Environmental Management (2010) 45:1286–1298
DOI 10.1007/s00267-010-9484-4

Defining Chlorophyll-*a* Reference Conditions in European Lakes

Sandra Poikāne · Maria Helena Alves · Christine Argillier · Marcel van den Berg · Fabio Buzzi · Eberhard Hoehn · Caridad de Hoyos · Ivan Karottki · Christophe Laplace-Treyture · Anne Lyche Solheim · José Ortiz-Casas · Ingmar Ott · Geoff Phillips · Ansa Pilke · João Pádua · Spela Remec-Rekar · Ursula Riedmüller · Jochen Schaumburg · Maria Luisa Serrano · Hanna Soszka · Deirdre Tierney · Gorazd Urbanič · Georg Wolfram

Received: 8 July 2009 / Accepted: 17 March 2010 / Published online: 18 April 2010

Abstract The concept of "reference conditions" describes the benchmark against which current conditions are compared when assessing the status of water bodies. In this paper we focus on the establishment of reference conditions for European lakes according to a phytoplankton biomass indicator—the concentration of chlorophyll-*a*. A mostly spatial approach (selection of existing lakes with no or minor human impact) was used to set the reference conditions for chlorophyll-*a* values, supplemented by historical data, paleolimnological investigations and modelling. The work resulted in definition of reference conditions and the boundary between "high" and "good" status for 15 main lake types and five ecoregions of Europe: Alpine, Atlantic, Central/Baltic, Mediterranean, and Northern. Additionally, empirical models were developed for estimating site-specific reference chlorophyll-*a* concentrations from a set of potential

S. Poikāne (✉)
Institute for Environment and Sustainability, European Commission Joint Research Centre, 21027 Ispra (VA), Italy
e-mail: sandra.poikane@jrc.ec.europa.eu

M. H. Alves · J. Pádua
Water National Institute, Av. Gago Coutinho 30, 1049-066 Lisbon, Portugal

C. Argillier
Research Institute for Agricultural and Environmental Engineering, CEMAGREF, 3275 Route de Cézanne, CS 40061 Aix-en-Provence, France

M. van den Berg
Centre for Water Management, Zuiderwagenplein 2, 8200 AA Lelystad, The Netherlands

F. Buzzi
Environmental Protection Agency of Lombardy, via I Maggio 21/B, 23848 Oggiono (LC), Italy

E. Hoehn
LBH, Glümerstr. 2a, 79102 Freiburg, Germany

C. de Hoyos
Centre for Hydrographic Studies, CEDEX, C/Paseo Bajo de la Virgen del Puerto 3, 28005 Madrid, Spain

I. Karottki
Ministry of Environment, Haraldsgade 53, 2100 Copenhagen, Denmark

C. Laplace-Treyture
Research Institute for Agricultural and Environmental Engineering, CEMAGREF, av de Verdun 50, 33612 Cestas-Gazinet, France

A. L. Solheim
Norwegian Institute for Water Research, Brekkeveien 19, 0411 Oslo, Norway

J. Ortiz-Casas · M. L. Serrano
Ministry of Environment, Pza San Juan de la Cruz, 28071 Madrid, Spain

I. Ott
Estonian University of Life Sciences, Kreutzwaldi 1, 51014 Tartu, Estonia

G. Phillips
Environment Agency, Kings Meadow Road, RG1 8DQ Reading, United Kingdom

A. Pilke
Finnish Environment Institute, P.O. Box 140, 00251 Helsinki, Finland

S. Remec-Rekar
Environmental Agency, Kidričeva 10, 4260 Bled, Slovenia

U. Riedmüller
BNÖ, Erlenweg 13, 79822 Titisee-Neustadt, Germany

predictor variables. The results were recently formulated into the EU legislation, marking the first attempt in international water policy to move from chemical quality standards to ecological quality targets.

Keywords Reference condition · Chlorophyll · Phytoplankton · Eutrophication · Water Framework Directive · Water assessment · Lakes

Introduction

This work focuses on the defining of reference conditions, which is one of the major keystones of ecological assessment of water body status required by the European Commission Water Framework Directive (WFD; EC 2000). Most biological assessment systems are based on the concept of comparing the current biological community to the "reference conditions"—a status of community observed in the absence of human disturbance or alteration (Bailey and others 2004). Therefore, reference conditions can serve as an important guide to set management aims, but it is important to emphasize that reference conditions are not the same as restoration goals (Egan and Howell 2001). However, the term "reference condition" has been used to refer to multiple, and often confusing and contradictory, concepts. For example, it can be used to refer to the condition of ecosystems at some point in the past; the best remaining conditions in a region heavily modified by human activity and so on (Stoddard and others 2006). In this article, the term "reference conditions" is used in the WFD sense which defined type-specific reference conditions as the biological conditions associated with no or very low human pressure (EC 2000).

Reference conditions can be defined in a number of different ways (Reynoldson and others 1997; Stoddard and others 2006). By far, the most common approach for estimating reference conditions is to quantify them at a set of sites relatively unexposed by human activity. This approach is widely known as the "reference site approach" (Hughes 1995; Bailey and others 2004) and presents a scientifically sound method for setting expectations, provided that the method of selecting reference sites is clearly defined. However, the spatial approach faces a practical obstacle because it is often difficult to find undisturbed sites. Establishment of reference sites is especially challenging in Europe (Moss and others 2003) for all but polar and mountain areas, and even there, climate warming and airborne pollutants make it unlikely that true reference sites exist. It may be especially difficult to find minimally impacted waters for some particular types, e.g., shallow lowland lakes (Bennion and others 2004).

The derivation of reference conditions from paleolimnological studies offers an alternative method in such cases. Paleolimnological research has a long tradition in Europe using various elements e.g., diatoms (Bennion and others 1995) and pigments (Kamenik and others 2000). Nevertheless, the reconstructions are often subject to variabilities of more than one order of magnitude (Sayer 2001), and this can obscure precise reconstruction of reference conditions.

Historical data probably give the best insight into how reference lakes looked. They often suffer, however, from poor quality, be it due to different sampling methods or different taxonomic resolution. The basic problems are the paucity of data available and the definition of the "reference period" which is usually considered the period before 1850 (Battarbee 1999) or before the Second World War if impacts from anthropogenic land use and urbanisation can be considered as negligible (Reichmann and Schulz 2004).

In situations without minimally disturbed sites and historical data, empirical models derived from associations between biological indicators and human-disturbance gradients can be extrapolated to infer conditions in the absence of human disturbance (Stoddard and others 2006). A promising approach to estimate the natural trophic state for lakes is the use of models that predict the trophic state of a lake from nutrient loading (e.g., Vollenweider 1976; OECD 1982), however, it must be borne in mind that any empirical model involving nutrient export coefficients bears various methodological errors (Ryding and Rust 1989).

It is acknowledged (Moss and others 2003) that, currently, the best approach is a combination of reference sites, modelling, paleolimnology and expert judgment. Integrating several approaches may lead to firmer, more defensible reference conditions, particularly if the conclusions derived from the different approaches are consistent (Stoddard and others 2006). The process depends on the accumulation of experience and integration from all of these lines of evidence rather than statistically rigorous

J. Schaumburg
Bavarian Environment Agency, Demollstraße 31, 82407 Wielenbach, Germany

H. Soszka
Institute of Environmental Protection, Kolektorska 4, 01692 Warsaw, Poland

D. Tierney
Environmental Protection Agency, Clonskeagh Road, 14 Dublin, Ireland

G. Urbanič
Institute for Water, Hajdrihova 28c, 1000 Ljubljana, Slovenia

procedures (Moss and others 2003). Such an approach was used for setting chlorophyll-*a* reference conditions in European water legislation and is presented in this article.

According to the Water Framework Directive, Member States are required to develop lake ecological status assessment systems based on various characteristics of phytoplankton, macrophytes, benthic invertebrates and fish fauna. An intercalibration exercise is foreseen to harmonise assessment systems of all EU and to ensure that the obligation to reach good status has the same meaning throughout Europe. The first stage of the intercalibration (2003–2008) aimed at setting of reference conditions and class boundaries for chlorophyll-*a* values for all lake types and all geographical regions of the EU. The work was carried out under the Common Implementation strategy of the Water Framework Directive (EC 2001) by joint effort of all European countries and greatly supported by several scientific studies of the European Framework 6 project REBECCA, e.g. setting of total phosphorus (TP) reference conditions (Cardoso and others 2007), preliminary analyses of chlorophyll-*a* values in reference lakes of Northern and Central regions (Carvalho and others 2008), development of chlorophyll-nutrient relationships (Phillips and others 2008) and phytoplankton responses to eutrophication (Ptacnik and others 2008).

The initial steps in development of reference conditions were (1) identification of anthropogenic pressures and selecting appropriate biological components and indicators to address their impact; (2) defining geographically homogenous regions and common types of lakes within them. This study has focused on the major pressure for lakes of Europe—eutrophication and the most relevant community detecting this pressure—phytoplankton. Chlorophyll-*a* was selected as a simple indicator for phytoplankton abundance with sufficient data availability across Europe. Five lake regions including all of the EU Member States and Norway were established based on Illies limnofaunistic division of Europe (1978)—Alpine, Atlantic, Central/Baltic, Mediterranean and Northern regions (EC 2005). Common lake typology with fifteen international lake types were selected for intercalibration (Table 1) based on natural abiotic characteristics—altitude, alkalinity

Table 1 Description of common international lake types included in analysis

Type code	Lake type characterisation	Altitude (m a.s.l.)	Mean depth (m)	Alkalinity (meq/l)	Additional characteristics
Alpine region					
AL3	Lowland or mid-altitude, deep, moderate to high alkalinity, large	50–800	>15	>1	Surface area >50 ha
AL4	Mid-altitude, shallow, moderate to high alkalinity, large	200–800	3–15	>1	Surface area >50 ha
Atlantic region					
A1/2	Lowland, shallow, calcareous	<200	3–15	>1	Non-humic
Central Baltic region					
CB1	Lowland, shallow, calcareous	<200	3–15	>1	Residence time 1–10 years
CB2	Lowland, very shallow, calcareous	<200	<3	>1	Residence time 0.1–1 years
CB3	Lowland, shallow, siliceous	<200	3–15	0.2–1	Residence time 1–10 years
Mediterranean region					
Msw	Reservoirs, deep, large siliceous, lowland, "wet areas"	0–800	>15	<1	Surface area >50 ha, annual mean precipitation 800 mm or annual mean T < 15°C, catchment area < 20 000km^2
Mc	Reservoirs, deep, large, calcareous	0–800	>15	>1	Surface area > 50 ha, catchment area < 20 000km^2
Northern region					
N1	Lowland, shallow, moderate alkalinity, non-humic	<200 m	3–15	0.2–1	Colour <30 mg Pt/l
N2a	Lowland, shallow, low alkalinity, non-humic	<200 m	3–15	<0.2	Colour <30 mg Pt/l
N2b	Lowland, deep, low alkalinity, non-humic	<200 m	>15	<0.2	Colour <30 mg Pt/l
N3a	Lowland, shallow, low alkalinity, humic	<200 m	3–15	<0.2	Colour 30–90 mg Pt/l
N5a	Mid-altitude, shallow, low alkalinity, non-humic	200–800 m	3–15	<0.2	Colour <30 mg Pt/l
N6a	Mid-altitude, shallow, low alkalinity, humic	200–800 m	3–15	<0.2	Colour 30–90 mg Pt/l
N8a	Lowland, shallow, moderate alkalinity, humic	<200 m	3–15	0.2–1	Colour 30–90 mg Pt/l

Lake type codes: *AL* Alpine; *A* Atlantic; *CB* Central Baltic; *M* Mediterranean; *N* Northern GIG and mean depth—which are important factors in determining the composition and abundance of biological communities (e.g., Kolada and others 2005). Additional factors were used in several regions, e.g., lake area (Alpine region), humic substances (Nordic region), climate factors (Mediterranean region), water retention time (Central region) the importance of which has been demonstrated in several studies (e.g., Miettinen and others 2005).

In short, this paper summarizes the evolution of type-specific chlorophyll-*a* reference conditions and describes the data and research used in their development. More specifically, we set the following objectives: (1) to describe and compare different approaches of selection of reference lakes and setting reference conditions and to evaluate consistency of the results; (2) to determine whether selected reference lakes may be considered representative of the whole lake population; (3) to explore relationships between chlorophyll-*a* values and environment variables and define the most important drivers of chlorophyll-*a* concentration in unimpacted lakes; (4) to develop empirical models for estimating site-specific reference chlorophyll-*a* values from a set of potential predictor variables.

Material and Methods

Data Sets

Altogether, data for approximately 1200 lakes and 2700 lake years were pooled from national datasets into intercalibration databases (see Table 2). These databases contained both basic data (altitude, surface area, mean depth, alkalinity), quality data (chlorophyll-*a*, nutrients, Secchi depth) and pressure data (land use, population density, other impacts).

Data were collected from environment agencies and scientific institutes including data both from national monitoring programs and several research projects. Inevitably, with such a large dataset of lakes from many countries, there were questions regarding data quality which were solved before the data analyses:

– Checking and correction of data were required before the data could be used: a common problem was erroneous units, values below detection limits coded in different ways, as well as numerous other irregularities;

Table 2 Description of lake data sets by region, type and country

Region	Lake type	Number of all lakes	Number of ref lakes	Number of reference lakes per country
Alpine	AL3	78	22	AT (14), DE (5), IT (2), SI (1)
	AL4	69	13	AT (10), DE (3)
Atlantic	A1/2	46	9	IE (8), UK (1)
Central Baltic	CB1	209	21	DE (3), DK (1), EE (1), LT (3), LV (6), NL (2), PL (5)
	CB2	138	5	LV (2), NL (2), UK (1)
	CB3	37	8	DK (1), EE (2), LV (3), PL (2)
	CB4	50	4	LV (3), NL (1)
Mediterranean	Mc	21	5	CY (1), ES (2), FR (1), RO (1)
	Msw	20	5	ES (2), GR (1), PT (2)
Northern	N1	69	19	FI (8), NO (10), UK (1)
	N2a	86	60	FI (26), NO (28), SE (1) UK (5)
	N2b	96	71	FI (2), NO (62), UK (7)
	N3a	98	46	FI (35), IE (2), NO (9)
	N3b[a]	42	16	FI (14), UK (2)
	N5a	49	37	FI (1), NO (22), SE (14)
	N6a	21	7	FI (3), NO (3), UK (1)
	N6b[a]	1	1	IE (1)
	N7[a]	3	2	SE (2)
	N8a	65	8	FI (6), NO (2)
	All	1197	359	AT (24), CY (1), DE (11), DK (2), EE (3), ES (4), FI (95), FR (1), GR (1), IE (11), IT (2), LT (5), LV (14), NL (3), NO (136), PL (7), PT (2), RO (1), SE (17), SI (1), UK (18)

AT austria; *BE* Belgium; *CY* Cyprus; *DE* Germany; *DK* Denmark; *EE* Estonia; *ES* Spain; *FI* Finland; *FR* France; *GR* Greece; *HU* Hungary; *IE* Ireland; *IT* Italy; *LT* Lithuania; *LV* Latvia; *NL* The Netherlands; *NO* Norway; *PL* Poland; *PT* Portugal; *RO* Romania; *SE* Sweden; *SI* Slovenia; *UK* United Kingdom

[a] Lake types not included in the final analyses due to low number of lakes

- Data quality was further checked by revealing outliers and testing well established relationships (e.g., between conductivity and alkalinity, chlorophyll-*a* and TP).

One problem was the heterogeneity of the data: due to different data origins, different sampling and analysis methods were used (except Mediterranean region, where sampling was carried out using an unified strategy). Despite the large heterogeneity of the data, some common patterns can be defined:

- Samples were mostly from the vegetation season; also winter/spring season samples included in the Alpine region;
- Approximately 4 sampling dates per season were used (ranging from 1–2 to 10);
- Most samples were taken from the epilimnion/surface layer; Mediterranean region—samples from the euphotic zone, defined as 2.5 Secchi depth;
- Spectrophotometry with ethanol/acetone extraction was used for chlorophyll-*a* measurements.

For our analyses, we used chlorophyll-*a* annual average values (Alpine region) or average values of vegetation season (all other regions).

Selection of Reference Lakes

A preferred approach to locate reference sites is to establish a set of criteria that, in total, describe the characteristics of sites in a region that are not or only minimally exposed to stressors (Stoddard and others 2006). It is advisable to use a set of reference criteria based on pressure data, not chemical and biological parameters which must be used only for confirmation of, not for selection of, reference sites because of the possibility of circularity and preconceived notions about the chemistry and biology at a "typical reference site" (Bailey and others 2004).

A list of criteria for the selection of reference sites was developed in every region (Table 3) based on criteria assessing the pressure from the catchment. Land use, point sources and population density were the main factors, but additional elements were included by several regions, such as the change of the natural regime, artificial modifications of the shoreline, introduction of fish and fish-farming activities, mass recreation, and invasive species. Some Member States (UK, Ireland, and Austria) additionally used paleolimnological data for confirmation of reference sites. For instance, Taylor and others (2006) found that diatom assemblages in the sediment core top samples from 11 of 34 candidate reference lakes in Ireland showed relatively little deviation from those in sediment core bottom samples. The core bottom samples appeared to pre-date ca. 1850, or the onset of agricultural intensification and major aforestation in the catchment, so those sites were selected as reference sites in the Atlantic region.

Historical quantitative data on phytoplankton were used only in the Alpine region, available from the 1930s for Carinthian lakes (Findenegg 1935; 1954) and for several lakes in the Northern Calcareous Alps (Ruttner 1937). Since these lakes were not affected by major anthropogenic pressure from industrialisation, intensive urbanisation or agriculture, the 1930s reflect reference conditions with insignificant anthropogenic impact.

A distinctive approach was taken in the Alpine region in which sites were accepted as reference sites if their actual trophic state did not deviate from the reference trophic state prior to industrialisation, intensive urbanisation or agriculture. From paleoreconstruction (e.g., Löffler 1972; Klee and others 1993) and theoretical considerations using the Vollenweider phosphorus loading model (Vollenweider 1976; OECD 1982), it was concluded that oligotrophy is the natural reference trophic state of deep Alpine lakes (AL3, mean depth >15 m). Lakes belonging to the lake type AL4 (mean depth 3–15 m), however, tend to have a higher reference trophic state at oligomesotrophic level. This is proved again by loading model calculations and paleoreconstruction (e.g., Lotter 2001; Schmidt and others 2002).

In summary, the reference lakes have been selected using the following approaches or a combination of these approaches:

- Criteria assessing the pressure from the catchment, e.g., predominantly (90%) natural land cover, absence of major point sources, population density (e.g. <10 inhabitants/km^2), were used in all regions for initial selection of candidate reference lakes;
- TP concentration corresponding to the defined natural trophic state was used in the Alpine region;
- Paleoreconstruction for selection or confirmation of reference sites was used in the Alpine, Atlantic and Northern regions;
- Historical data reflecting the reference state were used in Alpine region;
- Phosphorus loading model (Alpine and Nordic regions) was used for TP concentrations in the reference lakes;
- Additional screening by quality criteria (nutrient, chlorophyll-*a*) and expert judgment was broadly used in the final review of reference lake lists.

According to the reference criteria, 359 reference lakes were selected across the EU. Most of the reference lakes (267 lakes) belong to the Northern region (Table 2), dominated by lakes in Norway (136 lakes) and Finland (95 lakes), while the lowest numbers occur in the Central Baltic (38 lakes) and Mediterranean region (10 reservoirs),

Table 3 Pressure criteria used for reference lake selection

Region	Pressure criteria
ALP	Insignificant contribution of anthropogenic to total nutrient loading, validated by nutrient loading calculations
	No deviation from the natural trophic state:
	– natural trophic state of LAL3: oligotrophic (threshold value for the preselection of reference sites TP $\leq$8 μg/l)
	– natural trophic state of LAL4: oligomesotrophic (threshold value for the preselection of reference sites TP $\leq$12 μg/l)
	>80–90% natural forest, wasteland, moors, meadows, pasture
	No (or insignificant) urbanization or peri-urban areas
	No deterioration of associated wetland areas
	No (or insignificant) changes in the hydrological and sediment regime of the tributaries
	No direct inflow of (treated or untreated) waste water, no (or insignificant) diffuse discharges
	No (or insignificant) change of the natural regime (regulation, artificial rise or fall, withdrawal)
	No (or insignificant) artificial modifications of the shore line
	No introduction of fish where they were absent naturally (last decades) and fish-farming activities
	No mass recreation (camping, swimming, rowing)
	No exotic or proliferating species (any plant or animal group)
ATL	Absence of major modification to catchment e.g. intensive afforestation
	No discharges present that would impair ecological quality
	Water 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
	Dissolved oxygen: within range 80–120% saturation
	Oxygen depletion (66% of lake deoxygenated for a period >2 months) absent
	pH within range 6–9, salinity: <100 mg Cl/l
	TP <15 μg/l
CB	90% of catchment land use natural (or semi-natural)
	Population density <10 km^{-2}
	No point sources in the catchment:
	Criteria can be overruled if
	– clear and sound evidence from paleolimnological data, which is published or otherwise publicly available;
	– the direct related catchment of the lake is surrounded is for more than 90% of the area by natural land use and there are no signs of any disturbance;
	– the use of agricultural land is very extensive meaning, no artificial fertilizers are used;
	– the whole population in the catchment is connected to waste water treatment plants while the discharge is not connected to the candidate reference lake
MED	70% of the catchment area classified as "natural areas" (80% in Portugal, 90% in Cyprus and Greece)
	Very low occurrence of anthropogenic pressure in the catchment area
	Spain: Upstream accumulated demand of water for domestic use must be <3% of annual loading; <1.5% for industrial use; and <10% for agricultural irrigation
	Portugal: Low/moderate fishing and navigation pressures, low/moderate water level fluctuations
NOR	Agriculture: <10% in catchment (<5 Norway), mainly judged from visual observations of GIS land use data
	Population density <5 p.e./km (Norway), <10 p.e/km (Sweden) or absence of major settlements in catchment
	Absence of large industries in catchment
	Absence of major point sources in catchment
	Sweden and Norway: TP < 10 μg/l or higher if high colour
	Norway: chl-*a* <4 μg/l (low alkalinity clear types) or <6 μg/l (other types)
	UK and Ireland: confirmation with paleodata of diatoms

Alp Alpine; *ATL* Atlantic; *CB* Central Baltic; *MED* Mediterranean; *NOR* Northern which can be explained both by data availability and the level of anthropogenic pressure in those regions.

Setting of Reference Conditions and High/Good Status Boundary

In different regions chlorophyll-*a* reference values and the values corresponding to the boundary between the High and Good quality classes (H/G boundary) were established by following common principles (median of chlorophyll-*a* values in reference lake type specific populations was used for setting reference value, 75th–95th percentile—for setting H/G boundary), but slightly different methods.

In the Alpine region, class boundaries were set in two steps: (1) the reference conditions and boundaries were set for the annual mean total phytoplankton biovolume; (2) the reference value and boundaries for chlorophyll-*a* were derived from regression with total biovolume. The use of total biovolume is justified by the fact that historical data from the 1930s, which represent the best reference data, are available for total biovolume only, not for chlorophyll-*a* values. The reference value was calculated as the median of the values measured in the set of selected reference lakes, while H/G boundary—as 95th percentile of values in the reference lake population. The use of 95th percentile was justified by the strict criteria used for selection of reference sites and use of the arithmetic means of the lakes (1–19 years each) in the analyses, instead of single lake-years, in order to prevent a bias toward lakes with more data available.

In the Central-Baltic region, the median value of mean chlorophyll-*a* concentrations in reference lakes was used as the reference value for chlorophyll-*a* and the 75th percentile—as the H/G boundary. The 75th percentile was considered more appropriate for setting the H/G boundary than the 90th percentile which would result in a relatively high proportion of lakes that would be assessed to have high status but not assigned as reference lakes. To avoid the problem of insufficient data, the analysis was based on lake-years, not single lake averages. The results were compared with similar lake types from the Northern region and results of project REBECCA (Carvalho 2008) and found to be similar.

In the Mediterranean region, the median chlorophyll-*a* value in reference sites was taken for reference conditions (in fact, for Maximum Ecological Potential, as both Mediterranean types represented only reservoirs). The High/Good potential boundary is not required to be reported for heavily modified or artificial water bodies so it was not calculated. The reference lake number was small in this region, and future research is planned to revise the current results.

In the Northern region, the reference value was calculated as the median value of type-specific chlorophyll a of reference lakes, supplemented with expert judgments for types with insufficient data. H/G boundaries were set primarily at the 90th percentile of the distribution of the metric in reference lakes, thereafter, the values were compared with the response curves of phytoplankton taxonomic indicators (Ptacnik and others 2008) in conjunction with statistical analysis of Member State reference lake populations.

It is well known that no single value can represent reference conditions over all types of water bodies: ecosystems are complex and their characteristics mutually vary within large ranges, determined by external and internal factors (Moss and others 2003). Therefore, the final results of reference conditions and the H/G boundary were expressed as ranges, not fixed values for the following reasons: (1) a broad range of natural conditions within every common lake type (2) different monitoring practices in use, e.g., sampling depth, time and frequency, which have influences on chlorophyll-*a* data; (3) in the Mediterranean region, the main concern was interannual variability since the results were derived from one single sampling year dataset.

Statistical Analysis

Statistical analyses were carried out using the following methods:

- To derive type-specific reference conditions, descriptive statistics were used for chlorophyll-*a* for each lake type (medians, quartiles and percentiles);
- Cumulative frequency analyses were used for defining reference conditions (the cumulative distributions of the reference lake population were compared to the non-reference lake population);
- Analysis of Variance (ANOVA) was used to compare the mean chlorophyll-*a* concentrations among regions, types and Member states;
- Mann–Whitney U test was used to examine how representative the selected reference sites are of all lake populations. To address this issue, selected descriptors (altitude, depth, area, alkalinity, conductivity, TP, chlorophyll-*a*, Secchi depth) of type-specific reference lake populations were compared with impacted lake populations;
- General Linear Model (GLM) was used to estimate the best model to predict mean chlorophyll-*a* from several predictor variables. 2 types of predictors were used to estimate chlorophyll-*a* values: (1) altitude, alkalinity, mean depth, surface area (log transformed in order to obtain all normally distributed variables); (2) humic

type and region used as dummy variables—3 humic types (low, medium and high colour) and 5 regions (Alpine, Atlantic, Central/Baltic, Mediterranean and Northern).

Results and Discussion

Chlorophyll-*a* Concentrations in European Reference Lakes

The median type-specific chlorophyll-*a* concentrations in reference lake populations ranged from 1.4 to 7.8 µg/l (see basic statistics in Table 4) and in general followed the natural trophic gradient, influenced by depth, alkalinity and humic level (Fig. 1).

Depth was an important factor impacting chlorophyll-*a* reference values: the lowest values were found in deep Mediterranean reservoirs (1.4 and 1.8 µg/l), deep Alpine lake type AL3 (2.0 µg/l) and deep Northern lake type N2b (2.0 µg/l); there was no significant difference between the deep lake reference populations (ANOVA, $P > 0.1$). Conversely, the highest values were recorded for only one very shallow lake type, LCB2 (depth <3 m, median chlorophyll-*a* value 7.5 µg/l, which clearly differs from deep lakes (ANOVA, $P < 0.001$).

Surprisingly, the most important factor was water colour—all three humic lake types (colour 30–90 mg Pt/l) had significantly higher chlorophyll-*a* values compared to non-humic lake types, Mediterranean and Alpine lakes (Fig. 1, humic types N3a, N6a, N8a). The highest concentrations were found in shallow humic moderate alkalinity lake type (median value 7.8 µg/l) which was significantly higher compared with all other lake types (ANOVA, $P < 0.01$). Still, there were relatively few lakes in humic lake types N6a and N8a, so additional research is needed to draw clear conclusions.

Multiple Regression Model for Site-Specific Reference Chlorophyll-*a*

The results of the GLM model using independent predictors are presented in Table 5, revealing a significant effect of region, humic type, altitude, depth, and alkalinity, but not lake area. Overall, the variance explained by the model was 48.0% which is clearly acceptable and comparable with variability explained by TP model (51.4%, Cardoso and others 2007) or by diatom-inferred TP (47%, Bradshaw and Anderson 2001).

Our analyses has highlighted that a geographical region, humic type, altitude, depth and alkalinity all have a significant relationship with chlorophyll-*a* (Table 5) and several different regression models for predicting chlorophyll-*a* concentrations are required across Europe to take account these predictors (Table 6):

- The strongest gradients were observed for humic type: chlorophyll-*a* concentrations generally increased with increasing humic content ($P < 0.00001$);
- Depth ($r = -0.14$, $P = 0.0073$) and altitude ($r = -0.09$, $P = 0.0038$) had a negative correlation, while alkalinity was in positive correlation with chlorophyll-*a* values (0.11, $P = 0.043$);

Table 4 Chlorophyll-*a* concentration in European reference lakes: mean, median, minimum and maximum values, lower (25%) and upper (75%) quartile, standard deviation and number of lakes (n)

GIG	IC type	Mean	Median	Min	Max	25%	75%	St dev	n	Type description
Alpine	AL3	2.0	2.0	0.7	3.9	1.5	2.6	0.8	20	Lowland or mid-altitude, deep, high alkalinity, large
	AL4	3.1	3.3	1.6	4.5	2.2	3.8	1.0	13	Mid-altitude, shallow, high alkalinity, large
Atlantic	A1/2	4.7	2.7	1.4	12.7	2.3	5.1	4.3	9	Lowland, shallow, calcareous
Central Baltic	CB1	3.7	2.7	1.6	10.8	2.1	4.2	2.4	21	Lowland, shallow, calcareous
	CB2	6.2	7.5	1.4	11.3	1.7	9.0	4.4	5	Lowland, very shallow, calcareous,
	CB3	4.6	3.4	0.9	12.4	2.2	6.0	3.7	8	Lowland, shallow, small, moderate alkalinity
Mediterranean	LMc	1.4	1.8	0.4	1.8	0.5	1.6	0.6	5	Reservoirs, deep, large siliceous, lowland, "wet areas"
	LMsw	1.9	1.4	0.7	3.7	1.1	2.6	1.2	5	Reservoirs, deep, large, calcareous
Northern	N1	3.4	2.9	1.1	8.6	2.2	5.1	1.9	19	Lowland, shallow, moderate alkalinity, clear
	N2a	2.5	2.2	0.7	7.5	1.7	3.0	1.2	60	Lowland, shallow, low alkalinity, clear
	N2b	2.2	2.0	0.5	6.5	1.4	2.5	1.2	71	Lowland, deep, low alkalinity, clear
	N3a	5.0	4.2	1.1	21.5	3.0	6.3	3.6	46	Lowland, shallow, low alkalinity, humic
	N5a	1.9	1.7	0.8	7.3	1.1	2.2	1.1	37	Mid-altitude, shallow, low alkalinity, clear
	N6a	6.3	3.8	2.4	24.9	2.6	4.0	8.2	7	Mid-altitude, shallow, low alkalinity, humic
	N8a	10.8	7.8	3.7	25.5	4.8	16.0	8.3	8	Lowland, shallow, moderate alkalinity, humic

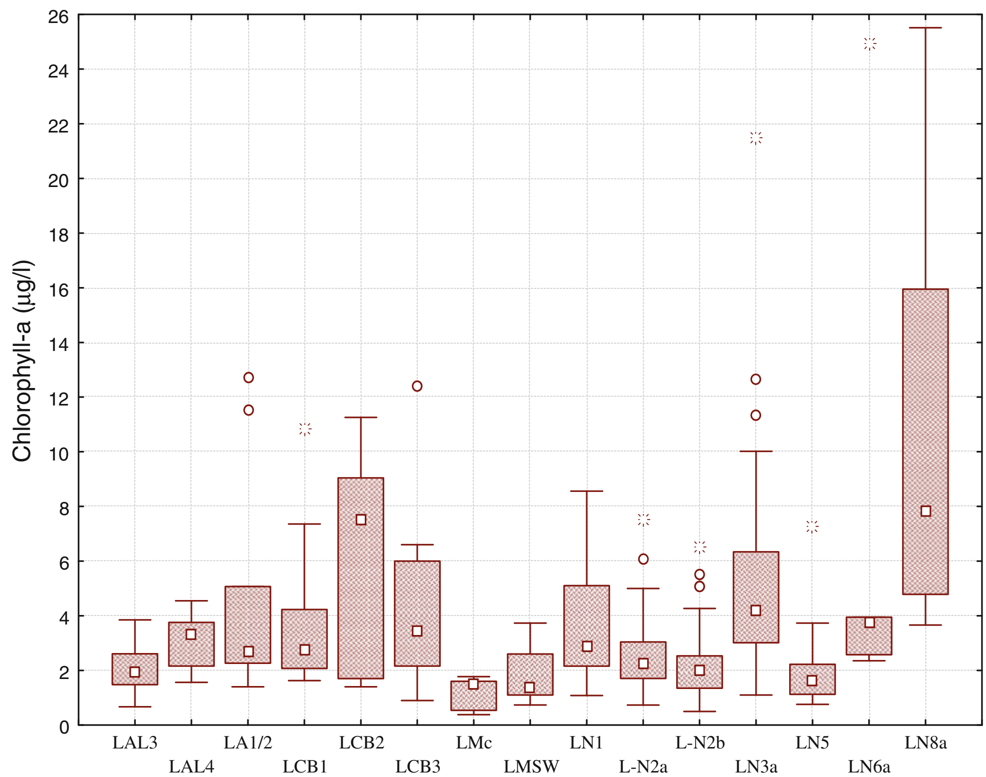

Fig. 1 Boxplots comparing chlorophyll reference conditions by different lake IC common types. The middle square indicates the median value, the top and bottom of the box are the upper (75%) and lower (25%) percentiles, the upper and lower line extend to the limits of non-outlier range, and the values beyond the lines represent outliers and extreme values

Table 5 General linear model result for chlorophyll-*a* variation in European reference lakes

	Sum of squares	Degree of freedom	Mean square	F	Significance
Intercept	34.61	1	34.61	113.86	**0.000**
GIG region	4.33	4	1.08	3.56	**0.007**
Humic class	38.51	2	19.25	63.34	**0.000**
Altitude	2.59	1	2.59	8.51	**0.004**
Depth	2.22	1	2.22	7.31	**0.007**
Area	1.07	1	1.07	3.52	0.061
Alkalinity	1.27	1	1.26	4.15	**0.042**
Error	84.19	277	0.30		

– As for the region effect, we found only two statistically significant classes: lakes in the Central Baltic region had on average more chlorophyll-*a*, but Mediterranean lakes less chlorophyll-*a* than lakes on the Alpine, Atlantic and Northern regions.

These findings are in accordance with well-established relationships between TP concentration and alkalinity (e.g., Vighi and Chiaudani 1985; Cardoso and others 2007), altitude (Müller and others 1998; Cardoso and others 2007) and depth (e.g., Ryder and others 1974). Our results only partly agree with Cardoso and others (2007) which found higher TP concentrations both in Central Baltic and Mediterranean region. The difference can be explained by the low number of Mediterranean lakes included in both studies and Mediterranean lake types which comprise reservoirs which biological characteristics differ from natural lakes. Higher chlorophyll-*a* values in the Central Baltic region can be explained by a type effect (lakes are shallower with higher alkalinity and lower altitude) and latitudinal effect related to temperature and its effects on mineralization in the catchment.

More controversial is the relationship between humic substances and chlorophyll-*a* values—for a long time, humic lakes were considered unproductive systems, as humic substances form complexes with phosphate ions and organophosphorus compounds, thereby reducing phosphorus availability to phytoplankton (Jones and others 1993). From the other side, it is now known from extensive recent experimental work that both natural ultraviolet as well as visible light induce major photolytic changes in complex organic molecules and generate large

Table 6 Equations predicting chlorophyll reference conditions in European lakes, using humic type, altitude (alt), alkalinity (alk) and mean depth (Z) as independent predictors

Region	Humic type	Equation predicting chlorophyll reference conditions
ALP	Low	Log (chl) = 1.70 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
ATL	Mod.	Log (chl) = 1.70 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
	Low	Log (chl) = 2.35 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
CB	Low	Log (chl) = 2.13 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
	Mod.	Log (chl) = 2.78 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
	High	Log (chl) = 3.79 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
MED	Low	Log (chl) = 1.22 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
N	Low	Log (chl) = 1.70 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
	Mod.	Log (chl) = 2.35 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)
	High	Log (chl) = 3.36 − 0.09 (0.03) log (alt) − 0.14 (0.05) log (Z) + 0.11 (0.05) log (alk)

Standard errors are in brackets. Humic lake types: low with colour values <30 mg Pt/l, moderate with colour values 30–90 mg Pt/l, high with colour values >90 mg Pt/l

ALP Alpine; *ATL* Atlantic; *CB* Central Baltic; *MED* Mediterranean; *N* Northern region

quantities of readily utilisable substrates for microbial metabolism (e.g., Winter and others 2007) which make available a huge reservoir of organic carbon and energy (e.g., Salonen and others 1992). Other possible mechanisms include light adaptation yielding higher Chl:biomas ratios (Phillips and others 2008) and the selective attenuation of ultraviolet light by humic substances that protects algae from photoinhibition in the surface layers (Moeller 1994) Several studies already have demonstrated higher chlorophyll-*a* values in humic lakes (Jasser 1997; Webster and others 2008), our data also confirm high productivity of humic ecosystems.

Representativeness of Reference Lakes

In general, the reference lake population represented all lake populations; there were no significant differences between reference and non-reference lakes by hydromorphological and physico-chemical (alkalinity, colour) parameters. Nevertheless, there were some exceptions where reference lake selection or type characteristics must be reconsidered:

- CB1 type reference lakes were significantly deeper than CB1 non-reference lakes (median mean depth values, 7.7 and 5.9 m respectively), less alkaline (median alkalinity values, 2.0 and 2.5 meq/l respectively) and with lower humic content (median for reference lakes, 18 mg Pt/l, and for non-reference lakes, 38 mg Pt/l);
- Also, N2a reference lakes were significantly deeper and less alkaline than non-reference lakes of this type, and N2b reference lakes possessed lower alkalinity than non-reference lakes;
- There were also differences in CB2 and CB3 lake types, but the small number of available reference lakes hinders drawing of meaningful conclusions.

As expected, in most lake types reference lakes differed significantly from non-reference lakes in chlorophyll-*a*, TP and Secchi depth. Nevertheless, in several types (AL4, CB3, N2b, N6a, N8a), reference lake chlorophyll-*a* distribution did not differ significantly from impacted lake chlorophyll-*a* distribution (N2b median value for reference lakes 2.0 μg/l, for non-reference lakes 2.5 μg/l; N6a median value for reference lakes 3.8 μg/l, for non-reference lakes 3.3 μg/l). In fact, in some lake types, reference lake populations outnumber impacted lakes; e.g., there are 71 reference and 25 non-reference lakes within the N2b type population. Even if some sound reasons for such homogeneity could be supposed (e.g., the whole type is relatively unimpacted), it is necessary to review the reference lake selection criteria and the sensitivity of the selected indicators (TP, chlorophyll-*a*, Secchi) to pressure factors occurring in these lake types.

Chlorophyll-*a* Reference Conditions in European Water Legislation

The final outcome of our work consists of reference conditions and establishment of a High/Good status boundary based on chlorophyll-*a* for the common lake types selected within the Geographical Intercalibration Groups for lakes. The chlorophyll-*a* values and ranges are given in Table 7.

Recently, the outcome of the work formulated into the EU legislation as *“Commission Decision establishing the values of the Member State monitoring system classifications as a result of the Intercalibration exercise*” (EC 2008), therefore marking the first attempt in international water policy to move from physico-chemical quality standards to ecological quality targets.

The Member States shall use the chlorophyll-*a* ranges defined for the common types to set the most suitable

Table 7 Chlorophyll-*a* reference conditions, High/Good boundary values and ranges by IC lake type defined and agreed in the WFD Intercalibration process

Lake type	Lake type characterisation	Reference conditions		High/Good boundary	
		Value	Range	Value	Range
AL3	Lowland or mid-altitude, deep, moderate to high alkalinity, large	1.9	1.5–1.9	2.7	2.1–2.7
AL4	Mid-altitude, shallow, moderate to high alkalinity, large	3.3	2.7–3.3	4.4	3.6–4.4
A1/2	Lowland, shallow, calcareous	3.2	2.6–3.8	5.8	4.6–7.0
CB1	Lowland, shallow, calcareous	3.2	2.6–3.8	5.8	4.6–7.0
CB2	Lowland, very shallow, calcareous	6.8	6.2–7.4	10.8	9.9–11.7
CB3	Lowland, shallow, siliceous	3.1	2.5–3.7	5.4	4.3–6.5
Msw	Reservoirs, deep, large, siliceous, lowland, "wet areas"	1.4	1.4–2.0	n.e.	n.e.
Mc	Reservoirs, deep, large, calcareous	1.8	1.8–2.6	n.e.	n.e.
N1	Lowland, shallow, moderate alkalinity, non-humic, large	3.0	2.5–3.5	6.0	5.0–7.0
N2a	Lowland, shallow, low alkalinity, non-humic, large	2.0	1.5–2.5	4.0	3.0–5.0
N2b	Lowland, deep, low alkalinity, non-humic, large	2.0	1.5–2.5	4.0	3.0–5.0
N3a	Lowland, shallow, low alkalinity, humic, large	3.0	2.5–3.5	6.0	5.0–7.0
N5a	Mid-altitude, shallow, low alkalinity, non-humic, large	1.5	1.0–2.0	3.0	2.0–4.0
N6a	Mid-altitude, shallow, low alkalinity, humic, large	2.5	2.0–3.0	5.0	4.0–6.0
N8a	Lowland, shallow, moderate alkalinity, humic, large	4.0	3.5–5.0	8.0	7.0–10.0

n.e. not established

boundaries for their national types according to the type characteristics, e.g., lakes types with low alkalinity, low humic matter, high altitude, high depth, and/or short retention time correspond with reference values close to the minimum of the range and vice versa.

Comparison with Chlorophyll-*a* Reference Values USA and Australia

Also, other nations have codified the concept of reference conditions in their legislation. For instance, in United States ecoregional reference conditions were established representing conditions minimally impacted by human activities (US EPA 2000). In general, the chlorophyll-*a* reference values are of the same magnitude as those for comparable lakes in Europe (1.9–4.9 μg/l). Although several approaches for setting reference conditions were proposed (historical data, predictive models, data of minimally impacted sites), in practice the 25th percentile of a sample distribution from the entire population of lakes was used to derive reference values. As a consequence, reference conditions are significantly higher for ecoregions with higher overall human impact (e.g., ecoregion VI, Western Corn Belt plains and ecoregion XIII, Southern Florida Coastal Plain).

Chlorophyll-*a* reference values for Australian freshwater lakes were derived using the statistical distribution of reference lake data collected within five geographical regions across Australia and New Zealand (ANZECC 2000). Although the term "reference condition" was used in a much less stringent way, allowing use of altered sites when it is not possible to find unimpacted sites, chlorophyll-*a* reference values for Australian lakes range from 3 to 5 μg/l and lie in the same magnitude as those found for lakes in Europe.

It can be concluded that lake chlorophyll-*a* reference conditions set in the European, USA and Australian legislation frameworks are broadly comparable, even if there are substantial differences both in theoretical background and practical application of the reference condition concept and setting environmental quality targets.

Methodological Concerns and Future Directions

There are several aspects which strongly influence the confidence of the chlorophyll-*a* reference values e.g., insufficient number of reference lakes, inherently large heterogeneity of data (different sampling and analyses methods); insufficient geographical coverage of the data; high natural variability within common lake types.

The main problem is that the present work has only focused on eutrophication pressure and only on quantitative part of phytoplankton while considering other pressures and taxonomic composition of phytoplankton are still the tasks for the nearest future.

We therefore believe that the work to be continued for the period of the next River Basin Management Plan, and that a longer period is needed to validate the present results and develop reference conditions both for phytoplankton biomass and taxonomic composition with higher confidence.

Conclusions

1. Mainly a “reference site” approach—selection of lakes with no or very minor human impact—was used for setting chlorophyll-*a* reference conditions in Europe. The selection of reference lakes based on criteria assessing the pressure from the catchment. (land-use, population density and absence of point sources). In some regions paleolimnological data, historical data and modelling of nutrient load were used to validate their choice of reference sites.
2. According to the reference criteria, 359 reference lakes were selected across the EU, representing fifteen common intercalibration types. In the majority of types, reference lakes may be considered type representative; nevertheless, in several cases reference lake selection or type characteristics must be reconsidered, for example, the Central-Baltic types and several Northern types. The dataset ideally needs further inclusion of lakes from the Central-Baltic (especially lake types LCB2 and LCB3) and Mediterranean region. Also, several Northern lake types have a low number of lakes and high variability of the data (LN6a and LN8a).
3. Reference conditions for 15 international lake types were calculated using a common principle: the median value of the measured metric at reference sites was used for reference conditions, while for the High/Good boundary, a percentile between the 75th and 95th was used.
4. Additionally, empirical models were developed for estimating site-specific reference chlorophyll-*a* concentrations from a set of potential predictor variables. Chlorophyll-*a* concentrations increased with humic level and alkalinity, decreased with lake depth and altitude, and varied with geographical region, while there was no clear relationship between chlorophyll-*a* and lake area.
5. A cross-region comparison of reference values and target value for chlorophyll-*a* shows a very good consistency between regions and types: high chlorophyll-*a* values are associated with low depth, high alkalinity, low altitude, and high water colour; conversely, the lowest values were defined for deep lakes with low content of humic matter.
6. The setting of ecological classification is included in the EU legislation (EC Decision), marking the first attempt in international water policy to move from physico-chemical quality standards to ecological quality targets.

Acknowledgments The work was carried out under the Common Implementation Strategy of Water Framework Directive, lead by Working Group ECOSTAT and coordinated by European Commission Joint Research Centre. The authors thank all Lake Intercalibration experts who contributed to the realization of the database and the formulation of ideas and concepts during intercalibration meetings: Luc Denys and Jeroen van Wichelen (Belgium), Andre - Sylvia Nicolaou and Gerald Dörflinger (Cyprus), Martin Søndergaard (Denmark), Helle Mäemets (Estonia), Liisa Lepistö (Finland), Julien de Bartoli and Vincent Bertrin (France), Jörg Schönfelder and Ute Mischke (Germany), Vasiliki Tsiaoussi (Greece), Wayne Trodd (Ireland), Antonio Dalmiglio and Aldo Marchetto (Italy), Mindaugas Gudas (Lithuania), Rob Portielje (the Netherlands), Dag Rosland and Tone Joeran Oredalen (Norway), Agnieszka Kolada (Poland), Ruxandra Maxim (Romania), Mikaela.Gonczi (Sweden), and Sian Davies (UK).

References

Australian and New Zealand Environment and Conservation Council (ANZECC) (2000) Australian and New Zealand guidelines for fresh and marine water quality. Auckland, New Zealand

Bailey RC, Norris RH, Reynoldson TB (2004) Bioassessment of freshwater ecosystems using the reference condition approach. Kluwer Academic Publishers, New York

Battarbee RW (1999) The importance of palaeolimnology to lake restoration. Hydrobiologia 395(396):149–159

Bennion H, Fluin J, Simpson GL (2004) Assessing eutrophication and reference conditions for Scottish freshwater lochs using subfossil diatoms. The Journal of Applied Ecology 41:124–138

Bennion H, Wunsam S, Schmidt R (1995) The validation of diatom-phosphorus transfer functions: an example from Mondsee, Austria. Freshwater Biology 34:271–283

Bradshaw EG, Anderson NJ (2001) Validation of a diatom-phosphorus calibration set for Sweden. Freshwater Biology 46:1035–1048

Cardoso AC, Solimini A, Premazzi G, Carvalho L, Lyche Solheim A, Rekolainen S (2007) Phosphorus reference concentrations in European lakes. Hydrobiologia 584:3–12

Carvalho L, Solimini A, Phillips G, van den Berg M, Pietiläinen OP, Lyche Solheim A, Poikane S, Mischke U (2008) Chlorophyll reference conditions for European lake types used for intercalibration of ecological status. Aquatic Ecology 42:203–211

EC (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23rd October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities, 22 December, L 327/1. European Commission, Brussels

EC (2001) Common implementation strategy for the water framework directive (2000/60/EC). Strategic document as agreed by the Water Directors, May 2, 2001. http://circa.europa.eu/Public/irc/env/wfd/library

EC (2005) Common implementation strategy for the water framework directive (2000/60/EC). Guidance on the Intercalibration process 2004–2006. Luxembourg, Office for Official publications of the European Communities. http://circa.europa.eu/Public/irc/env/wfd/library

EC (2008) Commission Decision of 30 October 2008 establishing, pursuant to Directive 2000/60/EC of the European Parliament and the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise 2008/915/EC. Official Journal of the European Communities, L332/20. European Commission, Brussels

Egan D, Howell EA (2001) The historical ecology handbook: a restorationist's guide to reference ecosystems. Island Press, Washington, USA

Findenegg I (1935) Limnologische Untersuchungen im Kärntner Seengebiet. Internationale Revue der gesamten. Hydrobiologie Hydrographie 32:408–415

Findenegg I (1954) Versuch einer soziologischen Gliederung der Kärntner Seen nach ihrem Phytoplankton. Angewandte Pflanzensoziologie, Festschrift Aichinger 1:299–309

Hughes RM (1995) Defining acceptable biological status by comparing with reference conditions. In: Davis WS, Simon T (eds) Biological assessment and criteria: tools for water resource planning and decision making. Lewis Publishers, Boca Raton, Florida, USA, pp 145–152

Illies J (1978) Limnofauna Europaea. A checklist of the animals inhabiting European inland waters, with account of their distribution and ecology. G. Fischer, Stuttgart and Swets & Zeitlinger, Amsterdam

Jasser I (1997) The dynamics and importance of picoplankton in a shallow, dystrophic lake in comparison with surface waters of two deep lakes with contrasting trophic status. Hydrobiologia 342(343):87–93

Jones RI, Salonen K, Haan H (1993) Phosphorus transformation in the epilinnion of humic lakes: abiotic interactions between dissolved humic materials and phosphate. Freshwater Biology 19:357–369

Kamenik C, Koinig KA, Schmidt R, Appleby PG, Dearing JA, Lami A, Thompson R, Psenner R (2000) Eight hundred years of environmental changes in a high Alpine lake inferred from sediment records. Journal of Limnology 59:43–52

Klee R, Schmidt R, Müller J (1993) Alleröd diatom assemblages in prealpine hardwater lakes of Bavaria and Austria as preserved by the Laacher See eruption event. Limnologica 23:131–143

Kolada A, Soszka H, Cydzik D, Golub M (2005) Abiotic typology of Polish lakes. Limnologica 35:145–150

Löffler H (1972) The distribution of subfossil ostracods and diatoms in pre-alpine lakes. Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie 18:1039–1050

Lotter AF (2001) The palaeolimnology of Soppensee (Central Switzerland), as evidenced by diatom, pollen and fossil-pigment analysis. Journal of Palaeolimnology 25:65–79

Miettinen JO, Kukkonen M, Simola H (2005) Hindcasting baseline values for water colour and total phosphorus concentration in lakes using sedimentary diatoms: implications for lake typology in Finland. Boreal Environment Research 10:31–43

Moeller RE (1994) Contribution of ultraviolet radiation (UV-A, UV-B) to photoinhibition of epilimnetic phytoplankton in lakes of differing UV transparency. Ergebnisse der Limnologie 43:157–170

Moss B, Stephen D, Alvarez C, Becares E, Bund van de W, Collings SE, Van Donk E, Eyto de E, Feldmann T, Fernández-*a*láez C, Fernandez-*a*laez M, Franken RJM, García-Criado F, Gross EM, Gyllström M, Hansson LA, Irvine K, Järvalt A, Jensen JP, Jeppesen E, Kairesalo T, Kornijów R, Krause T, Künnap H, Laas A, Lill E, Lorens B, Luup H, Miracle MR, Nõges P, Nõges T, Nykänen M, Ott I, Peczula W, Peeters ETHM, Phillips G, Romo S, Russell V, Salujõe J, Scheffer M, Siewertsen K, Smal H, Tesch C, Timm H, Tuvikene L, Tonno I, Virro T, Vicente E, Wilson D (2003) The determination of ecological status in shallow lakes—a tested system (ECOFRAME) for implementation of the European Water Framework Directive. Aquatic Conservation: Marine and Freshwater Ecosystems 13:507–549

Müller B, Lotter AF, Sturm M, Ammann A (1998) Influence of catchment quality and altitude on the water and sediment composition of 68 small lakes in Central Europe. Aquatic Sciences 60:316–337

OECD (1982) Eutrophication of waters—monitoring, assessment and control. Organization for Economic Cooperation and Development, Paris

Phillips G, OPietiläinen OP, Carvalho L, Solimini A, Lyche Solheim A, Cardoso AC (2008) Chlorophyll–nutrient relationships of different lake types using a large European dataset. Aquatic Ecology 42:213–226

Ptacnik R, Lepistö L, Willén E, Brettum P, Andersen T, Rekolainen S, Lyche Solheim A (2008) Quantitative responses of lake phytoplankton to eutrophication in Northern Europe. Aquatic Ecology 42:227–236

Reichmann M, Schulz L (2004) Typenspezifische Referenzbedingungen für die integrierende Bewertung des ökologischen Zustandes stehender Gewässer Österreichs gemäß der EU-Wasserrahmenrichtlinie. Projektstudie, Phase 3, Abschlußbericht Modul 2: Bewertung des Phytoplanktons anhand der Gruppen- bzw. Artverteilung. Bericht, Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, Wien

Reynoldson TB, Rosenberg DR, Day KE, Norris RH, Resh VH (1997) The reference condition: a comparison of multimetric and multivariate approaches to assess water quality impairment using benthic macroinvertebrates. Journal of the North American Benthological Society 16:833–852

Ruttner F (1937) Ökotypen mit verschiedener Vertikalverteilung im Plankton der Alpenseen. International Review of Hydrobiology 35:7–34

Ryder RA, Kerr SR, Loftus KH, Regier HA (1974) The morphoedaphic index, a fish yield estimator—review and evaluation. Journal of the Fisheries Research Board of Canada 31:663–688

Ryding SO, Rast W (1989) The control of eutrophication of lakes and reservoirs. Man and biosphere series. UNESCO, Paris and Parthenon Publishing, Carnforth

Salonen K, Kankaala P, Tulonen T, Hammar T, James M, Metsälä TR, Arvola L (1992) Planktonic food chains in a highly humic lakes. Hydrobiologia 229:143–157

Sayer C (2001) Problems with diatom-total phosphorus transfer functions: examples from a shallow English lake. Freshwater Biology 46:743–757

Schmidt R, Psenner R, Müller J, Indinger P, Kamenik C (2002) Impact of late glacial climate variations on stratification and trophic state of the meromictic lake Längsee (Austria): validation of a conceptual model by multi proxy studies. Journal of Limnology 61:49–60

Stoddard JL, Larsen DP, Hawkins CP, Johnson RK, Norris RH (2006) Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16(4):1267–1276

Taylor D, Dalton C, Leira M, Jordan P, Irvine K, Bennion H, McGee E, León-Vintró L (2006) Identification of reference status for Irish lake typologies using palaeolimnological methods and techniques. IN-SIGHT EPA/ERTDI Project # 2002-W-LS/7 Final Report. School of Natural Sciences. Trinity College, University of Dublin, Dublin

United States Environment Protection Agency (US EPA) (2000) Nutrient criteria technical guidance manual. Lakes and Reservoirs. EPAA 822-B-00–007 U.S. EPA, Washington, DC

Vighi M, Chiaudani G (1985) A simple method to estimate lake phosphorus concentrations resulting from natural background loading. Water Resources 10:987–991

Vollenweider RA (1976) Advances in defining critical loading levels for phosphorus in lake eutrophication. Memorie dell'Istituto Italiano di Idrobiologia 33:53–69

Webster KE, Soranno PA, Cheruvelil KS, Bremigan M, Downing A, Vaux P, Asplund T, Bacon LC, Connor J (2008) An empirical evaluation of the nutrient color paradigm for lakes. Limnology and Oceanography 53(3):1137–1148

Winter A, Fish T, Playle R, Smith DS, Curtis P (2007) Photodegradation of natural organic matter from diverse freshwater sources. Aquatic Toxicology 84:215–222