Vertical patterns of metabolism in three contrasting stratified lakes

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ABSTRACT

Recent advances in open water measurements suggest significant temporal and spatial variability of gross primary production (GPP), net ecosystem production (NEP), and respiration (R) with implications for understanding carbon cycling in lakes. This study applied high frequency depth profiles in three stratified lakes of different trophic status to investigate 1) the importance of vertical variations in metabolic rates, 2) the effects of changes in the depth of the mixed layer (Z_{mix}) and the photic zone (Z_{eu}), and 3) the photoacclimative responses of the aquatic autotrophs to changes in these conditions. Taking account of vertical differences in metabolism improved the reliability of whole-areal NEP estimates during stratification. Whereas the hypolimnion was always heterotrophic, and the epilimnion was mostly autotrophic, the metalimnion had NEP>0 when $Z_{eu}>Z_{mix}$. Although most of GPP and R occurred in the epilimnion, between 0-20% of GPP and 4-37% of R took place in the metalimnion. Areal metabolic estimates based on surface measurements deviated up to 60% for GPP and 80% for R when $Z_{eu} > Z_{mix}$. The vertical variability in metabolism was driven by available light in both the epi- and metalimnion. Coupling between GPP and R was low in all layers and indicated increasing background R with depth. Light utilization efficiency was significantly higher under low light conditions, indicating photophysiological acclimation of phytoplankton to decreasing light in the metalimnion.

Introduction

Measurements of the production and consumption of organic material has been a focus of aquatic science for more than 80 years during which an important aim has been to quantify the magnitude and understand the drivers of variability of metabolic rates and carbon processing. Estimating ecosystem primary production and respiration from measurements of diel, open water changes in dissolved oxygen (DO) concentrations has become a widely accepted method in aquatic science and has been particularly popular in lakes (Gelda and Effler 2002; Staehr et al. 2012*a*). The recent popularity of the diel open water technique among researchers and managers relates to awareness that it is important to capture the variable temporal dynamics in metabolic rates which can be obtained from sensors moored in lakes.

As the use of open-water techniques has expanded, new insights and new questions have emerged on the controls of the temporal and spatial variability of ecosystem gross primary production, (GPP), ecosystem respiration (R), and net ecosystem production (NEP = GPP – R) (Staehr and Sand-Jensen 2007; Van de Bogert et al. 2007; Solomon et al. 2013). Here, we investigate vertical patterns of lake metabolism in lakes deep enough to experience vertical temperature stratification. While this is not a new topic (Bella 1970; Hornberger and Kelly 1974; Melack 1982), technological advances in providing high frequency DO profiles, and analytical improvements have made it possible to determine depth specific production and respiration in detail (Staehr et al. 2012*b*) and evaluate if vertical day-to-day patterns in metabolism compare with those predicted from models of light and nutrient dependency.

In clear water lakes, the photic zone may extend below the thermocline resulting in elevated primary production and respiration below the upper mixed layer (Stefan et al. 1995). Such conditions make the metalimnion a zone of primary production when sufficient light is available and nutrient levels are depleted in the upper mixed layer (Staehr et al. 2012*b*). This agrees well with earlier findings that vertical patterns in water column productivity are controlled by variations in mixed layer depth (Z_{mix}) and water clarity through regulation of light and nutrient availability (Fee 1976; Grobbelaar 1985; Stefan et al. 1995). Under turbid

conditions where Z_{mix} exceeds the depth of the photic zone (Z_{eu}) phytoplankton cells may spend considerable time under non-productive dark conditions (Grobbelaar 1990). Under clear water conditions where $Z_{eu} > Z_{mix}$ deeper peaks in phytoplankton biomass and primary production can occur in the metalimnion, as sufficient light and nutrients are available. Experimental work (Grobbelaar et al. 1995; Mouget et al. 1999; Mellard et al. 2012) have furthermore shown that phytoplankton light utilization efficiency may increase under conditions of low light and high nutrient availability.

This study evaluated vertical patterns in lake metabolism and in photophysiological acclimation of phytoplankton by careful analysis of a dataset of high frequency oxygen, temperature and light profiles in three temperate lakes of different trophic status (from mesotrophic to hypereutrophic). Recent developments in the use of inverse modeling techniques to determine metabolic rates from diel changes in DO (Hanson et al. 2008; Batt and Carpenter 2012) allowed us to evaluate the variability in parameters describing the photophysiological state of the phytoplankton community, including the light utilization efficiency.

Our first objective was to quantify the importance of GPP, R, and NEP with depth and investigate relationships between R and GPP. From previous studies (Coloso et al. 2008; Sadro et al. 2011*a*; Staehr et al. 2012*b*) we hypothesize that i) metabolic rates will be higher in the epilimnion compared to the meta- and hypolimnion due to more available light and strong coupling between GPP and R, ii) GPP will decrease more than R with depth, and iii) the balance between GPP and R (~NEP) will be related to the prevailing light and mixing conditions causing the metalimnion to vary between net autotrophic and heterotrophic conditions on a daily basis. Furthermore we hypothesize that the relative importance of epi-, meta-, and hypolimnetic zones will reflect their spatial extent and volume specific rates, such that most of the areal production will occur in the epilimnion, especially in the eutrophic lake where light will be limited to the surface waters, while clear water lakes are hypothesized to have significant contributions to GPP, NEP, and R from the metalimnetic zone. With regard to the relationship between R and GPP, ecosystem respiration depends on both autochthonous (~GPP) and allochthonous (terrestrially derived) organic matter inputs (del Giorgio and Williams 2005). Recent studies

(Solomon et al. 2013) show a tighter relationship between R and GPP in oligotrophic than in eutrophic lakes, where substantial primary production escapes immediate respiration and becomes buried or exported (Caraco and Cole 2004). Within lakes, we therefore expect a stronger relationship between R and GPP in the epilimnetic zone (as compared to the meta- and hypolimnion zones) where high light availability stimulates autochthonous production. Furthermore, we hypothesize that background respiration (i.e., the rate of respiration at GPP = 0), will increase under conditions where the concentration of non-photosynthetic organic material is high (del Giorgio and Williams 2005). As depth increases and light availability decreases, we expect the relative contribution of background respiration to total respiration to increase.

Our second objective was to investigate the extent to which vertical patterns in water column primary production are controlled by variations in mixed layer depth (Z_{mix}) and the depth of the photic zone (Z_{eu}) together determining the ambient light availability. Assuming R to be relatively constant with depth and GPP to be strongly linked to light, the net ecosystem production (NEP) is hypothesized to show a strong light dependency. The light dependency is expected to be more pronounced in the metalimnion than the epilimnion, with significantly higher NEP in the metalimnion during periods where minimum light requirements are met (Stefan et al. 1995; Domingues et al. 2011). Assuming light to be the dominant driver of vertical changes in primary production, we finally want to investigate vertical changes in phytoplankton light acclimation. Field (Grobbelaar 1985; Kishino et al. 1986; MacIntyre et al. 2002) and laboratory studies (Grobbelaar 1995; Mouget et al. 1999) have previously shown a photoacclimative response to decreasing light by increasing the light utilization efficiency (α; slope of the initial part of the photosynthesis vs. light curve). Experimental studies (Staehr and Sand-Jensen 2006) have shown that α is higher under nutrient replete conditions. Therefore, within the photic zone (> 1% surface light) we hypothesize α to be higher in meta- than epilimnion due to less light and more nutrients available.

Methods

Study sites

This study was conducted in three Danish lakes which stratify most of the summer and differ in nutrient and dissolved organic carbon (DOC) loads and therefore in water clarity. The lakes in this study also differ in morphometry, residence time and mixed layer depth (Table 1). Lake Hampen (9.4ºE, 56.1ºN) is a mesotrophic polymictic lake, and was sampled from April to December 2007. Lake Vedsted (9.4ºE, 55.2ºN) is a small, eutrophic and dimictic lake, and was sampled from January to December 2008. Lake Castle (12.3ºE, 55.9ºN) is a hypereutrophic and polymictic lake, and was sampled from September to November 2006 (Fig. 1).

Monitoring stations

Continuous monitoring of oxygen concentration, electrical conductivity and temperature at different depths was performed through an automatic profiling mooring station equipped with a multiparametric sonde (Yellow Springs Instruments 6600), and placed at the deepest area in each lake. The DO sensor has a resolution of 0.01 mg L^{-1} with an accuracy of ± 0.1 mg L^{-1} and the temperature sensor has a resolution of 0.01ºC with an accuracy of ± 0.15ºC. Dissolved oxygen (DO) was measured with a self-cleaned optical sensor that was calibrated every 3 weeks during each deployment. No drifts in the sensor were observed between calibrations.

The sonde performed automatic profiles every 30 min, measuring data at specified depths from the surface to the bottom of the water column (Table 1). We allowed 3 min of sensor stabilisation prior to each measurement. We had a DO and temperature measure for each depth every 30 minutes during the deployment period. Wind speed (Onset® anemometer) and photosynthetic active radiation (2π Onset® light logger) were collected at 5 second intervals and recorded as 10 minute averages at 1.3 m above the water surface at the mooring location.

Underwater light conditions were determined every 10 minutes using a series of four underwater Onset® light sensors placed at 20, 40, 80, and 120 cm depth. The daily vertical light attenuation coefficient ($K_{D,}$ 400-700 nm) was determined as the slope of a linear regression model of irradiance (E_z) vs. depth (z): $\ln(E_z) = b + K_D z$. Continuous irradiance recordings integrated over 30 minute intervals, allowed 48 light profiles each day. We then calculated a daily mean of vertical light attenuation (during light hours) from K_D values estimated

from regressions with r^2 >0.8 according to Staehr et al. (2012*b*). Daily K_D values determined from HOBO sensors were finally corrected by linear regression analysis against biweekly K_D estimates based on underwater light profiles using a 4π LiCor photosynthetic available radiation (PAR) sensor. The depth of the photic zone (1% of surface light) was calculated for each day as 4.6 / K_D.

Vertical stratification

We divided the water column into three layers epilimnion, metalimnion and hypolimnion according to the procedure in Staehr et al. (2012*b*). We determined the depth of epilimnion as the shallowest depth with a water density gradient equal to or above a suitable threshold (around 0.07 kg m^{-3} m^{-1}) for each time step according to Read et al. (2011) and will refer to that depth as Z_{mix}. Water density was calculated from temperature (in ^oC) assuming negligible effects of solutes as in Read et al. (2011). The thermocline depth was defined as the depth with the maximum temperature gradient and the lower limit of the metalimnion was calculated as thermocline depth plus the distance between Z_{mix} and the thermocline. Here we assume a symmetric temperature gradient around the thermocline. The depths of each layer were calculated for every 30 min, from modeled temperature profiles according to the procedures described in Staehr et al. (2012*b*). Measured profiles of temperature were fitted to a continuous curve model (Rimmer et al. 2006), which we only used for determination of Z_{mix}, thermocline and metalimnion depths. The performance of the curve-fitting model was evaluated by comparing temperature profiles in another Danish lake measured at 0.25 m and 1 m depth intervals. Only small and non-significant (Student's *t*-test, t =1.82; df = 9; p = 0.08) differences were observed in the calculated vertical temperature profiles at 0.1 m depth resolution. We recognize that our smoothed profiles do not represent the actual fine-resolution variations in temperature or density that would be obtained from microstructure profiles (MacIntyre 1993, Imberger 1985*a*).

We restricted our analysis to the periods where temperature stratification prevailed (Table 1). Further details on the automatic profiling system and on the methods to estimate vertical stratification of the water column can be found in Staehr et al. (2012*b*).

Metabolic calculations

Data treatment prior to metabolic calculations included simple linear interpolation with depth to obtain DO and temperature values for every 30 minutes and every meter interval, followed by a temporal smoothing of the time series at each depth through a running average of four hours. The same procedures were applied to wind and light data.

Metabolic rates for each depth layer were calculated using a methodology that includes biological fluxes (metabolism), air-water gas exchange and DO exchange between depth layers driven by mixed-layer deepening and eddy diffusivity (Staehr et al. 2012*b*). The basic model assumes that the DO change between two consecutive time steps in a given depth layer *i* ($\frac{\Delta O_{2(i)}}{\Delta t}$, in mmol m^{-3} h^{-1}) is described by:

$$\frac{\Delta O_{2(i)}}{\Delta t} = NEP_i + D_{z(i)} - D_{v(i)} - D_{s(i)} \qquad (1)$$

where NEP_i is net ecosystem production, $D_{Z(i)}$ is the flux between layers driven by mixed-layer deepening, $D_{v(i)}$ is the flux between layers driven by eddy diffusivity, and $D_{S(i)}$ is the air-water gas exchange, all expressed in mmol m^{-3} h^{-1}.

Above Z_{mix} D_s was calculated as $D_{s(i)} = K_s(O_{2(i)} - O_{2sat(i)})/Z_{mix}$, where K_s is the gas transfer velocity at the in situ temperature (m h^{-1}), $O_{2(i)}$ is the measured DO concentration and $O_{2sat(i)}$ is the DO concentration in atmospheric equilibrium. Depth layers below Z_{mix} were considered to be isolated from atmosphere. K_s was calculated from the gas transfer velocity at a Schmidt number of 600, which was in turn calculated for the cooling and heating periods from wind speed data following the equations of MacIntyre et al. (2010). $D_{Z(i)}$ was calculated using changes in Z_{mix} (Staehr et al. 2012*b*), and $D_{v(i)}$ was calculated from the vertical turbulent diffusivity coefficient (K_v) following Hondzo and Stefan (1993). This parameterization of eddy diffusivity has significant limitations, especially if applied to diel variations, and has not been validated in comparison to other approaches based on theoretical and mechanistic understanding of the mixing processes (Imberger 1985*b*; MacIntyre et al. 1999; Yeates and Imberger 2004). Our measurements did not permit application of these

144 approaches. To evaluate the potential effect of uncertainties in K_v we performed a sensitivity analysis based on
145 allowing K_v to vary up to 3 orders of magnitude (from 10^{-4} to 10^{-6} m^2 s^{-1}, which is a large but reasonable range
146 for our lakes) and evaluating the variability in the derived R and GPP rates in epi-, meta-, and hypolimnion
147 during a 30 day period in Hampen Lake. We observed minor changes in the daily metabolic rates to changes in
148 K_v, thus supporting the validity of our results. Differences in R were always below 2 mmol O_2 m^{-3} d^{-1} (mean
149 coefficient of variation (CV) was below 8% in all layers). Differences in GPP were always below 1 mmol O_2 m^{-3} d^{-1} (mean CV 2% in epi- and metalimnion, and 13% in hypolimnion).

151 Metabolic rates were calculated using an inverse modeling procedure (Hanson et al. 2008) which
152 calculates NEP_i from photosynthetically active radiation (PAR_i, μmol m^{-2} s^{-1}) and temperature (T_i, ºC) at each
153 specific depth *i*. The model includes a saturating response of photosynthesis to PAR (Jassby and Platt 1976) and
154 a thermodependent respiration, and is described as:

155 $$NEP_i = P_{max} \tanh\left(\frac{\alpha PAR_i}{P_{max}}\right) - R_{20}\theta^{(T_i-20)} \qquad (2)$$

156 where P_{max} is the maximum photosynthetic rate at saturating light (mmol m^{-3} h^{-1}), α is the photosynthetic
157 efficiency (mmol O_2 m^{-3} h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$), R_{20} is the respiration rate at 20ºC (mmol m^{-3} h^{-1}) and θ is
158 a coefficient which stands for the thermal dependence of respiration (set to 1.07; Jorgensen and Bendoricchio
159 2001). The first term in Eq. 2 corresponds to gross primary production (GPP_i, mmol m^{-3} h^{-1}), and the second to
160 ecosystem respiration (R_i, mmol m^{-3} h^{-1}) for each depth layer. PAR_i, was obtained from continuous surface
161 PAR measurements and from the light attenuation coefficient in the water column (K_D, m^{-1}).

162 A model combining Eqs. 1 and 2 was fitted to the DO data for 24 hour periods using a numerical
163 minimization algorithm in the non-linear function in the Statistical Analysis System (SAS) software. Thus, by
164 fitting the model to the observed DO time series at each depth, we obtained, for every 24 h and every depth
165 layer, estimates of the parameters P_{max}, α, and R_{20}. We assessed model performance (i.e., how well the model
166 fitted the observed DO data) through the coefficient of determination (r^2).

By applying the parameters (P_{max}, α, and R_{20}) derived in Eq. 2 we calculated hourly metabolic rates for each depth (i) (NEP_i, GPP_i, and R_i, in mmol m^{-3} h^{-1}). The daily metabolic rates (in mmol m^{-3} d^{-1}) were calculated as the average hourly rates multiplied by 24 h. Whole-lake areal GPP, R, and NEP (mmol m^{-2} d^{-1}) were computed by multiplying the volumetric daily rates of each depth layer (mmol m^{-3} d^{-1}) by the water volume within each layer (m^3), and by summing these quantities and dividing by the surface lake area (m^2).

Water analysis

Biweekly water samples were collected from the epilimnion and measurements of chlorophyll a (Chl *a*), total phosphorous (TP), and colored dissolved organic matter (CDOM). Chl *a* samples were filtered through Advantech 0.7 μm filters, extracted in 96% ethanol for 24 h, and measured according to methods in Jespersen and Christoffersen (1987) using a Shimadzu ultraviolet (UV)-160AH spectrophotometer. Total phosphorus (TP) was determined by persulfate digestion according to Eaton et al. (1995) and further assayed according to Kragh and Sondergaard (2004). Absorbance of CDOM was measured in a GF/F filtrate (0.7 μm) at 360 nm through a 5 cm cuvette. In comprehensive measurements from Danish lakes and streams, DOC concentrations (mg C L^{-1}) increased linearly with CDOM absorbance at 360 nm (m^{-1}): $DOC = 0.454\ CDOM_{360} + 1.9$ (r^2 =0.80, n = 399, C. Stedmon unpubl.) permitting estimation of DOC from CDOM with an uncertainty of 20%.

Results

Vertical patterns in metabolism

As typically observed for Danish lakes (Staehr et al. 2010*b*), stratification commenced in all lakes when surface temperatures passed approximately 10°C in May (not shown). The duration and stability of the stratification however varied among the studied lakes. Mixing depth and extent of the metalimnetic zone was smaller and much more variable in the polymictic Hampen Lake compared to the dimictic Vedsted Lake (Fig. 1; Table 1). Vedsted Lake is the smallest lake and it is sheltered between hills and surrounding trees, causing the thermocline to be more stable compared to Hampen and Castle lakes which are situated in open and more wind exposed landscapes. In Hampen Lake the photic zone penetrated far into the metalimnion whereas the

191 metalimnion received much less light in Vedsted Lake and in Castle Lake the metalimnion did not have light
192 levels above 1% (Fig. 1; Table 1).

193 All lakes had net autotrophy (NEP > 0) in the photic zone and heterotrophy in the layers below the photic
194 depth (Figs. 2,3). Accordingly the autotrophic zone extended deeper in Hampen than in Vedsted Lake and was
195 limited to the upper meter in Castle Lake (Figs. 2,3). Hampen had larger variability in photic depth (5 to 9 m)
196 than the two other lakes thus resulting in variability in GPP and NEP within the metalimnion (Fig. 3) which was
197 higher than the variability in Vedsted and Castle Lake. The metalimnion was heterotrophic in Castle (NEP
198 range from -93.6 to -0.7 mmol m^{-3} d^{-1}; mean -23.1; Fig. 3), mostly heterotrophic in Vedsted (NEP range from -
199 84.1 to 63.7 mmol m^{-3} d^{-1}; mean -25.7), and varied from heterotrophic to slightly autotrophic in Hampen Lake
200 (NEP range from -96.5 to 81.3 mmol m^{-3} d^{-1}; mean -7.8). Whereas heterotrophy progressively increased with
201 depth in Hampen, the most negative NEP in Castle and Vedsted occurred in the metalimnion, not in the
202 hypolimnion.

203 GPP decreased with depth in all three lakes, and the highest GPP was observed in the epilimnion except
204 in Hampen, where GPP rates in the metalimnion occasionally exceeded GPP in epilimnion (Fig. 2 and 3). All
205 lakes had highest R rates in the upper epilimnion and the metalimnion. Differences in the physical structure and
206 light penetration between the three lakes were reflected in the contribution of the three depth strata to areal
207 metabolism (Fig. 4; Table 2). Despite the epilimnion being the main contributor to GPP and R, a considerable
208 contribution of non-epilimnetic waters was observed, especially in Hampen and Vedsted Lake (Table 2). In
209 these lakes, epilimnetic respiration accounted for 50-70% of total R. Most of the remaining R (about 30%)
210 occurred in the metalimnion (Table 2). The metalimnion accounted for up to 18% of the whole-column GPP on
211 average. In agreement with expectations no primary production occurred in the meta- and hypolimnetic waters
212 of Castle Lake, due to the shallow photic depth. Only 4% of areal ecosystem respiration occurred below the
213 upper mixed layer in Castle Lake.

Calculated areal rates of NEP based on depth specific estimates over the whole-column were lower than NEP calculated from a single sonde in the upper mixed layer (Fig. 4). Whereas the single-sonde approach provided positive values of areal NEP, the whole-column estimates were significantly lower than zero for both Vedsted ($t = -5.6$; $df = 62$; $p < 0.01$) and Castle ($t = -1.5$; $df = 31$; $p < 0.01$) lakes. The discrepancy was most evident in Vedsted Lake where the metalimnetic zone was generally net heterotrophic. Thus single-sonde estimates taken at 1m depth overestimated areal GPP, R, and NEP rates (Fig. 4). In Vedsted and Castle lakes the heterotrophic character of the water column was not observed when using metabolic estimates derived from single-sonde measurements. Despite that most of the metabolism occurred in the epilimnion, rates of GPP and R were significantly ($p<0.05$) overestimated when calculated from 1 meter depth.

Coupling between photosynthesis and respiration

Relationships between ecosystem respiration and gross primary production were generally weak, especially so for the hypereutrophic Castle Lake which had no significant relationships ($p>0.05$) based on a whole water column or strata specific comparison (Table 3). Using the coefficient of determination (r^2) of the relationship as a proxy of the direct coupling between R and GPP, suggested a stronger coupling in epilimnetic waters than in the deeper strata as expected. Background respiration rates were defined as the respiration rates in absence of primary production (i.e. respiration based on the organic pool of allocthonous organic material). These were determined as the y intercepts of the R vs. GPP relationships and were significantly lower for Hampen Lake than the two other lakes when comparing the whole water column ($p<0.01$), and the epilimnetic zone ($p<0.05$). This result agreed with the lower DOC concentrations in Hampen Lake (Kruskall-Wallis $p<0.01$) compared to the other lakes (Table 1). Within lakes there was a tendency for higher background R in deeper waters (meta- and hypolimnion) compared to the upper mixed layer in Hampen and Vedsted lakes, but for Castle Lake the tendency was opposite (Table 3).

Community response to light availability

We investigated if variations in light availability caused by changes in mixing depth and depth of the photic zone, determines the rate of primary production in the different zones of the water column. To test this we used GPP estimates as an indicator of primary production and divided data for each lake into measurements performed in epi-, meta- and hypolimnetic layers and whether they were in the photic or aphotic zone. Effects of depth and light zone were seen in Hampen Lake (Fig. 5) which had the largest variations in Z_{mix} and Z_{eu} (Table 1). A two way ANOVA showed that the aphotic layers in Hampen Lake had significantly lower primary production, thus confirming the decrease in GPP with depth seen in Fig. 3. Only epilimnion and metalimnion were analyzed as hypolimnion data had significantly different variances ($F_{2,62}$= 19, p<0.01) between the lakes. Pairwise comparisons with Tukey post hoc test showed that light conditions (photic or aphotic) had a significant effect on GPP in both epilimnion (p<0.01) and metalimnion (p<0.001). It also showed that for Hampen Lake, GPP was not significantly different between the photic zones of the epilimnion and metalimnion and likewise with aphotic zones (p>0.05). In Vedsted Lake pairwise comparisons in epi- and metalimnion showed that the epilimnion had higher GPP than the metalimnion, where there was no significant difference in GPP of the photic and aphotic zone (p>0.05). For Castle Lake only the epilimnion was considered since the photic zone did not penetrate below the mixed layer.

Given our observations that R varies less with depth than GPP which is strongly coupled to light, the net ecosystem production (NEP) is likely to have a strong light dependency. In agreement with these expectations, relationships between daily volumetric rates of NEP with depth and available light were stronger in the light exposed epi- and metalimnion than in the hypolimnion, where only Hampen Lake had a few days with light above 1% surface irradiance (Fig. 6; Table 4). Pooling data for all three lakes, we found a light dependency, well described in the epi- and metalimnetic layers by the commonly applied light vs. photosynthesis model of Jassby and Platt (1976). Overall the epilimnion was mostly (55%) net autotrophic, with a minimum daily light requirement of 37 μmol photons m^{-2} s^{-1}. In comparison, the minimum light requirement was lower in the metalimnion (15 μmol photons m^{-2} s^{-1}) but these conditions were rarely met, and the metalimnion was therefore dominated (86%) by net heterotrophic conditions (Fig. 6).

Given the observed dependence of volumetric rates of GPP and NEP on combinations of Z_{mix} and Z_{eu} (Figs. 2,3,5), we further analysed our data to determine if there was a common threshold in the ratio between Z_{mix} and Z_{eu} at which areal estimates of metabolism would differ significantly between water column integrated and those obtained from epilimnetic measurements (~single sonde) only. Combining data for all three lakes, we found an asymptotic relationship between the ratio of Z_{mix} to Z_{eu} and the relative contribution of the epilimnetic zone to whole water column GPP (Fig. 7A). Accordingly, epilimnetic measurements provide representative estimates of the entire water column when the mixing depth is deeper than the photic zone ($Z_{mix} > Z_{eu}$). However, for most of our measurements the photic zone extended beyond the mixing depth, causing areal GPP to be underestimated by up to 60% under conditions of high water clarity and a shallow mixed layer (Fig. 7A). A similar analysis for areal rates of respiration, indicated a hyperbolic relationship to $Z_{mix} : Z_{eu}$ with deviations up to 80% between R derived from epilimnetic and depth integrated measurements (Fig. 7B). The hyperbolic relationship was not as strong as for GPP, supporting previous findings that R is not strongly coupled to GPP via light, but is dependent on the allochthonous inputs which should not vary with depth.

For metabolic rates determined within the photic zone (> 1% surface light) we expected light utilization efficiency (α) to increase with depth due to acclimation to less light and higher levels of nutrients. To test this we compared mean values of α in the epi- and metalimnion, assuming no vertical gradients within each of these layers due to internal mixing. The selection criteria of > 1% surface light was met in the epi- and metalimnion in Hampen and Vedsted lakes, and only the epilimnion of Castle Lake. In both Hampen and Vedsted lakes α was significantly higher in the metalimnion ($\alpha = 0.30 \pm 0.18$ and 0.27 ± 0.14 in Hampen and Vedsted, respectively, mean ± standard deviation (SD)) compared to the epilimnion ($\alpha = 0.15 \pm 0.11$ and 0.14 ± 0.11, respectively) ($p<0.01$; Wilcoxon paired test).

Discussion

Vertical patterns in metabolism

285 The studied lakes became increasingly heterotrophic with depth during periods of thermal stratification.
286 This is because GPP is strongly light and therefore depth dependent while respiration is almost independent of
287 light (apart from photorespiration, and elevated respiration after sunset, following changes in the DOC pool,
288 Sadro et al. 2011*a*) and more constant over depth. These patterns are as expected and similar to other studies
289 (Stefan et al. 1995; Coloso et al. 2008; Staehr et al. 2012*b*). Respiration peaked around the thermocline as
290 described in other studies (Salonen et al. 1983; Stefan et al. 1995; Sadro et al. 2011*a*) and suggested to result
291 from accumulation of settling particles around the thermocline (Stefan et al. 1995; Staehr et al. 2012*b*).
292 However, from this study, it is evident that elevated net heterotrophy occurs in depths which are located both in
293 the metalimnic zone and below the photic zone (Fig. 2).

294 Variations in light availability and mixing depth had, as expected (Fee 1976; Abbott et al. 1984;
295 Grobbelaar 1985), a strong effect on depth specific rates of GPP in all three lakes (Figs. 3,5). Within both epi-
296 and metalimnion in Hampen Lake, GPP was higher on days when light availability was higher than 1% surface
297 irradiance. As respiration was not related to light conditions (Fig. 3), elevated GPP on days when strata were
298 within the photic zone, resulted in net autotrophy (NEP > 0), even in the metalimnion. Interestingly, GPP was at
299 comparable levels in the photic zone of epi- and metalimnion in Hampen Lake (Fig. 5), despite that the
300 epilimnion received three times more light than the photic zone in the metalimnion on average. This suggests
301 significantly higher light utilization efficiency (α) in the metalimnetic zone, as will be discussed later. In
302 comparison, significantly lower GPP was observed in the photic zone of the metalimnion than in the epilimnion
303 in Vedsted Lake (not shown). This is due to the definition of the photic zone as 1% of surface irradiance. Thus
304 if we compare the actual irradiance in the metalimnion, the maximum irradiance in Vedsted was 34 μmol
305 photons m^{-2} s^{-1} but up to 354 μmol photons m^{-2} s^{-1} in Hampen Lake. Therefore GPP can reach a higher level in
306 metalimnion in Hampen than in Vedsted with rates comparable to epilimnion. In Castle Lake GPP in
307 metalimnion is absent due to lack of light.

Since GPP were calculated with an inverse model using hourly light values as a driving variable, there could be a potential bias in our interpretation of light dependency of daily rates. Therefore we compared our light dependent model with both the standard book-keeping approach (BKA, Hanson et al. 2008; Staehr et al. 2010*a*) and an inverse modeling approach (IMA) using surface irradiance and not available irradiance as the forcing function at all depths. These three approaches confirmed the light dependency model. The IMA using surface irradiance as forcing function at all depths showed no differences in epilimnetic GPP in all three lakes (median difference < 0.1%), and the descending trend with depth in GPP was maintained. In the metalimnion the median difference between rates obtained using IMA and BKA was low in Hampen (median 0-4 mmol O_2 m^{-3} d^{-1}), higher in Vedsted (median 20-35 mmol O_2 m^{-3} d^{-1}) and relatively large in Castle lake (median 25-70 mmol O_2 m^{-3} d^{-1}), but production rates (up to 1000 mmol O_2 m^{-3} d^{-1}) in Hampen and Castle Lake following periods of mixing convinced us that these estimates were affected by physical fluxes and did not reflect a biological signal, as discussed below. The difference in hypolimnetic GPP rates was also relatively low in all the lakes (median 1-20 mmol O_2 m^{-3} d^{-1}). Comparing metabolic rates from the traditional BKA with those from our model (IMA using depth specific irradiance), we found almost identical daily NEP (epilimnetic data: r^2 = 0.9, p<0.01) but more variable GPP and R for the BKA, especially with depth. As a result, light dependencies of GPP based on the BKA were weaker than the corresponding analysis on GPP derived through IMA. Thus, while IMA-based rates of GPP confirms the conceptual model of combined effects of light availability and mixing depth (Fee 1976; Abbott et al. 1984; Grobbelaar 1985), we cannot exclude a bias from the inherent light dependency of the applied model though the overall pattern is maintained using model approaches independent of light extinction. Future evaluations of the regulating role of light in stratified lakes, would therefore benefit from in situ bottle incubations, and should include truly oligotrophic lakes, where it is expected that metalimnetic GPP should be even higher than in the epilimnion (Sadro et al. 2011*a*).

For metabolic rates calculated below the epilimnion physical variations resulting from internal waves and seiches may be problematic for dissolved oxygen measurements and compromise the metabolic estimates in the metalimnion, where these signal oscillations are often greatest (Monismith 1986). We examined the effects of

this issue on our estimates of metabolism through a wavelet analysis (Coloso et al. 2008), which identified significant diel oxygen signals around the metalimnion in both Hampen and Vedsted lakes. We did not register any significant DO signals at other periods than the diel and therefore we concluded that most of the variation in DO was due to metabolic processes and not internal water movements. Also, the fit of our model (Eq. 2) to observed data showed an increasing model error with depth, but with on average 30-50% variability in diel DO explained in the metalimnion. Because the IMA is less sensitive to the physical variability than the BKA (Batt and Carpenter 2012) we feel confident that the observed patterns of metabolism are valid. We actually found that with depth the BKA provided variable rates which poorly correlated with the IMA-based rates, whereas a good correlation between the two methods was observed only in the surface waters.

Contributions of depth layers to total metabolism

Epilimnetic measurements provide representative estimates of the entire water column when the epilimnion is well mixed and the mixing depth is deeper than the photic zone ($Z_{mix} > Z_{eu}$). When $Z_{eu} > Z_{mix}$ the deviation of areal metabolic estimates based on epilimnetic measurements only can be as high as 60% for GPP and 80% for R. Hence, we recommend estimating metabolism from vertically distributed DO measurements in lakes where the meta- and hypolimnion includes a substantial part of the total lake volume and where the photic depth is higher than or equal to the mixing depth.

Despite the relatively low volume of meta- and hypolimnion in the three lakes and the less favorable light conditions, the layers under epilimnion contribute significantly to the overall areal metabolism (Table 2). Only in Castle Lake where meta- and hypolimnion makes up less than 9% of the total volume and are in dark at all times, the contribution is insignificant. GPP therefore decreases strongly with depth in Castle Lake, and primary production is absent under the mixed layer due to lack of light (Fig. 3). Even the epilimnion seems to be separated into a more productive and a less productive zone. This can be related to the existence of secondary thermoclines within the mixed layer, or at least a mixing process which is slower than the production of oxygen in epilimnion and gives rise to deviation in single sonde estimates and depth integrated metabolism within epilimnion, as seen in other studies (Eckert et al. 2002; Coloso et al. 2008).

While all of the metabolic activity in Castle Lake occurs in the upper mixed layer, (Table 2) the strong vertical variability within the epilimnion would not be adequately measured by a single sonde. In comparison, Hampen and Vedsted had about 80% of areal GPP and 50% to 70% of respiration captured in the epilimnion (Fig. 4 and Table 2). Respiration in metalimnion represents 26% and 37% of the whole column areal respiration in Hampen and Vedsted lakes, respectively. This is more than the volume represented by this layer on average (11% and 18%). Importance of metabolism under the mixed layer has previously been investigated in an oligotrophic system (Sadro et al. 2011*a*) and a mesotrophic lake (Staehr et al. 2012*b*). Here we extend this analysis to eutrophic lakes and show how metabolism varies with depth in lakes differing in water clarity and trophic status. We generally confirm previous findings from relative clear water lakes that GPP decrease with depth while R is less depth dependent (Coloso et al. 2008; Staehr et al. 2012*b*). However periods with significant primary production and respiration below the thermocline were also observed (Figs. 2,3).

The small contribution of respiration in meta- and hypolimnion of Castle Lake can be explained by a combination of lake morphometry and anoxic conditions. Castle Lake is a shallow lake with a relatively small part of the volume in meta- and hypolimnion compared to the other lakes (Table 1). In addition there is anoxic condition in hypolimnion during the study period (maximum 4 % DO saturation and mean 0.3%) and therefore aerobic respiration is low or zero and respiration can only be carried out with other electron acceptors which we did not measure. Due to this we underestimate total respiration in hypolimnion in Castle Lake and cannot compare this with Hampen Lake where DO was present and Vedsted Lake where oxygen was usually present (only anoxic at 9 meters) and hence respiration will give rise to oxygen consumption.

Coupling between R and GPP

Coupling between GPP and R was weak in our lakes (maximum correlation was 0.49; Table 3) compared to previous studies in lakes (Sand-Jensen and Staehr 2009; Coloso et al. 2011; Laas et al. 2012) where correlations were generally higher than 0.9. Whereas our metabolic rates were obtained from DO diel data using IMA, previous studies have applied the traditional book-keeping approach, where daily GPP is

determined as daytime NEP plus total daily R (Hanson et al. 2003). Consequently, the GPP to R coupling in previous studies, are likely biased because GPP was not determined independently from calculated respiration values. To further evaluate this we compared GPP and R using the BKA with GPP and R based on IMA. We found a stronger correlation (r^2 between 0.19 and 0.96, $p<0.05$) between GPP and R based on the BKA compared to the rates found through IMA. Therefore it appears that previous studies using the BKA may have overestimated the dependency of R on GPP. This is further supported by a recent study by Solomon et al. (2013) who applied IMA and also found a weak coupling between R and GPP.

Consistent with expectations of substrate-limited bacterial R in oligotrophic lakes (Sadro et al. 2011*b*) we found the strongest coupling between respiration and primary production in the nutrient poorest mesotrophic Hampen Lake. This agrees with Solomon et al. (2013) who recently showed that the R-GPP coupling is higher in oligotrophic than eutrophic lakes. Interestingly, the correlation between GPP and R tended to decrease with depth in all three lakes while background respiration increased. This further supports the interpretation that lake respiration becomes less dependent on the activity of primary producers as autochthonous carbon is less available to heterotrophs. Background respiration for the whole water column was higher in Vedsted and Castle lakes compared to Hampen Lake (Table 3), which was consistent with the higher DOC level in these two lakes (Table 1). In addition we measured metabolism in a period following a large cyanobacterial bloom in Castle Lake. Subsequent degradation of this biomass would be interpreted as elevated background respiration throughout the water column. The lower rates of background respiration determined for the hypolimnetic waters of Castle Lake are therefore likely a result of absence of changes in oxygen concentrations caused by the anoxic conditions occurring near the bottom in this eutrophic lake as previously mentioned.

Acclimation to light

Further evidence for the importance of light was provided by the saturating light dependency of NEP for epi- and metalimnion for all lakes and hypolimnion for Hampen Lake (Fig. 6 and Table 4). Lack of light dependence in the hypolimnion of Vedsted and Castle Lake occurred because minimum light requirements (I_C)

406 to sustain positive net photosynthesis were not achieved. Saturating light dependency of NEP confirms the
407 findings in Staehr et al. (2012*b*) where NEP is shown to approach balanced (NEP = 0) or slightly autotrophic
408 (NEP > 0) conditions in metalimnion when more than 1% of surface light is available, and is heterotrophic
409 (NEP < 0) below this threshold. Interestingly, I_C values were lower in the metalimnetic zone suggesting
410 acclimation to lower light conditions through higher light utilization efficiency (α). This indication was
411 supported from the model derived estimates of α which were significantly higher in the metalimnion than in the
412 epilimnion in both Hampen and Vedsted lakes. Bottle incubations in Vedsted resulting in higher metalimnetic α
413 values further supported these results (data not shown). There are two explanations for an increase in α in
414 metalimnion. Either it can be due to physiological acclimation in the phytoplankton (Grobbelaar et al. 1995;
415 MacIntyre et al. 2002; Deblois et al. 2013) or it can be due to an increase in biomass of phytoplankton in this
416 zone (Amand and Carpenter 1993; Hamilton et al. 2010). To explore if the higher metalimnetic α values were
417 dictated by higher levels of phytoplankton biomass with depth, we examined daily profiles of Chl *a*
418 fluorescence measured in Vedsted Lake. During the stratified period we found no clear pattern with depth and
419 no significant differences between the epi- and metalimnion (p>0.05; Wilcoxon paired test). Furthermore, bi-
420 weekly measurements of Chl *a* above and below Z_{mix} in Hampen and Castle lakes (not shown), showed that for
421 both lakes, Chl *a* below Z_{mix}, was less than 50% of epilimnetic values during summer stratification. Thus higher
422 light utilization efficiency in the low light conditions of the metalimnion appears to be independent of biomass
423 accumulation. From our study it therefore appears that elevated light utilization efficiency by phytoplankton is a
424 common phenomenon occurring under low light conditions in the metalimnic zone, where nutrients are
425 generally more replete (Fee 1976; Abbott et al. 1984). This is similar to experimental conditions where overall
426 food chain efficiency of aquatic ecosystems has been found to be highest under low light conditions (Dickman
427 et al. 2008).

428 Our study underlines the importance of measuring depth-specific metabolism in stratified lakes to
429 reduce a bias towards water column net autotrophy when only surface processes are considered. In comparing
430 lakes of different trophic status, we found that although most of GPP and R occurred in the epilimnion, up to

20% and 37% of GPP and R took place in the metalimnion, having NEP>0 when the depth of the photic zone exceeded the depth of the upper mixed layer. Areal metabolic estimates based on epilimnetic measurements only may accordingly deviate significantly from depth integrated estimates when $Z_{eu} > Z_{mix}$. While the observed importance of light and mixing regime for vertical patterns in primary production and respiration is convincing and consistent with experimentally based models, further research is needed to support the suggested increase in background respiration and *in situ* light utilization efficiency with depth.

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TABLES

Table 1. Morphometrical and limnological descriptors of the three studied lakes (mean ± SD during the studied period).

	Hampen	Vedsted	Castle
Mean depth (m)	4.2	5.5	3.7
Maximum depth (m)	13	11	9
Volume (10^6 m^3)	3.688	0.460	0.827
Area (km^2)	0.76	0.08	0.22
Water residence time (y)	1.4	4.6	0.5
Chl ($\mu g\ L^{-1}$)	5.3 ± 4.3	41 ± 21	65 ± 67
TP ($\mu g\ L^{-1}$)	22.7 ± 5.8	19.5 ± 25.6	102 ± 34
K_d (m^{-1})	0.7 ± 0.1	0.8 ± 0.2	1.7 ± 0.7
DOC ($mg\ L^{-1}$)	2.97 ± 0.08	4.79 ± 0.56	3.46 ± 0.29
Studied period	30 May 2007 to 25 Aug 2007	01 Jun 2008 to 03 Aug 2008	08 Sep 2006 to 13 Oct 2006
Number of days	46 days	63 days	33 days
Sampled depths	1, 3, 5, 7, 9	0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9	0.5, 2, 4, 6, 8
Z_{mix} (m)	4.7 ± 1.7	3.8 ± 0.4	5.1 ± 1.0
Z_{eu} (m)	6.3 ± 0. 9	4.0 ± 0.7	2.2 ± 0.6
Metalimnetic width (m)	1.5 ± 0.7	1.8 ± 0.2	1.2 ± 0.4
Fraction of water volume in the epilimnion (%)	73 ± 13	55 ± 5	91 ± 5

Table 2. Relative contribution (%) of epilimnion (epi), metalimnion (meta), and hypolimnion (hypo) to total areal metabolism of the water column (mean ± SD for GPP and R).

		Hampen	Vedsted	Castle
GPP	epi	79 ± 17	83 ± 15	100 ± 0
	meta	20 ± 16	17 ± 15	0 ± 0
	hypo	1.1 ± 1.6	0.2 ± 0.6	0 ± 0
R	epi	69 ± 19	54 ± 17	96 ± 4
	meta	26 ± 18	37 ± 15	4 ± 4
	hypo	5 ± 4	9 ± 7	0.1 ± 0.1

Table 3. Summary statistics (slope, intercept, and r^2 of the equation R= β + aGPP) of the GPP vs. R relationships in the volumetric rates of epi-, meta-, and hypolimnion and in the total areal rates, for the three studied lakes. Note that β is the background respiration, expressed in mmol m^{-3} d^{-1}. Only the significant parameters of a Type II regression are shown (p<0.05). ns: not significant. Data are means ± standard error (SE)

	r^2	slope	*B*
Hampen			
epi	0.39	0.57±0.10	7.9±4.61
meta	-	ns	38.7±5.63
hypo	-	ns	30.3±3.47
whole column	0.49	0.68±0.10	6.1±3.9
Vedsted			
epi	0.19	0.39±0.11	17.4±3.7
meta	0.09	0.52±0.19	31.3±2.6
hypo	0.06	-3.66±1.7	23.6±1.9
whole column	0.21	0.45±0.11	21.5±2.7
Castle			
epi	-	ns	43.8±5.6
meta	-	ns	23.3±3.1
hypo	-	ns	6.8±2.1
whole column	-	ns	26.6±3.5

Table 4. Correlation (Pearson´s *r*) of the Jassby and Platt model relating mean daily volumetric NEP rates in epi-, meta-, and hypolimnion and the corresponding mean daily PAR in each layer. ns: not significant ($p<0.05$)

	Hampen	Vedsted	Castle	all lakes pooled
epi	0.55	0.28	0.44	0.59
meta	0.52	0.30	ns	0.43
hypo	0.53	ns	ns	Ns

FIGURES

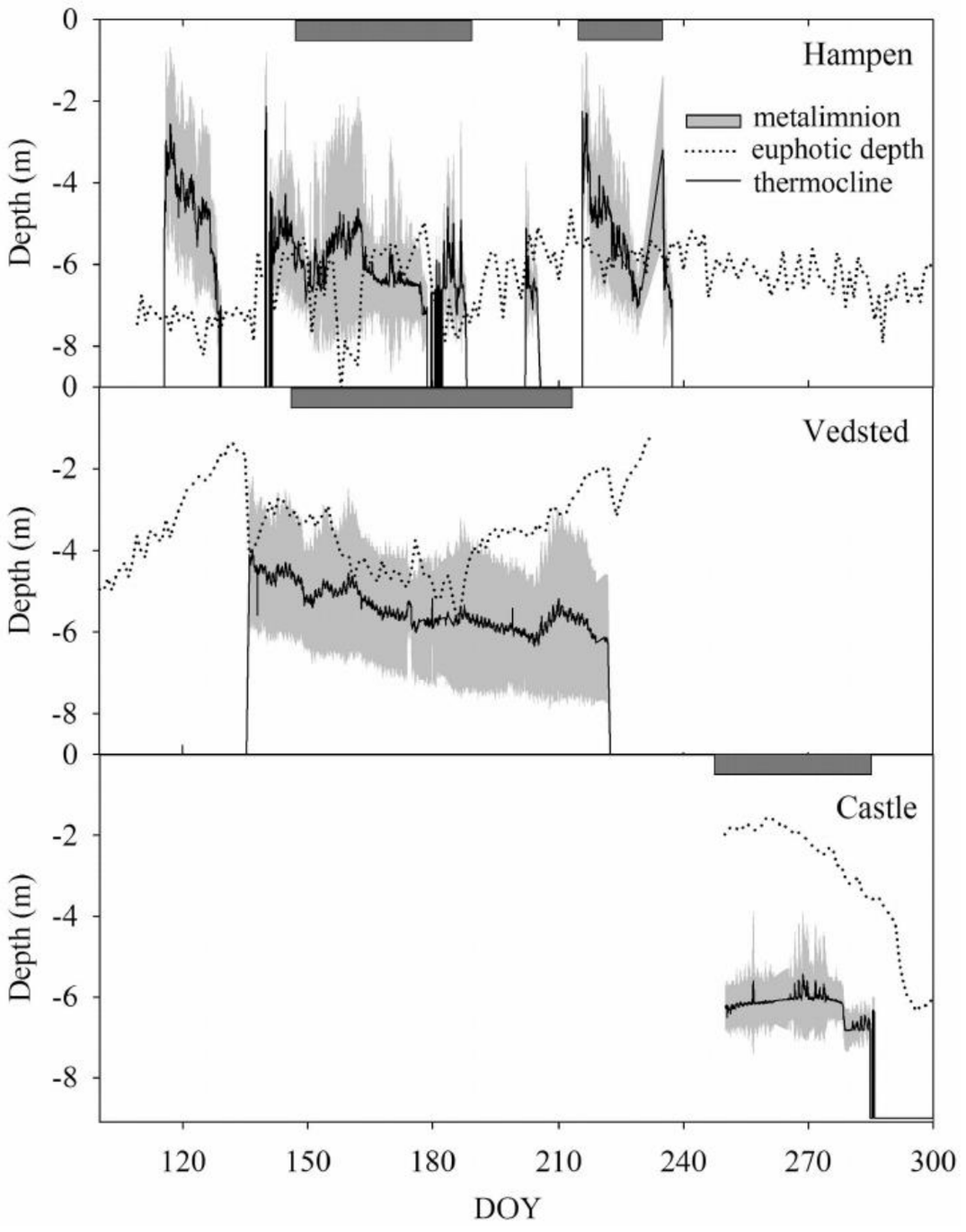

Figure 1. Seasonal variation in the physical structure of the water column and the depth of the photic zone (Z_{eu}, 1% of surface irradiance). Horizontal bars identify the studied periods in each lake. DOY – day of the year.

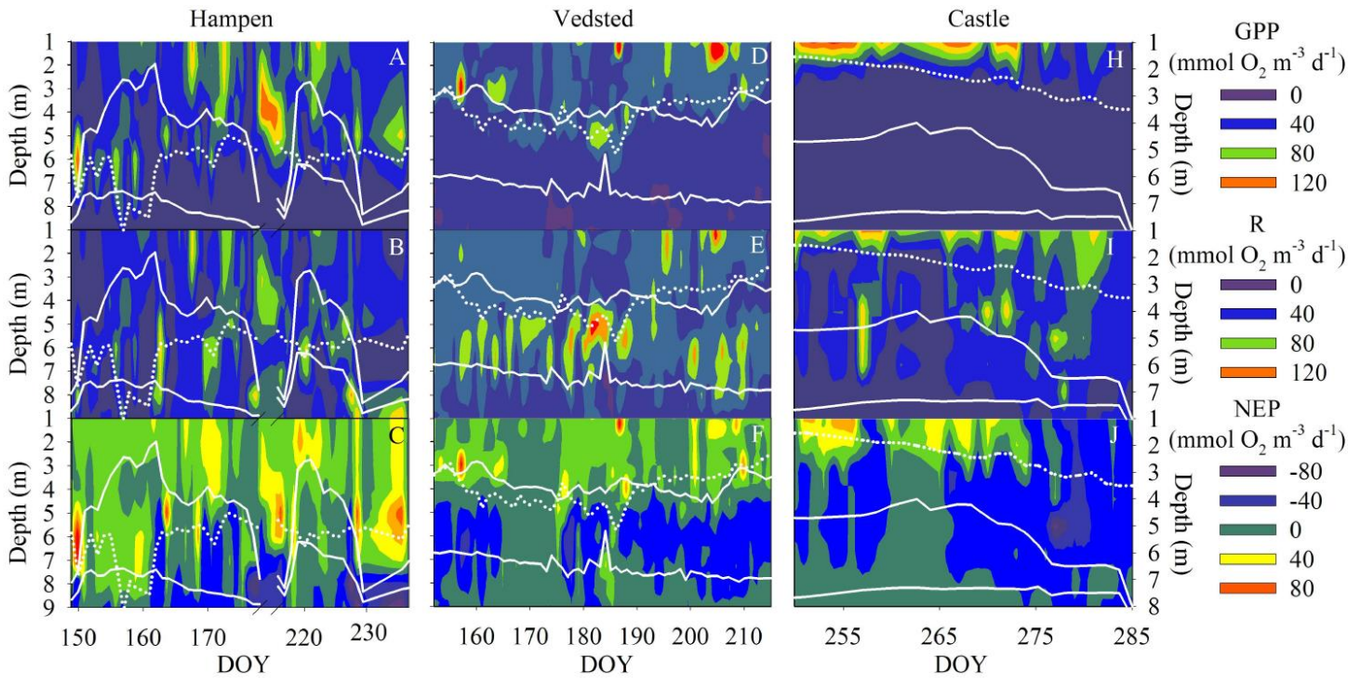

Figure 2. Depth specific daily rates (mmol O_2 m^{-3} d^{-1}) of gross primary production (GPP). respiration (R), and net ecosystem production (NEP) in (A-C) Hampen, (D-F) Vedsted, and (H-J) Castle Lake. The upper and lower limits of the metalimnetic zone are shown as solid white lines and the depth of the photic zone is shown as a dotted line. Time is shown as day of year (DOY) which is not the same for the three lakes (*see* Table 1).

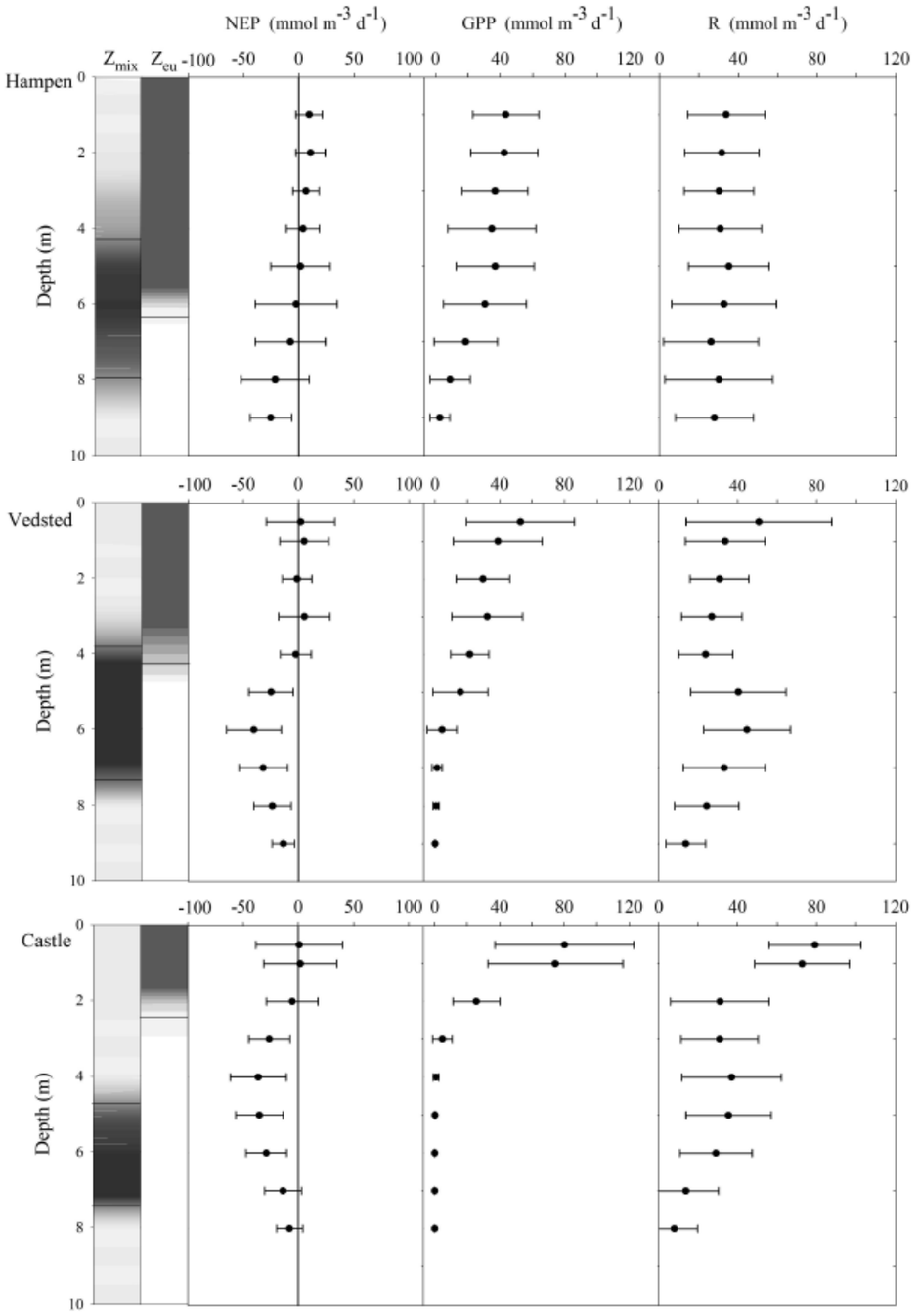

Figure 3. Depth profiles of specific NEP, GPP, and R (mmol m^{-3} d^{-1}) for the three studied lakes (mean ± SD). The depths of the metalimnion and of the photic zone are shown on the left boxes through a shading gradation (from black to white) that represents the frequency of a given depth of being within the metalimnion or the photic zone, respectively (the horizontal lines there correspond to the 90% confidence interval).

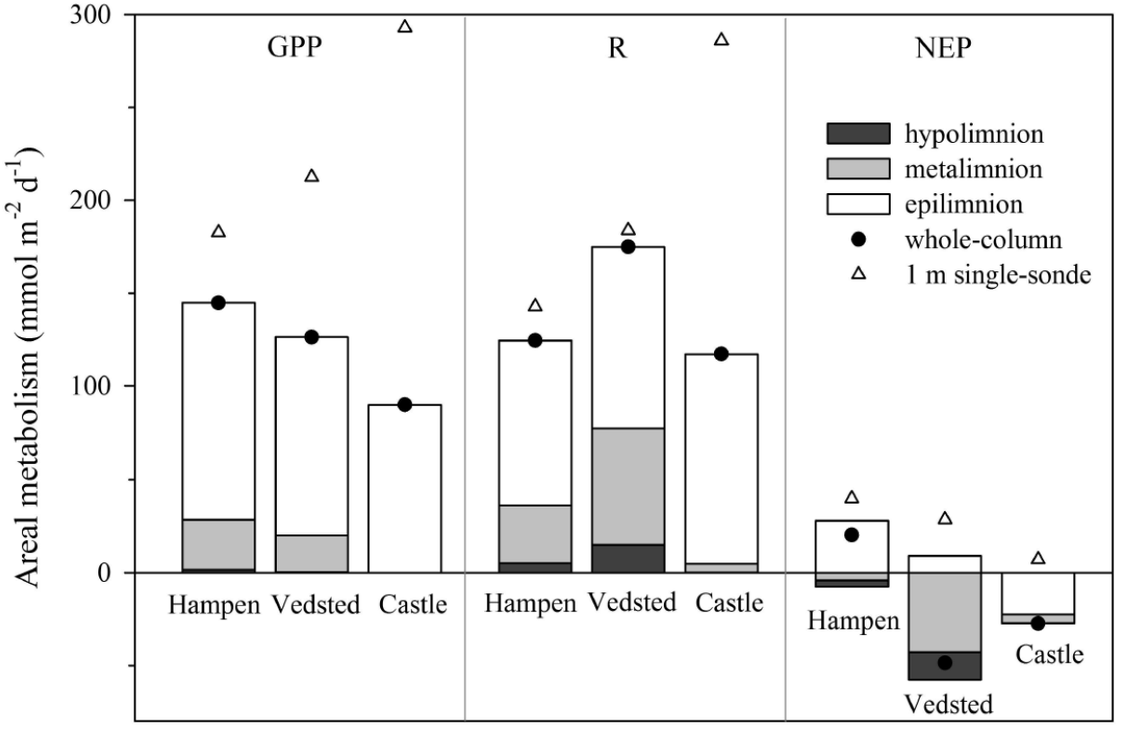

Figure 4. Mean areal rates (GPP, R, and NEP, in mmol m^{-2} d^{-1}) of the depth layers (bars) and integrated whole-column rates (circles) for the three studied lakes. The estimates derived from single-sonde (1 m depth) measurements are also shown (triangles).

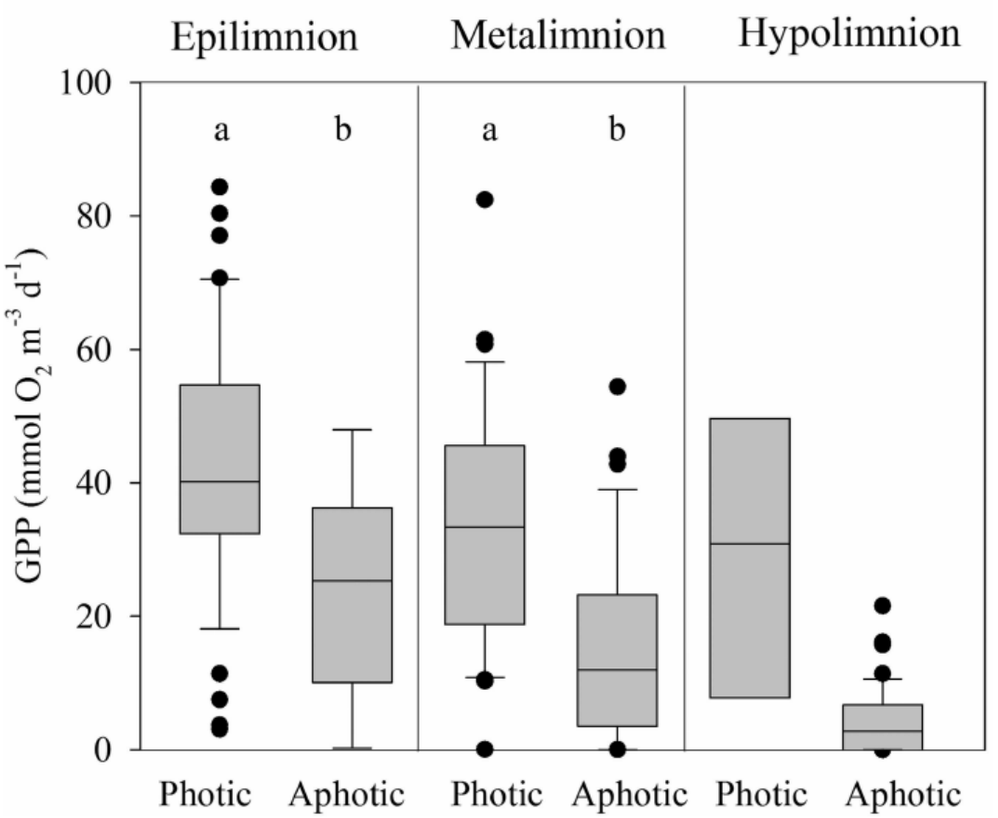

Figure 5. Median and 25th, 75th percentile boxes with 10th, 90th percentile whiskers of GPP (mmol O_2 m^{-3} d^{-1}) for the epi-, meta, and hypolimnion of Hampen Lake depending on whether the layers are or not in the photic zone. Dots are outliers and the lower case letters denotes the different groups according to Tukey post test. Hypolimnion data was left out of the analysis as they did not pass the test of variance homogeneity.

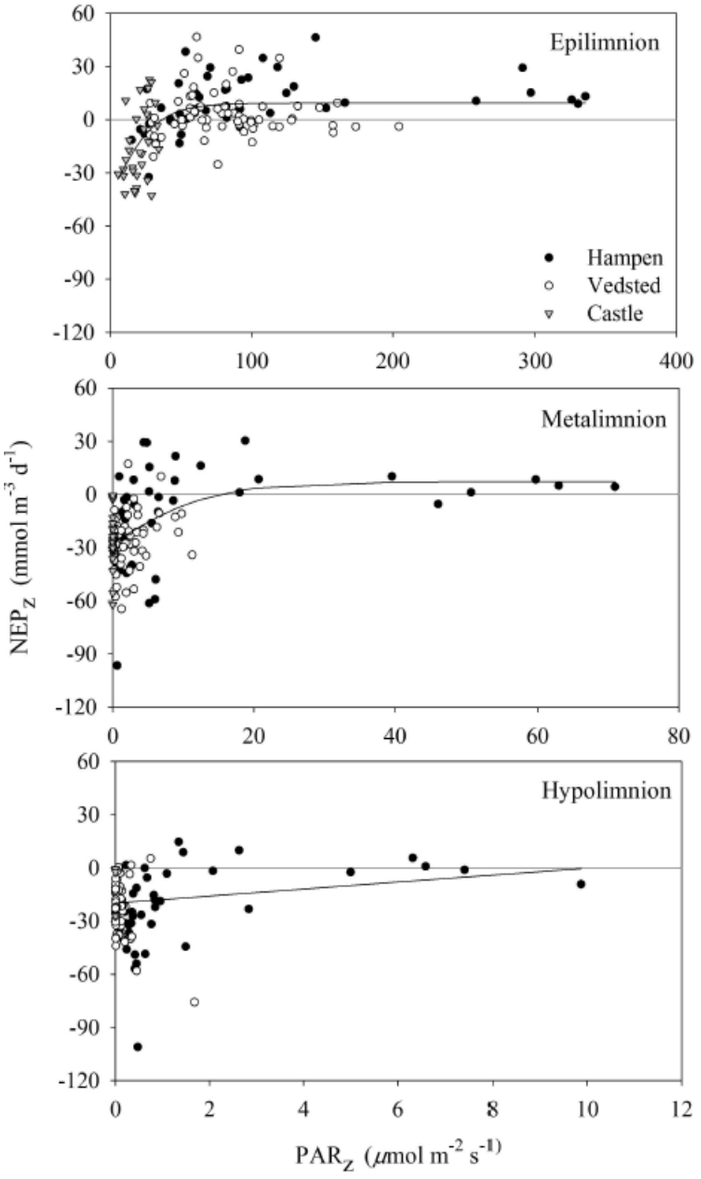

Figure 6. Relationship between daily specific NEP (mmol m^{-3} d^{-1}) and mean daily available PAR in each depth layer. The line corresponds to the fitted model at a daily scale, pooling the three lakes (*see* Table 4).

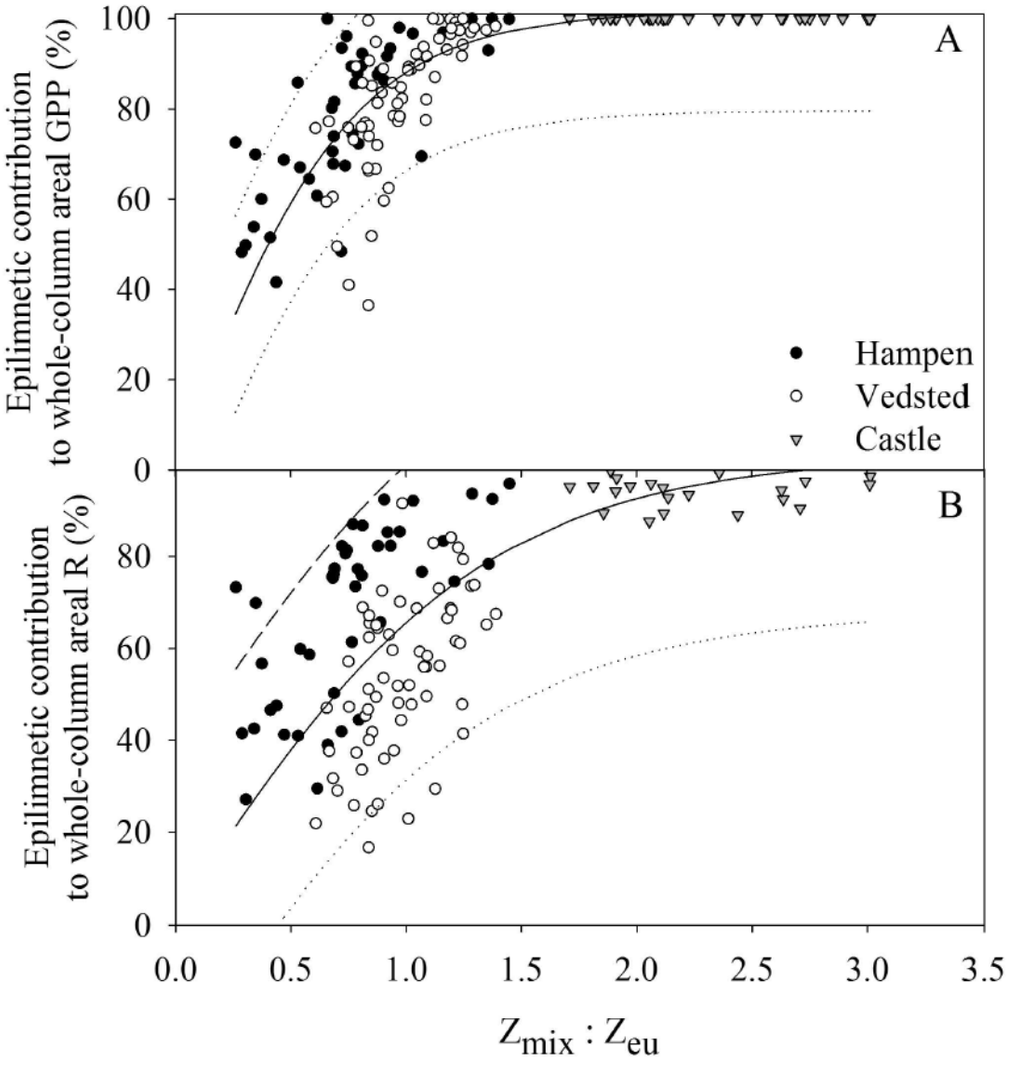

Figure 7. Relative contribution of epilimnetic metabolism to whole-column areal (A) GPP and (B) R in relation with the ratio $Z_{mix} : Z_{eu}$. The lines correspond to a fitted hyperbolic function with 95% confidence intervals (dotted).