Article

Quantifying pelagic phosphorus regeneration using three methods in lakes of varying productivity

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Abstract

Phosphorus (P) is often a limiting nutrient in freshwater ecosystems, and understanding P dynamics in lakes is critical for eutrophication management. Pelagic P regeneration can support a large fraction of primary production in stratified freshwaters. Various techniques have been used to quantify pelagic P regeneration including (1) P mass balance supply–demand, (2) regression using total P as a predictor, and, more recently, (3) whole-lake metabolism calculated from high-frequency dissolved oxygen (DO) data. To our knowledge no study comparing these methods in multiple lakes has been performed. To compare these 3 approaches, we investigated 3 Global Lake Ecological Observatory Network (GLEON) lakes that differ in productivity: Acton, a Midwestern USA hypereutrophic reservoir; and 2 Northeastern USA glacial lakes, oligotrophic Giles and mesotrophic/dystrophic Lacawac. In Acton, we used all 3 methods, but for Giles and Lacawac we used only the total P regression and metabolism techniques. Our results show the best agreement among methods in the mesotrophic lake, whereas the metabolism approach underestimated regeneration in the oligotrophic lake and overestimated regeneration in the hypereutrophic reservoir compared with other methods. P regeneration rates for the hypereutrophic reservoir were the most sensitive to the metabolism-based input parameters. Our study illustrates a novel use of high-frequency DO data, which are commonly collected on many GLEON buoys, to understand lake nutrient dynamics.

Key words: GLEON, high-frequency sensors, lakes, metabolism, nutrient recycling, phosphorus regeneration, primary production

Introduction

Early limnologists noted little variation in epilimnetic phosphorus (P) concentrations as summer progressed, whereas epilimnetic phytoplankton biomass was constant or even increased (Juday et al. 1927). This finding was unexpected because P limitation is prevalent in freshwater aquatic ecosystems (Schindler 1977, Smith 1979, Elser et al. 1990), and bioavailable P concentrations in lakes are often low during summer when phytoplankton biomass is maximal. Thus, efforts have been made to understand how primary production is maintained at high levels in the epilimnion of thermally stratified lakes during the summer, despite low new P inputs (e.g., from the catchment or deep P-rich waters). One important flux is P regeneration from zooplankton and pelagic bacteria. Nutrients regenerated (i.e., recycled, mineralized) by planktonic organisms are capable of supplying a quantitatively significant portion of the nutrients needed for phytoplankton growth (Sterner et al. 1995, Vanni 2002). Nutrient regeneration by smaller planktonic organisms, including heterotrophic microbes and zooplankton, can supply much of the epilimnetic

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dissolved P in low-nutrient lakes during summer thermal stratification (Dodds et al. 1991), and zooplankton nutrient excretion has been shown to support 4–58% of lake phytoplankton P demand (Vanni 2002).

Various techniques exist to quantify epilimnetic P regeneration. The radioisotope phosphate $^{33}PO_4$ has been used to label plankton communities to quantify the transfer of P from the particulate pool to the dissolved pool (i.e., P regeneration) via egestion, excretion, decay, cell lysis, cellular exudates, and sloppy feeding (Hudson and Taylor 1996, Hudson et al. 1999, Nowlin et al. 2007). In one radioisotope study, the authors developed a predictive regression for steady-state phosphate (hereafter $ssPO_4$-P) regeneration rates based on total phosphorus (TP) concentrations from lakes spanning a productivity gradient (Hudson et al. 1999). P budgets accounting for epilimnetic P supply and demand have also been used to estimate P regeneration (Caraco et al. 1992, Domine et al. 2010). These studies showed that both new P (i.e., from outside the euphotic or mixed zone) and regenerated P are important in sustaining phytoplankton production.

A new approach to quantify P regeneration takes advantage of high-frequency dissolved oxygen (DO) data used to estimate phytoplankton primary production as well as autotrophic and heterotrophic respiration (Kamarainen et al. 2009). Ecosystem respiration is measured directly and apportioned into that by autotrophs and heterotrophs. Once heterotrophic respiration is estimated, P regeneration can be calculated by assuming that planktonic heterotrophs regenerate P as a function of their respiration rate and the carbon to phosphorus ratios (C:P) in seston. P demand can also be calculated using estimated net primary production divided by the seston C:P ratio (which is assumed to reflect phytoplankton C:P). This newly developed metabolism method was used in a eutrophic lake (Kamarainen et al. 2009) but to our knowledge has not been tested in lakes of varying trophic status. Instrumented buoys capable of collecting high-frequency DO data that can be used to obtain metabolism estimates are becoming more common in lakes worldwide. Thus, this approach may prove useful for understanding the relative importance of nutrient regeneration in supporting phytoplankton growth in diverse lakes and how this varies seasonally.

We took advantage of 3 lakes with instrumented buoys within the Global Lake Ecological Observatory Network (GLEON), a network of lakes, high-frequency sensor data, and people (Weathers et al. 2013); many recent studies have contributed insights into lake ecosystem function using GLEON data in diverse lakes (Jennings et al. 2012, Klug et al. 2012, Read et al. 2012, Solomon et al. 2013). Using 3 lakes that represented a productivity gradient, we compared 3 methods of estimating P regeneration: (1) P mass-balance supply–demand (P budget), (2) regression using TP as a predictor, and, (3) whole-lake metabolism calculated from high-frequency DO data. We used the TP regression and metabolism approaches over 1 summer in 2014 for 2 glacial lakes in Northeastern USA, 1 oligotrophic, and 1 mesotrophic/dystrophic. For a hypereutrophic constructed reservoir in Midwestern USA, we used the first 2 approaches as well as a P budget over 2 summers in 2011 and 2012. We addressed 3 questions: (1) in the 2 natural lakes, how do P regeneration rates compare across the 2 methods (TP regression and metabolism); (2) in the hypereutrophic reservoir, how do P regeneration rates compare across the 3 methods; and (3) using the metabolism method, does estimated pelagic P regeneration supply an important proportion of estimated pelagic P demand, and does this vary among the 3 lakes? We were also interested in understanding how the approaches compare given that P regeneration derived from the metabolism method and budget methods is quantified as soluble reactive phosphorus (SRP) but as $ssPO_4$-P in the TP regression approach. Studies have shown that $ssPO_4$-P concentrations from steady state bioassays are ~2.5 orders of magnitude lower than corresponding SRP concentrations (Hudson et al. 2000, Nowlin et al. 2007). Our overarching objective was to better understand the utility of the new P regeneration approach in comparison to the other methods in lakes of varying productivity.

Methods

Study lakes

Acton Lake is a hypereutrophic reservoir in southwestern Ohio, USA (Table 1). The portion of the reservoir near the stream inflows is shallow (~1 m) and does not thermally stratify during the summer, whereas near the dam, the reservoir is deep enough to stratify during the summer months (~8 m when the lake is at full pool). Mean depth is ~3.9 m. For this study, we focused only on samples collected at a deep site near the dam, but we estimated fluxes for the entire mixed layer. As a productive reservoir, most primary production occurs in the upper portion of the reservoir, and the euphotic zone depth (as defined by the depth of 1% photosynthetically active radiation [PAR]) is generally shallower than or equal to the mixed layer. Acton's watershed is 89% agricultural land, the vast majority of which is cropland dominated by soy and corn (Knoll et al. 2003). Acton has 3 main inflow streams, Little Four Mile Creek, Four Mile Creek, and Marshall's Branch, that are gauged and collectively represent 86% of the watershed drainage (Vanni et al. 2001). Acton has one outflow point over a dam spillway. Phytoplankton in

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Table 1. General characteristics of the study lakes. Chlorophyll *a* and total phosphorus concentrations represent mean summer values.

Lake	Origin	Lake surface area (km^2)	Watershed surface area (km^2)	Maximum depth (m)	Chlorophyll *a* ($\mu g\ L^{-1}$)	Total phosphorus ($\mu g\ L^{-1}$)
Giles	Natural	0.48	1.3	24	0.8	3.5
Lacawac	Natural	0.21	0.48	13	3.8	9.0
Acton	Constructed	2.5	257	8	59	77.4

Acton are mostly P limited during the stratified period (Vanni et al. 2006a, Hayes et al. 2015).

Lake Giles and Lacawac are small, natural, glacially formed lakes in northeastern Pennsylvania, USA (Table 1). Giles is a deep oligotrophic lake that thermally stratifies each summer. The euphotic zone of Giles is much deeper than the mixed layer (~12 vs. ~4 m), and a sub-epilimnetic peak of phytoplankton biomass occurs during summer months. Giles has a forested watershed with minimal anthropogenic disturbance (Williamson et al. 2014). Giles receives water by rainfall, seepage, and to a lesser extent by a small stream draining a forested wetland, and has only one small outlet stream. Lake Lacawac is a mesotrophic and moderately dystrophic lake that thermally stratifies each summer. Similar to Giles, the euphotic zone of Lacawac is deeper than mixed layer (~5 vs. ~2 m), and at times a sub-epilimnetic peak of phytoplankton biomass occurs during summer months. Lacawac has a forested watershed located within a nature preserve and has been protected since the 1960s (Williamson et al. 2014). Lacawac receives water by rainfall and seepage and has one small outlet stream. Lacawac and Giles phytoplankton are at times co-limited by P and N (L.B. Knoll, unpubl. data).

TP regression approach – Giles, Lacawac, Acton

For all lakes, weekly TP samples were collected at the deepest spot of the waterbody using an integrated tube sampler when stratification was well established (Jun–mid-Aug). In Giles and Lacawac, samples were collected from the epilimnion as determined by a temperature profile (YSI-57, YSI Inc., Yellow Springs, OH, USA). In Acton, samples were collected in the euphotic zone as determined by a PAR profile (LI-COR spherical sensor, LI-COR Biosciences, Lincoln, NE, USA). TP was estimated on unfiltered and acidified (pH < 2) water samples preserved at 4 °C. Samples were analyzed on a Lachat autoanalyzer (QC 8000 FIA or QC 8500 FIA; Lachat Instruments, Loveland, CO, USA) using the acid molybdate method following potassium persulfate digestion. We estimated $ssPO_4$-P regeneration rates weekly from integrated TP concentrations and an established regression (Hudson et al. 1999):

$$\log_{10} \text{P regeneration rate (ng ssPO}_4\text{-P L}^{-1}\text{ h}^{-1}) = 1.0077(\log_{10}\text{TP } (\mu g\ L^{-1})) + 0.7206) \quad (1)$$

We then converted rates to $ssPO_4$-P mg m^{-2} d^{-1}. This regression was developed by Hudson et al. (1999) using a method in which the release of dissolved P by the plankton community is measured using plankton labeled with radiophosphate.

Metabolism approach – Giles, Lacawac, Acton

We used metabolic inference to calculate SRP regeneration from high-frequency measurements of whole-lake metabolism: gross primary production (GPP) and ecosystem respiration (ER; Kamarainen et al. 2009). We collected free-water DO measurements (at 1 m for Giles and Lacawac and at 1.5 m for Acton), water temperature profiles, PAR, and wind speed data with fully automated instrumented buoys at 15-minute intervals in Acton and at 10-minute intervals in Giles and Lacawac. In Acton, DO profiles were used to determine mixing depth, assumed to be where DO was >1 mg L^{-1}, and mixing depth was truncated at mean lake depth (3.9 m). For the 2 Pennsylvania lakes, mixing depth was determined by high-frequency temperature profile data. For all lakes, we used a maximum likelihood model within the metab function in the R package LakeMetabolizer to calculate GPP, NEP, and ER (R Development Core Team 2013, Winslow et al. 2015, 2016). Air–water gas exchange velocity and gas flux coefficient used in the calculation of GPP, ER, and NEP were calculated using the methods derived from Cole and Caraco (1998), as specified in the cole method in the LakeMetabolizer package (Winslow et al. 2016). Similar to other studies, data were screened before analysis to remove out-of-range or instrument error measurements (33–56% of data), and we aggregated daily values to the weekly scale (Staehr and Sand-Jensen 2007). GPP, ER, and NEP were converted from mg L^{-1} d^{-1} of O_2 to mg m^{-2} d−1 of C by multiplying by 0.375 (mass ratio of O_2 to C, assuming a photosynthetic quotient of 1) and then using the mean depth of the lake to convert rates to areal units (Kamarainen et al. 2009).

We estimated net primary production (NPP) by subtracting autotrophic respiration (R_{auto}) from GPP. R_{auto} varies among lakes, and its importance relative to ER has been shown to increase with lake productivity; Biddanda et al. (2001) found that R_{auto} is ~2–18% of planktonic ER in oligotrophic lakes and ~90% in eutrophic lakes. Another study estimated that R_{auto} can range from 35 to 60% of total respiration, with lower percentages in oligotrophic lakes and higher percentages in eutrophic lakes (del Giorgio and Peters 1993). For Acton, we estimated the relative contribution of R_{auto} to ER by comparing data from ^{14}C incubations (which provides rates close to NPP) with data from the O_2 method (which provides GPP) obtained simultaneously over 3 field seasons (2010–2012). ^{14}C-based NPP was usually estimated at 2-week intervals between late April and late September but occasionally more frequently. The ^{14}C method interpolates NPP between measurement dates using the photosynthesis–irradiance curves from the incubations and intervening data on depth-specific chlorophyll *a* (Chl-*a*) and PAR (Knoll et al. 2003). Thus, for each date on which NPP was measured using ^{14}C (41 dates), we obtained a weekly mean NPP estimate using the measurement date as well as 3 days before and after the rate measurement. Then we obtained mean GPP from the O_2 method using the same dates.

The 2 rates were significantly correlated, indicating that the methods were in good general agreement ($\log GPP = 1.689 + 0.693 * \log NPP$, $r^2 = 0.444$, $p < 0.0001$, $n = 37$). For each of these weekly estimates, we also calculated R_{auto} as GPP − NPP and then obtained the R_{auto}:ER ratio for each week. We also calculated this ratio as mean R_{auto} (using all dates) divided by mean ER (using all dates). These analyses showed that the R_{auto}:ER ratio was 0.688 (using the mean of weekly ratios) or 0.722 (using the ratio of mean R_{auto} to mean ER). Thus, we assumed R_{auto} was 70% for Acton, implying that heterotrophic respiration (R_{hetero}) is 30% of ER, which is within the range reported by Biddanda et al. (2001), and it brackets estimates for eutrophic lakes found by Biddanda et al. (2001) and del Giorgio and Peters (1993; ~10 and 40%, respectively). Because of these differences, however, we also present average regeneration rates over the summer based on 90% R_{auto} (Biddanda et al. 2001). We do not have NPP (^{14}C) data for the 2 natural lakes; therefore, based on previous studies we estimated that in the lower productivity Lacawac and Giles, R_{auto} was 30% of total respiration. Estimates vary for R_{auto} in lower productivity lakes, so we also present average regeneration rates over the summer based on a high estimate for R_{auto} of 50%. In all lakes, R_{hetero} was estimated as $ER - R_{auto}$.

We estimated P regeneration (equation 2) and P demand (i.e., uptake; equation 3) following Kamarainen et al. (2009) as:

$$\text{P regeneration} = R_{hetero} \div \text{seston C:P, and} \quad (2)$$

$$\text{P demand} = \text{NPP} \div \text{seston C:P,} \quad (3)$$

where NPP is net primary production from DO metabolism estimates, seston C:P is the seston carbon to phosphorus ratio, and R_{hetero} is the respiration attributed to heterotrophic bacteria. For P regeneration, this calculation assumes zero net heterotrophic growth and that the C:P of regeneration corresponds with the C:P of the seston available for mineralization (Kamarainen et al. 2009). The P demand calculation assumes that seston C:P represents the C:P of the phytoplankton community.

P budget approach - Acton

We also used an SRP budget supply–demand approach to estimate SRP regeneration for Acton in 2011 and 2012. New sources of SRP to the mixed layer included watershed loading, SRP excretion by sediment-feeding fish (gizzard shad [*Dorosoma cepedianum*]), and entrainment from anoxic water. SRP export via the dam outlet was considered a loss from the system. Phytoplankton P demand and bacteria P demand were also quantified. SRP regeneration was calculated as (Domine et al. 2010):

$$\text{SRP regeneration} = (\text{phytoplankton P demand} + \text{bacteria P demand} + \text{SRP loss from dam}) - (\text{SRP watershed loading} + \text{SRP gizzard shad excretion} + \text{SRP entrainment}). \quad (4)$$

We quantified Acton watershed loading on the 3 aforementioned streams that drain 86% of the watershed (Vanni et al. 2001, Renwick et al. 2008). Briefly, we employed a high-frequency, flow-dependent sampling regime utilizing ISCO automated water samplers (Teledyne ISCO, Lincoln, NE, USA) located on each stream. We collected samples ~3 times per week during baseflow and every 8 hours during storm events, when nutrient concentrations (including SRP) change rapidly (Vanni et al. 2001). Stream SRP samples were filtered through Gelman A/E glass fiber filters, acidified (pH < 2) and then preserved at 4 °C and analyzed as described above for TP. Stream stage was recorded every 10 minutes using data logging pressure transducers (Vanni et al. 2001, Renwick et al. 2008, Knoll et al. 2013). Hourly nutrient concentrations were obtained using flow-proportionate interpolation methods (Vanni et al. 2001). SRP loading was calculated from the product of hourly SRP concentration and hourly stream discharge, scaled to daily fluxes for the entire watershed (see Vanni et al. 2001 for detailed nutrient export calculations).

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Non-larval gizzard shad are facultative detritivores, and during mid to late summer young-of-year and adult gizzard shad are detritivorous in Ohio reservoirs (Higgins et al. 2006). These fish make up ~94% of the fish biomass and represent a quantitatively important flux of nutrients from the sediments to the pelagic zone of lakes during summer months (Vanni et al. 2006b). To estimate gizzard shad SRP excretion rates, we used size-specific data on fish abundance from hydroacoustics and per-fish excretion rates that are a function of fish wet mass and temperature (Vanni et al. 2006b).

SRP entrainment into the mixed layer from anoxic waters was estimated using weekly DO profiles and weekly depth-specific SRP concentrations taken at 1 m intervals with a Van Dorn water sampler. We used DO profiles rather than temperature profile data because lateral flows of water in reservoirs affect thermal stratification, and oxygen profiles offer a better estimate of mixed layer depth than temperature profiles (Melack 1978, Dickman et al. 2006). An entrainment event occurred when there was an increase in the mixed layer depth by 0.5 m or more. This change in mixing depth could cause an influx of hypolimnetic P representing a new input of P to the mixed layer. We used the volume of water represented by this increase and the SRP concentration of the entrained depths to calculate weekly entrainment SRP fluxes. SRP concentrations were quantified as described earlier.

SRP loss from the dam was quantified as the product of SRP concentration and discharge over the dam (Domine et al. 2010). Water for SRP was collected weekly from the outflow site in the 0–1 m layer using a Van Dorn water sampler. SRP was analyzed as described earlier. We linearly interpolated SRP concentrations for days when samples were not collected. Discharge over the dam was estimated hourly by using a water budget that takes into account all water inputs and outputs (Knoll et al. 2013). We considered the following water inputs: (1) stream discharge from the 3 gauged streams scaled up to the entire watershed, and (2) hourly precipitation; and the following water outputs: (1) potential evapotranspiration using hourly precipitation and temperature data, and (2) change in lake volume calculated using hourly lake level data (continuously recorded via a lake level gauge) and lake bathymetry.

Phytoplankton P demand was calculated using phytoplankton primary production from ^{14}C fixation rates and seston C:P ratios (Vanni et al. 2006b). We divided primary production rate (C: mg m^{-2} d^{-1}) by seston C:P (mg C to mg P) to estimate P demand (P: mg m^{-2} d^{-1}), assuming that seston C:P ratios reflect phytoplankton C:P ratios (Sterner and Elser 2002). Primary production experiments were conducted every other week using integrated water collected from the euphotic zone at the outflow site following previous methods (Knoll et al. 2003, Vanni et al. 2006b). Briefly, we quantified phytoplankton ^{14}C uptake at a range of PAR levels in an environmental chamber kept at lake temperatures. We generated Chl-*a*–specific photosynthesis–irradiance curves, which were then used with depth-specific in-lake PAR (every 0.5 m) and Chl-*a* concentrations (every 1 m) to obtain daily lake-wide primary production rates, corrected for lake morphometry.

Depth-specific water samples were collected for Chl-*a* concentration. Water was filtered onto Gelman A/E glass fiber filters, frozen in the dark until analysis. Chl-*a* was extracted with ethanol in the dark at 4 °C for 2–24 hours and quantified with a fluorometer (Turner Designs, Sunnyvale, CA, USA). Depth-specific PAR was collected as described earlier. Water for particulate C and particulate P was collected weekly with an integrated tube sampler from the euphotic zone at the outflow site and screened with a 63 μm mesh to remove large zooplankton. Particulate C samples were filtered onto pre-combusted 25 mm Gelman A/E glass fiber filters and particulate P onto pre-combusted 47 mm Gelman A/E glass fiber filters. Particulate C samples were analyzed on a CN elemental analyzer (CE Elantech Flash EA 1112, Lakewood, NJ, USA), and particulate P filters were digested with HCl and analyzed for SRP as described earlier.

We estimated bacteria P demand using previous data collected on Acton (Caston et al. 2009). Specifically, bacterial production was found to be ~10.4% of primary production, and the C:P of bacteria-sized particles (<1 μm) was 40.1 (mass:mass). Using primary production rates described earlier and these values, we estimated bacteria P demand (P: mg m^{-2} d^{-1}) by dividing estimated bacteria production (C: mg m^{-2} d^{-1}) by 40.1 (estimated C:P < 1 μm).

Statistics

Bootstrapping was used to provide error estimates on P regeneration rates calculated from the TP regression approach, metabolism approach, and the P budget approach. Bootstrap values represent bias corrected mean (±1 standard error) summer values (Jun–mid-Aug) generated by blocked time series bootstrapping (block length = time series length$^{1/3}$, iterations = 10 000).

All simulations were run using the using the tsboot function in the boot R package (Kunsch 1989, R Development Core Team 2013).

We also used a simple deterministic sensitivity analysis procedure (Loucks and van Beek 2005) to examine the sensitivity of P regeneration rates calculated from metabolism approach to changes in the input parameters (i.e., R_{hetero} and seston C:P). We restricted our

sensitivity analyses to only R_{hetero} and C:P parameters. We did not vary all of the parameters that go into metabolism estimates (e.g., wind, gas exchange) because understanding metabolism model assumptions is an active research area (Dugan et al. 2016). For our analyses, we varied each parameter individually by using all possible values in our dataset. For example, to examine the sensitivity of P regeneration to changes in C:P, we calculated the percent increase of all possible values on C:P from the mean of C:P for each lake. We then held R_{hetero} constant at the mean R_{hetero} value and calculated P regeneration (equation 2) for each variation of C:P across the range of values of that parameter within each lake. We ran sensitivity analyses twice for each lake and for each year of Acton data because, as described previously, the percentage of ER composed of R_{hetero} and R_{auto} varies. Thus, we ran analyses under 2 scenarios; where we assumed R_{hetero} was either 70 or 50% in Lacawac and Giles and either 30 or 10% in Acton. Note that these R_{hetero} scenarios are different from running the sensitivity analysis with variable R_{hetero}. The sensitivity analysis takes the range of R_{hetero} values in the dataset, and this range varies depending on whether we set the percentage of R_{hetero} to ER high (70% in Lacawac and Giles and 30% in Acton) or low (30% in Lacawac and Giles and 10% in Acton). All analyses were performed in R (R Development Core Team 2013).

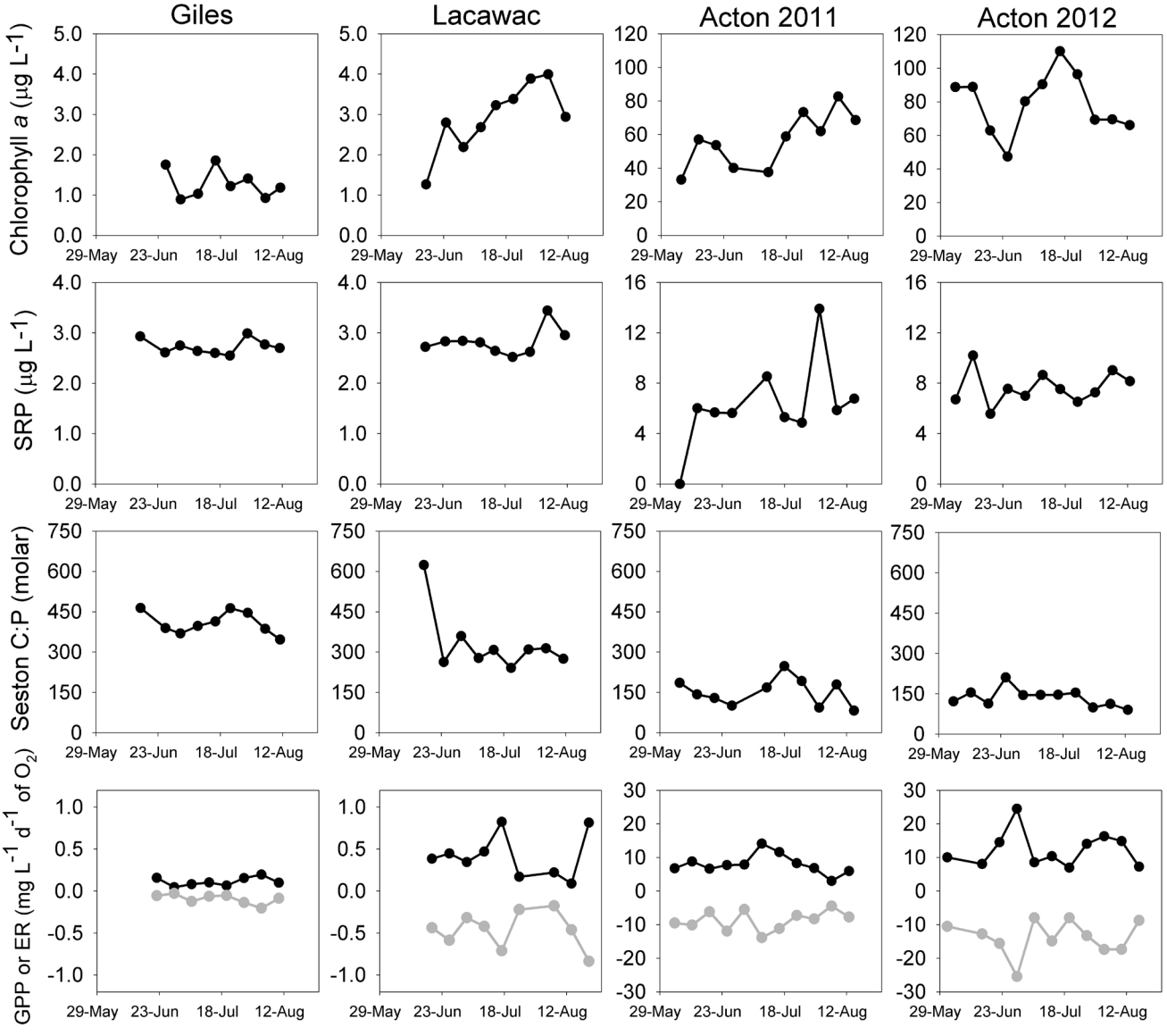

Fig. 1. Summer trends in chlorophyll *a*, SRP, seston C:P, gross primary production (GPP, black circles and line), and ecosystem respiration (ER, gray circles and line) for the 3 study lakes. Each circle represents a weekly average. Note the different y-axis scales.

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Results

In all 3 lakes over the well-stratified summer period, Chl-*a* and SRP concentrations were generally sustained or increased (Fig. 1). Chl-*a* was higher in Acton in 2012 than in 2011. Seston C:P was relatively constant within all lakes expect for one early date in Lacawac (Fig. 1). GPP and ER were low in Giles and Lacawac; Acton had much higher values, with the highest in 2012 (Fig. 1). Acton GPP and ER were similar to rates reported for this lake in 2008 (Solomon et al. 2013).

In the 2 natural lakes, P regeneration rates calculated from the metabolism and TP regression approaches were more similar in mesotrophic/dystrophic Lacawac than oligotrophic Giles (Table 2, Fig. 2). During summer, rates calculated by the 2 approaches followed the same temporal trends in Lacawac, but only toward late July did they begin to coincide in Giles (Fig. 2). For Giles, the TP regression predicted average summer P regeneration rates 2.7–3.9 times higher than the metabolism approach using R_{hetero} 70 and 50%, respectively, whereas in Lacawac, metabolism rates were similar to those from the TP regression using R_{hetero} 70% and 1.5 times higher using R_{hetero} 50% (Table 2).

For the P budget approach in hypereutrophic Acton, we were able to examine the relative importance of P regeneration versus the new fluxes of SRP from stream loading, entrainment, and excretion and nutrient translocation by a sediment-feeding fish. In both years, stream SRP loading was negligible by midsummer (Fig. 3 and 4). Average summer entrainment represented a small flux of SRP into the epilimnion (Fig. 4), but entrainment events provided pulses of SRP at high rates, particularly in 2012 (Fig. 3). In both years, SRP excretion by gizzard shad increased in magnitude and importance as the summer progressed (Fig. 3). Gizzard shad excretion represented the second largest average summer flux of SRP, with higher rates in 2012 than in 2011 (8.0 and 13.5 mg m^{-2} d^{-1}, respectively; Fig. 4). Average summer P regeneration was the largest SRP flux in both summers, and rates were higher in 2011 than in 2012 (Fig. 4). Temporally during summer, P regeneration often represented the largest SRP flux, but at times entrainment and excretion by gizzard shad were larger (Fig. 3). Phytoplankton P demand (calculated from ^{14}C primary production and seston C:P) was remarkably similar between years (36.7 and 36.3 mg m^{-2} d^{-1}), and bacterial P demand was slightly higher in 2011 than in 2012 (Fig. 4).

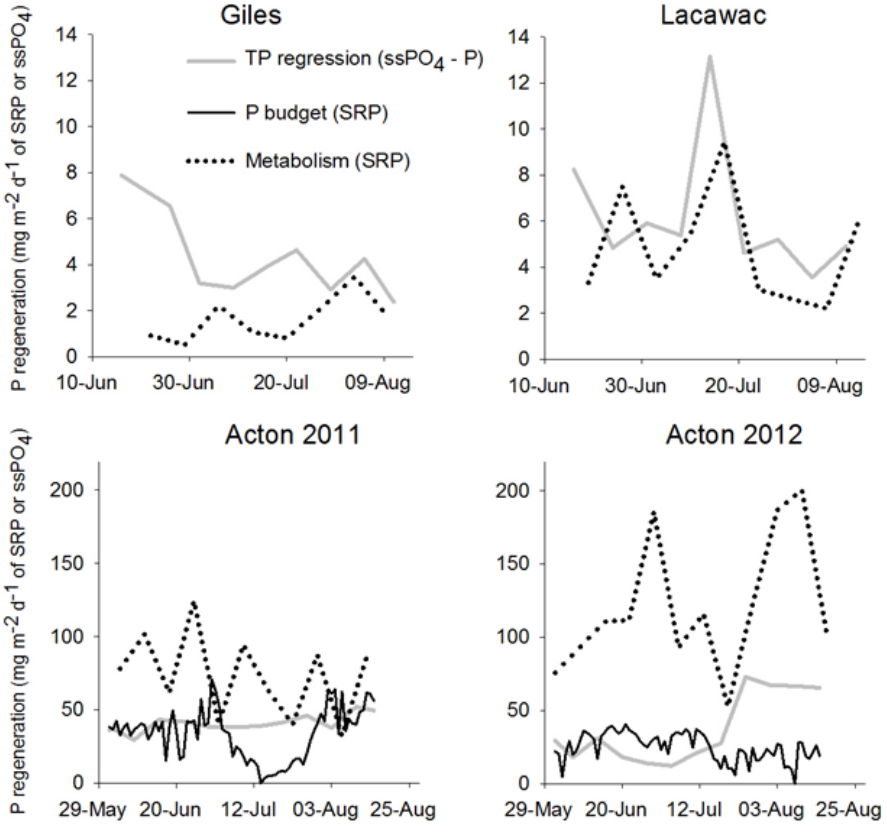

Fig. 2. P regeneration as quantified by TP regression (Hudson et al. 1999), P budget (Acton only; Domine et al. 2010), and a metabolism-based approach (Kamarainen et al. 2009) using 70% R_{hetero} for Giles and Lacawac and 30% R_{hetero} for Acton. Note the different y-axis scales.

Table 2. P regeneration as quantified by TP regression (Hudson et al. 1999), P budget (Acton only; Domine et al. 2010), and a metabolism-based approach (Kamarainen et al. 2009) using 2 sets of values for R_{hetero}:ER, as described in methods. P regeneration units for $ssPO_4$-P using the TP regression approach are mg m^{-2} d^{-1} and for SRP using the metabolism and P budget approach are mg m^{-2} d^{-1}. Values represent bias corrected mean (±1 standard error) summer values (June–mid-August).

Method	Giles P regeneration (mg m^{-2} d^{-1} of P)	Lacawac P regeneration (mg m^{-2} d^{-1} of P)	Acton (2011) P regeneration (mg m^{-2} d^{-1} of P)	Acton (2012) P regeneration (mg m^{-2} d^{-1} of P)
TP regression (as $ssPO_4$-P)	4.3 (0.5)	6.1 (0.7)	40.9 (1.0)	36.7 (9.8)
P budget (as SRP)	—	—	34.0 (5.1)	25.4 (2.9)
Metabolism R_{hetero} = 70%, 70%, 30%	1.6 (0.3)	5.8 (0.6)	73.7 (6.4)	123.2 (11.1)
Metabolism R_{hetero} = 50%, 50%, 10%	1.1 (0.2)	4.1 (0.4)	24.4 (2.3)	41.1 (3.7)

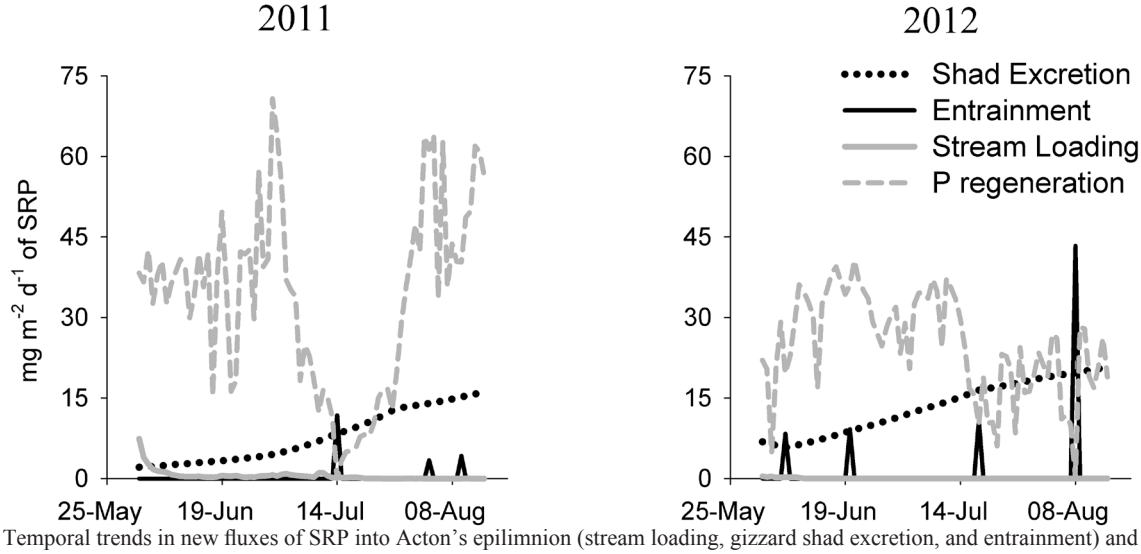

Fig. 3. Temporal trends in new fluxes of SRP into Acton's epilimnion (stream loading, gizzard shad excretion, and entrainment) and P regeneration as determined by P budgets for 2011 (left) and 2012 (right).

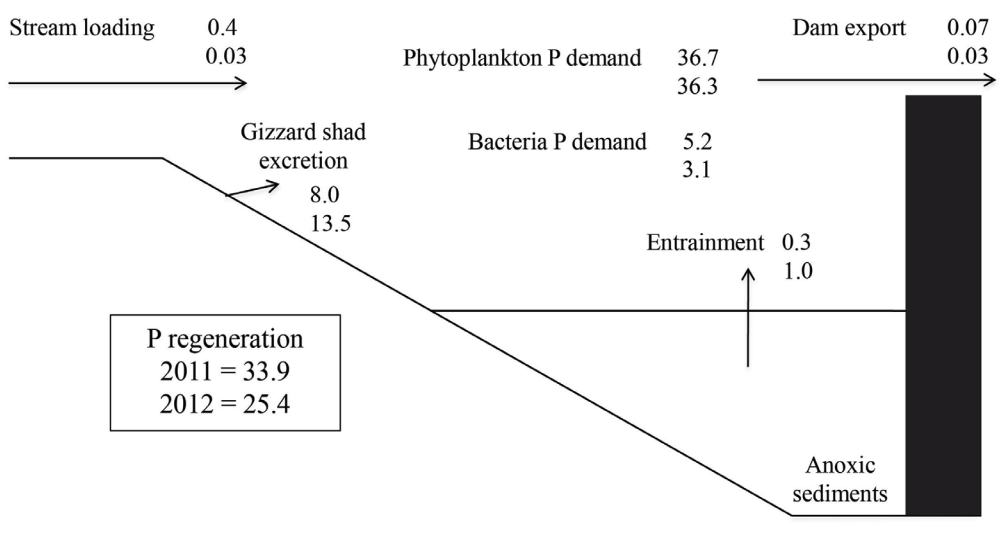

Fig. 4. Average summer fluxes of new SRP into Acton's epilimnion (stream loading, gizzard shad excretion, and entrainment) and P regeneration as determined by P budgets for 2011 (upper numbers) and 2012 (lower numbers). The horizontal line represents the thermocline. Units of SRP are in mg m^{-2} d^{-1}.

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In Acton, average summer P regeneration rates estimated from the 3 approaches were generally lowest using the P budget, with the exception of the metabolism method using 10% R_{hetero} (Table 2). Metabolism P regeneration rates estimated using 30% R_{hetero} and 10% R_{hetero} differed greatly (Table 2). For example in 2012, P regeneration was 123 mg m^{-2} d^{-1} assuming 30% R_{hetero} but was 41 mg m^{-2} d^{-1} when assuming 10% R_{hetero}. Compared to the TP regression and P budget approaches, P regeneration from the metabolism method was more similar using 10% R_{hetero} than 30% R_{hetero} (Table 2). Temporal P regeneration rates also show that the TP regression and P budget approaches were similar, with exceptions in mid-July in 2011 and late July to early August 2012 (Fig. 2). Metabolism estimates, however, were higher than the other methods, a mismatch especially pronounced in 2012 (shown with 30% R_{hetero}; Fig. 2).

Phytoplankton P demand calculated by the metabolism method (70% R_{auto} for Acton and 30% R_{auto} for Giles and Lacawac) was highest in Acton (max = 147 mg m^{-2} d^{-1}) followed by Lacawac (max = 11.5 mg m^{-2} d^{-1}) and then Giles (max = 3.3 mg m^{-2} d^{-1}). In Giles, P regeneration was estimated to supply a large portion of the phytoplankton P demand (28–200%), and in 2 of the 8 weeks, regeneration was estimated to fully support P demand (Fig. 5; i.e., when points fall along and above the 1:1 line, indicating that P regeneration can support P demand). Estimated P regeneration rates were high enough to fully support P demand in 3 of the 7 weeks in Lacawac (Fig. 5) and over the summer represented 73–151% of P demand. For Acton, 5 of the 22 weeks (over both years) resulted in estimates of no P demand (Fig. 5) because, for these weeks, R_{auto} was greater than NPP. Including the weeks for Acton with no demand, P

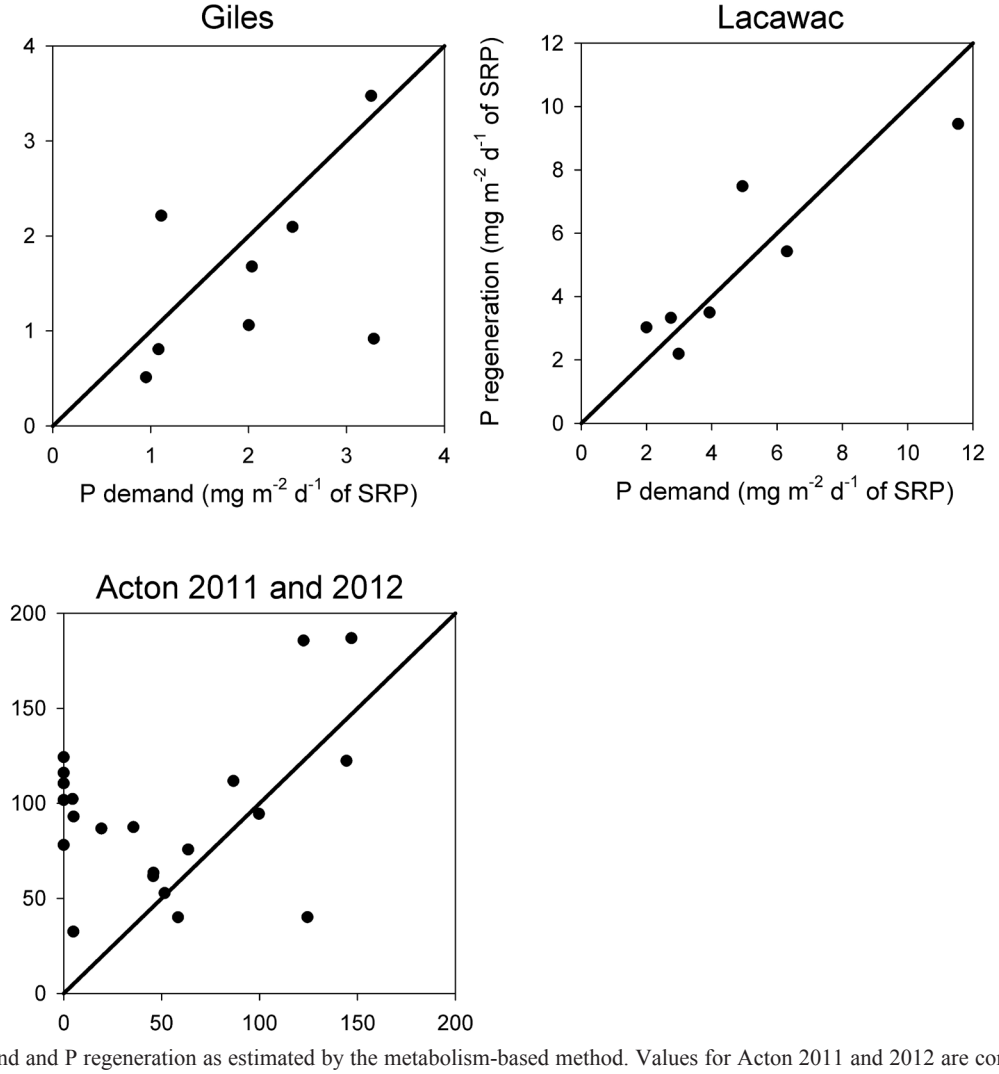

Fig. 5. P demand and P regeneration as estimated by the metabolism-based method. Values for Acton 2011 and 2012 are combined. The solid black line in each panel indicates the 1:1 line. Points that fall along and above the 1:1 line indicate that P regeneration can support P demand.

Table 3. Slopes as calculated from the sensitivity analysis of P regeneration when calculating the percent increase of all possible values R_{hetero} from the mean for each lake. Analyses were run twice for each lake because we assumed the percentage of ecosystem respiration (ER) attributed to R_{hetero} was either 70 or 50% in Lacawac and Giles and either 30 or 10% in Acton. Steeper slopes indicate a greater sensitivity of P regeneration to changes in R_{hetero} values within the dataset.

Lake	Slopes from P regeneration vs. percent change in R_{hetero}*	Slopes from P regeneration vs. percent change in R_{hetero}**
Giles	0.02	0.01
Lacawac	0.06	0.04
Acton 2011	0.71	0.24
Acton 2012	1.20	0.40

* Assumes R_{hetero} is 70% in Giles and Lacawac and 30% in Acton
** Assumes R_{hetero} is 50% in Giles and Lacawac and 10% in Acton

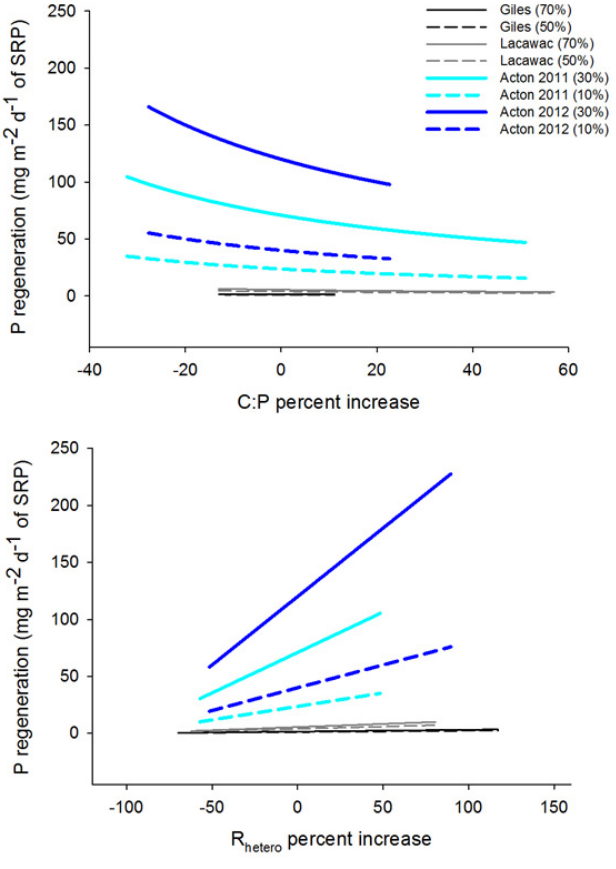

Fig. 6. Sensitivity analysis of P regeneration when calculating the percent increase of all possible values on C:P (upper panel) or R_{hetero} (lower panel) from the mean for each lake. We ran analyses for each lake twice because we assumed the percentage of ecosystem respiration attributed to R_{hetero} was either 70 or 50% in Lacawac and Giles and either 30 or 10% in Acton. Steeper slopes indicate higher sensitivity and also reveal whether relationships are nonlinear (as in upper panel) or linear (as in lower panel). X-axis values indicate the range of variation for that parameter in each dataset.

regeneration was estimated to fully support P demand in 13 of 22 weeks (Fig. 5) and over the summer represented a large range of P demand (0–2239%).

Our sensitivity analyses for the metabolism method revealed several interesting results about the influence of input parameters on estimated P regeneration rates. P regeneration versus the percent change in R_{hetero} displayed a linear relationship, whereas P regeneration versus the percent change in C:P was nonlinear (Fig. 6). Slopes from P regeneration versus the percent change in R_{hetero} showed highest values in Acton, followed by Lacawac and then Giles, suggesting that P regeneration rates in Acton are the most sensitive to changes in R_{hetero} and least sensitive in Giles (Fig. 6, Table 3). P regeneration, as influenced by variable C:P, also seems to be most sensitive in Acton. In general, changing the percentage of ER attributed to R_{hetero} (R_{hetero}:ER) influences P regeneration more in Acton than in either of the lower productivity Pennsylvania lakes. For example, the change in P regeneration rates was larger when considering R_{hetero} at 30% than at 10% R_{hetero} in Acton, whereas changes to R_{hetero}:ER in Giles and Lacawac had a smaller effect (Fig. 6, Table 3).

Discussion

P regeneration rates estimated from these methods show several important trends. When comparing within a lake, the TP regression and metabolism approaches produced similar P regeneration rates for the mesotrophic/dystrophic lake. In the hypereutrophic lake, metabolism-based rates were much higher or lower than either the TP regression or P budget rates, depending on percentage of ecosystem respiration attributed to R_{hetero}. In addition, sensitivity analyses showed that Acton P regeneration rates estimated by the metabolism method were the most sensitive to changes in R_{hetero}. Metabolism-based P regeneration rates in the oligotrophic lake were lower than with the TP regression approach. Sensitivity analyses showed that varying R_{hetero} in oligo-

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trophic Lake Giles had a relatively small effect on estimated P regeneration rates. These results highlight the potential for sensitivity based on the model assumptions of this method, suggesting that determining a realistic estimate for the contribution of R_{auto} and R_{hetero} to ER is important across productivity types and has the potential to greatly influence the absolute values of estimated P regeneration rates in a metabolically active system like hypereutrophic Acton. Interestingly, in a previous study on eutrophic Lake Mendota, the authors concluded that varying R_{auto} and R_{hetero} had a small influence on P regeneration rates (Kamarainen et al. 2009). Our sensitivity analyses revealed that this only seems to be the case in the 2 lower productivity lakes. Although GPP and ER rates are not presented for Lake Mendota in Kamarainen et al. (2009), they are provided in another study, and these O_2 rates did not exceed 9 mg L^{-1} d^{-1} for GPP and 5 mg L^{-1} d^{-1} for ER (Solomon et al. 2013). By comparison, GPP and ER (as O_2) in Acton were higher (up to 24 mg L^{-1} d^{-1} for GPP and 25 mg L^{-1} d^{-1} for ER), particularly so in 2012 when we documented a greater mismatch in P regeneration rates between the metabolism-based and other approaches. Seston C:P was relatively constant seasonally in Acton. Although work has been done to estimate R_{auto} and R_{hetero} in a variety of systems (del Giorgio and Peters 1993, Biddanda et al. 2001), more work is needed to improve the conversion of GPP calculated from free-water DO measurements to NPP.

The efficacy of the metabolism approach is influenced by several other factors. In the present study, we assumed that both the photosynthetic quotient (PQ) and respiratory quotient (RQ) were 1.0. This assumption is often made, but PQ has been shown to vary from 0.8 to 1.2 (del Giorgio and Peters 1993). In the Kamarainen et al. (2009) study, varying PQ and RQ influenced the magnitude of P demand and P regeneration but had a greater overall impact on the extent of P demand met by P regeneration. The metabolism method for estimating P regeneration also assumes that P is the limiting nutrient. This assumption is generally true for all 3 lakes; for Acton, nearly 10 years of data show that phytoplankton are usually P limited (Vanni et al. 2006a, Hayes et al. 2015), and both Lacawac and Giles phytoplankton are at times co-limited by P and N (L.B. Knoll, unpubl. data). Further, SRP concentrations in Acton are typically low and near detection limit except after storms, and P is incorporated into phytoplankton quickly (Vanni et al. 2006a). The metabolism approach also assumes that bacteria are not net consumers of P. If, however, bacteria are net consumers of P in these lakes, then P regeneration rates using the metabolism method will be overestimated (Kamarainen et al. 2009). In the Lake Mendota study using the metabolism approach, the authors support this bacteria assumption by illustrating how bacteria P uptake rates are likely near zero because Lake Mendota bacterial biomass in the summer is generally constant and because previous work suggested that bacteria are typically unable to store P (Cotner and Wetzel 1992, Kamarainen et al. 2009). Finally, the metabolism approach was developed using epilimnetic buoy and nutrient data. In lakes like Lacawac and Giles, with sub-epilimnetic peaks in phytoplankton biomass, using only epilimnetic oxygen data for the metabolism approach may misrepresent water column metabolism (Staehr et al. 2012, Obrador et al. 2014), and advances in automated profiling buoys may help to alleviate these issues and improve our ability to understand these systems with vertical heterogeneity (Brentrup et al. 2016).

P demand and P regeneration rates estimated from the metabolism method show that on a weekly basis, 0–2000% of P demand was supplied by regeneration; however, when considered for the entire summer, P regeneration did not meet all P demand requirements. We would not expect regeneration to fully support P demand over the long-term because other fluxes are known to be important in sustaining phytoplankton production (e.g., catchment inputs, entrainment). Data from the P budget in Acton highlight the relative importance of new sources of P in meeting P demands. Gizzard shad excretion represented a significant source of SRP, particularly in late summer. In the present study, P regeneration was 2.7–4.5 times greater than gizzard shad excretion, results similar to a previous Acton study (Domine et al. 2010). Note that SRP from gizzard shad excretion represents a new source of P, whereas regenerated P is recycled within the epilimnion. Thus, new sources of P are critical in sustaining phytoplankton production and also represent an increase in the P available to become part of the epilimnetic recycling P pool (Vanni 2002). In Acton, entrainment also represented a significant pulse of SRP during the summer, a finding supported by other studies (Auer et al. 1993, Soranno et al. 1997, Nowlin et al. 2005, Kamarainen et al. 2009). For Giles and Lacawac, we were unable to calculate P budgets, but we expect that entrainment may also represent an important new source of P, particularly in Lacawac. Historically, Giles has been well oxygenated throughout the water column in the summer, but in recent years the bottom waters have become anoxic in the summer with concomitant accumulation of P in the hypolimnion (L.B. Knoll, unpubli. data). Thus, entrainment in Giles is likely to support some P demand. In lakes like Lacawac and Giles, with sub-epilimnetic chlorophyll maxima, migrating zooplankton may also play a role in transporting nutrients to the epilimnion. These zooplankton feed

in deep chlorophyll maxima during the day and have the potential to transport SRP upward at night via excretion. Preliminary data in Giles suggest that this flux can supply ~3% of phytoplankton P demand during midsummer (T.H. Leach, unpubl. data). Without new SRP inputs, such as those by gizzard shad, entrainment, and migrating zooplankton, TP would decline in the epilimnion as phytoplankton die and become part of the sediment P pool (Baines and Pace 1994).

Our results show general agreement between the different methods used to estimate P regeneration in the 3 lakes of varying productivity, especially noteworthy considering the number of estimated parameters. However, our results also highlight the variability between the methods and the sensitivity of the metabolism approach to the relative contribution of R_{auto} and R_{hetero} to ER in a hypereutrophic lake. We also show that compared with the TP regression approach, the metabolism-based method underestimated P regeneration in the oligotrophic lake, was nearly equivalent in the mesotrophic/dystrophic lake, and overestimated P regeneration in the hypereutrophic lake. These results are intriguing given that the TP regression approach provides P regeneration as $ssPO_4$-P rather than SRP, and $ssPO_4$-P concentrations are often orders of magnitude lower than corresponding SRP concentrations (Hudson et al. 2000, Nowlin et al. 2007). Further, maximum TP values in the Hudson et al. (1999) regression were about 80 μg L^{-1}, Acton's mean summer TP concentrations in 2011 and 2012 were similar (77 μg L^{-1}), and the regression approach matches well with the P budget and metabolism approaches (when R_{hetero} is 10%).

Taken together, our results suggest that the metabolism approach may be easier to implement in oligotrophic to mesotrophic lakes (this study) and eutrophic lakes (e.g., Lake Mendota studied by Kamarainen et al. 2009) when using model assumptions based on current literature values. This method may be most sensitive to model assumptions in hypereutrophic lakes that are extremely biologically active; thus, more research is needed to fine-tune these assumptions in such systems. As high-frequency DO data become more common within GLEON and beyond, and as models for estimating GPP and ER improve along with technological advances in high-frequency measurements of other parameters like phosphorus concentrations, limnologists will be able continue efforts to understand lake dynamics on a finer temporal scale.

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References

Auer MT, Johnson NA, Penn MR, Effler SW. 1993. Measurement and verification of rates of sediment phosphorus release for a hypereutrophic urban lake. Hydrobiologia. 253:301–309.

Baines SB, Pace ML. 1994. Relationships between suspended particulate matter and sinking flux along a trophic gradient and implications for the fate of planktonic primary production. Can J Fish Aquat Sci. 51:25–36.

Biddanda B, Ogdahl M, Cotner J. 2001. Dominance of bacterial metabolism in oligotrophic relative to eutrophic waters. Limnol Oceanogr. 46:730–739.

Brentrup JA, Williamson CE, Colom-Montero W, Eckert W, de Eyto E, Grossart HP, Huot Y, Isles P, Knoll LB, Leach TH, et al. 2016. The potential of high-frequency profiling to assess vertical and seasonal patterns of phytoplankton dynamics in lakes: an extension of the Plankton Ecology Group (PEG) model. Inland Waters. 6:565–580.

Caraco NF, Cole JJ, Likens GE. 1992. New and recycled primary production in an oligotrophic lake - insights for summer phosphorus dynamics. Limnol Oceanogr. 37:590–602.

Caston CB, Nowlin WH, Gaulke A, Vanni MJ. 2009. The relative importance of heterotrophic bacteria to pelagic ecosystem dynamics varies with reservoir trophic state. Limnol Oceanogr. 54:2143–2156.

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.

Cotner JB, Wetzel RG. 1992. Uptake of dissolved inorganic and organic phosphorus-compounds by phytoplankton and bacterioplankton. Limnol Oceanogr. 37:232–243.

del Giorgio PA, Peters RH. 1993. Balance between phytoplankton production and plankton respiration in lakes. Can J Fish Aquat Sci. 50:282–289.

Dickman EM, Vanni MJ, Horgan MJ. 2006. Interactive effects of light and nutrients on phytoplankton stoichiometry. Oecologia. 149:676–689.

Dodds WK, Priscu JC, Ellis BK. 1991. Seasonal uptake and regeneration of inorganic nitrogen and phosphorus in a large oligotrophic lake - size-fractionation and antibiotic-treatment. J Plankton Res. 13:1339–1358.

 DOI: 10.5268/IW-6.4.866

Domine LM, Vanni MJ, Renwick WH. 2010. New and regenerated primary production in a productive reservoir ecosystem. Can J Fish Aquat Sci. 67:278–287.

Dugan HA, Woolway RI, Santosa AB, Corman JR, Jaimes A, Nodine ER, Patil VP, Zwart JA, Brentrup JA, Hetherington AL, et al. 2016. Consequences of gas flux model choice on the interpretation of metabolic balance across 15 lakes. Inland Waters. 6:581–592.

Elser JJ, Marzolf ER, Goldman CR. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the fresh-waters of North America - a review and critique of experimental enrichments. Can J Fish Aquat Sci. 47:1468–1477.

Hayes NM, Vanni MJ, Horgan MJ, Renwick WH. 2015. Climate and land use interactively affect lake phytoplankton nutrient limitation status. Ecology. 96:392–402.

Higgins KA, Vanni MJ, Gonzalez MJ. 2006. Detritivory and the stoichiometry of nutrient cycling by a dominant fish species in lakes of varying productivity. Oikos. 114:419–430.

Hudson JJ, Taylor WD. 1996. Measuring regeneration of dissolved phosphorous in planktonic communities. Limnol Oceanogr. 41:1560–1565.

Hudson JJ, Taylor WD, Schindler DW. 1999. Planktonic nutrient regeneration and cycling efficiency in temperate lakes. Nature. 400:659–661.

Hudson JJ, Taylor WD, Schindler DW. 2000. Phosphate concentration in lakes. Nature. 406:54–56.

Jennings E, Jones SE, Arvola L, Staehr PA, Gaiser E, Jones ID, Weathers KC, Weyhenmeyer GA, Chiu C-Y, De Eyto E. 2012. Effects of weather-related episodic events in lakes: an analysis based on high-frequency data. Freshwater Biol. 57:589–601.

Juday C, Birge EA, Kemmerer GI, Robinson RJ. 1927. Phosphorus content of lake waters of northeastern Wisconsin. T Wisc Acad Sci Arts Lett. 23:233–248.

Kamarainen AM, Penczykowski RM, Van de Bogert MC, Hanson PC, Carpenter SR. 2009. Phosphorus sources and demand during summer in a eutrophic lake. Aquat Sci. 71:214–227.

Klug JL, Richardson DC, Ewing HA, Hargreaves BR, Samal NR, Vachon D, Pierson DC, Lindsey AM, O'Donnell DM, Effler SW, et al. 2012. Ecosystem effects of a tropical cyclone on a network of lakes in northeastern North America. Environ Sci Technol. 46:11693–11701.

Knoll LB, Vanni MJ, Renwick WH. 2003. Phytoplankton primary production and photosynthetic parameters in reservoirs along a gradient of watershed land use. Limnol Oceanogr. 48:608–617.

Knoll LB, Vanni MJ, Renwick WH, Dittman EK, Gephart JA. 2013. Temperate reservoirs are large carbon sinks and small CO_2 sources: results from high-resolution carbon budgets. Glob Biogeochem Cy. 27:52–64.

Kunsch HR. 1989. The jackknife and the bootstrap for general stationary observations. Ann Stat. 17:1217–1241.

Loucks DP, van Beek E. 2005. Model sensitivity and uncertainty analysis. In: Water resources systems planning and management: an introduction to methods, models and applications. Paris (France): UNESCO. p. 255–290.

Melack JM. 1978. Morphometric, physical and chemical features of the volcanic crater lakes of western Uganda. Arch Hydrobiol. 84:430–453.

Nowlin WH, Davies JM, Mazumder A. 2007. Planktonic phosphorus pool sizes and cycling efficiency in coastal and interior British Columbia lakes. Freshwater Biol. 52:860–877.

Nowlin WH, Evarts JL, Vanni MJ. 2005. Release rates and potential fates of nitrogen and phosphorus from sediments in a eutrophic reservoir. Freshwater Biol. 50:301–322.

Obrador B, Staehr PA, Christensen JPC. 2014. Vertical patterns of metabolism in three contrasting stratified lakes. Limnol Oceanogr. 4:1228–1240.

R Development Core Team. 2013. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Read JS, Hamilton DP, Desai AR, Rose KC, MacIntyre S, Lenters JD, Smyth RL, Hanson PC, Cole JJ, Staehr PA, et al. 2012. Lake-size dependency of wind shear and convection as controls on gas exchange. Geophys Res Lett. 39.

Renwick WH, Vanni MJ, Zhang Q, Patton J. 2008. Water quality trends and changing agricultural practices in a Midwest US watershed, 1994–2006. J Environ Qual. 37:1862–1874.

Schindler DW. 1977. Evolution of phosphorus limitation in lakes. Science. 195:260–262.

Smith VH. 1979. Nutrient dependence of primary productivity in lakes. Limnol Oceanogr. 24:1051–1064.

Solomon CT, Bruesewitz DA, Richardson DC, Rose KC, Van de Bogert MC, Hanson PC, Kratz TK, Larget B, Adrian R, Babin BL, et al. 2013. Ecosystem respiration: drivers of daily variability and background respiration in lakes around the globe. Limnol Oceanogr. 58:849–866.

Soranno PA, Carpenter SR, Lathrop RC. 1997. Internal phosphorus loading in Lake Mendota: response to external loads and weather. Can J Fish Aquat Sci. 54:1883–1893.

Staehr PA, Christensen JPA, Batt RD, Read JS. 2012. Ecosystem metabolism in a stratified lake. Limonl Oceanogr. 57:1317–1330.

Staehr PA, Sand-Jensen K. 2007. Temporal dynamics and regulation of lake metabolism. Limnol Oceangr. 52:108–120.

Sterner RW, Chrzanowski TH, Elser JJ, George NB. 1995. Sources of nitrogen and phosphorus supporting the growth of bacterioplankton and phytoplankton in an oligotrophic Canadian shield lake. Limnol Oceanogr. 40:242–249.

Sterner RW, Elser JJ. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton (NJ): Princeton University Press.

Vanni MJ. 2002. Nutrient cycling by animals in freshwater ecosystems. Annu Rev Ecol Syst. 33:341–370.

Vanni MJ, Andrews RS, Renwick WH, Gonzalez MJ, Noble SE. 2006a. Nutrient and light limitation of reservoir phytoplankton in relation to storm-mediated pulses in stream discharge. Arch Hydrobiol. 167:421–445.

Vanni MJ, Bowling AM, Dickman EM, Hale RS, Higgins KA, Horgan MJ, Knoll LB, Renwick WH, Stein RA. 2006b. Nutrient cycling by

fish supports relatively more primary production as lake productivity increases. Ecology. 87:1696–1709.

Vanni MJ, Renwick WH, Headworth JL, Auch JD, Schaus MH. 2001. Dissolved and particulate nutrient flux from three adjacent agricultural watersheds: a five-year study. Biogeochemistry. 54:85–114.

Weathers KC, Hanson PC, Arzberger P, Brentrup J, Brooks J, Carey CC, Gaiser E, Hamilton DP, Hong GS, Ibelings B, et al. 2013. The Global Lake Ecological Observatory Network (GLEON): the evolution of grassroots network science. Limnol Oceanogr Bull. 22:71–73.

Williamson CE, Brentrup JA, Zhang J, Renwick WH, Hargreaves BR, Knoll LB, Overholt EP, Rose KC. 2014. Lakes as sensors in the landscape: optical metrics as scalable sentinel responses to climate change. Limnol Oceanogr. 59:840–850.

Winslow LA, Zwart JA, Batt RD, Dugan HA, Woolway RI, Corman JR, Hanson PC, Read JS. 2016. LakeMetabolizer: an R package for estimating lake metabolism from free-water oxygen using diverse statistical models. Inland Waters. 6:622–636

Winslow L, Zwart J, Batt R, Corman J, Dugan H, Hanson P, Holtgrieve G, Jaimes A, Read J, Woolway R. 2015. LakeMetabolizer. R package version 1.1 https://CRAN.R-project.org/package=LakeMetabolizer.

 DOI: 10.5268/IW-6.4.866