

Article

An estimation of diel metabolic rates of eight limnological archetypes from Estonia using high-frequency measurements

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Abstract

We employed a Bayesian model to assess the metabolic state of 8 Estonian lakes representing the 8 lake types according to the European Union Water Framework Directive. We hypothesized that long-term averages of light-related variables would be better predictors of lake metabolism than nutrient-related variables. Model input parameters were *in situ* high-frequency measurements of dissolved oxygen, temperature, and irradiance. Model simulations were conducted for several (5–12) diel cycles for each lake during the summer season. Accounting for uncertainty, the results from the Bayesian model revealed that 2 lakes were autotrophic for the duration of the experiment, 1 was heterotrophic, and 5 were balanced or had an ambiguous metabolic state. Cross-comparison with a traditional bookkeeping model showed that the majority of lakes were in metabolic balance. A strong coupling between primary production and respiration was observed, with the share of autochthonous primary production respired by consumers increasing with light extinction and nutrient-related variables. Unlike gross primary production, community respiration was strongly related to light extinction, dissolved organic carbon (DOC) and total phosphorus. These findings suggest that a drastic decrease in light-limited primary production along the DOC gradient counter-balanced nutrient supply in the darker lakes and thus blurred the relationship between primary production and nutrients. Thus, contrary to our hypothesis, both light and nutrient-related variables seemed to be good predictors of lake respiration and its coupling to lake primary production.

Key words: Bayesian modelling, hemiboreal region, high-frequency measurements, lake types, net ecosystem production, Water Framework Directive

Introduction

The metabolism of an ecosystem represents the integrated measure of its rates of production and consumption of organic matter (Odum 1971). The difference between gross primary production (GPP) and community respiration (CR) thus determines the net production (NEP) of an ecosystem (Howarth et al. 1996, Lovett et al. 2006). Consequently, an ecosystem can exhibit 3 different metabolic states depending of the value of its NEP: autotrophic if NEP is positive, balanced if NEP is close to zero, and heterotrophic when NEP is negative. Because lakes play a disproportionately important role in the global

carbon cycle (Tranvik et al. 2009, Heathcote et al. 2015) and have well-defined boundaries, lake metabolism has been actively studied in previous decades (Staeher et al. 2012). To date, however, there is still little agreement on the metabolic state of different lake types or what drives these metabolic states, especially for waterbodies at higher latitudes (Cremona et al. 2014a) because of the conflicting effects of light and nutrients, among other factors. In the hemiboreal region of the Northern Hemisphere, lakes commonly exhibit high nutrient (total phosphorus; TP > 20 $\mu\text{g L}^{-1}$) content, especially on the southern coast of the Baltic Sea stretching from Denmark to Estonia (Ott and Kõiv 1999, Jeppesen et al. 2000). Because nutrients,

phosphorus in particular, are limiting for freshwater primary producer growth, it has been hypothesized that TP-rich systems should be autotrophic (Hanson et al. 2003). In the shallow and turbid high-latitude waterbodies, seasonal nutrient replenishment has been accompanied by an increase of primary production, pushing lakes from a heterotrophic to an autotrophic metabolic state (del Giorgio and Peters 1994, Squires et al. 2009).

In addition to nutrients, several studies confirm that water clarity parameters play a crucial role in the metabolism of hemiboreal lakes. Northern lakes often receive substantial coloured dissolved organic carbon (DOC) loading from their catchments, which decreases light penetration in the water column and hence causes high vertical light extinction coefficients (K_d) in these lakes (Arst et al. 2008). Lakes of Estonia, for example, generally contain $\text{DOC} > 8 \text{ mg L}^{-1}$ and have a $K_d > 0.5 \text{ m}^{-1}$ (Ott and Kõiv 1999). High K_d and high DOC concentrations have been associated, respectively, with primary production limitation and heterotrophic activity

stimulation because bacteria use terrestrial carbon as a substratum and outcompete phytoplankton for nutrients (del Giorgio et al. 1997, Drakare et al. 2002, Godwin et al. 2014). High DOC concentrations in the water column are correlated with negative NEP, which suggests that the more coloured a lake, the more heterotrophic it should be (Prairie et al. 2002, Hanson et al. 2003, Ask et al. 2012).

In addition to cross-lake differences in metabolism, within-lake switches of metabolism are also common, and, being coupled to diel changes in temperature and solar radiation (Laas et al. 2012, Cremona et al. 2014a, 2014b), cannot be ascertained with a time-averaged approach. These findings suggest that to trace cross- and within-lake switches of metabolism, the variables related to average carbon and nutrient content of lakes (which determine background metabolism) should be complemented with high-frequency temperature and light measurements, which are better predictors of instantaneous metabolism. Free-water oxygen sondes have thus



Fig. 1. Location of the study lakes in Estonia (black dots) and the weather stations (grey triangles). With the exception of Vörtsjärv and Valguta Mustjärv, which shared the same weather station, meteorological data were obtained for each lake from the nearest located station.

Table 1. Limnological parameters of the 8 study lakes. All values are long-term averages except water temperature, which represents mean recorded value during the sampling period. When available, minimum and maximum values are displayed, respectively, in brackets.

	Äntu Sinijärv	Erastvere	Mullutu	Peipsi	Saadjärv	Valguta Mustjärv	Võrtsjärv	Ülemiste
WFD type	alkaliphilic	light-coloured softwater	coastal lake	Peipsi*	stratified with medium alkalinity	dark-coloured softwater	Võrtsjärv*	nonstratified with medium alkalinity
Geographical coordinates	59°06'N 26°24'E	57°97'N 26°78'E	58°25'N 22°35'E	58°84'N 26°95'E	58°53'N 26°64'E	58°20'N 26°14'E	58°12'N 26°08'E	59°41'N 24°77'E
Trophic state	oligotrophic	hypertrophic	eutrophic	eutrophic	mesotrophic	Hypertrophic	eutrophic	eutrophic
Mixing regime	polymictic	dimictic	polymictic	polymictic	dimictic	Polymictic	polymictic	polymictic
Lake area (km ²)	0.02	0.16	4.12	2611	7.24	0.2	270	9.44
Catchment to lake area	68	32	58	18	3.9	6.7	11.5	10.5
Mean depth (m)	6	3.5	<1	8.3	8	<1	2.8	2.5
Max depth (m)	8	9.7	1.7	12.9	25	1	6	4.2
Water T (°C)	15 (14.9–15.1)	18.1 (16.6–19.9)	23.4 (19.8–26)	17.1 (16.4–17.6)	18.7 (17.1–20.7)	25.2 (24.1–26.5)	25.1 (24–26.1)	21.3 (20.2–22.2)
TP (ug L ⁻¹)	9 (6–17)	45	60	41 (20–60)	30	242	40 (17–75)	48 (22–55)
TN (ug L ⁻¹)	3450	990 (730–1600)	1000	473	390 (370–1000)	670	900 (840–2100)	723
Chl- <i>a</i> (ug L ⁻¹)	1 (0.2–2)	62 (61–67)	9	13.4 (8–28)	7.2 (1–8)	23	51 (31–80)	24.7 (14–42)
DOC (mg L ⁻¹)	4.72	13	18.1	12	10	35.2	12.52	13.7
HCO ₃ ⁻ (mg L ⁻¹)	293	85	110	160	147	30	270	201.3
K _d (m ⁻¹)	0.25	2.96	0.58	1.6	0.42	10.34	2.76	3.5

* large lakes are their own type

facilitated recording of lake metabolic rates, which had previously been mostly achieved with more labour-consuming methods like ¹⁴C incubation (for GPP) or O₂ decline rates (for CR). Therefore, until recently, usually only time-averaged conditions for months or seasons were used rather than dynamic measurements for most of the monitored lakes (Staehr and Sand-Jensen 2007).

An increasingly popular method for assessing metabolic rates of lakes is based on measuring changes in dissolved oxygen (DO) concentrations in the upper mixed layer, corrected for O₂ exchange at the air–water interface (Staehr et al. 2012). Collecting these data has been greatly facilitated by the advent of cheap data loggers mounted on buoys and teleconnection systems to transfer large amounts of high-frequency (HF) data (Laas et al. 2012, Alfonso et al. 2015, Meinson et al. 2015). These advances in data collection have been paralleled by the development of performant metabolic models based on Bayesian calculations that can reconstruct metabolic rates based on diel measurements of input parameters (Solomon et al. 2013). These HF models can track metabolic switches that remained unnoticed by traditional measurements conducted at lower frequencies (Laas et al. 2012). One of these recent models, the Bayesian Metabolic Model (BaMM), can calculate GPP, respiration and their uncertainties based on diel curves of DO, water temperature (T), and photosynthetically active radiation (PAR; Holtgrieve et al. 2010, Cremona et al. 2014b). BaMM has been calibrated for 10–15 min time steps for use with HF data.

The objective of this research was to assess diel metabolic rates of 8 Estonian lakes, each representing a limnological archetype in line with the European Union Water Framework Directive (WFD), an operational tool adopted in 2000 to clean and prevent polluted waters (http://ec.europa.eu/environment/water/water-framework/info/intro_en.htm). Based on trophic state, the selected lakes were classified into 4 categories comprising 1 oligotrophic, 1 mesotrophic, 4 eutrophic, and 2 hypertrophic lakes. Water transparency varied widely among the lakes: 3 clear water ($K_d < 1$), 4 turbid water ($1 < K_d < 4$), and 1 dark water ($K_d > 10$). Our working hypothesis was that light-related variables would be better predictors of lake metabolism than nutrient-related variables because, on average, Estonian lakes are nutrient-rich and coloured (Ott and Kõiv 1999), meaning they are probably more light-than nutrient-limited. We thus expected our study lakes to range from autotrophic to heterotrophic along a light-extinction gradient.

Study sites

The study was conducted in 8 Estonian lakes (Fig. 1), all different lake types according to the WFD (Ministry of Environment 2009). All the lakes were relatively shallow but differed markedly by morphometry, DOC, and nutrient contents (Table 1). Lakes were sampled within a 2-month period from mid-July to mid-September 2014, a period with little variation in irradiance and lake water T (Table

Table 2. Weather conditions as recorded by closest meteorological station of each lake. For weather data, values in cells represent daily mean followed by minimum and maximum recorded values, respectively.

	Measurement period	Wind speed (m s ⁻¹)	Air temperature (°C)	PAR (μmol m ⁻² s ⁻¹)
Äntu Sinijärv	16–22 Jul 2014	2.4 (1.2–3.4)	18.7 (17.4–19.8)	517 (324–619)
Erastvere	20–27 Aug 2014	2.8 (1.4–4.4)	13.9 (12.4–15.7)	263 (55–391)
Mullutu	08–14 Aug 2014	4.5 (1.9–7)	20.4 (17.1–21.9)	358 (152–525)
Peipsi	10–16 Sep 2014	1.3 (0.6–2.8)	13.8 (9.9–16.6)	251 (179–350)
Saadjärv	19–27 Aug 2014	2.6 (1.7–4.1)	13.5 (13–14.3)	270 (55–397)
Valguta Mustjärv	24 Jul–5 Aug 2014	2.9 (2.5–3.4)	23.7 (22.6–24.7)	419 (337–452)
Võrtsjärv	24 Jul–5 Aug 2014	2.9 (2.5–3.4)	23.7 (22.6–24.7)	419 (337–452)
Ülemiste	17–23 Jul 2014	1.9 (1.6–2.1)	21.2 (20.1–22.2)	593 (578–608)

2), and were equipped with a fixed-point HF monitoring lake buoy for a 5 to 12 (full 24 h) day period. Manual water samples for laboratory analysis of DOC, TP, total nitrogen (TN), alkalinity (HCO₃⁻), and chlorophyll *a* (Chl-*a*) were taken once from all study lakes between deployment periods. Water samples were analysed at the Centre for Limnology using following methods: DOC with TOC-VCPH Analyzer, Shimadzu Scientific Instruments, according to ISO 8245 and Estonian national standard EN 1484; TP with standard ISO 15681-2; TN with standard EVS-EN ISO 11905-1; HCO₃⁻ with 0.02% methyl red test; and Chl-*a* concentration with 96% ethanol extract analysed spectrophotometrically and calculated according to Lorenzen (1967).

Methods

Collecting data

Before each deployment, T, DO, electrical conductivity, and pH profiles were measured with a handheld multisonde (Yellow Springs Instruments, YSI ProPlus) to determine the mixing depth (uppermost depth with a T gradient <1 °C m⁻¹). Continuous monitoring of DO concentration (mg L⁻¹) and T (°C) was performed by automated stations equipped with a multiparametric sonde (YSI 6600V42) at 1 m depth. YSI multisondes were equipped with a self-cleaning optical sensor for DO. Sensors were calibrated in saturated air, with a correction for barometric pressure at the beginning and at the end of the deployment. No drifts of the sensor were observed between calibrations. Sensors performed automatic measurements every 10 min in all lakes except Äntu Sinijärv, where a technical problem resulted in a 30 min frequency during half the measurement period. Automated stations were placed near the deepest area of each lake as generally recommended (Staehr et al. 2010a), except in Lake Peipsi where the buoy was placed in the northwest part of the lake near the shore of Mustvee for safety reasons.

For Võrtsjärv and Valguta Mustjärv, irradiance and wind speed were obtained with a Vaisala Weather Transmitter WXT520 and a Li-Cor pyranometer model 200R directly joined to the Võrtsjärv buoy. Global irradiance (Q) and wind speed (U) were measured 2 m above the lake surface. Wind speed was converted to wind speed at 10 m height (U₁₀) using the formula by Smith (1985):

$$U_{10} \text{ (m s}^{-1}\text{)} = U_z a \quad (1)$$

$$a = 1.4125 z^{-0.15}. \quad (2)$$

For the other lakes we used weather data from the Estonian Environment Agency (KAUR; Supplementary Material 1) for Tõravere, Tiirikoja, Tallin-Harku, Võru, Väike-Maarja, and Roomassaare stations (Fig. 1). KAUR measures hourly average Q only in Tõravere, Tiirikoja, Roomassaare, and Tallin-Harku, and we used values from the closest stations to each lake. We calculated hourly average PAR as $0.436 \times Q$ as described in Nõges et al. (2011) and Arst et al. (2012) and hourly average wind speed to calculate gas transfer velocity k_{600} and k (m h⁻¹) following the formulas of Cole and Caraco (1998):

$$k_{600} = (2.07 + 0.215 U_{10}^{1.7})/100, \quad (3)$$

and (Jähne et al. 1987):

$$k = k_{600} \times (Sc/600)^{-0.5}, \quad (4)$$

where *Sc* is the Schmidt coefficient based on water T (°C; Wanninkhof 1992):

$$Sc = -0.0476 T^3 + 3.7818 T^2 - 120.1 T + 1800.6. \quad (5)$$

Table 3. Environmental parameters and prior values employed for each lake in BaMM simulation. Values of k_{20} and beginning O_2 concentrations correspond to minimum and maximum inputs with a uniform distribution and were chosen according to measurements of wind speed and O_2 concentrations, respectively.

	Äntu Sinijärv	Erastvere	Mullutu	Peipsi	Saadjärv	Valguta Mustjärv	Võrtsjärv	Ülemiste
P_{\max}^* (O_2 : $mg\ m^{-2}\ h^{-1}$)	15	400	110	300	320	112	325	300
R_{20}^\dagger (O_2 : $mg\ m^{-2}\ h^{-1}$)	40	300	50	200	190	150	195	200
k_{20}^\ddagger ($m\ h^{-1}$)	0.001–0.01	0.02–0.06	0.009–0.15	0.02–0.06	0.02–0.06	0.02–0.3	0.001–0.01	0.001–0.003
Beginning O_2 conc. ($mg\ L^{-1}$)	6–17	7–11	7–13	6–12	6.5–12	1–7	6.5–13	7–13
Mixing depth (m)	6	4.9	1	8.3	10	1	3.2	2.5

* P_{\max} : maximum hourly primary production; $^\dagger R_{20}$: maximum hourly respiration; $^\ddagger k_{20}$: gas transfer velocity coefficient.

Modelling

To assess lake primary production and respiration and their uncertainties, we employed BaMM (Holtgrieve et al. 2010), a model that constructs a mass-balance of DO using a set of equations describing photosynthesis, respiration, and reaeration. BaMM compares the DO time series reconstructed from the mass balance with observed data using Bayesian methods to select the most efficient parameter values. The complete set of model equations can be found in Holtgrieve et al. (2010), and a conceptual representation of the model is available in Cremona et al. (2014b). We selected main prior ranges to employ for model simulations (Table 3). The output values consisted of instantaneous primary production (P) and respiration (R, expressed as O_2 in $mg\ m^{-2}$), and model-calculated GPP and CR, both integrated on a 24 h cycle (O_2 in $g\ m^{-2}\ d^{-1}$). The model package can be downloaded at no cost (<http://conserver.iugo-cafe.org/user/gholtgrieve/BaMM>).

For our simulations, we chose the most recent version of BaMM (8 Jun 2013), which runs in an Excel interface. Every simulation corresponded to a diel period from 0700 (day n) to 0650 h (day $n + 1$) for 5–12 days, depending on the lake. The modelling increment corresponded to the measurement frequency of the sensor (i.e., 10 min), yielding 144 values for each diel cycle. As explained previously, the sensors in Lake Äntu Sinijärv malfunctioned so that the increment was 30 min instead of 10 min for 4 of 5 diel cycles. Two of the high-frequency model input parameters (DO, T) were obtained from sensor measurements, whereas the hourly PAR values from weather stations were digitally split into 10 min increments using the built-in light model of BaMM (Holtgrieve et al. 2010). Our goal was to obtain a uniform distribution and a range sufficient to reflect the natural fluctuations of the gas transfer coefficient k measured *in situ*.

For practical purposes, the Automatic Differentiation Model Builder (ADMB) version of BaMM was preferred to the Visual Basic for Applications (VBA) version because ADMB is quicker and better characterizes the

posterior distributions than VBA (Holtgrieve et al. 2010). For each diel cycle, the model was run for 1 million iterations with a thinning factor of 1000. If a false-minimum value for P or R was obtained by the model, we adjusted starting parameters, especially k , until a positive value of P or R was attained for calibration (Holtgrieve et al. 2010).

Convergence of the distribution range of output values was checked with the JMP MCMC Diagnostics Add-in 10 (SAS Institute Inc., Cary, NC, 1989–2012), consisting of a visual inspection of chains and autocorrelation parameters, as recommended by Holtgrieve et al. (2010). The posterior distributions of instantaneous P and R were each displayed as mean values within 2.5 and 97.5% of credible intervals.

The uncertainties of P and R were assessed using the technique described in Cremona et al. (2014b). To summarize, minimum and maximum NEP (NEP_{\min} , NEP_{\max}) were calculated based on P and R credible intervals along every diel cycle increment for each lake. If both NEP values were positive or negative, the lake was considered “certainly” autotrophic or “certainly” heterotrophic, respectively. If NEP_{\max} was positive and NEP_{\min} negative, the lake was considered “likely” autotrophic or “likely” heterotrophic, depending on the absolute values of the 2 numbers. The total number of autotrophic and heterotrophic increments were then counted to assess the dominant metabolic state during the study period and its confidence level.

Model output metabolic values (GPP, CR, coupling) were compared to a range of limnological parameters related to light (DOC, K_d), nutrient content (Chl- a , TN, TP), and alkalinity (HCO_3^-) using linear regression analysis. The coupling of GPP and CR is defined as the slope of the GPP:CR relationship (Solomon et al. 2013). To assess the robustness of the results, we compared BaMM outputs of integrated NEP values with those of the “bookkeeping” method described by Cole et al. (2000) and modified by Laas et al. (2012) regarding mixing depth.

Results

Instantaneous primary production and respiration time series

Primary production (as O_2) exhibited a large diel variability in all the lakes, ranging from 0 mg m^{-2} at night to 3200 mg m^{-2} during daytime in Saadjärv in late August (Fig. 2). With the exception of Mullutu and Peipsi, the peak of P for the majority of the lakes was short-lived and restricted to a few hours per day. The reading of the P time-series revealed 2 categories of lakes: those in which the instantaneous P curves remained remarkably similar on a daily basis during most of the modelling period (e.g., Saadjärv) and those in which P followed no general pattern (e.g., Valguta Mustjärv and Vörtjärv).

Compared to P varying over 3 orders of magnitude, instantaneous R was much more stable and remained generally within 2 orders of magnitude. The greatest R value (as O_2) was reached in Valguta Mustjärv (3000 mg m^{-2}) in late July. In contrast to P, however, no peak of R was observed, but large plateaus often lasting several days occurred.

Coupling between gross primary production and community respiration

Coupling between mixed-layer GPP and CR was strong in all lakes except in hypertrophic Erastvere, where no relationship was found (Fig. 3). In eutrophic shallow lakes Vörtjärv and Ülemiste, P did not predict R ($p < 0.05$, $r^2 = 0.55$) as well as in other lakes ($p < 0.05$, $r^2 > 0.8$ in all

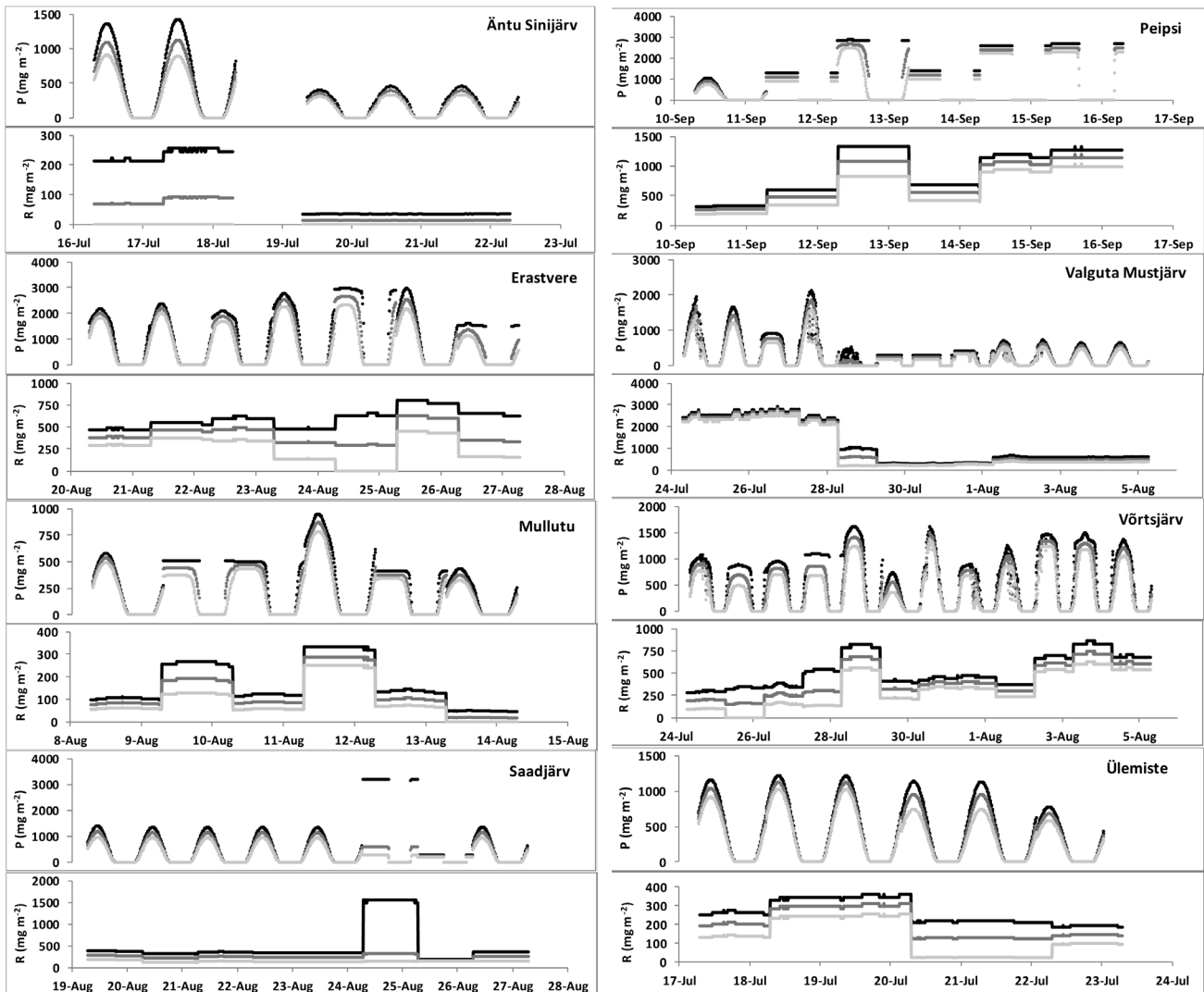


Fig. 2. Posterior distributions of instantaneous primary production (P) and respiration (R) time series of O_2 (mg m^{-2}) as modelled by BaMM for Estonian lakes. For each set of results, the black line represents the 97.5 percentile of credible intervals, the darker grey line corresponds to arithmetic mean values, and the lighter grey line signifies 2.5 percentiles of credible intervals.

other cases). The slope of the GPP:CR regression varied across the trophic gradient, with a greater proportion of autochthonous primary production respired within more eutrophic systems (Fig. 3). The coupling ranged from a slope value of 0.5 in oligotrophic and mesotrophic Äntu Sinijärv and Saadjärv to 3.8 in hypertrophic, DOC-rich Valguta Mustjärv. Eutrophic lakes Võrtsjärv, Ülemiste, and Peipsi exhibited a slope value slightly 1.

Metabolic state of lakes

According to the results of the modelling experiment with BaMM, the 8 studied lakes could be sorted into 3 categories depending on their NEP values and uncertainties (Table 4). The first category comprised autotrophic lakes Äntu Sinijärv and Mullutu; the second included

heterotrophic Valguta Mustjärv; and the third comprised lakes Erastvere, Peipsi, Saadjärv, Võrtsjärv, and Ülemiste, in which the certainly autotrophic or heterotrophic states lasted <math><50\%</math> of the modelled period, indicating that the metabolic state of these 3 lakes was ambiguous during the period considered. In Peipsi, the share of certainly autotrophic and certainly heterotrophic periods were both close to 50% with an uncertainty of only 4%, and thus the assumption that this lake was metabolically balanced during our study period was justified. In Saadjärv, both autotrophy (38%) and heterotrophy (46%) were not significantly different from 50% when considering the high uncertainty (14%); however, the certainly heterotrophic period dominated by a margin sufficient to conclude that this lake was probably balanced or weakly heterotrophic. By contrast, for a few other lakes, the gap between autotrophic and heterotrophic periods was so wide that their metabolic state was comparatively easier to define, as for Äntu Sinijärv and Valguta Mustjärv, which were autotrophic and heterotrophic, respectively.

Trophic state and metabolic state did not seem to be linked to each other because autotrophic lakes comprised both oligotrophic (e.g., Äntu Sinijärv) and eutrophic waterbodies (e.g., Mullutu and Ülemiste). Among the heterotrophic-leaning lakes were waterbodies as diverse as Valguta Mustjärv (shallow, hypertrophic) and Saadjärv (deep, mesotrophic).

Explaining variables

Variables reflecting underwater light (DOC, K_d) and nutrient (TP) conditions were strongly related with both CR and GPP:CR coupling (Fig. 4), defined as the slope of the GPP:CR relationship. The coefficient of determination of GPP:CR coupling was highest with TP ($p < 0.05$, $r^2 = 0.93$) and with DOC ($p < 0.05$, $r^2 = 0.89$). TN, Chl-*a* and alkalinity expressed no significant relationships with BaMM output variables (GPP, CR; $p > 0.05$), although Chl-*a* seemed slightly positively correlated with GPP.

Comparison with bookkeeping model

The modelling output values for integrated NEP obtained with BaMM and the bookkeeping model were largely within the same order of magnitude but were not correlated ($p = 0.049$, $r^2 = 0.06$, $df = 60$, $y = -0.2 + 0.9x$). When the 3 most negative outlier values of Valguta Mustjärv were removed from the analysis, a significant correlation was obtained, although with a low coefficient of determination ($p < 0.01$, $r^2 = 0.16$, $df = 57$, $y = 2.14 + 0.65x$). In a lake-wise comparison, the 2

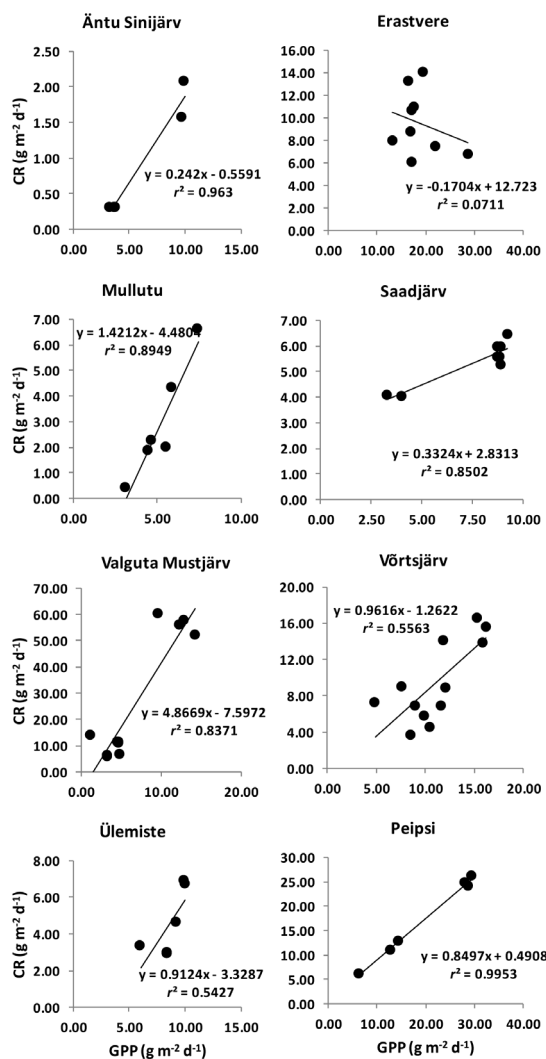


Fig. 3. Biplots of integrated GPP, CR, and their coupling (O_2 : $g\ m^{-2}\ d^{-1}$) outputs of BaMM for 8 Estonian lakes during the summer season. Coupling between GPP and CR was obtained by calculating the slope of the GPP:CR relationship.

Table 4. Autotrophy and heterotrophy duration (in % of modelled time), according to posterior distribution of metabolic parameters of BaMM, and their uncertainties for the 8 study lakes. Likely autotrophic and likely heterotrophic periods are considered uncertain, as described in the “modelling” section.

	Autotrophy		Heterotrophy		Uncertainty (%)
	Certainly ^a (%)	Likely ^b (%)	Certainly ^c (%)	Likely ^d (%)	
Äntu Sinijärv	56	7	28	9	16
Erastvere	45	9	44	2	11
Mullutu	50	3	39	8	11
Peipsi	47	3	49	1	4
Saadjärv	38	12	46	4	16
Valguta Mustjärv	0	21	69	10	31
Võrtsjärv	39	15	41	5	20
Ülemiste	49	6	39	6	12

^a $NEP_{min} > 0$; ^b $NEP_{max} + NEP_{min} > 0$, $NEP_{min} < 0$; ^c $NEP_{max} < 0$; ^d $NEP_{max} + NEP_{min} < 0$, $NEP_{max} > 0$

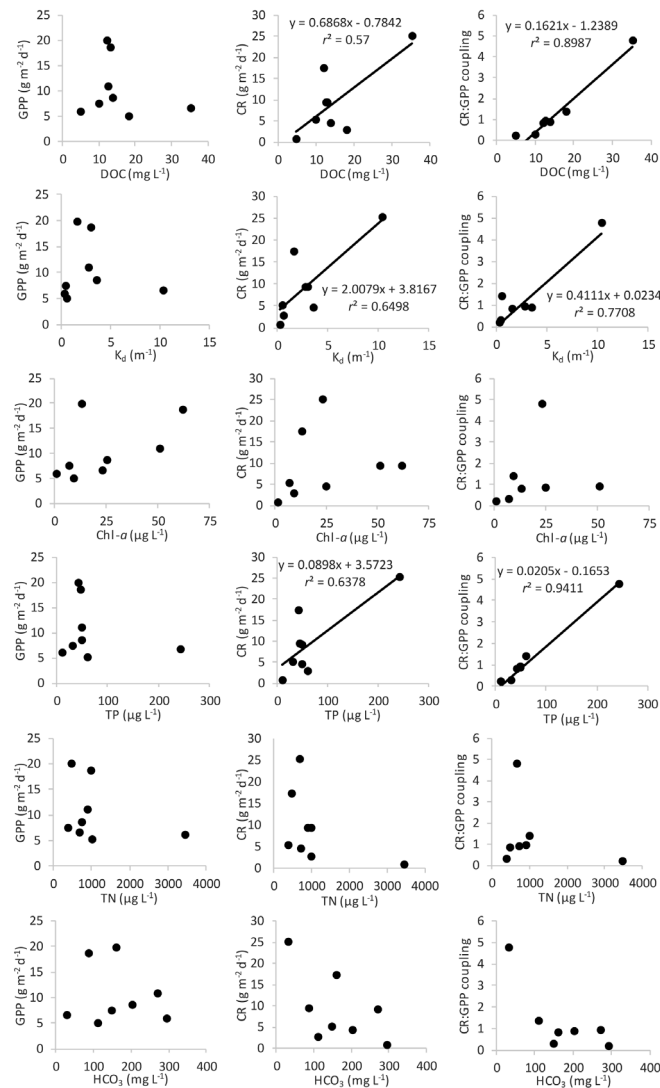


Fig. 4. Biplots of simulated metabolic rates integrated GPP and CR (O_2 : $g\ m^{-2}\ d^{-1}$) and their coupling with 6 variables related to light, trophic state, and alkalinity for 8 Estonian lakes during the summer season. Each row represents linear regression of respectively integrated GPP, CR, and their coupling (O_2 : $g\ m^{-2}\ d^{-1}$) vs. DOC, K_d , Chl- a , TP, TN, and HCO_3^- , successively. Coefficients of determination are displayed only for significant ($p < 0.05$) relationships.

models yielded similar NEP dynamics for lakes Äntu Sinijärv, Erastvere, Mullutu, and Võrtsjärv (Fig. 5), although the lakes were more heterotrophic based on the bookkeeping model. For the other 4 lakes, the 2 models yielded markedly different NEP dynamics, although, as for the earlier group of lakes, the bookkeeping model generally underestimated NEP relative to BaMM.

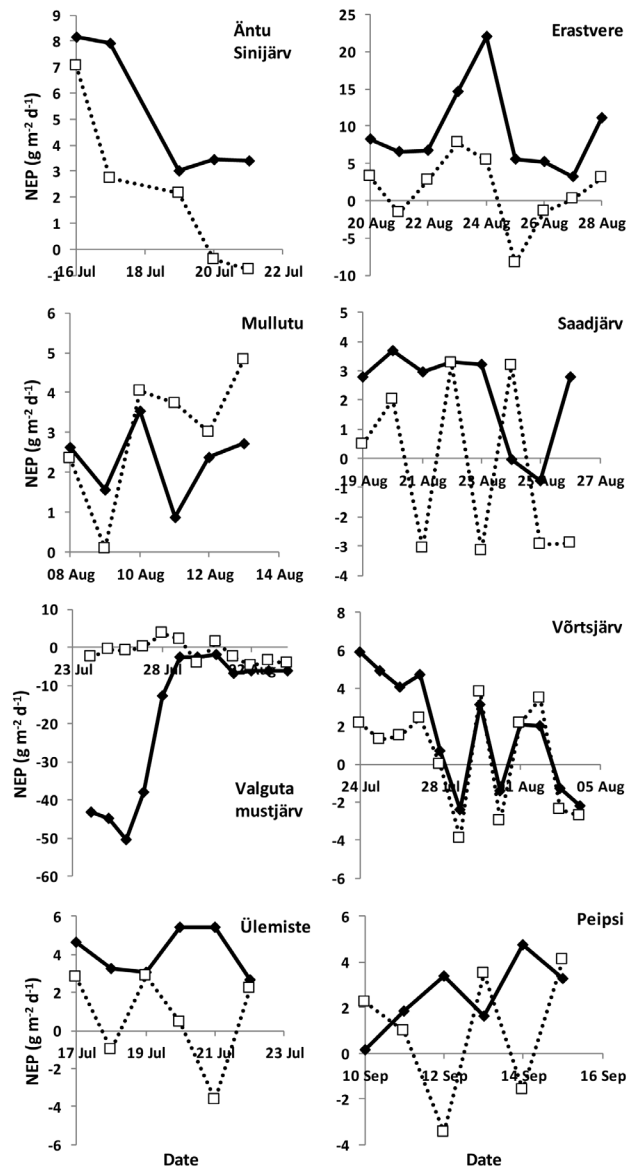


Fig. 5. Comparison of the outputs of BaMM (filled diamonds, solid line) and bookkeeping model (open squares, dotted line) for integrated net ecosystem production (NEP) time series in 8 Estonian lakes

Discussion

Majority of lakes in metabolic balance

Using the same model (BaMM) to estimate P and R for the 8 study lakes enables cross-comparison of the metabolic state of these systems. Only one lake in our study, the hypertrophic and DOC-rich Valguta Mustjärv, could be considered steadily heterotrophic. The other lakes were either autotrophic or of ambiguous metabolic state, but not heterotrophic given the relatively high percentage of autotrophic phases during the study period. In disagreement with our hypothesis, the metabolic parameters had statistically significant relationships both with light-related (DOC, K_d) and nutrient-related (TP) variables. The clearest lake (Äntu Sinijärv) was also the most autotrophic, whereas the darkest lake was the most heterotrophic. The connection between lake metabolic and trophic state was less straightforward; both oligotrophic (Äntu Sinijärv) and eutrophic (Mullutu) lakes were autotrophic, whereas other eutrophic lakes were neither autotrophic nor heterotrophic (Peipsi, Võrtsjärv, Ülemiste). The darker coloured lakes in our study were also most nutrient-rich, which explains why no correlation existed between GPP and nutrients, and why CR was positively correlated to TP. Trophic state can be positively correlated with whole-lake CO_2 emissions and sediment R (Kortelainen et al. 2006), and nutrients can stimulate directly heterotrophic microbial growth (Farjalla et al. 2002, Smith and Schindler 2009). This positive relationship between CR and TP is in agreement with Ask et al. (2012), who observed that the sharp decrease in light along the DOC gradient counter-balanced the nutrient availability in the darker lakes and hence obscured the relationships between nutrients and GPP. The covariance of DOC and nutrients and the simultaneous effects of DOC limiting GPP and nutrients stimulating GPP often result in a hump-shaped relationship between GPP and DOC (Fig. 4; Hanson et al. 2003, Thrane et al. 2014, Seekell et al. 2015). The uncertainty caused by the relatively low number of data points (Fig. 4), even though each represents thousands of time-integrated observations, suggests that adding more study lakes would increase the robustness of the relationship we observed between metabolic rates and other variables.

Tight coupling between GPP and CR in most lakes

Coupling between GPP and CR was strong in all study lakes but one. These results are broadly consistent with previous studies showing a clear relationship between GPP and CR, especially in the planktonic component of

the ecosystems (del Giorgio and Peters 1994, Solomon et al. 2013, Cremona et al. 2014b). The slope of the GPP:CR relationship, which can be employed as a proxy for the proportion of daily P consumed by heterotrophs (Solomon et al. 2013), seemed to scale up with the trophic state of the lake, ranging from 0.24 in oligotrophic Äntu Sinijärv to 4.8 in hypertrophic Valguta Mustjärv, with eutrophic lakes exhibiting intermediate values (0.8–1.4). These findings seem contradictory to reports to date because only in eutrophic systems were substantial proportions of GPP expected to escape CR (Carignan et al. 2000, del Giorgio and Williams 2005, Staehr and Sand-Jensen 2007, Solomon et al. 2013). Solomon et al. (2013) observed an average coupling between 0.6 and 1.2 for lakes with TP within the 20–50 $\mu\text{g L}^{-1}$ range, similar to that observed in our study. Recently, Alfonso et al. (2015) also reported a strong coupling between GPP and CR in a eutrophic shallow lake. The high (>3) coupling observed in Valguta Mustjärv can be easily explained for this hypertrophic but also dystrophic and coloured lake (mean [DOC] = 35 mg L^{-1}). The high DOC content of this lake, whether of autochthonous or allochthonous origin, fuels in-lake R so that organic matter mineralization exceeds P (Staehr and Sand-Jensen 2007, Staehr et al. 2010b). This explanation is corroborated by the significant positive relationship we observed between DOC and GPP:CR coupling in our study lakes. Our results indicate that DOC, which stimulates heterotrophic activity and attenuates light in the water column, might be a major driver of lake metabolism.

For Valguta Mustjärv and other eutrophic lakes, the strong coupling between P and R can additionally be caused by benthic R supplying P with CO_2 . Haargrave (1973) hypothesized that benthic R should be correlated to P and its components. In shallow eutrophic lakes such as Vörtsjärv, Ülemiste, Peipsi, and Mullutu, benthic R is responsible for a large fraction of the total oxygen consumption (Cornett and Rigler 1987, del Giorgio and Williams 2005). The CO_2 released from hypolimnetic carbon mineralization supplies P as soon as it reaches the photic zone and acts as a “primer” for photosynthesis and thus prevents carbon limitation of photosynthesis (Jansson et al. 2012).

Weak coupling in a few lakes

For 3 lakes (deep mesotrophic Saadjärv, shallow oligotrophic Äntu Sinijärv, and hypertrophic Erastvere) GPP:CR coupling was weaker than for the other lakes in our study, although the relatively low number of observations for Äntu Sinijärv and Saadjärv mitigates the robustness of the relationship. In deep stratified lakes like Saadjärv, most of the R occurring in the hypolimnion is

not connected to the upper layers, which thus seem to have low CR:GPP coupling (Laas et al. 2015). In shallow oligotrophic Äntu Sinijärv, the water column is fed by CO_2 -rich groundwater springs that might blur the relationship between CO_2 and metabolic rates (Laas et al. 2015). The absence of GPP:CR coupling in hypertrophic Lake Erastvere could be linked to an episode of cyanobacterial bloom during our experiment. Microscopic analyses conducted simultaneously to our HF measurements in Erastvere recorded a dominance of colonial cyanobacteria in this lake (Kersti Kangro, Estonian University of Life Sciences, pers. comm.). Previous studies have shown cyanobacteria blooms accompanied by large shifts in whole-lake metabolism caused by algae decay and a surge of heterotrophs activity (Cremona et al. 2014a).

Model differences

Because metabolic parameters cannot be measured independently (Chen et al. 2014), one procedure to assess the robustness of the model outputs is a cross-comparison with other methods of calculating metabolism. Similar trends of integrated NEP were obtained with BaMM and the bookkeeping model for half of our study lakes in the upper mixed layer, although the lakes' metabolic states seemed more autotrophic with BaMM. When using the bookkeeping approach, with the exception of Mullutu (which was autotrophic according to both models), all lakes were in metabolic balance. This difference can be explained by a greater sensitivity to input parameters for the bookkeeping model compared to BaMM (Cremona et al. 2014b). The bookkeeping approach applies nighttime R as a proxy for daytime R in the NEP calculations and has no correction mechanisms for removing aberrant results such as “negative” R, which occurs when water masses are replaced by more O_2 -rich masses (Supplementary Material 2; Laas et al. 2012). By comparison, BaMM calculates daytime R from temperature dynamics and real-time O_2 diffusion and then proceeds to a model-fitting procedure with GPP values.

BaMM is not without flaws, however. It shares with other Bayesian methods a subjectivity of prespecified distributions used as priors, which can influence posterior results (Lele and Dennis 2009). To increase the robustness of this type of model, a recent study showed that coupling of high-frequency chlorophyll fluorescence with DO is a sound method because algal biomass dynamics are often considered a good predictor of whole-lake GPP and CR (Honti et al. 2016). Other drawbacks are the sensitivity to water temperature for assessing R (which might have caused the negative NEP values calculated for Valguta Mustjärv; Table 2), the inability to calculate R of autotrophs, and the inability to trace component-specific

metabolism (Cremona et al. 2014b). Like most metabolic models, BaMM cannot disentangle benthic P or R from their pelagic counterparts, although benthic processes are expected to be more tightly coupled to DOC or K_d changes than the variables in the pelagic zone (Ask et al. 2012).

In conclusion, our modelling experiment revealed that the metabolic state of the surveyed lakes varied depending on the model, with the majority of lakes having a balanced metabolism, if we consider both model outputs, and one lake (Mullutu) being autotrophic. Contrary to our working hypothesis, variables related to light attenuation (K_d), carbon (DOC), and nutrient inputs (TP concentrations) were all good predictors of lake metabolism, with dark, nutrient-rich lakes generally displaying more heterotrophic behaviour than clearwater nutrient-poor lakes. The majority of lakes are in metabolic balance during summer when the autotrophic processes peak, suggesting these hemiboreal lakes might be heterotrophic the rest of the year. With the expected increase of in-lake light extinction, eutrophication, and turbidity caused by global climate change (Jeppesen et al. 2014), we anticipate the metabolic state of Estonian lakes to likely evolve toward a gradual reduction of autotrophic periods if no compensating mechanism arises.

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