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Delving deeper: Metabolic processes in the metalimnion of stratified lakes

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contributed substantially to revisions of and approved the final manuscript.

Abstract

Many lakes exhibit seasonal stratification with strong thermal and chemical gradients. An expansion of depth-integrated monitoring programs has provided insight into the importance of organic carbon processing that occurs below the upper mixed layer. However, the chemical and physical drivers of metabolism and metabolic coupling in the metalimnion remain unresolved. Sharp gradients in key resources such as light and temperature co-occur in the metalimnion with dynamic physical conditions that influence metabolic processes directly and simultaneously hamper the accurate tracing of biological activity. We evaluated the drivers of metalimnetic metabolism and its associated uncertainty across ten stratified lakes in Europe and North America. We hypothesized that the metalimnion would contribute highly to whole-lake functioning in clear oligotrophic lakes, and that rates would be highly variable in unstable polymictic lakes. Depth-integrated rates of gross primary production (GPP) and ecosystem respiration (ER) were modelled from diel dissolved oxygen curves using a Bayesian approach. Metabolic estimates were more uncertain below the epilimnion, but uncertainty was not consistently related to lake morphology or mixing regime. Metalimnetic rates exhibited high day-to-day variability in all trophic states, with metalimnetic contribution to daily whole-lake GPP and ER ranging from 0 to 87% and <1 to 92%, respectively. Nonetheless, the metalimnion of oligotrophic lakes contributed highly to whole-lake metabolism, driven by a collinear combination of light, phosphorous concentration and metalimnetic volume. Further, higher background respiration was observed in the metalimnion of eutrophic lakes. We show that a single-sensor approach does not necessarily reflect whole-ecosystem carbon dynamics in stratified lakes.

Introduction

Globally, many lakes exhibit thermal stratification for a substantial portion of the year, developing marked vertical gradients in physical and chemical properties (Boehrer and Schultze 2008). These gradients influence biological communities and biogeochemical processes such organic and inorganic matter cycling (Coloso et al. 2008; Van de Bogert et al. 2012), altering energy flow through lake food webs (Wilkinson et al. 2014). Consequently, patterns of stratification affect the important contribution that inland waters make to global carbon fluxes (Cole et al. 2007; Coloso et al. 2008; Staehr et al. 2012b). Vertical patterns of ecosystem metabolism vary among chemically and morphologically diverse lakes (Obrador et al. 2014), but information on the drivers of metabolism in the metalimnion is currently limited. However, a recent expansion of high-frequency and depth-integrated monitoring of lakes provides an opportunity to extend our understanding of lake metabolism below the upper mixed layer (epilimnion) (Meinson et al. 2015; Obrador et al. 2014; Staehr et al. 2010).

Light availability is a key driver of gross primary production (GPP) below the upper mixed layer (Obrador et al. 2014; Sadro et al. 2011a; Staehr et al. 2012b). Light availability in the metalimnion is determined by two physical factors; the thickness of the epilimnion (Z_{mix}) and light attenuation through the water column (K_D). These characteristics vary distinctly among lakes with different chemical characteristics, potentially exerting strong control on metalimnetic processes. Nutrient availability in surface waters controls phytoplankton biomass, so that trophic state and light availability at depth are correlated. Planktonic communities below the epilimnion in eutrophic lakes may be shaded by high plankton densities in upper layers, while stratification reinforces nutrient limitation and relative clarity of surface waters of oligotrophic lakes (Obrador et al. 2014). Nutrient availability may additionally induce physiological responses in phytoplankton (e.g. cell size and stoichiometry) that affect their activity rates (Finkel et al. 2010). The chemical influences on GPP propagate to other ecosystem functions. Metabolic coupling between GPP and

ecosystem respiration (ER) is pronounced under oligotrophic conditions where heterotrophs are substrate-limited and depend on labile photosynthetic exudates (Sadro et al. 2014; Sadro et al. 2011b; Solomon et al. 2013).

In addition to distinct variation among lakes, day-to-day variation in ecosystem metabolism within lakes is substantial (Solomon et al. 2013). The variability differs in magnitude among lakes, and may be related to physical processes determined by lake morphology and mixing regime (Solomon et al. 2013). Periods of stable stratification may facilitate the development of metalimnetic algal communities (Pannard et al. 2015), while unstable and short-term stratification patterns could alter nutrients and OM fluxes, affecting temporal and spatial patterns of metabolic activity. However, attributing biological activity at a specific place and time to physical processes in the metalimnion poses a considerable challenge (Coloso et al. 2011; Staehr et al. 2012a). Biological signals on diel timescales are dampened in the metalimnion, which is a zone characterized by sharp vertical gradients in physical conditions. Therefore, hydrologic processes caused by external forcing (e.g. internal waves and advection; Boegman et al. 2003; Sadro et al. 2011a) need only be minor to contribute substantial noise to diel patterns. Such physical processes alter the spatiotemporal footprint of sensors used to measure the free-water dissolved oxygen (DO) concentrations that are used to estimate metabolic rates (Odum 1956). Thus, physical processes in the metalimnion may simultaneously influence on metabolic processes biologically and affect our ability to accurately trace them with high-frequency measurements.

Understanding the chemical and physical drivers of metalimnetic metabolism and its uncertainty among a diverse lake ecosystems would allow for a more accurate classification of stratified lakes as carbon sources or sinks at the ecosystem scale. We investigated vertical patterns of lake metabolism, photosynthetic light-use efficiency, and the coupling relationship between ER and GPP using high-frequency and depth-specific data from ten lakes and reservoirs that ranged in nutrient concentration and thermal stratification patterns. We used a

modelling technique that accounted for uncertainty in the estimation of metabolic parameters (following e.g. Batt and Carpenter 2012; Cremona et al. 2014b; Grace et al. 2015; Hanson et al. 2008). This approach offers several advantages; overcoming sources of error present in earlier ‘book keeping’ methods (McNair et al. 2013), and allowing for the quantification of variation in diel DO that is not explained by the model. We assumed that unexplained variation in diel DO was predominantly attributable to process errors caused by lateral movements of water that are not incorporated in the depth-integrated framework.

We hypothesized that light availability, as described by the ratio of the mixing depth (Z_{mix}) to the photic depth (Z_{eu} ; determined by K_D) (i.e. $Z_{\text{mix}} : Z_{\text{eu}}$), would control biological processes in the metalimnion (Obrador et al. 2014; Sadro et al. 2011a; Staehr et al. 2012b). The relative contribution of the metalimnion to whole-lake metabolism was expected to be greatest in clear, oligotrophic lakes (i.e. low $Z_{\text{mix}}:Z_{\text{eu}}$). We sought to identify the conditions where depth-integrated estimates are useful to accurately estimate whole-lake NEP. It was expected that polymictic lakes with a more dynamic thermal structure would exhibit higher temporal variation explained by physical processes. Finally we utilized the depth specific metabolic estimates to investigate vertical patterns in the coupling between GPP and ER. Here we expected a tighter relation in the photic zone, especially in oligotrophic lakes, and ER to be more reliant on recalcitrant OM under low light conditions (Solomon et al. 2013; Obrador et al. 2014).

Methods

Study sites

We modelled open-water lake metabolism from high-frequency, depth-specific measurements of DO and water temperature (T) in ten lakes and reservoirs across Europe and North America. The dataset included monomictic, dimictic, and polymictic lakes with a range of morphological characteristics and chemical composition (Table 2). Concentration of nutrients,

dissolved organic carbon (DOC) and chlorophyll *a* (chl *a*) were determined from samples taken on 1-11 days at each lake during the stratified period (see Supplemental Text 1 for sampling and analytical methods). In addition, Chl-*a* concentration was estimated from high-frequency *in-situ* measurement using a fluorometer at some sites. Data from each lake represented a single year, for a period ranging from several weeks to many months. Only days when the water-column was thermally stratified (as described below) were included in analyses (17 to 69 days per lake).

The profiling systems or sensor chains recorded high-frequency measurements (10-60 min intervals) at 3 to 35 layers in each lake, with at least one measurement point in each depth zone (i.e. epi-, meta- and hypolimnion; Fig. 1; Table S1). We assumed that each measurement represented a horizontal layer of water centred on the measurement depth and having a thickness equal to the vertical resolution of measurements. Therefore, the deepest point of the profile was the depth of the deepest measurement plus half the vertical resolution of that layer. Meteorological data including photosynthetically active radiation (PAR), wind speed and barometric pressure were recorded at the same frequency as sub-surface measurements. Radiation measurements recorded as irradiance (E ; $W\ m^{-2}$) were converted to photon flux in the 400-700 nm range (PAR; $\mu mol\ m^{-2}\ s^{-1}$) as follows (Kirk 1994; McCree 1981):

$$PAR = E \times 4.6 \times 0.45. \quad (1)$$

Vertical stratification and underwater light regime

High vertical resolution (0.1 m) temperature profiles were modelled from available T data to determine if a lake was stratified on a given day and to delineate the metalimnion. T at each depth z was modelled as (Rimmer et al. 2005):

$$T(z) = T_h + (T_e - T_h) \left(\frac{1}{1 + (\alpha \times z)^n} \right)^{1 - \left(\frac{1}{n} \right)}, \quad (2)$$

where T_e and T_h are the maximum temperature in the epilimnion and hypolimnion, respectively, and α and n are model-estimated constants. Temperature curves were fitted in a Bayesian framework with JAGS (Plummer 2003) using normally distributed, maximum-entropy priors. The metalimnion extent (i.e. Z_{mix} to the top of the hypolimnion) was defined using water density (ρ), calculated as:

$$\rho = 1 - 6.63 \times 10^{-6}(T - 4)^2. \quad (3)$$

The gradient in density between adjacent points that defined Z_{mix} was selected by visual inspection (Fig. S1) for each lake individually, and ranged between 0.03 and 0.18 kg m⁻³ m⁻¹ (Read et al. 2011). The bottom of the metalimnion was similarly the deepest point with that density gradient. The sensitivity of metalimnetic volume and depth-zone specific metabolic rates to this gradient was examined at a subset of sites (Lake Ontario, Vedsted and Müggel; see Supplemental Text 2 and Table S2). The thermocline was defined between the points with the greatest density gradient (Read et al. 2011). Mean daily Z_{mix} and the bottom of the metalimnion were calculated as the mean values from all profiles on each day, and each layer was assigned to one depth zone (i.e. epi-, meta- or hypolimnion) for each day.

Water-column light attenuation (K_D) at each site was calculated from either measurements of underwater PAR at multiple depths, Secchi depth or other existing site-specific relationships (Table S1). Estimates of K_D were made from daily to biweekly intervals and were linearly interpolated between measurement days when necessary. Where underwater PAR measurements were available, K_D was estimated as the slope of the linear regression between log(PAR) and depth z . A mean daily K_D was calculated from the K_D of each profile during daylight ($PAR_0 > 5 \mu\text{mol m}^{-2} \text{s}^{-1}$) that had a linear regression fit with $r^2 > 0.80$ (Obrador et al. 2014; Staehr et al. 2012b). Where Secchi depth was available, K_D was calculated as $1.7/\text{Secchi depth}$ (Poole and Atkins 1929). The mean daily photic depth (Z_{eu}) was calculated as $4.6/K_D$. Following the determination of daily K_D at each site, PAR for each

time interval and depth z (PAR_z) was calculated from incident PAR (PAR_0) using the Beer-Lambert law:

$$PAR_z = PAR_0 \times e^{-K_D \times z}. \quad (4)$$

Model of depth-dependent metabolism

Gaps in the data series of up to 1 h were linearly interpolated before we applied a 4-h simple moving average smoothing to DO, PAR and wind speed measurements (following Giling et al. 2016; Obrador et al. 2014). To estimate mean rates of ecosystem metabolism at a daily scale, we used a Bayesian model with non-linear sub-models for GPP and ER (Giling et al. 2016). Changes in DO concentration between successive measurement times t for each depth were partitioned into the contribution by biotic and physical processes using a depth-specific diel oxygen model (Staehr et al. 2012b):

$$DO(t + 1) = DO(t) + NEP(t) - D_s(t) - D_v(t) + D_z(t); \quad (5)$$

where NEP is the rate of net ecosystem production ($\text{mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$), D_s is air-water exchange, D_v is diffusive vertical exchange between adjacent depths and D_z is metalimnetic exchange due to mixed-layer deepening. Atmospheric exchange (D_s) was applied only to layers in the epilimnion, and calculated as:

$$D_s(t) = K_s(t) \frac{DO_{mod}(t) - DO_{sat}(t)}{Z_{mix}(t)}. \quad (6)$$

The gas transfer velocity (K_s) was calculated at a Schmidt number of 600 (K_{600}) from wind speed standardized to 10 m height (U_{10}) according to Cole and Caraco (1998). In Equation 6, DO_{mod} is the modelled DO concentration following Song et al. (2016) and DO_{sat} is DO concentration at atmospheric equilibrium (calculated from temperature and barometric pressure). Vertical exchange between each depth and the adjacent depths due to turbulent diffusivity (D_v) was estimated using the Brunt-Väisälä buoyancy frequency ($N^2 [\text{s}^{-2}]$, a measure of local stability; Bohrer and Schultze 2010) to calculate the vertical eddy

diffusivity coefficient (K_v) according to Hondzo and Stefan (1993). Lake Ontario was an exception due to its surface area exceeding the applicable range of the Hondzo and Stefan (1993) model. The metalimnetic K_v for Ontario was set as $6 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$ based on measurements and theoretical work from Lakes Ontario and Erie (Bouffard et al. 2014; Sweers 1970). Epilimnetic and hypolimnetic K_v were expected to be higher than the metalimnetic estimate, so were set at Lake Ontario using a factor determined from the average relative difference between depth zones in the other nine lakes. The influence of K_v on metabolic rates at Ontario and two other lakes (Vedsted and Müggel) was assessed with a sensitivity analysis (for details see Supplemental Text 2 and Table S2). The flux D_z was calculated as proportional to the Z_{mix} deepening rate ($\Delta Z_{\text{mix}}/\Delta t$) and was applied to measurement points within the metalimnion and 1 m above or below (Obrador et al. 2014). At some sites, we set a threshold limiting the deepening rate to a maximum of 5 m h^{-1} to prevent short-term surface-water microstratification inaccurately affecting D_z in the metalimnion. For a full description of the model, see Staehr et al. (2012b).

Sub-models for the production and respiration components of *NEP* in Equation 5 were parameterized according to Grace et al. (2015). At each depth, GPP was modelled as a saturating function of PAR_z , while respiration was non-linearly dependent on T for each timestep t :

$$NEP(t) = A \times PAR(t)^p - R_{\bar{T}}(\theta^{(T(t)-\bar{T})}); \quad (7)$$

where the first component describes GPP ($\text{mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$), and A is a constant indicating production per unit light (i.e. photosynthetic efficiency), PAR is the depth-specific PAR (PAR_z), and p is an estimated exponent that represents the ability of producers to use light and accounts for saturating photosynthesis (when $p < 1$). The second component indicates DO consumption by ER ($\text{mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$); $R_{\bar{T}}$ is the respiration rate at \bar{T} , θ describes the temperature dependence of respiration (set to 1.072, corresponding to Q_{10} of 2; Wilcock et al. 1998), T is the water temperature at each timestep and \bar{T} is the mean daily temperature in that

layer. Respiration was estimated at \bar{T} and not standardized because we were interested in vertical patterns among the lakes at *in situ* conditions. Modelled rates were standardized to a temperature of 20°C (GPP₂₀ and ER₂₀; for results see Fig. S2) only for analysis of coupling of ER₂₀ to GPP₂₀ (Holtgrieve et al. 2010; Solomon et al. 2013). We estimated the parameters A , p and $R_{\bar{T}}$ in Equation 7 for each depth with a Bayesian approach in JAGS (Plummer 2003) using R code (R Development Core Team 2014) modified from Grace *et al.* (2015) following the rationale of Song et al. (2016). Prior distributions for the estimated parameters were uniformly distributed within known physical constraints as described by Grace *et al.* (2015). Mean daily metabolic estimates (and their propagated uncertainty) for each depth layer were calculated from estimated parameters as:

$$GPP = \sum_{t=1}^{measurements} A \times PAR(t)^p \quad (8)$$

$$ER = 86400 \times \frac{R_{\bar{T}}}{\Delta t} \quad (9)$$

where GPP is daily gross primary production (mg O₂ L⁻¹ d⁻¹), ER is daily ecosystem respiration at daily average temperature (mg O₂ L⁻¹ d⁻¹) and 86400 converts from seconds to days. Convergence and stationarity of Markov Chain Monte Carlo (MCMC) values were assessed with the Gelman-Rubin convergence statistic \hat{R} (Brooks and Gelman 1997). Models with $\hat{R} > 1.1$ (indicating unconverged chains) as well as poor fitting models ($r^2 < 0.40$) were excluded from further analyses. Excluding poor fitting models did not substantially alter vertical patterns of ecosystem metabolism or their uncertainty (Fig. S3). Example model fits for each lake are available in the supplementary material (Figs S7-S16) and example code for estimating depth-integrated metabolic rates is available online (github.com/dgiling).

Aggregating layer-specific metabolic rates

Mean daily rates in each depth zone (i.e. epi-, meta- and hypolimnion) were calculated by aggregating the appropriate daily estimates layer from each layer i . The standard deviation of

the aggregated depth zone rate (σ_{zone}) was propagated from the modelled uncertainties in layer i as follows:

$$\sigma_{zone} = \sqrt{\sum_i^n \sigma_i^2}, \quad (10)$$

where i to n are the layers belonging to that depth zone. The aggregated depth-zone estimates were used for further analysis of metabolic rates. Due to high day-to-day variability, only days when there was at least one adequate metabolic model fit (i.e. $\hat{R} < 1.1$ and $r^2 > 0.40$) from layers in each depth zone (epi-, meta- and hypolimnion when present) were considered to calculate the relative contribution of the metalimnion to whole-lake metabolism (total of 178 days; Table 2).

Whole-lake metabolism estimates

Whole-lake volume-weighted estimates (WLVW; Sadro et al. 2011a) were calculated by multiplying the daily depth-zone volumetric rates ($\text{g O}_2 \text{ m}^{-3} \text{ d}^{-1}$) by the total volume (m^3) of each depth zone before summing the three zones. Metalimnetic volume was calculated using surface areas from hypsographic data and mean daily thickness from high-resolution temperature profiles. Whole-lake areal estimates were then obtained by dividing the WLVW estimate by the lake surface area (m^2). Hypolimnetic volume and thus metabolic contribution will be underestimated in the small number of lakes that were considerably deeper than the available profile measurements (e.g. Lake Ontario; Table 2). The proportional contribution of the metalimnion to whole-lake metabolism was calculated as the metalimnetic volumetric rate divided by the WLVW estimate (termed ‘Prop_{metab}’). We also assessed whether the metalimnion disproportionately contributed metabolic activity for its size (volume) for each day in each lake. We did this by calculating the ratio between the proportion of WLVW metabolism attributable to the metalimnetic (Prop_{metab}) and the metalimnetic contribution to

whole-lake volume (termed 'Prop_{vol}'). Thus, the metalimnion contributed more to the whole lake metabolically than it did volumetrically when the ratio Prop_{metab}:Prop_{vol} was >1.

We assessed how the traditional metabolism estimates based on single sensors in the epilimnion compared to depth-integrated estimates. 'Single-sensor estimates' were calculated by taking the sensor placed at 1 m depth, the most widespread DO sensor deployment depth (Solomon et al. 2013), and calculating whole-lake areal metabolism as above assuming that this rate was constant over depth. The shallowest available probe was used when no sensor was available at 1 m depth.

The 'background respiration' of OM not recently fixed can be inferred from the intercept of the relationship between daily ER₂₀ and daily GPP₂₀, i.e. ER₂₀ when GPP₂₀ = 0 (del Giorgio and Williams 2005; Solomon et al. 2013). The slope of the relationship between daily ER₂₀ and GPP₂₀ describes the metabolic coupling, where a slope of 1 indicates a unit increase in ER₂₀ for each unit increase in GPP₂₀. The coefficient of determination (r^2) indicates the strength of the coupling (Obrador et al. 2014). We used estimates from layers where there were >5 days with successful fits to estimate coupling regression parameters. Models were excluded where the layer was nearly always dark (so that GPP₂₀ was zero or mean < 0.01 mg O₂ L⁻¹ d⁻¹) because the slope was either vertical (and therefore undefined) or highly outlying (20-40 times the mean slope) and not conceptually meaningful. Coupling estimates for depth zone were calculated as the mean of all intercept and slope estimates from layers belonging to that depth zone.

Statistical analyses

Comparisons among depth zones, correlations with ancillary variables, and ER₂₀-GPP₂₀ regressions were analyzed by fitting linear models or linear mixed models (LMMs) in R (R Development Core Team 2014). The LMMs included a random effect for site and layer and AR1 autocorrelation structure (nested within site) to account for repeated daily measurements

where appropriate. We compared a range of autocorrelation structures (including AR2 and AR3) with Akaike Information Criterion (AIC) values and found AR1 provided equivalent or better support for the models. Reported r^2 values refer to the variation explained by the fixed effects only. Variables were log-transformed when necessary. Principal components analysis (PCA) was used to reduce the number of collinear explanatory variables, and the resulting components were used as explanatory variables in LMMs to assess potential drivers of metalimnetic contribution.

Results

Uncertainty in model estimates and contribution of physical processes

The depth-integrated metabolic model provided a better description of diurnal changes in DO in the epilimnion (74% of models converged with adequate fit) than in the metalimnion (43%) or hypolimnion (32%; Fig. 2a). Furthermore, modelled metabolic estimates were on average more precise in the epilimnion (mean coefficient of variation [CV] for GPP and ER estimates = 0.14 and 0.23, respectively) than in the metalimnion (mean GPP and ER CV = 0.25 and 0.33, respectively) or hypolimnion (mean GPP and ER CV = 0.35 and 0.51, respectively) (Fig. 2b, c). There was high variability in model fit (R^2) and estimate certainty (CV) among days and depth zones (Fig. 2). In the metalimnion, R^2 was unrelated to water-column stability (assessed with the buoyancy frequency N^2 ; $F_{1,470} = 1.99$, $p = 0.159$; Fig. S4a). Accordingly, N^2 in successful models did not differ from N^2 in poor-fitting models (paired t-test, $t = -0.004$, $p = 0.997$; Fig. S4b). Variation in mean R^2 across all lakes and depth zones was also not explained by measurement frequency ($F_{2,25} = 0.445$, $p = 0.646$), the vertical resolution of measurements ($F_{1,26} = 0.000$, $p = 0.999$), Z_{\max} ($F_{1,26} = 2.11$, $p = 0.159$) or surface area ($F_{1,26} = 0.441$, $p = 0.513$; Fig. S5). CV for GPP and ER was also unaffected or only marginally affected by measurement frequency ($F_{2,24} = 1.71$, $p = 0.202$ and $F_{2,25} = 3.377$, $p = 0.050$) and the vertical resolution of measurements ($F_{1,26} = 0.105$, $p = 0.749$ and $F_{1,26} = 0.167$, $p = 0.898$).

The physical fluxes of DO (i.e. D_s , D_v and D_z) contributed a substantial proportion ($32 \pm 24\%$ across all estimates) of the total DO fluxes (i.e. sum of absolute NEP, D_s , D_v and D_z) (Table S3). In the epilimnion, a mean of 45% of DO changes were attributable to the diffusive components, mostly atmospheric exchange (D_s) *with a small contribution from D_v and D_z* . Transfer due to mixed layer deepening (D_z) contributed strongly to the metalimnion (mean 29% attributable to diffusive fluxes), and both D_z and D_v estimates were important in the hypolimnion (mean 16%; Table S3). The average magnitude of physical fluxes was unrelated to mean model R^2 ($F_{1,8} = 1.216$, $p = 0.302$) and CV of GPP and ER estimates among the ten lakes ($F_{1,8} = 3.499$, $p = 0.098$ and $F_{1,8} = 2.727$, $p = 0.173$). Further, we did not detect that the physical processes were not strongly affected by lake morphology. In the metalimnion, log-transformed lake area did not affect the balance between D_z , D_v and NEP with ($F_{1,8} = 1.218$, $p = 0.301$) or without ($F_{1,7} = 1.892$, $p = 0.211$) outlying Lake Ontario (Fig. S5). Sensitivity analysis demonstrated that the value of K_v did not strongly affect metabolic estimates, except for rates in the metalimnion and hypolimnion of polymictic Lake Müggel (Supplemental Text 2 and Table S2).

Metabolic estimates were characterized by high day-to-day variability in some lakes and layers (Fig. 1). For contiguous days with adequate model fits among the ten lakes, between 8-52%, 0-78% and 11-100% of epi-, meta- and hypolimnetic GPP estimates were within 2 standard deviations (SD) of the estimate from the previous day. Similarly, between 13-53%, 0-88% and 11-96% of epi-, meta- and hypolimnetic ER estimates were within 2 SD of the previous days' estimate. In the metalimnion, this did not appear to be strongly driven by PAR; the day-to-day shift in PAR_z did not consistently differ between consecutive days that had similar or disparate metabolic estimates. The proportion of days with estimates similar to the previous day was also not related to lake area (Fig. S5). Day-to-day variability in metalimnetic thickness as a proportion of Z_{max} was slightly higher in polymictic (SD in relative thickness = 0.08 ± 0.02 , $n = 4$ lakes) than in mono-/dimictic lakes (SD = 0.04 ± 0.02 ,

$n = 6$ lakes, $F_{1,8} = 6.33$, $p = 0.04$). However, metalimnetic local water-column stability did not differ among mixing regimes ($F_{1,8} = 3.26$, $p = 0.109$). The variability daily estimates of GPP and ER in the metalimnion (Table S3) was not related to lake mixing regime ($F_{2,7} = 0.753$, $p = 0.505$ and $F_{2,7} = 0.044$, $p = 0.957$) or trophic status ($F_{2,7} = 1.337$, $p = 0.223$ and $F_{2,7} = 0.178$, $p = 0.841$). We assessed whether process errors (e.g. lateral water movements) were responsible for high day-to-day variability by testing whether the SD among daily estimates differed when depth-zone means were aggregated from converged models with good ($R^2 > 0.4$) or poor fits ($R^2 < 0.4$). We found was no difference in propagated SD for metalimnetic GPP (paired t-test; $t_9 = 1.42$, $p = 0.190$) or ER ($t_9 = 0.772$, $p = 0.460$).

Depth-specific metabolic rates and photosynthetic efficiency

Vertical patterns of metabolism varied distinctly among the lakes, with mean GPP ranging from 0.00 to 5.98 mg O₂ L⁻¹ d⁻¹ and ER from 0.00 to 3.74 mg O₂ L⁻¹ d⁻¹ across all depth zones (Fig. 1; Table S3). Surface layers were most often autotrophic, whilst balanced to net heterotrophic conditions were prevalent in the metalimnion. Mean NEP was < 0 mg O₂ L⁻¹ d⁻¹ for 60% of the daily metalimnetic estimates across all the lakes and layers. Mean daily GPP in the epilimnion was positively correlated with mean epilimnetic TP concentration ($F_{1,7} = 8.75$, $p = 0.021$, $r^2 = 0.56$), as was epilimnetic ER ($F_{1,7} = 10.59$, $p = 0.014$, $r^2 = 0.60$). In the metalimnion, mean GPP and ER were not linearly correlated to epilimnetic TP concentration ($F_{1,7} = 2.04$, $p = 0.196$, $r^2 = 0.22$ and $F_{1,7} = 0.10$, $p = 0.404$, $r^2 = 0.10$, respectively; Fig. 3a,b).

We found evidence of photosynthetic activity down to *ca.* 0.1% of surface light. Increasing GPP with depth in some lakes was due to higher daily photosynthetic efficiency (i.e. parameter A from Equation 7; GPP [mg O₂ L⁻¹ d⁻¹] / PAR [μ mol m⁻² sec⁻¹]) in low-light conditions ($F_{1,792} = 105.17$, $p < 0.001$; Fig. S6). Correspondingly, photosynthetic efficiency varied by depth ($F_{2,809} = 58.55$, $p < 0.001$), and was lower in the epilimnion than in the metalimnion or hypolimnion ($p < 0.01$ in post-hoc pairwise comparisons), which did not

differ ($p = 0.905$). Photosynthetic efficiency was reduced in low nutrient conditions ($F_{2,23} = 25.72$, $p < 0.001$). Efficiency was significantly lower in oligotrophic lakes than in mesotrophic lakes (post-hoc comparison; $z = -4.54$, $p < 0.001$), which were lower again than eutrophic lakes ($z = -3.17$, $p = 0.004$).

Metalimnetic contribution to whole-lake metabolism

Across all sites, the proportional contribution of the metalimnion to WLWV metabolic estimates (i.e. $\text{Prop}_{\text{metab}}$) varied between 0 and 87% for daily GPP and between <1 and 92% for daily ER (Fig. 4a,b). The metalimnetic $\text{Prop}_{\text{metab}}$ was negatively correlated to epilimnetic TP concentration for GPP ($F_{1,7} = 6.09$, $p = 0.042$, $r^2 = 0.47$) and for ER ($F_{1,7} = 8.69$, $p = 0.021$, $r^2 = 0.55$), but these relationships were driven by Lake Castle (Fig. 3c,d). The proportion of whole-lake volume made up of the metalimnion (i.e. Prop_{vol}) was between 3 and 60% (mean 32%). The metalimnetic $\text{Prop}_{\text{metab}}$ was positively correlated with metalimnetic Prop_{vol} for both GPP (slope = 0.88 ± 0.12 , $F_{1,175} = 58.46$, $p < 0.001$, $r^2 = 0.25$) and ER (slope = 0.94 ± 0.14 , $F_{1,175} = 43.74$, $p < 0.001$, $r^2 = 0.20$). The metalimnetic $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$ was a mean 0.95 ± 0.67 SD for GPP and 1.06 ± 0.87 SD for ER. The ratio $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$ showed that the metalimnion disproportionately contributed metabolic activity for its size (i.e. had high $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$) in oligotrophic lakes (Fig. 3e,f). This was evidenced by a negative relationship between metalimnetic $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$ and mean epilimnetic TP concentration for GPP ($F_{1,7} = 15.31$, $p = 0.006$, $r^2 = 0.68$; Fig. 3e) and ER ($F_{1,7} = 12.46$, $p = 0.010$, $r^2 = 0.64$; Fig. 3f).

We observed a negative linear correlation between metalimnetic $\text{Prop}_{\text{metab}}$ and log-transformed $Z_{\text{mix}}:Z_{\text{eu}}$ for GPP ($F_{1,166} = 12.39$, $p < 0.001$; Fig. 4a). However, this trend was not robust to the exclusion of Lake Castle ($F_{1,151} = 2.47$, $p = 0.118$). A similar negative relationship was observed for metalimnetic ER $\text{Prop}_{\text{metab}}$ (Fig. 4b), with ($F_{1,166} = 18.58$, $p < 0.001$) or without ($F_{1,151} = 12.10$, $p < 0.001$) Lake Castle. Comparable trends were observed

for the effect of log-transformed $Z_{\text{mix}}:Z_{\text{eu}}$ on $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$, with a negative relationship for GPP ($F_{1,166} = 15.22$, $p < 0.001$; Fig. 4c), which was again not significant when Castle Lake was removed from the analysis ($F_{1,151} = 2.08$, $p = 0.151$). In contrast, the negative effect of log-transformed $Z_{\text{mix}}:Z_{\text{eu}}$ on $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$ for ER ($F_{1,166} = 12.46$, $p < 0.001$; Fig. 4d) was robust to the exclusion of Castle Lake ($F_{1,151} = 8.31$, $p = 0.005$). Metalimnetic Prop_{vol} tended to be high when Z_{mix} was shallow, meaning that $\log Z_{\text{mix}}:Z_{\text{eu}}$ and Prop_{vol} were negatively correlated ($F_{1,166} = 13.20$, $p < 0.001$, $r^2 = 0.11$). Hence, we used PCA to examine the collinear drivers of metalimnetic $\text{Prop}_{\text{metab}}$. Principal components (PCs) were derived from four variables representing abiotic resource availability and physical conditions (PAR_z , temperature, Prop_{vol} and N^2). The PC explaining the majority of variation (PC1) did not reduce the collinearity, as it was characterized by lower temperature, Prop_{vol} and N^2 (Table 3). Metalimnetic $\text{Prop}_{\text{metab}}$ for GPP and ER was negatively correlated to PC1 in linear mixed models (Table 3), suggesting a combined importance of these factors. A second component (PC2) was characterized mainly by low light and high local stability N^2 in the metalimnion, and was negatively correlated with $\text{Prop}_{\text{metab}}$ for GPP and ER (Table 3).

Depth-specific metabolic coupling

Coupling between ER_{20} and GPP_{20} varied among the lakes and depth zones. Background respiration (intercept) ranged from -0.16 to $2.70 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$, mean coupling slope ranged from -0.07 to 1.26 and mean r^2 varied between 0.05 and 0.94 (Table S4). There was an interaction between the effect of depth zone and epilimnetic TP concentration on mean background respiration ($F_{2,17} = 7.19$, $p = 0.005$). Mean epilimnetic TP did not affect mean background respiration in the epilimnion ($F_{1,7} = 4.64$, $p = 0.068$; Fig. 5a), but there was a positive linear correlation in both the metalimnion ($F_{1,7} = 26.21$, $p = 0.002$; Fig. 5b) and hypolimnion ($F_{1,7} = 19.19$, $p = 0.012$; Fig. 5c). The positive relationship in the hypolimnion was driven predominantly by Lake Müggel (Fig. 5c). Across all TP levels, background

respiration did not differ among depth zones ($F_{2,22} = 1.13$, $p = 0.340$). There was no interaction between the effect of epilimnetic TP and depth zone on the coupling slope ($F_{2,17} = 1.20$, $p = 0.324$), and no significant difference among depth zones ($F_{2,17} = 0.44$, $p = 0.653$). Across all depth zones, the coupling slope decreased with increasing epilimnetic TP concentration ($F_{1,17} = 6.48$, $p = 0.021$), but this relationship was not evident in any one depth zone individually ($p > 0.117$; Fig. 5d-f). For the strength of the coupling relationship (r^2) there was also no interaction between the effect of depth zone and mean epilimnetic TP ($F_{2,17} = 0.885$, $p = 0.431$), and no effect of depth zone ($F_{2,17} = 0.023$, $p = 0.977$). The strength of the coupling relationship decreased with increasing TP concentration ($F_{1,17} = 7.97$, $p = 0.012$) across all depth zones combined (Fig. 5g-i).

Effect of depth-integration on whole-lake areal NEP estimate

We assessed how WLWV estimates of GPP, ER and NEP made from a single epilimnetic sensor compared to the depth-integrated estimates. For mean GPP, the single-sensor approach over-estimated whole-lake metabolism at 9 of 10 sites, but the average difference from integrated estimates was variable (mean difference 3.17 ± 4.58 g O₂ m⁻¹ d⁻¹; Fig. 6a). In contrast, single-sensor estimates of ER did not consistently vary from integrated estimates (mean difference -0.37 ± 3.40 g O₂ m⁻¹ d⁻¹; Fig. 6b). Correspondingly, single-sensor estimates of whole-lake NEP generally were slight overestimates, but there was considerable variation both among lakes and among days within lakes (mean difference 3.55 ± 6.65 g O₂ m⁻¹ d⁻¹; Fig. 6c).

Discussion

We found contrasting patterns in the vertical structure of metabolic rates among the stratified lakes using a large depth-integrated and high frequency buoy dataset (Fig. 1). In clearer lakes, mean daily rates of GPP and ER were weakly associated with depth (e.g. Lake Bure) or

peaked in the metalimnion (e.g. Lakes Ontario, Stechlin), as reported before (Sadro et al. 2011a). In contrast, metabolic rates of some mesotrophic and eutrophic lakes were strongly depth-dependent (e.g. Lake Castle; Fig. 1). In lakes with high vertical variation in rates, single-sensor estimates of whole-lake metabolism deviated considerably from depth-integrated estimates on some days (Fig. 6). Despite dissimilar vertical profiles, we found that the metalimnion can contribute substantially to daily whole-lake metabolism across a broad range of lakes, with the exception of highly eutrophic conditions (Figs. 3 & 4). However, the relative contribution of the metalimnion varied substantially among days regardless of lake stratification pattern, and metalimnetic $\text{Prop}_{\text{metab}}$ was only moderately explained by changes in light availability (as indicated by $Z_{\text{mix}}:Z_{\text{eu}}$). Nonetheless, the metalimnion of oligotrophic lakes, with more light available at depth, contributed more on average to lake metabolism than expected from their metalimnetic volume ($\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}} > 1$; Fig. 3e,f).

Importance of physical processes to model uncertainty and day-to-day variability

Our depth-integrated analysis extends current understanding of the role that physical processes play in obtaining accurate metabolic estimates (Coloso et al. 2008; Staehr et al. 2012b; Van de Bogert et al. 2007). Combined with previous sensitivity analyses (Obrador et al. 2014), we show that daily estimates of metabolic rates are robust to uncertainties in vertical diffusive fluxes (D_v) under many circumstances, but there were exceptions in the deeper layers of a polymictic lake (Table S2). This increased sensitivity was likely due to the higher contribution of D_v and D_z to DO fluxes in deeper layers and corresponding decrease in the diel signal of DO, water temperature and PAR (Figs S7-S16), as well as model fit (Fig. 2). We expected that high variation in patterns of stratification or low water-column stability would be associated with poor fitting models and uncertainty in parameter estimates. However, within the metalimnion we found no evidence that polymictic lakes, or days and layers with low stability were associated with poor model convergence or fit. Furthermore,

model fit and uncertainty were similar among lakes and unrelated to mixing regime (Fig. 2). Hence, while the accuracy of K_v can influence metabolic results, it does not appear that the magnitude or accuracy of estimated diffusive fluxes are important factors in our ability to accurately describe diel variation in DO. This was supported by total diffusive fluxes (including D_s) being high in the epilimnion where model fits were often very good.

There are a range of physical processes that were not accounted for by the free-water framework utilized here, including horizontal advection, metalimnetic intrusions and internal seiches caused by wind forcing (Solomon et al. 2013; Van de Bogert et al. 2007). These process errors bring water masses influenced by heterogeneous chemical conditions and biological communities (Solomon et al. 2013; Van de Bogert et al. 2012; Van de Bogert et al. 2007). For example, Sadro *et al.* (2011a) found that pelagic rates were commonly influenced by littoral habitats due to advection and water-column stability. The patterns of model fits found among depth zones suggested physical process errors were a considerable issue in the meta- and hypolimnion. Because physical processes are a function of lake morphology, we expected the degree of uncertainty to vary among lakes. However, we did not observe any consistent trends in parameters of model fit or importance of diffusive fluxes across our gradient of lake areas (Fig S6), suggesting process errors did not systematically bias our interpretations. However, lake area is confounded by other variables in our dataset including measurement frequency (Table S1).

Physical processes in addition to diffusion may have contributed to the high day-to-day variability in the metabolic estimates because we found little evidence that variability in metalimnetic rates was driven by biological responses (e.g. due to differences in PAR). Conversely, high day-to-day variability is typical even of surface-water metabolic rates that are estimated with higher certainty and better model fits (Solomon et al. 2013; current study). Furthermore, selecting models with good or poor fit did not affect among-day variability or vertical patterns in metabolism (Fig S3). This suggested either that R^2 was a poor predictor of

the importance of process errors, or that process errors did not contribute highly to among-day variability. Quantifying the effect of physical processes on metabolic estimates using free-water measurements remains a considerable challenge and priority for future research. Ecological inferences made using free-water methods should remain coupled to this consideration (Sadro et al. 2011a) and be interpreted in the context of model fit and uncertainty (Cremona et al. 2014b).

Our results suggest we should remain cautious that patterns in metabolic activity are attributable to biological activity occurring at a certain place and time, especially when stratification periods are not prolonged. However, we employed a number of methods to assist in providing useful information on metabolic processes in the deeper layers of stratified lakes. These included analyzing weeks to months of data for each lake, and using methods such as temporal smoothing and calculating stratification and diffusive fluxes on sub-daily timescales (Coloso et al. 2011) was expected to. Further, where depth-integrated measures of chl-*a* concentration were available (Table S1), vertical patterns in metabolism generally matched algal biomass. In our study, the vertical resolution of measurements did not affect estimate certainty, but maintaining a high resolution will increase the accuracy of temperature profiles and stratification patterns. This is important for calculating the contribution of specific lake habitats, because metalimnetic contribution was sensitive to its thickness (Supplemental Text 2 and Table S2). Vertical resolution will also assist in cases such as Lake Castle where the development of hypolimnetic hypoxia (Fig. S9) could produce unrealistically sharp diffusive gradients if too few sensors are used.

Vertical patterns in efficiency of light utilization and metabolic coupling

The increase in light utilization efficiency in deeper depth zones explained the higher GPP rates in the metalimnion despite lower light availability. However, the mechanisms responsible for this pattern may vary among lakes. Chl-*a* concentration available at multiple

depths from subset of lakes showed that increases in metalimnetic photosynthetic efficiency at Lakes Hampen, Vedsted, Castle (Obrador et al. 2014) and El Gergal (Table 2) were not caused by higher algal biomass in the metalimnion. This suggests that the phytoplankton communities at depth were physiologically acclimated to the low-light conditions or benefited from the potentially higher nutrient availability and lower temperatures that reduce metabolic costs. In other lakes, such as oligotrophic Lakes Abant and Stechlin, and mesotrophic Lake Bure, chl-*a* concentration peaked in the metalimnion. Deep chlorophyll maxima (DCM) are a common occurrence in many lakes (e.g. Brentrup et al. in press; Hamilton et al. 2010), and their development will have a strong impact on vertical patterns of metabolism and metabolic coupling. Photosynthetic efficiency may also reflect the physiology of the species comprising the phytoplankton community. Rates of GPP and ER above Z_{eu} were conspicuously low at El Gergal for a mesotrophic system, potentially attributable to the dominance by a large, inefficient and slow-growing cyanobacterium (*Aphanizomenon flos-aquae*; Moreno-Ostos et al. 2016).

Our estimates corroborate with previous findings that heterotrophic to balanced conditions generally prevail in the metalimnion (e.g. Sadro et al. 2011a), even when it was presumably well lit (Fig. 4e). This has been previously ascribed to ER being less depth-dependent than GPP (Coloso et al. 2008). However, vertical patterns in ER were similar to GPP at many sites, at both *in situ* temperature and when standardized to 20°C (Figs. 1 & S2). Days of autotrophy in the metalimnion of lakes from all trophic states and mixing regimes interspersed the predominant state of heterotrophy (Wilkinson et al. 2015). Despite high variability in GPP and ER rates among sites, metalimnetic NEP was relatively stable (Fig. 4e), with a mean of $-0.08 \pm 0.26 \text{ mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$ among lakes when excluding Lake Müggel, which had outstandingly high autotrophy in the metalimnion (Fig. 1). This relative stability was likely due to the metalimnetic ER_{20} -to- GPP_{20} coupling gradient being close to 1 at many sites (Fig. 5e). Substrate limitation of heterotrophs is hypothesized to drive strong metabolic

coupling (Sadro et al. 2011b; Solomon et al. 2013), and may be pronounced at depth because the OM in deeper layers typically contains a greater proportion of recalcitrant molecules with low biodegradability (Ostrom et al. 1998). A small number of mean coupling slope estimates > 1 were observed in the meta- and hypolimnion (Fig. 5e, f), suggesting greater than unit increases in ER for each unit increase in GPP. Priming of recalcitrant OM (Guenet et al. 2010) could produce such a pattern but evidence for this process in freshwater systems is inconclusive (Catalán et al. 2015). As these high slope estimates all occurred at low GPP it is more likely that these estimates were artificially inflated by statistically influential data points with low GPP relative to ER.

Elevated background respiration in the meta- and hypolimnion of some mesotrophic and eutrophic sites suggests respiration of OM that was not recently or locally produced (Solomon et al. 2013). The metalimnion may be a zone with high degradation of particles that accumulate near the thermocline through a number of co-occurring mechanisms (Staehr et al. 2012b). This includes recently-produced organic material sinking from the epilimnion, combined with large pools of recalcitrant dissolved organic matter (DOM). Other particles such as zooplankton carcasses may be highly available in the metalimnion because they have higher residence times in stratified water columns and move slowly through the thermocline (Kirillin et al. 2012). Particulate organic matter (POM) accumulating below the mixed layer could also originate from resuspension of benthic material by internal seiches during stratification (Weyhenmeyer 1996) or external stream- and groundwater inputs that do not mix with the epilimnion. Higher nutrient availability below the epilimnion may also facilitate greater decomposition rates. For mean heterotrophic conditions, there must be a net subsidy of carbon to the metalimnion and hypolimnion. In addition to accumulating particles, diel vertical migrations could play a role in linking surface and deeper waters, which has been relatively unexplored compared to physical mechanisms. Daily rates of microbial background respiration in the metalimnion could be spatially subsidized by zooplankton that migrate to

surface waters at night and return to excrete epilimnion-derived DOM and POM at depth (Watras et al. 2015). Furthermore, some motile autotrophs and mixotrophs vertically migrate to the surface to photosynthesize during the day (Salonen et al. 1984), and so respire carbon in deeper waters that was fixed in the surface waters.

Drivers of metalimnetic contribution to whole-lake metabolism

Contrary to expectations, light conditions in the metalimnion, as measured by the relation between mixing depth and photic zone ($Z_{\text{mix}}:Z_{\text{eu}}$), did not explain a large portion of the variation in metalimnetic $\text{Prop}_{\text{metab}}$ or $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$ alone (Fig. 4). Instead, our results suggested a collinear combination of physical and chemical variables, including light, nutrient concentration, temperature, water-column stability and metalimnetic volume drives metalimnetic $\text{Prop}_{\text{metab}}$ (Figs. 3 & 4, Table 3). We did not find a strong association between mixing regime and metalimnetic $\text{Prop}_{\text{metab}}$, but the contribution will naturally be more temporally dynamic in polymictic lakes that have interspersed periods of mixed water columns. Concentration of TP in the epilimnion was negatively correlated with metalimnetic $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$, likely due to stimulating epilimnetic production that shades metalimnetic organisms (e.g. Laas et al. 2012). However, trends for GPP were highly influenced by the most eutrophic lakes, so that additional sites are required to confirm these patterns. Despite the decreasing metalimnetic $\text{Prop}_{\text{metab}}$, absolute rates in the metalimnion increased slowly with TP concentration, until a threshold where the metalimnion was shaded (Castle Lake; Fig. 3a,b). These results imply that eutrophication of lake ecosystems may shift primary production from the metalimnion to the epilimnion, as observed for coastal systems (Lyngsgaard et al. 2014).

The variability in metalimnetic contribution to whole-lake metabolism was highly evident in the range of 0.5 to 1.0 $Z_{\text{mix}}:Z_{\text{eu}}$ (Fig. 4). While interpolating K_D could introduce error, such variability was evident even at sites with sub-daily determination of K_D (e.g. Lake

Stechlin). The dynamic nature of metalimnetic volume on both daily and seasonal bases (Coloso et al. 2011) is likely to affect its contribution to the depth-integrated areal rates (Staeher et al. 2012b). Fluctuations in thermal structure represent not only shifts in light availability, but also potentially important fluxes of OM, nutrients and biota among layers or between sediments and the water column. These fluxes may partly account for the high variability in metabolic estimates at polymictic Lake Müggel, where the longest run of consecutive stratified days was seven. In addition, mixing caused by wind or rain might stimulate metabolism by providing a nutrient or OM subsidy (Giling et al. 2016; Johengen et al. 2008), or depress GPP by suspending OM (Sadro and Melack 2012; Tsai et al. 2008). A key priority to further understand the chemical and physical drivers of variability in the metabolism of stratified lakes is obtaining a greater vertical and temporal resolution of data on nutrient concentration and OM composition (e.g. Watras et al. 2015; Wilkinson et al. 2014).

The multiple interacting factors that influence processes below the upper mixed layer suggest that metabolic rates and metalimnetic contribution to whole-lake metabolism may be sensitive to a range of human influences. Anthropogenic changes pervasively affect patterns of thermal stratification and light attenuation in a multitude of ways (Adrian et al. 2009; Carpenter et al. 2011; Tanentzap et al. 2008). For example, climate change may reinforce the thermal stratification and gradually shift mixing regimes (Butcher et al. 2015; Kirillin 2010; Kraemer et al. 2015; Livingstone 2008), while extreme events will disrupt stratification (Jennings et al. 2012). Widespread brownification and cyanobacterial blooms will both shade the water column and alter food-web dynamics (Cremona et al. 2014a; Paerl and Paul 2012; Solomon et al. 2015). In some cases these shifts need only be minor to have a large impact on vertical patterns of metabolism. In particular, biological processes in the metalimnion of lakes with $Z_{\text{mix}}:Z_{\text{eu}}$ close to unity may have little resistance to altered light attenuation or thermal structure. These changes have implications for carbon efflux or storage at regional and even global scales (Tranvik et al. 2009).

In conclusion, we found that the metalimnion can contribute substantially whole-lake metabolism in many lakes using a depth-integrated approach. However, high variability in rates and collinearity among predictors meant that generalisations about the widespread importance of the metalimnion to water-column processes could not be made with broad lake categories such as trophic status, except for hypereutrophic lakes. Single sensors placed in the epilimnion sensors retain value, especially for investigating the drivers of day-to-day variation in long term records due to high reliability of estimates and relative freedom from process errors. However, single-sensor estimates may not necessarily reflect whole-lake functioning; and deviate from systematically from depth-integrated estimates of GPP. A depth-integrated approach enables enhanced understanding of how physical and biogeochemical processes influence functioning of lake ecosystems as a whole.

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Tables

Table 1. Description of acronyms and terms

Parameter or acronym	Description	Unit
OM	Organic Matter	
GPP	Gross Primary Production at in situ temperature	mg O ₂ L ⁻¹ d ⁻¹
GPP ₂₀	Gross Primary Production standardized to 20°C	mg O ₂ L ⁻¹ d ⁻¹
ER	Ecosystem Respiration at in situ temperature	mg O ₂ L ⁻¹ d ⁻¹
ER ₂₀	Ecosystem Respiration standardized to 20°C	mg O ₂ L ⁻¹ d ⁻¹
Background respiration	Respiration of OM not recently or locally fixed; the intercept of the correlation between ER ₂₀ and GPP ₂₀	mg O ₂ L ⁻¹ d ⁻¹
NEP	Net Ecosystem Production (GPP-ER); describes whether the layer or lake is net autotrophic or net heterotrophic	mg O ₂ L ⁻¹ d ⁻¹
TP	Total Phosphorous	µg L ⁻¹
TN	Total Nitrogen	µg L ⁻¹
DO	µg L ⁻¹	mg L ⁻¹
Z _{mix}	Mixing depth; bottom of the epilimnion and top of the metalimnion	m
Z _{eu}	Photic depth; equal to depth with 1% of surface light	m
Z _{mix} : Z _{eu}	Ratio of mixing to photic depth. Describes light availability in the metalimnion	
T	Temperature	°C
DOC	Dissolved Organic Carbon	mg L ⁻¹
Chl <i>a</i>	Chlorophyll- <i>a</i> concentration	µg L ⁻¹
DCM	Deep Chlorophyll Maximum	
PAR ₀	Incoming surface Photosynthetic Active Radiation	µmol m ⁻² s ⁻¹
PAR _z	Photosynthetic Active Radiation at depth z	µmol m ⁻² s ⁻¹
K _D	Light attenuation coefficient	m ⁻¹
U ₁₀	Wind speed at 10 m above surface of lake	m s ⁻¹
D _s	Atmospheric	mg O ₂ L ⁻¹ h ⁻¹
D _v	Vertical exchange between each depth and the adjacent depths due to turbulent diffusivity	mg O ₂ L ⁻¹ h ⁻¹
N ²	Brunt-Väisälä buoyancy frequency	s ⁻²
K _v	Vertical eddy diffusivity coefficient	
D _z	Exchange due to mixed-layer deepening	mg O ₂ L ⁻¹ h ⁻¹
WLWV	Whole-lake volume-weighted estimate of metabolism (Sadro et al. 2011a)	g O ₂ m ⁻³ d ⁻¹
Prop _{metab}	Proportion of WLWV metabolism occurring in a certain depth zone	
Prop _{vol}	Proportion of whole lake volume occurring in a certain depth zone	
Prop _{metab} : Prop _{vol}	Ratio of proportion metabolism to proportion volume; ratio is >1 when a depth zone contributes more metabolically than it does volumetrically	

Table 2. Site information and ancillary data for the ten study lakes. Chemical and biological data are mean measurements from 1-11 samples among sites during the period of stratification at each lake (Supplemental Text 1). ‘Epi.’ denotes the epilimnion (metalimnion and hypolimnion measurements are presented in Table S1). The column ‘# days (total)’ shows the total number of stratified days with metabolic estimates in each lake, with the number of days with at least one successful model fit in each depth zone (epi-, meta- and hypolimnion) in brackets.

Lake	Location	# days	Trophic status	Mixing regime	Max. depth (lake) m	Max. depth (profile) m	Mean Z_{mix} m	Mean Z_{eu} m	Mean meta. thickness m	Epi. TP $\mu\text{g L}^{-1}$	Epi. TN $\mu\text{g L}^{-1}$	Epi. DOC $\mu\text{g L}^{-1}$	Epi. Chl <i>a</i> $\mu\text{g L}^{-1}$
Abant	Turkey	17 (2)	oligo.	mono.	18	12.75	5.6	11.9	2.6	14.0	1.5		1.5
Ontario	United States	69 (17)	oligo.	mono.	244	35	10.3	23.2	10.3	6.4			0.7
Stechlin	Germany	41 (32)	oligo.	di.	69.5	17.25	6.8	12.7	5.1	12.2	0.4	5.0	1.9
Rappbode pre-dam	Germany	48 (19)	meso.	di.	17	15.75	2.6	4.7	4.7	25.2	0.7	4.8	9.4
Bure	Denmark	58 (29)	meso.	poly.	11	9.5	4.3	7.5	3.3	22.9	0.7		5.5
Hampen	Denmark	56 (15)	meso.	poly.	13	9.5	4.4	6.3	3.1	23.6	0.6	3.0	8.7
El Gergal	Spain	21 (17)	meso.	mono.	37	19.75	5.3	6.5	6.1				24.1
Vedsted	Denmark	60 (14)	eu.	di.	11	9.5	3.5	4.0	3.8	27.3	0.5	4.9	41.4
Müggel	Germany	28 (17)	eu.	poly.	7.7	5.25	1.5	3.0	2.0	63.5	0.8	7.2	35.3
Castle	Denmark	32 (16)	hypereu.	poly.	9	8.5	4.4	2.3	2.6	94.1	1.6	3.4	46.3

889

890 Table 3. Results of principal components analysis (PCA). ‘Linear mixed models’ rows indicate the results of linear models using the components as
 891 explanatory variables to explain metalimnetic contribution to whole-lake areal GPP and ER (Prop_{metab}). ‘ β ’ indicates the estimated slope of the
 892 linear model.

Principal component	PC1	PC2
% variation explained	36	32
Axis rotation		
Metalimnetic Prop _{vol}	-0.52	-0.42
Mean metalimnetic log PAR _z	-0.26	-0.65
Mean metalimnetic layer temperature	-0.68	0.19
Mean metalimnetic buoyancy frequency (N ²)	-0.43	0.60
Linear mixed models		
Prop _{metab} for GPP	$\beta = -0.11 \pm 0.01$ $p < 0.001, r^2 = 0.26$	$\beta = -0.02 \pm 0.02$ $p = 0.200, r^2 = 0.08$
Prop _{metab} for ER	$\beta = -0.07 \pm 0.02$ $p < 0.001, r^2 = 0.09$	$\beta = -0.10 \pm 0.02$ $p < 0.001, r^2 = 0.15$

Figure captions

Figure 1. Mean (\pm SD among days) depth-specific volumetric estimates of daily GPP (open white points) and ER (closed grey points) of adequately fit models over stratified days at each lake. The grey rectangle indicates the mean extent of metalimnion and the dashed black line indicates the mean daily Z_{eu} .

Figure 2. Scatterplots of mean (\pm SD) (a) R^2 , (b) coefficient of variation (CV) of GPP estimates, and (c) CV of ER estimates from all models across the study lakes and depth zones. White, grey and black points show epi-, meta- and hypolimnetic zones respectively. Value above the dashed horizontal line at $R^2 = 0.40$ in panel (a) were considered to have adequate model fit.

Figure 3. Scatterplots showing the correlation between mean (\pm SD) epilimnetic total phosphorus (TP) and metalimnetic GPP (left) and ER (right). Plots show mean (\pm SD) metalimnetic volumetric rates (a, b), mean relative contribution of the metalimnion to WLWV metabolic estimates (i.e. $Prop_{metab}$; c,d); and the ratio between $Prop_{metab}$ and the relative contribution of the metalimnetic volume to whole-lake volume ($Prop_{metab} : Prop_{vol}$; e,f). Solid black lines indicate significant linear relationships in linear mixed models. White, grey and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares/diamonds and triangles represent monomictic, dimictic and polymictic lakes, respectively.

Figure 4. Scatterplots showing the correlation between $Z_{mix}:Z_{eu}$ and metalimnetic metabolism. The top row shows the proportional contribution of the metalimnion to WLWV metabolic estimates ($Prop_{metab}$) for (a) GPP and (b) ER. The middle row shows the effect of $Z_{mix}:Z_{eu}$ on the ratio between $Prop_{metab}$ and the proportion contribution of the metalimnetic volume to

whole-lake volume ($\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$) for (c) GPP and (d) ER. At values higher than the dashed horizontal line at $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}} = 1$ the metalimnion contributes more to the whole lake metabolically than it does volumetrically. Panel (e) shows the volumetric rate of NEP as a function of mean daily $Z_{\text{mix}}:Z_{\text{eu}}$. Each point represents one day, with a point only for days with at least one appropriate model fit in each depth-zone shown for plots a-d. White, grey and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares and triangles represent monomictic, dimictic and polymictic lakes, respectively. In plots c and d some outlying high points at Lake Ontario were excluded to assist interpretation.

Figure 5. Scatterplots of the correlation between and nutrient concentration on $\text{ER}_{20}\text{-to-GPP}_{20}$ coupling in the epi- (left), meta- (centre) and hypolimnion (right). The top row shows the effect of mean ($\pm\text{SD}$) epilimnetic TP on mean ($\pm\text{SD}$) background respiration (i.e. intercept of correlation; $\text{mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$) in all depth zones. The dashed horizontal line indicate a background respiration of zero (conceptually no ER independent of GPP), and the solid lines show significant relationships in LMMs. The centre row shows the relationship of TP with coupling slope ($\text{mg O}_2 \text{ L}^{-1} \text{ d}^{-1}$), with the dashed line at a slope of unity (representing a unit increase in ER for each in GPP). The bottom row shows the correction of mean TP with coupling strength (r^2). White, grey and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares and triangles represent monomictic, dimictic and polymictic lakes, respectively.

Figure 6. Comparison of mean ($\pm\text{SD}$) daily whole-lake volume weighted (WLVW) metabolism between depth-integrated and single-sensor estimates of (a) GPP, (b) ER and (c) NEP. The dashed line indicates the 1:1 relationship. White, grey and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares and triangles represent monomictic, dimictic and polymictic lakes, respectively.