ANNEX 8 – LAKES – Phytoplankton – PLUTO

Contents

A1 Description of method

Three groups of indicators are used, phytoplankton abundance, taxonomic composition and the likelihood of cyanobacteria blooms.

Phytoplankton abundance is measured by proxy using chlorophyll a as a surrogate. The metric used is the mean1 annual chlorophyll a concentration, derived from samples collected monthly between January and December2.

Taxonomic composition is measured using the Plankton Trophic Index (PTI) calculated from samples collected monthly between July and September3.

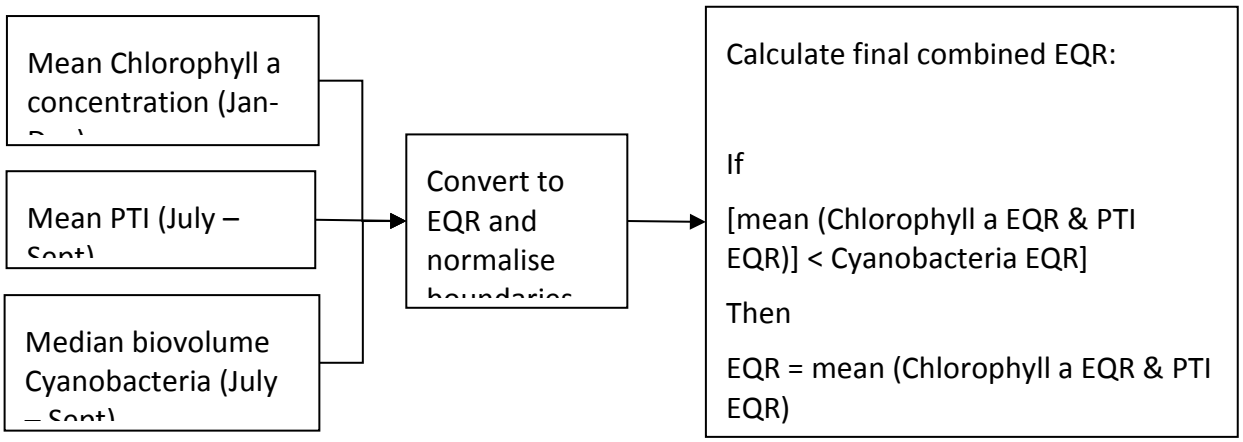
The likelihood of cyanobacteria blooms is calculated from the bio-volume of cyanobacteria present. The metric used is the geometric mean bio-volume of cyanobacteria in samples collected monthly between July and September.

Each of these metrics is converted to an EQR, using modelled estimates of reference conditions. These EQR are then normalised, so that the boundaries of each metric are on the same scale (0.8, 0.6, 0.4, 0.2), and then combined by averaging. The cyanobacteria EQR is excluded from the average if it is greater than the average of the chlorophyll and PTI EQR.

[1] Values are log transformed prior to averaging, so that the mean is a geometric mean. This allows uncertainty estimates to be made.

[2] January – December represents the growing season in the UK; in parts of the country significant biomass of phytoplankton are present in the winter months.

[3] July – September represents the late summer which is the most sensitive season for phytoplankton composition response to nutrient enrichment.



Monitoring system

Sampling for phytoplankton takes place from the lake shore, preferably near the outflow. It is critical that samples are not taken downstream of the outflow and that the edge samples are not contaminated by re-suspended sediment kicked up by the shore sampling. Phytoplankton samples should be taken a few metres away from the edge from sub-surface water (approximately 30 cm deep) to avoid any surface scums. As long as these criteria are applied, the precise methodology may vary between agencies. If the bottom sediments can be avoided (e.g. on a pier, wall or bridge at the outflow), sampling can be carried out using a bucket on a rope thrown from the edge. More ideally sampling should be carried out by throwing a weighted bottle attached to a float. The weight and float ensure the bottle sinks to approximately 30 cm below the water surface. Alternatively, water may be collected using an extendable pole at the end of which is attached a bottle. The length of the extendable pole should be long enough so as to minimise sediment disturbance at the lake edge. Approximately 1L water samples are needed for analysis of phytoplankton composition (for PTI and bloom metrics). Phytoplankton subsamples for composition are preserved immediately in the field with Lugol's Iodine and stored in the dark for subsequent microscope analysis in the laboratory. The addition of 5ml Lugol's Iodine solution per litre of sample is standard, although the final colour should be 'deep straw' or 'brandy' coloured. Phytoplankton composition analysis follows UK standard guidance (Brierley et al. 2007) which was based on draft CEN guidance (CEN, 2004).

Samples for chlorophyll analysis should be collected using the same sampling method and at the same time and also at the same location for the remaining monthly intervals when only chlorophyll is being analysed (Oct to Jun). To minimise sources of variability, it is recommended that the sampling location within an individual lake does not vary between sampling occasions, i.e. the same location at the edge or outflow is always sampled for a particular lake. As for phytoplankton composition, it is important to discard any samples that are clearly contaminated with sediment and other suspended matter from the littoral zone, and to resample.

The volume of water required for chlorophyll analysis varies seasonally but a minimum sampling volume of 0.5 L is recommended. Water samples for chlorophyll analysis should be filtered on the day of collection (ideally in the field) and the resulting filter papers should be wrapped in foil and stored in cold and dark conditions for short-term storage (<24 hrs), prior to analysis. Filter papers should be frozen if analysis does not take place within 24 hours. Chlorophyll analysis is undertaken following standard guidance developed by each Administration.

Samples for chlorophyll a are taken monthly throughout the year and are analysed at a central laboratory. As a minimum at least 4 quarterly samples are needed for classification, although monthly sampling is recommended to increase confidence in classification (Carvalho et al., 2006). Samples for taxanomic composition (and bloom metric) are taken monthly from July to September. The cells are counted with an inverted microscope by trained analysts following standard guidance (Brierley et al., 2007). [4]. Identification of taxa is generally to species, using a standardised list of taxa (Carvalho et al., 2007b). . Size measurements of a sub-sample of cells are taken to calculate bio-volume (μm^3 ml^{-1}).

Metric Details

Biomass Metric - Chlorophyll a

The biomass of phytoplankton is assessed by proxy using the chlorophyll a concentration as a surrogate. The annual geometric mean chlorophyll a concentration (*Chl*) is converted to an EQR using a modelled reference value (equation 1)

$$EQR_{Chl} = \frac{Chl_{\mathrm{Re}\,f}}{Chl} \qquad \text{.....equation 1}$$

Reference Chlorophyll

The reference chlorophyll a is predicted from a multiple regression model derived from 59 reference lakes (equation 2a).

$$Chl_{\mathrm{Re}\,f} = 10^{\left(0.223+0.166\times\log(Alk)+0.684\times\sqrt{1/Depth}\right)} \qquad \text{.....equation 2}$$

Where

Chl = geometric annual mean chlorophyll a concentration (µg/l)

$= 10^{LChl}$

LChl = mean of Log_{10} chlorophyll a values (µg/l)

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

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

The predicted reference chlorophyll a concentration is compared to a range of reference chlorophyll a concentrations which were set during Phase 1 of the intercalibration process (Poikane 2010). Where a value falls outside of this range, it is truncated to the upper or lower range limit (table 1). For lake types that have not been intercalibrated, site-specific reference chlorophyll values are constrained within the range of 1.3 – 6.0 µg/l.

[4] Analysts are subject to ring-tests and attend regular training sessions to ensure that their competency level is maintained

As the mean reference chlorophyll a values set during intercalibration are arithmetic, they are first transformed[5] to geometric means using a standard deviation estimated from a large EU data set (WISER), see equation 3

$$GeoChl = ArithChl \Big/ e^{(0.5\times(2.323\times SD)^2)}$$ …… equation 3

Where

GeoChl = Estimated geometric mean reference chlorophyll a defined during intercalibration

ArithChl = Arithmetic mean reference Chlorophyll defined during intercalibration

SD = standard deviation of $\log_{10}$Chl samples for a "typical" lake

= 0.213 for low and moderate alkalinity lakes (estimated from large EU data set)

= 0.285 for high alkalinity lakes (estimated from large EU data set)

To ensure that seasonal variability in chlorophyll a does not influence the estimate of the annual mean value, samples need to be taken at regular (monthly intervals). At least one monthly sample is required from each quarter of the year to classify a lake, although confidence in classification is increased if all 12 months are sampled (Carvalho et al., 2006). To avoid seasonal bias the mean annual chlorophyll value, samples are averaged by month , before calculating the annual average. If less than 6 samples are available, results should be averaged by month and then by quarter, prior to determining the annual average. This avoids introducing seasonal bias, as sampling is often less frequent in winter months due to poor weather or ice coverage.

Calculation of EQR and boundary setting

The approach to boundary setting is documented in the Phase 1 intercalibration reports, and the chlorophyll a EQR boundaries used here are those determined in that exercise (Table 1, and Poikane 2008). In the case of low alkalinity lakes (alkalinity < 0.2 mEq/l) the original chlorophyll a EQR boundaries were adjusted during harmonisation, and then normalised using piecewise linear transformation (equation 4)

$$ChlEQR_{Norm} = \left[\left(\frac{EQR_{Chl} - LowerBoundary}{ClassWidth}\right)\times 0.2\right] + LowerBoundary_{Norm}$$ …….equation 4

Where

$ChlEQR_{Norm}$ = Normalised EQR (e.g. HG = 0.80, GM = 0.60, MP = 0.40, PB =- 0.20)

LowerBoundary =lower un-normalised EQR boundary (see table1)

$LowerBoundary_{Norm}$ = lower normalised EQR boundary of class (e.g for Good = 0.60)

ClassWidth = Class width of non-normalised scale (e.g for Good = 0.55 – 0.32 = 0.23)

[5] For a log normal distribution the arithmetic and geometric means are related by AM = GM x exp($0.5SD^2$)

Lake Type	UK Type	IC Type (GIG)	Alkalinity (mEq/l)	Mean depth (m)	HG EQR	GM EQR	MP EQR	PB EQR	Min Ref Chl	Max Ref Chl
High alkalinity shallow	HAS	L-CB1	>1.0	3.0 - 15.0	0.55	0.32	0.16	0.05	2.1	3.1
High alkalinity very shallow	HAVS	L-CB2	>1.0	< 3.0	0.63	0.30	0.15	0.05	5.0	5.9
Moderate alkalinity deep	MAD		0.2 - 1.0	>15.0	0.50	0.33	0.17	0.05	1.3	6.0
Moderate alkalinity shallow	MAS	L-N1	0.2 - 1.0	3.0 - 15.0	0.50	0.33	0.17	0.05	2.2	3.1
Moderate alkalinity shallow humic	MAS	L-N8a	0.2 - 1.0	3.0 - 15.0	0.50	0.33	0.17	0.05	3.1	4.4
Moderate alkalinity very shallow	MAVS		0.2 - 1.0	< 3.0	0.63	0.30	0.15	0.05	1.3	6.0
Low alkalinity deep	LAD	L-N2b	<0.2	>15.0	0.64	0.33	0.17	0.05	1.3	2.2
Low alkalinity shallow	LAS	L-N2a	<0.2	3.0 - 15.0	0.64	0.29	0.15	0.05	1.3	2.2
Low alkalinity shallow humic	LAS	L-N3a	<0.2	3.0 - 15.0	0.64	0.29	0.15	0.05	1.3	2.2
Low alkalinity very shallow	LAVS		<0.2	< 3.0	0.63	0.30	0.15	0.05	2.2	3.1
Marl shallow	MarlS		>1.0	3.0 - 15.0	0.55	0.32	0.16	0.05	1.3	6.0
Marl very shallow	MarlVS		>1.0	< 3.0	0.63	0.30	0.15	0.05	1.3	6.0

Table 1 Lake type specific chlorophyll a EQR boundaries and range of constraining reference values used for UK phytoplankton classification

Taxonomic Metric – Plankton Trophic Index (PTI)

The Phytoplankton Trophic Index (PTI) was derived from a CCA ordination (univariate analysis) of the taxonomic data constrained by total phosphorus (log transformed). This single variable was most significantly related to the 1st axis of all the constrained ordinations tested and reflects the main pressure of concern in lake management, eutrophication. CCA reduces to a weighted average ordination in the case of a single variable (Braak and Looman 1986), and species axis 1 scores represent the $\log_{10}$ weighted average of total phosphorus. These scores were transformed to values between 0 (low pressure) and 1 (high pressure) by converting all the scores to positive values (by adding the lowest score), then dividing by the resulting maximum score.

The site PTI is calculated for each sample collected between July to September using equation 5. . The site PTI score is calculated by taking a mean value of all sample scores. To minimise uncertainty in classification, it is recommended that three monthly samples from July to September are collected each year for 3 years (i.e. 9 samples in total) (Carvalho et al., 2012), although as a bare minimum a classification result can be obtained with only three samples collected over 3 years.

Considering all PTI sample scores from all UK lakes, the resulting metric has a highly significant relationship with phosphorus and chlorophyll a (Figure 1).

$$PTI = \frac{\sum_{j=1}^{n} \log(a_j) s_j}{\sum_{j=1}^{n} \log(a_j)}$$ .....equation 5

Where:

a_j = biovolume of jth taxon in the sample ($\mu m^3\ ml^{-1}$) [6]

s_j = optimum of jth taxon in the sample (see table A1)

[6] The units are important due to the log transformation

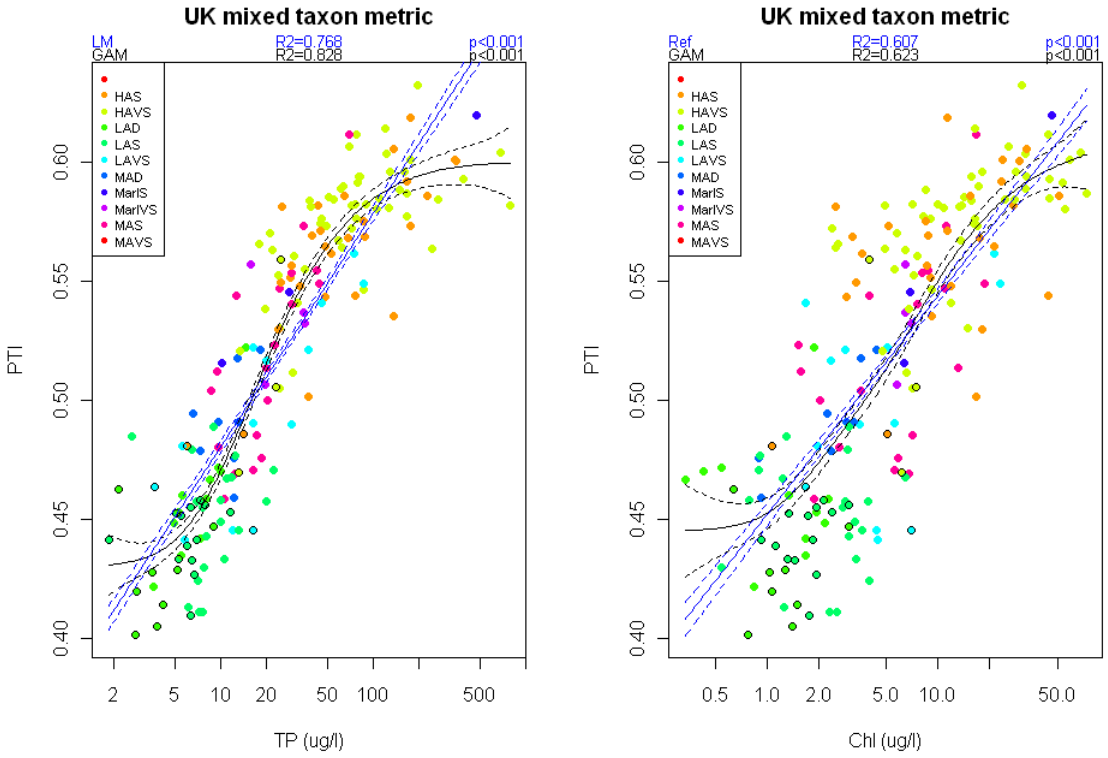


Figure 1. Relationship between PTI metric and a)mean annual total phosphorus, and b)mean annual chlorophyll a for UK lakes classified by waterbody type. Circles identify reference lakes

Correction of UK PTI during Intercalibration

The PTI metric calculated for UK sites in the intercalibration (WISER) database were different from those calculated for the same sites in the UK database due to the compromises in taxonomic nomenclature that were made for international harmonisation of the common (WISER) database. To compensate for this, NGIG[7] adjusted the PTI values calculated from the WISER intercalibration data set using the relationship between the scores calculated in the UK and those in the WISER database (PTI_{UK} = 0.889 PTI_{WISER} + 0.0589 R^2 = 0.977 $p<0.001$).

Reference PTI

The reference PTI is predicted from a multiple regression model derived from a sub-set (26) of reference lakes where taxonomic data were available at the time of method development (equation 6).

Reference PTI Model $PTI_{Ref} = 0.028 \times \log_{10}MEI + 0.498$ $R^2 = 0.688$ ...equation 6

Where

MEI = Alk/Depth (Morpho Edaphic Index)

If data for alkalinity or mean depth are not available, type specific reference PTI values should be used (Table 2)

[7] For CBGIG lakes UK EQR values were taken directly from the UK dataset and not from the WISER database.

Table 2. Median of site specific reference PTI values for UK lakes which are used as type specific reference values if data are not available to calculate a site specific reference PTI value

Lake Type	Ref PTI
High alkalinity shallow (HAS)	0.484
High alkalinity very shallow (HAVS)	0.501
Low alkalinity deep (LAD)	0.420
Low alkalinity shallow (LAS)	0.440
Low alkalinity very shallow (LAVS)	0.460
Moderate alkalinity deep (MAD)	0.443
Moderate alkalinity shallow (MAS)	0.465
Moderate alkalinity very shallow (MAVS)	0.484
Marl shallow (MarlS)	0.489
Marl very shallow (MarlVS)	0.499

Calculation of EQRPTI

Site specific reference PTI values are calculated for each lake, and then are used to convert the observed sample PTI to an EQR using equation 7

$$EQR_{PTI} = \left(\frac{PTI_{Obs} - PTI_{Max}}{PTI_{\mathrm{Re}\,f} - PTI_{Max}} \right)$$ .....equation 7

Where:

PTI_{Obs} = Sample PTI

PTI_{Max} = Maximum PTI score (*0.75)*

PTI_{Ref} = Reference PTI

Sample EQR_{PTI} are then averaged to obtain a water body EQR_{PTI}

Boundary setting for EQRPTI

EQR boundaries were initially set independently of the lake typology as the reference PTI are site specific and take into account alkalinity and depth (the key variables that have been found to determine the phytoplankton community; (Carvalho et al., 2007a; Phillips *et al.* 2010). The boundaries were subsequently reviewed in the light of type specific pressure responses and were also adjusted during the intercalibration process to ensure they were consistent with other European countries.

The High/Good EQR boundary was based on the 10th percentile of EQR_{PTI} values for reference lakes (H/G EQR_{PTI} = 0.93). The other EQR boundaries were set using changes in the proportion of taxa sensitivity

groups, split according to their nutrient optima and with reference to the bio-volume of eutrophic cyanobacteria taxa. The fractions of very sensitive and very tolerant taxa and the relationships between EQR_{PTI} and eutrophic cyanobacteria were examined and potential boundaries identified using GAM and quantile regression models. The Good/Moderate boundary was initially set at 0.82, the point at which 50% of lakes still have 20% of the very sensitive taxa and 90% of lakes have less than 10% of the very tolerant taxa. Cyanobacteria first show an increase in biomass at an EQR_{PTI} of 0.85 (Figure 2), a value that is below the proposed High/Good boundary and slightly above the proposed Good/Moderate boundary. At this point the response mainly occurs in high alkalinity lakes and although it represents more than a "slight" change in the phytoplankton community, it is clearly not a significant undesirable impact at this level. It is therefore consistent with good status, although the change in cyanobacterial response and the associated EQR_{PTI} value indicate that conditions are indeed approaching the Good/Moderate boundary. The Moderate/Poor boundary was set at 0.70, the point at which 50% of lakes have more than 5% of very tolerant taxa. The Poor/Bad boundary was set at 0.58, a value which provides the same class width for Poor as for Moderate (see Figure 3 for all modelled boundaries).

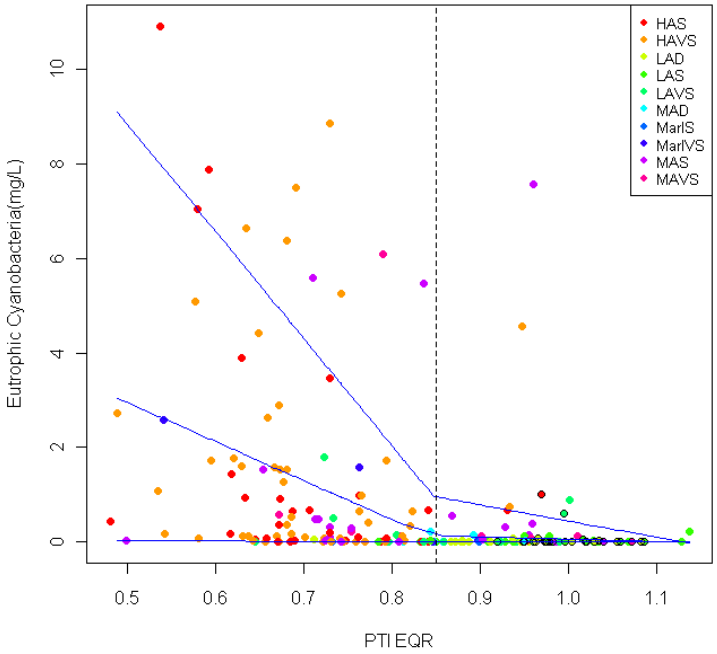


Figure 2: The relationship of EQR_{PTI} with the biovolume of eutrophic cyanobacteria. The 90th and 75th quantiles are given, reference sites are outlined and the potential EQR G/M boundary is shown at 0.85.

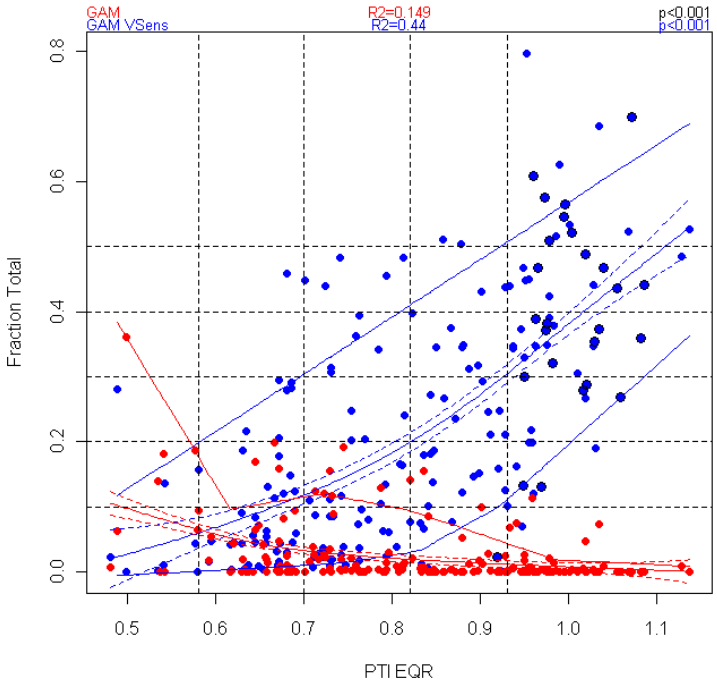


Figure 3. The relationship between EQR_{PTI} and the fraction of very sensitive taxa (blue spots) and very tolerant (red spots) together with 90th and 10th quantile regressions and GAM models. Reference sites are outlined and the potential boundaries at EQR_{PTI} 0.93, 0.82, 0.70 and 0.58 are shown.

Although it was initially intended to apply these EQR boundaries to all lake types, it was observed that the EQR from lakes of different alkalinity types had significantly different relationships with pressure despite the use of a site specific model to determine reference conditions. The importance of alkalinity on the phytoplankton community has also been identified in larger European data sets (Phillips *et al.* 2010). These different relationships were quantified using linear mixed models (Figure 4) with EQR_{PTI} as the dependent variable, log TP as a co-variable and type as a random variable. The model revealed significant differences in intercept between types, but not in slope. The model was repeated using fixed slopes and the resulting random effect values due to lake type (i.e. the differences in intercepts) were used to adjust the proposed EQR boundaries (Table 3).

Table 3. Random effect of lake geology type on relationship between PTI EQR and logTP for UK lakes, and the type specific EQR adjustments to account for this effect.

Lake Geology Type	Random effect of type on intercept of linear model	EQR adjustment
High Alkalinity	-0.021	-0.02
Moderate Alkalinity	-0.004	0.00
Low Alkalinity	+0.022	+0.02
Marl	+0.003	0.00

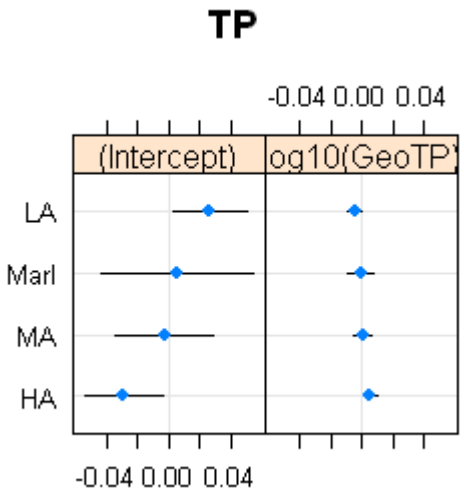


Figure 4. The range of intercept and slope values for linear mixed models between PTI EQR and logTP. Horizontal lines show confidence limits.

During the intercalibration process these boundaries were adjusted to ensure that the UK method was not less precautionary than other member states with similar lake types. Boundaries for other UK lake types that could not be intercalibrated were adjusted based on those that were. Very shallow lakes were assumed to have less stringent boundaries than shallow lakes and low alkalinity humic lakes to have less stringent boundaries than low alkalinity clear water lakes. The original and final harmonised EQR_{PTI} boundaries are shown in Table 4.

The EQR_{PTI} is normalised using quadratic functions of the form

$$PTIEQR_{Norm} = A \times EQR_{PTI}^{\ 2} - B \times EQR_{PTI} - C$$

Parameters used for each lake type are also given in Table 4.

Lake Type	Humic Type	UK Type	IC Type (GIG)	Type Parameter values			Original Boundaries				Harmonised Boundaries				Normalisation equation
				Alkalinity mEq/l	Mean depth m	Colour mgPt/l	HG EQR	GM EQR	MP EQR	PB EQR	HG EQR	GM EQR	MP EQR	PB EQR	
High alkalinity shallow		HAS	L-CB1	>1.0	3.0 - 15.0	not used	0.91	0.80	0.68	0.56	0.93	0.82	0.70	0.58	EQR_{Norm} = 1.228 x EQR^2 - 0.0898 x EQR - 0.1538
High alkalinity very shallow		HAVS	L-CB2	>1.0	< 3.0						0.91	0.80	0.68	0.56	EQR_{Norm} = 1.228 x EQR^2 - 0.0407 x EQR - 0.1551
Moderate alkalinity deep		MAD		0.2 - 1.0	>15.0		0.93	0.82	0.70	0.58	0.95	0.84	0.72	0.60	EQR_{Norm} = 1.228 x EQR^2 - 0.1389 x EQR - 0.1515
Moderate alkalinity shallow		MAS	L-N1, L-N8a	0.2 - 1.0	3.0 - 15.0										
Moderate alkalinity very shallow		MAVS		0.2 - 1.0	< 3.0						0.93	0.82	0.70	0.58	EQR_{Norm} = 1.228 x EQR^2 - 0.0898 x EQR - 0.1538
Low alkalinity deep	Clear	LADcl	L-N2b	<0.2	>15.0	≤ 30	0.95	0.84	0.72	0.60	0.98	0.87	0.75	0.63	EQR_{Norm} = 1.228 x EQR^2 - 0.2004 x EQR - 0.147
Low alkalinity deep humic	Humic	LADhm		<0.2	>15.0	> 30					0.95	0.84	0.72	0.60	EQR_{Norm} = 1.228 x EQR^2 - 0.1389 x EQR - 0.1515
Low alkalinity shallow	Clear	LAScl	L-N2a	<0.2	3.0 - 15.0	≤ 30					0.98	0.87	0.75	0.63	EQR_{Norm} = 1.228 x EQR^2 - 0.2004 x EQR - 0.147
Low alkalinity shallow humic	Humic	LAShm	L-N3a	<0.2	3.0 - 15.0	> 30					0.96	0.85	0.73	0.61	EQR_{Norm} = 1.228 x EQR^2 - 0.1512 x EQR - 0.1508
Low alkalinity very shallow	Clear	LAVScl		<0.2	< 3.0	≤ 30					0.95	0.84	0.72	0.60	EQR_{Norm} = 1.228 x EQR^2 - 0.1389 x EQR - 0.1515
Low alkalinity very shallow humic	Humic	LAVShm		<0.2	< 3.0	> 30					0.93	0.82	0.70	0.58	EQR_{Norm} = 1.228 x EQR^2 - 0.0898 x EQR - 0.1538
Marl shallow		MarlS		>1.0	3.0 - 15.0		0.93	0.82	0.70	0.58	0.95	0.84	0.72	0.60	EQR_{Norm} = 1.228 x EQR^2 - 0.1389 x EQR - 0.1515
Marl very shallow		MarlVS		>1.0	< 3.0						0.93	0.82	0.70	0.58	EQR_{Norm} = 1.228 x EQR^2 - 0.0898 x EQR - 0.1538

Table 4. EQR boundaries for Plankton Trophic Index (PTI). The harmonised boundaries are the final values used in the UK method following intercalibration. Equations for normalisation are also shown.

Bloom Intensity Metric – Cyanobacteria bio-volume

The WFD requires that the assessment of lake phytoplankton should include an assessment of the frequency and intensity of algal blooms. It does not define an algal bloom, but a definition emerging from the WFD intercalibration process is that it refers to an elevated biomass of cyanobacteria. Cyanobacteria are associated with enriched conditions in lakes and can produce a high biomass of potentially toxic algae which can restrict the use of a lake. This is a clear case of "undesirable disturbance" as defined by the WFD (European Commission 2009). Although increases in cyanobacteria are, in part, indicated by both an elevated biomass (chlorophyll concentration) and an increase in the PTI, the UK method now includes a direct assessment of cyanobacterial biomass using the geometric mean[8] biovolume of cyanobacteria.

Boundary Setting for Cyanobacteria biomass

The cyanobacteria metric assesses "undesirable disturbance" by indicating the risk of cyanobacterial blooms occurring, using the low and medium risk thresholds defined as by the World Health Organisation as 20,000 and 100,000 cells ml^{-1} respectively (WHO 1999). These values were converted to bio-volume thresholds of 1 and 5 $mm^3 l^{-1}$ by multiplication of a typical cell volume (based on a spherical cell such as *Microcystis* with a cell diameter of 4.5µm; (Hillebrand *et al.* 1999).

Status boundaries were set in accordance with the Eutrophication Guidance (European Commission 2009). This document proposes an increasing risk of undesirable disturbances, thus at Good status there should be a very low probability of blooms occurring. The likelihood increases through the Moderate class and is high at Poor status. The distribution of cyanobacteria biomass in a population of summer samples can be used to assess how often a particular lake exceeds these thresholds and consequently a classification can be derived. It is proposed that at the High/Good boundary 90% of samples from a particular lake would be below the 1 $mm^3 l^{-1}$ threshold, and at the Good/Moderate75% of samples would be below this threshold. The Moderate/Poor boundary was set where 75% of samples were above the 1 $mm^3 l^{-1}$ threshold and below 5 $mm^3 l^{-1}$, and the Poor/Bad boundary where at least 75% of samples exceeded the 5 $mm^3 l^{-1}$ threshold (Figure 5).

European lakes (EU FP7 WISER Project database) were classified according to the distribution of cyanobacteria using the above rules. The median summer cyanobacteria bio-volume (July – September) was calculated for each lake. The distribution of these median values in each class was determined and boundary values were set at the overlap between the upper and lower 25th percentiles of adjacent classes (Figure 6 and Table 5). The High/Good boundary median cyanobacteria biovolume is well below the WHO (1999) "vigilance" level (0.2 $mm^3 l^{-1}$), and the Good/Moderate boundary is below the low risk threshold and is therefore consistent with a low risks of "undesirable disturbance".

Table 5. Boundary values and EQRs for summer cyanobacteria biomass

[8] (Originally a median value was used, however when developing the uncertainty module for the phytoplankton classification it was found that the geometric mean was a more appropriate metric to summarise cyanobacteria. For a log-normal distribution the median is equal to the geometric mean and thus replacing the median with the geometric mean will not significantly change the classification.)

Boundary	Cyanobacteria biomass (July - September) samples	Geometric mean cyanobacteria bio-volume ($mm_3\ l^{-1}$)		EQR boundary values	
		Low & Moderate Alkalinity & Marl lakes	High alkalinity lakes	Low & Moderate Alkalinity & Marl lakes	High alkalinity lakes
Reference		0	0.01	1.00	1.00
High/Good	90th percentile < 1mg/l	0.08	0.20	0.47	0.63
Good/Moderate	75th percentile < 1mg/l	0.56	1.00	0.32	0.43
Moderate/Poor	25th percentile < 5mg/l	1.58	2.00	0.23	0.34
Poor/Bad	10th percentile >5 mg/l	5.62	5.62	0.13	0.21

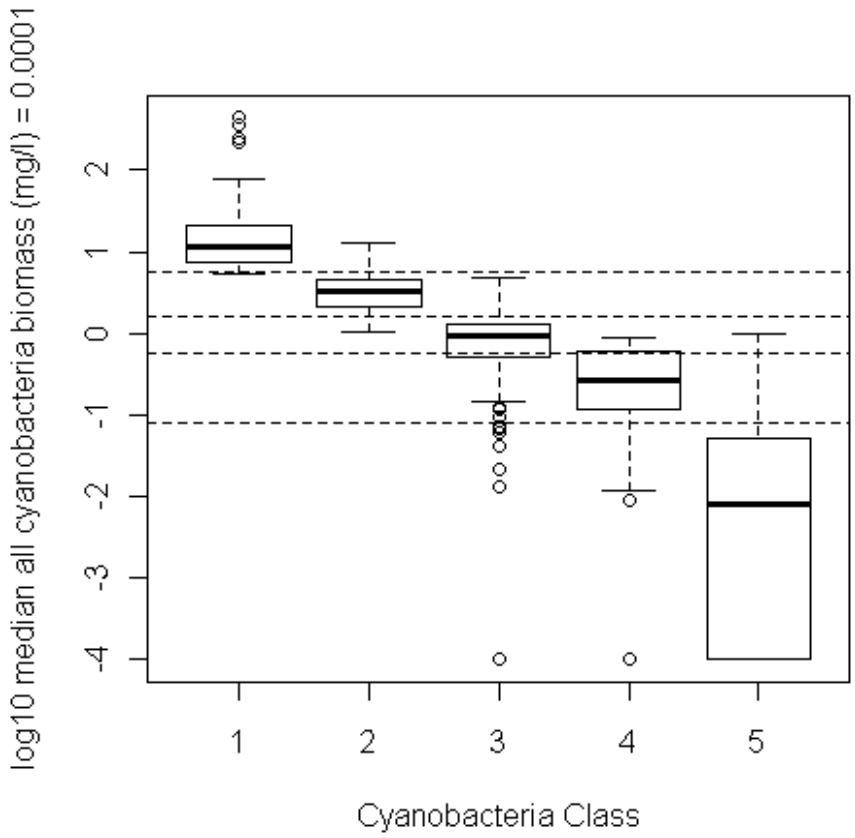


Figure 6, Distribution of median biomass of cyanobacteria in European lakes in different WFD classes (5 high, 4 good, 3 moderate, 2 poor, 1 bad). Boxes represent upper and lower 25th percentiles, lines 90th percentiles. Horizontal dotted lines mark boundary values for median summer cyanobacteria.

Conversion to EQR

The geometric mean cyanobacteria bio-volumes were converted to EQRs using the following equation[9].

$$EQR_{Cyan} = \frac{\log(BV_{Obs} + 0.0001) - \log(BV_{Max} + 0.0001)}{\log(BV_{\mathrm{Re}\,f} + 0.0001) - \log(BV_{Max} + 0.0001)} \quad \text{.....equation 8}$$

[9] Logarithms are used to create a realistic class width on the EQR scale

Where

BV_{Obs} = geometric mean bio-volume cyanobacteria ($mm^3\ l^{-1}$) [10]

BV_{Ref} = geometric mean bio-volume cyanobacteria in reference lakes ($mm^3\ l^{-1}$)

= 0.01 $mm^3\ l^{-1}$ for high alkalinity lakes

= 0.00 $mm^3\ l^{-1}$ for other lake types

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

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

The EQRCyan is then normalised using equation 8 for combination with other metrics

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

.....equation 9

For derivation of terms see equation 3

Combination of metrics

To calculate an overall EQR for the phytoplankton BQE ($PhytoEQR_{Norm}$), the normalised metric EQRs are combined by averaging.

The $ChlEQR_{Norm}$ and the $PTIEQR_{Norm}$ are first averaged to produce an interim EQR ($IntEQR_{Norm}$).

The cyanobacteria metric is only included in order to downgrade a lake status where blooms are likely; the absence of cyanobacteria should not upgrade the status of a lake, as cyanobacteria are generally very rare in certain lake types (e.g. low alkalinity and humic lakes). Consequently, only when the $CyanEQR_{Norm}$ is < $IntEQR_{Norm}$ is it averaged with $IntEQR_{Norm}$, otherwise the cyanobacteria metric is ignored.

The resulting overall EQR represent status on a standard scale with boundaries of HG= 0.80, GM=0.60, MP=0.40 and PB=0.20

Data checking & uncertainty estimation

Classification is normally based on data collected over the preceding three years.

Samples for Chlorophyll a must be collected evenly throughout the year (i.e. at the same time each month). Twelve monthly samples should be used, but at minimum of 1 sample from each quarter of the

[10] To convert from $\mu m^3\ ml^{-1}$ to $mm^3\ l^{-1}$ divide by 10^6

year is required to calculate a representative mean. Confidence of class is only calculated where there are 6 or more samples in the classification period, otherwise the classification is recorded as "uncertain".

Phytoplankton counts should be checked by comparing the calculated total sample bio-volume against a value predicted from the sample chlorophyll a value (equation 10). If the total sample bio-volume is outside of the predicted value ±95th percentile of the modelled residuals the sample should be marked as "suspect" and the results compared with other samples from the same lake and time of year, before the results for Cyanobacteria and PTI are included in the overall average EQR.

$$BV_{\mathrm{Pr}ed} = 10^{1.18\times\log(Chl)-1.11)} \qquad \text{.....equation 10}$$

$$UpperBV_{\mathrm{Pr}ed} = 10^{1.18\times\log(Chl)-1.11+0.5)}$$

$$LowerBV_{\mathrm{Pr}ed} = 10^{1.18\times\log(Chl)-1.11-0.5)}$$

Uncertainty assessment and example calculation

General assumptions

The confidence of class assessments assume that sampling is carried out in such a way as to provide representative and un-biased estimates of the metrics used to assess status. The following assessment is based on the following sampling strategy. Monthly samples, which are representative of the open water of the lake should be collected and chlorophyll a determined. During, July, August and September separate samples taken from the same location are counted to determine the number and size of phytoplankton taxa. For a single year this will provide 12 chlorophyll a values and 3 PTI and Cyanobacteria bio-volume estimates. For a classification period covering 3 years, these values are combined to provide 36 chlorophyll a values and 9 taxonomic metrics.

The confidence of class of each of the metrics is based on the metric EQR and estimates of its uncertainty, converted to a confidence of class following the approach outlined by (Ellis and Adriaenssens 2006), except that a logit transformation was not used, as the EQRs for phytoplankton are not bounded by a maximum value 1.0 and in practice never approach 0.0.

It is assumed that each of the metrics (Chla, PTI, Cyanobacteria) are independent and following the approach of (Davey 2009) the final EQR is given by

$$EQR_{Phyto} = \frac{\sum EQR_a}{a}$$

And the standard error of the combined metric is given by

$$SE(EQR_{Phyto}) = \frac{\sqrt{\sum_{a=1}^{a} SE(EQR_a)^2}}{a}$$

Where

EQR = Normalised EQR for each metric (Chlorophyll, PTI, Cyanobacteria)

SE = Standard error of normalised EQR for each metric

a = 2 or 3 depending on whether the first two or all 3 metrics are combined

The confidence of class for each metric and the combined metrics are calculated using the t- distribution approach where the probability of the observed EQR_{Norm} being less than the class EQR boundaries is calculated using

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

where

t denotes the cumulative Student t probability distribution (TDIST in Excel) with degrees of freedom = the number of individual EQR results contributing to the final EQR result.

(note degrees of freedom for the final combined EQR is estimated using the Welch-Satterthwaite equation, see below)

For classes 1 – 5 (Bad to High) the confidence of each class is calculated as

Confidence of class 5 (High) = $1 - p_5$

Confidence of class 4 (Good) = $p_5 - p_4$

Confidence of class 3 (Moderate) = $p_4 - p_3$

Confidence of class 2 (Poor) = $p_3 - p_2$

Confidence of class 1 (Bad) = p_1

Chlorophyll metric

An analysis of spatial and short-term (4 year) temporal variability in lakes (Carvalho et al., 2006; 2007a; 2012; Phillips 2012) demonstrates that monthly variation is a significant component of variability for chlorophyll a, year was less significant and sample location was not significantly different components. This is to be expected as the biomass of phytoplankton has a very strong seasonal component, and long-term change is relatively slow (Jeppesen *et al.* 2005). This was not the case for the taxonomic metrics, as they are collected during a short time window, representing the late summer phytoplankton. Thus

for the chlorophyll metric it is important that all months are equally represented in the annual mean value. To ensure that this is the case samples are averaged by month, prior to averaging of the whole data set. Where samples are not available for all calendar months data are averaged by month and then by quarter prior to calculating the annual mean.

In addition (Carvalho *et al.* 2006)showed that with regular sampling the true standard error (SE_{str}) of the resulting mean 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 Log10 the SE_{rand} could be reduced using the following function.

$$SE_{Str} = SE_{Rand} \times 1.0293 \times Sy^{-0.2379}$$

Where

Sy = number of regular samples per year

Example calculation for Low Alkalinity Deep lake

3 years of data from a low alkalinity deep lake (mean depth = 16.6m, Alkalinity = 0.057mEq l^{-1}

LChl = annual mean of Log_{10}Chl = 0.122

Chl = annual geometric mean Chl = $10^{0.122}$ = 1.32

SD_{LChl} = standard deviation of Log_{10}Chl = 0.310

SE_{LChl} = standard error of Log_{10}Chl = 0.310 / √32 = 0.055

N_{Chl} = number of samples = 32

Years = number of years sampled = 3

Sy = estimated samples per year = 32/3 = 11

Calculate the reference chlorophyll a value from alkalinity and depth using equation 2

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

$$Chl_{Ref} = 10^{\left(0.223+0.166\times\log(0.057)+0.684\times\sqrt{1/16.6}\right)}$$

$Chl_{Ref} = 1.53$ µg l^{-1} (check this value falls within range of values shown in table 1, re-set to min or max value shown in table 1)

Calculate the EQR for chlorophyll a (using equation 1)

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

$$EQR_{Chl} = \frac{1.53}{1.32} = 1.155$$

Normalise the EQR for chlorophyll a using equation 4 selecting the lower type specific EQR boundary values from those in table 1 and taking the normalised boundaries as 0.80, 0.60, 0.40, 0.20.

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

$$ChlEQR_{Norm} = \left[\left(\frac{1.155 - 0.64}{1.00 - 0.64}\right) \times 0.2\right] + 0.80$$

$$ChlEQR_{Norm} = \left[\left(\frac{0.515}{0.36}\right) \times 0.2\right] + 0.80 = 1.086$$

Thus the face value class for chlorophyll a is High as the normalised EQR is greater than 0.80

To determine the confidence of class it is first necessary to estimate the standard error of the normalised EQR for chlorophyll a. This is done following the method proposed by (Davey 2009). First determine the upper (U95) and lower (L95) 95% confidence limits of the observed mean chlorophyll and convert these values to EQRs

To do this first calculate an adjusted chlorophyll a standard error to allow for regular sampling.

$SE_{Str} = SE_{LChl} \times 1.0293 \times Sy^{-0.2379}$ ……. Adjust standard error for regular sampling

$$SE_{Str} = 0.055 \times 1.0293 \times 11^{-0.2379} = 0.032$$

then use this value to calculate U95 and L95 using the inverse of the Student t-distribution

$$L95_{Chl} = 10^{(LChl - TINV \times SE_{Str})}$$

Where TINV = inverse of the t-distribution where p=0.05 for N-1 degrees of freedom (31 in this example)

so

$$L95_{Chl} = 10^{(0.122 - 2.0395 \times 0.032)} = 1.140$$

$U95_{Chl} = 10^{(0.122+2.0395\times0.032)}$ = 1.538

Use these values to calculate EQRs for lower (L95EQR) and upper (U95EQR) confidence limits

$$L95EQR = \frac{Chl_{\mathrm{Re}f}}{U95_{Chl}} \quad \text{and} \quad U95EQR = \frac{Chl_{\mathrm{Re}f}}{L95_{Chl}}$$

$$L95EQR = \frac{1.530}{1.538} = 0.994 \quad \text{and} \quad U95EQR = \frac{1.530}{1.140} = 1.341$$

Normalise these EQR values as above

$$U95EQR_{Norm} = \left[\left(\frac{1.341-0.64}{1.00-0.64}\right)\times 0.2\right] + 0.80 = 1.190$$

$$L95EQR_{Norm} = \left[\left(\frac{0.991-0.64}{1.00-0.64}\right)\times 0.2\right] + 0.80 = 0.997$$

Estimate the standard error of the normalised chlorophyll EQR

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

$$SE(EQR_{Norm}) = \frac{1.190-0.997}{2\times 2.0395} = 0.047$$

Finally calculate the confidence of class for chlorophyll using the t distribution approach

Probability (p_i) of observed EQR_{Norm} being worse than class boundary EQR_i is given by

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

where

t denotes the cumulative Student t probability distribution (TDIST in Excel) with

so

Probability of this lake being worse than High is

$$p_{High} = t\left(\frac{0.80 - 1.086}{0.047)}\right)$$ = 0.000

Thus the confidence that the status is High is 1.00 – 0.000 = 1.000

Taxonomic Metrics

The plankton trophic index (PTI) and cyanobacteria biomass metrics are calculated for each sample in the classification period. These sample results are converted to normalised EQRs and the mean values used to determine confidence of class

Extending the example of the low alkalinity deep lake there were 4 taxonomic samples available for the period covered by chlorophyll (2009-2011), 28/09/09, 07/07/10, 19/08/10, 09/08/11. An example calculation for PTI and cyanobacteria are shown below for a single sample. Summary metrics for the other samples are then used to show how confidence of class of each metric and the overall class are determined

Determine PTI metric

An example for a single sample from lake taken in July is shown in Table 6.

Calculate the sample PTI using equation 5

$$PTI = \frac{\sum_{j=1}^{n} \log(a_j) s_j}{\sum_{j=1}^{n} \log(a_j)}$$ .....equation 5

Where:

a_j = biovolume of *j*th taxon in the sample (μm^3 ml^{-1}) [11]

s_j = optimum of *j*th taxon in the sample (see table A1)

The product term in the numerator of equation 5 (PTI Optima (s) x Log_{10} bio-volume (a)) is shown in Table 6 and the resulting PTI value for this sample is show below

$$PTI = \frac{35.687}{81.428}$$ = 0.438

Reference PTI is calculated from equation 5b

[11] The units are important due to the log transformation

Reference PTI Model $PTI_{Ref} = 0.028 \times \log_{10}MEI + 0.498$ ...equation 5b

where

MEI = Alk/Depth (Morpho Edaphic Index)

MEI = 0.057/16.6 = 0.0034

so

$PTI_{Ref} = 0.028 \times \log_{10}0.0034 + 0.498 = 0.429$

Calculate EQR for PTI (EQR_{PTI}) using equation 6

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

Where:

PTI_{Obs} = Sample PTI

PTI_{Max} = Maximum PTI score (*0.75)*

PTI_{Ref} = Reference PTI

$$EQR_{PTI} = \left(\frac{0.438 - 0.75}{0.429 - 0.75} \right) = 0.972$$

Calculate Normalise EQR for PTI ($PTIEQR_{Norm}$) using normalisation equations in Table 3

$$PTIEQR_{Norm} = A \times EQR_{PTI}^{\ 2} - B \times EQR_{PTI} - C$$

$$PTIEQR_{Norm} = 1.228 \times 0.972^2 - 0.2004 \times 0.972 - 0.147 \quad \text{(for a low alkalinity deep lake)}$$

$$PTIEQR_{Norm} = 0.818$$

Thus the face value class for PTI is High as the normalised EQR is greater than 0.80

Table 6 Taxa list for low alkalinity deep lake, sampled in July showing taxon PTI optima, as an example of calculating the observed plankton trophic index (PTI) for the lake.

SpeciesCode	Taxon name	PTI Optima	Bio Vol (µm3/ml)	Log BVol	Product (Optima x Log BVol)	Cyan BioVol (mm3/l)
01050000	Aphanocapsa	0.539	1131	3.05	1.646	0.0011
05040001	Cryptomonas (small) Length	0.53	39596	4.60	2.437	
05040002	Cryptomonas (medium) Length	0.533	6489	3.81	2.032	
05040003	Cryptomonas (large) Length	0.589	548	2.74	1.613	
05100000	Rhodomonas	0.539	83	1.92	1.035	
05100012	Rhodomonas lacustris var.	0.473	802	2.90	1.374	
06070000	Gymnodinium	0.46	125727	5.10	2.346	
06110050	Peridinium cinctum	0.485	20896	4.32	2.095	
08010000	Chrysochromulina	0.341	812	2.91	0.992	
09000000	Chrysophyceae	0.324	3153	3.50	1.134	
09030000	Bitrichia	0.288	227	2.36	0.678	
09230030	Dinobryon crenulatum	0.201	148	2.17	0.436	
09230050	Dinobryon divergens	0.392	1479	3.17	1.243	
09310000	Mallomonas	0.452	3654	3.56	1.610	
09430000	Pseudokephyrion	0.345	73	1.86	0.643	
09480000	Stichogloea	0.293	9477	3.98	1.165	
09559920	Pseudopedinella (big >5um)	0.37	592	2.77	1.026	
13820020	Tabellaria flocculosa	0.295	2874	3.46	1.020	
17000000	Chlorococcales	0.503	1227	3.09	1.554	
17580000	Monoraphidium	0.538	44	1.65	0.885	
17640000	Oocystis	0.54	1157	3.06	1.654	
25010000	Elakatothrix	0.437	75	1.88	0.820	
27370000	Staurastrum	0.458	47	1.67	0.767	
27390000	Staurodesmus	0.251	815	2.91	0.731	
90000000	Picoplankton - unidentified	0.539	129	2.11	1.137	
90000003	Nanoplankton - unidentified	0.532	4141	3.62	1.924	
90000005	Nanoplankton - unidentified	0.519	1803	3.26	1.690	
		Sum	227202	81.428	35.687	0.0011

Determine Cyanobacteria metric

Using the total bio-volume of cyanobacteria (BV_{Obs} = 0.0011 see table X) calculate the EQR for cyanobacteria using equation 7

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

Where

BV_{Obs} = geometric mean bio-volume cyanobacteria ($mm^3\ l^{-1}$) [12]

BV_{Ref} = geometric mean bio-volume cyanobacteria in reference lakes ($mm^3\ l^{-1}$)

= 0.01 $mm^3\ l^{-1}$ for high alkalinity lakes

[12] To convert from $\mu m^3\ ml^{-1}$ to $mm^3\ l^{-1}$ divide by 10^6

= 0.00 $mm^3\ l^{-1}$ for other lake types

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

$$EQR_{Cyan} = \frac{\log(0.0011+0.0001)-\log(30.0+0.0001)}{\log(0.0000+0.0001)-\log(30.0+0.0001)}$$

$$EQR_{Cyan} = \frac{-4.3869}{-5.4771} = 0.801$$

Normalise the EQR_{Cyan} using equation 8 and boundary values from table 4

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

$$CyanEQR_{Norm} = \left[\left(\frac{0.801-0.47}{1.00-0.47}\right)\times 0.2\right] + 0.80 = 0.925$$

Thus the face value class for cyanobacteria is High as the normalised EQR is greater than 0.80

Determine confidence of class for the taxonomic metrics

First calculate mean and standard deviation for PTI and Cyanobacteria normalised EQRs using the sample normalised EQR values (Table 7)

Table 7 Normalised sample EQR values for PTI and Cyanobacteria metrics for example lake and summary statistics for the period 2009 – 2011

Sample Date	28/09/09	07/07/10	19/08/10	09/08/11	Mean	Standard Deviation (StDev)	Samples (N)	Standard Error (StDev/Sqrt(N)
$PTIEQR_{Norm}$	0.952	0.818	0.909	0.839	0.880	0.062	4	0.031
$CyanEQR_{Norm}$	0.952	0.925	0.958	0.923	0.939	0.018	4	0.009

Determine confidence of class of each metric and the combined metrics using t-distribution method as for chlorophyll a

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

For PTI

P worse High for PTI = $t\left(\frac{0.80 - 0.879}{0.031}\right)$ = 0.041

Confidence PTI is High = 1 – 0.041 = 0.96

Confidence PTI is Good = 0.041- 0.000 = 0.04

For Cyanobacteria

P worse High for Cyanobacteria = $t\left(\frac{0.80 - 0.939}{0.009}\right)$ = 0.000

Confidence Cyanobacteria is High = 1 – 0.000 = 1.00

Combine metrics to determine overall class

First average the chlorophyll and PTI normalised EQRs and test to see if this less than the cyanobacteria normalised EQR

Mean $ChlEQR_{Norm}$ = 1.086 Mean PTI EQR_{Norm} = 0.880

Mean CyanEQRNorm = 0.939

Mean Chl and PTI = 0.983

As the cyanobacteria metric has a lower normalised EQR than the average of chlorophyll and EQR all three metric EQRs are averaged to determine the overall class

Mean Chl,PTI, Cyan = 0.968 which is a face value class of High status as value is > 0.80 the High/Good class boundary

To determine the confidence of class first calculate a combined standard error using the standard errors for each of the three normalised EQRs

$SE(ChlEQR_{Norm})$ = 0.047

$SE(PTIEQR_{Norm})$ = 0.031

SE(CyanEQR$_{Norm}$) = 0.009

$$SE(EQR_{Phyto}) = \frac{\sqrt{\sum_{a=1}^{a} SE(EQR_a)^2}}{a}$$

$$SE(EQR_{Phyto}) = \frac{\sqrt{0.047^2 + 0.030^2 + 0.009^2}}{3} = 0.019$$

Determine the confidence of class for the combined metric using the t-distribution. Calculate the degrees of freedom (n) for the combined metric using the Welch Satterthwaite estimate

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

Where

s_i^2 is estimated variance of each metric

n_i is degrees of freedom 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)

First estimate the standard deviation of ChlEQRNorm from standard error

$$s(ChlEQR_{Norm}) = SE(EQR_{Norm}) \times \sqrt{N}$$

$$s(ChlEQR_{Norm}) = 0.047 \times \sqrt{32} = 0.267$$

Determine the standard deviation of the normalised PTI and Cyanobacteria EQRs

s PTIEQR$_{Norm}$ = 0.062

s CyanEQR$_{Norm}$ = 0.018

Use these values to determine degrees of freedom (n)

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

$$n = \frac{\left\{0.267^2/_3 + 0.062^2/_3 + 0.018^2/_3\right\}^2}{\left\{\frac{\left(0.267^2/_3\right)^2}{(32-1)} + \frac{\left(0.062^2/_3\right)^2}{(4-1)} + \frac{\left(0.0.018^2/_3\right)^2}{(4-1)}\right\}} = 33.7$$

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

P worse High for Phytoplankton = $t\left(\frac{0.80 - 0.968}{0.019}\right)$ = 0.000

Confidence Phytoplankton is High = 1 – 0.000 = 1.00

Dealing with missing taxonomic metrics

Given the importance of the biomass response of phytoplankton it is considered essential to have chlorophyll a data available for classification. However, in some situations taxonomic data may not be available and in these situations a preliminary classification can be carried out using only chlorophyll data. However, comparing the Chlorophyll EQR with the Overall EQR from a large UK data set demonstrated that on average the inclusion of the other metrics lowers the final EQR. To allow for this, if classifying status using on chlorophyll a data, the Chlorophyll EQR is modified using a linear regression model derived from the above data.

$PhytoEQR_{Norm} = 0.829 \times ChlEQR_{Norm} - 0.004$

However, when determining a classification in this way it would not be appropriate to report the final class with high confidence (defined by UKTAG as > 95%) even if the statistical confidence calculated from the chlorophyll metric was this high and thus a maximum confidence of “uncertain” is reported in this situation.

A2 Summary of Changes between 1st and 2nd RBMP

In the first cycle of the RBMP, impacts on phytoplankton abundance were assessed on the basis of chlorophyll a concentrations and impacts on the natural composition of phytoplankton species were assessed using the percentage of eutrophic cyanobacteria. The new method uses a new indicator of impacts on composition; the Plankton Trophic Index (PTI) and assesses impacts on phytoplankton blooms by considering the abundance by biovolume of cyanobacteria. It also continues to assess impacts on phytoplankton abundance using chlorophyll a concentrations. In the new method, the reference values are predicted from alkalinity and depth rather than from total phosphorus.

A3 Consequences of changes

England

Table 8. Comparison of classifications of ecological status determined by original and revised (PLUTO) versions of the phytoplankton tool.

		Revised					
		High	Good	Moderate	Poor	Bad	Grand Total
Current	High	72	12				84
	Good	17	52	13			82
	Moderate		18	58	2		78
	Poor		1	25	55		81
	Bad			3	3	5	11
	Grand Total	89	83	99	60	5	336

Table 9. Percentage of water bodies in each class, determined using original and revised (PLUTO) versions of the phytoplankton tool.

Class	Current Method	Revised Method
High	25.0%	26.5%
Good	24.4%	24.7%
Moderate	23.2%	29.5%
Poor	24.1%	17.9%
Bad	3.3%	1.5%

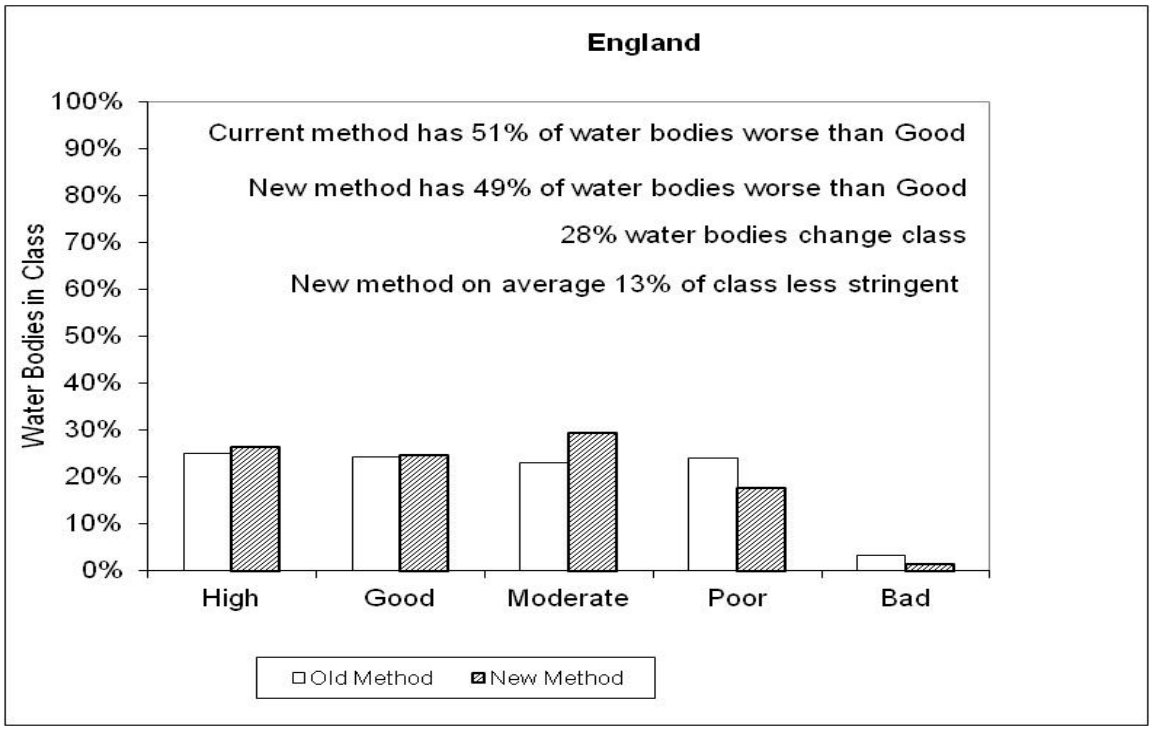


Figure 5. Percentage of water bodies in each class, determined using original and revised (PLUTO) versions of the phytoplankton tool.

Table 10. Number and percentage of water bodies that change class when using the revised version of the phytoplankton tool, PLUTO.

	Number	Percentage
Current 4 class worse	0	0.0%
Current 3 class worse	0	0.0%
Current 2 class worse	4	1.2%
Current 1 class worse	63	18.8%
Same class	242	72.0%
Revised 1 class worse	27	8.0%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%

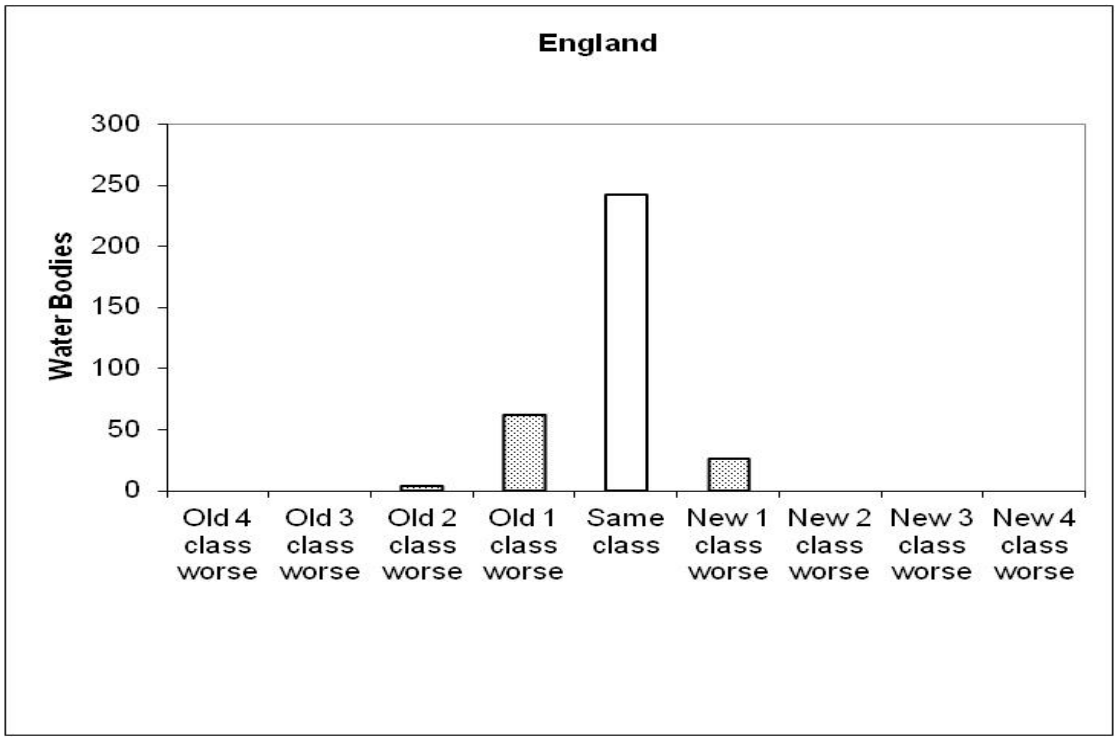


Figure 6. Number of water bodies that change class when using the revised version of the phytoplankton tool, PLUTO.

Wales

Table 11. Comparison of classifications of ecological status determined by original and revised (PLUTO) versions of the phytoplankton tool.

		Revised					
		High	Good	Moderate	Poor	Bad	Grand Total
Current	High	46	5				51
	Good	5	15	2			22
	Moderate	1	5	6			12
	Poor			1	2		3
	Bad					1	1
	Grand Total	52	25	9	2	1	89

Table 12. Percentage of water bodies in each class, determined using original and revised (PLUTO) versions of the phytoplankton tool.

Class	Current Method	Revised Method
High	57.3%	58.4%
Good	24.7%	28.1%
Moderate	13.5%	10.1%
Poor	3.4%	2.2%
Bad	1.1%	1.1%

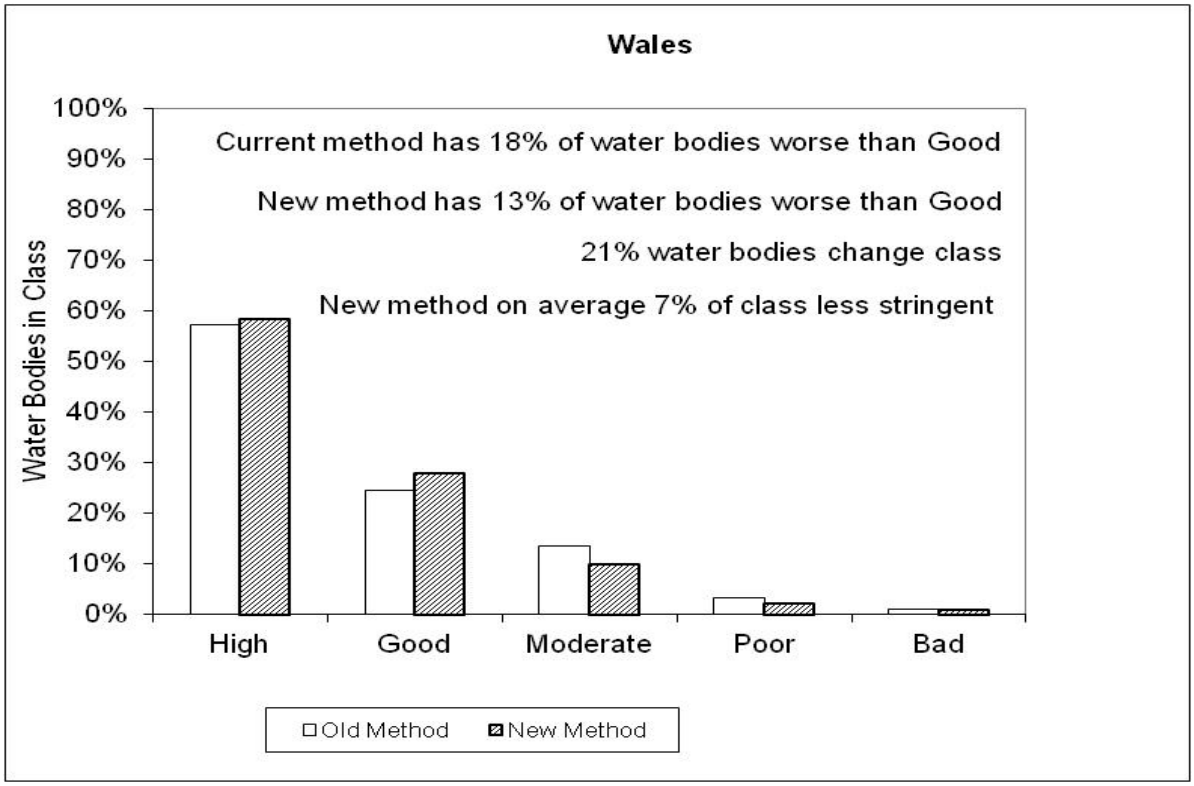


Figure 7. Percentage of water bodies in each class, determined using original and revised (PLUTO) versions of the phytoplankton tool.

Table 13. Number and percentage of water bodies that change class when using the revised version of the phytoplankton tool, PLUTO.

	Number	Percentage
Current 4 class worse	0	0.0%
Current 3 class worse	0	0.0%
Current 2 class worse	1	1.1%
Current 1 class worse	11	12.4%
Same class	70	78.7%
Revised 1 class worse	7	7.9%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%

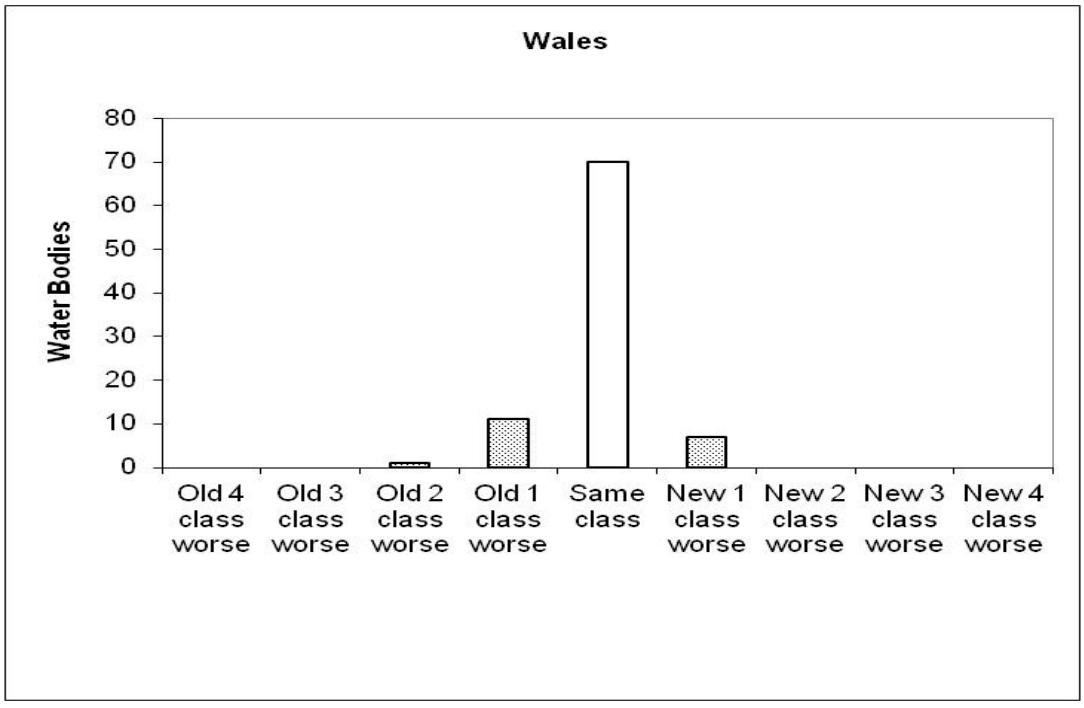


Figure 8. Number of water bodies that change class when using the revised version of the phytoplankton tool, PLUTO.

Scotland

Table 14. Comparison of classifications of ecological status determined by original and revised (PLUTO) versions of the phytoplankton tool.

		Revised					
		High	Good	Moderate	Poor	Bad	Grand Total
Current	High	35	5			35	40
	Good	11	8			11	19
	Moderate	2	3	5		2	10
	Poor	2	1	5	5	2	13
	Bad				2		2
	Grand Total	50	17	10	7	50	84

Table 15. Percentage of water bodies in each class, determined using original and revised (PLUTO) versions of the phytoplankton tool.

Class	Current Method	Revised Method
High	47.6%	59.5%
Good	22.6%	20.2%
Moderate	11.9%	11.9%
Poor	15.5%	8.3%
Bad	2.4%	0.0%

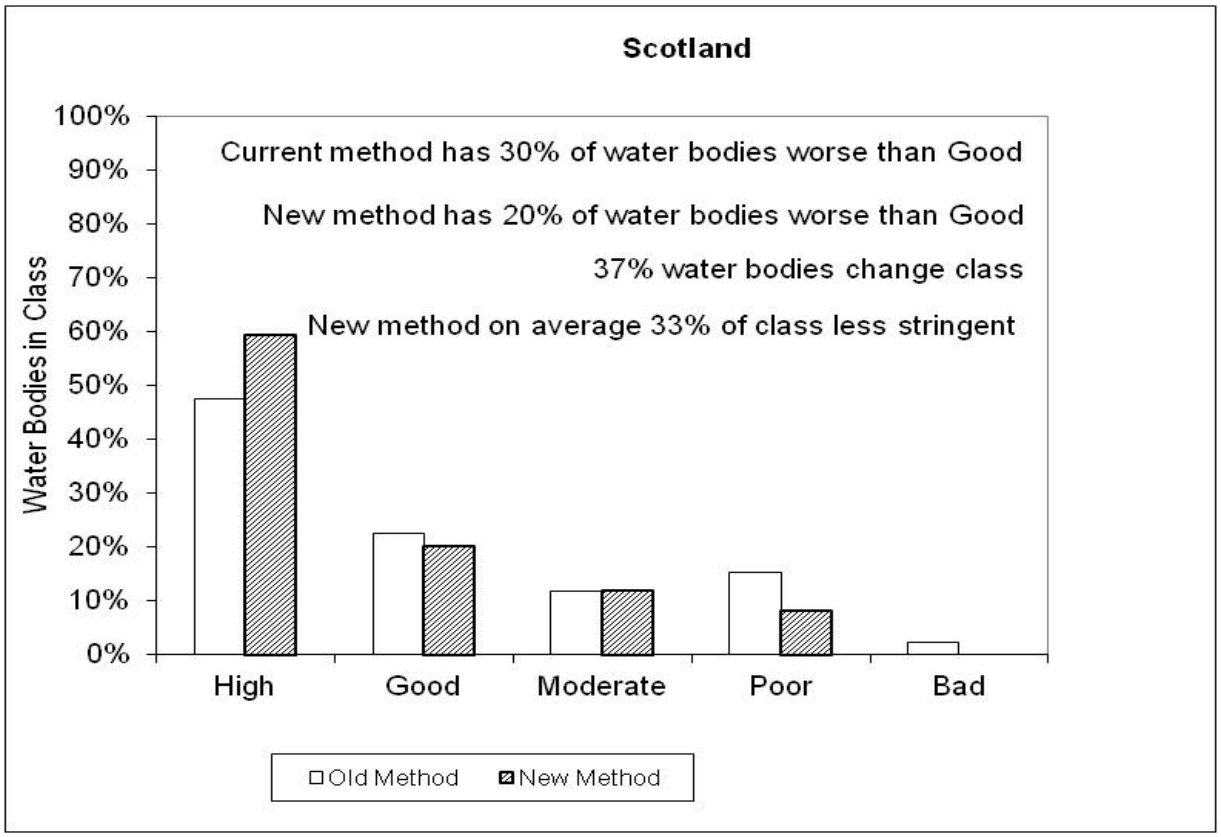


Figure 9. Percentage of water bodies in each class, determined using original and revised (PLUTO) versions of the phytoplankton tool.

Table 16. Number and percentage of water bodies that change class when using the revised version of the phytoplankton tool, PLUTO.

	Number	Percentage
Current 4 class worse	0	0.0%
Current 3 class worse	2	2.4%
Current 2 class worse	3	3.6%
Current 1 class worse	21	25.0%
Same class	53	63.1%
Revised 1 class worse	5	6.0%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%

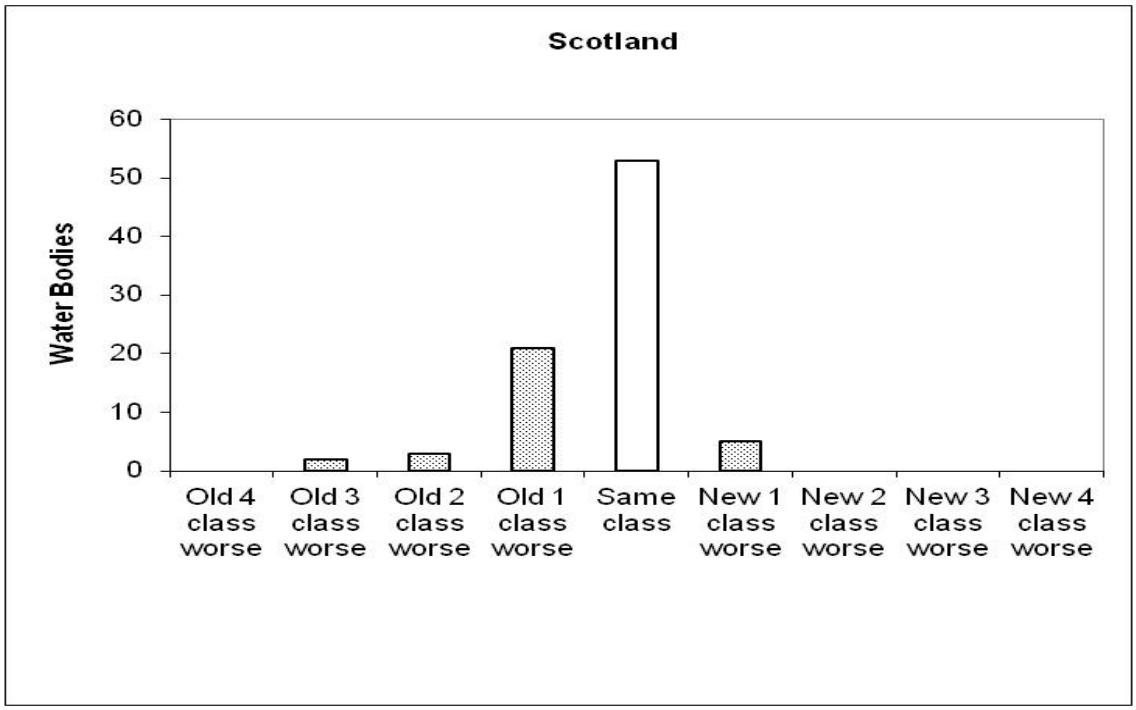


Figure 10. Number of water bodies that change class when using the revised version of the phytoplankton tool, PLUTO.

Northern Ireland

Table 17. Comparison of classifications of ecological status determined by original and revised (PLUTO) versions of the phytoplankton tool.

		Revised					
		High	Good	Moderate	Poor	Bad	Grand Total
Current	High	6	1				7
	Good	3	3	2			8
	Moderate			3			3
	Poor			1	8		9
	Bad						
	Grand Total	9	4	6	8		27

Table 18. Percentage of water bodies in each class, determined using original and revised (PLUTO) versions of the phytoplankton tool.

Class	Current Method	Revised Method
High	25.9%	33.3%
Good	29.6%	14.8%
Moderate	11.1%	22.2%
Poor	33.3%	29.6%
Bad	0.0%	0.0%

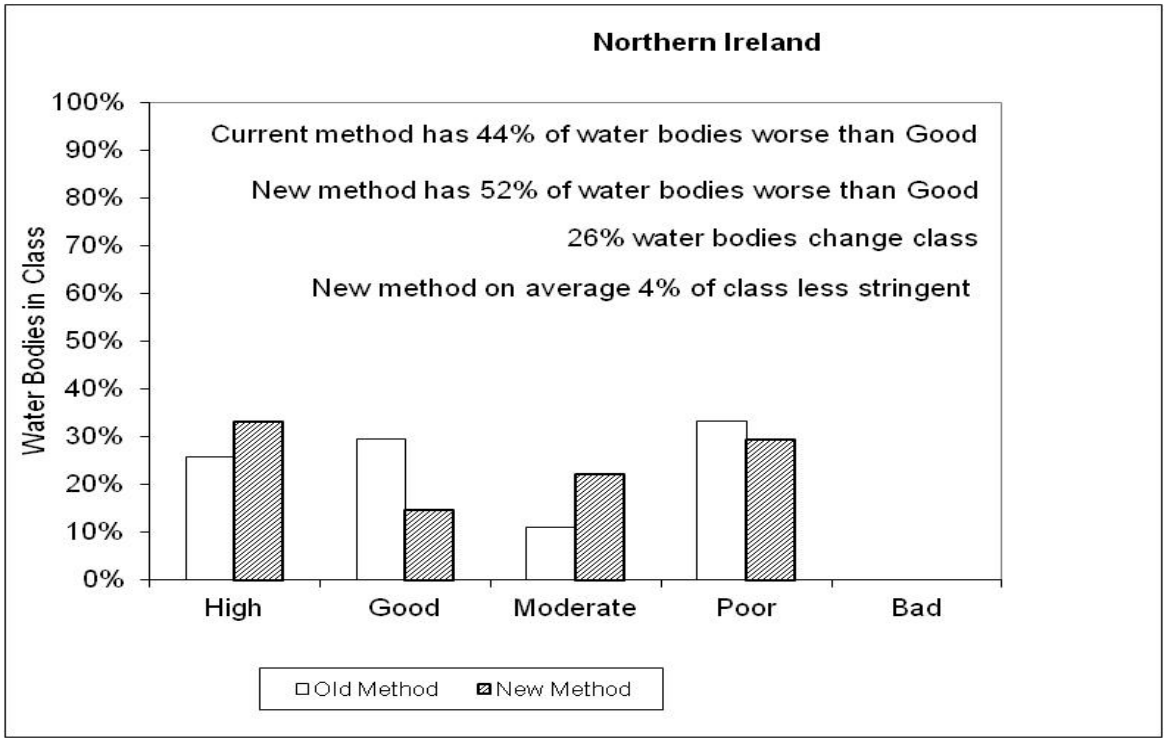


Figure 11. Percentage of water bodies in each class, determined using original and revised (PLUTO) versions of the phytoplankton tool.

Table 19. Number and percentage of water bodies that change class when using the revised version of the phytoplankton tool, PLUTO.

	Number	Percentage
Current 4 class worse	0	0.0%
Current 3 class worse	0	0.0%
Current 2 class worse	0	0.0%
Current 1 class worse	4	14.8%
Same class	20	74.1%
Revised 1 class worse	3	11.1%
Revised 2 class worse	0	0.0%
Revised 3 class worse	0	0.0%
Revised 4 class worse	0	0.0%

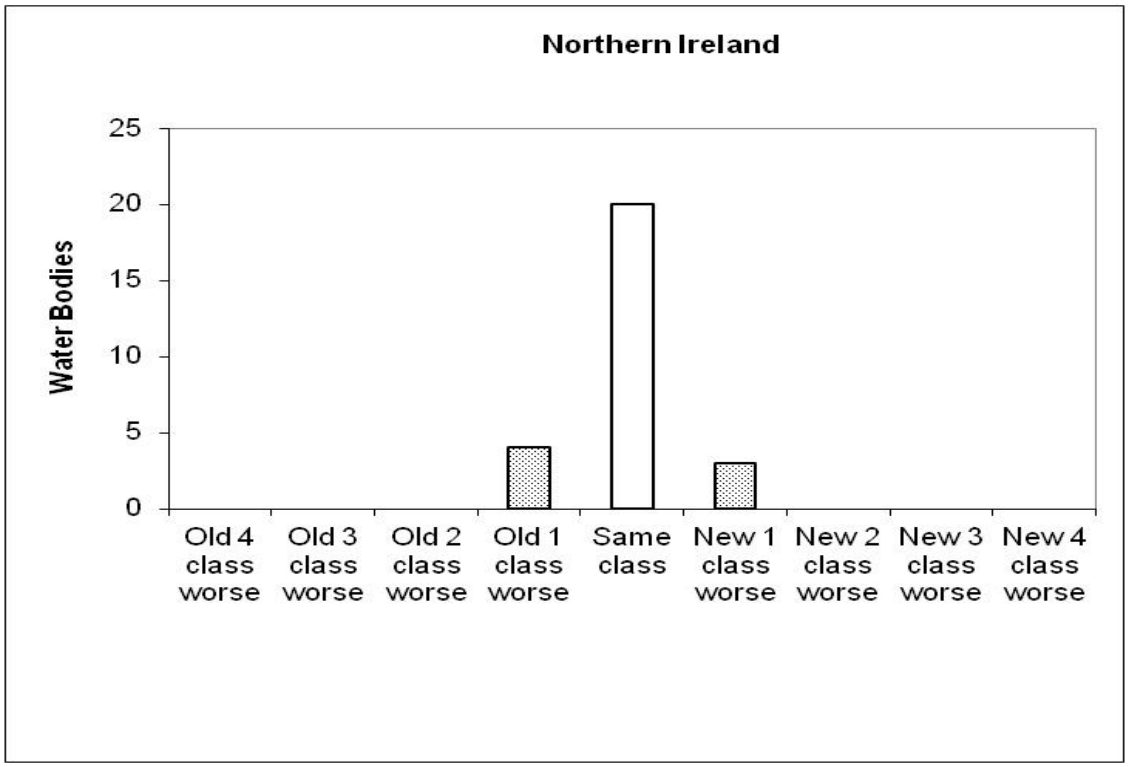


Figure 12. Number of water bodies that change class when using the revised version of the phytoplankton tool, PLUTO.

4 Key documents and References

Method statement	Detailed description of method used for 1st RBMP (chlorophyll and nuisance cyanobacteria metrics only)

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