Final Report **Project WFD80**

Phytoplankton Classification Tool (Phase 2)

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EXECUTIVE SUMMARY

WFD80: Phytoplankton Classification Tool (Phase 2) (June, 2007)

Project funders/partners: SNIFFER (WFD80) & Environment Agency

Background to research
The Environment Agency and SNIFFER have commissioned Phase 2 of this R & D project to develop the method to classify the ecological status of lakes on the basis of phytoplankton. As part of this assessment, metrics need to be developed for phytoplankton community composition.

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

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

Key findings and recommendations
Following collation of a dataset of matching phytoplankton and environmental data from 300 lake samples, a multivariate approach to metric development was adopted. CCA was used to develop a species-environment model for phytoplankton, with the main typology variables (alkalinity, altitude, mean depth) included as significant explanatory variables in the model alongside two variables indicative of eutrophication pressure (chlorophyll and TP concentrations). The CCA model indicated strong correlations between the eutrophication pressure gradients (Chlorophyll and TP) and alkalinity. This highlighted the potential problem of developing simple univariate optima of phytoplankton taxa against pressure gradients.

Optima were derived for 112 of the most common phytoplankton taxa (mixed Genus and species level) along both eutrophication gradients (chlorophyll and TP) using reciprocal averaging. Although this was still a univariate approach, the correlative effect with alkalinity is removed later through the calculation of an EQR by taking account of a site's alkalinity in the reference score. A metric, the Phytoplankton Index of Eutrophication (PIE) was developed which averages the taxa optima at a site, weighted by their abundance (log_{10} biovolume), to give an Observed PIE Score. Comparisons of various weightings of the community data clearly showed that this metric showed the strongest relationships with both log_{10}TP (r^2 = 0.60) and log_{10}Chlorophyll (r^2 = 0.64). The metric showed significant relationships with the two pressure gradients for all lake alkalinity types, although was weakest for low alkalinity lakes.

Three approaches were explored for establishing Expected, or Reference, PIE Scores for a site using data from 50 reference lake samples. Firstly, stepwise regression was carried out examining which typology variables explained significant variance in reference site PIE scores. Alkalinity was the only typology variable selected with a significant positive relationship between PIE score and alkalinity. Using this regression model, site-specific reference PIE scores can be predicted for any UK or Irish lake of known alkalinity. Type-specific reference PIE scores were also established with the regression model on the

basis of the median alkalinity measures in the UK and Irish lake dataset. It is recommended that, if possible, modelled site-specific reference conditions are adopted as they are ecologically more appropriate and correlate better with observed PIE scores at reference lakes than type-specific reference scores.

EQRs were calculated from the ratio of Observed to Expected PIE scores, which were then transformed, to produce an EQR ranging from 0 to 1. The High/Good (H/G) boundary was determined from the lower 25% of reference site EQRs. To derive the remaining boundaries, phytoplankton taxa were classified as "positive" (low eutrophication pressure) or "negative" (high eutrophication pressure) indicators of eutrophication pressure, by examining their optima and tolerances in a constrained CCA model. The % biovolume of positive and negative taxa was calculated for each sample and polynomial regression analysis was carried out to examine their relationships with EQR. The crossover point in these two relationships was chosen to represent the Good/Moderate (G/M) status class boundary. The 75% of residuals in the two equations were then used to identify the Moderate/Poor (M/P) status class boundary and the remaining Poor/Bad (P/B) boundary was derived from a division of the remaining EQR scale.

The PIE metric was applied to all UK and Irish lake phytoplankton samples. Observed and Expected PIE scores and resultant EQRs are given in Appendix 2. The mean variance in EQR scores between samples from different months for the same lake was relatively low. The relationship between observed EQR and variance in EQR was used to estimate the confidence in classification of results and mis-classification rate for a given EQR.

A number of sources of uncertainty, or error, in EQRs and associated constituent measures (observed PIE scores, sample biovolume, number of taxa) were examined, focusing on sample processing errors due to the combination of sub-sample and counter analytical error. The resultant estimates of the average within-site sampling/processing variability can also be used, in software such as STARBUGS, to derive estimates of uncertainty in assigning water bodies to a WFD ecological status class. These results are, however, preliminary, based on limited data and unstructured sampling. The effects of larger scale within-lake spatial variability on EQRs also needs to be examined, with estimates in more lakes over a wide range of ecological qualities using nested replicate samples and replicate sub-samples before these results can be considered reliable.

Sources of variation in chlorophyll data due to within-lake spatial variation and laboratory analytical variability were examined and compared with previous work on temporal variability. The largest source of variability in chlorophyll concentrations within a lake is temporal, but if sampling is carried out monthly then much of the seasonal variability is eliminated and becomes smaller than the estimated variance between replicate samples taken on the same day. If sampling is restricted to the outflow of lakes, the least variable location, then the estimate of replicate sampling variance becomes less than laboratory variance.

In terms of minimising uncertainty in both chlorophyll and composition classifications, it is recommended that sampling is carried out at a single specific location within a site, where the location is representative of the lake as a whole (outflow or centre of lake). Samples should be taken from July to September at a monthly frequency.

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

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1. INTRODUCTION

1.1 Phytoplankton in the Water Framework Directive

The EC Water Framework Directive (WFD) is the most significant piece of European water legislation for over twenty years. A key component of the Directive is the development of ecological classification tools for determining the ecological status of water bodies. Such tools need to be sensitive to specific environmental pressures. These include immediate pressures such as point and diffuse chemical inputs, and longer-term pressures such as climate change.

The Environment Agency and SNIFFER are funding the development of a package of new classification methods in order to satisfy the requirements of the WFD. WFD requires the ecological status of water bodies to be assessed on the condition of their biological quality elements (Article 8, annex V). For lakes this includes phytoplankton. For this purpose, SNIFFER have commissioned this R & D project (WFD 80) to further develop a method to classify the ecological status of lakes on the basis of phytoplankton communities. This follows on from Phase 1, the SNIFFER project WFD38 (Carvalho et al., 2006a).

Annex V of the WFD outlines three features of the phytoplankton quality element that need to be considered in the assessment of the ecological status of lakes. These three are:

1. Phytoplankton composition
2. Phytoplankton abundance and its effect on transparency conditions
3. Planktonic bloom frequency and intensity

Phytoplankton abundance and its effect on transparency conditions has already been considered and a classification scheme for phytoplankton chlorophyll$_a$ has been established for this purpose (Carvalho et al., 2006b). This report focuses on the first of these sub-elements, developing a classification scheme for phytoplankton composition. As part of this, the value of a % cyanobacteria metric, potentially in relation to planktonic bloom intensity is also considered.

Further relevant information in the WFD includes the normative definitions for phytoplankton in lakes associated with five ecological status classes. These definitions indicates that declining ecological quality is associated with increasing phytoplankton abundance, composition shifts and more frequent and intense phytoplankton blooms (Table 1.1)

Table 1.1 Qualitative criteria for assessing Ecological Status in terms of eutrophication impacts on phytoplankton (modified from ECOSTAT Eutrophication Guidance, 2005)

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

1.2 Phytoplankton and eutrophication

Eutrophication, or the enrichment of ecosystems with plant nutrients, is one of the most widespread pressures affecting European freshwaters. There are numerous socio-economic problems associated with eutrophication-related increases in phytoplankton abundance, particularly with increasing frequency and intensity of toxic cyanobacteria blooms. These include detrimental effects on drinking water quality, filtration costs for water supply, water-based activities, and conservation status (particularly sensitive fish species, such as salmonids and coregonids).

The fact that phytoplankton are short-lived and derive their nutrients from the water column makes this biological quality element the most direct and earliest indicator of impacts of changing nutrient conditions on lake ecosystems. Potentially, therefore, phytoplankton are ideal indicators of deteriorating ecological status associated with increasing nutrient status (eutrophication), or, of ecological recovery, or improving status, following reductions in nutrient loads.

The phytoplankton community is, however, notoriously diverse and dynamic. Developing an ecological classification specifically in relation to nutrient pressures requires minimising the effects of seasonal variability associated with the changing physical and biological structure of the water column and magnifying the signal related to nutrient pressures.

Individual species or taxa can be positive or negative indicators in relation to nutrient pressures. Widely recognised positive indicators include species of chrysophytes (e.g. *Dinobryon*), desmids (e.g. *Cosmarium*) and diatoms (e.g. *Cyclotella comensis*). Negative indicators include species of green algae (e.g. *Scenedesmus*), diatoms (e.g. *Stephanodiscus*) and many groups of cyanobacteria, such as the large colonial and filamentous genera *Microcystis*, *Aphanizomenon* and *Anabaena*. The latter are favoured by relatively stable stratification and high alkalinity and can, therefore, also form a

significant natural component of the phytoplankton community in deep alkaline lakes, i.e. they do not necessarily always indicate impacted conditions. As taxonomic status at the phylum/class level does not consistently represent positive or negative indicators, higher taxonomic resolution to genus or species level may be necessary for effective classification tool development.

1.3 Project Objectives

The objective of the project is to develop an operational tool for classifying ecological status in UK lakes on the basis of their phytoplankton community. To fit with the requirements of the WFD, the phytoplankton classification scheme needs to ensure that it:

- delivers a phytoplankton metric that is applicable to all UK lakes
- defines the expected reference condition for the phytoplankton metric for an individual site or lake type
- distinguishes 5 status classes High/Good/Moderate/Poor/Bad using reference-based Ecological Quality Ratios
- includes actual or relative abundance as well as composition
- reports the error or uncertainty in classification results

This report aims to deliver such a scheme for phytoplankton composition and illustrate its application to both UK and Irish lakes. Further uncertainty work on phytoplankton chlorophyll is also delivered.

Additional aspects associated with the work have included delivering:

- standardised sampling and counting guidance
- standardised recorder forms
- a comprehensive phytoplankton database for UK and Irish lakes, and
- a working classification tool

2. METHODS

2.1 Field sampling

Phytoplankton samples were collected from a range of locations: open water, edge and outflows of lakes. The type of sample collected was dependent on location, for example if an open water sample was collected using a boat, this was an integrated vertical sample. Edge and outflow samples were collected using a bottle. For future sampling, more standardised guidance is detailed below.

Number of sampling sites and replicate samples per water body

On the basis of an assessment of spatial and replicate sample variability (Chapters 7 & 8), one sample from one sampling station per lake appears to be generally sufficient. If there are clear sub- basins within a lake, these should be sampled separately, for example the 3 basins of Elter Water, Loch Lomond North, South and Central Basins and Windermere North and South Basin, to ensure sub-basins are adequately represented by a single sample.

Sampling frequency

The greatest source of variability in chlorophyll data (and potentially also phytoplankton composition data) is temporal variability. To minimise uncertainty in results, monthly sampling for both chlorophyll and phytoplankton composition is recommended. For phytoplankton composition, only 3 samples from July to September are required for model application.

Sampling location

A sample taken from the deepest part of the lake (or centre) is widely adopted across Europe as a standard and is generally thought to be representative for the majority of lakes. If boat access is not possible a sample taken by the lake outflow is the next best alternative.

To minimise sources of variability, it is recommended that sampling location within individual lakes does not vary between sampling occasions, e.g. either the lake centre or the outflow is always sampled for a particular lake. The chlorophyll uncertainty analysis suggested that the outflow was less variable than open water, probably due to its restricted spatial scale. It will, therefore, provide generally more precise measures of chlorophyll, although these may not necessarily be the most accurate representation of the lake basin as a whole.

Sampling Method

In open water, a vertical integrated water sample should be taken using a wide-bore hose-pipe. The depths at which samples are taken depend upon the lake. The following guidance is taken from Olrik et al. (1998):

- In shallow lakes (<4 m) with occasional stratification, collect vertical integrated samples down to 1 m above the sediment.
- In deeper lakes (>4 m) and during unstratified conditions, collect vertical integrated samples to cover the depth of the euphotic zone, estimated using a Secchi disc (approximately 2.5 x Secchi depth), but as a minimum down to 3 m.
- During stratified periods samples should be taken from the epilimnion.

For outflow samples, water should be collected using a bottle on a rope thrown from the edge. The bottle should have a weight and a float attached in order to sample about 30 cm below the surface. Care should be taken not to contaminate the sample with benthic algae from littoral sediments or vegetation.

The volume of water needed is dependent on phytoplankton abundance – 1 litre is generally sufficient, but may need more in very nutrient poor lochs or only 100 ml in very enriched lochs.

Samples should be preserved with acidified Lugol's solution for analysis and short-term storage (less than 1 year). It is recommended an additional sub-sample is taken and preserved with formaldehyde for longer-term storage. Lugol's iodine should be added in the field at a ratio of 100 ml of sample to 1 ml of Lugol's solution.

2.2 Sample analysis

Detailed guidance on analysing phytoplankton samples was developed during the project in collaboration with a counter workshop (Brierley & Carvalho, 2007; Appendix 1). Additionally a standardised counter form was developed to ensure consistency with data recording. All phytoplankton data used in the project were analysed following the first workshop, adopting the standard counter guidance. This was to ensure consistency in taxonomic identification, biovolume estimates and counting procedures.

This guidance is currently under review in order to further reduce sources of counter variability in phytoplankton composition data. It is recommended that EA & SEPA staff, or individuals contracted to count phytoplankton samples for the WFD, undergo training and testing in applying the counter guidance.

2.3 Datasets

The majority of data available were for the months July to September. For this reason, only a 'summer' model has been developed based on these three months. A model based on all samples, including the winter and spring samples, was rejected in order to reduce the effects of seasonality on composition. This left 300 samples available from 172 lake basins for model development, of which 45 samples (15%) were from reference sites. There was a more or less even spread of samples across these three months for both reference and non-reference lakes (Figure 2.1)

High alkalinity lakes were slightly better represented in the dataset than medium or low alkalinity lakes, with 41% of samples compared with 28% and 30% respectively (Table 2.1). Shallow and very shallow lakes were also better represented than deep lakes (Table 2.1). A similar representation of lake types was not, however, present in the set of reference lakes used in the model, with 62% of reference lake samples taken from low alkalinity lakes and only 24% and 14% from medium and high alkalinity lakes (Table 2.2). Further data from medium or high alkalinity reference lakes would be beneficial in order to provide a more balanced coverage across typology and pressure gradients.

Phytoplankton data were summarised for each sample as total biovolume per taxon, with most taxa recorded at either the genus or species level, although higher taxonomic units were used where genus level identification was not possible (e.g. unicellular centric diatoms, unidentified flagellates). Taxa occurring in less than 4 samples were excluded from the analysis as were a number of benthic diatom taxa (*Cocconeis*, *Cymbella*, *Gyrosigma*, *Navicula*).

Typology variables used in model development included: lake surface area, altitude, mean depth, mean alkalinity and water colour. Total phosphorus and chlorophyll concentrations were also included in model development as measures of eutrophication impact. Month was also included in the analysis to represent seasonal effects.

Typology, chemistry and phytoplankton biovolume data were all log transformed to normalise the data. Multivariate analysis (DCA and CCA) was carried out using CANOCO version 4.5.

Figure 2.1 Number of phytoplankton samples counted by month

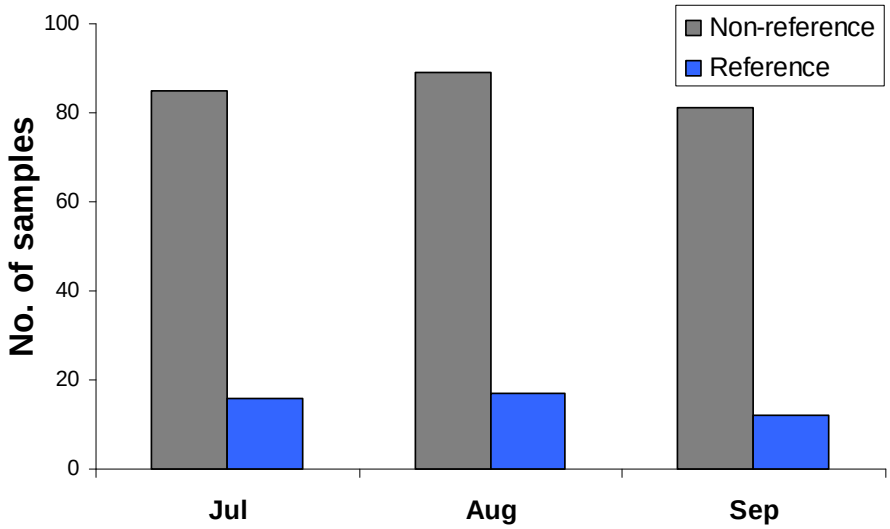


Table 2.1 Samples classified according to GB depth and alkalinity classes

	Alkalinity Type				
	Low	**Medium**	**High**	**Unknown**	**Total**
Very Shallow	22	20	75	1	118
Shallow	40	44	48	3	135
Deep	27	19			46
Unknown			1		1
Total	89	83	124	4	300

Table 2.2 Samples from reference lakes classified according to GB depth and alkalinity classes

	Alkalinity Type			
	Low	**Medium**	**High**	**Total**
Very Shallow	10	7	1	18
Shallow	16	4	6	26
Deep	5	1		6
Total	31	12	7	50

Data were also available from 670 Central European lake samples from 9 countries and these data (Central GIG dataset) were used to explore the value of certain metrics.

2.4 Project databases

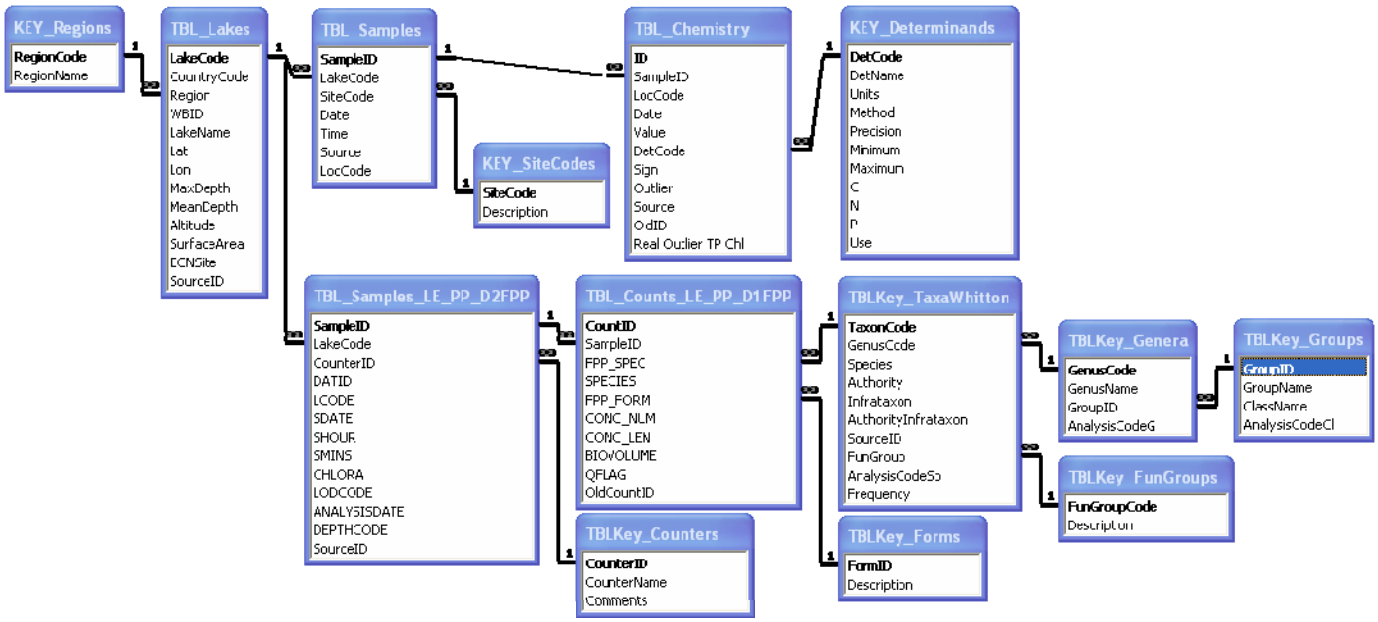
Microsoft Access was used to create two separate databases:

1) Lake typology and chemistry database for UK and Irish lakes
2) Phytoplankton composition database for UK and Irish lakes.

Tables and queries from these two databases are then combined in a third linked database. This third database has a user-friendly front-end form for data selection. The form runs a series of queries which allow output of raw data, data averaged over weeks, months, seasons or years. The phytoplankton data can also be output in terms of individual species, genera, phyla, at a mixed taxonomic level ("Analysis Code"), for example mixed genera and species level taxonomy. Data can also be output in terms of phytoplankton functional groups (c.f. Reynolds et al., 2002). The combined database also calculates expected scores for sites and results from application of the phytoplankton classification tool.

The structure of the phytoplankton database is illustrated in Figure 2.2. Taxonomic data are stored for individual samples using Whitton codes (Whitton et al., 1998 - see counter guidance Appendix II). The phytoplankton database is available on request from the EA Project Officer (Sian Davies).

Figure 2.2 Structure and relationships in the UK Phytoplankton Database



3. METRIC DEVELOPMENT: PHYTOPLANKTON INDEX OF EUTROPHICATION (PIE)

3.1 Introduction

Ecological classification for the WFD requires the comparison of the biological composition (species, genera, classes or functional groups) of an individual lake to an expected reference condition (site or type-specific). The approaches available for classification tool development are largely dependent on the type of data available and whether the pressure being assessed is correlated with another natural environmental gradient. Due to a lack of consistent quantitative empirical data in the past, previous attempts to classify phytoplankton in terms of nutrient pressures have been based on a combination of experimental evidence and expert knowledge/judgement (Reynolds in Carvalho et al., 2002).

As part of this project, a relatively harmonised dataset of phytoplankton composition data has been assembled alongside matched environmental and pressure data, enabling more objective quantitative approaches to be considered.

It is widely acknowledged, however, that nutrient pressures are correlated with the natural alkalinity of freshwaters; both being greatest in lowland areas, with their more alkaline geology, more intensive agriculture and higher population densities compared with upland areas. This correlation means that either a type-specific classification of phytoplankton must be considered, distinguishing responses in lakes of different alkalinity types, or a multivariate approach should be adopted.

Considering all this, a number of general points can be made on the philosophy adopted by this project for classification tool development:

- Type-specific classifications are restrictive and, in particular, problematic for sites close to type boundaries. The choice (and optima) of indicators will be restricted by where type boundaries are set. For this reason, a 'global' lake classification tool is preferred. Currently a global classification is necessary for phytoplankton due to data limitations within most lake types for UK lakes
- All taxa contain information, not just 'reference' or 'impact' taxa, or groups of recognised indicator value (e.g. cyanobacteria, chrysophytes or desmids)
- 'Species' optima contain more information if based on a continual scale along the impact gradient, rather than a binary system of reference/impact (or positive/negative)
- Using a community response is more robust as it does not necessarily rely on identifying all taxa in a sample and does not rely on the indicator values of just a few taxa that may not always be present

With community or assemblage data, there are three general approaches that can be adopted for developing a quality classification (US EPA, 1999):

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

These three general approaches are outlined and compared in a report by the US EPA (1999). They are not the only possible approaches and there are numerous possible variations of the three general approaches above. According to the US EPA (1999), approaches 1 & 2 are "easy to apply in a continuing operational monitoring program because data from an individual site are entered into a formula, and the site's deviation from reference conditions can be known immediately. The ordination approach (3) requires reanalysis of the reference data set for each new batch of monitoring sites." The metric approach is also the easiest to explain to managers and the public as it only requires simple mathematics to use and is highly pressure-specific.

In their favour, multivariate approaches (e.g. DCA, CCA, etc.) provide useful exploratory tools for investigating and visualising patterns in compositional data. They allow testing of which environmental variables help explain significant variance in the composition data. Unlike basic multimetric approaches, they also allow for correlations between typology and impact variables to be taken into account (i.e. the widely recognised correlation between alkalinity and nutrient pressures).

After much discussion and consideration of the various approaches, it was decided a combination multivariate-derived metric approach was most suitable for deriving a Phytoplankton Index of Eutrophication (PIE). The combination approach adopted is the "CBAS methodology" (Dodkins et al., 2005) and is described in detail in the methods section below.

A much simpler metric of "% cyanobacteria" was being developed by the Northern GIG and by some countries in Central Europe (Belgium) for use in WFD assessment. For this reason, in addition to the multivariate-derived metric, a much more simple "% cyanobacteria" metric was examined and compared with the multivariate-derived metric in terms of its effectiveness at representing the pressure gradient.

3.2 Methods

An agreed list of cyanobacteria taxa to include in a "% cyanobacteria" metric was provided by the Northern GIG (Eva Willen pers. comm.). This excluded all cyanobacteria genera in the Order Chroococcales, except *Microcystis*, *Coelosphaerium* and *Woronichinia*, and for this reason included most of the common large, bloom-forming taxa. It does, however, exclude cyanobacteria genera that are known to produce toxins harmful to human and animal health, such as *Merismopaedia*. The justification for the selection by the Northern Gig is unclear, except for the fact that those taxa omitted are more typical of low alkalinity waters.

For the Phytoplankton Index of Eutrophication (PIE), the "CBAS methodology" was adopted (Dodkins et al., 2005). CBAS is an acronym for CCA (Canonical Correspondence Analysis) Based Assessment System, developed initially for river macrophytes for the WFD (Dodkins et al., 2005). The methodology has undergone a number of refinements since the original published version, as documented in Dodkins & Rippey (2006).

The development of a CBAS methodology for lake phytoplankton can be summarised as requiring a number of steps:

1. Develop a multivariate model (CCA) using 'species' and environmental data, including lake typology and impact parameters that explain significant species variance.

2. Determine the univariate optima and tolerances of species along the impact gradients (through reciprocal averaging), in this case TP and chlorophyll$_a$ concentration gradients.
3. Calculate a 'metric score' at each reference site using the same approach as the Trophic Diatom Index (Kelly 1998), based on the optima, weighted by the abundance and potentially also the indicator value (derived from tolerance) of the species present (for each impact gradient), using equation 1 below.

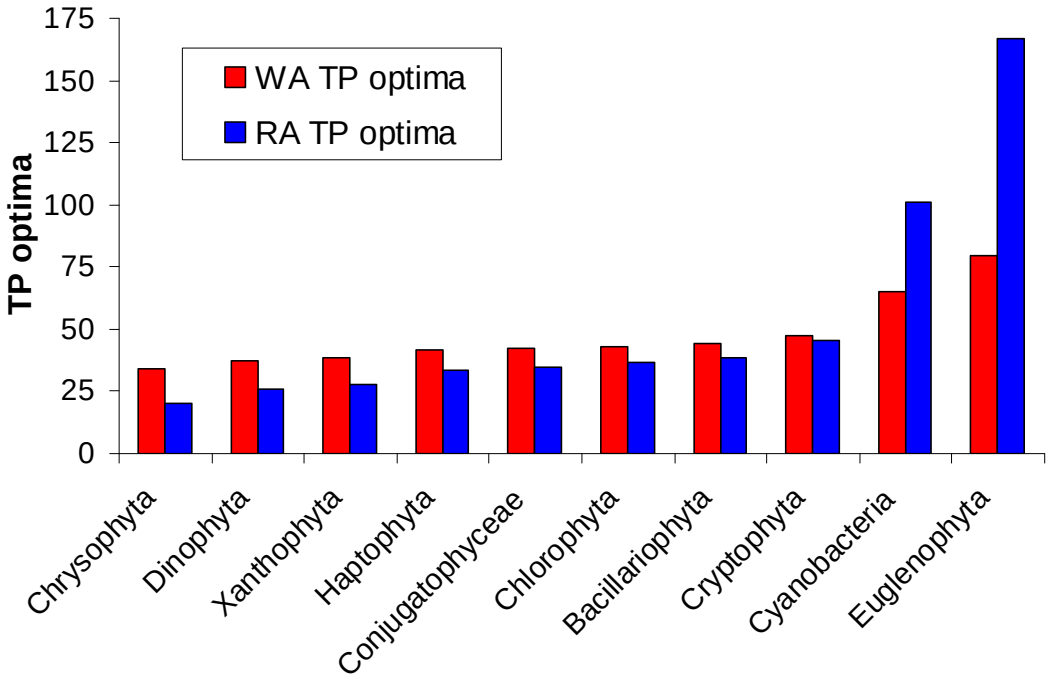
$$SiteScore = \frac{\sum a_i s_i v_i}{\sum a_i v_i} \qquad \text{(Equation 1)}$$

Where:
a_i = abundance of ith taxon at the site
s_i = optimum of ith taxon at the site
v_i = indicator value of ith taxon at the site
[latter is inverse of tolerance]

Reciprocal Averaging (RA) was used to derive simple species 'optima' and 'tolerances' to the pressure variables, rather than simple weighted-averaging (WA). RA maximises the spread of species optima along the impact gradient. This is illustrated in Figure 3.1 which compares TP optima derived by both RA and WA in the Central GIG dataset. RA produces much higher TP optima for cyanobacteria and Euglenophyta and lower TP optima in particular for Chrysophyta and Dinophyta (Figure 3.1).

Figure 3.1 Comparison of RA- and WA-TP optima for phytoplankton phyla

Optima are based on analysis of Central GIG dataset. RA-TP optima were derived by re-scaling CCA Axis 1 species scores using the relationship between site TP concentrations and Axis 1 site scores.



Another advantage of RA is that it can produce metrics to a range of pressures on the same ecological scale ('standard deviations of species turnover') and thus ecological responses to a number of pressures can potentially be added together (taking correlations into account).

The comparison of a site's metric score with a site's reference metric score can then be used to remove the correlation(s) between pressure and other significant environmental gradients, such as alkalinity. Chapter 4 describes the approach for defining reference conditions. Chapter 5 describes calculation of an EQR.

In terms of taxonomic level, analysis of the Central GIG dataset was carried out at phylum, genus and mixed genus/species level to examine which taxonomic level was most appropriate for future classification work.

3.3 % cyanobacteria metric

Preliminary analysis of the Central GIG dataset was carried out to examine the effectiveness of metrics based on different phytoplankton phyla. This revealed that individual phyla showed very poor relationships with proxies of eutrophication pressure (Table 3.1). The strongest relationship was with % cyanobacteria, although this was still not significant

Table 3.1 Correlation coefficients between % biovolume of phytoplankton phyla and chlorophyll and TP (log transformed) in Central GIG dataset

Phylum	log_{10}(Chl)	log_{10}(TP)
Cyanophyceae	0.13	0.08
Dinophyceae	0.09	0.05
Chrysophyceae	0.02	0.03
Raphidophyceae	0.01	0.01
Conjugatophyceae	0.01	0.00
Euglenophyceae	0.01	0.02
Cryptophyceae	0.00	0.00
Chlorophyceae	0.00	0.01
Bacillariophyceae	0.00	0.00

Despite this, a % cyanobacteria metric was explored for the UK dataset for its potential use as a phytoplankton metric. In the UK dataset the relationship with log_{10}Chlorophyll was a fraction stronger than found for the Central GIG (Figure 3.2) but weaker with log_{10}TP (Figures 3.3). The scatter in these relationships very clearly illustrates that this metric is too weak to be used as the main phytoplankton composition metric for determining ecological status classifications for the WFD. Potentially it could still be a useful metric for representing the sub-element 'phytoplankton bloom frequency and intensity' although further investigation of sources of variability in the metric are recommended.

Figure 3.2 Relationship between chlorophyll$_a$ and % cyanobacteria in UK lakes

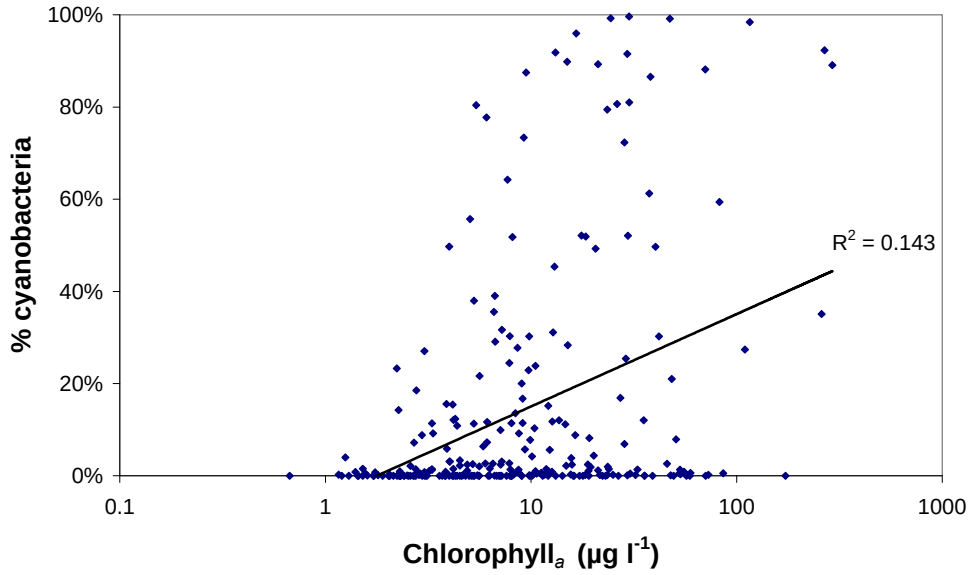
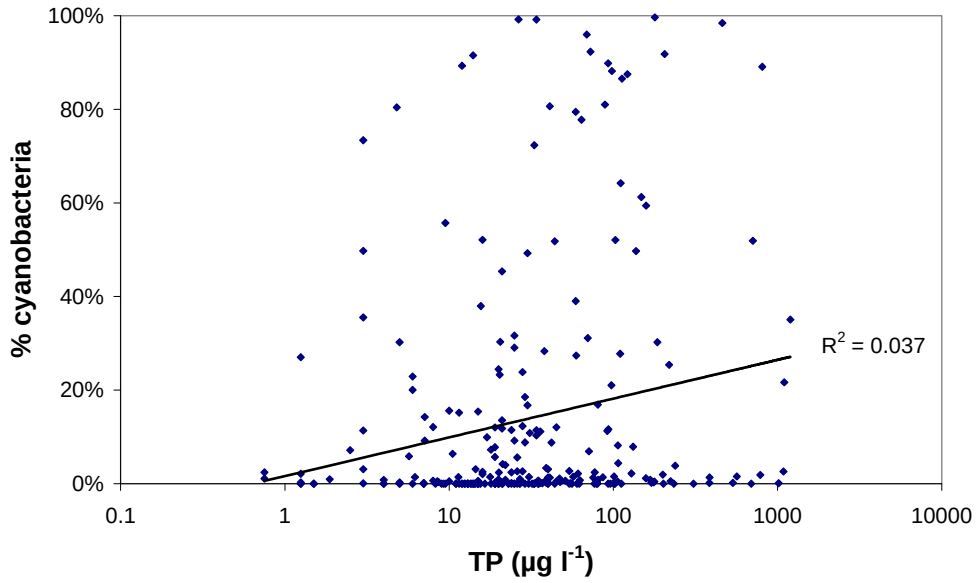


Figure 3.3 Relationship between TP and % cyanobacteria In UK lakes



3.4 Phytoplankton Index of Eutrophication (PIE)

Preliminary analysis of the Central GIG dataset was carried out to examine the effectiveness of a PIE based on different taxonomic resolution. This revealed that a phylum-level index was little better than the % cyanobacteria metric in terms of its relationships with TP concentrations (Figure 3.4). The relationship based on a genus-level index was much stronger (Figure 3.5). For this reason it was decided that to be an effective classification tool, the PIE should be based on at least genus-level taxonomic data, although where possible species-level identification would be beneficial for common, well-recognised species. A mixed-genus-level metric was, therefore, considered optimal.

Figure 3.4 **Relationship between log_{10}TP and site scores based on phylum-level indicator taxa**
Based on analysis of Central GIG dataset.

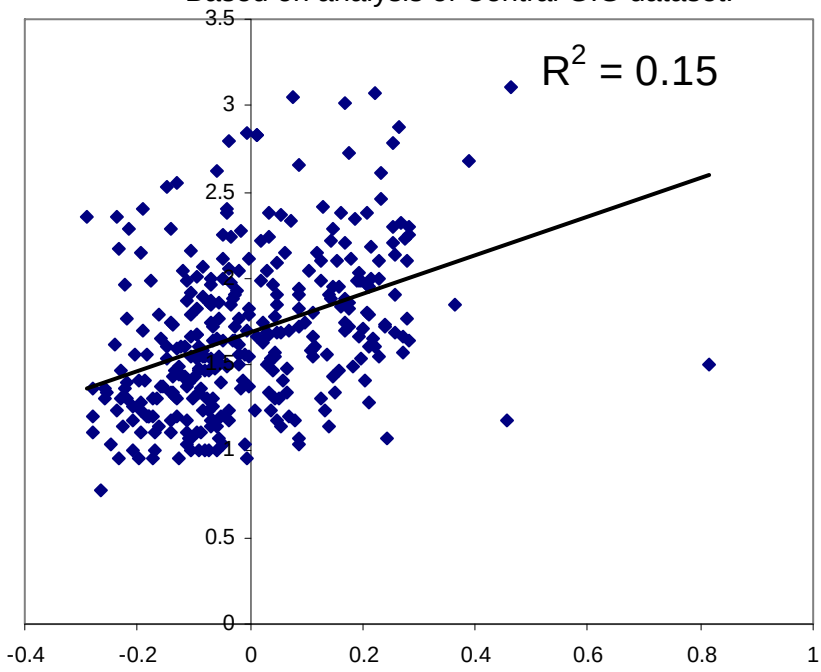
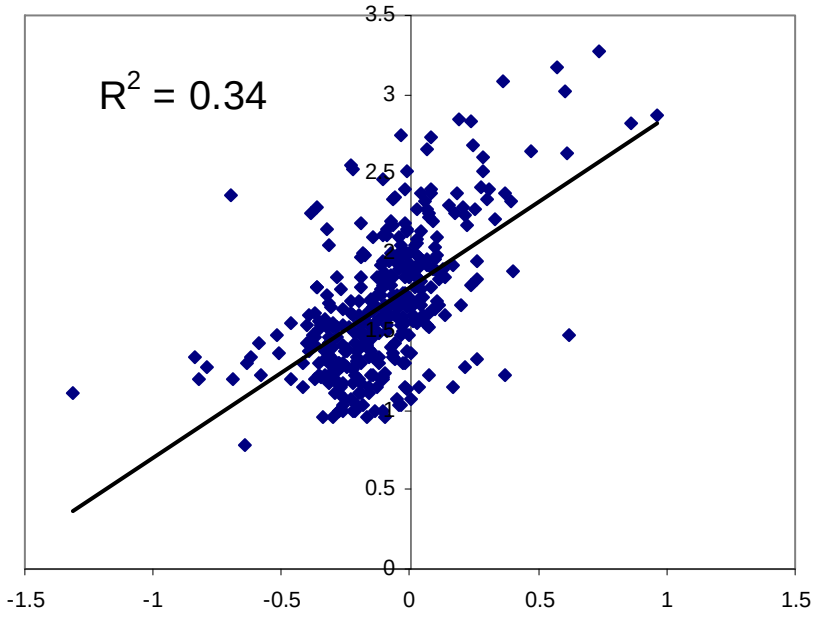


Figure 3.5 **Relationship between log_{10}TP and site scores based on genus-level indicator taxa**
Based on analysis of Central GIG dataset.



Analysis of the UK phytoplankton data, therefore, proceeded at a mixed genus-species taxonomic level. Particular well-recognised species, which were considered to have a useful indicator value, were selected by agreement at a Central GIG meeting of European phytoplankton experts. All other species were lumped at the genus level, where possible.

Initial DCA of the phytoplankton data alone revealed gradient lengths intermediate between those for which linear (<2) and unimodal (>3) responses would be expected (axis 1 gradient length 2.35 and axis 2 2.81). CCA was selected since it is a unimodal model which is also robust with linear gradients (Ter Braak and Šmilauer, 2002).

Stepwise manual forward selection was used in CANOCO to produce a species-environment model. The CCA analysis indicated log_chlorophyll explained the most variance in the phytoplankton composition data (2.3%) with log_depth, log_alkalinity and log_TP all explaining additional independent variance in the composition data. The analysis also indicated the absence of strong altitudinal or 'seasonal' effects in the July-September dataset, sample month being the variable that explained the least variance in the species data (0.4%).

The environmental variables included in the model explained only 5.1% of the variance in the phytoplankton data (Table 3.2). This is low, but typical of 'noisy' datasets with large numbers of taxa and rapidly varying biomass. The model was based on 292 samples with 112 active taxa and only 4 selected environmental variables This is relatively few samples and environmental variables, reducing the magnitude of variance explained.

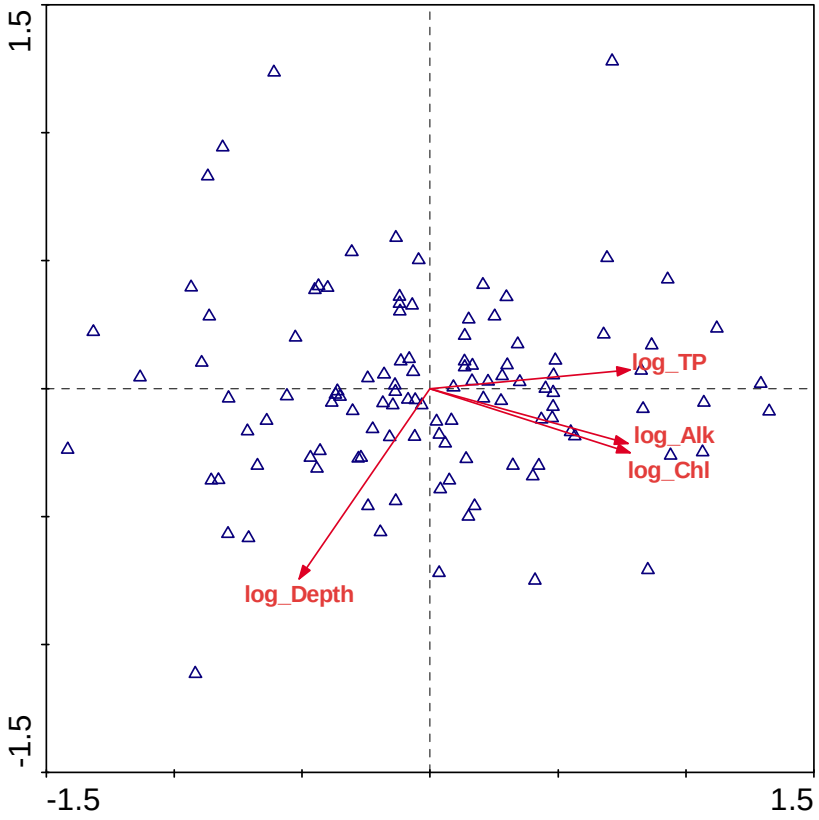
Table 3.2 Summary statistics for the first four CCA axes

CCA Axes	1	2	3	4
Eigenvalues	0.104	0.031	0.022	0.016
Species-environment correlations	0.771	0.587	0.549	0.480
Cumulative percentage variance				
of species data	3.0	3.9	4.6	5.1
of species-environment relation	60	78	91	100

The 'species'-environment biplot of the first two axes of the final CCA model is shown in Figure 3.6. This highlights the close correlation between alkalinity and chlorophyll$_a$ and to a lesser extent TP along the first axis, with mean depth more correlated with the 2nd axis. The fact that phytoplankton composition changes are likely to be similar in response to increasing alkalinity as they are with increasing nutrient gradients is well established (Lund 1961; Shapiro 1990).

The implications of this for a phytoplankton classification tool are that a simple univariate metric alone should not be used to assess eutrophication impact, as the phytoplankton may simply be representing a response to alkalinity. It is for this reason that the metric score must be compared with a reference metric score that takes into account a site's typology, particularly alkalinity, and that ideally this reference score should be site-specific. This must, however, be balanced against maintaining some signal of the pressure in the final EQR score.

Figure 3.6 Species-environment bi-plot of the first two axes of the CCA



3.4.1 Phytoplankton Indicator Scores

Optima and tolerances were obtained along both eutrophication pressure gradients (TP and Chlorophyll) by CCA analysis, using reciprocal averaging (RA) with each pressure gradient considered in the model individually (i.e. univariate analysis). These optima are equivalent to $\log_{10}$abundance weighted-averages (WA), iteratively adjusted to ensure site scores are weighted averages of taxa optima as well as taxa optima being the weighted averages of site scores. Hill's scaling was used to produce optima measured in standard deviations of species turnover.

Optima were transformed to values between 0 (low pressure) and 1 (high pressure) for use in the PIE metric and EQR assessment. This was carried out by converting all taxon scores to positive values (adding lowest score), then dividing by the resultant maximum score.

Indicator values were obtained from species 'tolerance' scores, by subtracting the tolerance score from the maximum tolerance score. This ensures that a small niche breadth (tolerance) produces a high indicator value.

Taxa optima and tolerances were determined in relation to chlorophyll (Table 3.3) and TP (Table 3.4) gradients in the dataset. Ranking the 112 indicators from low (1) to high (112) eutrophication pressure allows a comparison of the two sets of indicator scores (Table 3.5) and can help inform the choice of robust indicators of eutrophication pressure (i.e. little difference in ranks and high indicator value) from those taxa that appear less robust (i.e. high difference in ranks and low indicator value).

For example, Tables 3.3, 3.4 and 3.5 confirm widely-recognised indicators of low eutrophication pressure (and low alkalinity), such as the Chrysophyta (*Bitirichia*, *Chrysochromulina parva* and *Dinobryon*), certain diatom taxa (*Diatoma*, *Synedra ulna*, *Tabellaria* spp. and *Urosolenia*) and desmids (*Euastrum and Staurodesmus*).

Similarly, widely-recognised indicators of high eutrophication pressure (and high alkalinity) are confirmed, such as many Chlorophyta taxa (*Pteromonas*, *Treubaria*, *Coelastrum*, *Micractinium*, *Pediastrum*, and *Scenedesmus*), certain diatom taxa (*Aulacoseira granulata* and *Nitzschia acicularis*) and certain cyanobacteria (*Aphanizomenon flos-aquae*, *Anabaena flos-aquae*, *Microcystis* and *Pannus*) (Tables 3.3, 3.4 and 3.5).

There are some discrepancies between the indicators developed in relation to chlorophyll gradients (Table 3.3) compared with those for TP gradients (Table 3.4). For example, the cyanobacterium genus *Merismopaedia* and desmid *Closterium aciculare* appear to be indicators of low chlorophyll concentrations, but also indicators of relatively higher TP concentrations. The cyanobacterium genus *Gloeotrichia* and Chlorophyta *Gloeocystis*, *Golenkinia* and *Lagerheimia* appear to be indicators of high chlorophyll concentrations, but relatively lower TP concentrations. The optima and tolerances for *Gloeotrichia*, *Gloeocystis* and *Golenkinia* in particular are based on only 5 or less samples and are, therefore, particularly uncertain.

For this reason a smaller list of 42 taxa of the more robust indicators was established to examine whether this more limited taxa list represented pressure gradients more effectively (i.e. less noise).

Table 3.3 Phytoplankton taxa metrics: Optima and Indicator Value listed from lowest (least impact) to highest (most impact) in relation to chlorophyll

The number of samples (N) each taxon was recorded in is also indicated

Row	Taxon Name	N	Optima (0-1 scale)	Indicator Value	Row	Taxon Name	N	Optima (0-1 scale)	Indicator Value
1	Quadrigula	18	0.00	0.77	57	Diatoma tenue	10	0.44	0.53
2	Bitrichia	14	0.07	0.65	58	Staurastrum	92	0.45	0.29
3	Ulothrix	7	0.12	0.87	59	Coelosphaerium	41	0.45	0.45
4	Euastrum	14	0.12	0.74	60	Trachelomonas	98	0.45	0.34
5	Glenodinium	9	0.14	0.73	61	Closteriopsis	76	0.45	0.18
6	Mougeotia	18	0.15	0.67	62	Anabaena	91	0.46	0.37
7	Chrysochromulina parva	6	0.16	0.69	63	Peridinium willei	3	0.47	0.35
8	Ceratium furcoides	3	0.18	0.92	64	Sphaerocystis	46	0.48	0.22
9	Achnanthidium	10	0.19	0.71	65	Monoraphidium	178	0.48	0.33
10	Tabellaria flocculosa	60	0.20	0.64	66	Oscillatoria limnetica	86	0.48	0.41
11	Characium	20	0.21	0.72	67	Gomphosphaeria	50	0.48	0.41
12	Merismopedia	34	0.21	0.31	68	Chroomonas	63	0.48	0.29
13	Diatoma	8	0.24	0.67	69	Chroococcus	74	0.48	0.33
14	Staurodesmus	45	0.24	0.61	70	Fragilaria capucina	3	0.48	0.15
15	Urosolenia	52	0.26	0.66	71	Oscillatoria agardhii	69	0.49	0.30
16	Snowella lacustris	4	0.26	0.62	72	Planktosphaeria	10	0.50	0.59
17	Dinobryon	103	0.27	0.62	73	Ankistrodesmus	26	0.51	0.34
18	Closterium aciculare	11	0.27	0.91	74	Stephanodiscus	20	0.52	0.01
19	Gymnodinium	91	0.29	0.45	75	Phacus	27	0.52	0.29
20	Gonium	7	0.29	0.88	76	Golenkiniopsis	12	0.52	0.44
21	Crucigenia	48	0.29	0.20	77	Oscillatoria	27	0.52	0.48
22	Spondylosium	28	0.30	0.45	78	Dictyosphaerium	62	0.53	0.36
23	Xanthidium	9	0.31	0.57	79	Aphanocapsa	53	0.53	0.14
24	Synedra ulna	25	0.32	0.57	80	Aulacoseira	65	0.53	0.25
25	Carteria	11	0.32	0.43	81	Coenochloris	22	0.55	0.46
26	Peridinium	28	0.32	0.36	82	Anabaena catenula	27	0.55	0.38
27	Mallomonas	98	0.33	0.42	83	Tetrastrum	27	0.55	0.36
28	Radiococcus	5	0.34	0.45	84	Schroederia	28	0.55	0.23
29	Ochromonas	12	0.34	0.39	85	Synedra	30	0.56	0.44
30	Synura	8	0.34	0.85	86	Actinastrum	21	0.56	0.25
31	Tabellaria fenestrata	12	0.34	0.77	87	Peridinium cinctum	11	0.56	0.47
32	Fragilaria	24	0.34	0.55	88	Kirchneriella	13	0.57	0.25
33	Eudorina	31	0.34	0.43	89	Fragilaria crotonensis	46	0.57	0.53
34	Dinobryon divergens	22	0.35	0.40	90	Microcystis	42	0.57	0.09
35	Synedra acus	47	0.35	0.71	91	Ankyra	43	0.57	0.22
36	Closterium	29	0.35	0.30	92	Scenedesmus	141	0.58	0.33
37	Asterionella formosa	93	0.36	0.58	93	Cyclotella	12	0.58	0.41
38	Golenkinia radiata	22	0.37	0.31	94	Gloeocystis	5	0.58	0.71
39	Elakatothrix	69	0.38	0.32	95	Golenkinia	5	0.60	0.75
40	Chlorella	144	0.38	0.29	96	Tetraedron	61	0.62	0.23
41	Chlorococcum	19	0.38	0.43	97	Anabaena flos-aquae	38	0.64	0.28
42	Oscillatoria redekei	11	0.38	0.68	98	Pediastrum	70	0.65	0.33
43	Chlamydomonas	190	0.39	0.29	99	Goniochloris	6	0.66	0.66
44	Rhodomonas	228	0.39	0.38	100	Micractinium	11	0.67	0.32
45	Cosmarium	66	0.39	0.25	101	Coenococcus	9	0.68	0.38
46	Coenocystis	8	0.39	0.19	102	Coelastrum	43	0.69	0.30
47	Aphanothece	53	0.40	0.47	103	Nephrodiella	14	0.70	0.50
48	Pandorina	26	0.40	0.56	104	Gomphosphaeria lacustris	12	0.72	0.29
49	Unidentified	282	0.40	0.32	105	Lagerheimia	15	0.73	0.29
50	Volvox	7	0.40	0.54	106	Nitzschia acicularis	7	0.73	0.40
51	Gomphonema	10	0.40	0.19	107	Aphanizomenon flos-aquae	37	0.74	0.45
52	Cryptomonas	235	0.40	0.32	108	Pteromonas	5	0.76	0.92
53	Euglena	10	0.41	0.22	109	Treubaria	12	0.79	0.44
54	Ceratium hirundinella	89	0.42	0.39	110	Aulacoseira granulata	14	0.85	0.73
55	Closterium acutum	31	0.43	0.40	111	Pannus	6	0.86	0.85
56	Oocystis	122	0.44	0.24	112	Gloeotrichia	4	1.00	0.11

Table 3.4 Phytoplankton taxa metrics: Optima and Indicator Value listed from lowest (least impact) to highest (most impact) in relation to TP

The number of samples (N) each taxon was recorded in is also indicated

Row	Taxon Name	N	Optima (0-1 scale)	Indicator Value	Row	Taxon Name	N	Optima (0-1 scale)	Indicator Value
1	Ulothrix	7	0.00	0.67	57	Cryptomonas	235	0.41	0.57
2	Chrysochromulina parva	6	0.10	0.43	58	Golenkinia radiata	22	0.41	0.62
3	Gonium	7	0.17	0.60	59	Coelosphaerium	41	0.41	0.61
4	Characium	20	0.18	0.86	60	Unidentified	282	0.42	0.56
5	Tabellaria flocculosa	60	0.19	0.81	61	Oscillatoria agardhii	69	0.42	0.61
6	Radiococcus	5	0.20	0.50	62	Chlorella	144	0.42	0.45
7	Tabellaria fenestrata	12	0.21	0.92	63	Merismopedia	34	0.42	0.56
8	Carteria	11	0.21	0.74	64	Anabaena	91	0.42	0.61
9	Synura	8	0.22	0.89	65	Sphaerocystis	46	0.42	0.61
10	Staurodesmus	45	0.22	0.78	66	Chroococcus	74	0.43	0.37
11	Urosolenia	52	0.23	0.64	67	Oscillatoria limnetica	86	0.43	0.39
12	Spondylosium	28	0.23	0.74	68	Lagerheimia	15	0.44	0.33
13	Xanthidium	9	0.23	1.06	69	Trachelomonas	98	0.45	0.52
14	Ceratium furcoides	3	0.23	1.06	70	Anabaena catenula	27	0.45	0.79
15	Snowella lacustris	4	0.23	0.46	71	Monoraphidium	178	0.46	0.59
16	Dinobryon	103	0.24	0.84	72	Closteriopsis	76	0.46	0.63
17	Mougeotia	18	0.24	0.63	73	Oocystis	122	0.48	0.60
18	Gloeocystis	5	0.24	0.12	74	Dictyosphaerium	62	0.48	0.69
19	Bitrichia	14	0.26	1.10	75	Gloeotrichia	4	0.49	1.22
20	Synedra ulna	25	0.27	0.61	76	Aphanocapsa	53	0.49	0.55
21	Glenodinium	9	0.27	0.93	77	Coenochloris	22	0.49	0.47
22	Ochromonas	12	0.28	0.36	78	Volvox	7	0.49	1.11
23	Synedra acus	47	0.28	0.62	79	Gomphonema	10	0.49	0.18
24	Euastrum	14	0.28	0.85	80	Ankistrodesmus	26	0.49	0.76
25	Peridinium	28	0.29	0.65	81	Aulacoseira	65	0.49	0.48
26	Quadrigula	18	0.29	1.08	82	Tetrastrum	27	0.50	0.37
27	Eudorina	31	0.30	0.87	83	Microcystis	42	0.51	0.39
28	Euglena	10	0.30	0.49	84	Synedra	30	0.51	0.46
29	Oscillatoria redekei	11	0.30	0.68	85	Phacus	27	0.51	0.25
30	Diatoma	8	0.31	0.54	86	Peridinium cinctum	11	0.52	0.72
31	Achnanthidium	10	0.32	1.09	87	Micractinium	11	0.52	0.80
32	Mallomonas	98	0.32	0.75	88	Scenedesmus	141	0.53	0.57
33	Gymnodinium	91	0.33	0.61	89	Tetraedron	61	0.53	0.55
34	Pandorina	26	0.33	1.09	90	Planktosphaeria	10	0.54	1.06
35	Gomphosphaeria	50	0.34	0.84	91	Stephanodiscus	20	0.55	0.22
36	Chlorococcum	19	0.34	0.34	92	Kirchneriella	13	0.55	0.67
37	Crucigenia	48	0.34	0.50	93	Chroomonas	63	0.56	0.52
38	Dinobryon divergens	22	0.35	0.95	94	Cyclotella	12	0.57	0.69
39	Golenkiniopsis	12	0.36	0.79	95	Gomphosphaeria lacustris	12	0.58	0.51
40	Asterionella formosa	93	0.36	0.72	96	Actinastrum	21	0.58	0.25
41	Elakatothrix	69	0.36	0.50	97	Anabaena flos-aquae	38	0.60	0.43
42	Golenkinia	5	0.37	0.82	98	Pannus	6	0.60	0.86
43	Closterium acutum	31	0.37	0.95	99	Coenococcus	9	0.61	0.90
44	Fragilaria	24	0.37	0.63	100	Ankyra	43	0.61	0.40
45	Diatoma tenue	10	0.37	1.01	101	Treubaria	12	0.62	0.62
46	Rhodomonas	228	0.38	0.64	102	Nephrodiella	14	0.63	0.78
47	Oscillatoria	27	0.38	0.66	103	Pediastrum	70	0.63	0.55
48	Closterium	29	0.39	0.42	104	Coelastrum	43	0.65	0.80
49	Ceratium hirundinella	89	0.39	0.66	105	Nitzschia acicularis	7	0.65	0.49
50	Cosmarium	66	0.39	0.54	106	Fragilaria capucina	3	0.66	0.56
51	Closterium aciculare	11	0.39	0.31	107	Peridinium willei	3	0.69	0.00
52	Fragilaria crotonensis	46	0.39	0.70	108	Aphanizomenon flos-aquae	37	0.70	0.60
53	Coenocystis	8	0.40	0.10	109	Schroederia	28	0.72	0.46
54	Aphanothece	53	0.40	0.45	110	Goniochloris	6	0.83	0.35
55	Chlamydomonas	190	0.40	0.55	111	Aulacoseira granulata	14	0.86	0.56
56	Staurastrum	92	0.41	0.60	112	Pteromonas	5	1.00	1.14

Table 3.5 Phytoplankton taxa ordered in relation to differences in chlorophyll and TP indicator rank

The number of samples (N) each taxon was recorded in is also indicated

Taxon Name	N	Chl Rank	TP Rank	Difference in Rank	Taxon Name	N	Chl Rank	TP Rank	Difference in Rank
Gloeocystis	5	94	18	76	Sphaerocystis	46	64	65	-1
Golenkinia	5	95	42	53	Oscillatoria limnetica	86	66	67	-1
Golenkiniopsis	12	76	39	37	Aulacoseira	65	80	81	-1
Fragilaria crotonensis	46	89	52	37	Cyclotella	12	93	94	-1
Lagerheimia	15	105	68	37	Aphanizomenon flos-aquae	37	107	108	-1
Gloeotrichia	4	112	75	37	Aulacoseira granulata	14	110	111	-1
Gomphosphaeria	50	67	35	32	Elakatothrix	69	39	41	-2
Oscillatoria	27	77	47	30	Rhodomonas	228	44	46	-2
Euglena	10	53	28	25	Anabaena	91	62	64	-2
Tabellaria fenestrata	12	31	7	24	Coelastrum	43	102	104	-2
Radiococcus	5	28	6	22	Asterionella formosa	93	37	40	-3
Synura	8	30	9	21	Dinobryon divergens	22	34	38	-4
Gonium	7	20	3	17	Kirchneriella	13	88	92	-4
Carteria	11	25	8	17	Pteromonas	5	108	112	-4
Pandorina	26	48	34	14	Mallomonas	98	27	32	-5
Oscillatoria redekei	11	42	29	13	Cosmarium	66	45	50	-5
Micractinium	11	100	87	13	Cryptomonas	235	52	57	-5
Pannus	6	111	98	13	Pediastrum	70	98	103	-5
Synedra acus	47	35	23	12	Ceratium furcoides	3	8	14	-6
Closterium acutum	31	55	43	12	Monoraphidium	178	65	71	-6
Diatoma tenue	10	57	45	12	Coenocystis	8	46	53	-7
Anabaena catenula	27	82	70	12	Aphanothece	53	47	54	-7
Spondylosium	28	22	12	10	Ankistrodesmus	26	73	80	-7
Xanthidium	9	23	13	10	Trachelomonas	98	60	69	-9
Oscillatoria agardhii	69	71	61	10	Ankyra	43	91	100	-9
Gomphosphaeria lacustris	12	104	95	9	Phacus	27	75	85	-10
Treubaria	12	109	101	8	Actinastrum	21	86	96	-10
Characium	20	11	4	7	Mougeotia	18	6	17	-11
Ochromonas	12	29	22	7	Unidentified	282	49	60	-11
Microcystis	42	90	83	7	Closteriopsis	76	61	72	-11
Tetraedron	61	96	89	7	Goniochloris	6	99	110	-11
Eudorina	31	33	27	6	Fragilaria	24	32	44	-12
Chrysochromulina parva	6	7	2	5	Closterium	29	36	48	-12
Tabellaria flocculosa	60	10	5	5	Chlamydomonas	190	43	55	-12
Chlorococcum	19	41	36	5	Gymnodinium	91	19	33	-14
Ceratium hirundinella	89	54	49	5	Glenodinium	9	5	21	-16
Staurodesmus	45	14	10	4	Crucigenia	48	21	37	-16
Urosolenia	52	15	11	4	Bitrichia	14	2	19	-17
Synedra ulna	25	24	20	4	Diatoma	8	13	30	-17
Dictyosphaerium	62	78	74	4	Oocystis	122	56	73	-17
Coenochloris	22	81	77	4	Stephanodiscus	20	74	91	-17
Scenedesmus	141	92	88	4	Planktosphaeria	10	72	90	-18
Chroococcus	74	69	66	3	Euastrum	14	4	24	-20
Aphanocapsa	53	79	76	3	Golenkinia radiata	22	38	58	-20
Ulothrix	7	3	1	2	Achnanthidium	10	9	31	-22
Staurastrum	92	58	56	2	Chlorella	144	40	62	-22
Coenococcus	9	101	99	2	Quadrigula	18	1	26	-25
Snowella lacustris	4	16	15	1	Chroomonas	63	68	93	-25
Dinobryon	103	17	16	1	Schroederia	28	84	109	-25
Peridinium	28	26	25	1	Volvox	7	50	78	-28
Tetrastrum	27	83	82	1	Gomphonema	10	51	79	-28
Synedra	30	85	84	1	Closterium aciculare	11	18	51	-33
Peridinium cinctum	11	87	86	1	Fragilaria capucina	3	70	106	-36
Nephrodiella	14	103	102	1	Peridinium willei	3	63	107	-44
Nitzschia acicularis	7	106	105	1	Merismopedia	34	12	63	-51
Coelosphaerium	41	59	59	0					
Anabaena flos-aquae	38	97	97	0					

3.4.2 Model selection

PIE site scores were calculated using both the 112 and 42 taxa model by three different approaches using equation 1 (see Section 3.2):

1) No biovolume weighting with site scores based on optima only (i.e. presence/absence)
2) Site scores based on a weighted average of taxa optima. Weighting based on $\log_{10}$ of taxon biovolume
3) Site scores based on a weighted average of taxa optima. Weighting based on untransformed taxon biovolume

The correlations between the PIE site scores and log_{10} transformed alkalinity, TP and chlorophyll gradients were examined to determine which model appeared to be most effective. The model based on 42 taxa had much weaker relationships with both log_{10}Chlorphyll and log_{10}TP, so the 112 taxa model was selected.

Validation of the latter model, clearly showed that the model based on log_{10} biovolume-weighted optima showed the strongest relationships with both log_{10}TP (r^2 = 0.601) and log_{10}Chlorophyll (r^2 = 0.641) (Table 3.6). The model based on optima only, i.e. presence/absence data, was slightly less effective. The model based on optima weighted by untransformed biovolume data was clearly less effective, highlighting the increased noise, or uncertainty, associated with raw phytoplankton biovolume data. This finding is confirmed in the uncertainty analysis (Chapter 7) comparing biovolume estimates from different counters. The model based on 42 taxa had much weaker relationships with both log_{10}Chlorophyll (r^2 = 0.44) and log_{10}TP (r^2 = 0.42), so the 112 taxa model was selected.

Table 3.6 Correlation between site PIE Scores and alkalinity, TP and chlorophyll

Coefficients in bold indicate significant relationships

a) PIE Scores based on optima only

Lake Type		logAlk	logTP	logChl
LA	r^2	0.100	**0.249**	**0.323**
	p	0.359	0.018	0.002
MA	r^2	**0.345**	0.215	**0.480**
	p	0.008	0.100	<0.001
HA	r^2	0.003	**0.539**	**0.588**
	p	0.978	<0.001	<0.001
All samples	r^2	**0.594**	**0.589**	**0.636**
	p	<0.001	<0.001	<0.001

b) PIE Scores based on optima weighted by log biovolume

Lake Type		logAlk	logTP	logChl
LA	r^2	0.142	**0.289**	**0.331**
	p	0.192	0.006	0.001
MA	r^2	**0.365**	0.224	**0.508**
	p	0.005	0.085	<0.001
HA	r^2	-0.048	**0.530**	**0.581**
	p	0.642	<0.001	<0.001
All samples	r^2	**0.599**	**0.601**	**0.641**
	p	<0.001	<0.001	<0.001

c) PIE Scores based on optima weighted by biovolume

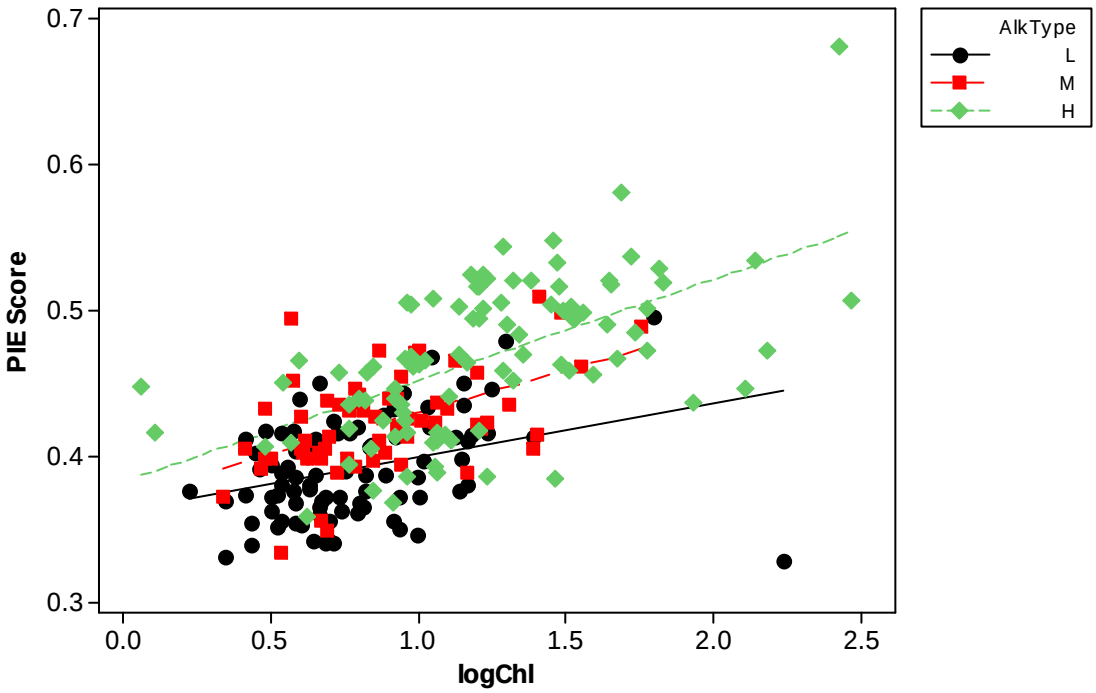
Lake Type		logAlk	logTP	logChl
LA	r^2	0.138	**0.28**	**0.258**
	p	0.206	0.007	0.014
MA	r^2	0.149	0.052	**0.462**
	p	0.269	0.692	<0.001
HA	r^2	-0.174	**0.364**	**0.384**
	p	0.091	<0.001	<0.001
All samples	r^2	**0.432**	**0.460**	**0.500**
	p	<0.001	<0.001	<0.001

The relationship between log_{10}Chlorophyll and the PIE metric (based on optima weighted by log_{10} of taxon biovolume) was examined separately for Low (LA), Medium (MA) and High (HA) alkalinity lakes (Table 3.6 and Figure 3.7). This shows that the relationship is weaker, but still highly significant, for low alkalinity lakes. A very similar pattern and similar correlative strengths were seen with the diatom classification tool (DALES) (Kelly et al., 2007)

With 1 LA outlier site excluded (Llyn Bodlynn: chlorophyll of 173 µg/l), the relationship is reasonably consistent between these three different lake types, supporting the development of a global 'all lake' model. The main difference between the three lake types is that the PIE score at low pressure (i.e. Figure 3.7 y-axis intercept) should be lower with decreasing alkalinity. This observation can be taken into account through development of an EQR assessment, with the reference condition dependent, at least in part, on the alkalinity type of the water body.

Figure 3.7 **Scatterplot showing relationship between logChlorophyll and PIE metric for three lake types: Low (L), Medium (M) and High (H) alkalinity**

(LA outlier Llyn Bodlyn excluded)



4. REFERENCE CONDITIONS

4.1 Introduction

The WFD prescribes the assessment of ecological quality of surface waters using an Ecological Quality Ratio (EQR). The EQR is defined as the relationship between the observed value and the reference condition for a given ecological quality element. The concepts of the EQR and reference conditions are first outlined in Annex V of the WFD:

"In order to ensure comparability of monitoring systems, the results of the systems operated by each Member State shall be expressed as ecological quality ratios for the purposes of classification of ecological status. These ratios shall represent the relationship between the values of the biological parameters observed for a given body of surface water and the values for these parameters in the reference conditions applicable to that body." (Annex V, Paragraph 1.4, WFD)

Further guidance for the WFD (REFCOND Guidance, 2003) defines reference conditions more explicitly as a state "corresponding to very low pressure, without the effects of major industrialisation, urbanisation and intensification agriculture and with only very minor modification of physico-chemistry, hydromorphology and biology". The WFD also acknowledges that reference conditions differ across lake types resulting from geographical differences of catchments (geology and altitude) and lake-specific factors (e.g. depth, area, water colour). To account for these differences, the WFD requires water bodies to be differentiated into 'ecotypes' within geographical regions and to establish type-specific reference conditions for the ecological quality elements.

4.2 Methods

A number of approaches can be used to establish reference conditions and these have been broadly summarised in the published guidance on reference conditions for the WFD (REFCOND Guidance, 2003). This outlines five general approaches available for defining chlorophyll reference conditions:

1. Survey data from a population of reference or minimally impacted lakes
2. Model-based prediction
3. Palaeolimnology
4. Historical data
5. Expert judgement

The guidance suggests that approach no. 1, a validated spatial network of reference or minimally impacted lakes is preferred if available and that approach 5 should really only be applied with validation from other approaches. For phytoplankton composition, method 3 (palaeolimnology) is only really possible for planktonic diatoms and method 4 (historical data) is limited by a lack of pre-disturbance historical data from all but a very few long-term study sites. For this reason only approaches 1 and 2 were considered in this project.

As part of the WFD Common Implementation Strategy, Member States developed a list of criteria for the selection of reference lakes, using a range of pressure criteria. In the UK, sites were selected if there were no major point sources in the catchment, 90% or more of catchment land-use was natural (or semi-natural) and catchment population density was <10 km^{-2}. The UK and Ireland additionally used palaeolimnology to validate choice of reference lakes – only selecting sites that show no significant change in diatom sub-fossil assemblages over the last 150 years or more (see Bennion et al., 2004 for more details). Some expert judgement was also used in the review of final reference site list.

Data from 50 reference lakes were collated for analysis (Table 4.1).

Table 4.1 UK and Irish reference lakes used to establish reference conditions

(PIE Scores are based on 112 taxa WA-log biovolume model)

Lake Code	Lake Name	Altitude (m a.s.l.)	Area (km^2)	Mean depth (m)	Alkalinity (m.equiv l^{-1})	PTI Score	Chl_a (µg l^{-1})	TP (µg l^{-1})
IEEA_07_270	Lough Bane	112	0.75	5.3	2.320	0.53	8.1	9.1
IESH_27_115	Lough Cullaun	16	0.50	6.3	3.016	0.39	6.0	7.5
IESH_27_94	Lough Muckanagh	17	0.96	5.6	3.136	0.37	3.2	7.9
IEWE_30_343	Lough Maumwee	46	0.28	2.6	0.041	0.39	2.8	7.3
IEWE_31_171	Lough Shindilla	38	0.70	7.2	0.052	0.37	1.6	9.7
IEWE_31_211	Lough Anaserd	8	0.87	0.9	0.432	0.43	3.2	8.9
IEWE_32_490	Lough Doo MO	30	1.55	12.6	0.122	0.38	2.8	7.4
IEZZ_00_006	Lough Barfinnihy	249	0.14	5.2	0.082	0.39	2.4	5.9
IEZZ_00_010	Lough Fin	28	0.00	0.4	0.110	0.38	3.6	8.3
IEZZ_00_018	Lough Veagh	40	2.61	10.7	0.160	0.36	1.2	7.9
UK2088	Loch of Mey	15	0.23	0.5	2.120	0.51	5.6	6.0
UK2490	Loch Hope	4	6.38	18.7	0.052	0.25	3.2	3.1
UK3904	Loch Loyal	114	6.46	19.9	0.133	0.20	2.3	1.5
UK4204	Loch Meadie	116	0.39	1.7	0.100	0.39	7.6	13.0
UK4974	Loch Syre	122	0.44	1.7	0.130	0.40	2.6	5.0
UK5222	Loch Meadie	146	2.11	6.3	0.063	0.40	3.6	1.9
UK5307	Loch Coulside	117	0.22	2.3	0.118	0.34	2.4	1.5
UK5350	Loch Stack	36	2.52	10.9	0.078	0.36	2.8	21.5
UK5714	Loch Rangag	117	0.32	**3.4**	0.680	0.39	6.1	10.0
UK6234	Loch Culaidh	137	0.11	**2.7**	0.049	0.30	7.2	4.0
UK6405	Loch Naver	73	5.59	11.9	0.061	0.27	3.2	6.0
UK8751	Loch Assynt	65	8.00	30.8	0.450	0.48	2.2	6.5
UK9669	Loch Culag	24	0.15	1.1	0.199	0.34	3.2	0.8
UK10934	Cam Loch	124	2.53	11.5	0.484	0.40	5.9	10.5
UK11189	Loch Osgaig	26	1.68	14.3	0.057	0.40	3.5	1.5
UK11338	Loch Ailsh	154	1.05	2.5	0.125	0.38	3.9	8.6
UK11611	Loch Brora	25	0.67	6.9	0.187	0.24	1.6	7.0
UK12578	Loch an Lagain	136	0.28	2.3	0.224	0.37	4.5	4.0
UK12733	Loch na Béiste	37	0.09	3.2	0.069	0.39	3.7	7.0
UK14057	Loch Maree	6	27.98	38.2	0.054	0.42	2.8	2.9
UK14403	Loch Achnacloich	117	0.07	**2.6**	0.720	0.45	8.2	18.0
UK15176	Loch a' Bhuird	6	0.32	3.2	0.056	0.35	3.8	0.8
UK16456	Loch Ussie	128	0.82	2.4	0.456	0.43	5.5	11.2
UK16530	Loch Gowan	156	0.18	2.1	0.116	0.41	1.9	6.0
UK17329	Loch Fada	145	0.33	**3.4**	0.432	0.39	4.8	3.0
UK17514	Loch Mór	58	0.17	**3.0**	0.940	0.45	24.3	21.0
UK18113	Loch Shnathaid	4	0.23	**4.7**	0.038	0.30	13.6	4.0
UK18305	Caslub	8	0.25	**1.6**	0.312	0.30	12.8	4.0
UK18682	Loch Druidibeag	7	2.57	3.5	0.119	0.39	5.6	8.4
UK22395	Lochan Lùnn Dà - Bhrà	156	0.26	2.6	0.460	0.35	6.4	3.0
UK24459	Loch Lubnaig	121	2.32	13.0	0.211	0.31	4.2	6.6
UK25899	Ardnave Loch	18	0.11	**0.6**	0.657	0.45	2.0	7.0
UK26178	Loch Ballygrant	77	0.27	**3.2**	1.553	0.42	0.3	5.0
UK26217	Loch Lossit	98	0.17	**3.0**	1.227	0.38	0.2	7.0
UK26944	Loch Kinnabus	77	0.44	**3.7**	0.756	0.47	3.6	4.0
UK29000	Crummock Water	96	2.50	26.7	0.051	0.49	5.9	1.3
UK29183	Wast Water	64	2.78	39.7	0.060	0.37	2.1	16.4
UK32761	Llyn yr Wyth-Eidion	68	0.01	6.0	4.170	0.43	8.2	35.0
UK33836	Llyn Idwal	370	0.13	3.4	0.106	0.33	1.7	9.2
UK46102	Little Sea	5	0.31	0.5	0.433	0.39	5.1	25.0

Stepwise Multiple Linear Regression was carried out in Minitab to derive a predictive equation relating PIE scores at reference sites to statistically significant lake typology variables. This predictive equation can then be used to derive site-specific expected PIE scores at new monitoring sites.

Type-specific reference conditions were initially established using approach 1: calculating simple descriptive statistics of PIE scores for each lake type. However, as few data were available for many combinations of depth/alkalinity types (Table 2.2), it was not possible to establish type-specific reference conditions for all possible type combinations. The CCA analysis indicated that the main typology gradient of concern was alkalinity (Figure 3.6). For these reasons, PIE scores were only summarised in terms of alkalinity type. Median values for a type were considered an appropriate measure for type-specific reference conditions, with the 75th or 90th percentile potentially being a suitable measure for defining the high/good status class boundary.

A second approach to establishing type-specific reference conditions was to apply the predictive equation relating PIE scores in reference sites to the median values within the typology range.

4.3 Site-specific reference conditions

Site scores for 50 reference lake samples were calculated with the 112 taxa PIE metric. Stepwise regression was then carried out to develop a model for predicting site-specific reference lake PIE scores. Mean depth, alkalinity and altitude were all considered as potential predictor variables in the regression analysis. Surface area was not included as it was highly correlated with mean depth.

The only typology variable selected in the model was $\log_{10}$alkalinity. There were 3 highly influential outlier samples – all Irish, high alkalinity reference lakes (Lough Bane, Lough Cullaun, Lough Muckanagh). Excluding these 3 sites improved the model considerably with the r^2 increasing from 0.25 to 0.45. The final model coefficients are provided in Table 4.2. Figure 4.1 clearly illustrates the significant positive relationship between PIE score and alkalinity.

Figure 4.1 Relationship between PIE score and alkalinity in reference lakes

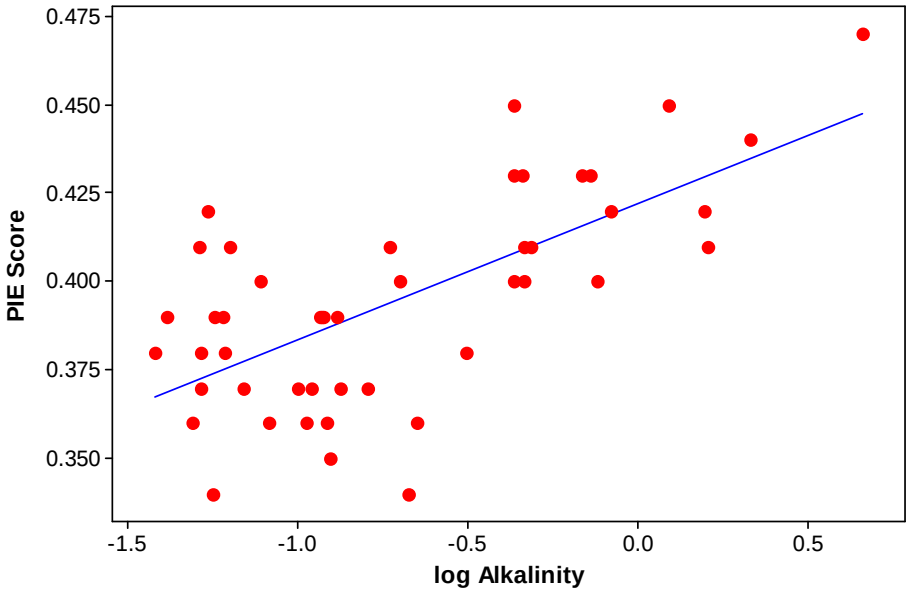


Table 4.2 Coefficients for PIE reference condition model

Predictor	Coef	SE_Coef	T	P
Constant	0.422	0.006	75.78	<0.001
log_Alk	0.038	0.006	6.07	<0.001

4.4 Type-specific reference conditions

Summary statistics for PIE scores for the three main lake alkalinity types (Table 4.3) also illustrate the general trend of increasing PIE scores with increasing alkalinity. The bias in the dataset towards higher numbers (N) of low alkalinity reference lakes is also evident (Table 4.3). Median values for a type are considered an appropriate measure for type-specific reference conditions.

Table 4.3 Measured PIE scores in reference lakes, summarised by lake alkalinity type

	Alkalinity Type		
	Low	Medium	High
N	28	15	7
Median	0.375	0.396	0.417
75%	0.392	0.446	0.469
90%	0.403	0.463	0.518

The second approach to establishing type-specific reference scores, involves application of the predictive model developed in Section 4.3. Using the median alkalinity measures in the whole UK and Irish dataset resulted in very similar type-specific reference conditions, with slightly higher values for all lake types (Table 4.4 compared with median values in Table 4.3)

Table 4.4 Modelled reference PIE scores summarised by lake alkalinity type

	Alkalinity Type		
	Low	Medium	High
Median Alkalinity	0.073	0.431	2.160
Modelled PTI Score	0.379	0.408	0.435

4.5 Discussion

The three approaches used to establish site- or type-specific reference conditions are all to some extent reliant on the same dataset of reference lakes. This dataset is still relatively small when compared with data available for setting reference conditions for river macroinvertebrates or lake macrophytes and, in particular, requires strengthening for medium and high alkalinity lakes. Nevertheless, type-specific reference PIE scores have been established to fulfil the requirements of the WFD.

The analysis revealed differences in reference PIE scores in relation to lake alkalinity types with increasing scores (indicative of higher pressure) associated with increasing alkalinity. The analysis did, however, highlight that type-specific reference conditions may not be ideal as the relationship with alkalinity is continuous, rather than abrupt at type boundaries. Sites that lie close to type boundaries may, therefore, be poorly represented and result in large errors in any type-specific, reference-based status assessment. It is, therefore, recommended that site-specific reference conditions are adopted as they are ecologically more appropriate.

If type-specific conditions have to be used, or at least reported, then the modelled PIE scores based on the median alkalinity values are recommended over the measured PIE scores from reference lakes, as these have a slightly better predictions of the PIE score at reference sites than the population-derived type values and also are likely to be more appropriate for a greater majority of lakes.

5. EQR, BOUNDARY SETTING AND STATUS CLASSES

5.1 Introduction

The WFD prescribes the assessment of ecological quality of surface waters using an Ecological Quality Ratio (EQR). The EQR is defined as the relationship between the observed value and the reference condition for a given ecological quality element. The concepts of the EQR is outlined in Annex V of the WFD:

"In order to ensure comparability of monitoring systems, the results of the systems operated by each Member State shall be expressed as ecological quality ratios for the purposes of classification of ecological status. These ratios shall represent the relationship between the values of the biological parameters observed for a given body of surface water and the values for these parameters in the reference conditions applicable to that body. The ratio shall be expressed as a numerical value between zero and one, with high ecological status represented by values close to one and bad ecological status by values close to zero" (Annex V, Paragraph 1.4.1, WFD)

5.2 EQR calculation

To calculate an EQR from lake phytoplankton community data involves a number of steps

Step 1) Observed PIE scores are calculated as the average of the taxa scores, weighted by the log_{10}-transformed abundance of each taxon. Currently the metric produces PIE values between 0.32 and 0.58, with a lower score (towards 0) being towards reference and a higher score (towards 1) representing a more impacted site, i.e. opposite of WFD EQR requirement.

Step 2) Expected PIE scores for samples are derived using a reference lake regression model (based on mean alkalinity) or a type-specific value (based on the modelled score for the median alkalinity of the lake alkalinity type) where no alkalinity data are available. Currently the reference condition model predicts PIE values between 0.32 and 0.45.

Step 3) Calculate EQR from the ratio of Observed to Expected PIE scores.
If EQR is calculated simply from O/E, EQRs in the UK and Irish datasets range from 0.11 to 1.52 (excluding Slapton Ley 2006 sample which had an EQR of -0.42)

Step 4) To produce an EQR ranging from 0 to 1, the EQRs produced in Step 3 need to be transformed by the minimum and maximum observed EQRs:

$$EQR_{0\text{-}1} = (O/E) - (\text{min'm } O/E) / (\text{max'm } O/E) - (\text{min'm } O/E)$$

To ensure the full EQR scale was used, outliers at either end of the EQR scale were not used; a minimum EQR value of 0.2 and a maximum EQR value of 1.4 were used for re-scaling. Any values obtained <0 were treated as zero and any >1 were treated as 1.

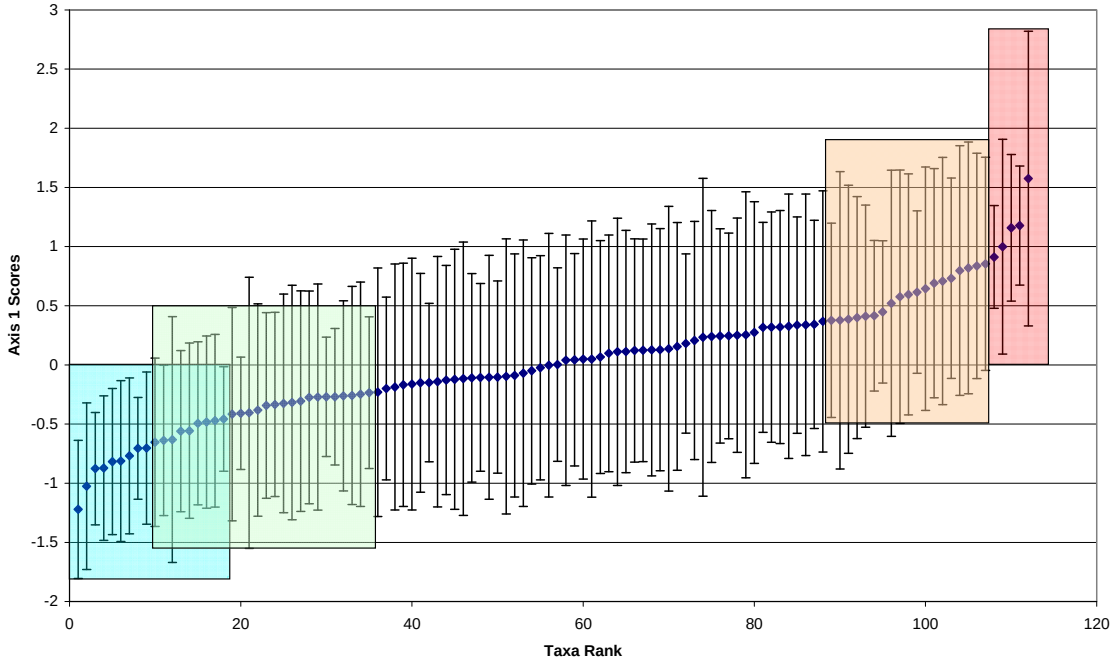
5.3 Boundary Setting

The High/Good (H/G) boundary was determined from the population of reference site EQRs. As several reference site samples appeared to have a flora indicative of eutrophication (EQRs as low as 0.48), the median of reference site EQRs was used to define the H/G boundary with an $EQR_{0\text{-}1}$ of 0.68.

To derive the remaining boundaries, phytoplankton taxa were classified as "positive" (low eutrophication pressure) or "negative" (high eutrophication pressure) indicators. This was

carried out by examining taxa optima and tolerances, in terms of their axis 1 scores from the CCA analysis (see Chapter 3). Taxa were classified as "positive" if their optima was <0 (i.e. less than the average pressure in the dataset) and their tolerance did not extend more than +0.5 species turnover units. If their tolerance did not extend above 0 (average pressure), they were classified as highly positive. Similarly, taxa were classified as "negative" if their optima was >0 (i.e. above average pressure in the dataset) and their tolerance did not extend less than -0.5 species turnover units (Figure 5.1, Table 5.1). If their tolerance did not extend below 0, they were classified as highly negative.

Figure 5.1 Identifying highly positive (blue), positive (green), negative (orange) and highly negative (red) phytoplankton taxa



The biovolumes for all these positive and negative indicator taxa were then summed and the %positive and %negative biovolume for each sample was calculated. Using Minitab, polynomial regression analysis was carried out on the %Positive versus $EQR_{0\text{-}1}$ and %Negative versus $EQR_{0\text{-}1}$ to give the following quadratic equations:

$$\%Positive = -0.064 + 0.181(EQR) + 0.173(EQR)^2$$
$$\%Negative = 0.619 - 1.490(EQR) + 0.896(EQR)^2$$

These quadratic equations were then solved to identify the crossover point, an EQR of 0.53, which was chosen to represent the Good/Moderate (G/M) status class boundary. This fits with the normative definition (Table 1.1) as it is the boundary between Good, defined as "slight changes in composition", and Moderate when "moderate change has occurred in composition" and "pollution tolerant species are more common". The 75% of residuals in the two equations were then used to identify the lower confidence band in the crossover point, which was taken to be the Moderate/Poor (M/P) status class boundary (Table 5.2), again fitting with the normative definition that pollution sensitive species should no longer be common. The remaining Poor/Bad (P/B) boundary was derived from a division of the remaining EQR scale between 0 and M/P.

Table 5.1 Positive and negative phytoplankton taxa used in boundary-setting

Taxa were classified as highly positive (blue), positive (green), negative (orange) or highly negative (red) as described in Section 5.3

Taxon Name	Positive Indicator	Negative Indicator
Quadrigula	-0.64	
Bitrichia	-0.32	
Ulothrix	-0.40	
Euastrum	-0.26	
Glenodinium	-0.20	
Mougeotia	-0.13	
Chrysochromulina parva	-0.11	
Ceratium furcoides	-0.27	
Achnanthidium	-0.06	
Tabellaria flocculosa	0.06	
Characium	0.00	
Merismopedia	0.41	
Diatoma	0.12	
Staurodesmus	0.18	
Urosolenia	0.20	
Snowella lacustris	0.25	
Dinobryon	0.26	
Closterium aciculare	-0.02	
Gymnodinium	0.49	
Gonium	0.07	
Xanthidium	0.44	
Synedra ulna	0.44	
Synura	0.24	
Tabellaria fenestrata	0.31	
Synedra acus	0.41	
Oscillatoria agardhii		-0.89
Fragilaria crotonensis		-0.45
Gloeocystis		-0.22
Golenkinia		-0.15
Anabaena flos-aquae		-0.49
Pediastrum		-0.42
Goniochloris		-0.07
Micractinium		-0.38
Coenococcus		-0.28
Coelastrum		-0.34
Nephrodiella		-0.11
Gomphosphaeria lacustris		-0.26
Lagerheimia		-0.24
Nitzschia acicularis		-0.12
Aphanizomenon flos-aquae		-0.05
Pteromonas		0.48
Treubaria		0.09
Aulacoseira granulata		0.54
Pannus		0.67
Gloeotrichia		0.33

Table 5.2 Proposed Interim EQR boundary values

Class Boundary	EQR
H/G	0.68
G/M	0.53
M/P	0.31
P/B	0.16

5.4 Application to UK lakes

EQR values were derived for all the monthly phytoplankton samples to derive a status class based on phytoplankton composition. Full results for all UK and Irish monthly samples are given in Appendix 2. A comparison of the summary results from all UK and Irish samples, reveals that the site-specific approach is slightly less stringent than the type-specific approach, with 62% of samples of good status or higher compared with 53% (Table 5.3). Irish sites appear to be generally of higher status than UK lakes (Table 5.4). The summary results also indicate that only 49% of samples from reference lakes are classified as high status, although only 9% of reference lake samples were less than good status (Table 5.4).

Table 5.3 Summary results of status classes for site- and type-specific approaches

Status class	Site-specific Total	Type-specific Total
High	93	61
Good	91	98
Moderate	75	105
Poor	30	29
Bad	9	5

Table 5.4 Summary results of status classes for UK, Irish and reference lakes

Status class	UK	Ireland	Reference Lakes
High	71	22	22
Good	77	14	19
Moderate	73	2	4
Poor	30		
Bad	9		

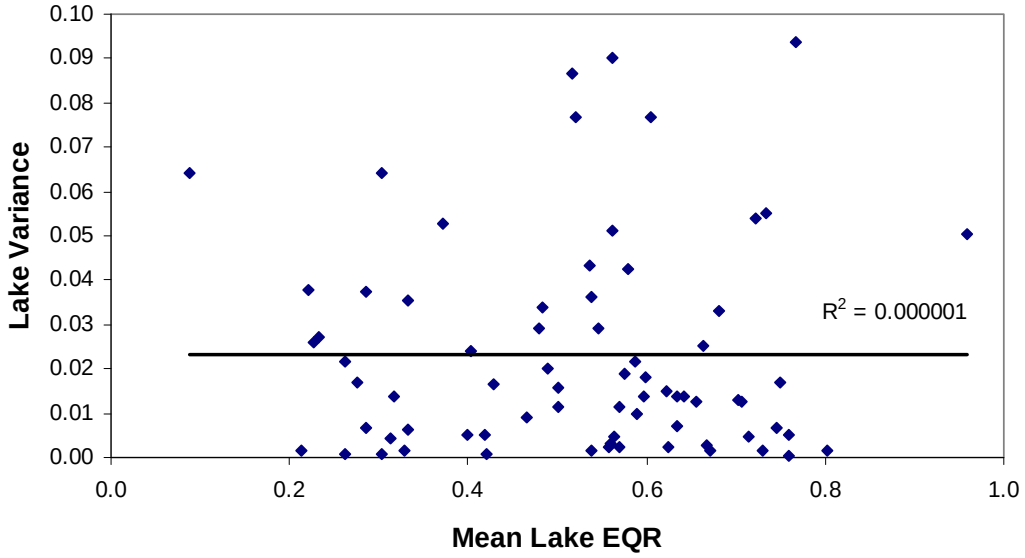
5.5 Confidence in Classification

The mean variance between samples from different months for the same lake was relatively low, and there appeared to be little relationship with sample frequency (Table 5.5). There was also no relationship between mean EQR for a lake and the variance in EQR (Figure 5.2). This means that an average estimate of within-lake S.D. of 0.13 could be used in assessing confidence in classification. The sources of this variance, and implications for sampling and analysis to minimise uncertainty, are examined further in Chapter 6.

Table 5.5 Mean standard deviation and variance of EQR scores in relation to sample frequency

Sample Frequency	N	Mean S.D.	Mean Variance
2	31	0.14	0.03
3	25	0.12	0.02
4	14	0.14	0.02
5	1	0.12	0.01

Figure 5.2 Relationship between mean lake EQR and lake variance



If the relationship between observed mean EQR and S.D. EQR is accepted as an acceptable estimate of the error associated with a given EQR (i.e. accounts for sampling, temporal and spatial sources of variation), we can then combine this with information on class boundaries to predict the confidence with which a site can be assigned to a given class. The procedure for calculating confidence of class is outlined by Ellis (2006). The risk of face-value misclassification (i.e. of assigning a site to the wrong class) is then computed as the sum of confidences of membership of all classes except for the observed class. It should be noted that this approach differs slightly from that using the STARBUGS software (Clarke, 2004; Clarke & Hering, 2006).

The first stage in the procedure outlined by Ellis (2006) is fitting a power curve model to the data to best explain the relationship between mean and S.D. of EQR values (Figure 5.3). For this, it has been assumed that the model has anchor points at EQR 0 and 1. Based on this relationship and the boundary values outlined in Table 5.2, Figure 5.4 illustrates the confidence that a site belongs to an observed class for a given EQR value. This is based on 9 monthly summer samplings carried out over a six-year monitoring cycle. This analysis highlights that in the middle of a status class, the confidence that a lake belongs to that class is at least 90%. In terms of the risk of misclassification (Figure 5.4), this will always be at least 50% for an EQR that lies exactly on a class boundary, but will fall to a minimum moving towards the middle of that class. For example, the risk of misclassification for a site with an EQR in the middle of the good status class is just less than 10%, where there is either about 5% chance the site is in moderate status or about a

5% chance that it is in high status (Figure 5.3). The analysis does show that the high and moderate status class widths are possibly too wide and should be reviewed.

Figure 5.3 Fitted relationship between mean lake EQR and lake variance

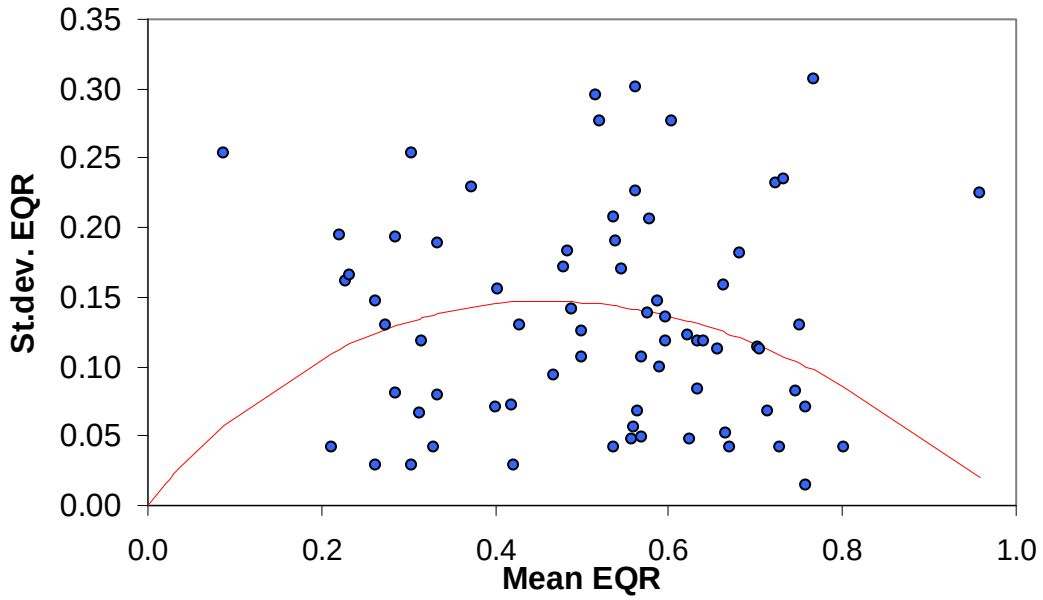


Figure 5.4 Confidence of class (%) for a given EQR

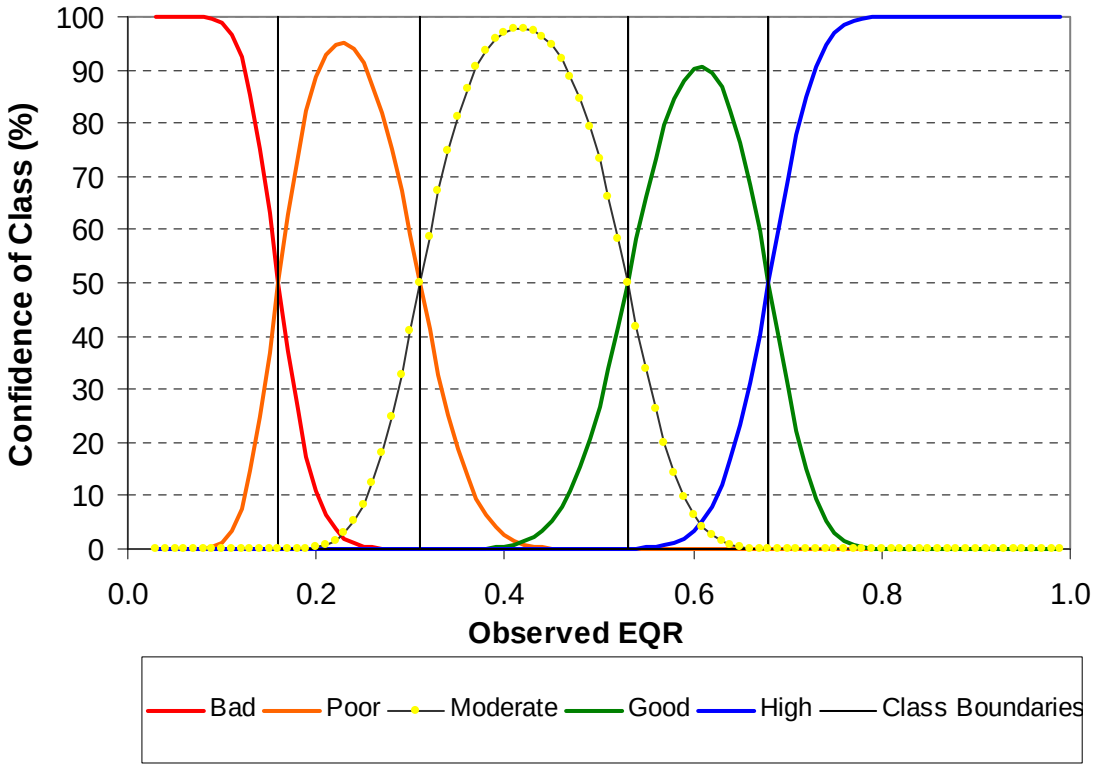
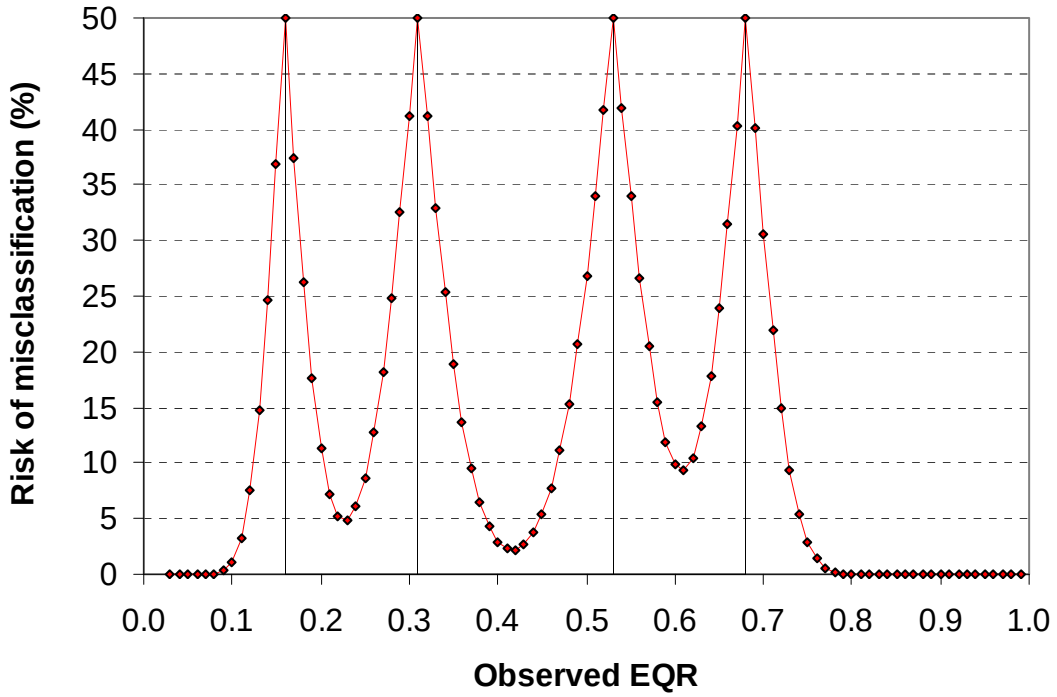


Figure 5.5 Risk of mis-classification (%) for a given EQR



6. UNCERTAINTY IN PHYTOPLANKTON COMPOSITION

Ralph Clarke, Iwan Jones and Laurence Carvalho

Note: EQR values used in this chapter were based upon preliminary untransformed EQRs. The analysis needs to be repeated on the final agreed EQR_{0-1} scale to be applicable. The relative magnitudes of sources of error are, however, informative for developing future sampling and counting guidance.

6.1 Introduction

There are a number of sources of uncertainty or error in applying the PIE metric or any other metric based on phytoplankton composition data. The main components of variability are:

- Variation between sampling locations within a given water body (spatial variability) – both horizontal and vertical variability
- Variation between sub-samples of a sample and fields of view observed under a microscope (sub-sample variability)
- Variation between years (inter-annual variability)
- Temporal variation within a year (seasonal, monthly, daily and diurnal changes in composition) (in this report referred to as "temporal variability")
- Differences due to observer or analytical error (referred to as "counter variability" in this report)

To effectively decompose the variability in PIE scores for a given lake into these components would require a well-structured, hierarchical sampling design, as outlined in Jones et al. (2006). It has not been possible to gather such a nested dataset to investigate all these sources of variability for phytoplankton composition. For this reason, the project focused on collecting data on what was considered to be the greatest source of error or variability, the combination of sub-sample and counter variability. This was carried out by conducting two counter "ring-tests".

The aim of the phytoplankton ring tests were to assess and quantify the variation in phytoplankton metric values and uncertainty in derived ecological status classes which can arise purely from the effect of a different counter taking, identifying and enumerating a different sub-sample from a field sample of the phytoplankton community. One ring-test was carried out before a counter workshop and one after. Comparison of the results could, therefore, also inform how effectively training could be used to minimise uncertainty.

Many of the other aspects of variability have been examined in terms of phytoplankton chlorophyll$_a$ concentrations (see Chapter 8), which although not the same as PIE scores, will be affected to some extent by the same drivers (such as horizontal patchiness associated with wind-driven surface water movements). For this reason, the chlorophyll uncertainty estimates may give some indication as to their relative importance in terms of phytoplankton community composition, and certainly of variability in total biovolume.

6.2 Methods

A sample of the phytoplankton was obtained from each of six "lakes" selected to cover a range of depths, alkalinities and perceived ecological quality classes (Table 6.1). A sub-sample from each main sample was taken and the phytoplankton taxa identified and enumerated independently by each of up to six counters (identified by the first letter of

their surname B, F, G, K, P and S). Loch of Clunie, Grasmere, Elter Water and Upton Broad were enumerated by all six counters, while Barton Broad was not counted by counter K and Ennerdale Water was not counted by either counter K or S.

The within lake variation incorporates variation due to:

- differences between the sub-samples of the main sample (one sub-sample taken by each counter),
- differences between the actual fields of view observed by the counters (sub-sub-samples of the main sample),
- differences between the numbers of fields of view observed for each taxa by the counters,
- differences in the identification and enumeration of the different taxa by the different counters (i.e. in terms of the number of taxa recorded and/or the type of taxa recorded and perhaps missed or mis-identified).

Whilst it is not necessary (or possible with these data) to isolate the impact of these individual sources of variation on the overall uncertainty in the procedure, it maybe useful, in future studies, to separate the uncertainty associated with sub-sampling from that associated with processing in order to identify areas where improvements can be made to the technique (this would require different people to process the same sub-sample and/or same fields of view).

Although this experimental design is not quite balanced (i.e. not all counters sub-sampled each water body), it was still possible to use unbalanced analysis of variance (ANOVA) techniques (procedure GLM in MINITAB) to test for systematic differences between the results obtained by the six counters (i.e. after allowing for differences between lakes in average metric value).

As only one main sample was taken in the field from each lake, there is actually no replication within lakes with which to assess the larger scale spatial variability of phytoplankton community composition within lakes. It is not, therefore, possible to do a proper statistical test for differences in metric values between lakes, as any inferred differences could just be due to the fact that each lake is a different field sample, regardless of whether it is from the same or a different lake.

However, to assess the relative size of sub-sampling variability and counter differences, we used random effects ANOVA to estimate the:

1. variance (VC) due to systematic differences in counter average values (i.e. due to variation in counter biases)
2. average variance (VS) in metric values between sub-samples (adjusted for any systematic counter biases) within a lake
3. variance (VB) due to differences between lakes (and/or main field samples).

From these variance component estimates, we can estimate:

$V_W = V_S + V_C$ = total within-lake variance due to sample processing (i.e. sub-sampling + counter effects)

$P_C = 100\ V_C / V_W$ = percentage of sample processing variance (V_W) due to systematic counter biases

$V_T = V_S + V_C + V_B$ = Total study variance within and between lakes

$P_W = 100\ V_W / V_T$ = percentage of total study variance due to sample processing effects

Five phytoplankton metrics were analysed:

1. Observed value of PIE Score
2. Ecological Quality Ratio (EQR), where Observed PIE Score has been divided by the estimate of the Expected PIE Score for that lake type. To get an EQR scale from 0 -1, both observed and expected scores were transformed by subtracting from the maximum observed score.
3. Total biovolume of phytoplankton per ml of water
4. Total number of taxa recorded in the sub-sample (species richness)
5. Total number of taxa recorded which were involved in determining the PIE Score (referred to as Taxa Matched)

The ANOVA techniques are used to estimate the average, across all the lakes, of the variance in a metric's values within a lake (ANOVA statistical tests are based on an assumption of equal within-lake variability). These types of overall estimate of the average within-site sampling/processing variability are also used in uncertainty assessment software, such as STARBUGS (STARBUGS system (STAR Bioassessment Uncertainty Guidance Software; Clarke 2005, Clarke & Herring 2006)) to derive estimates of these process effects on uncertainty in assigning water bodies to a WFD ecological status class. For the single estimate of the average within-lake variance to be appropriate across all other lakes for which it is applied, it is important to try to minimise any dependence of within-lake variability in a metric's values on the average, or general level, of the metric for a particular lake. In particular, the within-lake variance tends to increase naturally with the within-lake mean value for counts or biovolumes of individual taxa or groups of taxa. In this study, total variability between counters in estimates of total biovolume was much greater for Upton Broad and especially Barton Broad, which had average total biovolumes orders of magnitude higher than the other study lakes.

By assessing the relationship between variance and mean, it is often possible to transform the metric so that, on the transformed scale (square roots or logarithmic), the within-site variability in a metric's values is relatively less variable between sites and thus it is more appropriate and valid to assume the estimate of average within-site variance will apply to other sites for which we may just have a single sub-sample (see Clarke *et al* 2002, Clarke *et al*. 2006a, 2006b for further details). In this study, we used Taylor's Power law regressions (Clarke *et al* 2002) of log within-lake variance on log within-lake mean to determine whether there was any significant relationship and to determine, where needed, the best transformation of each metric (part (b) of Figures 6.1-6.5).

6.3 Results

The variation in values of each metric within each water body is summarised in Table 6.1 and Figure 6.1-6.5.

Variation in Observed Score was greatest for Ennerdale Water and Barton Broad but there was no overall tendency for the variance to increase with the average Observed score for a lake (Figure 6.1). A similar pattern and lack of significant variance-to-mean relationship was found for the standardised EQR Score, although variation between sub-sample values was greatest for the poor quality Barton Broad water body which had the lowest average EQR Score (Figure 6.2).

In contrast, total biovolume still showed a tendency for within-lake variance to increase with average total biovolume for a lake when analysed on a logarithmic (log to base 10) scale (Figure 6.3). From the limited study site data available, the best transformation of

total biovolume to make the within-site variability most equitable was the double-log transformation $\log_{10}(\log_{10}(X))$ (Figure 6.6).

The within-lake variances in the total number of taxa recorded and the number of matched taxa recorded both increase with the average level for a lake (Figure 6.4-6.5). Working with the logarithms ($\log_{10}$) of these two taxonomic richness metrics appears to eliminate any variance-to-mean relationship (Figure 6.7-6.8); this should make any estimate of average within-lake variance on this transformed scale more appropriate to use for other lakes in assessing uncertainty in their ecological status based on these and perhaps other metrics.

The estimates of the within-lake variance, between-lake variance and percentage within-lake variance for each metric on its perceived optimally-transformed scale are given in Table 6.2.

Table 6.1 Mean, standard deviation (SD), standard error of mean (S.E.), minimum, median, maximum and range of values of selected phytoplankton community metrics within each of the six study lakes

(a)Observed Score							
LakeName	Mean	S.E.	S.D.	Min	Median	Max	Range
Ennerdale Water	0.358	0.017	0.033	0.334	0.346	0.406	0.072
Barton Broad	0.512	0.019	0.041	0.454	0.524	0.561	0.107
Loch of Clunie	0.437	0.010	0.023	0.417	0.430	0.479	0.062
Grasmere	0.383	0.004	0.010	0.368	0.385	0.395	0.027
Elter Water	0.400	0.010	0.024	0.362	0.398	0.426	0.064
Upton Broad	0.458	0.010	0.025	0.433	0.454	0.505	0.072

(b) EQR Score (type-specific Expected Score)							
LakeName	Mean	S.E.	S.D.	Min	Median	Max	Range
Ennerdale Water	1.123	0.077	0.154	0.900	1.180	1.230	0.330
Barton Broad	0.530	0.112	0.251	0.230	0.460	0.880	0.650
Loch of Clunie	0.877	0.051	0.126	0.650	0.915	0.980	0.330
Grasmere	1.003	0.019	0.047	0.950	0.995	1.070	0.120
Elter Water	1.075	0.052	0.127	0.940	1.085	1.280	0.340
Upton Broad	0.860	0.061	0.149	0.580	0.885	1.010	0.430

(c) Total Biovolume						
LakeName	Mean	S.E.	S.D.	Min	Median	Max
Ennerdale Water	106899	24245	48490	60456	96131	174880
Barton Broad	359451338	322618099	721395999	16499767	46759288	1649500684
Loch of Clunie	1107336	425376	1041954	255046	584221	2876776
Grasmere	1111351	328512	804687	66796	1032208	2298543
Elter Water	436473	175409	429663	47813	260371	1017067
Upton Broad	9798218	8485596	20785381	857021	1214983	52215195

(d) Total Taxa recorded							
LakeName	Mean	S.E.	S.D.	Min	Median	Max	Range
Ennerdale Water	15.75	1.70	3.40	11	16.5	19	8
Barton Broad	29.40	2.77	6.19	21	32.0	36	15
Loch of Clunie	21.50	1.20	2.95	18	20.5	26	8
Grasmere	17.33	1.36	3.33	13	17.0	22	9
Elter Water	14.17	1.01	2.48	11	14.5	18	7
Upton Broad	10.83	0.95	2.32	7	11.0	14	7

(e) Taxa Matched (i.e. used to calculate observed Score)							
LakeName	Mean	S.E.	S.D.	Min	Median	Max	Range
Ennerdale Water	12.25	1.84	3.69	8	12.0	17	9
Barton Broad	24.20	2.22	4.97	19	22.0	30	11
Loch of Clunie	17.00	1.32	3.22	14	15.5	22	8
Grasmere	12.83	1.11	2.71	10	12.0	17	7
Elter Water	10.33	0.96	2.34	7	11.0	13	6
Upton Broad	8.50	0.67	1.64	6	8.5	11	5

Table 6.2 Estimates of the components of variance in selected phytoplankton community metrics

Variability due to sub-sampling (V_S), systematic counter biases (V_C) and between-lake (and/or field sample) differences (V_B). $V_W = V_S + V_C$ = total within-lake variance due to sample processing effects, $P_C = 100\ V_C / V_W$ = % of sampling processing variance due to systematic counter biases, $P_W = 100\ V_W / (V_W + V_B)$ = % of total study variance due to sample processing effects; $SD_W = \sqrt{V_W}$ = sample processing standard deviation. Estimates based on the optimally-transformed scale, where appropriate.

Metric	V_S	V_C	V_W	V_B	P_C	P_W	SD_W
Observed Score	0.00064	0.00007	0.00071	0.00274	10	21	0.027
EQR Score	0.01999	0.0024	0.02239	0.03847	89	37	0.150
$Log_{10}Log_{10}$Total Biovolume	0.00146	0.00002	0.00148	0.00367	1	29	0.038
Log_{10} Total Taxa recorded	0.00653	0.00096	0.00749	0.02296	13	25	0.087
Log_{10} Taxa Matched	0.0064	0.00284	0.00924	0.02664	31	26	0.096

The apparent differences between lakes were statistically significant (all test $p < 0.001$) for all five metrics, but, as was pointed out above, because only one field sample was taken from each lake, this could just be due to spatial variation between field samples, regardless of lake.

Systematic differences between counters (i.e. a consistent tendency for one or more counters to record higher values than other counters) were not statistically significant for Observed Score ($p = 0.197$), EQR Score ($p = 0.189$), $Log_{10}Log_{10}$ Total Biovolume ($p = 0.393$) or Log_{10} Total Taxa Recorded ($p = 0.156$), but were for Log_{10} Taxa Matched ($p = 0.020$). This significant result occurred primarily because counter F recorded fewer Taxa Matched than counters G and K at each of the six and four lakes respectively where comparisons could be made (Figure 6.8(a)).

Variation due to systematic counter biases (due to consistent differences in the number of taxa recorded and/or the type of taxa recorded and perhaps missed or mis-identified) accounted for at most an estimated 13% of overall sampling processing variance in all metrics, except for the metric Log_{10} Taxa Matched for which systematic counter differences explained 31% of sample processing variance (Table 6.2).

Thus the vast majority (at least 69%) of the total variance in metric values due to sample processing (i.e. what you do with the field sample) is due to the combined effect of sub-sampling and inconsistencies among the counters such as the number of fields of view observed.

Overall sample processing variance (V_W) accounted for between 21%-37% of the total variance in metric values within this study. The percentage sample processing variance (P_W) was lowest for observed score (21%), but this was partly because Observed Score varies naturally between lake types; once standardised (i.e. “corrected”) for lake type by expressing it as EQR Score, sample processing variance increased to 37% of total study variance (Table 6.2).

The estimated average standard deviation (SD_W) of EQR Score due to sample processing errors is 0.150 (Table 6.2), which indicates that different sub-samples could lead to estimates of EQR Score for the same field sample which vary by plus or minus twice 0.15 or roughly over a range of 0.6, which is rather large given the potential range of EQR

values across all lakes and ecological status classes. However, the range of EQR Scores values observed amongst the 4-6 counters for each lake was actually considerably less than this for five of the lakes, varying from 0.12 at Grasmere to 0.43 at Upton Broad; but at poorer quality Barton Broad the EQR Score values obtained from the five counters varied from 0.23 to 0.88, a range of 0.65 (Figure 6.2 and Table 6.1)

6.3 Application to status class classification

If any of these metrics were to be used in the assessment the ecological status of lakes, then the estimate of the typical standard deviation of metrics values within a site due to the combined effects of inter-counter differences and sub-sampling variability obtained from this study (namely the SDW estimates in Table 6.2) could be used in software such as STARBUGS as preliminary estimates of the consequences of this source of variability on uncertainty in assignment to ecological status class based on one or more of these phytoplankton metrics.

6.4 Recommendations for sampling

If the composition also varies spatially within the lake, then the metric must be defined as the EQR at a specific location within the lake (e.g. outflow or centre of lake), ideally where the community is mixed and representative of the average of the lake as a whole (e.g. integrated vertical sample).

To cover the temporal variability adequately, it is recommended that samples should be taken at the same location throughout summer at a monthly frequency (July, August & September). This will help reduce uncertainty in the metric (PIE Score) and hence in the final EQR assessment of the lake.

6.5 Further research

Within the resources available, it has not been possible to take more than one field sample from around each lake to assess and quantify the effects of larger scale within-lake spatial variability in phytoplankton community composition on derived metric values and thus estimates of WFD ecological status class. Obviously, if the sampling protocol is always to sample at say the lake “outfall”, then, in one sense, it does not matter how much the lake varies elsewhere. However, if the aim is to characterise the ecological quality of the whole lake, then the ecological status derived from the sample or samples taken is intended to represent the status of the whole lake and it is important to understand what the quality is around the lake and whether it makes much difference where the sample(s) are taken.

These preliminary analyses of variability hint that this new EQR Score metric may be more susceptible to sub-sampling (and perhaps field sampling variation) at poorer quality lakes which have greater but highly variable sub-sample total biovolumes and greater but highly variable total numbers of taxa and taxa actively involved in determining Observed Score and thus EQR Score for a lake. However, more lakes of a wide range of ecological qualities need to be assessed using replicate samples and replicate sub-samples for this to be confirmed.

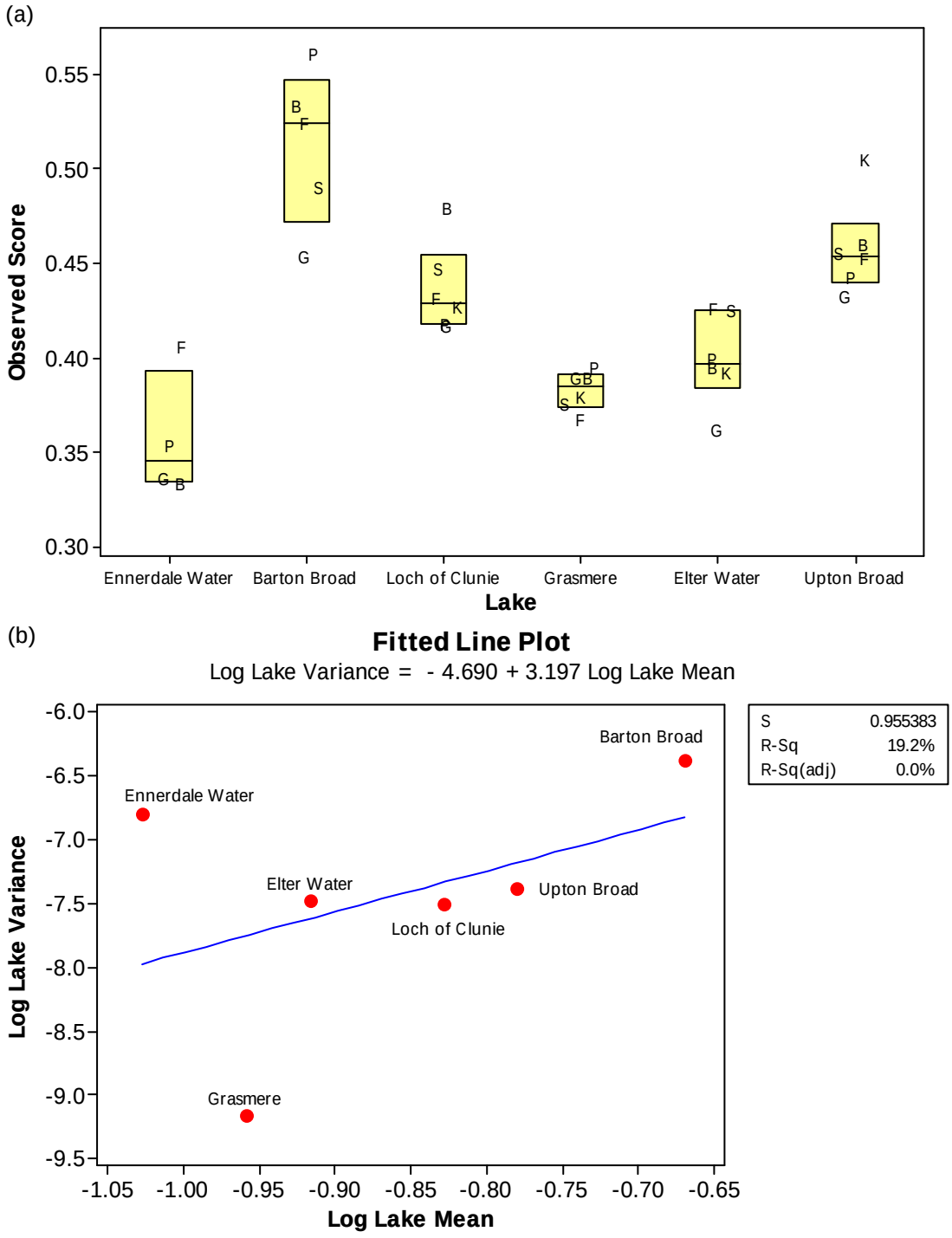


Figure 6.1 **Observed Score: (a) boxplot of scores within each lake (letters denote individual counters, boxes indicate inter-quartile range and median); (b) regression plot of log variance against log mean**

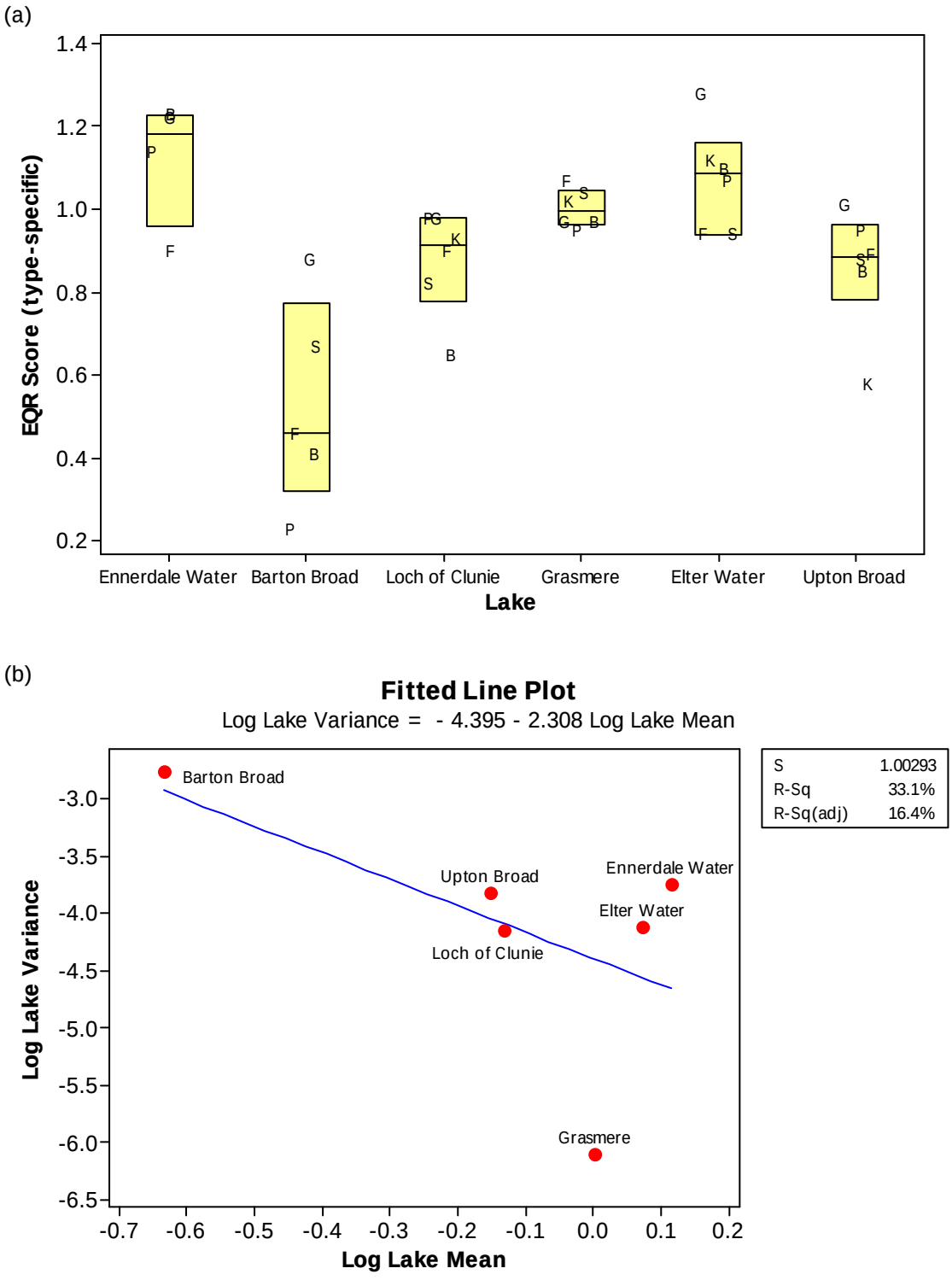


Figure 6.2 **Ecological Quality ratio (EQR) Score: (a) boxplot of scores within each lake (letters denote individual counters, boxes indicate inter-quartile range and median); (b) regression plot of log variance against log mean**

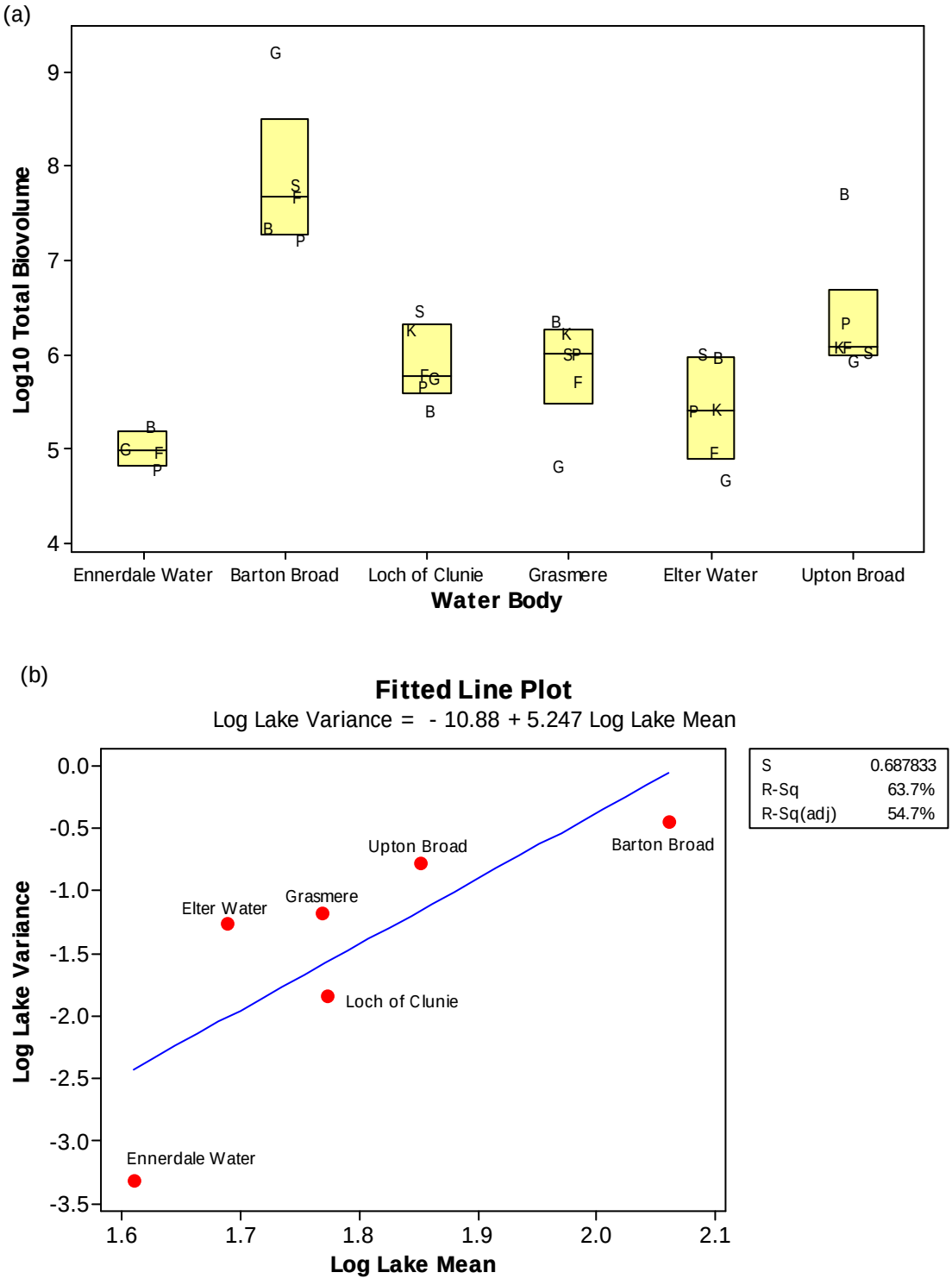


Figure 6.3 **Log_{10} total phytoplankton biovolume within each lake: (a) boxplot of scores within each lake (boxes indicate inter-quartile range and median); (b) regression plot of log variance against log mean**

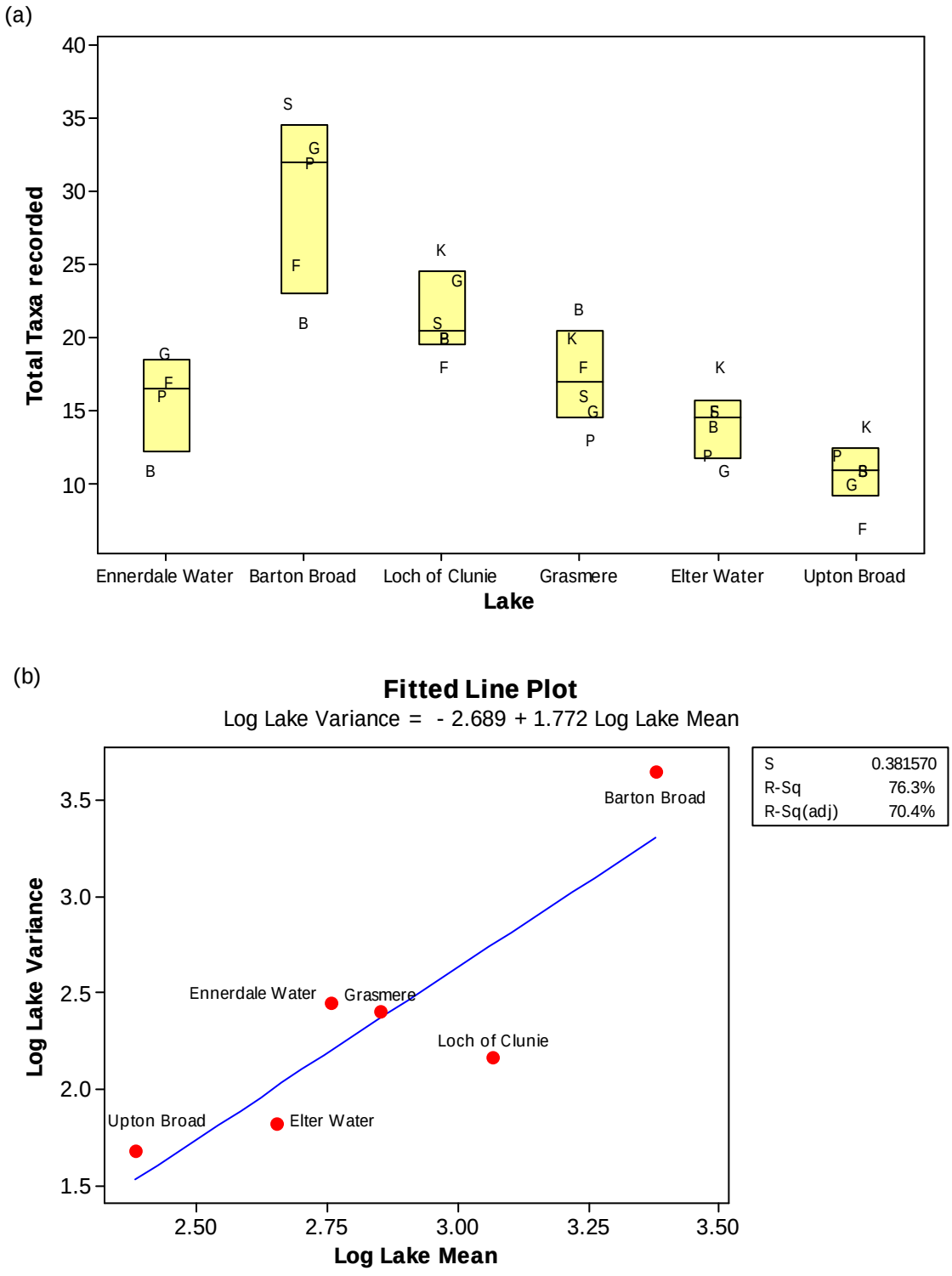


Figure 6.4 **Total taxa recorded: (a) boxplot of scores within each lake (boxes indicate inter-quartile range and median); (b) regression plot of log variance against log mean**

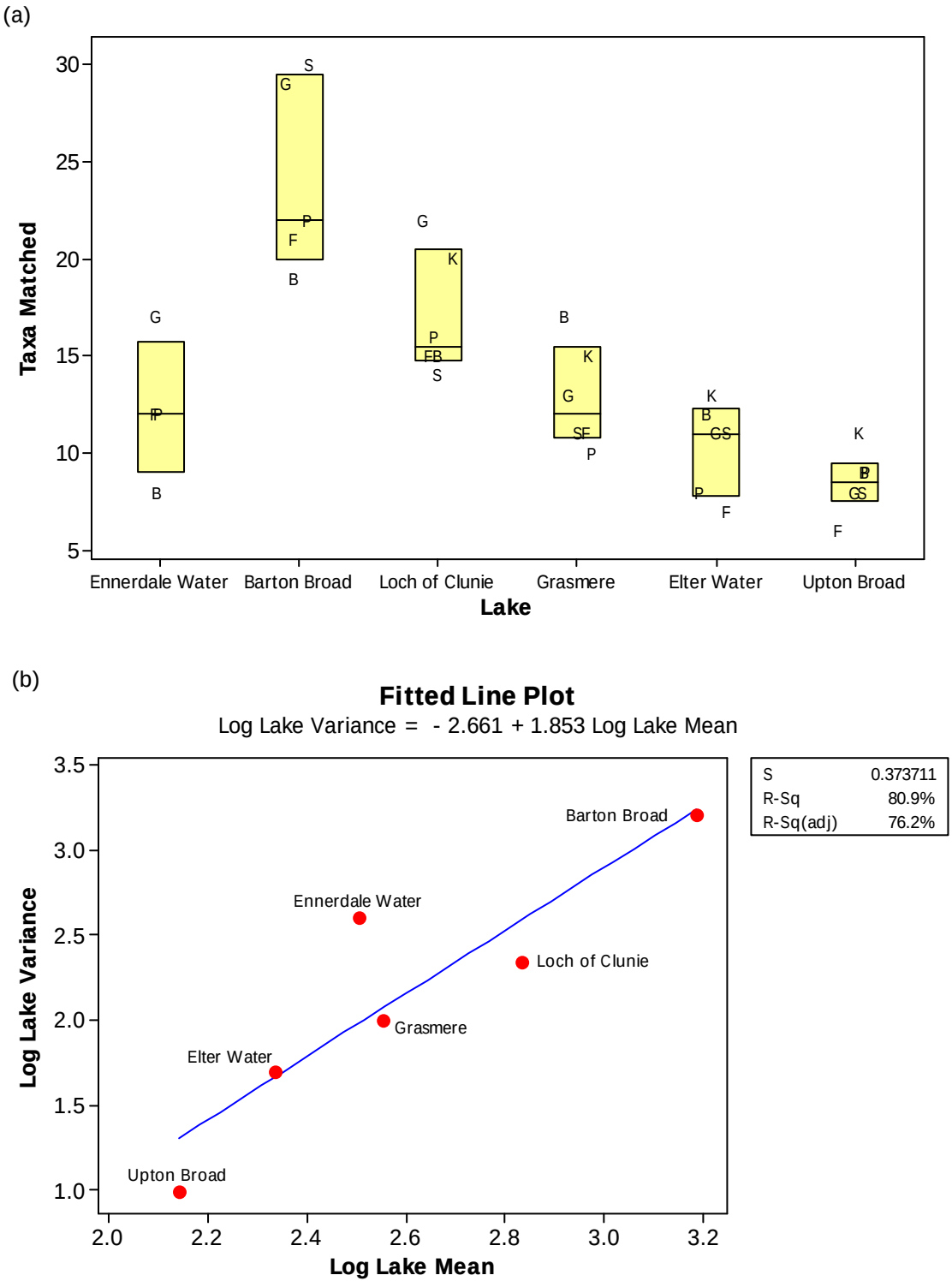


Figure 6.5 **Taxa Matched: (a) boxplot of scores within each lake (boxes indicate inter-quartile range and median); (b) regression plot of log variance against log mean**

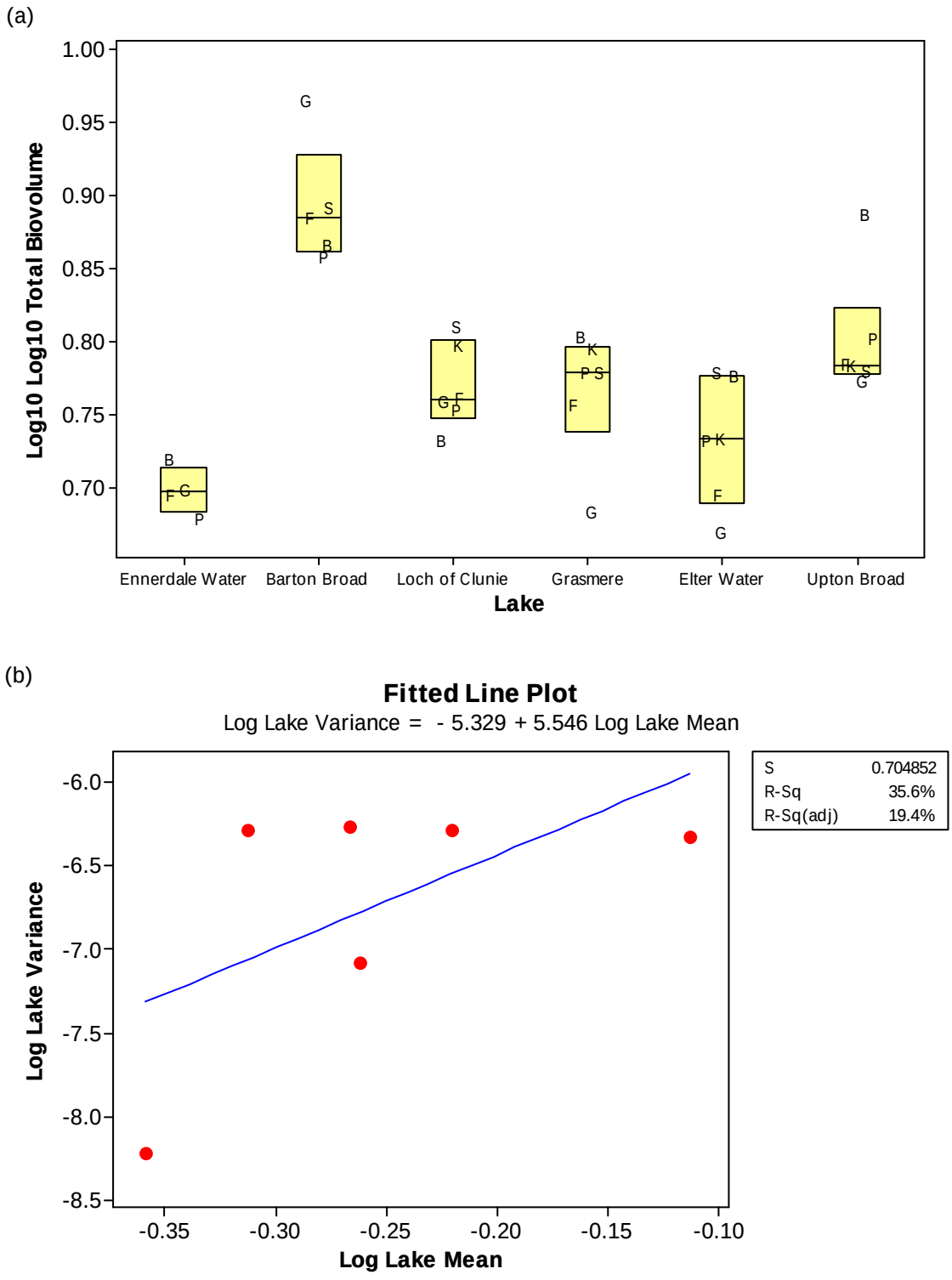


Figure 6.6 **Double logarithm ($Log_{10}Log_{10}$) of total phytoplankton biovolume within each lake: (a) boxplot of values within each lake (boxes indicate inter-quartile range and median); (b) regression plot of log variance against log mean**

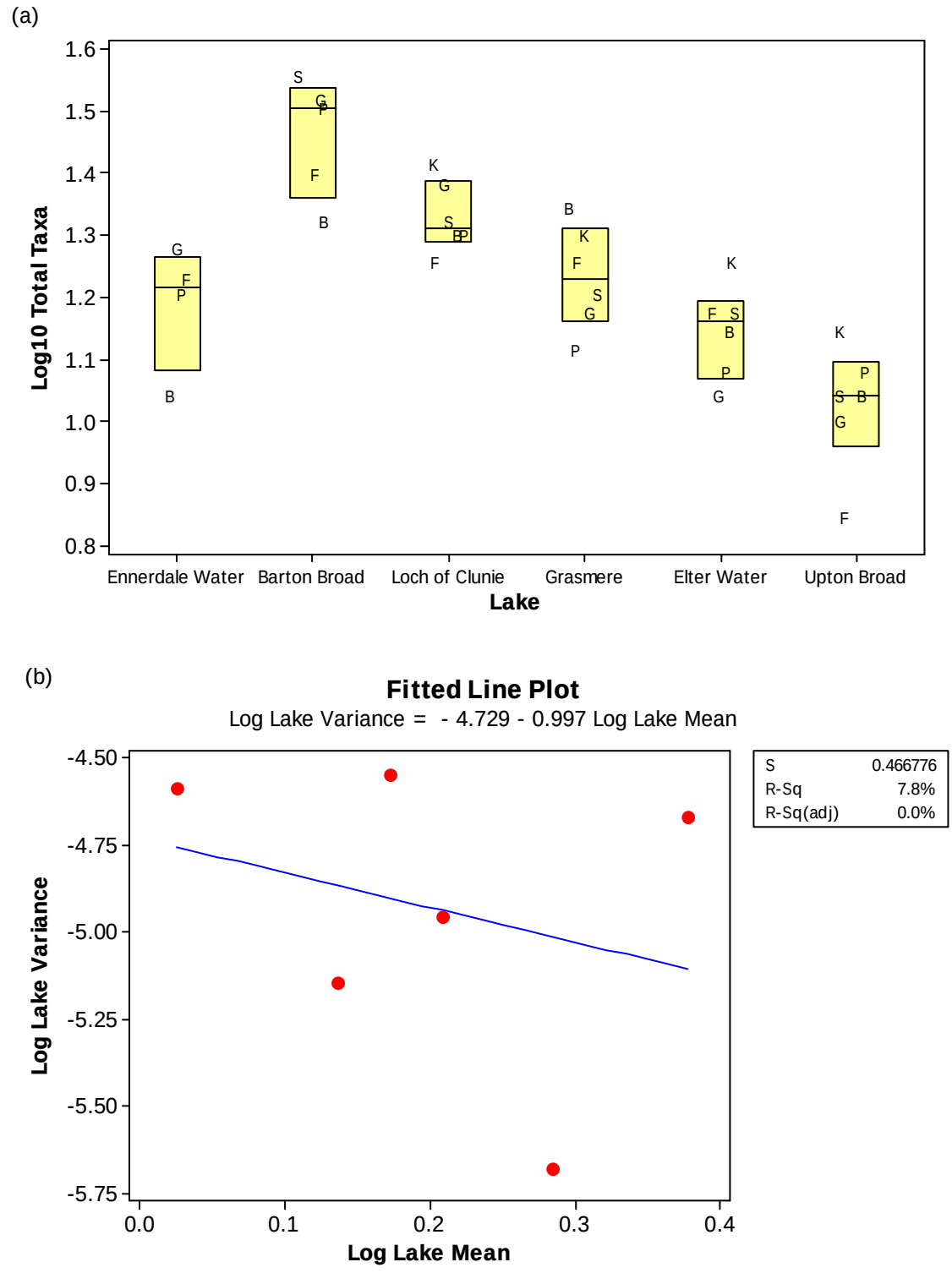


Figure 6.7 **Log_{10} Total taxa recorded: (a) boxplot of values within each lake (boxes indicate inter-quartile range and median); (b) regression plot of log variance against log mean**

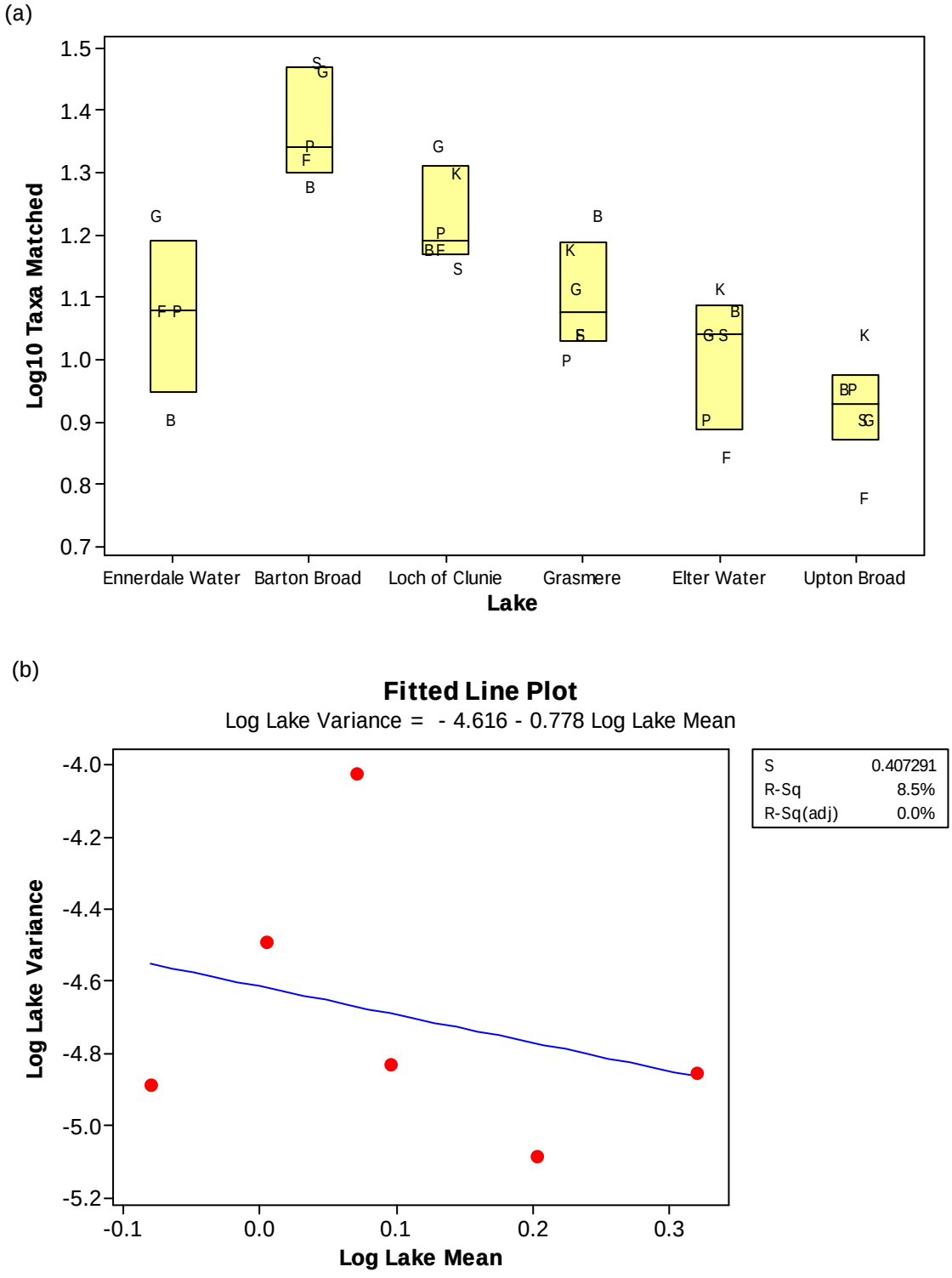


Figure 6.8 **Log_{10} Taxa Matched: (a) boxplot of values within each lake (boxes indicate inter-quartile range and median); (b) regression plot of log variance against log mean**

7. UNCERTAINTY IN CHLOROPHYLL METRIC

7.1 Introduction

The aim of this work package was to examine sources of variation in chlorophyll data. The focus of this work was on within-lake spatial variation and laboratory analytical variability. The results are then summarised and compared with previous work on temporal variability (Clarke et al., 2006). Finally the overall implications for sampling and status classification are considered

7.2 Spatial variability in chlorophyll concentrations

Lakes are well known to show spatial heterogeneity or patchiness (Downing 1991). At large spatial and temporal scales this can be related to varying conditions such as temperature, light, hydraulic conditions or nutrient availability. In small lakes and shorter time scales, heterogeneity of phytoplankton populations is normally a consequence of interactions between wind speed and direction and the thermal stability of the water column (Reynolds, 1984, 1997, 2006; George 1993; Webster & Hutchinson 1994; Marce et al 2007). Reynolds (1997) suggested a 'conveyor belt' analogy for the distribution of different algal types as a result of wind and water movement. When the wind blows in one direction, positively buoyant species such as cyanobacteria will concentrate at the downwind shore and negatively buoyant species such as diatoms will accumulate at the opposite shore while neutrally buoyant species will be randomly distributed. An example of the wind-driven patchiness of phytoplankton is illustrated in George & Edwards (1976) and Heaney (1976).

To examine spatial variability, from each of 12 lakes a number of chlorophyll samples were collected from the open water zone (5 replicates), the edge of the lake (5 replicates) and the outflow (3 replicates).

The data were first visualised by calculating mean and standard deviation for the 12 lakes and three sampling locations. In some lakes, such as Barton Broad (Figure 7.1) there was a close agreement between estimates made on samples collected from different locations, but at other lakes, such as Elterwater, samples from the open water and the edge were both very variable and there were large differences in mean values from the three collection locations. The overall grand total means were very similar for the different sampling locations when averaged over the twelve lakes.

The data were analysed to answer two key questions:

Q1. Are chlorophyll concentrations significantly different in the three locations?
Q2. In which location do we find the least variable chlorophyll concentrations?

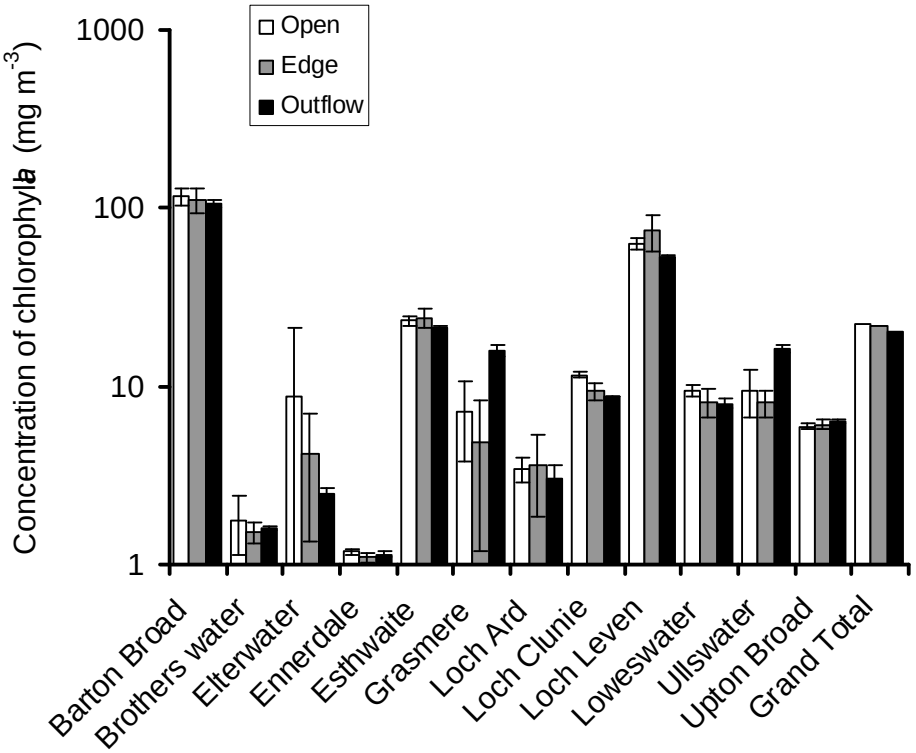


Figure 7.1 Summary of mean chlorophyll a concentration in samples collected from the open water, lake edge and outflow at twelve lakes in summer 2006

Error bars denote one standard deviation. Note the logarithmic scale on the y-axis.

Q1. Are chlorophyll concentrations significantly different in the three locations?

To answer this question a two-way ANOVA within interaction (procedure GLM in MINITAB) was used with chlorophyll *a* concentration as the response variable and with lake and location (open water, edge, outflow) as the two explanatory factors. Because the lakes selected are just a sample of all the UK lakes about which we would like to apply the results, lake was treated as a random factor in the ANOVA. The idea was to control statistically for among-lake variations in chlorophyll *a* concentration whilst testing for effects of location.

An initial ANOVA was carried out on these data and was examined for evidence of such problems. This analysis showed a very significant effect ($p < 0.001$) of lake on the raw chlorophyll *a* concentration, no significant overall differences in average chlorophyll *a* between locations ($p=0.530$), but a significant interaction between location and lake ($p=0.021$) (Table 7.1). The two predictor variables and their interaction jointly explained an estimated 97% of the variation in chlorophyll *a*, which indicates that variation between replicates at the same location on the same lake contribute only 3% of the total variation in chlorophyll *a* amongst the study lakes.

Table 7.1 Analysis of variance table for a two-way ANOVA within interaction of raw chlorophyll a concentration on lake and location

SS = sum of squares, MS = mean square.

Source	df	Adjusted SS	Adjusted MS	F	P
Lake	11	159424	13371	361.55	<0.001
Location	2	48	24	0.64	0.530
Interaction (Lake x Location)	22	1489	68	1.83	0.021
Replicates	117	4327	37		

An initial examination of the chlorophyll *a* data showed that it was heavily skewed: comprising many low values and a small number of very large values, chiefly from Loch Leven and Barton Broad. Furthermore, residual variations from the ANOVA on the untransformed chlorophyll *a* data did not meet the ANOVA assumption of normality and homogeneity of variability between replicates in each lake-location combination (Figure 7.2). It is common for variability between replicates to increase with the mean value of the replicates, in which situations a log transformation of values can remove or reduce both skewness and heteroscedasticity.

The chlorophyll *a* data were therefore $\log_{10}$ transformed and the analysis was run again with these transformed data (Table 7.2).

Table 7.2 Results of a two-way ANOVA with interaction of $\log_{10}$ transformed chlorophyll a concentration on lake and location

SS = sum of squares, MS = mean square.

Source	df	Adjusted SS	Adjusted MS	F	P
Lake	11	45.843	4.1675	86.17	<0.001
Location	2	0.094	0.0468	0.95	0.400
Interaction (Lake x Location)	22	1.080	0.0491	2	0.01
Replicates	117	2.869	0.0245		

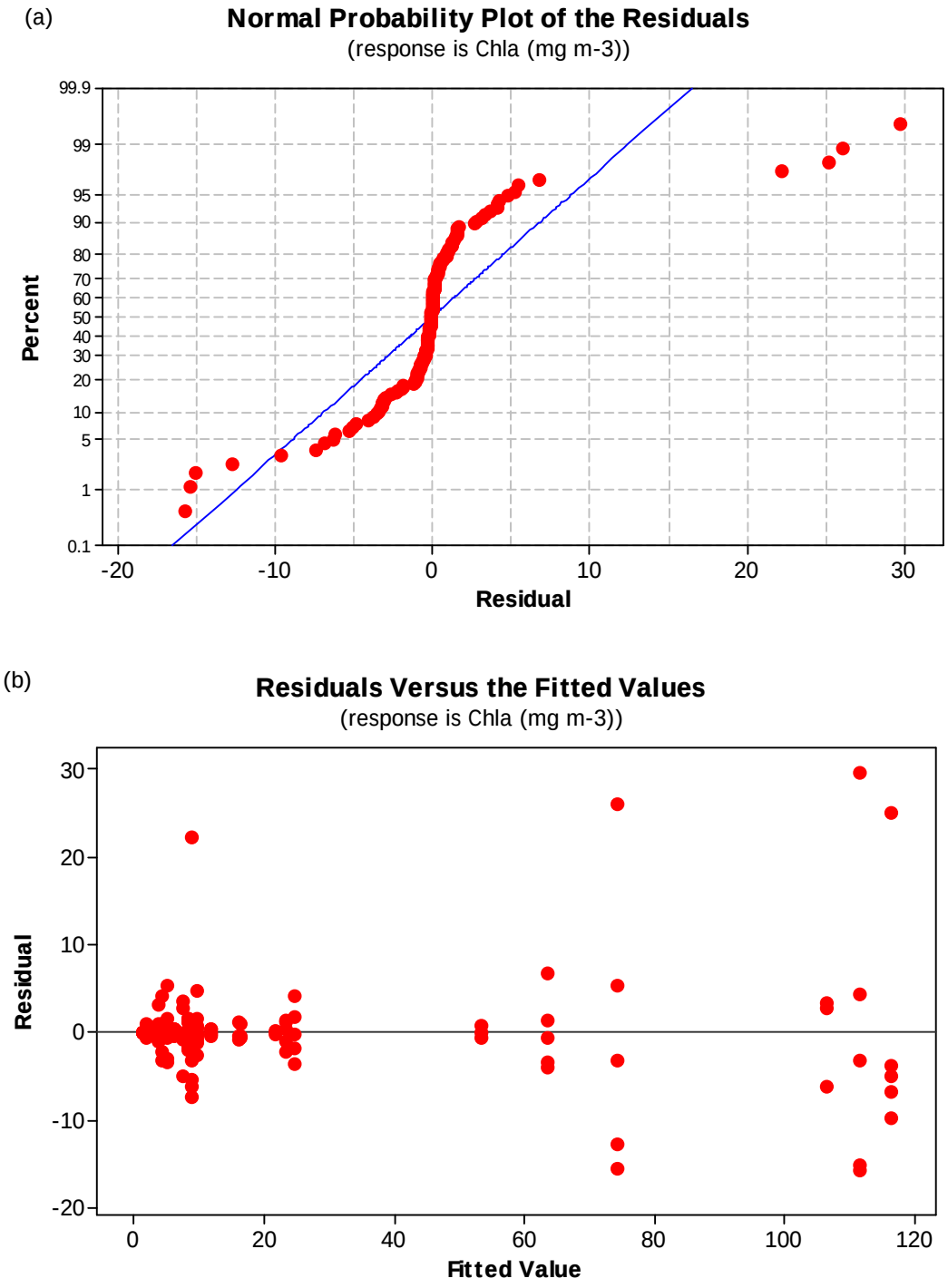


Figure 7.2 **a) Normal probability plot and b) residual versus fit plot from the analysis of the raw chlorophyll a data**

This analysis showed, as before, that lake had a very significant effect (p<0.001) on chlorophyll *a* concentration. There was still no statistically significant overall difference in average chlorophyll *a* concentrations between the three locations (p=0.400), but the size of differences between locations on individual lakes did vary between lakes (i.e. the lake-location interaction was significant (p=0.010)). In this log-based ANOVA model, lake and location (and their interaction) jointly explained an estimated 93% of the total variation in $\log_{10}$ chlorophyll *a* within the study lakes.

For the ANOVA interaction between lake and location to be significant, but the main effect of location (i.e. average across sites) to not be significant usually indicates that the location with the highest average chlorophyll *a* concentration varies between lakes. One way ANOVA of $\log_{10}$ chlorophyll *a* concentration on location was therefore carried out on each lake separately and the pattern of inter-location differences summarised in Table 7.3. Chlorophyll concentrations are significantly highest at the outflow sampling location for Grasmere and Ullswater, but in open water for Loch Clunie, and at the edge of Loch Leven.

Table 7.3 One-way ANOVA and Kruskal-Wallis test p vales for difference in $\log_{10}$ chlorophyll$_a$ concentrations between locations within each lake

Where significant (p<0.05, highlighted in bold), the lowest, middle and highest location means are coloured blue, green and red respectively to highlight how location differences vary between lakes.

Lake	ANOVA test *p*	Kruskal-Wallis test *p*	Edge		Open		Outflow	
			Mean	S.D.	Mean	S.D.	Mean	S.D.
Barton Broad	0.663	0.495	2.043	0.069	2.063	0.050	2.027	0.022
Brothers water	0.773	0.837	0.178	0.062	0.228	0.147	0.203	0.009
Elterwater	0.666	0.755	0.520	0.357	0.661	0.517	0.400	0.032
Ennerdale	0.098	0.082	0.037	0.027	0.073	0.018	0.058	0.019
Esthwaite	0.247	0.290	1.385	0.053	1.366	0.030	1.334	0.003
Grasmere	**0.042**	**0.023**	**0.568**	**0.364**	**0.801**	**0.281**	**1.199**	**0.029**
Loch Ard	0.791	0.545	0.525	0.176	0.535	0.065	0.474	0.078
Loch Clunie	**0.001**	**0.013**	**0.973**	**0.047**	**1.063**	**0.014**	**0.943**	**0.004**
Loch Leven	**0.040**	**0.031**	**1.862**	**0.094**	**1.802**	**0.029**	**1.726**	**0.006**
Loweswater	0.140	0.093	0.904	0.080	0.973	0.033	0.903	0.025
Ullswater	**0.003**	**0.034**	**0.900**	**0.080**	**0.963**	**0.119**	**1.209**	**0.025**
Upton Broad	0.171	0.141	0.784	0.025	0.772	0.016	0.802	0.013

Estimates of the variance in ($\log_{10}$) chlorophyll *a* concentrations due to lake, location and the interaction between lake and location were estimated by treating both lake and location as random factors (Table 7.4). Inter-lake differences amongst the 12 study lakes account for over 90% of total variance in ($\log_{10}$) concentrations. However, on average within lakes, the overall variance of the varying extent of differences between locations accounted for an estimated 19% of the total within-lake variance, the majority (81%) being due to differences between replicate samples from the same location type with a lake (Table 7.4).

Table 7.4 Estimates of variance in ($\log_{10}$) chlorophyll a concentrations due to lake, location and the interaction between lake and location

(SD = square root of variance)

Source	Variance	% of total variance	% of total variance within lake	SD
Lake	0.3409	91%		0.584
Location	0.0000	0%	0%	0.000
Lake-Location interaction	0.0059	2%	19%	0.077
Replicate samples (within a location)	0.0245	7%	81%	0.157

The results of this analysis indicated that:

1. there were significant variations in the mean (log_{10}) chlorophyll ***a*** concentration among lakes
2. there was no significant overall difference among locations in their mean (log_{10}) chlorophyll ***a*** concentration averaged across the sites
3. there was a significant lake by location interaction
4. there were significant differences between locations in (log_{10}) chlorophyll ***a*** concentration at each of four lakes (Grasmere and Ullswater (outflow highest concentration), Loch Clunie (open water highest) and Loch Leven (edge highest).

Q2. In which location do we find the least variable chlorophyll concentrations?

In order to address question 2, the coefficient of variation (CV) was used to quantify variability in chlorophyll ***a*** concentrations at each location within each lake. The CV was calculated as:

$$\text{Coefficient of variation (\%)} = \text{CV} \left(\frac{SD}{\bar{x}} \right) * 100$$

Where *SD* is the standard deviation of the chlorophyll ***a*** concentration values at a particular location, within a specific lake and $\bar{x}$ is the mean chlorophyll ***a*** concentration at a particular location, within a specific lake. It is more appropriate to use the coefficient of variation than the SD in untransformed chlorophyll ***a*** concentration, since the latter will be affected by differences in the mean chlorophyll ***a*** concentrations among locations and sites. We also calculated and compared the standard deviations (SD_{Log}) of the log_{10} concentrations at each location of each lake, as transformation to logarithms aims to eliminate (or at least reduce) dependence of replicate variability on replicate mean.

Friedman two-way analysis of ranks test was used to test whether either the *CV* or the SD_{Log} tended to be consistently higher at one location than another within lakes (after allowing for any overall differences in *CV* or SD_{Log} between lakes). This non-parametric test was used because neither *CV* nor SD_{Log} are likely to be normally distributed. Friedman's test effectively ranks the three values (*CV* or SD_{Log}) separately within each of the 12 lakes and assesses whether the sum of ranks for a location is lower or higher than might be expected by chance.

The Friedman test showed that there were significant (p=0.001) differences between locations in coefficient of variation (CV%) in chlorophyll ***a*** concentrations for replicate samples; due to generally lower CV amongst outflow samples (Table 7.5). The average rank of the coefficient of variation values from the outflow samples was only 1.17, much less than that from the other two locations (>2.0); in fact the CV was lowest for the outflow samples at all except two (Ennerdale and Loch Ard) of the 12 lakes (Table 7.5). When variation was expressed in terms of SD_{Log} , identical results were obtained (Table 7.5).

When the Friedman analysis was run again, after removing the outflow data, in order to compare just open water and edge samples, there were no remaining significant differences in either median *CV* or median SD_{Log} (both test p = 0.083). Thus there is no evidence that replicate sample chlorophyll ***a*** concentrations from these locations differed consistently in variability.

Table 7.5 Freidman tests for differences between locations within lake

Tests based on percentage coefficient of variation (*CV*) in chlorophyll *a* concentration or standard deviation (SD_{Log}) of log_{10} concentration amongst replicate samples within a location within a lake.

Lake	*CV* %			SD_{Log}		
	Edge	Open	Outflow	Edge	Open	Outflow
Barton Broad	16.7	12.2	5.0	0.069	0.050	0.022
Brothers water	14.2	36.8	2.2	0.062	0.147	0.009
Elterwater	68.0	142.4	7.2	0.357	0.517	0.032
Ennerdale	6.2	4.0	4.4	0.027	0.018	0.019
Esthwaite	12.3	6.7	0.6	0.053	0.030	0.003
Grasmere	75.5	47.4	6.7	0.364	0.281	0.029
Loch Ard	48.8	16.4	18.8	0.176	0.065	0.078
Loch Clunie	11.1	3.2	1.0	0.047	0.014	0.004
Loch Leven	22.6	6.9	1.3	0.094	0.029	0.006
Loweswater	17.9	7.6	5.7	0.080	0.033	0.025
Ullswater	17.8	29.9	5.7	0.080	0.119	0.025
Upton Broad	5.7	3.6	3.1	0.025	0.016	0.013
Median value	17.2	9.9	4.7	0.074	0.041	0.021
Friedman test						
Average rank within lakes	2.75	2.08	1.17	2.75	2.08	1.17
test *p*	0.001			0.001		

In summary, these analyses showed that replicate sample chlorophyll *a* concentrations from outflow locations were less variable than those from the open water or edge locations (which do not differ significantly from each other in levels of variability). The lower variability in chlorophyll *a* concentrations of samples from lake outflows could be partly due to increased water flow and thus greater mixing of phytoplankton near outflows. But it could also be simply because the area of the lakes which can be considered “outflow”, and from which the outflow samples were taken, was generally much less than the spatial coverage of edge and open water sampling locations.

Sampling chlorophyll *a* concentrations at outflows could therefore give less variable results and thus increased sampling precision in estimating ecological status. However, we have already shown in answering Q1, that for some lakes, average chlorophyll *a* concentrations at outflows can be different to those in other parts of the lakes, so concentrations, and perhaps resulting estimates of ecological status, obtained from outflow samples may not be representative and may be biased for other parts of the lake or the lake as a whole. For any particular lake, it is difficult to determine the adequacy of using just outflow sampling without a prior study to assess the spatial pattern in concentrations, and more importantly, ecological status around the lake. If different parts of lakes have different reference conditions (based on factors such as depth, distance from edge, etc), then differences in observed concentrations around the lake may not necessarily lead to differences in estimates of ecological status when the observed concentrations are standardised to Ecological Quality Ratios (EQR) using appropriate reference condition concentrations for that part and type of lake.

7.3 Analytical variability in chlorophyll concentrations

In order to examine the variability in chlorophyll *a* concentrations due to differences in analytical methodology among Environment Agency laboratories, 10 replicate samples from Barton Broad were analysed at each of 4 different laboratories. These data were analysed to examine differences in mean chlorophyll *a* concentrations determined at each laboratory. Two-way ANOVA was used for the analysis with chlorophyll *a* concentration as the response variable and laboratory and replicate as the explanatory factors. Including replicate as a factor allows for any differences in concentrations between the replicate field samples to be eliminated from the comparison of laboratories. Tukey tests were used to test for pair-wise differences between laboratories.

A visual estimation of the extent of inter-laboratory measurements of ten samples taken from the open water of Barton Broad suggests that there are some systematic differences in the concentration of phytoplankton chlorophyll *a* made at the four laboratories (Figure 7.3). The estimates from Haddiscoe were greater than in the three other laboratories in each of the ten replicates. Overall, the mean value from Haddiscoe was 1.6-times greater than those from Llanelli, 1.3 times greater than those from Starcross and 1.4-times greater than those from Nottingham (Figure 7.4).

Figure 7.3 Estimates of chlorophyll$_a$ from ten replicates taken from Barton Broad and analysed in four different laboratories

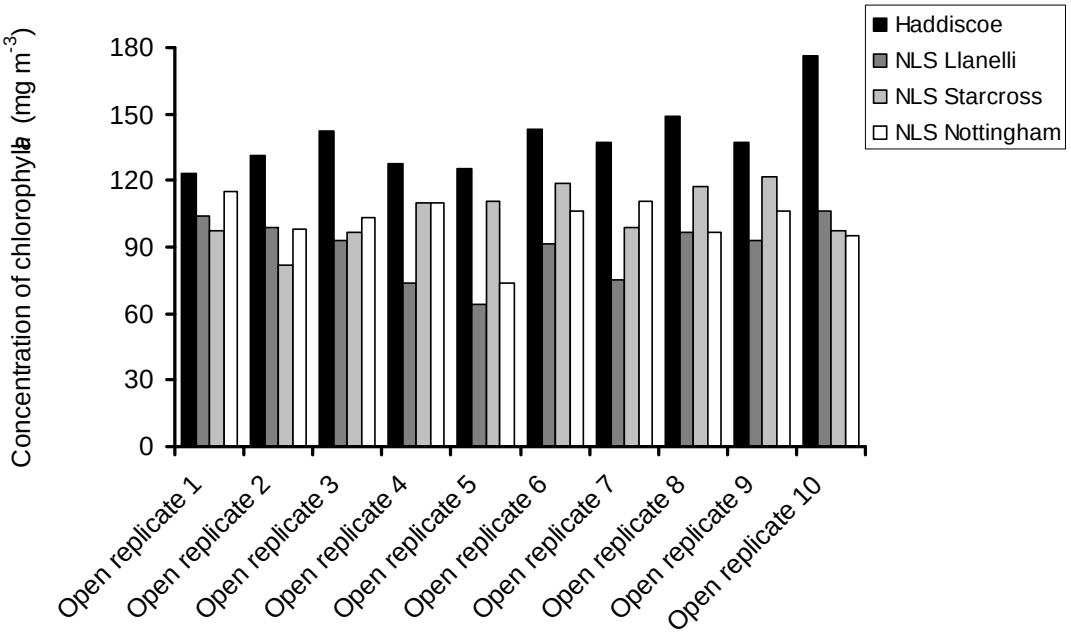
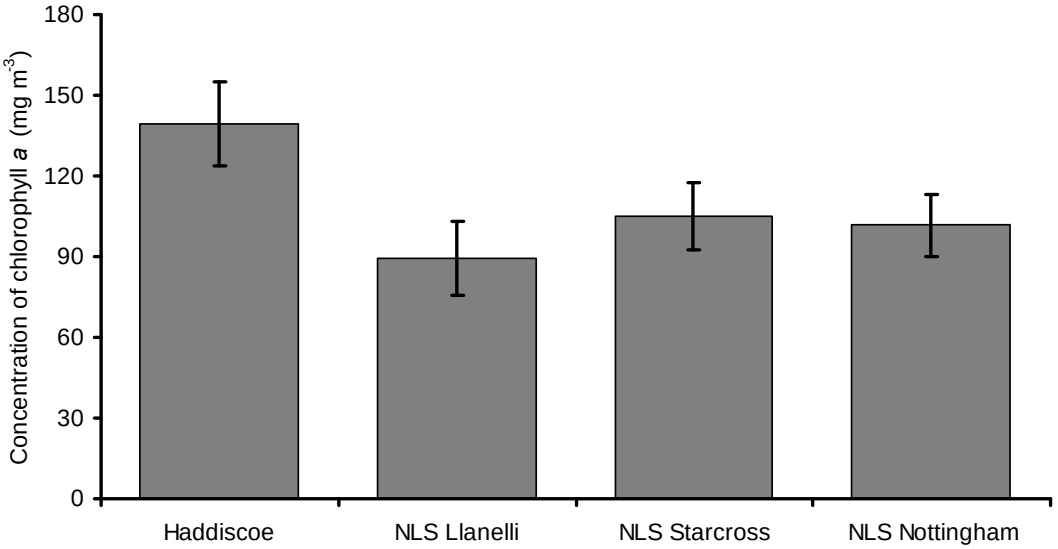


Figure 7.4 Mean and standard deviation of chlorophyll$_a$ based on ten replicates from Barton Broad analysed in four different laboratories



The ANOVA results confirmed that there were significant differences (p<0.001) in the chlorophyll values determined by the different laboratories (Table 7.6). This was largely due to the high values produced by the Haddiscoe laboratory compared to the others (Tukey test, p<0.001, Table 7.7). However, in a separate ANOVA on just the other three sites, there is also a suggestion that values produced from the Llanelli laboratory were significantly lower than the other two sites (p=0.035).

Table 7.6 Analysis of variance table for a two-way ANOVA of chlorophyll a concentration on laboratory and replicate sample

Source	df	Sum of squares	Mean square	F	*p*
Replicate	9	1987	221	1.31	0.277
Laboratory	3	13627	4542	26.98	0.000
Error	36	4545	168		
Total	39	20159			

Table 7.7 Means and standard deviations of chlorophyll a concentrations from each Environment Agency laboratory

Source	N	Mean	Standard deviation
Haddiscoe	10	139.2	15.5
Llanelli	10	89.5	13.9
Nottingham	10	101.6	11.6
Starcross	10	105.2	12.6

In an attempt to compare the relative size of different sources and components of variance in chlorophyll *a* concentration values for a lake across different studies, the data were re-analysed treating both replicate and laboratory as random factors in a two-way ANOVA of $\log_{10}$ concentrations. Within this study of Barton Broad, replicate samples

accounted for very little variance, while inter-laboratory differences accounted for two-thirds (66%) of the total variance in (log_{10}) chlorophyll *a* concentrations (Table 7.8).

Table 7.8 Estimates of components of variance in log_{10} chlorophyll$_a$ concentrations amongst laboratories and replicate samples from Barton Broad

(SD = square root of variance)

Source	Variance	% of total variance	SD
Replicate samples	0.0003	3%	0.017
Laboratory	0.0064	66%	0.080
Residual	0.0030	31%	0.054

The results showed that there was significant analytical variability in chlorophyll *a* concentration among laboratories. Specifically, the Haddiscoe laboratory produced significantly higher values than the other three laboratories.

Integrating and comparing variance component estimates from different studies

We have attempted to compare the relative size of the estimates of variance in log_{10} chlorophyll *a* concentrations due to various factors studied here with those from an earlier study (Clarke et al., 2006) within Phase 1 of this project on temporal variability in concentrations over a whole year (Table 7.9). The estimate of temporal variance over a year and within a month are based on the estimates of SD_{Log} derived from the recent sampling period 2000-2004 at Loch Leven as reported in Table 6.1 of Clarke et al. (2006).

Table 7.9 Synthesis of estimates of components of variance in log_{10} chlorophyll$_a$ concentrations from studies within Phase I and II of project

Source of variance	Variance	SD	Reference
Replicate samples (within a location on the same day) Estimate averaged over all locations	0.02452	0.157	Table 4 in this report
Replicate samples (within a location on the same day) Estimate based on outflow SD only	0.00044	0.021	median SD_{Log} for outflow samples from Table 4 in this report
Laboratory	0.00641	0.08	Table 8 in this report
Location within a lake (including interaction with lake)	0.00592	0.077	Table 4 in this report
Total variability over a year (includes replicate variance)	0.10304	0.321	SD from Table 4 in Clarke et al. 2006
residual variance within months (includes replicate variance)	0.0392	0.163	SD from Table 4 in Clarke et al. 2006
Temporal variance within a year (excluding replicate variance)	0.07852	0.28	0.10304 – 0.02452
Temporal variance within a month (excluding replicate variance)	0.01468	0.121	0.0392 – 0.02452

When put into context with natural spatial and temporal variability within a lake, variance due to laboratory differences are fairly small (variance estimate of 0.00641).

Replicate samples from the same (or nearby) spots taken on the same day contribute more variability when averaged over all locations (outflow, edge and open waters

(variance estimate of 0.02452). But if restricted to the replicate variability in the outflow locations of lakes, which are less variable (Table 7.5), then the estimate of replicate sampling variance (based on the square of the median SD_{Log} for outflow locations at the 12 study lakes (Table 7.5)), is only 0.00044, which is less than laboratory variance. This is an example of the problem of comparing estimates in an appropriate manner. The choice of estimates depends on the situations (lakes/locations/labs) to which they are intended to be applicable.

However, the largest source of variability in concentrations within a lake is temporal with an estimate of true annual temporal variance (i.e. eliminating replicate sampling variance) of 0.07852.

But if sampling was carried out monthly, then much of the seasonal variability is eliminated from errors in estimating annual mean concentration and the remaining within-month temporal (i.e. non-replicate) variance is much smaller (estimate of variances is 0.01468; this is now less than the estimated variance between replicate samples taken on the same day.

Finally it must be pointed out this attempt to provide an integrate assessment and comparison of the different sources of variance is necessarily crude, but based on the limited study information available. The individual studies were based on one or more different lakes, at different times, often using different analytical laboratories.

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APPENDIX I PHYTOPLANKTON COUNTING GUIDANCE

Guidance on the quantitative analysis of phytoplankton in Freshwater Samples

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Contents

1. Introduction

Phytoplankton are increasingly being used to monitor the ecological quality and health of the water environment and also to measure the effectiveness of management or restoration programmes or regulatory actions.

The European Water Framework Directive (2000/60/EC) requires member states to monitor phytoplankton abundance and composition and a uniform procedure has been developed by CEN.

The following guidance has been developed with reference to the CEN standard "Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)" (CEN 2004), Test Methods and Procedures: Freshwater Phytoplankton NRA (1995) and "PL100 Quantitative and qualitative phytoplankton analysis" (SYKE) as well as reference texts such as Utermöhl (1958) and Lund , Kipling and LeCren (1958).

Analysis should be carried out using sedimentation chambers with an inverted microscope (Utermöhl technique).

This method is suitable for studies investigating the abundance, composition and biovolume of phytoplankton in rivers and lakes.

2. Terms and definitions

The terms and definitions used are as those as described in "Water quality - Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)" CEN 2004.

3. Principles

The quantitative analysis described here includes the identification, enumeration and calculation of biovolumes of Lugol's iodine preserved water samples.

The preserved sample is thoroughly mixed and a sub-sample of known volume is placed in a sedimentation chamber. When the algae have settled to the bottom of the chamber, they are counted and identified using an inverted microscope.

The statistical reliability of the analysis depends upon the distribution of algal units/cells within the sedimentation chamber and assumes that the algae are randomly distributed within the chamber. If the algae are randomly distributed (and comply with a Poisson distribution) then a 95% confidence limit of +_ 20% can be achieved by counting about 100 algal units (Lund, Kipling and LeCren, 1958). Note that random distributions are not always achieved in sedimentation chambers so alternative protocols or methods may have to be used.

The counts for individual taxa are converted to algal biomass by using the cell/unit volume of the count units. The volumes are based on measurements made during counting.

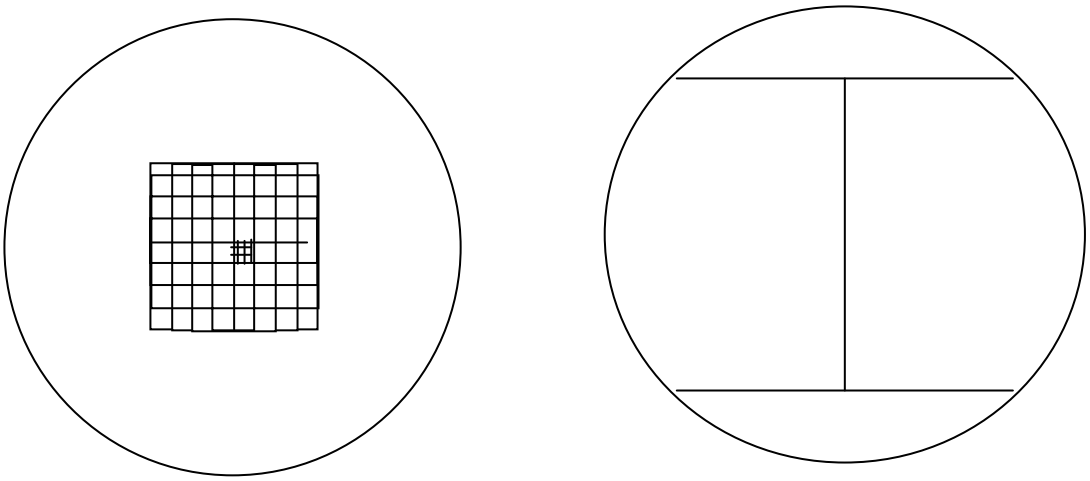
4. Equipment

- Sedimentation chambers of 5 to100ml capacity (Hydro-Bios plankton chambers or similar are recommended). Sedimentation chambers with volumes greater than 10mls are usually combination chambers and consist of a base plate and upper removable column which is slid aside once the algae have settled.

- Inverted microscope with phase contrast (and/or DIC/Normarski) including:
  - long working distance condenser with numerical aperture of >0.5
  - 10x or 12.5x binocular eyepieces, one with a square grid e.g. Whipple eyepiece graticule, Miller Square or similar, and another with a cross-hair graticule (Figure 4.1)
  - low power objective (5x or 10x)
  - 10x, 20x, 40x and 100x oil immersion, phase &/or DIC objectives
  - ideally the microscope should be fitted with a (digital) camera
  - a mechanical stage

Figure 4.1 Eyepiece graticules

(a) Whipple graticule **(b) cross-hair graticule**



- Variety of pipettes with wide bore tips

- Glass cylinders for initial sedimentation

- Supply of ultra high purity or membrane filtered water is required for topping up, diluting and general cleaning.

- Supply of acidified Lugol's iodine. Make up by dissolving 100g of KI (potassium iodide) in 1 l of distilled water then adding 50g I (iodine). Shake until all dissolved and add 100g of glacial acetic acid. Store in dark bottle.

- Computer with algal counting spreadsheet.

Calibration of equipment

- Each counting chamber should be marked with a unique mark or number and a note made of the counting chamber area. This is calculated by measuring the cover slip aperture (rather than the chamber itself) using either a vernier gauge or the microscope stage vernier if one is present. The mean of 5 diameters should be taken and the area of the chamber calculated using the formula πr^2. Both the measurements of the diameters and the chamber volume should be recorded against the individual counting chamber in a log book.

- All eyepiece/graticule and objective combinations should be calibrated with a stage micrometer (e.g. 100µm x 10µm divisions) and the dimensions and areas of counting fields, transects and the whole chamber area should be calculated for each of the magnifications used and recorded in a log book.

5. Preparation of samples

5.1 Acclimatisation

Stored and preserved samples, sedimentation chambers and all equipment used should be allowed to acclimatise to the same (room) temperature for at least 12 hours (preferably 24 hours). This has been found to be one of the most important factors in achieving a random distribution of algal cells in the chambers.

5.2 Sample mixing

Just before taking a sub-sample to fill the sedimentation chamber, the sample must be thoroughly mixed. It is recommended that the mixing is done manually and that this is standardised; the sample should be mixed using a combination of alternating horizontal rolling and vertical tumbling (turning upside down) of the sample bottle for 2 minutes. These actions should be **gentle** and not involve any vigorous shaking.

5.3 Sub-sample preparation and setting up chambers

After thorough mixing, a known volume of sample is used to fill the sedimentation chamber. The method and care taken to fill the chambers is crucial as it determines the final distribution of settled algae in the chamber. If care is taken then a random distribution allows uniform counting strategies and statistical methods to be used. If a random distribution is not achieved then alternative and often more complex methods must be employed.

The exact volume of sample used to fill the chamber depends on the phytoplankton density. Large volumes of up to 100 ml may be required for oligotrophic waters whilst at high phytoplankton densities dilution may be required.

Ideally, enough sample should be taken to completely fill the chamber in one addition, either directly pouring from the sample bottle or using a wide-bore pipette. Fill a little more than needed and allow a little to over-spill the chamber when you slide the lid across.

This recommendation, to fill the chamber in one addition, raises a number of difficulties for samples with either very low or very high phytoplankton densities. A number of options are available for dealing with varying densities of phytoplankton:

1) Use a sedimentation chamber of an appropriate size depending on how abundant the algae are (chlorophyll concentrations may be used as a guide if available). For example use a 2.5 ml chamber if densities are high or a 10 ml chamber if densities are low.

2) For very low densities, a pre-concentration step may be necessary. Let sample settle in a measuring cylinder - usually 250 ml is sufficient. Leave for 3 days, then draw off top water leaving 25 ml at bottom of cylinder (i.e. x10 concentration). If needed this can be repeated with up to 4 250 ml cylinders and the 4 lots of 25 ml then poured into a 100 ml measuring cylinder for a second pre-concentration to 10 ml (i.e. x100 concentration).

3) For very high densities, where 2.5 or 5 ml of sample is too much it may be necessary to add a smaller measured volume. Use an accurate wide-bore pipette and add 0.5 or 1 ml of sample to the chamber, then top up with distilled water. You must be very careful not to add too much water - so none spills over. The alternative is to count fields at x100 magnification.

A general rule is to aim for about four counting units per field of view at high (×400) magnification.

The following points should be noted:
- ensure all equipment and sample are acclimatised to room temperature and be as constant as possible.

- place the sedimentation chamber on a horizontal flat surface – a perspex or thin acrylic board (which is a poor heat conductor) is ideal – and it should be placed away from strong heat, light and vibration sources.

- take enough sample, either directly from the bottle or with a pipette, to completely fill the chamber in one addition.

- close the chamber with a thick cover slip, making sure air bubbles are avoided.

- make a note of the sample volume, sample site and date next to the chamber or label the flat sedimentation board.

- allow contents to settle, undisturbed, for at least 4 hours per cm height of chamber. For 10 ml HydroBios chambers settle for at least 12 hours and for 50 ml chambers at least 48 hours settling time is recommended.

- if there are large numbers of buoyant cyanobacteria present you can add either a drop of diluted detergent or glacial acetic acid to the chamber before closing the chamber with the cover slide.

- after sedimentation if combination chambers are used, then slide the chamber column aside and replace it with a thick cover slide. With both combination chambers and 5 or 10 ml HydroBios type chambers, check for and try to avoid introducing any air bubbles at this stage. This can be eliminated by carefully topping up with UHP or membrane filtered water from a dropper pipette whilst sliding the cover slide back into place.

- the sedimentation chamber should be gently moved to the microscope stage. Open chambers should not be moved as the settled algae will be easily disturbed.

After the appropriate settlement period and before counting two checks need to be made:

1. the overall distribution pattern of particles should be checked using a stereo zoom or inverted microscope at very low power (4x or 10x objectives). A random (poisson) distribution is required and this is recognised by the irregular pattern, often with open spaces. If particles are not randomly distributed and for example are concentrated in one area of the chamber or found in concentric rings towards the edge of the chamber then a new sample should be set up. The distribution of particles/algal cells or units should be checked from time to time and this can be done using the methods outlined in Annex F of the CEN method. The simplest of these being to undertake a count of one taxa and calculate the variance to mean ratio – this approximates the Chi squared distribution for n-1 df – the result is then checked using a goodness to fit test for Chi squared.

2. If the algal density is too low or too high then another sample should be set up and the volume adjusted accordingly. It can sometimes be extremely difficult to judge the correct volume but the general advice is

- if there are too many particles then they may not settle independently and pile up, also it can be very difficult to count and can lead to inaccuracies from "fatigue"
- if there are too few particles, the errors increase especially when counting random fields or transects and large areas of the chamber need to be observed. The density of detritus or non-algal particles is also important especially if algal densities are low, and skill is needed to judge the ideal volume to sediment.

6. Counting

6.1 General

The counting procedure involves recording the taxa observed and the number of algal units (objects) for each taxon in a known area of the counting chamber. As the volume of sample added and area of the whole chamber observed is recorded, the concentration of each individual taxon can then be calculated.

The observed taxa are identified to the required taxonomic level (see section 6.3). **It is very important to remember that it is better to correctly identify algae to lower taxonomic level than misidentify to a higher level**.

It is useful to scan the sample at a variety of magnifications before the quantitative analysis is undertaken and to compile a taxa list before beginning the count.

If there is evidence of significant benthic contamination or littoral taxa present (eg periphyton) such that the open water taxa are obscured, then it may not be worth undertaking a full count.

Where small numbers of littoral or benthic taxa such as *Surirella* and *Nostoc*, are present, they should not be counted.

6.2 Counting procedure

The count should be carried out in the following manner:
- a low magnification (e.g. x 40 or x100), whole chamber count to pick up large taxa, followed by;

- transect counts at an intermediate magnification (x250), which are helpful to enumerate "intermediate-sized" taxa that are too small for the low-magnification count but too large to be reasonably counted using fields of view at high magnification, followed by;

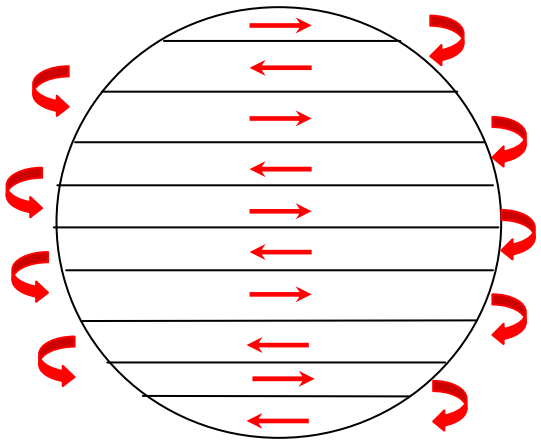
- a high magnification count (x400 or greater) using fields of view. This picks up the small taxa. Aim to count 100 fields of view (i.e. about 400 units assuming the recommended sample concentration)

Details are provided in sections 6.2.1 to 6.2.3 below.

6.2.1 Counting the whole chamber at low magnification for large taxa.

Working at low power (x40 to x100) the whole chamber should be scanned in a series of horizontal transects (figure 6.1) and the larger taxa (e.g. *Ceratium*), large colonial or filamentous forms (e.g. *Microcystis*, *Fragilaria*) counted. A cross-hair graticule eyepiece (figure 4.1) is used when counting the whole chamber. Algae that lie between the two horizontal lines are counted as they pass the horizontal line. Algal objects that cross the top line are included whilst those crossing the bottom line are not and will be counted on the next transect (or vice versa).

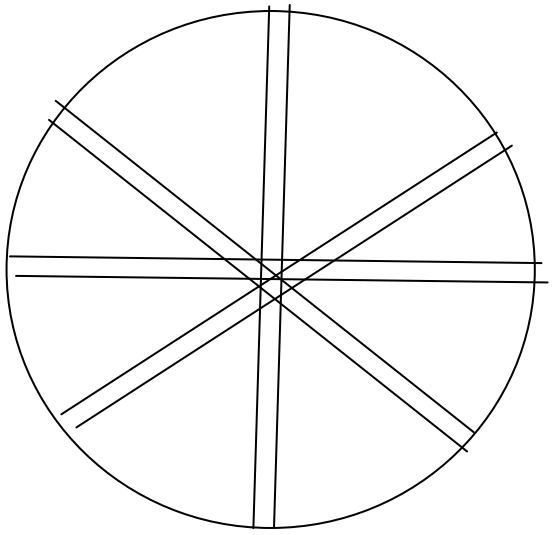
Figure 6.1 Counting method for whole chamber.



6.2.2 Counting transects.

Algal objects larger than approximately 20 µm (small *Cryptomonas*) can be counted at a magnification of approximately x200 in 3 - 5 randomly chosen diameter transects of the counting chamber (figure 6.2). The cross-hair eyepiece and method for counting algal objects described in the section above is used also. The chamber is rotated between transect to randomly chosen positions.

Figure 6.2 Counting method for diameter transects.



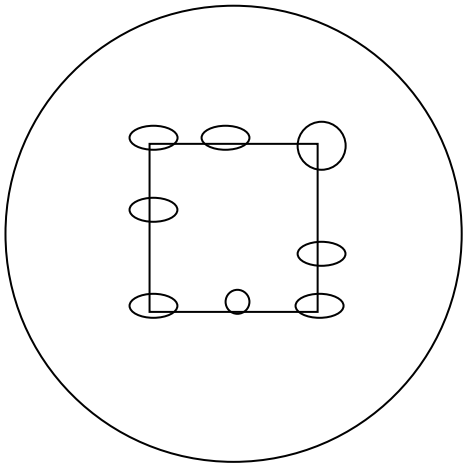
6.2.3 Counting randomly selected fields.

Small algae, less than about 20 µm (e.g. *Rhodomonas*, small centric diatoms), should be counted in 100 (or more) randomly selected fields at x400 magnification (or greater) using a square or Whipple graticule, Miller Square or similar in the ocular eyepiece to delineate the counting area. Fields can be selected either in a pseudo-random way by the counter or using a mechanical stage with a vernier that allows random positions to be found from random number coordinates or using an electronic stage with built in random position control.

A tally of the number of fields counted is required as well as the counts of individual identified algal units (cells, colonies or filaments).

When counting random fields it is important to take a consistent approach to decide whether algal objects lying across the grid lines are counted in or out. A simple rule should be adopted as described in the CEN method (2004) e.g. algal objects (cells, colonies or filaments) crossing both the top and the left hand side of the grid are not counted whilst those crossing the bottom or right hand side of the grid are counted (see Figure 6.3).

Figure 6.3 Example of rule for counting cells on edge of field



Y - counted

N – not counted

6.2.4 Point to consider when counting

• **Algal objects and counting units**: Algal objects or counting units are independent algal cells, colonies or filaments/trichomes. One species or taxa may be present in the sample as different counting units and may be counted at different magnifications. For example, *Microcystis* colonies will probably be counted in the whole-chamber or transect but individual *Microcystis* cells (which may be present if colonies are disintegrating) will be counted in random fields. Similarly *Dinobryon* colonies are most likely to be counted in diameter transects and single *Dinobryon* cells will be counted in random fields.
Other examples of counting/algal units include:
– Colonies e.g. *Aphanothece, Coelosphaerium, Sphaerocystis*
– Algal cells which can occur as single cells but also form colonies, e.g. *Aulacoseira, Dinobryon, Melosira*.
– Colonies which have more or less permanent cell numbers, e.g. *Desmodesmus/Scenedesmus* (2, 4 or 8 cells), *Pandorina* (16 cells) *Crucigenia* (4 cells)
– Filaments or trichomes e.g. *Anabaena, Aphanizomenon, Oscillatoria, Planktothrix*
– Colonies where the size and shape vary e.g. *Microcystis*

• **Calculating cells per colony/filament** – it may be necessary to estimate the numbers of cells per colony or filament and if this is the case then the colonies or

filaments should be treated as individual algal objects or units as described above. For some taxa the cell numbers per colony may be consistent or have several modes as illustrated above whilst for others the cell numbers do not have a consistent distribution e.g. *Microcystis* where the number of cells per colony can vary from a few cells to several million cells.

For estimating biovolumes of colonies or coenobia:-

- Using cell volumes – make direct counts of cells in 'sub-colonies' or small areas. These can then be multiplied up by number of 'sub-colonies' or the ratio of small area to whole colony to get the total cell numbers, e.g. *Microcystis, Woronichinia*, etc.
- Using colony/coenobium measurements – measure colony width and depth e.g. *Pediatrum*, *Microcystis* (using Reynolds & Jaworski's formula embedded in counter spreadsheet)

For estimating biovolumes of filaments:-

- Using filament measurements – calculate mean dimensions by measuring the length and diameter of at least 30 filaments. For high-magnification random field of view counts, only the lengths of the filaments lying within the grid should be measured. For whole chamber or transect counts at low or intermediate magnification whole filament lengths can be measured.
- Using cell volumes – combine counting of filaments with the mean numbers of cells per filament, e.g. *Aphanizomenon*
  - Count the number of filaments in the normal way (transects or random fields) and measure the length of at least thirty filaments (using a calibrated eyepiece graticule e.g. Whipple) to calculate the average length.
  - From up to 10 filaments, calculate the average number of cells per unit length (e.g. 20 µm). This can be measured at a higher magnification if the cells are small or hard to distinguish easily (e.g. some species of *Oscillatoria)*.
  - Then the number of cells per filament is calculated by multiplying up the average filament length by the average number of cells per unit length.
- Where the algae form spiral filaments e.g. *Anabaena circinalis,* the average the number of cells per gyre is counted and then the number of gyres per filament is estimated. The two numbers are multiplied together to give the estimated number of cells per filament.

6.3 Identification and coding

Appendix A provides a list of taxa which is to be used to guide the required level of identification. It includes Whitton Codes, accepted names, biovolume formulae and biovolume ranges, where available. If taxa can be identified but are not included within this list, photographs and drawings (including measurements) should be taken and the inclusion of the 'new' taxa to the list should be checked with the Project Manager.

The standard flora for identification is the Freshwater Algal Flora of the British Isles (Whitton et al., 2003) but other identification guides are also available and may be used if they prove more helpful for certain taxonomic groups (see Section 10).

It is very important to remember that it is better to correctly identify algae to lower taxonomic level than misidentify to a higher level.

The following codes and accepted names have been adopted for the purposes of WFD phytoplankton enumeration for 'difficult' taxa following a workshop of many of the UK analysts (Table 6.1). These have been incorporated into the taxa list in Appendix 1.A.

Table 6.1 New codes agreed for taxa of specific size classes or unidentified taxa groups commonly recorded by UK counters

Whitton Code	Accepted name
12000001	Small centric diatom (5-<10 µm diameter)
12000002	Medium centric diatom (10-20 µm diameter)
12000003	Large centric diatom (>20 µm diameter)
12000004	Very small centric diatom (<5 µm diameter)
13000001	Small pennate diatom (Length <10 µm)
13000002	Medium pennate diatom (Length 11-20 µm)
13000003	Large pennate diatom (Length >21µm)
17000000	Unidentified small green round cells (sgrt)
17000000	Unidentified colonial green
05040001	*Cryptomonas* sp. (small) Length <20 µm
05040002	*Cryptomonas* sp. (medium) Length 20-30 µm
05040003	*Cryptomonas* sp. (large) Length >30 µm
90000000	Picoplankton - unidentified single cells <2 µm diameter
90000003	Nanoplankton - unidentified non-flagellate cells, 2–20 µm length
90000004	Unidentified cells >20 µm diameter
90000005	Nanoplankton - Unidentified flagellates 2–20 µm length

Verification of species identification should be carried out for any difficult species, especially those of cyanobacteria, chrysophytes or green algae by sending samples with drawings, photographs and measurements to taxonomic experts.

Intra- and inter-laboratory identification comparisons should be carried out on a regular basis to avoid and minimise identification difficulties. Quality assurance and validation of counts is described in detail in section 8 below.

7. Calculation of phytoplankton biovolume

Biovolumes must be measured for all taxa and is done by assigning simple geometric shapes to each cell, filament or colony, measuring the appropriate dimensions and inputting these into formulae to calculate the cell volume.

The counting spreadsheet which will accompany this guidance includes, for all the taxa listed in Appendix A, a fixed, pre-determined, formula for the biovolume of each taxon. All that is required is for the appropriate average dimensions to be input to the spreadsheet so that the biovolume can be calculated automatically (see points listed below).

Measurements of the required cell dimensions (length, width, diameter) are made at an appropriate magnification using a calibrated ocular eyepiece, e.g. a Whipple Graticule. The eyepiece is rotated so that the scale is put over the required cell dimension and the measurement made by taking the ocular measurement and multiplying by the calibration factor for that magnification and eyepiece combination.

The following points should be noted:

- it is important to measure the linear dimensions of at least ten individual units of all taxa observed in the sample and for taxa of more variable size, at least 20 individuals should be measured to estimate mean dimensions. If the cells are very variable then up to 50 cells should be measured.

- for some species with external skeletons much larger than cell contents, e.g. *Dinobryon*, *Rhizosolenia*, the dimensions of the plasma/organic cell contents should be measured, not the external skeleton dimensions.

- for filamentous taxa, the average biovolume can be estimated using the method described in 6.2.4 for estimating number of cells per filament/colony, except for biovolume it is only necessary to measure average filament length of at least 30 filaments and average diameter of 3 to 5 filaments.

- for colonial taxa count cell numbers and multiply by mean cell dimensions (often single measure of dimensions needed). If the colony is very large or cells are very small, mean cell numbers may have to be estimated. This is best done by estimating cell numbers in a more restricted area of the colony and estimating how many similar areas are contained within the counting field.

A new CEN standard is being prepared currently for calculating cell volumes of phytoplankton (CEN 2007)

8 Data entry

An Excel spreadsheet will be provided for data entry. It contains the fixed taxon list and provides biovolume formulae for each. It also allows the raw data to be summarised. All required details must be recorded on the counting sheet or in the counter's notebook and should be input into the counting spreadsheet according to the accompanying instructions.

Data to be entered will include information on the sample site and date of collection, date of analysis, who carried out the count, information on the chamber and counting areas and the volume of sample used. For each taxa found, the number of units counted, the number of fields of view (or equivalent for whole chamber or diameter transects) in which it was counted and average dimensions of the taxa will be recorded. For taxa which are counted in more than one form, e.g. individual cells and filaments/colonies, it is important to fill in one row for cells counted and the other for filaments or colonies.

Cells/ml and biovolumes for each taxa are automatically calculated.

The range of biovolumes for many taxa (from the published literature) are included in the spreadsheet so that calculated biovolumes can be validated against published ranges. If the calculated biovolumes are significantly different

to the published ranges then measurements of taxa dimensions and the calibration of eyepiece graticules should be checked.

9 Quality Assurance and validation of counts

Detailed quality assurance methodology and validation of counts are given in CEN (2004), NRA (1995) and Environment Agency (1998).

The following should be noted:

- Details of microscopes, chambers (individually identified and calibrated), calibration of all ocular/objective combinations should be recorded in a note book and kept for reference. If fixed volume pipettes these should be calibrated annually.

- Checks for random distribution of sample should be done visually at low magnification for each sample, whereas a more detailed check using simple Chi squared test should be done if a sample does not appear to be randomly sedimented or 1 sample every 3 months or so.

- Intra (same chamber and sample) and inter (replicate subsamples from same sample) chamber counts should be carried out at regular intervals by the same analyst and if possible by further analysts.

In addition, it is recommended that

- where ring-tests are undertaken, a staged approach should be adopted:
  1) determining mainly counting errors – group of analysts to count limited number of named taxa (1 to 3) or latex particles/pollen grains in set fields – can be done using photographs or videos
  2) repeat transect or field counts by 2 or more analysts on real sample to check identification and counting errors.
  3) Full count comparisons

- regular workshops should be held (3 - 4 times per annum) to carry out identification and ring tests, possibly combined with ½-1 day taught workshop on difficult groups

10 Literature

Methodology

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Key UK identification guides/coded lists

John D.M., Whitton B.A. and Brook A.J. (Eds), 2003. *The Freshwater Algal Flora of the British Isles: an identification guide to freshwater and terrestrial algae*, Cambridge University Press

Whitton et al., 1998. *A Coded List of Freshwater Algae of the British Isles* Second Edition. vailable from: http://www.ceh.ac.uk/data/dict/algae/

Other useful identification keys/guides

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Appendix 1.A

Whitton Code	Accepted name	Colony biovolume formula	Cell biovolume formula	Minimum Biovolume	Typical Biovolume	Maximum Biovolume
12010010	Acanthoceras zachariasii		Sphere			
17020010	Actinastrum hantzschii		Cone			
01020040	Anabaena catenula	Circle based ellipse	Circle based ellipse			
01020042	Anabaena catenula var. solitaria	Circle based ellipse	Sphere			
01020050	Anabaena circinalis	Circle based ellipse	Sphere			
01020090	Anabaena flos-aquae	Circle based ellipse	Circle based ellipse			
01020000	Anabaena sp.	Circle based ellipse	Circle based ellipse			
01020140	Anabaena spiroides	Circle based ellipse	Sphere			
01020190	Anabaena viguieri	Circle based ellipse	Sphere			
17050030	Ankistrodesmus falcatus		Cone			
17050050	Ankistrodesmus fusiformis		Cone			
17050000	Ankistrodesmus sp.		Cone			
17060020	Ankyra judayi		Cone	234	1021	1299
01040020	Aphanizomenon flos-aquae	Circle based ellipse	Circle based cylinder - long			
01040040	Aphanizomenon issatschenkoi	Circle based ellipse	Circle based cylinder - long		309	
01040000	Aphanizomenon sp.	Circle based ellipse	Circle based cylinder - long			
01050020	Aphanocapsa delicatissima	0.5 sphere	Sphere			
01050030	Aphanocapsa elachista	Sphere	Sphere			
01050000	Aphanocapsa sp.	0.5 sphere	Sphere			
01060020	Aphanothece clathrata	0.5 sphere	Circle based ellipse		105	
01060000	Aphanothece sp.	0.5 sphere	Circle based ellipse			
13080010	Asterionella formosa		Cuboid/rectangle		270	1400
12030060	Aulacoseira granulata	Circle based cylinder - long	Circle based cylinder - long	46		260
12030062	Aulacoseira granulata v. angustissima	Circle based cylinder - long	Circle based cylinder - long			
12030070	Aulacoseira islandica	Circle based cylinder - long	Circle based cylinder - long			

12030080	Aulacoseira italica	Circle based cylinder - long	Circle based cylinder - long			
12030084	Aulacoseira italica v. tenuissima	Circle based cylinder - long	Circle based cylinder - long	80		400
12030000	Aulacoseira sp.	Circle based cylinder - long	Circle based cylinder - long	20		180
09030010	Bitrichia chodatii		Circle based ellipse			
09030020	Bitrichia longispina		Circle based ellipse			
09030000	Bitrichia sp.		Circle based ellipse		2.15	
17080010	Botryococcus braunii	Circle based ellipse	Circle based ellipse			
17080000	Botryococcus sp.	Circle based ellipse	Circle based ellipse	0.4		3.3
16060000	Carteria sp.		Circle based ellipse			
06020020	Ceratium cornutum			31		504
06020030	Ceratium furcoides				30	
06020040	Ceratium hirundinella					
16180000	Chlamydomonas sp.		Circle based ellipse	11	14.3	17
09050030	Chromulina nebulosa		Circle based ellipse			
09050000	Chromulina sp.		Circle based ellipse			
01130020	Chroococcus dispersus		Sphere			
01130060	Chroococcus minutus		Sphere			
01130000	Chroococcus sp.		Sphere	30		280
05020010	Chroomonas acuta		Oval based ellipse	20		70
05020000	Chroomonas sp.		Oval based ellipse	30		180
08010010	Chrysochromulina parva		Oval based ellipse			
09130000	Chrysococcus sp.		Sphere	30		120
09150000	Chrysolykos sp.		Circle based ellipse	100		430
09170000	Chrysopyxis sp.		Circle based ellipse	10	34	70
17170010	Closteriopsis acicularis		Cone	236		860
17170020	Closteriopsis longissima		Cone	10		70
17170000	Closteriopsis sp.		Cone			
27040030	Closterium aciculare		Cone	3		30
27040040	Closterium acutum		Cone			
27040044	Closterium acutum v. variabile		Cone	32		91
27040340	Closterium kuetzingii		Cone			
27040500	Closterium parvulum		Cone			
27040000	Closterium sp.		Cone			

17200010	Coelastrum astroideum	Sphere	Circle based ellipse			
17200020	Coelastrum microporum	Sphere	Sphere		301	
17200000	Coelastrum sp.	Sphere	Sphere			
17200070	Coelastrum sphaericum	Sphere	Sphere			
01150010	Coelosphaerium kuetzingianum	0.2 sphere	Sphere			
01150000	Coelosphaerium sp.	0.2 sphere	Sphere			
17210010	Coenochloris fottii		Circle based ellipse			
17230020	Coenocystis planktonica		Circle based ellipse			
27050000	Cosmarium sp		Oval based ellipse			
17250000	Crucigenia sp.		Oval based ellipse			
17250030	Crucigenia tetrapedia		Cuboid/rectangle	84	128	150
05040030	Cryptomonas erosa		Oval based ellipse	35	105	183
05040040	Cryptomonas marssonii		Oval based ellipse		25560	
05040050	Cryptomonas ovata		Oval based ellipse		18600	
05040000	Cryptomonas sp.		Oval based ellipse		44000	70000
05040003	Cryptomonas sp. (large) Length >30µm		Oval based ellipse			
05040002	Cryptomonas sp. (medium) Length 20-30 µm		Oval based ellipse			
05040001	Cryptomonas sp. (small) L<20µm		Oval based ellipse	9905		25000
12070000	Cyclotella sp.		Circle based cylinder - short			
13260042	Diatoma elongatum		Cuboid/rectangle	21120	57000	99700
13260000	Diatoma sp.		Cuboid/rectangle	41000	64500	103000
13260040	Diatoma tenuis		Cuboid/rectangle		18560	
17330040	Dictyosphaerium pulchellum	0.25 sphere	Sphere	40	75	125
17340000	Didymocystis sp		Circle based ellipse			
17350020	Didymogenes palatina		Circle based ellipse	467	887	970
09230030	Dinbryon crenulatum		Circle based ellipse	99	112	158
09230010	Dinobryon bavaricum		Circle based ellipse			
09230050	Dinobryon divergens		Circle based ellipse		183	
09230070	Dinobryon sertularia		Circle based ellipse			
09230080	Dinobryon sociale		Circle based ellipse			

09230000	Dinobryon sp.		Circle based ellipse			
09230090	Dinobryon suecicum		Circle based ellipse			
25010010	Elakatothrix gelatinosa		Cone + hemisphere			
09250000	Epipyxsis sp.		Circle based ellipse			
27110000	Euastrum sp.		Oval based ellipse			
16260010	Eudorina elegans	0.25 sphere	Sphere			
04020000	Euglena sp.		Oval based ellipse			
13370030	Fragilaria capucina		Cuboid/rectangle			
13370040	Fragilaria crotonensis		Cuboid/rectangle			
13370000	Fragilaria sp.		Cuboid/rectangle			
06050000	Glenodinium sp		Oval based ellipse			
17420000	Gloeocystis sp.		Sphere	22		1000
17430020	Golenkinia radiata		Sphere	1000		20000
17430000	Golenkinia sp.		Sphere			
17440020	Golenkiniopsis longispina		Sphere			
01320010	Gomphosphaeria aponina	0.75 * sphere	Circle based ellipse			
01320000	Gomphosphaeria sp.	0.75 * sphere	Circle based ellipse			
27130000	Gonatozygon sp.		Circle based cylinder - long			
07010010	Gonyostomum semen		Cone + hemisphere			
06070110	Gymnodinium helveticum		Oval based ellipse			
06070000	Gymnodinium sp.		Oval based ellipse	329	580	? 2200
09290000	Kephyrion sp.		Circle based ellipse			
25030010	Koliella longiseta		Cone	424	597	3816
25030000	Koliella sp.		Cone		377	575
17540040	Lagerheimia genevensis		Circle based cylinder - long	254	487	? 3185
17540000	Lagerheimia sp.		Circle based cylinder - long	35	540	5828
12000003	Large centric diatom (>20 µm diam.)		Circle based cylinder - short	377		615
13000003	Large pennate diatom >20 µm		Cuboid/rectangle			
09310030	Mallomonas akrokomos		Cone + hemisphere			
09310080	Mallomonas caudata		Cone + hemisphere	1501	4671	14223
09310000	Mallomonas sp.		Circle based ellipse			

12000002	Medium centric diatom 10-20µm diam.		Circle based cylinder - short			
13000002	Medium pennate diatom 10-20 µm		Cuboid/rectangle	141		1884
12110000	Melosira sp.	Circle based cylinder - long	Circle based cylinder - long	1207		3418
12110080	Melosira varians	Circle based cylinder - long	Circle based cylinder - long	114	480	983
01460000	Merismopedia sp.	Cuboid/rectangle	Circle based ellipse			
17570010	Micractinium pusillum		Sphere	60	204	2993
17570000	Micractinium sp		Sphere	320	550	1482
01490010	Microcystis aeruginosa		Sphere			
01490020	Microcystis flos-aquae		Sphere	81	388	1011
01490000	Microcystis sp.		Sphere	169	640	2228
01490030	Microcystis wesenbergii		Sphere			
17580010	Monoraphidium arcuatum		Cone			
17580020	Monoraphidium contortum		Cone	37	200	912
17580030	Monoraphidium convolutum		Cone			
17580040	Monoraphidium griffithii		Cone	544	880	2700
17580050	Monoraphidium irregulare		Cone		158	
17580070	Monoraphidium komarkovae		Cone	920	1600	9800
17580080	Monoraphidium minutum		Cone			
17580110	Monoraphidium pusillum		Cone	828		2185
17580000	Monoraphidium sp.		Cone	440	1185	7349
17580120	Monoraphidium tortile		Cone		2402	
90000003	Nanoplankton - unidentified single cells, 2–20 µm diam.		Sphere			
13520000	Navicula sp.		Cuboid/rectangle			
13540020	Nitzschia acicularis		Cuboid/rectangle * 0.5			
13540000	Nitzschia sp.		Cuboid/rectangle			
09350000	Ochromonas sp.		Circle based ellipse	49		2078
17640130	Oocystis borgei		Circle based ellipse		509	
17640050	Oocystis lacustris		Circle based ellipse	31		205
17640000	Oocystis sp.		Circle based ellipse			
01530010	Oscillatoria agardhii	Circle based cylinder - long	Circle based cylinder - long	17		181

01530012	Oscillatoria agardhii var. isothrix	Circle based cylinder - long	Circle based cylinder - long	16		132
01530160	Oscillatoria limnetica	Circle based cylinder - long	Circle based cylinder - long	3		71
01530170	Oscillatoria limosa	Circle based cylinder - long	Circle based cylinder - long			
01530230	Oscillatoria redekei	Circle based cylinder - long	Circle based cylinder - long		52	
01530000	Oscillatoria sp.	Circle based cylinder - long	Circle based cylinder - long			
16470010	Pandorina morum	Sphere	Sphere			
16470000	Pandorina sp.	Sphere	Sphere			
17680020	Pediastrum biradiatum	Circle based cylinder - short				
17680030	Pediastrum boryanum	Circle based cylinder - short		31	258	716
17680050	Pediastrum duplex	Circle based cylinder - short		19		293
17680080	Pediastrum simplex	Circle based cylinder - short		58	95	130
17680000	Pediastrum sp.	Circle based cylinder - short				
17680090	Pediastrum tetras	Circle based cylinder - short		4000		32226
09360000	Pedinella sp.		Oval based ellipse			
06110050	Peridinium cinctum		Oval based ellipse	15		189
06110000	Peridinium sp.		Oval based ellipse	15		167
06110100	Peridinium willei		Oval based ellipse			
04070000	Phacus sp.		Oval based ellipse	19	21	24
90000000 .	Picoplankton - unidentified single cells <2 µm diam.		Sphere			
17690010	Planktosphaeria gelatinosa		Sphere			
09430000	Pseudokephyrion sp.		Circle based ellipse	448		1732
Whitton code required	Pseudopedinella sp.		Sphere		523	
17780000	Quadrigula sp.		Cone			

05100010	Rhodomonas lacustris		Cone + hemisphere	12	35	144
05100012	Rhodomonas lacustris var nannoplanctica		Cone + hemisphere			
Whitton code required	Rhodomonas lens		Cone + hemisphere	31		485
05100000	Rhodomonas sp		Cone + hemisphere			
17810030	Scenedesmus acuminatus		Circle based ellipse	26		121
17810080	Scenedesmus armatus		Circle based ellipse	4		68
17810220	Scenedesmus falcatus		Circle based ellipse	44		107
17810340	Scenedesmus opoliensis		Circle based ellipse	31		421
Whitton code required	Scenedesmus quadricauda		Circle based ellipse		353	
17810000	Scenedesmus sp.		Circle based ellipse	44		283
17830030	Schroederia setigera		Cone	32		103
17830000	Schroederia sp.		Cone		124	
12000004	Very small centric diatom (<5 µm diam.)		Circle based cylinder - short	3		102
12000001	Small centric diatom (5-<10 µm diam.)		Circle based cylinder - short			
13000001	Small pennate diatom <10 µm		Cuboid/rectangle	73	110	411
01750010	Snowella lacustris	0.75 * sphere	Circle based ellipse		1415	
01750000	Snowella sp.	Sphere	Sphere			
17910020	Sphaerocystis schroeteri		Sphere			
17910000	Sphaerocystis sp.		Sphere			
09450000	Spinifertomonas sp.		Sphere			
27360040	Spondylosium planum		Oval based ellipse			
27380330	Staurastrum cingulum			187	643	8181
27380840	Staurastrum longipes					
27380860	Staurastrum lunatum					
27380000	Staurastrum ophiura			11		394
27381120	Staurastrum planctonicum			19		289
27370000	Staurastrum sp.					
27381460	Staurastrum tetracerum			28		430
27390190	Staurodesmus incus					
27390000	Staurodesmus sp.			36	147	793
12180000	Stephanodiscus sp.		Circle based cylinder -			

			short			
09480000	Stichoglea sp.		Circle based ellipse		69	
13810010	Synedra acus		Cuboid/rectangle			
13810120	Synedra nana		Cuboid/rectangle	139		905
13810000	Synedra sp.		Cuboid/rectangle	30		387
13810180	Synedra ulna		Cuboid/rectangle			
09530000	Synura sp.		Circle based ellipse	9		113
13820010	Tabellaria fenestrata		Cuboid/rectangle	29		157
13820020	Tabellaria flocculosa		Cuboid/rectangle			
13820022	Tabellaria flocculosa var. asterionelloides		Cuboid/rectangle	41	218	247
13820000	Tabellaria sp.		Cuboid/rectangle			
17960010	Tetraedron caudatum		Cuboid/rectangle	11	45	130
17960030	Tetraedron minimum		Cuboid/rectangle		570	916
17960000	Tetraedron sp.		Cuboid/rectangle		377	
17970000	Tetrastrum sp.		Cone + hemisphere	21436		95529
17970050	Tetrastrum staurogeniaeforme		Cone + hemisphere	8150		33809
17970060	Tetrastrum triangulare		Cone + hemisphere			
04100000	Trachelomonas sp.		Circle based ellipse			
18010010	Treubaria setigera		Circle based cylinder - short			
90000004 .	Unidentified cells >20 µm diam.		Sphere	129	154	262
17000001	Unidentified colonial greens.		Sphere		22763	
01000000	Unidentified cyanophytes - colonial algae <2 µm diameter.		Sphere	1916		15215
90000005	Unidentified flagellates 2 – 20 µm diam.		Sphere		1767	
17000000	Unidentified small green round cells (sgrt)		Sphere		7503	
09540000	Uroglena sp.		Circle based ellipse		3031	
12200000	Urosolenia		Cone		1608	
12200010	Urosolenia eriensis		Cone			
12200020	Urosolenia longiseta		Cone		48444	

16770010	Volvox aureus		Circle based ellipse			
16770010	Volvox sp.		Circle based ellipse			
01780010	Woronichinia naegeliana	0.2 sphere	Circle based ellipse			
01780000	Woronichinia sp.	0.2 sphere	Circle based ellipse			
27430020	Xanthidium antilopaeum		Oval based ellipse	163	323	696
27430000	Xanthidium sp.		Oval based ellipse			

Algal Biovolume formulae and names:

Biovolume shape	Formula	Taxon examples
CIRCLE BASED CYLINDER - LONG	3.141592654*L*D*D/4	*Aphanizomenon, Aulocolsaera*
CIRCLE BASED CYLINDER - SHORT	3.141592654*H*D*D/4	Centric diatoms,
CIRCLE BASED ELLIPSE	3.141592654*L*D*D/6	
OVAL BASED CYLINDER	3.141592654*L*D*H/4	
OVAL BASED ELLIPSE	3.141592654*L*D*H/6	
CONE	3.141592654*L*D*D/12	*Mallamonas akrokomos*, horn of *Staurastrum*
CONE + HEMISPHERE	(3.141592654*D*D)12*(D/2+L)	*Rhodomonas, Mallamonas caudata*
DOUBLE CONE	3.141592654*L*D*D/12	*Ankistrodesmus, Closterium*
CUBOID/RECTANGLE	L*D*H	*Tabellaria*, pennate diatoms, *Merismopedia*
CUBOID/RECTANGLE * 0.5	0.5*L*D*H	*Nitzschia acicularis*
SPHERE	3.141592654*D*D*D/6	*Microcystis, Sphaerocytis*, picoplankton cells
0.2 SPHERE	0.2*3.141592654*D*D*D/6	*Woronichinia*
0.25 SPHERE	0.25*3.141592654*D*D*D/6	*Eudorina*
0.5 SPHERE	0.5*3.141592654*D*D*D/6	*Aphanothece, Aphanocapsa*
0.75 * SPHERE	0.75*3.141592654*D*D*D/6	*Snowella, Gomphosphaeria*

L = length (µm)
D = Diameter or width (µm)
H = Depth or height (µm)
P = Numbers of arms/branches in *Staurastrum* half cell

APPENDIX II PIE RESULTS FOR UK AND IRISH LAKES

Lake Code	LakeName	Date	Observed PTI Score	Expected PTI Score (site-specific)	EQR_{0-1} (site-specific)	Status class (site-specific)
IEEA_07_270	Lough Bane	2005/08	0.39	0.44	0.93	High
IEEA_07_274	Lough Lene	2005/08	0.42	0.43	0.73	High
IENS_35_160	Lough Melvin	2005/07	0.46	0.42	0.52	Moderate
IENS_36_648	Lough Garadice	2005/08	0.44	0.43	0.61	Good
IENS_38_692	Lough Dunglow	2005/07	0.42	0.39	0.58	Good
IESH_25_188	Lough Ennell	2005/08	0.42	0.44	0.77	High
IESH_25_190	Lough Graney	2005/07	0.41	0.41	0.67	High
IESH_26_693	Lough O'Flynn	2005/08	0.40	0.44	0.88	High
IESH_26_703	Lough Owel	2005/08	0.41	0.43	0.75	High
IESH_27_115	Lough Cullaun	2005/07	0.38	0.44	1.00	High
IESH_27_130	Lough Inchiquin	2005/07	0.39	0.44	0.95	High
IESH_27_94	Lough Muckanagh	2005/07	0.36	0.44	1.10	High
IESH_28_82	Lough Doo CE	2005/08	0.42	0.39	0.56	Good
IESW_22_172	Lough Guitane	2005/07	0.42	0.38	0.53	Good
IEWE_30_343	Lough Maumwee	2005/07	0.39	0.37	0.60	Good
IEWE_31_171	Lough Shindilla	2005/07	0.37	0.37	0.67	High
IEWE_31_208	Lough Nahasleam	2005/07	0.37	0.38	0.71	High
IEWE_31_211	Lough Anaserd	2005/08	0.40	0.41	0.70	High
IEWE_32_136	Lough Easky	2005/07	0.37	0.37	0.66	High
IEWE_32_479	Lough Ballynakill	2005/08	0.41	0.41	0.64	High
IEWE_32_490	Lough Doo MO	2005/08	0.36	0.39	0.79	High
IEZZ_00_001	Lough Acrow	2005/08	0.40	0.32	0.43	Moderate
IEZZ_00_002	Lough Akibbon	2005/07	0.41	0.42	0.73	High
IEZZ_00_003	Lough Annaghmakerig	2005/08	0.44	0.43	0.63	High
IEZZ_00_004	Lough Atorick	2005/08	0.36	0.39	0.81	High
IEZZ_00_005	Lough Ballycullinan	2005/07	0.39	0.44	0.95	High
IEZZ_00_006	Lough Barfinnihy	2005/07	0.36	0.38	0.76	High
IEZZ_00_007	Lough Columbkille	2005/07	0.33	0.40	0.92	High
IEZZ_00_009	Lough Dromore	2005/08	0.41	0.44	0.83	High
IEZZ_00_010	Lough Fin	2005/08	0.37	0.39	0.74	High
IEZZ_00_011	Lough Lattone	2005/07	0.42	0.42	0.68	High
IEZZ_00_012	Lough Lickeen	2005/07	0.44	0.41	0.55	Good
IEZZ_00_013	Lough MacNean lower	2005/07	0.42	0.43	0.72	High
IEZZ_00_014	Lough MacNean upper	2005/07	0.42	0.42	0.63	High
IEZZ_00_015	Lough Moher	2005/07	0.36	0.41	0.89	High
IEZZ_00_016	Lough More	2005/08	0.41	0.43	0.78	High
IEZZ_00_017	Lough Naminn	2005/07	0.40	0.40	0.68	High
IEZZ_00_018	Lough Veagh	2005/07	0.37	0.39	0.75	High
UK1678	Loch of Swannay	2006/09	0.44	0.43	0.63	High
UK2088	Loch of Mey	2004/09	0.44	0.43	0.64	High
UK2358	Loch Calder	2003/09	0.43	0.42	0.66	High
UK2490	Loch Hope	2004/07	0.38	0.38	0.67	High
UK2499	Loch Scarmclate	2004/08	0.41	0.44	0.81	High
UK3458	Loch Craggie	2006/08	0.38	0.38	0.70	High
UK4204	Loch Meadie	2004/09	0.37	0.38	0.68	High
UK5222	Loch Meadie	2004/07	0.41	0.37	0.53	Good
UK5350	Loch Stack	2004/07	0.40	0.38	0.57	Good
UK6234	Loch Culaidh	2004/09	0.36	0.37	0.73	High
UK6405	Loch Naver	2004/07	0.38	0.38	0.65	High
UK8751	Loch Assynt	2006/07	0.37	0.41	0.84	High
UK8751	Loch Assynt	2006/08	0.41	0.41	0.66	High

Lake Code	LakeName	Date	Observed PTI Score	Expected PTI Score (site-specific)	EQR_{0-1} (site-specific)	Status class (site-specific)
UK10934	Cam Loch	2006/08	0.41	0.39	0.62	High
UK11189	Loch Osgaig	2003/09	0.39	0.37	0.61	Good
UK11338	Loch Ailsh	2006/08	0.35	0.40	0.87	High
UK11611	Loch Brora	2004/08	0.41	0.39	0.58	Good
UK12578	Loch an Lagain	2004/09	0.36	0.39	0.78	High
UK14057	Loch Maree	2004/09	0.42	0.37	0.50	Moderate
UK16456	Loch Ussie	2004/09	0.43	0.41	0.57	Good
UK18682	Loch Druidibeag	2004/08	0.39	0.39	0.68	High
UK20860	Loch Insh	2003/08	0.39	0.39	0.69	High
UK22839	Loch Laidon	2004/07	0.41	0.38	0.56	Good
UK23559	Loch of Lowes	2004/09	0.41	0.41	0.68	High
UK24132	Loch Earn	2004/07	0.40	0.40	0.67	High
UK24459	Loch Lubnaig	2003/08	0.34	0.39	0.88	High
UK24919	Lake of Menteith	2004/07	0.46	0.41	0.47	Moderate
UK25899	Ardnave Loch	2006/08	0.42	0.42	0.63	High
UK26168	Loch Gorm	2006/08	0.43	0.41	0.58	Good
UK26257	Loch Skerrols	2006/08	0.37	0.42	0.89	High
UK27568	Catcleugh Reservoir	2006/08	0.44	0.42	0.56	Good
UK27698	Kielder Water	2005/07	0.40	0.41	0.70	High
UK27698	Kielder Water	2006/07	0.41	0.41	0.64	High
UK28165	Greenlee Lough	2004/07	0.45	0.42	0.53	Moderate
UK28165	Greenlee Lough	2004/08	0.46	0.42	0.48	Moderate
UK28165	Greenlee Lough	2004/09	0.54	0.42	0.11	Bad
UK28172	Broomlee Lough	2005/07	0.45	0.43	0.57	Good
UK28172	Broomlee Lough	2005/09	0.46	0.43	0.51	Moderate
UK28200	Woodhall Loch	2004/09	0.47	0.40	0.38	Moderate
UK28220	Crag Lough	2005/07	0.43	0.43	0.69	High
UK28220	Crag Lough	2005/09	0.46	0.43	0.52	Moderate
UK28220	Crag Lough	2006/08	0.46	0.43	0.50	Moderate
UK28386	Talkin Tarn	2004/08	0.47	0.42	0.44	Moderate
UK28386	Talkin Tarn	2004/09	0.48	0.42	0.40	Moderate
UK28519	Derwent Reservoir	2006/07	0.44	0.42	0.58	Good
UK28847	Bassenthwaite Lake	2004/07	0.40	0.40	0.67	High
UK28847	Bassenthwaite Lake	2004/09	0.42	0.40	0.60	Good
UK28847	Bassenthwaite Lake	2005/07	0.50	0.40	0.27	Poor
UK28847	Bassenthwaite Lake	2005/09	0.46	0.40	0.40	Moderate
UK28955	Ullswater	2004/07	0.43	0.40	0.58	Good
UK28955	Ullswater	2005/07	0.42	0.40	0.61	Good
UK28955	Ullswater	2005/09	0.38	0.40	0.78	High
UK28965	Derwent Water	2004/08	0.37	0.39	0.77	High
UK28965	Derwent Water	2005/07	0.37	0.39	0.77	High
UK28965	Derwent Water	2005/09	0.37	0.39	0.74	High
UK28986	Loweswater	2004/08	0.44	0.40	0.52	Moderate
UK28986	Loweswater	2004/09	0.43	0.40	0.53	Good
UK28986	Loweswater	2005/07	0.38	0.40	0.73	High
UK28986	Loweswater	2005/09	0.42	0.40	0.58	Good
UK29000	Crummock Water	2004/09	0.43	0.38	0.48	Moderate
UK29000	Crummock Water	2005/07	0.36	0.38	0.77	High
UK29000	Crummock Water	2005/09	0.31	0.38	0.95	High

Lake Code	LakeName	Date	Observed PTI Score	Expected PTI Score (site-specific)	EQR_{0-1} (site-specific)	Status class (site-specific)
UK29021	Thirlmere	2004/09	0.40	0.38	0.58	Good
UK29021	Thirlmere	2005/07	0.35	0.38	0.79	High
UK29021	Thirlmere	2005/09	0.36	0.38	0.74	High
UK29052	Buttermere	2004/09	0.37	0.38	0.68	High
UK29052	Buttermere	2005/07	0.34	0.38	0.79	High
UK29052	Buttermere	2005/09	0.38	0.38	0.67	High
UK29062	Ennerdale Water	2004/08	0.37	0.38	0.73	High
UK29062	Ennerdale Water	2004/09	0.37	0.38	0.69	High
UK29062	Ennerdale Water	2005/07	0.37	0.38	0.70	High
UK29062	Ennerdale Water	2005/09	0.33	0.38	0.87	High
UK29178	Sunbiggin Tarn	2006/08	0.51	0.44	0.32	Moderate
UK29183	Wast Water	2004/07	0.40	0.38	0.58	Good
UK29183	Wast Water	2004/09	0.36	0.38	0.76	High
UK29183	Wast Water	2005/07	0.40	0.38	0.58	Good
UK29183	Wast Water	2005/09	0.40	0.38	0.61	Good
UK29184	Grasmere	2004/07	0.44	0.40	0.48	Moderate
UK29184	Grasmere	2004/08	0.42	0.40	0.58	Good
UK29184	Grasmere	2005/07	0.42	0.40	0.57	Good
UK29184	Grasmere	2005/09	0.41	0.40	0.62	High
UK29222	Elter Water	2004/07	0.43	0.40	0.55	Good
UK29222	Elter Water	2004/08	0.41	0.40	0.63	High
UK29222	Elter Water	2004/09	0.43	0.40	0.53	Good
UK29233	Windermere	2004/07	0.45	0.40	0.46	Moderate
UK29233	Windermere	2004/09	0.48	0.40	0.34	Moderate
UK29233	Windermere	2005/07	0.42	0.40	0.60	Good
UK29233	Windermere	2005/09	0.42	0.40	0.60	Good
UK29270	Blelham Tarn	2004/07	0.47	0.41	0.41	Moderate
UK29270	Blelham Tarn	2004/09	0.38	0.41	0.80	High
UK29321	Coniston Water	2004/07	0.46	0.40	0.43	Moderate
UK29321	Coniston Water	2004/08	0.42	0.40	0.58	Good
UK29321	Coniston Water	2004/09	0.40	0.40	0.68	High
UK29321	Coniston Water	2005/07	0.39	0.40	0.73	High
UK29321	Coniston Water	2005/09	0.39	0.40	0.70	High
UK29328	Esthwaite	2004/07	0.55	0.42	0.08	Bad
UK29328	Esthwaite	2004/08	0.48	0.42	0.36	Moderate
UK29479	Semer Water	2006/08	0.52	0.43	0.21	Poor
UK29647	Hawes Water	2004/07	0.44	0.45	0.70	High
UK29647	Hawes Water	2004/09	0.36	0.45	1.10	High
UK29647	Hawes Water	2005/09	0.37	0.45	1.08	High
UK29844	Malham Tarn	2004/07	0.42	0.44	0.78	High
UK29844	Malham Tarn	2005/09	0.50	0.44	0.35	Moderate
UK30030	Stocks Reservoir	2004/08	0.41	0.42	0.70	High
UK30030	Stocks Reservoir	2004/09	0.40	0.42	0.76	High
UK30244	Hornsea Mere	2005/09	0.45	0.44	0.62	High
UK30604	Widdop Reservoir	2006/07	0.39	0.40	0.71	High
UK31104	White Holme Reservoir	2006/07	0.42	0.40	0.60	Good
UK32359	Derwent Reservoir	2004/07	0.39	0.41	0.78	High
UK32435	Llyn Llygeirian	2004/08	0.46	0.42	0.48	Moderate
UK32435	Llyn Llygeirian	2004/09	0.42	0.42	0.69	High
UK32459	Ladybower Reservoir	2004/07	0.39	0.41	0.75	High
UK32459	Ladybower Reservoir	2004/08	0.46	0.41	0.43	Moderate
UK32459	Ladybower Reservoir	2004/09	0.36	0.41	0.85	High
UK32459	Ladybower Reservoir	2005/09	0.40	0.41	0.70	High

Lake Code	LakeName	Date	Observed PTI Score	Expected PTI Score (site-specific)	EQR_{0-1} (site-specific)	Status class (site-specific)
UK32538	Llyn Alaw	2004/07	0.42	0.42	0.65	High
UK32538	Llyn Alaw	2004/08	0.48	0.42	0.38	Moderate
UK32538	Llyn Alaw	2004/09	0.49	0.42	0.36	Moderate
UK32538	Llyn Alaw	2005/08	0.44	0.42	0.57	Good
UK32650	Rostherne Mere	2004/07	0.49	0.44	0.42	Moderate
UK32650	Rostherne Mere	2004/08	0.50	0.44	0.33	Moderate
UK32650	Rostherne Mere	2004/09	0.52	0.44	0.26	Poor
UK32744	Mere Mere	2004/07	0.53	0.43	0.18	Poor
UK32744	Mere Mere	2004/09	0.49	0.43	0.37	Moderate
UK32761	Llyn yr Wyth-Eidion	2004/07	0.43	0.45	0.78	High
UK32761	Llyn yr Wyth-Eidion	2005/08	0.47	0.45	0.55	Good
UK32804	Tatton Mere	2004/07	0.50	0.44	0.34	Moderate
UK32804	Tatton Mere	2004/09	0.48	0.44	0.43	Moderate
UK32804	Tatton Mere	2005/08	0.47	0.44	0.48	Moderate
UK32948	Llyn Dinam	2004/08	0.47	0.43	0.47	Moderate
UK32960	Tabley Mere	2004/08	0.51	0.44	0.29	Poor
UK32961	Llyn Helyg	2004/07	0.45	0.42	0.52	Moderate
UK32961	Llyn Helyg	2004/08	0.45	0.42	0.53	Good
UK32961	Llyn Helyg	2004/09	0.42	0.42	0.64	High
UK32968	Llyn Penrhyn	2004/07	0.47	0.43	0.51	Moderate
UK32968	Llyn Penrhyn	2004/08	0.52	0.43	0.23	Poor
UK32968	Llyn Penrhyn	2004/09	0.47	0.43	0.48	Moderate
UK33337	Llyn Coron	2004/07	0.48	0.43	0.41	Moderate
UK33337	Llyn Coron	2004/09	0.49	0.43	0.36	Moderate
UK33337	Llyn Coron	2005/08	0.53	0.43	0.18	Poor
UK33474	Oak Mere	2004/08	0.53	0.39	0.12	Bad
UK33474	Oak Mere	2004/09	0.47	0.39	0.35	Moderate
UK33627	Llyn Rhos-ddu	2004/07	0.43	0.44	0.69	High
UK33627	Llyn Rhos-ddu	2004/08	0.51	0.44	0.30	Poor
UK33627	Llyn Rhos-ddu	2004/09	0.45	0.44	0.62	High
UK33730	Llyn Padarn	2004/07	0.45	0.39	0.44	Moderate
UK33730	Llyn Padarn	2004/08	0.45	0.39	0.42	Moderate
UK33730	Llyn Padarn	2005/08	0.37	0.39	0.76	High
UK33784	Rudyard Reservoir	2004/08	0.50	0.43	0.30	Poor
UK33784	Rudyard Reservoir	2004/09	0.49	0.43	0.36	Moderate
UK33803	Llyn Ogwen	2004/08	0.46	0.38	0.35	Moderate
UK33803	Llyn Ogwen	2004/09	0.41	0.38	0.53	Good
UK33803	Llyn Ogwen	2005/08	0.34	0.38	0.80	High
UK33836	Llyn Idwal	2004/08	0.36	0.38	0.77	High
UK34002	Llyn Cwellyn	2004/07	0.42	0.38	0.53	Good
UK34002	Llyn Cwellyn	2004/08	0.42	0.38	0.52	Moderate
UK34002	Llyn Cwellyn	2004/09	0.41	0.38	0.54	Good
UK34002	Llyn Cwellyn	2005/08	0.35	0.38	0.80	High
UK34400	Llyn Conwy	2004/07	0.38	0.37	0.61	Good
UK34400	Llyn Conwy	2004/09	0.36	0.37	0.71	High
UK34400	Llyn Conwy	2005/08	0.36	0.37	0.68	High
UK34480	Comber Mere	2006/07	0.47	0.44	0.53	Good

Lake Code	LakeName	Date	Observed PTI Score	Expected PTI Score (site-specific)	EQR_{0-1} (site-specific)	Status class (site-specific)
UK34622	Llyn Glasfryn	2004/07	0.46	0.41	0.43	Moderate
UK34622	Llyn Glasfryn	2004/09	0.43	0.41	0.58	Good
UK34780	Hanmer Mere	2005/08	0.47	0.43	0.46	Moderate
UK34987	Llyn Tegid or Bala Lake	2004/08	0.42	0.39	0.53	Good
UK34987	Llyn Tegid or Bala Lake	2004/09	0.42	0.39	0.53	Moderate
UK34987	Llyn Tegid or Bala Lake	2005/08	0.37	0.39	0.73	High
UK34990	Ellesmere	2004/07	0.47	0.44	0.48	Moderate
UK34990	Ellesmere	2004/09	0.54	0.44	0.13	Bad
UK35091	White Mere	2004/07	0.51	0.43	0.27	Poor
UK35091	White Mere	2004/09	0.58	0.43	-0.09	Bad
UK35211	Crose Mere	2004/07	0.45	0.44	0.59	Good
UK35211	Crose Mere	2004/09	0.47	0.44	0.53	Moderate
UK35561	Llyn Bodlyn	2006/07	0.33	0.37	0.83	High
UK35568	Lake Vyrnwy / Llyn Efyrnwy	2005/08	0.35	0.38	0.79	High
UK35640	Hickling Broad	2004/07	0.46	0.44	0.56	Good
UK35640	Hickling Broad	2004/08	0.43	0.44	0.73	High
UK35724	Aqualate Mere	2004/07	0.54	0.44	0.18	Poor
UK35724	Aqualate Mere	2004/08	0.52	0.44	0.24	Poor
UK35953	Wroxham Broad	2004/09	0.44	0.45	0.72	High
UK35981	Rollesby Broad	2004/07	0.48	0.44	0.45	Moderate
UK35981	Rollesby Broad	2004/08	0.50	0.44	0.35	Moderate
UK36202	Upton Broad	2004/07	0.44	0.44	0.66	High
UK36202	Upton Broad	2004/08	0.46	0.44	0.59	Good
UK36405	Tal-y-llyn Lake	2004/09	0.41	0.40	0.63	High
UK36479	Rutland Water	2005/08	0.52	0.44	0.27	Poor
UK36523	Chasewater	2004/08	0.53	0.43	0.20	Poor
UK36523	Chasewater	2004/09	0.47	0.43	0.47	Moderate
UK36544	Bomere Pool	2004/07	0.50	0.43	0.33	Moderate
UK36566	Betton Pool	2004/08	0.48	0.44	0.42	Moderate
UK38214	Craig Goch Reservoir	2004/07	0.37	0.37	0.68	High
UK38214	Craig Goch Reservoir	2004/08	0.35	0.37	0.77	High
UK38214	Craig Goch Reservoir	2004/09	0.33	0.37	0.83	High
UK38310	Grafham Water	2004/07	0.54	0.45	0.15	Bad
UK38310	Grafham Water	2004/08	0.51	0.45	0.31	Poor
UK38310	Grafham Water	2004/09	0.54	0.45	0.13	Bad
UK38310	Grafham Water	2005/08	0.47	0.45	0.55	Good
UK38390	Llyn Teifi	2006/08	0.38	0.37	0.66	High
UK38394	Llyn Hîr	2006/08	0.33	0.34	0.68	High
UK38409	Llyn Egnant	2004/07	0.36	0.37	0.72	High
UK38409	Llyn Egnant	2004/09	0.46	0.37	0.33	Moderate
UK38422	Llyn Eiddwen	2004/07	0.46	0.39	0.37	Moderate
UK38422	Llyn Eiddwen	2004/08	0.52	0.39	0.16	Bad
UK38525	Llyn Gynon	2004/07	0.32	0.37	0.83	High
UK38525	Llyn Gynon	2004/08	0.37	0.37	0.66	High
UK38525	Llyn Gynon	2004/09	0.38	0.37	0.63	High
UK38907	Llyn Berwyn	2004/09	0.45	0.38	0.40	Moderate
UK38907	Llyn Berwyn	2005/08	0.42	0.38	0.53	Good
UK39450	Stewartby Lake	2004/07	0.51	0.43	0.30	Poor
UK39450	Stewartby Lake	2004/08	0.46	0.43	0.56	Good
UK39450	Stewartby Lake	2004/09	0.48	0.43	0.43	Moderate

Lake Code	LakeName	Date	Observed PTI Score	Expected PTI Score (site-specific)	EQR_{0-1} (site-specific)	Status class (site-specific)
UK39967	Usk Reservoir	2004/07	0.47	0.41	0.42	Moderate
UK39967	Usk Reservoir	2004/08	0.41	0.41	0.67	High
UK39967	Usk Reservoir	2004/09	0.41	0.41	0.64	High
UK40067	Llangorse Lake	2004/07	0.49	0.44	0.39	Moderate
UK40067	Llangorse Lake	2004/08	0.51	0.44	0.29	Poor
UK40067	Llangorse Lake	2004/09	0.52	0.44	0.23	Poor
UK40067	Llangorse Lake	2005/08	0.50	0.44	0.33	Moderate
UK40755	Stanborough Lake	2004/09	0.53	0.44	0.18	Poor
UK41011	Farmoor Reservoir	2006/08	0.51	0.45	0.33	Moderate
UK41427	Hanningfield Reservoir	2004/09	0.52	0.45	0.27	Poor
UK41559	Cotswold Park Lake 12	2004/09	0.46	0.43	0.51	Moderate
UK41602	Lily Ponds	2004/07	0.48	0.44	0.48	Moderate
UK41602	Lily Ponds	2004/08	0.46	0.44	0.58	Good
UK41602	Lily Ponds	2004/09	0.40	0.44	0.91	High
UK41602	Lily Ponds	2005/08	0.39	0.44	0.93	High
UK42170	Kenfig Pool	2004/09	0.45	0.43	0.60	Good
UK42170	Kenfig Pool	2005/08	0.50	0.43	0.36	Moderate
UK43096	Chew Valley lake	2004/09	0.52	0.44	0.26	Poor
UK43348	Cheddar Reservoir	2004/08	0.42	0.44	0.78	High
UK43348	Cheddar Reservoir	2004/09	0.42	0.44	0.78	High
UK43348	Cheddar Reservoir	2005/08	0.41	0.44	0.85	High
UK43602	Bough Beech Reservoir	2004/09	0.51	0.43	0.29	Poor
UK43909	Shear Water	2004/07	0.52	0.43	0.24	Poor
UK43909	Shear Water	2004/08	0.51	0.43	0.28	Poor
UK43943	Frensham Little Pond	2004/07	0.49	0.43	0.38	Moderate
UK43943	Frensham Little Pond	2004/08	0.52	0.43	0.22	Poor
UK43943	Frensham Little Pond	2004/09	0.51	0.43	0.27	Poor
UK44031	Frensham Great Pond	2004/07	0.52	0.43	0.23	Poor
UK44031	Frensham Great Pond	2004/08	0.49	0.43	0.36	Moderate
UK44031	Frensham Great Pond	2004/09	0.50	0.43	0.33	Moderate
UK44031	Frensham Great Pond	2005/08	0.58	0.43	0.00	Bad
UK44471	Wimbleball Lake	2006/08	0.50	0.41	0.28	Poor
UK44518	Fonthill Lake	2004/07	0.44	0.45	0.73	High
UK44518	Fonthill Lake	2004/09	0.51	0.45	0.31	Poor
UK45108	Burton Mill Pond	2004/09	0.47	0.45	0.55	Good
UK45108	Burton Mill Pond	2005/08	0.39	0.45	0.98	High
UK45652	Hatchet Pond	2004/09	0.44	0.40	0.51	Moderate
UK46102	Little Sea	2004/07	0.44	0.40	0.52	Moderate
UK46102	Little Sea	2004/08	0.48	0.40	0.32	Moderate
UK46102	Little Sea	2004/09	0.40	0.40	0.66	High
UK46102	Little Sea	2005/08	0.39	0.40	0.69	High
UK46232	Dozmary Pool	2004/09	0.43	0.39	0.50	Moderate
UK46279	Burrator Reservoir	2004/09	0.42	0.39	0.55	Good
UK46279	Burrator Reservoir	2005/08	0.37	0.39	0.72	High
UK46501	Stithians Reservoir	2004/09	0.46	0.40	0.43	Moderate
UK46501	Stithians Reservoir	2005/08	0.39	0.40	0.73	High
UK46556	The Loe	2004/09	0.49	0.41	0.33	Moderate
UK46556	The Loe	2005/08	0.50	0.41	0.28	Poor