Physical and biological contributions to metalimnetic oxygen maxima in lakes

Grace M. Wilkinson,[1]* Jonathan J. Cole,[2] Michael L. Pace,[1] Robert A. Johnson,[1†] Maxwell J. Kleinhans[3]

[1]Department of Environmental Science, University of Virginia, Charlottesville, Virginia
[2]Cary Institute of Ecosystem Studies, Millbrook, New York
[3]Center for Limnology, University of Wisconsin, Madison, Wisconsin

Abstract

Many lakes have positive, heterograde vertical oxygen (O_2) profiles with a metalimnetic maximum usually assumed to be the result of biological O_2 production. However, supersaturated metalimnetic O_2 maxima are formed by biological processes (net photosynthetic production of O_2) and physical processes (warming of gasses trapped below the thermocline). Although the mechanisms contributing to supersaturated metalimnetic O_2 peaks are understood, the contribution of biological vs. physical processes is not well known in lakes. To examine these contributions, we measured O_2 and argon (Ar) saturation anomalies in the metalimnia of 17 lakes. Unlike O_2, Ar is biologically inert and, therefore, can be used to quantify physical processes. There was a positive Ar anomaly at the depth of the O_2 maximum in every lake. On average, only 14% of the O_2 maximum could be attributed solely to biological production of O_2, but this percentage varied widely among lakes. Additionally, in a subset of lakes, the relative saturation of O_2 at the metalimnetic maximum was lower than Ar due to net biological consumption, creating a weaker O_2 maximum than would be expected based on the physical processes alone. Some lakes were sampled multiple times during summer and net ecosystem production (NEP) was also calculated. There were many instances of positive NEP in the metalimnion; however, net autotrophy was usually transient instead of persistent. Overall, biological production of O_2 alone is not responsible for metalimnetic O_2 maxima as both physical and biological processes contribute substantially to the formation and persistence of O_2 maxima in lakes.

The vertical distribution of oxygen (O_2) is a fundamental property of lakes. In stratified lakes, atmospheric exchange at the surface and respiratory consumption of O_2 in the hypolimnion lead to a classic clinograde profile (Kalff 2002). However, numerous lakes have positive, heterograde profiles with the O_2 maximum occurring in the metalimnion. Metalimnetic O_2 maxima can be striking features, reaching saturation values greater than 300% (Eberly 1964) which are usually assumed to be the result of high primary production (Stefan et al. 1995; Wetzel 2001). These depth layers are also ecologically important as metalimnetic primary production may be an important resource for zooplankton (Francis et al. 2011; Batt et al. 2012) and are an important refuge for fish that require colder temperatures and O_2 rich waters (Morbey et al. 2006; Blair et al. 2013).

While it is commonly assumed that metalimnetic O_2 maxima are the result of photosynthetic O_2 production, two distinct mechanisms contribute to their formation. First, colder water has a greater dissolved oxygen (DO) concentration at equilibrium. As stratification develops in the spring in north temperate lakes, colder, O_2 rich waters are trapped under the thermocline and cut off from atmospheric exchange (Cole and Pace 1998). Subsequent solar warming at depth can then lead to supersaturated conditions. The second process is positive net ecosystem production (NEP) creating an excess of biologically produced O_2 below the thermocline. Metalimnetic O_2 maxima often co-occur with chlorophyll *a* maxima in lakes (Parker et al. 1991; Matthews and DeLuna 2008) or at the depth of maximum macrophyte growth (Dubay and Simmons 1979) suggesting significant contributions of O_2 from photosynthesis.

Although the processes that lead to the formation and persistence of metalimnetic O_2 maxima are well known, the contribution of biological production and physical trapping of O_2 has not been evaluated extensively in lakes. Therefore, it is unclear if metalimnetic O_2 maxima are largely biological

*Correspondence: gmw6yy@virginia.edu

[†]Present address: Department of Biology, University of Florida, Gainesville, Florida

phenomena as is usually assumed, or if physical processes play a larger role in both the formation and persistence of metalimnetic O_2 maxima. Hutchinson (1957) recognized the role of physical processes in the formation of metalimnetic O_2 maxima, but categorized physical contributions as minor and only important in very oligotrophic lakes. Instead, metalimnetic supersaturation in most lakes has been attributed to photosynthesis in that layer (Hutchinson 1957; Eberly 1964). To date, there has been no systematic effort to quantify the contribution of biological and physical processes to the formation of metalimnetic O_2 maxima among lakes.

Concurrent measurement of biologically inert, noble gasses such as argon (Ar) with O_2 is a common oceanographic method for quantifying physical and biological effects on O_2 concentrations (Emerson et al. 1995; Kaiser et al. 2005). Supersaturation of Ar at the depth of the O_2 maximum indicates physical trapping of gases below the thermocline (Emerson et al. 2012) as would occur with the development of stratification and subsequent metalimnetic warming. Supersaturation of O_2 in excess of Ar indicates biological production of O_2, or positive NEP, contributing to supersaturation at that depth (Craig and Hayward 1987; Craig et al. 1992). Although common in oceanography, the measurement of noble gases in limnology is rare, and largely restricted to a few very large, deep lakes (Craig et al. 1992; Tomonaga et al. 2012).

Comparing concentrations at one point in time of O_2 and Ar at the O_2 maximum can yield information about the metabolic balance in a lake. In addition, changes in O_2 concentration over time at the metalimnetic O_2 maximum is an estimate of the average rate of NEP. While much is known about epilimnetic metabolism (Hanson 2003; Pace and Prairie 2005; Solomon et al. 2013), there is less information about the metabolic balance of the metalimnetic layer (Stefan et al. 1995). In some lakes, primary production in the metalimnion, coinciding with the metalimnetic O_2 maximum, contributes substantially to the gross primary production (GPP) of the whole system (Batt and Carpenter 2012; Staehr et al. 2012). However, it is unclear if the metalimnetic contribution is generally large in lakes with an O_2 maximum or even if an O_2 maximum always signifies high rates of primary production in that layer.

Here, we evaluate the contribution of physical and biological processes to the formation and persistence of metalimnetic O_2 maxima in lakes. We measured the absolute concentrations of O_2 and Ar throughout the water column in a number of lakes using a membrane inlet mass spectrometer (MIMS; Kana et al. 1994), and calculated gas saturation anomalies (Craig and Hayward 1987; Kaiser et al. 2005) of both gases. Many lakes were sampled on multiple occasions during the ice-free period to evaluate whether the metalimnion was net autotrophic, heterotrophic, or in metabolic balance between sampling events. Given the rapid development of strong thermoclines in lakes of the region we studied, we hypothesized that there would be both significant physical as well as biological (positive NEP) contributions to metalimnetic O_2 maxima.

Methods

Survey sampling

We sampled 24 lakes located in the Northern Highlands Lake District (NHLD) of Wisconsin and Michigan during the ice-free period of 2013. The NHLD is a lake-rich region with numerous glacial kettle lakes surrounded by largely forested watersheds (Magnuson et al. 2006). Sampled lakes were selected based on previous observations of a metalimnetic O_2 maximum. Of the 24 lakes that were sampled, 7 were sampled once, 11 were sampled twice, 5 were sampled three times, and 1 lake (Paul Lake) was sampled nine times (total profiles = 52).

Sampling occurred over the deepest point in each lake determined from bathymetric maps. Temperature and DO profiles were taken at half-meter intervals using an YSI Incorporated (Yellow Springs) handheld optical DO sensor to determine six sampling depths for discrete water samples in each lake (Fig. 1A,C). The reported temperature and DO accuracy for the sensor is $\pm 0.2^\circ$C and ± 0.1 mg L^{-1}, respectively, and the resolution is 0.01°C and 0.01 mg L^{-1}, respectively. Secchi depth was also recorded. Discrete water samples were taken to determine absolute O_2 and Ar concentrations; the DO probe was used only to select sampling depths. In general discrete samples were taken at the surface, above the thermocline, below the thermocline, at the peak of the O_2 maximum, and above and below the peak. If there was no metalimnetic O_2 maximum present, samples were not taken. Water samples were collected from the appropriate depths using a Van Dorn sampler. Uncapping one end of the Van Dorn, water was slowly drawn into a 25 mL Finnpipette Stepper pipette and then transferred without aeration to a 12 mL borosilicate vial containing 200 μL of saturated mercuric chloride solution for preservation. The vials were capped with a gas-tight screw cap with no head space and kept on ice in the field. Three replicate vials were taken for each sample depth. Samples were stored at 4°C and analyzed for O_2 and Ar concentrations on a MIMS within seven days of collection.

Water samples were analyzed using the MIMS at or below their in situ temperature (Kana et al. 1994) to minimize off-gassing and bubble formation. A standard of deionized water held at a constant temperature and equilibrated with the atmosphere via gentle, constant stirring was analyzed between every 9 and 12 sample vials. Changes in the standard water measurement during the run were used to linearly correct the samples for instrument drift. Standard water was also continuously pumped through the line for at least 10 min to flush the system after super-saturated or under-saturated samples were analyzed. The drift-corrected O_2 and
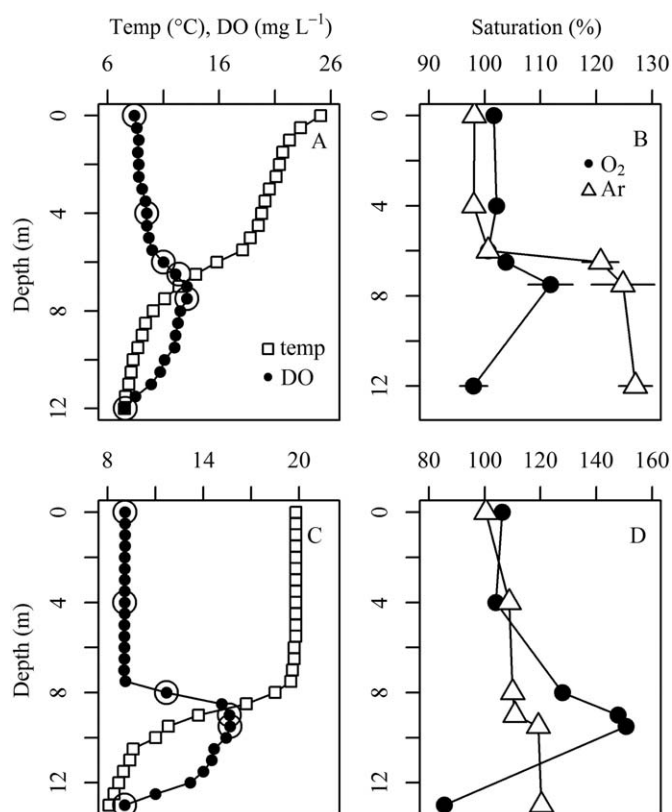
Fig. 1. Representative O_2 and Ar profiles for the survey lakes as well as temperature (temp) and DO profiles measured with an optical probe sensor. Circles around DO values indicate the depth at which samples were taken for O_2 and Ar analysis with MIMS. Error bars are the SD, which in many cases is smaller than the symbol. (A,C) DO and temperature profiles in a lake with higher Ar than O_2 saturation at the maximum (Lac du Lune, day of year (DOY) 224) and lower Ar than O_2 saturation at the maximum (Stormy, DOY 226), respectively. (B,D) O_2 and Ar saturation (%) profiles in Lac du Lune (DOY 224) and Stormy (DOY 226), respectively.

Ar absolute concentrations from the three replicate samples were then averaged for each depth. The coefficient of variation of replicate O_2 and Ar samples was typically less than 0.45% and 0.75%, respectively. Percent gas saturation was calculated as the measured absolute concentration over the expected absolute concentration based on temperature, barometric pressure, and pressure at depth. The lakes had dilute water (1–167 μS cm^{-1}), so no correction for salinity was made.

Metalimnion metabolism incubations

Weekly light and dark bottle incubations were carried out in the metalimnion of Paul Lake during the ice-free period of 2013. The change in O_2 concentration was used to calculate metabolic rates in the bottle incubations. Paul Lake is a small, oligotrophic glacial kettle lake with a persistent, seasonal metalimnetic O_2 maximum at approximately four

meter depth. Initial samples ($n = 5$) of O_2:Ar were taken at the beginning of the incubation. Five light and five dark 300 mL glass bottles with ground glass stoppers were incubated at four meter for 10–12 h, depending on the photic period. At the end of the incubation period, water was drawn from the bottles using a Finnpipette Stepper pipette and preserved for analysis in the same manner as described above. The change in O_2:Ar from the initial to the final samples of the light bottles was used to calculate NEP. Estimates of NEP for the 24 h period were calculated by adding the change in O_2:Ar from the dark bottles to the change in O_2:Ar from the light bottles. The light and dark bottle estimates were prorated for the number of daylight and non-daylight hours on the day of the incubation.

When the survey lakes were sampled on more than one occasion during the season, the change in absolute O_2 concentration at the O_2 maximum between visits was used to estimate average NEP during that time. Average NEP was estimated as

$$\text{NEP} = \frac{O_{2,t2} - O_{2,t1}}{t_2 - t_1} \qquad (1)$$

where t_1 and t_2 are the two sampling dates and the respective O_2 concentrations (μmol L^{-1}) determined with a MIMS (Staehr et al. 2010). Diffusive exchange was assumed negligible if the O_2 maximum occurred below the thermocline. The O_2 maximum depth in Paul Lake varied (± 0.5 m) over the course of the summer. As such, time-averaged NEP values were also calculated for the four meter depth from the sampling events in Paul Lake to match the incubation depth which was always four meter. The four meter time-averaged NEP estimates were then compared to the weekly bottle incubation NEP measurements in Paul Lake.

Gas saturation anomalies

Gas saturation anomalies were calculated following Craig and Hayward (1987) as

$$\Delta i(\%) = \left(\frac{C_i}{C_i^*} - 1 \right) \times 100 \qquad (2)$$

where i denotes either O_2 or Ar, C is the measured concentration (μmol L^{-1}) of i, and C^* is the expected concentration (μmol L^{-1}) of C_i based on temperature and barometric pressure. Craig and Hayward (1987) used saturation anomalies to estimate biological production of O_2 in the ocean. In the ocean air injection (bubble formation) must also be considered. However, in this dataset air injection (i.e., inputs of atmospheric gas from waves causing bubble formation) was assumed to be negligible given that the sampled lakes were small (mean size = 1.4 km^2).

If the gas saturation anomalies of O_2 and Ar (ΔO_2 and ΔAr, respectively) are equivalent at the metalimnetic O_2 maximum, then the O_2 maximum is purely the result of

Table 1. The percentage of lakes with O_2 maxima in survey studies in the NHLD.

Survey	Number of lakes profiled	% lakes with O_2 maxima
Carpenter and Kratz 2006	62	434
Wilkinson et al. 2013	40	38
Hrabik et al. 2005	44	46

physical processes and the biological processes are in metabolic balance (NEP = 0). If the gas saturation anomalies are not equivalent, then the metalimnion is not in metabolic balance and positive or negative NEP has created a disparity in the saturation anomalies. To quantify the metabolic balance, either positive or negative NEP, at the metalimnetic O_2 maximum (max), we quantified the disparity in anomalies as a percentage (F) of the larger anomaly such that

$$F(\%) = \left(\frac{\Delta O_2 - \Delta Ar}{\max(\Delta O_2 \text{ or } \Delta Ar)} \right) \times 100 \qquad (3)$$

where max (ΔO_2 or ΔAr) is the value of the larger gas saturation anomaly at the O_2 maximum. When NEP is positive ($\Delta O_2 > \Delta Ar$), F is positive and is equivalent to the percentage of the metalimnetic O_2 maximum that is attributable to biological processes alone. When NEP is negative ($\Delta Ar > \Delta O_2$), F is negative and is equivalent to the O_2 deficit (as a percentage of ΔAr) of what ΔO_2 should be based on physical conditions alone.

Gas saturation anomalies of O_2 and Ar were also used in a regression analysis (R Software Development Team 2013) to determine if any lake characteristics predicted anomaly strength. Variables considered included lake size, maximum depth, depth of the thermocline, Secchi depth, and sampling date. The thermocline depth was defined as the top of the vertical 0.5 m interval with the greatest change in temperature. The depth of the mixed layer was calculated as the top of the first 0.5 m interval over which temperature changed by more than 1°C.

The frequency of metalimnetic O_2 maxima occurrence

We did not use a randomized design when selecting lakes to sample in the NHLD region as the goal of the survey was to quantify the contribution of processes forming metalimnetic O_2 maxima, not to quantify the prevalence of O_2 maxima in lakes. However, lake surveys previously conducted in the region provided a means to estimate the percentage of lakes with a metalimnetic O_2 maximum in the NHLD. Specifically, we analyzed DO profiles that were taken during the ice-free period from three comparative lake surveys (Hrabik et al. 2005; Carpenter and Kratz 2006; Wilkinson et al. 2013). Each of these surveys measured vertical profiles of

temperature and DO using handheld DO probes. We tabulated the number of lakes with a temperature and DO profile that indicated a metalimnetic O_2 maximum (similar to Fig. 1A,C). The percentage of lakes with a metalimnetic O_2 maximum in each survey was calculated by dividing the number of lakes with a metalimnetic O_2 maximum by the total number of lakes sampled in the survey.

Results

Based on prior surveys, on average, 42% of lakes in the NHLD have a metalimnetic O_2 maximum at some time during the ice-free season (Table 1). In our survey of 24 lakes, 17 had a metalimnetic O_2 maximum during one or multiple sampling events. For 17 lakes sampled in this study with an O_2 maximum, the O_2 and Ar percent saturation profiles were in one of two categories: O_2 saturation was greater than Ar saturation at the peak of the metalimnetic O_2 maximum (Fig. 1B; Table 2), and O_2 saturation was less than or equal to Ar saturation at the peak of the metalimnetic O_2 maximum (Fig. 1D; Table 2). Of the 52 sampling events, 19 were in the category of greater O_2 saturation at the peak, and 16 were in the category of greater Ar saturation at the peak. Of the 35 sampling events with a metalimnetic O_2 maximum, the maximum always occurred below the thermocline.

The O_2 saturation anomalies (ΔO_2) at the peak of the metalimnetic maxima ranged from −8.2% to 91.7% with a mean (±standard deviation [SD]) of 24.1% ± 21.7% (Fig. 2). The Ar saturation anomalies (ΔAr) ranged from −5.8% to 75.3% with a mean (±SD) of 19.12% ± 17.5%. The variance in ΔO_2 and ΔAr among samples for a lake was typically quite small except for two lakes in which the variance of ΔO_2 was quite high, likely due to sampling error. There was no system in which ΔO_2 was positive and ΔAr was zero. ΔO_2 was predicted by ΔAr ($\Delta O_2 = 1.0 \times \Delta Ar + 5.0$; p-value < 0.001) but there was considerable residual variability ($R^2 = 0.65$) suggesting that different processes affect ΔO_2 relative to ΔAr.

There was a significant relationship between the gas saturation anomalies and lake size, depth of the thermocline, and the sampling date (Table 3). There was no relationship between gas saturation anomaly strength and Secchi depth. The slopes of the regressions between sampling date and the anomalies were not significantly different from each other (t-test, $t = 0.29$, p-value = 0.77, degrees of freedom [df] = 62). Similarly, the slopes of the regressions between thermocline depth and the gas saturation anomalies were not significantly different (t-test, $t = 0.47$, p-value = 0.64, df = 62). Finally, the independent variables used in the regression analysis with the gas saturation anomalies were also significantly related (Table 3). Most notably, the relationships between sampling date and lake size were significantly related to thermocline depth ($R^2 = 0.54$ and 0.62, respectively). The

Table 2. Sampling events with a metalimnetic O$_2$ maximum and associated lake characteristics. O$_2$ % sat and Ar % sat are the percent saturation in the metalimnion. DOY is day of year.

Lake	DOY	Depth of O$_2$ max (m)	O$_2$ % sat	Ar % sat	Surface area (km^2)	Maximum depth (m)	Thermocline depth (m)	Latitude (°N)	Longitude (°W)
Arrowhead	177	4.5	145.0	113.4	0.40	13.1	2.0	45.908167	−89.689833
Arrowhead	222	5.5	139.7	123.4	0.40	13.1	5.5	45.908167	−89.689833
Bay	162	4.0	100.6	113.3	0.67	12.2	3.5	46.242641	−89.495966
Brandy	177	3.5	133.2	111.9	0.45	13.4	3.0	45.908644	−89.700028
Brandy	222	4.5	106.5	116.7	0.45	13.4	4.5	45.908644	−89.700028
Crampton	138	3.5	91.8	103.4	0.26	15.3	1.0	46.210398	−89.473650
Crampton	176	4.0	115.6	115.5	0.26	15.3	3.0	46.210398	−89.473650
Crampton	213	5.5	102.7	109.9	0.26	15.3	4.5	46.210398	−89.473650
Diamond	192	8.0	123.6	118.6	0.50	12.2	5.0	46.044225	−89.715520
Diamond	235	8.0	161.3	157.3	0.50	12.2	8.0	46.044225	−89.715520
Emeline	195	6.0	112.1	106.3	0.50	23.5	4.0	46.240771	−89.479786
Lac du Lune	176	7.5	114.2	112.1	1.72	20.7	5.5	46.134661	−89.387751
Lac du Lune	224	8.5	111.8	120.8	1.72	20.7	8.0	46.134661	−89.387751
Long	182	3.0	108.3	113.5	0.03	17.0	2.0	46.235636	−89.502274
Moosehead	178	2.5	105.3	110.8	0.22	11.9	2.5	46.241899	−89.608618
Paul	139	1.0	110.0	112.5	0.02	12.0	1.0	46.251648	−89.503519
Paul	146	4.0	103.6	94.1	0.02	12.0	1.5	46.251648	−89.503519
Paul	161	3.5	111.4	101.0	0.02	12.0	2.5	46.251648	−89.503519
Paul	176	4.0	112.3	103.3	0.02	12.0	2.0	46.251648	−89.503519
Paul	200	4.5	114.7	109.3	0.02	12.0	2.0	46.251648	−89.503519
Paul	225	4.5	127.0	119.5	0.02	12.0	3.0	46.251648	−89.503519
Paul	239	5.0	126.3	132.1	0.02	12.0	3.0	46.251648	−89.503519
Presque Isle	211	8.0	115.2	115.1	5.18	24.4	7.5	46.224147	−89.782003
Raspberry	137	2.0	107.8	101.2	0.05	6.1	2.0	46.250209	−89.506523
Raspberry	182	2.0	136.8	146.1	0.05	6.1	2.0	46.250209	−89.506523
Roach	169	5.0	116.1	110.3	0.45	10.0	4.0	46.232103	−89.549954
Sparkling	167	6.5	121.1	109.8	0.82	20.0	5.0	46.009422	−89.700721
Sparkling	218	9.0	139.1	150.5	0.82	20.0	6.0	46.009422	−89.700721
Sparkling	235	8.0	191.7	175.3	0.82	20.0	3.5	46.009422	−89.700721
Stormy	193	7.0	150.7	120.3	2.11	19.2	5.5	46.053429	−89.322735
Stormy	226	9.0	158.5	118.4	2.11	19.2	8.5	46.053429	−89.322735
Trout	192	11.5	139.3	131.3	16.08	35.7	6.0	46.035884	−89.666045
Trout	233	10.5	157.8	148.8	16.08	35.7	5.5	46.035884	−89.666045
Tuesday	136	1.0	99.4	104.3	0.01	15.0	0.0	46.251870	−89.497167
Tuesday	146	1.5	133.8	119.1	0.01	15.0	1.0	46.251870	−89.497167

regression between lake size and Secchi depth was also significant ($R^2 = 0.48$; Table 3).

Estimates of NEP

The percentage of the metalimnetic O$_2$ maximum attributable to NEP (F), whether positive or negative, was calculated for 32 of the sampling events. Three sampling events with observed O$_2$ maxima were excluded as either ΔO$_2$ and/or ΔAr were less than zero. The mean ($\pm$ SD) of F was 14.2% $\pm$ 48.0% (Fig. 3). The most positive value of F was 91.0% but the majority of positive values (> 70% of the estimates) were in the range of 0–50% (Fig. 3). For 10 lakes F was negative

indicating net biological consumption (e.g., negative NEP) caused the O$_2$ maxima to be lower than expected by physical processes alone. The most negative value of F was −95.9% but the majority (70%) of negative values were in the range of 0–50%. F was not significantly related to any of the variables listed in Table 3.

Based on repeat samplings, 18 estimates of average NEP at the metalimnetic O$_2$ maxima between visits were calculated. The estimates of average NEP between sampling events in lakes ranged from −1.76 μmol L^{-1} d^{-1} to +7.58 μmol L^{-1} d^{-1} (Fig. 4A). The mean ($\pm$ SD) of NEP between sampling events in a Paul Lake was 0.7 μmol L^{-1} d^{-1} $\pm$ 2.8 μmol L^{-1} d^{-1}. This is

similar to the average weekly estimate of NEP from the bottle incubations in Paul which was 0.95 μmol L^{-1} d^{-1} ± 2.7 μmol L^{-1} d^{-1} (Fig. 4B).

Discussion

Of the 35 sampling events where a metalimnetic O$_2$ maximum occurred, there were no instances in which ΔO_2 was positive and ΔAr was zero. Thus, in no case was the metalimnetic O$_2$ maximum created by biological production of O$_2$ alone. Trapping of cold, gas rich, water layers during thermocline formation, and subsequent solar warming is likely the physical process that contributed to the

Fig. 2. The Ar and O$_2$ saturation (%) anomalies at the peak of the metalimnetic maximum for the 35 sampling events with a metalimnetic O$_2$ maximum. Error bars are the SD of replicate samples.

supersaturated O$_2$ in all lakes with an O$_2$ maximum. If biological production of O$_2$ was the only process causing supersaturation of O$_2$ in the metalimnion, we would expect the plot of ΔAr vs. ΔO_2 to have no slope with all of the ΔAr values clustered at zero. Instead, ΔO_2 is predicted by ΔAr indicating that the O$_2$ maximum in every lake was partially the result of physical processes. Interestingly, the slope of the relationship is equal to one, indicating that ΔO_2 and ΔAr increase proportionally.

Both ΔO_2 and ΔAr were significantly related to the sampling date such that the lakes sampled later in the season had greater gas saturation anomalies. As both ΔO_2 and ΔAr increased over time and at a similar rate (slopes of the regressions were not significantly different) the relationship with sampling date was likely not due an increase in positive NEP. Later in the season the thermocline depth increased (became deeper) and gas saturation anomalies also significantly increased with the depth of the thermocline. The depth of the thermocline was also strongly and positively associated with lake size which was weakly related to ΔO_2 and ΔAr. Supersaturation of Ar in the metalimnion could have been influenced by groundwater inflow as soil pore water is supersaturated in Ar (Kipfer et al. 2002). However, groundwater contributes about 10% annually to the total volume of water in seepage lakes in the region (Anderson et al. 1993) in wet years, so the influence of supersaturated groundwater in the metalimnion is likely minor.

Eberly (1964) observed that smaller lakes tended to have metalimnetic O$_2$ maxima while larger lakes did not. We found that for lakes with an O$_2$ maximum, larger lakes had a greater O$_2$ maximum (larger ΔO_2) than smaller lakes. However, this distinction for large lakes does not imply the O$_2$ maximum in these lakes was due to relatively greater metalimnetic primary production that caused net oxygen production. The significant regressions between lake morphometric variables and the lack of a relationship between the percentage of the O$_2$ maxima from NEP (*F*) and morphometry underscores the importance of the physical processes
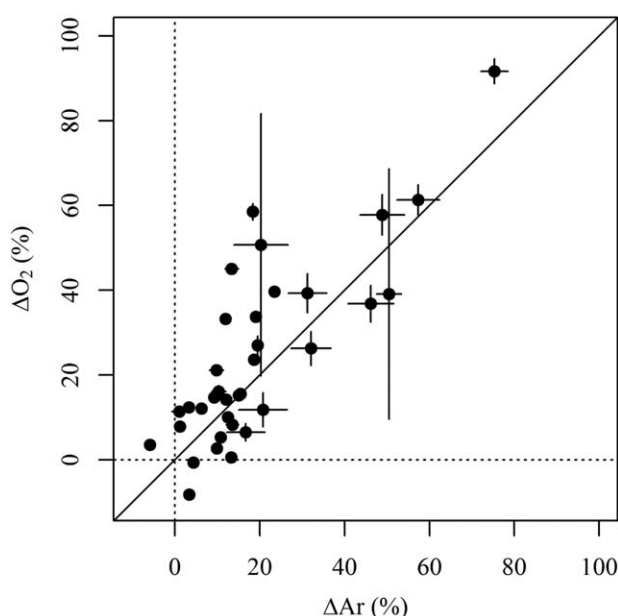
Table 3. Significant ($\alpha = 0.05$) results of the regression analysis among lake characteristics and gas anomalies.

Independent variable	Dependent variable	Slope	Y-intercept	R^2	p-value
Sampling date	ΔO_2	0.39	−49.3	0.33	<0.001
Sampling date	ΔAr	0.36	−47.8	0.42	<0.001
Thermocline depth	ΔO_2	4.14	8.2	0.18	0.013
Thermocline depth	ΔAr	3.18	6.5	0.16	0.018
Log (surface area)	ΔO_2	4.09	11.4	0.15	0.020
Log (surface area)	ΔAr	2.95	9.9	0.12	0.040
Secchi depth	Thermocline depth	1.13	−1.0	0.48	<0.001
Sampling date	Secchi depth	0.02	0.3	0.22	0.020
Sampling date	Thermocline depth	0.05	−6.0	0.54	<0.001
Log (surface area)	Secchi depth	1.16	−2.1	0.48	<0.001
Log (surface area)	Thermocline depth	0.86	1.1	0.62	<0.001

contributing to the formation and persistence of metalimnetic O_2 maxima. This is further underscored by a lack of a relationship between F and Secchi depth. Secchi depth is a proxy for the light environment in the water column, influencing primary production and presumably biological gas balance in the metalimnion.
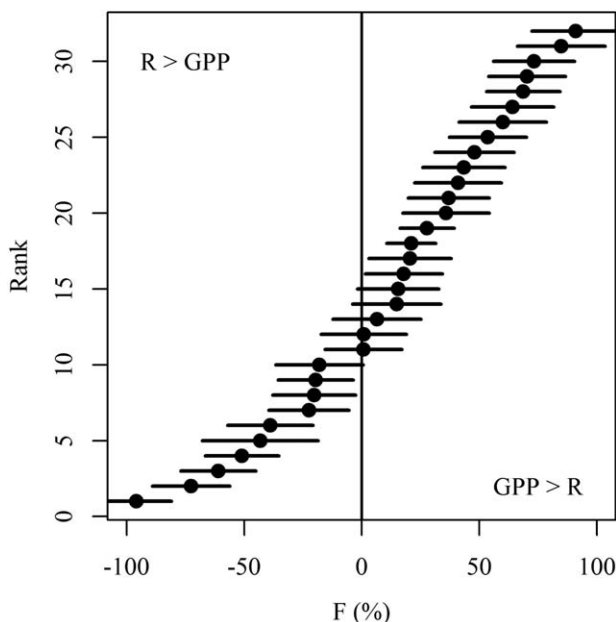

Fig. 3. The percentage that the O_2 anomaly deviates from only physical forcing (when $\Delta O_2 = \Delta Ar$) due to biology. F is positive when ΔO_2 is greater than ΔAr (GPP > R, NEP is positive). F is negative when ΔO_2 is less than ΔAr (R > GPP, NEP is negative). The 35 sampling events with an O_2 maximum are ranked from most negative NEP to most positive. Error bars are SD.

Estimates of NEP

Net metabolic balance (F) can be inferred from the difference in gas saturation anomalies at the metalimnetic O_2 maximum. Although overall, physical processes contributed substantially to the O_2 maximum in each lake, the biological contribution to O_2 supersaturation was also substantial in many lakes. For 22 of the sampling events net autotrophy (positive NEP) was responsible for approximately 40% of the metalimnetic O_2 maximum. The range of positive F values is similar to the values observed by Craig and Hayward (1987) for the O_2 maximum in the Pacific Ocean using the same approach. The depletion of O_2 by net heterotrophy (negative NEP) in lakes relative to ΔAr at the O_2 peak was also approximately 40%. For the systems with a negative F, it is impossible to infer if there was a previous instance of positive NEP that formed the metalimnetic O_2 maximum or if the O_2 maximum formed from physical trapping of cold, O_2-rich water below the thermocline and NEP was always negative at that depth.

Time-averaged NEP values estimated between sampling events were more commonly negative than positive. This tendency is consistent with the observed net heterotrophy of most lakes (Kratz et al. 1997; Cole et al. 2000) as well as the estimates of NEP in lakes in this region (Hanson et al. 2003; Batt and Carpenter 2012). Additionally, the more common occurrence of net heterotrophy supports the recent findings that metalimnetic primary production is often a minor resource supporting zooplankton in many lakes (Cole et al. 2011; Armengol et al. 2012; Wilkinson et al. 2014). However, the average NEP value of all estimates was slightly net autotrophic as the positive rates were much higher, even though they were less common. Given the light environment in the metalimnion of lakes, it is possible that strong pulses of
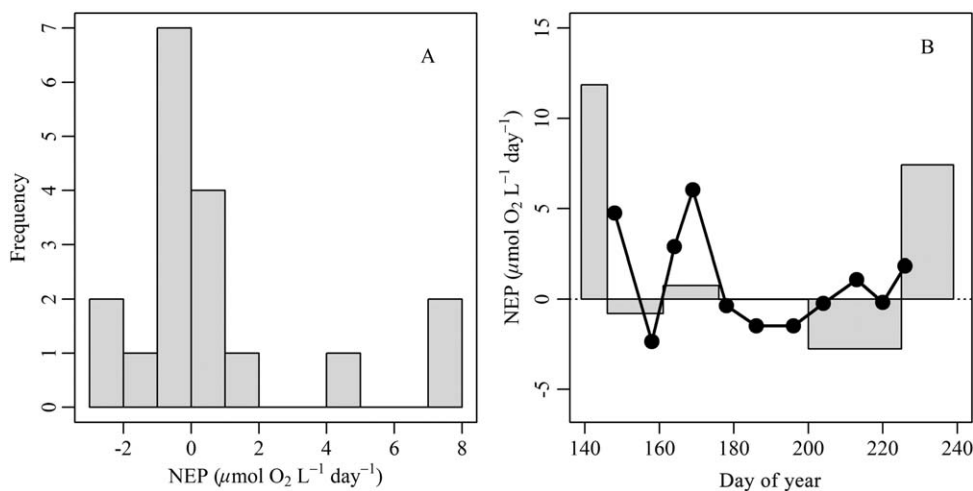

Fig. 4. Estimates of NEP at the metalimnetic O_2 maxima in lakes. (A) Estimates of NEP between sampling events in lakes and (B) estimates of NEP at four meter depth in Paul Lake between sampling events (gray bars) compared to weekly bottle incubations at four meter (circles). The rate from day of year 176–200 was -0.03 μmol L^{-1} d^{-1} and is therefore not visible on the graph.

autotrophic production occur periodically under favorable conditions causing the high rates of observed NEP, but heterotrophy is the more common metabolic state in the metalimnion. This is the pattern observed in the more detailed Paul Lake time series for the bottle incubation method. Net heterotrophy was more commonly observed at the metalimnetic O_2 peak in Paul, however, there were times during the season where net autotrophy occurred and was relatively large. This pattern was also observed in neighboring Peter Lake in 2012 (Wilkinson et al. 2014).

To calculate time-averaged NEP in the metalimnion, we assumed that vertical diffusion was negligible. We know from several tracer experiments in some of these lakes that solutes or dissolved gases added to the epilimnion (lithium bromide or sulfur hexafluoride) do not mix below the thermocline until the thermocline itself deepens during mixing (Cole and Pace 1998; Cole et al. 2010). Further, solutes added to the metalimnion (dissolved inorganic ^{13}C) stay in the narrow depth zone to which they were added, again until thermocline deepening (Wilkinson et al. 2014). The assumption of negligible vertical mixing is likely valid for a large portion of the lakes in our dataset as the vertical diffusion coefficient (K_Z) is generally near the molecular rate in small, sheltered lakes, and strongly stratified lakes (MacIntyre and Melack 2009, but see Sadro et al. 2011). In the larger lakes such as Trout, often there is a stable thermocline except when storm fronts pass over the region leading to increased wind speeds and upwelling (MacIntyre and Melack 2009). If K_Z was near the molecular rate during the period between sampling events in the lakes in this dataset, the assumption of negligible vertical exchange holds. If there was mixing or a substantial flux of O_2 into the hypolimnion (Sadro et al. 2011) between sampling periods, the assumption would be violated and the NEP estimates could be erroneous. For this analysis, we also assumed that bubble injection was negligible (Craig and Hayward 1987) as wind speeds were below 5 m s^{-1} on most of the lakes and the metalimnion is not directly exposed to surface wave action.

Our study demonstrates that it would be incorrect to assume that the presence of an O_2 maximum is caused solely by biological production of O_2. In the lakes we surveyed, the O_2 maximum was never simply the result of net autotrophy alone. There was a substantial physical component to the metalimnetic O_2 maxima in all instances indicated by a positive Ar anomaly in all lakes. Additionally, in a large subset of the surveyed lakes, the saturation of O_2 was less than expected due to physical processes alone. Net biological consumption at the O_2 max lowered O_2 and created a weaker O_2 maximum in these lakes than would be caused by physical processes alone. Although there were many instances of positive NEP at the depth of the O_2 maximum, time integrated estimates of NEP and more detailed time series revealed that net autotrophy is usually transient instead of persistent.

Overall, both physical and biological processes are important in the formation and persistence of metalimnetic O_2 maxima in lakes.

References

Anderson, M. P., and X. Cheng. 1993. Long- and short-term transience in a groundwater/lake system in Wisconsin, U.S.A. J. Hydrol. **145**: 1–18. doi:10.1016/0022-1694(93)90217-W

Armengol, X., W. A. Wurtsbaugh, A. Camacho, and M. R. Miracle. 2012. Pseudo-diel vertical migration in zooplankton: A whole-lake ^{15}N tracer experiment. J. Plankton Res. **34**: 976–986. doi:10.1093/plankt/fbs058

Batt, R. D., and S. R. Carpenter. 2012. Free water lake metabolism: Addressing noisy time series with a Kalman filter. Limnol. Oceanogr. **10**: 20–30. doi:10.4319/lom.2012.10.20

Batt, R. D., S. R. Carpenter, J. J. Cole, M. L. Pace, T. J. Cline, R. A. Johnson, and D. A. Seekell. 2012. Resources supporting the food web of a naturally productive lake. Limnol. Oceanogr. **57**: 1443–1452. doi:10.4319/lo.2012.57.5.1443

Blair, J. M., I. Ostrovsky, B. J. Hicks. R. J. Pitkethley, and P. Scholes. 2013. Growth of rainbow trout (*Oncorhynchus mykiss*) in warm-temperate lakes: Implications for environmental change. Can. J. Fish. Aquat. Sci. **70**: 815–823. doi:10.1139/cjfas-2012-0409

Carpenter, S. R., and T. Kratz. 2006. Biocomplexity at North Temperate Lakes LTER; Coordinated field studies: Lakes 2001–2004 [accessed Jan 2014]. Available from http://tropical.lternet.edu/knb/metacat/knb-lter-ntl.209.4/lter.

Cole, J. J., D. L. Bade, D. Bastviken, M. L. Pace, and M. Van de Bogert. 2010. Multiple approaches to estimating air-water gas exchange in small lakes. Limnol. Oceanogr. **8**: 285–293. doi:10.4319/lom.2010.8.285

Cole J. J., S. R. Carpenter, J. F. Kitchell, M. L. Pace, C. T. Solomon, and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proc. Natl. Acad. Sci. USA **108**: 1957–1980. doi:10.1073/pnas.1012807108

Cole, J. J., and M. L. Pace. 1998. Hydrological variability of small, northern lakes measured by the addition of tracers. Ecosystems **1**: 310–320. doi:10.1007/s100219900024

Cole, J. J., M. L. Pace, 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. doi:10.4319/lo.2000.45.8.1718

Craig, H., and T. Hayward. 1987. Oxygen supersaturation in the ocean: Biological versus physical contributions. Science **235**: 199–202. doi:10.1126/science.235.4785.199

Craig, H., R. A. Wharton, and C. P. McKay. 1992. Oxygen supersaturation in ice covered Antarctic lakes—biological versus physical contributions. Science **255**: 318–321. doi:10.1126/science.11539819

Dubay, C. I., and G. M. Simmons. 1979. The contribution of macrophytes to the metalimnetic oxygen maximum in a

Montana, oligotrophic lake. Am. Midl. Nat. **101**: 108–117. doi:10.2307/2424906

Eberly, W. R. 1964. Further studies on the metalimnetic oxygen maximum, with special reference to its occurrence throughout the world. Invest. Indiana Lakes Streams **5**: 103–139.

Emerson, S., T. Ito, and R. C. Hamme. 2012. Argon supersaturation indicates low decadal-scale vertical mixing in the ocean thermocline. Geophys. Res. Let. **39**: L18610. doi:10.1029/2012GL053054

Emerson, S., P. D. Quay, C. Stump, D. Wilbur, and R. Schudlich. 1995. Chemical tracers of productivity and respiration in the subtropical Pacific Ocean. J. Geophys. Res. **100**: 15873–15887. doi:10.1029/95JC01333

Francis, T. B., D. E. Schindler, G. W. Holtgrieve, E. R. Larson, M. D. Scheuerell, B. X. Semmens, and E. J. Ward. 2011. Habitat structure determines resource use by zooplankton in temperate lakes. Ecol. Lett. **14**: 364–372. doi:10.1111/j.1461-0248.2011.01597.x

Hanson, P. C., D. L. Bade, S. R. Carpenter, and T. K. Kratz. 2003. Lake metabolism: Relationship with dissolved organic carbon and phosphorus. Limnol. Oceanogr. **48**: 1112–1119. doi:10.4319/lo.2003.48.3.1112

Hrabik, T. R., B. K. Greenfield, D. B. Lewis, A. I. Pollard, K. A. Wilson, and T. K. Kratz. 2005. Landscape scale variation in taxonomic diversity in four groups of aquatic organisms: The influence of physical, chemical, and biological properties. Ecosystems **8**: 301–317. doi:10.1007/s10021-002-0270-x

Hutchinson, G. E. 1957. A treatise on limnology: Geography, physics, and chemistry, v. 1. Wiley.

Imberger, J., and J. C. Patterson. 1990. Physical limnology. Adv. Appl. Mech. **27**: 303–473. doi:10.1016/S0065-2156(08)70199-6

Kaiser, J., M. K. Reuer, B. Barnett, and M. L. Bender. 2005. Marine productivity estimates from continuous O2/Ar ratio measurements by membrane inlet mass spectrometry. Geophys. Res. Lett. **32**: L19605. doi:10.1029/2005GL023459

Kalff, J. 2002. Limnology: Inland water ecosystems. Prentice Hall.

Kana, T. M., C. Darkangelo, M. D. Hunt, J. B. Oldham, G. E. Bennett, and J. C. Cornwell. 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N_2, O_2 and Ar in environmental water samples. Anal. Chem. **66**: 4166–4170. doi:10.1021/ac00095a009

Kipfer, R., W. Aeschbach-Hertig, F. Peeters, and M. Stute. 2002. Noble gasses in lakes and ground waters, in noble gases in geochemistry and cosmochemistry. Rev. Mineral. Geochem. **47**: 615–700. doi:10.2138/rmg.2002.47.14

Kratz, T. K., J. Schindler, D. Hope, J. L. Riera, and C. J. Bowser. 1997. Average annual carbon dioxide concentrations in eight neighboring lakes in northern Wisconsin. Verh. Int. Ver. Limnol. **26**: 335–338.

MacIntyre, S., and J. M. Melack. 2009. Mixing dynamics in lakes across climactic zones, p. 603–612. In G. E. Likens [ed.], Encyclopedia of inland waters. Elsevier.

Magnuson, J. J., and others. 2006. Origin, operation, evolution and challenges, p. 280–322. In J. J. Magnuson, T. K. Kratz, and B. J. Benson [eds.], Long-Term Dynamics of Lakes in the Landscape: Long-Term Ecological Research on North Temperate Lakes. Oxford Univ. Press.

Matthews, R., and E. DeLuna. 2008. Metalimnetic oxygen and ammonium maxima in Lake Whatcom, Washington (USA). Northwest Sci. **82**: 18–29. doi:10.3955/0029-344X-82.1.18

Morbey, Y. E., P. Addison, B. J. Shuter, and K. Vascotto. 2006. Within-population heterogeneity of habitat use by lake trout *Salvelinus namaycush*. J. Fish. Biol. **69**: 1675–1696. doi:10.1111/j.1095-8649.2006.01236.x

Pace, M. L., and Y. T. Prairie. 2005. Respiration in lakes, p. 103–121. In P. J. leB. Williams and P. A. del Giorgio [eds.], Respiration in aquatic ecosystems. Oxford Univ. Press.

Parker, B. C., L. J. Wenkert, and M. J. Parson. 1991. Cause of the metalimnetic oxygen maximum in Mountain Lake, Virginia. J. Freshw. Ecol. **6**: 293–303. doi:10.1080/02705060.1991.9665306

Sadro, S., J. M. Melack, and S. MacIntyre. 2011. Depth-integrated estimates of ecosystem metabolism in a high-elevation lake (Emerald Lake, Sierra Nevada, California). Limnol. Oceangr. **56**: 1764–1780. doi:10.4319/lo.2011.56.5.1764

Solomon, C. T., and others. 2013. Ecosystem respiration: Drivers of daily variability and background respiration in lakes around the globe. Limnol. Oceanogr. **58**: 849–866. doi:10.4319/lo.2013.58.3.0849

Staehr, P. A., J. P. A. Christensen, R. D. Batt, and J. A. Read. 2012. Ecosystem metabolism in a stratified lake. Limnol. Oceanogr. **57**: 1317–1330. doi:10.4319/lo.2012.57.5.1317

Staehr, P. A., and others. 2010. Lake metabolism and the diel oxygen technique: State of the science. Limnol. Oceanogr. **8**: 628–644. doi:10.4319/lom.2010.8.628

Stefan, H. G., X. Fang, D. Wright, J. G. Eaton, and J. H. McCormick. 1995. Simulation of dissolved oxygen profiles in a transparent, dimictic lake. Limnol. Oceanogr. **40**: 105–118. doi:10.4319/lo.1995.40.1.0105

Tomonaga, Y., R. Blattler, M. S. Brennwald, and R. Kipfer. 2012. Interpreting noble gas concentrations as proxies for salinity and temperature in the world's largest soda lake (Lake Van, Turkey). J. Asian Earth Sci. **59**: 99–107. doi:10.1016/j.jseaes.2012.05.011

Wetzel, R. G. 2001. Limnology: Lake and river ecosystems, 3rd ed. Academic Press.

Wilkinson, G. M., S. R. Carpenter, J. J. Cole, M. L. Pace. 2013. Terrestrial support of pelagic consumers: Patterns and variability revealed by a multi-lake study. Freshw. Biol. **58**: 2037–2049. doi:10.1111/fwb.12189

Wilkinson, G. M., S. R. Carpenter, J. J. Cole, and M. L. Pace. 2014. Use of deep autochthonous resources by zooplankton: Results of a metalimnetic addition of ^{13}C to a small lake. Limnol. Oceanogr. **59**: 986–996. doi:10.4319/lo.2014.59.3.0986