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RESTORATION OF EUTROPHIC LAKES

Calibration of in situ chlorophyll fluorometers for organic matter

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Abstract Organic matter (OM) other than living phytoplankton is known to affect fluorometric in situ assessments of chlorophyll in lakes. For this reason, calibrating fluorometric measurements for OM error is important. In this study, chlorophyll (Chl) fluorescence was measured in situ in multiple Finnish lakes using two sondes equipped with Chl fluorometers (ex.470/em.650–700 nm). OM absorbance (A_{420}) was measured from water samples, and one of the two sondes was also equipped with in situ fluorometer for OM (ex.350/em.430 nm). The sonde with Chl and OM fluorometers was also deployed continuously on an automated water quality monitoring station on Lake Konnevesi. For data from multiple lakes, inclusion of water colour estimates into the calibration model improved the predictability of Chl assessments markedly. When OM absorbance or in situ OM fluorescence was used in the calibration model, predictability between the in situ Chl and laboratory Chl a assessments was also enhanced. However, correction was not superior to the one done with the water colour estimate. Our results demonstrated that correction with water colour assessments or in situ measurements of OM fluorescence offers practical means to overcome the variation due to OM when assessing Chl in humic lakes in situ.

Keywords Automated monitoring · Chlorophyll a · Fluorescence · Organic matter · Optical sensors · Water colour

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Introduction

Phytoplankton biomass is widely used as an indicator of eutrophication in the status assessment of surface waters. Chlorophyll *a* concentration is used as a proxy for phytoplankton biomass, traditionally quantified in laboratory from water samples by ethanol extraction followed by spectrophotometric measurement at wavelengths 665 and 750 nm (Lorenzen, 1967); ISO 10260, 1992). The shortcomings of this protocol are also well known: it requires large sample volumes, sample transportation, storage and handling. Therefore, spatial and temporal coverage and representativeness of traditional measurements are generally limited. During the last two decades, field

spectrofluorometers to measure chlorophyll in situ have become increasingly common worldwide (Meinson et al., 2015). In the text, we use "Chl *a*" to refer to chlorophyll *a* concentration measured in laboratory, in contrast to "Chl" that refers to in situ fluorescence of chlorophyll.

Due to the ease of measuring, Chl fluorometers are frequently used to study phytoplankton distribution, activity and population dynamics in situ (Proctor & Roesler, 2010; Zeng & Li, 2015). Fluorometric quantification of Chl is generally cost-effective and allows frequent observations during sudden phenomena such as mixing events or short-lived algal blooms (Jennings et al., 2012; Klug et al., 2012). On the other hand, interpretation of in situ Chl fluorometer data is not straightforward. Values yielded by the fluorometers are arbitrary and need to be calibrated to, for example, traditional Chl *a* concentrations in order to interpret the measurements, and calibration is affected by several sources of variation. In addition to phytoplankton biomass, the intensity of Chl fluorescence is dependent on the pigment composition and physiological reactions of phytoplankton (Williams & Bridges, 1964; Seppälä & Balode, 1998; Richardson et al., 2010). Physical and chemical conditions in the water also affect the in situ Chl fluorescence. Such variables include changes in the underwater light and temperature conditions, back scattering and absorption of light by other particles than phytoplankton (Strickland, 1968; Carder et al., 1991; Serra et al., 2009; Downing et al., 2012; Ostrowska, 2012). Most prominently, Chl fluorescence signal is affected by fluorescence of organic matter (OM) related to something else than living phytoplankton (Proctor & Roesler, 2010; Twiss, 2011).

Practices on how to control the sources of error due to OM are under a vigorous scientific discussion. OM is a broad term, including DOM (dissolved organic matter) and POM (particulate organic matter). In literature, OM correction has been conducted by using concepts of soluble fluorescence (Carlson & Shapiro, 1981), yellow substances (Twiss, 2011), fluorescent or chromophoric dissolved organic matter (CDOM; Proctor & Roesler, 2010) or humic substances (Carlson & Shapiro, 1981). In this study, we use the general term OM to describe organic material other than phytoplankton in the lake water, because we did not fractionate or characterize the different OM components. Previous studies have shown that the OM signal is generally linked to allochthonous dissolved humic substances in boreal lakes (Carlson & Shapiro, 1981; Twiss, 2011). In the marine environment, phytoplankton degradation products may comprise a larger part of total OM (Xing et al., 2017).

The variety of proposed measures to control the OM signal is broad, indicating its importance in Chl fluorescence problematics. Carlson & Shapiro (1981) filtered a sample water to subtract OM background from Chl fluorescence in North American lakes and Leppä et al. (1995) followed the same protocol in Finnish lakes. Twiss (2011) and Proctor & Roesler (2010) used multiple waveband sensors to measure fluorescent OM in lakes and to establish a calibration model between Chl and OM, whereas Xing et al. (2017) used a single waveband sensor for fluorescent OM to correct in situ Chl fluorescence values in deep vertical profiles from marine environment. In situ Chl fluorometers are becoming increasingly common in the monitoring of inland waters, but international standards for field operation are still under development (e.g. Lavigne et al., 2012; Cremella et al., 2018). In the near future, increase in water colour may become more important due to browning of boreal lakes (Monteith et al., 2007; Evans et al., 2012; Kritzberg & Ekström, 2012). Corrections of Chl for OM fluorescence would thus become increasingly relevant in monitoring.

In boreal lakes, the connection between dissolved OM and water colour is typically strong and rather uniform due to the presence of humic substances (Carlson & Shapiro, 1981; Hessen & Tranvik, 1998; Keskitalo & Eloranta, 1999). Results by Leppä et al. (1995) indicated good correlation between water colour and background fluorescence in fourteen lakes in Eastern Finland. For this reason, and the simplicity of water colour measurement, the main aim of this study was to scrutinize water colour as a proxy for OM in correction in humic lakes. In this study, we tested an in situ OM fluorescence sensor to correct Chl results. Major fluorescence peaks for humic substances in water consist of excitation between 330 and 390 nm and emission between 420 and 500 nm wavelengths (Matilainen et al., 2011), and the OM sensor deployed in this study covered the same wavelength range. We hypothesized that correcting fluorometric Chl data with (1) average or median water colour, (2) water colour at the measurement depth assessed via absorbance (A_{420}), or (3) in situ fluorometric

measurements of OM improves, and equally well, the predictability between fluorometric Chl and traditional laboratory Chl *a* assessment. We aimed also to obtain information of the seasonal OM variation within one lake and to determine whether continuous monitoring of Chl could benefit from the OM calibration.

Materials and methods

Study lakes

This study was conducted on lakes located in Central and Southern Finland (N-Europe) varying in size, nutrient concentration and water colour (Table 1). To understand lake-specific effects of different calibration models, Lakes Konnevesi and Jyväsjärvi in Central Finland and Lakes Vesijärvi and Vanajavesi in Southern Finland were scrutinized separately. Of the lakes, Jyväsjärvi and Vanajavesi represent small and large humic lakes, respectively. Based on their typical Chl *a* and total phosphorus concentrations (Table 1), these lakes are oligo-mesotrophic. Lakes Konnevesi and Vesijärvi represent large clearwater lakes, the latter being oligotrophic and the former a more eutrophic lake.

In situ Chl measurements and Chl a laboratory analysis

In situ measurements on the selected lakes were done with two separate YSI6600 multiparameter sondes (type V2-4; YSI Inc., Yellow Springs, OH, USA; hereafter Sondes 1 and 2) that were equipped with Chl fluorometers (ex.470/em.650–700 nm) without temperature correction. Sonde 1 was used to measure Chl fluorescence on six of the study lakes including both humic and clearwater lakes (Table 1). Samples for the laboratory analysis of Chl *a* were taken at the depth of 1 m and several other depths from the lakes measured with Sonde 1 (see the link in Data availability for values). Samples were taken with a 4.4 l Limnos-tube sampler and stored in cool (6°C) and dark until the analysis within 24 h after the collection. Chl *a* (SFS-ISO 10260:1992) was measured in the laboratory after filtration of 0.5–1.0 l of sample water through GF/C filters. Chl *a* was measured using the cold ethanol extraction method and water colour spectrophotometrically. Wavelengths 665 and 750 nm were measured

Table 1 Locations and basic limnological characteristics of the study sites

Lake	Coordinates (~ WGS84)		Area (ha)	Depth (m)		Tot-P, (μg l^{-1})	Chl *a*, (μg l^{-1})	Water colour, (mg Pt l^{-1})	Sonde
	Lat	Lon		Max	Mean				
Alvajärvi	62° 18′ 50.004″	25° 43′ 11.655″	210	16.5	3.8	29	16.5	80	1
Jyväsjärvi	62° 14′ 11.671″	25° 46′ 2.868″	314	25.0	7.0	25	10.8	70	1
Konnevesi	62° 37′ 57.366″	26° 36′ 16.504″	19,028	57.1	10.6	6	4.2	25	1
Ruokojärvi	62° 15′ 32.902″	27° 18′ 41.28″	464	8.2	1.3	30	10.6	110	1
Vanajavesi	61° 9′ 19.713″	24° 13′ 44.982″	10,261	23.9	7.7	24	16.0	50	1
Vesijärvi	61° 3′ 1.662″	25° 35′ 4.926″	6,471	40.0	6.1	27	9.6	10	1
Alasenjärvi	61° 0′ 46.564″	25° 44′ 35.29″	275	15.2	6.1	14	4.5	10	2
Alinen Rautjärvi	61° 11′ 43.098″	25° 6′ 1.241″	50	12.0	No data	23	23.0	100	2
Arkiomaanjärvi	61° 3′ 14.659″	25° 44′ 26.692″	208	20.2	5.1	17	5.3	25	2
Joutjärvi	60° 58′ 36.406″	25° 42′ 6.567″	40	5.0	3.3	25	24.0	20	2
Merrasjärvi	61° 1′ 3.695″	25° 41′ 4.868″	24	2.6	1.5	31	19.3	50	2
Pääjärvi	61° 3′ 48.76″	25° 7′ 56.998″	1,352	85.0	14.8	12	5.5	70	2
Ruuhijärvi	61° 1′ 38.011″	26° 0′ 28.054″	573	18.7	5.6	18	10.8	30	2
Työtjärvi	60° 59′ 44.515″	25° 28′ 2.635″	56	7.0	< 1.5	23	No data	50	2

Limnological data from the database of Finnish Environmental Institute (SYKE) or Dolman et al. (2015)

Sonde refers to either of the two multiparameter sondes (Sonde 1 and Sonde 2) that was deployed on the lake

Tot-P total phosphorus concentration, *Chl a* chlorophyll *a* concentration

with the Shimadzu UV-1800 spectrophotometer (Shimadzu Co., Kyoto, Japan) in 5-cm quartz cuvettes.

Sonde 2 was used to measure Chl fluorescence in eight lakes, also including humic and clearwater lakes (Table 1). Vertical profiles were recorded, as for Sonde 1, and integrated (0–2 m) samples for Chl *a* collected with a Limnos sampler. Chl *a* samples were filtered using Whatman GF/F filters and stored in a freezer (− 20°C) until measurement within 1–3 weeks after sampling using a hot ethanol extraction method and spectrophotometer (SFS-ISO 10260, 1992; Dolman et al., 2015). The methods for Chl *a* extraction were different for the two datasets, because the extractions were carried out in two different institutes (Lammi Biological Station of University of Helsinki and University of Jyväskylä). However, both methods are general and well-established procedures in laboratory analysis of Chl *a*.

Water colour of the study lakes ($colour_{typical}$)

In this study, we first tested the epilimnetic water colour of the study lakes as a parameter (hereafter $colour_{typical}$) to correct Chl measurements. For most of the study lakes, these colour values, also used for lake typology classification, were available from the database of Finnish Environment Institute (SYKE, https://wwwp2.ymparisto.fi/scripts/kirjaudu.asp, requires free registration and use of website translator). Water colour was analysed either with a Hellige/AVM Neocomparator or spectrophotometrically (wavelength 410 nm) according to SFS-EN-ISO 7887:2011. The water colour of the methods are highly comparable. For four of the study lakes, $colour_{typical}$ value was taken from Dolman et al. (2015), and were determined using spectrophotometric analysis from 0.45 µm prefiltered samples (SFS-EN-ISO 7887, 2011, 410 nm).

On site water colour measurements ($colour_{Lab}$)

From the lakes measured with Sonde 1, samples for the laboratory analysis of water colour ($colour_{Lab}$) were taken at the same depths as samples for laboratory analysis of Chl *a* (see the link in Data availability for values). Samples were taken as subsamples from the 4.4-l Limnos-tube sampler and stored in cool and dark conditions until the analysis within 24 h after the collection. Water colour was measured in the laboratory after filtration of 0.5–1.0 l of sample water through GF/C filters. Absorbance at 420 nm wavelength was measured with the Shimadzu UV-1800 spectrophotometer using 1-cm quartz cuvettes and converted to mg Pt l^{-1} (SFS-EN-ISO 7887, 2011).

In situ fluorescence of organic matter

Sonde 1 was equipped with Cyclops-7 organic matter (OM) fluorometer (ex.350/em.430 nm; Turner Designs Inc., Sunnyvale, CA, USA). The OM fluorometer was calibrated for water temperature as in Watras et al. (2011), for which the temperature coefficient (ρ) of − 0.009 was used to transform data to a reference temperature of 20°C (RFU_{20}).

Continuous water quality monitoring on Lake Konnevesi

To test the performance of different calibration methods and to study seasonal variation in Chl and OM, Sonde 1 was continuously also deployed in Lake Konnevesi during the open water season in 2013 (June–October). One profile in every three hours at 0.5-m step was recorded from the 42-m-deep water column. Daily averages of Chl and OM from 1.5 to 2.0 m depth were taken into analysis. Surface (0– m) Chl *a* data from 2013 were collected by the regional environmental agency KES-ELY during the open water period (June–October, $n = 5$, Database of Finnish Environment Institute) and supplemented by our Chl *a* samplings ($n = 4$) from 1 m and 2 m depths with the Limnos sampler. Regression models (Table 2a, b) developed for the multiple lakes dataset were tested to calibrate the continuous Chl data.

Comparison of the calibration methods

We compared Chl fluorometer data and corresponding Chl *a* results from laboratory analyses and established calibration equations for the Chl fluorometer by fitting a linear regression model. Then we added water colour ($colour_{Typical}$) into the calibration equation. For Sonde 1, we also used $colour_{Lab}$ taken at the time of profile measurements and included of in situ OM fluorometer data into the calibration models. Surface (average of 1–2 m) Chl and OM fluorometer results from Lake Konnevesi (Central Finland) were corrected with each of the established calibration equations, excluding

Table 2 Univariate and multivariate linear regression models for the calibration of the Sonde 1 (a) and Sonde 2 (b) fluorometers using the laboratory measured chlorophyll *a*

a. Model equation for Sonde 1	*n*	R^2	*P*
(1) Chl *a* = 1.717(Chl) − 1.944	71	0.537	< 0.001
(2) Chl *a* = 3.024(Chl) − 0.162($colour_{typical}$) − 1.877	71	0.914	< 0.001
(3) Chl *a* = 2.843(Chl) − 0.133($colour_{Lab}$) − 1.067	71	0.816	< 0.001
(4) Chl *a* = 2.288(Chl) − 5.109(OM) + 3.613	71	0.879	< 0.001
b. Model equation for Sonde 2	*n*	R^2	*P*
(1) Chl *a* = 0.811(Chl) − 4.902	71	0.537	< 0.001
(2) Chl *a* = 1.076(Chl) − 0.165($colour_{typical}$) − 2.938	71	0.914	< 0.001

(Chl *a*) concentrations (μg l^{-1}) and water colour ($colour_{typical}$, $colour_{Lab}$, mg Pt l^{-1}) and in situ fluorescent organic matter OM (RFU). *n* number of observations (samplings on lakes) used to construct the models

$colour_{Lab}$, to illustrate the feasibility of the calibration for automated water quality monitoring.

Calculations of modelling efficiency (ME = $1 - \sum(y_o - y_p)^2/\sum(y_o - y_m)^2$), where y_o represents observed values, y_p predicted values, and y_m the mean of observed values, and mean absolute percentage error (MAE(%) = $100[\sum(|y_o - y_p|/y_o|)]/n$,), where *n* is the number of pairs, were conducted according to Mayer & Butler (1993) using Microsoft Excel (2010, Microsoft Co., Redmond, WA, USA). Linear regressions and statistical tests were conducted with SPSS software (version 22.0, IBM Co., Chicago, IL, USA). Results with $P < 0.05$ were reported significant.

Results

In the lakes measured with Sonde 1 (see Table 1), manually sampled Chl *a* concentration varied between 0.5 and 17.4 μg l^{-1}, and in the lakes measured with Sonde 2 between 3.6 and 43.7 μg l^{-1} (Fig. 1). After

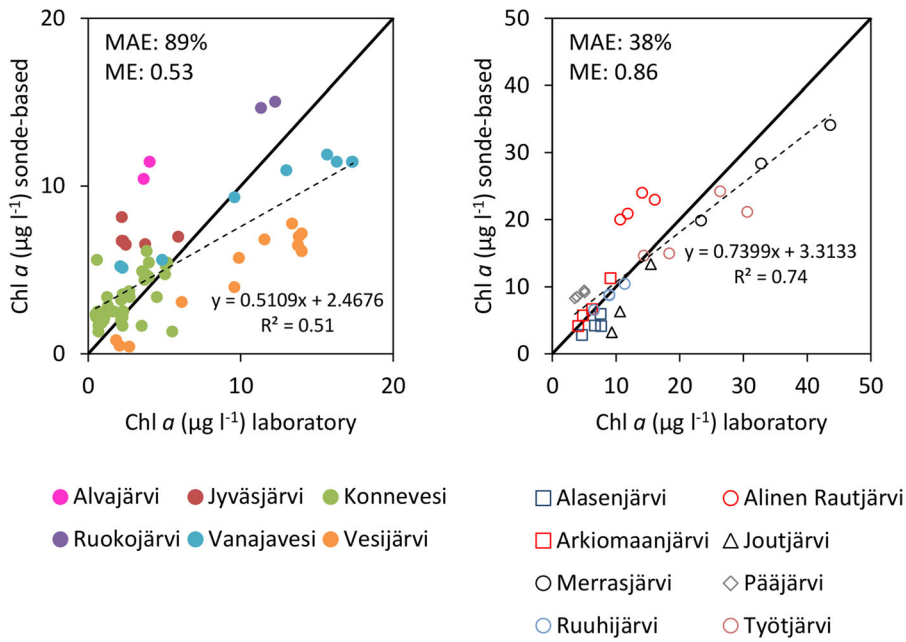

Fig. 1 Linear regression (broken line) between the observed Chl *a* concentrations and estimated Chl *a* concentrations based on the calibration with measured Chl *a* in the study lakes (model 1; see Table 2a, b). Solid line = 1:1 line. Dots—Sonde 1 data; open markers—Sonde 2 data; *MAE* mean absolute percentage error (%), *ME* modelling efficiency (%)

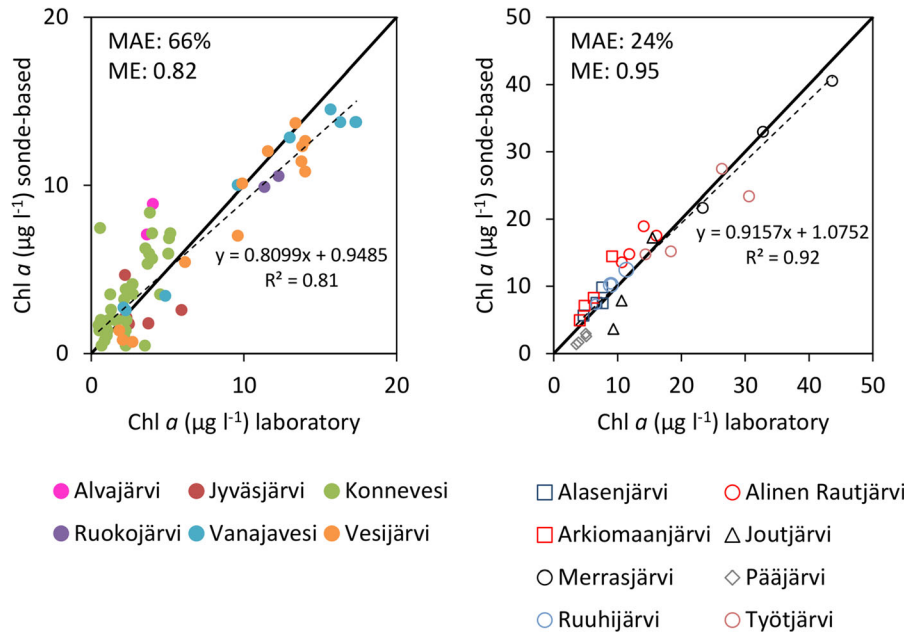

Fig. 2 Linear regression (broken line) between the observed Chl *a* concentrations and estimated Chl *a* concentrations based on the calibration with measured Chl *a* and water colour estimate ($colour_{typical}$, Table 1). Regression model 2 was used here (see Table 2a, b). Solid line = 1:1 line. Dots—Sonde 1 data; open markers—Sonde 2 data; *MAE* mean absolute percentage error (%), *ME* modelling efficiency (%)

calibrating the fluorometer readings only with the Chl *a* concentrations (model 1, Table 2a, b), the regression coefficients, modelling efficiency (ME) and mean absolute percentage errors (MAE) were rather weak (R^2 = 0.51 and 0.74, MAE = 89% and 38%, ME = 0.53 and 0.86, for Sondes 1 and 2, respectively, Fig. 1).

The respective predictabilities of model 2, including both Chl *a* and water colour ($colour_{typical}$) as correcting variables, were notably better for both sondes (R^2 = 0.81 and 0.92, MAE = 66% and 24%, ME = 0.82 and 0.95, for Sondes 1 and 2, respectively, Fig. 2).

For Sonde 1, we also constructed models 3 and 4, including Chl *a* and $colour_{Lab}$ or OM fluorescence, respectively. Model 3 yielded slightly better predictability (R^2 = 0.85, MAE = 64%, ME = 0.86) than the water colour ($colour_{typical}$, Fig. 3). For model 4, introducing OM fluorescence as a calibration factor was relatively effective (R^2 = 0.77, MAE = 65%, ME = 0.78), but not superior to models 2 or 3. Chl *a* values yielded by different models did not differ significantly from each other (Related Samples Wilcoxon Signed Rank Test, $P > 0.2$ for each combination pair of models).

The relationship between $colour_{Lab}$ and OM fluorescence (RFU_{20}) was logarithmic (Fig. 4): OM fluorescence first increased rapidly along $colour_{Lab}$, but in lakes with $colour_{Lab} > 50$ mg Pt l^{-1} it levelled off (Fig. 4).

Scrutinization of the performance of each model for a sub-set of lakes corroborated the general overview and demonstrated that, prior to any OM correction, the variation in the fluorometer readings calibrated only with the Chl *a* varied notably from the Chl *a* measurements even inside one lake (Figs. 5, 6). In humic Lakes Jyväsjärvi and Vanajavesi, the predictability between the two was moderate, but mean absolute error was high (R^2 = 0.88, MAE = 97%, ME = 0.83, Fig. 5). In clear water lakes Konnevesi and Vesijärvi, the predictability without any OM correction was weaker but mean absolute error slightly lower (R^2 = 0.56, MAE = 86%, ME = 0.71, Fig. 6). However, when any of the OM correction models (models 2–4 in Table 2a, b) was applied, the predictabilities increased in all four lakes. In Jyväsjärvi and Vanajavesi, the regression coefficients, mean absolute errors and the modelling efficiencies of models 2–4 varied only little (R^2 = 0.93–0.97, MAE = 25–36%%, ME = 0.93–0.97, Fig. 5). Similarly, clear water lakes Konnevesi and Vesijärvi benefited from each of the calibration methods rather equally (R^2 = 0.74–0.76, MAE = 74–79%, ME = 0.85–0.87, Fig. 6).

In the oligotrophic, humic Lake Konnevesi, Chl *a* varied between 1.8 and 6.2 µg l^{-1} in June–October 2013, $colour_{typical}$ was 25 mg Pt l^{-1} and OM

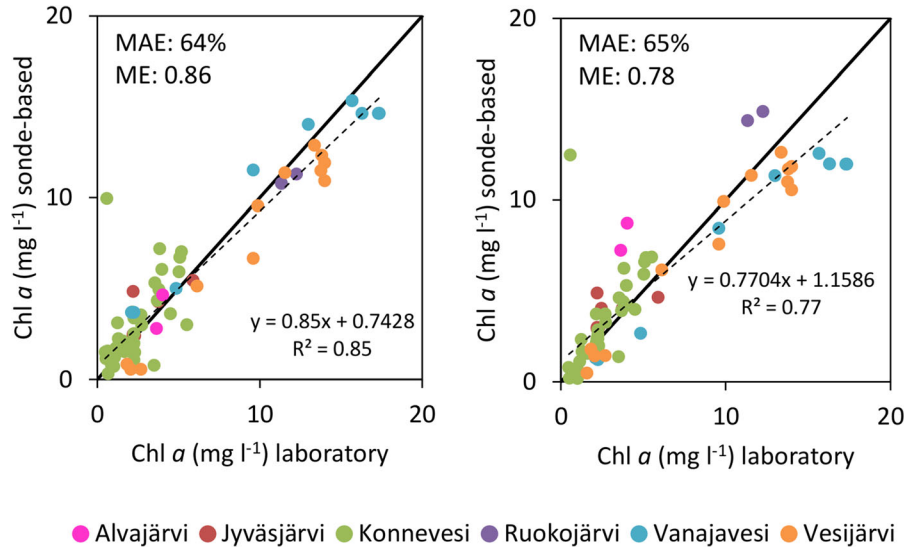

Fig. 3 Linear regression (broken line) between the observed Chl *a* concentrations and estimated Chl *a* concentrations based on the calibration with measured Chl *a* and absorbance-based water colour (A_{420}, left panel, model 3 in Table 2b) or in situ OM fluorescence (right panel, model 4 in Table 2b). Solid line = 1:1 line; *MAE* mean absolute percentage error (%), *ME* modelling efficiency (%)

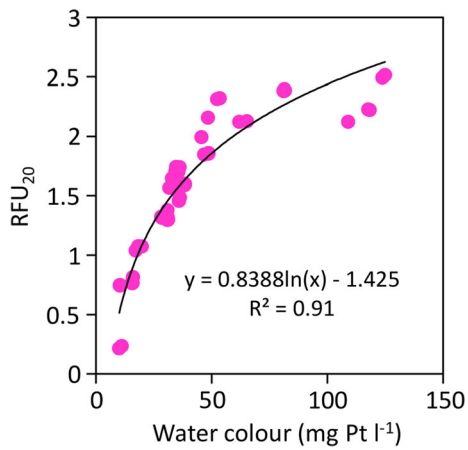

Fig. 4 Relationship between water colour and OM fluorescence (RFU_{20}) in the study lakes

fluorescence (RFU_{20}) varied between 0.95 and 58 (Fig. 7). When models 1, 2 and 4 (Table 2a) were used to correct the automated fluorometer monitoring data, only little differences between the modelled Chl *a* results were found (Fig. 7). However, the decrease of water colour in autumn 2013, monitored with in situ OM, produced a period with higher Chl fluorescence in September than the models using correction with only Chl *a* or Chl *a* and $colour_{typical}$ (Fig. 7).

Discussion

Our study demonstrated the use of water colour as an estimate of OM in controlling the OM fluorescence in Chl in situ fluorometer data. The applicability of a typical colour value of the lake in calibration was validated by comparing it with the calibrations done using simultaneously measured water colour and in situ OM fluorescence. As we expected, inclusion of any of these parameters into the calibration model improved the predictability between the in situ Chl fluorescence and Chl *a*.

A non-linear relationship between OM and water colour is well documented (Watras et al., 2011; Coble et al., 2014). High concentrations of dissolved organic material in the water column affect the OM fluorescence measurements by suppressing the values at higher dissolved OM concentrations (Watras et al., 2011). We also observed this in our study lakes (Fig. 4), and it may explain why the in situ OM calibration was not a superior method over the more simplistic water colour calibration. Proctor & Roesler (2010) outlined similarly that OM may lead to an underestimation of Chl *a* by absorbing excitation or emission wavelengths or, on the other hand, OM may cause seemingly intensified Chl emission by contributing to the signal detected by Chl fluorometers. In

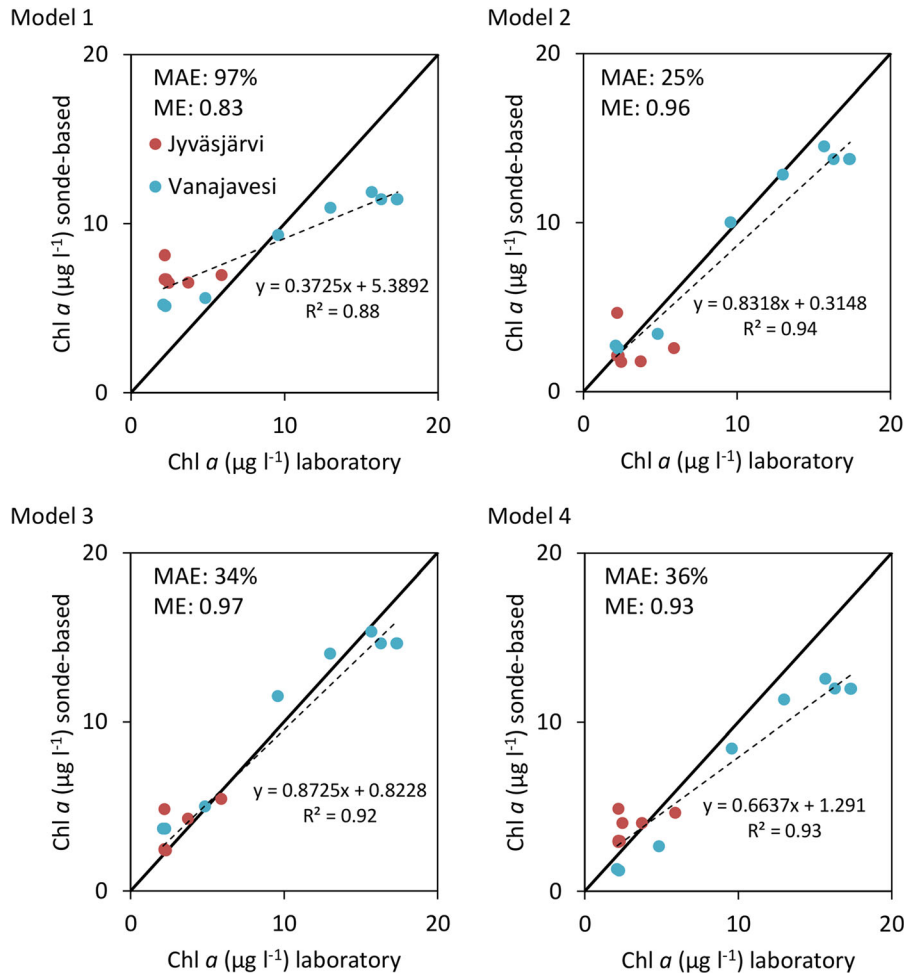

Fig. 5 Performance of the calibration equations in humic Lakes Jyväsjärvi and Vanajavesi (see Table 1 for lake characteristics). Broken line represents linear regression between the observed Chl *a* concentrations and estimated Chl *a* concentrations based on the calibration with measured Chl *a* (model 1, see Table 2a, b for the models) or measured Chl *a* and water colour estimate (model 2), absorbance-based water colour (A_{420}, model 3) or in situ OM fluorescence (model 4). Solid line = 1:1 line; *MAE* mean absolute percentage error (%), *ME* modelling efficiency (%)

our data, the latter was typical for most of the studied lakes, except for the eutrophic and most clearwater lakes (colour $\leq$ 10 mg Pt l^{-1}).

Our results agree with previous studies stating that without OM calibration, the interpretation of in situ Chl results is generally misleading. Proctor & Roesler (2010) collected water samples from a lake, pond, stream and a bog in Maine (USA) with varying OM levels and measured phytoplankton and OM fluorescence with the multiple waveband fluorometers in the laboratory. They observed a linearly increasing OM signal along the OM concentration in the dilution series determined fluorometrically and spectrophotometrically. Based on the linear congruence between the OM and algae multiexciter signals, they stated that the quantity of OM is of central importance. Goldman et al. (2013) also showed significant overestimation of Chl concentration with increased OM concentrations in an estuary. Leppä et al. (1995) found a high background fluorescence in humic lakes of South-Eastern Finland, caused by water colour, which lead to recommendations for the Chl fluorometry calibration against the OM background.

In our study, the improvement due to calibration was not that clear in the continuously measured fluorometer data of Lake Konnevesi. However, in the end of July 2013, OM fluorescence decreased and the OM calibrated Chl fluorescence (model 4) increased leading to a better congruence with the measured Chl *a*, which might indicate that the continuous on-line Chl measurements could benefit from the OM calibration (Fig. 7). Likely, the significance of in situ OM correction is emphasized, compared to laboratory analyses, when Chl is measured with a high frequency in real time. In Lake Konnevesi, the automated system recorded a decrease of OM fluorescence towards the end of the summer, which is a known phenomenon in boreal lakes and associated with photochemical

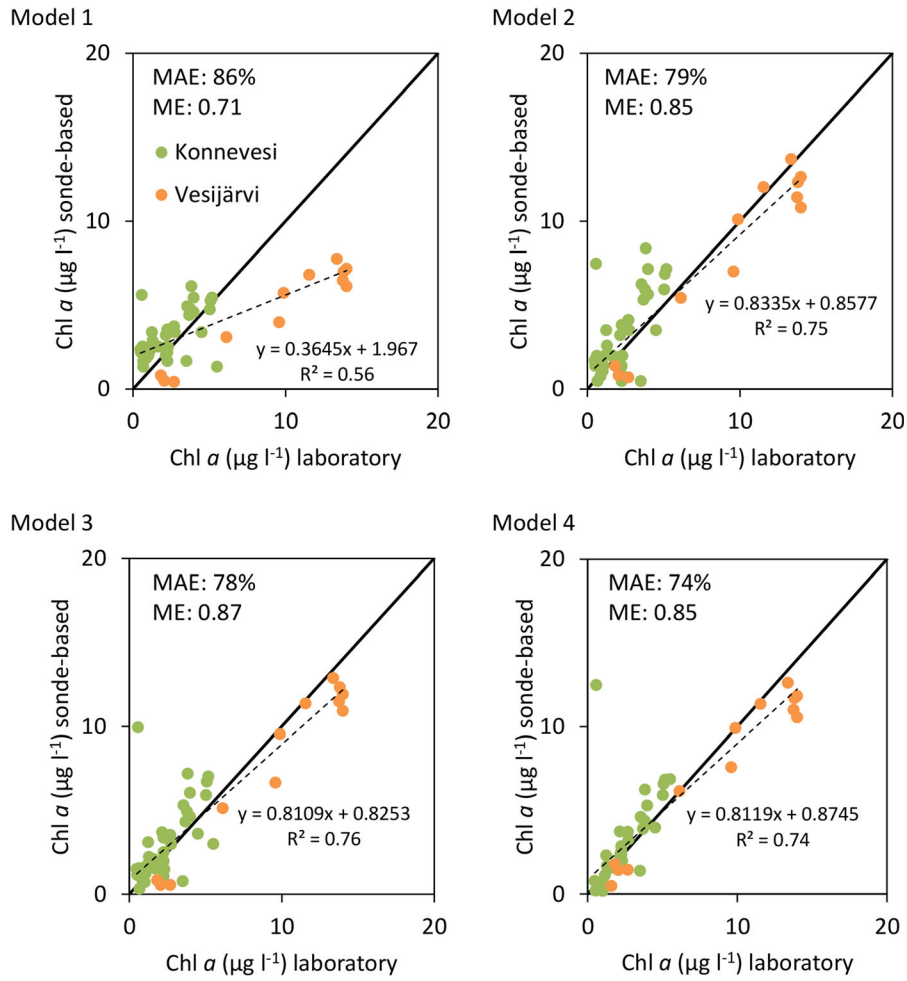

Fig. 6 Performance of the calibration equations in clear water Lakes Konnevesi and Vesijärvi (see Table 1 for lake characteristics). Broken line represents linear regression between the observed Chl *a* concentrations and estimated Chl *a* concentrations based on the calibration with measured Chl *a* (model 1, see Table 2a, b for the models) or measured Chl *a* and water colour estimate (model 2), absorbance-based water colour (A_{420}, model 3) or in situ OM fluorescence (model 4). Solid line = 1:1 line; *MAE* mean absolute percentage error (%), *ME* modelling efficiency (%)

degradation and lower import of CDOM (e.g. Müller et al., 2014). Deploying, for example, an on-line UV Vis spectrophotometer in conjunction with the continuous Chl measurements could reveal more details in the changes of OM quality during the growing season.

As demonstrated by the scrutinization of two humic and two clear water lakes, predictability between Chl *a* analysed in laboratory and measured in situ varied slightly between lakes after OM calibrations. In the clearwater lakes, predictability stayed lower than in humic lakes, likely due to interfering factors other than organic matter. It has been shown that in one of the clear water lakes, Lake Vesijärvi, Chl fluorescence is influenced by the high amount of cyanobacteria, and therefore the calibration of the Chl fluorometer with microscopic counts of cyanobacteria biomass was found effective for increasing the accuracy of Chl fluorometer results (compared to Chl *a* alone extracted in the laboratory, (Anttila et al., 2012). In this study, we did not study algal pigment abundance or algal community composition. Chl fluorescence might vary between day and night as well, being higher at night when photosynthetic activity is lower (Aiken, 1981). In clear water lakes, phytoplankton may have to protect their photosystems from photobleaching through non-photochemical quenching processes (Suggett et al., 2010). The consequence of this is a suppression of fluorescence, which should be corrected in cases of high irradiance. In Finnish lakes, this situation is typically limited to very shallow depths at the surface and to a greater extent, to only few lakes and few days of summer. Watras et al. (2011) demonstrated temperature quenching of Chl readings that could be overcome by temperature calibration of the fluorometer data. As we did our measurements in rather uniform summer temperatures, temperature quenching is likely a minor source of variation in our data.

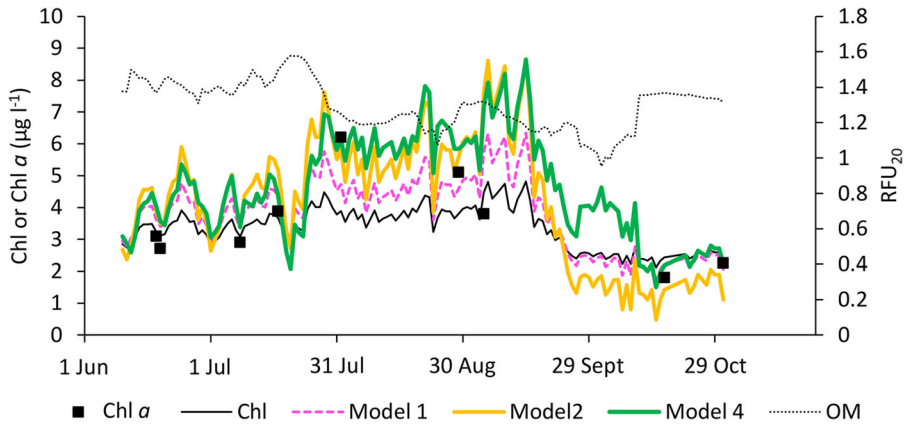

Fig. 7 On-line in situ Chl (µg l^{-1}) and OM fluorescence (RFU_{20}) in Lake Konnevesi in 2013. Chl was yielded by the fluorometer (Sonde 1) and models 1–2 and 4 are described in Table 2. Chl *a* refers to laboratory analysis of Chl *a* from samples

Conclusions

Comparison of Chl fluorescence is generally incorrect in water bodies with different humic contents if the calibration procedures for OM are not conducted. OM fluorescence causes errors in estimation of Chl that are not consistent between lakes or even within lakes. Our calibration procedures, including water colour, laboratory assessments or in situ OM fluorescence, were each demonstrated to be promising and practical methods for the in situ Chl fluorometer data calibration for boreal humic waters. Our rather limited results from the continuously monitored Lake Konnevesi suggest that changes in OM quantity in a single water body may not affect the calibration in great extent, but this requires further scrutinization.

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Data availability The datasets generated and analysed during this study are available in the University of Jyväskylä JYX repository with https://doi.org/10.17011/jyx/dataset/62634 and URI: http://urn.fi/URN:NBN:fi:jyu-201901291351

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