the DBI-2006 (Biological Diatom Index version 2006, here after DBI).
This index is based on the weighted average equation of Zelinka & Marva (1961). DBI is based on the ecological profile of 209 'apparent' taxa and their relative abundance, weighted by their indicative value. An apparent taxon incorporates one or more associated taxa that are difficult to distinguish morphologically.
To compute the DBI, seven water quality classes were defined. For each class, the relative abundance of each taxon is multiplied by its probability of presence and by its indicative value. This product is summed over all taxa and standardized to get a 'F' value per water quality class. The seven F values (one per quality class) are then weighted and standardized to get a score which is rescaled between 0 and 20, the DBI score.

Additional abiotic data recorded

No additional data needed to compute the DBI.

Method features compliant with WFD DBI can be derived in 5 classes as required by the WFD but is not based on the direct comparison with observable communities in reference conditions. Nonetheless, the departure from the taxonomic composition that corresponds to nearly undisturbed conditions is evaluated.
Rules to define ecological classes and reference conditions Ecological classes are defined from the IBD values. H: IBD ≥ 17.0 G: 13.0 ≤ IBD < 17.0 M: 9.0 ≤ IBD < 13.0 P: 5.0 ≤ IBD < 9.0 B: IBD < 5.0

Literature Reference AFNOR. 2007. Norme NF T 90-354 Coste, M., S. Boutry, J. Tison-Rosebery, and F. Delmas. 2009. Improvements of the Biological Diatom Index (BDI): Description and efficiency of the new version (BDI-2006). Ecological Indicators 9:621-650. Lenoir A. & Coste M, 1996. Development of a practical diatom index of overall water quality applicable to the french national water board network. In: Whiton B.A. & Rott E. (eds). Use of algae for monitoring rivers II, Innsbruck Austria 17-19 sept 95, Studia Student. Gmbh : 29-43.

Method reported by: **Name: Maxime Logez** **Institute: AFB/Irstea**

Country: France
Category: Lake
Biological Quality Element: Fish

Short description of sampling procedure

Fish are sampled with benthic gillnets, 1.5m height and 30m length, with 2.5m panels for each mesh sizes (5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55mm), following the CEN standard (CEN 2015). The number of nets depends of the lake area and depth. Pelagic gillnets are also used for deep lakes, but only data collected in the benthic gillnets are used to assess the ecological status of lakes. The sampling design is a depth stratified sampling, which implies that the lake is divided in depth strata (0-5.9m, 6-11.9m, etc.), that each strata has to be sampled by a given number of nets and that the gillnets have to be randomly deployed in each strata.

Gillnets are deployed during one night (≈ 12h) and the sampling period has to cover the sunset and the sunrise peaks of activity of fish. Gillnets are generally deployed the same day, in the afternoon and lifted in the next morning. If it is not possible to sample the whole lake in one day, then the sampling must be undertaken on consecutive days and should not be interrupted.

All individuals are identified at the species levels, sized and counted. Small fish from the same species and caught in the same mesh size of a given net, could be grouped before weighting and only a subsample can be sized. This to limit the amount of time spends on the field.

Sampling period/frequency

Sampling in summer/early autumn and once per WFD managing plan (per 6 years).

Characterisation of representative sampling site

The whole lake.

Short description of processing method and evaluation (e.g. metrics, level of identification)

Individuals are identified at the species levels.

Two indices are used depending of the origin of the lake: natural or artificial (reservoirs) but these two indices were developed with the same methodology.

For natural lakes, the IIL (Indice Ichtyofaune Lacustre equivalent to the European Lake Fish Index) is based on three metrics: catch per unit effort, CPUE, (total number of fish standardized by unit effort, gillnet area and sampling time), biomass per unit effort, BPUE, and catch per unit effort of omnivorous individuals, CPUE_OMNI.

For reservoirs, the IIR (Indice Ichtyofaune retenue) is also based on three metrics: biomass per unit effort, biomass of planktivorous individuals BPUE_PLAN and the biomass of no native species (excluding the salmonids) BPUE_NN_NS.

Additional abiotic data recorded

To define reference values it is necessary to have these abiotic variables:

Maximum depth, lake area, catchment area, altitude, mean air annual temperature and temperature amplitude (amplitude between the warmest month and the coolest month), retention time (reservoirs), global index of slope (Danis et al. 2012).
Method features compliant with WFD The sampling protocol used is the normalized European sampling protocol for fish in lakes. The two indices integrated different aspect of fish communities: density, biomass and species tolerance (omnivory). The scores are based on the deviation between observed and reference values, derived into EQRs and the index scores can be expressed in ecological classes following WFD requirements. The index for natural lakes has been intercalibrated in the Central-Baltic GIG.
Rules to define ecological classes and reference conditions Reference conditions are defined by an hindcasting method, which consists in taking into account both environmental variables and stressors when modelling metric values. Reference values are predicted by setting stressor values to values observable in not or slightly impacted lakes (for instance low values of total phosphorous). Reference values are predicted from linear functions. Observed values are matched to the reference values, and these deviations are transformed in EQRs that are finally aggregated to obtain the ILL value. For the IIL (natural lakes) the ecological classes are obtained from the following thresholds: H: IIL ≥ 0.733 G: 0.494 ≤ IIL < 0.733 M: 0.350 ≤ IIL < 0.494 P: 0.175 ≤ IIL < 0.350 B: IIL < 0.175 Different thresholds are used for the IIR (reservoirs).

Literature Reference Argillier, C. et aL. 2013. Development of a fish-based index to assess the eutrophication status of European lakes. Hydrobiologia 704:193-211. Ritterbusch, D. et al. 2017. Water Framework Directive intercalibration: Central-Baltic lake fish fauna ecological assessment methods; Part B: Development of the intercalibration common metric; Part C: Intercalibration., Joint Research Centre (JRC), the European Commission's science and knowledge service. CEN. 2015. Water quality - guidance on the estimation of fish abundance with multi-mesh gillnets. European standard. European Committee for Standardisation. Ref. No. EN 14757:2015. Logez et al. 2018. Monte-Carlo methods to assess the uncertainty related to the use of predictive multimetric indices. Ecological Indicators.

Danis P-A, Ferrer R, Gevrey M, Argillier C (2012) Seuils des paramètres physicochimiques soutenant la biologie - Plans d'eau naturels - Rapport d'avancement.

Comments
The uncertainty associated with the scores of these two indices could be estimated to assess the probability that a given lake could be in the different ecological status (Logez et al. 2019).
The index for the reservoir is almost finished and would be usable for the next period of the WFD.

Method reported by:
Name: Maxime Logez
Institute: AFB/Irstea

Country: France
Category: River
Biological Quality Element: Fish

Short description of sampling procedure

The protocol follows the recommendations of the CEN (2003).
Fish are sampled by electrofishing either by wading or by boat depending of depth. Depending of the width and of the depth it could either point sampling (at least 75 points) or complete sampling. For small and/or shallow rivers, multiple runs could be performed to sample fish. The stream reach is sample for downstream to upstream. Several anodes could be used simultaneously if possible. All individuals are identified at the species levels, counted, sized and weighted. As for lakes, group of small individuals from the spaces species could be weighted together and a subsample of individuals sized to limit the sampling time. Larger individuals must be sized and weighted separately. For large rivers, only the shores are sampled. Shores are sampled consecutively.
All samples must be realized the same day.

Sampling period/frequency

A standardized electrofishing sampling protocol is conducted during low flow period, between May and October and by day. Only one sampling occasion is performed per year for a given station. Some station are sampled each year, or one year or two, or at least once during a managing plan (6 years).

Characterisation of representative sampling site

A sampling site is a homogeneous stream reach in terms of geomorphology and biotypology. Its length varies with its width (station of at least 60m length if width < 3m, station length is 20-times the stream width if this latest is comprised between 3 and 30m, etc.).

Stream width	Minimal Length
< 3m	At least 60m
3<width<30m	20x stream width
> 30m	10x stream width

Short description of processing method and evaluation (e.g. metrics, level of identification)

Individuals are identified at the species levels. IPR the French index to assess the ecological status of streams by fish is based on 7 metrics, three based on the number of species and 4 based on fish densities:
- Total number of Species
- Number of rheophilic species
- Number of lithophilic species
- Tolerant species individuals
- Invertivorous species individuals

- Omnivorous species individuals
- Total density of individuals

Additional abiotic data recorded

Several abiotic data are required to compute the values that would be observed in reference conditions: fishing area, catchment area, distance from source, wetted width, slope, depth, altitude, mean air temperature of July, mean air temperature of January, hydrographic unit.

Method features compliant with WFD

Different aspect of fish communities are used, richness, densities, species trophic regime, spawning habitat, rheophily and tolerance.

The metric scores are computed as the deviation between metric observed values and metric reference values. These scores are transformed in EQR and the final score is derived in 5 ecological classes.

This index has been successfully intercalibrated.

Rules to define ecological classes and reference conditions

Reference conditions are established by generalized linear models that were calibrated on a 'reference' data set. Reference sites were not pristine sites but minimally disturb sites.

For a given site, once the abiotic data gathered, the reference values could be computed through logistic and linear functions.

The ecological classes are determined from the IPR values with the following rules:

High: IPR < 5

Good: 5 ≤ IPR < 16

Moderate: 16 ≤ IPR < 25

Poor: 25 ≤ IPR < 36

Bad: IPR > 36

Literature Reference

Oberdorff, T., D. Pont, B. Hugueny, and J.-P. Porcher. 2002. Development and validation of a fish-based index for the assessment of 'river health' in France. Freshwater Biology 47:1720-1734.

Jepsen, N. and D. Pont. 2007. Intercalibration of Fish-based Methods to evaluate River Ecological Quality. JRC scientific and technical report, JRC, Ispra.

Comments

A newest version of this index was already developped and published (Marzin et al. 2014) but it is only considered as an additional diagnostic tool for the moment. The IPR+ index integrates an estimation of the uncertainty associated with reference values.

Method reported by:
Name: Maxime Logez
Institute: AFB/Irstea

Water quality assessment-National methods

Germany

Country: Germany **Category: Lake** **Biological Quality Element: phytoplankton**

Short description of sampling procedure At the deepest point of the lake one integrated sample is taken: in polymictic lakes sub-samples from all water depth in 0.5 or 1m steps and in stratified lakes when Zeu< Zepi, than from epilimnion zone and if Zeu > Zepi, than from the euphotic zone (= Zepi = 2.5 x Secchi depth) in (March) April to October. Mixed sample should be taken and at least 2 l.
Sampling period/frequency At least April to October – additionally in Bavaria monthly from January to December
Characterisation of representative sampling site Deepest point
Short description of processing method and evaluation (e.g. metrics, level of identification) a) Sub-sampling of the Lugol fixed sample (usually 25ml) for chamber sedimentation. Counting and identification (species / species groups) follow the Utermöhl-technique standard EN 15204 (2006) with at least 20 taxa and 400 objects and two magnifications at inverse microscope. Taxa biovolumes (mm3/l) are calculated based on cell counts and specific cell volumes according the standard EN 16695. b) Unfixed water sample (1-2Liter) are filtered on a glas fibre filter for photometric measurement of pigment chlorophyll_a (total biomass) after ethanol extraction.
Additional abiotic data recorded Total phosphorus, Secchi depth, water temperature, SRP, Si, total nitrogen, nitrate-N, ammonia-N
Method features compliant with WFD All compliant – Name of method: Phyto-See-Index
Rules to define ecological classes and reference conditions Existing near-natural reference sites Other reference source: palaeo-limnological studies

Alp region: oligotrophic (AL 3) resp. oligo-mesotrophic lakes (Al 4), in high status by pre classification (see Wolfram et al. 2009)

Literature Reference

Mischke, U., Riedmüller, U., Hoehn, E., Nixdorf, B. (2016): Method Description of the Assessment of Lakes and Reservoirs with Phytoplankton and the Phyto-See-Index in Germany. User Handbook. Excerpt of original version December 2016. Electronic publication. http://www.gewaesser-bewertung.de/files/english_handbook_german_lake_assessment_method_description_psi_dec2016-1.pdf

EN 16695 (*EN 16695*. Water quality - Guidance on the estimation of phytoplankton biovolume

EN 15204. Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique

Wolfram et al. 2009: Reference conditions and WFD compliant class boundaries for phytoplankton biomass and chlorophyll-a in Alpine lakes. Hydrobiologia 633: 45–58.

Comments
In Bavaria depth range of integral sampling is given as 0-20m for stratified Alpine lakes, which corresponds in most cases to euphotic zone.

Method reported by:
Name: Ute Mischke
Institute: Bavarian Environment Agency

Country: Germany **Category: Lake** **Biological Quality Element: benthic diatoms**

Short description of sampling procedure The German method PHYLIB assess the bio-component “marcophytes/phytobenthos” and covers two biological groups “macrophytes” and “benthic diatoms” (Schaumburg et al. 2015). For benthic diatoms a minimum of five subsamples are taken all over the sampling site in a water depth of about 30cm, representing the different occurring natural substrata. Cobbles and stones are preferred and avoid organic sustratum wherever applicable. The biofilm is taken from those cobbles/stones etc. with a spoon, teeth brush or similar. Samples are fixed with ethanol with final concentration of 60 %.
Sampling period/frequency Summer, July until middle of August. One occasion per sampling season.
Characterisation of representative sampling site Homogenous section regarding flow velocity, shading and sediment conditions. Number of transects according shore length up to 20 - 50 transects per lake and same as for macrophytes (100m). No inflows (e.g. tributaries, drainages) within sampling section! In case of the large pilot site Starnberger See out of 27 transects analysed in former PHYLIB investigations, a selection of 10 transects accordingly highest taxonomic bio-diversity will be studied for EcoAlpsWater sampling.
Short description of processing method and evaluation (e.g. metrics, level of identification) Diatoms: after chemical oxidation of the material 500 objects of diatoms are determined and enumerated. Identification of organisms on species / subspecies level.
Additional abiotic data recorded Information about the shoreline, morphology of the littoral-zone, structure of the sediment and substratum
Method features compliant with WFD All compliant Method name: German Assessment System for Macrophytes and Phytobenthos according to the EU WFD
Rules to define ecological classes and reference conditions According to WFD: “Normative definitions of ecological status classifications”, Annex V, Chapter 1.2

Literature Reference

Schaumburg, J., Schranz, C., Stelzer, D., Vogel, A., (2015): Instruction Manual for the Assessment of Lake Ecological Status in Accordance with the Requirements of the EUWater Framework Directive: Macrophytes and Phytobenthos Phylib. Status February 2014. Bayerisches Landesamt für Umwelt. Im Auftrag der LAWA (Projekt Nr. O 10.10), 137 S., Augsburg/Wielenbach
Download:
https://www.lfu.bayern.de/wasser/gewaesserqualitaet_seen/phylib_englisch/instruction_protocols/index.htm

Comments

You find a full method description (in German language) while checking also linked pages- start link:
http://www.gewaesser-bewertung.de/index.php?article_id=74&clang=0

Method reported by:
Name Ute Mischke und Christine Schranz
Institute Bavarian Environmental Agency

Country: Germany **Category: River** **Biological Quality Element: benthic diatoms**

Short description of sampling procedure The German method PHYLIB assess the bio-component “marcophytes/phytobenthos” and covers three biological groups “macrophytes”, “benthic diatoms” and “phytobenthos without diatoms = PoD” in rivers (Schaumburg et al. 2015). For benthic diatoms a minimum of five subsamples are taken all over the sampling site in a water depth of about 30cm, representing the different occurring natural substrata. Cobbles and stones are preferred and avoid organic sustratum wherever applicable. The biofilm is taken from those cobbles/stones etc. with a spoon, teeth brush or similar. Site selection according expert knowledge, including surveys of the waterbody in the years before.
Sampling period/frequency Summer, July until middle of August. One occasion per sampling season.
Characterisation of representative sampling site
Short description of processing method and evaluation (e.g. metrics, level of identification) Diatoms: after chemical oxidation of the material 500 objects of diatoms are determined and enumerated. Identification of organisms on species / subspecies level.
Additional abiotic data recorded Information about the river channel e.g. morphology, structure, sediment and substratum, velocity
Method features compliant with WFD All compliant Method name: German Assessment System for Macrophytes and Phytobenthos according to the EU WFD
Rules to define ecological classes and reference conditions According to WFD: “Normative definitions of ecological status classifications”, Annex V, Chapter 1.2

Literature Reference

Schaumburg, J., Schranz, C., Stelzer, D., Vogel, A., Gutowski, A. (2012): Verfahrensanleitung für die ökologische Bewertung von Fließgewässern zur Umsetzung der EU-Wasserrahmenrichtlinie: Makrophyten und Phytobenthos . Stand Februar 2012. Bayerisches Landesamt für Umwelt. Im Auftrag des Umweltbundesamtes(FKZ 3707 28 201), 137 S., Augsburg/Wielenbach

Comments

You find a full method description (in German language) while checking also linked pages- start link:
http://www.gewaesser-bewertung.de/index.php?article_id=74&clang=0

Method reported by:
Name Ute Mischke und Christine Schranz
Institute Bavarian Environmental Agency

Country: Germany
Category: Lake
Biological Quality Element: Fish

Short description of sampling procedure

The assessment of Lake Starnberg consists of a combination of 4 standardised methods: Electrofishing along the shoreline, benthic and pelagic gillnet fishing and hydroacoustic surveys.

Electrofishing:

Electrofishing in the littoral zone is used to detect fish inhabiting the lakeshore and to detect fish that have a low probability to get caught in gill-nets. The number of sites to be fished is dependent on the lake surface area (see section on characterization of representative sampling sites). Sampling is done by boat electrofishing. From the slow-moving boat (constant depth of approx. 1.5m), the anode is immersed in the water, and all stunned fish are caught as soon as possible and put into an oxygenated holding tank. Fishing is performed at each site, if possible over a period of 15 min; yet this period may also be shorter depending on habitat size. The length of the section is, in addition, measured via GPS, and the site is photographed (Gassner et al. 2015). Fish are measured (total length), counted and determined to species level. If necessary, fish may be sedated to enable quicker processing of the samples. Fish larvae and 0+ fish may be preserved in alcohol for the purpose of later identification in the laboratory. The remaining fish should be released at the capture site, after complete recovery from sedation.

Benthic gillnet fishing:

Sampling in the benthic zone is used to detect fish species inhabiting the littoral and the benthic zone. The number of gillnet nights necessary for sampling depends on the maximum depth and the surface area of the lake (Table 1, European standard EN 14757:2015). Detailed information on the sampling effort in relation to surface area and depth can be found in the appendix. Nets placed at a depth of 0 to 3 m, should be attached to well-visible buoys in order to prevent danger to swimmers. In addition, designated bathing sites, diving sites and landing sites must be avoided with these nets.

Pelagic gillnet fishing:

Sampling in the pelagic zone is used to detect fish species inhabiting the pelagic zone and their relative abundances and biomass. In contrast to existing standards, the Austrian assessment approach uses more sampling sites than required in the European standard. According to CEN (2005, 2015) only the deepest location of the lake should be sampled with pelagic gillnets. However, in Germany the following rules are applied: For lakes with a surface area of ≤ 5km² one sampling site (deepest point) should be used, for lakes between 5 - 10 km² the deepest point and an additional random site should be sampled. For lakes > 10km² the deepest point as well as two random sites should be sampled. As given in EN 14757:2005 the sampling is done covering the full depth range at

the deepest point starting at the 0-6 m depth and lowering the 6x27,5 m (height x length) nets in 6 m intervals down to the bottom. This standard was later modified for deep lakes to cover a depth range of 0 - 70 m only.

Gillnet (benthic and pelagic) sample handling:
On the shore, bins containing fish are cooled with ice. Sample processing is performed per net. Net sheet by net sheet, the fish are removed from the net, sorted according to mesh widths, and then provided in labelled bowls (mesh width, site, net number, net depth, date, time) for further treatment. Individuals are numbered, the species is determined, and total length and full wet weight are measured. Furthermore, scale or otolith samples are drawn from sentinel fish species for the purpose of age determination. All these data are immediately entered in a data sheet and stored in the PC. After removing the fish, the nets are cleaned from branches, leaves etc. and rinsed in soapy water. After that, they are taken up in order to make them ready-to-use again.

Hydroacoustic surveys:
Hydroacoustic surveys are used to estimate the overall fish biomass, to survey the spatial-temporal fish distribution and to validate gill-net catches. Hydroacoustic surveying has to be performed with a scientific split-beam echosounder. Surveys must be conducted exclusively during night hours along zigzag transects. Because of the patchy distribution of fish in lakes the quality of a biomass estimation increases with accumulating number of transects and the degree of coverage has to be >5. Position is continuously controlled and logged using a GPS system. Boat speed should be maintained at 5–7 km per hour. The spherical transducer is mounted 0.2 – 0.5 m below the water surface and oriented vertically. Ping rate has to be as fast as possible depending on the water depth, and pulse duration has to be set to 0.064 ms. The threshold for volume backscattering strength (Sv) has to be set at -70 dB, and the single echo detector of the echosounder should accept echoes with minimum target strength (TS) of -61 dB. Minimum and maximum echo lengths are 0.5 and 1.9 of the transmitted pulse length. Before each survey, a standard target test should be performed, and if necessary, the hydroacoustic system must be calibrated using a standard copper sphere (diameter 23 mm). Raw data and positioning data (GPS device) are continuously recorded and stored on the operating computer (Gassner et al. 2015).

Sampling period/frequency
Electrofishing:
Sampling should be done between July and mid of October.
Benthic & Pelagic gillnet fishing:
In order with a catch duration of appr. 12 hours according to CEN (2005), the nets are set in the evening (between 5 p.m. and 8 p.m.) and lifted in the morning (between 5 a.m. and 8 a.m.).
Sampling should take place between July and mid October, when the surface water temperature is still > 15° (CEN 2005).
Hydroacoustics:
The optimal season for hydroacoustic surveys is between September and January.

Characterization of representative sampling site

Electrofishing:

Lakes < 4km² surface area need at least 4 lakeshore sites, lakes > 4km² surface area need 1 site for each km². The sampling sites should represent all the different lakeshore habitats (depending on sediment composition, submerged and shoreline vegetation, entrance of tributaries and natural vs. artificial shorelines) that can be found in the waterbody of interest. According to EN 14011:2003, a minimum of 50 meters needs to be sampled per site.

Benthic gillnet fishing:

Number of sampling sites and nets used depends on surface area and depth. Detailed information on the sampling effort in relation of these factors can be found in the appendix.

Pelagic gillnet fishing:

For lakes with a surface area of ≤ 5km² one sampling site (deepest point) should be used, for lakes between 5 - 10 km² the deepest point and an additional random site should be sampled. For lakes > 10km² the deepest point as well as two random sites should be sampled.

Hydroacoustic surveys:

All offshore areas >15 m depth (CEN 2014) of the investigated lake should be covered.

Short description of processing method and evaluation (e.g. metrics, level of identification)

To determine the ecological status of lakes based on the biological quality element fish, the DeLFI-Index is used. The German ecological assessment method for lakes is based on two modules called the Site-Modul and the Type Modul. Lakes larger than 1000 ha will be analyzed with the Site-Modul. The DeLFI-Index is defined by metrics which will be rated and subsequent an EQR-value (ecological quality ratio) will be calculated. The individual metrics and, consequentially, also the total EQR) can constitute a value between 0 and 1, with 1 representing the reference status and each smaller value expressing the respective deviation from the reference status.

Additional abiotic data recorded

Temperature, conductivity, surface area, turbidity, depth, latitude, pH, dissolved oxygen

Method features

Method is compliant with WFD.

Ecological classes and reference conditions

German ecological assessment for lakes is described in “Schriften des Instituts für Binnenfischerei e.V. Potsdam-Sacrow, Verfahrensvorschlag zur Bewertung des ökologischen Zustandes von Seen anhand der Fische, Band 41“.

The ecological status is defined by 5 classes (high, good, moderate, poor, bad). With the requirements of the Water Framework Directive the calculated EQR will be allocated to one of five ecological assessment classes (shown in table).

Ecological status class	EQR (German DeLFI-Index)
high	≥ 0,85
good	< 0.85
moderate	< 0.69
poor	< 0,50
bad	< 0.25

References

CEN (2003): EN 14011 Water quality — Sampling of fish with electricity.

CEN (2005): EN 14757 Water quality — Sampling of fish with multi-mesh gillnets

CEN (2014): EN 15910 Water quality — Guidance on the estimation of fish abundance with mobile hydroacoustic methods

CEN (2015): EN 14757 Water quality — Sampling of fish with multi-mesh gillnets

GASSNER, H., D. ACHLEITNER, M. LUGER (2015) Guidance on surveying the biological quality elements. Part B1 – Fish. Published by: Austrian Federal Ministry of Agriculture and Forestry,Environment and Water Management (ISBN: 978-3-85174-063-9).

Ritterbusch, D., Brämick, U. (2015): Verfahrensvorschlag zur Bewertung des ökologischen Zustands von Seen anhand der Fische, Schriften des Instituts für Binnenfischerei e.V. Band 41., Potsdam-Sacrow.

Method reported by:
Name: Michael Schubert, Christian Vogelmann
Institute: Bavarian State Research Center for Agriculture

Appendix

	Depth stratum m	Maximum depth m						
		< 6	6 to 11,9	12 to 19,9	20 to 34,9	35 to 49,9	50 to 75	> 75
Lake area < 20 ha	< 3	4	3	4	4	3		
	3 to 5,9	4	3	4	3	3		
	6 to 11,9		2	4	3	3		
	12 to 19,9			4	3	3		
	20 to 34,9				3	2		
	35 to 49,9					2		
Total number of gillnet-nights		8	8	16	16	16		
Lake area 21 ha to 50 ha	<3	4	5	5	5	5		
	3 to 5,9	4	6	5	5	5		
	6 to 11,9		5	3	5	6		
	12 to 19,9			3	5	6		
	20 to 34,9				4	6		
	35 to 49,9					4		
Total number of gillnet-nights		8	16	16	24	32		
Lake area 51 ha to 100 ha	< 3	8	8	7	7	7	7	
	3 to 5,9	8	8	7	7	7	7	
	6 to 11,9		8	5	9	7	10	
	12 to 19,9			5	6	4	4	
	20 to 34,9				3	4	4	
	35 to 49,9					3	4	
	50 to 75						4	
Total number of gillnet-nights		16	24	24	32	32	40	
Lake area 101 ha to 250 ha	< 3	8	8	8	7	7	7	
	3 to 5,9	8	8	8	7	7	7	
	6 to 11,9		8	8	10	10	6	
	12 to 19,9			8	8	6	6	
	20 to 34,9				8	6	6	
	35 to 49,9					4	4	
	50 to 75						4	
Total number of gillnet-nights		16	24	32	40	40	40	

	Depth stratum m	Maximum depth m						
		<6	6 to 11,9	12 to 19,9	20 to 34,9	35 to 49,9	50 to 75	>75
Lake area 251 ha to 1 000 ha	< 3	12	11	10	10	10	10	10
	3 to 5,9	12	11	10	10	10	10	10
	6 to 11,9		10	10	10	10	10	10
	12 to 19,9			10	10	8	8	8
	20 to 34,9				8	6	8	5
	35 to 49,9					4	6	5
	50 to 75						4	4
Optional	>75							0 or 4
Total number of gillnet-nights		24	32	40	48	48	56	52 to 56
Lake area 1 001 ha to 5 000 ha	<3	12	11	10	10	10	10	10
	3 to 5,9	12	11	10	10	10	10	10
	6 to 11,9		10	10	12	12	10	10
	12 to 19,9			10	12	9	10	10
	20 to 34,9				12	9	10	10
	35 to 49,9					6	10	6
	50 to 75						4	4
Optional	>75							0 or 4
Total number of gillnet-nights		24	32	40	56	56	64	60 to 64

Table 1 Detailed information on number of benthic gillnet nights used within each depth stratum depending on the surface area of the lake

Country: Germany
Category: River
Biological Quality Element: Fish

Short description of sampling procedure

The German Water quality assessment method for rivers is based on the "fischbasiertes Bewertungssystem (fiBS). The quality assessment compares fish faunistic reference conditions and representative fishmonitoring data. In exceptional cases dummy data will be used, if there is evidence of presence e.g. from catch statistics from commercial or recreational fisheries.

Only rivers with a catchment area more than 10 km² are sampled. These rivers are analyzed concerning the geographical, morphological and hydrological characteristics to determine appropriate sampling strategies. The fishing methods and equipment differs depending on water depth and river width. The minimum sampling stretch is 200 m and the maximum is 3000 m (40/50 x river width in wadable/non- wadbale river). If the river is wider than 20 m the use of two anodes are recommended. A representative sampling area should consider the existing habitats proportional to the whole monitored water body. All sampling stretches have to be sampled two or three times during 6 years (official WDF term). Furthermore the samplings have to be taken in different years. The operators fish upstream so that turbidity, caused by wading has no negative affect to the efficiency and that the stunned fishes are drifted out of the electric field. The equipment especially the power generator is placed in a boat or carried as a backpack depending on size.

<u>Sample processing for both methods:</u>

Fish affected by the electric current are removed from the electric field after the identification as quickly as possible. Fish are transferred to suitable holding containers with oxygen. A sufficient supply of oxygen is necessary until release. Fish are estimated by size classes (0<2, 2<5, 5<10, 10<15, 15<20, 20<25, 25<30, 30<40, 40<50, 50<60, 60<70, ≥70 cm). They are counted and determined to species level. The remaining fishes should be released near the capture site.

Sampling period/frequency

The timing of sampling is linked to the life cycle strategies of the target species. In most circumstances sampling is carried out towards the end of the growing season when juveniles (0+) have reached a sufficient large size to be caught by electro fishing and can easily be identified. Due to reduced fish activity and therefore reduced sampling efficiency as well as to avoid stress to the fish, sampling shouldn`t be done at water-temperatures < 5 °C

Characterization of representative sampling site

A representative sampling area should consider the existing habitats proportional to the whole river.

Additional abiotic data recorded

River bed width (min., max., mean), discharge, water temperature, water depth (min., max., mean), conductivity, turbidity, (pH, oxygen)

Method features compliant with WFD

All requirements for the WFD are covered (species composition, abundance, age structure) and the ecological status is defined by 5 classes (high, good, moderate, poor, bad).

Rules to define ecological classes and reference conditions/evaluation and description of processing method

Biological attributes of ecological quality (metrics) are selected with respect to their sensitivity to particular types of environmental stress. The German concept follows an integrated approach using the following types of information: reference sites, historical fish data, historical maps, reference models and expert judgement.

To determine the ecological status of rivers based on the biological quality element fish, the fish index fiBS is used (Dußling, 2009). fiBS compares the reference with the monitored data from a river and estimates the ecological status class using 6 defined quality features with different metrics: fish region, age structure, indicator species, migration, species and guild inventory, species and guild abundance. The calculation of the ecological assessment is based on the results of the metrics and the quality features. The result constitutes an Index value from 1 to 5. With 5 representing the reference status and each smaller value expressing the respective deviation from the reference status.

Rules to define ecological status classes of rivers:

Ecological status class	Fish Index Germany (EQR)
high	> 3,75-5
good	> 2,50-3,75
moderate	> 2,00-2,50
poor	> 1,50-2,00
bad	≤ 1,50

Literature Reference

Dußling, U. (2009): Handbuch zu fiBS. – Schriftenreihe des Verbandes Deutscher Fischereiverwaltungsbeamter und Fischereiwissenschaftler e.V., Heft 15

Gewässerbewertung: http://www.gewaesser-bewertung.de/index.php?article_id=129&clang=0

CEN (2003): EN 14011 Water quality – Sampling of fish with electricity.

Method reported by:
Name: Michael Schubert, Christian Vogelmann
Institute: Bavarian State Research Center for Agriculture

Water quality assessment-National methods

Country: Italy
Category: Lake
Biological Quality Element: Phytoplankton

Short description of sampling procedure

In Veneto, the analised parameters for phytoplankton monitoring include species composition, biovolume and Clorophyll-a concentration.
Phytoplankton samples are taken according with the Italian Manuals and Guidelines 111/2014 Biological methods for inland surface waters performed by ISPRA.
In Lake Garda water sample collected for phytoplankton analyses is referred to the integrate 0-20m zone, the historical sampling euphotic depth on which long term records are based (Normally for the other lakes euphotic zone (Z_{eu}) is estimated by multiplying the Secchi disk measurements by 2.5).
Sampling system to obtain the integrated sample is tube sampler (or with a Niskin bottle for smaller lakes).
From the final sampling water representative of the euphotic zone are obtained:

- n°1 subsample (200ml) for phytoplankton analysis (in a dark glass bottle)
- n°1 subsample (2L) for chlorophyll *a* (in a dark plastic bottle)

In the field, samples in the dark and in a cool place are stored and with Lugol's solution are preserved.

Sampling period/frequency

Samples are collected one each month in two stations (24 samples a year) every year.

Characterisation of representative sampling site

Lake Garda is divided into two basins:

- the west basin (large and deep, Z_{max}= 350m)
- the east basin (smaller, Z_{max}= 80m)

Field samplings and measurements are carried out at two stations referring at the deepest location in the West Basin (Brenzone) and East Basin (Bardolino).

Short description of processing method and evaluation (e.g. metrics, level of identification)

Phytoplankton analysis is described in the UNI EN 15204:2006 Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)
After manual homogenisation, a known volume of sample, in relation with chlorophyll values, is used to fill counting chamber for a time of 4 hours for each centimeter of height of the column

Once the settling time has elapsed, the column is removed and the chamber is ready for the use with the inverted microscope.
The procedure for quantitative analysis involves recording the taxa observed and the number of algal objects for each taxon, in a known area of the counting chamber.
At least 100 objects of the main taxon (more present) are counted.
Depending on the composition and density of the phytoplankton, three alternative count strategies for each taxon are used:

1. counting a number of randomly-selected fields (usually 400X)
2. counting transects (usually 200X)
3. counting the whole chamber (100 or 200X)

Most of the taxa are determined to species level.
Species-level densities are joined in major phytoplanktonic groups and expressed as

- ind/l (colonial algae)
- cell/l
- mm3/m3 (estimation of bio-volumes)

The Chlorophyll-a concentration is determined with spectrophotometric analysis (APHA Standard Methods for the Examination of Water and Wastewater ed. 23rd 2017 10200 H).

Additional abiotic data recorded

Transparence (Secchi depth), temperature, pH, conductivity, dissolved oxygen, total phosphorus, total nitrogen

Method features compliant with WFD

Rules to define ecological classes and reference conditions

According with the Report CNR-ISE, 02.13 (updated with Alpine GIGs results developed for Alpine lakes), the metrics included in the Italian phytoplankton assessment method are:

- the biomass metrics chlorophyll a
- total biovolume and the taxonomic composition metric (PTIot - Phytoplankton Trophic Index)

Literature Reference

Italian Manuals and Guidelines 111/2014 *Biological methods for inland surface waters* performed by ISPRA http://admin.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/MLG__111_2014_Metodi_Biologici_acque.pdf

Report CNR-ISE, 02.13 Indici per la valutazione della qualità ecologica dei laghi. Versione 2018 (Report on Italian Methods for the evaluation of the ecological quality of lake waterbodies, 2013)
http://www.ise.cnr.it/images/seminar/Report/Report_2013_02_indici.pdf

http://www.ise.cnr.it/images/wfd/en/phytoplankton.pdf

UNI EN 15204:2006 Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)

APHA Standard Methods for the Examination of Water and Wastewater ed. 23rd 2017 10200 H

D.Lgs.152/2006 Norme in materia ambientale, con le sue successive modifiche ed integrazioni, recepisce formalmente la Direttiva 2000/60/CE

Decreto 260/2010 Criteri tecnici per la classificazione dello stato dei corpi idrici superficiali – Modifica norme tecniche D.Lgs. 152/2006 (Technical criteria for the classification of the status of surface water bodies)

Comments
Concerning the management of bathing water quality (D.Lgs 30/05/2008, n. 116, Dm 30 marzo 2010 and DIRECTIVE 2006/7/EC) the proliferation of cyanobacteria and cyanobacterial toxins are monitored
Cyanobacteria analysis is described in the UNI EN 15204:2006. Samples in summer are monitored and between 0-30cm and in bathing water are taken.
Cyanobacterial toxins with LC-MS/MS method are analyzed (Demethyl microcystin-LR (dmMC-LR), Demethyl microcystin-RR (dmMC-RR), Microcystin-LR (MC-LR), Microcystin-RR (MC-RR), Microcystin-YR (MC-YR), Microcystin-LY (MC-LY), Microcystin-LW (MC-LW), Microcystin-LF (MC-LF), Nodularin (NOD))

Method reported by:
Federica Giacomazzi, Chiara Zampieri
ARPAV

Country: Italy
Category: Lakes
Biological Quality Element: Diatoms

Short description of sampling procedure

Diatom samples are taken according to the European Standard: UNI EN 13946:2014 *Water quality- Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes*
Wherever possible, lake littoral zone with naturally occurring moveable hard surface (large pebbles, cobbles and boulders) is sampled, in particular:

- a selection of 5 cobbles or 5 small boulders or 10 pebbles is collected (better without filamentous algae, otherwise these are removed)
- any loosely attached surface contamination is removed by washing the substratum briefly in the river or lake water
- substrata are placed in a tray, along with approximately 50ml of distilled water
- upper surface of substrata is brushed using a clean stiff toothbrush to remove the diatom film
- minimal surface of 100 cm2 at 20-50cm depth is scrapped
- the toothbrush is periodically rinsed in the water
- water from the tray into the sample jar is transferred

Samples with use of artificial substrata are also collected (not for Lake Garda sampling):

- substrata with heterogeneous surfaces are preferred, usually pebbles placed in a net with a 2 cm mesh are used
- substrata at least 4 weeks in lake are left at 30-50 cm depth

Samples with use of submerged or emerged macrophytes and macroalgae are also collected (not for Lake Garda sampling):

- 5 to 6 entire plants or stems are cut and transferred directly in a plastic bag
- by agitating the plants vigorously in some distilled water diatoms are removed and put in a baker
- or diatoms from the stem by stirring, scraping and gentle brushing are removed

Sampling period/frequency

- sampling period is July, August and September, months with high light intensity and mild tempering and with the greatest diversity of species
- frequency is once a year every 3 years in the case of operational monitoring (e.g. Lake Garda) and every 6 years in the case of surveillance monitoring (Directive 2000/60/EC)

Characterisation of representative sampling site

A segment of lake shore that has substrata suitable for sampling is selected, usually 10 m in length or more within a morphological and physical uniformity of the sampling site
According with the Italian Manuals and Guidelines 111/2014 *Biological methods for inland surface waters* performed by ISPRA:

- 3 sampling sites approximately equidistant per body of water are chosen
- for the large south alpine lakes, e.g. Lake Garda, 9 sites are selected
- sampling sites correspond to macrophytes sampling sites (where the assessment of the macrophyte community is carried out)

Short description of processing method and evaluation (e.g. metrics, level of identification)

- method for cleaning diatoms consists in the exposure to strong oxidizing agents (5-10ml sample + 20ml hydrogen peroxide in a baker) and in the heat on a hotplate at about 90±5°C until all organic material has been oxidized. The addition of few drops of hydrochloric acid is recommended in our region where carbonates are usual to be present. Washing process with distilled water and centrifuge is needed to remove all traces of hydrogen peroxide
- samples are centrifuged 10minutes at 1500rpm
- The cleaned diatom suspension for the collection of slides with Naphrax® is used
- The sample under microscope is checked: a concentration of 10-15 valves per field at a magnification of 1000x is recommended
- Identification of diatoms is species-level
- For enumeration of diatoms valves are used as basic units
- In order to obtain a representative distribution, 400-450 valves are determinated in a prepared slide
- For enumeration of diatoms a slow vertical or horizontal traverse is performed

Additional abiotic data recorded

With multiparametric probes: ph, conducibility, temperature and oxygen

Method features compliant with WFD

Rules to define ecological classes and reference conditions

According with the Report CNR-ISE, 02.13 (Report on Italian Methods for the evaluation of the ecological quality of lake waterbodies):

- the EPI-L is the method used to assess lake water quality on the basis of the composition of the benthic diatom communities and is calibrated against eutrophication (http://www.ise.cnr.it/images/seminar/Report/Report_2014_01_epi_l.pdf)
- EPI-L is a new method correlated to the trophic pressure in the Italian lakes but not yet in the inter_GIG calibrated

Literature Reference

UNI EN 13946:2014 *Water quality-Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes*

Italian Manuals and Guidelines 111/2014 *Biological methods for inland surface waters* performed by ISPRA http://admin.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/MLG__111_2014_Metodi_Biologici_acque.pdf

Report CNR-ISE, 02.13 Indici per la valutazione della qualità ecologica dei laghi. Versione 2018 (Report on Italian Methods for the evaluation of the ecological quality of lake waterbodies, 2013) http://www.ise.cnr.it/images/seminar/Report/Report_2013_02_indici.pdf

Method reported by:
Federica Giacomazzi
ARPAV

Country: Italy
Category: Rivers
Biological Quality Element: Diatoms

Short description of sampling procedure

Diatom samples are taken according to the European Standard: UNI EN 13946:2014 *Water quality- Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes*
Wherever possible, lake littoral zone with naturally occurring moveable hard surface (large pebbles, cobbles and boulders) is sampled, in particular:

- a selection of 5 cobbles or 5 small boulders or 10 pebbles is collected (better without filamentous algae, otherwise these are removed)
- any loosely attached surface contamination is removed by washing the substratum briefly in the river or lake water
- substrata are placed in a tray, along with approximately 50ml of distilled water
- upper surface of substrata is brushed using a clean stiff toothbrush to remove the diatom film
- minimal surface of 100 cm2 at 20-50cm depth is scrapped
- the toothbrush is periodically rinsed in the water
- water from the tray into the sample jar is transferred

Samples with use of artificial substrata are also collected (e.g. River Adige):

- substrata with heterogeneous surfaces are preferred, usually pebbles placed in a net with a 2 cm mesh are used
- substrata at least 4 weeks in lake are left at 30-50 cm depth

Samples with use of submerged or emerged macrophytes and macroalgae are also collected:

- 5 to 6 entire plants or stems are cut and transferred directly in a plastic bag
- by agitating the plants vigorously in some distilled water diatoms are removed and put in a baker
- or diatoms from the stem by stirring, scraping and gentle brushing are removed

Sampling period/frequency

- The most suitable period for the Alpine area corresponds to the hydrological regime of low and normal flow levels (January-February or August-September for the low and April-May for the normal flow levels);
- frequency is twice a year every three years in the case of operational monitoring and every 6 years in the case of surveillance monitoring, e.g. River Adige (Directive 2000/60/EC)

Characterisation of representative sampling site

Area of the river bed that has substrata suitable for sampling is selected, usually 10 m in length or more within a morphological and physical uniformity of the sampling site. In rivers “riffles” are preferred, but “runs” and “glides” are also suitable.

Short description of processing method and evaluation (e.g. metrics, level of identification)

- method for cleaning diatoms consists in the exposure to strong oxidizing agents (5-10ml sample + 20ml hydrogen peroxide in a baker) and in the heat on a hotplate at about 90±5°C until all organic material has been oxidized. The addition of few drops of hydrochloric acid is recommended in our region where carbonates are usual to be present. Washing process with distilled water and centrifuge is needed to remove all traces of hydrogen peroxide
- samples are centrifuged 10minutes at 1500rpm
- The cleaned diatom suspension for the collection of slides with Naphrax® is used
- The sample under microscope is checked: a concentration of 10-15 valves per field at a magnification of 1000x is recommended
- Identification of diatoms is species-level
- For enumeration of diatoms valves are used as basic units
- In order to obtain a representative distribution, 400-450 valves are determinated in a prepared slide
- For enumeration of diatoms a slow vertical or horizontal traverse is performed

Additional abiotic data recorded

With multiparametric probes: ph, conducibility, temperature and oxygen

Method features compliant with WFD

Rules to define ecological classes and reference conditions

- For the assessment of ecological status, using diatoms communities, the Multimeter Intercalibration Index (ICMi) is applied. The ICMi is based on the IPS Pollutant Sensitivity Index and the TI Trophy Index.
- Identification of diatoms is species-level

Literature Reference

UNI EN 13946:2014 *Water quality-Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes*

Italian Manuals and Guidelines 111/2014 *Biological methods for inland surface waters* performed by ISPRA http://admin.isprambiente.gov.it/files/pubblicazioni/manuali-lineeguida/MLG__111_2014_Metodi_Biologici_acque.pdf

D.Lgs.152/2006 Norme in materia ambientale, con le sue successive modifiche ed integrazioni, recepisce formalmente la Direttiva 2000/60/CE

Decreto 260/2010 Criteri tecnici per la classificazione dello stato dei corpi idrici superficiali – Modifica norme tecniche D.Lgs. 152/2006 (Technical criteria for the classification of the status of surface water bodies)

Method reported by:
Federica Giacomazzi
ARPAV

Country: Italy
Category: Lake
Biological Quality Element: Fish

Short description of sampling procedure

The method contemplates three approaches depending on the sampling depth: Electrofishing, gillage multi-mesh bottom nets, gillage mesopelagic multi-mesh nets

1) Electrofishing must be used in a littoral environment (max 1.5 m depth). The technical information is specific for the methodology and therefore we refer to the reference protocol UNI - EN 14011, 2003. Water quality - Sampling of fish with electricity. It is recommended to perform sampling using Electrofishing in the same period of sampling with multi-mesh networks. It is recommended to use a generator with a power of at least 5,000 W. The sampling with electrofishing must be done by points (Point Abundance Sampling Electrofishing-PASE) using the generator in the "direct current" mode. in the same period of sampling with multi-mesh networks. It is recommended to use a generator with a power of at least 5,000 W.
2) Gillage Multi-mesh nets are passive capture tools, since they are based on the fact that moving fish remains stored in the net at the gill region. Each net consists of a series of standard-sized panels, each characterized by a different mesh size so that different sized fish can be caught.The RM can be divided into two categories: "bottom" or "bentiche" (RMB) and "mesopelagiche" (RMP) in relation to the type of installation: the first anchored and laid on the bottom, the second raised with respect to the lake bottom.

The used number of bentic nets depends on lake surface and maximum water depth. The number of pelagic nets depends on the area of the lake.

・The shoreline is sampled by Point abundance electrofishingSampling method;

・Data processing: the following parameters are taken: fish total length, fish total weigth, scale for age reading.

・Identification level: species.

Sampling period/frequency

Sampling must be performed between the month of July and the month of October.The nets must be laid at sunset, approximately between 18:00 and 20:00, and set sail the following morning between 6:00 am and 8:00 am. A residence time in water of about 12 hours is recommended, but in the case of eutrophic or hypereutrophic lakes the shutter speed can be reduced.

Characterisation of representative sampling site

The proposed method is based on a stratified sampling of the water column and on the random definition of the sampling stations.

Short description of processing method and evaluation (e.g. metrics, level of identification)

The I-LFI (Italian Lake Fish Index) has 5 metrics:

Metric 1 – abundance of key species: number of individuals of each fish species in the standard catches (CEN gillnetting + point abundance sampling electric fishing). Five classes of abundance. Five score (from 10 to 2)
Metric 2 – Population structure of key species: evaluated by means of the length based structural index PSD-Proportional Stock Density Index (Anderson & Neumann 1996). Four classes, four score (from 10 to 0).
Metric 3 – Reproductive success (as % on the total indicator species for each lake type) of the key and accompanying fish species: 0+ or 1+ individuals in the standard catches for each species. Five classes. Five scores (from 10 to 2).
Metric 4 – Decrease (%) of the number of indicator species (as % on the total indicator species for each lake type). Five classes. Five scores (from 10 to 2).
Metric 5 – % Ratio among the number of alien species and the total number of species in the catches. Five classes. Five scores (from 10 to 2).

Additional abiotic data recorded

none

Method features compliant with WFD

The present version of the Italian assessment method includes five metrics and covers all requirements (species composition, abundance, age structure) of the WFD:

1. Ecological status is classified by one of five classes (high, good, moderate, poor and bad).
2. High, good and moderate ecological status are set in line with the WFD's normative definitions (Boundary setting procedure)
3. All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A combination rule to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole
4. Assessment is not adapted to intercalibration common types defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT because is used a site specific approach.
5. The water body is assessed against type-specific near-natural reference conditions
6. Assessment results are expressed as EQRs
7. Sampling procedure allows for representative information about water body quality/ecological status in space and time
8. All data relevant for assessing the biological parameters specified in the WFD's normative definitions are covered by the sampling procedure
9. Selected taxonomic level achieves adequate confidence and precision in classification

Rules to define ecological classes and reference conditions

EQR calculation = Sum of metric scores/50.
The ecological status of a lake is classified by 5 classes. As a first step the boundaries for the EQRvalues were set equidistantly (class width: 0.2).

Literature Reference

Gassner H., Achleitner D., Luger M., Ritterbusch D., Schubert M., Volta P. 2014. Water Framework

Directive Intercalibration Technical Report. Alpine Lake Fish fauna ecological assessment methods. 68

p. S. Poikane (ed.). (JRC Technical Reports, vol. EUR 26506 EN). Ispra: Publications Office of the European Union, 2014.

Volta P. 2011. Indice per la valutazione della qualità ecologica dei laghi a partire dalla composizione della comunità ittica: Lake Fish Index (LFI). In:Indici per la valutazione della qualità ecologica dei laghi (CNR-ISE Ed.). REPORT CNR-ISE 03.13

Volta P., Oggioni A., Bettinetti R & E. Jeppesen. 2011. Assessing lake typologies and indicator fish species for Italian natural lakes using past fish richness and assemblages. Hydrobiologia 671, 227-240.

Volta, P. & A. Oggioni, 2010. Key- and type- specific fish species in natural lakes of italian Alpine Ecoregion reconstructed from historical data: a preliminary index to assess the quality status of fish fauna according to WFD 2000/60/CE. (In Italian). Studi Trentini Scienze Naturali 87, 97-104.

Volta, P., 2010. Analysis of the population structure of Coregonus lavaretus (Linneus 1758) in three deep Italian lakes using stock density indexes. Studi Trentini Scienze Naturali 87, 257-260.

Method reported by:
Stefano Macchio
ISPRA

Country: **Category: River** **Biological Quality Element: Fish**

Short description of sampling procedure The application of the sampling procedure is specific to small-medium sized wading rivers. The relief of the fish community is aimed at defining the composition, abundance and age structure of the fish fauna. Sampling is carried out exclusively by electro-fishing, using an electromechanger capable of emitting both direct current (DC) and pulsed direct current (PDC). The sampling is carried out on 2 consecutive sections, the first of which must be sampled with a quantitative approach and the second with a qualitative approach.
Sampling period/frequency Sampling must be carried out in a period in which the hydrological flows allow safe access to the station, the conditions of water transparency are the best possible, while avoiding interfering with the breeding periods and with the biological needs of the species present. The sampling frequency is 1 time per year.
Characterisation of representative sampling site The sampling site should be identified in order to avoid the sampling of communities subjected in recent times (at least one year) to biomanipulation interventions (introductions, repopulations, mass selective withdrawals), which could lead to incorrect assessments on composition, abundance and structure of the natural community. At each site, one or more representative sampling stations must be identified, always based on expert judgment. The information necessary for the location of the monitoring station must be reported on the field sheet form. Environmental and support data can be obtained with 3 modes: A. Detected / estimated in the field by appropriate instrumentation. B. Detected / estimated a posteriori from standardized field shots. C. A priori or a posteriori findings of sampling from bibliography, cartography, SIT / GIS, aerial photos, web.
Short description of processing method and evaluation (e.g. metrics, level of identification) Once the electropolishing operations have been completed, the containers are collected with the enclosed fish to proceed to the determination and measurement of individuals. Alternatively, measurements can be made in the different collection points. The data collected must be distinguished by a single pass (or, at least, by keeping those of the first passed from those of the following), to allow the estimation of the abundance, of the efficiency of capture, of the species-specific and specific-size catchability rate, either by single increment. For each of the individuals captured must be noted: 1) Species

2) Total length (in mm)
3) Weight (measured at the minimum precision of 1 gram).
4) Possible external anomalies of tail, dorsal fin, anal fin, pectoral fins, body, head, eyes, nostrils, lips, operculum, barbels, such as:
- abnormal pigmentation;
- anomalous form;
- abnormal reduction (eg, shortening of the operculum);
- abnormal increase (eg exophthalmia: swollen, protruded eye);
- abnormal absence (eg absence of barbs);
- erosion of the fins;
- growths, swellings, swellings, nodules;
- injuries;
- emboli (gaseous vesicles);
- bleeding;
- presence of mushrooms;
- presence of worms.

The NISECI is a multimetric index that uses as main criteria for the assessment of the ecological status of a given watercourse:
- the naturalness of the fishing community (understood as completeness of the composition in indigenous species expected in relation to the zoogeographic and ecological framework);
- the biological condition of the populations present (positively quantified for the indigenous species expected and negatively for the aliens), in terms of abundance and population structure such as to guarantee the ability to self-reproduce and have normal ecological-evolutionary dynamics.

The multimetric index formulation, whose value varies, as well as that of all constitutive metrics and sub-metrics, between 0 and 1, is given by:

$\mathbf{NISECI}$ =0.1 x10.5+0.1 x20.5+0.8 (x1×x2)−0.1 (1− x3)×(0.1 x10.5+0.1 x20.5+0.8 (x1×x2))

where: x1 = metric "presence / absence of indigenous species"
x2 = metric "biological condition of populations of native species"
x3 = metric "presence of alien or hybrid species, structure of relative populations and ratio numerical compared to indigenous species "

Additional abiotic data recorded

Temperature, pH, Condctivity, Dissolved oxygen

Method features compliant with WFD

The method is based on composition, abundance and population structure parameters as required by WFD.

Rules to define ecological classes and reference conditions

The reference condition (corresponding to the high ecological status) is represented by a community in which all the awaited native species are present, with populations in good biological condition, and are absent alien species or hybrids.

The relationship between NISECI and RQE-NISECI was obtained by simulating 21000 cases, during which the 3 metrics of the index were varied from 0 to 1 in increments of 0.1:

$\mathbf{RQE_{NISECI}}$= ($log\ NISECI$+1.1283)/1.0603

Since the classification of the ecological status must be expressed in 5 classes, the NISECI threshold values have been calculated so as to define RQE intervals of equal amplitude for each class.

The EU-IC process, concluded at the beginning of 2017 (Macchio et al., 2017), determined the need to partially modify the class limits for the Alpine area.

Literature Reference

Italy Legislative Decree No. 152 approving the Code on the Environment. Gazzetta Ufficiale della Repubblica Italiana No. 88, 14 April 2006

ISPRA. Biological methods for internal surface waters. Rome. 2014 (Manuali e Linee Gu

ISPRA. Nuovo Indice dello Stato Ecologico delle Comunità Ittiche (NISECI). Manuali e Linee Guida 159/2017 ida,Vol. 111/2014).

UNI-EN 14011:2003 - Campionamento di pesci mediante elettricità.

UNI-EN 14962:2006 - Linee guida sullo scopo e la selezione dei metodi di campionamento di pesci.

UNI-EN 14996:2006 - Linee guida per assicurare la qualità delle valutazioni biologiche ed ecologiche nell'ambiente acquatico.

Zerunian A., Goltara A., Schipani I., Boz B., 2009. Adeguamento dell'Indice dello Stato Ecologico delle Comunità Ittiche alla Direttiva Quadro sulle Acque 2000/60/CE. Biologia Ambientale 23(2): 1-16.

Method reported by:
Cristina Martone
ISPRA

Water quality assessment-National methods
Slovenia

Country: Slovenia
Category: Lake
Biological Quality Element: Phytoplankton

Short description of sampling procedure

Quantitative and **qualitative** phytoplankton samples for the **ecological status assessment** with phytoplankton in lakes and reservoirs are taken in accordance with the standardized sampling method (**EN 16698:2015).**

Qualitative samples are taken with the Electronic integrating water sampler (Hydrobios) from the surface to the defined sampling depth. Sampling depth depends on lake depth and stratification period. In order to define the phytoplankton sampling zone it is necessary to measure Secchi depth (SIST EN ISO 7027:2000) and temperature-depth profiles before sampling.

In clear deep alpine lakes (**$Z_{max} \leq 15$ m)** quantitative phytoplankton samples are collected from the euphotic zone (euphotic zone = 2,5 x Secchi depth) or exceptionally from the epilimnetic zone (the Table below). During the homothermy sampling is always performed from the surface to the depth of 20 m. In the case where a deep chlorophyll maximum (DCM) occurs, it is essential to take a sample from the corresponding depth too.

Qualitative samples are taken with a phytoplankton net (20 µm) from the same profile as qualitative samples.

Samples preservation, transport and storing according to Utermöhl (EN 15204) standard.

Homothermy	Stratification
$Z_{euf} > 20$ m → $Z_s = 20$ m **$Z_{euf} < 20$ m → $Z_s = 20$ m**	**$Z_{euf} > 20$m → $Z_s = 20$ m (+Z_{DCM})** **$Z_{euf} < Z_{epi} \rightarrow Z_s = Z_{epi}$** **$Z_{euf} > Z_{epi} \rightarrow Z_s = Z_{euf}$ (+Z_{DCM})**

Z_S - sampling depth
Z_{euf} - euphotic depth = Secchi depth x 2,5
Z_{epi} - the first thermoclina depth

Sampling period/frequency

Minimal sampling frequency for ecological status assessment with phytoplankton is four 4 - times per year, once in each of the limnological period i.e. early spring homotermy, beginning of stratification, strong summer stratification and autumn homotermy, during three following years.

Characterisation of representative sampling site

Samples are collected at the deepest lake point.

Short description of processing method and evaluation (e.g. metrics, level of identification)

Slovene Phytoplankton Assesment Method for the Ecological status of Lakes [Metodologija vrednotenja ekoloskega stanja jezer na podlagi fitoplanktona] is adopted AT Phytoplankton Assessment method for the Alpine lakes, which was developed during the WFD itercalibration process by Wolfram G. and other coworkers in the Lake Alpine GIG (2009 - 2012).

Basic parameters for phytoplankton frequency, abundance and spesies composition determination in this Method are :

Total phytoplankton biovolume ($mm^3 \cdot L^{-1}$) determined according to - SIST EN 15204:2007- Utermöhl technic and SIST EN 16695:2015 - Guidance on the estimation of microalgal biovolume

Chlorophyll-a ($\mu g \cdot L^{-1}$) procedure is following SIST ISO 10260:2001 for chlorophyll determination

Brettum index - index for evaluation phytoplankton species community (Brettum, 1989; Dokulil in Teubner, 2006). List of indicatory species for alpine lakes was formed in the framework of the Alpine Lake GIG. Identification to the species level is needed.

Basic parameter values are transformed with reference values, typical for the lake type into EQR values. Normalised EQR values are used for the **multimetric phytoplankton index (MMI_FPL)** calculation for ecological status determination.

$$\mathbf{MMI\_FPL_i} = \frac{(\mathbf{n\ EQR\_BVi} + \mathbf{n\ EQR\ \_Chla_i})/2 + \mathbf{n\ EQR\_BI_i}}{\mathbf{2}}$$

MMI_FPL_i–multimetric phytoplankton value in the i-year
$nREK\_BV_i$ – normalized EQR value for the annual phytoplankton biovolumen in the i-year
$nREK\_Chla_i$ – normalized EQR value for the annual chlorophyll-a concentration in the v i the i-year
$nREK\_BI_i$ – normalized EQR value for the annual Brettum index in the i-year

MMI_FPL	Ecological status classes
1,00-0,80	very good
0,79-0,60	good
0,59–0,40	moderate
0,39–0,20	bad
0,19	very bad

Additional abiotic data recorded

Beside Secchi depth (EN ISO 7027) also temperature, pH, dissolved oxygen, conductivity and chlorophyll-a depth profile is measured (Hydrolab multivariate water probe MS5).

Samples for phytoplankton, chlorophyll-a, nutrients and other common physical and chemical parameters are taken at the same time. Samples for nutients are taken from the whole lake depth profile and separately from epilimnion, meta and hypolimnion.

We measure total phosphorus (TP), soluble reactive orthophosphate (SRP), total nitrogen (TN), ammonium (NH_4^+), nitrates (NO_3^-); dissolved organic carbon (DOC) and m-alkalinity.

Method features compliant with WFD

Method is compliant with WFD, defining eutrophication level in correlation with annual total phosphorus concentration (TP).

Rules to define ecological classes and reference conditions

Rules were defined during the WFD intercalibration process and presented in the article Wolfram G., Argillier C., de Bortoli J., Buzzi F., Dalmiglio A., Dokulil M. T., Hoehn E., Marchetto A., Martinez P.-J., Morabito G., Reichmann M., Remec-Rekar Š., Riedmüller U., Rioury C., Schaumburg J., Schulz L. & Urbanič G. (2009). Reference conditions and WFD compliant class boundaries for phytoplankton biomass and chlorophyll-a in Alpine lakes. Hydrobiologia 633: 45–58.

Literature Reference

Wolfram G., Donabaum K., Dokulil M., GUIDANCE ON THE MONITORING OF THE BIOLOGICAL QUALITY ELEMENTS PART B2 – PHYTOPLANKTON, 2013, Federal Ministry of Agriculture and Forestry Environment and Water Management, A - 1012 Vienna

Method reported by:
Name: Spela Remec-Rekar
Institute: Slovenian Environment Agency

ARSO ENVIRONMENT
Slovenian Environment Agency

Country: Slovenia
Category: Lake
Biological Quality Element: Phytobenthos

Short description of sampling procedure

Different habitats are sampled in the ratio of their coverage and are united in one sample. Sampling method is chosen according to habitat type:

- Substrate covered with phytobenthos which can be transferred from the water is scrubbed into plastic container with a knife or a toothbrush.
- Soft sediments are sampled with a spoon. Upper millimeters of sediment are lifted up carefully and transferred into a plastic container.
- Filamentous organisms are picked up with hand into a plastic container.

Sample in plastic container is gently mixed and poured into accurate marked plastic bottle. Samples are fixed with ethanol.

Sampling period/frequency

Phytobenthos is sampled in stable hydrological conditions in summer period (June - September). Frequency of sampling in once per year.

Characterisation of representative sampling site

Three segments length of 100 meters are selected in the lake. Littoral zone with naturally occurring movable hard surfaces are recommended. In each segment one sampling site (length of 50 m) with various habitats which are representative (type of substrate, water depth and shading) for this water body is selected.

Short description of processing method and evaluation (e.g. metrics, level of identification)

The preparation clears the diatom sample from organic matter and limestone with nitrogen acid. Diatoms are determined and counted with an optical microscope at a magnification of 1000x and immersion oil, following the Süsswasserflora von Mitteleuropa (Krammer and Lange-Bertalot 1997-2004). At least 500 valves should be counted in transects.
For ecological status assessment only diatoms are used, although other classes of phytobenthic community are also determined.

Additional abiotic data recorded

Temperature, pH, dissolved oxygen, oxygen saturation and conductivity are measured with portable multimeter.

Method features compliant with WFD

GIG: Alpine

Relevant intercalibration types: Alpine GIG, L-AL3

Rules to define ecological classes and reference conditions

Ecological status assessment is determined by Trophic index (Rott et al., 1999) based on diatoms and Slovenian index for lake ecosystem ecological status assessment based on macrofites (Melzer 1999).

Scope of reference conditions: Existing near-natural reference sites, Expert knowledge

Literature Reference

Methodology for ecological status assessment on the basis of phytobenthos and macrophythes [Metodologija vrednotenja ekološkega stanja jezer na podlagi fitobentosa in makrofitov] (in Slovene) http://www.mop.gov.si/si/delovna_podrocja/voda/ekolosko_stanje_povrsinskih_voda/

EN 13946 Water quality – Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes.

Melzer A. (1999). Aquatic Macrophytes as tools for lake management. Hydrobiologia 395(396): 181-190.

Rott E., Pipp E., Pfister P., van Dam H., Ortler K., Binder N., Pall K. (1999). Indikationslisten für Aufwuchsalgen. Teil 2: Trophieindikation. Bundesministerium für Land- und Forstwirtschaft, Wien.

Method reported by:
Name: Tadeja Balanč, Aleksandra Krivograd Klemenčič
Institute: Sovenian Environment Agency

Country: Slovenia
Category: River
Biological Quality Element: Phytobenthos

Short description of sampling procedure

Different habitats defined by substrate, water velocity, water depth, and shading are sampled regarding on the ratio of habitat coverage. Samples from different habitats are combined in one sample.

Substrate with a well-developed phytobenthos community such as stones, sand, macrophythes, wood or artificial materials is transferred into plastic container and scrubbed with a knife or a toothbrush.

In the case that substrate is not possible to transfer from the water (e.g. big rocks in alpine rivers), the substrate is scrubbed in the water and phytobentos is caught in planktonic net.

Soft sediment is sampled with a spoon or other appropriate object. Filamentous algae can be sampled with toothbrush or fingers.

Samples are fixed with alcohol.

Length of survey stretch is:

- 25 m, if surface of drainage basin till sampling site is 10-100 km^2
- 50 m, if surface of drainage basin till sampling site is 100-1000 km^2
- 100 m, if surface of drainage basin till sampling site is 1000-2500 km^2
- 250 m for category large rivers

Sampling period/frequency

Frequency of sampling is from once per year (e.g. border rivers) to every six years (once in every River basin management plan period)

Phytobentos is sampled at the end of low flow period when hydrological conditions are stable, or at least 2 weeks after flooding. Based on hydrological conditions and characteristics of watercourses in Slovenia recommended periods for phytobenthos sampling are as follows:

a) Large rivers: winter (December-February) or summer period (June-September)
b) Rivers which dry up: spring period (March-May), before river dries up
c) All other rivers: summer period (June-September)

Characterisation of representative sampling site

Sampling is conducted at least 1 m from the river banks to avoid areas with standing or slowly flowing water, or at least 10% of river width away from the river banks for small watercourses. A maximum sampling depth of 0.6 m should not be exceeded. On the field the following data, which characterize sampling site, must be entered in the field protocol:

1) Name of the river investigated
2) Name of the sampling site
3) Nearest settlement, place

4) Gauss-Krueger coordinates
5) Survey section/area (length, width, running water cross-section [m])
6) Natural or artificial river bed
7) Visibility of river bed (turbidity estimation)
8) Mean flow velocity, mean depth, type of substrate, shading (estimation in %)
9) Water level (estimation)
10) Coverage of river bed with algae (estimation from 1-6)

Short description of processing method and evaluation (e.g. metrics, level of identification)

Trophic index (Rott et al., 1999) and Saprobic index (Rott et al., 1997). Level of identification according to Rott et al. (1997, 1999).

Only diatoms are used for ecological status assessment, although other classes of phytobenthic community are also determined. Level of identification for non-diatom taxa is according to national algae database.

Regarding ecological status assessment phytobenthos and macrophytes are combined as one biological quality element.

The preparation clears the diatom sample from organic matter and limestone with nitrogen acid. Diatoms are determined and counted with an optical microscope at a magnification of 1000x and immersion oil, following the Süsswasserflora von Mitteleuropa (Krammer and Lange-Bertalot 1997-2004). At least 500 valves should be counted in transects.

Additional abiotic data recorded

Basic physical and chemical parameters (pH, water temperature, electric conductivity, dissolved oxygen, and saturation) are measured onsite with portable multimeter.

With frequency 6x per year are measured: total phosphorous, ortho-phosphorous, total nitrogen, ammonium, nitrite, nitrate, alkalinity, total suspended solids, total hardness, water level, flow, air temperature, COD, BOD_5, DOC

Method features compliant with WFD

GIG: Alpine, Eastern Continental, Mediterranean

Relevant intercalibration types:

Alpine: R-A, Eastern Continental: R-E4 ,R-EX5, R-EX6, Mediterranean: R-M1, R-M2, R-M5

Rules to define ecological classes and reference conditions

Combination rule for multi-metrics: Worst metric score

Scope of reference conditions: Existing near-natural reference sites, Expert knowledge, Modelling (extrapolating model results)

Literature Reference

Methodology for ecological status assessment on the basis of phytobenthos and macrophythes [Metodologija vrednotenja ekološkega stanja vodotokov na podlagi fitobentosa in makrofitov] (in Slovene) http://www.mop.gov.si/si/delovna_podrocja/voda/ekolosko_stanje_povrsinskih_voda/

EN ISO 5667-3 Water quality - Sampling - Part 3: Preservation and handling of water samples

EN 15708 Water quality - Guidance standard for the surveying, sampling and laboratory analysis of phytobenthos in shallow running waters

EN 13946 Water quality - Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes

EN 14407 Water quality - Guidance for the identification and enumeration of benthic diatom samples from rivers and lakes

Rott E., Hofmann G., Pall K., Pfister P., Pipp E. (1997). Indikationslisten für Aufwuchsalgen. Teil 1: Saprobielle Indikation. Bundesministerium für Land- und Forstwirtschaft, Wien.

Rott E., Pipp E., Pfister P., van Dam H., Ortler K., Binder N., Pall K. (1999). Indikationslisten für Aufwuchsalgen. Teil 2: Trophieindikation. Bundesministerium für Land- und Forstwirtschaft, Wien.

Method reported by:
Name: dr. Aleksandra Krivograd Klemenčič
Institute: Slovenian Environment Agency

Country: Slovenia
Category: Lake
Biological Quality Element: Fish

Short description of sampling procedure
(SIST EN 14757:2015) Water Quality – Sampling of fish with multi-mesh gillnets
Random sampling/surveying, stratified sampling
According to standard the number of bentic nets depends on lake surface and maximum water depth and the number of pelagic nets depends on the lake area.

Standardized sampling of fish community for the ecological status assessment comprise three different ways of sampling:

- Pelagic gillnets
- Benthic gillnets (if Z_{max} > 10m)
- The shoreline is sampled by Point abundance Electrofishing
-

Sampling period/frequency
Minimal sampling frequency for ecological status assessment with fish fauna is one sampling per 6 year, between July and October.

Characterisation of representative sampling site
Sampling is random, at standard depth profile and standard location. Abaut 40 – 50 point samplings depend on habitat types in litotal to 1,5 m depth.

Short description of processing method and evaluation (e.g. metrics, level of identification)
Each sampled fish is numbered, the sampling location and sampling depth of each sampled fish is defined; species determination, total length (mm), total weight (g).

Method comprise 5 metricks which express:

- species composition, (species determination)
- spesies abundance, (individual counts - absolute abundance and absolute biomass/area; Relative abundance (NPUE, BPUE)
- reproductive effectiveness,
- population age structure (life-size) and
- presence of allian species

Method for the ecological status assesment of lakes with fish in Slovenia based on modified Italian metod (Italian Lake fish indeks (I-LFI)).

SI − LFI = (M1 + M2+ M3+ M4 + M5)
5

SI-LFI index for ecological status assesment
Mi (i=1-5) EQR values for 5 metrics

M1 = Nuber of catch dominant fish species on the unit of effort (NPUE)
M2 = PSD (Proportional Stock Density Index)
M3 = reproductive effectiveness % of dominant and accompany species
M4 = percentage decline of dominant and accompany species
M5 = percentage of allian species

Detecting preasures: eutrophication, hydromorphological pressure, biological degradation, fisheries exploitation

Additional abiotic data recorded

Beside Secchi depth (EN ISO 7027) also temperature, pH, dissolved oxygen and conductivity depth profile is measured (Hydrolab multivariate water probe MS5).

Method features compliant with WFD

Method is compliant with WFD, Intercalibratable finalized method

Rules to define ecological classes and reference conditions

Slovenian assessment method (Metodologija vrednotenja ekološkega stanja jezer na podlagi rib) is an adapted Italian method (I-LFI), what means that same metrics are used, but type (lake) specific reference conditions with specific guiding and accompanying species are defined. Pressure-impact relationship is tested against pressure gradient defined in the Alpine GIG fish group. Available alpine GIG data and relationships were used (Gassner et al 2014) in order to compare position of Slovenian data in the global alpine dataset. It was confirmed that slovenain data fits well with established relationships. Thus, in the intercalibration process harmonised boundary values of the italian method (I-LFI) were used. In Slovenia we use a standardised boundary values system with equidistant boundaries i.e. 0.8, 0.6, 0.4 and 0.2 and thus transformation equations of original Slovenian asssessment method values are provided. Reference conditions were defined according to historical data recorded before the first quarter of the 20th century. Historical data until 1926, based on evidence specimens in museums, historical literature, and historical catch statistics.

Literature Reference

Gassner H., Achleitner D., Luger M., Ritterbusch D., Schubert M., Volta P., Poikane S. (ur.) (2014). Water Framework Directive Intercalibration Technical Report: Alpine Lake Fish fauna ecological assessment methods. European Commission. Joint Research Centre – Institute for Environment and Sustainability. 68 pp.

Podgornik S., Urbanič G., Koren Š., Šiling R., Petkovska . (2016). Razvoj metodologije vrednotenja ekološkega stanja in razvrščanja vodnih teles jezer na podlagi rib. Zavod za ribištvo Slovenije, Inštuitut za vode Republike Slovenije, Sp. Gameljne, Ljubljana, 69 str.

SIST EN 14757:2015. Kakovost vode – Vzorčenje rib s pomočjo zabodnih mrež (gillnets). Slovenski institut za standardizacijo.

Volta P., Oggioni A., Bettinetti R., Jeppesen E. (2011). Assessing lake typologies and indicator fish species for Italian natural lakes using past fish richness and assemblages. Hydrobiologia 671, 227-240.

Volta P. 2011. Indice per la valutazione della qualità ecologica dei laghi a partire dalla composizione della comunità ittica: Lake Fish Index (LFI). In:Indici per la valutazione della qualità ecologica dei laghi (CNR-ISE Ed.). REPORT CNR-ISE 03.11

Method reported by:
Name Špela Remec-Rekar
Institute: Slovenian Environment Agency

Country: Slovenia
Category: River
Biological Quality Element: Fish

Short description of sampling procedure

Electric fishing method is used for taxonomic identification, counting, measurement of biological parameters (length, weight) and examination of fish for external anomalies. The selection of waveform DC or PDC depends on the water conductivity, the dimensions of the water body and the fish species to be expected. Depending on river width and depth, two different sampling methods are used:

- By wading for small and shallow rivers across the entire width. DC or PDC wave form may be used. One anode per 5 m width is used. Fishing is carried out slowly, with covering the habitat with a sweeping movement of the anodes and attempt to draw fish out of hiding. In fast flowing water catching net is hold in the wake of the anode. For absolute estimation stop nets are used to delimit survey zones. For relative estimation partial barriers (e.g. shallow riffles) are used. Fish are collected in a bucket filled with water.
- By boat in deeper large rivers, where wading is not possible. The DC wave form is used. There are 7 anodes hanging from the boat. The boat is moved downstream to facilitate good coverage of the habitat, especially where weed beds are present or hiding places of any kind are likely to conceal fish, or upstream if the flow is high. In rapid water is important to allow boat to travel at the same speed as the water flow, only using outboard motor or paddles for maneuvering, such that the boat remains close to immobilized fish. Every stripe is sampled only once. Fish are collected in a bucked filled with water.

Before measurement fish are narcotized with ethylene glycol monophenyl ether. After measurement fish are transformed into bucket with fresh water and when they woke up from narcosis, they are released in the littoral zone with slow flowing water.

Sampling period/frequency

The timing of sampling is linked to an understanding of the life history strategies of the target species. Sampling is carried out towards the end of the growing season. However, electric fishing shall not be made at temperatures below 5 °C, at time with high or extremely low waters.

Characterisation of representative sampling site

The selection of sites shall be representative of habitats within the watershed, but shall not include sites downstream bigger artificial barriers.

- Wadable rivers:

Sampling length is at least ten times longer than the river width, but not less than 100 meters.

- Non-wadable rivers:

Whether water is not deeper than 2.5 meters, stripe method is used (Schmutz et. al., 2001). If water is deeper, sections which correspond with this criterion are chosen. Sampling is carried out stratified in all representative habitats in stipe method. River channel is divided into littoral and inner zone in the transverse direction and into pools, riffles and runs in the longitudinal direction. Recommended number of stripes is at least 8 for less diverse sections (less than 3 habitats) and at least 12 for more diverse sections (3 or more habitats). Stripes are chosen in relation to habitat occurrence in chosen section. Section length is not prescribed. In general each habitat is sampled at least 3 times. Stripes length is 50-100 m in littoral zone and 100-300 m in inner zone.

Short description of processing method and evaluation (e.g. metrics, level of identification)

Fish are determined to the species by morphological characters. In case of unclear characters, sub-samples are brought to the laboratory for further examination. Fish length is recorded in millimeters and weight in grams. Age is identified in the site if possible, otherwise a sample of scales is taken.

In wadable rives species list, number of individuals and biomass for each species is made separately for each catch. Abundance is evaluated with Seber and Le Cren (1967) method.

In non-wadable rivers species list, number of individuals and biomass for each species is made separately for each stripe. Abundance of each species per catch is reported as total recorded numbers and as numbers per 100 m^2.

Age structure is reported for each species, as mean length per age group together with standard deviation and number of fish per sample. For abundant species the number in each young age class (e.g. 0+, 1+, <1+) is reported.

Additional abiotic data recorded

Water depth, water temperature, pH, dissolved oxygen, oxygen saturation and conductivity.

Method features compliant with WFD

Method is compliant with WFD.

Rules to define ecological classes and reference conditions

Ecological status is evaluated with Slovenian index for ecological status assessment of fish fauna in rivers (SIFAIR). Index is developed for rivers ecological status assessment in hydroecoregion in Alps ($SIFAIR_{AL}$) and in Pannonian lowland ($SIFAIR_{PN}$).

Literature Reference

Schmutz, S., Zauner, G., Eberstaller, J., Jungwirth, M. (2001). Die Streifenbefischungs-methode: eine Methode zur Quantifizierung von Fischbeständen mittelgroßer Fließgewässer. Österreichs Fischerei Jg. 54, Heft 1/2001: 14-27.

Seber, G.A., Le Cren, E.D. (1967). Estimating population parameters from catches large relative to the population. Journal of Animal Ecology 36: 631–643.

EN 14011:2003. Water quality – Sampling of fish with electricity

Methodology for ecological status assessment on the basis of fish fauna in rivers [Metodologija vrednotenja ekološkega stanja vodotokov na podlagi rib] (in Slovene) http://www.mop.gov.si/si/delovna_podrocja/voda/ekolosko_stanje_povrsinskih_voda/

Method reported by:
Name: Tadeja Balanč, Aleksandra Krivograd Klemenčič
Institute: Slovenian Environment Agency

Water quality assesment-National methods Switzerland

Country: Ticino, Switzerland **Category: Lake** **Biological Quality Element: Phytoplankton**

Short description of sampling procedure In Ticino, the parameters analised for phytoplankton monitoring include species composition, biomass and Clorophyll-a concentration. The presence of cyanobacterial toxins is not assessed. On Lake Lugano, phytoplankton is counted from integrated samples from 0 to 20 m depth, which corresponds to the lower limit of the euphotic zone (PAR is measured monthly with an underwater quantum sensor). Integrated samples are collected with a Schröder bottle and two subsamples of 200 ml are fixed with Lugol and Formalin (final concentration 1%). The Chlorophyll-a is measured from both an integrated sample (0-20 m) and 11 samples collected at discrete depths from 0 to 20 m with a Niskin bottle.
Sampling period/frequency The integrated samples for phytoplankton counting are collected monthly from July to February, and fortnightly from March to June (16 samples a year). The integrated samples for Chlorophyll-a are collected fortnightly (24 samples a year), while the discrete samples are collected monthly from November to February, and fortnightly from March to October (20 samples a year).
Characterisation of representative sampling site Lake Lugano is divided into two basins (north and south) with specific characteristics by a morainic front with an artificial damn, and the south basin is further divided in two main sub-basins. Therefore, there are three sampling stations representative of each basin/sub-basin, one in the north basin and two in the south basin. Each sampling site is located near the deepest point of the respective basin/sub-basin.
Short description of processing method and evaluation (e.g. metrics, level of identification) Phytoplankton counts are carried out on samples fixed with Lugol and placed in a sedimentation chamber (Utermöhl-method UNI EN 15204:2006). At least 200 cells per sample are counted with an inverted microscope at various magnifications (100X, 250X, 400X). Large algae are counted in the whole chamber (100x), small algae in transects or fields at 250x and 400x. The subsample fixed with formalin is used as replicate and for the determination of colonial algae.

Most of the taxa are determined to species level. Species-level densities are joined in major phytoplanktonic groups and expressed as biomass (g m^{-3}) and biovolume (mm^3 L^{-1}). The Chlorophyll-a concentration is determined with spectrophotometric analysis (ISO-10260 1992 E).
Additional abiotic data recorded Transparence (Secchi depth), light, temperature, pH, conductivity, dissolved oxygen, total phosphorus, total nitrogen
Method features compliant with WFD The status classes developed within the Swiss Modular Stepwise Procedure are comparable with the system of ecological classes defined in the WFD.
Rules to define ecological classes and reference conditions Swiss Modular Stepwise Procedure for studying and assessing surface waters refers to a reference status close to the natural status. The objective to be achieved is subdivided into hierarchical sub-objectives. The achievement of objectives is measured on a continuous scale ranging from 0 (for a very bad state) to 1 (close to the natural state). Quantification is associated with discrete state classes (bad, poor, moderate, good, very good) which indicate the achievement of the ecological objectives (threshold: 0.6 - class good).. The International Commission for the Protection of Waters between Italy and Switzerland (CIPAIS) set the objective to achieve to a “good” status.

Literature Reference

DFI 1982. Recommandations pour l’analyse des eaux superficielles en Suisse. Département fédéral de l’intérieur. Berne.

ISO 10260:1992. Water quality– Measurement of biochemical parameters – Spectrometric determination of the chlorophyll-a concentration

UNI EN 15204 : 2006. Water quality- Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique).

OFEV, 2013. Système d’analyse et d’appréciation des lacs en Suisse. Guide pour l’élaboration de modules d’appréciation. Berne

Comments
The Federal Office for the Environment (OFEV), in collaboration with Eawag, defined recommendations for the analysis and assessment of lakes (Système d'analyse et d'appréciation des lacs en Suisse, 2013), as part of the Modular Stepwise Procedure, which served as a basis for specialized cantonal services to develop their monitoring programs. A standardized method of analysis has not been developed for Swiss lakes.
The procedures used on Lake Lugano have been developed in agreement with recommendations from the administration of Canton Ticino and CIPAIS.

Method reported by:
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Institute SUPSI