Final Report   **Project WFD38**

WP1 Report on Potential Phytoplankton
Pressure Sensitive Metrics

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EXECUTIVE SUMMARY

WFD38: Phytoplankton Classification Tool for UK Lakes: WP1 Report on Potential Phytoplankton Pressure Sensitive Metrics (February, 2006)

Project funders/partners: SNIFFER (WFD38) & Environment Agency (EMC(03)07)

Background to research
The Environment Agency and SNIFFER have commissioned this R & D project to develop a method to classify the ecological status of lakes on the basis of phytoplankton. As part of this assessment, metrics need to be developed for phytoplankton community composition.

Objectives of research
Specific objectives for the project were to develop a robust classification, incorporating:

1. Prediction of reference scores for UK lakes based on phytoplankton composition
2. Developing criteria for defining the good/moderate boundary
3. Classifying the ecological status of a water body in to one of five status classes (High/Good/Moderate/Poor/Bad), based on the calculation of an Ecological Quality Ratio (EQR). An EQR being calculated from the relationship between current observed and reference phytoplankton community composition for a site
4. Determining uncertainty associated with the classification result, based on statistical confidence or probability of class

Key findings and recommendations
This report provides an initial review of potential metrics available for assessing the ecological status of lakes using phytoplankton. Additionally, it outlines the data availability at the time of the start of the project. Guidance on sample collection and phytoplankton counting is also documented

Key words: phytoplankton, WFD, classification, lake, ecological status, database

Acknowledgements

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1.   INTRODUCTION

The phytoplankton community is widely considered the first biological community to respond to eutrophication pressures and is the most direct indicator of all the Biological Quality Elements (BQEs) of nutrient concentrations in the water column. There are numerous socio-economic problems associated with increases in phytoplankton abundance, particularly with increasing frequencies and intensities of toxic cyanobacteria blooms. These include detrimental effects on drinking water quality, filtration costs for water supply (industrial and domestic), water-based activities and conservation status (sensitive pelagic fish species, such as coregonids). In some contexts, however, increasing phytoplankton abundance can be considered as a positive feature, for example, in increasing fisheries productivity, and some phytoplankton taxa can be considered indicative of high ecological status.

Annex V of the WFD outlines three features of the phytoplankton quality element that need to be considered in the assessment of the ecological status of lakes and for which there is, therefore, the need to relate in quantitative terms to nutrient conditions. These three are:

- Phytoplankton composition
- Phytoplankton abundance and its effect on transparency conditions
- Planktonic bloom frequency and intensity

The WFD normative definition for phytoplankton in lakes indicates that declining ecological quality is associated with increasing phytoplankton abundance, composition shifts and more frequent and intense phytoplankton blooms (Table 1).

Table 1.   Qualitative criteria for assessing Ecological Status in terms of eutrophication impacts (modified from ECOSTAT Eutrophication Guidance, 2005)

Ecological Status	WFD normative definition	Primary impacts on phytoplankton	Secondary impacts (e.g. transparency and O_2 deficiency)
High	Undisturbed conditions or minor changes	None	None
Good	Slight change	Slight changes in composition, abundance or frequency and intensity of blooms	None
Moderate	Moderate change	Moderate change in composition and abundance begins to have significant undesirable disturbance. Persistent blooms may occur in summer. Pollution tolerant species more common	Occasional impacts on other biological elements, transparency and oxygen
Poor	Major change	Pollution sensitive species no longer common. Persistent blooms of pollution tolerant species	Secondary impacts common & occasionally severe.
Bad	Severe change	Totally dominated by pollution tolerant species	Severe impacts common

Report Objectives
This report has three main objectives:
1. Review potential pressure-sensitive metrics for the three phytoplankton sub-quality elements: composition, abundance and bloom frequency and intensity
2. Identify and collate existing UK phytoplankton data sets and create an electronic database to store the phytoplankton and supporting environmental data
3. Make recommendations on sample collection and analysis

2. PHYTOPLANKTON METRICS

Phytoplankton composition
The phytoplankton community is notoriously diverse and variable. Developing an ecological classification specifically in relation to nutrient pressures requires overcoming the large seasonal and inter-annual variability associated with the changing physical structure of the water column and grazing pressure from zooplankton, and magnifying the signal related to nutrient concentrations. For this purpose, a phytoplankton index based on key indicators is likely to be most appropriate.

Individual species or taxa can be considered as positive, negative or indifferent indicators in relation to nutrient pressures. Positive indicators include species of chrysophytes (e.g. *Dinobryon*), desmids (e.g. *Cosmarium*) and diatoms (e.g. *Cyclotella comensis*). Negative indicators include species of green algae (e.g. *Scenedesmus*), diatoms (e.g. *Stephanodiscus*) and many groups of cyanobacteria, such as the large colonial and filamentous genera *Microcystis*, *Aphanizomenon* and *Anabaena*. The latter are favoured by relatively stable stratification and high alkalinity and can, therefore, form a significant natural component of the phytoplankton community in deep alkaline lakes. As taxonomic status at the phylum/class level does not consistently represent positive or negative indicators, higher taxonomic resolution to genus or species level may be necessary for classification tool development.

Alternatively, phytoplankton composition may be considered in terms of functional groups (c.f. Reynolds et al., 2002). Such functional groupings have been developed using a combination of experimental evidence, empirical data and expert opinion to group species with consistent functional properties or attributes. In principle, functional groups are more predictable than individual species or genera in terms of their response to nutrient conditions under a broad set of physical conditions and, therefore, can potentially be developed to indicate impacts of nutrient pressures more consistently (Reynolds et al., in Carvalho et al., 2004). Assigning taxa to functional group does, however, still require taxonomic resolution to the genus level, and species-level identification is required for some taxa.

The relative abundance or balance of positive and negative indicators can be used to construct a measure of ecological status in terms of nutrient pressures. A predominance of positive indicators (taxonomic or functional group) of low nutrient pressure in a given lake type should be representative of reference conditions – although more widely tolerant taxa may be common and negative indicators may also be present in low abundance. Positive and negative indicators (taxa or functional groups) can be identified through expert opinion (Reynolds in Carvalho et al., 2004), although ideally this opinion needs to be validated using empirical data, palaeolimnology or modelling.

Ecological classification for the WFD requires the comparison of the biological composition of an individual lake to an expected reference condition (site or type-specific). Three general approaches can be adopted (US EPA, 1999):

1. Multimetric assessment using an index that is the sum of several metrics. This is the basis of the Index of Biotic Integrity (IBI) (Karr et al. 1986).
2. Multimetric assessment using an index that is developed from a multivariate discriminant model to discriminate reference from impaired sites. This is the basis of the estuarine invertebrate indices developed by the EMAP-Estuaries program (USEPA 1993).
3. Assessment using multivariate ordination of species abundances. This methodology has been used widely in the assessment of UK rivers and streams, through the development of RIVPACS (e.g., Wright et al. 1984).

These approaches are outlined and compared in a report by the US EPA (1999). They summarise their applicability as follows: "both the multimetric index and the discriminant model index (approaches 1 and 2) are easy to apply in a continuing operational monitoring program because data from an individual site are entered into a formula, and the site's deviation from reference conditions can be known immediately (Gerritsen 1995). The ordination approach (3) requires reanalysis of the entire reference data set for each new batch of monitoring sites. The multimetric index (approach 1) is the easiest to explain to managers and the public because it does not rely on specialized concepts such as multivariate statistics. The ordination approach (3) may be most cost-effective if the biological survey is a single event—a large number of sites are surveyed once, and there is no plan to continue monitoring or to survey new sites."

One taxonomic metric approach being considered by Norway for the WFD examines the proportion of cyanobacteria of the total phytoplankton biomass (Figure 1; Solheim et al., 2004). Other Member States are also developing metrics based on the relative abundance of positive and negative phytoplankton groups (e.g. Denmark - Sondergaard et al, 2003; Hungary? - Padisak et al., 2005). Both taxonomic (Figure 1) and functional group (Figure 2) metrics need to be examined and validated further.

Multivariate approaches (e.g. DCA, CCA, etc.) provide useful exploratory tools for investigating patterns in compositional data and can also be used to derive indicator taxa/groups and develop ecological assessment schemes for WFD (e.g. RIVPACS refs; Dodkins et al., 2005). Multivariate approaches tend, however, to be less explicitly pressure-specific and, therefore, less easily interpreted in terms of ecological impacts related to a specific pressure, such as nutrients. Classification schemes based on multivariate analysis can also be more strongly influenced by outliers in the data compared with multimetric indices, so data used need to be carefully selected across all the main environmental gradients.

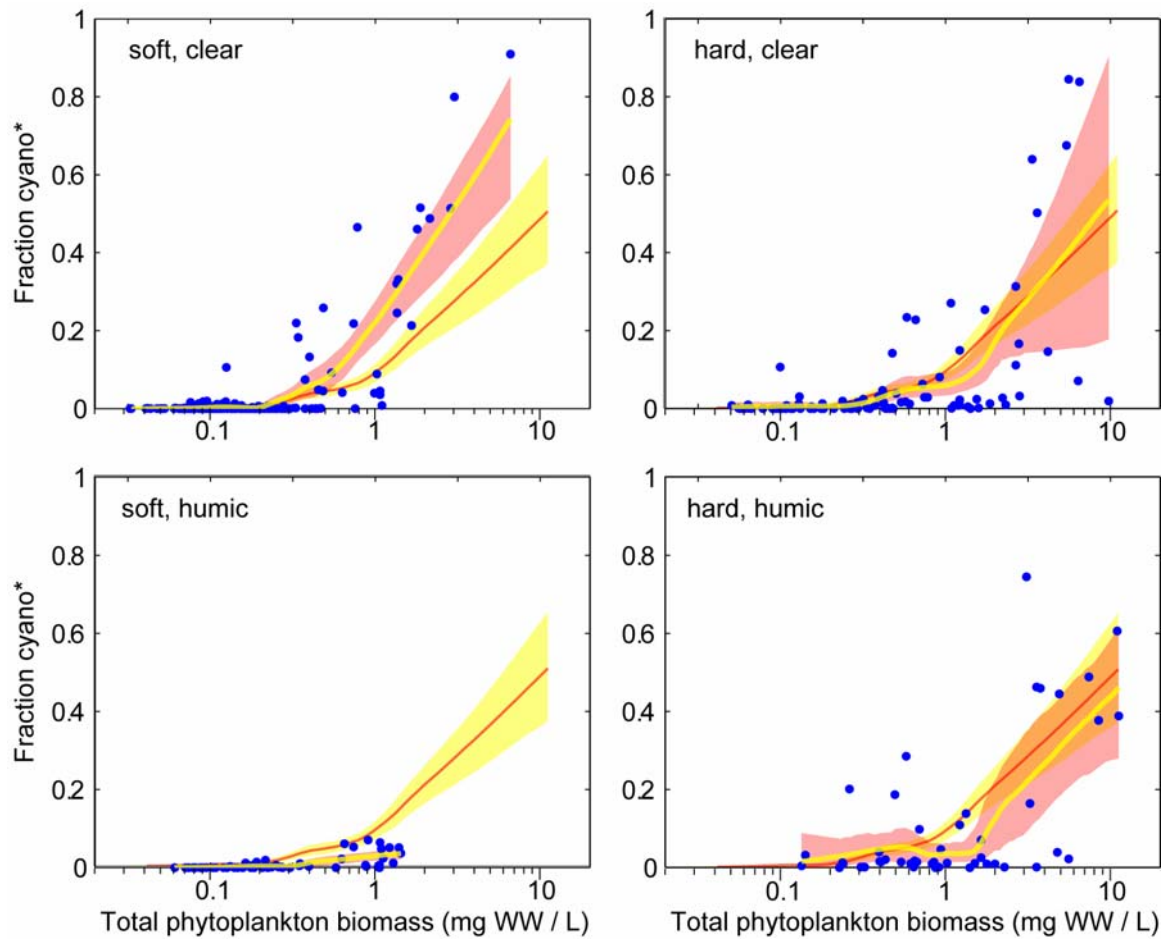**Figure 1**   **Relationship between total phytoplankton biomass (WW= Wet Weight) and impact taxonomic group "cyanobacteria" in Norweigian lakes**
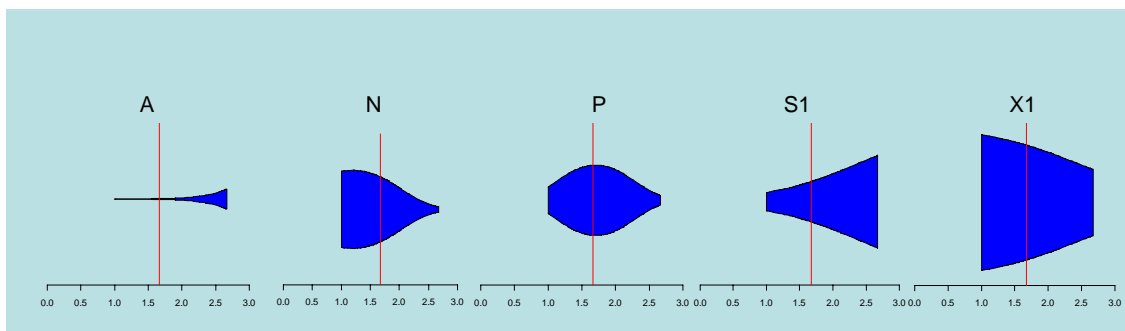
(Source: Solheim et al., 2004)

Figure 2   **Relationship between biomass of phytoplankton functional groups (c.f. Reynolds et a. 2002) along the total phosphorus gradient in very shallow lakes**

(Source: Ptacnik, unpublished)

Phytoplankton abundance and its effect on transparency conditions
In general, as nutrient concentrations increase, phytoplankton abundance shows more frequent and sustained peaks throughout summer, and consequently transparency declines. The abundance of phytoplankton can be expressed in three ways:

- Density of individual organisms (numbers of individual cells/filaments/colonies per ml)
- Volume of these cells as a fraction of the volume of water (biovolume in $mm^3\ m^{-3}$)
- Chlorophyll-a concentration expressed as a concentration in the water ($\mu g\ l^{-1}$)

All of these approaches have their pros and cons. For WFD monitoring purposes, many Member States are choosing to use chlorophyll-a concentration, as it is a relatively robust and simple measure widely used in all Member States (Phillips 2005 (LTT84); Sondergaard et al, 2003, 2005; van der Berg, 2004). In addition to chlorophyll metrics, biovolume estimates are widely being used across Europe, but are incorporated in the analysis of the compositional data.

Transparency is widely used as an indirect or surrogate estimate of the amount of phytoplankton or chlorophyll-a, and, therefore, as an indicator of eutrophication. The optical properties of lakes are, however, not just controlled by the amount of phytoplankton other factors, such as dissolved colour, suspended inorganic and organic particles may contribute significantly in some lakes (Tilzer 1988).

Transparency is mentioned in Annex V of the WFD as a general physico-chemical factor supporting the biological elements. In the normative definition of ecological status (Annex V, 1.2.2) it is stated that, under high status conditions, *the average phytoplankton biomass is consistent with the type-specific physico-chemical conditions and is not such as to significantly alter the type-specific transparency conditions*.

Transparency of water is commonly estimated using a Secchi disk, less commonly it is measured using remote sensing methods. The Secchi disk measurement is obtained for a very restricted area (a lake or more specifically discrete points in a lake) while remote sensing methods can cover a larger area at once (up to hundreds of lakes). A Secchi disk is a circular, 20 cm diameter, black and white disk that disappears when lowered into water and reappears again when raised. The actual photosynthetic layer of water is approximately three times the Secchi disk depth. The Secchi disk method is a very simple, useful and cost-effective way to monitor and assess the status of surface waters. Furthermore, this method is a useful tool for monitoring by citizens to enhance public participation in the WFD. The use of satellite remote sensing is, however, a cost-effective method to assess the transparency of surface waters (turbidity, chlorophyll *a*) on a large scale.

As well as transparency, there is a need to relate phytoplankton abundance to supporting chemistry, particularly phosphorus and nitrogen concentrations. Quantitative relationships have been developed relating total phosphorus (TP) concentrations with phytoplankton biomass (chlorophyll-a). The most widely reported relationships were developed by Dillon & Rigler (1974) and Vollenweider/OECD (OECD, 1982). The latter relationship (Figure 3) was developed for a set of, predominantly large, temperate lakes, not ecotype specific. There is a great deal of scatter in all the published relationships highlighting the fact that a number of sensitivity factors are involved, such as water colour and flushing rate, of which the latter can be altered by hydromorphological pressures such as flow regulation. There is, therefore, a need to examine more complex regression models that incorporate these sensitivity factors. Alternatively, and more appropriate for the WFD, many of the lake typology factors (depth, altitude, colour) affect how effectively nutrients are transformed into algal biomass, highlighting a need to examine lake-type specific models.
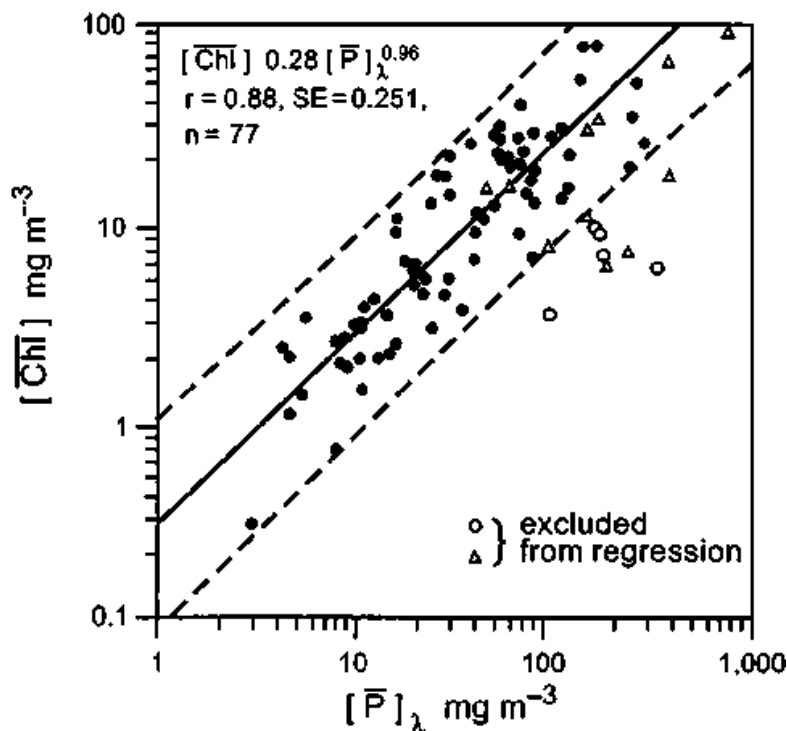
Figure 3. **Regression of annual mean phosphorus concentration with phytoplankton biomass (as annual mean chlorophyll a) (OECD, 1982)**

Planktonic bloom frequency and intensity
The term "planktonic bloom" is poorly defined, but in general refers to the phenomenon when phytoplankton populations greatly increase in numbers to densities much higher than the average for the lake. Almost by definition a bloom is usually composed of only one or possibly two species that dominate when conditions are particularly suitable for them. All species of algae can "bloom" when suitable ecological conditions occur, but most concern focuses on when these blooms are composed of large, colonial cyanobacteria as these taxa are potentially toxic and so can have a significanct impact on water use and activities. Additionally, these colonies can accumulate at the surface and may then concentrate on a downwind shore accumulating to such an extent as to form a surface "scum". When blue-green algal cells start to die and break up, any toxins that may be present are released into the surrounding water. Cell pigments are also released resulting in a scum often resembling turquoise emulsion paint. Not all blue-green algal scums are, however, this typical colour; they can range from black through dark greens to blues, reds and pinks.

There are only a few other types of algae (notably the green alga *Botryococcus braunii* and the flagellate *Euglena*) that will occasionally form surface scums. Benthic blue-green algae can also form algal mats that can occasionally detach from the lake bed, rise to the surface, and may then be washed up on the shore. These detached mats are often very different in appearance to planktonic forms. They are usually very dark in colour (black, dark brown or green) and are much more cohesive in nature than planktonic scum.

As toxic cyanobacteria blooms are of most concern, the project will explicitly target the development of a classification scheme for cyanobacteria bloom frequency and intensity. Dense crops of other algae are represented elsewhere in the classifications for phytoplankton composition and abundance which consider all phytoplankton and average abundance.

Bloom-forming cyanobacteria can be filamentous or colonial and are distributed across a range of genera, but all contain gas-vacuoles that are used to regulate buoyancy (Reynolds and Walsby, 1975). The sudden appearance of surface blooms, or scums, is associated with filaments or colonies migrating to the surface rapidly following the onset of calm weather. For WFD classification purposes, a bloom will be defined here as a 'large' population of cyanobacteria, whether or not surface scums have developed.

The two major environmental factors affecting cyanobacterial bloom frequency and intensity are the stability of thermal stratification (favouring buoyant or motile algae) and declining nutrient availability in the epilimnion (but typically rich sources in hypolimnion or sediment (Reynolds, 1987; Oliver & Ganf, 2000). Stable stratification is not, however, a pre-requisite as *Oscillatoria* species can form dense populations in shallow, well-mixed nutrient-rich lakes.

No quantified relationships have been described in the literature detailing how bloom frequency or intensity is related to nutrient conditions. Increasing frequency of surface blooms is more likely to be related to meteorological or climatic changes, but, increasing abundance (intensity), is widely accepted to be related to increasing enrichment. Cyanobacteria can, however, form a significant natural component of the phytoplankton community in deep alkaline lakes (McGowan et al., 1999) although increasing nutrient concentrations are likely to result in their higher abundance in these lakes too.

There is no agreed European quantitative limit for defining simply when an algal or cyanobacterial bloom is present. Different organisations and Member States use a variety of thresholds depending on the use of the water, the species dominating and the measure taken: cyanobacteria densities, chlorophyll-a concentrations or amounts of toxin (e.g. microcystin) are all used. In the UK, WHO guidance levels have been adopted relating the cyanobacteria concentrations (units per ml) equivalent to 10µg of chlorophyll-a per litre (Table 2).

In Finland, a more practical approach to monitoring bloom frequency has been taken with no strict quantitative definition of a bloom. A nation-wide observation system has been set up to provide up-to-date information on cyanobacterial occurrence/blooming across the country. The monitoring programme is a joint-venture of local and regional authorities and the Finnish Environment Institute (SYKE). Observation sites, selected by regional environment centres, particularly focus on waters in the vicinity of cities or public beaches. In summer 2004 the observation network included 262 sites in lakes and rivers. The observations are made between June and August. Municipal health or environmental authorities or volunteer citizens visit the sites weekly and estimate cyanobacterial abundance by visually examining the water area from the shore. In order to harmonize the estimates, the observers receive prior training from SYKE. The observations are classified into four classes (0-3) (Table 3).

Table 2   The nature and size of the units typically formed by different blue-green algal species and the concentration of these typically equivalent to the lower WHO guidance level for chl$_a$
(Scottish Executive Health Department, 2002: Derived from: "Environment Agency Policy on Blue Green Algal Monitoring and Management of Incidents").

Taxon	Assume "Units" are	Concentration (units per ml) equivalent to 10μg of chlorophyll-a per litre
Anabaena circinalis *Anabaena spiroides* *f. spiroides*	18 cells/gyre	1,000 - 1,400 gyres
Anabaena flos - aquae	26 cells/gyre	760 - 2,300 gyres
Anabaena solitaria	50 cells/filament	40 -160 filaments
Anabaena spiroides *f. crassa*	50 cells/filament	70 - 80 filaments
Aphanizomenon *flos-aquae*	60 cells, single filaments 60 - 70 filaments	1,200 - 4,200 filaments 20 - 60 flakes
Aphanothece/ *Aphanocapsa*	40μm colonies (= 100 cells) 80μm colonies (= 1,000 cells)	4,000 colonies 240 colonies
Coelosphaerium *kutzingianum*	50μm colonies (= 300 cells) 80μm colonies (= 1,000 cells)	350 colonies 60 colonies
Gloeotrichia echinulata	500 μm filaments	120 -200 filaments or 1 - 2 colonies
Gomphosphaeria *naegeliana*	50μm colonies (= 300 cells) 80μm colonies (= 1,000 cells)	160 colonies 40 colonies
Merismopedia sp.	30μm^2 'plates' =64 cells	2,000 - 42,000 plates
Microcystis aeruginosa	90μm colonies (= 1,000 cells) 200μm colonies (= 10,000 cells)	40 colonies 3 colonies
Nodularia spumigena	15 cells/filament	2,000 - 8,000 filaments
Oscillatoria agardhii	300 μm filaments	250 - 600 filaments
Oscillatoria isothrix	1,000 μm filaments	60 - 160 filaments
Oscillatoria redekei	300 μm filaments	1,300 - 3,500 filaments
Oscillatoria rubescens	1,000 μm filaments	120 - 360 filaments
Pseudanabaena sp.	300 μm filaments	600 - 700 filaments
Synechococcus sp.	Unicellular	0.5 - 25 x 10^6 cells

Table 3   Finnish visual classification of algal blooms
                (Source: Lepisto, unpublished)

0	No algae. No algae on the water surface or on the shore line. The Secchi depth is normal.
1	Observed. Greenish flakes detected in the water or when taken into a transparent container, or narrow stripes on the shore. The Secchi depth is reduced by algae.
2	Abundant. The water is clearly coloured by algae, small surface scums or cyanobacterial mass on the beach are detected.
3	Very abundant. Wide and heavy surface scums or thick aggregates of cyanobacteria are detected on the shore.

Note: the term "algae" is used to encompass all algae, not just cyanobacteria

The network produces information for health authorities for decision making, information on the ecological condition of the water bodies, and it also meets the needs of informing the public. Health authorities have been given guidelines to monitor cyanobacteria in public beaches and place warning signs if cyanobacteria have been detected. If cyanobacteria are estimated as abundant or very abundant a water sample is taken for further microscopical investigation. The species composition and abundance of the cyanobacteria are then recorded in a national database of harmful algae.

Neither the WHO guideline of 10 µg/l or the Finnish classification are WFD-compliant as neither are reference-based. A more widely accepted definition of what magnitude of change in abundance of cyanobacteria, or 'intensity' constitutes a phytoplankton bloom is needed. The relationship between the mean and standard deviation may provide a useful measure. Once a definition has been agreed, any relationship with nutrient pressures can be explored. Data will be needed at a high sampling frequency to assess what magnitude of change can be considered an increasing trend in intensity or frequency from reference condition. For this reason, the analysis is likely to be limited to a few well-monitored lakes with sufficient data.

One approach for incorporating defined bloom thresholds with a probability of occurrence (or accepted risk) is through the application of quantile regression (Figure 4).
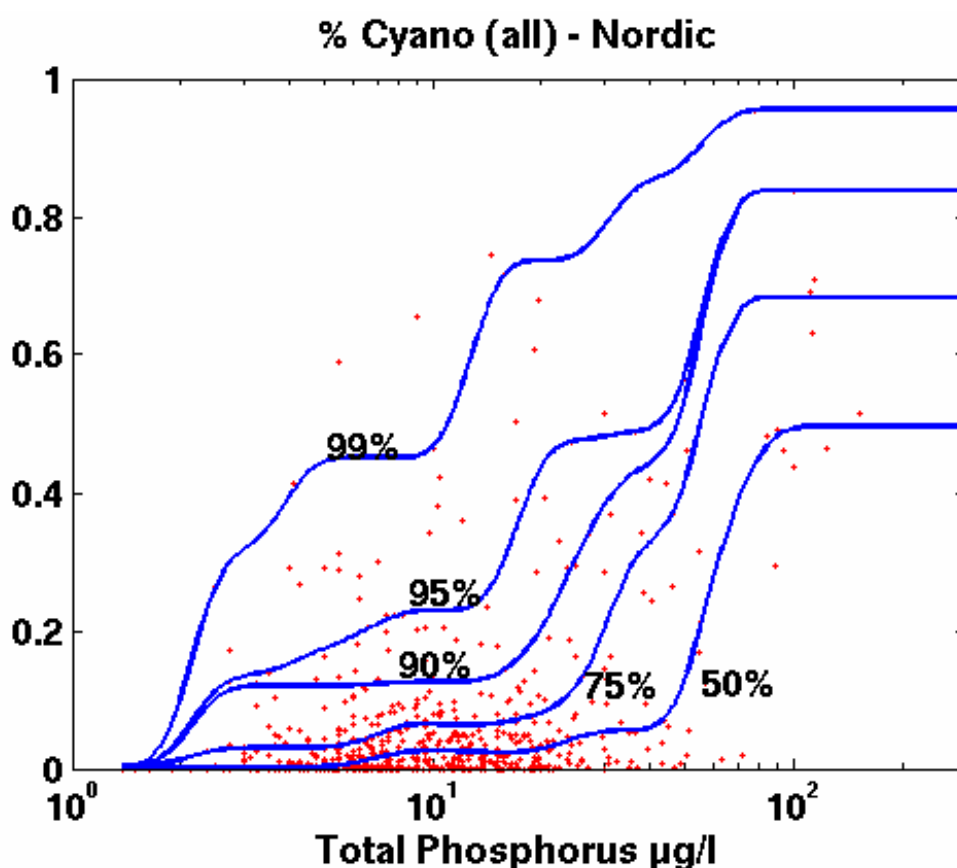
Figure 4.   Quantile regression plot of proportion of cyanobacteria in Nordic lakes along a total phosphorus gradient

Percentile regression lines are shown for 50%, 75%, 90%, 95% and 99% of sites (Ptacnik, unpublished)

Project issues:

- Focus explicitly on cyanobacteria bloom-frequency and intensity
- Need to define what proportional change in abundance, or "intensity", over reference conditions constitutes a cyanobacteria bloom
- Need to obtain sufficient data of high sampling frequency to develop quantitative relationships between cyanobacteria bloom frequency and nutrient pressure

3.   DATA AVAILABILITY AND DATABASE STRUCTURE

A number of sources of chemistry, chlorophyll and phytoplankton composition data have been collated as part of the project. These include GB data from EA, SEPA, CEH, CCW and the University of Liverpool. Data from Irish lakes were provided by the EPA, DARD and the University of Ulster.

A further update to the project database is planned in early 2006 to incorporate new data from EA, SEPA. Data from NI and Irish lakes will also be incorporated with a focus on those lakes having data from several sampling occasions within a growth season.

Lake typology factors

Data largely sourced from GB lakes database (http://ecrc.geog.ucl.ac.uk/gblakes/) and EA and SEPA chemistry databases. GB lakes geological type were frequently overridden from alkalinity data provided by EA and SEPA. Information on Irish lakes were provided by EPA, DARD and the University of Ulster.

GB lakes

In total the database contains 378 lake basins from GB (Windermere and Loch Lomond both constitute two basins) with typology data for most of these (Table X). Colour data are only available for lakes in England & Wales.

Table X. No. of lakes in the GB chemistry/typology database classified according to GB depth and alkalinity classes:

	VShallow	Shallow	Deep	Unknown	Total
Low Alk	35	71	60	23	**189**
Med. Alk	22	39	19	8	**88**
High Alk	56	35	7	2	**100**
Unknown Alk	1				**1**
Total	**114**	**145**	**86**	**33**	**378**

Chlorophyll and water chemistry

A number of sources of chemistry and chlorophyll data have been collated as part of the project. These include GB data from EA, SEPA, CEH, CCW and the University of Liverpool. Data from Irish lakes were provided by the EPA, DARD and the University of Ulster.

GB lakes

Chemistry data from GB lakes have been collated together by CEH in a single Microsoft Access chemistry database with accompanying lake typology data. This has been made available to the Project Board and all other UK WFD classification tool projects to ensure consistency in the chemistry data used.

Of these GB lakes, 366 lake basins have chlorophyll data from the growing season (April to September), with 310 basins having chlorophyll data from at least 3 months in a single growing season.

NI Lakes

DARD provided fortnightly chemistry and phytoplankton monitoring data from 4 lakes (Melvin, Brantry, White, Macnean). Typology data (mean depth and alkalinity) still to collate.

University of Ulster provided summary chemistry and typology data from 20 small (possibly reference) lakes covered in the NILS – 3 seasonal (spring, summer, autumn) samples taken per year

Phytoplankton composition data
Existing data was provided to the project from a number of sources including CEH, University of Liverpool, the Irish EPA and DARD. This existing data was not, however, utilised by the project for a number of reasons:

1) Data did not include biovolume measurements
2) No taxonomic harmonisation between previous counters and with new count data being collected for the project
3) Practical difficulties in entering data into appropriate electronic format

For these reasons it was decided to base the compositional tool development solely on new samples being analysed specifically for the project. The data obtained was counted following a ring-test of samples, a follow-up taxonomic and counting workshop and clear counting guidance (see recommended methods).   Standardised recording forms were constructed to allow straightforward transfer of data to a database.

The new phytoplankton count data was collated together in a single Oracle database using a similar structure to the Environmental Change Network (ECN) database and using Whitton codes (Whitton et al., 1998). A Microsoft Access front-end has also been constructed with basic queries to deliver data summaries. The phytoplankton database includes accompanying lake typology data but no chemistry data.

Of the GB lakes, 384 samples have been counted from 169 lake basins with the vast majority of these samples spanning the months July to September (Fig. 5). Several sites have samples from spring and summer to allow for analysis of seasonal differences in compositional metrics and a few sites have two samples taken from different sampling locations (edge and open water) to allow for analysis of spatial variability in metrics.
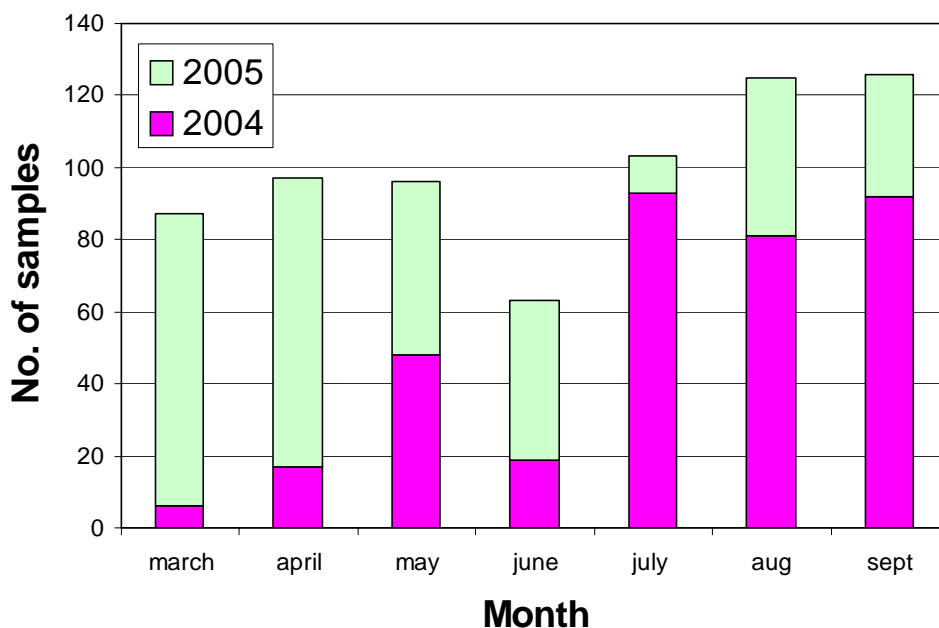
Figure 5   Number of phytoplankton samples counted by month

Table 4   Number of lakes in the UK phytoplankton database classified according to GB depth and alkalinity classes

	LA	MA	HA	Marl	P	Total
Deep	32	36	36	5	10	119
Shallow	10	8	29	1	0	48
Total	42	44	65	6	10	167

Data gaps

Chemistry and chlorophyll
Lacking data from HA and MA deep lakes
Lacking HA and MA very shallow reference lakes

SEPA: Mean or maximum depth data from several sites
        Colour data (Hazen units) from all sites
        Regular (monthly) chemistry monitoring of chlorophyll and nutrients absent from many sites – particularly reference sites

NI: raw data from NILS
        Alkalinity data for DARD four detailed lakes

Phytoplankton
- Data from very shallow LA, MA and peaty lakes limited (Table X: old GB typology shallow = very shallow IC typology)
- Need to analyse by new GB depth types
- Reference lake coverage by type – still to check

4.   PHYTOPLANKTON SAMPLE COLLECTION AND ANALYSIS

Chlorophyll
Sampling   Open water, integrated sample or bottle on rope thrown from edge – by outflow, pier
Volume of water – dependent on phytoplankton abundance – 1 litre generally sufficient, but may need more in very nutrient poor lochs (refer to SEPA data – lots of very low / zero values – UK stands out in intercalibration)
Regular sampling – recommended monthly sampling minimum from uncertainty work

Storage   Cold, alkaline storage for short-term (24 hrs). Frozen if longer.

Analysis   [Cold, alkaline, acetone extraction with grinding. Corrected for degradation products] – to be reviewed

Phytoplankton Composition
Sampling   Open water, integrated sample or bottle on rope thrown from edge – by outflow, pier
Volume of water – dependent on phytoplankton abundance – 1 litre generally sufficient
Regular sampling – recommended monthly during growth season (April to September)
Storage   Preservative – acidified lugol's for short-term storage only (less than 1 year). Recommend additional sample taken and preserved with formaldehyde for longer-term storage

5.   UK GUIDANCE ON COUNTING AND ANALYSIS OF FRESHWATER PHYTOPLANKTON SAMPLES

Laurence Carvalho

Introduction
The following guidance has been developed with reference to the CEN water quality guidance standard for the routine analysis of phytoplankton abundance and composition (CEN, 2004), standard operating procedures developed for Irish lakes (Donnelly, 2004) and lakes in Northern Ireland (Girvan, 2003), phytoplankton methods summarised in Wetzel and Likens (2000) and comments from Bill Brierly (Environment Agency).

Two accepted methods are described for counting phytoplankton:
  1) Using sedimentation chambers on an inverted microscope (Utermöhl technique)
  2) Using a Lund Chamber with a conventional compound microscope

CEN guidance (CEN, 2004) is focused on the use of sedimentation chambers with inverted microscopy, although much of the guidance is applicable to other counting methods. The use of Lund Chambers with conventional compound microscopes is detailed in Annex E of the CEN guidance document.

The CEN guidance does not explicitly state that one method is more suitable than another, although implicit in its focus on sedimentation chambers, is that these are more widely adopted and accepted. CEN guidance does describe a number of advantages and disadvantages for both methods. The main advantage of the sedimentation chamber is that their use may circumvent the need for an initial sample concentration step (unless algal densities are low), reducing the errors associated with concentrating samples. Sedimentation chambers are also closed during analysis and so evaporation does not occur during counting, whereas evaporation can occur from the open ends of the Lund Chamber during counting; the effect of this needs to be minimised by sufficiently quick counting or counting a smaller number of fields in several re-fills of the Lund Chamber. The other advantage of sedimentation chambers is that there should be no size bias with counts of both small and large algae, whereas very large colonial algae can be restricted entering Lund Chambers through the bore of pipette and/or the depth of the cell.

The advantage of using a Lund Chamber is that they can be viewed using objectives of up to x40 magnification on conventional compound microscopes, although these still require microscope objectives with long working distances. The other advantage of using a Lund Chamber is that it is quick to set up with a settling time of 2-5 minutes, although a prior concentration step is usually required (CEN, 2004: Annex E.4). This compares favourably with 4-24 hours using sedimentation chambers varying between 1 to 6 cm in depth (CEN, 2004: Section 6.5). CEN (2004) also highlights that because of the shallow nature of a Lund Chamber, random distribution of phytoplankton cells is more likely than with the use of deeper sedimentation chambers. Clumping around the edge is more common in sedimentation chambers, making random counting methods less appropriate.

It is unlikely that many counters will be able to switch from one counting chamber to another as each requires a different microscope, so either chamber is considered acceptable.

Sample preparation and Filling
Sample preparation and filling of the chambers should follow CEN (2004). To promote random distribution of cells in counting chambers, it is very important that samples and chambers are first allowed to acclimatise to room temperature over a period of 12 hours or so. Just before

filling chambers, samples should be well mixed through <u>gentle</u> shaking (rolling and turning upside down) for at least 1 minute.

Sedimentation Chambers (Utermöhl technique)
The description below is modified from Donnelly (2004), incorporating aspects of CEN (2004).

1. Using a wide-bore pipette, or pouring into a measuring cylinder if dilution is required, dispense the temperature-acclimatized, well-mixed sample into the sedimentation chamber.
2. Fill the chamber completely in one instance, then slide a thick cover glass over the top of the chamber to close it, making sure you avoid any air bubbles.
3. Allow contents to settle on a flat surface for at least 4 hours per cm height of chamber before examination. Usually 16 hours is sufficient (CEN, 2004).
4. After settling, place carefully on microscope stage.

Lund Chambers
The description below is modified from CEN (2004: Annex E.4).

1. Place a coverslip diagonally across the rim of the chamber
2. Using a Pasteur/large-bore pipette, without a pipette bulb, dispense very carefully by capillarity, the temperature-acclimatized, well-mixed sample into the chamber from one of the open ends. Slide the coverslip into position and fill completely ensuring no air bubbles are trapped. When full the coverslip should fit tightly, not slipping if pushed.
3. Take care not to overfill - as removing excess liquid with a pipette or tissue will interfere with cell distribution. Overfilled Lund cells are better emptied and re-filled.
4. Place chamber on the microscope stage and allow contents to settle for 5 minutes (with the illumination off to reduce evaporation).

Counting Procedure

1. Under low magnification (x4 or x10 objectives) check that the phytoplankton appear randomly distributed (e.g. large forms are not concentrated near edges of counting chamber), scan the chamber, and make a list of the dominant taxa – in particular noting large algae that may be rarer and require counting under low magnification (x10 objective)
2. Under high power (x40 or x50 objectives), check that the abundance of the phytoplankton cells in the field of view is neither too over-crowded or too sparse (if so, adjust sub-sample accordingly). Aim to have around 3-5 algal units per field of view

Counts of all taxa at high magnification
3. It is recommended that random fields of view are counted, counting a minimum of 100 fields of view. For sedimentation chambers, transect counting across the widest part of the chamber can be carried out if there is a suspicion of clumping around the edges, If clumping is very obvious, chambers should be re-filled.
4. Count all live cells, filaments or colonies of identified plankton per field until 100 fields of view have been observed, ideally >400 units (cells, filaments, or colonies) should be counted in total. If cells have lost >50% of their cell contents they should be considered dead and not counted. Note that for some diatom species, such as *Rhizosolenia*, cell contents can only take up a small proportion of the frustule and should not be considered dead (cf. Fig 10h, Cox, 1996).
5. For cells/colonies/filaments that cross the edges of the counting field apply a consistent rule as to whether it is included in the count (e.g. count cells crossing left and bottom

boundaries only - see Fig. 2 in CEN (2004) guidance). Note guidance below on estimating biovolume of filaments or colonies that are not entirely in the counting field.

6. If counting using transects in sedimentation chambers, continue until a full transect has been completed and >400 units (cells, filaments, or colonies) have been counted. Several transects may be required and chambers can be turned between transects so new areas can be counted.

7. If 100 individual units (cells, filaments or colonies) of an individual taxon have been reached, then counting can be stopped for that specific taxon – but it is very important to note down the number of fields of view counted for this specific taxon (including fields of view where absent). If transect counting, full transects should always be completed before counting of an individual taxon is stopped – total transect length should be noted.

Counts of larger taxa at low magnification

8. Large species that are identifiable at lower magnification are often uncommon under high magnification, but can contribute proportionally more to total biovolume. For this reason, counts of large taxa should also be carried out at low magnification (e.g. x10 objective lens) to ensure sufficient numbers are observed. Whole chamber counts should be carried out.

Biovolume measurements

9. To estimate biovolumes, it is important to measure linear dimensions of at least three individual of all taxa observed in the sample. For taxa of more variable size, at least 10 individuals should be measured to estimate mean dimensions. For some species with external skeletons much larger than cell contents, e.g. *Dinobryon*, *Rhizosolenia*, etc. the dimensions of the organic cell contents should be measured, not the external skeleton dimensions.

10. For filamentous taxa, filament lengths should be measured for <u>all</u> filaments observed. Filament width/diameter is normally relatively fixed and generally only needs to be measured once. Only measure the filament length that is contained within the counting field. **Do not measure the whole filament length if it extends outside the counting field**

11. For colonial taxa count cell numbers and multiply by mean cell dimensions (often single measure of dimensions needed). If the colony is very large or cells are very small, mean cell numbers may have to be estimated. This is best done by estimating cell numbers in a more restricted area of the colony and estimating how many similar areas are contained within the counting field. Remember to take into account in estimates colony depth and hidden cells. **Do not measure the whole colony volume if it extends outside the counting field.**

12. Use representative formulae to estimate biovolume as illustrated in Hillebrand et al (1999) or Wetzel and Likens (2000: Figure 10.9). Check biovolume estimate with published biovolumes in spreadsheets provided. If biovolume estimates are very different with published literature for many species, check the calibration of your microscope.

Recording data

13. Make a log of all results on counting sheets (copies to be sent with results to Sian) and input into the standard spreadsheet provided to calculate cells/ml and biovolume/ml for individual taxa and main phyla

14. When counts are based on low magnification observations of the whole chamber enter the equivalent fields of view in the spreadsheet (= total area of chamber / area of field of view)

15. If some identifications were uncertain these should be flagged on the spreadsheet

16. Produce a summary count spreadsheet for inputting into the phytoplankton database by using an auto-filter (Data/Filter) on the "count" column to remove rows where the count was zero (filter for "NonBlanks") and copy to the separate "Species list" worksheet
17. Send all completed spreadsheets to Sian and Laurence.

Identification and Coding
The standard flora for identification is the Freshwater Algal Flora of the British Isles (Whitton et al., 2003) and use of the blue-green and green algae CD-ROMs produced for the Environment Agency. Please record species codes noted in the Whitton et al. (2003) flora on the recording sheet – please also note old codes (in brackets) where appropriate as currently the phytoplankton database is using the old Whitton et al. (1998) codes.

Identification should be carried out to the highest possible taxonomic level, although for monitoring purposes the genus level is often sufficient for placing taxa within a functional group. The exceptions to this are *Peridinium*, *Staurastrum* and certain diatom (*Aulacoseira*, *Cyclotella*, *Fragilaria*, *Stephanodiscus*, *Synedra* and *Tabellaria*) and cyanobacteria (*Anabaena*, *Microcystis*, *Oscillatoria* and *Planktothrix*) genera which, if possible, should be identified to species level. For centric diatoms this may only be possible if dead cells are visible, for which specialist floras are currently required (series by Krammer & Lange-Bertalot), although *Aulacoseira* species can sometimes be identified from 'live' material if characteristic spines are present (Cox, 1996)

Photosynthetic picoplankton are not distinguishable from non-photosynthetic prokaryotes in Lugol's-preserved samples (Brian Whitton, pers. comm.) and are not consistently counted across Europe (In Finland counted "when abundant" Liisa Lepisto, pers. comm.). It has yet to be decided what, if anything, can be concluded from picoplankton counts, but for the time being it is recommended that picoplankton <u>are</u> routinely counted and measured to include in the total biovolume estimates. Small unidentified cells of <2 µm size without a flagella or obvious cell structures should be recorded as prokaryotic picoplankton (code 90000002) and those with flagella are recorded as small unidentified flagellates or eukaryotic picoplankton (code 90000001)

If possible, for all unidentified taxa that are relatively abundant, digital/photographic images should be taken for circulation amongst counting teams and storage in an image database associated with the phytoplankton database.

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