UKTAG Lake Assessment Method

Phytoplankton

Phytoplankton Lake Assessment Tool with Uncertainty Module (PLUTO)

by

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

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Health and safety statement

WARNING. Working in or around water is inherently dangerous; persons using this standard should be familiar with normal laboratory and field practice. This published monitoring system does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory guidelines.

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.

Erratum

Equation 5 on page 12 should read as follows:

$$PTI_{Ref} = 0.028 \times \log_{10} MEI + 0.498 \qquad \textbf{Equation 5}$$

UKTAG Guide to Phytoplankton in Lakes
Phytoplankton Classification with Uncertainty Tool (PLUTO)

1 Introduction

This classification method enables the assessment of phytoplankton in lakes according to the requirements of the Water Framework Directive (WFD). It encompasses phytoplankton abundance, taxonomic composition and cyanobacterial bloom intensity. It replaces the phytoplankton classification tool used for the classifications in the first river basin planning cycle. This method is known as PLUTO; Phyto**PL**ankton Classification with **U**ncertainty **TO**ol.

1.1 Metrics

The classification comprises three metrics which are assessed separately then combined to provide an overall classification;

- **Phytoplankton abundance**, measured using chlorophyll *a*.
- **Phytoplankton species composition**, assessed using a metric called the Plankton Trophic Index (PTI), derived from biovolume of taxa present in the late summer.
- **Bloom intensity**, assessed from the biovolume of cyanobacteria

Ecological Quality Ratios (EQRs) are derived from each of the three metrics, based on observed data and predicted reference (expected) values. The values are then normalised so they use the same scale and finally combined by averaging to provide an overall EQR representing an ecological status class; High, Good, Moderate, Poor or Bad.

1.2 Environmental pressures to which the method is sensitive

This assessment method is based on the impact of eutrophication. It is primarily indicating response to nutrients, particularly phosphorus. However, the combined metric assesses the status of the phytoplankton which can be influenced by other factors such as grazing by zooplankton, flushing rates and nitrate limitation. These may influence the overall classification result.

1.3 Geographic application

This assessment method is appropriate for all lakes, natural and modified which occur in the UK and which are assessed in accordance with the Water Framework Directive.

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. phytoplankton, macrophytes) to ensure similar levels are set across all countries. The process involved some adjustments of class boundary values for many of the classification tools and this process has influenced class boundaries used in the PLUTO method. 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 focussed on the EQRs which define the class boundaries between High and Good and Good and Moderate, the H/G and G/M class boundaries respectively.

2 Data collection

2.1 Sample collection – location, frequency, sampling period and sample volume

Samples need to be collected for analysis of chlorophyll *a* content and measurement of species composition and bio-volume. If not already known, information should also be obtained on the alkalinity and water colour of the lake which is needed to derive reference conditions (see section 2.3).

The following section defines the way the samples should be collected and subsequently analysed to obtain data on both chlorophyll *a* and phytoplankton species composition.

2.1.1 Location

Samples must be representative of open water conditions in the lake being studied. Samples can be collected directly from the open water in the deepest part of a lake, using a boat, or can be collected from the lake shore or a suitably located pier or jetty. If lake shore samples are used these should, if at all possible, be collected at or close to the lake outflow. Care must be taken to ensure samples are not contaminated with benthic material. Samples taken from a boat on the open water should be taken sub-surface (30cm below the surface) or from an integrated sample of the epilimnion.

Lake shore samples should be collected using a bottle on the end of a long pole or a weighted bottle attached to a float (30cm from the bottle) on a long rope. The bottle and float is thrown as far into the lake as possible (15m is possible), the bottle sinks 30cm below the water surface to fill.

Samples should not be collected close to inflowing watercourses or potential pollution sources such as drains or sewer pipes.

All samples for chlorophyll and species composition should be collected from the same location and ideally when species composition samples are collected, they are collected at the same time as the chlorophyll samples.

2.1.2 Frequency

The frequency and total number of samples required differs according to the parameter being assessed.

Chlorophyll *a* – samples for chlorophyll *a* analysis should be collected at monthly intervals from January to December, giving a total of 12 samples per year. This encompasses the full extent of the algal growing season in UK lakes, some of which can have high phytoplankton biomass in the winter.

It is important to take samples at even intervals throughout the year to ensure proper representation of the natural seasonal variation in phytoplankton biomass.

Ideally, the monitoring should be continued over three full years, such that 36 chlorophyll samples are collected in total.

Species composition – these samples should be collected at monthly intervals in the period from July to September. These samples represent the late summer phytoplankton

composition which is the period when the maximum impact from eutrophication is likely to be seen. Samples taken outside of this period should not be used to determine the PTI metric.

2.1.3 Sample volume

There is not a precise volume of sample required for either chlorophyll or species composition, as the final analysis depends on the concentration of chlorophyll or phytoplankton within each sample. This will differ between lakes and across seasons. The following is a recommendation only;

Chlorophyll *a* – A minimum volume of 0.5L is recommended. 1L samples are recommended from lakes where the water is very clear.

Species composition - A minimum volume of 0.5L is recommended. 1L samples are recommended from very clear lakes.

2.2 Sample preservation and analysis

The samples collected for chlorophyll require a chemical analysis to extract and measure the pigment content. Samples taken for taxonomic composition need to be preserved and then inspected using an inverted microscope to indentify, enumerate and measure the volume of the species present. The following provides recommendations on how this should be done.

Chlorophyll *a* – these samples need to undergo chemical analysis to extract and measure chlorophyll *a* pigment content. This needs to be done in an analytical chemistry laboratory. For most chemical analysis methods the chlorophyll needs to be extracted from the sample by filtering a known volume of sample onto a glass fibre filter paper, then wrapping and refrigerating this until such time as the chlorophyll pigment can be extracted and measured. The pigment will still degrade even if refrigerated, so filters should not be held for more than a few days before being analysed. Ideally, the sample should be filtered at the time of collection.

If samples cannot be filtered at the time of collection they should be kept refrigerated and dark and analysed as soon as possible after collection.

Species composition – these samples must be preserved and assessed as follows;

Sample preservation. Each sample should be preserved using Lugol's iodine solution. Preservation should take place at the time of sample collection by adding sufficient Lugol's Iodine to the sample to turn it the colour of brandy or straw.

Species composition and biovolume. These assessments need to be carried out on the preserved samples using standard methods. Accompanying this document is a spreadsheet (PPTool_v02.02.xls) used by the UK environment agencies which allows phytoplankton species to be recorded and their biovolumes calculated. Using this spreadsheet will ensure the correct geometry is used for determining biovolume of each taxa. It also enables a simple export of the count data in spreadsheet form for use in the PLUTO method. Also accompanying this document is a guidance document on the enumeration of phytoplankton using an inverted microscope. It is written for the benefit of the UK environment agencies but is based on standard methods and should be followed as far as possible. It is called "Guidance_phytoplanton counting_Feb2014"

Phytoplankton species composition assessment is not advised on samples which have been poorly preserved or are degraded. This might be indicated by no observable colouration from the Lugols iodine, aquatic mould growing in the samples, or evidence of living material when observed through a microscope. Heavy contamination of the sample by benthic material will mask planktonic phytoplankton taxa; benthic taxa do not form part of the classification tool.

2.3 Other data requirements

In addition to chlorophyll and phytoplankton species composition other information is necessary to enable the PLUTO assessment method to be carried out. These data enable the prediction of reference (expected) conditions which are needed to calculate the EQRs. These data, referred to as *predictor variables,* represent the reference conditions for the lake and should not vary with time. They also allow the lake to be assigned to a type (see below).

2.3.1 Alkalinity

A subset of the sample collected for chlorophyll should be analysed for alkalinity, measured in mEq/L. The average alkalinity from a minimum of 12 samples should be used. In some lakes alkalinity fluctuates seasonally so it is important that the average value represents the full year.

Note: This method uses alkalinity expressed as milliequivalents (mEq) per litre. Alkalinity is commonly reported as mg/L $CaCO_3$ (strictly speaking this is carbonate alkalinity). An alkalinity value in mg/L $CaCO_3$ can be converted to mEq/l by multiplying by 20 and dividing by 1000.

2.3.2 Water colour

A subset of the sample collected for chlorophyll should be analysed for colour, measured in Hazen units, mgPt/L. The average colour value from a minimum of 12 samples should be used.

2.3.3 Mean lake depth

Measured in meters.

2.4 Minimum data requirements

The data requirements for application of the PLUTO classification tool have some flexibility. The UK Environment Agencies use, where possible, three years worth of data for each lake. Classifications can be determined using fewer data points, although with fewer data the interpretation of results can be more difficult and there are certain minimum data requirements needed to calculate statistical confidence of the results.

Chlorophyll classifications are ideally carried out using monthly data from a three year period, giving 36 samples, taken at monthly intervals. The minimum data requirement is 6 data points, with at least one from each quarter in a year.

If samples are not taken at evenly spaced intervals resulting in more than one sample in a month, then chlorophyll concentrations should be averaged by month, prior to calculating the overall average.

For species composition, three samples collected at the required times each year over a three year period are ideal. The minimum which should be used is one sample from each year over three years.

If there are no taxonomic samples it is possible to estimate the status of a lake from the chlorophyll concentration, however this is not an application of the full classification method and the resulting classification should be considered to be uncertain.

2.5 Lake "typology"

To apply the classification method, lakes need to be assigned to "types" based on their mean depth, alkalinity and water colour (see table 1) although PLUTO also uses lake specific predictor variables for parts of the calculation.

However, if lake-specific predictor variables are not available then the types can be used to provide indicative values.

Table 1: Combinations of alkalinity, mean depth and colour defining the lake "types" found across the UK

Lake Type	Alkalinity, mEq/L	Mean Depth, m	Humic lakes, (colour as Hazen units), mgPt/L
High alkalinity, deep	>1.0	>15.0	<30
High alkalinity, shallow	>1.0	3.0-15.0	<30
High alkalinity, shallow, humic	>1.0	3.0-15.0	>30
High alkalinity, very shallow	>1.0	<3.0	<30
High alkalinity, very shallow, humic	>1.0	<3.0	>30
Moderate alkalinity, deep	0.2-1.0	>15.0	<30
Moderate alkalinity, deep, humic	0.2-1.0	>15.0	>30
Moderate alkalinity, shallow	0.2-1.0	3.0-15.0	<30
Moderate alkalinity, shallow, humic	0.2-1.0	3.0-15.0	>30
Moderate alkalinity, very shallow	0.2-1.0	<3.0	<30
Moderate alkalinity, very shallow, humic	0.2-1.0	<3.0	>30
Low alkalinity, deep	<0.2	>15.0	<30
Low alkalinity, deep, humic	<0.2	>15.0	>30
Low alkalinity, shallow	<0.2	3.0-15.0	<30
Low alkalinity, shallow, humic	<0.2	3.0-15.0	>30
Low alkalinity, very shallow	<0.2	<3.0	<30
Low alkalinity, very shallow, humic	<0.2	<3.0	>30
Marl, shallow	>1.0	3.0-15.0	<30
Marl, shallow, humic	>1.0	3.0-15.0	>30
Marl, very shallow	>1.0	<3.0	<30
Marl, very shallow, humic	>1.0	<3.0	>30

3 Procedures for calculating metric EQRs

EQRs are a ratio of observed and expected data. *Observed values* are taken from samples collected in the field and *expected values* (also referred to as reference values) are predicted from alkalinity, mean depth and colour (the predictor variables), or lake type.

The EQR boundary values for each metric were set independently to reflect different manifestation of impact. As a result they need to be transformed to a uniform scale where boundary values have a standard common value before they can be combined. This process is called normalisation and uses either a linear or polynomial scaling method. The normalised EQR scale enables combination of metrics and the assessment of status class indicated by each metric. The normalised boundary values are as follows;

Status class	Normalised EQR Boundary
High	>0.8
Good	>0.6 <=0.8
Moderate	>0.4 <=0.6
Poor	>0.2 <=0.4
Bad	<=0.2

The following sections outline how the three phytoplankton metric EQRs are calculated. Each section comprises a step by step process allowing the calculation of observed metric values, expected (reference) metric values, calculation of the EQR then “normalisation” of the EQR.

Once the EQRs have been calculated, an assessment of the statistical confidence of these values can be made. This is referred to as Confidence of Class as the procedure allows the calculation of statistical confidence of a given EQR falling into any of the five status classes.

The procedures for calculating the phytoplankton metric EQRs, the overall EQR and the Confidence of Class can be carried out using a calculator and MX Excel. However, the task can be made simpler by using the accompanying spreadsheet calculator (PLUTOsinglesiteV4f.xls). This is an Excel spreadsheet with a series of worksheets for data input and calculation of all components of the classification, including Confidence of Class.

Note that corrections or modifications are likely to be made to the accompanying calculators and guidance documents, and updated over time. It is important to check that the most recent version is being used.

3.1 Calculating EQR for Phytoplankton Biomass – chlorophyll a

3.1.1 Determine lake type

Use measured or modelled data for the lake being assessed to assign it to a lake type. The lake typologies are set out in Table 1.

3.1.2 Calculate observed chlorophyll *a* concentration, *Chl*, ug/L

This is a *geometric mean* value. It is calculated using data from 1-3 full years of data, so is an annual (or N year) mean.

Calculate as follows;

Step 1 Convert each chlorophyll *a* value to $\log_{10}$.

If sampling is not evenly spaced and multiple samples are collected in a month these values need to be averaged before averaging to determine the observed chlorophyll metric. Some judgement can be used; for example, if no samples are collected in May, but two samples are from June, including one at the very start of June, this early June sample could be used as the May sample, in which case you would not average the two June samples first.

Step 2 Calculate the mean of the $\log_{10}$ values, after averaging by month

= mean $\log_{10}$ chl*a*

Step 3 Calculate the geometric mean Chl*a*

= $10^{(\text{mean } \log_{10} \text{ chla})}$

= Observed chlorophyll concentration, ug/L

3.1.3 Calculate expected (reference) chlorophyll *a* concentration, ug/L

Expected (reference) chlorophyll *a* is predicted according to Equation 1, using the predictor variables, alkalinity and mean depth.

$$Chl_{Ref} = 10^{\left(0.223+0.166\times\log10(Alk)+0.684\times\sqrt{1/Depth}\right)}$$

Equation 1

Where

Chl$_{ref}$ = geometric annual mean chlorophyll a concentration (µg/l)

Alk = alkalinity (mEq/l) (minimum value of 0.005)

Depth = mean depth (m) (minimum value of 1.0)

Substitute into Equation 1, alkalinity and mean depth data from the lake being studied to derive an expected chlorophyll concentration. Note that the resulting expected (reference) value is a *geometric* annual mean value.

The expected chlorophyll concentration needs to fall within the type-specific ranges listed in Table 2. If the expected chlorophyll concentration falls outside of the value in Table 2 it needs to be truncated to the upper or lower range limit such that expected values above the upper range limit are reduced to the maximum value of the range limit, expected values less than the lower range limit are increased to the minimum value of the range.

Table 2: Minimum and maximum geometric mean chlorophyll concentrations for all the lake types listed in Table 1. They indicate the ranges within which expected chlorophyll concentrations must fall, or be constrained to if they are outside the range.

Lake Type	Minimum reference (expected) geometric mean chl, ug/L	Maximum reference (expected) geometric mean chl, ug/L
High alkalinity, deep	1.3	6.0
High alkalinity, shallow	2.1	3.1
High alkalinity, shallow, humic	2.1	3.1
High alkalinity, very shallow	5.0	5.9
High alkalinity, very shallow, humic	5.0	5.9
Moderate alkalinity, deep	1.3	6.0
Moderate alkalinity, deep, humic	1.3	6.0
Moderate alkalinity, shallow	2.2	3.1
Moderate alkalinity, shallow, humic	3.1	4.4
Moderate alkalinity, very shallow	1.3	6.0
Moderate alkalinity, very shallow, humic	1.3	6.0
Low alkalinity, deep	1.3	2.2
Low alkalinity, deep, humic	1.3	6.0
Low alkalinity, shallow	1.3	2.2
Low alkalinity, shallow, humic	2.2	3.1
Low alkalinity, very shallow	1.3	6.0
Low alkalinity, very shallow, humic	1.3	6.0
Marl, shallow	1.3	6.0
Marl, shallow, humic	1.3	6.0
Marl, very shallow	1.3	6.0
Marl, very shallow, humic	1.3	6.0

3.1.4 Calculate chlorophyll *a* EQR (EQR_{Chl})

Into Equation 2, substitute the observed chlorophyll concentration, *Chl* (from **3.1.2**) and the calculated expected chlorophyll concentration, Chl_{ref} (from **3.1.3**) to derive the chlorophyll EQR (EQR_{Chl}).

$$EQR_{Chl} = \frac{Chl_{\mathrm{Re}\,f}}{Chl}$$

Equation 2

The result is a ratio which represents the chlorophyll classification for the lake. Table 3 shows the EQR_{Chl} class boundary values for each lake type.

Table 3: The EQRs for chlorophyll which delineate the High/Good, Good/Moderate, Moderate/Poor and Poor/Bad class boundaries for each of the lake types. EQRs exceeding the High/Good boundary value represent High status, those less than the Poor/Bad boundary value represent Bad status.

Lake Type	Chlorophyll EQR (EQR_{Chl}) Boundary values			
	High/Good	Good /Moderate	Moderate /Poor	Poor/Bad
High alkalinity, deep	0.55	0.32	0.16	0.05
High alkalinity, shallow	0.55	0.32	0.16	0.05
High alkalinity, shallow, humic	0.55	0.32	0.16	0.05
High alkalinity, very shallow	0.63	0.30	0.15	0.05
High alkalinity, very shallow, humic	0.63	0.30	0.15	0.05
Moderate alkalinity, deep	0.50	0.33	0.17	0.05
Moderate alkalinity, deep, humic	0.50	0.33	0.17	0.05
Moderate alkalinity, shallow	0.50	0.33	0.17	0.05
Moderate alkalinity, shallow, humic	0.50	0.33	0.17	0.05
Moderate alkalinity, very shallow	0.63	0.30	0.15	0.05
Moderate alkalinity, very shallow, humic	0.63	0.30	0.15	0.05
Low alkalinity, deep	0.64	0.33	0.17	0.05
Low alkalinity, deep, humic	0.64	0.33	0.15	0.05
Low alkalinity, shallow	0.64	0.29	0.15	0.05
Low alkalinity, shallow, humic	0.64	0.29	0.15	0.05
Low alkalinity, very shallow	0.63	0.30	0.15	0.05
Low alkalinity, very shallow, humic	0.63	0.30	0.15	0.05
Marl, shallow	0.55	0.32	0.16	0.05

Marl, shallow, humic	0.55	0.32	0.16	0.05
Marl, very shallow	0.63	0.30	0.15	0.05
Marl, very shallow, humic	0.63	0.30	0.15	0.05
All lake types – normalised EQR boundary values	**0.80**	**0.60**	**0.40**	**0.20**

3.1.5 Normalise the EQR

This process allows the calculation of a class boundary of 0.80 for High/Good, 0.60 for Good/Moderate, 0.40 for Moderate/Poor and 0.20 for Poor/Bad.

Step 1 Identify the typology of the lake in question using Table 1 (**3.1.1**)

Step 2 Note the non-normalised boundary values for that lake type from Table 3. Check the EQR boundary values which fall either side of the calculated EQR_{Chl} and note the lower of these two values (lower boundary). Also record the class that this represents (High, Good, Moderate, Poor or Bad).

e.g. if EQR_{Chl} = 0.41 and the lake type is Moderate Alkalinity Deep, the EQRs which fall either side of this are 0.50 and 0.33 and the lower value is 0.33. The class this EQR_{chl} represents is Good.

For EQRs which represent High status, the upper boundary value is 1. For EQRs which represent Bad status, the lower boundary value is 0.

Step 3 Note the lower *normalised* EQR for the class selected in **3.1.5 Step 2**. See the bottom row of Table 3.

e.g for the Moderate Alkalinity deep lake, the EQR_{chl} of 0.41 represents Good status. The lower normalised EQR for Good status is 0.60.

For normalised EQRs which represent Bad status, the lower boundary value is 0.

Step 4 Note the class width of the non-normalised EQR for the class represented by EQRchl for the lake type in question.

e.g. if EQR_{Chl} = 0.41 and the lake type is Moderate Alkalinity deep, the EQRs which fall either side of this are 0.50 and 0.33 and the class width is 0.50-0.33 = 0.17

For EQRs which represent High status, the upper boundary value is 1. For EQRs which represent Bad status, the lower boundary value is 0.

Step 5 Substitute the EQR_{Chl} calculated from equation 2, plus the boundary EQR value information from **3.1.5 Steps 2-4** into equation 3 to calculate a normalised EQR ($ChlEQR_{norm}$).

$$ChlEQR_{Norm} = \left[\left(\frac{EQR_{Chl} - LowerBoundary}{ClassWidth} \right) \times 0.2 \right] + LowerBoundary_{Norm}$$

Equation 3

Where:

$ChlEQR_{Norm}$	= Normalised EQR
LowerBoundary	= lower un-normalised EQR boundary (**3.1.5 Step 2**)
$LowerBoundary_{Norm}$	= lower normalised EQR boundary of class (**3.1.5 Step 3**)
ClassWidth	= Class width of non-normalised scale (**3.1.5 Step 4**)

Use the normalised EQR to determine the status class of the lake being studied, according to chlorophyll.

If the EQR calculated is exactly the same as a boundary EQR listed in Table 3, simply read the normalised EQR off from the table.

Follow the next two sections to calculate the Taxonomic and Bloom Intensity metrics to produce an overall phytoplankton classification for the lake.

3.2 Calculating EQR for the Taxomonic Metric – Plankton Trophic Index (PTI)

This EQR is based on the phytoplankton species composition and phytoplankton cell biovolume of samples collected from the lake. It combines the biovolume of each taxon and a taxon-specific score (optima) representative of that species' sensitivity or tolerance to elevated phosphorus concentrations. It is calculated as follows;

3.2.1 Determine lake type

Use the lake type determined in step 1 of the chlorophyll a EQR calculation.

3.2.2 Calculate observed PTI

This is carried out for each sample initially, before combining data for multiple samples at the end of the calculation.

Step 1 For each sample, determine the total biovolume for each taxon. It may be necessary to add together biovolumes for a taxon such as microcystis, where biovolume may be determined for both colonies and single cells within the same sample. It is important to carry out this step before further use of the data.

Step 2 Calculate $\log_{10}$ of the biovolume measurement for each taxon

Step 3 For each taxon identified in each sample, assign the correct optima to that taxon. Appendix A contains a list of all the phytoplankton taxa which have been recorded in UK lakes during the development of PLUTO and their associated optima.

Step 4 Calculate observed PTI for each phytoplankton sample by inserting $\log_{10}$ biovolume and optima data into equation 4.

$$PTIobs = \frac{\sum_{j=1}^{n} \log(a_j) s_j}{\sum_{j=1}^{n} \log(a_j)}$$

Equation 4

Where:

a_j = biovolume of *j*th taxon in the sample ($\mu m^3\ ml^{-1}$)

s_j = optimum of *j*th taxon in the sample

3.2.3 Calculate expected (reference) PTI

The expected PTI is predicted from a multiple regression model (equation 5). To calculate expected PTI, substitute values for alkalinity and depth in to equation 5 as shown.

$$PTI_{Ref} = 0.028 \times log_{10}MEI + 0.$$

Equation 5

Where

MEI = Alk/Depth (Morpho Edaphic Index)

Where

Alk = lake mean alkalinity, mEq/L

depth = mean lake depth, m

These data should be the same as those used to determine lake type and the chlorophyll expected value. If data for alkalinity or mean depth are not available, type specific reference PTI values should be used and can be selected from Table 4. The reference PTI for humic lakes is the same as the equivalent for clear lakes so if not listed, use the equivalent clear lake type.

Table 4: Type-specific reference PTI values for UK lakes which should be used if data are not available to calculate a site specific reference PTI value.

Lake Type	Reference PTI
High alkalinity, shallow	0.484
High alkalinity, very shallow	0.5005
Moderate alkalinity, deep	0.4425
Moderate alkalinity, shallow	0.465
Moderate alkalinity, shallow, humic	0.465
Moderate alkalinity, very shallow	0.4835
Low alkalinity, deep	0.420
Low alkalinity, deep, humic	0.420
Low alkalinity, shallow	0.440
Low alkalinity, shallow, humic	0.440
Low alkalinity, very shallow	0.460
Low alkalinity, very shallow, humic	0.460
Marl, shallow	0.489
Marl, very shallow	0.499

3.2.4 Calculate PTI EQR per sample

Calculate the PTI EQR for each phytoplankton sample using the observed PTI (from step 2) and the expected PTI (from step 3) and substituting these values into equation 6.

$$EQR_{PTI} = \left(\frac{PTI_{Obs} - PTI_{Max}}{PTI_{\mathrm{Re}f} - PTI_{Max}} \right)$$

Equation 6

Where:

PTI_{Obs} = Observed PTI

PTI_{Max} = Maximum PTI score (*0.75*). *This is a fixed value used to ensure the EQR is not negative*

PTI_{Ref} = Reference (expected) PTI

3.2.5 Normalise the EQR

To normalise the PTI EQR it is necessary to insert the PTI EQR value into the appropriate quadratic equation for the lake type. The lake type-specific quadratic equations are listed in table 5.

3.2.6 Calculate the lake PTI EQR

Finally, calculate the lake PTI EQR by taking the average of the normalised EQRs from each sample.

Use the normalised EQR to assess the status class of the lake being studied according to PTI.

Follow the next section to calculate the Bloom Intensity metric.

Table 5: PTI EQR boundaries and equations for normalising EQRs for each lake type.

Lake Type	PTI EQR Boundary values				Normalisation Equation
	High/ Good	Good/ Moderate	Moderate/ Poor	Poor/ Bad	
High alkalinity, deep	0.93	0.82	0.70	0.58	$EQR_{norm} = (1.228 \times EQR^2) - (0.0898 \times EQR) - 0.1538$
High alkalinity, shallow	0.93	0.82	0.70	0.58	$EQR_{norm} = (1.228 \times EQR^2) - (0.0898 \times EQR) - 0.1538$
High alkalinity, shallow, humic	0.93	0.82	0.70	0.58	$EQR_{norm} = (1.228 \times EQR^2) - (0.0898 \times EQR) - 0.1538$
High alkalinity, very shallow	0.91	0.80	0.68	0.56	$EQR_{norm} = (1.228 \times EQR^2) - (0.0407 \times EQR) - 0.1551$
High alkalinity, very shallow, humic	0.91	0.80	0.68	0.56	$EQR_{norm} = (1.228 \times EQR^2) - (0.0407 \times EQR) - 0.1551$
Moderate alkalinity, deep	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.1389 \times EQR) - 0.1515$
Moderate alkalinity, deep, humic	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.1389 \times EQR) - 0.1515$
Moderate alkalinity, shallow	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.1389 \times EQR) - 0.1515$
Moderate alkalinity,	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.1389 \times EQR) - 0.1515$

shallow, humic					
Moderate alkalinity, very shallow	0.93	0.82	0.70	0.58	$EQR_{norm} = (1.228 \times EQR^2) - (0.0898 \times EQR) - 0.1538$
Moderate alkalinity, very shallow, humic	0.93	0.82	0.70	0.58	$EQR_{norm} = (1.228 \times EQR^2) - (0.0898 \times EQR) - 0.1538$
Low alkalinity, deep	0.98	0.87	0.75	0.63	$EQR_{norm} = (1.228 \times EQR^2) - (0.2004 \times EQR) - 0.147$
Low alkalinity, deep, humic	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.1389 \times EQR) - 0.1515$
Low alkalinity, shallow	0.98	0.87	0.75	0.63	$EQR_{norm} = (1.228 \times EQR^2) - (0.2004 \times EQR) - 0.147$
Low alkalinity, shallow, humic	0.96	0.85	0.73	0.61	$EQR_{norm} = (1.228 \times EQR^2) - (0.1512 \times EQR) - 0.1508$
Low alkalinity, very shallow	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.1389 \times EQR) - 0.1515$
Low alkalinity, very shallow, humic	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.0898 \times EQR) - 0.1538$
Marl, shallow	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.1389 \times EQR) - 0.1515$
Marl, shallow, humic	0.95	0.84	0.72	0.60	$EQR_{norm} = (1.228 \times EQR^2) - (0.1389 \times EQR) - 0.1515$
Marl, very shallow	0.93	0.82	0.70	0.58	$EQR_{norm} = (1.228 \times EQR^2) - (0.0898 \times EQR) - 0.1538$
Marl, very shallow, humic	0.93	0.82	0.70	0.58	$EQR_{norm} = (1.228 \times EQR^2) - (0.0898 \times EQR) - 0.1538$
All lake types – normalised EQRs	**0.80**	**0.60**	**0.40**	**0.20**	

3.3 Calculating EQR for the Bloom intensity Metric – Cyanobacteria biovolume

This is carried out for each sample initially, before combining data for multiple samples at the end of the calculation.

It is calculated from the total biovolume of all cyanobacteria taxa in the samples collected. Calculate the cyanobacteria EQR as follows;

3.3.1 Calculate observed cyanobacterial biovolume

Step 1 Identify all cyanobacteria in each sample. This can be done using the taxon coding. According to the Whitton coding system, all cyanobacteria have a taxon code starting "01".

Step 2 Convert the biovolume value for each cyanobacterial taxon from $\mu m^3/mL$ to mm^3/L;

$$\mu m^3/mL \div 1{,}000{,}000 = mm^3/L$$

Step 3 Sum the converted cyanobacterial biovolumes for each sample. This gives the observed cyanobacterial biovolume in units of mm^3/L per sample.

3.3.2 Calculate expected cyanobacterial biovolume

Select from the following the value appropriate for the lake type being assessed;

a) 0.01 mm^3/L for High Alkalinity lakes

b) 0.00 mm^3/L for all other lake types

Note that these two expected values cover all lake depths and degree of colour.

3.3.3 Calculate the cyanobacterial EQR

Use Equation 7 to calculate the cyanobacterial EQR for each sample.

$$EQR_{Cyan} = \frac{\log(BV_{Obs} + 0.0001) - \log(BV_{Max} + 0.0001)}{\log(BV_{\mathrm{Re}f} + 0.0001) - \log(BV_{Max} + 0.0001)}$$

Equation 7

Where

BV_{Obs} = total bio-volume of cyanobacteria in each sample (mm^3/L)

BV_{Ref} = expected bio-volume of cyanobacteria ($mm^3\ l^{-1}$)

BV_{Max} = maximum bio-volume (a fixed value taken as 30.0 $mm^3\ l^{-1}$)

If $BV_{Obs} > BV_{Max}$ then EQR_{Cyan} defaults to 0.0

3.3.4 Normalise the cyanobacterial EQR

Use equation 8 to normalise the cyanobacterial EQR for each sample. As with chlorophyll EQR normalisation, choose the class represented by the cyanobacterial EQR, record it's lower boundary value and class width (see Table 6).

$$CyanEQR_{Norm} = \left[\left(\frac{EQR_{Cyan} - LowerBoundary}{ClassWidth} \right) \times 0.2 \right] + LowerBoundary_{Norm}$$

Equation 8

For EQRs which are High, take the class width to be 1-H/G boundary. For EQRs which are Bad, take the lower class boundary to be zero.

Table 6 : The cyanobacterial EQR boundary values for the different lake types.

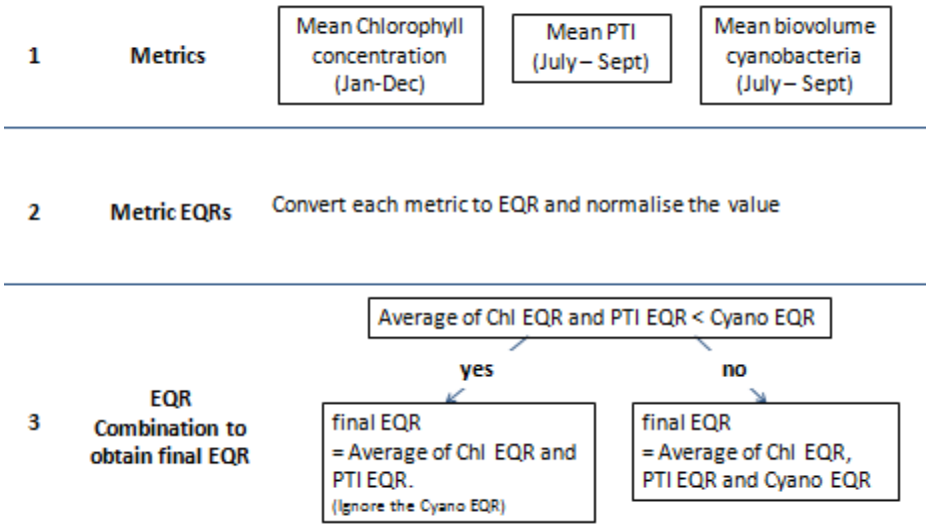
Lake Type	Cyanobacteria EQR (EQR_{Cyan}) Boundary values			
	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
Low alkalinity, Moderate alkalinity and Marl lakes – all depths and colour types	0.47	0.32	0.23	0.13
High alkalinity lakes – all depths and colour types	0.63	0.43	0.34	0.21
All lake types – normalised EQR boundary values	**0.80**	**0.60**	**0.40**	**0.20**

3.3.5 Calculate the lake cyanobacterial EQR

Finally, calculate the lake cyanobacterial EQR by taking the average of the normalised EQRs from each sample.

3.4 Combination of all three metric EQRs to calculate an overall EQR and classification for phytoplankton

The diagram below shows the steps of the process and the final rules for combining EQRs.



3.4.1 Calculate the average of the normalised Chl EQR and the normalised PTI EQR. This gives an interim EQR (*IntEQRnorm*)

3.4.2 If *CyanEQRnorm* < *IntEQRnorm*, calculate the average of all three normalised EQRs (chlorophyll, PTI and Cyanobacteria). Otherwise, ignore the cyanobacterial EQR. This step provides an overall normalised EQR for phytoplankton.

This final step is carried out so that the cyanobacterial EQR is only used to lower a lake status where blooms are likely to occur; it does not increase a lake status in the absence of cyanobacteria for the reason that in some lake types (low alkalinity and humic lakes) cyanobacteria are rare regardless of status.

3.4.3 The overall normalised phytoplankton EQR can then be assessed on the normalised EQR scale, to determine the phytoplankton class where;

Status class	Normalised EQR
High	>0.8
Good	>0.6 <=0.8
Moderate	>0.4 <=0.6
Poor	>0.2 <=0.4
Bad	<=0.2

4 Procedures for calculating statistical confidence in metrics and overall classifications

The assessment of statistical error associated with each phytoplankton metric is calculated using the standard error and is expressed as a "confidence of class", i.e. the statistical confidence we have of any metric (or combined metric) falling into each of the five classes, from High to Bad. This also makes it possible to determine the statistical confidence of the lake classifying as "worse than Good status".

The standard error of each metric is calculated then the confidence of class for each metric calculated using the t-distribution as follows;

$$\text{Confidence of class} = t\left(\frac{EQR_{NormBoundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

Where;

t denotes the cumulative Student t probability distribution (TDIST in Excel). The degrees of freedom is taken from either the number of individual data points contributing to an individual metric EQR, or the data points contributing to the combined metric, estimated using the Welch-Satterthwaite equation.

$EQR_{NormBoundary}$ = the normalised boundary value for which confidence of class is being assessed

EQR_{Norm} = the normalised EQR of the metric (or combined metrics)

$SE(EQR_{Norm})$ = the standard error of the normalised metric EQR (or combined metric EQRs)

For classes High to Bad the confidence of each class is calculated as

Confidence of class High (5)	= $1 - p_5$
Confidence of class Good (4)	= $p_5 - p_4$
Confidence of class Moderate (3)	= $p_4 - p_3$
Confidence of class Poor (2)	= $p_3 - p_2$
Confidence of class Bad (1)	= p_1

The procedures for calculating confidence of class differ for each metric. The following sections outline the procedures for each metric followed by the confidence of class for the

combined metrics. Note that the accompanying spreadsheet, PLUTOsinglesiteV4f.xls also enables calculation of Confidence of Class for each metric and the overall classification.

4.1 Calculating confidence of class for chlorophyll a

It is first necessary to estimate the standard error of the normalised EQR for chlorophyll. This is done following the method of Davey 2009, by determining the upper and lower 95% confidence limits of the observed mean chlorophyll then converting these values to EQRs.

Estimating the standard error of chlorophyll, when the data are collected from one or more full years, is prone to bias because of the natural seasonal fluctuations in chlorophyll. (Carvalho et al., 2006; 2007a; 2012; Phillips 2012) demonstrate that monthly variation is a significant component of variability for chlorophyll a. In addition (Carvalho *et al.* 2006) showed that with regular sampling the true standard error (SE_{str}) of the resulting mean chlorophyll concentration is less than a standard error taken from random sampling (SE_{rand}). Subsequent analysis of data from 8 lakes with at least fortnightly sampling frequencies showed that for chlorophyll a data transformed by Log_{10} the SE_{rand} could be reduced using the following function (equation 9).

$$SE_{Str} = SE_{Rand} \times 1.0293 \times Sy^{-0.2379} \qquad \textbf{Equation 9}$$

Where

S_y = number of regular samples per year

SE_{str} = the corrected standard error

SE_{rand} = the standard error calculated from the raw data

As a result, datasets which are composed of one or more full years worth of data collected at regular, monthly intervals, can have their standard error lowered by applying this correction factor.

Calculate the upper and lower 95% confidence limits (U95 and L95) using the inverse of the Student t-distribution as follows;

$L95 = 10^{(LChl - TINV \times SEStr)}$

$U95 = 10^{(LChl + TINV \times SEStr)}$

Where *LChl* = annual mean of Log_{10} Chl

SE_{Str} = corrected standard error (by application of equation 9)

TINV = inverse of the t-distribution where P=0.05 for N-1 degrees of freedom (calculate degrees of freedom from the number of samples contributing to LChl)

Use these values to calculate EQRs for L95 and U95 as follows;

$$L95EQR = \frac{Chl_{Ref}}{U95} \quad \text{and} \quad U95EQR = \frac{Chl_{Ref}}{L95}$$

Where

Chl$_{ref}$ = the reference (expected) chlorophyll concentration (geometric mean) used to calculate the chlorophyll EQR.

Normalise these EQRs according to the normalisation equation used to calculate the chlorophyll EQR (based on equation 3). The U95EQR and L95EQRs are assigned to status classes based on the boundary values of the lake type in question. The lower boundary value, class width and lower normalised boundary values are determined based on these;

$$U95EQR_{Norm} = \left[\left(\frac{U95EQR - LowerBoundary}{ClassWidth}\right) \times 0.2\right] + LowerBoundary_{Norm}$$

Where

LowerBoundary = the chlorophyll boundary value below the U95 EQR

ClassWidth = the width of the class into which the U95EQR falls

LowerBoundary$_{norm}$ = the lower normalised boundary represented by the class into which the U95EQR falls.

and

$$L95EQR_{Norm} = \left[\left(\frac{L95EQR - LowerBoundary}{ClassWidth}\right) \times 0.2\right] + LowerBoundary_{Norm}$$

Where

LowerBoundary = the chlorophyll boundary value below the L95 EQR

ClassWidth = the width of the class into which the L95EQR falls

LowerBoundary$_{norm}$ = the lower normalised boundary represented by the class into which the L95EQR falls.

Having determined the normalised L95 and U95EQRs, calculate the standard error as follows;

$$SE(EQR_{Norm}) = \frac{U95EQR_{Norm} - L95EQR_{Norm}}{2 \times TINV}$$

Finally, calculate the confidence of class for chlorophyll using the t-distribution where the probability (p) of the normalised chlorophyll EQR being worse than a given class boundary is given by;

$$p = t\left(\frac{EQR_{Boundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

Where

t = the Student t probability distribution (TDIST in excel, with degrees of freedom determined from 1- the number of samples contributing to the chlorophyll EQR, and using a 1-tailed test)

$EQR_{Boundary}$ = the class boundary against which the normalised chlorophyll EQR is being assessed

EQR_{Norm} and $SE(EQR_{Norm})$ are the normalised chlorophyll EQR and the standard error of the normalised EQR respectively

4.2 Calculating confidence of class for PTI and Cyanobacteria

As the EQR is calculated for each individual sample, calculating standard error for these metrics is a relatively simple process.

For each metric (PTI or cyanobacteria), calculate the mean, standard deviation and standard error from the normalised EQRs contributing to the metric. Ideally there will be 9 EQRs for each metric, that is three samples from each of three years, although the value maybe less according to data availability. Fewer than 3 EQRs for either metric will give very unreliable estimates of confidence of class.

Calculate the confidence of class for each metric using the t-distribution where the probability (p) of the normalised chlorophyll EQR being worse than a given class boundary is given by;

$$p = t\left(\frac{EQR_{Boundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

Where

t = the Student t probability distribution (TDIST in excel, with degrees of freedom determined from 1- the number of samples contributing to the PTI or Cyanobacteria EQR, and using a 1-tailed test)

$EQR_{Boundary}$ = the class boundary against which the normalised PTI or cyanobacteria EQR is being assessed

EQR_{Norm} and $SE(EQR_{Norm})$ are the normalised PTI or Cyanobacteria EQRs and the standard errors of the normalised EQRs respectively

4.3 Calculate confidence of class for the combined metrics

This will be calculated for chlorophyll and PTI or chlorophyll, PTI and cyanobacteria, depending on whether or not cyanobacteria was excluded from the final combined metric (see section 3.4.2).

First determine the combined standard error of the two or three normalised EQRs;

$$SE(EQR_{Phyto}) = \frac{\sqrt{SE(ChlEQR_{norm})^2 + SE(PTIEQR_{norm})^2}}{2}$$

Or

$$SE(EQR_{Phyto}) = \frac{\sqrt{SE(ChlEQR_{norm})^2 + SE(PTIEQR_{norm})^2 + SE(CyanoEQR_{norm})^2}}{3}$$

Where

$SE(ChlEQR_{norm})$ = standard error of the normalised chlorophyll EQR

$SE(PTIEQR_{norm})$ = standard error of the normalised PTI EQR

$SE(CyanoEQR_{norm})$ = standard error of the normalised cyanobacteria EQR

The confidence of overall class is determined using the same formula as for the individual metrics;

$$p = t\left(\frac{EQR_{Boundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

However, to enable use of the t-distribution, it is necessary to know the degrees of freedom of the combined metrics. This can be calculated using the Welch-Satterthwaite formula;

$$n = \frac{\{\sum_{a=1}^{a} k_i \times s_i^2\}^2}{\sum_{a=1}^{a} \{(k_i \times s_i^2)^2 / n_i\}}$$

Where

n_i is degrees of freedom of each metric

s_i^2 is estimated standard deviation of each metric

k is a weight in the sum derived as 1/a variables (1/2 or 1/3 for 2 or 3 variables respectively), i.e. for chlorophyll and PTI metrics(2 variables) or chlorophyll, PTI and cyanobacteria metrics (3 variables).

For calculating degrees of freedom where chlorophyll and PTI EQRs are used to calculate the overall EQR, the following equation should be used

$$n = \frac{\left\{\frac{sChlEQRnorm^2}{2} + \frac{sPTIEQRnorm^2}{2}\right\}^2}{\left\{\frac{\left(sChlEQRnorm^2/2\right)^2}{(Nchl-1)} + \frac{\left(sPTIEQRnorm^2/2\right)^2}{(NPTI-1)}\right\}}$$

Where

sChlEQRnorm = the standard deviation of the normalised chlorophyll EQRs

sPTIEQRnorm = the standard deviation of the normalised PTIEQRs

NChl = the number of samples used to calculate mean $\log_{10}$ chlorophyll

NPTI = the number of samples used to calculate PTI

For calculating degrees of freedom where chlorophyll, PTI and cyanobacterial EQRs are used to calculate the overall EQR, the following equation should be used

$$n = \frac{\left\{\frac{sChlEQRnorm^2}{3} + \frac{sPTIEQRnorm^2}{3} + \frac{sCyanEQRnorm^2}{3}\right\}^2}{\left\{\frac{\left(sChlEQRnorm^2/3\right)^2}{(Nchl-1)} + \frac{\left(sPTIEQRnorm^2/3\right)^2}{(NPTI-1)} + \frac{\left(sCyanEQRnorm^2/3\right)^2}{(NCyano-1)}\right\}}$$

Where

sChlEQRnorm = the standard deviation of the normalised chlorophyll EQRs (calculated as standard deviation = SE x SQRT(N))

sPTIEQRnorm = the standard deviation of the normalised PTIEQRs

sCyanEQRnorm = the standard deviation of the normalised cyanobacterial EQR

NChl = the number of samples used to calculate mean $\log_{10}$ chlorophyll

NPTI = the number of samples used to calculate PTI

NCyano = the number of samples used to calculate the cyanobacterial metric (should be the same number as for PTI)

Finally, calculate the confidence of class for the combined metric using the t-distribution where the probability (p) of the normalised combined EQR being worse than a given class boundary is given by;

$$p = t\left(\frac{EQR_{Boundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

Where

t = the Student t probability distribution (TDIST in excel, with degrees of freedom determined from the Welsh-Satterthwaite estimate, and using a 1-tailed test)

$EQR_{Boundary}$ = the class boundary against which the normalised combined EQR is being assessed

EQR_{Norm} and $SE(EQR_{Norm})$ are the normalised combined EQRs and the standard errors of the normalised combined EQRs respectively

5 Worked example

5.1 Calculating EQR for Phytoplankton Biomass – chlorophyll a

5.1.1 Determine lake type

A dataset from Loweswater is used as an example, consisting of three years of chlorophyll data collected at monthly intervals, plus 7 phytoplankton samples taken over the same three years.

Loweswater; Mean alkalinity = 0.198 mEq/L, mean depth = 8.37m.

Lake type = Low alkalinity, shallow

5.1.2 Calculate observed chlorophyll a concentration, ug/L

36 samples collected at monthly intervals from January 2010 to December 2012.

Any values which are reported as "less than", i.e. "<" are halved.

Mean $\log_{10}$ chl = 0.67968

Geometric mean *Chl* = $10^{(\text{mean } \log_{10} \text{chl})}$

= $10^{(0.67968)}$ = 4.78579 ug/L (observed annual geometric mean chlorophyll concentration

5.1.3 Calculate expected (reference) chlorophyll *a* concentration, ug/L

Expected (reference) chlorophyll *a* is predicted according to Equation 1, using the predictor variables, alkalinity and mean depth.

$$Chl_{Ref} = 10^{\left(0.223+0.166\times\log(Alk)+0.684\times\sqrt{1/Depth}\right)} \qquad \textbf{Equation 1}$$

Alk = 0.198 mEq/L, Depth = 8.37m

Chl_{ref} = $10^{(0.223 + (0.166\log(0.198) + 0.684 \times \text{Sqrt}(1/\text{Depth}))}$

Chl_{ref} = 2.2013 ug/L

For a Low alkalinity, shallow lake, the range of expected chlorophyll values is 1.3 to 2.2 ug/L (see Table 2). The calculated expected chlorophyll concentration is slightly higher than the upper range for this lake type, so is truncated to 2.2ug/L, the value used to calculate the EQR.

5.1.4 Calculate chlorophyll a EQR

Into Equation 2, substitute the observed chlorophyll concentration (4.78279ug/L) and the calculated expected chlorophyll concentration (2.2ug/L) to derive the chlorophyll EQR (EQR_{Chl}).

$$EQR_{Chl} = \frac{Chl_{Ref}}{Chl} \qquad \textbf{Equation 2}$$

EQR_{Chl} = 2.2/4.78279

=0.460

5.1.5 Normalise the EQR

From Table 3, note the boundaries for a low alkalinity, shallow lake. These are;

Lake Type	Chlorophyll EQR (EQR_{Chl}) Boundary values			
	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
Low alkalinity, shallow	0.64	0.29	0.15	0.05
All lake types – normalised EQR boundary values	**0.8**	**0.6**	**0.4**	**0.2**

Note the non-normalised boundary values for that lake type from Table 3. Check the EQR boundary values which fall either side of the calculated EQR_{Chl} and note the lower of these two values (lower boundary). Also record the class that this represents (High, Good, Moderate, Poor or Bad).

For Loweswater, the EQR of 0.46 falls between the High/Good and Good/Moderate boundaries, and represents Good status. The lower boundary value is 0.29. The lower normalised boundary value for Good status is 0.6. The class width of the non-normalised boundaries is 0.64 – 0.29 = 0.35

Substitute these values into Equation 3 as follows;

$$ChlEQR_{Norm} = \left[\left(\frac{EQR_{Chl} - LowerBoundary}{ClassWidth}\right) \times 0.2\right] + LowerBoundary_{Norm}$$ **Equation 3**

$ChlEQR_{norm}$ =(((0.459982 – 0.29)/0.35)x0.2) + 0.6

= 0.697

5.2 Calculating EQR for the Taxomonic Metric – Plankton Trophic Index (PTI)

Table 7 shows algal counts from one sample taken from Loweswater on 13/09/2010. It shows the information necessary to perform the PTI calculation for each sample collected.

Table 7: Phytoplankton count data from one sample from Loweswater, collected 13/09/2010, showing taxon optima, and calculated values of a_j and s_j (see equation 4) for each taxon

Species Code	Species Name	Biovolume, um^3/mL	Biovolume, um^3/mL – duplicate records summed	Optima	Log_{10}Biovol x Optima	Log_{10} Biovol
01020000	Anabaena	52,104	84,306	0.568	2.679185067	4.716875
01020000	Anabaena	32,201		0.568		
01530000	Oscillatoria	30,338	30,338	0.567	2.541289702	4.481992
01780000	Woronichinia	1,347,920	1,347,920	0.503	3.08322113	6.129664
04100000	Trachelomonas	45,465	45,465	0.621	2.892414886	4.657673
05040001	Cryptomonas (small) Length <20 µm	2,257	2,257	0.53	1.777351752	3.353494
05040002	Cryptomonas (medium) Length 20-30 µm	110,568	110,568	0.533	2.688255263	5.043631
05100012	Rhodomonas lacustris var. nannoplanctica	20,301	20,301	0.473	2.037453788	4.307513
06020040	Ceratium hirundinella	278,035	278,035	0.493	2.683940894	5.444099
06110050	Peridinium cinctum	51,156	51,156	0.485	2.283813208	4.708893
08010000	Chrysochromulina	15,902	15,902	0.341	1.43269262	4.201445
09230050	Dinobryon divergens	61,610	61,610	0.392	1.877543132	4.789651
09310000	Mallomonas	4,339	4,339	0.452	1.644109812	3.637411
12000002	Medium centric diatom (10-20 µm diam.)	24,486	24,486	0.568	2.492904288	4.388916
12030000	Aulacoseira	5,572	5,572	0.606	2.270099543	3.746039
13080010	Asterionella formosa	2,554	2,554	0.491	1.672960471	3.407251
17000000	Unidentified green	18,952	18,952	0.503	2.151661025	4.277656
17080000	Botryococcus	67,597	67,597	0.313	1.511768275	4.829931
17580020	Monoraphidium contortum	250	250	0.596	1.429023195	2.39769
17780000	Quadrigula	202	202	0.271	0.624748999	2.305347
17960030	Tetraedron minimum	1,909	1,909	0.581	1.906116502	3.280751
24170000	Gloeotila	2,477	2,477	0.264	0.895978071	3.393856
25010000	Elakatothrix	111	111	0.437	0.894569085	2.047069
27370000	Staurastrum	1,255	1,255	0.458	1.419205233	3.098701
90000000	"Picoplankton" - unidentified single cells <2 µm diam.	20,299	20,299	0.539	2.321730501	4.307478

90000005	"Nanoplankton" - unidentified flagellates 2–20 µm diameter	2,827	2,827	0.519	1.791236174	3.451322
				SUM	**49.122**	**100.613**

5.2.1 Calculate observed PTI

Calculate observed PTI for each phytoplankton sample by inserting $\log_{10}$ biovolume and optima data(from table 7) into equation 4.

$$PTIobs = \frac{\sum_{j=1}^{n} \log(a_j) s_j}{\sum_{j=1}^{n} \log(a_j)}$$ **Equation 4**

Where:

a_j = biovolume of *j*th taxon in the sample ($\mu m^3\ ml^{-1}$)

s_j = optimum of *j*th taxon in the sample

For the sample from Loweswater;

PTI_{obs} = 49.122/100.613

= 0.488

5.2.2 Calculate expected (reference) PTI

Use equation 5 to calculate the expected PTI

$PTI_{Ref} = 0.028 \times log_{10}MEI + 0.498$ **Equation 5**

Alk = 0.198 mEq/L, Depth = 8.37m,

MEI = *Alk*/*Depth* = 0.198/8.37

= 0.02366

PTI_{ref} = 0.028 x $\log_{10}$0.02366 + 0.498

=0.452

5.2.3 Calculate PTI EQR per sample

Calculate the PTI EQR for each phytoplankton sample using equation 6.

$$EQR_{PTI} = \left(\frac{PTI_{Obs} - PTI_{Max}}{PTI_{\mathrm{Re}f} - PTI_{Max}} \right)$$ **Equation 6**

EQR_{PTI} = (0.488 – 0.75)/(0.452 – 0.75)

= -0.262/-0.298

= 0.879

5.2.4 Normalise the EQR

To normalise the EQR, insert EQR_{PTI} into the lake type specific normalisation equation taken from Table 5.

For Loweswater, a low alkalinity, shallow lake, the normalisation equation is

EQR_{norm} = (1.228 x EQR^2) – (0.2004 x EQR) – 0.147

EQR_{norm} = (1.288 x 0.879 x 0.879) – (0.2004 x 0.879) – 0.147

= 0.9488 – 0.176 – 0.147

= 0.672

This normalised EQR indicates Good status.

5.2.5 Calculate the lake PTI EQR

Finally, calculate the lake PTI EQR by taking the average of the normalised EQRs from each sample.

For the Loweswater dataset, the average normalised PTI EQR from the 7 samples is 0.549

5.3 Calculating EQR for the Bloom intensity Metric – Cyanobacteria biovolume

5.3.1 Calculate observed cyanobacterial biovolume

Identify all cyanobacterial taxa from the sample (see Table 7 for the cyanobacterial counts from the Loweswater sample). Convert the biovolume unit to mm^3/L, and calculate the sum of all cyanobacteria for the sample.

Species Code	Species Name	Biovolume, um^3/mL	Biovolume, um^3/mL – duplicate records summed	Biovolume as mm^3/L
01020000	Anabaena	52,104	84,306	0.084306
01020000	Anabaena	32,201		
01530000	Oscillatoria	30,338	30,338	0.030338
01780000	Woronichinia	1,347,920	1,347,920	1.347920
			SUM	**1.463**

5.3.2 Calculate expected cyanobacterial biovolume

This value is 0.00 for Low alkalinity lake types of all depths.

5.3.3 Calculate the cyanobacterial EQR

Insert the values for observed and expected cyanobacterial EQR into Equation 7.

$$EQR_{Cyan} = \frac{\log(BV_{Obs} + 0.0001) - \log(BV_{Max} + 0.0001)}{\log(BV_{Ref} + 0.0001) - \log(BV_{Max} + 0.0001)}$$

Equation 7

EQR_{Cyan} = ($\log_{10}$(1.4626 + 0.0001) – $\log_{10}$(30.0 + 0.0001))/($\log_{10}$(0.00 + 0.0001) – $\log_{10}$(30.0 + 0.0001))

= (0.1652 – 1.477)/(-4 – 1.477)

= -1.31166/-5.47712

= 0.2395

5.3.4 Normalise the cyanobacterial EQR

Insert the appropriate values into Equation 8 to normalise the cyanobacteria EQR

$$CyanEQR_{Norm} = \left[\left(\frac{EQR_{Cyan} - LowerBoundary}{ClassWidth}\right) \times 0.2\right] + LowerBoundary_{Norm}$$ **Equation 8**

EQR_{Cyan} of 0.2395 represents Moderate status, the lower boundary value is 0.23, the class width is 0.32 – 0.23 = 0.09(from Table 6), the lower normalised boundary value is 0.4

$CyanoEQR_{Norm}$ =(((0.2395 – 0.23)/0.09) x 0.2) + 0.4

= (0.10556 x 0.2) + 0.4

= 0.02111 + 0.4

= 0.4211

This EQR represents Moderate status

The average of all normalised EQRs for cyanobacteria for Loweswater is 0.485

5.4 Combination of all three metric EQRs to calculate an overall EQR and classification for phytoplankton

Calculate the average of the normalised Chl EQR and the normalised PTI EQR. This gives an interim EQR ($IntEQR_{norm}$)

$IntEQR_{norm}$ = (0.697 + 0.549)/2

= 0.623

If CyanEQRnorm < IntEQRnorm, repeat the calculation averaging all three normalised EQR values. Otherwise, ignore the cyanobacterial EQR. This step provides an overall normalised EQR for phytoplankton.

The normalised cyanobacterial EQR for Loweswater is 0.485. This is less than the average of chlorophyll and PTI EQRs, therefore;

Overall normalised EQR = (0.697+ 0.549 + 0.485)/3 = 0.577

The overall normalised EQR for Loweswater from all the data available is 0.577, which represents **Moderate** status.

5.5 Calculate statistical confidence of class

Confidence of class is calculated for each metric, then overall confidence of the classification is determined.

5.5.1 Confidence of class for the chlorophyll EQR

It is necessary to have the following information for this calculation;

LChl	=	annual mean of Log_{10}Chl	= 0.680
Chl	=	annual geometric mean Chl	= $10^{0.680}$ = 4.78
SD_{LChl}	=	standard deviation of Log_{10}Chl	= 0.331
SE_{LChl}	=	standard error of Log_{10}Chl	= 0.331 / √36 = 0.055
N_{Chl}	=	number of samples	= 36
Years	=	number of years sampled	= 3
Sy	=	estimated samples per year	= 36/3 = 12

The samples collected from Loweswater used in this example are collected at regular, monthly intervals over a three year period. For this reason it is valid to adjust the standard error, using Equation 9.

$$SE_{Str} = SE_{Rand} \times 1.0293 \times Sy^{-0.2379} \qquad \textbf{Equation 9}$$

SE_{Str} = 0.055 x 1.0293 x $12^{-0.2379}$

=0.031

Calculate the upper and lower 95% confidence limits for chlorophyll

$L95 = 10^{(LChl - TINV \times SEStr)}$

TINV = 2.030, where p=0.05, degrees of freedom = 36-1

L95 = $10^{(0.680 - 2.030 \times 0.031)}$

= $10^{0.61707}$

= 4.140

U95 = $10^{(LChl + TINV \times SEStr)}$

=$10^{(0.680 + 2.030 \times 0.031)}$

=$10^{0.7492}$

=5.613

Convert these values to EQRs

$$L95EQR = \frac{Chl_{Ref}}{U95} \quad \text{and} \quad U95EQR = \frac{Chl_{Ref}}{L95}$$

L95EQR = 2.2/5.613 = 0.392

U95EQR = 2.2/4.140 = 0.531

Normalise the EQRs according to the following equation substituting the upper or lower 95% confidence EQRs as appropriate;

$$U95EQR_{Norm} = \left[\left(\frac{U95EQR - LowerBoundary}{ClassWidth}\right) \times 0.2\right] + LowerBoundary_{Norm}$$

Both U95EQR and L95EQR values fall between the High/Good and Good/Moderate boundaries for this lake type which means;

LowerBoundary = 0.29,

$LowerBoundary_{norm}$ = 0.60

Class width = 0.64 – 0.29 = 0.35

Lake Type	Chlorophyll EQR (EQR_{Chl}) Boundary values			
	High/Good	Good/Moderate	Moderate/Poor	Poor/Bad
Low alkalinity, shallow	0.64	0.29	0.15	0.05
All lake types – normalised EQR boundary values	**0.80**	**0.60**	**0.40**	**0.20**

$U95EQR_{norm}$ = (((0.531 - 0.29)/0.35) x 0.2) + 0.6

= 0.7377

$L95EQR_{norm}$ = (((0.3919 - 0.29)/0.35) x 0.2) + 0.6

= 0.6582

Use the normalised EQRs to calculate the standard error of the EQRs using the following formula

$$SE(EQR_{Norm}) = \frac{U95EQR_{Norm} - L95EQR_{Norm}}{2 \times TINV}$$

$SEEQR_{norm}$ = (0.7377 – 0.6582)/(2 x 2.030)

= 0.019

Calculate the confidence of class for the chlorophyll metric (note, it is necessary to use the absolute values for estimation of t, and where $EQR_{boundary}$>EQR_{norm}, use 1-p, otherwise use p);

$$p = t\left(\frac{EQR_{Boundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

p < High	= t((0.8 - 0.697)/0.019)	= 1 – 2.2×10^{-6}	= 1.00
p < Good	= t((0.6 - 0.697)/0.019)	= 5.8×10^{-6}	= 0.00
p < Moderate	= t((0.4 - 0.697)/0.019)	= 1.1×10^{-17}	= 0.00
p < Poor	= t((0.2 - 0.697)/0.019)	= 7.2×10^{-25}	= 0.00

Confidence of Class is therefore;

Confidence of H	= 1 – p<H	= 1 – 1.00	= 0.00
Confidence of G	= p<H – p<G	= 1.00 – 0.00	= 1.00
Confidence of M	= p<G – p<M	= 0.00 - 0.00	= 0.00
Confidence of P	= p<M – p<P	= 0.00 - 0.00	= 0.00
Confidence of B	= p<P		= 0.00

5.5.2 Confidence of class for PTI and Cyanobacterial metrics

For each metric calculate the mean, standard deviation and standard error from the normalised EQRs which contribute to the overall metric.

Sample date	07/07/2010	12/08/2010	13/09/2010	27/09/2011	24/07/2012	21/08/2012	26/09/2012	**Mean**	**Standard Deviation**	**Standard Error**	**N**	**Degrees of freedom**
PTI EQR_{Norm}	0.45	0.66	0.63	0.43	0.61	0.44	0.63	0.55	0.10	0.04	7	6
Cyan EQR_{Norm}	0.54	0.51	0.42	0.67	0.40	0.34	0.52	0.49	0.11	0.04	7	6

Calculate the confidence of class for the PTI and cyanobacterial metrics (note, it is necessary to use the absolute values for estimation of t, and where $EQR_{boundary}$>EQR_{norm}, use 1-p, otherwise use p);

For PTI;

$$p = t\left(\frac{EQR_{Boundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

p < High	= t((0.8 - 0.55)/0.04)	= 1 – 0.0003	= 1.00
p < Good	= t((0.6 - 0.55)/0.04)	= 1 - 0.12	= 0.88
p < Moderate	= t((0.4 - 0.55)/0.04)	= 0.005	= 0.01
p < Poor	= t((0.2 - 0.55)/0.04)	= 6.2×10^{-5}	= 0.00

Confidence of Class is therefore;

Confidence of H	= 1 – p<H	= 1- 1.00	= 0.00
Confidence of G	= p<H – p<G	= 1.00 – 0.88	= 0.12
Confidence of M	= p<G – p<M	= 0.88 - 0.00	= 0.88
Confidence of P	= p<M – p<P	= 0.00 - 0.00	= 0.00
Confidence of B	= p<P		= 0.00

For Cyanobacteria;

$$p = t\left(\frac{EQR_{Boundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

p < High	= t((0.8 - 0.485)/0.04)	= 1 – 0.0001	= 1.00
p < Good	= t((0.6 - 0.485)/0.04)	= 1 - 0.015	= 0.985
p < Moderate	= t((0.4 - 0.485)/0.04)	= 0.042	= 0.042
p < Poor	= t((0.2 - 0.485)/0.04)	= 0.0002	= 0.00

Confidence of Class is therefore;

Confidence of H	= 1 – p<H	= 1- 1.00	= 0.00
Confidence of G	= p<H – p<G	= 1.00 – 0.985	= 0.015
Confidence of M	= p<G – p<M	= 0.985 - 0.042	= 0.943
Confidence of P	= p<M – p<P	= 0.042 - 0.00	= 0.042
Confidence of B	= p<P		= 0.00

5.5.3 Calculation of confidence of class for the combined metrics

First determine the combined standard error of the three metrics used for the overall phytoplankton assessment. Note that the cyanobacterial EQR was used in this assessment. In other circumstances, where there is no need to include the cyanobacterial metric, the standard error of the combined metrics would only be determined from chlorophyll and PTI.

$$SE(EQR_{Phyto}) = \frac{\sqrt{SE(ChlEQR_{norm})^2 + SE(PTIEQR_{norm})^2 + SE(CyanoEQR_{norm})^2}}{3}$$

SE(EQRphyto) = (SQRT(0.019^2 + 0.039^2 + 0.041^2))/3

= SQRT(0.003563)/3

=0.02

To calculate the confidence of class using the t-distribution, as has been done for the individual metrics, it is necessary to know the degrees of freedom. For the combined metrics

this is estimated using the Welch-Satterthwaite formula which, for three metrics, is as follows;

$$n = \frac{\left\{\frac{sChlEQRnorm^2}{3} + \frac{sPTIEQRnorm^2}{3} + \frac{sCyanEQRnorm^2}{3}\right\}^2}{\left\{\frac{\left(sChlEQRnorm^2/3\right)^2}{(Nchl-1)} + \frac{\left(sPTIEQRnorm^2/3\right)^2}{(NPTI-1)} + \frac{\left(sCyanEQRnorm/3\right)^2}{(NCyano-1)}\right\}}$$

Substituting the standard deviation of each of the normalised metrics give the following estimate of degrees of freedom;

= $(0.115^2/3 + 0.103^2/3 + 0.109^2/3)^2/(0.115^2/3)^2/35 + (0.103^2/3)^2/6 + (0.109^2/3)^2/6$

= $0.00014/5.2536 \times 10^{-6}$

= 27

Finally, calculate the confidence of class for the combined metrics as above, using the estimated degrees of freedom

$$p = t\left(\frac{EQR_{Boundary} - EQR_{Norm}}{SE(EQR_{Norm})}\right)$$

p < High	= t((0.8 - 0.577)/0.02)	= $1 - 6.5 \times 10^{-12}$	= 1.00
p < Good	= t((0.6 - 0.577)/0.02)	= 1 - 0.130	= 0.87
p < Moderate	= t((0.4 - 0.577)/0.02)	= 9.1×10^{-10}	= 0.00
p < Poor	= t((0.2 - 0.577)/0.02)	= 2.3×10^{-17}	= 0.00

Confidence of Class is therefore;

Confidence of H	= 1 – p<H	= 1 – 1.00	= 0.00
Confidence of G	= p<H – p<G	= 1.00 – 0.87	= 0.13
Confidence of M	= p<G – p<M	= 0.87 - 0.00	= 0.87
Confidence of P	= p<M – p<P	= 0.00 - 0.00	= 0.00
Confidence of B	= p<P		= 0.00

6 References

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Appendix A

The following table lists optima for the phytoplankton taxa which form the basis of the PLUTO classification system. Provided is the Whitton Code (John *et al* 2011) which is the recognised coding system used for UK phytoplankton and the taxon name. The optima are provided for each taxon and the optima type indicates whether the optima are based on the species response, the genus response or a higher taxonomic level. Genus optima will therefore be the same for a number of species within that genus, unless particular species have stood out as having clearly different optima to the genus. The final column indicates which taxa are listed on the UK phytoplankton counter spreadsheet, which contains all but the most infrequently recorded taxa from the UK lakes which are monitored. Any species or sub-species which are identified but are not listed here should be recorded at the next highest taxonomic level which does have an optima.

Note that Whitton codes and taxa names are subject to occasional review and some may change. The coding in this document may not keep pace with these changes. However, the optima will remain unchanged until such time as a formal review of the classification tool is carried out.

Note that some taxa do not have a Whitton code. Others have "functional" codes which take the form of the Whitton coding system but have been created separately to provide codes under which to lump taxa, usually as size classes, which cannot be identified more specifically. These are denoted by *.

Whitton code (* denotes "functional" code)	**Phytoplankton taxon name**	**Optima**	**Optima type**	**Listed on UK phytoplankton count spreadsheet**
01000000	Unidentified Cyanophyte	0.581	group	yes
01020000	Anabaena	0.568	genus	yes
01020040	Anabaena catenula	0.713	species	yes
01020042	Anabaena catenula var. solitaria	0.701	species	yes
01020050	Anabaena circinalis	0.743	species	yes
01020090	Anabaena flos-aquae	0.652	species	yes
01020130	Anabaena planctonica	0.618	species	
01020140	Anabaena spiroides	0.631	species	yes
01030000	Anabaenopsis	0.729	genus	
01040000	Aphanizomenon	0.717	genus	yes
01040020	Aphanizomenon flos-aquae	0.748	species	yes
01040040	Aphanizomenon issatschenkoi	0.717	genus	yes
01050000	Aphanocapsa	0.539	genus	yes
01050020	Aphanocapsa delicatissima	0.539	genus	yes
01050030	Aphanocapsa elachista	0.405	species	yes
01060000	Aphanothece	0.459	genus	yes
01060020	Aphanothece clathrata	0.459	genus	yes

Whitton code (* denotes "functional" code)	**Phytoplankton taxon name**	**Optima**	**Optima type**	**Listed on UK phytoplankton count spreadsheet**
01060080	Aphanothece minutissima	0.301	species	yes
01130000	Chroococcus	0.438	genus	yes
01130020	Chroococcus dispersus	0.438	genus	yes
01130060	Chroococcus minutus	0.349	species	yes
01150000	Coelosphaerium	0.496	genus	yes
01150010	Coelosphaerium kuetzingianum	0.495	species	yes
	Coelosphaerium subarcticum	0.233	species	
01170000	Cyanodictyon	0.325	genus	
01170010	Cyanodictyon imperfectum	0.418	species	
01170020	Cyanodictyon planctonicum	0.322	species	yes
01200000	Cylindrospermum	0.193	genus	
01300000	Gloeothece	0.365	genus	
01310000	Gloeotrichia	0.602	genus	
01320000	Gomphosphaeria	0.46	genus	yes
01320010	Gomphosphaeria aponina	0.436	species	yes
01430000	Lyngbya	0.71	genus	yes
01430050	Lyngbya contorta	0.71	genus	yes
01460000	Merismopedia	0.48	genus	yes
01460070	Merismopedia warmingiana	0.225	species	yes
01490000	Microcystis	0.672	genus	yes
01490010	Microcystis aeruginosa	0.672	genus	yes
01490020	Microcystis flos-aquae	0.672	genus	yes
01490030	Microcystis wesenbergii	0.672	genus	yes
01530000	Oscillatoria	0.567	genus	yes
01530010	Oscillatoria agardhii	0.552	species	yes
01530012	Oscillatoria agardhii var. isothrix	0.322	species	yes
01530160	Oscillatoria limnetica	0.643	species	yes
01530170	Oscillatoria limosa	0.567	genus	yes
01530230	Oscillatoria redekei	0.567	genus	yes
01550000	Phormidium	0.188	genus	
01570000	Pleurocapsa	0.492	genus	
01580000	Pseudanabaena	0.503	genus	yes
01610000	Rhabdoderma	0.211	genus	
01690000	Synechococcus	0.458	genus	
01700000	Synechocystis	0.337	genus	
01750000	Snowella	0.513	genus	yes
01750000	Snowella	0.513	genus	yes
01750010	Snowella lacustris	0.639	species	yes
01750010	Snowella lacustris	0.639	species	yes

Whitton code (* denotes "functional" code)	**Phytoplankton taxon name**	**Optima**	**Optima type**	**Listed on UK phytoplankton count spreadsheet**
01750020	Snowella septentrionalis	0.309	species	yes
	Snowella atomus	0.311	species	yes
01790000	Radiocystis	0.187	genus	
01790010	Radiocystis geminata	0.173	species	
01780000	Woronichinia	0.503	genus	yes
01780010	Woronichinia naegeliana	0.526	species	yes
04020000	Euglena	0.587	genus	yes
04070000	Phacus	0.715	genus	yes
04090000	Strombomonas	0.633	genus	yes
04100000	Trachelomonas	0.621	genus	yes
04100150	Trachelomonas hispida	0.451	species	
04100160	Trachelomonas intermedia	0.488	species	
04100360	Trachelomonas volvocinopsis	0.506	species	
05020000	Chroomonas	0.544	genus	yes
05020010	Chroomonas acuta	0.502	species	yes
05040000	Cryptomonas	0.547	genus	yes
05040001*	Cryptomonas (small) Length <20 µm	0.53	species	yes
05040002*	Cryptomonas (medium) Length 20-30 µm	0.533	species	yes
05040003*	Cryptomonas (large) Length >30 µm	0.589	species	yes
05040030	Cryptomonas erosa	0.547	genus	yes
05040040	Cryptomonas marssonii	0.631	species	yes
05040050	Cryptomonas ovata	0.508	species	yes
05060000	Cyathomonas	0.606	genus	
05100000	Rhodomonas	0.539	genus	yes
05100010	Rhodomonas lacustris	0.358	species	yes
05100012	Rhodomonas lacustris var. nannoplanctica	0.473	species	yes
05109910	Rhodomonas lens	0.539	genus	yes
05110000	Plagioselmis	0.355	genus	
06000000	dinoflagellate	0.288	group	
06020000	Ceratium	0.505	genus	
06020010	Ceratium carolinianum	0.505	genus	yes
06020020	Ceratium cornutum	0.505	genus	yes
06020030	Ceratium furcoides	0.644	species	yes
06020040	Ceratium hirundinella	0.493	species	yes
06050000	Glenodinium	0.561	genus	yes
06070000	Gymnodinium	0.46	genus	yes
06070110	Gymnodinium helveticum	0.479	species	yes

Whitton code (* denotes "functional" code)	**Phytoplankton taxon name**	**Optima**	**Optima type**	**Listed on UK phytoplankton count spreadsheet**
06110000	Peridinium	0.485	genus	yes
06110040	Peridinium bipes	0.293	species	
06110050	Peridinium cinctum	0.485	genus	yes
06110090	Peridinium umbonatum	0.229	species	yes
06110100	Peridinium willei	0.332	species	yes
07010000	Gonyostomum	0.297	genus	
07010010	Gonyostomum semen	0.297	genus	yes
08010000	Chrysochromulina	0.341	genus	yes
08010010	Chrysochromulina parva	0.348	species	yes
08040000	Prymnesium	0.838	genus	yes
09000000	Chrysophyceae	0.324	genus	yes
09030000	Bitrichia	0.288	genus	yes
09030010	Bitrichia chodatii	0.235	species	yes
09030020	Bitrichia longispina	0.288	genus	yes
09050000	Chromulina	0.41	genus	yes
09050030	Chromulina nebulosa	0.41	genus	yes
09060000	Chrysamoeba	0.256	genus	yes
09080000	Chrysidiastrum	0.276	genus	yes
09080010	Chrysidiastrum catenatum	0.267	species	yes
09130000	Chrysococcus	0.427	genus	yes
09130010	Chrysococcus cordiformis	0.157	species	
09150000	Chrysolykos	0.233	genus	yes
09150010	Chrysolykos planctonicus	0.245	species	yes
09150020	Chrysolykos skujae	0.122	species	
09230000	Dinobryon	0.411	genus	yes
09230010	Dinobryon bavaricum	0.328	species	yes
09230030	Dinobryon crenulatum	0.201	species	yes
09230040	Dinobryon cylindricum	0.211	species	yes
09230050	Dinobryon divergens	0.392	species	yes
09230052	Dinobryon divergens var. schauinslandii	0.318	species	yes
09230070	Dinobryon sertularia	0.411	genus	yes
09230080	Dinobryon sociale	0.41	species	yes
09230090	Dinobryon suecicum	0.233	species	yes
09230110	Dinobryon borgei	0.205	species	yes
09250000	Epipyxis	0.411	genus	yes
09290000	Kephyrion	0.434	genus	
09290040	Kephyrion boreale	0.178	species	
09290050	Kephyrion cupuliforme	0.121	species	
09290060	Kephyrion skujae	0.231	species	

Whitton code (* denotes "functional" code)	**Phytoplankton taxon name**	**Optima**	**Optima type**	**Listed on UK phytoplankton count spreadsheet**
09310000	Mallomonas	0.452	genus	yes
09310030	Mallomonas akrokomos	0.473	species	yes
09310080	Mallomonas caudata	0.346	species	yes
09330000	Monochrysis	0.23	genus	
09350000	Ochromonas	0.409	genus	yes
09370000	Phaeaster	0.252	genus	yes
	Phaeaster aphanaster	0.283	species	
09430000	Pseudokephyrion	0.345	genus	yes
	Pseudokephyrion tatricum	0.179	species	
09450000	Spiniferomonas	0.211	genus	yes
09480000	Stichogloea	0.293	genus	yes
09530000	Synura	0.365	genus	yes
09540000	Uroglena	0.443	genus	yes
09570000	Pseudopedinella	0.372	genus	
09570010*	Pseudopedinella (small <5um)	0.471	species	yes
09570020*	Pseudopedinella (big >5um)	0.37	species	yes
10050000	Centritractus	0.326	genus	yes
10090000	Goniochloris	0.764	genus	yes
10110000	Isthmochloron	0.184	genus	yes
10140000	Ophiocytium	0.545	genus	yes
10160000	Pseudostaurastrum	0.714	genus	yes
10180000	Tetraedriella	0.138	genus	
10180010	Tetraedriella jovetti	0.137	species	
10190000	Tribonema	0.366	genus	
10220000	Gloeobotrys	0.312	genus	
12000000	Centric diatoms	0.571	group	
12000001*	Small centric diatom (5 - <10 µm diam.)	0.573	group	yes
12000002*	Medium centric diatom (10-20 µm diam.)	0.568	group	yes
12000003*	Large centric diatom (>20 µm diam.)	0.599	group	yes
12000004*	Very small centric diatom (<5 µm diam.)	0.574	group	yes
12010000	Acanthoceras	0.721	genus	
12010010	Acanthoceras zachariasi	0.716	species	yes
12030000	Aulacoseira	0.606	genus	yes
12030020	Aulacoseira ambigua	0.606	genus	yes
12030050	Aulacoseira distans	0.155	species	
12030060	Aulacoseira granulata	0.717	species	yes
12030062	Aulacoseira granulata var. angustissima	0.719	species	yes

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12030070	Aulacoseira islandica	0.663	species	
12030080	Aulacoseira italica	0.475	species	yes
12030084	Aulacoseira italica v.tenuissima	0.435	species	yes
12030150	Aulacoseira subarctica	0.563	species	yes
12040000	Chaetoceros	1	genus	
12040010	Chaetoceros muelleri	1	genus	yes
12070000	Cyclotella	0.355	genus	yes
12070370	Cyclotella comta	0.349	species	
12110000	Melosira	0.71	genus	yes
12110080	Melosira varians	0.722	species	yes
12160000	Skeletonema	0.8	genus	
12180000	Stephanodiscus	0.634	genus	yes
12180090	Stephanodiscus hantzschii	0.616	species	
12200000	Urosolenia	0.3	genus	yes
12200010	Urosolenia eriensis	0.258	species	yes
12200020	Urosolenia longiseta	0.346	species	yes
13000000	Pennate diatoms	0.418	group	
13000001*	Small pennate diatom <10 µm diam	0.356	group	yes
13000002*	Medium pennate diatom 10-20 µm diam	0.349	group	yes
13000003*	Large pennate diatom >20 µm diam	0.379	group	yes
13050000	Amphora	0.305	genus	
13080000	Asterionella	0.492	genus	
13080010	Asterionella formosa	0.491	species	yes
13200000	Cylindrotheca	1	genus	
13210000	Cymatopleura	0.57	genus	
13260000	Diatoma	0.567	genus	yes
13260040	Diatoma tenuis	0.573	species	yes
13280000	Didymosphaenia	0.203	genus	
13370000	Fragilaria	0.44	genus	yes
13370030	Fragilaria capucina	0.534	species	yes
13370040	Fragilaria crotonensis	0.467	species	yes
13420000	Gyrosigma	0.712	genus	
13520000	Navicula	0.497	genus	yes
13540000	Nitzschia	0.628	genus	yes
13540020	Nitzschia acicularis	0.673	species	yes
13770000	Staurosira	0.35	genus	
13770013	Fragilaria construens var. exigua	0.347	species	yes

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13800000	Surirella	0.708	genus	
13810000	Synedra	0.506	genus	yes
13810010	Synedra acus	0.544	species	yes
13810120	Synedra nana	0.506	genus	yes
13810180	Synedra ulna	0.381	species	yes
13820000	Tabellaria	0.327	genus	yes
13820010	Tabellaria fenestrata	0.297	species	yes
13820020	Tabellaria flocculosa	0.295	species	yes
13820022	Tabellaria flocculosa var. asterionelloides	0.333	species	yes
15030000	Monomastix	0.305	genus	
15050000	Neproselsmis	0.402	genus	
15110000	Pyramimonas	0.503	genus	
15120000	Scourfieldia	0.529	genus	
16000000	Unidentified green	0.285	genus	
16020000	Asterococcus	0.313	genus	
16060000	Carteria	0.586	genus	yes
16170000	Chlamydocapsa	0.275	genus	
16180000	Chlamydomonas	0.511	genus	yes
16190000	Chlorogonium	0.492	genus	yes
16260000	Eudorina	0.544	genus	
16260010	Eudorina elegans	0.539	species	yes
16330000	Gonium	0.292	genus	yes
16470000	Pandorina	0.683	genus	yes
16470010	Pandorina morum	0.652	species	yes
16490000	Paulschulzia	0.304	genus	
16590010	Pseudosphaerocystis lacustris	0.299	species	yes
16600000	Pteromonas	0.902	genus	yes
16680000	Spermatozopsis	0.582	genus	
16740000	Tetraspora	0.448	genus	
16770000	Volvocales	0.544	genus	
16770010	Volvox aureus	0.664	species	yes
17000000	Chlorococcales	0.503	genus	yes
17020000	Actinastrum	0.803	genus	
17020010	Actinastrum hantzschii	0.789	species	yes
17050000	Ankistrodesmus	0.425	genus	yes
17050030	Ankistrodesmus falcatus	0.482	species	yes
17050050	Ankistrodesmus fusiformis	0.348	species	yes
17050060	Ankistrodesmus spiralis	0.443	species	yes
17060000	Ankyra	0.627	genus	yes

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17060020	Ankyra judayi	0.625	species	yes
17080000	Botryococcus	0.313	genus	yes
17080010	Botryococcus braunii	0.344	species	yes
	Botryococcus terribilis	0.167	species	
17170000	Closteriopsis	0.696	genus	yes
17170010	Closteriopsis acicularis	0.696	genus	yes
17170020	Closteriopsis longissima	0.484	species	yes
17200000	Coelastrum	0.699	genus	yes
17200010	Coelastrum astroideum	0.726	species	yes
17200020	Coelastrum microporum	0.718	species	yes
17200070	Coelastrum sphaericum	0.699	genus	yes
17210000	Coenochloris	0.437	genus	
17210010	Coenochloris fottii	0.437	genus	yes
17220000	Coenococcus	0.13	genus	
17230000	Coenocystis	0.247	genus	
17230020	Coenocystis planctonica	0.247	genus	yes
17250000	Crucigenia	0.552	genus	yes
17250030	Crucigenia tetrapedia	0.535	species	yes
17260000	Crucigeniella	0.504	genus	yes
17300000	Dichotomococcus	0.452	genus	
17330000	Dictyosphaerium	0.556	genus	yes
17330040	Dictyosphaerium pulchellum	0.618	species	yes
17340000	Didymocystis	0.424	genus	yes
17350020	Didymogenes palatina	0.492	genus	yes
17410000	Franceia	0.593	genus	
17420000	Gloeocystis	0.82	genus	yes
17430000	Golenkinia	0.618	genus	yes
17430020	Golenkinia radiata	0.618	genus	yes
17440000	Golenkiniopsis	0.337	genus	
17440020	Golenkiniopsis longispina	0.337	genus	yes
17500000	Keratococcus	0.337	genus	
17510000	Kirchneriella	0.605	genus	yes
17530000	Korshikoviella	0.601	genus	yes
17540000	Lagerheimia	0.778	genus	yes
17540040	Lagerheimia genevensis	0.767	species	yes
17570000	Micractinium	0.629	genus	yes
17570010	Micractinium pusillum	0.629	genus	yes
17580000	Monoraphidium	0.538	genus	yes
17580010	Monoraphidium arcuatum	0.713	species	yes
17580020	Monoraphidium contortum	0.596	species	yes

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17580030	Monoraphidium convolutum	0.685	species	yes
17580040	Monoraphidium griffithii	0.553	species	yes
17580050	Monoraphidium irregulare	0.64	species	yes
17580070	Monoraphidium komarkovae	0.614	species	yes
17580080	Monoraphidium minutum	0.623	species	yes
17580090	Monoraphidium mirabile	0.31	species	
17580120	Monoraphidium tortile	0.538	genus	yes
17580130	Monoraphidium dybowskii	0.419	species	yes
17620000	Nephrochlamys	0.331	genus	
17630000	Nephrocytium	0.436	genus	
17630010	Nephrocytium agardhianum	0.255	species	
17640000	Oocystis	0.54	genus	yes
17640030	Oocystis borgei	0.406	species	
17640050	Oocystis lacustris	0.542	species	yes
17640120	Oocystis parva	0.432	species	yes
17670000	Palmodictyon	0.223	genus	
17680000	Pediastrum	0.686	genus	yes
17680020	Pediastrum biradiatum	0.686	genus	yes
17680030	Pediastrum boryanum	0.706	species	yes
17680032	Pediastrum boryanum var. cornutum	0.566	species	
17680050	Pediastrum duplex	0.726	species	yes
17680080	Pediastrum simplex	0.686	genus	yes
17680090	Pediastrum tetras	0.628	species	yes
17690010	Planktosphaeria gelatinosa	0.309	species	yes
17740000	Dictyosphaerium	0.151	genus	
17780000	Quadrigula	0.271	genus	yes
17780010	Quadrigula closterioides	0.252	species	
17780020	Quadrigula pfitzeri	0.186	species	yes
17800000	Raphidocelis	0.511	genus	yes
17800000	Kirchneriella	0.511	genus	
17810000	Scenedesmus	0.616	genus	yes
17810010	Scenedesmus abundans	0.593	species	
17810020	Scenedesmus aculeolatus	0.275	species	
17810080	Scenedesmus armatus	0.603	species	yes
17810160	Scenedesmus communis	0.701	species	yes
17810220	Scenedesmus falcatus	0.859	species	yes
17810340	Scenedesmus opoliensis	0.817	species	yes
17830000	Schroederia	0.748	genus	

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17830020	Schroederia robusta	0.683	species	yes
17830030	Schroederia setigera	0.757	species	yes
17860000	Selenastrum	0.723	genus	yes
17870000	Siderocelis	0.257	genus	
17910000	Sphaerocystis	0.558	genus	yes
17910020	Sphaerocystis schroeteri	0.479	species	yes
17940000	Tetrachlorella	0.331	genus	
17960000	Tetraedron	0.621	genus	yes
17960010	Tetraedron caudatum	0.633	species	yes
17960030	Tetraedron minimum	0.581	species	yes
17970000	Tetrastrum	0.658	genus	
17970010	Tetrastrum elegans	0.658	genus	yes
17970040	Crucigenia quadrata	0.358	species	yes
17970050	Tetrastrum staurogeniaeforme	0.834	species	yes
17970060	Tetrastrum triangulare	0.658	genus	yes
18010000	Treubaria	0.666	genus	
18010010	Treubaria setigera	0.666	genus	yes
18030000	Westella	0.442	genus	
24170000	Gloeotila	0.264	genus	yes
24340000	Stichococcus	0.365	genus	
24380000	Ulothrix	0.305	genus	
25010000	Elakatothrix	0.437	genus	yes
25010010	Elakatothrix gelatinosa	0.436	species	yes
25010030	Elakatothrix genevensis	0.342	species	yes
25030000	Koliella	0.439	genus	yes
25030010	Koliella longiseta	0.53	species	yes
25030020	Koliella spiculiformis	0.271	species	yes
27010000	Actinotaenium	0.638	genus	
27040000	Closterium	0.592	genus	yes
27040030	Closterium aciculare	0.613	species	yes
27040040	Closterium acutum	0.594	species	yes
27040044	Closterium acutum var. variabile	0.579	species	yes
27040260	Closterium gracile	0.382	species	
27040340	Closterium kuetzingii	0.316	species	yes
27040500	Closterium parvulum	0.499	species	yes
27040760	Closterium venus	0.27	species	
27050000	Cosmarium	0.456	genus	yes
27050160	Cosmarium bioculatum	0.241	species	
27050380	Cosmarium contractum	0.256	species	

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27050900	Cosmarium humile	0.216	species	
27051080	Cosmarium margaritiferum	0.224	species	
27051420	Cosmarium phaseolus	0.336	species	
27051650	Cosmarium punctulatum	0.388	species	yes
27051860	Cosmarium reniforme	0.301	species	
27052120	Cosmarium subcrenatum	0.205	species	yes
27060000	Cosmocladium	0.33	genus	
27070000	Cylindrocystis	0.193	genus	
27070020	Cylindrocystis brebissonii	0.202	species	
27110000	Euastrum	0.367	genus	yes
27120000	Genicularia	0.232	genus	
27130000	Gonatozygon	0.203	genus	yes
27180000	Hyalotheca	0.23	genus	
27230000	Netrium	0.19	genus	
27240000	Onychonema	0.337	genus	
27260060	Penium	0.136	genus	
27280000	Pleurotaenium	0.183	genus	
27360040	Spondylosium planum	0.339	species	yes
27380000	Staurastrum	0.458	genus	yes
27380060	Staurastrum anatinum	0.458	genus	yes
27380330	Staurastrum cingulum	0.451	species	yes
27380840	Staurastrum longipes	0.388	species	yes
27380860	Staurastrum lunatum	0.338	species	yes
27381030	Staurastrum paradoxum	0.412	species	yes
27381110	Staurastrum pingue	0.309	species	yes
27381120	Staurastrum planctonicum	0.27	species	yes
27381160	Stauratsrum pseudopelagicum	0.249	species	yes
27381190	Staurastrum punctulatum	0.158	species	
27381460	Staurastrum tetracerum	0.458	genus	yes
27390000	Staurodesmus	0.251	genus	yes
27390030	Staurodesmus aversus	0.265	species	
27390130	Staurodesmus cuspidatus	0.255	species	yes
27390140	Staurodesmus dejectus	0.279	species	
27390190	Staurodesmus incus	0.178	species	
27390290	Staurodesmus indentatus	0.17	species	
27390420	Staurodesmus subtriangularis	0.098	species	
27390440	Staurodesmus triangularis	0.169	species	yes
27400000	Teilingia	0.271	genus	
27400020	Teilingia granulata	0.248	species	
27420000	Tetmemorus	0.188	genus	

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27430000	Xanthidium	0.34	genus	yes
27430020	Xanthidium antilopaeum	0.302	species	yes
27440000	Zygnema	0.202	genus	
90000000*	Picoplankton - unidentified single cells <2 µm diam.	0.539	genus	yes
90000003*	Nanoplankton - unidentified single cells 2-20 µm diameter	0.532	group	yes
90000004*	Unidentified cells >20 µm diam.	0.6	group	yes
90000005*	Nanoplankton - unidentified flagellates 2-20 µm diameter	0.519	group	yes