

# *Delving deeper: metabolic processes in the metalimnion of stratified lakes*

Article

Accepted Version

Giling, Darren P., Staehr, Peter A., Grossart, Hans Peter, Andersen, Mikkel René, Boehrer, Bertram, Escot, Carmelo, Evrendilek, Fatih, Gómez-Gener, Lluís, Honti, Mark, Jones, Ian D., Karakaya, Nusret, Laas, Alo, Moreno-Ostos, Enrique, Rinke, Karsten, Scharfenberger, Ulrike, Schmidt, Silke R., Weber, Michael, Woolway, R. Iestyn ORCID logo ORCID: <https://orcid.org/0000-0003-0498-7968>, Zwart, Jacob A. and Obrador, Biel (2017) Delving deeper: metabolic processes in the metalimnion of stratified lakes. *Limnology and Oceanography*, 62 (3). pp. 1288-1306. ISSN 0024-3590 doi: <https://doi.org/10.1002/lno.10504> Available at <https://centaur.reading.ac.uk/70399/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

Published version at: <http://dx.doi.org/10.1002/lno.10504>

To link to this article DOI: <http://dx.doi.org/10.1002/lno.10504>

Publisher: Wiley

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in

the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

## **CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

1 **Delving deeper: Metabolic processes in the metalimnion of stratified lakes**

2 Darren P. Giling<sup>1,\*</sup>, Peter A. Staehr<sup>2</sup>, Hans Peter Grossart<sup>1,3</sup>, Mikkel René Andersen<sup>4</sup>, Bertram  
3 Boehrer<sup>5</sup>, Carmelo Escot<sup>6</sup>, Fatih Evrendilek<sup>7</sup>, Lluís Gómez-Gener<sup>8</sup>, Mark Honti<sup>9</sup>, Ian D  
4 Jones<sup>10</sup>, Nusret Karakaya<sup>7</sup>, Alo Laas<sup>11</sup>, Enrique Moreno-Ostos<sup>12</sup>, Karsten Rinke<sup>5</sup>, Ulrike  
5 Scharfenberger<sup>13,14</sup>, Silke R. Schmidt<sup>13,15</sup>, Michael Weber<sup>5</sup>, R. Iestyn Woolway<sup>16</sup>, Jacob A.  
6 Zwart<sup>17</sup>, Biel Obrador<sup>8</sup>

7

8 <sup>1</sup> Department of Experimental Limnology, Leibniz-Institute of Freshwater Ecology and  
9 Inland Fisheries (IGB), Alte Fischerhütte 2, 16775 Stechlin, Germany

10 <sup>2</sup> Aarhus University, Department of Bioscience, Frederiksborgvej 399, Box 358, 4000  
11 Roskilde, Denmark

12 <sup>3</sup> Institute of Biochemistry and Biology, Potsdam University, Maulbeerallee 2, 14469  
13 Potsdam, Germany

14 <sup>4</sup> Freshwater Biological Laboratory, Faculty of Science, University of Copenhagen,  
15 Universitetsparken 4, 2100 Copenhagen, Denmark

16 <sup>5</sup> Helmholtz-Centre for Environmental Research, Department of Lake Research,  
17 Brueckstrasse 3a, 39114 Magdeburg, Germany.

18 <sup>6</sup> Empresa Metropolitana de Abastecimiento y Saneamiento de Aguas de Sevilla, S.A.  
19 EMASESA. C/ Escuelas Pías 1. 41003. Sevilla, Spain

20 <sup>7</sup> Department of Environmental Engineering, Abant Izzet Baysal University, 14280 Bolu,  
21 Turkey

22 <sup>8</sup> Department of Ecology, University of Barcelona, Diagonal 643, 08028 Barcelona, Spain

23 <sup>9</sup> MTA-BME Water Research Group, Hungarian Academy of Sciences, 1111  
24 Budapest, Hungary

25 <sup>10</sup> Lake Ecosystems Group, Centre for Ecology & Hydrology, Lancaster Environment Centre,  
26 Library Avenue, Bailrigg, Lancaster, LA1 4AP, UK

27 <sup>11</sup> Estonian University of Life Sciences, Centre for Limnology, Kreutzwaldi 5, Tartu, Estonia

28 <sup>12</sup> Department of Ecology, Marine Ecology and Limnology Research Group, University of  
29 Málaga, Campus Universitario de Teatinos S/N. 29071 Málaga, Spain.

30 <sup>13</sup> Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Department of  
31 Ecosystem Research, Müggelseedamm 301, 12587 Berlin, Germany

32 <sup>14</sup> Freie Universität Berlin, Department of Biology, Chemistry and Pharmacy, Takustraße 3,  
33 14195 Berlin, Germany

34 <sup>15</sup> University of Potsdam, Institute of Earth and Environmental Sciences, Karl-Liebknecht-  
35 Str. 24-25, 14476 Potsdam, Germany

36 <sup>16</sup> Department of Meteorology, University of Reading, Reading, RG6 6BB, UK.

37 <sup>17</sup> Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana,  
38 46556 USA

39

40 \* Corresponding author. Present address: German Centre for Integrative Biodiversity  
41 Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany, and  
42 Institute of Biology, Leipzig University, Johannisallee 21, 04103 Leipzig, Germany  
43 Phone: +49 34197 33122, E-mail: darren.giling@idiv.de

44

45 **Running title:** Metabolism in stratified lakes

46 **Key-words:** lake metabolism, gross primary production, ecosystem respiration, ecosystem  
47 processes, ecosystem function, organic matter, lake monitoring, high-frequency, buoy data

48

49 **Authorship statement:** PAS, BO, DPG, JAZ, LGG, IDJ, RIW, MRA, MH, US, AL & HPG  
50 contributed to the conception of the study; PAS, BO, DPG, NK, FE, JAZ, KR, MW, BB, EM-  
51 O, CE, US & SRS acquired the data; DPG analyzed the data and wrote the first draft; all  
52 contributed substantially to revisions of and approved the final manuscript.

53 **Abstract**

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

75

## 76 **Introduction**

77 Globally, many lakes exhibit thermal stratification for a substantial portion of the year,  
78 developing marked vertical gradients in physical and chemical properties (Boehrer and  
79 Schultze 2008). These gradients influence biological communities and biogeochemical  
80 processes such organic and inorganic matter cycling (Coloso et al. 2008; Van de Bogert et al.  
81 2012), altering energy flow through lake food webs (Wilkinson et al. 2014). Consequently,  
82 patterns of stratification affect the important contribution that inland waters make to global  
83 carbon fluxes (Cole et al. 2007; Coloso et al. 2008; Staehr et al. 2012b). Vertical patterns of  
84 ecosystem metabolism vary among chemically and morphologically diverse lakes (Obrador et  
85 al. 2014), but information on the drivers of metabolism in the metalimnion is currently  
86 limited. However, a recent expansion of high-frequency and depth-integrated monitoring of  
87 lakes provides an opportunity to extend our understanding of lake metabolism below the  
88 upper mixed layer (epilimnion) (Meinson et al. 2015; Obrador et al. 2014; Staehr et al. 2010).

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

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

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

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

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

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

147

## 148 **Methods**

### 149 *Study sites*

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



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

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

$$171 \quad PAR = E \times 4.6 \times 0.45. \quad (1)$$

172

### 173 *Vertical stratification and underwater light regime*

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

$$177 \quad T(z) = T_h + (T_e - T_h) \left( \frac{1}{1 + (\alpha \times z)^n} \right)^{1 - \left( \frac{1}{n} \right)}, \quad (2)$$

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

$$183 \quad \rho = 1 - 6.63 \times 10^{-6}(T - 4)^2. \quad (3)$$

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

193 Water-column light attenuation ( $K_D$ ) at each site was calculated from either  
194 measurements of underwater PAR at multiple depths, Secchi depth or other existing site-  
195 specific relationships (Table S1). Estimates of  $K_D$  were made from daily to biweekly intervals  
196 and were linearly interpolated between measurement days when necessary. Where underwater  
197 PAR measurements were available,  $K_D$  was estimated as the slope of the linear regression  
198 between  $\log(\text{PAR})$  and depth  $z$ . A mean daily  $K_D$  was calculated from the  $K_D$  of each profile  
199 during daylight ( $\text{PAR}_0 > 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) that had a linear regression fit with  $r^2 > 0.80$   
200 (Obrador et al. 2014; Staehr et al. 2012b). Where Secchi depth was available,  $K_D$  was  
201 calculated as  $1.7/\text{Secchi depth}$  (Poole and Atkins 1929). The mean daily photic depth ( $Z_{\text{eu}}$ )  
202 was calculated as  $4.6/K_D$ . Following the determination of daily  $K_D$  at each site, PAR for each

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

$$205 \quad PAR_z = PAR_0 \times e^{-K_D \times z}. \quad (4)$$

206

### 207 *Model of depth-dependent metabolism*

208 Gaps in the data series of up to 1 h were linearly interpolated before we applied a 4-h simple  
209 moving average smoothing to DO, PAR and wind speed measurements (following Giling et  
210 al. 2016; Obrador et al. 2014). To estimate mean rates of ecosystem metabolism at a daily  
211 scale, we used a Bayesian model with non-linear sub-models for GPP and ER (Giling et al.  
212 2016). Changes in DO concentration between successive measurement times  $t$  for each depth  
213 were partitioned into the contribution by biotic and physical processes using a depth-specific  
214 diel oxygen model (Staeher et al. 2012b):

$$215 \quad DO(t + 1) = DO(t) + NEP(t) - D_s(t) - D_v(t) + D_z(t); \quad (5)$$

216 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,  
217  $D_v$  is diffusive vertical exchange between adjacent depths and  $D_z$  is metalimnetic exchange  
218 due to mixed-layer deepening. Atmospheric exchange ( $D_s$ ) was applied only to layers in the  
219 epilimnion, and calculated as:

$$220 \quad D_s(t) = K_s(t) \frac{DO_{mod}(t) - DO_{sat}(t)}{Z_{mix}(t)}. \quad (6)$$

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

228 diffusivity coefficient ( $K_v$ ) according to Hondzo and Stefan (1993). Lake Ontario was an  
 229 exception due to its surface area exceeding the applicable range of the Hondzo and Stefan  
 230 (1993) model. The metalimnetic  $K_v$  for Ontario was set as  $6 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$  based on  
 231 measurements and theoretical work from Lakes Ontario and Erie (Bouffard et al. 2014;  
 232 Sweers 1970). Epilimnetic and hypolimnetic  $K_v$  were expected to be higher than the  
 233 metalimnetic estimate, so were set at Lake Ontario using a factor determined from the average  
 234 relative difference between depth zones in the other nine lakes. The influence of  $K_v$  on  
 235 metabolic rates at Ontario and two other lakes (Vedsted and Müggel) was assessed with a  
 236 sensitivity analysis (for details see Supplemental Text 2 and Table S2). The flux  $D_z$  was  
 237 calculated as proportional to the  $Z_{\text{mix}}$  deepening rate ( $\Delta Z_{\text{mix}}/\Delta t$ ) and was applied to  
 238 measurement points within the metalimnion and 1 m above or below (Obrador et al. 2014). At  
 239 some sites, we set a threshold limiting the deepening rate to a maximum of  $5 \text{ m h}^{-1}$  to prevent  
 240 short-term surface-water microstratification inaccurately affecting  $D_z$  in the metalimnion. For  
 241 a full description of the model, see Staehr et al. (2012b).

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

$$246 \quad NEP(t) = A \times PAR(t)^p - R_{\bar{T}}(\theta^{(T(t)-\bar{T})}); \quad (7)$$

247 where the first component describes GPP ( $\text{mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$ ), and  $A$  is a constant indicating  
 248 production per unit light (i.e. photosynthetic efficiency),  $PAR$  is the depth-specific PAR  
 249 ( $PAR_z$ ), and  $p$  is an estimated exponent that represents the ability of producers to use light and  
 250 accounts for saturating photosynthesis (when  $p < 1$ ). The second component indicates DO  
 251 consumption by ER ( $\text{mg O}_2 \text{ L}^{-1} \text{ h}^{-1}$ );  $R_{\bar{T}}$  is the respiration rate at  $\bar{T}$ ,  $\theta$  describes the  
 252 temperature dependence of respiration (set to 1.072, corresponding to  $Q_{10}$  of 2; Wilcock et al.  
 253 1998),  $T$  is the water temperature at each timestep and  $\bar{T}$  is the mean daily temperature in that

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

$$264 \quad GPP = \sum_{t=1}^{measurements} A \times PAR(t)^p \quad (8)$$

$$265 \quad ER = 86400 \times \frac{R_{\bar{T}}}{\Delta t} \quad (9)$$

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

275

### 276 *Aggregating layer-specific metabolic rates*

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

279 the aggregated depth zone rate ( $\sigma_{zone}$ ) was propagated from the modelled uncertainties in  
280 layer  $i$  as follows:

$$281 \quad \sigma_{zone} = \sqrt{\sum_i^n \sigma_i^2}, \quad (10)$$

282 where  $i$  to  $n$  are the layers belonging to that depth zone. The aggregated depth-zone estimates  
283 were used for further analysis of metabolic rates. Due to high day-to-day variability, only  
284 days when there was at least one adequate metabolic model fit (i.e.  $\hat{R} < 1.1$  and  $r^2 > 0.40$ )  
285 from layers in each depth zone (epi-, meta- and hypolimnion when present) were considered  
286 to calculate the relative contribution of the metalimnion to whole-lake metabolism (total of  
287 178 days; Table 2).

288

#### 289 *Whole-lake metabolism estimates*

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

303 whole-lake volume (termed 'Prop<sub>vol</sub>'). Thus, the metalimnion contributed more to the whole  
304 lake metabolically than it did volumetrically when the ratio Prop<sub>metab</sub>:Prop<sub>vol</sub> was >1.

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

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

323

### 324 *Statistical analyses*

325 Comparisons among depth zones, correlations with ancillary variables, and ER<sub>20</sub>-GPP<sub>20</sub>  
326 regressions were analyzed by fitting linear models or linear mixed models (LMMs) in R (R  
327 Development Core Team 2014). The LMMs included a random effect for site and layer and  
328 AR1 autocorrelation structure (nested within site) to account for repeated daily measurements

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

336

## 337 **Results**

### 338 *Uncertainty in model estimates and contribution of physical processes*

339 The depth-integrated metabolic model provided a better description of diurnal changes in DO  
340 in the epilimnion (74% of models converged with adequate fit) than in the metalimnion (43%)  
341 or hypolimnion (32%; Fig. 2a). Furthermore, modelled metabolic estimates were on average  
342 more precise in the epilimnion (mean coefficient of variation [CV] for GPP and ER estimates  
343 = 0.14 and 0.23, respectively) than in the metalimnion (mean GPP and ER CV = 0.25 and  
344 0.33, respectively) or hypolimnion (mean GPP and ER CV = 0.35 and 0.51, respectively)  
345 (Fig. 2b, c). There was high variability in model fit ( $R^2$ ) and estimate certainty (CV) among  
346 days and depth zones (Fig. 2). In the metalimnion,  $R^2$  was unrelated to water-column stability  
347 (assessed with the buoyancy frequency  $N^2$ ;  $F_{1,470} = 1.99$ ,  $p = 0.159$ ; Fig. S4a). Accordingly,  $N^2$   
348 in successful models did not differ from  $N^2$  in poor-fitting models (paired t-test,  $t = -0.004$ ,  $p$   
349 = 0.997; Fig. S4b). Variation in mean  $R^2$  across all lakes and depth zones was also not  
350 explained by measurement frequency ( $F_{2,25} = 0.445$ ,  $p = 0.646$ ), the vertical resolution of  
351 measurements ( $F_{1,26} = 0.000$ ,  $p = 0.999$ ),  $Z_{\max}$  ( $F_{1,26} = 2.11$ ,  $p = 0.159$ ) or surface area ( $F_{1,26} =$   
352 0.441,  $p = 0.513$ ; Fig. S5). CV for GPP and ER was also unaffected or only marginally  
353 affected by measurement frequency ( $F_{2,24} = 1.71$ ,  $p = 0.202$  and  $F_{2,25} = 3.377$ ,  $p = 0.050$ ) and  
354 the vertical resolution of measurements ( $F_{1,26} = 0.105$ ,  $p = 0.749$  and  $F_{1,26} = 0.167$ ,  $p = 0.898$ ).



355 The physical fluxes of DO (i.e.  $D_s$ ,  $D_v$  and  $D_z$ ) contributed a substantial proportion ( $32 \pm$   
356 24% across all estimates) of the total DO fluxes (i.e. sum of absolute NEP,  $D_s$ ,  $D_v$  and  $D_z$ )  
357 (Table S3). In the epilimnion, a mean of 45% of DO changes were attributable to the diffusive  
358 components, mostly atmospheric exchange ( $D_s$ ) with a small contribution from  $D_v$  and  $D_z$ .  
359 Transfer due to mixed layer deepening ( $D_z$ ) contributed strongly to the metalimnion (mean  
360 29% attributable to diffusive fluxes), and both  $D_z$  and  $D_v$  estimates were important in the  
361 hypolimnion (mean 16%; Table S3). The average magnitude of physical fluxes was unrelated  
362 to mean model  $R^2$  ( $F_{1,8} = 1.216$ ,  $p = 0.302$ ) and CV of GPP and ER estimates among the ten  
363 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  
364 physical processes were not strongly affected by lake morphology. In the metalimnion, log-  
365 transformed lake area did not affect the balance between  $D_z$ ,  $D_v$  and NEP with ( $F_{1,8} = 1.218$ ,  
366  $p = 0.301$ ) or without ( $F_{1,7} = 1.892$ ,  $p = 0.211$ ) outlying Lake Ontario (Fig. S5). Sensitivity  
367 analysis demonstrated that the value of  $K_v$  did not strongly affect metabolic estimates, except  
368 for rates in the metalimnion and hypolimnion of polymictic Lake Müggel (Supplemental Text  
369 2 and Table S2).

370 Metabolic estimates were characterized by high day-to-day variability in some lakes and  
371 layers (Fig. 1). For contiguous days with adequate model fits among the ten lakes, between 8-  
372 52%, 0-78% and 11-100% of epi-, meta- and hypolimnetic GPP estimates were within 2  
373 standard deviations (SD) of the estimate from the previous day. Similarly, between 13-53%,  
374 0-88% and 11-96% of epi-, meta- and hypolimnetic ER estimates were within 2 SD of the  
375 previous days' estimate. In the metalimnion, this did not appear to be strongly driven by PAR;  
376 the day-to-day shift in  $PAR_z$  did not consistently differ between consecutive days that had  
377 similar or disparate metabolic estimates. The proportion of days with estimates similar to the  
378 previous day was also not related to lake area (Fig. S5). Day-to-day variability in  
379 metalimnetic thickness as a proportion of  $Z_{max}$  was slightly higher in polymictic (SD in  
380 relative thickness =  $0.08 \pm 0.02$ ,  $n = 4$  lakes) than in mono-/dimictic lakes (SD =  $0.04 \pm 0.02$ ,

381  $n = 6$  lakes,  $F_{1,8} = 6.33$ ,  $p = 0.04$ ). However, metalimnetic local water-column stability did not  
382 differ among mixing regimes ( $F_{1,8} = 3.26$ ,  $p = 0.109$ ). The variability daily estimates of GPP  
383 and ER in the metalimnion (Table S3) was not related to lake mixing regime ( $F_{2,7} = 0.753$ ,  $p =$   
384  $0.505$  and  $F_{2,7} = 0.044$ ,  $p = 0.957$ ) or trophic status ( $F_{2,7} = 1.337$ ,  $p = 0.223$  and  $F_{2,7} = 0.178$ ,  $p$   
385  $= 0.841$ ). We assessed whether process errors (e.g. lateral water movements) were responsible  
386 for high day-to-day variability by testing whether the SD among daily estimates differed when  
387 depth-zone means were aggregated from converged models with good ( $R^2 > 0.4$ ) or poor fits  
388 ( $R^2 < 0.4$ ). We found was no difference in propagated SD for metalimnetic GPP (paired t-test;  
389  $t_9 = 1.42$ ,  $p = 0.190$ ) or ER ( $t_9 = 0.772$ ,  $p = 0.460$ ).

390

#### 391 *Depth-specific metabolic rates and photosynthetic efficiency*

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

401 We found evidence of photosynthetic activity down to *ca.* 0.1% of surface light.

402 Increasing GPP with depth in some lakes was due to higher daily photosynthetic efficiency  
403 (i.e. parameter A from Equation 7;  $\text{GPP} [\text{mg O}_2 \text{ L}^{-1} \text{ d}^{-1}] / \text{PAR} [\mu\text{mol m}^{-2} \text{ sec}^{-1}]$ ) in low-light  
404 conditions ( $F_{1,792} = 105.17$ ,  $p < 0.001$ ; Fig. S6). Correspondingly, photosynthetic efficiency  
405 varied by depth ( $F_{2,809} = 58.55$ ,  $p < 0.001$ ), and was lower in the epilimnion than in the  
406 metalimnion or hypolimnion ( $p < 0.01$  in post-hoc pairwise comparisons), which did not

407 differ ( $p = 0.905$ ). Photosynthetic efficiency was reduced in low nutrient conditions ( $F_{2,23} =$   
408  $25.72, p < 0.001$ ). Efficiency was significantly lower in oligotrophic lakes than in  
409 mesotrophic lakes (post-hoc comparison;  $z = -4.54, p < 0.001$ ), which were lower again than  
410 eutrophic lakes ( $z = -3.17, p = 0.004$ ).

411

#### 412 *Metalimnetic contribution to whole-lake metabolism*

413 Across all sites, the proportional contribution of the metalimnion to WLWV metabolic  
414 estimates (i.e.  $\text{Prop}_{\text{metab}}$ ) varied between 0 and 87% for daily GPP and between <1 and 92%  
415 for daily ER (Fig. 4a,b). The metalimnetic  $\text{Prop}_{\text{metab}}$  was negatively correlated to epilimnetic  
416 TP concentration for GPP ( $F_{1,7} = 6.09, p = 0.042, r^2 = 0.47$ ) and for ER ( $F_{1,7} = 8.69, p = 0.021,$   
417  $r^2 = 0.55$ ), but these relationships were driven by Lake Castle (Fig. 3c,d). The proportion of  
418 whole-lake volume made up of the metalimnion (i.e.  $\text{Prop}_{\text{vol}}$ ) was between 3 and 60% (mean  
419 32%). The metalimnetic  $\text{Prop}_{\text{metab}}$  was positively correlated with metalimnetic  $\text{Prop}_{\text{vol}}$  for both  
420 GPP (slope =  $0.88 \pm 0.12, F_{1,175} = 58.46, p < 0.001, r^2 = 0.25$ ) and ER (slope =  $0.94 \pm 0.14,$   
421  $F_{1,175} = 43.74, p < 0.001, r^2 = 0.20$ ). The metalimnetic  $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$  was a mean  $0.95 \pm$   
422  $0.67$  SD for GPP and  $1.06 \pm 0.87$  SD for ER. The ratio  $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$  showed that the  
423 metalimnion disproportionately contributed metabolic activity for its size (i.e. had high  
424  $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$ ) in oligotrophic lakes (Fig. 3e,f). This was evidenced by a negative  
425 relationship between metalimnetic  $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$  and mean epilimnetic TP concentration  
426 for GPP ( $F_{1,7} = 15.31, p = 0.006, r^2 = 0.68$ ; Fig. 3e) and ER ( $F_{1,7} = 12.46, p = 0.010, r^2 = 0.64$ ;  
427 Fig. 3f).

428 We observed a negative linear correlation between metalimnetic  $\text{Prop}_{\text{metab}}$  and log-  
429 transformed  $Z_{\text{mix}}:Z_{\text{eu}}$  for GPP ( $F_{1,166} = 12.39, p < 0.001$ ; Fig. 4a). However, this trend was not  
430 robust to the exclusion of Lake Castle ( $F_{1,151} = 2.47, p = 0.118$ ). A similar negative  
431 relationship was observed for metalimnetic ER  $\text{Prop}_{\text{metab}}$  (Fig. 4b), with ( $F_{1,166} = 18.58, p <$   
432  $0.001$ ) or without ( $F_{1,151} = 12.10, p < 0.001$ ) Lake Castle. Comparable trends were observed

433 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  
434 GPP ( $F_{1,166} = 15.22$ ,  $p < 0.001$ ; Fig. 4c), which was again not significant when Castle Lake  
435 was removed from the analysis ( $F_{1,151} = 2.08$ ,  $p = 0.151$ ). In contrast, the negative effect of  
436 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  
437 robust to the exclusion of Castle Lake ( $F_{1,151} = 8.31$ ,  $p = 0.005$ ). Metalimnetic  $\text{Prop}_{\text{vol}}$  tended  
438 to be high when  $Z_{\text{mix}}$  was shallow, meaning that  $\log Z_{\text{mix}}:Z_{\text{eu}}$  and  $\text{Prop}_{\text{vol}}$  were negatively  
439 correlated ( $F_{1,166} = 13.20$ ,  $p < 0.001$ ,  $r^2 = 0.11$ ). Hence, we used PCA to examine the collinear  
440 drivers of metalimnetic  $\text{Prop}_{\text{metab}}$ . Principal components (PCs) were derived from four  
441 variables representing abiotic resource availability and physical conditions ( $\text{PAR}_z$ ,  
442 temperature,  $\text{Prop}_{\text{vol}}$  and  $N^2$ ). The PC explaining the majority of variation (PC1) did not  
443 reduce the collinearity, as it was characterized by lower temperature,  $\text{Prop}_{\text{vol}}$  and  $N^2$  (Table 3).  
444 Metalimnetic  $\text{Prop}_{\text{metab}}$  for GPP and ER was negatively correlated to PC1 in linear mixed  
445 models (Table 3), suggesting a combined importance of these factors. A second component  
446 (PC2) was characterized mainly by low light and high local stability  $N^2$  in the metalimnion,  
447 and was negatively correlated with  $\text{Prop}_{\text{metab}}$  for GPP and ER (Table 3).

448

#### 449 *Depth-specific metabolic coupling*

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

459 respiration did not differ among depth zones ( $F_{2,22} = 1.13$ ,  $p = 0.340$ ). There was no  
460 interaction between the effect of epilimnetic TP and depth zone on the coupling slope ( $F_{2,17} =$   
461  $1.20$ ,  $p = 0.324$ ), and no significant difference among depth zones ( $F_{2,17} = 0.44$ ,  $p = 0.653$ ).  
462 Across all depth zones, the coupling slope decreased with increasing epilimnetic TP  
463 concentration ( $F_{1,17} = 6.48$ ,  $p = 0.021$ ), but this relationship was not evident in any one depth  
464 zone individually ( $p > 0.117$ ; Fig. 5d-f). For the strength of the coupling relationship ( $r^2$ ) there  
465 was also no interaction between the effect of depth zone and mean epilimnetic TP ( $F_{2,17} =$   
466  $0.885$ ,  $p = 0.431$ ), and no effect of depth zone ( $F_{2,17} = 0.023$ ,  $p = 0.977$ ). The strength of the  
467 coupling relationship decreased with increasing TP concentration ( $F_{1,17} = 7.97$ ,  $p = 0.012$ )  
468 across all depth zones combined (Fig. 5g-i).

469

#### 470 *Effect of depth-integration on whole-lake areal NEP estimate*

471 We assessed how WLWV estimates of GPP, ER and NEP made from a single epilimnetic  
472 sensor compared to the depth-integrated estimates. For mean GPP, the single-sensor approach  
473 over-estimated whole-lake metabolism at 9 of 10 sites, but the average difference from  
474 integrated estimates was variable (mean difference  $3.17 \pm 4.58$  g O<sub>2</sub> m<sup>-1</sup> d<sup>-1</sup>; Fig. 6a). In  
475 contrast, single-sensor estimates of ER did not consistently vary from integrated estimates  
476 (mean difference  $-0.37 \pm 3.40$  g O<sub>2</sub> m<sup>-1</sup> d<sup>-1</sup>; Fig. 6b). Correspondingly, single-sensor estimates  
477 of whole-lake NEP generally were slight overestimates, but there was considerable variation  
478 both among lakes and among days within lakes (mean difference  $3.55 \pm 6.65$  g O<sub>2</sub> m<sup>-1</sup> d<sup>-1</sup>; Fig.  
479 6c).

480

## 481 **Discussion**

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

485 peaked in the metalimnion (e.g. Lakes Ontario, Stechlin), as reported before (Sadro et al.  
486 2011a). In contrast, metabolic rates of some mesotrophic and eutrophic lakes were strongly  
487 depth-dependent (e.g. Lake Castle; Fig. 1). In lakes with high vertical variation in rates,  
488 single-sensor estimates of whole-lake metabolism deviated considerably from depth-  
489 integrated estimates on some days (Fig. 6). Despite dissimilar vertical profiles, we found that  
490 the metalimnion can contribute substantially to daily whole-lake metabolism across a broad  
491 range of lakes, with the exception of highly eutrophic conditions (Figs. 3 & 4). However, the  
492 relative contribution of the metalimnion varied substantially among days regardless of lake  
493 stratification pattern, and metalimnetic  $\text{Prop}_{\text{metab}}$  was only moderately explained by changes in  
494 light availability (as indicated by  $Z_{\text{mix}}:Z_{\text{eu}}$ ). Nonetheless, the metalimnion of oligotrophic  
495 lakes, with more light available at depth, contributed more on average to lake metabolism  
496 than expected from their metalimnetic volume ( $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}} > 1$ ; Fig. 3e,f).

497

#### 498 *Importance of physical processes to model uncertainty and day-to-day variability*

499 Our depth-integrated analysis extends current understanding of the role that physical  
500 processes play in obtaining accurate metabolic estimates (Coloso et al. 2008; Staehr et al.  
501 2012b; Van de Bogert et al. 2007). Combined with previous sensitivity analyses (Obrador et  
502 al. 2014), we show that daily estimates of metabolic rates are robust to uncertainties in  
503 vertical diffusive fluxes ( $D_v$ ) under many circumstances, but there were exceptions in the  
504 deeper layers of a polymictic lake (Table S2). This increased sensitivity was likely due to the  
505 higher contribution of  $D_v$  and  $D_z$  to DO fluxes in deeper layers and corresponding decrease in  
506 the diel signal of DO, water temperature and PAR (Figs S7-S16), as well as model fit (Fig. 2).  
507 We expected that high variation in patterns of stratification or low water-column stability  
508 would be associated with poor fitting models and uncertainty in parameter estimates.  
509 However, within the metalimnion we found no evidence that polymictic lakes, or days and  
510 layers with low stability were associated with poor model convergence or fit. Furthermore,

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

516 There are a range of physical processes that were not accounted for by the free-water  
517 framework utilized here, including horizontal advection, metalimnetic intrusions and internal  
518 seiches caused by wind forcing (Solomon et al. 2013; Van de Bogert et al. 2007). These  
519 process errors bring water masses influenced by heterogeneous chemical conditions and  
520 biological communities (Solomon et al. 2013; Van de Bogert et al. 2012; Van de Bogert et al.  
521 2007). For example, Sadro *et al.* (2011a) found that pelagic rates were commonly influenced  
522 by littoral habitats due to advection and water-column stability. The patterns of model fits  
523 found among depth zones suggested physical process errors were a considerable issue in the  
524 meta- and hypolimnion. Because physical processes are a function of lake morphology, we  
525 expected the degree of uncertainty to vary among lakes. However, we did not observe any  
526 consistent trends in parameters of model fit or importance of diffusive fluxes across our  
527 gradient of lake areas (Fig S6), suggesting process areas did not systematically bias our  
528 interpretations. However, lake area is confounded by other variables in our dataset including  
529 measurement frequency (Table S1).

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

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

543 Our results suggest we should remain cautious that patterns in metabolic activity are  
544 attributable to biological activity occurring at a certain place and time, especially when  
545 stratification periods are not prolonged. However, we employed a number of methods to assist  
546 in providing useful information on metabolic processes in the deeper layers of stratified lakes.  
547 These included analyzing weeks to months of data for each lake, and using methods such as  
548 temporal smoothing and calculating stratification and diffusive fluxes on sub-daily timescales  
549 (Coloso et al. 2011) was expected to. Further, where depth-integrated measures of chl-*a*  
550 concentration were available (Table S1), vertical patterns in metabolism generally matched  
551 algal biomass. In our study, the vertical resolution of measurements did not affect estimate  
552 certainty, but maintaining a high resolution will increase the accuracy of temperature profiles  
553 and stratification patterns. This is important for calculating the contribution of specific lake  
554 habitats, because metalimnetic contribution was sensitive to its thickness (Supplemental Text  
555 2 and Table S2). Vertical resolution will also assist in cases such as Lake Castle where the  
556 development of hypolimnetic hypoxia (Fig. S9) could produce unrealistically sharp diffusive  
557 gradients if too few sensors are used.

558

### 559 *Vertical patterns in efficiency of light utilization and metabolic coupling*

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



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

577 Our estimates corroborate with previous findings that heterotrophic to balanced  
578 conditions generally prevail in the metalimnion (e.g. Sadro et al. 2011a), even when it was  
579 presumably well lit (Fig. 4e). This has been previously ascribed to ER being less depth-  
580 dependent than GPP (Coloso et al. 2008). However, vertical patterns in ER were similar to  
581 GPP at many sites, at both *in situ* temperature and when standardized to 20°C (Figs. 1 & S2).  
582 Days of autotrophy in the metalimnion of lakes from all trophic states and mixing regimes  
583 interspersed the predominant state of heterotrophy (Wilkinson et al. 2015). Despite high  
584 variability in GPP and ER rates among sites, metalimnetic NEP was relatively stable (Fig. 4e),  
585 with a mean of  $-0.08 \pm 0.26$  mg O<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup> among lakes when excluding Lake Müggel, which  
586 had outstandingly high autotrophy in the metalimnion (Fig. 1). This relative stability was  
587 likely due to the metalimnetic ER<sub>20</sub>-to-GPP<sub>20</sub> coupling gradient being close to 1 at many sites  
588 (Fig. 5e). Substrate limitation of heterotrophs is hypothesized to drive strong metabolic

589 coupling (Sadro et al. 2011b; Solomon et al. 2013), and may be pronounced at depth because  
590 the OM in deeper layers typically contains a greater proportion of recalcitrant molecules with  
591 low biodegradability (Ostrom et al. 1998). A small number of mean coupling slope estimates  
592  $> 1$  were observed in the meta- and hypolimnion (Fig. 5e, f), suggesting greater than unit  
593 increases in ER for each unit increase in GPP. Priming of recalcitrant OM (Guenet et al.  
594 2010) could produce such a pattern but evidence for this process in freshwater systems is  
595 inconclusive (Catalán et al. 2015). As these high slope estimates all occurred at low GPP it is  
596 more likely that these estimates were artificially inflated by statistically influential data points  
597 with low GPP relative to ER.

598         Elevated background respiration in the meta- and hypolimnion of some mesotrophic and  
599 eutrophic sites suggests respiration of OM that was not recently or locally produced (Solomon  
600 et al. 2013). The metalimnion may be a zone with high degradation of particles that  
601 accumulate near the thermocline through a number of co-occurring mechanisms (Staeher et al.  
602 2012b). This includes recently-produced organic material sinking from the epilimnion,  
603 combined with large pools of recalcitrant dissolved organic matter (DOM). Other particles  
604 such as zooplankton carcasses may be highly available in the metalimnion because they have  
605 higher residence times in stratified water columns and move slowly through the thermocline  
606 (Kirillin et al. 2012). Particulate organic matter (POM) accumulating below the mixed layer  
607 could also originate from resuspension of benthic material by internal seiches during  
608 stratification (Weyhenmeyer 1996) or external stream- and groundwater inputs that do not  
609 mix with the epilimnion. Higher nutrient availability below the epilimnion may also facilitate  
610 greater decomposition rates. For mean heterotrophic conditions, there must be a net subsidy of  
611 carbon to the metalimnion and hypolimnion. In addition to accumulating particles, diel  
612 vertical migrations could play a role in linking surface and deeper waters, which has been  
613 relatively unexplored compared to physical mechanisms. Daily rates of microbial background  
614 respiration in the metalimnion could be spatially subsidized by zooplankton that migrate to

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

619

#### 620 *Drivers of metalimnetic contribution to whole-lake metabolism*

621 Contrary to expectations, light conditions in the metalimnion, as measured by the relation  
622 between mixing depth and photic zone ( $Z_{\text{mix}}:Z_{\text{eu}}$ ), did not explain a large portion of the  
623 variation in metalimnetic  $\text{Prop}_{\text{metab}}$  or  $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$  alone (Fig. 4). Instead, our results  
624 suggested a collinear combination of physical and chemical variables, including light, nutrient  
625 concentration, temperature, water-column stability and metalimnetic volume drives  
626 metalimnetic  $\text{Prop}_{\text{metab}}$  (Figs. 3 & 4, Table 3). We did not find a strong association between  
627 mixing regime and metalimnetic  $\text{Prop}_{\text{metab}}$ , but the contribution will naturally be more  
628 temporally dynamic in polymictic lakes that have interspersed periods of mixed water  
629 columns. Concentration of TP in the epilimnion was negatively correlated with metalimnetic  
630  $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$ , likely due to stimulating epilimnetic production that shades metalimnetic  
631 organisms (e.g. Laas et al. 2012). However, trends for GPP were highly influenced by the  
632 most eutrophic lakes, so that additional sites are required to confirm these patterns. Despite  
633 the decreasing metalimnetic  $\text{Prop}_{\text{metab}}$ , absolute rates in the metalimnion increased slowly with  
634 TP concentration, until a threshold where the metalimnion was shaded (Castle Lake; Fig.  
635 3a,b). These results imply that eutrophication of lake ecosystems may shift primary  
636 production from the metalimnion to the epilimnion, as observed for coastal systems  
637 (Lyngsgaard et al. 2014).

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

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

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

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

678

679 **References**

- 680 Adrian, R. and others 2009. Lakes as sentinels of climate change. *Limnol Oceanogr* **54**: 2283-  
681 2297.
- 682 Batt, R. D., and S. R. Carpenter. 2012. Free-water lake metabolism: addressing noisy time  
683 series with a Kalman filter. *Limnol. Oceanogr. Methods* **10**: 20-30.
- 684 Boegman, L., J. Imberger, G. N. Ivey, and J. P. Antenucci. 2003. High-frequency internal  
685 waves in large stratified lakes. *Limnol Oceanogr* **48**: 895-919.
- 686 Boehrer, B., and M. Schultze. 2008. Stratification of lakes. *Rev. Geophys.* **46**: RG2005.
- 687 Boehrer, B., and M. Schultze. 2010. Density Stratification and Stability. *In* G. E. Likens [ed.],  
688 *Lake Ecosystem Ecology: A Global Perspective*. Academic Press.
- 689 Bouffard, D., L. Boegman, J. D. Ackerman, R. Valipour, and Y. R. Rao. 2014. Near-inertial  
690 wave driven dissolved oxygen transfer through the thermocline of a large lake. *J Gt*  
691 *Lakes Res* **40**: 300-307.
- 692 Brentrup, J. A. and others in press. Extending the Plankton Ecology Group (PEG) model: The  
693 potential of high-frequency profiling to assess vertical and seasonal patterns of  
694 phytoplankton dynamics in lakes. *Inland Waters*.
- 695 Brooks, S. P., and A. Gelman. 1997. General methods for monitoring convergence of iterative  
696 simulations. *Journal of Computational and Graphical Statistics* **7**: 434-455.
- 697 Butcher, J. B., D. Nover, T. E. Johnson, and C. M. Clark. 2015. Sensitivity of lake thermal  
698 and mixing dynamics to climate change. *Clim Change* **129**: 295-305.
- 699 Carpenter, S. R., E. H. Stanley, and M. J. Vander Zanden. 2011. State of the world's  
700 freshwater ecosystems: Physical, chemical, and biological changes. *Annual Review of*  
701 *Environment and Resources* **36**: 75-99.
- 702 Catalán, N., A. M. Kellerman, H. Peter, F. Carmona, and L. J. Tranvik. 2015. Absence of a  
703 priming effect on dissolved organic carbon degradation in lake water. *Limnol*  
704 *Oceanogr* **60**: 159-168.

705 Cole, J. and others 2007. Plumbing the global carbon cycle: Integrating inland waters into the  
706 terrestrial carbon budget. *Ecosystems* **10**: 172-185.

707 Cole, J. J., and N. F. Caraco. 1998. Atmospheric exchange of carbon dioxide in a low-wind  
708 oligotrophic lake measured by the addition of SF<sub>6</sub>. *Limnol Oceanogr* **43**: 647-656.

709 Coloso, J., J. Cole, and M. Pace. 2011. Short-term variation in thermal stratification  
710 complicates estimation of lake metabolism. *Aquat Sci* **73**: 305-315.

711 Coloso, J. J., J. J. Cole, P. C. Hanson, and M. L. Pace. 2008. Depth-integrated, continuous  
712 estimates of metabolism in a clear-water lake. *Can J Fish Aquat Sci* **65**: 712-722.

713 Cremona, F. and others 2014a. From bacteria to piscivorous fish: estimates of whole-lake and  
714 component-specific metabolism with an ecosystem approach. *PLoS ONE* **9**: e101845.

715 Cremona, F., A. Laas, P. Nõges, and T. Nõges. 2014b. High-frequency data within a  
716 modeling framework: On the benefit of assessing uncertainties of lake metabolism.  
717 *Ecol Modell* **294**: 27-35.

718 Del Giorgio, P. A., and P. J. L. B. Williams. 2005. The global significance of respiration in  
719 aquatic ecosystems: From single cells to the biosphere. *In* P. A. del Giorgio and P. J.  
720 L. B. Williams [eds.], *Respiration in aquatic ecosystems*. Oxford University Press.

721 Finkel, Z. V., J. Beardall, K. J. Flynn, A. Quigg, T. a. V. Rees, and J. A. Raven. 2010.  
722 Phytoplankton in a changing world: cell size and elemental stoichiometry. *J Plankton*  
723 *Res* **32**: 119-137.

724 Giling, D. P. and others 2016. Thermocline deepening boosts ecosystem metabolism:  
725 Evidence from a large-scale lake enclosure experiment simulating a summer storm.  
726 *Glob Change Biol* **in press**.

727 Grace, M. R., D. P. Giling, S. Hladyz, V. Caron, R. M. Thompson, and R. Mac Nally. 2015.  
728 Fast processing of diel oxygen curves: estimating stream metabolism with BASE  
729 (BAYesian Single-station Estimation). *Limnol. Oceanogr. Methods* **13**: 103-114.

730 Guenet, B., M. Danger, L. Abbadie, and G. Lacroix. 2010. Priming effect: bridging the gap  
731 between terrestrial and aquatic ecology. *Ecology* **91**: 2850-2861.

732 Hamilton, D. P., K. R. O'brien, M. A. Burford, J. D. Brookes, and C. G. McBride. 2010.  
733 Vertical distributions of chlorophyll in deep, warm monomictic lakes. *Aquat Sci* **72**:  
734 295-307.

735 Hanson, P. C., S. R. Carpenter, N. Kimura, C. Wu, S. P. Cornelius, and T. K. Kratz. 2008.  
736 Evaluation of metabolism models for free-water dissolved oxygen methods in lakes.  
737 *Limnol. Oceanogr. Methods* **6**: 454-465.

738 Holtgrieve, G. W., D. E. Schindler, T. A. Branch, and Z. T. A'mar. 2010. Simultaneous  
739 quantification of aquatic ecosystem metabolism and reaeration using a Bayesian  
740 statistical model of oxygen dynamics. *Limnol Oceanogr* **55**: 1047-1063.

741 Hondzo, M., and H. G. Stefan. 1993. Lake water temperature simulation model. *Journal of*  
742 *Hydraulic Engineering* **119**: 1251-1273.

743 Jennings, E. and others 2012. Effects of weather-related episodic events in lakes: an analysis  
744 based on high-frequency data. *Freshwater Biol* **57**: 589-601.

745 Johengen, T. H., B. A. Biddanda, and J. B. Cotner. 2008. Stimulation of Lake Michigan  
746 plankton metabolism by sediment resuspension and river runoff. *J Gt Lakes Res* **34**:  
747 213-227.

748 Kirillin, G. 2010. Modeling the impact of global warming on water temperature and seasonal  
749 mixing regimes in small temperate lakes. *Boreal Environ Res* **15**: 279-293.

750 Kirillin, G., H.-P. Grossart, and K. W. Tang. 2012. Modeling sinking rate of zooplankton  
751 carcasses: Effects of stratification and mixing. *Limnol Oceanogr* **57**: 881-894.

752 Kirk, J. T. 1994. *Light and photosynthesis in aquatic ecosystems*. Cambridge University  
753 Press.

754 Kraemer, B. M. and others 2015. Morphometry and average temperature affect lake  
755 stratification responses to climate change. *Geophys. Res. Lett.* **42**: 2015GL064097.



756 Laas, A., P. Nõges, T. Kõiv, and T. Nõges. 2012. High-frequency metabolism study in a large  
757 and shallow temperate lake reveals seasonal switching between net autotrophy and net  
758 heterotrophy. *Hydrobiologia* **694**: 57-74.

759 Livingstone, D. M. 2008. A Change of Climate Provokes a Change of Paradigm: Taking  
760 Leave of Two Tacit Assumptions about Physical Lake Forcing. *Int Rev Hydrobiol* **93**:  
761 404-414.

762 Lyngsgaard, M. M., S. Markager, and K. Richardson. 2014. Changes in the vertical  
763 distribution of primary production in response to land-based nitrogen loading. *Limnol*  
764 *Oceanogr* **59**: 1679-1690.

765 Mccree, K. J. 1981. Photosynthetically active radiation. *In* O. L. Lang, P. Novel, B. Osmond  
766 and H. Ziegler [eds.], *Physiological plant ecology*. Encyclopedia of plant physiology  
767 (new series). Springer-Verlag.

768 McNair, J. N., L. C. Gereaux, A. D. Weinke, M. R. Sesselmann, S. T. Kendall, and B. A.  
769 Biddanda. 2013. New methods for estimating components of lake metabolism based  
770 on free-water dissolved-oxygen dynamics. *Ecol Modell* **263**: 251-263.

771 Meinson, P., A. Idrizaj, P. Nõges, T. Nõges, and A. Laas. 2015. Continuous and high-  
772 frequency measurements in limnology: history, applications, and future challenges.  
773 *Environ Rev* **24**: 52-62.

774 Moreno-Ostos, E., R. L. Palomino-Torres, C. Escot, and J. M. Blanco. 2016. Planktonic  
775 metabolism in a Mediterranean reservoir during a near-surface cyanobacterial bloom.  
776 *Limnetica* **35**: 117-130.

777 Obrador, B., P. A. Staehr, and J. P. C. Christensen. 2014. Vertical patterns of metabolism in  
778 three contrasting stratified lakes. *Limnol Oceanogr* **59**: 1228-1240.

779 Odum, H. T. 1956. Primary production in flowing waters. *Limnol Oceanogr* **1**: 102-117.

780 Ostrom, N. E., D. T. Long, E. M. Bell, and T. Beals. 1998. The origin and cycling of  
781 particulate and sedimentary organic matter and nitrate in Lake Superior. *Chemical*  
782 *Geology* **152**: 13-28.

783 Paerl, H. W., and V. J. Paul. 2012. Climate change: Links to global expansion of harmful  
784 cyanobacteria. *Water Res.* **46**: 1349-1363.

785 Pannard, A., D. Planas, and B. E. Beisner. 2015. Macrozooplankton and the persistence of the  
786 deep chlorophyll maximum in a stratified lake. *Freshwater Biol* **60**: 1717-1733.

787 Plummer, M. 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs  
788 sampling. *Proceedings of the 3rd International Workshop on Distributed Statistical*  
789 *Computing (DSC 2003)*.

790 Poole, H. H., and W. R. G. Atkins. 1929. Photoelectric measurements of submarine  
791 illumination throughout the year. *J Mar Biol Assoc U K* **16**: 297–324.

792 R Development Core Team. 2014. R: A language and environment for statistical computing.  
793 R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL  
794 <http://www.R-project.org>.

795 Read, J. S. and others 2011. Derivation of lake mixing and stratification indices from high-  
796 resolution lake buoy data. *Environmental Modelling and Software* **26**: 1325-1336.

797 Rimmer, A., Y. Aota, M. Kumagai, and W. Eckert. 2005. Chemical stratification in thermally  
798 stratified lakes: A chloride mass balance model. *Limnol Oceanogr* **50**: 147-157.

799 Sadro, S., G. W. Holtgrieve, C. T. Solomon, and G. R. Koch. 2014. Widespread variability in  
800 overnight patterns of ecosystem respiration linked to gradients in dissolved organic  
801 matter, residence time, and productivity in a global set of lakes. *Limnol Oceanogr* **59**:  
802 1666-1678.

803 Sadro, S., and J. Melack. 2012. The effect of an extreme rain event on the biogeochemistry  
804 and ecosystem metabolism of an oligotrophic high-elevation lake. *Arct. Antarct. Alp.*  
805 *Res.* **44**: 222-231.

806 Sadro, S., J. M. Melack, and S. Macintyre. 2011a. Depth-integrated estimates of ecosystem  
807 metabolism in a high-elevation lake (Emerald Lake, Sierra Nevada, California).  
808 *Limnol Oceanogr* **56**: 1764-1780.

809 Sadro, S., C. E. Nelson, and J. M. Melack. 2011b. Linking diel patterns in community  
810 respiration to bacterioplankton in an oligotrophic high-elevation lake. *Limnol Oceanogr*  
811 **56**: 540-550.

812 Salonen, K., R. I. Jones, and L. Arvola. 1984. Hypolimnetic phosphorus retrieval by diel  
813 vertical migrations of lake phytoplankton. *Freshwater Biol* **14**: 431-438.

814 Solomon, C. and others 2015. Ecosystem consequences of changing inputs of terrestrial  
815 dissolved organic matter to lakes: Current knowledge and future challenges.  
816 *Ecosystems* **18**: 376-389.

817 Solomon, C. T. and others 2013. Ecosystem respiration: Drivers of daily variability and  
818 background respiration in lakes around the globe. *Limnol Oceanogr* **58**: 849-866.

819 Song, C., W. K. Dodds, M. T. Trentman, J. Rüegg, and F. Ballantyne. 2016. Methods of  
820 approximation influence aquatic ecosystem metabolism estimates. *Limnol. Oceanogr.*  
821 *Methods* **14**: 557–569.

822 Staehr, P., J. Testa, W. Kemp, J. Cole, K. Sand-Jensen, and S. Smith. 2012a. The metabolism  
823 of aquatic ecosystems: history, applications, and future challenges. *Aquat Sci* **74**: 15-  
824 29.

825 Staehr, P. A. and others 2010. Lake metabolism and the diel oxygen technique: State of the  
826 science. *Limnol. Oceanogr. Methods* **8**: 628-644.

827 Staehr, P. A., J. P. A. Christensen, R. D. Batt, and J. S. Read. 2012b. Ecosystem metabolism  
828 in a stratified lake. *Limnol Oceanogr* **57**: 1317-1330.

829 Sweers, H. E. 1970. Vertical diffusivity coefficient in a thermocline. *Limnol Oceanogr* **15**:  
830 273-280.

831 Tanentzap, A. J. and others 2008. Cooling lakes while the world warms: Effects of forest  
832 regrowth and increased dissolved organic matter on the thermal regime of a temperate,  
833 urban lake. *Limnol Oceanogr* **53**: 404-410.

834 Tranvik, L. J. and others 2009. Lakes and reservoirs as regulators of carbon cycling and  
835 climate. *Limnol Oceanogr* **54**: 2298-2314.

836 Tsai, J.-W. and others 2008. Seasonal dynamics, typhoons and the regulation of lake  
837 metabolism in a subtropical humic lake. *Freshwater Biol* **53**: 1929-1941.

838 Van De Bogert, M. C. and others 2012. Spatial heterogeneity strongly affects estimates of  
839 ecosystem metabolism in two north temperate lakes. *Limnol Oceanogr* **57**: 1689-1700.

840 Van De Bogert, M. C., S. R. Carpenter, J. J. Cole, and M. L. Pace. 2007. Assessing pelagic  
841 and benthic metabolism using free water measurements. *Limnol. Oceanogr. Methods*  
842 **5**: 145-155.

843 Watras, C. J., K. A. Morrison, J. T. Crawford, C. P. McDonald, S. K. Oliver, and P. C.  
844 Hanson. 2015. Diel cycles in the fluorescence of dissolved organic matter in  
845 dystrophic Wisconsin seepage lakes: Implications for carbon turnover. *Limnol*  
846 *Oceanogr* **60**: 482-496.

847 Weyhenmeyer, G. A. 1996. The influence of stratification on the amount and distribution of  
848 different settling particles in Lake Erken. *Canadian Journal of Fisheries and Aquatic*  
849 *Sciences* **53**: 1254-1262.

850 Wilcock, R. J. J. W. N., G. B. McBride, K. J. Collier, B. T. Wilson, and B. A. Huser. 1998.  
851 Characterisation of lowland streams using a single-station diel curve analysis model  
852 with continuous monitoring data for dissolved oxygen. *New Zealand Journal of Marine*  
853 *and Freshwater Research* **32**: 67-79.

854 Wilkinson, G. M., S. R. Carpenter, J. J. Cole, and M. L. Pace. 2014. Use of deep  
855 autochthonous resources by zooplankton: Results of a metalimnetic addition of <sup>13</sup>C to  
856 a small lake. *Limnol Oceanogr* **59**: 986-996.

857 Wilkinson, G. M., J. J. Cole, M. L. Pace, R. A. Johnson, and M. J. Kleinhans. 2015. Physical  
858 and biological contributions to metalimnetic oxygen maxima in lakes. *Limnol*  
859 *Oceanogr* **60**: 242-251.  
860

861 **Acknowledgements**

862 The collaboration was made possible by the activities of Networking Lake Observations in  
863 Europe (NETLAKE), funded by the European Corporation in Science and Technology  
864 (COST) action ES1201. Ideas for this manuscript were initiated at a NETLAKE workshop  
865 organized by PAS, BO and E. Jennings in Roskilde, Denmark. Acquisition of Lake Stechlin  
866 data was facilitated by grants from the German Federal Ministry of Education and Research  
867 (BMBF; no. 033L041B) and the German Research Foundation (DFG Core Facility; no. GE  
868 1775/2-1) to M.O. Gessner, and from the Leibniz Association (project ‘TemBi’; SAW-2011-  
869 IGB-2) to P. Casper and HPG. We thank S. A. Berger, C. Engelhardt, M.O. Gessner, M.  
870 Lentz, J. C. Nejtgaard and A. Penske for acquisition of ancillary data from Lake Stechlin.  
871 Data from Danish lakes were supported by the Danish Centre for Lake Restoration (CLEAR).  
872 Data collection from Lake Abant was funded by the Scientific and Technological Research  
873 Council of Turkey (TUBITAK) (Grant No: 111Y059). Data collection for El Gergal  
874 Reservoir was supported by EMASESA and projects EU- ENV/UK/000604 and CGL2005-  
875 04070. Lake Ontario data was provided by B. Weidel and M. Paufve and funding for data  
876 collection at Lake Ontario came from the Great Lakes Restoration Initiative, Great Lakes  
877 Fishery Commission, and New York State Department of Conservation. Two anonymous  
878 reviewers provided comments that greatly improved this manuscript.

879

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

882

883

884

885 Table 2. Site information and ancillary data for the ten study lakes. Chemical and biological data are mean measurements from 1-11 samples among  
886 sites during the period of stratification at each lake (Supplemental Text 1). ‘Epi.’ denotes the epilimnion (metalimnion and hypolimnion  
887 measurements are presented in Table S1). The column ‘# days (total)’ shows the total number of stratified days with metabolic estimates in each  
888 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<sub>metab</sub>). ‘ $\beta$ ’ indicates the estimated slope of the  
 892 linear model.

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

893 **Figure captions**

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

898

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

904

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

914

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

919 whole-lake volume ( $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}}$ ) for (c) GPP and (d) ER. At values higher than the  
920 dashed horizontal line at  $\text{Prop}_{\text{metab}}:\text{Prop}_{\text{vol}} = 1$  the metalimnion contributes more to the whole  
921 lake metabolically than it does volumetrically. Panel (e) shows the volumetric rate of NEP as  
922 a function of mean daily  $Z_{\text{mix}}:Z_{\text{eu}}$ . Each point represents one day, with a point only for days  
923 with at least one appropriate model fit in each depth-zone shown for plots a-d. White, grey  
924 and black points indicate oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles,  
925 squares and triangles represent monomictic, dimictic and polymictic lakes, respectively. In  
926 plots c and d some outlying high points at Lake Ontario were excluded to assist interpretation.  
927

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

939  
940 Figure 6. Comparison of mean ( $\pm\text{SD}$ ) daily whole-lake volume weighted (WLWV)  
941 metabolism between depth-integrated and single-sensor estimates of (a) GPP, (b) ER and (c)  
942 NEP. The dashed line indicates the 1:1 relationship. White, grey and black points indicate  
943 oligotrophic, mesotrophic and eutrophic lakes, respectively. Circles, squares and triangles  
944 represent monomictic, dimictic and polymictic lakes, respectively.