Estimating primary production in lakes: Comparison of 14C incubation and free-water O2 approaches

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: All data and code are available for peer review at: location. Data will be made public with associated DOI if/when the manuscript is accepted and finalized.

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**Running Header**: Comparing 14C and in situ O2 primary production estimates

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# Abstract

Historically, estimates of pelagic primary production in lake ecosystems were made by measuring the uptake of 14C labeled inorganic carbon in samples incubated under laboratory or in situ conditions. However, incubation approaches are increasingly being replaced by methods that analyze diel changes in high-frequency *in situ* data such as free-water dissolved oxygen (O2). While there is a rich literature on the comparison of approaches for estimating primary production using incubations (e.g., 14C and O2 bottle experiments), as well for approaches using high-frequency data (e.g., diel O2 and CO2 metabolism models), there are few direct comparisons of 14C incubations and free-water O2 approaches for estimating primary production. We used 20 lake-years of concurrent measurements of primary production quantified from high frequency free-water O2 data and 14C incubations in four different lakes (4 - 7 years per lake) to compare these different approaches. Across all lakes, 61% of the 14C production estimates were within the 95% credible intervals of the free-water O2 production estimates. Error-in-variable regressions support the assumption that 14C methods estimate a production value between gross primary production and net primary production and the bottle effect is constant across the entire range of production values considered here. There was little evidence that daily pelagic, epilimnetic estimates of primary production differed substantially based on the selection of free-water O2 or 14C approaches in these lakes.

# Introduction

Primary production, the production of organic matter by autotrophs, is a fundamental process that in most ecosystems determines the amount of energy available to higher trophic levels. In the pelagic regions of lakes and reservoirs, oxygenic primary production is typically determined using variants of two basic techniques: measuring the uptake of dissolved inorganic carbon or the production of dissolved oxygen (Hall, Thomas, and Gaiser (2007), but see Peeters et al. (2016; Peeters, Hofmann, and Fernández 2019) as examples of other approaches). These techniques can be applied either in situ or in laboratory incubations and can include measurements in bottles or open water.

Primary production estimates based on uptake of inorganic carbon usually involve labeling a sample of lake water with a known amount of inorganic 14C tracer, incubating the labeled sample under known temperature and light conditions (either in the lake or in the laboratory), and measuring the amount of labeled carbon that is fixed into organic forms by phytoplankton via photosynthesis during the incubation (E. J. Fee 1973; Peterson 1980). This 14C technique has multiple advantages. It can be used in low production systems because the abundance of 14C can be measured precisely (Hall, Thomas, and Gaiser 2007). Samples can be incubated at multiple light levels allowing calculation of production versus light relationships that can be used to estimate pelagic primary production at multiple depths in the lake. Repeated measurements within a year allow for the estimation of annual primary production and dynamics of primary production over time. For these reasons many research programs have compiled long time series of production measurements based on this technique.

The assumptions underlying the 14C approach pose challenges for interpretation of the data and inferences drawn about ecosystems. First, this approach estimates production only of the water placed in the bottle under the environmental conditions in which it is incubated. Consequently, extrapolating production to a region of the lake or the whole ecosystem entails assumptions about the representativeness of those incubations for the region to which they are being extrapolated. Further, because some carbon fixed during the incubation can be respired (Vollenweider, Talling, and Westlake 1974; Peterson 1980), the technique is commonly presumed to underestimate gross primary production (GPP) and may be closer to Net Primary Production (NPP). The magnitude of the underestimation is dependent on incubation time and algal turnover rates (Hall, Thomas, and Gaiser 2007), although there are approaches to account for this underestimation (Legendre et al. 1983). Additionally, the costs associated with specialized training and certifications to handle isotopes, management of radioactive waste, and the time-consuming nature of the incubations constrain the extent to which the methods are applied in most research programs. Nonetheless, this technique has been the standard by which all other approaches have been compared (Peterson 1980).

Over the past 2-3 decades, free-water (e.g., Vachon et al. (2020)) dissolved oxygen (O2) techniques have emerged as a common approach to estimate production of aquatic ecosystems because the data can be readily obtained at high frequencies using *in situ* sensors. While O2 techniques have long been used to estimate production in aquatic systems (Sargent and Austin 1949; Odum 1956; Staehr et al. 2010), the advent of automated sensors capable of making in situ, high frequency measurements of O2 greatly reduced the labor associated with this technique along with providing opportunities to gather the data during difficult sampling conditions, such as storm events or ice breakup. Several models can be used to estimate metabolism from sensor data (Cole et al. 2000; Hanson et al. 2008; Holtgrieve et al. 2013; Batt and Carpenter 2012; Solomon et al. 2013; Phillips 2020). These approaches all assume that biological production and atmospheric exchange drive changes in oxygen (Odum 1956). A major advantage of the free-water O2 approach is that it allows multiple components of metabolism (GPP, ecosystem respiration [R], and net ecosystem production [NEP]) to be estimated simultaneously. Additionally, free-water O2 metabolism estimates can integrate across habitats (e.g., benthic and pelagic production) when the sensor is located in a well-mixed parcel of water that is in contact with these habitats (Van de Bogert et al. 2007). Because of the relative ease of measurement using this technique, many researchers and research groups, such as the Global Lake Ecological Observatory Network (GLEON; Weathers et al. (2013)) have adopted this technique (Solomon et al. 2013). However, estimates based on the free-water O2 approach can be difficult to interpret, because metabolic rates exhibit substantial vertical (Staehr, Christensen, et al. 2012) and horizontal (Van de Bogert et al. 2012) heterogeneity within a lake, and movement of water parcels past the sensor can cause oxygen levels recorded by the sensor to change even in the absence of biological processes (Rose et al. 2014). Furthermore, along with spatial differences in processes, how models account for atmospheric gas exchange (e.g., Dugan et al. (2016)) can lead to noisy high-frequency observations (Batt and Carpenter 2012) or to large and significant changes in estimated metabolism rates between days (Solomon et al. 2013). Therefore, heterogeneity complicates the interpretation of the results and potentially compromises their accuracy at short temporal scales.

As the free-water O2 and other approaches based on high-frequency in situ data continue to gain popularity over the 14C incubations, much is yet to be learned about how estimates of GPP compare between the two approaches. There is a long history of comparing 14C incubations to O2 production from light/dark bottle incubations to determine production levels in marine and aquatic environments (Williams et al. 1983; Bender et al. 1987; Gazeau et al. 2007). Similarly, studies in marine ecosystems have compared 14C incubations to steady-state, sample-based oxygen methods such as 18O labeling, triple-isotope, 17, O2/Ar, and others, and have generally found that the 14C methods produce lower estimates (Juranek and Quay 2005; Quay et al. 2010; Hamme et al. 2012; Regaudie-de-Gioux et al. 2014). To our knowledge, no direct comparison of the free-water O2 and bottle 14C methods across multiple lakes and years have been made (but see Lauster, Hanson, and Kratz (2006) for free-water and O2 bottle comparisons). Here we use 20 lake-years of data from four lakes that differ in trophic status to assess how similar in situ free-water O2 pelagic epilimnetic production estimates are to concurrent pelagic epilimnetic estimates made using 14C incubations.

# Materials and Procedures

## Study Lakes

Daily lake 14C pelagic production, high-frequency dissolved oxygen (O2), water temperature, and meteorological data were collected as part of an ongoing long-term research projects in northern Wisconsin (North Temperate Lakes [NTL] Long-Term Ecological Research Program[[2]](#footnote-23); Trout and Sparkling Lakes), California (Castle Lake Environmental Research and Education Program[[3]](#footnote-24); Castle Lake), and Ohio (Center for Aquatic & Watershed Sciences[[4]](#footnote-25); Acton Lake). Sparkling and Trout lakes are embedded in a landscape that is predominantly a mix of deciduous and coniferous forest (54%), lakes (13%), and wetlands (28%) (Magnuson, Kratz, and Benson 2006). Both study lakes are oligotrophic/mesotrophic with relatively low nutrient and chlorophyll concentrations (Table [1](#tab:table1)). Castle Lake is a meso-oligotrophic, subalpine (1646 m. a.s.l.) lake (Vander Zanden et al. 2006) located in northern California with similar nutrient and chlorophyll concentrations as Trout and Sparkling lakes (Table [1](#tab:table1)). Acton Lake is a hypereutrophic reservoir (Table [1](#tab:table1)) that was created in 1957 by damming a creek for recreational use. Watershed landuse is primarily row crop agriculture (80%, Vanni et al. (2001)).

## 14C Production Methods

The approaches for estimating primary production in the study lakes using 14C incubations differed slightly between the three research programs, but each estimated daily epilimnetic pelagic production (mmol C m-3 d-1). In NTL lakes, integrated samples of water from the surface of the lake to the bottom of the epilimnion were collected between 2007 and 2013 using a 1.5 inch PVC tube approximately every two weeks during the open water season (first described in these lakes by Adams, Meinke, and Kratz (1993)). Samples were labeled with inorganic 14C in the form of NaHCO3 and then incubated in the lab for 3-hr across a range of light intensities with additional dark bottles to correct for non-uptake sorption of 14C at ambient epilimnetic water temperature. The resultant photosynthesis-irradiance (P-I) data were used to derive P-I curves by fitting a 3-parameter photosynthesis light-inhibition model (Platt, Gallegos, and Harrison 1980) to these data. The P-I curves were coupled with concurrent, high-frequency photosynthetically active radiation (mol m-2 s-1; PAR) measurements and water column light extinction data (m-1) to estimate daily primary production (mmol C m-3 d-1) in both Sparkling and Trout Lake. Over this time period, the availability of data for 14C production varied due to sporadic sample contamination and equipment failures.

Methods for 14C incubations in Acton Lake were similar to those in NTL lakes. Integrated samples were collected from the euphotic zone (usually equal to the epilimnion) and incubated in the lab for 1-2 hr with NaH14CO3 at a range of light intensities (including dark bottles; E. Fee (1990)). Incubations were usually done every two weeks (23 of 55 experiments over the four years) or more frequently (24 experiments); only 8 experiments were done at intervals >2 weeks. As in NTL lakes, P-I curves were coupled with high-frequency PAR measurements, and water column light extinction data collected at weekly intervals. Detailed methods 14C are described in Knoll, Vanni, and Renwick (2003).

At Castle Lake, vertical water collections were made from 13 depths between the surface and 30 m; duplicate light and 1 dark bottle samples from each each depth were labeled with inorganic 14C in the form of NaHCO3 and then incubated in situ at the depth of collection for 4 hours. Detailed methods are described elsewhere (Charles R. Goldman, Mason, and Wood 1963; C. R. Goldman 1968). Total daily incident solar radiation was measured throughout the summer with a LI-COR Li-200 pyrheliometer. Light profiles at the height of the solar day are measured using a Biospherical Instruments 2104P radiometer. Daily phytoplankton productivity rates were calculated by dividing productivity measured during the incubation period by the fraction of the total daily PAR received during the incubation.

## Free-water O2 Metabolism Methods

The same approach was used to estimate pelagic primary production (mmol C m-3 d-1; GPP) in all lakes using in situ time series of dissolved oxygen data (O2). Free-water O2 production estimates were based on high frequency measurements of dissolved oxygen (mg L-1), water temperature (), PAR (mol m-2 s-1), wind speed (m s-1), and barometric pressure (mbar). Data frequencies varied from 1 to 15 minutes based on the research program and the year data were collected. The raw, high-frequency time series of dissolved oxygen and water temperature were filtered to remove outliers by excluding values that were greater than 3 and 5 standard deviations respectively from a 7-day running average (Appendix [5](#Appendix:appendix_trimmed)A,B; *sensu* Phillips (2020)). The choice of sampling frequencies has implications for the processes influencing dissolved oxygen patterns and the amount of data needed to characterize those processes (Staehr et al. 2010). In general, frequencies between 30 minutes and 3 hours are optimal for capturing changes driven by biological processes (Staehr et al. 2010). Thus, we extracted hourly time series for all high frequency data by averaging observations (mean value) on the hour of observation (n = 4-60 depending on frequency of raw data) centered on the hour (Phillips 2020) for use in metabolism models.

Epilimnetic depth (m) was quantified from either high-frequency thermistor string data (Trout, Sparkling, and Acton Lakes) or discrete temperature profiles (Castle Lake). The high frequency data were filtered for outliers as outlined above and epilimnetic depth determined using the rLakeAnalyzer package (Read et al. 2011; L. Winslow et al. 2019) at the temporal frequency of the raw data. Hourly aggregate data were then extracted based on a 1-day running average to reduce the significant amount of noise that existed in these estimates (Appendix [5](#Appendix:appendix_trimmed)C). rLakeAnalyzer was also used to quantify epilimnetic depth from bi-monthly water temperature profile data in Castle Lake and linearly interpolated at hourly time steps between observations.

Exchange of dissolved gas with the atmosphere is a critical component of metabolism models, and, while there are a number of different models for estimating piston velocities in lentic ecosystems (Dugan et al. 2016), the model proposed by Vachon and Prairie (2013) is robust across multiple different types of lakes (Dugan et al. 2016) and the metabolism model used in this study (see below) is quite robust to choice in parameterization of piston velocities (Phillips 2020). Piston velocities (m hr-1) were calculated using the LakeMetabolizer R package (L. A. Winslow et al. 2016) and the parameterization proposed by Vachon and Prairie (2013). Light extinction coefficients (m-1), which were typically quantified bimonthly in all lakes, were linearly interpolated at hourly time steps between observations, and combined with epilimnetic depth and PAR to estimate the average light levels within the epilimnion of each lake (Staehr, Baastrup-Spohr, et al. 2012; Phillips 2020)

The data described above was used to generate daily estimates (mmol O2 m-3 d-1) of gross primary production (GPP), respiration (R), and net ecosystem production (NEP; NEP = GPP - R) using a time-varying ecosystem metabolism model (Phillips 2020). This model differs from many of the more commonly used metabolism models (e.g., L. A. Winslow et al. (2016)) in that the model is not fit iteratively over a daily time scale, but rather characterizes changes across all time periods (hourly measurements across 4-7 years of data) for a given lake in a single model fit, as well as constraining GPP and R to positive and negative values respectively (i.e., ecologically feasible ranges; Phillips (2020)). This takes advantage of the fact that the physical and biological processes governing ecosystem metabolism and other aspects of DO dynamics are autocorrelated through time, which means that this shared information can be used to inform the parameter estimates across all time points. Furthermore, this method is statistically unified because it uses all data to fit a single model, which facilitates characterizing the uncertainty in the ecosystem metabolism estimates (Phillips 2020).

The model used here differs slightly from that presented in Phillips (2020) in that we used a photoinhibition P-I curve (Steele 1962) to describe GPP (*sensu* Staehr et al. (2016)) instead of a light saturating curve:

where is the production rate at light intensity *I*, is the maximum production rate, and is the optimal light intensity. This photoinhibition model was chosen because recent work by Staehr et al. (2016) found that photoinhibition often occurred in lakes. The model by Steele (1962) is one of the simplest photoinhibition models (two-parameter), and, regardless of the P-I curve formulation chosen, it is often difficult to distinguish significant differences in model fits between different models (Aalderink and Jovin 1997). Both and , along with the model coefficient associated with R, (see Phillips (2020)) were allowed to vary through time at a daily time scale. The degree of auto-correlation in the parameters through time was constrained by hierarchical variance parameters in the random walk components of the model. Attempts to fit these parameters were unsuccessful, which is unsurprising as hierarchical variances often have poor identifiability. Thus, the random walk variances were treated as a “tuning parameters” and were selected manually such that the model converged while producing meaningful temporal smoothing in the parameters of the photoinhibition curve.

Observed dissolved oxygen time series were fit to all years (Trout: 2007-2010, 2012; Sparkling: 2007-2013; Castle: 2014-2017; Acton: 2010-2012, 2014) simultaneously for each lake individually (i.e., lake-specific metabolism model fitting). Missing values in the model input data time series left some days with fewer than 24 observations. Although the metabolism model is robust to missing data because it fits the entire time series simultaneously instead of in discrete daily time steps, we did not estimate metabolism parameters for an individual day if more than two hours of data were missing for that day (Phillips 2020). The model was fit via Stan (Stan Development Team 2020) run in R (R Core Team 2020) using the rstan package (STAN Citation) as described in (Phillips 2020). Posterior median values were used for daily GPP values along with the 0.025 and 0.975 quantiles of the posterior values to characterize the 95% credible intervals. Model fits were validated by checking effective sample size, , tree depth, energy Bayesian Fraction of Missing Information, and divergence (see Betancourt (2007)). Metabolism parameters were not estimated when the epilimnetic depth was shallower than the dissolved oxygen sensor (0.5m in Trout, Sparkling, Acton; 3m Castle; 4% of all observations). Gross primary production values (mmol O2 m-3 d-1) were converted to units of carbon (mmol C m-3 d-1) assuming a photosynthetic quotient (O2:CO2) of 1.25 (Bott 1996; Hanson et al. 2003; Wielgat-Rychert et al. 2017).

Data and code associated with the analyses included in this manuscript (Lottig et al. reference updated upon acceptance)

# Assessment

The goal of the analyses presented here is to compare 14C and free-water O2 daily primary production estimates to determine how interchangeable these two approaches are. We specifically tailor our analyses to identify two potential biases. First, given the commonly presumed bias of 14C to slightly underestimate GPP (Peterson 1980; Hall, Thomas, and Gaiser 2007), we wanted to know if there are constant differences in the magnitude of daily production values between the two approaches (i.e., we expected free-water O2 approach to yield higher estimates than the 14C approach). Second, we wanted to know if there were any fixed biases (i.e., intercept of linear regression different from zero) and/or proportional biases between the two methods (i.e., slope of linear regression different from 1). Our assumption was that free-water O2 estimates of GPP would be slightly higher than 14C (i.e., fixed bias) but the two methods should yield proportionally similar results. If there were no significant fixed or proportional biases, we interpret the results to mean that the methods are interchangeable for the lakes considered in this study.

Across the four lakes included in this study, we had 20 lake-years of concurrent 14C and free-water O2 pelagic, epilimnetic primary production estimates (Acton Lake: 4 yrs, Castle Lake: 4 yrs, Sparkling Lake: 7 yrs, Trout Lake: 5 yrs, Fig. [1](#fig:timeseries)). Direct comparisons between production estimates were available on 101 discrete days. In most cases (61% for all lakes; 75% excluding Castle Lake), 14C estimates of production were contained within the 95% credible intervals of the free-water O2 estimates and the seasonal patterns were similar between the two approaches (Fig. [1](#fig:timeseries), but see Castle Lake).

To assess potential biases between free-water O2 and 14C daily production estimates, epilimnetic production was compared by regressing 14C daily production values against free-water O2 daily production values. We assume that a slope of one and intercept of zero indicates that no significant difference exists between the two approaches (i.e., the methods are interchangeable). Intercept values significantly different from zero would indicate potential fixed biases, and slope values significantly different than one would indicate a proportional bias. Because both 14C and O2 estimates contain measurement errors (Macedo 2001; Pemberton, Clarke, and Joint 2006; Solomon et al. 2013), we used robust principal components error-in-variables regression (Passing and Bablok 1983) implemented in the ’mcr’ r package (Manuilova, Schuetzenmeister, and Model 2014). While the data were log-transformed in some cases prior to analysis to increase the normality of the observations, we also include results from regressions on non-log-transformed data.

A strong linear relationship was observed between the two approaches for estimating in-lake production across the approximate 200 mmol C m-3 d-1 pelagic epilimnetic production gradient observed in this study (Fig. [2](#fig:pointestimates)). Error-in-variable regression using all discrete observations (n=101) included the line of equality suggesting across large gradients of pelagic epilimnetic production, there was no proportional difference between the two approaches for measuring production in the lakes examined here (Table [2](#tab:table2)). The slight rightward shift in the distribution of free-water O2 as well as an intercept significantly greater than 0 in the error-in-variables (log transformed) regression is consistent with the general assumption that 14C production methods typically quantify a value slightly lower than GPP (Peterson 1980; Hall, Thomas, and Gaiser 2007). The linear relationship between the two approaches was consistent for both log-transformed and untransformed data (Table [2](#tab:table2)). The increase in slope coefficient values in the non-logged analyses for all lakes is largely due to the low productivity systems serving as a leverage point in the regression. Confidence intervals of non-transformed error-in-variable regression for Acton Lake (high productivity) and low productivity lakes included the line of equality (Table [2](#tab:table2)).

The linear relationships between 14C and free-water O2 within lakes were not as strong as the relationships observed both across lakes and across wide gradients in pelagic epilimnetic production (Fig. [3](#fig:lakevalues), Table [3](#tab:table3)). For example, in Trout Lake there is a significant negative relationship (Table [3](#tab:table3)). The lack of a strong 1:1 linear relationship in lakes that have a limited range of 14C estimates is likely due to the limited range of observed production values within a given lake combined with the uncertainty of both 14C (not quantified) and free-water O2 production estimates (quantified). Despite the narrow range of 14C production observed in the different lakes, most of the points cluster around the 1:1 line and a majority (61%; 75% excluding Castle Lake) of the 95% credible intervals of the free-water O2 estimates intersect the 1:1 line. We note that Castle Lake is unique among lakes in this study in that the data suggests a consistently lower production (1.7 mmol C m-3 d-1) value estimated with the 14C approach relative to the free-water O2 approach (Table [3](#tab:table3)).

Free-water O2 metabolism estimates can vary substantially from day-to-day (Staehr and Sand-Jensen 2007; Staehr et al. 2010; Coloso, Cole, and Pace 2011; Van de Bogert et al. 2012; Solomon et al. 2013). As with many other studies, the time series of O2 daily production in this study exhibited high day-to-day variation, especially in the hypereutrophic system (Acton Lake; Fig. [1](#fig:timeseries)). Because both spatial and temporal averaging can reduce variability in metabolism estimates (Staehr et al. 2010; Van de Bogert et al. 2012; Richardson et al. 2017; Zwart et al. 2017), we explored whether averaging (median value) free-water daily production estimates over 7 days (weekly) centered on the day of 14C incubations strengthened the relationship between O2 daily production and 14C daily production. Overall, comparing 14C estimates to the median value of O2 production did not alter the conclusions drawn from comparing measurements made on the same day, but the results suggest that comparing the weekly median value reduced any proportional bias in the data and increased the likelihood of a magnitude bias in the data as we and others hypothesized might exist (Table [3](#tab:table3), Fig. [4](#fig:medianvalues)).

# Discussion

Our results suggest that the pelagic, epilimnetic 14C and free-water O2 production approaches examined here can be interpreted similarly for the lakes considered in this study. Across gradients in production from oligotrophic to hypereutrophric systems, both of these approaches provide production estimates that are very similar in magnitude. Comparison of results between both methods indicated no statistically significant deviation from the 1:1 relationship, although there is evidence that, as expected, 14C estimates may be slightly lower than free-water O2 estimates of pelagic epilimnetic production. Unlike other studies (e.g., Staehr et al. (2010)), temporal aggregation of free-water O2 estimates did not have a major impact on the relationships observed in this study, likely because the metabolism model smooths parameter estimates through time (Phillips 2020).

A priori, we anticipated that free-water O2 estimates would be proportional to 14C estimates and that 14C estimates would be, on average, slightly lower than free-water O2, because 14C estimates tend to lie between GPP and net primary production (NPP; Peterson (1980)). In general, the results confirmed our expectations. The lack of strong statistical evidence across all lakes of lower 14C relative to O2 estimates in our study may reflect the considerable uncertainty in both estimates, which we explicitly incorporated in our analysis via the error-in-variables approach. Additionally, the research programs responsible for generating the 14C production estimates specifically targeted short incubation periods to generate estimates that closely approximated GPP (Hall, Thomas, and Gaiser 2007). Thus, even though we observed slight lower 14C production estimates relative to the free-water O2 estimates, the lack of strong statistical significance across all analyses is not necessarily surprising given approaches employed by the research programs collecting the 14C production data.

The specific results for Castle Lake are an exception to the conclusions based on results drawn from the full data set spanning all lakes. There is strong evidence that 14C estimates are significantly lower than free-water O2 estimates for this lake alone, even though the two approaches are proportionally similar. We consider two potential reasons for this pattern in Castle Lake, one of which is methodological and the other is consistent with hypotheses presented here. First, the degree to which 14C production estimates approximate GPP relative to NPP is influenced by incubation time (Hall, Thomas, and Gaiser 2007), whereby shorter incubations tend to estimate a value closer to GPP, represented in this study as free-water O2 estimates. Castle Lakes incubations were the longest (4 hours) of any of the three programs that collected 14C data, and thus it might be expected that the relative differences between the approaches was greatest for this program compared to the other two programs that collect 14C data. The other potential issue relates to how the 14C data from Castle Lake were generated (see Methods above). Briefly- samples were incubated in situ for 4 hours from 10:00 - 14:00 hours (time period of maximum solar insolation), and the relationship between production and solar insolation (i.e., P-I curve) was assumed to be linear; whereas, a relationship that includes photoinhibition is likely more accurate (Huovinen 1999). Because the incubations were conducted when solar insolation was near maximal, this approach has the potential to significantly underestimate production rates at lower light levels regardless of the shape of the true P-I curve. Nearly all of the lower 14C epilimnetic production estimates in Castle Lake were in samples that were incubated directly at the lake’s surface where solar insulation is much greater relative to insulation deeper in the water column. Samples incubated at deeper depths had higher production estimates and would be consistent with P-I curves characterized by strong photoinhibition. Thus, while not influencing the overall patterns across all lakes, Castle Lake serves as a strong reminder that it will be important to account for potential differences in how both 14C and free-water O2 production data are generated.

While we suggest that free-water O2 and 14C epilimnetic daily pelagic production approaches are largely interchangeable, it is important to emphasize that there is still substantial variability between the methods. It can be difficult to reliably fit free-water metabolism models in some systems, especially low productivity systems, because physical processes influencing O2 often dominate the dissolved oxygen temporal patterns and/or errors in accounting for these physical processes influencing O2 result in unrepresentative results. While physical processes are less of a concern for incubations, there is a suite of other concerns (Hall, Thomas, and Gaiser 2007). At the hypereutrophic end of the productivity spectrum, high variability in daily free-water O2 estimates are common (Williamson, Vanni, and Renwick 2020), and bottle incubations for 14C production may miss important temporal and/or spatial variability that is captured by the free-water O2 approach. Although averaging free-water O2 production estimates did not alter the relationships observed between 14C and free-water O2 production in these lakes, averaging free-water O2 did reduce the variability around the line of equality (see Fig. [4](#fig:medianvalues)). Thus, averaging free-water O2 production estimates over several days may reduce the uncertainty in the 14C and O2 relationship, especially in more productive systems.

Estimating metabolism parameters, including primary production from free-water O2 data can be challenging in low productivity systems (e.g., Richardson et al. (2017; McNair et al. 2015; Honti and Istvánovics 2019)) where 14C is generally considered optimal (Hall, Thomas, and Gaiser 2007). Given that a majority of the lakes in this study are characterized by low productivity, it is likely that the ability of the Phillips (2020) model to leverage all possible data across multiple years to fit the models contributed to strong agreement between the free-water O2 and 14C estimates. While not the focus of this study, an exploration of how different free-water O2 models perform may lead to a better understanding of when and where different model formulations should be leveraged (e.g., Honti et al. (2016; Staehr et al. 2016; McNair et al. 2015)).

Multi-site comparisons like this study are critical for gaining a better understanding of lake daily production measurements generated by these two widely used methods. Each method has unique advantages and disadvantages that may influence the choice of methods for particular research applications. For example, we would expect large differences between free-water and bottle estimates in freshwaters where littoral and benthic production contribute substantially to total metabolism (Lauster, Hanson, and Kratz 2006; Van de Bogert et al. 2007). Thus, it is likely that both methods will continue to be used and there will be an ongoing need to compare results across methods. Analyses conducted here provide little evidence of systematic differences in estimates of epilimnetic lake daily production based on free-water O2 or 14C methods across a wide gradient in lake trophic status.

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# Tables and Figures

Study lake characteristics

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Trout Lake | Sparkling Lake | Castle Lake | Acton Lake |
| Location (lat,lon) | 46.03,-89.67 | 46.01,-89.70 | 41.23,-122.38 | 39.58,-84.76 |
| Area (ha) | 1565.1 | 63.7 | 19 | 232 |
| Mean Depth (m) | 14.6 | 10.9 | 11.4 | 3.9 |
| Total Nitrogen (mg L-1) | 0.247 | 0.371 | 0.15 | 3.36 |
| Total Phosphorus (mg L-1) | 0.014 | 0.015 | 0.01 | 0.10 |
| Chlorophyll *a* (g L-1) | 3.1. | 2.2 | 2.4 | 64.8 |

Error-in-variable regression results for all lakes as well as lakes separated by productivity class.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Estimate | SE | Lower CI | Upper CI |
| **All Lakes (log transformed)** |  |  |  |  |  |
|  | Intercept | 0.201 | 0.039 | 0.103 | 0.257 |
|  | Slope | 0.926 | 0.041 | 0.849 | 1.003 |
| **All Lakes (non transformed)** |  |  |  |  |  |
|  | Intercept | 1.000 | 0.418 | -0.060 | 1.770 |
|  | Slope | 1.244 | 0.134 | 0.962 | 1.578 |
| **All Lakes Averaged (non transformed)** |  |  |  |  |  |
|  | Intercept | 1.529 | 0.516 | 0.211 | 2.552 |
|  | Slope | 0.992 | 0.152 | 0.622 | 1.142 |
| **High Productivity Lake (non-transformed)** |  |  |  |  |  |
|  | Intercept | -194.6 | 266.4 | -765.8 | 68.14 |
|  | Slope | 2.445 | 1.691 | 0.987 | 6.215 |
| **Low Productivity Lakes (non-transformed)** |  |  |  |  |  |
|  | Intercept | 1.464 | 0.736 | -0.071 | 2.311 |
|  | Slope | 1.019 | 0.276 | 0.636 | 1.559 |

Error-in-variable regression results for each individual lake. Data were not transformed prior to analyses.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | Estimate | SE | Lower CI | Upper CI |
| **Acton Lake)** |  |  |  |  |  |
|  | Intercept | -194.6 | 266.4 | -765.8 | 68.14 |
|  | Slope | 2.445 | 1.691 | 0.987 | 6.215 |
| **Castle Lake** |  |  |  |  |  |
|  | Intercept | 1.671 | 0.376 | 1.089 | 2.381 |
|  | Slope | 1.115 | 0.251 | 0.640 | 1.717 |
| **Trout Lake** |  |  |  |  |  |
|  | Intercept | 8.157 | 3.260 | 5.689 | 16.66 |
|  | Slope | -0.735 | 0.794 | -2.928 | -0.137 |
| **Sparkling Lake** |  |  |  |  |  |
|  | Intercept | 4.846 | 9.591 | -45.54 | 1.210 |
|  | Slope | 2.817 | 2.889 | 0.783 | 16.17 |

![Time series of pelagic epilimnetic primary production determined from high frequency in situ dissolved oxygen data and discrete measurements of epilimnetic primary production determined from 14C incubations in 4 lakes that range in trophic status from ologitrophic to hypereutrophic. Light blue shaded areas represent the 95% credible interval of the free-water O2 estimate.](data:application/pdf;base64,)

Time series of pelagic epilimnetic primary production determined from high frequency in situ dissolved oxygen data and discrete measurements of epilimnetic primary production determined from 14C incubations in 4 lakes that range in trophic status from ologitrophic to hypereutrophic. Light blue shaded areas represent the 95% credible interval of the free-water O2 estimate.

![Point estimate (A) and distribution (B) comparisons of 14C and free-water O2 production (mmol C m-3 d-1) estimates from concurrent observations in four lakes of varying trophic status. Error bars (A) are 95% credible intervals from bayesian metabolism model. (B) Blue is the free-water O2 estimate, black is the 14C estimate. O2 production values in B are posterior median daily values from Bayesian metabolism model](data:application/pdf;base64,)

Point estimate (A) and distribution (B) comparisons of 14C and free-water O2 production (mmol C m-3 d-1) estimates from concurrent observations in four lakes of varying trophic status. Error bars (A) are 95% credible intervals from bayesian metabolism model. (B) Blue is the free-water O2 estimate, black is the 14C estimate. O2 production values in B are posterior median daily values from Bayesian metabolism model

![Point estimate comparisons of 14C and free-water O2 production estimates from concurrent observations in four lakes of varying trophic status.](data:application/pdf;base64,)

Point estimate comparisons of 14C and free-water O2 production estimates from concurrent observations in four lakes of varying trophic status.

![Comparison of daily estimates of 14C production and weekly median free-water O2 production in four lakes of varying trophic status. Light grey points are non-averaged original estimates for each day.](data:application/pdf;base64,)

Comparison of daily estimates of 14C production and weekly median free-water O2 production in four lakes of varying trophic status. Light grey points are non-averaged original estimates for each day.

# Appendix

![Time series of data from Acton Lake (2010) showing the raw (green), outlier free (blue), and hourly estimates (red) for dissolved oxygen (A), water temperature at the dissolved oxygen sensor depth (B) and epilimnetic depth or mixed layer depth (C). Hourly values for epilimnetic depth is based on a hourly rolling mean over a 24 hour time period while hourly data in other plots is the simple average value for each discrete hour.](data:application/pdf;base64,)

Time series of data from Acton Lake (2010) showing the raw (green), outlier free (blue), and hourly estimates (red) for dissolved oxygen (A), water temperature at the dissolved oxygen sensor depth (B) and epilimnetic depth or mixed layer depth (C). Hourly values for epilimnetic depth is based on a hourly rolling mean over a 24 hour time period while hourly data in other plots is the simple average value for each discrete hour.

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