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RESEARCH ARTICLE

Short-term variation in thermal stratification complicates estimation of lake metabolism

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Abstract Previous studies have used sondes to measure diel changes in dissolved oxygen and thereby estimate gross primary production (GPP), ecosystem respiration (R), and net ecosystem production (NEP). Most of these studies estimate rates for the surface layer and require knowing the depth of the mixed layer (Z_{mix}), which is usually determined from discrete daily or weekly temperature profiles. However, Z_{mix} is dynamic, as the thermal structure of lakes may change at scales of minutes rather than days or weeks. We studied two thermally stratified lakes that exhibited intermittent microstratification in the mixed layer. We combined sonde-based estimates of metabolism with high-frequency measurements of stratification using thermistor chains to determine how the short-term dynamics of stratification affect metabolic rates. We calculated estimates of metabolism using time series of Z_{mix} measured at seasonal, weekly, daily, and 5-min intervals. Areal rates of GPP and R were up to 24 and 29% less, respectively, using the 5-min measurements of Z_{mix} rather than weekly Z_{mix}, while NEP was not significantly different. These reduced areal rates are mostly the consequence of the reduction in the depth of the mixed layer. Microstratification occurred frequently in both lakes and affected volumetric rates in one lake where R was significantly lower, NEP was significantly higher, and GPP was marginally lower compared to days without microstratification. Hence, microstratification not only affects the depth of the mixed layer, but also alters the processes that influence photosynthesis and respiration. Future studies should consider microstratification and possibly employ multiple sondes with thermistor chains that enable integrating metabolic rates to a specific depth, rather than assuming a stable upper mixed layer as the basis for calculations.

Keywords Lake metabolism · Gross primary production · Respiration · Net ecosystem production · Stratification

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Introduction

Rates of ecosystem metabolism are important parameters that integrate biologic activity in the system and respond to changes in key system drivers. Ecosystem metabolism consists of gross primary production (GPP), ecosystem respiration (R), and net ecosystem production where net ecosystem production (NEP) = GPP − R (Odum 1956; Lovett et al. 2006). While lake ecosystem metabolism was commonly estimated using bottle incubations, free-water techniques using high frequency data from dissolved oxygen (DO) sondes have generally replaced those methods because they are less cumbersome, more integrative across the entire ecosystem, and avoid artifacts caused by containment (Lauster et al. 2006). Furthermore, sondes are able to sample at frequencies of minutes instead of hours or days as with bottle methods. Consequently, high-frequency sampling allows for greater accuracy than discrete bottle measurements. Due to limited sampling, discrete measurements may not detect short-term variability and

therefore miss important events, which could potentially cause erroneous conclusions about rates as well as assessment of whether a lake is net autotrophic or heterotrophic (Staehr and Sand-Jensen 2007). Furthermore, the use of sondes has allowed researchers to measure metabolism in a wide variety of aquatic systems (e.g. Caraco and Cole 2002; Hanson et al. 2003; Hagerthey et al. 2010).

To date, most studies using sonde methods have focused on the upper mixed layer of lakes because this zone usually has the highest rates of metabolism and detectable diel oxygen signals (Coloso et al. 2008). The calculation of metabolism from sondes requires several components: the change in DO concentration during a time interval; an estimate of gas exchange across the air–water interface; and the depth of the layer over which the sondes are able to detect changes in DO (Z_{meas}). Sondes deployed within the upper mixed layer of a lake are typically assumed to measure that entire layer such that Z_{meas} equals the depth of the mixed layer (Z_{mix}). In metabolism calculations, the volumetric rate of DO change is converted to an areal rate by multiplying by Z_{mix}. This conversion is required so that gas exchange, which is inherently an areal process, can be accounted for as a non-metabolic cause of DO change. Rates of GPP, R, and NEP are then either expressed on an areal basis or converted back to volumetric rates by dividing by Z_{mix}. Therefore, Z_{mix} is an essential parameter for estimating metabolic rates within the upper mixed layer because it defines the layer of measurement and affects the calculation of gas exchange.

Despite the importance of Z_{mix}, previous studies have often used discrete weekly temperature profiles to assess mixing depth (Lauster et al. 2006; Van de Bogert et al. 2007; Coloso et al. 2008), although the use of high-frequency temperature profiles is increasing (Staehr and Sand-Jensen 2007; Hanson et al. 2008; Staehr et al. 2010). Weekly estimates of Z_{mix} are interpolated to daily or sub-daily time series for use in a model to estimate daily rates of metabolism. However, the problem with using discrete measurements of Z_{mix} in metabolic calculations is that lake stratification is dynamic and changes can occur over time scales of minutes (Imberger 1985; MacIntyre et al. 2006). Discrete measures of thermal stratification miss the deepening or shallowing of the mixed layer that can occur on a diel basis (Imberger 1985). Furthermore, discrete measures will also miss any temporary, small-scale stratification that can develop within the more stably stratified mixed layer (Imberger 1985; Ishikawa and Tanaka 1993; MacIntyre 1993). This phenomenon, referred to here as microstratification, can have important impacts on water movement within the mixed layer as well as gas exchange with the atmosphere (Ishikawa and Tanaka 1993; MacIntyre 1993). For example, microstratification influences CO_2 concentrations in surface waters (Åberg et al. 2010). While thermal stratification is known to be dynamic, it is unknown how that dynamic nature affects rates of metabolism estimated from diel changes in oxygen concentrations.

Dynamic stratification should impact the calculation of areal rates, and it could also influence volumetric rates by altering the processes controlling metabolic rates. Estimates of areal rates will differ simply due to the use of a different measure of depth over which metabolism estimates are integrated. Furthermore, prior studies have generally assumed that a sonde was always in the mixed layer (e.g. Cole et al. 2000), when in reality the sonde may have been below the mixed layer for periods of hours or even days. Consequently, inaccurate measurements of thermal stratification may have caused errors in estimates of areal metabolism. High-frequency changes in thermal structure due to microstratification could affect the volumetric rates of metabolism due to changes in the phytoplankton light regime, nutrient availability, and gas exchange with the atmosphere. Accounting for these short-term changes in lake stratification when estimating rates of lake metabolism should allow for more accurate estimates, which can be used to better understand the underlying drivers of ecosystem processes.

In this study, we measured the thermal structure of two small temperate lakes at weekly, daily, and 5-min intervals and evaluated the impacts of these different measures of thermal structure on metabolic rates. Z_{mix} for each time scale was used, along with continuous data on DO, in a metabolism model to examine what sampling frequency is required to detect the short-term dynamics of thermal stratification and how those dynamics affect the calculation of metabolic rates. Additionally, we investigated the importance of microstratification to metabolic rates and gas exchange with the atmosphere. Specifically, we tested the hypothesis that volumetric rates of GPP, R, and NEP differ between sampling dates with and without microstratification.

Methods

Study site

We sampled Peter and Paul lakes during the summer of 2009. These lakes are located at the University of Notre Dame Environmental Research Center in Michigan's Upper Peninsula (89°30.2′W, 46°15.1′N). Peter and Paul are small (2.6 and 1.7 ha, respectively) but relatively deep lakes with maximum depths of 19.6 and 15.0 m, respectively. The lakes, originally two lobes of an hour-glass shaped single lake, were separated by an impermeable dike built in 1951. Both lakes are strongly stratified during the summer season and have sparse to no submerged

macrophytes. In both lakes, the euphotic zone extends beyond the depth of the upper mixed layer. Mean summertime mixed-layer values from 2005 to 2009 indicate that Peter and Paul lakes have moderate concentrations of chlorophyll *a* (5.1 and 4.0 μg L^{-1}, respectively), low nutrients (9.7 and 8.8 μg L^{-1} total phosphorus, respectively), and moderate levels of dissolved organic carbon (5.6 and 4.5 mg C L^{-1}, respectively). These lakes are described in greater detail by Carpenter and Kitchell (1993).

Thermal stratification

High-frequency measurements of thermal stratification were made using thermistor chains composed of NexSens T-node thermistors and data logger (model SDL500). The chains were deployed from a buoy moored near the center of each lake. Thermistors were placed at half meter intervals from 0.5 to 4 m with an additional thermistor at 5 m. The maximum depth of the mixed layer during summer is typically 4 m in both lakes, thus the thermistor chains were designed to continuously capture the dynamics of the mixed layer. The thermistor chains recorded temperature profiles at 5-min intervals. Additionally, manual temperature profiles were taken once a week at approximately 09:00 using a YSI Professional Plus meter.

The depth of the mixed layer (Z_{mix}) was calculated as the depth at which the temperature changed at least 1°C per half meter depth interval. We chose this depth interval based on experience with the temperature profiles in these lakes. Other definitions of Z_{mix} are possible, but we did not compare alternatives as our main goal was to evaluate how changes in the mixed layer, by whatever definition, impacted metabolic rates. In Peter and Paul lakes, stratification often occurred at multiple depths. In these instances, the shallowest stratified depth was considered to be Z_{mix} as it isolates the surface water from the rest of the lake. From the weekly and high-frequency temperature profiles, time series of Z_{mix} were created at four different time scales for use in the metabolism model (described below). The first time series was a seasonal time series created by holding Z_{mix} constant at 2.6 m, which was the mean of the weekly values of Z_{mix}. The second time series was the manually sampled weekly values of Z_{mix} that were linearly interpolated to daily values as has been done in previous studies (Cole et al. 2000; Lauster et al. 2006; Coloso et al. 2008). The third times series comprised daily Z_{mix} values created by extracting daily profiles from the high-frequency temperature data to simulate a manually collected profile and measurement of Z_{mix}. We used the profile sampled at 09:00, which is similar to the time of the weekly profiles. The fourth time series was the high-frequency values of Z_{mix} determined directly from the 5-min temperature profiles.

The thermal structure of the lakes was also examined by calculating water column stability (E). Stability is a measure of the resistance of a water molecule to vertical movement within the water column (Knauss 1997) and was calculated for each depth as

$$E = \frac{1}{\rho_0}\frac{\partial \rho}{\partial z} - \frac{g}{c^2} \quad (1)$$

Where ρ_0 is a reference density (1 kg L^{-1}), ρ is the density at depth z, g is gravitational acceleration, and c is the speed of sound in water. Density was calculated for each depth from the temperature using the following equation (Kalff 2002)

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

where T is the water temperature in °C. High values of stability indicate stratification, whereas low stability signifies mixing.

Dissolved oxygen and metabolism model

Daily estimates of metabolism were made from high-frequency measurements of DO. The metabolism model used to calculate metabolic rates from DO is described below (Coloso et al. 2008). DO measurements were made using YSI 6600 V2 sondes fitted with optical DO probes (model 6150) and combined temperature and conductivity probes (model 6560). The DO probes were calibrated in air-saturated water before and after sonde deployment. These calibrations were used to compensate for sensor drift, which was assumed to be linear. The sondes were deployed at a depth of 0.7 m from the same buoys as the thermistor chains. Each week the sondes were removed from the lakes for calibration, cleaning, data retrieval, and other maintenance. In order to maintain continuous data collection, a second sonde was deployed at the same time as the first sonde was removed from each lake. Therefore, four sondes were used to provide continuous sampling at the two lakes. The deployment of each sonde was randomized to prevent any potential bias from an individual sonde. The sondes were programmed to record DO and temperature every 5 min for the duration of the summer.

High-frequency DO measurements were used to calculate estimates of GPP, ecosystem R, and NEP using a model written in MATLAB (The MathWorks Inc., version 7.6). The model is outlined by Cole et al. (2000), which uses the basic approach originally described by Odum (1956) in which

$$\frac{dDO}{dt} = GPP - R + Ex \quad (3)$$

Where $\frac{dDO}{dt}$ is the change in DO over each time interval (converted to areal units by dividing by Z_{mix}), and *Ex* is the

diffusive exchange of oxygen with the atmosphere. All terms in the equation are expressed in areal units (mmol O_2 m^{-2} min^{-1}). This model has been used extensively over the last 50 years (McIntire et al. 1964; Bott et al. 1985; Hagerthey et al. 2010) and, despite its simplicity, provides estimates of metabolic rates that compare well with more complicated models (Hanson et al. 2008).

From Eq. 3, NEP (NEP = GPP − R) can be calculated for each time interval if *Ex* is known. *Ex* can be calculated using the following equation.

$$Ex = k(DO_{sat} - DO_w) \quad (4)$$

Ex is a function of the piston velocity (*k*) and the difference between the equilibrium DO concentration (DO_{sat}) and the actual DO concentration of the lake (DO_w). The piston velocity, *k*, drives the rate of gas exchange between the lake and the atmosphere. For each time interval, *k* is derived from k_{600} (Jahne et al. 1987), which is a normalized piston velocity that is calculated directly from wind speed (Cole and Caraco 1998).

As mentioned above, in order to calculate NEP, $\frac{dDO}{dt}$ must be converted from a volumetric rate to an areal rate so that *Ex*, which is an areal rate, can be subtracted. To make this conversion, the depth over which the sonde measures oxygen (Z_{meas}) must be determined. This conversion is required even when using Bayesian or other similar statistical models to estimate metabolism (Holtgrieve et al. 2010). Since the sondes in this study were deployed in the mixed layer, we assumed the sondes measured the oxygen concentration of the entire mixed layer from the surface to Z_{mix} (i.e. $Z_{meas} = Z_{mix}$). In some instances the sondes were below the mixed layer such that Z_{meas} was not equal to Z_{mix} (see below). In either case, $\frac{dDO}{dt}$ is multiplied by Z_{meas} to get areal rates, which can then be divided by Z_{meas} to get volumetric rates. Therefore, the depth that the sonde is assumed to measure affects areal but not volumetric rates of metabolism.

Once NEP is calculated for each time interval, the rate of nighttime respiration (R_{night}) can be calculated by assuming that, because there is no nighttime GPP, nighttime NEP is equal to R_{night}. Daytime respiration (R_{day}) cannot be directly measured because during the day NEP is due to both GPP and R. Therefore, we assumed that R_{day} is equal to R_{night} (Cole et al. 2000; Lauster et al. 2006; Coloso et al. 2008) despite the fact that R_{day} is likely greater than R_{night} (Pace and Prairie 2005). This difference between R_{day} and R_{night} would have caused us to equally underestimate both GPP and R, but it would not have affected our estimates of NEP. The rates of R_{day} and R_{night} for every 5-min interval were averaged and then multiplied by the hours of daylight or darkness to get a daily (24 h) rate of R. For each day, GPP was calculated as the sum of R_{day} and daytime NEP. The daily value of NEP was then calculated as the difference between the daily rates of GPP and R.

One consequence of using the 5-min Z_{mix} values in the metabolism model is that often Z_{mix} was shallower than the depth of the sondes. This means that during those periods the sondes were below the apparent mixed layer and therefore Z_{meas} was different than Z_{mix}. In these instances, Z_{meas} was set to be the distance between Z_{mix} and the next depth where the change in temperature was at least 1°C over a half meter. Additionally, when the sondes were below the mixed layer they were cut off from the atmosphere, resulting in reduced or no gas exchange with the atmosphere. To account for this limitation on diffusion, the metabolism model was run a second time with the 5-min Z_{mix} data using a switch that set the gas exchange to zero whenever Z_{mix} was shallower than the sonde depth.

Estimates of metabolism made here only included GPP and R that occurred within the apparent mixed layer of the lake because the sondes were only deployed in the surface waters. However, GPP and R also occur below the mixed layer (Coloso et al. 2008). Furthermore, the centrally located sondes in this study may have missed some portion of littoral metabolism (Van de Bogert et al. 2007). Hence, the values reported in this study represent mixed layer metabolism for the pelagic portion of the lake.

Statistical analysis

Estimates of GPP, R, and NEP in both Peter and Paul lakes were made using each of the four time scales described above (5 min, daily, weekly, seasonal). Analysis of variance (ANOVA) was used to test for differences between the estimates made with differing Z_{mix}. Separate ANOVAs were run for each metabolic parameter in each lake and significant results were examined further through pairwise multiple comparisons using Tukey's tests. *t* tests were used to compare estimates of metabolism made with and without the switch to turn off gas exchange whenever Z_{mix} was less than the sonde depth. Finally, *t* tests were used to compare metabolic rates on days when microstratification did and did not occur. Days when microstratification occurred were determined to be those days during which Z_{mix} was shallower than the sonde depth for at least one consecutive hour. All statistical analyses were done in SAS (SAS Institute, version 9.1) using the MIXED and TTEST procedures. The dependent variable in each test (GPP, R, NEP) was square root or log transformed when necessary to meet normality and homogeneity of variance assumptions.

The above tests frequently had moderate levels of positive autocorrelation in their residuals (autocorrelations at lag 1 in the range of 0.1–0.7 with a mean of 0.4), which

suggests the data were not independent. Positive autocorrelation can be a problem as it can increase the probability of a type 1 error (Box 1954). We ran a simulation to assess the potential effect of the autocorrelation on the statistical tests. We resampled the data by randomly assigning which days were microstratified while maintaining the original ratio of microstratified days to days without microstratification (i.e. we did not change the number of days with microstratification, but rather simply changed which days were microstratified). The dependent variable (GPP, R, NEP) was unchanged and thus the residuals remained autocorrelated. We then calculated the difference between the randomly assigned microstratified and non-microstratified groups of the dependent variable for each resampling. The resampling process was iterated 10,000 times to create a distribution of the among group differences. The resulting distribution was always highly normal despite the presence of autocorrelation. Furthermore, the probability of a difference in the simulated distribution as large as the observed difference was very similar to the observed p value from the standard ANOVA or t test initially employed. If the autocorrelation did increase the probability of a type 1 error, the simulated distribution would not be normal (it would have fatter tails indicating a higher probability of finding significantly large differences) and the observed p value would be significantly smaller than the simulated p value. Thus, the autocorrelation in the data did not increase the probability of a type 1 error, so all statistical tests were evaluated at the standard alpha value of 0.05.

Results

Microstratification

Both Peter and Paul Lakes were stably stratified for the entire study period, in that conventional vertical stratification persisted throughout the summer without any periods of whole-lake mixing. However, the stratification in the upper waters of these lakes was not static, but rather was quite dynamic at multiple time scales. For example, high-frequency temperature profiles and values of water column stability during a particularly dynamic period in Peter Lake (Fig. 1) fluctuated in the shallow depths at the diel scale with warming during the day and cooling at night. The surface water microstratified between the 0.5 and 1 m depths and alternated between stable, stratified conditions during the day and unstable mixing at night. Microstratification occurred on 72 and 55% of the days in Paul and Peter Lakes, respectively. The temperature and stability of the shallow depths were also dynamic at the time scale of several days to a week as the lakes underwent warming or cooling periods associated with weather conditions. The deeper depths (below 2.5 m in Peter Lake, Fig. 1) continually warmed for the majority of the summer and were unaffected by the diel or multi-day dynamics. The thermal dynamics in Paul Lake were similar to those in Peter Lake during the period highlighted in Fig. 1 and throughout the summer.

Estimates of Z_{mix} reflected the dynamic nature of the lake thermal structure seen in the temperature and stability

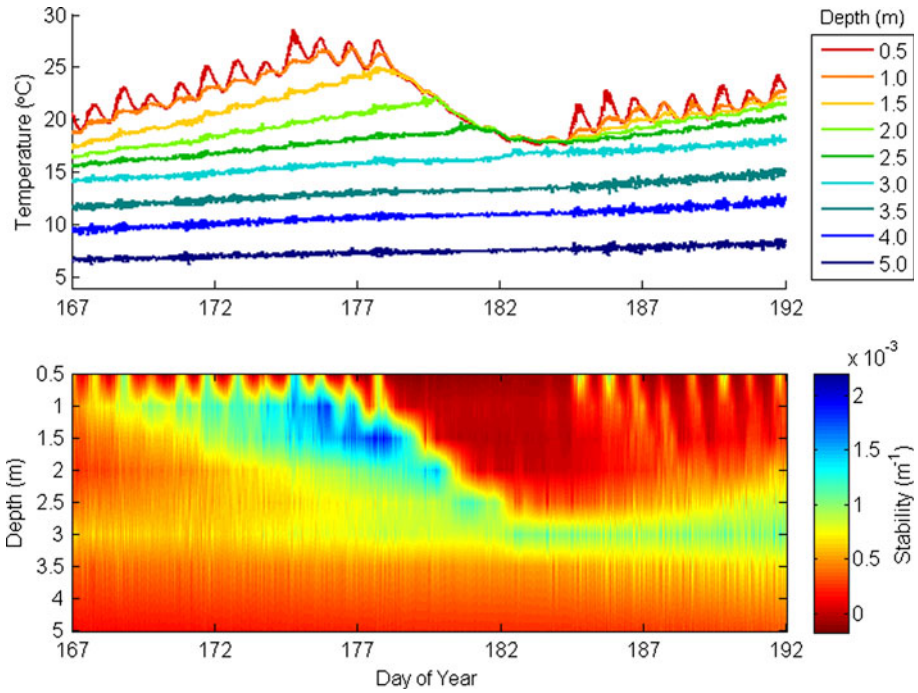

Fig. 1 High-frequency temperature profiles (*top panel*) and water column stability (*bottom panel*) of Peter Lake from June 16 to July 11, 2009. Depths with equal temperatures indicate mixing. Low values of stability (shown here in *red*) indicate mixing while high values of stability (*green* to *blue*) indicate stratification

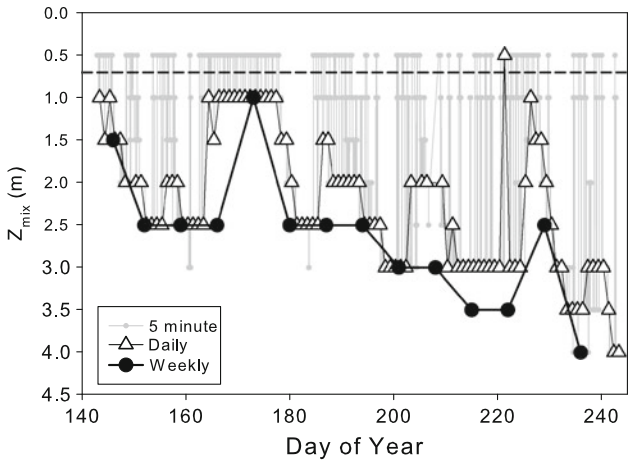

Fig. 2 Measurements of the depth of the mixed layer (Z_{mix}) at various time scales in Peter Lake. The *dashed line* indicates the depth that the sondes were deployed

profiles. However, values of Z_{mix} were quite different depending on the sampling frequency of the temperature profiles (Fig. 2). Weekly measurements of Z_{mix} ranged from 1 to 4 m while daily and 5-min measurements of Z_{mix} ranged from 0.5 to 4 m. All three time scales of Z_{mix} showed a similar deepening trend throughout the summer. Although the three estimates of Z_{mix} agreed on the range and overall trend, they did not equally capture the short-term dynamics. Particularly, weekly Z_{mix} missed most of the periods where Z_{mix} became shallower. Thus, weekly Z_{mix} best represents the maximum depth of the mixed layer over the course of the summer. Daily Z_{mix} captured more of the short-term variation in mixing depth, but still missed much of the short-term decreases. Consequently, the mean 5-min Z_{mix} for the entire dataset was significantly shallower than both the weekly Z_{mix} (0.9 m shallower) and the daily Z_{mix} (0.4–0.5 m shallower) in both Peter and Paul Lakes (t tests, $p < 0.001$).

Effects of Z_{mix} on areal estimates of metabolism

Mean rates of metabolism for the summer were higher in Paul Lake than in Peter Lake, independent of the time scale for Z_{mix}. Using the weekly values of Z_{mix}, metabolic rates in Paul Lake were 35.0, 41.5, and −6.5 mmol O_2 m^{-2} day^{-1} for GPP, R, and NEP, respectively. In Peter Lake, GPP was 30.1, R was 29.3, and NEP was 0.8 mmol O_2 m^{-2} day^{-1}. Metabolic rates in both lakes were quite variable. The coefficient of variation (CV) of daily GPP was 46 and 63% in Paul and Peter Lake, respectively. Rates of R were slightly more variable with CVs of 41% in Paul Lake and 71% in Peter Lake. Rates of NEP were also variable and fluctuated between positive and negative values in both lakes. Thus, although Paul Lake was net heterotrophic and Peter Lake was net autotrophic at the seasonal scale, those distinctions were not as clear at the daily time scale as the lakes could be net autotrophic one day and net heterotrophic the next day. While the rates of metabolism were variable at the daily time scale, there were no strong seasonal trends in the metabolic rates of either lake.

Comparison of areal metabolic rates using values of Z_{mix} measured at different time scales in both lakes resulted in significantly different rates of GPP and R, but not NEP (Fig. 3; ANOVA, Paul: $n = 96$, $p < 0.01$; Peter: $n = 100$, $p < 0.01$). Tukey's pairwise multiple comparisons tests revealed that for both GPP and R in Peter and Paul Lakes, the estimates using 5-min Z_{mix} were significantly less than the estimates using weekly or seasonal Z_{mix} data ($p < 0.02$; Fig. 3). Estimates of GPP and R from both lakes using Z_{mix} calculated on daily, weekly, and seasonal time scales were not significantly different. As expected, volumetric rates of metabolism made using the various values of Z_{mix} were not significantly different because Z_{mix} was factored out of the volumetric calculation (ANOVA, Paul: $n = 96$, $p > 0.93$; Peter: $n = 100$, $p > 0.64$).

In both Peter and Paul Lakes, areal rates of GPP and R were significantly less on days when microstratification occurred (Fig. 4; t test, Paul: $n = 96$, $p < 0.001$; Peter: $n = 100$, $p < 0.0001$). Rates of NEP were not affected by microstratification in either lake. Much of the difference in areal rates of GPP and R was simply due to differences in the depth over which the sondes measured (Z_{meas}) and hence the depth over which rates were integrated to obtain an areal value. On days when microstratification occurred, the mean Z_{meas} (including times when the sondes were within and below the mixed layer) was 1.6 m, while it was 2.4 m on days when microstratification did not occur.

Effects of Z_{mix} on volumetric estimates of metabolism

In Peter Lake, the volumetric rates of R and NEP, which are not affected by Z_{meas}, were significantly different on days when microstratification occurred (Fig. 5; t test, $n = 100$, $p < 0.01$). Volumetric R was nearly twice as large on days without microstratification than on days with microstratification. The volumetric rate of GPP in Peter Lake was less when microstratification occurred, and the difference approached statistical significance at the 0.05 criteria level (t test, $n = 100$, $p = 0.07$). As a result of reduced R, the rate of NEP in Peter Lake was much larger on days with microstratification, suggesting relative greater autotrophic production. These differences in metabolic rates were not consistent between lakes as the volumetric rates in Paul Lake were not significantly different on days with microstratification.

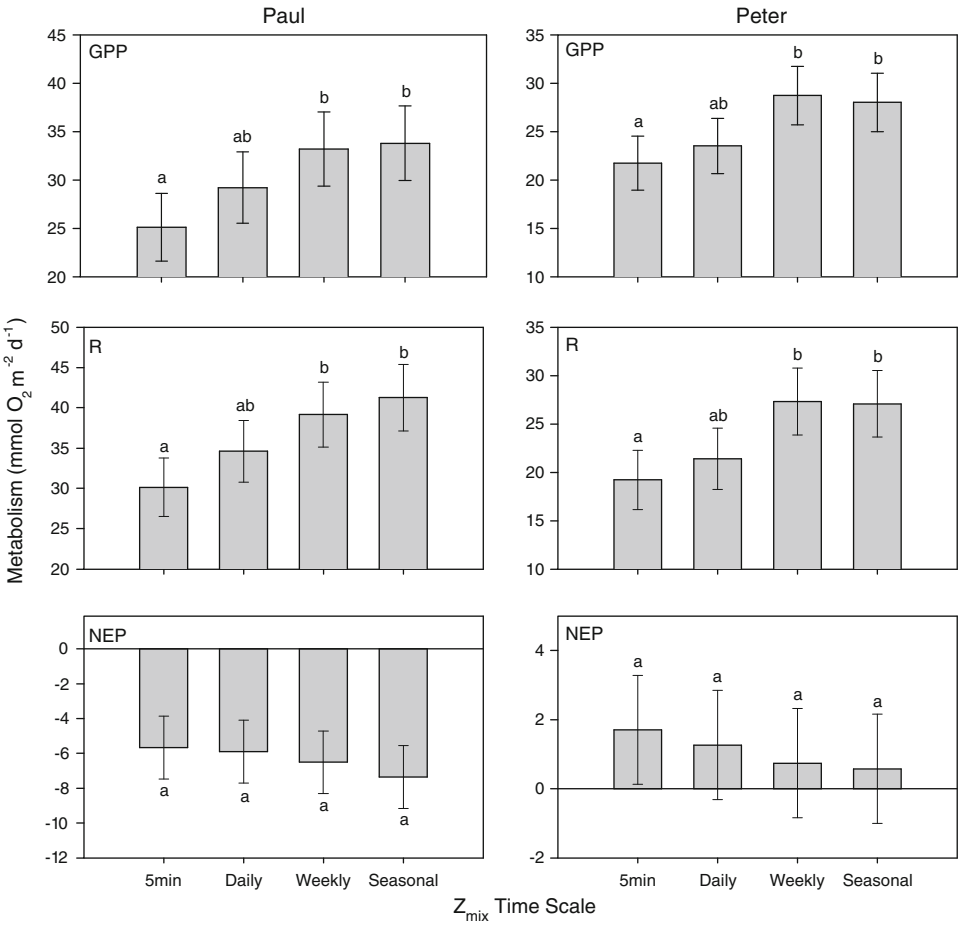

Fig. 3 Mean areal rates of metabolism for Paul and Peter Lakes using the various time scales of Z_{mix}. The *right column* of *panels* show estimates from Paul Lake while the *left column* is from Peter Lake. *Error bars* indicate 95% confidence intervals. *Bars* with the *different letters* are significantly different ($p < 0.05$, ANOVA, Tukey's multiple comparison tests)

The differences in the volumetric rates of metabolism associated with microstratification in Peter Lake were not the result of changes in gas exchange with the atmosphere. Estimates of metabolism made with and without the switch in the model that turned off gas exchange whenever microstratification occurred were not significantly different in either lake. When using the switch, mean gas exchange was less on days when microstratification occurred (0.29 compared to 0.52 mmol O_2 m^{-2} day^{-1}). However, this difference did not significantly affect the estimates of metabolism because gas exchange in these lakes is small compared to metabolic rates.

Discussion

High-frequency temperature profiles revealed the dynamic nature of the thermal structure of both Peter and Paul Lakes, which had not previously been documented in these lakes using discrete weekly temperature data. While weekly and daily temperature profiles were able to capture the seasonal range and trend in Z_{mix}, and give a general idea of the maximum mixing depth, these metrics did not provide an accurate picture of the lake thermal structure at any given time. Furthermore, discrete temperature profiles are affected by the time of day that they are measured. The morning profiles used in this study tended to capture the deepest Z_{mix} of the day. However, if profiles were taken in the late afternoon when surface temperatures were typically at their highest, estimates of Z_{mix} would be much shallower. In Peter Lake, the mean of daily Z_{mix} values calculated from profiles taken at 17:00 was 1.3 m, which is 0.9 m shallower than the mean of the daily 09:00 Z_{mix} values and 0.5 m shallower than the mean of the 5-min Z_{mix} data. Rates of GPP and R calculated using the 17:00 daily Z_{mix} data are significantly less than the rates calculated from the 09:00 daily Z_{mix} (t test, $n = 100$, $p \leq 0.0001$). Furthermore, using the 17:00 daily Z_{mix} data would have altered the results of this study by producing significantly lower rates of GPP and R than rates using the 5-min Z_{mix} data (t test, $n = 100$, $p = 0.006$ and $p = 0.0045$ for GPP and R, respectively). Thus, discrete measurements are dependent on the time of sampling and are not good estimates of Z_{mix}. If high-frequency measures of Z_{mix} are unavailable, averaging two discrete weekly measurements of Z_{mix} taken on the same day during

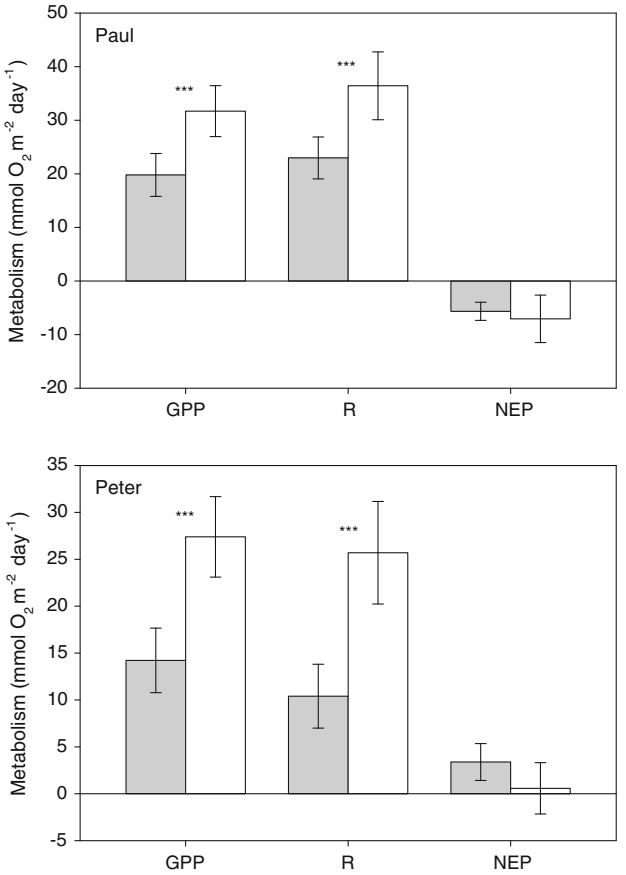

Fig. 4 Mean areal rates of metabolism in Paul and Peter Lakes on days with microstratification (*shaded bars*) and without microstratification (*open bars*). *Error bars* indicate 95% confidence intervals. Significant differences in metabolic rates between days with and without microstratification are indicated by $***p < 0.001$

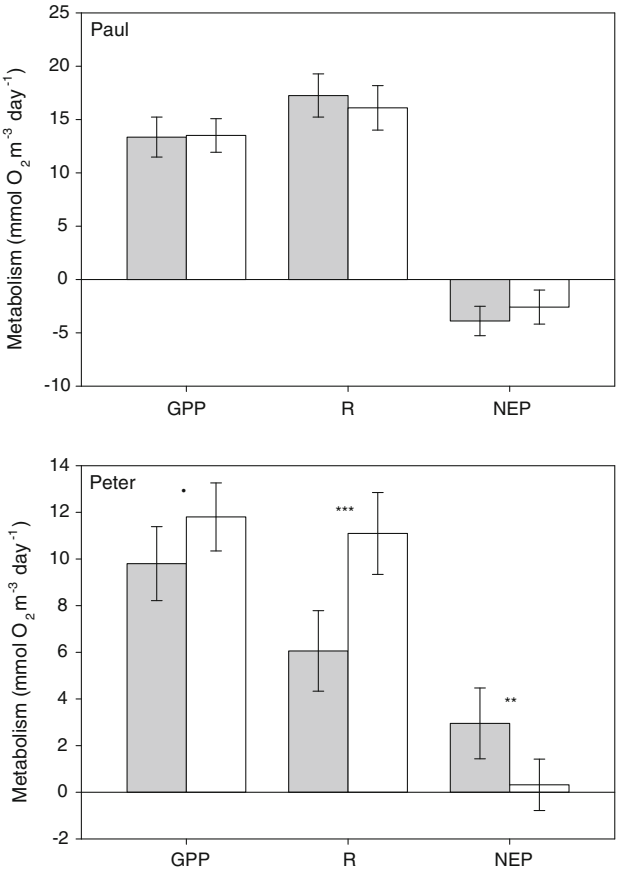

Fig. 5 Mean volumetric rates of metabolism in Paul and Peter Lakes on days with microstratification (*shaded bars*) and without microstratification (*open bars*). *Error bars* indicate 95% confidence intervals. Significant differences in metabolic rates between days with and without microstratification are indicated by *filled circle* ($0.05 < p < 0.1$), $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$

maximum and minimum mixing depths (at 09:00 and 17:00, respectively, in this case) may suffice, as it resulted in similar estimates of GPP, R, and NEP as the estimates using high-frequency Z_{mix} (t test, $n = 100$, $p \geq 0.46$). However, high-frequency measurements are needed to ensure the most accurate picture of lake thermal structure, which is important for estimating lake metabolism.

The dynamic nature of thermal stratification can affect the estimates of metabolism in three ways. The first two of these simply affect the computation of metabolism in the upper mixed layer. First, in the simplest case, if the short-term mixed layer is deeper or shallower than that assumed from longer-term profiles, then areal metabolism (the product of a volumetric measurement of the rate of DO change and the depth of the mixed layer) will be directly affected. Second, microstratification in the surface waters can isolate the sonde from the atmosphere and prevent gas exchange from occurring. This would result in less gas exchange than what would be assumed using discrete temperature sampling and would cause errors in the metabolism estimates. Third, changes in the depth of the mixed layer are actually associated with changes in the rate of metabolism within the upper mixed layer. This latter change would be caused by effects from mixing that influence rates, for example changes in light or nutrient concentrations. In this study we saw all three effects, although the effect of gas exchange was negligible as discussed below.

Areal estimates of mixed-layer metabolism are dependent on the frequency at which Z_{mix} is measured. In both Peter and Paul lakes, areal rates of GPP and R were significantly less when 5-min values of Z_{mix} were used rather than the traditional weekly Z_{mix}. Using 5-min Z_{mix} resulted in estimates of GPP that were 24% lower and estimates of R that were 23–29% lower in the two lakes. These decreased areal rates occur because weekly Z_{mix} overestimated the size of the mixed layer and therefore the sonde measured less water than what was assumed. These results are similar to those of Gelda and Effler (2002) who used

several methods to determine the depth over which they integrated metabolism and found that areal rates of metabolism are lower when the depth of integration decreases. Using high-frequency measures of Z_{mix} decreased the depth of integration as the mean Z_{meas} for the summer was about 0.75 m shallower when 5-min Z_{mix} was used than with weekly Z_{mix}. Surprisingly, estimates of metabolism using the constant seasonal value of Z_{mix} were nearly identical to those made using weekly values of Z_{mix}. This indicates that the weekly changes in Z_{mix} have little impact on the daily rates of metabolism.

Microstratification isolates the sonde from the atmosphere and thus reduces or prevents gas exchange from occurring, which could alter estimates of metabolism. However, rates of metabolism were not significantly different when calculated with and without a switch in the model that turned off gas exchange whenever microstratification occurred. The on/off nature of the switch used in the model was chosen to produce the maximum possible effect. Therefore, other more complicated switches would have resulted in even less of a difference in metabolic rates. In Peter and Paul Lakes, gas exchange with the atmosphere was negligible as rates were over an order of magnitude less than rates of metabolism. This is because the piston velocity was small due to low wind speeds, and DO concentrations were typically close to saturation. Peter and Paul are small lakes that are sheltered from the wind by forested watersheds and thus they have small fetches. The maximum 5 min averaged wind speed observed on these lakes was only 2.1 m s^{-1}, while the mean summer wind speed was 1.1 m s^{-1}. Gas exchange may be more important in other lakes that are larger and receive more wind (MacIntyre et al. 1995), and thus the effect of microstratification on gas exchange and estimates of metabolism may be significant.

Changes in the mixing depth due to microstratification had a significant impact on estimates of volumetric metabolism in Peter Lake. The volumetric rate of R was significantly less with microstratification, indicating that not only was there less R because the sondes measured less water, but also the actual rate of R was lower when microstratification occurred. The decrease in metabolic rates was unrelated to changes in gas exchange caused by microstratification as discussed above. This finding suggests that extrapolating rates measured for Z_{meas} to weekly Z_{mix} on dates of microstratification would lead to inaccurate estimates of R and possibly GPP (as the difference was nearly significant) in Peter Lake. Microstratification had no effect on volumetric rates of metabolism in Paul Lake, suggesting that the change in areal metabolism was solely due to the change in Z_{meas}. In Paul Lake, extrapolating rates for Z_{meas} to weekly Z_{mix} on days of microstratification might be reasonable especially if trial measurements of rates at more than one depth in the mixed layer proved similar.

The decreased volumetric rates of R that occurred in Peter Lake on days of microstratification likely occurred due to several processes. When the mixed layer deepens it entrains portions of the lower epilimnion where nutrients and microbial heterotrophic activity are often higher (Eckert et al. 2002), causing higher rates of R. Microstratification increases water column stability which reduces the mixing scales and prevents deeper mixing and the associated fluxes of nutrients and microbial substrates (Imberger 1985; Ishikawa and Tanaka 1993), thus leading to lower rates of R. Furthermore, deeper mixing results in a larger surface area of sediments within the mixed layer. When Z_{mix} in Peter lake deepens from 2 to 3 m, the area of sediment within the mixed layer increases by 41%. Benthic rates of metabolism are substantial and have been found to be a significant part of whole-lake metabolism for lakes of the size considered in this study (Vadeboncoeur et al. 2002, 2008). When microstratification occurs, there is less sediment in the mixed layer and thus the rate of R is lower. It is likely that the lack of deep mixing during microstratification also caused the decrease in GPP that was observed in Peter Lake, although that difference was smaller than the difference in R and was only border-line significant. In addition to reduced benthic production and lower nutrient availability, the decrease in GPP could be due to changes in light. Microstratification confines phytoplankton in the surface waters under a higher and relatively constant light regime. Photosynthetic rates can be higher with a fluctuating light regime that occurs with deeper mixing (Marra 1978a, b). This is possible in Peter and Paul Lakes because their photic zones always penetrate below Z_{mix} (mean depth of 1% surface light is 6 m, while maximum Z_{mix} was 4 m), so deeper mixing does not remove phytoplankton from the photic zone. The significant increase of NEP in Peter Lake was due to the larger rates of R relative to GPP that occurred with microstratification.

The different response to microstratification between Peter and Paul Lakes may be due to differences in morphometry. Peter Lake deepens quite linearly, whereas the margins of Paul Lake contain a shallow shelf in many places, which results in an overall shallower littoral zone. Consequently, most of the sediments in the littoral zone of Paul Lake are always within the mixed layer. When Z_{mix} deepens from 2 to 3 m, the area of the littoral zone increases by only 29% in Paul Lake, compared to 41% in Peter Lake. Therefore, the fluctuations in Z_{mix} caused by microstratification have less of an impact on the surface area of sediments within the mixed layer, and thus the rates of metabolism, in Paul Lake than in Peter Lake.

The results of this study suggest that previous studies using discrete measurements of Z_{mix} may have

overestimated the depth of the mixed layer and consequently overestimated the amount of GPP and R that occur within the mixed layer. Some of the GPP and R measured in earlier work likely occurred below the mixed layer. The effect of high-frequency fluctuations of Z_{mix} on whole-lake metabolism (i.e. rates integrated across the entire water column) is uncertain. It is possible that decreases in mixed-layer metabolism due to shallowing of the mixed layer were balanced by increases in metabolism below the mixed layer. Coloso et al. (2008) used sondes deployed at multiple depths to produce depth-integrated measurements of metabolism and reported that a large portion of whole-lake metabolism can occur below the mixed layer (up to 28% of GPP and 43% of R). If they had used high-frequency measurements of Z_{mix} rather than weekly measurements, their deeper sondes may not have been in the mixed layer as often as they thought. This may have caused them to find an even larger portion of whole-lake metabolism occurring below the mixed layer. However, Coloso et al. (2008) studied a larger lake (25 ha) where thermal stratification may not have been as dynamic. Microstratification may be less frequent and more transitory in larger lakes with greater fetches relative to the small, low-wind systems considered in this study (Hanson et al. 2008). Nonetheless, in addition to deploying multiple sondes over depth, we suggest that future studies use high-frequency measurements of thermal stratification when calculating lake metabolism. We also recommend that future studies avoid using the upper-mixed layer as the basis for calculations and instead use data from multiple sondes to report metabolism integrated to fixed depths (e.g. the seasonal mean Z_{mix}) or defined depths such as the 1 or 10% light level.

It is clear that short-term dynamics in the thermal structure of a lake can have a significant impact on both volumetric metabolic rates and on the calculation of areal rates when the upper-mixed layer is used as the basis of the calculation. Models of metabolism may require a better integration of key physical processes within a lake. Processes like boundary mixing, internal waves, and microstratification can alter the movement of phytoplankton, the vertical and horizontal fluxes of nutrients and dissolved gasses, and gas exchange with the atmosphere (Imberger 1985; MacIntyre et al. 1999; Eckert et al. 2002). These physical dynamics will also vary in significance in relation to lake size and morphometry (Fee et al. 1996). By developing new models that couple these physical processes with the biological processes, we can achieve more integrated and accurate estimates of whole-lake metabolism, which can allow us to better examine the important underlying drivers of these ecosystem processes.

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References

Åberg J, Jansson M, Jonsson A (2010) Importance of water temperature and thermal stratification dynamics for temporal variation of surface water CO_2 in a boreal lake. J Geophys Res 115:G02024. doi:10.1029/2009JG001085

Bott TL, Brock JT, Dunn CS, Naiman RJ, Ovink RW, Peterson RC (1985) Benthic community metabolism in four temperate stream systems: an inter-biome comparison and evaluation of the river continuum concept. Hydrobiologia 123:3–45

Box GEP (1954) Some theorems on quadratic forms applied in the study of analysis of variance problems, II. Effects of inequality of variance and of correlation between errors in the two-way classification. Ann Math Stat 25:484–498

Caraco NF, Cole JJ (2002) Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. Ecol Appl 12:1496–1509

Carpenter SR, Kitchell JF (1993) The trophic cascade in lakes. Cambridge University Press, London

Cole JJ, Caraco NF (1998) Atmospheric exchange of carbon dioxide in a low-wind oligotrophic lake measured by the addition of SF6. Limnol Oceanogr 43:647–656

Cole JJ, Pace ML, Carpenter SR, Kitchell JF (2000) Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. Limnol Oceanogr 45:1718–1730

Coloso JJ, Cole JJ, Hanson PC, Pace ML (2008) Depth-integrated, continuous estimates of metabolism in a clear-water lake. Can J Fish Aquat Sci 65:712–722

Eckert WJ, Imberger J, Saggio A (2002) Biogeochemical response to physical forcing in the water column of a warm monomictic lake. Biogeochemistry 61:291–307

Fee EJ, Hecky RE, Kasian SEM, Cruikshank DR (1996) Effects of lake size, water clarity, and climatic variability on mixing depths in Canadian Shield lakes. Limnol Oceanogr 41:912–920

Gelda RK, Effler SW (2002) Metabolic rate estimates for a eutrophic lake from diel dissolved oxygen signals. Hydrobiologia 485:51–66

Hagerthey SE, Cole JJ, Kilbane D (2010) Aquatic metabolism in the Everglades: dominance of water column heterotrophy. Limnol Oceanogr 55:653–666

Hanson PC, Bade DL, Carpenter SR, Kratz TK (2003) Lake metabolism: relationships with dissolved organic carbon and phosphorus. Limnol Oceanogr 48:1112–1119

Hanson PC, Carpenter SR, Kimura N, Chin W, Cornelius SP, Kratz TK (2008) Evaluation of metabolism models for free-water dissolved oxygen methods in lakes. Limnol Oceanogr Methods 6:454–465

Holtgrieve GW, Schindler DE, Branch TA, A'mara ZT (2010) Simultaneous quantification of aquatic ecosystem metabolism and reaeration using a Bayesian statistical model of oxygen dynamics. Limnol Oceanogr 55:1047–1063

Imberger J (1985) The diurnal mixed layer. Limnol Oceanogr 30:737–770

Ishikawa T, Tanaka M (1993) Diurnal stratification and its effects on wind-induced currents and water qualities in Lake Kasumigaura, Japan. J Hydraulic Res 31:307–322

Jahne B, Munnich KO, Bosinger R, Dutzi A, Huber W, Libner P (1987) On the parameters influencing air–water gas-exchange. J Geophys Res Oceans 92:1937–1949

Kalff J (2002) Limnology: inland water ecosystems. Prentice-Hall, USA

Knauss JA (1997) Introduction to physical oceanography, 2nd edn. Prentice-Hall, USA

Lauster GH, Hanson PC, Kratz TK (2006) Gross primary production and respiration differences among littoral and pelagic habitats in northern Wisconsin lakes. Can J Fish Aquat Sci 63:1130–1141

Lovett G, Cole J, Pace M (2006) Is net ecosystem production equal to ecosystem carbon accumulation? Ecosystems 9:152–155

MacIntyre S (1993) Vertical mixing in a shallow, eutrophic lake—possible consequences for the light climate of phytoplankton. Limnol Oceanogr 38:798–817

MacIntyre S, Wanninkhof R, Chanton JP (1995) Trace gas exchange across the air-water interface in freshwater and coastal marine environments. In: Matson PA, Harriss RC (eds) Methods in ecology biogenic trace gasses: measuring emissions from soil and water. Blackwell Science, Oxford, pp 52–97

MacIntyre S, Flynn KM, Jellison R, Romero JR (1999) Boundary mixing and nutrient fluxes in Mono Lake, California. Limnol Oceanogr 44:512–529

MacIntyre S, Sickman JO, Goldthwait SA, Kling GW (2006) Physical pathways of nutrient supply in a small, ultraoligotrophic arctic lake during summer stratification. Limnol Oceanogr 51:1107–1124

Marra J (1978a) Phytoplankton photosynthetic response to vertical movement in a mixed layer. Mar Biol 46:203–208

Marra J (1978b) Effect of short-term variations in light intensity on photosynthesis of a marine phytoplankter: a laboratory simulation study. Mar Biol 46:191–202

McIntire CD, Garrison RL, Phinney HK, Warren CE (1964) Primary production in laboratory streams. Limnol Oceanogr 9:92–102

Odum HT (1956) Primary production in flowing waters. Limnol Oceanogr 1:102–117

Pace ML, Prairie YT (2005) Respiration in lakes. In: del Giorgio PA, Williams PJLB (eds) Respiration in aquatic ecosystems. Oxford University Press, USA, pp 103–121

Staehr P, Sand-Jensen K (2007) Temporal dynamics and regulation of lake metabolism. Limnol Oceanogr 52:108–120

Staehr PA, Sand-Jensen K, Raun AL, Nilsson B, Kidmosec J (2010) Drivers of metabolism and net heterotrophy in contrasting lakes. Limnol Oceanogr 55:817–830

Vadeboncoeur Y, Vander Zanden MJ, Lodge DM (2002) Putting the lake back together: reintegrating benthic pathways into lake food web models. Bioscience 52:44–54

Vadeboncoeur Y, Peterson G, Vander Zanden MJ, Kalff J (2008) Benthic algal production across lake size gradients: interactions among morphometry, nutrients, and light. Ecology 89:2542–2552

Van de Bogert MC, Carpenter SR, Cole JJ, Pace ML (2007) Assessing pelagic and benthic metabolism using free water measurements. Limnol Oceanogr Methods 5:145–155