UKTAG River Assessment Method Macrophytes and Phytobenthos

Phytobenthos – Diatoms for Assessing River and Lake Ecological Quality (River DARLEQ2)

by

Water Framework Directive – United Kingdom Technical Advisory Group (WFD-UKTAG)



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It is also the responsibility of the user if seeking to practise the method outlined here, to gain appropriate permissions for access to water courses and their biological sampling.



UKTAG Guide to Phytobenthos in Rivers Diatoms for Assessing River and Lake Ecological Quality (DARLEQ2)

1 Introduction

This classification method enables the assessment of phytobenthos in rivers according to the requirements of the Water Framework Directive (WFD). Phytobenthos refers to a mostly microscopic group of organisms called algae found attached to submerged surfaces such as stones and plant stems. Assessment focuses on the diatoms, a large and diverse group of algae using a tool called "DARLEQ2" (Diatoms for Assessing River and Lake Ecological Quality, based on a metric called the Trophic Diatom Index (TDI).

DARLEQ2 forms one part of the quality element "macrophytes and phytobenthos". Macrophytes are assessed separately with a method called LEAFPACS2. An earlier version of DARLEQ was evaluated and revised to ensure that in combination with the revised macrophyte tool, it provides an appropriate assessment to the overall quality element of macrophytes and phytobenthos. The revised method is known as DARLEQ2.

DARLEQ2 and LEAFPACS2 results are combined to produce an overall classification for macrophytes and phytobenthos, using the worst class of either sub-element. In addition, the final combined class can be modified by taking into account the presence of bacterial tufts.

1.1 Metrics

The metric used to classify phytobenthos in rivers is the TDI, and the version used in DARLEQ2 is TDI4. Diatom taxa are each assigned a score from 1 (nutrient sensitive) to 5 (nutrient tolerant) and the computed TDI4 scores range from 0 (very low nutrients) to 100 (very high nutrients). The WFD requires derivation of ecological status as an EQR (Ecological Quality Ratio). The TDI4 EQR is calculated based on observed data and predicted reference values, resulting in an overall EQR representing an ecological status class of either High, Good, Moderate, Poor or Bad. The EQR scale ranges from 0 (bad ecological status) to 1 (high ecological status).

1.2 Environmental pressures to which the method is sensitive

The method is used to detect the impact of nutrient enrichment, primarily indicating response to phosphorus. However, other factors such as grazing by invertebrates and hydromorphological regimes such as flow can influence diatom abundance and composition. These may influence the overall classification result, but are not built into the classification method.

1.3 Geographic application

This assessment method is appropriate for all rivers in the UK.

1.4 Intercalibration

This is a process whereby all European Member States were required to compare WFD class boundary values for each biological quality element (e.g. phytobenthos, phytoplankton) to ensure similar levels are set across all countries. Once a classification method has been intercalibrated, the method must be adhered to by Member States for the purposes of WFD assessment and reporting. Intercalibration mainly focussed on the EQRs which define the



class boundaries between High and Good, and Good and Moderate. DARLEQ2 was successfully intercalibrated for rivers. The current DARLEQ2 EQR boundaries are:

High/good status	0.80
Good/moderate status	0.60
Moderate/poor status	0.40
Poor/bad status	0.20

2 Data collection

2.1 Sample collection – location, frequency and sample volume

Samples of the biofilm which covers the upper surfaces of submerged cobbles or small boulders in rivers are collected by brushing or scraping with a clean toothbrush. Cobbles and small boulders are the preferred sampling substrate, but if there are no cobbles or small boulders present at the sampling site, the submerged stems of emergent macrophytes, such as *Phragmites australis*, *Sparganium erectum*, *Glyceria maxima* or *Typha* species, or leaves and stems of submerged macrophytes such as *Ranunculus* species and *Potamogeton* species are sampled instead. The sampling method is fully detailed and conforms to European Standards (CEN, 2014). Samples should be collected from mid-stream using a clean toothbrush, ensuring substrata have been submerged for at least 4 weeks prior to sampling.

2.1.1 Location

Samples must be representative of conditions in the river being studied, and should be collected from habitats with similar physical conditions at all sites, especially when collecting samples on repeat visits. Samples are ideally taken from riffles, runs, glides in rivers, from sites representative of the water body as a whole, and away from obvious human impacts. Location of sites near visible discharges should be far enough downstream for the discharge to have mixed fully with the river, and one should avoid sites where direct effects of organic pollution are obvious on the invertebrate fauna. This will obviously differ between catchments, but generally a site 200-500m downstream of a discharge may be most suitable. Samples should not be taken immediately after prolonged low flows or following periods of heavy rain.

2.1.2 Timing and Frequency

Two samples per year should be collected, one during spring (between March and May) and one during autumn (September to November). If this is not possible, summer sampling (June to August) is an alternative option as analysis of results has demonstrated that seasonal effects are not significant. Samples should be collected not less than two months apart.

2.1.3 Sample volume

Vigorous brushing of the substrate with a clean toothbrush in a plastic tray removes the diatom film from the surface of the substrate. In situations where submerged macrophytes have been sampled, cut random lengths are put into clean sampling bottles or bags and vigorously agitated to dislodge the attached diatoms. This process results in a nominal volume of sample collected that is easily transferred to a plastic container (volume 50-



100mls). There is not a precise volume of sample required, but larger volumes should be avoided.

2.2 Sample analysis

If samples cannot be analysed soon after collection they should be preserved as soon as possible with Lugol's iodine (adding approximately 5-10% by volume). Samples are then digested to remove all internal contents of the diatom cell (the frustule), leaving clean 'valves' (digestion of the frustule generally results in separation of two valves). Permanent slides are prepared using Naphrax as a diatom mountant, and at least 300 undamaged valves of non-planktic taxa should be identified and counted using a high power microscope (x1000 magnification). The presence and number of valves is recorded of each diatom taxa present. The analytical method is fully detailed and conforms to European Standards (CEN, 2014).

2.3 Other data requirements

Alkalinity data are required as a predictor of reference (expected) conditions for the DARLEQ2 EQR. Data should be obtained from analysis of samples from the water body taken at monthly intervals over a period of at least one year, reported as mg/L CaCO₃. Alkalinity is the observed annual mean alkalinity of the site unless the observed annual mean alkalinity is <5 mg L⁻¹ CaCO₃, in which case the value is set at "5"; or ≥250 mg L⁻¹ CaCO₃, in which case the value is set at "250". It is recommended that the same alkalinity value is used as a predictor variable for both macrophyte and phytobenthos classifications.

Analysis carried out for the UKTAG (UKTAG, 2013) has confirmed that a DARLEQ2 based assessment alone would generally give a reliable classification if alkalinity is < 75 mg L⁻¹ CaCO₃ (because the diatom-based status is consistently lower than the macrophyte-based status over this range of alkalinity and will thus define the overall classification), whilst a LEAFPACS2 based assessment alone is adequate at >200 mg L⁻¹ CaCO₃ (because the macrophyte-based status is then consistently lower than the diatom-based status). In the middle range, both components are necessary (because neither one is consistently lower than the other) although, on average, an assessment based on DARLEQ2 alone will be more reliable guide than LEAFPACS2 alone at alkalinities up to ~120 mg L⁻¹ CaCO₃ , whereas LEAFPACS2 alone will be a more reliable guide at alkalinities above this.

2.3.1 Bacterial tufts

The normative definition in the WFD (Annex V) refers to the "displacement" of macrophytes and phytobenthos by bacterial tufts and coats at moderate status, implying a need to recognise a state where the organic loading is so high that heterotrophic organisms can outcompete phototrophic organisms. Bacterial tufts and coats are generally referred to as "sewage fungus", which include a mixture of heterotrophic bacteria, fungi and protozoans.

Presence of sewage fungus should be recorded in the field on all occasions a diatom sample is collected, recording cover: occasional (<30% of surface area); widespread (30-60% of surface area) or extensive (>61% of surface area), and density: trace (but only just detectable); thin (obvious presence but substrate not obscured); thick (thick enough to fully obscure substrate) or massive (occupies a significant proportion of the water column). Sewage fungus should only have the potential to downgrade from Moderate or worse class.



Currently UK agencies implement this where appropriate. A weight of evidence approach is adopted, where sewage fungus coverage and density (as an average over the assessment period) should over-ride the results of LEAFPACS and DARLEQ assessments to determine final status. This occurs in situations where LEAFPACS and DARLEQ assessments suggest status is Moderate or lower, and where the sewage fungus assessment suggests a lower class than the combined LEAFPACS and DARLEQ assessment.

2.4 Minimum data requirements

Confidence of classification will depend on the number of samples taken, and the proximity of the resulting EQR to a class boundary. Ideally a total of 6 samples (over 3 years) are recommended. In practice fewer are used to produce a classification, although with a reduced confidence of class.

2.5 Typology

Typology defines waterbodies by factors which have a strong influence on their ecology; for calculation of river phytobenthos EQR, sites require input of annual mean alkalinity as detailed above.



3 Procedures for calculating metric EQR

The phytobenthos EQR is a ratio of observed to expected values. *Observed values* are taken from samples collected in the field and *expected values* (reference values) are predicted from alkalinity.

3.1 Calculating EQR for DARLEQ

3.1.1 Status class boundaries

The status class boundaries were derived during the intercalibration process to give final values as:

High/good status	0.80
Good/moderate status	0.60
Moderate/poor status	0.40
Poor/bad status	0.20

3.1.2 Manual calculation of EQR and status class

Calculation of phytobenthos EQR can be carried out manually as detailed below.

All diatom taxa are assigned a score from 1 (nutrient sensitive) to 5 (nutrient tolerant). The list of diatom taxa and their nutrient sensitivity scores is detailed in Appendix A.

The observed TDI for each sample is calculated using equations 1 and 2:

Observed value of river trophic diatom = $(W \times 25) - 25$ index

Equation 1

where:

"W" is given by the equation:

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

Equation 2

where:

 a_{j} is the number of values of taxon j, and

"s_j" is the nutrient sensitivity score in Appendix A corresponding to the taxon represented by j.The expected (reference) value of the TDI (eTDI) is predicted from a regression equation derived from a subset of reference sites characterized by very low levels of human pressure. The model uses alkalinity, an environmental variable that reflects background geology and fertility.



The value for the expected TDI (eTDI) at reference conditions applicable to the river should be calculated using the following equation:

$$eTDI = 9.933 * Exp(Log_{10}(alkalinity)*0.81)$$

Equation 3

where:

"alkalinity" is the observed annual mean alkalinity of the river in mg/I CaCO₃ unless the observed annual mean alkalinity is $< 5 \text{ mg L}^{-1} \text{ CaCO}_3$, in which case the value is set at "5"; or $\geq 250 \text{ mg L}^{-1} \text{ CaCO}_3$, in which case the value is set at "250".

The EQR is calculated using the following equation:

Equation 4

The final EQR value for each sample is then normalized so that it conforms to a scale from 0-1. To normalize the EQR, multiply the EQR value by 0.8. This normalization step allows the confidence of class to be calculated correctly.

Where the calculated EQR is > 1.0, its value should be set to "1".

3.1.3 Worked example

The following details a manual worked example from an analysis of a real sample collected from a site in the English midlands:

Taxon name	Abundance (a = number of valves)	Nutrient sensitivity score (s)	axs
Achnanthidium minutissimum type	12	2	24
Amphora montana	1	5	5
Amphora pediculus	1	5	5
Cocconeis pediculus	5	4	20
Cocconeis placentula	61	3.1	189.1
Cyclotella atomus	1	0	0
Cyclotella meneghiniana	3	0	0
Diatoma vulgare	1	5	5
Encyonema minutum (E. "ventricosum")	1	2.6	2.6
Encyonema silesiacum (E. "ventricosum")	14	2.6	36.4
Fistulifera / Mayamaea	1	3.9	3.9
Fragilaria vaucheriae	1	2	2
Gomphonema "intricatum" type	3	3.6	10.8



Taxon name	Abundance (a = number of valves)	Nutrient sensitivity score (s)	axs
Gomphonema clavatum	1	2	2
Gomphonema parvulum	3	3	9
Melosira varians	3	4	12
Navicula capitatoradiata	54	4	216
Navicula cryptotenella	107	4	428
Navicula gregaria	2	4	8
Navicula lanceolata	1	4	4
Navicula menisculus	1	4	4
Navicula minima	1	3	3
Navicula reichardtiana	1	4	4
Navicula tripunctata	2	5	10
Nitzschia fonticola	2	4	8
Nitzschia gracilis	1	3	3
Nitzschia palea	7	4	28
Nitzschia paleacea	3	3	9
Nitzschia sp.	1	3	3
Planothidium frequentissimum	2	3	6
Reimeria sinuata ("Reimeria sp.")	6	3	18
Reimeria uniseriata ("Reimeria sp.")	4	3	12
Rhoicosphenia abbreviata	7	4	28
Unidentified pennate diatom	1	3	3
Σa =	315	Σ as =	1121.8

The observed value for the river trophic diatom index for this sample is calculated as follows:

sum as for all taxa in sample = 1121.8

sum a for all taxa in sample = 315

Calculate W = sum as / sum a = 3.56

Calculate the observed value of TDI using equations 1 and 2:

 $(W \times 25) - 25 = (3.74 \times 25) - 25 = 64.0$

The mean total alkalinity for this sample was 88.5 mg/l CaCO₃.

The reference value for river TDI4 for the sample is:



 $-25.36 + [56.83 \times \log_{10}88.5] - [12.96 \times \log_{10}(88.5)^{2}] + [3.21 \times 1] = 38.03$ 9.933 * Exp(Log₁₀(88.5)*0.81) = 48.1

The EQR for the sample is:

(100 - 64.0) / (100 - 48.1) = 36 / 51.9 = 0.69

Normalise the EQR by multiplying by 0.8 to give a final EQR of;

0.69 x 0.8 = 0.552

An EQR of 0.552 gives a face value class of Moderate status.

An average of EQRs obtained over a year provides an annual EQR.

3.1.4 Automated calculation of EQR and status class

An MS Excel spreadsheet has been produced with a series of worksheets for data input and calculation of all components of the classification, including Confidence of Class. Instructions for use are provided within the spreadsheet calculator. The most recent version of the spreadsheet calculator can be found on the UKTAG website. Automated data entry and calculation of diatom EQR using the DARLEQ2 calculator spreadsheet is recommended for large data sets.

NB As revisions are likely to be made and the calculator updated over time, it is important to check that the most recent version is being used.



4 Procedures for calculating statistical confidence in metric

The assessment of statistical error associated with each EQR entry is calculated within DARLEQ2 and is expressed as a "confidence of class", i.e. the statistical confidence we have of the metric falling into each of the five classes, from High to Bad. This also makes it possible to determine the statistical confidence of the river classifying as "worse than Good status".

5 References

CEN (2014). Water quality - Guidance standard for the routine sampling and preparation of *benthic diatoms from rivers and lakes.* EN 13946: 2014. Comité European de Normalisation, Geneva.

CEN (2014). Water quality – Guidance for the identification and enumeration of benthic diatom samples from rivers and lakes. EN 14407: 2014. Comité European de Normalisation, Geneva.

Environment Agency (2012). A streamlined taxonomy for the Trophic Diatom Index. Environment Agency Report SC070034/TR1.

UKTAG (2013) Final recommendations on new and updated biological standards. September 2013. UKTAG 2013. <u>http://www.wfduk.org/sites/default/files/Media/UKTAG%20Final%20recommendations%20on</u> %20biological%20stds_20131030.PDF



Appendix A

The following table lists the UK streamlined diatom taxa where recorded maximum relative abundance was $\geq 2\%$ (EA, 2012) for use in TDI4.

Taxa name	Sensitivity score
Achnanthes clevei	5
Achnanthes exigua	3
Achnanthes oblongella	1
Achnanthes sp.	3
Achnanthidium eutrophilum	2
Achnanthidium microcephalum.	1
Achnanthidium minutissimum	2
Achnanthidium pyrenaicum	2
Achnanthidium sp	2
Achnanthidium subatomus	2
Adlafia minuscula	3
Adlafia minuscula var. muralis	5
Amphipleura pellucida	3
Amphora libyca.	4
Amphora montana	5
Amphora ovalis	5
Amphora pediculus agg.	5
Amphora sp	4
Amphora veneta.	5
Bacillaria paradoxa	5
Brachysira brebissonii	1
Brachysira vitrea	1
Caloneis bacillum	4
Caloneis hyalina	5
Caloneis silicula	4
Caloneis sp.	3
Cavinula cocconeiformis	2
Cavinula variostriata	3
Cocconeis diminuta	5
Cocconeis pediculus	4
Cocconeis placentula	3.1
Cocconeis scutellum	3
Cocconeis sp.	3
Craticula accomoda	4
Craticula halophila	4
Craticula molestiformis	5
Ctenophora pulchella	2
Cymatopleura solea	5
Cymbella affinis	2
Cymbella cistula	2
Cymbella helvetica	2

T	Sensitivity
Taxa name	score
Cymbella lanceolata	4
<i>Cymbella</i> sp.	3
Cymbellonitzschia diluviana	4
Delicata delicatula	1
Denticula tenuis	2
Diadesmis contenta	5
Diadesmis contenta fo. biceps	3
Diadesmis gallica	3
Diatoma ehrenbergii	1
Diatoma mesodon	1
Diatoma moniliformis	1
Diatoma problematica	2
Diatoma sp.	2
Diatoma tenue	1
Diatoma vulgare	5
Didymosphenia geminata	1
Diploneis elliptica	5
Diploneis marginestriata	5
Diploneis oblongella	5
Ellerbeckia arenaria	4
Encyonema 'ventricosum' agg.	2.6
Encyonema caespitosum	4
Encyonema gracile	1
Encyonema hebridicum	1
Encyonema prostratum	5
Encyonema sp.	4
Encyonopsis cesatii	2
Encyonopsis falaisensis	1
Encyonopsis microcephala	2
Eolimna minima	3
Eolimna subminuscula	4
Eolimna submuralis	5
Epithemia adnata	5
Epithemia sorex	3
Epithemia sp.	2
Epithemia turgida	1
Eucocconeis flexella	1
Eucocconeis laevis	1
Eunotia sp.	1.4
Fallacia helensis	5
Fallacia indifferens	2
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Fallacia lenzii5Fallacia pygmaea5Fallacia subhamulata5Fistulifera/Mayamaea spp.3.9Fragilaria bidens3Fragilaria capucina1.2Fragilaria fasciculata4Fragilaria fasciculata4Fragilaria nitzschioides3Fragilaria vaucheriae2Fragilaria vaucheriae2Fragilaria vaucheriae2Fragilaria vaucheriae2Frustulia krammeri1Frustulia krammeri2Frustulia vulgaris2Geissleria acceptata4Geissleria schoenfeldii3Gomphonema intricatum' type3.6Gomphonema acuminatum2Gomphonema constrictum var.2Gomphonema constrictum var.2Gomphonema pracile1Gomphonema pracile1Gomphonema pracile1Gomphonema pracile3Gomphonema pracile3Gomphonema pracile3Gomphonema pracile3Gomphonema pracile1Gomphonema pracile3Gomphonema provulum3Gomphonema provulum3Gomphonema provulum3Gomphonema provulum3Gomphonema provulum3Gomphonema provulum3Gomphonema trucatum3Gomphonema trucatum3Gomphonema trucatum3Gomphonema trucatum5Gomphonema trucatum5Gomphonema tru	Taxa name	Sensitivity score
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Fragilaria sp.2Fragilaria vaucheriae2Fragilariforma sp.2.5Frustulia krammeri1I1Frustulia sp.5Frustulia vulgaris2Geissleria acceptata4Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema angustatum3Gomphonema angustatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema gracile1Gomphonema noisigne5Gomphonema pasudoaugur3Gomphonema pasudoaugur5Gomphonema sp.3Gomphonema tergestinum4Gomphonema sp.3Gomphonema tergestinum4Gomphonema sp.3Gomphonema sp.3Gomphonema tergestinum4Gomphonema sp.3Gomphonema tergestinum4Gomphonema sp.3Gomphonema turncatum3Gomphonema sp.3Gomphonema turncatum5Gyrosigma acuminatum5Gyrosigma calproides5Hannaea arcus1Hantzschia abundans5Hantzschia abundans5Hantzschia abundans5Kolbesia ploenensis5	Fragilaria fasciculata	4
Fragilaria vaucheriae2Fragilaria vaucheriae2Fragilariforma sp.2.5Frustulia krammeri1Frustulia sp.5Frustulia vulgaris2Geissleria acceptata4Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema acuminatum2Gomphonema angustatum3Gomphonema angustatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema gracile1Gomphonema olivaceoides2Gomphonema parvulum3Gomphonema parvulum3Gomphonema sp.3Gomphonema tergestinum4Gomphonema sp.3Gomphonema tergestinum4Gomphonema sp.3Gomphonema sp.3Gomphonema turucatum3Gomphonema turucatum5Gorphonema acuminatum5Gorphonema acuminatum5Gorphonema sp.3Gomphonema turucatum3Gomphonema turucatum5Gyrosigma acuminatum5Gyrosigma acuminatum5Hannaea arcus1Hantzschia abundans5Hannaea arcus1Hantzschia abundans5Hantzschia abundans5Si ploenensis5	Fragilaria nitzschioides	3
Fragilariforma sp.2.5Frustulia krammeri1Frustulia sp.5Frustulia vulgaris2Geissleria acceptata4Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema auuminatum2Gomphonema angustatum3Gomphonema angustatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema gracile1Gomphonema parvulum3Gomphonema parvulum3Gomphonema parvulum3Gomphonema sp.3Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphonema ventricosum5Gyrosigma attenuatum5Gyrosigma attenuatum5Hantzschia abundans5Hantzschia abundans5Hantzschia aploenensis5	Fragilaria sp.	2
Frustulia krammeri1Frustulia sp.5Frustulia vulgaris2Geissleria acceptata4Geissleria ignota4Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema acuminatum2Gomphonema angustatum3Gomphonema anoenum4Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema gracile1Gomphonema olivaceoides2Gomphonema pavulum3Gomphonema pavulum3Gomphonema tergestinum4Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphonema arcus1Hantzschia abundans5Hantzschia abundans5Hantzschia applioxys4Karayevia laterostrata3Kolbesia ploenensis5	Fragilaria vaucheriae	2
Frustulia sp.5Frustulia vulgaris2Geissleria acceptata4Geissleria ignota4Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema acuminatum2Gomphonema angustatum3Gomphonema angustatum3Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema olivaceoides2Gomphonema parvulum3Gomphonema parvulum3Gomphonema sp.3Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphonema ventricosum5Gyrosigma acuminatum5Gyrosigma acalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Fragilariforma sp.	2.5
Frustulia vulgaris2Geissleria acceptata4Geissleria ignota4Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema acuminatum2Gomphonema angustatum3Gomphonema anoenum4Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema gracile1Gomphonema olivaceoides2Gomphonema olivaceum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphonema ventricosum5Gyrosigma acuminatum5Gyrosigma acuminatum5Hannaea arcus1Hantzschia abundans5Hantzschia applioxys4Karayevia laterostrata3Kolbesia ploenensis5	Frustulia krammeri	1
Geissleria acceptata4Geissleria ignota4Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema acuminatum2Gomphonema angustatum3Gomphonema angustatum2Gomphonema angustatum2Gomphonema anoenum4Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema olivaceoides2Gomphonema olivaceum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema turncatum3Gomphonema ventricosum1Gomphonema ventricosum1Gorphosphenia grovei4Gyrosigma acuminatum5Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia applioxys4Karayevia laterostrata3Kolbesia ploenensis5	Frustulia sp.	5
Geissleria ignota4Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema acuminatum2Gomphonema angustatum3Gomphonema anoenum4Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema olivaceoides2Gomphonema olivaceoides2Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphonema ventricosum1Gorphosphenia grovei4Gyrosigma acuminatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia abundans5Hantzschia abundans5Karayevia laterostrata3Kolbesia ploenensis5	Frustulia vulgaris	2
Geissleria schoenfeldii3Gomphonema 'intricatum' type3.6Gomphonema acuminatum2Gomphonema angustatum3Gomphonema anoenum4Gomphonema anoenum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema gracile1Gomphonema olivaceoides2Gomphonema olivaceoides2Gomphonema parvulum3Gomphonema parvulum3Gomphonema paseudoaugur5Gomphonema truncatum3Gomphonema truncatum3Gomphonema truncatum5Gyrosigma acuminatum5Gyrosigma acuminatum5Gyrosigma acuminatum5Hannaea arcus1Hantzschia abundans5Hantzschia aphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Geissleria acceptata	4
Gomphonema 'intricatum' type3.6Gomphonema acuminatum2Gomphonema angustatum3Gomphonema anoenum4Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum2Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema olivaceoides2Gomphonema olivaceoides2Gomphonema parvulum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema truncatum3Gomphonema truncatum5Gyrosigma acuminatum5Gyrosigma acuminatum5Gyrosigma acuminatum5Hannaea arcus1Hantzschia abundans5Hantzschia aphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Geissleria ignota	4
Gomphonema acuminatum2Gomphonema angustatum3Gomphonema anoenum4Gomphonema clavatum2Gomphonema clavatum1Gomphonema clevei1Gomphonema constrictum var.2capitatum1Gomphonema gracile1Gomphonema olivaceoides2Gomphonema olivaceoides2Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma nodiferum5Hannaea arcus1Hantzschia abundans5Hantzschia abundans5Karayevia laterostrata3Kolbesia ploenensis5	Geissleria schoenfeldii	3
Gomphonema angustatum3Gomphonema anoenum4Gomphonema clavatum2Gomphonema clavatum1Gomphonema constrictum var. capitatum2Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema gracile1Gomphonema olivaceoides2Gomphonema olivaceum3Gomphonema parvulum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphonema ventricosum5Gyrosigma acuminatum5Gyrosigma nodiferum4Gyrosigma nodiferum1Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema 'intricatum' type	3.6
Gomphonema anoenum4Gomphonema clavatum2Gomphonema clavatum2Gomphonema clavatum1Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema gracile1Gomphonema olivaceoides2Gomphonema olivaceoides2Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma nodiferum4Gyrosigma nodiferum1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema acuminatum	2
Gomphonema clavatum2Gomphonema clevei1Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema insigne5Gomphonema olivaceoides2Gomphonema olivaceum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphonema acuminatum5Gyrosigma acuminatum5Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema angustatum	3
Gomphonema clevei1Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema insigne5Gomphonema olivaceoides2Gomphonema olivaceum3Gomphonema parvulum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema anoenum	4
Gomphonema constrictum var. capitatum2Gomphonema gracile1Gomphonema insigne5Gomphonema olivaceoides2Gomphonema olivaceum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema tergestinum4Gomphonema ventricosum1Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Kolbesia ploenensis5	Gomphonema clavatum	2
capitatumGomphonema gracile1Gomphonema insigne5Gomphonema olivaceoides2Gomphonema olivaceum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema sp.3Gomphonema tergestinum4Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema clevei	1
Gomphonema gracile1Gomphonema insigne5Gomphonema olivaceoides2Gomphonema olivaceum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema pseudoaugur5Gomphonema sp.3Gomphonema tergestinum4Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5		2
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Gomphonema olivaceum3Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema sp.3Gomphonema tergestinum4Gomphonema truncatum3Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema insigne	5
Gomphonema parvulum3Gomphonema pseudoaugur5Gomphonema sp.3Gomphonema tergestinum4Gomphonema truncatum3Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema olivaceoides	2
Gomphonema pseudoaugur5Gomphonema sp.3Gomphonema tergestinum4Gomphonema truncatum3Gomphonema truncatum1Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia abundans5Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema olivaceum	3
Gomphonema sp.3Gomphonema tergestinum4Gomphonema truncatum3Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema parvulum	3
Gomphonema tergestinum4Gomphonema truncatum3Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema pseudoaugur	5
Gomphonema truncatum3Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema sp.	3
Gomphonema ventricosum1Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema tergestinum	4
Gomphosphenia grovei4Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema truncatum	3
Gyrosigma acuminatum5Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphonema ventricosum	1
Gyrosigma attenuatum5Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gomphosphenia grovei	4
Gyrosigma nodiferum4Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gyrosigma acuminatum	5
Gyrosigma scalproides5Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gyrosigma attenuatum	5
Hannaea arcus1Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gyrosigma nodiferum	4
Hantzschia abundans5Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Gyrosigma scalproides	5
Hantzschia amphioxys4Karayevia laterostrata3Kolbesia ploenensis5	Hannaea arcus	1
Karayevia laterostrata3Kolbesia ploenensis5	Hantzschia abundans	5
Kolbesia ploenensis 5	Hantzschia amphioxys	4
	Karayevia laterostrata	3
Lemnicola hungarica 5	Kolbesia ploenensis	5
	Lemnicola hungarica	5



Taxa name	Sensitivity score
Luticola goeppertiana	5
Luticola mutica	3
Luticola sp.	4
Luticola ventricosa	3
Melosira varians	4
Meridion circulare	2
Meridion circulare var. constrictum	1
Navicula [small species]	4
Navicula angusta	1
Navicula capitata	4
Navicula capitatoradiata	4
Navicula cari	4
Navicula carteri	3
Navicula cincta	4
Navicula claytonii	3
Navicula cryptocephala	3
Navicula cryptotenella	4
Navicula digitoradiata	4
Navicula gregaria	4
Navicula hungarica	5
Navicula integra	4
Navicula lanceolata	4
Navicula menisculus	4
Navicula modica	5
Navicula oblonga	4
Navicula radiosa	3
Navicula recens	5
Navicula reichardtiana	4
Navicula reinhardtii	4
Navicula rhynchocephala	2
Navicula schroeterii	5
Navicula slesvicensis	3
Navicula sp.	4
Navicula subrhynchocephala	4
Navicula subrotundata	5
Navicula tenelloides	4
Navicula tripunctata	5
Navicula trivialis	4
Navicula veneta	4
Navicula viridula	4
Nitzschia acicularioides	5
Nitzschia acicularis	3
Nitzschia amphibia	5
Nitzschia archibaldii	2
Nitzschia brevissima	2

Taxa name	Sensitivity score
Nitzschia capitellata	4
Nitzschia clausii	4
Nitzschia communis	5
Nitzschia disputata	2
Nitzschia dissipata	3
Nitzschia dissipata subsp. media	3
Nitzschia filiformis	5
Nitzschia flexa	3
Nitzschia fonticola	4
Nitzschia fossilis	5
Nitzschia frustulum	3
Nitzschia gracilis	3
Nitzschia hantzschiana	2
Nitzschia heufleriana	4
Nitzschia inconspicua	4
Nitzschia lacuum	2
Nitzschia liebetruthii	1
Nitzschia linearis	4
Nitzschia littoralis	4
Nitzschia microcephala	3
Nitzschia palea	4
Nitzschia paleacea	3
Nitzschia paleaeformis	3
Nitzschia perminuta	3
Nitzschia perspicua	3
Nitzschia pusilla	4
Nitzschia recta	4
Nitzschia sigma	4
Nitzschia sigmoidea	3
Nitzschia sociabilis	4
Nitzschia sp.	3
Nitzschia subacicularis	4
Nitzschia sublinearis	4
Nitzschia supralitorea	5
Nitzschia tubicola	4
Nitzschia vermicularis	4
Parlibellus protracta	4
Peronia fibula	1
Pinnularia sp.	2.2
Placoneis clementis	4
Placoneis elginensis	5
Planothidium bioretti	2
Planothidium delicatulum	5
Planothidium dubium	3
Planothidium ellipticum	3



Taxa name	Sensitivity score
Planothidium frequentissimum	3
Planothidium granum	5
Planothidium lanceolatum	4
Planothidium rostratum	5
Planothidium sp.	4
Platessa conspicua	5
Psammothidium chlidanos	2
Psammothidium grishunun fo. daonensis	2
Psammothidium helveticum	2
Psammothidium lauenburgianum	5
Psammothidium levanderi	3
Psammothidium marginulatum	2
Psammothidium scoticum	2
Psammothidium sp.	2
Psammothidium subatomoides	2
Pseudostaurosira/Staurosira agg.	3.7
<i>Reimeria</i> sp.	3
Rhoicosphenia abbreviata	4
Rossithidium sp.	1
Sellaphora joubaudii	4
Sellaphora pupula	4
Sellaphora seminulum	4
Simonsenia delognei	5
Stauroneis anceps	2
Stauroneis kriegeri	2
Stauroneis sp.	3
Stauroneis thermicola	3
Staurosirella leptostauron	4
Staurosirella pinnata	4
Surirella angusta	3
Surirella brebissonii	3
Surirella crumena	3
Surirella islandica	3
Surirella linearis	1
Surirella minuta	4
Surirella ovalis	5
Surirella roba	2
<i>Surirella</i> sp.	2
Surirella terricola	4
Synedella parasticia	5
Synedra acus	3
Synedra famelica	2
Synedra tenera	1
Synedra ulna	2
<i>Tabellaria</i> sp.	1



Taxa name	Sensitivity score
Tryblionella acuminata	5
Tryblionella apiculata	5
Tryblionella debilis	4

Taxa name	Sensitivity score
Tryblionella hungarica	4
<i>Tryblionella</i> sp.	4