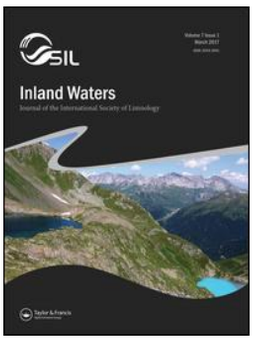

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Phytoplankton response to the summer 2015 heat wave – a case study from prealpine Lake Mondsee, Austria

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ABSTRACT

Phytoplankton response to a heat wave in deep prealpine Lake Mondsee, Austria, was minor overall, although temperature significantly affected the epilimnetic phytoplankton assemblage at the deepest site of the lake. We detected no significant effect of nutrients. Using 3 complementary optical methods—light microscopy (Utermöhl technique), FlowCAM, and acoustic flow cytometry (AFC)—we found relatively low horizontal variation in the epilimnion but significant changes in phytoplankton community composition integrated over the 0–20 m water layer at the central station of the lake. These changes were mainly caused by a vertical shift in the *Planktothrix rubescens* peak from 11 to 16 m during the heat wave. Air temperatures reaching or exceeding 30 °C were measured in the area during 6 consecutive days at the end of June to the beginning of July, the first of 3 heat waves recorded during summer 2015; in-shore surface water temperature exceeded 27 °C. We sampled 9 stations across Lake Mondsee on 4 occasions during the heat wave and analysed temperature, nutrient levels, conductivity, pH, and the phytoplankton community. In addition to reporting the implications of increasing water temperatures for the algal assemblage in a deep stratified lake, we discuss the pros and cons of the different optical methods for phytoplankton identification and counting. For future field campaigns similar to the present study, we recommend using light microscopy to assess large or rare species, such as *Ceratium hirundinella*, and AFC, FlowCAM, or similar semi-automated devices for abundant small- to medium-sized species.

KEYWORDS

Acoustic flow cytometry; *Cryptomonas* sp.; FlowCAM; heat wave; Lake Mondsee; phytoplankton; *Planktothrix rubescens*

Introduction

Extreme meteorological events such as heat waves are predicted to increase in frequency as a result of the current global warming scenario in central Europe and North America (Meehl and Tebaldi 2004, Team et al. 2007). These weather-related episodic events are typically unpredictable, short-termed, and are therefore often missed in low-frequency monitoring programmes such as the every 4-month sampling requested by the European Water Framework Directive (WFD; 2000/60/EC). It is crucial, however, for conservation biology and water management to understand the impact of these extreme short-term events on the plankton composition of inland lakes. In particular, the sensitivity of the phytoplankton community to sudden increases in irradiance and water temperature, typically concomitant with reduced nutrient load from the catchment (Reynolds 1984), needs to be assessed.

Most studies on climate warming focus on increases of mean temperatures, either by conducting microcosm or mesocosm experiments (e.g., Staehr and Sand-Jensen 2006, Burgmer and Hillebrand 2011, Yvon-Durocher et al. 2011) or by comparing warmer with average temperate periods using data derived from long-term studies (Gallina et al. 2011). Although some authors have used a modelling approach to predict the effects of increased water temperature on the phytoplankton community (Elliott et al. 2006, Huber et al. 2008, Elliott 2012a, 2012b), few studies have investigated heat waves *in situ*. Adrian and colleagues (Wagner and Adrian 2009, Huber et al. 2012) reported that heat waves led to shifts in phytoplankton communities, favouring cyanobacteria in a shallow polymictic lake in Germany. Similarly, Jöhnk et al. (2008) reported that heat waves promoted cyanobacterial blooms in a eutrophic lake in the Netherlands. Microcosm experiments simulating summer heat waves revealed significant shifts in the phytoplankton assemblage, mainly driven by temperature-sensitive species such as *Cryptomonas* spp. and *Oocystis pusilla* (Moss et al. 2003, Weisse et al. 2016). Moss et al. (2003) found no particular pattern in the taxonomy or biological characteristics of species affected by their temperature/nutrient treatments. Laboratory experiments with single species, however, revealed that

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growth rates of common colonial cyanobacteria such as *Microcystis aeruginosa* and several *Anabaena* species reach their optima at temperatures >25 °C, whereas growth rates of many eukaryotic taxa such as the diatoms *Asterionella formosa* and *Aulacoseira subarctica*, the chrysophyte *Dinobryon divergens*, and several cryptophyte species of the genus *Cryptomonas* level off or decline in the vicinity of 25 °C (Butterwick et al. 2005, Lürling et al. 2013, Weisse et al. 2016).

Little is known about immediate changes in the phytoplankton community in relation to heat waves in deep lakes (Gallina et al. 2011, 2013; reviewed by Dokulil 2013). To this end, we investigated the effects of a summer heat wave on the phytoplankton community composition of deep prealpine Lake Mondsee in July 2015. Summer 2015 was the hottest summer ever recorded in the northern hemisphere (NASA 2016), and July temperatures were 3.7 °C higher than the long-term average in Austria (ZAMG 2015). Because temperate prealpine deep lakes have different mixing regimes than shallow lakes and are strongly stratified during the summer period, we assumed that the effect of heat waves is mainly restricted to the epilimnion. Several definitions of heat waves are available in the literature; we followed Kyselý's criteria for central Europe, who defined a heat wave as "at least 3 days with maximum air temperature (T_{max}) >30.0 °C, the mean T_{max} over the whole period is ≥30.0 °C, and T_{max} must not drop below 25.0 °C" (Kyselý et al. 2000).

We analysed phytoplankton species composition and biomass at 9 sampling stations on 4 occasions during the heat wave to assess possible temporal and spatial changes of the phytoplankton community. We expected to record significant increases of water temperature and irradiance, along with decreasing nutrient load (soluble reactive phosphorus [SRP], silicate, and nitrate) from the tributaries. We hypothesised that, because of species-specific temperature sensitivity, the phytoplankton assemblage would significantly change during the heat wave. Based on the previously reported field observations and experimental findings, we anticipated that colonial cyanobacteria would increase and cryptophytes would decrease in near-surface waters.

Along with investigating changes in the phytoplankton community composition during a heat wave, we also used this study to compare 3 methods for phytoplankton identification and enumeration: inverted light microscopy (Utermöhl 1958) and 2 advanced optical techniques, the semi-automated FlowCAM (flow cytometer and microscope) and the Attune Acoustic Focusing Cytometer. In contrast to light microscopy, live samples can be counted with these techniques to avoid potential bias originating from fixation. The combination of light microscopy, FlowCAM, and flow cytometry allows coverage of a wide spectrum of the phytoplankton community, ranging from ~0.1 µm (flow cytometry) to 1000 µm (light microscopy). The FlowCAM version and objective we used measures a size spectrum from ~5 to 200 µm. We hypothesised that all 3 methods can complement each other and that the accuracy of each method is comparable.

Study site

Prealpine Lake Mondsee, located in the Salzkammergut Lake District in central Austria (47°50′N, 13°23′E), is an oligo-mesotrophic, deep, stratified lake with dimictic and monomictic mixing regimes. It has a mean depth of 34 m, a maximum depth of 68 m, a surface area of 13.78 km², and a theoretical water retention time of 1.7 years (Beiwl and Mühlemann 2008). The lake is flushed by 3 main tributaries, Fuschler Ache, Wangauer Ache, and Zeller Ache (Fig. 1); its only outflow, the Seeache, drains into Lake Attersee. The summer phytoplankton biomass in Lake Mondsee is dominated by diatoms, dinoflagellates, and cryptophytes; chrysophytes and colonial cyanobacteria other than the deep-living *Planktothrix rubescens* are of minor importance (Dokulil and Teubner 2012). Single-celled, phycoerythrin-rich picocyanobacteria occur in high numbers (>10^5 cells mL^{-1}) in the epilimnion during summer (Crosbie et al. 2003; T. Weisse, Research Institute for Limnology, Mondsee, unpubl. data). Below the thermocline, phytoplankton is dominated by the filamentous cyanobacterium *P. rubescens* (up to 85% of total biovolume), whereas this species is almost absent in the epilimnion (Dokulil and Teubner 2012).

Methods

Sampling

We sampled 9 stations distributed across Lake Mondsee (Fig. 1) on 30 June and 2, 6, and 9 July 2015. Samples were taken with a 5 L volume water sampler from distinct depths (Schröder 1969) and with a 0.8 L volume integrating water sampler (Züllig, Rheineck, Switzerland). Integrated samples (0–20 m) were taken from sampling stations S3, S6, and S8. At the shallow station S1 (depth 13.5 m) in the vicinity of the outflow of Lake Mondsee, integrated samples were taken from 0 to 12 m. Surface samples were taken from S3, S4, S5, and S9. At the central station (S3) located at the deepest point of the lake, discrete samples were also taken from 0, 5, 10, 15, and 20 m. Temperature, conductivity, pH, and nutrient levels were analysed from all sampling stations within the lake. Nutrients were either measured by ion chromatography (nitrate [NO_3^-] and ammonium [NH_4^+]; Dionex ICS-1100, Thermo Fisher Scientific Inc, USA) or spectrophotometrically (SRP and dissolved reactive silicate

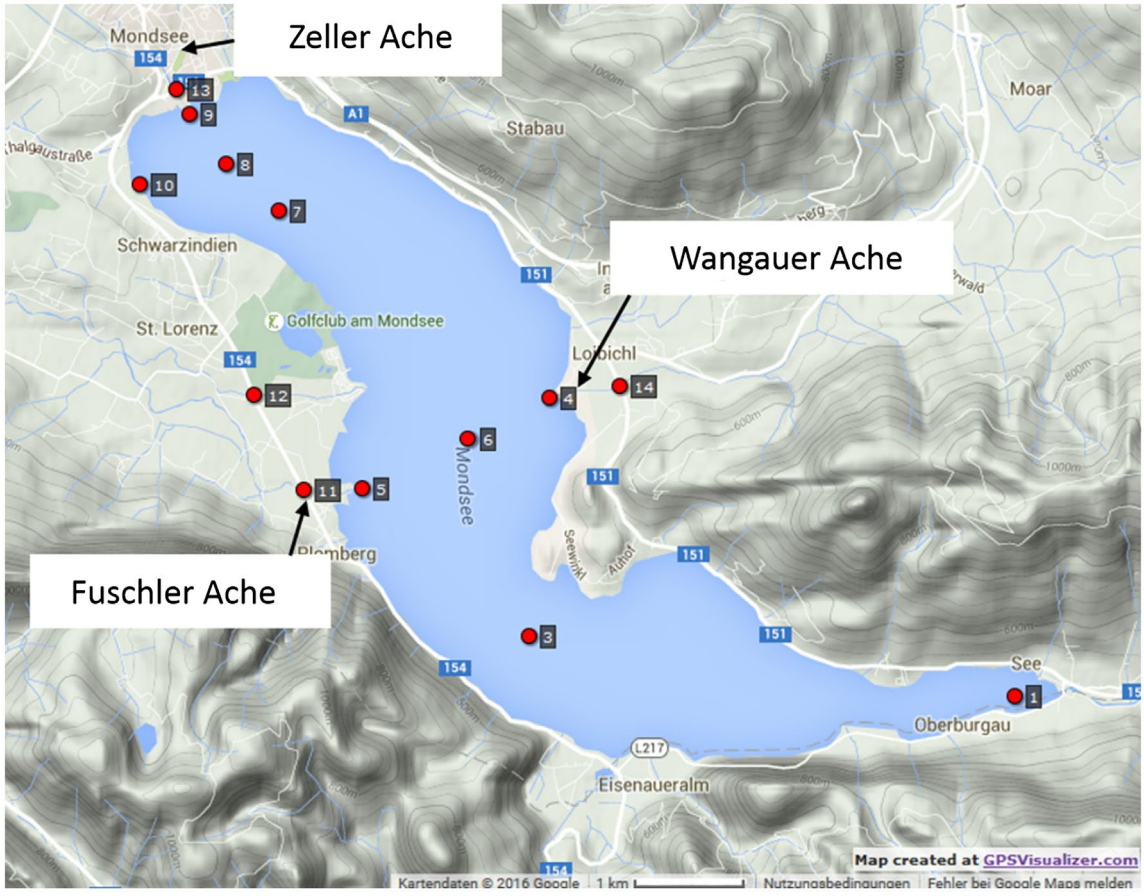

Figure 1. Sampling stations in Lake Mondsee. Stations 1–10 were sampled 4 times each during the heat wave (30 June to 9 July 2015). Profiles of ambient parameters (multiparameter sonde, light) were taken at the central station S3. The main tributaries (Fuschler Ache, Wangauer Ache, and Zeller Ache) of Lake Mondsee (S11–S14) were sampled twice during the heat wave.

[DRSi]) after 0.2 µm filtration using standard operating procedures (Bendschneider and Robinson 1952, Vogler 1966, Smith and Milne 1981). Spectrophotometric chlorophyll *a* (Chl-*a*) measurements were conducted after extraction with hot ethanol following ISO 10260 (ISO 1992). Additionally, we profiled Chl-*a* in relative fluorescence units using a multiparameter probe (YSI 6600 V2, Yellow Springs Instruments, USA) from 0 to 60 m at the central station of the lake; relative fluorescence units were automatically converted to Chl-*a* units of µg L^{-1} using the calibration provided by the manufacturer. Similarly, profiles of phycoerythrin (PE) fluorescence (in relative fluorescence units, PE-RFU), temperature, dissolved oxygen, conductivity, and pH were measured. Light profiles were recorded with a scalar quantum sensor (LI 190 SB) connected to an integrating quantum meter (LI 188, LI-COR Inc, USA) at the central station (S3) only. The main tributaries of Lake Mondsee (sampling stations S11–S14; Fig. 1) were sampled on 2 and 9 July. Phytoplankton community composition was qualified and quantified using 3 methods: light microscopy, FlowCAM, and acoustic flow cytometry (AFC). Cell and colony counts were obtained with FlowCAM and AFC, whereas biomass estimations were derived from light microscopy only.

Light microscopy

Samples for microscopic analyses were fixed with acidic Lugol's solution (0.2% final concentration) immediately after sampling and stored in amber glass bottles in the dark until counting. Counts were conducted at S3 (0 and 5 m, 0–20 integrated), S6, and S8 for all 4 sampling days during the heat wave. Samples from S3 from 10, 15, and 20 m were only analysed for 30 June and 9 July. Cells were counted within 4 months after sampling. Algal cells were counted and measured using a combined plate chamber (Hydro-Bios No. 435025) containing 50 mL sample volume. Phytoplankton cell counts and biovolume estimations were conducted according to Brierley et al. (2007).

Flow CAM

The FlowCAM (flow cytometer and microscope, Fluid Imaging Technology, Yarmouth, ME, USA), a combination

of a flow cytometer and a microscope with a digital camera attached, characterises each particle within a sample with >30 morphological and fluorescence parameters (Sieracki 1998). Subsamples from all sampling stations were filtered through a 200 µm mesh to prevent clogging of the 80 µm-wide FC80FV flow cell and analysed using a 10× objective at a flow rate of 0.15 mL min^{-1}. Samples were analysed within 6 h after sampling. Measurements were conducted in trigger mode, using the green laser installed (532 nm) to excite cellular Chl-*a* and accessory pigments such as PE. The classification of all samples was conducted using the Visual Spreadsheet 3.7.5 software.

Flow cytometry

An Attune Acoustic Focusing Cytometer (Life Technologies, Thermo Fisher Scientific) equipped with violet (405 nm, 50 mW), blue (488 nm, 50 mW), and yellow (561 nm, 50 mW) lasers was used to count live phytoplankton samples. The instrument is based on a recently granted US patent (Kaduchak et al. 2012); the principle of acoustic particle concentration and positioning in suspensions has been known for >10 years (Goddard and Kaduchak 2005). Acoustic-assisted hydrodynamic focusing uses ultrasonic radiation pressure (>2 MHz) to transport particles into the centre of the sample stream. This pre-focused stream is then injected into the sheath stream (focusing fluid), which supplies an additional hydrodynamic pressure to the sample without high-velocity or high-volumetric sheath fluid.

Analyses were conducted within 4 h after sampling. Instrument settings were optimised using the AttuneR Cytometric Software. Forward scatter (FSC) and BL3 autofluorescence (595/40 nm) were used as triggers with low thresholds (FSC = 0.5×10^3, BL3 = 0.1×10^3) for all measurements. A sample volume of 0.5 mL was analysed at a flow rate of 0.1 mL min^{-1}. Analyses of the data files (fcs format) were conducted with the software programme FlowJo 10.0.8r1.

Statistical analyses

Statistical analyses were conducted using the software R (R Development Core Team 2011) and SigmaPlot 12.5. Results reported in the following represent means with 1 standard deviation (SD). All data were tested for homogeneity of variances (Levene's test, car package) and normal distribution (Shapiro-Wilk normality test) to meet analysis of variance (ANOVA) assumptions. Data were log-transformed in cases of non-normal distribution or heterogeneity of variances. If the ANOVA assumptions were met, one-way ANOVA and Tukey's post hoc tests were conducted to test for (1) temperature differences in the epilimnion at the central sampling station, (2) differences of the phytoplankton community composition at the various sampling stations, (3) differences of the phytoplankton community during the heat wave (pooled surface samples and pooled integrated samples), and (4) differences in cryptophyte cell counts conducted by the 3 instruments used. The nonparametric Kruskal-Wallis or Mann-Whitney rank sum tests were used for datasets that did not pass the tests for normality and equal variance, even after log-transformation of the raw data.

The relationship between the taxon-specific phytoplankton biomass and environmental variables (temperature and nitrate concentration) at the central station (epilimnetic samples, 0 and 5 m) was explored by performing a canonical correspondence analysis (CCA; cca function, vegan package in R). Not all tested environmental variables were used (i.e., temperature, nitrate, silicate, conductivity, and pH) because of the low number of samples available (2 epilimnetic samples × 4 sampling days = 8 samples total), which can lead to a higher explained variance of the CCA (100% if there are more environmental variables than actual samples). To avoid misinterpretations, we report only the CCA with the most important environmental variables that influence the phytoplankton community during the heat wave (i.e., temperature and nitrate concentration). We then tested which of the environmental variables were significantly related to phytoplankton community composition by 999 unrestricted Monte Carlo permutations (anova.cca, vegan package).

Results

Ambient parameters

Air temperature reached or exceeded a daily maximum of 30 °C during 1–7 July 2015, thus meeting Kyselý's definition of a heat wave (Kyselý et al. 2000). The last sampling (9 July) was conducted at the breakdown of the heat wave. Water temperature, pH, and conductivity recorded at the sampling stations on the 4 sampling occasions (Supplemental Table S1; Fig. 2, which refers to the central station S3) show that surface water temperature generally increased from 30 June to 6 July 2015 and then slightly declined on the last day of our observation (9 July). Surface temperature increase measured during the first week varied between 2.9 °C (S10) and 3.4 °C (S9). The highest water temperature (27.2 °C) was recorded at the lake shore next to the Research Institute for Limnology, Mondsee (data not shown).

The lake was strongly stratified during the period of observation, with the epilimnion extending from the surface to 4–6 m (Fig. 2a). The average temperature in the upper 5 m at the central station (S3) increased significantly

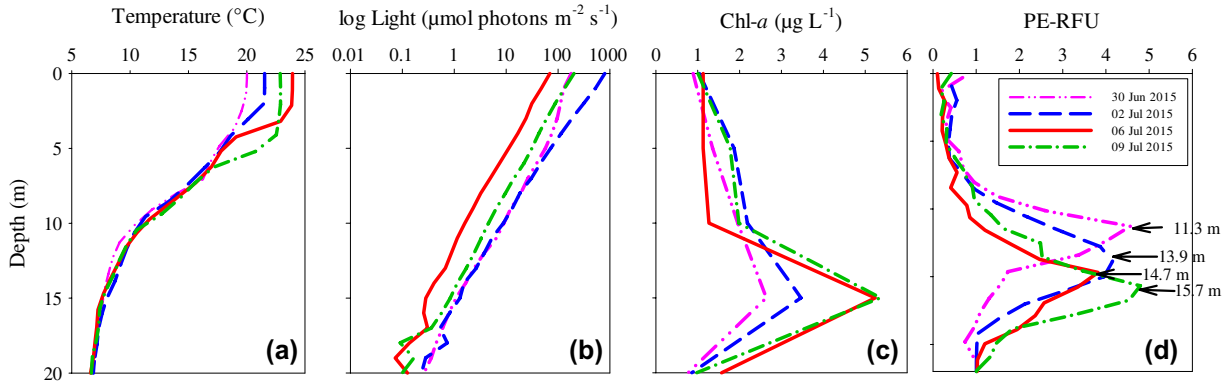

Figure 2. (a) Temperature, (b) light, (c) chlorophyll *a*, and (d) relative phycoerythrin (PE) fluorescence profiles at the central station (S3) in Lake Mondsee during the heat wave. RFU = relative fluorescent units.

(one-way ANOVA, $P = 0.018$) from 19.4 ± 0.9 °C recorded on 30 June to 22.4 ± 0.8 °C measured on 9 July 2015. Only minor temperature increases were recorded in the metalimnion (~6–12 m), upper hypolimnion (12–20 m), and in integrated samples (Supplemental Table S1). Parallel to the temperature changes, pH decreased from 30 June to 6 July but was higher on the last day of our investigation (Supplemental Table S1). Conductivity was relatively constant at the various sampling stations and significantly higher (Mann-Whitney rank sum test, $P < 0.001$) in the tributaries than in the lake (Supplemental Table S1). Light levels were recorded at station S3 between 0830 and 0930 h, and are therefore relatively low (Fig. 2b). Higher irradiances (in photons) ranging from 1400 to 1750 µmol m^{-2} s^{-1} were measured at noon at another station close to S8 in Lake Mondsee between 30 June and 6 July 2015 (E. Entfellner, PhD student, Research Institute for Limnology, Mondsee, July 2015, unpubl. data).

Nutrient concentrations varied depending on sampling station and depth (Supplemental Table S2). Silicate and nitrate levels were highest in the tributaries and also significantly higher in the metalimnion and hypolimnion than in the epilimnion of the lake (Kruskal-Wallis rank sum test, $P < 0.001$). Nutrient levels remained relatively constant for the individual sampling stations during the heat wave; SRP was under the detection limit for all sampling stations within the lake. In the tributaries (S11–S14), SRP concentration ranged from 3.1 µg L^{-1} (S14, 0 m, 9 July) to 8.5 µg L^{-1} (S12, 0 m, 2 July). If all tributary sampling stations were pooled, SRP concentration tended to decrease during the heat wave, but this decline was not significant (Kruskal-Wallis rank sum test, $P = 0.25$).

Chlorophyll a and fluorescence profiles

Spectrophotometrically measured Chl-*a* ranged from 0.77 µg L^{-1} (S5, 0 m, 6 July) to 5.36 µg L^{-1} (S3, 15 m, 6 July) during our investigation (Fig. 2c; Supplemental Fig. S1). Chl-*a* concentration measured in the integrated samples at stations S1, S3, S6, and S8 were not different (one-way ANOVA, $P = 0.15$). Significantly lower values were recorded at the inshore stations S4, S5, and S9 in the plumes of the tributaries (one-way ANOVA, $P < 0.01$ in all 3 cases). If all data were pooled for each sampling occasion, mean Chl-*a* concentration increased continuously from 1.32 to 1.95 µg L^{-1} from 30 June to 9 July 2015, but this change was not significant (one-way ANOVA, $P = 0.41$).

The phycoerythrin sensor of the YSI probe clearly showed the *P. rubescens* peak in the lower metalimnion/upper hypolimnion (Fig. 2d). This peak migrated downward from 11.3 to 15.7 m during the study period. Light intensity (in photons) at the depth of the *P. rubescens* peak ranged from 0.32 µmol m^{-2} s^{-1} (6 July) to 5.63 µmol m^{-2} s^{-1} (30 June), corresponding to 2.25–0.24% of surface light irradiance. The lowest relative light intensity at the *P. rubescens* peak was measured at the end of this investigation.

Phytoplankton community changes measured using three optical methods

We were able to analyse all samples from all sampling stations using FlowCAM and flow cytometry; however, for logistic restrictions we only analysed the integrated samples (S3, S6, S8) and the discrete samples from the central station (S3) using light microscopy.

Light microscopic analyses

Altogether, we identified 46 algal taxa by light microscopy: 24 taxa at the species level and the remaining 22 at the genus level.

When integrated samples from stations S3, S6, and S8 were pooled for each sampling occasion, cyanobacterial biomass varied between 0.136 ± 0.006 mm^3 L^{-1} on 9 July

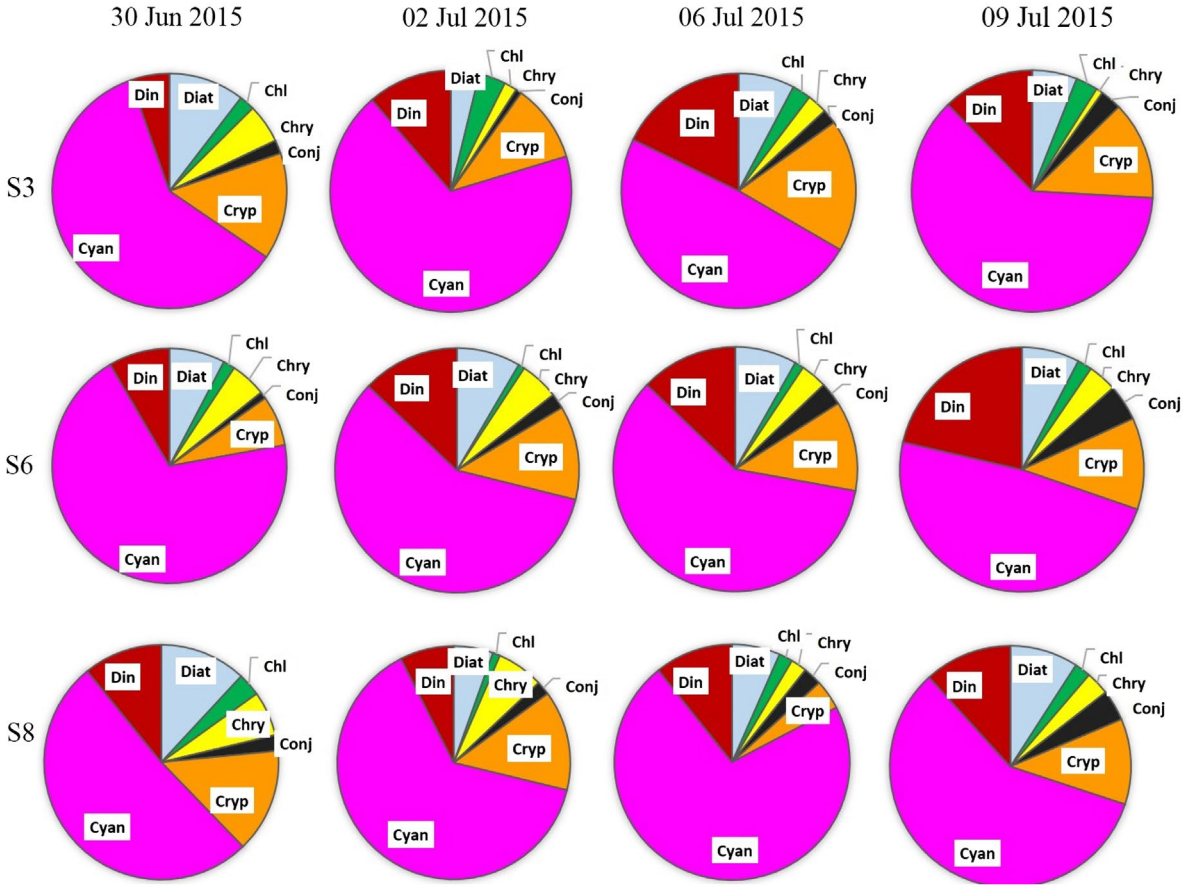

Figure 3. Relative composition of phytoplankton biomass during a heat wave in 2015 at 3 sampling stations (S3, S6, S8) across Lake Mondsee. Phytoplankton composition was derived from integrated samples (0–20m). *Diat* = Diatoms, *Chl* = chlorophytes, *Chry* = chrysophytes, *Conj* = Conjugatophyceae, *Cryp* = cryptophytes, *Cyan* = Cyanophyceae, *Din* = dinoflagellates.

Table 1. Total phytoplankton biovolume of sampling stations S3, S6, and S8 (0–20 m, integrated) calculated using light microscopy.

	Total biovolume [mm³ L⁻¹]			
Site	30 Jun 2015	2 Jul 2015	6 Jul 2015	9 Jul 2015
S3	0.2086	0.2966	0.1701	0.2590
S6	0.2914	0.2451	0.2286	0.1879
S8	0.1371	0.1948	0.2577	0.2198

and 0.156 ± 0.008 mm³ L^{-1} on 2 July, but there was no significant spatial or temporal (site and sampling day, respectively) variation (Kruskal-Wallis-rank-sum-test, $P > 0.05$). Biomass of eukaryotes seemed to increase slightly from 30 June (0.080 ± 0.009 mm³ L^{-1}) to 9 July (0.096 ± 0.003 mm³ L^{-1}), but, again, this increase was not significant (one-way ANOVA, $P = 0.4$). Within the integrated samples (S3, S6, S8), cyanobacteria (mainly *Planktothrix rubescens*) were the most abundant class (Fig. 3). Dinoflagellates contributed most to total biomass of eukaryotic algae (0.0263 ± 0.008 mm³ L^{-1}), followed by cryptophytes (0.0258 ± 0.006 mm³ L^{-1}), diatoms (0.017 ± 0.004 mm³ L^{-1}), and chrysophytes (0.008 ± 0.004 mm³ L^{-1}). Integrated (0–20 m) total phytoplankton biovolume ranged from 0.14 mm³ L^{-1} (S8, 30 June) to 0.30 mm³ L^{-1} (S3, 2 July), with no obvious changes during the heat wave at each sampling station (Table 1); similarly, integrated samples (S3, S6, S8) showed no significant changes in biomass of the various phytoplankton classes.

At the central station, colonial cyanobacterial biovolume was negligible at 0 and 5 m (Fig. 4). At 10 m, the contribution of *P. rubescens* to total biovolume decreased from 43% at the beginning of the heat wave to only 4% on the last sampling day. An inverse trend was obvious in the upper hypolimnion; at 15 m, the share of *P. rubescens* increased from 78% at the beginning to 94% at the end of the heat wave. At 20 m, the proportion of cyanobacterial biovolume increased from 40% to 50% during the heat wave (Fig. 2d and 4). The contribution to total biovolume at 0, 5, and 10 m by the other taxa was 16–45% for dinoflagellates, 18–44% for cryptophytes, and 6–27% for chrysophytes; diatoms (3–36%) and cryptophytes (2–23%) were prominent at 15 and 20 m depth.

To assess the effect of the 2015 heat wave relative to meteorologically "average" years, we compared the biomass calculated for each algal class from 2 July 2015 to

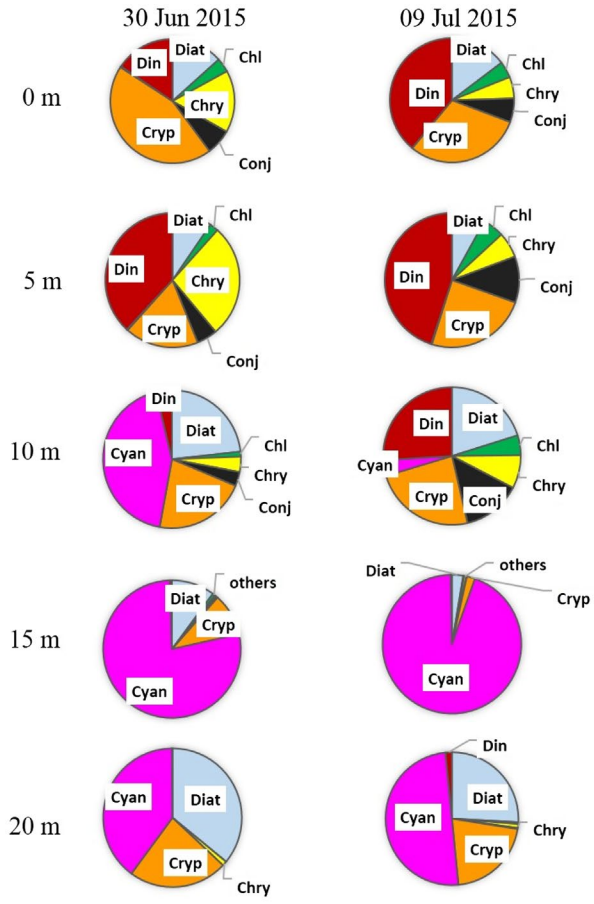

Figure 4. Phytoplankton biomass composition at different depths at the beginning and the end of the heat wave. Samples were taken above the deepest point of Lake Mondsee (S3) at 0, 5, 10, 15, and 20 m. *Diat* = Diatoms, *Chl* = chlorophytes, *Chry* = chrysophytes, *Conj* = Conjugatophyceae, *Cryp* = cryptophytes, *Cyan* = Cyanophyceae, *Din* = dinoflagellates.

the estimates made at the beginning of July from 2009 to 2014, respectively, and at the beginning of August 2007 and 2008 (Fig. 5; for these 2 years, no biomass data are available for early July). Phytoplankton biomass generally decreased over the years, and we recorded low biomasses for all algal classes during the heat wave, except for Cyanophyceae, which increased in July 2015 compared to previous years. Linear regression analysis revealed that cyanobacterial biovolumes were positively correlated to average July air temperatures from 2007 to 2015 (r^2 = 0.657; Supplemental Fig. S2). For all other phytoplankton classes the temperature effect was insignificant (data not shown).

CCA explained 57.6% of the total variance associated with the phytoplankton–environment (temperature and nitrate) relationship for epilimnetic samples (0 and 5 m) at the central station (Supplemental Fig. S3). The first canonical axis alone explained 41.9% of the variation (Table 2). Axis 1 was mainly composed of the environmental factor temperature (r = −0.863), and axis 2 consisted mainly of the environmental factor nitrate (r = 0.7309; Table 2, Supplemental Fig. S3). The Monte Carlo permutation test confirmed that temperature significantly affected phytoplankton biomass (P = 0.028) at the central station, whereas the effect of nitrate was less clear (P = 0.095; Supplemental Table S3); silicate, conductivity, and pH had no effect (data not shown).

FlowCAM analyses

We identified 48 algal taxa by FlowCAM: 12 taxa at the species level and the remaining 36 at the genus level. Most genera belonged to Chlorophyceae (17), followed by Bacillariophyceae (8). The class Conjugatophyceae comprised only one genus, *Cosmarium* sp.

Cryptophytes were the most numerous eukaryotic algae >5 µm measured with FlowCAM (average 329.8 ± 162.2 cells mL^{-1}; Supplemental Fig. S4e). Pooled over the 4 sampling occasions, dinoflagellates (9.9 ± 9.8 cells mL^{-1}), chlorophytes (13.3 ± 9.6 particles mL^{-1}), chrysophytes (11.3 ± 8.6 particles mL^{-1}), and diatoms (9.9 ± 9.8 particles mL^{-1}) were nearly equally abundant (Supplemental Fig. S4).

When the data were pooled for all stations and each sampling occasion, mean cyanobacterial particle (i.e., mainly colonies), numbers declined from 106 ± 98 to 51 ± 39 particles mL^{-1} from 30 June to 9 July, but this seeming decline was insignificant (one-way ANOVA, P = 0.36). When the data from all near-surface sampling stations S3 (0 and 5 m), S4, S5, S9, and S10 were pooled, total particle numbers decreased toward the end of the study period, and total cryptomonad cell numbers were negatively related to increasing water temperatures (Supplemental Fig. S5).

Similar to microscopic analyses, the integrated samples (S1, S3, S6, and S8) showed little variation during the heat wave. Although their contribution to total phytoplankton abundance was low, a significant increase was found in colony numbers of chrysophytes (one-way ANOVA, P = 0.018). Cyanobacterial particle numbers (mostly *Aphanothece* sp. colonies) decreased significantly during the heat wave (one-way ANOVA, P = 0.02).

Flow cytometric analyses

The AFC was able to discriminate >20 clearly defined phototrophic populations. Primarily based on their relative phycobiliprotein content (mainly PE) and cell size (forward and sideward scatter: FSC and SSC), half of those populations (11) were identified as single-celled cyanobacteria or microcolonies (Supplemental Fig. S6). Cell numbers of filamentous cyanobacteria and larger colonies of coccoid cyanobacteria are underestimated because

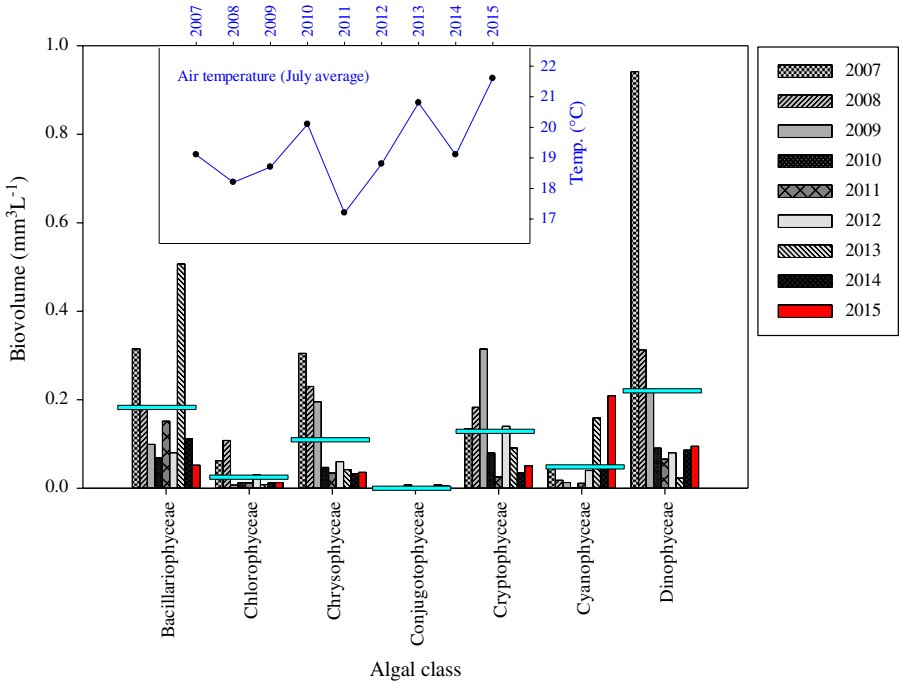

Figure 5. Phytoplankton biomass in July (2009–2015) and August (2007–2008) at the central station of Lake Mondsee derived from an integrated sample (0–21 m). The 2015 sample (red bar) was taken 2 July during the heat wave investigated. All samples were analysed using microscopy. Blue bars indicate mean values of all samples taken from 2007–2014 (data online available from www.land-oberoesterreich.gv.at/files/publikationen/gzuev_ooe_phytoplankton.pdf). Inset shows average July air temperatures from 2007 to 2015 (temperature data online available from http://www.zamg.ac.at/cms/en/climate/climate-overview/current_climate/monthly_climate/klimawerte/).

Table 2. Summary of canonical correspondence analysis of the phytoplankton composition in relation to the strongest environmental variables tested (temperature and nitrate concentration).

Total inertia		0.13103
Sum of all constrained eigenvalues		0.07548
Proportion of all constrained eigenvalues		0.576
	Axis 1	Axis 2
Eigenvalues	0.05424	0.02124
Proportion of explained eigenvalues	0.41391	0.16212
Species-environment correlation	0.86339	0.76249

the AFC measures particles (i.e., does not capture single cells in colonies and filaments). Despite this conservative estimate, (pico)cyanobacteria occurred in high numbers (0.54×10^5 to 2.36×10^5 mL^{-1}) at all stations throughout the investigation period. Variation in (pico)cyanobacterial particle numbers at each station and depth were minor during the study period. Near-surface samples from stations S1, S3 (0 m and 5 m), S4, S5, and S9 were not different (one-way ANOVA, $P = 0.30$); significantly higher cell numbers were found at station S10 (one-way ANOVA and Tukey test, $P = 0.02$). At this station, the mean picocyanobacterial numbers were 14–25% higher than at the other epilimnetic stations. When the data were pooled for all stations and each sampling occasion, mean cyanobacterial particle numbers declined from $1.54 \pm 0.41 \times 10^5$ mL^{-1} to $1.39 \pm 0.48 \times 10^5$ mL^{-1} from 30 June to 9 July 2015, but this change was insignificant.

Diatoms, chrysophytes, and cryptophytes were the most numerous eukaryotic algae measured by the AFC (data not shown). Collectively, eukaryote particle numbers were 2 orders of magnitude lower than those of (pico) cyanobacteria, ranging from 0.64×10^3 to 6.63×10^3 mL^{-1}. Mean eukaryote particle numbers (i.e., pooled for all stations/depths and each sampling occasion) were virtually constant, ranging from $3.3 \pm 1.4 \times 10^3$ mL^{-1} (30 June) to $3.7 \pm 1.7 \times 10^3$ mL^{-1} (2 July).

Similar to (pico)cyanobacteria, changes in eukaryotic cell numbers at each station and depth were minor during the study period; near-surface samples from stations S1, S3, S4, S5, and S9 were not different (one-way ANOVA, $P = 0.51$), whereas eukaryotic cell numbers were significantly higher at station S10 (one-way ANOVA and Tukey test, $P = 0.003$). Relative changes between the stations and depths, however, were larger for eukaryotes than for picocyanobacteria; at S10, the mean eukaryote cell numbers were 26–45% higher than at the other epilimnetic stations. Lowest absolute eukaryote cell numbers were recorded at station S3 at 15 and 20 m depth, where they remained almost constant throughout the study period.

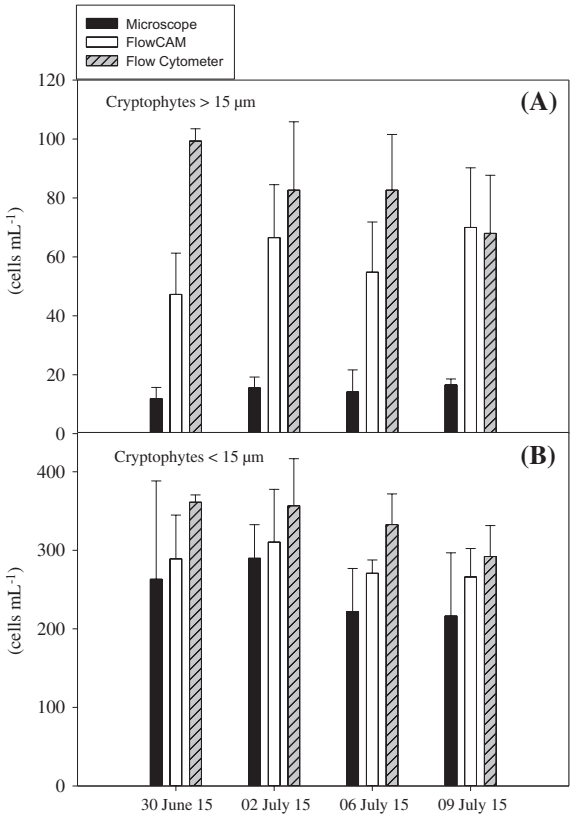

Figure 6. Comparison of cell counts of (a) medium-sized (~15–50 µm) and (b) small (<15 µm) cryptophytes using light microscopy, FlowCAM, and flow cytometry. Each bar represents the pooled cells numbers from the integrated samples from station S3, S6, and S8. Error bars represent 1 SD.

Methodological comparison

Light microscopy, FlowCAM, and AFC were complementary tools used during this field study. The accuracy of the 3 methods was tested by comparing cryptophyte cell numbers. Cryptophytes were abundant (~300 cells mL^{-1}) at all stations and, with cell sizes ranging from 6 to 50 µm, fell into the size range captured by all 3 instruments. We were able to distinguish small-sized species (<15 µm, mainly *Rhodomonas* spp. according to microscopic and FlowCAM identification) from medium-sized species (~15–50 µm, *Cryptomonas* spp.) using all 3 methods. Because AFC does not take images of particles, flow cytometric discrimination was primarily based on FSC, a measure of cell size.

Cell counts of medium-sized cryptophytes (Fig. 6a) were significantly different among all 3 methods (Kruskal-Wallis rank sum test, $P < 0.001$). FlowCAM and AFC cell counts were 4–8 times higher than light microscopic estimates.

Cell counts of small cryptophytes (Fig. 6b) were significantly higher using AFC compared to light microscopy (one-way ANOVA and Tukey test, $P < 0.002$). FlowCAM counts did not differ significantly from microscopic (one-way ANOVA and Tukey test, $P = 0.28$) and flow cytometric (one-way ANOVA and Tukey test, $P = 0.09$) analyses.

Discussion

We assessed possible changes in the phytoplankton community distribution within a deep prealpine lake during a summer heat wave in 2015. Nutrient levels remained low during the heat wave and, in accordance with Reynolds (1984), SRP levels decreased within the main tributaries of Lake Mondsee because of a reduced nutrient load from the catchment. In the lake sampling stations, SRP levels were below the detection limit on all occasions. As expected, we recorded a significant increase in water temperature during the heat wave, and this increase was restricted to the epilimnion. Our statistical analyses revealed that temperature significantly affected the phytoplankton community at the central station in the lake, whereas nutrient changes over time were minor and had little effect on the phytoplankton community. Some caution is needed with this interpretation, however, because we could not include SRP in our statistical analyses.

Heat wave effects on the algal community in Lake Mondsee

Although the phytoplankton community in the epilimnion was significantly affected by temperature, we observed only minor or insignificant changes overall in the total phytoplankton biomass during the heat wave. This finding holds true mainly for the temporal/spatial variation of prokaryotic and eukaryotic phytoplankton measured across the lake. For instance, Chl-*a* concentration measured in the integrated samples at 4 stations were not different; however, we observed significant changes in the phytoplankton community in the metalimnion and upper hypolimnion in Lake Mondsee. In particular, the peak of the filamentous cyanobacterium *Planktothrix rubescens* shifted from 11 to 16 m depth during the heat wave (Fig. 2d and 4). According to Dokulil and Teubner (2012), this species prefers dim light (<1%) and cold (5–12 °C) conditions in Lake Mondsee. Our data suggest that the temperature of the *P. rubescens* peak may be restricted to 7–9 °C in Lake Mondsee, but this may be coincidental; although the occurrence of *P. rubescens* in central European lakes suggests it is a cold-water stenotherm species (Reynolds 1984), its optimum temperature for growth is >20 °C (Davis and Walsby 2002), and the main driver for the vertical distribution of *P. rubescens* is light quantity and quality (Bright and Walsby 2000, Walsby and Schanz 2002, Oberhaus et al. 2007). While SRP was at or below the detection limit, nitrate and silicate increased significantly

with increasing depth at the central station; therefore, the vertical shift of the *Planktothrix* peak was possibly affected by increasing nitrate levels because *Planktothrix* strains are known to take advantage of increased dissolved inorganic and organic nitrogen levels under low light conditions (Zotina et al. 2003, Walsby and Jüttner 2006, Davis et al. 2015).

We recorded low biomasses for all algal classes during the heat wave, except for Cyanophyceae, which increased in July 2015 compared to previous years (Fig. 5). The annual average biovolume of *P. rubescens* in Lake Mondsee was 0.12 $mm^3 L^{-1}$ in 2015 compared to only 0.02 $mm^3 L^{-1}$ in 2014, leading to a degradation of the ecological status of the lake from "high" to "good" (Schafferer and Pfister 2016). The positive correlation between average July temperatures and cyanobacterial biomass in Lake Mondsee (Supplemental Fig. S2) further confirms the general conjecture that lake warming, directly or indirectly, favours cyanobacteria (Moss et al. 2011, O'Neil et al. 2012, Paerl and Paul 2012). Most likely, the decrease in eukaryote phytoplankton biomass in Lake Mondsee over the years primarily reflects the ongoing reoligotrophication process of Lake Mondsee. Dokulil and Teubner (2005) noted that, different from eukaryotic algae, growth of *P. rubescens* has been largely uncoupled from the phosphorus concentration in the lake since ~1990.

Our CCA suggests that cyanobacteria and chrysophytes benefitted from increased temperature (Supplemental Fig. S3). Despite these specific changes, we conclude that low nutrient inputs from the tributaries, concomitant with minor nutrient changes in the epilimnion of the lake, and stable weather conditions during the heat wave affect the (epilimnetic) phytoplankton community in oligo-mesotrophic, deep, stratified lakes less than in shallow eutrophic or polymictic lakes (Jöhnk et al. 2008, Wilhelm and Adrian 2008). Undoubtedly, the nutrient load is the main driver for seasonal phytoplankton community dynamics, although this factor can be enhanced by increasing temperatures (Elliott 2012b). We infer that the temperature effect on phytoplankton communities during heat waves is minor in the epilimnion of deep oligotrophic lakes relative to nutrient-rich lakes, where the temperature effect may interact with (the greater availability of) nutrients. The climate effect on the metalimnetic peak of *P. rubescens* in deep (pre)alpine lakes awaits further study.

Methodological comparison

Flow cytometric and FlowCAM analyses primarily yield the number of particles (cells and colonies) measured, linked with various fluorescence parameters. Microscopic counts are reliable for rarer and larger species and cell volume calculations. The data obtained with all 3 methods mostly complemented each other (different size classes for different methods) or corresponded to each other.

We found only minor differences in cell counts of the small cryptophytes (<15 µm; Fig. 6b) but significant differences among all 3 methods for the larger species (Fig. 6a); light microscopy seemed to underestimate cell counts of species ranging in size from ~15–50 µm by a factor of up to 8. Flow cytometry may have overestimated the cell number of medium- and larger-sized cryptophytes slightly because some cyanobacterial colonies show scatter and fluorescence characteristics similar to medium- and larger-sized cryptophytes. By contrast, the gate characterizing the small cryptophytes was unequivocally defined. The discrepancy between FlowCAM and microscopic cell counts of medium-sized cryptophytes was primarily caused by cells in the 15–20 µm size range of the live samples. Some of the cells falling into this category may have been identified as medium-sized cryptophytes with FlowCAM and as small-sized cryptophytes with light microscopy because these particles may shrink in Lugol's preserved samples. Lugol's fixation may lead to cell swelling, shrinking, or breakage, and the fixation artefacts may be species-specific (Menden-Deuer et al. 2001, Zarauz and Irigoien 2008). Comparisons of fresh samples analysed with FlowCAM with Lugol's fixed samples measured microscopically from natural marine phytoplankton led to the conclusion that traditional microscopy underestimates the abundance and biomass due to preservation, in particular cell numbers of nanoplankton (<20 µm; Zarauz and Irigoien 2008, Álvarez et al. 2014).

In a previous study conducted in our laboratory, Lugol's fixed microscopic cell counts of *Cryptomonas* sp. strain no. 26.80 (average cell volume ~200 $µm^3$; Weisse and Stadler 2006) did not differ from live FlowCAM measurements and live, as well as formalin-fixed flow cytometric estimates (Bergkemper and Weisse, unplubl.), suggesting that fixation did not affect estimates of cell numbers of small cryptophytes. We used the same preservative in the previous laboratory and the (present) field study, but the time span between fixation and analysis was shorter in the laboratory experiment (1 week compared to up to 4 months in the present study); we assume that the larger cryptophytes may be less well preserved with Lugol's than the small species (i.e., underestimation of their true cell numbers may increase with time). If this conclusion is correct, it may have significant implications on estimates of phytoplankton biomass within monitoring studies and should therefore be investigated in more detail.

Ideally, we recommend using all 3 complementary methods to assess abundance and biomass of all algal taxa ranging in size from minute coccoid picocyanobacteria to large cyanobacterial filaments and other large and rare taxa. We were only able to sample all stations

across the lake because of the fast sample processing by flow cytometry and FlowCAM. Furthermore, FlowCAM and AFC provide analyses of live samples, avoiding fixation/preservation artefacts. We agree with Àlvarez et al. (2014) that FlowCAM complements traditional techniques by (1) improving the sampling resolution available for study of highly dynamic communities, (2) providing detailed descriptions of particle-size spectra necessary for many ecological studies, and (3) providing objective criteria of taxon identification irrespective of human factors such as fatigue and expertise of the operator. Flow cytometry is ideally suited to yield fast and statistically sound estimates of the smallest and usually most abundant pico- and nano-sized autotrophs that cannot be quantified with the Utermöhl (1958) and FlowCAM methods, but it lacks comparable taxonomic resolution. Therefore, for future field campaigns similar to the present study, we recommend counting large and rare species (e.g., *Ceratium hirundinella*) by light microscopy and using AFC and FlowCAM or advanced imaging cytometers for all other samples, allowing many more samples to be processed with only minor loss of taxonomic resolution. This option has practical implications for lake monitoring; in particular, when more samples will be analysed to adequately assess future effects of meteorological extreme events such as heat waves and heavy precipitation that seem to increase in central Europe as a consequence of global warming.

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