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Chlorophyll *a* concentration across a trophic gradient of lakes: An estimator of phytoplankton biomass?

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Dedicated to Prof. Jürgen Benndorf on the occasion of his 65th birthday.

Abstract

Chlorophyll *a* (chl*a*) concentration was evaluated as a predictor of phytoplankton biomass across a broad trophic gradient of lakes (oligotrophic – highly eutrophic). First, a literature survey was conducted to collect information on the proportion of chl*a* in phytoplankton biomass. As a result of this study ($n = 21$) a mean value of 0.505% ± 0.197 S.D. chl*a* per unit wet weight of phytoplankton was calculated. Second, analyses were performed on 756 paired measurements from an unpublished database where the specific chl*a* content of phytoplankton biomass was regressed against phytoplankton standing stocks and chl*a* concentration. Within an interval of 0.1–50 g m^{-3} of phytoplankton wet weight, a substantial decrease in chl*a* proportion from approximately 2.5% to 0.18% was found. Likewise, the proportion in phytoplankton wet weight decreased from 0.7% to 0.15% across a chl*a* concentration interval of 0.001–0.150 g m^{-3}. These results had a significant impact both on chl*a*-based biomass calculations and the subsequent comparison with phytoplankton biomasses resulting from microscopic counts. Assuming the microscopic method was a measure of the "true" phytoplankton standing stocks, then the precision by which phytoplankton biomass might be predicted based on chl*a* measurements is clearly better when using variable proportions as compared to a constant conversion factor. The same holds for temporal coherence between annual records of phytoplankton biomass. The temporal fit was apparently better when relating the results of microscopic counts and biomass estimation based on variable proportions of chl*a* in phytoplankton biomass. Nevertheless, this effect diminished as the tropic status of the lakes increased. Because of their variable specific chl*a* content, separate taxonomic groups of phytoplankton differently affected the proportion of chl*a* in total

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phytoplankton wet weight. Chlorophyceae, Cryptophyceae and cyanobacteria had a high impact, while Bacillariophyceae, Dinophyceae and Chrysophyceae were of lesser importance.

Keywords: Phytoplankton; Biomass estimation; Comparison of methods; Microscopic counts; Specific chlorophyll *a* proportion; Conversion factors; Lakes; Trophic gradient

Introduction

Estimating phytoplankton biomass is one of the most useful measurements in limnology and oceanography. Although frequently performed, the approach is not trivial and the results are sometimes hard to interpret (Tolstoy, 1977; Wasmund, 1984; Stich and Brinker, 2005). This is especially true if information from various methods is being compared (Hallegraeff, 1977; Halfson, 1984; Schmid et al., 1998).

Methods to determine phytoplankton standing crops have been developed for quite some time and can be categorised into two general groups: (1) particle counting (Utermöhl, 1923, 1958), and (2) measurement of chemical constituents (Richards and Thompson, 1952; Strickland and Parsons, 1960) with flow-cytometry being a combination of both (Töpel et al., 2004). Over the past decades both approaches were heavily refined and modified. Nevertheless, some of the basic methodological problems have not been resolved (Padisák et al., 1999; Wright et al., 1997). The most important are: (i) methodological flaws, (ii) variable chl*a* proportions per unit phytoplankton biomass, (iii) the taxonomic composition of the phytoplankton community and finally (iv) seasonal aspects.

Microscopic examination and counting of phytoplankton species in collected samples is time-consuming and requires extensive taxonomic experience by the investigator (Banse, 1977; Krienitz et al., 1996). Chemical preservation of the samples can alter the size frequency distribution of the phytoplankton cells (Verity et al., 1992). Moreover, autotrophic picoplankton (APP) may sometimes contribute significantly to total phytoplankton biomass but are not often recorded (Padisák et al., 1997). To overcome these problems, particle counters and image analysis systems have been utilised, but their performance in estimating phytoplankton biomass as compared to microscopic methods is still questioned (Hillebrand et al., 1999).

Concerning chl*a* extraction and the subsequent photometric or HPLC measurements, several authors have shown that there is no ideal protocol (Pápista et al., 2002; Stich and Brinker, 2005). Depending on the taxonomic structure of the phytoplankton sample being analysed, different extraction solvents may have different extraction efficiencies (Vollenweider, 1974; Wright et al., 1997).

Finally, various studies have found that chl*a* content per unit of phytoplankton biomass decreases as phytoplankton standing stocks increase (Desortova, 1981; Ahlgren, 1983; Wojciechowska, 1989; Watson et al., 1992; Talling, 1993; Chow-Fraser et al., 1994; Schmid et al., 1998; Felip and Catalan, 2000; Sandu et al., 2003; Kiss et al., 2006). This phenomenon may be influenced by lake trophic status (Harris, 1986), phytoplankton community structure (Bursche, 1961; Nusch and Palme, 1975), the size frequency distribution of the algal cells (Watson and McCauley, 1988), and by seasonal shifts within the plankton community (Loth, 1985; Vanni et al., 1993).

Notwithstanding these problems and limitations, we examined whether chl*a* concentration across a trophic gradient of lakes (oligotrophic – eutrophic) can be used as a predictor of phytoplankton biomass. Chl*a*-based calculations of phytoplankton biomass were performed by applying constant conversion factors as determined from the literature and by using variable ratios gained from a comprehensive database of the Leibniz-Institute of Freshwater Ecology & Inland Fisheries (IGB, Neuglobsow, Germany). Moreover, we tested the precision and temporal coherence by which time series of phytoplankton biomass of various lakes can be predicted using these conversion factors as compared to the results of microscopic counts.

Material and methods

Investigation sites

The five lakes included in this study are located within the eastern part of Germany's glacial Baltic lake region (53°15′N, 13°10′E) approximately 100 km north of Berlin. They are seepage lakes with ground water and rainfall being the major sources of water. The lakes thermally stratify from May until at least September. Mean temperature of the mixed layer varies between 4 °C (January) and 20 °C (August). Global radiation ranges between 200 J cm^{-2} d^{-1} (December) and 1700 J cm^{-2} d^{-1} (June; German Weather Service, unpublished results). The lakes have significantly different morphometric and chemical characteristics. Their trophic status spans from oligotrophic to highly eutrophic (cf. Table 1). For more information about the five study lakes see Casper (1985), Kasprzak et al.

Table 1. Morphometric and chemical characteristics of the investigation sites according to Koschel et al. (1985), Casper (1985), Koschel and Kasprzak (1994) and unpublished data of the authors

Feature	Stechlin	Kleiner Väter	Großer Väter	Tiefwaren	Feldberger Haus
Area (km^2)	4.23	0.10	0.12	1.41	1.36
Volume (10^6 m^3)	98.70	0.51	0.63	13.80	8.15
Mean depth (m)	23.3	5.0	5.2	9.6	6.0
Maximum depth (m)	69.5	13.3	11.5	23.0	12.0
Sampled layer (m)	0–20	0–6	0–6	0–5	0–5
Total P (μg L^{-1})	8.6–16.7	16.2–22.7	18.6–29.2	24.8–124.6	93.1–1292.5
Total N (μg L^{-1})	300–585	750–820	860–950	980–1200	1300–1900
Chlorophyll *a* (mean) (μg L^{-1})	0.7–9.5 (2.3)	1.1–8.1 (4.0)	0.9–15.6 (4.6)	1.4–29.5 (6.2)	1.3–175.9 (24.2)
Sechi transparency (m)	8.4	4.3	3.9	4.7	1.8
Trophic status	Oligotrophic	Mesotrophic	Mesotrophic	Meso-eutrophic	Eutrophic

Total phosphorus and nitrogen concentrations represent the minimum and maximum of annual means, respectively, within the mixed layer of the lakes. Concerning chlorophyll *a* concentration, the grand mean of all observations is additionally denoted in parenthesis. The classification of the trophic status was conducted following OECD recommendations (Premazzi and Chiaudani, 1992). Water clarity was calculated based on all available Secchi readings.

(2000), Kasprzak et al. (2003), Koschel and Adams (2003) and Koschel et al. (2006).

Field sampling

Data used in this study were collected during 1985–2006 from the deepest location of each lake. However, the number of investigation years and consequently the number of samples were different (min. Tiefwarensee, $n = 74$, max. Feldberger Haussee, $n = 265$, cf. Table 3). During most years, samples were taken at least once a month although samples were also collected biweekly from May until September in a number of years. Samples were either taken at discrete depths or as composite samples, and as such roughly represented the euphotic zone of the study lakes (cf. Table 1). All samples were split for chl*a* and phytoplankton biomass measurements.

Field sampling and sample treatment followed widely accepted protocols (see below). Still, over the years technical equipment (e.g. microscopes, sedimentation chambers, photometers and chemicals) varied and several people performed the analyses (especially phytoplankton counts). For this study, we did not compare results produced during various periods or by different investigators. Rather, we assumed that the methods had no major bias and correctly reflected the magnitude of each value in the time series.

Chlorophyll *a* determination

Chl*a* samples were processed following recommendations of the German Standard Methods of Water and Wastewater Analyses (Deutsche Einheitsverfahren, 1983–1985). Appropriate aliquots (200–2000 ml) were either filtered through membrane filters (pore size 1.2 μm; Lake Stechlin, Tiefwarensee, Feldberger Haussee) or through Whatman GF/C glass fibre filters (Kleiner Vätersee, Großer Vätersee). If not analysed immediately, the two types of filters were frozen at −20 and −80 °C, respectively, until further treatment. Prior to extraction, filters were homogenised to enhance extraction efficiency. Extraction was performed by either adding 99.8% acetone (Lake Stechlin, Tiefwarensee, Feldberger Haussee) or 90% hot ethanol (78 °C; Kleiner Vätersee, Großer Vätersee) followed by either filtration or centrifugation to produce a supernatant with minimal turbidity. For Lake Stechlin, Tiefwarensee and Feldberger Haussee, the absorbance of the processed samples was recorded at three different wavelengths (630, 665 and 750 nm) following the protocol of Strickland and Parsons (1960) for calculating chl*a* concentration. For Kleiner Vätersee and Großer Vätersee, chl*a* concentration was determined using the absorption maximum of 665 nm exclusively.

Phytoplankton biomass estimation

Phytoplankton biomass was estimated in four separate ways: (1) by microscopic counts, (2) based on a constant chl*a* proportion in phytoplankton wet weight, (3) by applying variable chl*a* ratios in phytoplankton wet weight as related to phytoplankton biomass and (4) by using variable chl*a* ratios in phytoplankton wet weight as related to chl*a* concentration.

Microscopic counts

Phytoplankton samples were preserved in Lugol's solution and counted under an inverted microscope (Utermöhl, 1958). Biovolume was calculated based on cell- or colony volumes suggested by several authors (Willén, 1992; Hamilton, 1990; Gosselain and Hamilton, 2000; Hoehn et al., 1998) and subsequently converted into wet weight assuming a specific gravity of $1\,g\,cm^{-3}$ (Rott, 1978). APP was preferably counted immediately after sampling in unpreserved samples. If counting was not possible, either unpreserved samples were deep-frozen and enumerated within one month or sub-samples preserved in 0.2% formaldehyde were filtered on to 0.4 μm pore size polycarbonate membranes and processed later. APP was only estimated for Lake Stechlin, Kleiner Vätersee and Großer Vätersee. Although its biomass may increase as phytoplankton standing stocks rise, the proportion of APP to total phytoplankton tended to diminish as the lake trophic status increased from oligotrophic to eutrophic (see Fig. 2c, p. 72, in Callieri and Stockner, 2002). With respect to the total phosphorus concentration of Tiefwarensee and highly eutrophic Feldberger Haussee, the proportion of APP within total phytoplankton may range between 3% and 5%. We therefore decided to not correct any of the phytoplankton measurements collected from these lakes. For further details concerning phytoplankton analysis see Krienitz et al. (1996), Gervais et al. (1997), Padisák et al. (1997) and Padisák et al. (2003).

Constant chl*a*/wet weight ratio as related to phytoplankton biomass

Based on the results of 21 studies reported in the literature a mean proportion of chl*a* in phytoplankton wet weight was estimated. Using this factor the chl*a* data considered in our study were converted into phytoplankton wet weight. Numbers on specific chl*a* content originate from North American and European lakes and reservoirs, respectively. Their trophic status represents a broad spectrum ranging from oligotrophic to eutrophic conditions. In two instances information collected from algal cultures was integrated.

Variable chl*a*/wet weight ratio as related to phytoplankton biomass

A total of 756 paired observations (IGB database) on the ratio of chl*a*/phytoplankton wet weight was regressed over phytoplankton wet weight. The regression function was subsequently used to calculate phytoplankton biomass.

Variable chl*a*/wet weight ratio as related to chl*a* concentration

Based on the same collection of 756 paired observations (IGB database) the ratio of chl*a*/phytoplankton wet weight was regressed against chl*a* concentration. By applying the regression function, phytoplankton biomass was estimated.

Microscopic estimates of phytoplankton biomass are designated BM_{count}. Phytoplankton biomass calculated by constant or variable per wet weight chl*a* proportions are denoted $BM_{chla\text{-}con}$, $BM_{chla\text{-}var(1)}$, and $BM_{chla\text{-}var(2)}$, respectively.

Statistical analyses

To qualify and compare different ways of calculating phytoplankton biomass, Pearsons's product-moment regression coefficient (r), mean systematic deviation (MSD, bias) and standard deviation (S.D.) were calculated. Regression analysis was performed in order to relate the results of phytoplankton calculations based on chl*a* measurements ($BM_{chla\text{-}con}$, $BM_{chla\text{-}var(1)}$, $BM_{chla\text{-}var(2)}$) and microscopic counts (BM_{count}). A graphical check for homoscedasticity indicated variances were not homogeneous. Therefore, prior to regression analysis both chl*a* concentration and phytoplankton biomass values were $\log_{10}$-transformed. Kolmogorov–Smirnov one-sample-test indicated the residuals of the $\log_{10}$-transformed data to be approximately normal distributed. Spearman rank correlation coefficient was applied to comprehend the impact of separate taxonomic groups of phytoplankton on the relation of chla/BM_{count} as related to BM_{count}. All statistical calculations were performed using SPSS 9.0 (SPSS Inc., Chicago, Bühl and Zöfel, 2000).

Results

The three ways of calculating the proportion of chl*a* in phytoplankton wet weight (chla/BM_{count}) led to remarkably different findings. The literature survey results indicated the chla/BM_{count} content of several phytoplankton populations (Table 2). Depending on the parameter used for the calculation, the proportion amounted to 0.505% (mean) and 0.447% (median). Overall the results varied between 0.158% (minimum) and 0.900% (maximum).

In Fig. 1 (upper panel), the ratio of chla/BM_{count} is plotted against BM_{count} for the 756 paired observations obtained from the IGB data collection. Over a range of BM_{count} roughly spanning between 0.1 and $50\,g\,ww\,m^{-3}$, a substantial decrease in specific chl*a* content from approximately 2.5% to 0.18% was found (cf. Eq. (1)). Likewise, within a concentration interval of $0.001–0.150\,g\,m^{-3}$ chl*a* (Fig. 1, lower panel) the proportion of chla/BM_{count} decreased from approximately 0.7% to 0.15% (cf. Eq. (2)):

$$\log\left(\frac{\text{chl}a}{BM_{count}}\right) = -0.403 - 0.482 \log BM_{count} + 0.229(\log BM_{count})^2 - 0.040(\log BM_{count})^3$$
$$(r^2 = 0.550,\ \text{S.D.} = 0.243,\ P<0.001,\ \text{chl}a - g\,m^{-3},\ BM_{count} - g\,ww\,m^{-3}), \quad (1)$$

Table 2. Proportion of chlorophyll *a* (chl*a*) in phytoplankton wet weight (ww) calculated based on published literature information

chl*a*/ww [%]	*n*	Parameter	Reference
0.900	25	Median	Nicholls and Dillon (1978, p. 146), Table 3, lakes, natural phytoplankton
0.560	4	Mean	Tolstoy (1977, p. 16), lakes, natural phytoplankton (10–90 μg L^{-1})
0.420	7	Median	Vörös and Padisák (1991, p. 113), Table 1, lakes, natural phytoplankton (0.1–238 μg L^{-1})
0.691	19	Median	Reynolds (1984, p. 30), Table 4, lakes, single species of natural populations
0.630	4	Mean	Rott (1978, p. 16), Table 1, lakes, natural phytoplankton (3.2–5.5 μg L^{-1})
0.230	16	Median	Wojciechowska (1989, p. 66), Table 1, lakes, natural phytoplankton (3–12 μg L^{-1})
0.690	4	Mean	Loth (1985, p. 323), Table 1, reservoirs, natural phytoplankton (1–8 μg L^{-1})
0.720	11	Median	Desortova (1981, p. 160), Table 1, reservoirs, natural phytoplankton (2.4–23.1 μg L^{-1})
0.364	1	–	Schellenberger et al. (1985, p. 219), lakes, natural phytoplankton
0.447	14	Median	Chow-Fraser et al. (1994, pp. 2060–2061), Figs. 6b and 9, lakes, natural phytoplankton (0.6–100 μg L^{-1})
0.355	6	Median	Watson et al. (1992, p. 2607), Fig. 1b, lakes, natural phytoplankton (1–100 μg L^{-1})
0.390	96	Median	Bursche (1961, pp. 617–645), Tables. 2–11, laboratory cultures, various species (10–70 μg L^{-1})
0.280	33	Median	Montagnes et al. (1994, p. 1047), Table 1, laboratory cultures, various species (0.1–100 μg L^{-1})
0.780	23	Median	Ahlgren (1983, p. 497), Table 3, lakes, natural phytoplankton (6–118 μg L^{-1})
0.600	17	Median	Schmidt et al. (1998, p. 1658), Fig. 1a, lakes, natural phytoplankton (5–25 μg L^{-1})
0.158	38	Median	Sandu et al. (2003, S. 393), Table 2, lakes, natural phytoplankton (13.3–125.3 μg L^{-1})
0.643	1	–	Felip and Catalan (2000, p. 91–105), lake, natural phytoplankton (1.1–5.7 μg L^{-1})
0.619	20	Median	Kiss et al. (2006, p. 2052), Table 1, lakes, natural phytoplankton (8.7–64.8 μg L^{-1})
0.400	5	Mean	Lampert and Schober (1978, p. 370), Abb. 2b, lake, natural phytoplankton (10–35 μg L^{-1})
0.420	24	Median	Talling (1993, p. 90), Table 3, lakes, natural phytoplankton (1–120 μg L^{-1})
0.300	1	–	Padisák et al. (1999, p. 369)
0.505	Mean		
0.197	S.D.		
0.447	Median		
0.158	Minimum		
0.900	Maximum		

n refers to the number of observations derived from the references cited. Regardless of *n*, the results concerning chl*a*/ww [%] were not frequency-weighted but used as separate numbers to calculate grand mean, median and standard deviation. In many cases the values have been estimated from chlorophyll *a* and cell volume data given in figures and tables. When provided, the interval of chlorophyll *a* concentration is given in parenthesis.

$$\log\left(\frac{\text{chl}a}{\text{BM}_{\text{count}}}\right) = -0.979 - 0.282 \log \text{BM}_{\text{count}}$$
$$(r^2 = 0.164,\ \text{S.D.} = 0.027,\ P<0.001,$$
$$\text{chl}a - \text{g m}^{-3},\ \text{BM}_{\text{count}} - \text{g ww m}^{-3}). \quad (2)$$

Spearman's rank correlation coefficient pointed to different effects of separate taxonomic groups of phytoplankton to the changes in the ratio of chl*a*/BM_{count}. Except for chrysophyceans all other taxa significantly affected the specific chl*a* content of phytoplankton wet weight ($p<0.001$; Chlorophyceae: −0.436, Cryptophyceae: −0.268, cyanobacteria: −0.214, Bacillariophyceae: −0.208, Dinophyceae: −0.170 and Chrysophyceae −0.062).

The results portrayed above are clearly reflected in Fig. 2 where phytoplankton wet weight was calculated based on chl*a* content ($\text{BM}_{\text{chl}a\text{-con}}$, $\text{BM}_{\text{chl}a\text{-var(1)}}$, $\text{BM}_{\text{chl}a\text{-var(2)}}$) and regressed against the results of microscopic counts (BM_{count}). If related to BM_{count}, the before mentioned constant proportion of 0.505% chl*a* per unit phytoplankton wet weight resulted in a significant underestimation of phytoplankton biomass and a greater variability along the regression line (Fig. 2, upper panel, slope 0.617, $p<0.0001$, S.E. 0.013, adjusted r^2 0.729). When applying variable proportions of chl*a* per unit phytoplankton wet weight (Eq. (1)), the ratio between $\text{BM}_{\text{chl}a\text{-var(1)}}$ and BM_{count}, respectively, was close to 1, and the variability along the regression line was smaller (Fig. 2, central panel, slope 1.036, $p<0.0001$, S.E. 0.012, adjusted r^2 0.906). Concerning the results calculated using Eq. (2), the difference between $\text{BM}_{\text{chl}a\text{-var(2)}}$ and BM_{count} was somewhat smaller (Fig. 2, lower panel, slope 0.781, $p<0.0001$, S.E. 0.027, adjusted r^2 0.722) as was found for $\text{BM}_{\text{chl}a\text{-con}}$. Still, the estimated phytoplankton biomass was clearly lower then BM_{count}.

The predictive power of $\text{BM}_{\text{chl}a\text{-con}}$, $\text{BM}_{\text{chl}a\text{-var(1)}}$ and $\text{BM}_{\text{chl}a\text{-var(2)}}$ as related to BM_{count} are summarised in Table 3. Looking at the results obtained from all lakes, the closest relationship was found between BM_{count} and $\text{BM}_{\text{chl}a\text{-var(1)}}$. This was true for correlation (r 0.952), MSD (−0.003) and variability (S.D. 0.217). The quality of the prediction of phytoplankton biomass based on $\text{BM}_{\text{chl}a\text{-con}}$ and $\text{BM}_{\text{chl}a\text{-var(2)}}$ was less precise.

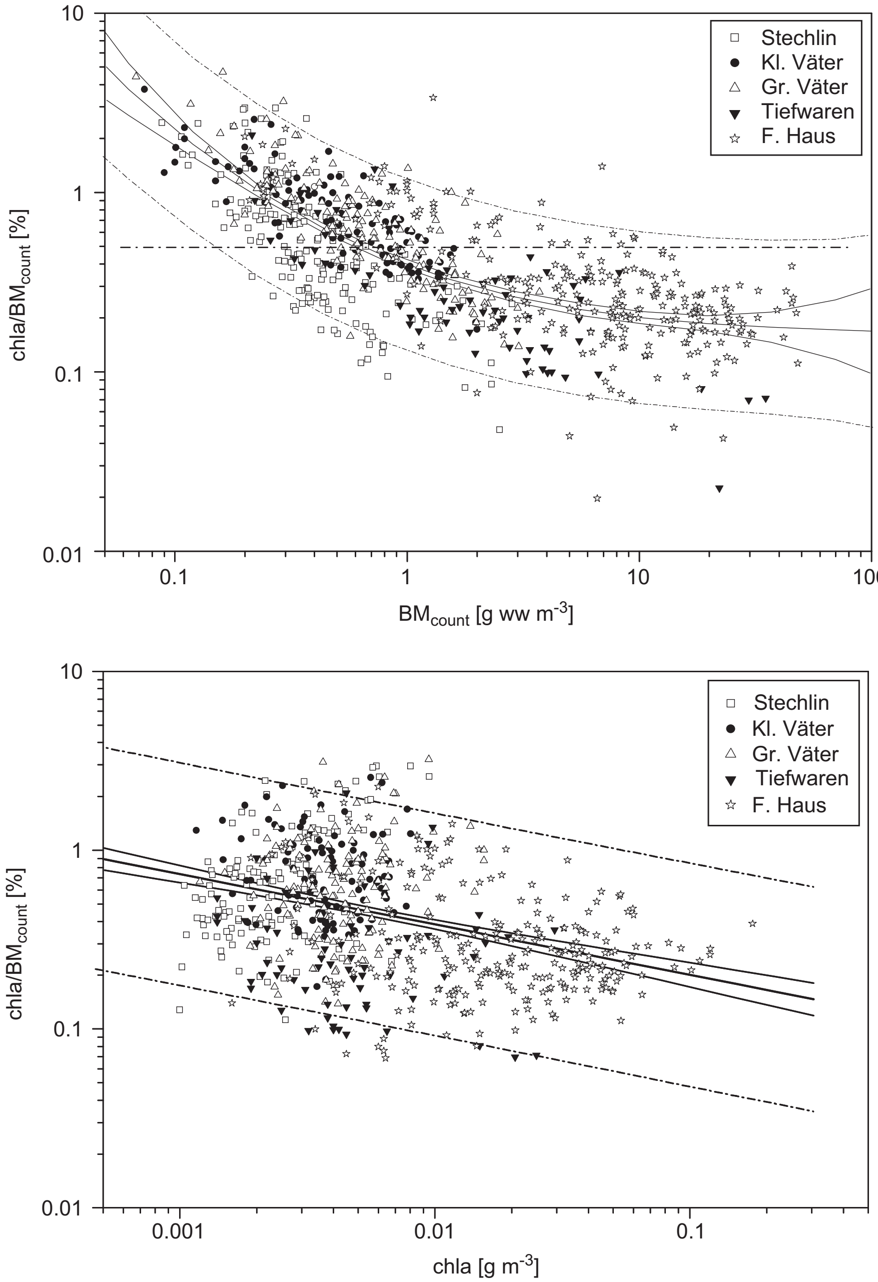

Fig. 1. Proportion of chlorophyll *a* per phytoplankton wet weight (chl*a*/BM_{count}) regressed against phytoplankton wet weight (BM_{count}, upper panel) as obtained from microscopic counts and against chl*a* concentration (lower panel, including 95% confidence bands and prediction bands of the regression line, cf. Eqs. (1) and (2)). The dotted horizontal line in the upper panel indicates the 0.505% constant ratio of per wet weight chlorophyll *a* content in phytoplankton biomass (cf. Table 2).

Furthermore, the difference in the predictive power of $BM_{chla\text{-}con}$ and $BM_{chla\text{-}var(2)}$ was small. Clear differences were only found concerning MSD where $BM_{chla\text{-}var(2)}$ was superior to $BM_{chla\text{-}con}$. Looking at separate lakes, the results were varying. Nevertheless, even then a clear tendency of better correlation, smaller deviation and lesser variation was detected when phytoplankton biomass was calculated based on $BM_{chla\text{-}var(1)}$ as compared to the use of $BM_{chla\text{-}con}$ and $BM_{chla\text{-}var(2)}$.

Qualified by Pearson's product-moment correlation coefficient (*r*), Fig. 3 rates the temporal coherence between BM_{count} and $BM_{chla\text{-}con}$, $BM_{chla\text{-}var(1)}$ and $BM_{chla\text{-}var(2)}$, respectively, as was found for the time series of separate lakes. It is obvious that by using $BM_{chla\text{-}var(1)}$ a higher temporal coherence could have been achieved as compared to the application of $BM_{chla\text{-}con}$ or $BM_{chla\text{-}var(2)}$. Moreover, this tendency was less pronounced in eutrophic lakes.

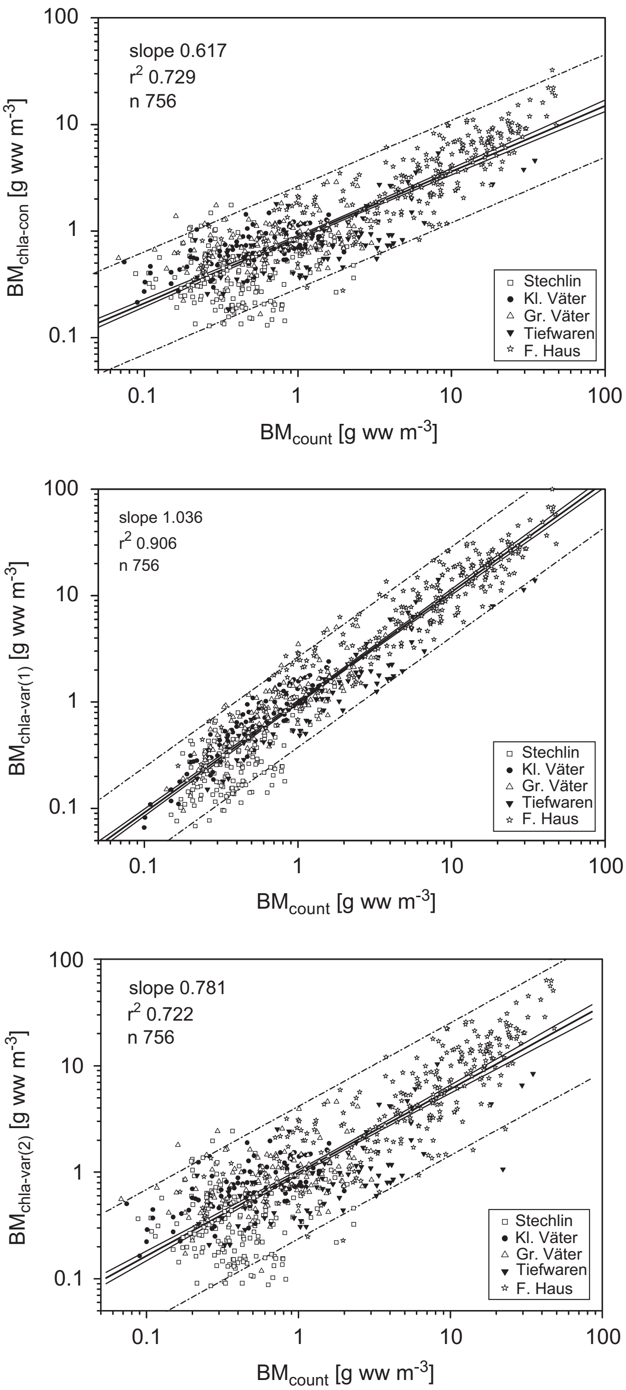

Fig. 2. Relation between phytoplankton wet weight based on microscopic counts (BM_{count}) and phytoplankton wet weight calculated using constant ($BM_{chl\mathit{a}\text{-}con}$, upper panel) and variable ($BM_{chl\mathit{a}\text{-}var(1)}$, central penal, $BM_{chl\mathit{a}\text{-}var(2)}$, lower panel) proportions within phytoplankton biomass, respectively.

Finally, the example of three separate lakes (Fig. 4) provides specific information on the annual record of temporal coherence between BM_{count}, $BM_{chl\mathit{a}\text{-}con}$, $BM_{chl\mathit{a}\text{-}var(1)}$ and $BM_{chl\mathit{a}\text{-}var(2)}$. Again, as indicated by Pearson's product-moment correlation coefficient (*r*), there was a general tendency of higher temporal coherence when using, $BM_{chl\mathit{a}\text{-}var(1)}$ as compared to $BM_{chl\mathit{a}\text{-}con}$ and $BM_{chl\mathit{a}\text{-}var(2)}$. The best fit of annual records in individual lakes was found for Feldberger Haussee in the year 2000 (*r* 0.989).

Overall the conclusion seems reasonable that $BM_{chl\mathit{a}\text{-}var(1)}$ was clearly a better estimator of phytoplankton biomass than $BM_{chl\mathit{a}\text{-}con}$ or $BM_{chl\mathit{a}\text{-}var(2)}$. Calculations based on $BM_{chl\mathit{a}\text{-}var(1)}$ and microscopic counts (BM_{count}) lead to similar results both with respect to precision and temporal coherence. In contrast, estimation of phytoplankton biomass using $BM_{chl\mathit{a}\text{-}con}$ resulted in significant over- (oligotrophic lakes) and underestimation (mesotrophic, eutrophic lakes) of phytoplankton biomass, respectively. As compared to $BM_{chl\mathit{a}\text{-}con}$, phytoplankton biomass calculated from $BM_{chl\mathit{a}\text{-}var(2)}$ was more similar to the results of microscopic counts. Still, the deviations in terms of precision and temporal coherence were clearly greater as was found for $BM_{chl\mathit{a}\text{-}var(1)}$. Of course, these conclusions can only be considered valid if BM_{count} represented the "true" phytoplankton biomass.

Discussion

The microscopic elaboration of phytoplankton samples including subsequent calculation of algal biomass are labour-intensive and require sound taxonomic skills of the investigator (Utermöhl, 1958; Hillebrand et al., 1999). Consequently, chl*a* concentration began being used as a quick and easy-to-measure surrogate of phytoplankton biomass (e.g. Richards and Thompson, 1952; Strickland and Parsons, 1960; Kamoto, 1966; Dillon and Rigler, 1974). Nevertheless, besides the fact that chl*a* measurements lack any information on phytoplankton community structure, our investigations indicate that constant conversion factors are inappropriate for calculating phytoplankton biomass. In oligotrophic lakes, chl*a*-based phytoplankton biomass calculations tend to be higher then biomass derived from microscopic counts, while an increasing trend of underestimation was found for mesotrophic and eutrophic lakes, respectively. Using constant conversion factors, in a study on the relation of chl*a* content and phytoplankton biomass in eutrophic Danube delta lakes, Sandu et al. (2003) found significantly higher results by microscopic counts as compared to estimates based on constant chl*a* proportions.

Taking into account the 756 IGB paired observations of chl*a*/BM_{count}, the conclusion instead seems reasonable for a functional relationship between chl*a* and BM_{count}. The higher BM_{count} the lower is the proportion of chl*a* per unit of BM_{count}. Beyond the above-mentioned multitude of variables (cf. Introduction section), there are two major factors responsible for dropping chl*a*/BM_{count} as BM_{count} increases.

Table 3. Statistical indices qualifying the results of various approaches to calculate phytoplankton biomass based on chl*a* measurements as compared to the results of microscopic counts

Lake	chl*a*/BM_{count} (%) min., max. (mean)	BM_{count} vs. $BM_{chla\text{-}con}$			BM_{count} vs. $BM_{chla\text{-}var(1)}$			BM_{count} vs. $BM_{chla\text{-}var(2)}$			*n*
		r	MSD	S.D.	*r*	MSD	S.D.	*r*	MSD	S.D.	
Stechlin (o)	0.047–2.954 (0.699)	0.045	0.038	0.361	0.624	0.172	0.229	0.126	0.087	0.408	170
K. Väter (m)	0.173–3.757 (0.860)	0.639	−0.126	0.248	0.918	−0.069	0.128	0.499	−0.154	0.246	103
G. Väter (m)	0.138–4.676 (0.796)	0.443	−0.053	0.305	0.847	−0.071	0.166	0.387	−0.095	0.319	144
Tiefwaren (eu)	0.023–2.093 (0.344)	0.562	0.188	0.647	0.930	0.147	0.173	0.582	0.133	0.637	74
F. Haus (eu)	0.020–3.372 (0.380)	0.801	0.286	0.319	0.898	−0.085	0.189	0.798	0.076	0.331	265
All lakes	0.020–4.676 (0.594)	0.853	0.100	0.396	0.952	−0.003	0.217	0.850	0.020	0.390	756

BM_{count} – phytoplankton wet weight obtained from microscopic counts, $BM_{chla\text{-}con}$ – phytoplankton wet weight assuming a constant ratio between chlorophyll *a* and phytoplankton wet weight (chl*a*/ww = 0.505%, cf. Table 2), $BM_{chla\text{-}var(1)}$ – phytoplankton wet weight assuming a variable ratio between chlorophyll *a* and phytoplankton wet weight (cf. Fig. 1, upper panel, Eq. (1)), $BM_{chla\text{-}var(2)}$ – phytoplankton wet weight assuming a variable ratio between chlorophyll *a* and phytoplankton wet weight (cf. Fig. 1, lower panel, Eq. (2)), *r* – Pearson's product moment correlation coefficient, MSD – mean systematic deviation (bias), S.D. – standard deviation, *n* – number of observations. The acronyms in the first column of the table refer to oligotrophic (o), mesotrophic (m) and eutrophic (eu).

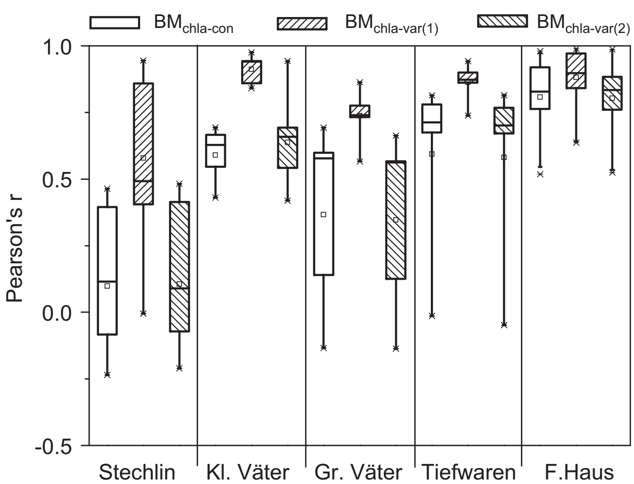

Fig. 3. Temporal coherence as qualified by Pearson's product-moment correlation coefficient (*r*) of annual phytoplankton biomass records, estimation based on microscopic counts (BM_{count}) and on the assumption of constant ($BM_{chla\text{-}con}$) and variable ($BM_{chla\text{-}var(1)}$, $BM_{chla\text{-}var(2)}$) ratios of per wet weight chlorophyll *a* content in phytoplankton biomass calculated for separate lakes. Number of recorded lake years: Lake Stechlin – 10, Kleiner Vätersee – 6, Großer Vätersee – 8, Tiefwarensee – 5 and Feldberger Haussee – 18. Box-whisker-plots indicate minimum, maximum, percentiles (10, 25, 75, 90%), mean and median of (*r*).

First, some studies provide convincing evidence that the proportion of chl*a* per unit of BM_{count} is inversely related to algal cell- or colony volume (Harris, 1986; Wojciechowska, 1989; Vörös and Padisák, 1991). Therefore, a given biomass unit of "small" algal cells is likely to contain more chl*a* then does the same amount of "big" phytoplankton cells. Because increasing mean cell- or colony volume is characteristic of eutrophic lakes (Watson and McCauley, 1988), decreasing chl*a*/BM_{count} is an apparent consequence of nutrient enrichment (Watson et al., 1992; Chow-Fraser et al., 1994). Also, because mean phytoplankton cell size typically increases during seasonal succession (Sommer et al., 1986), seasonal aspects might be relevant as well.

Furthermore, besides our own investigation a number of other studies indicate decreasing chl*a*/BM_{count} in natural phytoplankton populations as BM_{count} increases (Desortova, 1981; Wojciechowska, 1989; Talling, 1993; Schmid et al., 1998; Felip and Catalan, 2000; Kiss et al., 2006). This trend does seem to be valid both for natural phytoplankton communities but also for laboratory cultures of certain species as well (*Planktothrix agardhii*; Ahlgren, 1983). Reynolds (2006) argues that a high surface-to-volume ratio is beneficial for nutrient acquisition. Because nutrient limitation tends to diminish as nutrient concentrations rise, eutrophication might be the ultimate factor allowing the mean particle size of natural phytoplankton communities to increase. Thus, growing phytoplankton biomass in concert with increasing mean cell- or colony volume might necessarily result in decreasing chl*a*/BM_{count} ratio as the lakes develop from oligotrophic into eutrophic conditions.

The impact of species-specific differences on chl*a* per unit of phytoplankton biomass is controversial. Reynolds (1984, p. 38) reported on significant differences in volume-related chl*a* proportion between different taxonomic groups of phytoplankton. Working with laboratory cultures, Bursche (1961) and Nusch and Palme (1975) found significant differences between systematic groups as well with highest proportions in green algae and lowest in cyanobacteria. Desortova (1981) and Talling (1993) confirmed these result for natural phytoplankton communities during those periods when one or another taxonomic group dominated. Kohl and Nicklisch (1988) concluded that because of the structure of the light-harvesting pigment–protein complex, the specific chl*a* content is high in green algae, low

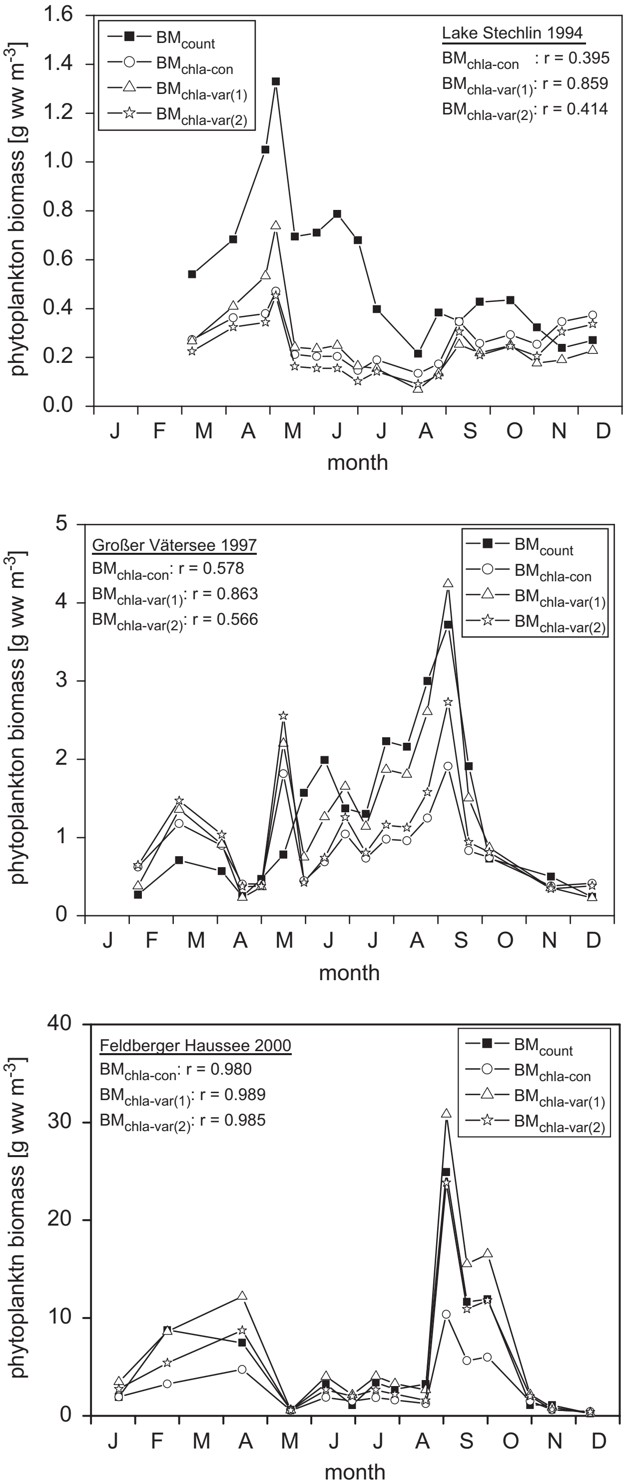

Fig. 4. Selected examples of annual records of phytoplankton standing stocks in various lakes estimated based on microscopic counts (BM_{count}) and on the assumption of constant ($BM_{chla\text{-}con}$) and variable ($BM_{chla\text{-}var(1)}$, $BM_{chla\text{-}var(2)}$) ratios of per wet weight chlorophyll *a* content in phytoplankton biomass, respectively. Pearson's product-moment correlation coefficient (*r*) has been used to qualify temporal coherence.

in cyanobacteria and intermediate in chromophyta. However, other studies doubt the impact of taxonomic composition of natural phytoplankton communities on chl*a*/BM_{count} ratio (Schellenberger et al., 1985; Vörös and Padisák, 1991).

Nutrient enrichment of lakes is usually accompanied by characteristic shifts within the phytoplankton community. During eutrophication small flagellated taxa are replaced by increasing proportions of green algae, with cyanobacteria finally predominating (Reynolds, 1984; Richman et al., 1984; McQueen et al., 1986). From algological studies concerning the lakes under consideration it is known that the phytoplankton population of eutrophic Feldberger Haussee during a number of years was occupied by filamentous cyanobacteria (Kasprzak et al., 1993). Especially during the early 1990s, sometimes 90% of total phytoplankton biomass was represented by this group (Krienitz et al., 1996). Therefore, a high percentage of cyanobacteria within total phytoplankton biomass may additionally have contributed to low chl*a*/BM_{count} when BM_{count} was high. Nevertheless, our study at least from a statistical point of view indicates that the proportion of other major taxonomic groups (Chlorophyceae, Cryptophyceae) may also significantly affect the ratio of chl*a*/BM_{count}. The missing impact of chrysophyceans, however, might be a result of mixotrophic nutrition (Jones and Rees, 1994) and large relative proportions during the clear-water phase (Fott et al., 1980). Within such periods, photosynthesis is not the principal source of carbon acquisition of the phytoplankton community. Therefore, a minor influence of this group on chl*a*/BM_{count} is likely.

Our results also point out a closer temporal coherence between BM_{count} on one hand, and $BM_{chla\text{-}con}$, $BM_{chla\text{-}var(1)}$, and $BM_{chla\text{-}var(2)}$ on the other hand, as the lakes develop from oligotrophic into eutrophic conditions. Moreover, temporal coherence clearly increased when phytoplankton biomass was calculated by variable chl*a* proportions as opposed to constant ratios. At least to some extent these differences might be a result of methodological shortcomings. Some authors criticised the extraction method of chl*a* estimation in combination with photometric extinction measurements for being imprecise when chl*a* concentration was low (Vollenweider, 1974; Hallegraeff, 1977; Schmid et al., 1998). Additionally, our study has shown that the proportion of chl*a* within phytoplankton biomass may significantly decrease as phytoplankton biomass increases. Since chl*a* represents only a minor proportion of phytoplankton biomass, biased measurements may result in significant miscalculations of phytoplankton standing stocks (Tolstoy, 1977; Ahlgren, 1983). Furthermore, methodological problems related to microscopic examinations of phytoplankton samples in concert with incorrect calculation of phytoplankton biovolume may additionally contribute to poor temporal coherence and precision of the measurements (Utermöhl, 1958; Verity et al., 1992; Hillebrand et al., 1999).

In summary we conclude that chlorophyll *a* concentration might be used with caution as a predictor of phytoplankton biomass. Regardless of whether constant or variable proportions of chl*a* have been applied to calculate phytoplankton standing stocks ($BM_{chla\text{-con}}$, $BM_{chla\text{-var(1)}}$, $BM_{chla\text{-var(2)}}$), for extensive numbers of observations ($n = 756$) collected across a broad trophic gradient of lakes (oligotrophic – eutrophic) we found a close statistical correlation with the results of microscopic biomass estimation ($r^2 = 0.729$, 0.906 and 0.722). However, if the results of microscopic estimates (BM_{count}) are considered the "true" phytoplankton biomass, constant proportions of chlorophyll *a* ($BM_{chla\text{-con}}$) tend to either over- or underestimate BM_{count} depending on the trophic status of the lakes. In contrast, estimations based on variable proportions of chlorophyll *a* ($BM_{chla\text{-var(1)}}$) and BM_{count} were found to be similar. Moreover, using BM_{count} as a reference, our results applied to annual records of phytoplankton in lakes of various trophic status clearly indicate, that $BM_{chla\text{-var(1)}}$ is a much better predictor then $BM_{chla\text{-con}}$. The results using $BM_{chla\text{-var(2)}}$ are ambiguous. Although the correlation with BM_{count} is somewhat better as compared to $BM_{chla\text{-con}}$, the calculated biomass is clearly lower as was found for microscopic counts. Nevertheless, both precision and temporal coherence of the prediction are the better the higher the trophic status of a given lake no matter if it is based on $BM_{chla\text{-con}}$, $BM_{chla\text{-var(1)}}$ or $BM_{chla\text{-var(2)}}$.

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