Final Report **Project WFD38**

Chlorophyll and Phosphorus Classifications for UK Lakes

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EXECUTIVE SUMMARY

WFD38: Phytoplankton Classification Tool: Chlorophyll and Phosphorus Classifications for UK Lakes (October, 2006)

Project funders/partners: SNIFFER & Environment Agency

Background to research

The Environment Agency and SNIFFER have commissioned this R & D project to develop a method to classify the ecological status of lakes on the basis of phytoplankton communities. As part of this assessment, chlorophyll concentrations have been chosen as a measure of phytoplankton abundance. Chlorophyll has also been selected as a key measure for the WFD Intercalibration process for lakes. Countries need to provide lake-type specific chlorophyll concentrations for reference conditions and the High/Good (H/G) and Good/Moderate (G/M) boundary values for comparison between Member States.

Objectives of research

Specific objectives for the project were to develop a robust chlorophyll classification, incorporating:

1. Predictions of reference chlorophyll concentrations in UK lakes
2. Developing ecological criteria for defining the good/moderate chlorophyll 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 chlorophyll concentrations for a site
4. Defining supporting phosphorus concentrations for reference conditions and the high/good and good/moderate boundaries
5. Determining uncertainty associated with the classification result, based on statistical confidence or probability of class

Key findings and recommendations

The findings and recommendations reported here result from a close collaboration between the project team with the EC REBECCA project and the EC Intercalibration process and have been reliant on extensive datasets collated from lakes across Europe.

Type-specific chlorophyll reference conditions have been defined for a number of lake types using a large European dataset of 540 reference lakes. Mean chlorophyll concentrations from the phytoplankton growing season (April to September) have been collated for all reference lakes. Using these data, median values and the 75th percentile for several lake types have been established. Median values are recommended as a summary statistic for the reference condition and the 75% is recommended for the H/G boundary.

Regression models for predicting site–specific chlorophyll and total phosphorus reference conditions from typology variables (mean depth, alkalinity, altitude and colour) have also been developed. It is recommended that, where applicable, these models are used in preference to type-specific reference conditions as they will lead to less error in classification, particularly for sites that lie close to lake type boundaries. Regression models derived solely from UK reference lakes, had much greater predictive strength compared with European models, and so may be more appropriate in a UK context, although were based on much more limited datasets in terms of lake numbers and representation of typology gradients.

UK modelled chlorophyll reference values were, however, generally higher than limits agreed by intercalibration. For this reason, an alternative approach to predict reference chlorophyll values from reference TP concentrations and TP-chlorophyll relationships was developed. This approach predicted reference chlorophyll values in much closer agreement with other Member States and following discussion with the Lake Task Team, it was agreed to adopt this approach.

During intercalibration the High/Good boundary was determined as an upper percentile of a range of European reference sites. The EQR resulting from this boundary is, therefore, defined by intercalibration and will be used to establish the H/G boundary for all UK lake types subject to intercalibration. For lake types not subject to intercalibration, it is recommended that the EQR for the same depth type of intercalibrated lakes is used.

In line with WFD normative definitions, it is recommended that the G/M boundary should be set based on the secondary effects of elevated phytoplankton biomass. This project considered the effects of chlorophyll on the depth distribution of macrophytes to establish appropriate type-specific G/M boundary values. A method was also outlined to establish site-specific values based on reference chlorophyll conditions and background light attenuation. Further work carried out during intercalibration has subsequently validated the UK model and combined results from other European studies to set appropriate type-specific G/M boundary values. It is recommended that the G/M boundary for TP should be derived from the GM boundary for chlorophyll a using the TP-Chlorophyll regression relationships derived from the REBECCA and Intercalibration data.

The amount of precaution that is used to establish appropriate TP boundary values from the TP-Chlorophyll regression relationships is a matter of judgement. However, it is important to understand the level of uncertainty and to use an appropriate precautionary value. The resulting values need to be compared with other quality elements sensitive to phosphorus, such as macrophyte and phytoplankton composition.

The error associated with classification was examined for Loch Leven and Windermere. Estimates of the error in annual mean measures of TP and chlorophyll associated with sampling frequency were calculated. This indicated that TP was less variable than chlorophyll, but for both regular monthly sampling was recommended as this reduced error and mis-classification considerably compared with quarterly monitoring.

Currently there is great bias in both the UK and European reference lake datasets towards shallow and deep low alkalinity lakes. For this reason, more effort is needed to identify and sample sufficient numbers of sites (reference and non-reference) of these under-represented lake types to improve models and ensure they are truly representative across typology gradients. In the UK, most effort should probably be focused on very shallow lake types (low, medium and high alkalinity) and medium and high alkalinity lakes in general (all depth types). Peaty lochs may also require targeted attention. Measurements of the mean depth of many lakes and the humic content of Scottish lochs is also needed for characterising lake types accurately and developing improved models for classification. Protocols for sample collection, storage and analysis are reported here, although further analysis of variability associated with sampling and analytical methods are required to more accurately estimate error and risk of misclassification

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1. Introduction

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 classification tools for determining the ecological status of surface waters. The Environment Agency and SNIFFER have commissioned this R & D project to develop a method to classify the ecological status of lakes on the basis of phytoplankton communities.

As part of this assessment, many European countries are choosing chlorophyll concentrations as a measure of phytoplankton abundance. Chlorophyll *a* is the primary light-harvesting pigment in photosynthesis and is present in all photosynthetic plants and algae including cyanobacteria (Kirk, 1994). The concentration of Chlorophyll *a* is widely used as a convenient surrogate for phytoplankton biomass and as a simple measure of water quality in response to eutrophication pressures (e.g. OECD 1982).

A large scale formal assessment of the comparability of assessment schemes across EC Member States is also being carried out as part of the implementation of the WFD - a process known as Intercalibration. Chlorophyll has been selected as a key measure for this Intercalibration process for lakes because of its recognition as a good general measure of ecological impact and because data are widely available across Europe. Member States need to provide lake-type specific chlorophyll concentrations for reference conditions and H/G and G/M boundary values for comparison in the Intercalibration process and provide a quantitative measure of the uncertainty of these values and the confidence in classification.

In addition to the requirement to establish a classification for chlorophyll concentrations, this project aims to refine relationships between chlorophyll and key supporting variables, specifically to derive supporting phosphorus standards.

1.1 Project Objectives

Specific objectives for the project were to develop a robust chlorophyll classification, incorporating:

1. Predictions of the expected (reference condition) chlorophyll concentrations for UK lake types, or site-specific reference conditions.
2. Developing ecological criteria for defining the good/moderate chlorophyll boundary
3. Classifying the ecological status of a water body into 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 chlorophyll concentrations for a site.
4. Defining supporting phosphorus concentrations for reference conditions and the high/good and good/moderate boundaries for UK lake types (or site-specific values).
5. Determining uncertainty associated with the classification result, based on statistical confidence or probability of class

1.2 Lake types

Lakes types were defined using “system A” descriptors, altitude, depth, surface area and geology, as outlined in the WFD, with boundaries between types identified largely following UKTAG (2004) and Intercalibration guidance (Van de Bund et al., 2004). Alkalinity and colour (or humic type) were used as proxy descriptors for geology as they were felt to differentiate more specific, ecologically-relevant types, than broad geological types outlined in system A. The boundaries between the 12 core lake types used in this study are indicated in Table 1.1 although these types could be further sub-divided by altitude, area or specific geologies (e.g. marl lakes in limestone catchments) when ecologically-relevant.

Table 1.1 Core lake types used in the phytoplankton classification project

Lake Type Code	Description	Broad Geology	Alkalinity (m equiv. l^{-1})	Mean depth (m)
LA_VSh	Low alkalinity, very shallow	Siliceous	<0.2	<3
LA_Sh	Low alkalinity, shallow	Siliceous		3-15
LA_D	Low alkalinity, deep	Siliceous		>15
MA_VSh	Medium alkalinity, very shallow	Siliceous	0.2 - 1.0	<3
MA_Sh	Medium alkalinity, shallow	Siliceous		3-15
MA_D	Medium alkalinity, deep	Siliceous		>15
HA_VSh	High alkalinity, very shallow	Calcareous	>1.0	<3
HA_Sh	High alkalinity, shallow	Calcareous		3-15
HA_D	High alkalinity, deep	Calcareous		>15

As part of the WFD Common Implementation Strategy, Geographical Intercalibration Groups (GIGs) have been created to develop consistent approaches to classification and boundary-setting (see Van de Bund et al, 2004). There are five lake GIG regions (Northern, Central-Baltic, Atlantic, Alpine, and Mediterranean). UK lakes fall within the former three GIGs. In some sections of the report, lake types used in the Intercalibration process within these three GIGs are referred to. The overlap between these GIG types and the core UK typology is detailed in Table 1.2. (see Van de Bund et al., 2004 for a more detailed description of GIG lake types). Note that there is overlap between the Northern GIG type L-N1 and the Central GIG type L-CB3 and the Atlantic GIG types L-A1 and L-A2 (now combined into a single type for Intercalibration, L-A1/2)and the Central GIG type L-CB1.

Table 1.2 GIG lake types and their overlap with UK core lake types

GIG Type	UK Core Type	Altitude (m a.s.l.)	Mean depth (m)	Humic content (mg Pt/l)	Alkalinity (meq/l)	Lake area (km^2)
L-N1	MA Sh	< 200	3-15	<30	0.2 - 1	> 0.5
L-N2a	LA Sh	< 200	3-15	<30	< 0.2	> 0.5
L-N2b	LA D	< 200	>15	<30	< 0.2	> 0.5
L-N3a		< 200	3-15	30-90	< 0.2	> 0.5
L-N3b		< 200	3-15	>90	< 0.2	> 0.5
L-N5		200-800	3-15	<30	< 0.2	> 0.5
L-N6a		200-800	3-15	30-90	< 0.2	> 0.5
L-N6b		200-800	3-15	>90	< 0.2	> 0.5
L-N7		>800	3-15	<30	< 0.2	> 0.5
L-N8a		<200	3-15	<30	0.2 - 1	Unspecified
L-A1	HA Sh	<200	3-15	30-90	>1	<0.5
L-A2	HA Sh	<200	3-15	<30	>1	>0.5
L-A3		<200	3-15	30-90	Unspecified	<0.5
L-CB1	HA Sh	<200	3-15	<30	>1	Unspecified
L-CB2	HA VSh	<200	<3	<30	>1	Unspecified
L-CB3	MA Sh	<200	<15	<30	0.2 - 1	Unspecified

2. Chlorophyll reference conditions and the high/good boundary

Laurence Carvalho, Geoff Phillips and Stephen Maberly

2.1 Introduction

The estimation of reference conditions is crucial in any ecological assessment programme (e.g. Moss et al., 1996; US EPA 2000). These provide the baseline from which to determine change with time, and are necessary to evaluate a site's current status or potential for change. The WFD acknowledges this, prescribing the assessment of ecological quality of surface waters using an Ecological Quality Ratio (EQR). The EQR is defined as the relationship between the current observed value and the reference condition value for a given ecological quality element, such as chlorophyll or phosphorus concentrations.

Reference conditions for quality elements are expected to change across Europe resulting from geographical differences of catchments (geology and altitude), and individual lake 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 derive type-specific reference conditions for the appropriate ecological quality elements. As part of the chlorophyll classification, it is, therefore, essential to determine chlorophyll reference conditions for all European lake types found within the UK, or alternatively, models for predicting reference chlorophyll concentrations on a site-specific basis.

What do reference conditions represent?

Reference conditions are a state corresponding to very low pressure, without the effects of major industrialisation, urbanisation and intensification of agriculture. The EC REFCOND Project, which developed guidance on reference conditions, defines them as a state with "no, or only very minor, evidence of disturbance of physico-chemical, hydro-morphological and biological quality elements" (Anonymous 2003). There is, however, still wide debate amongst scientific and political communities what exactly represents minor disturbance. Some papers have suggested pragmatic approaches for setting reference conditions based on sites with very little intensive agriculture and urbanisation (Johnes, Moss and Phillips, 1996, although opinions vary with some authors interpreting a more uncompromising view of a more or less pristine, undisturbed systems (e.g. Moss, 2006). The definition of 'minor' change is clearly subjective and, therefore, needs to be compared with major change to at least establish some sense of what minor is in relation to.

A number of approaches can be used to establish reference conditions (Hughes, 1995; Reynoldson et al., 1997, Nielsen et al., 2003) and these have been broadly summarised in the published guidance for the WFD (Anonymous, 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 EC guidance (Anonymous, 2003) suggests that the decision of which method/approach to take for the determination of reference conditions is dependent on the condition of the sites available for a certain lake type: a) where undisturbed or nearly undisturbed conditions prevail, a validated spatial network is preferred; b) if degraded conditions prevail then a modelling approach is preferred; c) expert judgement should be used as the last resort and be accompanied by an acceptable validation process.

Historical data prior to catchment disturbance and palaeolimnology (transfer functions) are generally unavailable for establishing reference chlorophyll concentrations, so the approach in this project is based on 1 and 2, both of which require a validated set of reference lakes.

For this study, we collated data from UK reference lakes and have also used data from >500 European reference lakes collected as part of Intercalibration and the EC Rebecca Project. This large European dataset provides sufficient coverage to estimate type-specific reference conditions (Approach 1). Furthermore, we explore the relationships between chlorophyll concentrations and potential predictor variables (e.g. alkalinity, depth, altitude, area) in order to develop empirical regression models for predicting reference chlorophyll concentrations in lakes on a site-specific basis (Approach 2).

2.2 Methods

2.2.1 Criteria for reference site selection

In order to guarantee a common understanding of a reference site, a common view of what is accepted as minor degree of change in anthropogenic pressure was necessary. As part of the WFD Common Implementation Strategy, Geographical Intercalibration Groups (GIGs) have been created (see Van de Bund et al, 2004). There are five lake GIG regions (Northern, Central-Baltic, Atlantic, Alpine, and Mediterranean). Each GIG has developed a list of criteria for the selection of reference sites, using a range of pressure criteria such as % low intensity land use, absence of major point sources in catchment and low population density (Table 2.1). Despite some differences in specific values for each pressure criterion between GIGs, and even individual countries, all follow the REFCOND guidelines in general, that very little industrialization, intensive urbanization or agriculture should be present in the catchment (Anonymous, 2003). Some Member States (including UK & Ireland) have additionally used palaeolimnology to validate choice of reference sites – 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). Many countries additionally used expert judgement in the review of final site lists.

Some European countries selected sites that locally may be considered in very good condition biologically, but had high nutrient concentrations compared with other countries with lakes of a similar type. For this reason a threshold mean TP concentration of 100 μg l^{-1} was used as a final criterion, above which sites were removed from this analysis. This resulted in 5 sites, all actually having TP concentrations >150μg l^{-1}, being excluded out of a total of 545 sites (i.e. <1%). The TP concentrations in the remaining dataset of 540 reference lakes were all lower than 70 μg l^{-1}, with only three sites having concentrations >50 μg l^{-1}.

Table 2.1 Pressure criteria used to validate reference site selection

GIG	Pressure criteria
Alpine	• Insignificant contribution of anthropogenic to total nutrient loading, validated by nutrient loading calculations
Atlantic	• Absence of major modification to catchment e.g. intensive afforestation • No discharges present that would impair ecological quality. • Abstraction at level that would not interfere with ecological quality • Water level fluctuation: within natural range. • Absence of shoreline alteration e.g. roads and harbours • Groundwater connectivity within natural range. • No impairment by invasive plant or animal species • Stocking of non- indigenous fish not significantly affecting the structure and functioning of the ecosystem. • No impact from fish farming. • No intensive use for recreation purposes
Central-Baltic	• 90% of catchment land-use natural (or semi-natural) • Population density <10 km^{-2} • no point sources in the catchment
Mediterranean	• 70% of the catchment area classified as “natural areas” (80 % in Portugal) • very low occurrence of anthropogenic pressure in the catchment area • Upstream accumulated demand of water for domestic use must be <3% of annual loading; <1.5% for industrial use; and <10% for agricultural irrigation • Low/moderate fishing and navigation pressures • low/moderate water level fluctuations
Nordic	• Agriculture: <5-20 % in catchment (<5 % Norway, <10 % Sweden and UK, 7-20% Finland) • Population density <5 p.e. km^{-2} (Norway), < 10 p.e km^{-2} (Sweden) or absence of major settlements in catchment • Absence of large industries in catchment • Absence of major point sources in catchment

2.2.2 UK data

No reference lakes have been formally identified in Northern Ireland. Of the 61 reference lake basins identified in Great Britain (GB), only 55 have chlorophyll data and many of these for only a single sampling occasion To minimise noise in the dataset, lakes were only included in the analysis if they had three or more samples from different months between the period April to September. If data from several years were provided for an individual lake, these growth season means were averaged over the years.

Only 23 GB reference lakes had sufficient chlorophyll data with at least 3 samples in the growing season (Appendix 1). Data from these reference sites were collated on chlorophyll and phosphorus concentrations, altitude, surface area, mean depth, alkalinity, humic type, and GIG region. Data were gathered from national datasets from EA, SEPA, CCW, CEH and the UK lakes database (see http://www.uklakes.net/).

2.2.3 European data

As with the UK data, European reference sites were only selected for analysis of chlorophyll reference conditions if at least 3 separate monthly records existed for a single growing season (April to September). Data from 540 reference lakes met this condition, with 483 (89%) of these sites coming from the Northern GIG region (Table 2.2). Figure 2.1 illustrates clearly that a large proportion of these lakes are of low alkalinity (70% <0.2 m.equiv l^{-1}) and relatively shallow (68% <15 m mean depth).

Inevitably with such a large dataset of lakes from many countries there are questions over the quality of the data. To minimise noise in the dataset, lakes were only included in the analysis if they had three or more samples from different months between the period April to September (a 'growing period' in all lakes in the dataset). If data from several years were provided for an individual lake, these growth season means were averaged over the years. If data from several sites within a lake were provided (particularly an issue with Finnish lakes), these site means were averaged to give a whole lake mean, to ensure no bias was given to any particular lake.

The high representation of Northern GIG sites compared with all other GIGs is probably a relatively true representation of the fact that this region is generally less impacted, with lower population densities, less industry and less intensive agriculture. The European dataset may also be biased by greater sampling of lakes occurring in Norway and Finalnd in particular.

European lake data were gathered from national datasets from individual Member States through partners in the EC REBECCA Project (see http://www.environment.fi/syke/rebecca) and from GIG coordinators.

Table 2.2 Number of reference lakes by country and GIG region

	GIG region					
Country	**Atlantic**	**Alpine**	**Central-Baltic**	**Mediterranean**	**Northern**	**Total**
Norway					252	252
Finland					174	174
Sweden					31	31
UK	1		1		21	23
Germany		11	3			14
Latvia			14			14
Ireland	6				5	11
Poland			7			7
Netherlands			5			5
Estonia			3			3
Lithuania			3			3
Denmark			2			2
Italy				1		1
Total	**7**	**11**	**38**	**1**	**483**	**540**

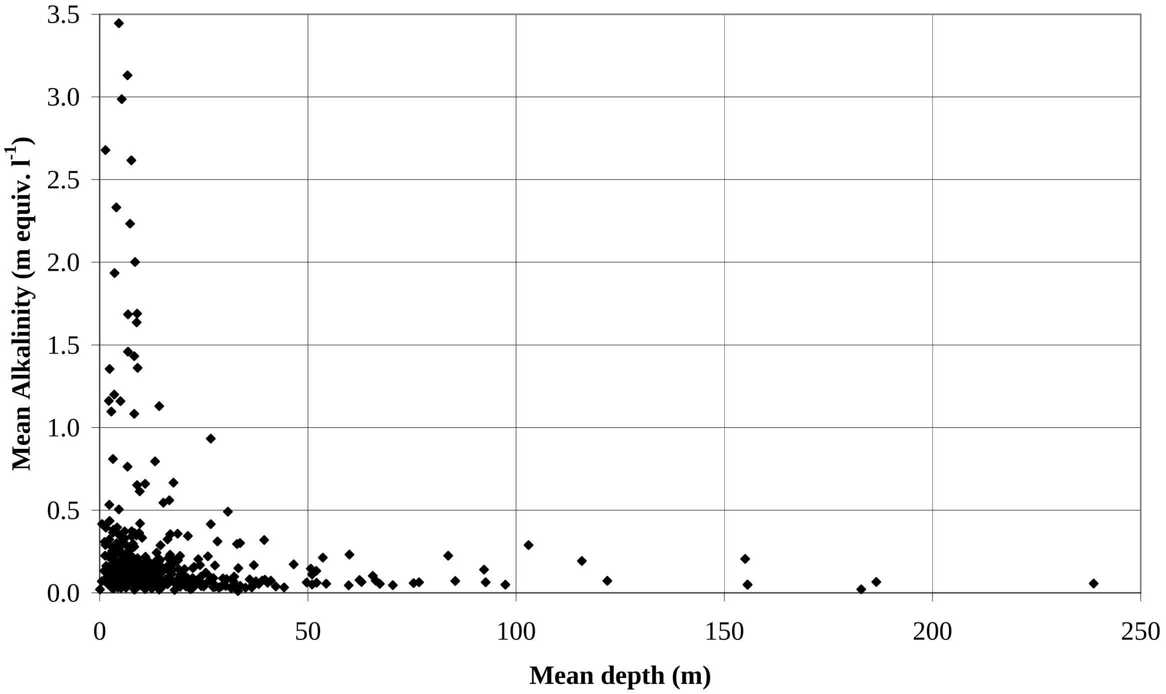


Figure 2.1 **Distribution of European reference lakes by mean depth and mean alkalinity**

2.2.4 Descriptive statistics by lake type

The first approach to deriving reference chlorophyll concentrations is to examine chlorophyll data from a population of reference lakes and compute a summary statistic to represent the type-specific reference value. REFCOND guidance highlights the median value or arithmetic mean as possible statistics (Anonymous, 2003). Means are influenced by extreme values and, therefore, median values are generally preferable. Median values were calculated for each GIG type and for core depth and alkalinity types (see Table 2.3). In addition to this, 75^{th} and 90^{th} percentiles of each type-specific population were calculated, as potential statistics for defining the High/Good boundary.

2.2.5 Correlation and regression models

Prior to model development, Pearson correlations were computed for each pair of variables to identify correlations between potential predictor variables. Stepwise regression was then carried out to determine which predictor variables (altitude, lake area, colour, mean depth and mean alkalinity) should remain in the final regression model, with consideration given to significant correlations between predictor variables. All parameters were $\log_{(10)}$ transformed to stabilise variance. All the statistical analyses were performed with the software Minitab (Release 14.1).

2.3. Results

2.3.1 Descriptive statistics

Only 23 UK reference lakes had sufficient chlorophyll data, with at least 3 samples in the growing season (Appendix 1). Of these 23 sites, only two of the nine core lake types had data from five or more sites (Table 2.3). Clearly insufficient data were available from UK sites for identifying reliable type-specific reference conditions and their associated variability within a type, as recommended by REFCOND guidance (Anonymous, 2003).

Table 2.3 Number of UK reference lakes by core typology

	Alkalinity Type			
Depth Type	**Low**	**Medium**	**High**	**Total**
Very Shallow		2	2	4
Shallow	9	1		10
Deep	8	1		9
Total	17	4	2	23

Of the 540 European reference lakes with growth season chlorophyll data, 335 can be assigned to a specific GIG type. Of these, many GIG types have sufficient data (≥5 sites) for estimating median values and an estimate of the variability (75th and 90th percentiles) (Table 2.4)[1]. Boxplots for the chlorophyll values for Northern and Central-Baltic GIG types are shown in Figs 2.2 and 2.3. Results for the Mediterranean and Alpine GIGs are not presented as only limited data were included in the REBECCA database.

Table 2.4 Number of lakes (N) by GIG type and corresponding median, 75th and 90th percentile values for chlorophyll-a (Apr-Sep mean)

GIG Region	IC Type	N	Median	75th %	90th %
Atlantic	L-A1	1	3.1		
	L-A2	4	3.3	4.3	
	L-A3	0			
Central-Baltic	L-CB1	20	2.8	4.7	6.8
	L-CB2	5	6.9	9.0	10.4
	L-CB3	12	4.8	6.3	11.8
Northern	L-N1	22	2.9	4.5	5.6
	L-N2a	61	2.3	3.1	4.1
	L-N2b	74	2.0	2.6	4.0
	L-N3a	48	4.1	6.3	8.6
	L-N3b	16	13.8	17.9	20.9
	L-N5	40	1.6	2.2	2.6
	L-N6a	8	3.3	3.8	10.2
	L-N6b	1	1.5		
	L-N7	2	0.5		
	L-N8a	9	7.0	10.0	22.6

[1] Values estimated in this report differ from those reported in GIG reports due to differences in REBECCA and GIG datasets and also differences in the approach used to summarise data (above analysis averaging data across years for the same lake

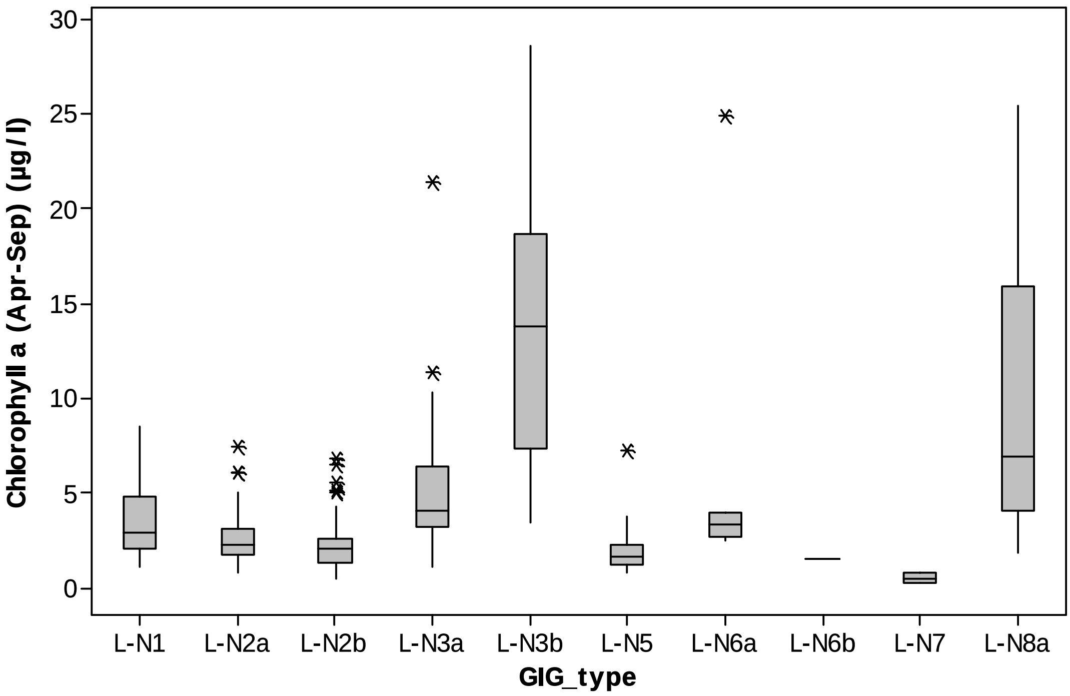


Figure 2.2 Boxplots of chlorophyll-a concentrations by Northern GIG types
The mid-line of the box indicates the median value and the top and bottom of the box indicate the 75th and 25th percentile respectively

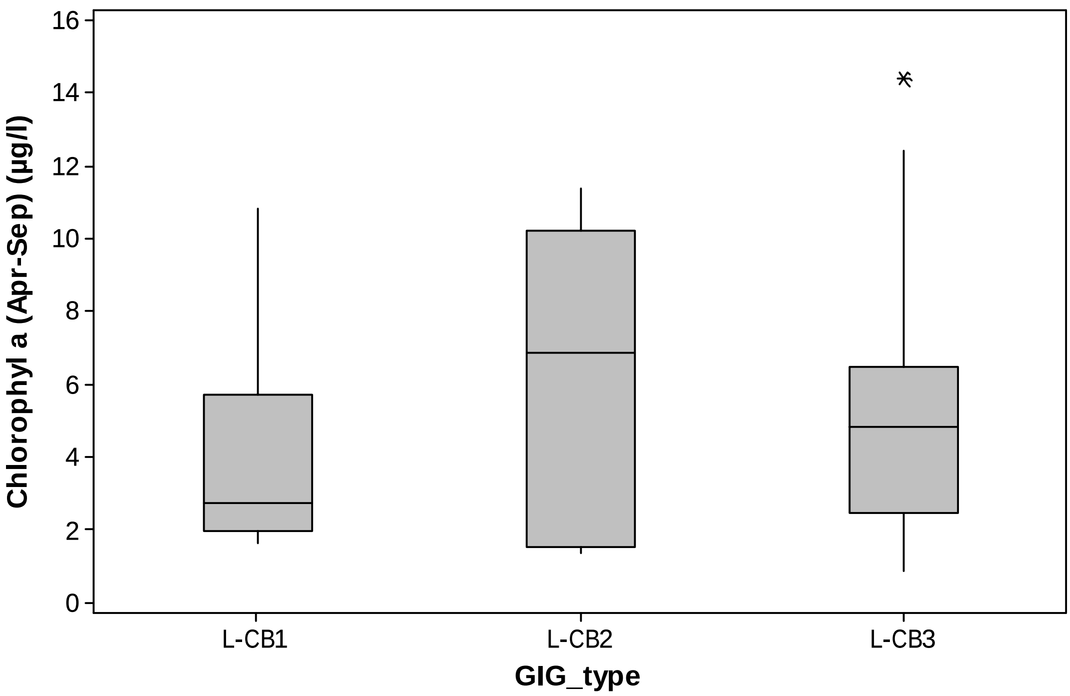


Figure 2.3 Boxplots of chlorophyll-a concentrations by Central-Baltic GIG types
The mid-line of the box indicates the median value and the top and bottom of the box indicate the 75th and 25th percentile respectively

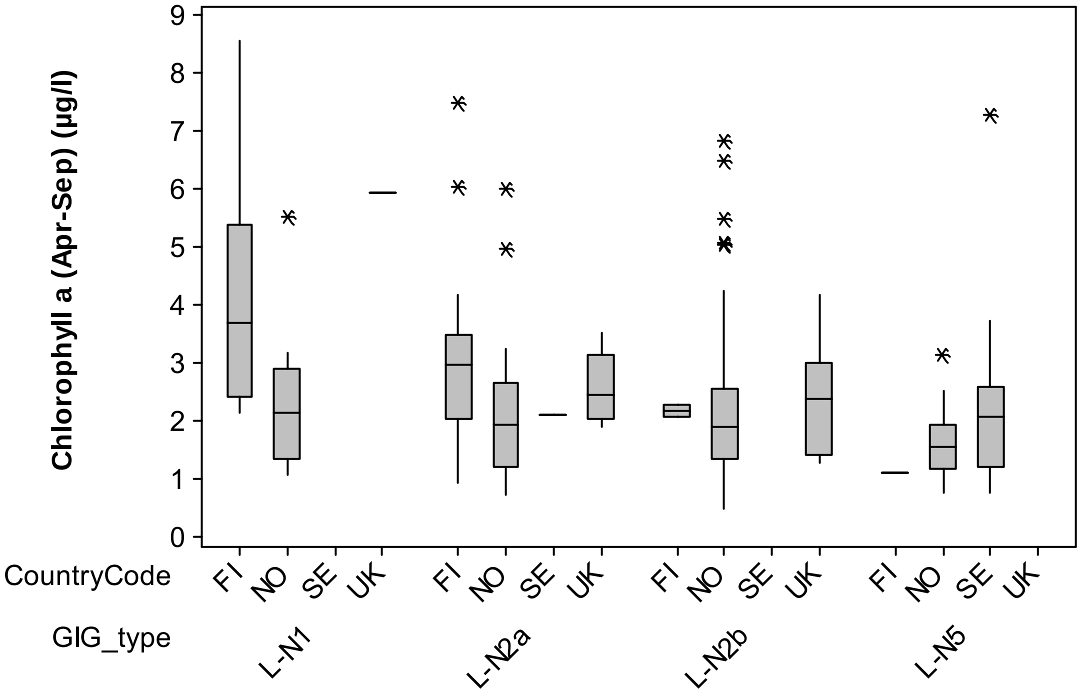


Figure 2.4a Boxplots of chlorophyll-a concentrations by non-humic Northern GIG lake types and by country

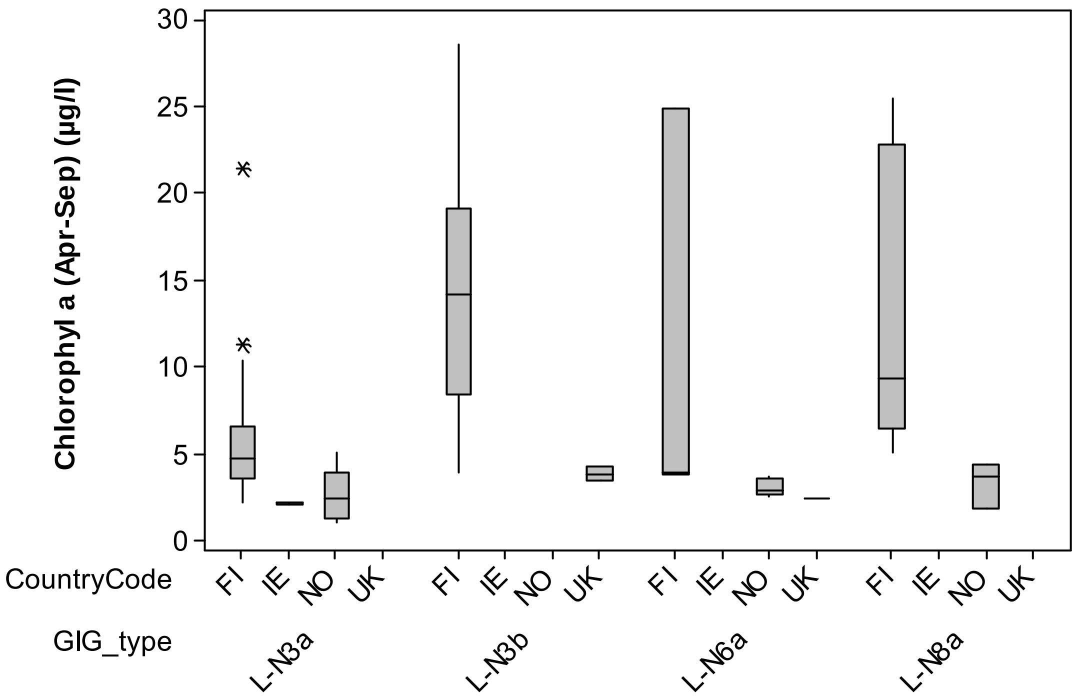


Figure 2.4b Boxplots of chlorophyll-a concentrations by humic Northern GIG lake types and by country

The mid-line of the boxes indicate the median value and the top and bottom of the boxes indicate the 75th and 25th percentile respectively

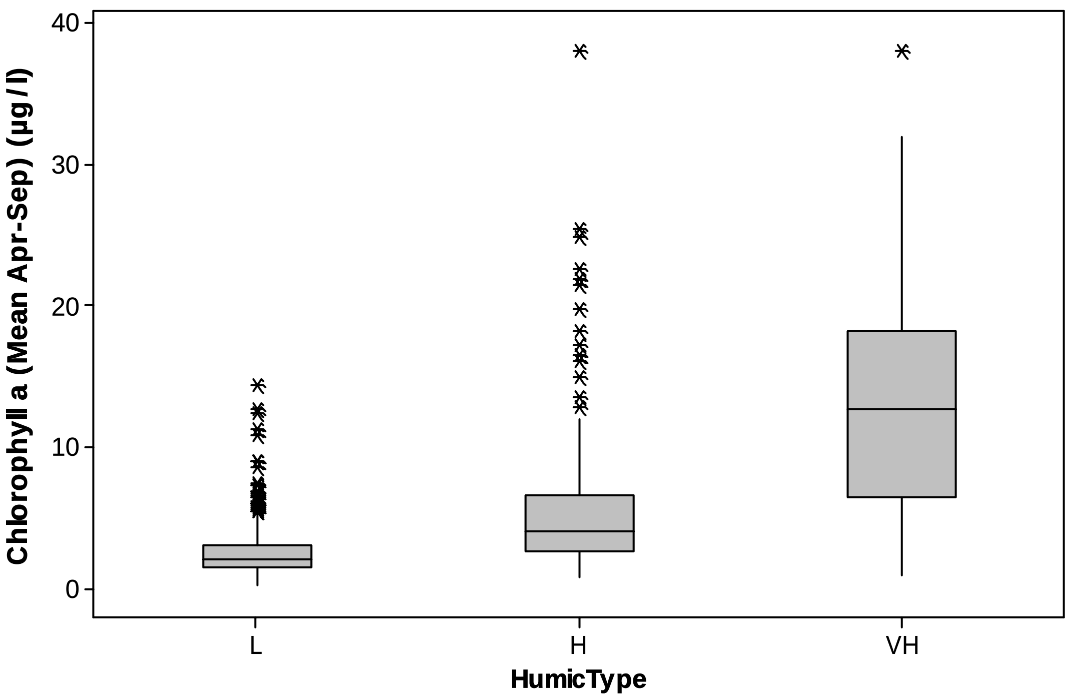
For most lake types in the Northern GIG, there is a clear difference in the median values of humic and non-humic lake types, with the former all >3 µg l^{-1} (particularly L-N3b and L-N8a) and the latter all <3 µg l^{-1}, the only exception being the humic lake type, L-N6b, for which data were available from only 1 reference lake. Of the non-humic lakes, highest chlorophyll concentrations were recorded in moderate alkalinity lakes (L-N1), compared with the other low alkalinity lake types

Median values for Central-Baltic GIG lake types were generally higher than those of Northern GIG lake types, with highest concentrations recorded for the very shallow lake type, L-CB2. Data were too limited from other GIGs (except Alpine L-AL3) to have any great confidence in reference values

A breakdown of the Northern GIG lake types by country is illustrated for non-humic and humic lake types in Figs 2.4a and 2.4b, respectively. This reveals that there is reasonable consistency with values from the UK and other Northern GIG countries. The exceptions to this are the Finnish data from humic lake types (Fig. 2.4b), which show much higher median values and greater variability than other Northern GIG countries.

Instead of specific GIG types, an analysis of reference lakes classified into humic types is shown in Fig. 2.5 These boxplots highlight increasing chlorophyll concentrations associated with lake types of increasing water colour (low, high and very high humic content).

Figure 2.5 Boxplots of chlorophyll-a concentrations by humic types
The mid-line of the box indicates the median value and the top and bottom of the box indicate the 75th and 25th percentile respectively.



A similar analysis, but with reference lakes classified into humic, depth and alkalinity types is shown in Table 2.5. This analysis indicates increasing chlorophyll concentrations associated with decreasing lake depth for all humic types, but a less consistent relationship with alkalinity. Boxplots of low-humic lakes only also illustrate how variability in chlorophyll reference conditions increases with decreasing lake depth (Figure 2.6).

Table 2.5 Number of lakes (N) by humic, depth and alkalinity type and corresponding median, 75th and 90th percentile values for chlorophyll-a (Apr-Sep mean)

Humic type	Depth type	Alkalinity type	N	Median	75th %	90th %
Low	Deep	Low	100	1.8	2.4	3.8
		Medium	23	1.5	2.3	3.6
		High	0			
	Shallow	Low	105	2.0	2.9	3.7
		Medium	39	2.8	4.4	5.5
		High	22	2.6	4.7	6.1
	Very Shallow	Low	1	2.0		
		Medium	3	6.8		
		High	9	6.0	9.0	9.5
High	Deep	Low	20	2.8	3.7	6.7
		Medium	5	2.8	4.3	
		High	0			
	Shallow	Low	71	3.8	5.5	8.7
		Medium	21	4.6	6.6	10.0
		High	2	4.2		
	Very Shallow	Low	19	7.3	11.7	16.2
		Medium	7	11.9	18.5	27.1
		High	1	4.6		
Very High	Deep	Low	0			
		Medium	0			
		High	0			
	Shallow	Low	16	12.4	16.9	19.2
		Medium	2	9.0		
		High	0			
	Very Shallow	Low	9	18.0	21.0	31.2
		Medium	3	6.2		
		High	1	1.4		

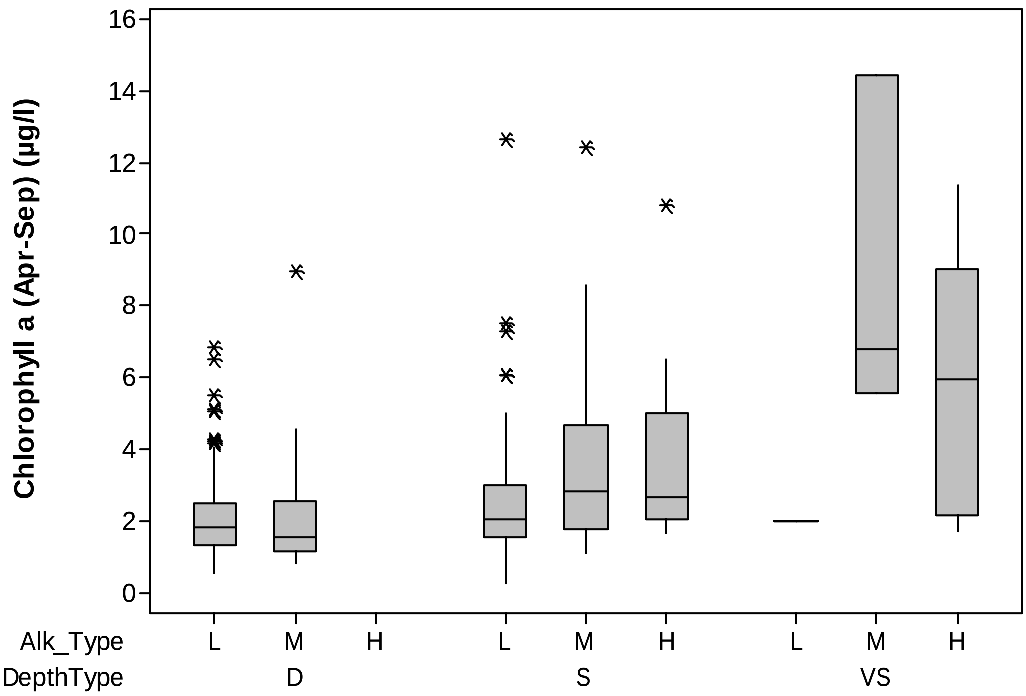


Figure 2.6 Boxplots of chl$_a$ concentrations for low-humic lakes by depth and alkalinity types

The mid-line of the box indicates the median value and the top and bottom of the box indicate the 75th and 25th percentile respectively.

2.3.2 UK model

The relationships between chlorophyll and potential typology predictor variables for the 23 UK reference lakes are shown in Fig. 2.7. Correlation analysis indicated that mean depth, alkalinity and lake surface area were all significantly related to chlorophyll concentrations and, therefore, potentially good predictor variables (Table 2.6). These three typology variables were, however, highly significantly correlated with each other (Table 2.6). Altitude and colour were not significantly related to chlorophyll concentrations, or to any of the typology variables. This was not surprising given the fact that gradients in these two variables were limited, with most of the 23 reference lakes situated at low altitude (<200m) and of relatively low colour (most <40 Pt l^{-1}).

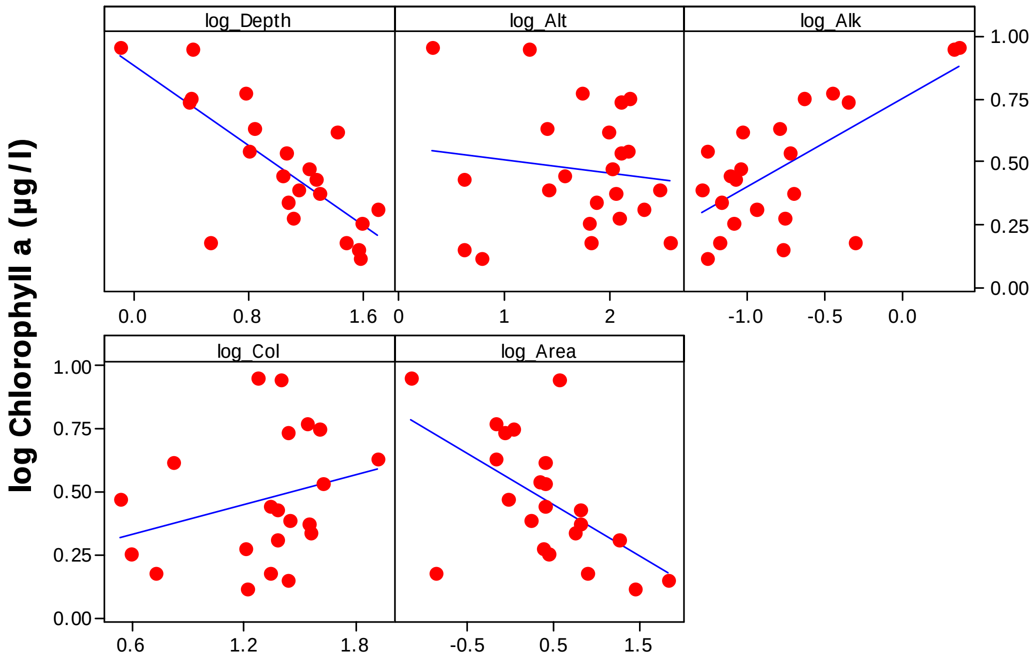


Figure 2.7 Scatterplots of chl_a vs potential predictor variables

Table 2.6 Results of Pearson correlation analysis between log(chl_a) and predictor variables for 23 UK reference lakes

		log_Chl	log_Depth	log_Alk	log_Area	log_Col
log_Depth	r	-0.728				
	p	<0.001				
log_Alk	r	0.651	-0.574			
	p	0.001	0.004			
log_Area	r	-0.554	0.771	-0.285		
	p	0.006	0.000	0.187		
log_Col	r	0.258	-0.247	0.269	0.152	
	p	0.247	0.269	0.225	0.499	
log_Alt	r	-0.130	-0.015	-0.266	-0.170	-0.125
	p	0.554	0.945	0.221	0.437	0.579

Initially all typology variables were included in the stepwise regression. This highlighted log(depth) and log(alkalinity) as the only variables to incorporate in a predictive model for log(chlorophyll). The fact that log(area) was significantly correlated with these two typology variables was considered, but it was felt, chlorophyll concentrations were more likely to be a direct response to depth and alkalinity than lake area, so no manual selection of area over either depth or alkalinity was considered appropriate.

As colour was not significant, it was removed from the stepwise regression procedure and the analysis was re-run to include an additional site (Loch Meadie) which had no measured or predicted colour data. This produced a similar model (Table 2.7), but with slightly better predictive strength (r^2_{adj} = 0.57, r^2_{pred} = 0.44).

Table 2.7 Regression model coefficients for predicting $\log_{10}$ chl_a reference conditions in UK lakes

Standard errors for coefficients, t-statistics and significance level are also shown.

Predictor	Coef	SE Coef	T	P
Constant	0.894	0.083	10.82	<0.001
$\log_{10}$ Depth	-0.273	0.088	-3.11	0.006
$\log_{10}$ Alkalinity	0.186	0.091	2.04	0.054

The two sites with the highest altitude (Llyn Idwal and Lochindorb) were identified as outliers in the regression model. The regression analysis was, therefore, re-run excluding these two sites. This produced a simpler model with log(depth) as the only variable to incorporate in a predictive model (Table 2.8). This model had a much improved predictive strength (r^2_{adj} = 0.76, r^2_{pred} = 0.72) than the previous model incorporating the two mid-altitude sites.

Table 2.8 Regression model coefficients for predicting $\log_{10}$ chl_a reference conditions in low altitude UK lakes

Predictor	Coef	SE Coef	T	P
Constant	0.969	0.066	14.68	<0.001
$\log_{10}$ Mean depth	-0.455	0.057	-7.94	<0.001

Using the former regression model (Table 2.7), reference chlorophyll concentrations can be directly estimated for other GB lakes with known mean depth and alkalinity. Applying the coefficients in Table 2.7) to idealised lakes with minimum, maxiumum and mid-point mean depth and alkalinity values illustrates the range of site-specific reference conditions (Table 2.9). The modelled chlorophyll reference conditions in response to depth and alkalinity are illustrated in Figure 2.8.

Table 2.9 Minimum, mid- and maximum potential chlorophyll reference conditions for UK lakes classified by type

Min'm and max'm depths used were 0.3 and 132 m respectively. Mid-point for deep lake depths was 27 m. Mid-point and max'm alkalinity for high alkalinity lakes was 2.24 and 4.26 m equiv./l respectively.

	LA_D	LA_S	LA_VSh	MA_D	MA_S	MA_VSh	HA_D	HA_S	HA_VSh
Min'm possible	0.6	1.0	1.6	1.5	2.8	4.3	2.1	3.7	5.8
Mid-typology	2.1	2.8	4.6	2.9	3.9	6.4	3.7	5.0	8.1
Max'm possible	2.8	4.3	8.1	3.7	5.8	10.9	4.9	7.6	14.3

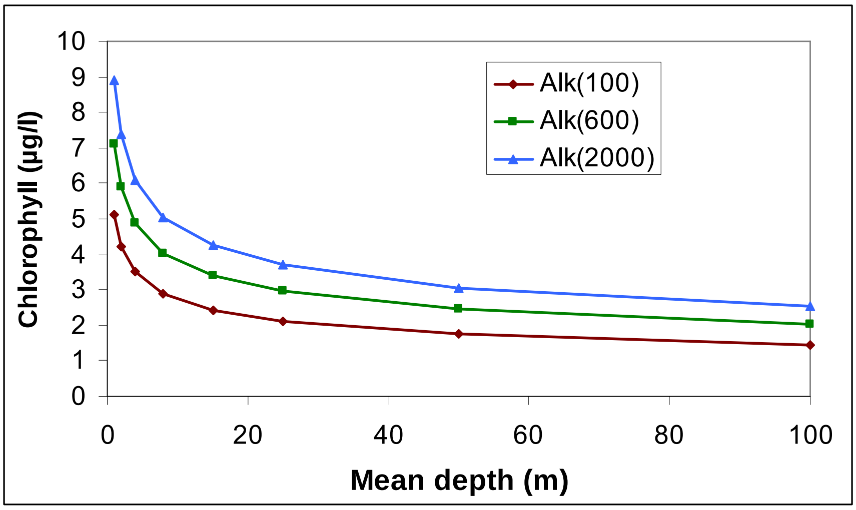


Figure 2.8 Modelled chlorophyll reference conditions in response to depth for 3 levels of alkalinity (100, 600 and 2000 µequiv. l^{-1})

2.3.3 European models

The relationships between chlorophyll and potential typology predictor variables for the 540 European reference lakes are shown in Fig. 2.9. Correlation analysis indicated that colour, mean depth and alkalinity were likely to be the key potential predictor variables, with altitude almost significant (Table 2.10). As lake area showed little correlation with chlorophyll-a, but was significantly correlated with mean depth and alkalinity, it was removed from regression model development. Despite colour being the most strongly correlated variable with chlorophyll concentrations, measured colour data were only available for about half the sites, although almost all sites were placed into a humic type. For this reason regression analysis was carried out separately, without colour as a predictor variable, for two datasets: 1) low-humic and 2) high-humic lakes.

Figure 2.9 Scatterplots of chl_a vs potential predictor variables in 540 European reference lakes

Chlorophyll, altitude (Alt), area, depth, alkalinity (Alk) and colour (Col) were all log transformed.

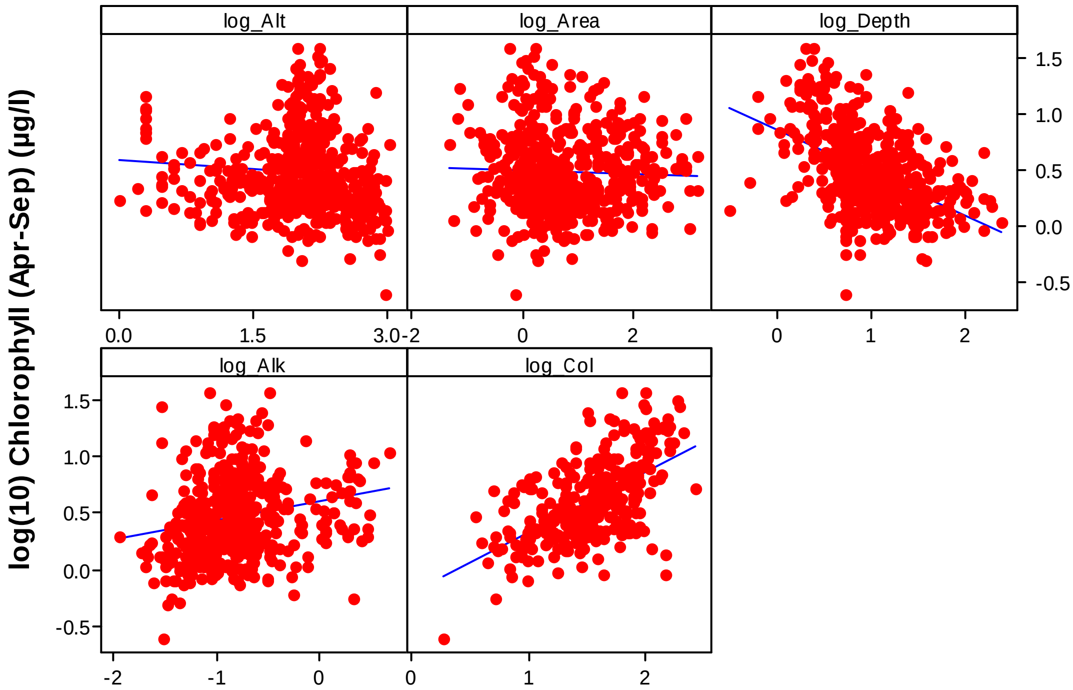


Table 2.10 Results of Pearson correlation analysis between $log_{10}Chl_a$ and predictor variables in 540 European reference lakes

		log_Chl	log_Depth	log_Alk	log_Area	log_Col
log_Depth	r	-0.497				
	p	<0.001				
log_Alk	r	0.212	-0.313			
	p	<0.001	<0.001			
log_Area	r	-0.040	0.355	-0.095		
	p	0.364	<0.001	0.037		
log_Col	r	0.587	-0.447	-0.265	0.047	
	p	<0.001	<0.001	<0.001	0.419	
log_Alt	r	-0.075	0.061	-0.171	0.089	0.054
	p	0.083	0.177	<0.001	0.042	0.351

Low Humic lakes:

279 low-humic reference lakes were used in model development. This produced a model (r^2_{adj} = 0.27, r^2_{pred} = 0.26) with depth, alkalinity and altitude all identified as highly significant predictor variables (Table 2.11).

Table 2.11 Regression model coefficients for predicting $\log_{10}$ chl$_a$ reference conditions in non-humic European lakes

Standard errors for coefficients, t-statistics and significance levels are also shown

Predictor	Coef	SE Coeff	T	P
Constant	0.851	0.056	15.06	<0.001
log_Depth	-0.164	0.036	-4.5	<0.001
log_Alt	-0.110	0.022	-4.92	<0.001
log_Alk	0.130	0.033	3.93	<0.001

.

High Humic lakes:

Initial regression analysis included 26 polyhumic lakes (>90 Pt/l). Polyhumic lakes were, however, absent from the UK dataset and were largely confined to Finland. As they weakened the predictive power of the model greatly, they were removed from the analysis, leaving 135 reference lakes with a high humic content (30-90 Pt/l) used in model development. This produced a model (r^2_{adj} = 0.30, r^2_{pred} = 0.27) with depth and alkalinity as highly significant predictor variables (Table 2.12). This model differs from the low-humic lake model by the absence of altitude as a predictor variable, but also the remaining coefficients are clearly significantly different between the two models (>2 standard errors).

Table 2.12 Regression model results for chl$_a$ response in humic European reference lakes

Predictor	Coef	SE Coeff	T	P
Constant	1.188	0.078	15.23	<0.001
log_Depth	-0.326	0.061	-5.34	<0.001
log_Alk	0.325	0.078	4.19	<0.001

2.4 Discussion

2.4.1 Descriptive Statistics

Because of the limited number of UK reference lakes with reliable chlorophyll data, the "spatial network", or 'population of reference lakes' approach was only possible through the amalgamation with reference lake datasets from other European countries. This was made possible by the development of large lake databases through the EC Rebecca Project and the WFD Intercalibration process. UK lake types were generally well represented in these datasets with particularly good coverage of low and medium alkalinity lakes in the Northern and Central-Baltic GIGs, ensuring reasonable confidence in the results.

For the Northern GIG, there were sufficient data to compare reference conditions within a GIG type by country. Where these comparisons were possible, chlorophyll concentrations in UK reference lakes were comparable with other Northern European countries. One country-specific difference was apparent, median values for Finnish very high humic lakes were consistently higher than those from other countries, although data on these lake types were limited from countries other than Finland. One explanation for this is more stringent criteria for selection of reference lakes in Ireland, Norway and the UK. A second explanation is that for some reason Finland has naturally more fertile waters within these lake types.

As there are possible issues over the selection of reference sites by all Member States, it is recommended that the 75% would be a more appropriate value to use for the H/G boundary compared with the 90%, the latter being more greatly affected by outliers.

The population approach cannot currently be used reliably for all UK/European lake types, where too few existing reference lakes are available for estimating median values and variability in chlorophyll reference conditions (e.g. Atlantic GIG lake types). In particular there appear to be very few, very shallow, low, medium and high alkalinity reference lakes in Europe, despite these being relatively common lake types in the UK. A recent analysis estimated that there are >3200 high alkalinity, >900 medium alkalinity and >500 low alkalinity very shallow lakes in the UK. Of these 145, 53 and 98, respectively, are estimated to be at high status (Carvalho et al., 2005). Even if these figures are not wholly reliable, they indicate that reference sites are needed for these lake types and probably sufficient numbers exist in the UK for targeted monitoring. Deep, high alkalinity lakes were also absent from the European reference lake dataset, although this may be largely because it is a very rare lake type.

Table 2.13 indicates lake types with currently insufficient data for estimating variability in reference values. Focused assessments of lakes (and their catchments) that typify these under-represented lake types could help identify sufficient numbers of acceptable reference sites for future data collection. In the UK, most effort should probably be focused on very shallow lake types (low, medium and high alkalinity) and possibly deep, high alkalinity low-humic lakes. Some evidence from reference lakes in Ireland also suggests that marl lakes, high alkalinity sites in limestone catchments, may also require specific targets because of the effect of calcium carbonate on the availability of phosphorus.

Improved monitoring of the colour of peaty lakes may also reveal additional requirements for coverage of high and very high-humic lake types in the UK, although these are likely to be of low alkalinity. Peaty, machair lochs may be an exception to this and will probably need specific targets.

Table 2.13 Numbers of reference lakes (N) by humic, depth and alkalinity type

Unshaded rows are lake types with insufficient data for estimating reference values

Humic type	Depth type	Alkalinity type	N
Low	Deep	Low	100
		Medium	23
		High	0
	Shallow	Low	105
		Medium	39
		High	22
	Very Shallow	Low	1
		Medium	3
		High	9
High	Deep	Low	20
		Medium	5
		High	0
	Shallow	Low	71
		Medium	21
		High	2
	Very Shallow	Low	19
		Medium	7
		High	1
Very High	Deep	Low	0
		Medium	0
		High	0
	Shallow	Low	16
		Medium	2
		High	0
	Very Shallow	Low	9
		Medium	3
		High	1

What the descriptive statistics do show are that chlorophyll reference conditions appear to increase with increasing humic content and decreasing depth in particular. For these reasons, the very shallow lake type L-CB2 and humic lake types L-N3 and L-N8 had the highest median chlorophyll values. Further discussion of the reasons for these relationships is covered in the later discussion on predictive models.

As chlorophyll concentrations in reference lakes appear to show gradients in response to the typology factors, a site-specific modelling approach may be more suitable for setting reference conditions. This is particularly true for lakes falling near a type boundary, for which reference chlorophyll concentrations based on the median of either type would be inappropriate. Any large error in reference condition will result in a large error in the EQR and low confidence in classification. For these reasons, it is highly recommended that site-specific regression models are used to derive site-specific chlorophyll reference conditions. Another advantage for establishing predictive models is that many lakes do not fall within a particular lake type for which sufficient reference sites exist, so the development of regression models for setting reference conditions may be the only available approach.

2.4.2 UK chlorophyll model

As there are currently too few data from UK reference lakes to derive chlorophyll reference conditions using a type-specific population approach, it was necessary to consider a regression modelling approach that used data from all UK lake types. The development of comparable regression models for setting reference conditions is well established for phosphorus, with the Morphoedaphic Index model. This is a simple model that uses mean depth and alkalinity data from a limited set of undisturbed lakes to predict reference TP concentrations (Vighi & Chiaudani, 1985). Despite the relative success of the MEI model, no similar models have been published for setting chlorophyll reference conditions.

Preliminary analysis in this project examined predictive models for chlorophyll based on annual mean measurements; models based on mean chlorophyll values over the phytoplankton 'growing season', defined in this project from April to September, have, however, been much more robust with higher predictive capacity. Chlorophyll concentrations over this period are also practically more relevant as phytoplankton abundance is frequently an issue of public interest over summer and also has greatest impact on macrophyte communities during the spring and summer periods too.

The regression analysis of UK lake data alone, identified both mean depth and alkalinity as potential predictor variables (r^2_{pred} = 0.44). There are acceptable ecological reasons for a causal relationship between these two predictor variables and chlorophyll concentrations. Increasing chlorophyll with decreasing mean depth is most readily explained in terms of greater light availability for phytoplankton in shallower waters. Even stratified lakes generally show increased mixing depths in their stratified period with increasing mean depth. The positive relationship with alkalinity can be explained by naturally higher nutrient concentrations in more alkaline lakes, as highlighted in the literature on the MEI model (Vighi & Chiaudani, 1985).

A simpler model using just mean depth had improved predictive strength (r^2_{pred} = 0.72). This model is, however, only applicable to lakes of low altitude (<210m) and of relatively low colour (most <40 Pt l^{-1}) and also suggests that all lakes of a given depth have similar chlorophyll reference conditions, despite differences in their alkalinity (or geology). It is well established, however, that lakes of higher alkalinity have naturally higher nutrient concentrations (Vighi & Chiaudani, 1985) and so it would be expected that reference chlorophyll concentrations would consequently be higher. In reference lakes, it may be possible that processes such as grazing or competition for nutrients with macrophytes buffer any phytoplankton response. In conclusion, however, the more complex model including both depth and alkalinity is considered more ecologically realistic and should be used in preference.

The limited data available for model development is likely to result in bias and large influence of individual outlier sites in the model. It is, therefore, recommended that the direct approach of modelling site-specific chlorophyll reference conditions is not applied until more representative data become available for further model development.

Two other options are possible for developing site-specific reference conditions:
1) Derive model from a larger dataset of European reference lakes, or;
2) Derive UK chlorophyll reference conditions from UK TP reference conditions using European TP-chlorophyll regression models (see Chapter 7 for discussion).

The first option is considered below.

2.4.3 European chlorophyll models

The larger European dataset allows much greater coverage of the gradients in all the typology variables compared with the UK reference lake dataset. The significantly different relationship between chlorophyll-a and predictor variables for low- and high- humic lakes highlighted the need to develop two separate models for these lake classes.

The explanation for why reference lakes had higher chlorophyll concentrations with increasing humic content is not obvious. Boreal lake surveys have generally shown that phytoplankton biomass is lowest in clear-water lakes (Arvola et al, 1999), but intuitively, chlorophyll concentrations would be expected to decrease with increasing colour, due to the decreasing light availability to phytoplankton. Photo-adaption of phytoplankton cells to produce more chlorophyll in darker waters is one possible explanation. Hutchinson (1957) and Wetzel (1983) also both refer to higher P concentrations in humic lakes than in comparable clear water lakes and this has been confirmed in more recent work by Jones (1990; 1992), although the latter work does suggest that these nutrients are not easily available to phytoplankton. Other possible explanations are that sampling is more restricted to the uppermost metre(s) of the water column in high humic lakes as phytoplankton often concentrate at the surface; areal biomass may actually be higher in low-humic lakes (Arvola et al., 1999). Another plausible explanation is that large mixotrophic phytoplankton that can utilise dissolved organic matter often dominate humic lakes, and these algae maintain high chlorophyll concentrations to exploit light resources too.

The low-humic lake model also incorporated altitude as a predictor variable, explaining additional independent variance from depth and alkalinity. A causal negative relationship between altitude and chlorophyll concentrations can be explained by lower temperatures at higher altitudes, higher flushing rates or even impacts of higher UV levels.

Although both low- and high- humic lake models were highly significant ($p<0.001$), overall the variance explained was not that high, with low predictive strength for both (r^2_{pred} = 0.26 and 0.27 for low- and high- humic lake models respectively). In relation to the UK, the regression models derived solely from UK reference lakes, had much greater predictive strength compared with the European models, and so may be more appropriate in a UK context. It should, however, be noted that all the regression models should only be applied to lakes whose typology factors fall within the range covered by the model. For this reason the European models, with their greater coverage of alkalinity, depth and altitude gradients, will be more applicable outside the range of the UK models.

2.5 Summary and Recommendations

Type-specific chlorophyll reference conditions have been defined for a number of lake types using a large European dataset of 540 reference lakes. Mean chlorophyll concentrations from the phytoplankton growing season (April to September) have been collated for all reference lakes. Using these data, median values and the 75th percentile for several lake types have been established.

Median values are recommended as a summary statistic for the reference condition and the 75% is recommended for the H/G boundary.

Regression models for predicting site–specific reference conditions from typology variables (colour, depth, alkalinity and altitude) have also been developed. Ideally, these models should be used in preference to type-specific reference conditions. However, it is recommended that the direct approach of modelling site-specific chlorophyll reference conditions is not applied until improved models become available.

Regression models derived solely from UK reference lakes, had much greater predictive strength compared with European models, and so may be more appropriate in a UK context, but they were based on a very limited dataset and it should be noted that the models should only be applied to lakes whose typology factors fall within the range covered by the model.

Currently there is great bias in the UK and European reference lake datasets towards shallow and deep low alkalinity lakes. The limited chlorophyll and phosphorus data available from some lake types bias the regression models and result in uncertain estimates of variability in reference values for a number of lake types. For this reason, more effort is needed to identify and sample sufficient numbers of acceptable reference sites of these under-represented lake types. In the UK, most effort should probably be focused on very shallow lake types (low, medium and high alkalinity) and medium and high alkalinity lakes in general (all depth types). Peaty lochs may also require targeted attention.

3. Phosphorus reference conditions and the high/good boundary

Laurence Carvalho & Geoff Phillips

3.1 Introduction

Phosphorus concentrations are widely recognised as a key limiting nutrient controlling primary production in lakes, particularly phytoplankton production (e.g. Schindler, 1977). For this reason, most river basin management activities aimed at reducing eutrophication pressures in lakes, target phosphorus concentrations.

Phosphorus classifications and quality standards, for lakes have been widely developed (e.g. OECD, 1982; Cardoso 2001). These schemes are, however, not WFD-compliant, in that they do not assess status in terms of deviation from a reference state. Although phosphorus is a supporting quality element in the WFD, Annex II of the directive outlines a requirement to establish type-specific reference conditions for supporting physicochemical (and hydromorphological) quality elements. The key ecological importance of phosphorus and its focus for catchment management, also mean that the UK has set a requirement for WFD-compliant environmental standards for phosphorus in freshwaters.

Previous research established provisional reference conditions and boundary values for total phosphorus for GB lake types (Carvalho et al., 2005). Five approaches were used to identify reference conditions, using approaches outlined in REFCOND guidance (Anonymous, 2003):

1) Survey data: lower 25th percentile of contemporary observations
2) Model-based prediction: Morphoedaphic index (MEI) (Vighi and Chiaudani, 1985)
3) Palaeolimnology: diatom-inferred TP (e.g. Bennion et al., 2004a)
4) Model-based prediction: PLUS export coefficient model (Scotland only) (Ferrier et al., 1996)
5) Model-based prediction: Land Use Regions (LUR) export coefficient model (England and Wales only) (Johnes et al., 1996)

The first approach is, however, generally not recommended as it assumes 25% of sites have had only minor influence from anthropogenic nutrient sources. In some cases this was clearly not true (e.g. very shallow, medium and high alkalinity lakes; Carvalho et al., 2005). A more suitable ‘population’ approach, recommended by REFCOND guidance, is to consider TP concentrations in a population of reference lakes only. This chapter examines this approach.

The second approach, the TP-MEI model, is widely being adopted across Europe and the USA for setting phosphorus reference conditions (e.g. US EPA, 2000). The TP-MEI model is simple, requiring only data on lake mean depth and alkalinity or conductivity. The MEI-TP model of Vighi & Chiaudani (1985) is, however, based on only 53 lakes, of which only 12 were in Europe, most were of low alkalinity and none were shallow (mean depth was 36.6m (±26.4 m)). For this model to be widely applied to UK lakes, a more robust model, or type-specific models, covering greater depth and alkalinity gradients are required.

The other approaches considered in Carvalho et al. (2005) are not so easily applied to large numbers of lakes, and so were considered unworkable for a national assessment scheme. The palaeolimnological approach is, however, an excellent tool for validating the choice of reference sites (Bennion et al., 2004b), needed for approaches 1) and 2) above.

This chapter examines setting TP reference conditions and H/G boundary values using two approaches:

1) Population' approach, using a large dataset of European reference lakes
2) Developing regression models to predict TP reference conditions using explanatory typology variables (alkalinity, depth, altitude, etc.). As for chlorophyll, models based on UK and European data are both examined.

Results from these analyses have been previously reported in Phillips (2005) and Lyche-Solheim (2005). For this reason only a brief summary is provided here.

3.2 Methods

3.2.1 UK data

The selection of reference sites was carried out as described for chlorophyll. No reference lakes have been formally identified in Northern Ireland. Of the 61 reference lake basins identified in Great Britain (GB), only 25 have reliable annual mean TP estimates based on four quarterly samples. If data from several years were provided for an individual lake, these annual means were averaged over the years.

Data from the reference sites were collated on phosphorus concentrations, altitude, surface area, mean depth, alkalinity, humic type, and GIG region. Data were gathered from national datasets from EA, SEPA, CCW, CEH and the UK lakes database (see http://www.uklakes.net/).

3.2.2 European data

The selection of reference sites was carried out as described for chlorophyll. European reference sites were only selected for analysis if at least 3 separate monthly records existed for a single growing season (April to September). If data from several years were provided for an individual lake, these growth season means were averaged over the years. If data from several sites within a lake were provided (particularly an issue with Finnish lakes), these site means were averaged to give a whole lake mean, to ensure no bias was given to any particular lake. Data from 567 reference lakes met these conditions, with a large proportion of these sites coming from the Northern GIG region and of low alkalinity.

European lake data were gathered from national datasets from individual Member States through partners in the EC REBECCA Project (see http://www.environment.fi/syke/rebecca) and from GIG coordinators.

3.2.3 Descriptive statistics

TP data was summarised from a population of European reference lakes and the median value within a lake type was adopted as the type-specific reference value, following REFCOND guidance (Anonymous, 2003). In addition to this, 75th and 90th percentiles of each type-specific population were calculated, as potential statistics for defining the High/Good boundary.

3.2.4 Correlation and regression models

Prior to model development, Pearson correlations were computed for each pair of variables to identify correlations between potential predictor variables. Stepwise regression was then carried out to determine which predictor variables (altitude, lake area, colour, mean depth and mean alkalinity) should remain in the final regression model, with consideration given to significant correlations between predictor variables. All parameters were $\log_{10}$ transformed to stabilise variance. The statistical analyses were performed with the software Minitab (Release 14.1) and SPSS.

The MEI was calculated using alkalinity and depth data according to the following equation:

MEI_{alk} = alkalinity (m.equiv. l^{-1}) / mean depth (m)

3.3 Results

3.3.1 Descriptive statistics

Table 3.1 Total phosphorus concentrations in European reference lakes
Descriptive statistics by Geographical Intercalibration Group (GIG) and lake type is reported. IC= intercalibration; 75perc= 75th percentile, N= Number of reference lakes.

GIG region	IC type	median	75perc	N	description
Atlantic	L-A1	4.0	12.2	3	Lowland, shallow, calcareous, small
	L-A2	8.0	11.5	9	Lowland, shallow, calcareous, large
	L-A3	9.0	12.0	10	Lowland, shallow, peat, small
	L-AX	9.0	12.0	4	Outside: Atlantic IC-types
Alpine	L-AL3	4.0	6.0	19	Lowland or mid-altitude, deep, moderate to high alkalinity. (alpine influence), large
	L-AL4	10.0	10.5	5	Mid-altitude, shallow, moderate to high alkalinity (alpine influence), large
	L-ALX	10.0	14.2	21	Outside: Alpine IC-types
Central Baltic	L-CB1	18.8	29.0	35	Lowland, shallow, stratified, calcareous
	L-CB2	17.8	30.6	12	Lowland, very shallow, calcareous
	L-CB3	15.8	21.0	16	Lowland,shallow , siliceous, vegetation dominated by Lobelia
Mediterranean	L-MX	16.6	17.4	3	Outside: Mediterranean IC-types
Northern	L-N1	9.1	13.5	17	Lowland, shallow, moderate alkalinity, clear large.
	L-N2a	6.7	8.8	51	Lowland, shallow, low alkalinity, clear large.
	L-N2b	5.6	7.5	48	Lowland, deep, low alkalinity, clear, large.
	L-N3	11.3	16.3	47	Lowland, shallow, low alkalinity, humic, large
	L-N5	6.3	7.3	25	Mid-altitude, shallow, low alkalinity,clear, large
	L-N6	9.1	11.5	17	Mid-altitude, shallow, low alkalinity, humic, large
	L-N8	12.7	16.7	9	Lowland, shallow, moderate alkalinity, humic large
	L-NX	8.8	15.5	183	Outside: Northern IC-types

3.3.2 UK Model

25 UK reference sites were used to derive a regression model for total phosphorus based on the morpho edaphic index:

Log TP = 1.284 (±0.109) + 0.27 (±0.053) log (MEI) $r^2 = 0.527$ $p<0.001$ n = 25

Where MEI is alkalinity (mEq/l) / mean depth (m)

3.3.3 European models

Models were developed using independent predictors (Table 3.2) and using MEI and altitude as predictors (Table 3.3)

Table 3.2 Equations predicting TP reference concentrations in European lakes using humic type, altitude, alkalinity and depth as independent predictors
Standard errors are in brackets. N= Northern; A= Atlantic; Al= Alpine; CB= Central- Baltic; alt= altitude; alk=alkalinity.

Humic type	GIG region	Equation
Humic	N	Log(TP)=1.58(0.06)-0.08(0.02)Log(alt)-0.13(.04)Log (depth)+0.24 (0.03)Log(alk)
Non humic	N	Log(TP)=1.35(0.01) -0.08(0.02)Log(alt)-0.13(.04)Log (depth)+0.24 (0.03) Log(alk)
Humic	A	Log(TP)=1.29(0.01) -0.08(0.02)Log(alt)-0.13(.04)Log (depth)+0.24 (0.03) Log(alk)
Non humic	A	Log(TP)=1.13(0.01) -0.08(0.02)Log(alt)-0.13(.04)Log (depth)+0.24 (0.03) Log(alk)
Non humic	AL	Log(TP)=1.03(0.01) -0.08(0.02)Log(alt)-0.13(.04)Log (depth)+0.24 (0.03) Log(alk)
Humic	CB	Log(TP)=1.65(0.01) -0.08(0.02)Log(alt)-0.13(.04)Log (depth)+0.24 (0.03) Log(alk)
Non humic	CB	Log(TP)=1.49(0.01) -0.08(0.02)Log(alt)-0.13(.04)Log (depth)+0.24 (0.03) Log(alk)

Table 3.3 Equations predicting TP reference concentrations in European lakes lakes using humic type, altitude and MEIalk as independent predictors
Standard errors are in brackets. N= Northern; A= Atlantic; Al= Alpine; CB= Central- Baltic.

Humic type	GIG region	Equation
Humic	A, N	Log (TP)=1.62 (0.12) -0.09 (0.02) Log (altitude)+ 0.24 (0.06) Log (MEIalk)
Non humic	A, N	Log (TP)=1.36 (0.03) -0.09 (0.02) Log (altitude)+ 0.24 (0.06) Log (MEIalk)
Non humic	AL	Log (TP)=0.93 (0.06) -0.09 (0.02) Log (altitude)+ 0.24 (0.06) Log (MEIalk)
Humic	CB	Log (TP)=1.81 (0.02) -0.09 (0.02) Log (altitude)+ 0.24 (0.06) Log (MEIalk)
Non humic	CB	Log (TP)=1.55 (0.04) -0.09 (0.02) Log (altitude)+ 0.24 (0.06) Log (MEIalk)

3.4 Discussion

All analyses indicate that TP reference conditions increase with increasing alkalinity and decreasing depth (Carvalho et al., 2005; Lyche-Solheim et al., 2005). Regression models derived solely from UK reference lakes, had much greater predictive strength compared with European models, and so may be more appropriate in a UK context. It should, however, be noted that the regression model should only be applied to lakes whose typology factors fall within the range covered by the model. For this reason the European models, with their greater coverage of humic type, alkalinity, depth and altitude gradients, will be more applicable for UK lakes that fall outside the range of the current UK model.

Currently there is great bias in the UK and European reference lake datasets towards shallow and deep low alkalinity lakes. The limited phosphorus data available from some lake types bias the regression models and result in uncertain estimates of variability in reference values. As for chlorophyll reference conditions, more effort is needed to identify and sample sufficient numbers of acceptable reference sites of these under-represented lake types (Table 2.13) e.g. very shallow lake types (low, medium and high alkalinity), marl lakes and peaty lakes.

3.5 Recommendations

1. Site-specific rather than type-specific reference conditions should be used where adequate data are available.
2. Reference total phosphorus concentrations should be determined from alkalinity and mean depth using a UK regression model. Site-specific values should be checked against diatom-inferred TP values (palaeolimnology) where available.
3. The H/G boundary for phosphorus should include 90% of modelled reference values (90th percentile of residuals) giving an EQR for the TP H/G boundary of 0.7. The G/M boundary will be based on the G/M chlorophyll boundary and the likelihood of secondary impacts of eutrophication (see Chapter 4), although the final position on boundaries will be determined during the Intercalibration process.
4. Further data collection should be carried out from UK reference lakes to better represent typology gradients and improve the predictive strength of the model.

4. Chlorophyll and light: defining the good/moderate boundary

Stephen Maberly, Geoff Phillips, Sian Davies and Laurence Carvalho

4.1 Introduction

Annex V of the WFD outlines definitions for the biological quality elements at high, good and moderate status, known as normative definitions. The definitions outlined for phytoplankton abundance can be interpreted to help establish the Good/Moderate (G/M) boundary. At good status, Annex V states that slight changes in the abundance of phytoplankton should not result in undesirable disturbances to the balance of organisms present in the water body or to the physico-chemical quality of the water or sediment. EC Eutrophication guidance (Ecostat, 2005) and later GIG reports (e.g. van den Berg et al., 2006) have provided further interpretation of what would effects would constitute an undesirable disturbance associated with increasing phytoplankton abundance, also known as secondary effects [of eutrophication].

Three undesirable secondary effects are the main focus of intercalibration:

1. a decrease in the abundance of submerged macrophytes and phytobenthos
2. a decrease in the maximum colonised depth of macrophytes
3. an increase in the proportion of cyanobacteria

Secondary effects of increased phytoplankton on consumers (fish, macro-invertebrates, birds) have not yet been considered.

In Annex V of the WFD, an undesirable effect is defined as ‘likely to occur in poor status’ and ‘unlikely to occur in good status’. In addition, the deviation from reference conditions, as well as the probability of having poor status, can be taken into consideration for setting the G/M boundary. For this reason, the G/M boundary could be set simply by splitting the EQR scale into equal classes between H/G values and the worst value (theoretically zero). This chapter, however, focuses, on developing robust ecological criteria for the setting of the G/M boundary through the secondary effects of enhanced phytoplankton abundance. Specifically, this is examined in terms of the effect on light attenuation, and subsequent effect on the depth limit of aquatic macrophytes.

Macrophyte depth limits are usually controlled by light (Spence, 1982; here ‘light’ refers to photosynthetically available radiation, 400 – 700 nm). Laboratory studies in Denmark have shown, at 7 ^{o}C and a 16h light: 8h dark photoperiod, that the average growth compensation point for macrophytes is 6.1 μmol m^{-2} s^{-1}. This equates to about 128 mol m^{-2} y^{-1}, which is about 1.8 % of the typical surface light in Denmark of 6930 mol m^{-2} y^{-1} (Sand-Jensen & Madsen, 1991). Surveys of macrophyte depth limit as a function of sub-surface light suggest a higher light requirement in a lake, probably as a result of other ecological factors being sub-optimal and because loss processes will also occur. For example, Chambers & Kalff (1985) reported a range of values from 3.3 to 37% of surface PAR. Part of this variation relates to the type of macrophyte (charophyte, bryophyte or angiosperm) and equations have been developed to relate maximum depth to secchi depth for these three groups (Chambers & Kalff, 1985). Part of this variation also derives from variation with latitude (Duarte & Kalff, 1987) and an inconstant relationship between secchi depth and light attenuation: since widely available secchi disc depth has been used to derive light attenuation values (Middleboe & Markager, 1997). In a recent extensive survey, Middelboe & Markager (1997) quote average percent sub-surface light at depth limits of 2.2% for bryophytes, 5% for charophytes, 16.3% for isoetid macrophytes and 12.9% for elodeid macrophytes, the latter benefiting from an ability to employ shoot extension as a way of 'foraging' for more light higher in the water column, and these values will be used here.

4.1.1 Light attenuation

Light attenuation through a uniform water column follows Beer's law (strictly this refers to a single wavelength):

$$I_Z = I_0 \exp^{-(K_d z)} \tag{1}$$

Where I_z is the light at depth, I_0 is the light at the surface (or more strictly the sub-surface), K_d is the downward attenuation coefficient (m^{-1}) and z is depth (m). This can be re-arranged to calculate the depth at which a particular proportion of sub-surface light is found which could, for example, relate to one of the suggested average depth limits of Middleboe & Markager (1997):

$$z = \frac{\ln I_0 - \ln I_z}{K_d} \tag{2}$$

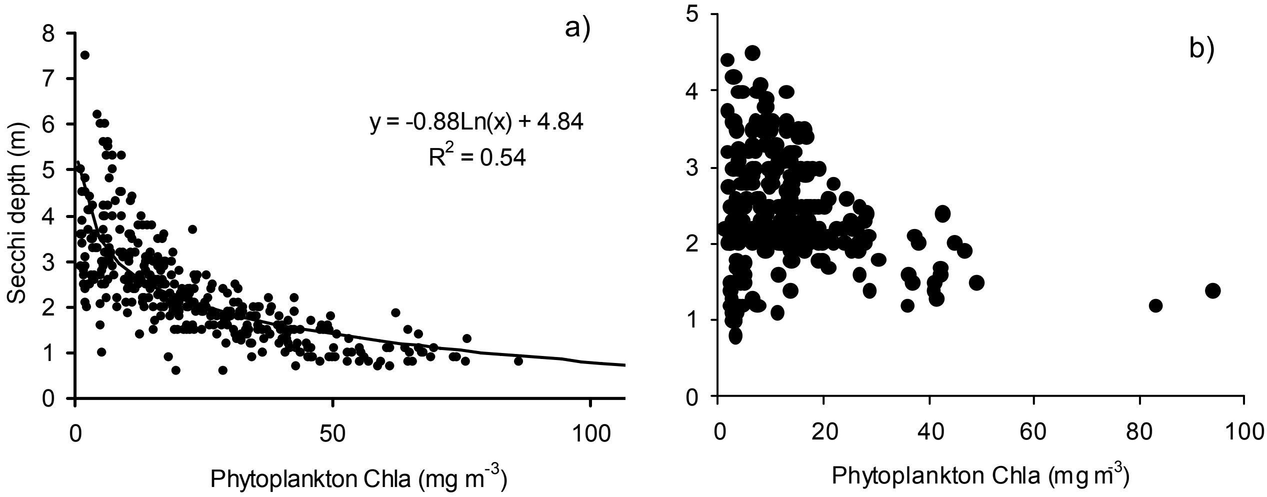
4.1.2 The causes of attenuation

Attenuation is caused by the absorption and scattering of light within the water column. Components of attenuation include the water, dissolved humic substances (gilvin), non-living particles (tripton) as well as the phytoplankton. In order to separate attenuation by phytoplankton from the other sources, one can attribute the total attenuation (K_d) into two components: that caused by the phytoplankton (K_{chl}) and background attenuation (K_B) that comprises attenuation by all the other components (equn 3).

$$K_d = K_B + K_{chl} \tag{3}$$

Strictly speaking, this approach implies that K_d is an inherent optical property (Kirk, 1994) i.e. it is independent of the light field. In reality, K_d is an apparent optical property (Kirk, 1994; Gallegos, 2001) and so is affected by factors such as surface waves, solar angle of incidence, water depth and cloud cover. As a consequence, it is not strictly valid to partition the downward coefficient into a linear sum of its components as suggested in equation 3. The consequences of this assumption for the depth distribution of submerged aquatic vegetation in estuaries are explored in detail by Gallegos (2001) but this level of complexity cannot be dealt with in the broad brush approach necessary to implement the Water Framework Directive, particularly as it would require site-specific data on concentration of dissolved organic carbon and total suspended solids and the use of equations that relate attenuation to the concentration of these variables (e.g. Gallegos, 2001; Squires & Lesack, 2003). Nevertheless, some sites will have much greater light attenuation for a given phytoplankton chlorophyll where suspended solids or dissolved organic carbon is high. For example, in Esthwaite Water phytoplankton is the major cause of light attenuation and there is a reasonable relationship between Secchi depth (very approximately, K_d = 1.44/Secchi depth) and phytoplankton chlorophyll *a* (Fig. 4.1a). In contrast, Bassenthwaite Lake is a shallow lake where the sediments are susceptible to wind disturbance and so total suspended solids can be high. As a result, Secchi depth can be very low even where there is little phytoplankton chlorophyll (Fig. 4.1b).

Figure 4.1 Long-term relationships between Secchi depth and phytoplankton chlorophyll a in a) Esthwaite Water and b) Bassenthwaite Lake



4.2 Methods

In the absence of detailed site information on the contributors to K_B, I suggest using three possible values, 0.2, 0.5 and 1.5 m^{-1}, which represent values associated with clear, moderate and turbid lakes respectively, although extremely turbid lakes can have even higher values (Squires & Lesack, 2003). In order to deal with the lack of information on background attenuation values are allocated to the different lake typologies at reference condition in a 'expert judgement' (Table 4.1). The logic of this allocation is that at reference condition all peaty water lakes will have a high background attenuation because of the dissolved organic carbon and this will be independent of lake depth. All very shallow lakes will be susceptible to wind and wave action disturbing particles on the lake bottom and these have all been allocated to a moderate background attenuation. The remaining lakes were allocated to a low background attenuation at reference condition.

Table 4.1 Background attenuation coefficient in lakes of different typology. L = 0.2, M = 0.5, H = 1.5 m^{-1}

Depth category	Low alkalinity-Clear	Low alkalinity - Peaty	Medium alkalinity	High alkalinity	Marl
Very shallow (mean depth < 3 m)	M	H	M	M	M
Shallow (mean depth 3 – 15 m)	L	H	L	L	L
Deep (mean depth > 15 m)	L	H	L	L	L

3.2.1 Chlorophyll-specific attenuation

The attenuation caused by phytoplankton K_{chl} (m^{-1}) can be estimated from the product of the phytoplankton chlorophyll *a* concentration (mg m^{-3}) and the chlorophyll-specific attenuation coefficient (k_c, m^2 mg^{-1}). However, k_c is not constant because of variability in the amounts of accessory pigments and because different 'packing' of chlorophyll in cells of different sizes affects attenuation (sieve effect, see Kirk 1994). Nevertheless, published values for the chlorophyll-specific attenuation coefficient are relatively constrained between about 0.01 and 0.03 m^2 mg^{-1} Chl *a*, with an average of about 0.02 m^2 mg^{-1} Chl *a* (Kirk, 1994). More recently, Krause-Jensen & Sand-Jensen (1998) have reviewed data from 32 values in the literature and suggest an average of 0.015 m^2 mg^{-1} for phytoplankton chlorophyll *a*, and this is the value used here.

4.3 Results: phytoplankton chlorophyll concentration & macrophyte depth limit

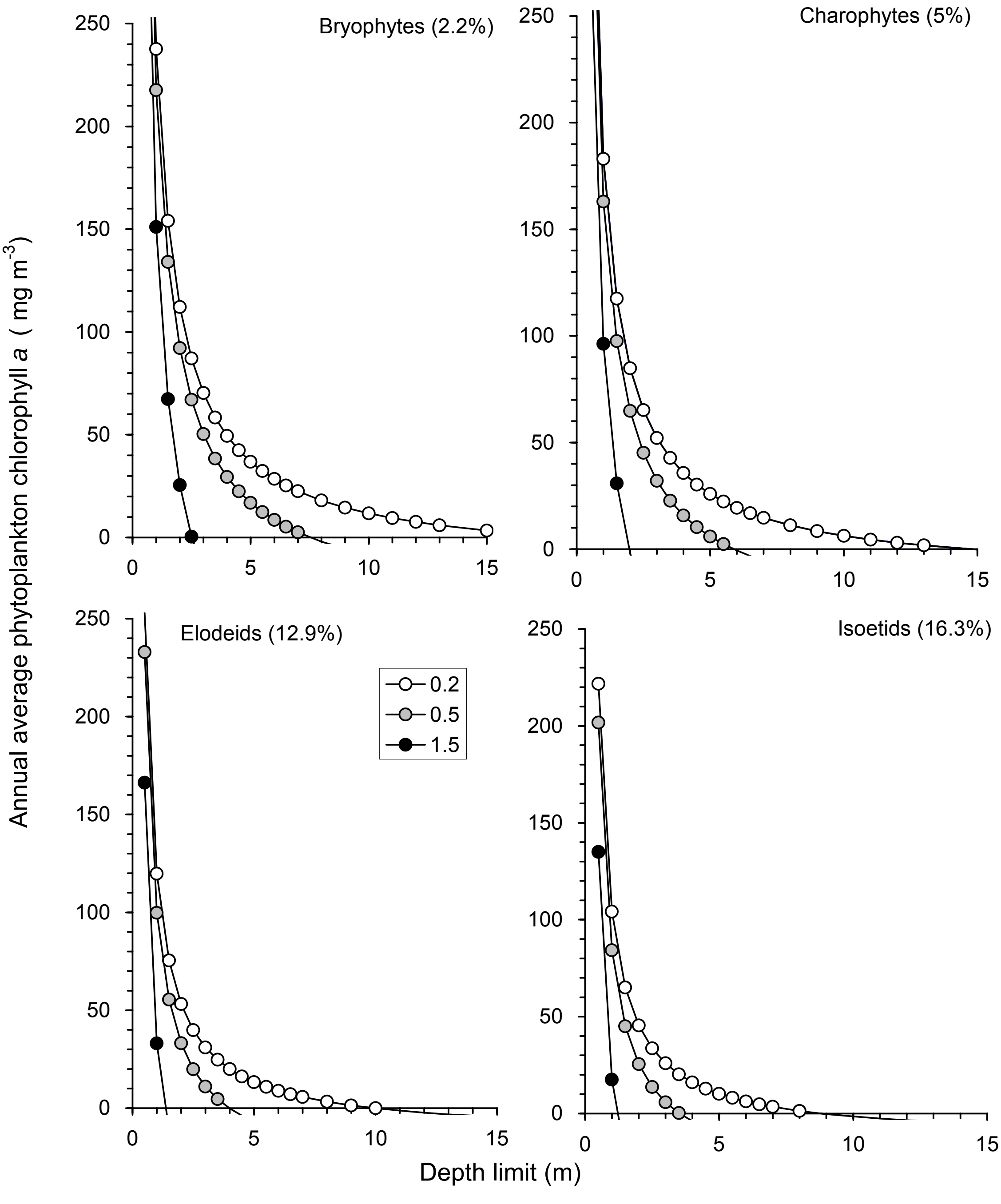
The phytoplankton chlorophyll *a* concentration as an average over a growing season (Middleboe & Markager, 1997) that allows a particular depth limit for bryophytes, charophytes, elodeids and isoetids can be calculated by combining equations 2 and 3 (equn 4), where I_x is the macrophyte group-specific percent of surface light at maximum colonisable and Z_{max} is the maximum depth of colonisation. Ln 95 is used because, following Middleboe & Markager (1997) from whom the depth limits are derived, a 5% surface reflection is assumed.

$$Chla = \frac{\left(\frac{(Ln95 - LnI_x)}{z_{\max}}\right) - K_B}{k_c} \qquad (4)$$

These calculations show (Fig. 4.2) that in the absence of phytoplankton, background attenuation will place depth limits of between 1.2 and 2.5 m for high background attenuation, between 3.5 and 7.5 m for the moderate background attenuation and between 8.8 and 18.8 m (data not shown) for low background attenuation, depending on the macrophyte-group. The presence of phytoplankton will then reduce these depth limits further (Fig. 4.2).

Figure 4.2 Effect of phytoplankton chlorophyll a concentration during the growing season on macrophyte depth limits

Four types of submerged macrophytes are illustrated with different light requirements and three levels of background attenuation representing low background attenuation (0.2 m^{-1}, open circles), moderate background attenuation (0.5 m^{-1}, grey circles) and relatively high background attenuation (1.5 m^{-1}, black circles)



This approach needs to be evaluated by comparing depth limits of different groups of macrophytes with estimates of phytoplankton chlorophyll *a* concentration. For example in Loch Borralie, Durness, charophytes are recorded to 15 m and elodeids to 6.5 m (Spence et al., 1984). Using a background attenuation of 0.2 m^{-1} (Table 3.1), this would be achievable with average growing season phytoplankton chlorophyll *a* of 0 and 7 mg m^{-3} for charophytes and elodeids respectively. These values are reasonable but illustrate the uncertainty inherent in this method, particularly in setting K_B, although this could be done precautionarily.

4.4 Discussion: setting the good-moderate boundary

In order to avoid a circular argument, a suggested way to set the phytoplankton chlorophyll *a* at the good-moderate boundary for a given lake type is set out below.

1. Set the background attenuation coefficient for different lake types as suggested in Table 3.1;
2. Use the reference condition phytoplankton chlorophyll *a* concentration with the background attenuation to set a reference depth limit for the different categories of macrophyte;
3. Set a reduction in depth limit that represents the good/moderate boundary using 'expert judgement';
4. Calculate the concentration of phytoplankton chlorophyll *a* that would allow this depth limit.

Provisional reference concentrations of phytoplankton chlorophyll *a* based on median values for reference lakes are shown in Table 4.2, as are those at the good-moderate boundary which represent a doubling of the reference concentrations.

Table 4.2 Provisional mean annual phytoplankton chlorophyll *a* concentration (mg m^{-3}) at reference and good/moderate boundary for lakes in the UK
Insufficient UK data to produce a value is indicated by '-'

	Low alkalinity		Peaty		Medium alkalinity		High alkalinity		Marl	
	Ref	G/M	Ref	G/M	Ref	G/M	Ref	G/M	Ref	G/M
Very shallow	4.5	8.9	-	-	5.8	11.7	11.0	21.9	10.1	20.3
Shallow	2.3	4.7	3.5	7.0	4.4	8.7	7.3	14.6	7.6	15.2
Deep	2.1	4.2	-	-	2.7	5.4	5.1	10.1	-	-

In Table 4.3 the reference concentration of phytoplankton chlorophyll *a* are used to calculate reference depth limits for the different types of lake and macrophyte. These range from 1.4 m for isoetids in peaty water to 16.3 m for bryophytes in deep, low alkalinity lakes (Table 4.3). The values in Table 4.3 are broadly consistent with known depth limits for lakes which are at or close to reference condition. For example, using the tabulated depth limits in Spence (1982), Borralie, Wastwater and Ennerdale Water which are probably at reference condition show a relatively good agreement between observed and calculated reference depth although there is a tendency for the reference depth to be greater than the observed depth, particularly for the elodeids (Table 4.4). This may not be particularly unusual because it is possible that factors other than light may influence depth distribution (Spence, 1982). In the case of Derwentwater, the reference depths are substantially greater than the observed depths suggesting that this lake may not be at reference condition and the discrepancy is marked for Esthwaite Water and Loch Leven that are known to have been impacted by nutrient enrichment. There are signs of an increase in macrophyte colonisation depth in L. Leven in recent years although the observed/reference depth is still on 0.42 for Elodeids and 0.26 for charophytes (Table 4.4).

Table 4.3 Calculated depth limits (m) for different types of macrophyte in different lake categories

Using the background attenuation coefficients in Table 4.1, reference phytoplankton chlorophyll *a* concentration in Table 4.2 and equation 4. Insufficient UK data to produce a value is indicated by '-' .

		Reference depth limit (m)				
Depth category	Macrophyte type	Low alkalinity	Peaty	Medium alkalinity	High alkalinity	Marl
Very shallow	Bryophyte	6.6	-	6.4	5.7	5.8
	Charophyte	5.2	-	5.0	4.4	4.5
	Elodeid	3.5	-	3.4	3.0	3.1
	Isoetid	3.1	-	3.0	2.7	2.7
Shallow	Bryophyte	16.1	3.0	14.2	12.2	12.0
	Charophyte	12.6	2.4	11.1	9.5	9.4
	Elodeid	8.5	1.6	7.5	6.5	6.4
	Isoetid	7.5	1.4	6.6	5.7	5.6
Deep	Bryophyte	16.3	-	15.7	13.6	-
	Charophyte	12.7	-	12.2	10.6	-
	Elodeid	8.6	-	8.3	7.2	-
	Isoetid	7.6	-	7.3	6.4	-

Table 4.4 Observed and calculated reference depth (m) for Elodeids and Charophytes in six lakes

Four lakes at or close to reference condition and two lakes substantially impacted by nutrient enrichment. Observed depths from Spence (1982) apart from L. Leven in 2005- CEH unpublished

		Elodeid			Charophyte		
Lake	Category	Observed	Reference	O/R	Observed	Reference	O/R
Borralie	HA-shallow	6.0	6.5	0.92	15.5	9.5	1.63
Wastwater	LA-deep	6.0	8.6	0.70	12.0	12.7	0.94
Ennerdale	LA-deep	-	-		10.0	12.7	0.79
Derwentwater	LA-shallow	5.0	8.5	0.59	6.0	12.6	0.48
Esthwaite Water	MA-shallow	3.1	7.5	0.41	2.8	11.1	0.25
Leven (1974) Leven (2005)	HA-shallow	1.0 2.7	6.5 6.5	0.15 0.42	1.0 2.5	9.5 9.5	0.11 0.26

There are several ways of carrying out step 3 above. One way is to set a proportional reduction in depth, such as 0.75 of the reference depth, to represent the good/moderate boundary. A second way is to set an actual depth reduction for all types of lakes and a third way is to set an actual depth reduction categorised by lake depth, so it takes some account of the effect on the lake and is somewhat intermediate between the first and second approaches. The second approach where a depth of 2 m was used produced very high chlorophyll concentrations, particularly in very shallow lakes, and the results of the calculations are not show here. Table 3.5 shows a 0.75 reduction in reference depth (Method 1) and a reduction of 1, 2 or 3 m for very shallow, shallow and deep lakes respectively (Method 2).

These estimated phytoplankton chlorophyll *a* concentrations at the good-moderate boundary can be compared directly with those suggested simply by doubling the reference chlorophyll concentration (Table 4.2). The results suggest that there is a good, although not perfect, agreement between the chlorophyll *a* concentration at the good-moderate boundary estimated by the two methods (Fig. 4.3). This is encouraging because, although both based on the same reference chlorophyll value, the good-moderate boundary is established in two independent ways. In general, the good-moderate boundary based on macrophyte depth zonation is slightly less restrictive- i.e. it has higher chlorophyll *a* values- than that based on doubling reference chlorophyll. For all the macrophyte types, the slope of response is close to one (ranging between 0.90 and 1.18) but the intercept varies between 2.45 and 3.98 (Fig. 4.3). The only data in Table 3.5 that were not included in Figure 3.3 were the set of data from peaty lakes (only shallow lakes available) because the method based on macrophyte colonisation produced high estimates of chlorophyll concentration at the good-moderate boundary. This is probably because this lake type was allocated a high background attenuation because of the water colour and as a result the reference depth limit is fairly shallow (Table 4.3) and so high chlorophyll concentrations are needed to reduce it further. The relatively high chlorophyll concentrations at the good-moderate boundary for the elodeids and isoetids are for a similar reason: because of the higher light requirements of these two groups their depth limit is shallower and so a higher chlorophyll concentration is needed to achieve the same degree of shading. This reflects the ecological reality that plants in shallow water are less susceptible to shading by phytoplankton than plants in deep water. This approach does not take into account other forms of competition, such as for inorganic carbon, nor does it include competition by periphyton. This latter component may be important in shading macrophytes, particularly in shallow water (Phillips et al., 1978).

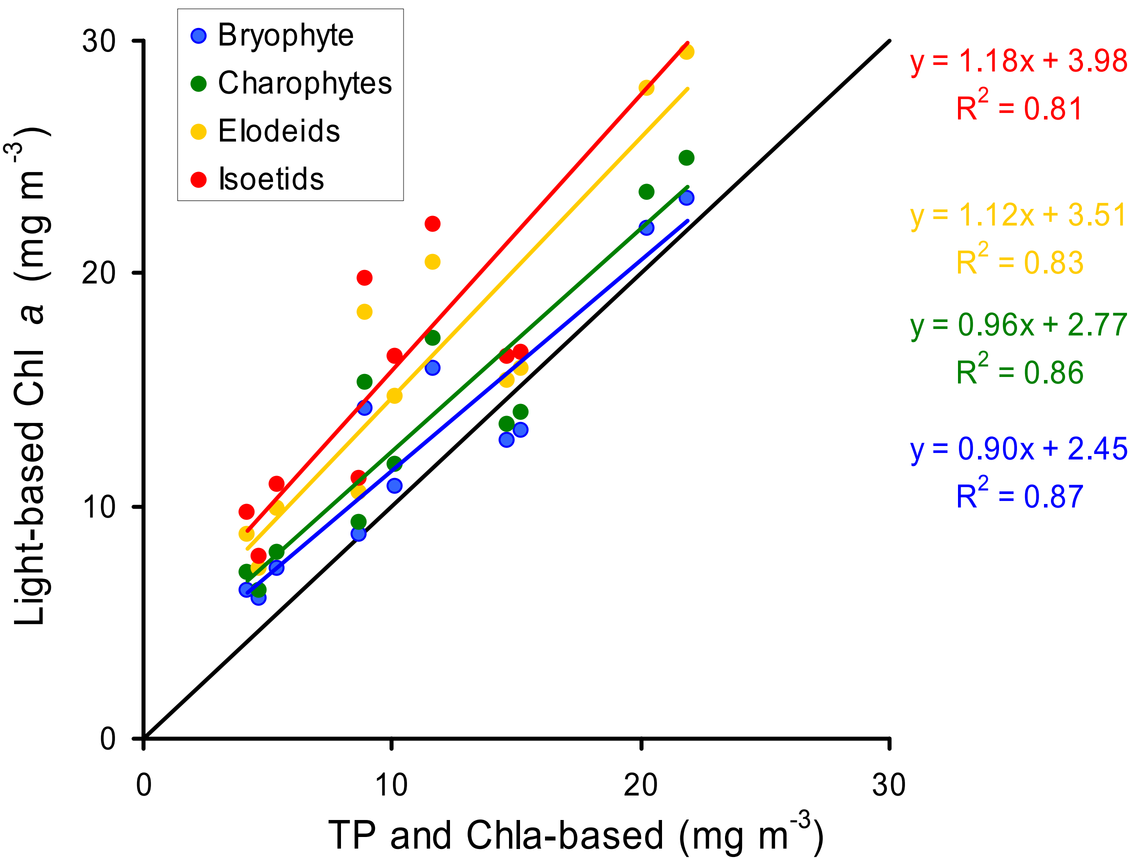


Figure 4.3 Comparison of two approaches for assessing annual mean concentration of chl$_a$ at the good-moderate boundary

Values based on reduction in macrophyte colonisation depth (Table 3.5) and a doubling of the reference chlorophyll concentration (Table 3.2). The macrophyte values vary with different types of macrophyte. Lines of best fit and regression equation for each macrophyte type is given. The diagonal black line is the 1:1 relationship. The figure excludes the single value for peaty water- see text.

Table 4.5 Suggested phytoplankton chlorophyll *a* concentrations (mg m^{-3}) at the good-moderate boundary for different types of lake and macrophyte

Method 1 = good-moderate depth is 0.75 of reference depth; Method 2 good-moderate depth is 1 m less than reference depth for very shallow lakes, 2 m less for shallow lakes and 3 m less for deep lakes. No reference chlorophyll value is indicated by '-'. If it is not possible to produce sufficient shading, even at very high chlorophyll values, this is indicated by 'NA'.

Depth category	Macrophyte type	Method	Low alkalinity	Peaty	Medium alkalinity	High alkalinity	Marl
Very shallow	Bryophyte	1	17.1	-	18.1	25.8	24.6
		2	11.2	-	13.0	20.5	19.2
		Mean	**14.2**	**-**	**15.9**	**23.2**	**21.9**
	Charophyte	1	17.1	-	18.8	25.8	24.6
		2	13.5	-	15.5	23.9	22.4
		Mean	**15.3**	**-**	**17.2**	**24.9**	**23.5**
	Elodeid	1	17.1	-	18.8	25.8	24.6
		2	19.5	-	22.1	33.1	31.1
		Mean	**18.3**	**-**	**20.5**	**29.5**	**27.9**
	Isoetid	1	17.1	-	18.8	25.5	24.6
		2	22.5	-	25.3	37.9	35.6
		Mean	**19.8**	**-**	**22.1**	**31.7**	**30.1**
Shallow	Bryophyte	1	7.5	31.3	10.3	14.2	14.6
		2	4.5	170	7.3	11.4	11.8
		Mean	**6.0**	**84.9**	**8.8**	**12.8**	**13.2**
	Charophyte	1	7.5	31.3	10.3	14.2	14.6
		2	5.3	480	8.3	12.8	13.3
		Mean	**6.4**	**256**	**9.3**	**13.5**	**14.0**
	Elodeid	1	7.5	31.3	10.3	14.2	14.6
		2	7.1	NA	10.8	16.6	17.2
		Mean	**7.3**	**31.3**	**10.6**	**15.4**	**15.9**
	Isoetid	1	7.5	31.3	10.3	14.2	14.6
		2	8.0	NA	12.1	18.5	19.2
		Mean	**7.8**	**31.3**	**11.2**	**16.4**	**16.6**
Deep	Bryophyte	1	7.2	-	8.0	11.2	-
		2	5.6	-	6.5	10.3	-
		Mean	**6.4**	**-**	**7.3**	**10.8**	**-**
	Charophyte	1	7.2	-	8.0	11.2	-
		2	6.9	-	7.9	12.3	-
		Mean	**7.1**	**-**	**8.0**	**11.8**	**-**
	Elodeid	1	7.2	-	8.0	11.2	-
		2	10.3	-	11.8	18.2	-
		Mean	**8.8**	**-**	**9.9**	**14.7**	**-**
	Isoetid	1	7.2	-	8.0	11.2	-
		2	7.6	-	8.7	13.5	-
		Mean	**9.7**	**-**	**10.9**	**16.4**	**-**

4.5 Conclusions

The approach of setting a good-moderate boundary for chlorophyll based on the secondary effect of reducing the macrophyte depth limit through shading is examined in comparison to the approach to that achieved by simply doubling the reference chlorophyll concentration. These two methods cannot be expected to give exactly the same chlorophyll *a* concentration in all lake types. Nevertheless, the two approaches are generally in rather good agreement, with the exception of peaty lakes where macrophyte depths limits are likely to be shallow and, therefore, only weakly susceptible to further shading by phytoplankton.

If this approach is used then, an approach needs to be agreed of dealing with the slightly different results for the different types of macrophyte, with their different tolerance to shade. One suggestion is to use the type(s) of macrophyte that is most relevant to a particular lake type. For example, one could use the macrophyte type with the greatest colonisation depth in a particular lake and compare this with the calculated reference depth for the appropriate lake type (the mean values in Table 4.5). This approach would minimise the risk of not having detected the greatest colonisation depth of a particular type of macrophyte.

The method is sensitive to the background attenuation values selected in Table 4.1. In reality, this will be a continuous variable among lake types. If the concentration of dissolved organic carbon and total suspended solids are known then background attenuation can be estimated using equations, such as those in Squires & Lesack (2003). Consideration should be made to collect these data more routinely in lakes.

5. Relationships between phosphorus, nitrogen and chlorophyll a concentrations

Geoff Phillips, Olli-Pekka Pietiläinen, Laurence Carvalho & Stephen Maberly[2]

5.1 Introduction

Phytoplankton production can be regulated by many environmental factors such as temperature and light conditions. However, during the growing season phytoplankton communities are often limited by nutrients, especially phosphorus and/or nitrogen.

The relative role of nitrogen and phosphorus as potential controlling factors for primary production has been studied and discussed for decades. The major conclusion has been that phosphorus appears to be the main nutrient controlling primary production in lakes and nitrogen in oceans. However, this is a generalisation, and in some cases nitrogen, or a combination of nutrients, can limit algal growth in inland waters during all or part of the growth season (Maberly et al., 2002).

In the longer-term (years, decades, centuries) and at larger spatial scales (regional, continental, global) phosphorus is generally regarded as being in shortest supply and thus the nutrient most likely to be the limiting factor ("ultimate limiting nutrient"). At shorter temporal (seasonal, annual) and smaller spatial scales (local, regional) nitrogen could be more likely to be a limiting nutrient because of its ability to reach equilibrium with the availability of phosphorus ("proximate limiting nutrient"). Accordingly, if we consider broader temporal and spatial scales, it is likely that both phosphorus and nitrogen loading should be reduced to avoid eutrophication of lakes, rivers, coastal and open sea waters.

Previous studies have quantified the relationships between nutrients and chlorophyll (e.g. OECD 1982) but these relationships have often been based on data predominantly from North America. The basic idea of this study was to examine these relationships for European lakes and to identify if there were any differences in the relationships for different geographic regions or for lake types. In doing this we have provided mathematical expression for these relationships which can be used to assist with the implementation of the EU Water Framework Directive. In particular, they allow supporting nutrient standards to be developed for the newly established chlorophyll standards and in the longer-term can be used, in reverse, to estimate the likely ecological benefits of catchment measures aimed at reducing nutrient loading, and hence in-lake concentrations. This study examines both nitrogen and phosphorus, evaluating of the relative role of phosphorus and nitrogen as potential algal growth controlling factors to assist with the development of appropriate water protection measures.

5.2 Methods

The source data used for analysis were from the Rebecca database version 23 November 2005, with updated typology tables 20 January 2006, provided by Jannicke Moe. Mean monthly total phosphorus (TP), total nitrogen (TN) and chlorophyll *a* (Chla) concentrations were calculated for all sites and years. A growing season (April – September) mean was then determined and the number of months contributing to this mean recorded. Only growing season mean values for TP, TN and Chla for the same site and year were related. Site/year data for the following analysis was only selected where there were at least 2 months of data contributing to the growing season mean and averaging these determinands for all dates and sites within a single lake to provide an overall lake growing season mean. Where sites within a lake were allocated to a different typology, or were identified as being at

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[2] This chapter is largely copied from an unpublished report written by Geoff Phillips and Olli-Pekka Pietilainen for REBECCA/Intercalibration (draft dated 2 January 2006)

reference state the site remained separate and in the analysis was treated as an independent "lake". Thus the analysis was based on seasonal means derived from paired Chl TP and Chl TN for each lake.

To determine relationships between Chlorphyll a and the supporting elements TP and TN regression analysis was used (using SPSS). All data were log transformed for analysis to ensure error terms conformed to normal distributions and to minimise heteroscedasity. To reduce the influence of lakes where TP is unlikely to be a limiting nutrient analysis, the datasets was restricted to sites with values where TP was <100 μgL^{-1} and Chl was ≥1μgL^{-1}. To provide type-specific relationships lakes were grouped by both the core intercalibration typology of alkalinity and depth (Table 1.1)[3] and by GIG types (Table 1.2). Significance of differences between type-specific relationships and the effects of other categorical variables, such as lake colour, were tested using the general linear model routine. Prior to analysis scatter plots of data by lake type were examined to determine obvious outliers which were removed from the analysis. For TN analysis all data from UK were excluded as this determinand has not been routinely used in the UK and the few data which were available were from new, unvalidated methods.

5.3 Results

In total 1577 TP v Chl, 1340 TN v TP and 1304 combined TP TN v Chl records were available for analysis, although of these not all had been allocated to type and/or GIG region. Records were available from 16 countries, with the majority being from Norway, Finland, UK (England, Wales and Scotland only) and Germany (Figure 5.1).

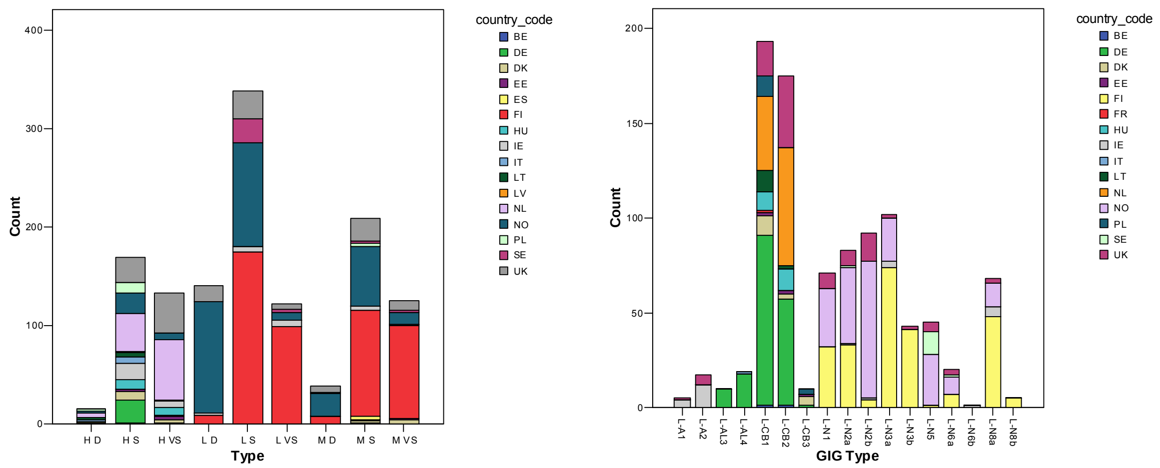

Figure 5.1 No. of lakes with available data, grouped by (a) core depth and alkalinity typology and (b) GIG types

5.3.1 Descriptive statistics

Chlorophyll, phosphorus and nitrogen concentrations ranged over 2 orders of magnitude decreasingly markedly with depth, and slightly with decreasing alkalinity (Figure 5.2). The resulting TN:TP ratios were similar for each alkalinity type but decreased with depth (Fig 5.2d). From the TN:TP ratio it seems that phytoplankton biomass in the majority of European lakes in the Rebecca data set are likely to be P limited (Forsberg et al. 1978).

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[3] Analysis of the core typology included data from all GIGs.

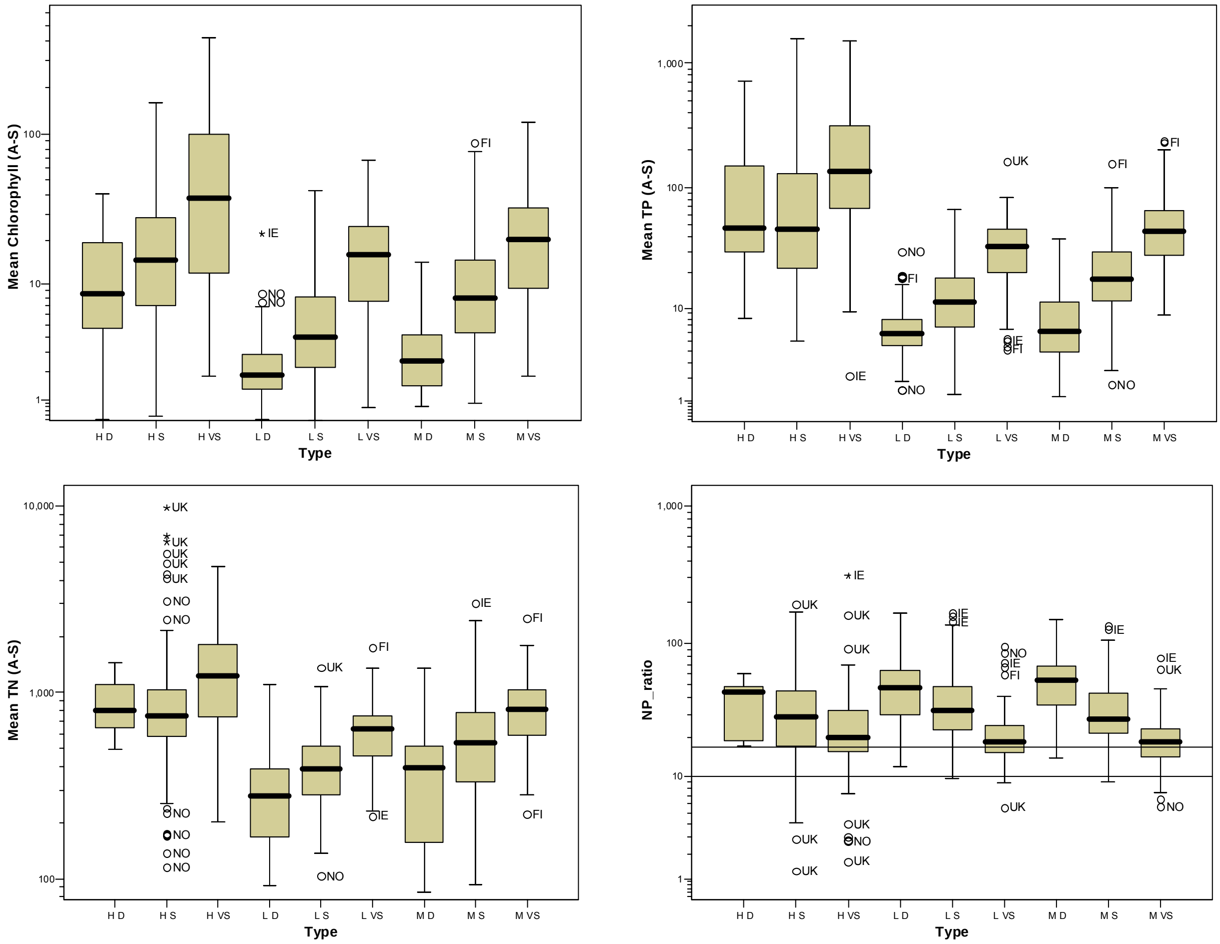


Figure 5.2 Range of mean growing season (April – September) for (a) chlorophyll a (µg L^{-1}), (b) total phosphorus (µg L^{-1}), (c) total nitrogen (µg L^{-1}) and (d) total nitrogen to total phosphorus ratio, for core lake types

Box plots show median, inter-quartile range, min, max and outliers. Lines on Fig 5.2d represent the TN:TP ratio of 17 and 10 marking boundaries of potential P, N or P and N limitation respectively (Forsberg et al. 1978).

TP and TN concentrations were correlated with each other in all lake types, except high alkalinity deep lakes[4], and as expected chlorophyll concentration increased with both TP and TN (Figure 5.3), although lower categories of chlorophyll remain at higher TN levels in comparison to TP. The ratio of TN:TP decreases with chlorophyll concentration with the lowest concentrations being associated with high N:P ratios (Fig 5.4). This is due to proportionally lower P content and subsequent P limitation and occurs most clearly in deep lakes (Fig 5.2). N or P limitation may occur at intermediate chlorophyll concentrations, but it is only at the highest chlorophyll values that N limitation is likely to occur. N limitation is also reported to be possible in very oligotrophic lakes with low TP and TN concentrations. However, in these cases water quality problems should not arise and thus there should be no need to control either P or N loading.

[4] in this case there were only 6 records

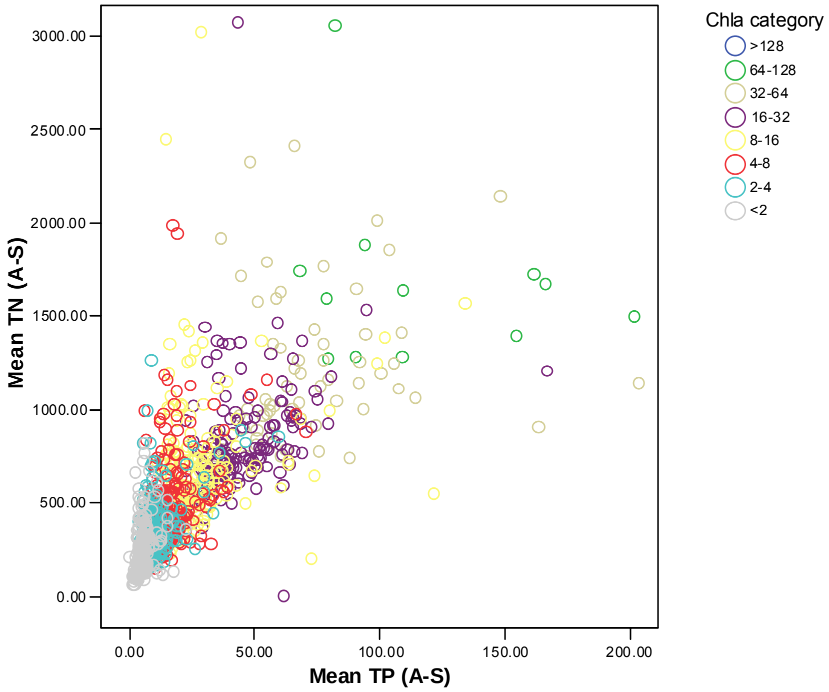


Fig 5.3 Relationship between total phosphorus (µgL-1) and total nitrogen (µgL-1) showing categorised mean growing season chlorophyll a concentrations (µgL-1) for all lake types

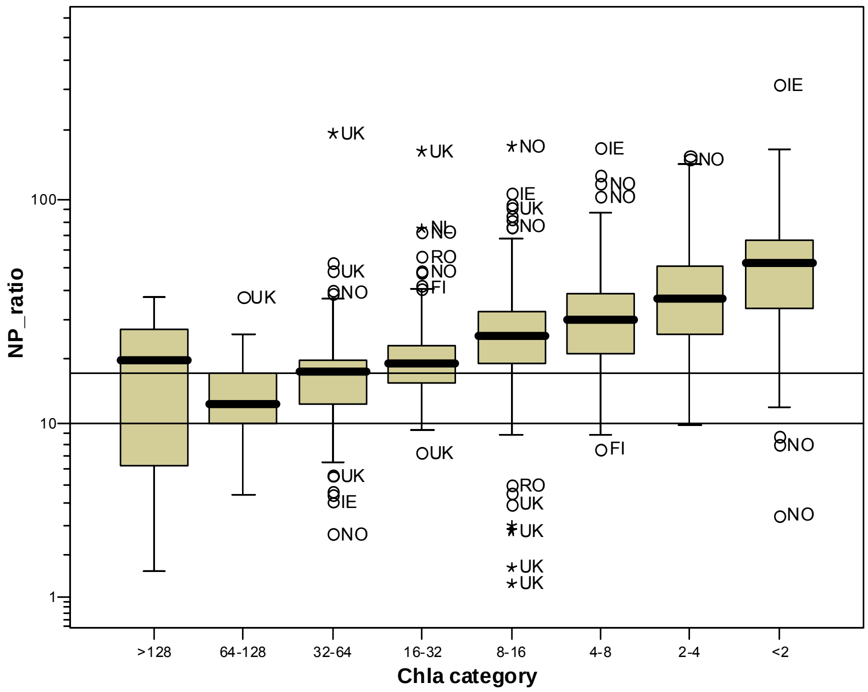


Fig 5.4 Range of N:P ratio for geometric series of mean growing season chlorophyll a (μgL^{-1}) categories

Box plots show median, inter-quartile range, min, max and outliers, lines mark N:P ratio of 17 and 10

5.3.2 Regression relationships for core IC types

Significant regressions for both TP Chl and TN Chl were found for all lakes, although the r^2 values for TP were greater than those for TN (Table 5.1). Multiple regression of Chl with TP and TN only increased the proportion of explained variation by 1%. There is a relatively wide scatter of chlorophyll in these relationships and for the purpose of boundary setting for the Water Framework Directive the boundaries of the scatter also provide useful information. These boundaries were determined from the 90th and 10th percentiles of the residuals of the log regressions and are reported for the TP relationship (Table 5.1). A scatter plot for total phosphorus and chlorophyll, showing the all-lake regression line and upper and lower boundaries is shown in Figure 5.5

Table 5.1 Regression equations for chlorophyll relationship with total phosphorus and total nitrogen

Equations for lines enclosing 90% and 10% of data points for the chlorophyll total phosphorus relationship are also included

Type		r^2	n	p
All Lakes	Log Chl = -0.420(±0.020) + 1.004(±0.015) Log TP	0.732	1577	<0.001
	Log Chl = -2.563(±0.085) + 1.273(±0.031) Log TN	0.554	1340	<0.001
	Log Chl = -1.031(±0.065) + 0.933(±0.022) Log TP + 0.270(±0.031) Log TN	0.794	1304	<0.001
Upper 90th %tile	Log Chl = -0.420(±0.020) + 1.004(±0.015) Log TP + 0.262			
Lower 10th %tile	Log Chl = -0.420(±0.020) + 1.004(±0.015) Log TP - 0.300			

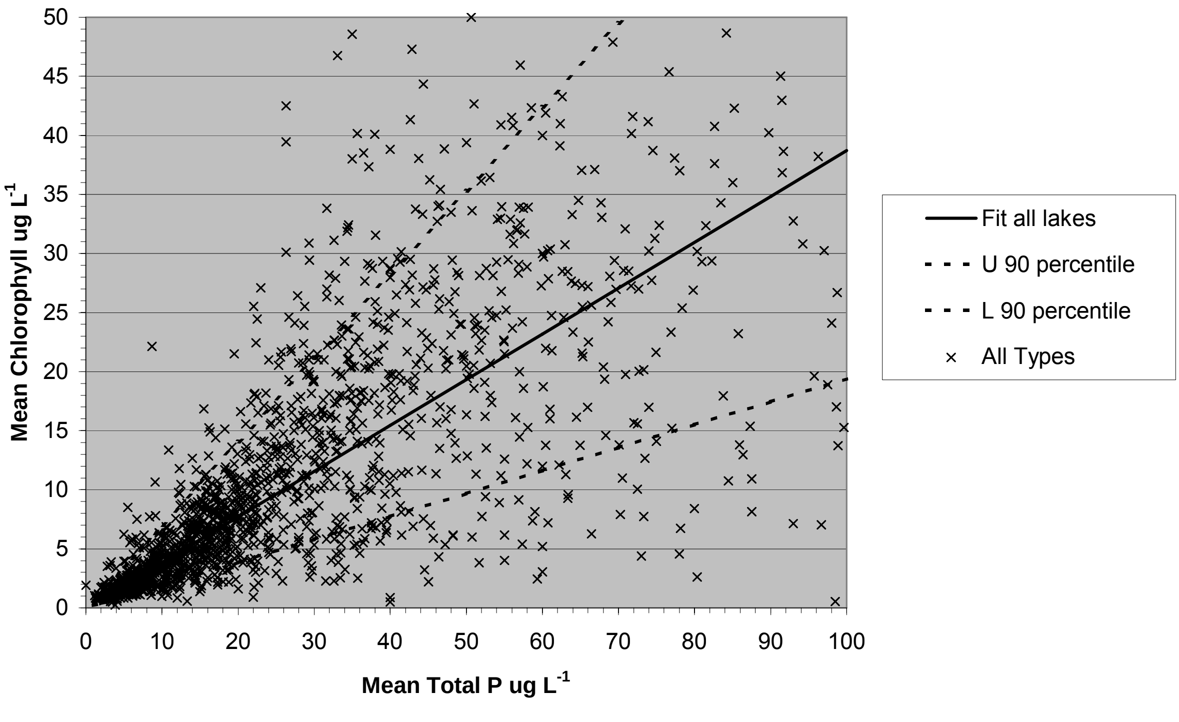


Fig 5.5 Scatter plot of mean growing season TP and chlorophyll for all lakes

log regression and boundaries for the upper and lower 90th and 10th percentiles of points are also shown

Regression relationships between total phosphorus and total nitrogen were determined for each of the core intercalibration types as shown in Table 5.2. Using analysis of variance (SPSS General Linear Model) interactions with depth and alkalinity types were investigated. For both phosphorus and nitrogen significant effects of depth and alkalinity were found, the most significant being that of depth.

Table 5.2 Regression equations for relationships between mean growing season chlorophyll a and total phosphorus and total nitrogen for core intercalibration types

Type		r^2	n	p
High Alk Deep	***Log Chl = -0.186(±0.513) + 0.701(±0.347) Log TP***	0.255	9	0.078
	Log Chl = -5.010(±1.533) + 2.018(±0.525) Log TN	0.696	6	0.012
High Alk Shallow	*Log Chl = -0.143(±0.121) + 0.767(±0.082) Log TP*	0.436	112	<0.001
	Log Chl = -1.178(±0.359) + 0.928(±0.126) Log TN	0.434	70	<0.001
High Alk Very Shallow	*Log Chl = -0.500(±0.163) + 0.994(±0.099) Log TP*	0.676	48	<0.001
	Log Chl = -4.567(±1.018) + 1.882(±0.350) Log TN	0.621	17	<0.001
Mod Alk Deep	*Log Chl = -0.220(±0.058) + 0.800(±0.062) Log TP*	0.820	36	<0.001
	Log Chl = -1.445(±0.370) + 0.751(±0.142) Log TN	0.443	34	<0.001
Mod Alk Shallow	*Log Chl = -0.398(±0.055) + 1.039(±0.043) Log TP*	0.745	202	<0.001
	Log Chl = -2.125(±0.183) + 1.113(±0.067) Log TN	0.598	186	<0.001
Mod Alk Very Shallow	*Log Chl = -0.496(±0.152) + 1.088(±0.095) Log TP*	0.535	112	<0.001
	Log Chl = -3.434(±0.313) + 1.630(±0.108) Log TN	0.667	114	<0.001
Low Alk Deep	*Log Chl = -0.190(±0.059) + 0.673(±0.071) Log TP*	0.389	139	<0.001
	Log Chl = -0.716(±0.194) + 0.431(±0.080) Log TN	0.187	123	<0.001
Low Alk Shallow	*Log Chl = -0.544(±0.041) + 1.115(±0.037) Log TP*	0.733	336	<0.001
	Log Chl = -2.803(±0.199) + 1.344(±0.076) Log TN	0.477	337	<0.001
Low Alk Very Shallow	*Log Chl = -0.518(±0.111) + 1.116(±0.074) Log TP*	0.656	119	<0.001
	Log Chl = -3.642(±0.347) + 1.723(±0.125) Log TN	0.620	116	<0.001

Several of the above type specific regressions are similar, slight differences being due to the particular distribution of observed data. It is useful to determine which lake types show similar relationships as this may not only provide information on lake functioning, but a more general relationship useful for prediction from a larger data set covering a wider range of conditions. Using the type-specific regression equations as a guide, lake types were grouped so that there were no significant effects of depth and alkalinity within the group. For phosphorus, the low and moderate alkalinity shallow and very shallow lakes were found to have a generally steeper gradient (Group P1). All the deep lakes had a shallow gradient (Group P3) and the high alkalinity shallow and very shallow lakes were found to be intermediate between the other two groups (Group P2). For nitrogen there were clear effects of depth, with a decreasing gradient between nitrogen and chlorophyll as depth increased (Groups N1 – N3). When lakes were divided into the 3 depth types a significant effect of alkalinity was found in deep and shallow lakes. This was attributed to the effect of the high alkalinity lakes and thus these lakes were placed in a separate group (N4). Regression equations were then determined for each of these groups (Table 5.3).

Using the typology groups no significant effects of GIG region[5] were found, but for phosphorus there was a significant effect of altitude for Group P1 lakes. There were too few lakes within the high altitude type to determine regression relationships, but the response of chlorophyll to phosphorus was significantly different between moderate and low altitude lakes. This group was thus split into 2 sub-groups (Table 5.3). Scatter plots for each grouped phosphorus regression are shown on linear scales in figs 5.6-5.8. Based on the

[5] Only Northern and Central Baltic GIGs were tested as too few sites were available for the Alpine, Atlantic and Mediterranean GIGs

available data it is recommended that the type specific relationships in Table 5.3 are used to link the supporting elements total phosphorus and total nitrogen with chlorophyll.

Table 5.3 Regression equations for relationships between mean growing season chlorophyll a and total phosphorus and total nitrogen for lakes categorised by grouped typology factors

Regression equations for lake types which did not have significantly different regression equations for total phosphorus.				
Group P1 Mod Alk Shallow Mod Alk Very Shallow Low Alk Shallow Low Alk Very Shallow	*Log Chl = -0.512(±0.028) + 1.105(±0.021) Log TP*	0.777	772	<0.001
Group P1a Moderate Altitude	*Log Chl = -0.534(±0.067) + 0.998(±0.060) Log TP*	0.681	127	<0.001
Group P1b Low Altidude	*Log Chl = -0.439(±0.029) + 1.069(±0.022) Log TP*	0.795	633	<0.001
Group P2 High Alk Shallow High Alk Very Shallow	*Log Chl = -0.249(±0.096) + 0.840(±0.062) Log TP*	0.528	161	<0.001
Group P3 High Alk Deep Mod Alk Deep Low Alk Deep	*Log Chl = -0.220(±0.041) + 0.731(±0.046) Log TP*	0.574	186	<0.001
Regression equation for lake types which did not have significantly different regression equations for total nitrogen				
Group N1 High Alk Very Shallow Mod Alk Very Shallow Low Alk Very Shallow	*Log Chl =-3.350(±0.239) +1.599(±0.084) Log TN*	0.592	249	<0.001
Group N2 Mod Alk Shallow Low Alk Shallow	*Log Chl =-2.546(±0.132) +1.253(±0.050) Log TN*	0.546	524	<0.001
Group N3 Mod Alk Deep Low Alk Deep	*Log Chl =-1.048(±0.168) +0.575(±0.068) Log TN*	0.309	158	<0.001
Group N4 High Alk Deep High Alk Shallow	*Log Chl =-1.718(±0.359) +0.928(±0.126) Log TN*	0.434	70	<0.001

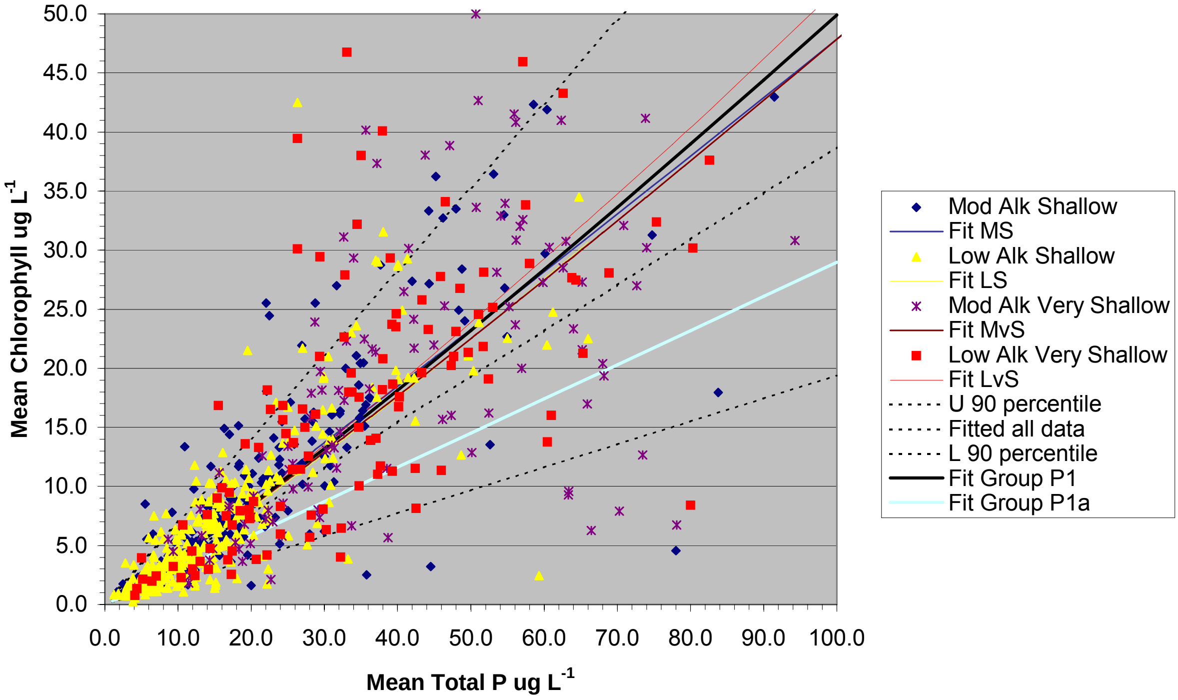


Fig 5.6 Scatter plot of mean growing season TP and chlorophyll concentration for low and moderate alkalinity shallow and very shallow lakes (Group P1)

Solid lines represent log log regressions for each lake type, the bold line the log log regression for all lakes within the group. The lower (light blue) line the regression for the significantly different sub-type of moderate altitude low and moderate alkalinity shallow and very shallow lakes (Group P1a). The dotted lines are the regression line and upper and lower 90th and 10th percentiles of points for all lakes.

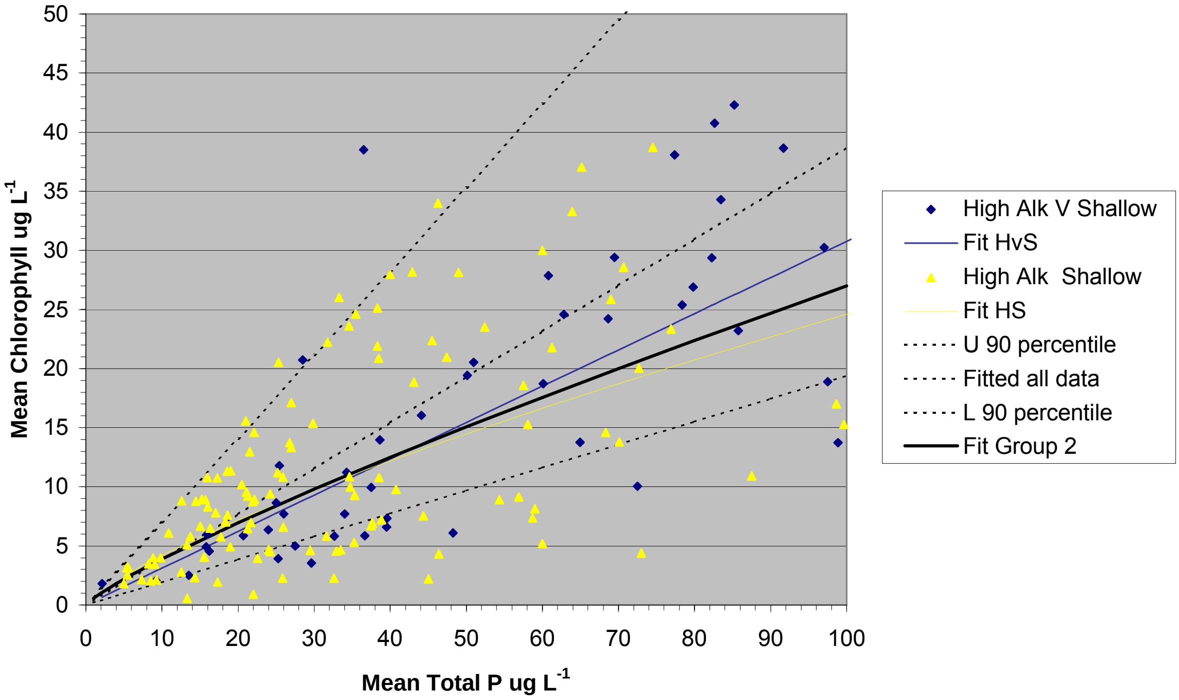


Fig 5.7 Scatter plot of mean growing season total phosphorus and chlorophyll a concentration for high alkalinity shallow and very shallow lakes (Group P2)

Solid lines represent log log regressions for each lake type, the bold line the log log regression for all lakes within the group. The dotted lines show the regression line and upper and lower 90th and 10th percentiles of points for all lakes.

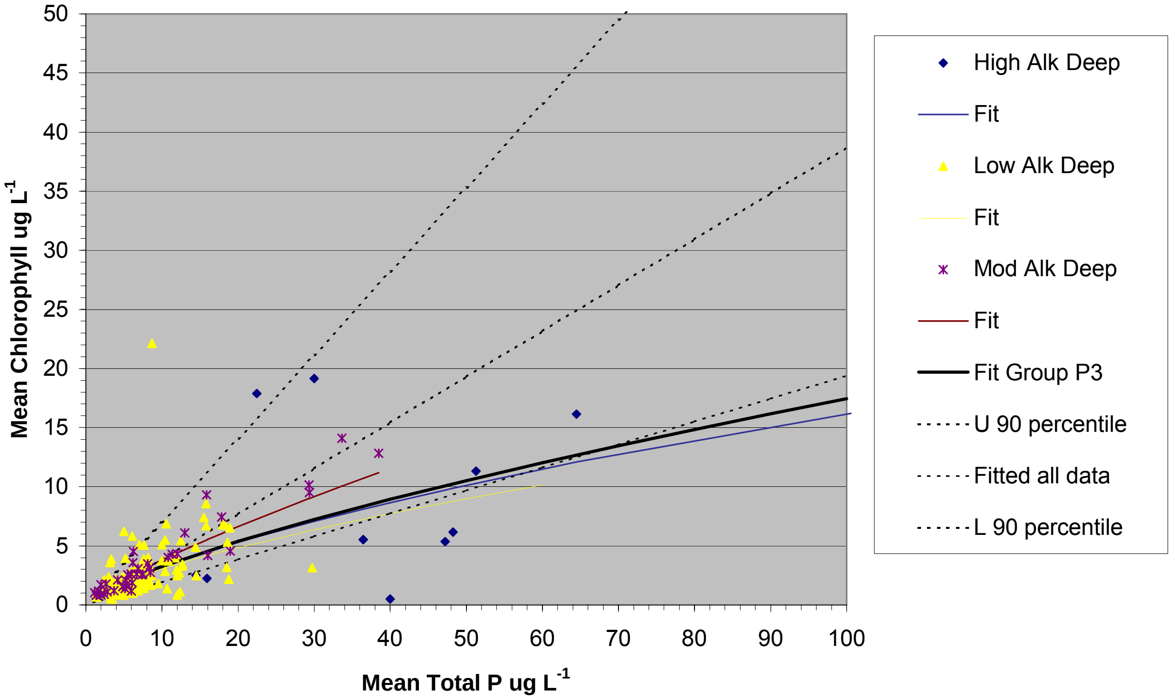


Fig 5.8 Scatter plot of mean growing season TP and chlorophyll a concentration for high, moderate and low alkalinity deep lakes (Group P3)
Solid lines represent log log regressions for each lake type, the bold line the log log regression for all lakes within the group. The dotted lines show the regression line and upper and lower 90th and 10th percentiles of points for all lakes.

5.3.3 Regression relationships for GIG types

As the effect of GIG type on the above reationships was shown to be not significant it is possible to determine the relationship between any lake from the above relationships. However, for information, GIG type specific relationships have also been provided The range of chlorophyll, total phosphorus and total nitrogen are shown in Figures 5.9-5.11 and regression equations for each GIG type are shown in Table 5.4.

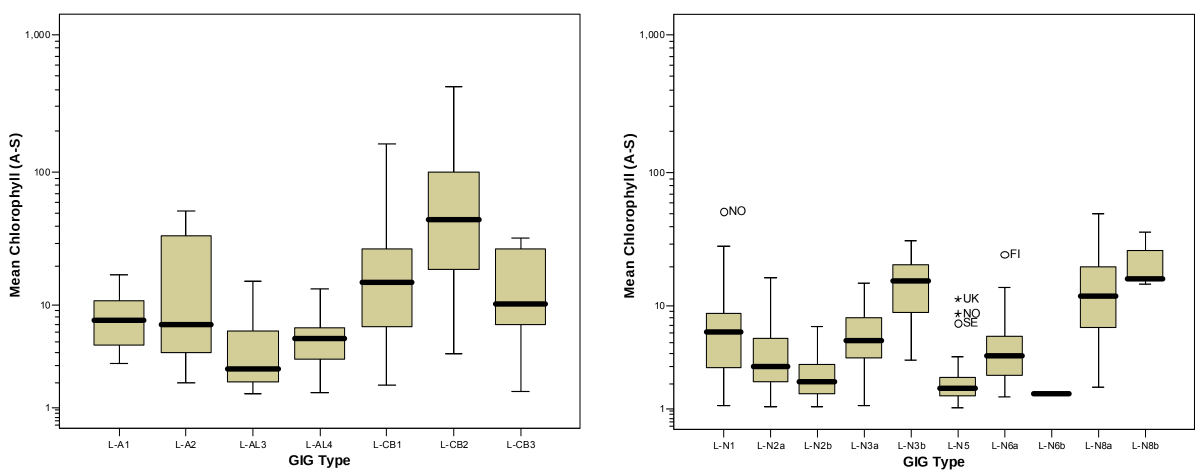


Fig 5.9 Range of mean growing season (April – September) chlorophyll a (μgL^{-1}) for GIG types
Box plots show median, inter-quartile range, min, max and outliers.

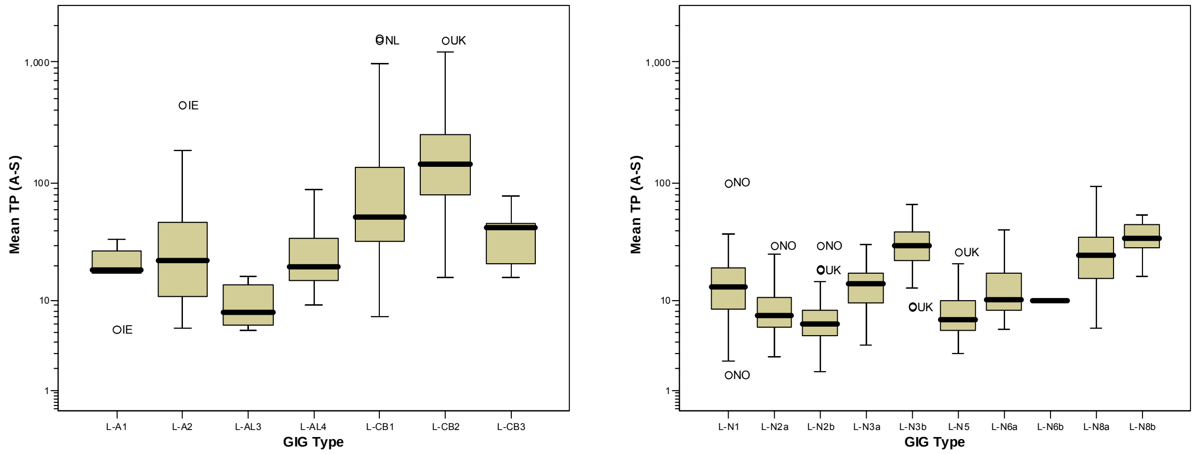


Figure 5.10 Range of mean growing season total phosphorus (µg L^{-1})
Box plots show median, inter-quartile range, min, max and outliers

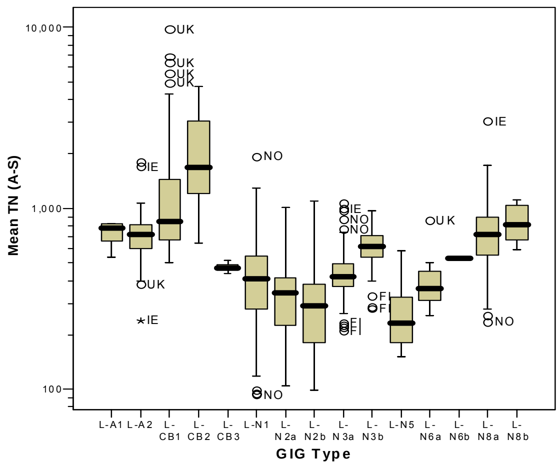
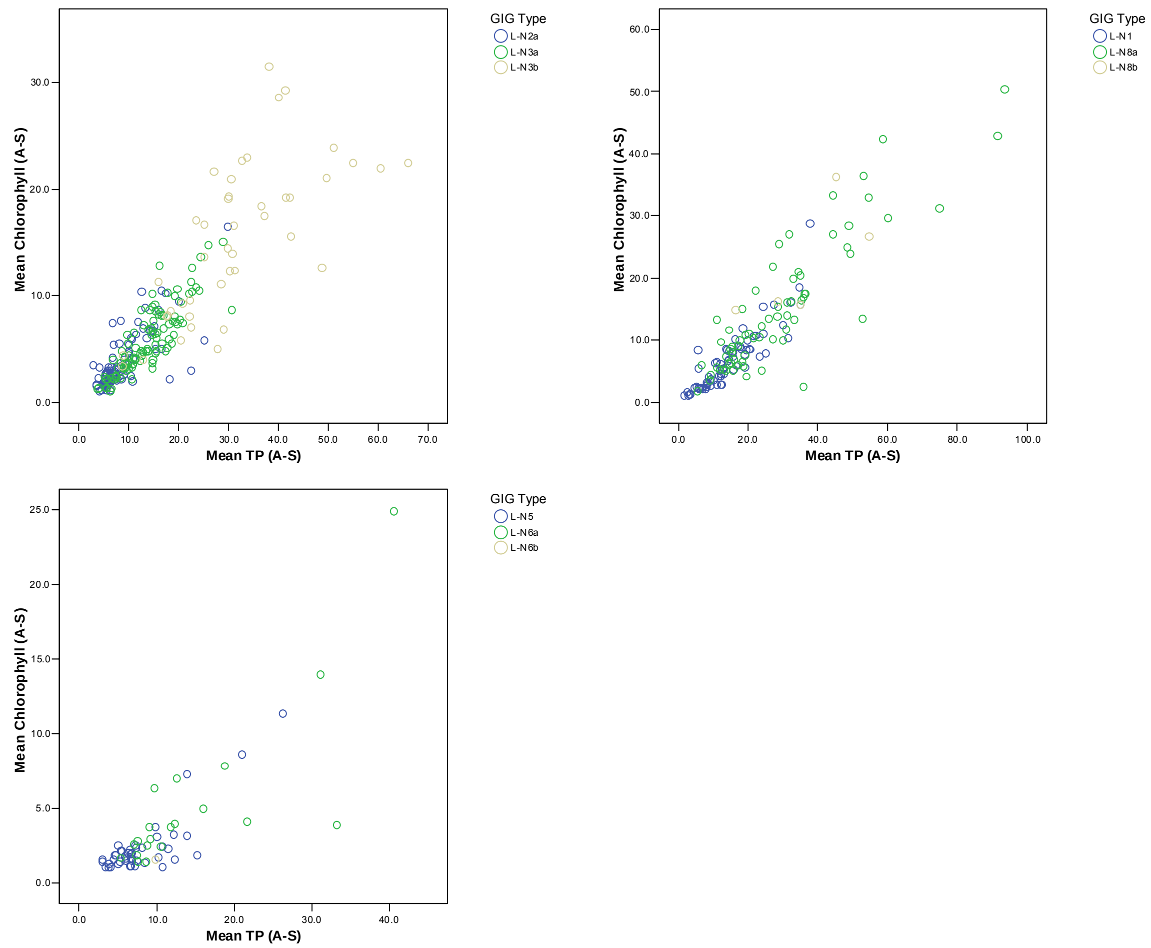


Figure 5.11 Range of mean growing season total nitrogen (µg L^{-1})
Box plots show median, inter-quartile range, min, max and outliers.

As for analysis of the core lake types, regression analysis of variance was used to investigate differences between GIG types. In particular the effect of humic substances was investigated in the shallow low and moderate alkalinity lakes. In all of these types humic lakes contained significantly higher total phosphorus, total nitrogen and chlorophyll a concentrations, but the regression relationships were not significantly different (Table 4). Scatter plots comparing the humic and non-humic types are shown in Figure 5.12. No effect of humic type was found during the analysis of the core typology and there is, therefore, no evidence that humic substances modify the relative amount of chlorophyll produced for a given amount of phosphorus. For these lakes, the Northern GIG groups N1, N2, N3 are suggested as the most robust GIG specific regressions. Alternatively the core typology regressions from Table 5.3 could be used.



Figs 5.12 Scatter plot of mean growing season total phosphorus and chlorophyll a concentration for different lake types

(a) low alkalinity shallow low altitude clear water, humic and polyhumic lakes; (b) moderate alkalinity shallow low altitude clear water, humic and polyhumic lakes; and (c) low alkalinity shallow mid altitude clear water, humic and polyhumic lakes.

For the Atlantic GIG no significant differences were found between the regression equations for the small (L-A1) and large (L-A2) high alkalinity shallow lakes and thus for this GIG a combined regression is appropriate. It should also be noted that there was no significant difference between this combined Atlantic GIG group and the high alkalinity shallow lakes from the Central GIG (L-CB1). This confirms the conclusions from the analysis of the core types where no effect of GIG region was found.

Table 5.4 Regression equations for relationships between mean growing season chlorophyll a and total phosphorus and total nitrogen for lakes categorised by grouped typology factors

Type		r^2	N	P
L-A1	Not significant	0.093	4	0.321
L-A2	*Log Chl = -0.581(±0.159) + 1.166(±0.117)Log TP* *Log Chl =-17.083(±6.324) + 0.037(±0.007)Log TN*	0.875 0.538	14 13	<0.001 <0.001
L-AL3	*Log Chl = -0.922(±0.217) + 1.577(±0.227)log TP*	0.840	9	<0.001 <0.001
L-AL4	*Log Chl = -0.225(±0.202)+ 0.664(±0.148)logTP*	0.516	18	<0.001 <0.001
L-CB1	*Log Chl = -0.386(±0.153) + 0.187(±0.097) Log TP* *Log Chl =-3.232(±1.123) + 1.485(±0.391) Log TN*	0.380 0.340	130 26	<0.001 <0.001
L-CB2	*Log Chl = -0.664(±0.265) + 1.132(±0.155) Log TP*	0.496	53	<0.001 <0.001
L-CB3	Not significant	0.076 0.965	9 2	0.239 0.084
L-N1	Log Chl = -0.327(±0.063)+ 0.978(±0.057) Log TP Log Chl =-1.625(±0.263) + 0.901(±0.101)Log TN	0.812 0.544	69 66	<0.001 <0.001
L-N2a	Log Chl = -0.307(±0.087) + 0.915(±0.094) Log TP Log Chl =-0.908(±0.403) + 0.569(±0.159)Log TN	0.534 0.131	82 78	<0.001 <0.001
L-N2b	Log Chl = -0.117(±0.064) + 0.583(±0.078) Log TP Log Chl =-1.96(±0.254) + 0.218(±0.105)Log TN	0.375 0.041	91 77	<0.001 0.026
L-N3a	Log Chl = -0.595(±0.069) + 1.182(±0.062) Log Tp Log Chl =-1.538(±0.444) + 0.857(±0.168)Log TN	0.784 0.180	100 113	<0.001 <0.001
L-N3b	Log Chl = -0.331(±0.173) + 1.000(±0.117) Log TP Log Chl =-2.721(±0.436) + 1.398(±0.157)Log TN	0.630 0.626	42 47	<0.001 <0.001
L-N5	Log Chl = -0.356(±0.103) + 0.759(±0.118) Log TP Log Chl =-2.294(±0.498) + 1.087(±0.208)Log TN	0.479 0.397	44 40	<0.001 <0.001
L-N6a	Log Chl = -0.539(±0.205) + 1.034(±0.184) Log TP Log Chl =-3.811(±2.031) + 1.735(±0.792)Log TN	0.616 0.182	19 17	<0.001 0.019
L-N8a	Log Chl = -0.275(±0.117) + 0.976(±0.083) Log TP Log Chl =-1.443(±0.421) + 0.885(±0.147)Log TN	0.673 0.327	67 72	<0.001 <0.001
L-N8b	Not significant	0.437	4	0.136
Regression equations for lake types which did not have significantly different regression equations for total phosphorus.				
Group N1 Mod Alk Shallow Low Altitude L-N1 8a 8b	Log Chl = -0.354(±0.054) + 1.023(±0.042)Log TP Log Chl = -1.938(±0.209) + 1.046(±0.076)LogTN	0.806 0.564	142 145	<0.001 <0.001
Group N2 Low Alk Shallow Low Altitude L-N2a 3a 3b	Log Chl = -0.451(±0.040) + 1.066(±0.036) Log TP Log Chl = -2.479(±0.261) + 1.227(±0.099) Log TN	0.797 0.389	226 240	<0.001 <0.001
Group N3 Low Alk Shallow Mid Altitude L-N5 L-N6a	Log Chl = -0.485(±0.088) + 0.933(±0.092) Log TP Log Chl = -2.658(±0.488) + 1.250(±0.199)Log TN	0.618 0.395	65 59	<0.001 <0.001
Group N1 & N2 Siliceous, Shallow, Low Alt	Log Chl =-0.433(±0.032) + 1.065(±0.027) Log TP	0.812	369	<0.001
Group A1 High Alk, Shallow L-A1 A2	Log Chl = -0.443(±0.174) + 1.059(±0.130) Log TP	0.774	19	<0.001
LA1 A2 CB1	Log Chl = -0.303(±0.124) + 0.833(±0.080 Log TP)	0.415	150	<0.001
L-N1 L-CB3	Log Chl = -0.254(±0.077) + 0.899(±0.065) Log TP Log Chl = -0.336(±0.068) + 0.981(±0.059) Log TP	0.707 0.783	78 77	<0.001

5.4 Discussion

The transformation of total phosphorus into phytoplankton chlorophyll *a* is influenced by the stoichiometric relationships between phosphorus and chlorophyll (Reynolds & Maberly 2002) which is probably variable among different types of algae. Within a lake, the degree to which phosphorus is limiting will also influence the extent to which phosphorus is translated into chlorophyll. For example, in a deep lake, light availability may limit the efficiency to which phosphorus is converted to chlorophyll. Similarly, in lakes that are nitrogen-limited or co-limited by nitrogen and phosphorus (e.g. Maberly et al., 2002) less chlorophyll is likely to be produced per unit phosphorus. Another example is in lakes with a substantial amount of macrophytes: they may make use of available phosphorus while suppressing phytoplankton growth. A final example might be a very rapidly flushed lake where hydraulic losses prevent phytoplankton to develop to the extent that would be permitted by the availability of phosphorus. Despite all these examples, there is a very broad relationship over several orders of magnitude between the concentration of total phosphorus and chlorophyll embodied in relationships such as those of Vollenweider & Kerekes (1980) and this present study.

6. Uncertainty in Chlorophyll-a and Total Phosphorus Classifications

Ralph Clarke, Laurence Carvalho and Stephen Maberly

6.1 Introduction and approach

As one component of developing a phytoplankton-based tool for assessing the ecological status class of lakes, it is proposed to use a measure or “parameter” of the chlorophyll a concentrations (µg chl l^{-1}) in the lakes. Total phosphorus concentration (µg TP l^{-1}) is another proposed measure of lake status.

Whatever biological metric, index or parameter is chosen to assess the ecological status class of a lake (or other water body), it is vital to have some idea of the effects of sampling variation and other errors on the value of the measure for any particular lake. A measure of ecological quality is of little value without some knowledge of its levels of ‘uncertainty’ (Clarke, 2000). There is a need for some form of confidence limits for the estimated value of the measure of any site, rather than just a form of statistical test of whether it difference from the expected reference condition. There is also a long-term need for methods of assessing whether there is a statistically significant or real difference in the ecological quality estimates obtained for two samples, whether from the same site at two points in time or for two different sites (Clarke, 2000).

In assigning a lake to a status class on the basis of the estimated value of the parameter for the lake, it is crucial to have some estimate of the chance or probability that, because of natural sampling variation, the true status class of the lake could be better or worse than the estimated class. This is the subject of this section of the report.

The precise measure of chlorophyll (or TP) to be used will affect the spatial and temporal frequency and timing of sampling required to get an adequate estimate of the parameter. For example, if the parameter is the annual mean, then with seasonal variation in chlorophyll concentrations, samples need to be taken at intervals throughout the year to encompass this seasonal variation, whose precise pattern and timing will often vary between years. If concentrations were negligible during the winter months, then sampling could be done in just the other seasons and treat the winter concentrations as zero or some small constant. If the chosen parameter was average concentration during the period May-November, then obviously sampling should be restricted to this period each year.

This discussion is based on coping with temporal variation only. If the chlorophyll (or TP) concentration also varies spatially within the lake, then either the parameter must be defined as the average concentration at say the outflow where concentrations could be expected to be more mixed and representative of the average of the lake as a whole, or a sampling needs to be carried out at several points within the lake intended to adequately cover the spatial variability. Further discussion will concentrate on coping with temporal variability, and assume the aim is to estimate the annual mean concentration.

Because chlorophyll (or TP) concentration is changing from day to day or week to week to varying extents, any estimate of say the true annual mean concentration based on a limited set of samples will not be correct, but have an error, perhaps unknown. With almost any sampling scheme, the error in the estimate of the parameter will decrease with the number of samples on which the estimate is based.

Assume there is some seasonal pattern of chlorophyll (or TP) concentrations within a lake, where the pattern may be unknown and vary between years.

Assume n samples, $x_1, x_2, ..., x_n$, are taken during the year

to give an average value of $\bar{x} = \sum_{i=1}^{n} x_i / n$

The simple sample standard deviation (SD) of the n values is $s = \sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2 / (n-1)}$

The simple sample standard error of the mean is calculated as $SE_{ran} = s / \sqrt{n}$

If n samples were to be taken at random times during the year, then the mean value $\bar{x}$ would have a standard error (SE) equal to SE_{ran} .

However, if as is usual, samples are taken systematically at regular intervals throughout the year, then the true standard error of the resulting mean $\bar{x}$ will be less than the simple standard error SE_{ran} .

The true standard error of any estimator of a parameter is defined to be the simple standard deviation of the estimates that would be obtained if the sampling procedure could be repeated many times. For example with a procedure based on taking one sample at monthly intervals, all possible dates and times within a month could be used as the regular sampling time. Each different sampling time would lead to (at least slightly) different estimates of the annual mean concentration. The standard deviation of all of these possible estimates gives us the true standard error (SE) of the estimator and procedure.

When there a strong seasonal pattern to concentrations, the SE of an estimate based on sampling at regular intervals can be much less than that implied by the simple SE SE_{ran} .

For any lake, the seasonal variation over any one year can be removed to varying extents by sampling at regular intervals during the year. This can be crudely thought of as a form of stratified sampling whereby the year is subdivided into strata of consecutive time periods. A sample is then taken within each time stratum. The sampling is not strictly a stratified random sampling scheme as the samples will usually be taken at regular intervals and hence fixed times within each period (rather than at independent random times within each period), but the analogy is useful in a practical sense. (In statistical jargon, regular sampling through time is a form of systematic sampling). If a sample is then taken in each period and averaged to obtain an estimate of the annual mean, then the true SE of the estimate only depends on the variability in concentrations within each period/stratum.

Dividing the year into more periods and taking one sample per period will obviously lead to a larger sample size, but also reduce the residual uncontrolled (i.e. short-term temporal) variation within a period.

Long term data was available on chlorophyll concentrations (mg l^{-1}) and/or total phosphorus (TP mg l^{-1}) for each of the several lakes (Table 1).

6.2 Loch Leven

For example, chlorophyll samples were available from the outfall of Loch Leven over the period 1968-2004 (see Boxplots below by year and by month). In general a single sample was taken at weekly intervals, but there are numerous missing periods of one or more weeks and no data for 1984, 1986 and 1987. The statistical approach used is first demonstrated using this chlorphyll-a data for Loch leven.

To assess the effect of different sampling frequencies, we stratified the time series of chlorophyll or TP concentration values into strata based on one of the following:

- whole years,
- quarters of each year in turn (e.g. Jan-March 1968,, Apr-Jun 1968, etc),
- two-months of each year in turn (e.g. Jan-Feb 1968, Mar-Apr 1968, ...),
- months of each year each turn (e.g. Jan 1968, Feb 1968, ...
- two-week periods of each year in turn (e.g.1-14 Jan 1968, 15-28 Jan 1968, ...)

One-way analysis of variance ANOVA of individual chlorophyll values on the stratum factor was then used to provide an estimate of the average standard deviation SD of values within any one stratum (denoted by SD_W). ANOVA correctly allows for the reduction in degrees of freedom as the number of strata is increased. This SD roughly determines the variability in values we could have obtained for any single period if we had been able to take more than one sample in the period. Variation within periods is now the only source of error in our estimates of the annual mean.

Table 2 shows the estimates for each the average within-period SD in Chlorophyll-a concentrations (SD_W) for Loch Leven for each length of period of between samples.

Assuming that one sample is taken per period giving a total of n_{str} samples per year, then the standard error (SE_{str}) of the mean value $\bar{x}$ for a year can be estimated by

$$SE_{str} = SD_W / \sqrt{n_{str}}$$

The standard error (SE_{str}) of an estimate of mean based on taking a sampling within each period of the year is actually dependent on the variances in values within each of the separate periods of the year and concentrations may be more variable in some periods of the year than others. However, because the SE of the estimate of annual mean involves the simple sum of the within-stratum variances over all periods of the year, then effectively it only involves the average of the within-stratum variances, which is estimated using one-way ANOVA by $(SD_W)^2$.

The simple standard error SE_{ran} of the mean $\bar{x}$ which ignores the systematic stratified nature of the sampling can, for detailed datasets, be approximated by the SD of all values within a year ($SD_{W(\text{year})}$) divided by the n_{str}, namely:

$$SE_{ran} \approx SD_{W(year)} / \sqrt{n_{str}}$$

This shows how sampling at regular intervals eliminates that part of the annual variation in concentration which is seasonal, and leads to more precise estimates than calculated from the simple SE of the observed mean

The great advantage of this statistical approach, which is just based on one-way ANOVA, is that it can cope with having irregular-spaced and missing values, including having only one sampling in some sampling periods (i.e. no within period replication) and even with having no data for some periods. Providing there are enough sampling periods throughout the whole time series with more than sample within the sampling period, then the average within-period variation in values is used to estimate SD_W. Bias in estimating SE_{str} would only arise if multiple samples within a period were only or mostly available at certain times of the year, so that the variability in these periods dominated the estimate of average within-period variability, which could be more or less at other times of the year.

Table 6.1 Average within-stratum standard deviation (*SD_W*) in Chlorophyll ($\mu g\ l^{-1}$) and Log_{10} Chlorophyll concentrations for Loch Leven over the period 1968-2004
%SD_W = SD_W expressed as a percentage of SD_W for the whole year period

Stratum/ Period	samples per year	Chlorophyll				Log_{10} Chlorophyll				
		SD_W	% SD_W	SE_{ran}	SE_{str}	SD_W	% SD_W	SE_{ran}	SE_{str}	CV_{str}
Whole year	1	33.5	100%	33.5	33.5	0.332	100%	0.332	0.332	115%
3-months	4	25.8	77%	16.8	12.9	0.276	83%	0.166	0.138	37%
2-months	6	21.9	65%	13.7	8.9	0.239	72%	0.136	0.098	25%
Monthly	12	18.3	55%	9.7	5.3	0.198	60%	0.096	0.057	14%
Fortnightly	26	15.0	45%	6.6	2.9	0.139	42%	0.065	0.027	6%

As the intensity of sampling of Loch Leven was never more than weekly, it was not possible to derive an estimate of average variability which can occur within any one-week period and thus it is not possible to derive an appropriate estimate of the SE of a estimator of annual mean concentration based on weekly sampling. However, an upper limit for the SE based on weekly sampling can be obtained as the within-fortnight period SD divided by the square root of 52, namely $SE_{str} = 15.0/\sqrt{52} = 2.1$.

Because variability in concentrations can be non-normal and skewed with occasional (short-term) high values and because the within-stratum variability is likely to be greater when concentrations are higher, the data were also analysed on the $\log_{10}$ scale – for which the within stratum SD is more likely to be roughly constant and hence the average SD obtained from the ANOVA (i.e. *SD_W*) is likely to be more informative (Table 6.1).

Based on the estimates of SE_{str} using the $\log_{10}$ transformed concentrations, the back-transformed estimate (i.e. the geometric mean) of $10^{\bar{x}}$ has an estimated constant percentage SE equivalent to a coefficient of variation (CV) of : $CV = 100(10^{SE_{str}} - 1)$.

As an example, the percentage standard error (CV_{str}) values in Table 2 give a rough initial guide to the level of error likely to be associated with estimates of annual mean chlorophyll based on 1,4,6,12 or 26 regular interval samples per year. For this lake, sampling at monthly intervals reduces the average SE of the estimate of annual mean chlorophyll to 5.3 or, when analysed on a $\log_{10}$-scale, to 0.057, equivalent to a percentage SE of 14.0% (i.e. 100 ($10^{0.057}$ -1)). Assuming approximate normality on the log scale and a $\log_{10}$ annual sample mean of $\bar{x}$, then the estimate annual mean chlorophyll is $10^{\bar{x}} = \bar{X}$ with approximate 95% confidence intervals of $(10^{-2\times0.057}\bar{X}, 10^{2\times0.057}\bar{X})$, which equals $(0.77\bar{X}, 1.30\bar{X})$.

Table 6.2 Average within-stratum standard deviation (SD_W) in total phosphorus ($\mu g\ l^{-1}$) and Log_{10} TP concentrations for Loch Leven over the period 1968-2004
$\%SD_W = SD_W$ expressed as a percentage of SD_W for the whole year period

Stratum/ Period	samples per year	TP				Log_{10} TP				
		SD_W	% SD_W	SE_{ran}	SE_{str}	SD_W	% SD_W	SE_{ran}	SE_{str}	CV_{str}
Whole year	1	35.4	100%	35.4	35.4	0.185	100%	0.185	0.185	53%
3-months	4	28.9	82%	17.7	14.5	0.139	75%	0.093	0.070	17%
2-months	6	26.8	76%	14.5	10.9	0.128	69%	0.076	0.052	13%
Monthly	12	23.2	66%	10.2	6.7	0.109	59%	0.053	0.031	8%
Fortnightly	26	20.9	59%	6.9	4.1	0.097	52%	0.036	0.019	4%

Table 6.2 gives the equivalent uncertainty estimates for the same range of sampling frequencies for TP for Loch Leven based on average variability of the same period 1968-2004. In most years, total phosphorus values were obtained weekly or at least fortnightly, but with gaps and irregularities, and few if any values were obtained in 1978, 1979, 1981, 1984 and 1986.

Seasonal patterns and short-term variability in concentrations of both chlorophyll and TP may have changed over the past four decades. Therefore the analyses were repeated using data from just 2000-2004 to derive estimates of the precision of estimates of the annual mean concentrations which may be more appropriate for recent and forthcoming years. Within-period variability and hence estimates of SD_W and SD_{str} for different sampling frequencies in recent years appears to be less than for earlier periods (1968-1999) at Loch Leven (Tables 6.3 and 6.4).

Table 6.3 Average within-stratum standard deviation (SDW) in Chlorophyll (µg l^{-1}) and Log10 Chlorophyll concentrations for Loch Leven over each of the periods 1968-1999 and 2000-2004

%SD_W = *SD_W* expressed as a percentage of *SD_W* for the whole year period

Stratum/ Period	samples per year	Chlorophyll				Log_{10} Chlorophyll				
		1968-1999		2000-2004		1968-1999		2000-2004		
		SD_W	SE_{str}	SD_W	SE_{str}	SD_W	SE_{str}	SD_W	SE_{str}	CV_{str}
Whole year	1	34.3	34.3	24.8	24.8	0.333	0.333	0.321	0.321	109%
3-months	4	26.4	13.2	19.7	9.9	0.279	0.140	0.242	0.121	32%
2-months	6	22.2	9.1	18.9	7.7	0.242	0.099	0.207	0.085	21%
Monthly	12	18.6	5.4	14.7	4.2	0.201	0.058	0.163	0.047	11%
Fortnightly	26	15.3	3.0	5.3	1.0	0.140	0.027	0.107	0.021	5%

Table 6.4 Average within-stratum standard deviation (*SD_W*) in total phosphorus (µg l^{-1}) and Log_{10} TP concentrations for Loch Leven over each of the periods 1968-1999 and 2000-2004

%SD_W = *SD_W* expressed as a percentage of *SD_W* for the whole year period.

Stratum/ Period	samples per year	TP				Log_{10} TP				
		1968-1999		2000-2004		1968-1999		2000-2004		
		SD_W	SE_{str}	SD_W	SE_{str}	SD_W	SE_{str}	SD_W	SE_{str}	CV_{str}
Whole year	1	36.9	36.9	23.0	23.0	0.187	0.187	0.170	0.170	48%
3-months	4	30.3	15.2	15.9	8.0	0.142	0.071	0.113	0.057	14%
2-months	6	28.1	11.5	15.5	6.3	0.131	0.053	0.099	0.040	10%
Monthly	12	24.3	7.0	10.3	3.0	0.112	0.032	0.073	0.021	5%
Fortnightly	26	21.4	4.2	9.0	1.8	0.098	0.019	0.059	0.012	3%

6.3 South Basin of Windermere

Chlorophyll and total phosphorous sample values were obtained for the South Basin of Windermere at approximately fortnightly intervals over the period 1992-2004. The same statistical methods described above for Loch Leven were used estimate the likely sampling precision of estimates of annual mean concentration for South Basin (Tables 6.5 and 6.6). Estimates were based on within-period variability over the whole time series and based on the most recent five years only (2000-2004). August to October 2001 had much higher (and more variable) levels of chlorophyll-a than at any time before or after (Figure 6.1) and this influences the estimates of average SE for each sampling regime. Obviously, with only one sample per fortnight, it was not possible to derive an estimate of the SD of variability within any two-week period. Although the within-fortnight variability is likely to be less than the within-month variability, the latter can be used as an upper estimate to derive an approximate estimate of the precision of an annual mean concentration based on a regular fortnightly sampling scheme (given in brackets in Tables 6.5 and 6.6).

Table 6.5 Average within-stratum standard deviation (SD_W) in Chlorophyll (μg chl l^{-1}) and Log_{10} Chlorophyll concentrations for South Basin of Windermere over each of the periods 1992—2004 and 2000-2004

%SD_W = SD_W expressed as a percentage of SD_W for the whole year period.

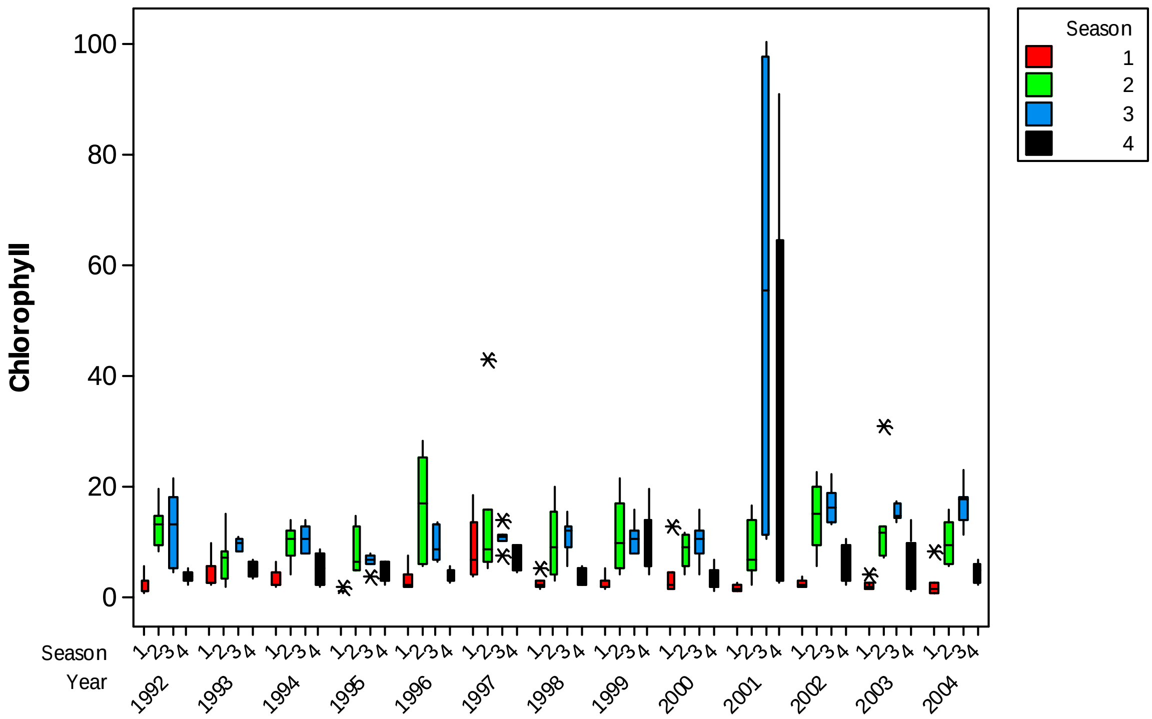
Stratum/ Period	samples per year	Chlorophyll				Log_{10} Chlorophyll				
		1992-2004		2000-2004		1992-2004		2000-2004		
		SD_W	SE_{str}	SD_W	SE_{str}	SD_W	SE_{str}	SD_W	SE_{str}	CV_{str}
Whole year	1	10.51	10.51	15.4	15.4	0.398	0.398	0.462	0.462	190%
3-months	4	8.36	4.18	12.4	6.2	0.238	0.119	0.283	0.142	39%
2-months	6	6.60	2.69	9.2	3.8	0.218	0.089	0.249	0.102	26%
Monthly	12	5.31	1.53	7.1	2.1	0.170	0.049	0.188	0.054	13%
Fortnightly	26	-	-	-	(1.4)	-	-	-	-	-

Table 6.6 Average within-stratum standard deviation (SD_W) in total phosphorus (μg TP l^{-1}) and Log_{10} TP concentrations for South Basin of Windermere over each of the periods 1992-2004 2000-2004

%SD_W = SD_W expressed as a percentage of SD_W for the whole year period

Stratum/ Period	samples per year	TP				Log_{10} TP				
		1992-2004		2000-2004		1992-2004		2000-2004		
		SD_W	SE_{str}	SD_W	SE_{str}	SD_W	SE_{str}	SD_W	SE_{str}	CV_{str}
Whole year	1	5.59	5.59	5.05	5.05	0.187	0.187	0.118	0.118	31%
3-months	4	4.73	2.37	4.55	2.28	0.142	0.071	0.106	0.053	13%
2-months	6	4.45	1.82	4.42	1.80	0.131	0.053	0.102	0.042	10%
Monthly	12	4.32	1.25	4.01	1.16	0.112	0.032	0.092	0.027	6%
Fortnightly	26	-	-	-	(0.79)	-	-	-	-	-

Figure 6.1 Boxplots of chlorophyll concentrations (µg chl l^{-1}) in South Basin of Windermere for each season over the period 1992-2004
(1=Jan-Mar, 2=Apr-Jun, 3=Jul-Sep, 4=Oct-Dec)



6.4 Implications for assignment of lakes to ecological status classes

The WFD specifies that lakes (and other water bodies) should be assigned to ecological status classes ("high", "good", "moderate", "poor" or "bad") on the basis of their selected biological metrics or other appropriate parameters. The UK Technical Advisory Group (UKTAG) assisting with the implementation of the WFD for lakes have derived provisional status class boundary values for both chlorophyll and total phosphorus. The critical values are given for the high/good (H/G) and good/moderate (G/M) boundary and are specific to each type of lake (Table 6.7). In addition, it has been suggested that the moderate/poor (M/P) and poor/bad (P/B) boundary values could be set at two and four times the G/M boundary value respectively; these values have been used in the analyses below assessing uncertainty in status class assignment.

Table 6.7 Ecological status class high/good (H/G) and good/moderate (G/M) boundary values for chlorophyll (μg l^{-1}) and total phosphorus (μg l^{-1})

Values given are for a range of lakes, based on lake type and using the 90% percentile method and values given in Table 1 of the 'UKTAG Technical Report on Environmental Standards'. M/P boundary = 2 x G/M; P/B boundary = 4 x G/M. Reference condition values are also given where available.

Lake	Lake Type	Chlorophyll		Total Phosphorus		
		H/G	G/M	Reference (MEI_TP)	H/G	G/M
Loch Leven	HA, Sh	11	18	13.9	29	46
South Basin (Windermere)	MA, D	4	6	5.7	10	22
North Basin (Windermere)	MA, D	4	6		10	22
Bassenthwaite Lake	MA, S	8	12		19	30
Blelham Tarn	MA, S	8	12		19	30
Derwent Water	LA, S	4	6		10	16
Esthwaite water	MA, S	8	12		19	30
Grasmere	LA, S	4	6		10	16

If the class boundaries for a metric or parameter are known, together with an estimate of the sampling precision of the metric/parameter, then its possible to calculate the statistical uncertainty associated with assigning a lake to a ecological status class on the basis of its estimated value of the metric/parameter. Clarke *et al*. (1996) and Clarke (2000) discuss the general principles of assessing uncertainty in assignment of sites and water bodies to biological quality grades and ecological status classes and give the statistical mathematics to calculate mis-classification rates.

More specifically, for the current study of lakes, for any particular value for the estimate of the annual mean concentration of chlorophyll (or total phosphorus), the estimates of standard error (SE_{str} in Table 6.3-6.6) can be used to calculate the probability that the true status class of the lake is different form its observed class based on the estimate of annual mean. This mis-classification probability (P_M) depends the standard error (SE_{str}) of the estimate of annual mean, which depends on the sampling frequency over the year. Figures 6.2-6.5 show the probabilities of mis-classification using the five WFD status classes associated with each of a wide range of values of estimated annual mean concentration of chlorophyll and total phosphorus for Loch Leven and the South Basin of Windermere. (These calculations are based on assuming a normal distribution for the sampling distribution of the estimator of the annual mean with a constant variance, regardless of the value of the estimate).

In these initial analyses, the mis-classification rates are the overall probabilities that the true status class of a lake is different from its observed status class. For example, if the observed status of a lake is 'good' based on the estimate of its annual mean concentration, then the misclassification rate is the sum of the probability that the true status could be 'high' and the probability that the true status could be 'moderate'. However, the practical consequences of being 'moderate' could be much greater than of being high – further analyses of uncertainty based on using just the good/moderate boundary to divide lakes into two major classes are given below.

As a explanation of Figures 6.2-6.5, consider the uncertainty in estimates of status class for Loch Leven based on an estimate of annual mean chlorophyll concentration shown in Figure 6.2.
The H/G and G/M boundary values are 11 and 18 μg chl_a l^{-1} for this type of lake (Table 6.7). If the estimate of annual mean is 14.5 μg chl_a l^{-1}, then although this places the lake in the middle of the 'good' status class, if the estimate was based on a single sample during the year, the standard error is so great (SE_{str} = 24.8) that the true class is very likely to be either better or worse, with probability P_M = 89% (Figure 6.2). If the same estimate of annual mean is based on quarterly, two-monthly, monthly or fortnightly samples, then the mis-classification rate reduces to 72%, 65%, 40% and almost zero respectively. The estimated great reduction in uncertainty in moving from monthly to fortnightly sampling is because the latter sampling frequency greatly reduces the within-period variation (SD_W reduces from 14.7 to 5.3 when averaged over the most recent five years) and thus when combined with the increase in number of sample, the estimate SE of the estimate of annual mean is reduced from 4.2 to 1.0 μg chl_a l^{-1} (Table 6.3).

Mis-classification rates are generally less for the poorer quality status classes becuase their class widths cover a wider range of concentrations, but in these analyses the sampling standard error (SE_{str}) is assumed to be the same for all estimates of annual mean concentration.

It may be more appropriate to assume that sampling variability is more nearly constant on a logarithmic scale and use the estimate of SE_{str} in Tables 6.3-6.6 based on the $\log_{10}$ transformed concentrations.

Although, the above analyses assess the overall uncertainty with assigning lakes to one of the five WFD status classes, 'high', 'good', 'moderate', 'poor' and 'bad', most current interest is in assessing whether a water body is in 'good' or better ecological status, or whether it is of 'moderate' or worse status. Obviously sites whose condition places them on or very near the good/moderate (G/M) border in terms of the critical parameter must have at least a 50% chance of being assignment to the wrong status class. One aim is to determine how far the estimate of annual mean concentration needs to be below the boundary value to be confident that it is truly of good or better status; and equivalently how far above the boundary to be confident that it is really of moderate or worse status.

Therefore the above analyses to quantify mis-classification rates were repeated using only the G/M boundary and just two classes 'good or better' and 'moderate or worse'. Figures 6.6-6.9 and accompanying Tables 6.8-6.11 show the probability of mis-classifying sites either side of the G/M boundary in relation to their observed sample value for the annual concentration mean of chlorophyll and total phosphorus for Loch Leven and the South Basin of Windermere.

Figure 6.2 Probability (P_M) of mis-classifying the ecological status of Loch Leven in relation to the estimate of annual mean chlorophyll concentration ($\mu g\ l^{-1}$)
Probabilities based on 1, 4, 6, 12 and 26 regular samples per year. Status class boundary values as in Table 6.7.

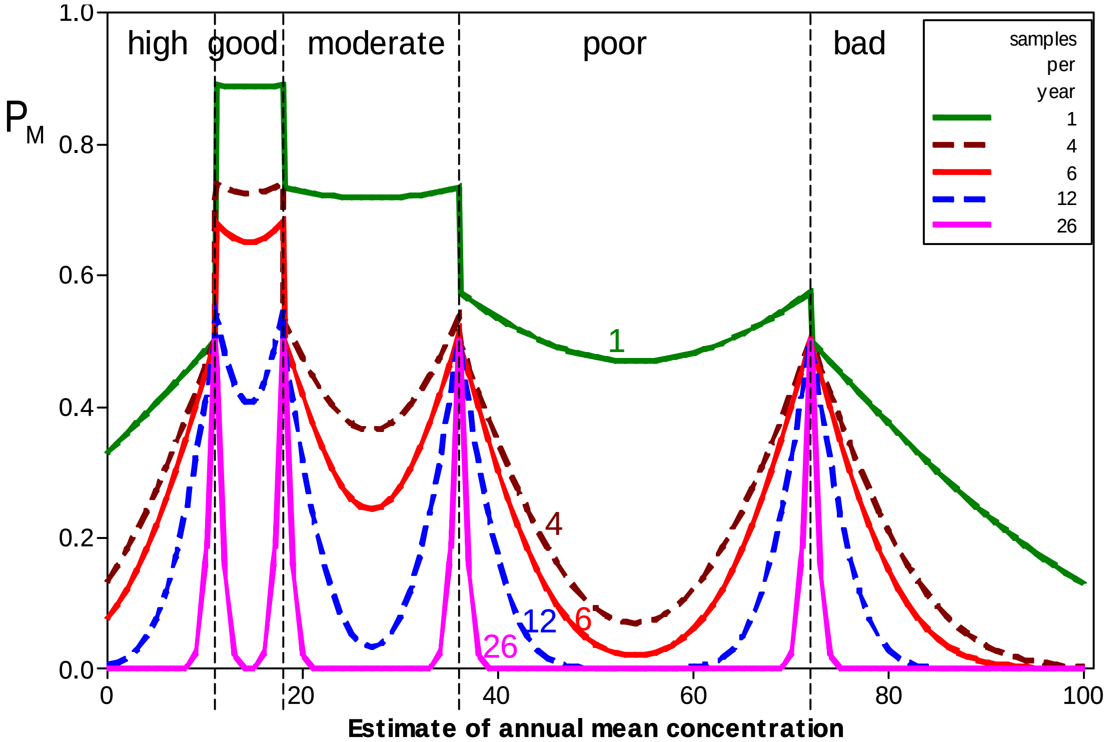


Figure 6.3 Probability (P_M) of mis-classifying the ecological status of Loch Leven in relation to the estimate of annual mean TP concentration ($\mu g\ l^{-1}$)
Probabilities based on 1, 4, 6, 12 and 26 regular samples per year. Status class boundary values as in Table 6.7

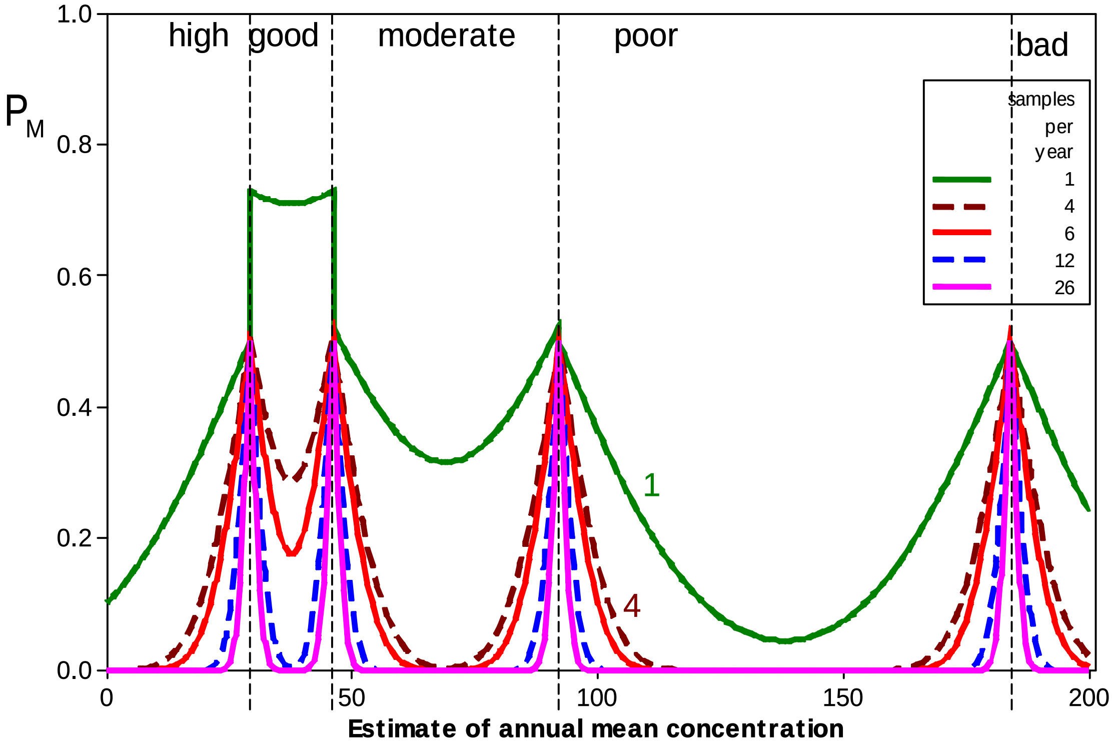


Figure 6.4 Probability (P_M) of mis-classifying the ecological status of South Basin of Windermere in relation to the estimate of annual mean chl$_a$ concentration (µg l^{-1})
Probabilities based on 1, 4, 6, 12 and 26 regular samples per year. Status class boundaries as in Table 6.7

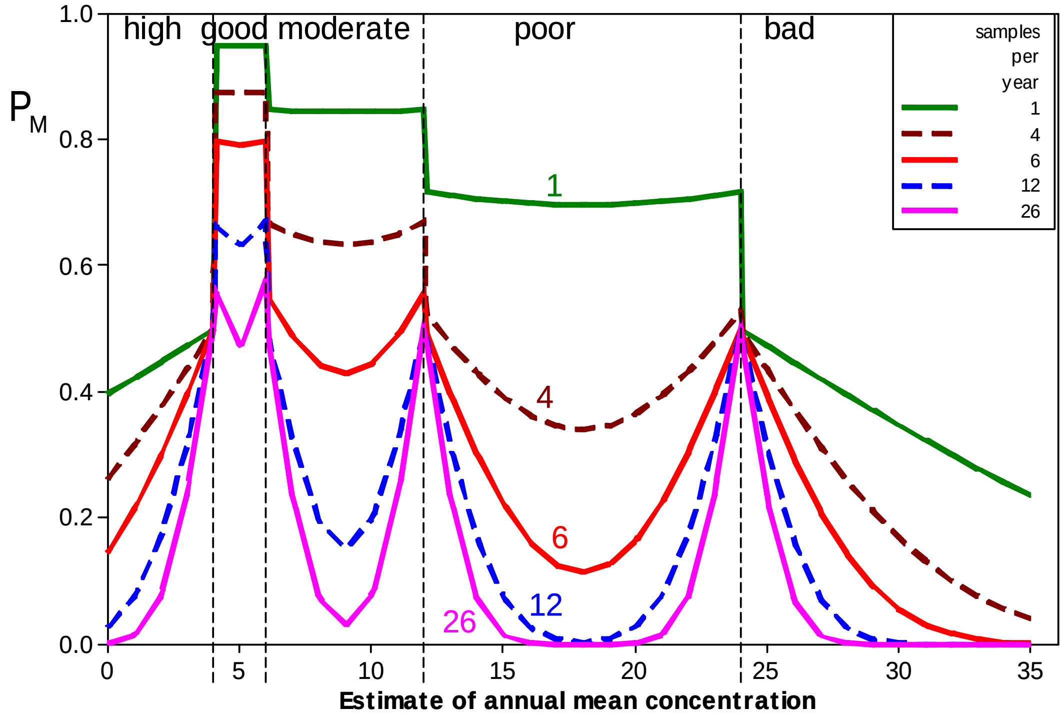


Figure 6.5 Probability (P_M) of mis-classifying the ecological status of South Basin of Windermere in relation to the estimate of annual mean TP concentration (µg l^{-1})
Probabilities based on 1, 4, 6, 12 and 26 regular samples per year. Status class boundaries as in Table 6.7

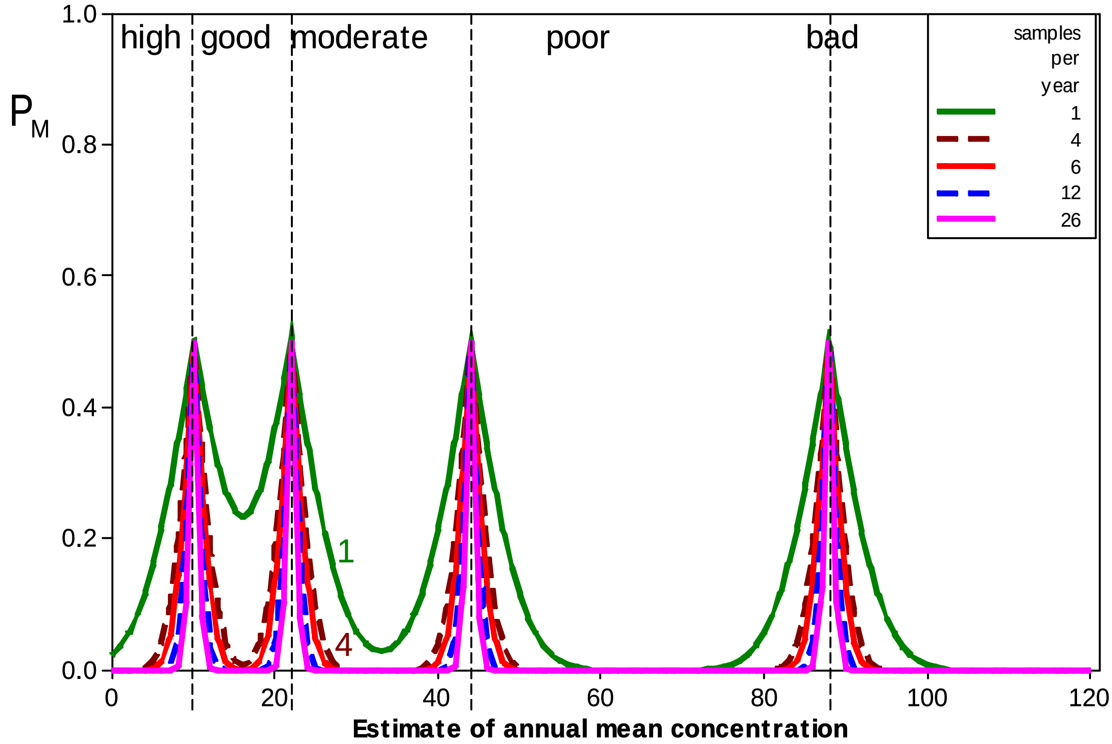


Figure 6.6 Probability (P_M) of mis-classifying the ecological status of Loch Leven in relation to the estimate of annual mean chlorophyll concentration (µg l^{-1})
Based on 1, 4, 6, 12 and 26 regular samples per year. G/M boundary =18 µg chl_a l^{-1}

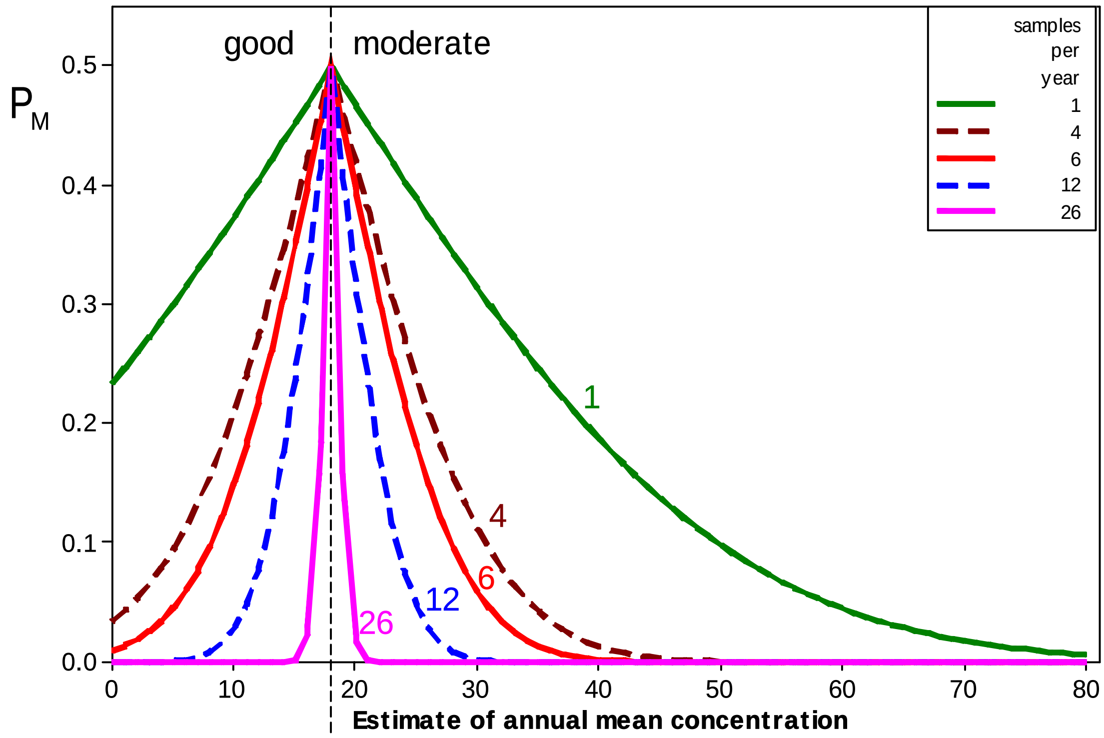


Table 6.8 Probability (P_M) of mis-classifying the ecological status (Good or better versus Moderate or worse) of Loch Leven in relation to the estimate of annual mean chlorophyll concentration (µg l^{-1})
Based on 1, 4, 6, 12 and 26 regular samples per year. G/M boundary =18 µg chl_a l^{-1}

Estimate of annual mean concentration	samples per year					Estimated status
	1	4	6	12	26	
1	0.247	0.043	0.014	0.000	0.000	high
5	0.300	0.095	0.046	0.001	0.000	
8	0.343	0.156	0.097	0.009	0.000	
11	0.389	0.240	0.182	0.048	0.000	
15	0.452	0.381	0.348	0.238	0.001	good
17	0.484	0.460	0.448	0.406	0.159	
17.99	0.500	0.500	0.500	0.500	0.500	
18.01	0.500	0.500	0.500	0.500	0.500	moderate
19	0.484	0.460	0.448	0.406	0.159	
21	0.452	0.381	0.348	0.238	0.001	
22	0.436	0.343	0.302	0.170	0.000	
25	0.389	0.240	0.182	0.048	0.000	
28	0.343	0.156	0.097	0.009	0.000	
35	0.247	0.043	0.014	0.000	0.000	
40	0.188	0.013	0.002	0.000	0.000	
45	0.138	0.003	0.000	0.000	0.000	
50	0.098	0.001	0.000	0.000	0.000	
60	0.045	0.000	0.000	0.000	0.000	
70	0.018	0.000	0.000	0.000	0.000	

Figure 6.7 Probability (P_M) of mis-classifying the ecological status of Loch Leven in relation to the estimate of annual mean TP concentration ($\mu g\ l^{-1}$)
Based on 1, 4, 6, 12 and 26 regular samples per year. G/M boundary = 46 $\mu g\ l^{-1}$

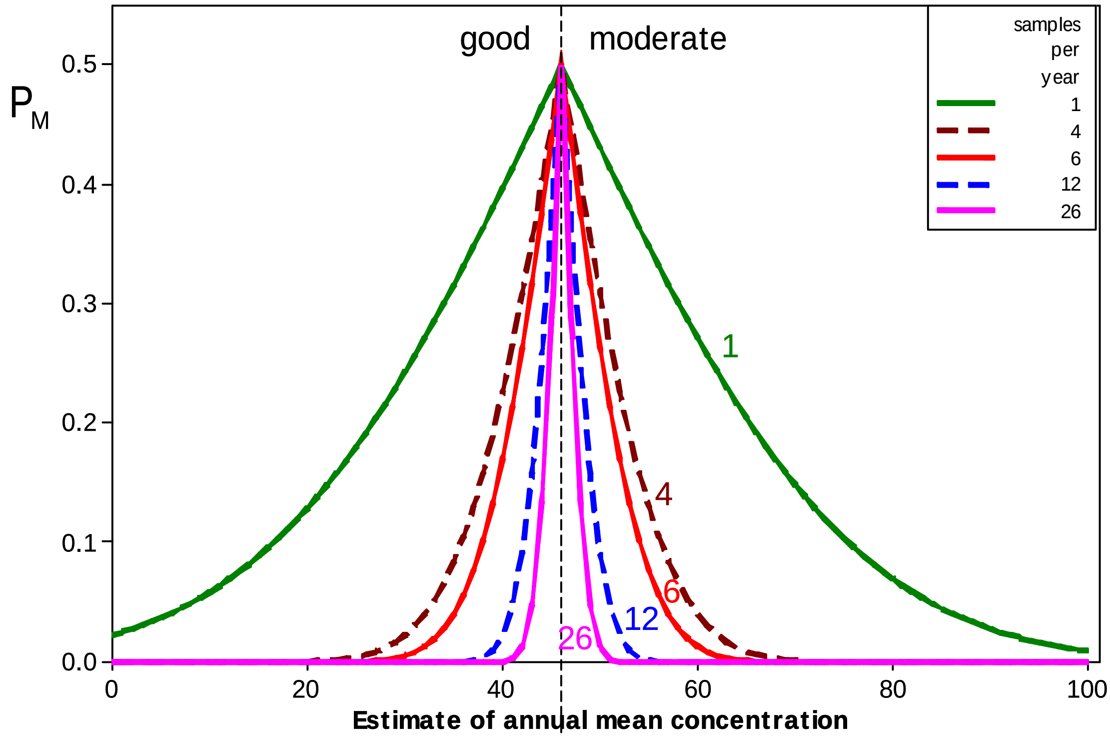


Table 6.9 Probability (P_M) of mis-classifying the ecological status (Good or better versus Moderate or worse) of Loch Leven in relation to the estimate of annual mean TP concentration ($\mu g\ l^{-1}$)
Based on 1, 4, 6, 12 and 26 regular samples per year. G/M boundary = 46 $\mu g\ l^{-1}$

Estimate of annual mean concentration	samples per year					Estimated status
	1	4	6	12	26	
20	0.129	0.001	0.000	0.000	0.000	high
29	0.230	0.017	0.003	0.000	0.000	
35	0.316	0.085	0.040	0.000	0.000	good
40	0.397	0.227	0.170	0.023	0.000	
41	0.414	0.266	0.214	0.048	0.003	
42	0.431	0.309	0.263	0.091	0.013	
43	0.448	0.354	0.317	0.159	0.048	
44	0.465	0.401	0.375	0.252	0.133	
45	0.483	0.450	0.437	0.369	0.289	
45.99	0.500	0.500	0.500	0.500	0.500	
46.01	0.500	0.500	0.500	0.500	0.500	moderate
47	0.483	0.450	0.437	0.369	0.289	
48	0.465	0.401	0.375	0.252	0.133	
49	0.448	0.354	0.317	0.159	0.048	
50	0.431	0.309	0.263	0.091	0.013	
51	0.414	0.266	0.214	0.048	0.003	
52	0.397	0.227	0.170	0.023	0.000	
53	0.380	0.191	0.133	0.010	0.000	
60	0.271	0.040	0.013	0.000	0.000	

Figure 6.8 Probability (P_M) of mis-classifying the ecological status of South Basin of Windermere in relation to the estimate of annual mean chl$_a$ concentration (µg l^{-1})
Based on 1, 4, 6, 12 and 26 regular samples per year. G/M boundary = 6 µg l^{-1}

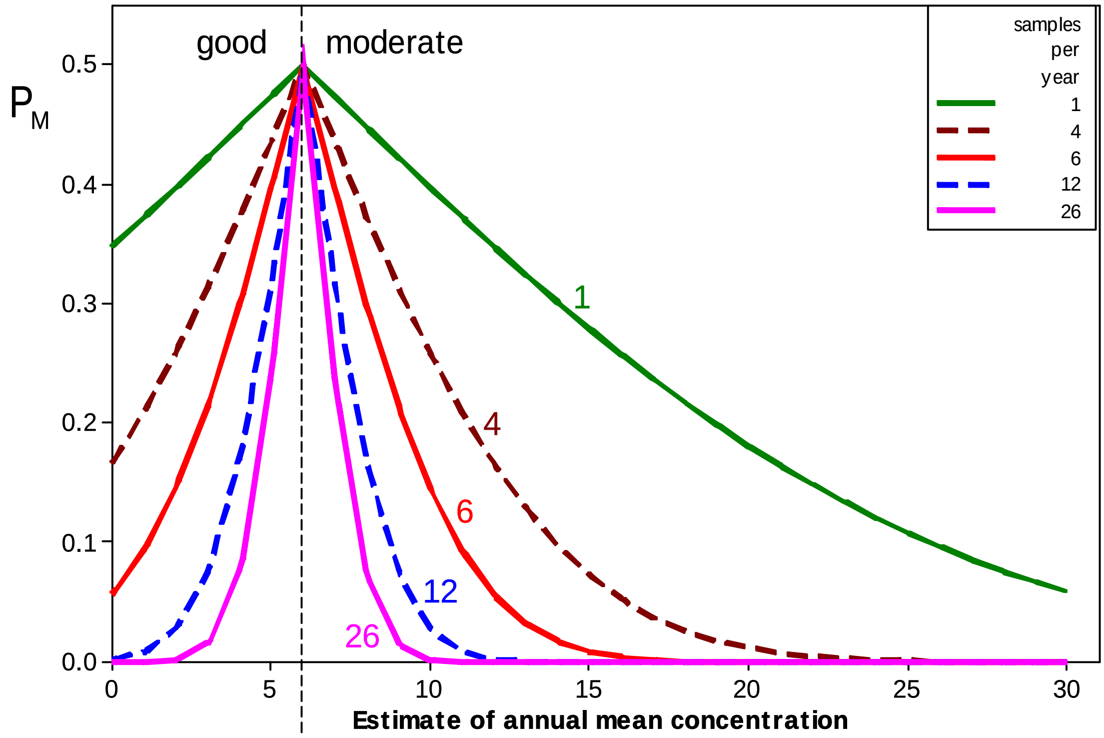


Table 6.10 Probability (P_M) of mis-classifying the ecological status (Good or better versus Moderate or worse) of South Basin of Windermere in relation to the estimate of annual mean chlorophyll concentration (µg l^{-1})
Based on 1, 4, 6, 12 and 26 regular samples per year. G/M boundary =6 µg chl$_a$ l^{-1}.

Estimate of annual mean concentration	samples per year					Estimated status
	1	4	6	12	26	
1	0.373	0.210	0.094	0.009	0.000	high
2	0.398	0.259	0.146	0.028	0.002	
3	0.423	0.314	0.215	0.077	0.016	
4	0.448	0.374	0.299	0.170	0.077	
4.5	0.461	0.404	0.347	0.238	0.142	good
5	0.474	0.436	0.396	0.317	0.238	
5.5	0.487	0.468	0.448	0.406	0.360	
5.99	0.500	0.500	0.500	0.500	0.500	
6.01	0.500	0.500	0.500	0.500	0.500	moderate
6.5	0.487	0.468	0.448	0.406	0.360	
7	0.474	0.436	0.396	0.317	0.238	
7.5	0.461	0.404	0.347	0.238	0.142	
8	0.448	0.374	0.299	0.170	0.077	
9	0.423	0.314	0.215	0.077	0.016	
10	0.398	0.259	0.146	0.028	0.002	
11	0.373	0.210	0.094	0.009	0.000	
12	0.348	0.167	0.057	0.002	0.000	
15	0.279	0.073	0.009	0.000	0.000	
20	0.182	0.012	0.000	0.000	0.000	

Figure 6.9 Probability (P_M) of mis-classifying the ecological status of South Basin of Windermere in relation to the estimate of annual mean TP concentration (µg l^{-1})
Based on 1, 4, 6, 12 and 26 regular samples per year. G/M boundary =22 µg TP l^{-1}

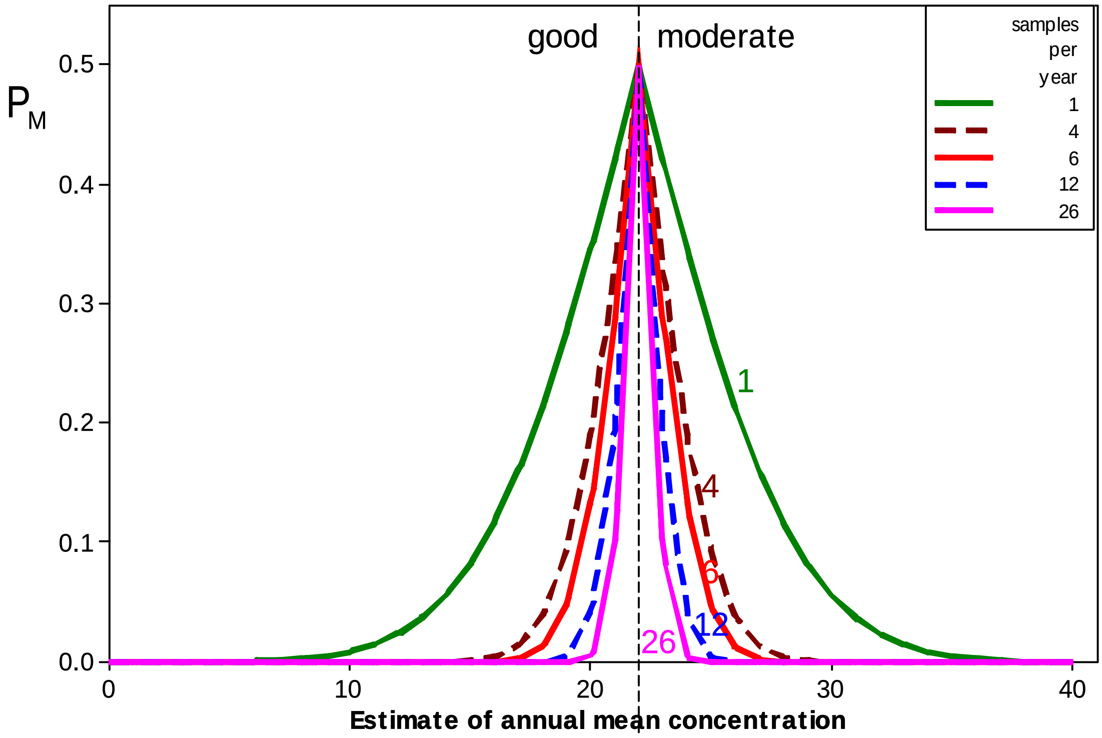


Table 6.11 Probability (P_M) of mis-classifying the ecological status (Good or better versus Moderate or worse) of South Basin of Windermere in relation to the estimate of annual mean TP concentration (µg l^{-1})
Based on 1, 4, 6, 12 and 26 regular samples per year. G/M boundary = 22 µg TP l^{-1}

Estimate of annual mean concentration	samples per year					Estimated status
	1	4	6	12	26	
10	0.009	0.000	0.000	0.000	0.000	high
15	0.083	0.001	0.000	0.000	0.000	good
17	0.161	0.014	0.003	0.000	0.000	
18	0.214	0.040	0.013	0.000	0.000	
19	0.276	0.094	0.048	0.005	0.000	
20	0.346	0.190	0.133	0.042	0.006	
21	0.422	0.330	0.289	0.194	0.103	
21.5	0.461	0.413	0.391	0.333	0.263	
21.99	0.500	0.500	0.500	0.500	0.500	
22.01	0.500	0.500	0.500	0.500	0.500	moderate
22.5	0.461	0.413	0.391	0.333	0.263	
23	0.422	0.330	0.289	0.194	0.103	
23.5	0.383	0.255	0.202	0.098	0.029	
24	0.346	0.190	0.133	0.042	0.006	
25	0.276	0.094	0.048	0.005	0.000	
26	0.214	0.040	0.013	0.000	0.000	
27	0.161	0.014	0.003	0.000	0.000	
28	0.117	0.004	0.000	0.000	0.000	
30	0.057	0.000	0.000	0.000	0.000	

6.5 Use of STARBUGS

If the class boundaries are converted to log scale, then it is possible to use the above estimates of the standard error (SE_{str}) on the log scale to estimate the uncertainty associated with the assignment of a particular lake to one or more status classes. This could be done using the STARBUGS (STAR Bioassessment Uncertainty Guidance Software) which CEH Dorset has developed within the EU STAR project led by CEH Dorset (www.eu-star.at).

STARBUGS can use the SE_{str} value to simulate the range of possible values that could have been obtained by this sampling scheme and hence deduce estimates of the probabilities that the lake could have been assigned to each status class.

For example, with log concentrations boundaries of 'high'/'good' of 1.30 (= 20µg l^{-1}) and of 'good'/moderate' of 1.60 (= 20µg l^{-1}), an observed estimate of (log) annual mean of 1.54 (= 35µg l^{-1}) and a SE_{str} of 0.139 (4 samples per year in Table 1) would have say a 5% probability of being assigned to 'high', an 80% probability of being classed in the observed class of 'good' and a 15% probability of being classed as 'moderate'. With single parameter/metric assessments, these probabilities could be done by hand, but the real power of STARBUGS is to assess uncertainty in status class assignment based on two or more metrics (e.g. chlorophyll and total phosphorus) and multi-metric rules (e.g. worst of the classes based on individual parameters/metrics). Obviously external estimates of individual parameter SE are still needed.

6.6 Estimating uncertainty for other lakes

The problem for other lakes is when we do not have a weekly regular sampling data over several years and hence we do not a decent estimate of the underlying true pattern of temporal variation. The choices are either to sample intensively for one or more years before making a decision on sampling frequency, or to learn from other lakes (ideally of similar physical and biological dynamic properties) with long-term historical data.

7. Summary and application of the classifications

Geoff Phillips, Laurence Carvalho and Sian Davies

7.1 Reference Conditions

The analysis clearly showed that chlorophyll and phosphorus reference conditions at a site are a response to a number of factors, with significant relationships with mean lake depth and alkalinity. Because of this gradient in response, type-specific reference conditions are not recommended, as they will lead to large errors in classification for sites near typology boundaries. It is, therefore, recommended that site-specific reference conditions are derived using a modelling approach. Where these data are not available it is proposed that the reference value is based on the type value agreed by the GIGs, or the median value of all GB modelled data for lake types not subject to intercalibration (Table 7.1)

The project concluded that the ideal site-specific model to use would have been a single model that predicts reference chlorophyll from mean depth and alkalinity directly (Section 2.3.2). However, this model predicted reference values which were generally greater than the range of type specific reference values agreed by the GIGs, particularly for high alkalinity shallow and very shallow lakes. The reasons for the differences between modelled UK reference values and those agreed by the GIGs are due to the type specific approach adopted by the GIGs with only a limited range of reference values. The assumption made by the GIG was that other values reflected varying degrees of impact, rather than natural variability caused by a range of typology variables.

For example, the range of values allowed by the Central GIG for L-CB1 (HA_S lakes) are 2.6 to 4.2 µg/l (Table 7.1), despite the fact that the GIG dataset of lakes of this type highlight that 25% of reference lakes have values higher than 5.8 µg/l. The GIG proposed minimum and maximum values show a large discontinuity between the two lake types. On this basis a lake of depth 3.1 m is likely to have a much more stringent reference condition than a lake of 2.9 m. Figure 7.1 highlights how the UK modelling approach shows a smooth gradient in potential reference conditions across the depth boundary compared with the L-CB1 and L-CB2 limits.

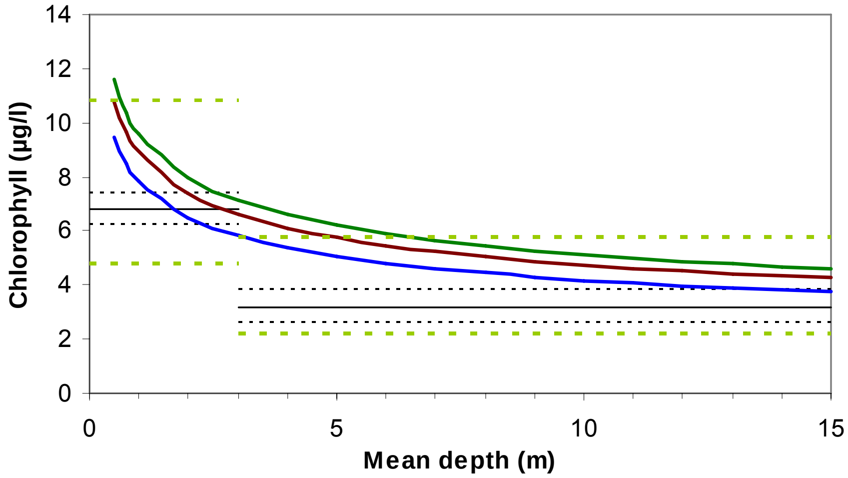


Figure 7.1 Predicted reference chlorophyll values for lakes across a depth range (shallow and very shallow) based on the UK model

Lines represent three high alkalinity values (1, 2 and 3 m equiv./l, blue, red, green solid lines respectively). Values are compared against the equivalent minimum and maximum (black, dotted lines) Central GIG limits for L-CB1 (3-15 m depth range) and L-CB2 lakes (0-3 m depth range). Median (black, solid line) and 25th and 75th % (green, dashed line) of reference population of L-CB1 and L-CB2 lakes are also shown.

Another reason that the UK model-derived values are generally higher is almost certainly due to the very limited data available from UK reference lakes, with no shallow high alkalinity lakes in the model and only two very shallow, high alkalinity lakes, so UK model-derived reference conditions for any high alkalinity lake are likely to be greatly influenced by these two points. Additionally, there may also be a bias in the GIG datasets in terms of typology coverage and in terms of the choice of reference sites; with only sites of very low chlorophyll concentrations being accepted as reference sites.

An alternative approach for the UK to predict site-specific reference chlorophyll values are from reference TP concentrations. This involves using the GB calibrated Morpho-Edaphic Index model (Section 3.3.2) to derive site-specific reference TP concentrations, which can then converted to reference chlorophyll concentrations using the type-specific TP-chlorophyll regression equations derived from the large European REBECCA and GIG datasets. This approach predicted lower reference chlorophyll values in much closer agreement with other Member States and for this reason, following discussion with the Lake Task Team, it was agreed to adopt this approach. The range of lake specific reference chlorophyll values that results from this method are shown in Table 7.1.

For shallow and very shallow lakes it was noted that this approach also tended to predict reference chlorophyll values that were greater than those defined by the GIGs. In these lake types there was considerable scatter in the TP chlorophyll regression, with several lakes showing low chlorophyll relative to TP. In shallow and very shallow lakes it is well established that grazing and interactions with macrophytes can limit phytoplankton biomass. These interactions would be particularly important when a lake is in reference condition and thus when modelling reference chlorophyll from reference phosphorus in these lake types an equation based on the lower 25th percentile of the TP-Chlorophyll regression residuals has been used (Box 1). In all cases, the modelled site specific reference values are compared with those established by Intercalibration and values are truncated to remain within the agreed GIG range.[6]

Box 1. Equations describing the relationships between chlorophyll and total phosphorus concentrations in different lake types

All deep lakes: $Log\ Chl = -0.220(\pm0.041) + 0.731(\pm0.046)\ Log\ TP$ $r^2 = 0.57$[7]

Low & moderate alkalinity shallow and very shallow lakes:
$Log\ Chl = -0.512(\pm0.028) + 1.105(\pm0.021)\ Log\ TP - 0.095$ $r^2 = 0.78$[8]

High alkalinity shallow lakes:
$Log\ Chl = -0.436(\pm0.073) + 0.862(\pm0.045)\ Log\ TP - 0.206$ $r^2 = 0.32$[9]

High alkalinity very shallow lakes:
$Log\ Chl = -0.500(\pm0.163) + 0.994(\pm0.099)\ Log\ TP - 0.181$ $r^2 = 0.68$[10]

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[6] At the time of writing it is still uncertain if the commission will accept a range of reference value for each lake type. If this is not accepted the use of site specific reference values will not be possible.

[7] Regression for all deep lakes (Group P3) in Rebecca data set

[8] Regression for low & moderate alkalinity shallow & very shallow lakes (Group P1) in Rebecca data set

[9] High alkalinty shallow lakes from Central GIG data set (Phillips, 2006 GIG report)

[10] High alkalinity very shallow lakes in Rebecca data set

7.2 High/Good Chlorophyll Boundary

Initially it was proposed that the H/G boundary should be based on the 75th percentile of the modelled residuals of all UK lakes within each type. However, as these models did not conform sufficiently closely to the range of reference values agreed during intercalibration this approach was not recommended for use. During intercalibration the HG boundary was determined as an upper percentile (75th or 90th depending on type) of a range of European reference sites (see Section 2.3.1). The EQR resulting from this boundary is thus defined by intercalibration and will be used to establish the H/G boundary for all of the UK lake types subject to intercalibration. For lake types not subject to intercalibration the EQR for the same depth type of intercalibrated lakes was used.

7.3 Good/Moderate Chlorophyll Boundary

In line with WFD normative definitions, it is recommended that the G/M boundary should be set based on the secondary effects of elevated phytoplankton biomass. A range of effects could be considered, including the reduction in the depth distribution of submerged macrophytes, changes in composition of macrophytes and phytoplankton, particularly the abundance of cyanobacteria. This project was only able to consider the effects of chlorophyll on the depth distribution of macrophytes (Chapter 4). Values in the range of 14-30 µg/L and 6-16 µg/L were proposed for very shallow and shallow/deep lakes respectively. These values were based on modelled relationships. Subsequently further work carried out during intercalibration (Phillips 2006) tested the model on larger European data sets and concluded that for very shallow and shallow lakes values of 28 µg/L and 13 µg/L respectively were most appropriate as type-specific boundary values.

An alternative approach to boundary setting would be to determine essentially arbitrary boundaries, based on a regular division along the pressure gradient. This approach would be necessary where interactions with other quality elements cannot be determined, due to lack of data or lack of obvious discontinuities. Although it is proposed that sufficient data has now been made available, via this project and the intercalibration process, a comparison of the resulting boundaries with this simple approach was considered appropriate. The REBECCA database was used to determine the maximum or the 95th percentile of the growing season Chlorophyll a values for lakes in each of the core types. This was assumed to represent a 'worst case' scenario, and for most lake types assumed to be bad status. The proposed H/G boundary was then taken and distance between these divided into logarithmic intervals (Table 7.2)

Further work investigating relationships between chlorophyll a and cover of macrophytes in very shallow lakes, and phytoplankton species composition in all lake types, carried out during intercalibration, are reported in the Geographic Intercalibration Group Milestone 6 reports (Phillips et al., 2006; van den Berg et al., 2006). The boundaries agreed for the intercalibrated types are shown in table 7.1 together with values for the lake types not subject to intercalibration. For the non intercalibrated lake types EQR values were those derived from geometric divisions of the REBECCA data (Table 7.2).

7.4 Total Phosphorus Boundaries

It is proposed that the H/G boundary for TP should be based on the 75th percentile of the residuals of the GB calibrated MEI model. Thus the H/G boundary is given by

Log TP = 1.284 + 0.270 log(MEI) +0.137 which produces an EQR of 0.73

It is proposed that the G/M boundary for TP should be derived from the GM boundary for chlorophyll a using the TP-Chlorophyll regression relationships derived from the REBECCA and GIG data (Box 1). Different levels of precaution can be obtained by determining the TP value from the regression line or from lines based on different percentiles of the residuals of the regression. If the TP value is derived from the regression + the upper 75th percentile of the residuals and a particular boundary value for Chlorophyll then 75% of sites are likely to achieve this chlorophyll value for that specified TP concentration. A TP value based on the regression line would represent 50% and the regression + the lower 25th percentile, 25%. Equations used to calculate these values are shown in Table 7.3, together with the TP EQRs that would result, given the proposed EQRs for chlorophyll. Achieving the proposed TP HG EQR of 0.73 (see above) would on average, mean that 60-90% of lakes would achieve High status for chlorophyll. Currently SEPA use an EQR for TP on 0.50 to assess lake quality which would on average result in 60-90% of lakes being at Good status.

The amount of precaution that is used to establish appropriate TP boundary values is a matter of judgement. However, it is important to understand the level of uncertainty and to use an appropriate value. Based on the relationship between TP and chlorophyll and EQR of 0.50 for the GM boundary would provide a varying level of precaution for different lake types. Deep lakes would be highly protected with only a risk that 10% might not be at good status based on chlorophyll if the GM TP boundary was achieved. This might be appropriate to protect such lakes from deterioration, but would also result in significant numbers of lakes being at Good status on the basis of phytoplankton biomass, but at worse than Good status based on phosphorus. Such a lake may be at worse than Good status due to other quality elements sensitive to phosphorus, or it may be at risk of further change, particularly if phosphorus load remains high. It is thus difficult to provide a clear recommendation on TP boundary values that can be used to "protect" lake status and to "classify" status.

Given the current data and level of understanding it is suggested that to achieve the chlorophyll boundaries an EQR of 0.73 and 0.50 could be used with site specific reference TP values to establish site specific TP boundary values. However, the resulting values need to be compared with other quality elements sensitive to phosphorus such as macrophytes. To illustrate the use of these EQRs the resulting TP values are shown in Table 7.4.

7.5 Uncertainty and Data Issues

Currently there is great bias in the UK and European lake datasets towards shallow and deep low alkalinity lakes. The limited data available from some lake types biases the regression models and results in uncertain estimates of variability within lake types. For this reason, more effort is needed to identify and sample sufficient numbers of these under-represented lake types, particularly reference lakes. In the UK, most effort should probably be focused on very shallow lake types (low, medium and high alkalinity) and medium and high alkalinity lakes in general (all depth types). Peaty lochs may also require targeted attention. Measurements of the mean depth of many lakes and the humic content of Scottish lochs are also needed for characterising lake types accurately and developing improved models for classification.

Further work is required to determine variation in chlorophyll a summary statistics. This should include comparison of sampling methods (surface dip, integrated samples of photic zone, whole water column etc), analytical methods and season over which results are summarised. The analysis carried out for REBECCA used data summarised as April - September means as these were the most widely available data sets. The boundaries established by the GIGs are described as "Growing Season" means and were generally derived from data summarised between March - October or April - September. It is clear that in different parts of Europe climatic factors define substantially different growing seasons and it was the intention of the GIGs to allow member states to define what their particular growing season is based on climatic factors. Although April to September means are likely to be more appropriate to public water quality issues and secondary impacts on macrophytes and fish, examination of data from the UK demonstrates that significant chlorophyll concentrations can occur in all months of the year, including January and December in some shallow lakes.

Comparing annual with April - September mean values produced the following relationship:

April - Sept mean = 1.26 x Annual mean ($r^2 = 0.97$, $p<0.001$)

It is thus proposed that, for the UK, chlorophyll a is summarised as an annual metric, but that for comparison with GIG boundaries a conversion based on the above equation is used when assessing compliance.

7.6 Summary tables of reference conditions and boundary values

Table 7.1 Comparison of proposed UK and GIG mean chlorophyll a reference values, H/G and G/M boundaries and EQRs (GIG values shown are for growth season and equivalent values for annual means based on a conversion factor of 1.27) with proposed annual mean values for UK derived from modelled reference TP and EQRs agreed during intercalibration or (for non-intercalibrated types) from equal log divisions. Ranges for GIGs are those proposed in Milestone 6 reports, those for UK the 5th and 95th percentiles of modelled data. UK type values are the GIG type value for intercalibrated types or the median of modelled data for non intercalibrated types.

Lake Type		**Reference**		**High Good Boundary**			**Good Moderate Boundary**		
		Range	**Type**	**Range**	**Type**	**EQR**	**Range**	**Type**	**EQR**
High Alk Deep	Too few lakes to determine								
High Alk Shallow	L-CB1 growth season (L-CB1 annual) **UK annual**	2.6-4.2 2.0-3.3 **2.0-2.9**	3.2 2.5 **2.5**	4.7-7.6 3.7-6.0 **3.7-5.4**	5.8 4.6 **4.6**	**0.53**	8-13 6.3-10.2 **6.4-9.2**	10 7.9 **7.5**	**0.32**
High alk shallow on limestone or in N. Ireland	L-A1/2 growth season (L-A1/2 annual) **UK annual**	Type specific only	3 **2.4**	Type specific only	6 **4.8**	**0.50**	Type specific only	10 **7.9**	**0.30**
High Alk Vshallow	L-CB2 growth season *(L-CB2 annual)* **UK annual**	6.2-7.4 4.8-5.8 **4.8-5.8**	6.8 5.4 **5.4**	9.9-11.8 7.8-9.8 **7.8-9.2**	10.8 8.7 **8.6**	**0.63**	21-25 16.5-19.7 **16.5-19.4**	21 16.5 **16.5**	**0.32**
Mod Alk Deep (clear)	**UK**	**1.8-2.3**	**2.2**	**3.6-4.6**	**4.4**	**0.50**	**5.5-7.0**	**6.7**	**0.33**
Mod Alk Shallow	L-N1 growth season (L-N1 annual) **UK annual**	2.5-3.5 2.0-2.8 **2.2-2.8**	3 2.4 **2.4**	5-7 3.9-5.5 **4.3-5.5**	6 4.7 **4.7**	**0.50**	7.5-10.5 6.0-8.3 **6.5-8.4**	**9** 7.1 **7.2**	**0.33**
Mod Alk VShallow	**UK annual**	**3.8-7.6**	**5.2**	**6.0-12.1**	**8.3**	**0.63**	**11.2-22.4**	**15.3**	**0.34**
Low Alk Deep	L-N2b growth season (L-N2bannual) **UK annual**	1.5-2.5 1.2-2.0 **1.2-2.0**	2 1.6 **1.6**	3-5 2.4-4.0 **2.4-3.9**	4 3.2 **3.2**	**0.50**	4.5-7.5 3.6-6.0 **3.6-6.0**	**6** 4.8 **4.8**	**0.33**
Low Alk Shallow	L-N2a growth season (L-N2a annual) **UK annual**	1.5-2.5 1.2-2.0 **1.2-2.0**	2 1.6 **1.6**	3-5 2.4-3.9 **2.4-4.0**	4 3.2 **3.2**	**0.50**	5-8.5 3.9-6.8 **4.1-6.8**	7 5.5 **5.5**	**0.29**
Low Alk VShallow	**UK annual**	**1.3-4.3**	**2.6**	**2.1-6.3**	**4.1**	**0.63**	**3.9-12.1**	**7.9**	**0.33**

Table 7.2 Potential chlorophyll and EQR boundaries for all lake types

Based on geometric divisions of chlorophyll between the H/G boundary (derived from agreed intercalibration values) and the maximum observed mean chlorophyll value in the REBECCA summary database. All data represent growing season means (April - September). Maximum value for high alkalinity very shallow lakes (L-CB2 IC Type) was the 95th percentile rather than the maximum

					Boundaries at geometric intervals									
					Ref	HG	GM	MP	PB		HG	GM	MP	PB
Lake Type		**N all lakes**	**N Ref Lakes**	**Max**	**Chlorophyll a (µg/L)**						**EQR**			
Deep Lakes	Low alk deep clear L-N2b	96	71	25	2	4	6.3	10.0	15.8		0.50	0.32	0.20	0.13
	Mod alk deep			14	2.8	5.6	7	9	11		0.50	0.32	0.32	0.25
Shallow lakes	Low alk lowland shallow clear L-N2a	89	61	35	2.3	4.0	6.9	11.7	20.1		0.58	0.34	0.20	0.11
	Low alk lowland shallow humic LN3a	104	48	25	4.2	6.5	9.1	12.7	17.7		0.65	0.46	0.33	0.24
	Low alk lowland shallow polyhumic LN3b	43	16	32	13.8	18.8	24.3	31.5	40.8		0.73	0.57	0.44	0.34
	Low alk mid altitude shallow clear LN5	49	37	20	1.7	3.1	4.9	7.8	12.5		0.55	0.34	0.22	0.14
	Low alk mid altitude shallow humic LN6a	21	7	25	3.8	5.4	7.9	11.5	17.0		0.70	0.48	0.32	0.22
	Mod alk shallow L-N1	73	21	52	2.9	5.9	10.2	17.5	30.3		0.49	0.28	0.17	0.10
	Mod alk shallow humic L-N8a	68	8	50	7.8	11.1	16.3	23.7	34.6		0.70	0.48	0.33	0.23
	Mod alk lobelia L-CB3	10	18	33	3.1	5.4	8.5	13.3	21		0.57	0.37	0.23	0.15
	High alk shallow L-CB1	195	96	160	3.1	5.8	13.3	30.4	70		0.53	0.23	0.10	0.04
Very Shallow Lakes	Low alk very shallow			68	3.3	5.2	9.9	18.8	36		0.63	0.33	0.18	0.09
	Mod alk very shallow			119	6.6	10.5	19.2	35.4	65		0.63	0.34	0.19	0.10
	High alk very shallow L-CB2	177	40	219	6.8	10.8	22.9	48.6	103		0.63	0.30	0.14	0.07

Table 7.3 Equations used to predict total phosphorus from chlorophyll boundaries and resulting EQR values for total phosphorus

Values calculated using residuals of TP Chl regression to provide estimates of the likely number of lakes to achieve the desired chl_a boundary value for a given level of phosphorus.

Lake Type	Proportion protected	HG	GM	Equation used
Deep Lakes	50%	0.39	0.22	Log TP = (Log Chl +0.22)/0.731
	75%	0.52	0.29	Log TP = (Log Chl +0.22-0.093)/0.731
	90%	0.78	0.44	Log TP = (Log Chl +0.22-0.222)/0.731
Low Alk Shallow	50%	0.53	0.33	Log TP = (Log Chl +0.512)/1.105
	75%	0.85	0.52	Log TP = (Log Chl +0.512 -0.127)/1.105
	90%	1.04	0.63	Log TP = (Log Chl +0.512 -0.223)/1.105
Mod Alk Shallow	50%	0.53	0.37	
	75%	0.85	0.58	
	90%	1.04	0.71	
Low & Mod Alk very Shallow	50%	0.66	0.37	
	75%	1.05	0.58	
	90%	1.28	0.71	
High Alk Shallow	50%	0.48	0.27	Log TP = (Log Chl +0.436)/0.862
	75%	1.47	0.82	Log TP = (Log Chl +0.436-0.214)/0.862
	90%	2.42	1.35	Log TP = (Log Chl +0.436-0.400)/0.862
High Alk very Shallow	50%	0.63	0.32	Log TP = (Log Chl +0.500)/0.994
	75%	1.29	0.65	Log TP = (Log Chl +0.500-0.131)/0.994
	90%	2.09	1.06	Log TP = (Log Chl +0.500-0.338)/0.994

Table 7.4 Distribution of modelled reference annual mean total phosphorus values and resulting, high/good and good/moderate boundaries

Based on EQRs of 0.73 for H/G and 0.50 for GM. Range shows 5th and 95th percentiles of type population, type value is derived from the median value. (Values in brackets are draft values published in UKTAG environmental standards report)

Lake Type	Reference		High Good Boundary		Good Moderate Boundary	
	Range	Type	Range	Type	Range	Type
High Alk Deep	There are too few lakes of this type					
High Alk Shallow N=50	**13-20**	**16**	(16-23) **17-27**	(20) **22**	(28-40) **25-39**	(34) **32**
High Alk Vshallow, N=88	**16-32**	**23**	(20-36) **22-43**	(28) **31**	(33-56) **32-64**	(43) **46**
Mod Alk Deep N=12	**5-7**	**6**	(6-8) **6-9**	(7) **8**	(10-14) **9-13**	(13) **12**
Mod Alk Shallow, N=64	**7-13**	**9**	(9-15) **10-17**	(12) **13**	(13-21) **14-25**	(17) **19**
Mod Alk Vshallow, N=30	**11-25**	**16**	(15-25) **15-34**	(20) **22**	(21-36) **22-50**	(28) **32**
Low Alk Deep N=49	**3-5**	**4**	(4-6) **3-7**	(5) **5**	(6-11) **5-10**	(9) **8**
Low Alk Shallow, N=125	**3-8**	**6**	(5-9) **4-10**	(7) **8**	(7-13) **6-15**	(10) **11**
Low Alk Vshallow, N=35	**4-13**	**9**	(6-15) **6-18**	(11) **12**	(8-21) **9-27**	(15) **17**
Marl	As for Moderate Alkalinity					
Peat	As for Alkalinity type until further data can be obtained for humic lakes					

7.8 Recommendations for further work

Review standard sampling, storage and analysis protocols and provide detailed standard operating procedure

Following further data acquisition, revise regression models for estimating chlorophyll and TP reference conditions and H/G boundaries

Update guidance in line with WFD Intercalibration. In particular review chlorophyll (and TP) G/M boundary following further work on secondary impacts of chlorophyll on frequency/intensity of cyanobacteria blooms and on changes to macrophyte composition

Develop uncertainty/confidence in class work further – examining spatial sampling variability and analytical error

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Appendix 1: UK Reference lakes with sufficient chlorophyll data

Lake Code	Lake Name	GIG region	GIG type	Altitude (m a.s.l.)	Area (km^2)	Mean Depth (m)	Mean Alkalinity (m equiv. l^{-1})	Colour (Pt units)	Altitude Type	Area Type	Depth Type	Alkalinity Type	Humic Type
2490	Loch Hope	N	L-N2b	4	6.38	18.7	0.081	24	L	L	D	L	L
2712	Loch Watten	A	L-CB2	17	3.73	2.6	2.133	25	L	L	VS	H	L
3904	Loch Loyal	N	L-N2b	114	6.46	19.9	0.193	36	L	L	D	L	H
5222	Loch Meadie	N	L-N2a	146	2.11	6.3	0.054		L	L	S	L	L
5350	Loch Stack	N	L-N2a	36	2.52	10.9	0.076	22	L	L	S	L	L
6405	Loch Naver	N	L-N2a	73	5.59	11.9	0.067	37	L	L	S	L	H
8751	Loch Assynt	N	L-NX	65	8.00	30.8	0.491	22	L	L	D	M	L
10934	Cam Loch	N	L-N3b	124	2.53	11.5	0.182	43	L	L	S	L	H
11189	Loch Osgaig	N	L-N2a	26	1.68	14.3	0.050	28	L	L	S	L	L
11338	Loch Ailsh	N	L-NX	154	1.05	2.5	0.228	40	L	L	VS	M	H
11611	Loch Brora	N	L-N3b	25	0.67	6.9	0.159	84	L	L	S	L	H
14057	Loch Maree	N	L-N2b	6	27.98	38.2	0.053	17	L	L	D	L	L
16456	Loch Ussie	N	L-NX	128	0.82	2.4	0.435	27	L	L	VS	M	L
18825	Lochindorb	N	L-N6a	296	2.15	3.8	0.188	45	M	L	S	L	H
22782	Loch Rannoch	N	L-NX	206	18.81	51.0	0.112	24	M	L	D	L	L
24447	Loch Lomond	N	L-N2b	4	70.73	37.0	0.167	27	L	L	D	L	L
24459	Loch Lubnaig	N	L-N2a	121	2.32	13.0	0.172	16	L	L	S	L	L
28200	Woodhall Loch	N	L-N1	54	0.65	6.0	0.349	35	L	L	S	M	H
29000	Crummock Water	N	L-N2b	96	2.50	26.7	0.092	7	L	L	D	L	L
29052	Buttermere	N	L-N2b	103	0.91	16.6	0.088	3	L	L	D	L	L
29183	Wast Water	N	L-N2b	64	2.78	39.7	0.081	4	L	L	D	L	L
33836	Llyn Idwal	N	L-NX	370	0.13	3.4	0.064	5	M	S	S	L	L
36202	Upton Broad	CB	L-CB2	2	0.07	0.8	2.291	19	L	S	VS	H	L

Appendix 2: Protocols for sample collection, storage and analysis

It is recommended that a standard operating procedure is developed for collection, storage and analysis of samples for chlorophyll and TP. These should follow CEN guidance where available (e.g. CEN 1993a; 1993b; 2003) . APHA standards should also be considered (APHA, 1992)

A brief summary of appropriate methods for chlorophyll is described below:

Sampling	Open water, integrated sample preferred sampled from a boat If boat is not available, edge sampling is possible, preferably from the outflow. For the latter, a weighted bottle on a rope thrown from edge should be used to avoid edge/bottom sediment contamination Volume of water sampled is dependent on phytoplankton abundance – 1 litre generally sufficient, but may need more in very nutrient poor lochs when phytoplankton is sparse, or less in nutrient-rich waters in summer when phytoplankton is more abundant Regular sampling – recommended monthly sampling between April to September to calculate growing season mean and an additional winter sample taken in December or January to calculate a geometric annual mean
Storage	Filter on day of collection and store filter paper in cold, dark and alkaline conditons for short-term (24 hrs). Frozen if longer.
Analysis	Standard spectrophotometric techniques following methods outlined by Lorenzen (1967) and Strickland & Parsons (1968). 90%, cold, acetone extraction with grinding is recommended, with an acidification step to correct for degradation products – see APHA, 1992)

There has been much debate about the variability obtained by the spectrophotometric analytical methods, with HPLC considered more reliable (see Wiltshire et al., 1998)

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