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Chlorophyll reference conditions for European lake types used for intercalibration of ecological status

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Abstract The Water Framework Directive (WFD), requires European Member States to assess the "ecological status" of surface waters. As part of this, many European countries have developed an ecological quality classification scheme for chlorophyll concentrations as a measure of phytoplankton abundance. The assessment of ecological quality must be based on the degree of divergence of a water body from an appropriate baseline, or 'reference condition'. It is, therefore, necessary to determine chlorophyll reference conditions for all European lake types. This involves examining how chlorophyll concentrations vary by lake type, in the absence of any nutrient pressures from agriculture or wastewater. For this purpose, a dataset of 540 European lakes considered to be in a relatively undisturbed reference condition has been assembled, including data on chlorophyll concentration, altitude, mean depth, alkalinity, humic content, surface area and geographical region. Chlorophyll was found to vary with lake type and geographical region, and to be naturally highest in low-altitude, very shallow, high alkalinity and humic lake types and naturally lowest in clear, deep, low alkalinity lakes. The results suggest that light and mineral availability are important drivers of chlorophyll concentrations in undisturbed lakes. Descriptive statistics (median and percentiles) of chlorophyll concentrations were calculated from populations of lakes in this reference lake dataset and used to derive lake-type specific reference chlorophyll concentrations. These reference conditions can be applied, through a comparison with observed chlorophyll concentrations at a site, in the assessments of ecological status and provide a consistent baseline to adopt for European countries.

Keywords Lake · Phytoplankton · Water Framework Directive · Baseline · Rebecca

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Introduction

The use of chlorophyll concentrations as a general measure of lake water quality is widely adopted around the world. Yet, there has been very little scientific research examining how chlorophyll concentrations vary naturally, in the absence of nutrient pressures. The European Directive 2000/60/EC, commonly referred to as the Water Framework Directive (WFD), challenges this lack of understanding. The WFD prescribes the assessment of ecological quality of surface waters using an Ecological Quality Ratio (EQR). The EQR is defined as the relationship between the current observed value and the reference condition value for a given ecological quality element. Reference conditions are a state corresponding to very low pressure, with only minimal human impacts from industrialisation, urbanisation and intensive agriculture. Reference conditions differ across Europe resulting from geographical differences of catchments (geology and altitude) and 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 a part of the assessment of ecological quality, many European countries have chosen chlorophyll concentrations as a part of the ecological quality element phytoplankton. A large scale formal assessment of the comparability of national assessment schemes is also being carried out as a part of the implementation of the WFD—a process known as Intercalibration. Chlorophyll has been selected as a key parameter for this intercalibration process for lakes because of its recognition as a good general measure of ecological impact of eutrophication and wide-availability of its data. It is, therefore, essential to determine chlorophyll reference conditions for all WFD European lake types. The WFD requirement to define type-specific reference conditions, therefore, makes this analysis of the factors determining chlorophyll concentrations in the absence of nutrient pressure (i.e. reference conditions), of high topical interest to both freshwater scientists and policy makers across Europe.

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

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

The EC guidance (Anonymous 2003) suggests that approach no. 1, a validated spatial network of reference or minimally impacted lakes is preferred. For this study, we collated data from >500 European reference lakes (Moe et al. 2008, this issue). We aim to use this large dataset to estimate type-specific chlorophyll reference conditions for many intercalibration lake types (see Van de Bund et al. 2004). These reference conditions can be applied, through a comparison with observed chlorophyll concentrations at a site, in the assessments of ecological status for WFD purposes and we hope may provide consistent baseline measures for European Member States to adopt. By examining chlorophyll concentrations in such a large data set of undisturbed lakes, we also aim to describe the factors that determine natural, background concentrations of chlorophyll in the absence of nutrient pressure, a topic of high topical interest to freshwater scientists in general.

Materials and methods

Criteria for reference lake selection

In order to guarantee a common understanding of a reference lake, a common view of accepted minor degree of change in the natural conditions was necessary. As a 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 regional GIG has developed a list of criteria for the selection of reference lakes, using a range of pressure criteria such as a low % of intensive agriculture, absence of major point sources in catchment and low population density (Table 1). Despite some differences in specific values for each pressure criterion

Table 1 Nutrient pressure criteria used to validate reference lake 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 are 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	• Intensive agriculture (arable or intensely grazed): <10% in catchment (<5% Norway, <10% Sweden and UK, 7–20% Finland depending on type of agriculture and proximity to water body) • Population density <5 km^{-2} (Norway), <10 km^{-2} (Sweden) or absence of major settlements in catchment • Absence of large industries in catchment • Absence of major point sources in catchment

among 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). Three Member States (UK, Ireland, Austria) have also used palaeolimnology to validate the choice of reference lakes—only selecting sites that show no significant change in diatom sub-fossil assemblages over the last 150 years or more (see Bennion et al. 2004 for more details).

Many countries additionally used expert judgement in the review of final site lists. Some countries selected sites that locally may be considered in very good condition biologically, but had high nutrient concentrations compared with lakes of a similar type in other countries. For this reason, a threshold mean TP concentration of 100 µg l^{-1} was used as a further criterion, above which sites were removed from this analysis. This resulted in 5 sites, all actually having TP concentrations >150 µg 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 <70 µg l^{-1}, with only three sites having concentrations >50 µg l^{-1}.

The final dataset highlights a significantly higher number of reference lakes from the northern GIG than all other GIGs (Table 2). This is probably a true representation of the fact that these other regions are generally more impacted by higher population densities, industry and more intensive agriculture.

Table 2 Numbers of reference lakes with chlorophyll data by country and by Geographical Intercalibration Group (GIG) region

Country	GIG region					
	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

Data

Data from reference lakes were collated on chlorophyll concentration, altitude, surface area, mean depth, alkalinity, humic content and GIG region. These 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 the GIG coordinators (see Moe et al. this issue for details).

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.

Statistical analysis

To derive type-specific reference chlorophyll concentrations, descriptive statistics were produced for chlorophyll by each lake type. As the dataset of reference lakes was carefully selected using relatively strict and consistent environmental and other pressure criteria, type-specific reference conditions should represent chlorophyll concentrations in all reference lakes within a type. A statistic representing average conditions was, therefore, considered more appropriate than an extreme percentile. The median statistic was chosen in preference to the mean as it is less affected by possible outliers in the dataset. This was considered relevant as some reference sites may have fitted within the pressure criteria but may still be impacted by local or undocumented nutrient pressures.

Standard deviations of medians and percentiles were obtained by a bootstrapping procedure (Maindonald and Braun 2007), which resampled with replacement of the original dataset, estimating the median and percentile statistics and the resulting standard deviations. Analysis of Variance (ANOVA) was used to compare the mean chlorophyll concentration among GIG regions, GIG types and Member States.

If reference conditions were based on a lower quartile statistic of the chlorophyll data in reference sites, the reference condition established would not then be met by most reference lakes. A high percentile statistic of the chlorophyll data, such as the 75th or 90th percentile, is, however, potentially a suitable measure for defining the high/good status class boundary as this would mean that, appropriately, a high proportion of reference lakes would be classified as high status. Analysis of type-specific values was only carried out for those lake types from which data existed from 4 or more lakes (Table 4).

Results

Reference conditions by GIG types and GIG region

Out of the 540 reference lakes for which chlorophyll data for the growth season are available, 335 can be assigned to a specific GIG type. Of these, 13 GIG types have sufficient data (≥4 sites) for estimating reference chlorophyll conditions (median values) and potential high/good boundary values (75th or 90th percentiles). Minimum and maximum reference chlorophyll values for individual lakes ranged between 0.3 and 38.0 μg l^{-1}. Type-specific reference conditions generally ranged between 2.0 and 7.0 μg l^{-1} except for L-N3b, the northern GIG polyhumic lake type, which had a much higher value (14 μg l^{-1}) (Table 4).

For most lake types in the northern GIG, the median chlorophyll values of humic (L-N3a, L-N3b, L-N6a and L-N8a) and non-humic (L-N1, L-N2a, L-N2b and L-N5) clearly differ: lake types (ANOVA, $P < 0.01$), with the former all >3 μg l^{-1} and the latter all <3 μg l^{-1}. In the non-humic lakes, highest chlorophyll concentrations were recorded in moderately alkaline lakes (L-N1; ANOVA, $P < 0.01$), and lowest concentrations in other low alkalinity lake types. The lowest chlorophyll concentration was observed in L-N5 and was significantly lower than the others (ANOVA, $P < 0.05$). In the non-humic lakes, chlorophyll concentrations were also greater in shallow low alkalinity lakes (L-N2a) than in deep low alkalinity lakes (L-N2b) although this was not statistically significant. In humic lakes of the Nordic

Table 3 Characteristics of Lake Geographical Intercalibration Group (GIG) types included in analysis. For GIG Type L = Lake, AL = Alpine, A = Atlantic, CB = Central-Baltic, N = Northern

GIG region	GIG Type	Lake characterisation	Altitude (m a.s.l.)	Mean depth (m)	Humic content (mg Pt l^{-1})	Alkalinity (mequiv. l^{-1})	Lake area (km^2)
Alpine	L-AL3	Low-mid altitude, deep, high alkalinity, large	50–800	>15	<30	>1	>0.5
Atlantic	L-A2	Lowland, shallow, calcareous, large	<200	3–15	<30	>1	>0.5
Central-Baltic	L-CB1	Lowland, shallow, high alkalinity	<200	3–15	<30	>1	Unspecified
	L-CB2	Lowland, very shallow, high alkalinity	<200	<3	<30	>1	Unspecified
	L-CB3	Lowland, shallow, moderate alkalinity	<200	<15	<30	0.2–1	Unspecified
Northern	L-N1	Lowland, shallow, moderate alkalinity, large	<200	3–15	<30	0.2–1	>0.5
	L-N2a	Lowland, deep. Low alkalinity, large	<200	3–15	<30	<0.2	>0.5
	L-N2b	Lowland, deep, low alkalinity, large	<200	>15	<30	<0.2	>0.5
	L-N3a	Lowland, shallow, humic, low alkalinity, large	<200	3–15	30–90	<0.2	>0.5
	L-N3b	polyhumic, low alkalinity, large	<200	3–15	>90	<0.2	>0.5
	L-N5	Boreal, shallow, low alkalinity, large	200–800	3–15	<30	<0.2	>0.5
	L-N6a	Boreal, shallow, humic, large	200–800	3–15	30–90	<0.2	<0.5
	L-N8a	Lowland, shallow, humic, moderate alkalinity	<200	3–15	30–90	0.2–1	Unspecified

Table 4 Number of lakes (*N*) by GIG type and corresponding median, 75th and 90th percentile values for chlorophyll *a* (Apr-Sep means). Standard errors (S.E.) are also given for the three statistics

GIG Region	IC Type	*N*	Median	S.E. Median	75th %	S.E. 75th %	90th %	S.E. 90th %
Alpine	L-AL3	9	2.8	1.5	6.1	3.0	9.0	3.8
Atlantic	L-A2	4	3.3	0.9	4.3	0.9	4.8	0.7
Central-Baltic	L-CB1	20	2.8	0.5	4.7	1.5	6.8	1.5
	L-CB2	5	6.9	3.2	9.0	2.6	10.4	1.8
	L-CB3	12	4.8	1.1	6.3	2.3	11.8	3.3
Northern	L-N1	22	2.9	0.5	4.5	0.9	5.6	0.9
	L-N2a	61	2.3	0.2	3.1	0.2	4.1	0.6
	L-N2b	74	2.0	0.1	2.6	0.2	4.0	0.5
	L-N3a	48	4.1	0.3	6.3	0.7	8.6	1.2
	L-N3b	16	13.8	0.3	17.9	0.7	20.9	1.2
	L-N5	40	1.6	0.1	2.2	0.2	2.6	0.4
	L-N6a	8	3.3	1.2	3.8	5.4	10.2	8.5
	L-N8a	9	7.0	3.0	10.0	6.5	22.6	6.2

GIG, median chlorophyll values were more heterogeneous (L-N3a differed from L-N3b and L-N8a, L-N3b from L-N6a; ANOVA, $P < 0.05$).

Median values for Central-Baltic GIG lake types were higher than those for northern GIG lake types (ANOVA, $P < 0.01$), even for L-CB3 which is an equivalent lake type to L-N1 (median values of 4.8 μg l^{-1} and 2.9 μg l^{-1}, respectively), although the data from these two lake types did not significantly differ ($P = 0.09$). Chlorophyll values did not differ significantly among Central-Baltic lake types (ANOVA, $P = 0.39$).

The Central-Baltic GIG lake type L-CB1 is the same as the Atlantic GIG type L-A2 (lowland, shallow, high alkalinity). Both median and 75th percentile values were similar and were not significantly different ($P = 0.73$).

In summary, chlorophyll reference conditions differed by humic content, depth type and alkalinity type. They were lowest in deep and clear water low alkalinity lake types, and conversely, highest for very shallow, high alkalinity and humic lake types.

Comparison of reference conditions by Member States

For a few northern GIG lake types, there were sufficient data to compare median reference conditions across Member States for the same lake type. This is illustrated for non-humic and humic lake types in Figs. 1 and 2, respectively. This reveals that there was reasonable consistency for reference chlorophyll values among northern GIG countries. In the clear water lake types L-N1 and L-N2a, median values for Norway were significantly lower than the others (ANOVA, $P < 0.05$). In humic lake types, Finnish lakes had consistently higher median values (ANOVA, $P < 0.05$) and consistently greater variability (Fig. 2). The bias in numbers of reference lakes from Norway and Finland could skew the statistics for Northern lake types. For clear water lake types (L-N2a and L-N2b), where Sweden and the UK had reasonable representation there was no evidence of bias (Fig. 1). Bias is more in evidence for humic lake types (L-N3b, L-N6a and L-N8a) where the UK and Norway had very limited representation and different median statistics from Finland (Fig. 2). For this lake type, Member States may want to consider whether national targets are more appropriate with current data availability.

Discussion

Differences between GIG types

The collation of the data highlighted that reference chlorophyll concentrations can potentially span quite

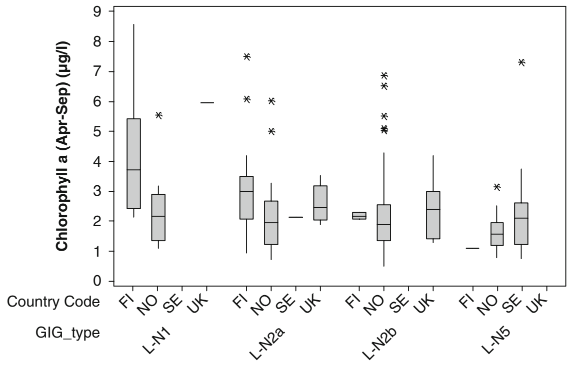

Fig. 1 Boxplots comparing chlorophyll reference conditions by Member States for different northern GIG clear water lake types. (FI: Finland; NO: Norway; SE: Sweden; UK: United Kingdom). The middle horizontal line indicates the median value, the top and bottom of the box are the third (75%) and first quartile (25%), respectively. The upper and lower whiskers extend to the limits defined by adding or subtracting the inter-quartile range. The values beyond whiskers (asterisks) represent extreme outliers

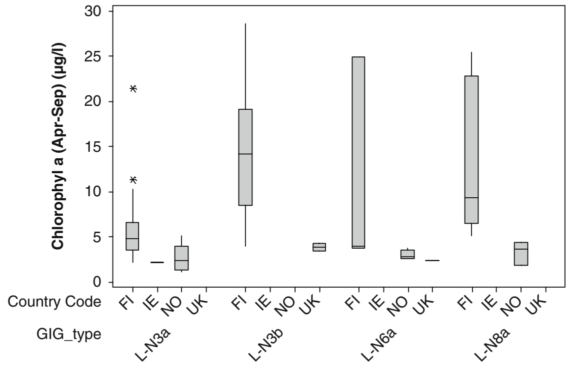

Fig. 2 Boxplots comparing chlorophyll reference conditions by Member States for different northern GIG humic lake types. The middle horizontal line indicates the median value, the top and bottom of the box are the third (75%) and first quartile (25%). The upper and lower whiskers extend to the limits defined by adding or subtracting the inter-quartile range. The values beyond whiskers (asterisks) represent extreme outliers

a large range across all GIG types, although values were frequently less than 4 µg l^{-1} and generally lower than 7 µg l^{-1}. The humic and non-humic lake types differed clearly, in particular the polyhumic lake type L-N3b which had a much higher median value than any other lake type. Analysis of total phosphorus concentrations in humic reference lakes (Cardoso et al. 2007) has highlighted that nutrient concentrations are consistently higher, and it is plausible that these are simply a response to these higher nutrient concentrations. Some studies of humic lakes support these findings (Nürnberg and Shaw 1999), whilst others suggest that the opposite is true—lower phytoplankton production in humic lakes associated with the low light availability and low concentrations of nutrients that are readily bioavailable (Münster 1999). The results observed in this study for humic lake types are based largely on data from Finnish lakes (14 out of 16 sites for L-N3b). They could, therefore, be due to sampling or analytical biases or real biogeographical or ecological differences in Finland (see later discussion on Member States differences). A wide number of Boreal lake surveys across Finland have reported that phytoplankton biomass is generally lower in clear water lakes, although within meso- or polyhumic lakes the relationship between water colour and phytoplankton biomass is more variable (Arvola et al. 1999).

Consistent patterns were also recorded with chlorophyll concentrations increasing with decreasing depth class, with all other typology variables remaining equal. For example, this pattern is observed when comparing L-N2b (deep) versus L-N2a (shallow) and L-CB1 (shallow) versus L-CB2 (very shallow) lake types. These differences were, however, relatively small and not statistically significant. Higher chlorophyll concentrations would be expected with decreasing depth class reflecting the well established positive relationships between algal biomass and light availability (Scheffer 1998). Less consistent was the pattern with alkalinity type. Shallow, moderate alkalinity lakes (L-N1) did have significantly higher median chlorophyll concentrations than equivalent low alkalinity lakes (L-N2a and L-N5), but the shallow high alkalinity lake type (L-CB1) had concentrations that were not significantly different from an equivalent moderate alkalinity type (L-N1). The latter finding was surprising, as it is well established that background nutrient availability is generally greater with increasing alkalinity (Dillon and Kirchner 1975; Vighi and Chiaudani 1985; Cardoso et al. 2007). One possible explanation for the low chlorophyll concentrations in the high alkalinity lake type is that, as undisturbed lakes of this type are rarer, expert judgement may have been used more widely to select reference lakes. This may have led to the mistaken exclusion of sites with naturally higher phosphorus and chlorophyll concentrations.

Differences between GIG regions and Member States

The fact that there were no statistically significant differences in reference conditions for the same lake types in different GIG regions is encouraging and suggests that the criteria for reference lake selection among different GIGs were more or less consistent, and that there are no region-specific differences in reference chlorophyll, at least within northern and central Europe.

The analysis revealed that reference conditions in most countries were relatively comparable for a particular GIG lake type. There did, however, appear to be consistent differences across humic lake types for different countries in the northern GIG with Finland consistently higher than Norway. Explanations involving different criteria for selection of reference lakes or different sampling and analytical methods were discounted following checks with national representatives across Scandinavia, which indicated very consistent approaches. It may, therefore, highlight real biogeographical or ecological differences between Finnish humic lakes and those in other Northern European countries. The greater variability in chlorophyll concentrations in Finnish humic lakes may be a true reflection of the greater humic gradients present in Finland, which contributed the bulk of the humic sites. In very humic waters, higher chlorophyll concentrations could be a response to phytoplankton becoming adapted to the low light availability by producing more chlorophyll per unit biomass or simply due to the known compositional shifts to large mixotrophic species, such as *Gonyostomum*, that are known to occur (Arvola et al. 1999; Salonen et al. 2002).

One country in the Central-Baltic GIG had all its reference lakes excluded from the analysis on the basis of TP concentrations >150 μg/l. This highlights the fact that differences can exist between how countries select reference lakes. Although these sites did have indications of a high ecological status based on diverse macrophyte communities, their nutrient pressures exceeded the WFD recommended guideline, and so were rejected as reference lakes for analysis of reference chlorophyll conditions.

General issues

The analysis has led to statistically robust, WFD-compliant chlorophyll reference conditions for many European intercalibration lake types. It has also highlighted that there appears to be generally very good consistency between Member States and GIG regions in the criteria used to select reference sites in relation to nutrient pressures.

There are clear differences in chlorophyll reference conditions among lake types with increasing concentrations associated with increasing water colour and decreasing depth; No single fixed value for chlorophyll reference conditions is, therefore, appropriate across all lake types. The analysis does, however, highlight that even type-specific reference chlorophyll concentrations may not be ideal as the effects of certain factors such as water colour and depth are really continuous, rather than abrupt differences among types. Sites that lie close to type boundaries may, therefore, be poorly represented and lead to large errors in any type-specific, reference-based status assessment. Site-specific reference conditions may, therefore, be ecologically more appropriate and could be developed by establishing empirical regression models using the raw typology data available from the lakes used in this study (c.f. MEI model: Vighi and Chiaudani 1985). Another advantage of developing site-specific regression models is that reference conditions could be established for lakes that do not fall strictly into the intercalibration lake types reported here.

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