

## Lake metabolism: Relationships with dissolved organic carbon and phosphorus

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### *Abstract*

Recent literature has suggested that for many lakes and rivers, the respiratory breakdown of organic matter (R) exceeds production of organic matter by photosynthesis (gross primary production [GPP]) within the water body. This metabolic balance ( $GPP < R$ ; “heterotrophy”) implies that allochthonous organic matter supports a portion of the aquatic ecosystem’s respiration. Evidence that many lakes are heterotrophic comes from diverse approaches, and debate remains over the circumstances in which heterotrophy exists. The methods used to estimate GPP and R and the limited extent of lake types studied, especially with respect to dissolved organic carbon (DOC) and total phosphorus (TP) concentrations, are two reasons for differing conclusions. We deployed  $O_2$  and  $CO_2$  sondes to measure diel gas dynamics in the surface waters of 25 lakes. From these data, we calculated GPP, R, and net ecosystem production ( $NEP = GPP - R$ ). Over the broad range in TP and DOC among the lakes, diel  $CO_2$  and  $O_2$  changed on a near 1:1 molar ratio. Metabolism estimates from the two gases were comparable, except at high pH. Most lakes in our data set had negative NEP, but GPP and R appeared to be controlled by different factors. TP correlated strongly with GPP, whereas DOC correlated with R. At low DOC concentrations, GPP and R were nearly equal, but, at higher DOC, GPP and R uncoupled and lakes had negative NEP. Strong correlations between lake metabolism and landscape related variables suggest that allochthonous carbon influences lake metabolism.

Research in lake metabolism has provided intriguing but sometimes contradictory insights into the balance between gross primary production (GPP) and respiration (R) for surface waters in north temperate lakes (del Giorgio and Peters 1994; Carignan et al. 2000; Prairie et al. 2002). The ambiguity in the interpretation of results may be due to the diversity of lakes that have been compared and to the methods used to estimate GPP and R (Carignan et al. 2000; Prairie et al. 2002).

The balance between GPP and R, or net ecosystem production ( $NEP = GPP - R$ ), can be used to define lake trophic classification, with positive NEP equaling autotrophy and negative NEP equaling heterotrophy. Lakes with high total phosphorus (TP) concentrations and low dissolved organic carbon (DOC) concentrations tend to be autotrophic, whereas lakes with low TP and high DOC tend to be heterotrophic (Cole et al. 2000). Although north temperate lakes span broad ranges in TP and DOC, many have moderate concentrations and do not fit the extreme TP and DOC profiles of lakes that clearly belong in one of the two trophic classifications.

Estimating GPP and R in lakes and explaining their variability among lakes have emerged as topics of debate (Car-

ignan et al. 2000; Prairie et al. 2002). Interpretation of the results of two comparative studies (del Giorgio et al. 1994; Carignan et al. 2000) led to divergent views on the relative importance of autochthonous and allochthonous carbon to metabolism in oligotrophic lakes. Prairie et al. (2002) explained these differences as resulting from slightly differing DOC ranges. Most of the lakes in the aforementioned studies (del Giorgio et al. 1994; Carignan et al. 2000; Prairie et al. 2002) were oligotrophic and do not represent the full range of productivity and DOC in north temperate lakes. Their results leave unanswered questions about the importance of allochthonous versus autochthonous carbon to metabolism in the full spectrum of north temperate lakes. One study (Cole et al. 2000), in which broad ranges in DOC and TP were included, found lakes to be heterotrophic unless nutrients and the food web were manipulated, but only four lakes were included in that study.

The method used for estimating GPP and R also can lead to different conclusions regarding lake trophic status. Variations on traditional bottle incubations (Fee 1990) and extrapolation of results to whole-lake values has been critically addressed in previous work (Schindler 1977; Fee 1980). One alternative to bottle techniques is measuring in situ changes in free water gases (Odum 1956), but studies using free water diel dissolved  $O_2$  (DO) in lakes are not common (Carpenter and Gasith 1978; Cole and Fisher 1978; Bachmann et al. 2000; Cole et al. 2000), and the use of DO probes for metabolic estimates has come under scrutiny for its inadequate sensitivity in unproductive waters (Carignan 1998). Metabolism, as measured by free water  $CO_2$ , should corroborate DO estimates. Although some researchers have built free water  $CO_2$  measurement systems (DeGrandpre et al. 1995; Sellers et al. 1995; Carignan 1998), no published estimates of metabolism using these devices in a variety of

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Table 1. Chemical characteristics and mean metabolic estimates for surface waters of lakes in the study. Metabolic values are estimated from diel gas dynamics.  $R_{CO_2}$ ,  $R_{O_2}$ ,  $GPP_{CO_2}$ , and  $GPP_{O_2}$  are in  $mmol\ m^{-3}\ d^{-1}$ .

Lake	Area (ha)	Total P ( $mg\ m^{-3}$ )	DOC ( $g\ m^{-3}$ )	Chlorophyll ( $mg\ m^{-3}$ )	Thermocline depth (m)	$R_{CO_2}$	$R_{O_2}$	$GPP_{CO_2}$	$GPP_{O_2}$
Allequash	168.4	39.8	3.7	9.6	5.8	9.8	39.6	10.4	34.2
Big Muskellunge	396.3	9.4	4.5	4.5	10.0	7.2	20.0	4.4	11.8
Bog Pot	1.8	41.5	13.5	37.5	1.5	48.8	95.7	48.5	74.9
Brown	32.9	55.9	9.1	13.2	2.5	5.8	39.0	4.1	36.9
Crampton	25.8	13.2	4.0	4.1	5.3	12.4	13.8	10.6	3.8
Cranberry Bog	1.4	17.9	11.5	22.6	1.8	59.3	49.4	44.1	34.4
Crystal	36.7	4.4	1.6	2.7	7.5	3.5	5.6	4.6	6.1
Diamond	49.4	7.1	1.9	2.5	7.8	9.5	12.9	7.6	10.0
East Long	2.3	23.2	12.4	14.7	2.5	38.8	45.9	25.4	25.5
Helmet	2.8	19.9	20.3	3.5	1.5	24.7	51.0	1.01	7.8
Hiawatha	14.6	20.2	17.5	13.6	3.5	14.1	27.5	3.6	13.3
Hummingbird	0.8	34.3	20.3	19.8	1.5	61.3	145.8	-15.5	15.0
Kickapoo	7.9	34.9	14.2	14.3	1.0	119.8	121.0	75.3	77.9
Little Arbor Vitae	216.1	105.3	3.2	56.9	5.0	0.5	112.1	2.3	144.3
Mary	1.2	18.6	21.7	25.1	2.0	50.7	47.3	31.6	35.0
Muskellunge	110.0	78.3	5.0	18.4	4.0	14.6	41.6	17.3	47.9
Northgate Bog	0.3	15.3	24.6	2.7	1.5	83.5	77.6	3.1	7.1
Peter	2.7	21.3	6.4	30.2	3.5	-0.3	46.1	1.0	60.8
Plum	91.0	28.0	4.7	10.3	7.0	5.6	41.8	8.6	28.3
Reddington Bog	1.2	34.0	23.1	17.6	1.5	93.8	110.0	-2.3	13.3
Tenderfoot	165.2	42.5	7.8	17.3	5.0	14.3	39.4	13.4	30.5
Trout Bog	1.1	29.3	17.0	38.8	1.5	33.1	35.2	39.4	49.1
Trout Lake	1,090.9	25.2	2.2	3.0	11.0	1.3	7.4	1.3	7.3
Ward	2.7	28.1	7.0	5.8	3.0	28.3	35.4	22.7	32.1
West Long	5.5	13.8	6.6	7.6	3.5	33.0	39.3	30.2	29.7

lakes are available. Besides technological hurdles, free water methods have inherent limitations and assumptions. Changes in dissolved gas concentrations are due not only to metabolic activity but also to groundwater loading, physicochemical interactions, photorespiration of DOC, and flux with the atmosphere.

In the present study, we address the relative importance of DOC and TP concentrations as drivers of GPP, R, and NEP in northern Wisconsin lakes. The lakes span broad and orthogonal ranges in DOC and TP, which allows for independent estimates of the significance of these drivers on metabolism. We also investigate the influence on metabolism of other limnological variables, such as chlorophyll, thermocline depth, and water color. We measure in situ diel dissolved  $CO_2$  and  $O_2$  and use metabolism estimates from the former to corroborate those from the latter. Finally, we assess the trophic status of these lakes across the DOC and TP gradients and discuss the implications of using free water methods versus more traditional bottle techniques.

## Methods

*Study sites*—We sampled surface waters of 25 lakes in the Northern Highland Lake district of Wisconsin and the Upper Peninsula of Michigan during July and August of 2000 (Table 1). The lakes were chosen to span wide and orthogonal ranges in DOC and TP concentrations and for their close proximity to the Trout Lake Station in Vilas county, Wisconsin. The order in which the lakes were sampled was randomized.

*Limnological samples*—Limnological samples were collected once for each lake at 0.5 m depth as follows. DOC samples were collected as the filtrate through Whatman GF/F filters and were analyzed on a Shimadzu model 5050 high-temperature total organic carbon analyzer. Color was also measured from this filtrate as absorbance at 440 nm on a Spectronic Genesys 2 spectrophotometer using 10-cm quartz cuvettes.

Chlorophyll *a* was collected by filtering 200 ml of lake water and then freezing filters for at least 24 h, followed by methanol extraction for 24 h. Fluorescence was determined before and after acidification, to correct for pheopigments (Marker et al. 1980). TP was analyzed on a Lachat autoanalyzer after persulfate digestion of a whole water sample.

Dissolved inorganic carbon (DIC) and  $CO_2$  partial pressure ( $pCO_2$ ) were measured on a Shimadzu GC-8AIT (TCD detector) gas chromatograph. DIC was determined using the method of Stainton (1973), where headspace from acidified samples was injected into the GC.  $pCO_2$  was determined by the method of Cole et al. (1994), in which 2 liters of water is equilibrated with 60 ml of air in a polycarbonate bottle. The equilibrated headspace gas was drawn into two 20-ml syringes and taken to the lab for analysis on the GC. Two replicate equilibrations were performed at each lake.

pH was measured using an Orion digital pH meter with an automatic temperature-compensating electrode. Temperature and DO profiles were measured using a YSI temperature/DO meter. Spot measurements of surface water DO were made on quadruplicate samples, using Winkler titrations as described in Bade et al. (1998).

**Buoys**—We deployed a buoy that sampled dissolved CO<sub>2</sub>, DO, water temperature, photosynthetically active radiation (PAR), and wind speed for 2–4 d on each lake. All water measurements were made at a depth of 0.5 m. Wind speed was measured 1 m above the lake, using an RM Young model 03001, and PAR was measured 10 cm above the lake surface using a Li-Cor model 190SA quantum sensor. Electronic control and data collection were managed by a Campbell Scientific CR10X data logger. DO and water temperature were measured with a YSI model 600-XLM sonde fitted with a Rapid Pulse oxygen probe (model 6562) and temperature sensor. The sonde was attached to the buoy at the opposite end from the CO<sub>2</sub> equilibration chamber (described below). Before deployment, the DO probe was calibrated in air, with a correction for barometric pressure. Calibrations were repeated after retrieval, and data were corrected by assuming linear drift between calibrations. We performed additional calibrations during deployments by taking discrete water samples for Winkler titration. During deployment, DO and water temperature were sampled every 15 min and PAR and wind speed every minute. We averaged these data for the 30 min prior to the CO<sub>2</sub> sampling time, to synchronize data.

We measured dissolved CO<sub>2</sub> independently from DO. Our CO<sub>2</sub> equilibration system was based on the Carignan (1998) design but with modifications. We equilibrated a closed loop of atmospheric gas in an equilibration chamber submerged to 0.5 m. The chamber was made from a PVC cylinder (0.152 × 0.584 m) and contained 100 thin-walled silicon tubes (outer diameter, 1.27 mm; inner diameter, 1.07 mm; length, 260 cm) that converged on common entry and exit points. The equilibrated gas volume was ~234 ml, compared with 38 ml in Carignan's (1998) system; however, the surface area: volume ratios in the exchange systems were similar. The large sample volume accommodated infrared gas analyzer (IRGA) (Li-Cor model 800) response time. We recirculated gas for the last 10 min of every 30-min period, with a flow rate of ~9 ml s<sup>-1</sup>. A pump exchanged lake water every minute during equilibration. Laboratory tests of this system demonstrated 90% equilibration over a 1500-ppm range in 7 min. Equilibrated gas was diverted to the IRGA, equipped with a 14-cm sample cell for lakes with CO<sub>2</sub> concentration <2,000 ppm or a 5-cm sample cell for lakes with CO<sub>2</sub> concentration between 2,000 and 20,000 ppm. After the analysis of equilibrated gas, solenoids were activated to route atmospheric gas (taken 10 cm above the water) for CO<sub>2</sub> analysis. Prior to deployment, the IRGA was calibrated using pure nitrogen and 1,012 ppm CO<sub>2</sub> gas for the longer sample cell or 10,100 ppm CO<sub>2</sub> for the shorter sample cell. Calibrations were repeated after buoy retrieval, and data were corrected by assuming linear drift. During deployment, we performed additional calibrations by taking discrete water samples and analyzing for CO<sub>2</sub> using headspace analysis.

**Metabolism models**—We used the models described in Cole et al. (2000) for calculating NEP and R from diel O<sub>2</sub> and CO<sub>2</sub> curves. During darkness, the change in gas concentration for each 30-min interval is assumed to be due to R and flux with the atmosphere (F). During daylight hours,

changes in gas concentrations are assumed to be due to R, F, and GPP.

Our methods differed from Cole et al. (2000) in that we measured dissolved CO<sub>2</sub> as well as DO, and our sampling frequencies were different. The subscript "gas" in the following paragraphs refers to either CO<sub>2</sub> or O<sub>2</sub>. We calculated R<sub>gas</sub> for each 30-min interval from 30 min past dusk until 30 min before dawn. These half-hour buffers helped reduce erroneous values of R that can occur during the inflection in gas curves that occurs near dawn and dusk. The results were divided by the day fraction to produce estimates for 24-h R<sub>gas</sub>, which then was averaged to produce reported values for each lake (R<sub>O<sub>2</sub></sub> and R<sub>CO<sub>2</sub></sub>; Table 1).

We calculated NEP<sub>daylight</sub> for each 30-min interval for each gas from 30 min past dawn until 30 min before dusk. The results were divided by the day fraction, which in turn were multiplied by the fraction of the day during which light was >10 μmol m<sup>-2</sup> s<sup>-1</sup>, which was the irradiance that corresponded well with inflections in gas concentrations. This produced estimates for NEP<sub>daylight</sub>. GPP<sub>gas</sub> was the sum of NEP<sub>daylight</sub> and R<sub>daylight</sub>. R<sub>daylight</sub> was estimated by multiplying R<sub>gas</sub> by the fraction of the day during which light was >10 μmol m<sup>-2</sup> s<sup>-1</sup>. We assumed that daytime R<sub>gas</sub> equaled nighttime R<sub>gas</sub>, in keeping with the literature (Carignan et al. 2000; Cole et al. 2000). Individual estimates of GPP<sub>gas</sub> were averaged for each lake to produce reported values (GPP<sub>CO<sub>2</sub></sub> and GPP<sub>O<sub>2</sub></sub>; Table 1). The 24-h estimates for NEP<sub>gas</sub> were calculated as the differences between GPP<sub>gas</sub> and R<sub>gas</sub>.

**Statistical analyses**—All variables except pH were transformed (natural log) to normalize distributions and linearize relationships. Because most NEP values were negative, we created a new variate, Y, which was the sum of negative NEP and a constant (33 mmol m<sup>-3</sup> d<sup>-1</sup>) required to make all values of Y positive. Correlations were performed with Y; however, in reporting the correlations, we reversed their signs so that the relationships between correlates and NEP would be more intuitive. TP and DOC each were correlated with GPP, R, and Y. The combined effects of TP and DOC on metabolism were removed by fitting linear regression models, using TP and DOC as predictor variables. Models were fitted separately for the three response variables, GPP, R, and Y. The partial correlations of the regression residuals were calculated for lake color, chlorophyll, pH, thermocline depth, and lake area.

## Results

Physical and chemical characteristics of the 25 study lakes covered ranges typical of northern Wisconsin (Table 1). Nineteen of the lakes were strongly stratified, with the remaining six having thermoclines within 2 m of the bottom.

Buoy measures of free water gases agreed well with manual measures. Simple linear regression between the manual and buoy pCO<sub>2</sub> showed a slope of 1.03 ± 0.037 (±SE) (n = 57, r<sup>2</sup> = 0.935, p < 0.00001) and intercept of -0.313 ± 0.245. The IRGA showed minimal drift, with a mean of -0.28 ± 2.0% (SD) of calibrated value per day. When sonde DO values were regressed against Winkler values, the slope was 1.10 ± 0.036 (±SE) (n = 81, r<sup>2</sup> = 0.913, p < 0.00001)

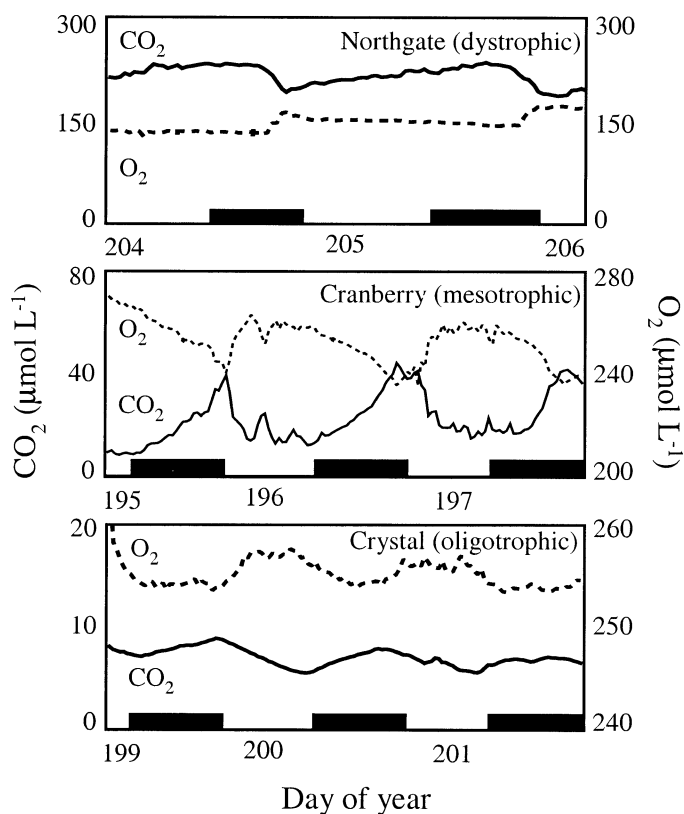


Fig. 1. Continuous dissolved gas measurements in three representative lakes in this study. In each graph, the dashed line is dissolved  $O_2$  ( $\mu\text{mol L}^{-1}$ ), the solid line is dissolved  $CO_2$  ( $\mu\text{mol L}^{-1}$ ), and the horizontal black bars indicate nighttime hours. The concentrations of  $CO_2$  and  $O_2$  decrease and increase respectively from Northgate (dystrophic) to Cranberry (mesotrophic) and Crystal (oligotrophic) lakes.

with an intercept of  $-0.1751 \pm 0.075$ . By comparing initial and final calibrations, we determined drift in the DO probe averaged  $0.02 \pm 0.37$  (SD)  $\text{mg } O_2 \text{ L}^{-1} \text{ d}^{-1}$ . For both  $CO_2$  and DO regressions, no patterns were evident in the residuals, which suggests that buoy measures were unbiased estimates of manual measures. To test whether the DO corrected to Winkler values resulted in different metabolism answers, we calculated GPP and R using both data sets but found no qualitative differences in results. We used the drift-corrected DO values in the calculations of all reported estimates.

**Metabolism models**—Diel changes of  $CO_2$  are near equal but opposite in direction from those of  $O_2$  when compared on a molar basis (Fig. 1). In lakes with high pH, especially Little Arbor Vitae and Peter lakes, the amplitude of the diel  $CO_2$  cycle was dampened compared with the  $O_2$  cycle, probably because of interactions of  $CO_2$  with the carbonate system. Because of the influence of pH on  $CO_2$  measurements, we presented many of the figures using  $O_2$  metabolic values. Metabolic measures based on the two different gases were closely related (Fig. 2). When  $R_{O_2}$  was regressed with  $R_{CO_2}$ , the slope was  $0.941 \pm 0.14$  ( $\pm\text{SE}$ ) ( $p < 0.0001$ ), with an intercept of  $18 \text{ mmol m}^{-3} \text{ d}^{-1} \pm 6.6$  ( $p < 0.02$ ). When

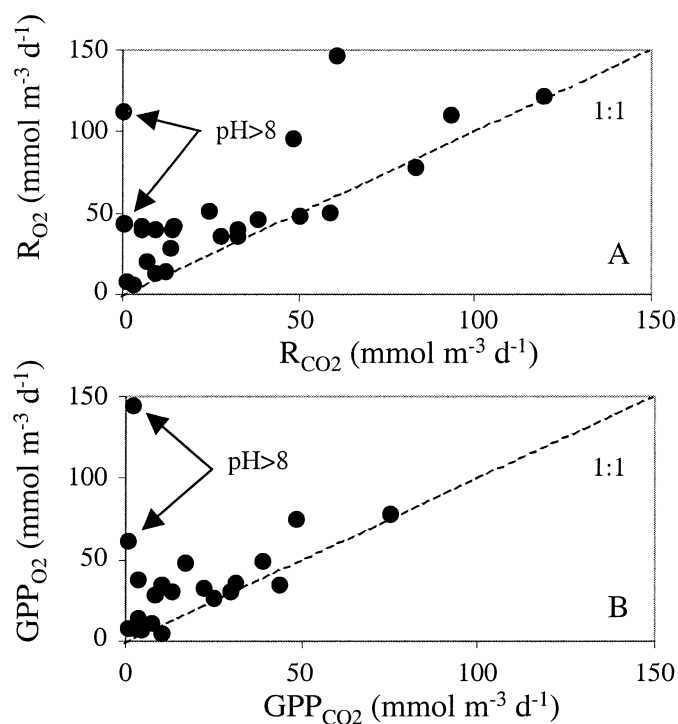


Fig. 2. (A) Mean lake respiration estimates based on  $O_2$  measurements ( $R_{O_2}$ ) versus those based on  $CO_2$  measurements ( $R_{CO_2}$ ). The dashed line is  $Y = X$ . (B) Mean lake gross primary production estimates based on  $O_2$  measurements ( $GPP_{O_2}$ ) versus those based on  $CO_2$  measurements ( $GPP_{CO_2}$ ). The dashed line is  $Y = X$ .

$GPP_{O_2}$  was regressed with  $GPP_{CO_2}$ , the slope was  $0.835 \pm 0.12$  ( $\pm\text{SE}$ ) ( $p < 0.00001$ ), and the intercept was  $13.3 \text{ mmol m}^{-3} \text{ d}^{-1} \pm 3.2$  ( $p < 0.00001$ ).

We calculated correlation coefficients between DO metabolism and lake TP and DOC. TP was positively correlated with GPP ( $r = 0.68$ ,  $p < 0.001$ ), R ( $r = 0.63$ ,  $p < 0.005$ ), and NEP ( $r = 0.33$ ,  $p = 0.11$ ). DOC was positively correlated with R ( $r = 0.70$ ,  $p < 0.001$ ) and negatively with NEP ( $r = -0.68$ ,  $p < 0.05$ ) but was not significantly correlated with GPP ( $r = 0.14$ ,  $p = 0.51$ ). Plots of R and NEP versus DOC (Fig. 3A,B) show that, as DOC increases, respiration increases and NEP decreases. Increasing TP is associated with increasing GPP and increasing NEP (Fig. 3C,D). The lakes with the most negative NEP were highest in DOC, and the lakes with the highest NEP were low in DOC and moderate to high in TP (Fig. 4). When TP and DOC were combined as parameters in linear regression, they explained significant pattern in GPP ( $R^2 = 0.47$ ,  $p < 0.005$ ), R ( $R^2 = 0.71$ ,  $p < 0.005$ ) and NEP ( $R^2 = 0.44$ ,  $p < 0.005$ ). When the residuals of the regressions were correlated with lake color, chlorophyll concentration, pH, thermocline depth and lake area, only chlorophyll showed significance at the  $0.1\alpha$  level. It was positively correlated with residuals from GPP ( $r = 0.50$ ,  $p < 0.05$ ) and NEP ( $r = 0.35$ ,  $p < 0.1$ ).

The relationship among TP, GPP, and R changed over a gradient of DOC. We graphed GPP versus DOC (Fig. 5A) and observed a unimodal pattern with a hump at about  $\text{DOC} = 10 \text{ mg L}^{-1}$ . Below this DOC concentration, GPP and R rise, but, above it, R continues to rise slightly and GPP de-

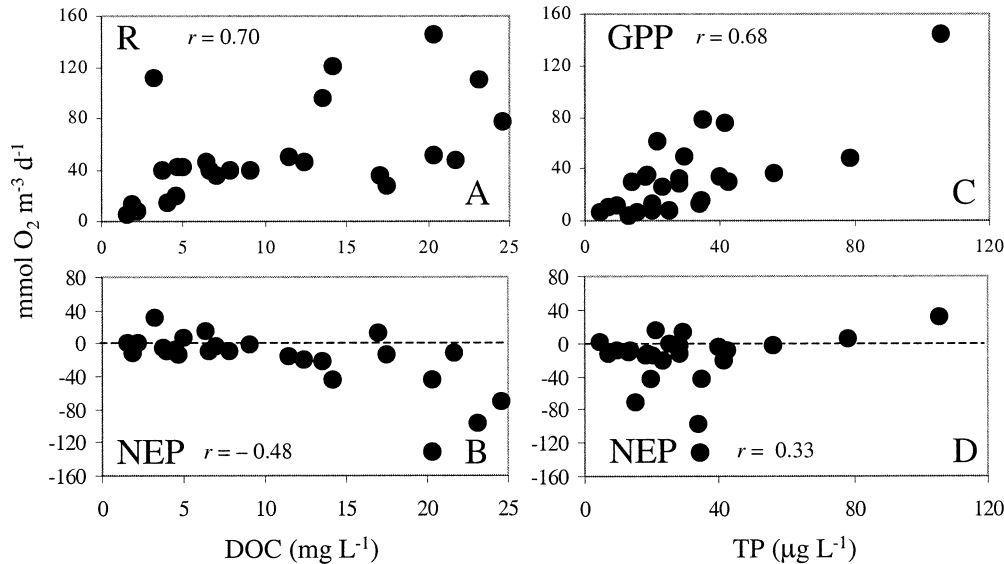


Fig. 3. The relationships of DOC and TP with epilimnetic metabolism in this study. (A and B) Increasing R and decreasing NEP are associated with increasing DOC. (C) Increasing GPP is associated with increasing TP, and (D) increasing NEP is weakly associated with increasing TP. Correlation coefficients are from transformed data.

clines, resulting in negative NEP. Below  $\text{DOC} = 10 \text{ mg L}^{-1}$ , TP was correlated with GPP ( $r = 0.77$ ;  $p < 0.005$ ;  $n = 14$ ), and GPP predicted R ( $r^2 = 0.86$ ;  $p < 0.00001$ ), with a significantly positive intercept ( $0.96 \text{ mmol m}^{-3} \text{ d}^{-1}$ ;  $p < 0.05$ ) and a slope  $< 1$  ( $0.75$ ;  $p < 0.0001$ ). Three of the lakes in this range had positive NEP, and the others were nearly in balance or slightly negative. Above  $\text{DOC} = 10 \text{ mg L}^{-1}$ , the correlation between DOC and GPP ( $r = -0.66$ ;  $p < 0.03$ ;  $n = 11$ ) was stronger than that between TP and GPP ( $r = 0.53$ ;  $p < 0.1$ ), GPP no longer predicted R ( $p = 0.82$ ), and GPP was inversely related to DOC (slope,  $-2.14$ ;  $p < 0.05$ ). All the high DOC lakes had  $\text{NEP} < 0$ , with the exception of Trout Bog, which had unusually high chlorophyll for a dystrophic lake (Table 1). The trend toward negative NEP with increasing DOC concentration was accompanied by a trend

in DO toward undersaturation across the same DOC gradient (Fig. 5B).

## Discussion

*Diel  $\text{O}_2$  and  $\text{CO}_2$* — $\text{O}_2$  and  $\text{CO}_2$  sondes provided similar estimates for lake metabolism, although differences were evident in two respects. First,  $\text{GPP}_{\text{O}_2}$  and  $\text{R}_{\text{O}_2}$  were greater than  $\text{GPP}_{\text{CO}_2}$  and  $\text{R}_{\text{CO}_2}$  in most cases (Fig. 2). One explanation for this could be photosynthetic quotients (PQ) ( $\text{mol O}_2/\text{mol CO}_2$ )  $> 1$  and respiratory quotients (RQ) ( $\text{mol CO}_2/\text{mol O}_2$ )  $< 1$ . For neutral to low pH lakes in our study ( $\text{pH} \leq 7$ ) (see below), the mean PQ was  $\sim 1.25$ ; however, it was not significantly different from 1 ( $p = 0.35$ ). RQ for these lakes was  $\sim 0.77$ , but, unlike PQ, the mean value was different from 1 ( $p < 0.005$ ). Although PQ was not significantly correlated with any other variables in the study, RQ was weakly correlated with lake area ( $r = -0.45$ ;  $p < 0.1$ ) and pH ( $r = -0.44$ ;  $p < 0.1$ ). Our PQ and RQ values are similar to those reported by Wetzel and Likens (1991). Second, lakes with  $\text{pH} > 8$  (Fig. 2) showed much higher metabolic estimates on the basis of diel DO. Enhanced diffusion of  $\text{CO}_2$  at high pH (Wanninkhof and Knox 1996) could explain the underestimate of GPP. However, if diffusion was enhanced, we would expect an overestimate of R in lakes undersaturated in  $\text{CO}_2$ , but we found the opposite trend. The discrepancies between estimates are more likely a function of  $\text{CO}_2$  interactions with the carbonate system at high pH, in which case a significant portion of the C for GPP and R exchanges directly with bicarbonate.

To estimate the effect of the carbonate system on measurable  $\text{CO}_2$  at high pH, we simulated the addition and removal of DIC in a system with constant acid-neutralizing capacity. During photosynthesis, primary producers lower

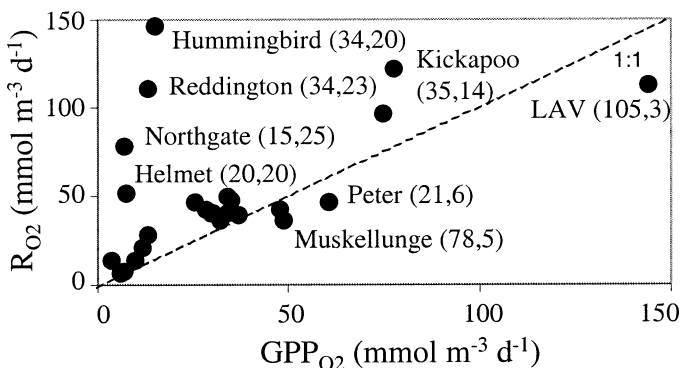


Fig. 4. Estimates of GPP and R for 25 lakes in northern Wisconsin based on diel  $\text{O}_2$  show that surface waters of most lakes are slightly net heterotrophic. Numbers in parentheses indicate TP and DOC concentrations in  $\mu\text{g L}^{-1}$  and  $\text{mg L}^{-1}$ , respectively. LAV, Little Arbor Vitae lake.

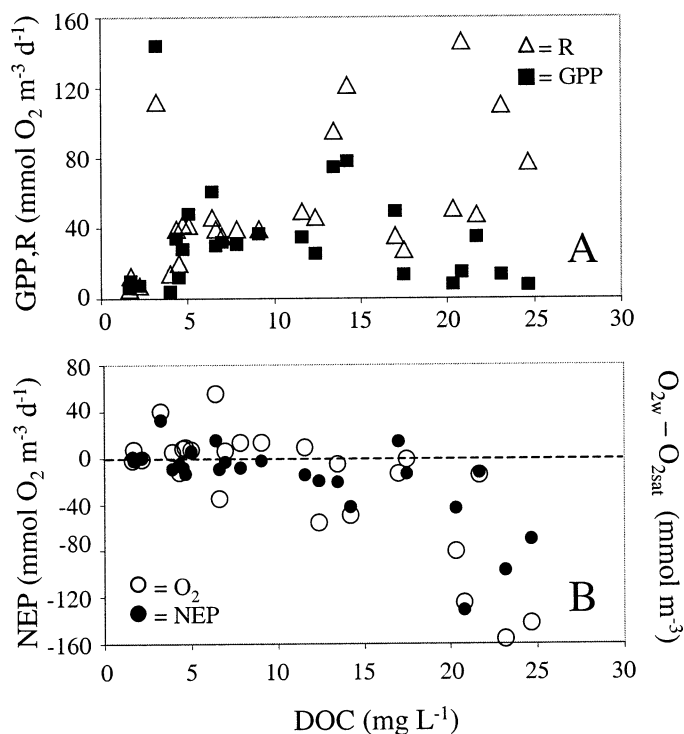


Fig. 5. (A) Estimates of GPP and R for lakes in this study plotted against DOC. Below DOC = 10 mg L<sup>-1</sup>, GPP and R are near equal and linearly related, but above this point, R usually exceeds GPP. (B) Estimates for NEP and direct measures of mean dissolved O<sub>2</sub> minus the O<sub>2</sub> concentration at saturation with the atmosphere (O<sub>2w</sub> - O<sub>2sat</sub>). Below DOC = 10 mg L<sup>-1</sup>, NEP is near zero and dissolved O<sub>2</sub> is near saturation, but above this DOC concentration, NEP becomes negative and dissolved O<sub>2</sub> becomes undersaturated.

DIC concentration, which raises the pH and redistributes carbonate species according to their thermodynamic equilibria. As pH rises above ~8, changes in the DIC pool reflect changes in HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>, with changes in CO<sub>2</sub> becoming less apparent. The GPP signal, as evidenced by changes in dissolved CO<sub>2</sub>, is lost once pH of ~9 is reached. The same is true for R in that changes in CO<sub>2</sub> will not be detected until enough has been added to lower the pH below ~9. Once pH has dropped to ~8, 90% of the free CO<sub>2</sub> added remains as free CO<sub>2</sub>.

Although both DO and CO<sub>2</sub> methods provide similar estimates of metabolism, the more reliable and cost-effective method is DO. However, concern has been expressed that the accuracy of Clark-type electrodes ( $\pm 0.2$  mg L<sup>-1</sup>) is similar to the diel excursion of DO in oligotrophic waters (Carignan 1998). We found this concern mediated by three factors: the high number of measurements used to estimate metabolic values reduced the error, CO<sub>2</sub> metabolism generally corroborated DO metabolism in unproductive waters, and sonde and manual samples agreed well. The disadvantage of the O<sub>2</sub> method is that it requires assumed values for PQ and RQ to convert metabolism to carbon units. Debate over appropriate values for PQ and RQ over a broad range of lakes remains unresolved.

**Metabolism**—We found that both TP and DOC concentrations were important drivers of metabolism, which is consistent with results in other studies (del Giorgio and Peters 1994; Cole et al. 2000). The response of metabolism to the two variables became clearer when GPP and R were viewed across a DOC gradient (Fig. 5A). At low DOC concentrations, GPP was comparable to R, which suggests that autochthonous carbon provides most of the substrate for system respiration. Low-DOC lakes tended to be in near metabolic balance, with 4 lakes having NEP > 0 and 10 lakes having slightly negative NEP. At DOC > 10 mg L<sup>-1</sup>, most lakes had negative NEP. This finding is comparable to that of Prairie et al. (2002), who described lakes as becoming heterotrophic when DOC concentration was > 6 mg L<sup>-1</sup>.

DO data in these lakes corroborated the divergence between GPP and R at DOC = 10 mg L<sup>-1</sup> (Fig. 5B). Lakes showed trends toward negative NEP at DOC > 10 mg L<sup>-1</sup>, and mean DO, as measured by sondes, shifted from supersaturated to undersaturated. Mean CO<sub>2</sub> showed a similar but opposite trend (data not shown), changing from undersaturated to supersaturated. Others have noted similar trends in gas saturation levels above DOC 4–6 mg L<sup>-1</sup>, with CO<sub>2</sub> becoming supersaturated (Hope et al. 1996) and DO becoming undersaturated (Prairie et al. 2002). Although our shift to negative NEP occurred at a higher DOC concentration than the heterotrophic threshold (DOC = 4–6 mg L<sup>-1</sup>) described by Prairie et al. (2002), the difference might be explained by differing TP concentrations. In their study, when DOC was < 10 mg L<sup>-1</sup>, TP concentrations generally were < 20  $\mu$ g L<sup>-1</sup>, whereas, in our study, TP concentrations generally were < 40  $\mu$ g L<sup>-1</sup> over the same DOC range.

**Net production in lakes**—Ample evidence links allochthonous carbon with metabolic processes in lakes. For example, most lakes are supersaturated with CO<sub>2</sub> (Cole et al. 1994), pCO<sub>2</sub> increases with increasing DOC (Hope et al. 1996; Riera et al. 1999), and high DOC is associated with high bacterial production (Hessen 1992). The relative contributions of CO<sub>2</sub> loading versus excess CO<sub>2</sub> due to heterotrophy are debated. DOC budgets suggest that DOC from watersheds is respired in lakes (Dillon and Molot 1997) and that DOC loading can greatly exceed autochthonous production (Caraco and Cole 2000). However, the empirical evidence for heterotrophy is mixed. Some studies have found that unproductive waters are heterotrophic (Hessen 1992; del Giorgio et al. 1997), whereas others have found them to be autotrophic (Carignan et al. 2000) or some mix of the two, depending on DOC concentration (Prairie et al. 2002). Further confounding estimates of heterotrophy is the contribution of DOC photodegradation to the DIC pool (Bertilsson and Tranvik 2000), which tends to bias estimates toward negative NEP. In the following discussion, we address two topics germane to the debate over metabolism and lake trophic classification: the role of CO<sub>2</sub> loading versus in situ metabolism and NEP as assessed by bottles versus sondes.

We estimated the contribution of groundwater CO<sub>2</sub> to calculated lake metabolism in this study as follows. We assumed the mean residence time for lakes in this region to be 2.8 yr (Cole and Pace 1998) and that all water flowing into the lakes was groundwater. Groundwater CO<sub>2</sub> was set

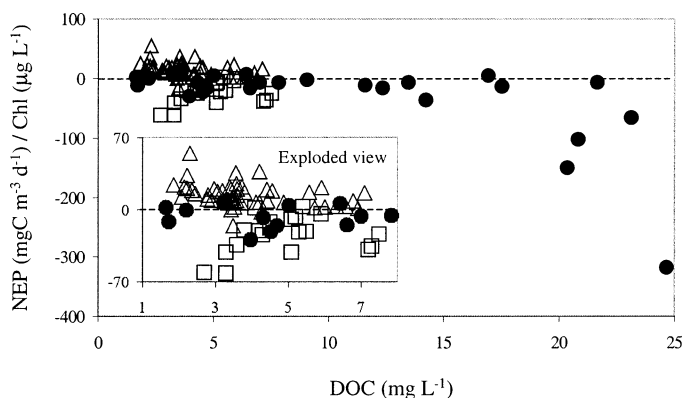


Fig. 6. NEP values normalized for chlorophyll plotted against DOC from three studies, del Giorgio and Peters (1994) (open squares), Carignan et al. (2000) (open triangles), and the present study (filled circles).

to the mean peak hyporheic value ( $250 \mu\text{mol L}^{-1}$ ) measured in the region (Schindler and Krabbenhoft 1998). On the basis of these parameters, a generous estimate of the groundwater contribution to lake  $\text{CO}_2$  was  $0.21 \text{ mmol m}^{-3} \text{ d}^{-1}$ , which was  $\sim 0.8\%–3.7\%$  of measured respiration in the study. These calculations are of the same magnitude as those made by Cole et al. (2000). A notable exception to these assumptions could be dystrophic lakes, for which  $\text{CO}_2$  in the interstitial waters of the surrounding peat has been measured as high as  $2,600 \mu\text{mol L}^{-1}$  (Kratz unpubl. data). In this case,  $\text{CO}_2$  loading could contribute  $2.3\%–7.2\%$  of the R estimate for bog lakes in the study. It is unlikely that groundwater contributions of dissolved gas alone explain R that is in excess of GPP in lakes. The concentrations of dissolved gases in these lakes probably are a direct result of in-lake processes.

Our estimates for GPP and R generally are in agreement with those measured by del Giorgio and Peters (1994) and Carignan et al. (2000), and our estimates for NEP were between theirs. To compare directly their results and ours, we reduced the dimensionality of the data by normalizing NEP for chlorophyll and then plotted the normalized NEP against DOC (Fig. 6). Data from Carignan et al. (2000) suggest that the pelagia of oligotrophic lakes are autotrophic but tend significantly ( $p < 0.05$ ) toward metabolic balance as DOC increases, whereas data from del Giorgio and Peters (1994) show they are heterotrophic with no DOC relationship ( $p = 0.25$ ). Our data generally lie between the two for oligotrophic lakes but then become more negative in NEP with increasing DOC. Prairie et al. (2002) described their data as belonging to the same continuum as Carignan et al.'s (2000), with autotrophy below  $\text{DOC} \sim 6 \text{ mg L}^{-1}$  and heterotrophy at DOC concentrations above that. Our results follow roughly the same pattern.

An important difference between our study and previous work (del Giorgio and Peters 1994; Carignan et al. 2000) is our use of free-water measurements to estimate metabolism. The free-water method avoids the ambiguities of the  $^{14}\text{C}$  method (Peterson 1980) and avoids the debate over the merits of bottle  $\text{O}_2$  versus bottle  $^{14}\text{C}$  methods for estimating production. Furthermore, the integrative nature of free water

measurements allows for systems-level estimates of metabolism.

There is no reason to expect sonde and bottle estimates of lake metabolism to be the same, because they measure different components of lake metabolism. Bottles measure metabolism due to plankton. Sondes measure free-water changes in gas concentrations, which include the contributions of pelagia, sediments, and ground and surface waters, as well as some mixing with the hypolimnion. According to sondes, the surface waters of most northern Wisconsin lakes have negative NEP, especially at higher DOC concentrations. Bottle estimates of NEP tend to differ from those made by sondes for lakes with similar TP and DOC, and, because bottle measures represent only pelagic processes, the differences between sonde and bottle measures could provide insights into the contributions of nonpelagic sources to whole-lake metabolism.

## References

- BACHMANN, R. W., M. V. HOYER, AND D. E. CANFIELD. 2000. Internal heterotrophy following the switch from macrophytes to algae in Lake Apopka, Florida. *Hydrobiologia* **418**: 217–227.
- BADE, D., J. HOUSER, AND S. SCANGA. 1998. Methods of the cascading trophic interactions project. 5th ed. Center for Limnology, University of Wisconsin–Madison.
- BERTILSSON, S., AND L. J. TRANVIK. 2000. Photochemical transformation of dissolved organic matter in lakes. *Limnol. Oceanogr.* **45**: 753–762.
- CARACO, N. F., AND J. J. COLE. 2000. When terrestrial organic matter is sent down the river: Importance of allochthonous C inputs to the metabolism in lakes and rivers. In G. Polis and M. E. Power [eds.], *Food webs at the landscape level*. Univ. Chicago Press.
- CARIGNAN, R. 1998. Automated determination of carbon dioxide, oxygen, and nitrogen partial pressures in surface waters. *Limnol. Oceanogr.* **43**: 969–975.
- , P. DOLORS, AND C. VIS. 2000. Planktonic production and respiration in oligotrophic Shield lakes. *Limnol. Oceanogr.* **45**: 189–199.
- CARPENTER, S. R., AND A. GASITH. 1978. Mechanical cutting of submersed macrophytes: Immediate effects on littoral water chemistry and metabolism. *Water Res.* **12**: 55–57.
- COLE, J. J., N. F. CARACO, G. W. KLING, AND T. K. KRATZ. 1994. Carbon dioxide supersaturation in the surface waters of lakes. *Science* **265**: 1568–1570.
- , AND S. G. FISHER. 1978. Annual metabolism of a temporary pond ecosystem. *Am. Midl. Nat.* **100**: 15–22.
- , AND M. L. PACE. 1998. Hydrologic variability of small, northern Michigan lakes measured by the addition of tracers. *Ecosystems* **1**: 310–320.
- , ———, S. R. CARPENTER, AND J. F. KITCHELL. 2000. Persistence of net heterotrophy in lakes during nutrient addition and food web manipulations. *Limnol. Oceanogr.* **45**: 1718–1730.
- DEL GIORGIO, P. A., J. J. COLE, AND A. CIMBLERIS. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* **385**: 148–151.
- , AND R. H. PETERS. 1994. Patterns in planktonic P:R ratios in lakes: Influence of lake trophy and dissolved organic carbon. *Limnol. Oceanogr.* **39**: 772–787.
- DEGRANDPRE, M. D., T. R. HAMMAR, S. P. SMITH, AND F. L. SAYLES. 1995. In situ measurements of seawater  $\text{pCO}_2$ . *Limnol. Oceanogr.* **40**: 969–975.

- DILLON, P. J., AND L. A. MOLOT. 1997. Dissolved organic and inorganic carbon mass balances in central Ontario lakes. *Biogeochemistry* **36**: 29–42.
- FEE, E. J. 1980. Important factors for estimating annual phytoplankton production in the experimental lakes area. *Can. J. Fish. Aquat. Sci.* **37**: 513–522.
- . 1990. Computer programs for calculating in situ phytoplankton photosynthesis. *Can. Tech. Rep. Fish. Aquat. Sci.* **1740**: 1–27.
- HESSEN, D. O. 1992. Dissolved organic carbon in a humic lake: Effects on bacterial production and respiration. *Hydrobiologia* **229**: 115–123.
- HOPE, D., T. K. KRATZ, AND J. L. RIERA. 1996. Relationship between pCO<sub>2</sub> and dissolved organic carbon in northern Wisconsin lakes. *J. Environ. Qual.* **25**: 1442–1445.
- MARKER, A. F. H., C. A. CROWTHER, AND R. J. M. GUNN. 1980. Methanol and acetone as solvents for estimating chlorophyll and pheopigments by spectrophotometry. *Ergeb. Limnol.* **14**: 52–69.
- ODUM, H. T. 1956. Primary production in flowing waters. *Limnol. Oceanogr.* **1**: 103–117.
- PETERSON, B. J. 1980. Aquatic primary productivity and the <sup>14</sup>C-CO<sub>2</sub> method: A history of the productivity problem. *Annu. Rev. Ecol. Syst.* **11**: 359–285.
- PRAIRIE, Y. T., D. F. BIRD, AND J. J. COLE. 2002. The summer metabolic balance in the epilimnion of southeastern Quebec lakes. *Limnol. Oceanogr.* **47**: 316–321.
- RIERA, J. L., J. E. SCHINDLER, AND T. K. KRATZ. 1999. Seasonal dynamics of carbon dioxide and methane in two clear-water lakes and two bog lakes in northern Wisconsin, U.S.A. *Can. J. Fish. Aquat. Sci.* **56**: 265–274.
- SCHINDLER, D. W. 1977. Evolution of phosphorus limitation in lakes. *Science*. **195**: 260–262.
- SCHINDLER, J. E., AND D. P. KRABBENHOFT. 1998. The hyporheic zone as a source of dissolved organic carbon and carbon gases to a temperate forested stream. *Biogeochemistry* **43**: 157–174.
- SELLERS, P., R. H. HESSELEIN, AND C. A. KELLY. 1995. Continuous measurement of CO<sub>2</sub> for estimation of air-water fluxes in lakes: An in situ technique. *Limnol. Oceanogr.* **40**: 575–581.
- STANTON, M. P. 1973. A syringe gas-stripping procedure for gas-chromatographic determination of dissolved inorganic and organic carbon in freshwater and carbonates in sediments. *Journal of the Fisheries Research Board of Canada* **30**: 1441–1445.
- WANNINKHOF, R., AND M. KNOX. 1996. Chemical enhancement of CO<sub>2</sub> exchange in natural waters. *Limnol. Oceanogr.* **41**: 689–697.
- WETZEL, R. G., AND G. E. LIKENS. 1991. *Limnological analyses*, 2nd ed. Springer.

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