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Effect of reducing allochthonous P load on biomass and alkaline phosphatase activity of phytoplankton in an urbanized watershed, Michigan

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Abstract

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Phosphorus (P) concentrations have declined in the Huron River in southeast Michigan subsequent to first a municipal ordinance, and later state legislation, restricting the application of commercial fertilizers containing phosphate. One management objective of the environmental policy was to reduce eutrophication of surface waters and nuisance blooms of cyanobacteria, particularly in large impoundments along the Huron River. Alternative management theory contended that nuisance algal conditions resulted from internal loading of P within the lakes rather than the allochthonous nutrient load. In summer 2012 during conditions of relative drought, we had an opportunity to test whether reduced river P was intensifying P limitation among phytoplankton in impoundments receiving the river water. We compared chlorophyll concentrations and alkaline phosphatase activity measured in 2012 to measurements at the same sites obtained using identical methods in 2004 and 2005, before P reductions were implemented. A large bloom of *Aphanizomenon* developed in 2012 after a polymictic episode mixed high concentrations of P from the anoxic hypolimnion throughout the lake. Algal biomass was higher and alkaline phosphatase activity levels in 2012 were significantly lower at all sites than in 2004 and 2005, despite reduced supply rate of riverine P. The results confirm that reducing nonpoint source P runoff in the upstream catchment alone is not an efficacious management strategy for these lakes.

Key words: algae, alkaline phosphatase, cyanobacteria, eutrophication, P limitation

Seasonal algal blooms in impoundments of the Huron River near Ann Arbor, Michigan, have been recognized as an environmental problem for many years. In an effort to reduce nonpoint source loading of phosphorus (P) to the river, the city of Ann Arbor enacted an ordinance in 2007 banning the use of lawn fertilizer containing P unless soil test data identified a specific chemical need for the element (Lehman et al. 2009). Similar legislation was later adopted statewide in Michigan as Public Act 299 of 2010. Statistical analyses after 3 years of post-ordinance observation revealed significant reductions in river P (Lehman et al. 2011).

Meanwhile, studies in Lake Ford, the Huron River impoundment historically producing the largest nuisance cyanobacteria blooms, indicated that internal loading of P from anoxic sediments rather than those from river inputs was the greatest factor inducing the blooms (Lehman 2011). An experimental program of hypolimnetic water discharge prior to development of anoxia proved to effectively eliminate midsummer cyanobacteria blooms and replaced them with a diatom community (Ferris and Lehman 2007, Lehman et al. 2009, Goldenberg and Lehman 2012). The results prompted the municipality with jurisdiction over the lake and its outlet hydroelectric dam to manage the lake by hypolimnetic withdrawal in subsequent summers, and water quality was greatly improved from 2008 to 2011.

Scientific interpretations were confounded, however, by the coincidence that the ordinance restricting lawn fertilizers containing P had been enacted in the upstream drainage in 2007. It remained unresolved whether the P restrictions and reduced allochthonous loading rates had been responsible, at least in part, for increased P limitation of lake

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algae and reduced nuisance conditions. Bioassay experiments performed in 2005 demonstrated that P was the principal element limiting algal biomass development in Ford Lake (Ferris and Lehman 2007), and bioassays in 2008 revealed that *Fragilaria* achieved dominance over *Aphanizomenon* through superior competition for limiting P (McDonald and Lehman 2013).

Drought conditions across the Midwest in 2012 prevented the use of hypolimnetic discharge as a management tool in 2012, and Ford Lake again developed cyanobacteria blooms. We took this opportunity to use an existing archive of water chemistry data, including alkaline phosphatase (AP) activity rates, to assess the biochemical evidence for P limitation of post-ordinance algal communities in the absence of whole lake manipulation. Baseline data for AP activity, chlorophyll (Chl-*a*), and water chemistry measured in 2004–2005 (pre-ordinance) were compared with data collected by identical methods in 2012. We also compared AP activity in 2012 with 2006, which was the first year that experimental trials of hypolimnetic withdrawal were conducted (Ferris and Lehman 2007). Because AP production is induced by phosphate limitation, the enzyme is a useful quantitative index of P limitation (Healey and Hendzel 1979, Gage and Gorham 1985). Chrost and Overbeck (1987) found that during summer, AP activity in the photic zone of a eutrophic lake was mainly produced by phytoplankton rather than bacterioplankton. They also found that AP activity was inversely related to available dissolved inorganic P and positively related to algal biomass, measured as adenosine triphosphate (ATP) in their study. Other authors have found similar patterns between AP activity, inorganic P, and biomass measured as Chl-*a* (Labry et al. 2005). With this foundation, our aim was to test 2 *a priori* hypotheses:

Null Hypothesis: AP activity in 2012 was unchanged from baseline levels measured in 2004 and 2005 despite significant reductions in P loading into the lakes from the river, signifying no change in the P limitation status of phytoplankton.

Causation Hypothesis: Lake phytoplankton exhibited significantly increased AP activity in 2012, signifying increased P limitation within the algal community subsequent to efforts reducing nonpoint source loading of P from the upstream catchment.

Application of the scientific method required that we look for the existence of evidence that explicitly should not exist if the test theories were correct. In this case, the management theory being tested was that successful reduction of P levels in the Huron River (reduced external loading) had reduced eutrophic conditions and that it both increased the phosphorus deficiency and reduced the biomass of nuisance algae in Ford Lake. We therefore would not expect to find increased cyanobacteria biomass and reduced P limitation by those algae if the causation theory was correct.

Materials and methods

Field site

Our field site was the middle portion of the Huron River catchment in southeastern Michigan (United States Geological Survey [USGS] Cataloging Unit 04090005; Fig. 1). We studied the 2 largest impoundments, Ford Lake and Belleville Lake. Ford Lake (42.21°N, 83.56°W) has a surface area of 4.039 km^2 and mean depth of 4.3 m; Belleville Lake (42.21°N, 83.49°W) has surface area of 3.91 km^2 and mean depth of 5.1 m (Nriagu et al. 2001). Both impoundments were constructed in the early 20th century for hydroelectric power production, and they continue to produce power commercially. The impoundments are operated as run-of-the-river, meaning that stage heights are held nearly constant by controlling water flow through turbines and automated hydraulic flood gates.

Each of the impoundments was sampled at or near its outlet dam at stations designated F3 and B2 for Ford Lake and Belleville Lake, respectively. Each lake was also sampled at an additional site, designated F2 and B1, respectively (Fig. 1). The Huron River was sampled at 3 sites where runoff was within the jurisdiction of the lawn fertilizer ordinance, designated A, B, and F, as well as an upstream site, named CTL, to provide control information about river water quality entering the study region (Fig. 1).

Water sampling and analysis

Water samples were collected weekly at each sampling site from May to September 2012. Only immediate subsurface (0 m) samples were used for this study. Archival data existed at weekly or shorter intervals for each site from prior years. Again, only immediate subsurface samples were used for comparison. Nutrient chemical analyses were identical to those described by Ferris and Lehman (2007), Lehman et al. (2009), and Lehman (2011) and included soluble molybdate-reactive P (SRP), total dissolved P (DP), total P (TP), soluble molybdate-reactive Si (SRSi), and nitrate (NO_3^-). The SRP was measured as molybdate-reactive phosphate in filtrate (10 cm path length at 885 nm, 3 replicates, detection limit 0.2 μg/L as P); DP and TP were measured as SRP after first oxidizing filtrate (DP) or unfiltered water (TP) with potassium persulfate at 105 C for 1 h.

Samples collected from lake sites were also measured for Chl-*a* and AP activity. For Chl-*a*, pigment was extracted

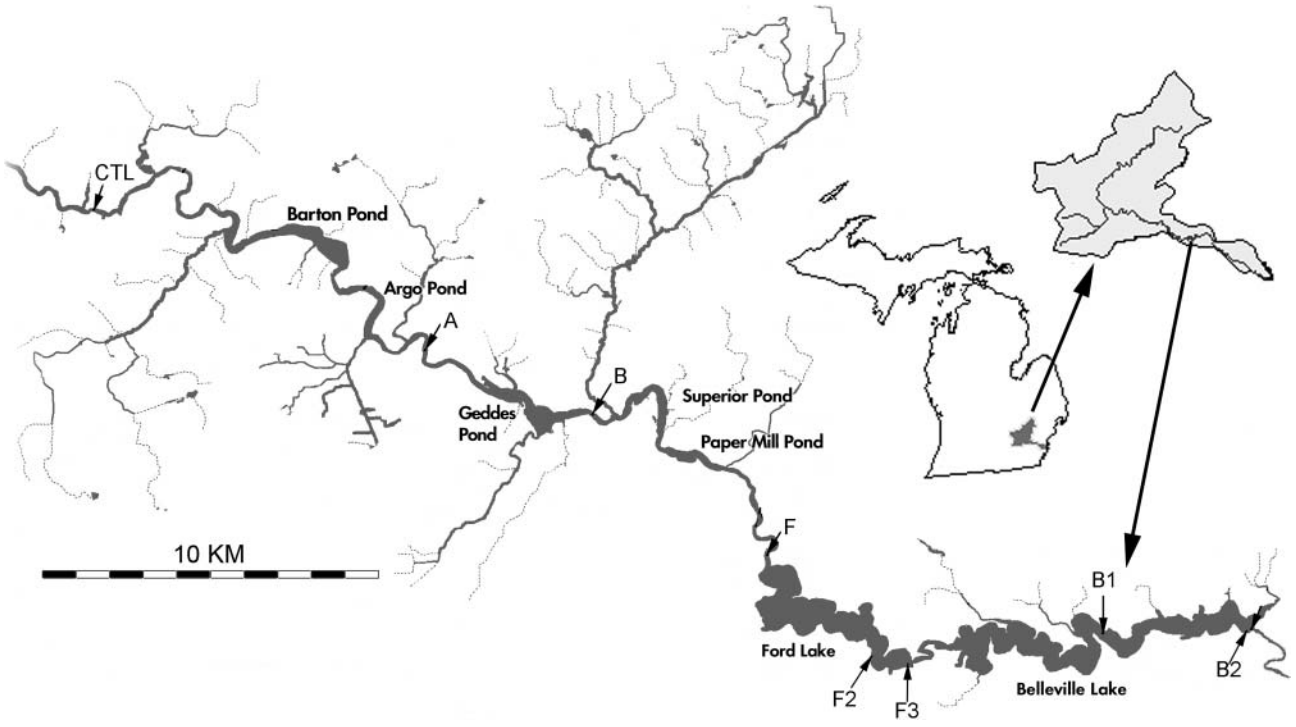

Figure 1.-Site map with river and lake sampling sites identified.

by macerating Whatman GF/C filters in ice-cold 90% v/v acetone by tissue grinder then filtering the slurry through a Whatman GF/D filter. Chl-*a* was measured fluorometrically using a Turner Designs TD700 fluorometer with 436 nm excitation filter and 680 nm emission filter. AP activity was measured using a modification of Turner Designs application method 998-2679. We added 1 mL aliquots of 36 μM 4-methylumbelliferyl phosphate (MUP) in 50 mM pH 8.0 TRIS buffer to 4 mL raw water at 22 C, and both initial and time series fluorescence were measured with a Turner Designs Model 10 fluorometer using the long wavelength UV filter kit (P/N 10-302R) with 310–390 nm excitation filter and 410–600 nm emission filter. Fluorescence changes were converted to rate of substrate hydrolysis using reference standards of 2 μM 4-methylumbelliferone as sodium salt. Enzyme activity was expressed as nmol MUP hydrolyzed per hour.

Ford Lake sampling and water flow

YSI temperature sensors and sondes have been moored at F3 since 2008 to provide temperature at 2 m intervals to 8 m depth as well as dissolved oxygen (DO) at 3, 6, and 8 m every 30 min with data accessible through online data radiotelemetry. Prior to 2008, vertical profiles of temperature and DO were measured weekly by boat. Discharge from the lake epilimnion through hydroelectric turbines and from flood gates at the base of the dam (11 m) were obtained from logs maintained by the dam operator.

Statistical analyses

All analytes exhibited lognormal distributions and consequently were logarithmically transformed prior to statistical analysis. AP activity was the only variable that included values of zero; therefore 1 nM/h (roughly our detection limit) was added to all values before transformation. Univariate or multivariate analyses of variance (AOV) were performed with IBM SPSS version 19.

Results

Precision of measurements

As reported previously (Lehman et al. 2011), the quality control of our water chemistry measurements resulted in standard errors <5% of mean values among replicates. AP activity, however, proved to be much more intrinsically variable. Although all assays were performed in triplicate, the mean ratio of standard error to sample mean was 0.20 (SD = 0.22, n = 104). Thus, the largest component of uncertainty

Table 1.-Mean concentrations (and SE) of SRP, DP, and TP (μg/L as P) at site F, inlet to Ford Lake from May to Sep of years listed. n = number of observations.

Years (n)	SRP	DP	TP
2004–2005 (44)	19.0 (1.7)	34.4 (2.0)	60.3 (2.8)
2008–2010 (66)	16.2 (0.8)	27.8 (1.2)	51.8 (1.4)
2012 (21)	16.5 (1.7)	29.9 (2.3)	50.3 (3.9)

in our statistical analyses traces to the AP measurements more so than the associated variables.

Huron River P

Concentrations of P in the Huron River were significantly reduced from 2008 to 2010 compared with 2003 to 2005 following a ban on the use of lawn fertilizers containing P (Lehman et al. 2011). To test whether P concentrations in 2012 continued at the reduced level, multivariate AOV tests were performed using the analytes SRP, DP, and TP as dependent variables, and period (2008–2010 vs. 2012) and month of year as factors. Our previous research had already established that river P concentrations vary significantly month to month. No significant effect of period (years) was detected at river sites A and F ($P > 0.27$), but there were significant differences at sites CTL ($P = 0.016$) and B ($P = 0.006$). Inspection of the data revealed, however, that concentrations in 2012 were lower than those from 2008 to 2010 at those 2 sites. Nevertheless, concentrations entering Ford Lake (site F) were fully comparable to 2008–2010, and lower than preordinance conditions (Table 1; $P = 0.02$ for TP).

Inside the lakes, however, P concentrations were comparable to the 2004–2005 period. Multivariate AOV of SRP, DP, and TP detected no significant differences between 2012 and 2004–2005 at F2 ($P > 0.9$), F3 ($P > 0.3$), or B2 ($P > 0.1$). A significant difference was detected only at B1 ($P = 0.018$); inspection of the data revealed that SRP and DP were lower in 2012 than in 2004–2005, but TP concentrations were virtually identical. Concentrations of Chl-*a*, however, were substantially elevated at all sites in 2012 compared to earlier, preordinance years (Table 2). Chl-*a* was significantly elevated at F3, B1, and B2 ($P < 0.03$ in each case) but only marginally so at F2 ($P = 0.067$). Chl-*a* was also significantly elevated at all sites in 2012 ($P < 0.01$) compared to 2006, the first year of experimental selective withdrawal treatments, and also a preordinance year.

River discharge and Ford Lake

The year 2012 was marked by drought across the Midwest. River discharge measured at Ann Arbor (USGS 04174500) was similar to 2005 and lower than 2004 and 2006 (Fig. 2), which are the 3 years when baseline measurements of AP activity were made. Mean volumetric retention time from May to September in 2012 (34 d) was almost the same as 2005 (36 d) and considerably longer than 2004 (13 d) and 2006 (17 d). As a consequence of these low flow conditions in 2012, there was insufficient water available at Ford Lake for the operator to continuously discharge 300,000 m^3/d from the base of the dam, which is the volume required to destabilize the water column and prevent anoxia (Ferris and Lehman 2007). Instead, water was discharged mainly from the epilimnion to generate hydroelectricity (Fig. 3, third panel), and Ford Lake developed and maintained thermal stratification in June and July (Fig. 3, upper panel). Anoxia developed below 6 m (Fig. 3, second panel), and when a storm induced polymixis in early August, oxygen became uniformly depressed, P concentrations increased (from 2.2 μg/L SRP, as mass of P, on 6 Aug to 43.4 μg/L SRP on 10 Aug), and a massive bloom of *Aphanizomenon flos-aquae* developed as stratification reestablished (Fig. 3, lower panel).

AP activity versus specific activity

Although AP is commonly reported as specific activity (catalysis rate per unit biomass, e.g., nmol/h/μg Chl-*a*), that practice can implicitly suggest a direct, linear relationship between enzyme activity and biomass. Such was not the case in this study. The mean slope of ln(AP) versus ln(Chl-*a*) for all sites and years reported in this study was 0.28 (SE = 0.14, n = 11; B1 was not measured in 2006), significantly different from a slope of 1.0 that would indicate linearity in untransformed data.

Our *a priori* expectation was that AP activity would covary positively with Chl-*a* and negatively with SRP; consequently, we evaluated AOV for AP activity at each lake site using SRP and Chl-*a* as covariates. Our *a priori* expectation was confirmed in Ford Lake but not in Belleville Lake (Table 3). The effect of time period was statistically strong in both lakes, however. Post hoc Bonferroni comparisons of marginal means revealed that AP activity in 2004–2005 and 2006 were not significantly different but that AP activity was significantly reduced in 2012 at all sites. A graphic example of the data variability and the effects of Period, SRP, and Chl-*a* is provided for station F3 (Fig. 4). For comparison with other studies, the highest specific rate of AP activity (activity per unit biomass) encountered in this study was 59.1 nmol/h/μg Chl-*a* at station F2 on 11 July 2005; however, as presented earlier, specific activity does not represent a suitable variable for statistical tests owing to the nonlinear effects of independent variables (e.g., both SRP and Chl-*a*) on expressed enzyme activity.

Table 2.-Mean concentrations of SRP and TP (as μg/L P) and Chl-*a* (μg/L) from May to Sep at lake sampling sites for years listed. na = no data available.

Lake–Site	2004–2005 SRP	2006 SRP	2012 SRP	2004–2005 TP	2006 TP	2012 TP	2004–2005 Chl-*a*	2006 Chl-*a*	2012 Chl-*a*
Ford–F2	3.7	3.9	3.7	41.0	37.7	na	20.0	11.8	35.0
Ford–F3	3.4	2.7	3.8	32.5	37.0	36.3	16.6	12.3	28.2
Belleville–B1	5.0	na	3.6	51.4	na	50.6	22.6	na	35.4
Belleville–B2	3.6	2.1	3.2	40.1	40.9	36.5	18.1	16.6	26.4

Table 3.-AOV *P*-values for AP at lake sites, with AP activity as the dependent variable, Period (2004–2005, 2006, or 2012) as an independent factor, and SRP and Chl *a* as covariates. Marginal means for AP activity, antilog-transformed to nM MUP/h, are listed for 2004–5, 2006, and 2012. NS = not statistically significant at $\alpha = 0.05$; na = not available.

Lake–Site	Period	SRP	Chl-*a*	r^2	2004–2005	2006	2012
Ford–F2	0.006	0.001	<0.0005	0.31	136	159	48
Ford–F3	0.003	<0.0005	<0.0005	0.28	136	110	42
Belleville–B1	<0.0005	0.41; NS	0.46; NS	0.25	192	na	61
Belleville–B2	0.001	0.09; NS	0.19; NS	0.23	151	178	46

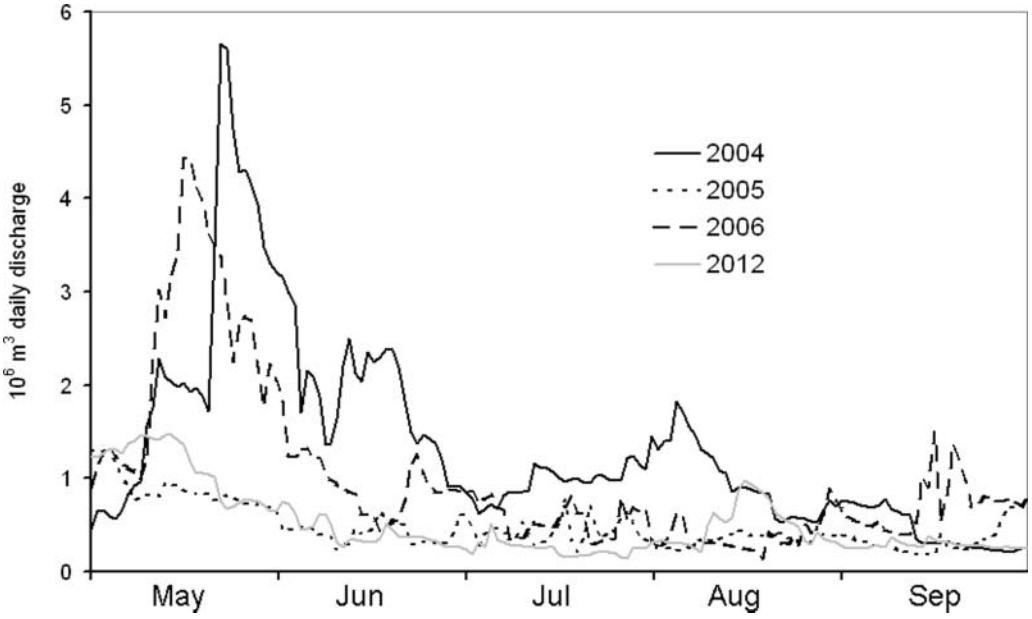

Figure 2.-Daily fluvial discharge of the Huron River at Ann Arbor, MI (USGS 041745000) for the 4 years relevant to this report.

Discussion

Both of our *a priori* hypotheses were rejected by the evidence we collected. Neither was AP activity in 2012 the same as 2004–2005 levels, nor did AP activity increase, signifying increased P limitation. Instead, AP activity in both Ford and Belleville lakes was lower in 2012 and algal biomass measured as Chl-*a* was higher than that measured in any of the baseline years (2004–2005 or 2006) even though incoming river water had lower concentrations of P reflective of increased control of nonpoint source P runoff.

The results refute both the Null and Causation hypotheses but do not refute the contention that internal loading of P from anoxic sediments is the factor controlling nuisance bloom formation in Ford Lake (Lehman 2011). They also serve as a reminder that efforts to curb P loading from suburban landscapes, while certainly environmentally conscious and probably salutary in many cases, are not a panacea for all lake eutrophication problems.

It might seem enigmatic that P limitation as measured by AP activity was not elevated in 2006, the first year in which we used whole-lake manipulation by hypolimnetic withdrawal to artificially mix Ford Lake to prevent midsummer anoxia; however, our manipulations transformed species composition of the algal community (Lehman et al. 2009). Bioassay experiments revealed that the diatoms that dominated the transformed community showed no evidence of P limitation, while growth rates of sympatric subdominant cyanobacteria were strongly P limited (McDonald and Lehman 2013). Others have used enzyme-labeled fluorometry (ELF) to investigate variations in AP activity at the cellular level (e.g., Duhamel et al. 2008, 2009, Gonzalez-Gil et al. 1998, Telford et al. 2001, Rengefors et al. 2001, 2003, Nedoma et al. 2003), sometimes using optical microscopy (Dyhrman and Palenik 1999, Dyhrman et al. 2002) but more commonly using flow cytometry. Differences in P limitation status have been identified both interspecifically and among cells or colonies of a single species; thus, it seems plausible that under competition for P, the superior competitors (e.g., diatoms) might evince less physiological evidence of P stress than would the species they outcompeted. The maximum rate of AP activity registered per unit Chl-*a*, 59 nmol/h/μg Chl-*a*, is only 26% lower than the maximum specific activity induced by Ferris and Lehman (2007) in bioassay experiments performed with Ford Lake water incubated for 6 d without added P (Table 5 in Ferris and Lehman 2007) but is considerably less than the maximum Labry et al. (2005) reported for the Bay of Biscay (Figure 5b in Labry et al. 2005). Using their assumed stoichiometry of 50 C:1 Chl-*a* by mass, their maximum AP specific activity at zero measurable SRP exceeded 150 nmol/h/μg Chl-*a*. At the opposite extreme, Smith and Kalff (1981) reported maximum average June to September AP specific activity from oligotrophic regions of Lake Memphremagog ($\sim$10 μg/L TP concentration) of approximately 1.5 μg 3-O-methylfluorocein/h/μg Chl-*a*. Given the substrate's formula weight of 346.33, this represents 4 nmol/h/μg Chl-*a*. In continuous cultures of lake phytoplankton maintained at low P-limited growth rates, they achieved at most 14 nmol/h/μg Chl-*a* (Figure 3 in Smith

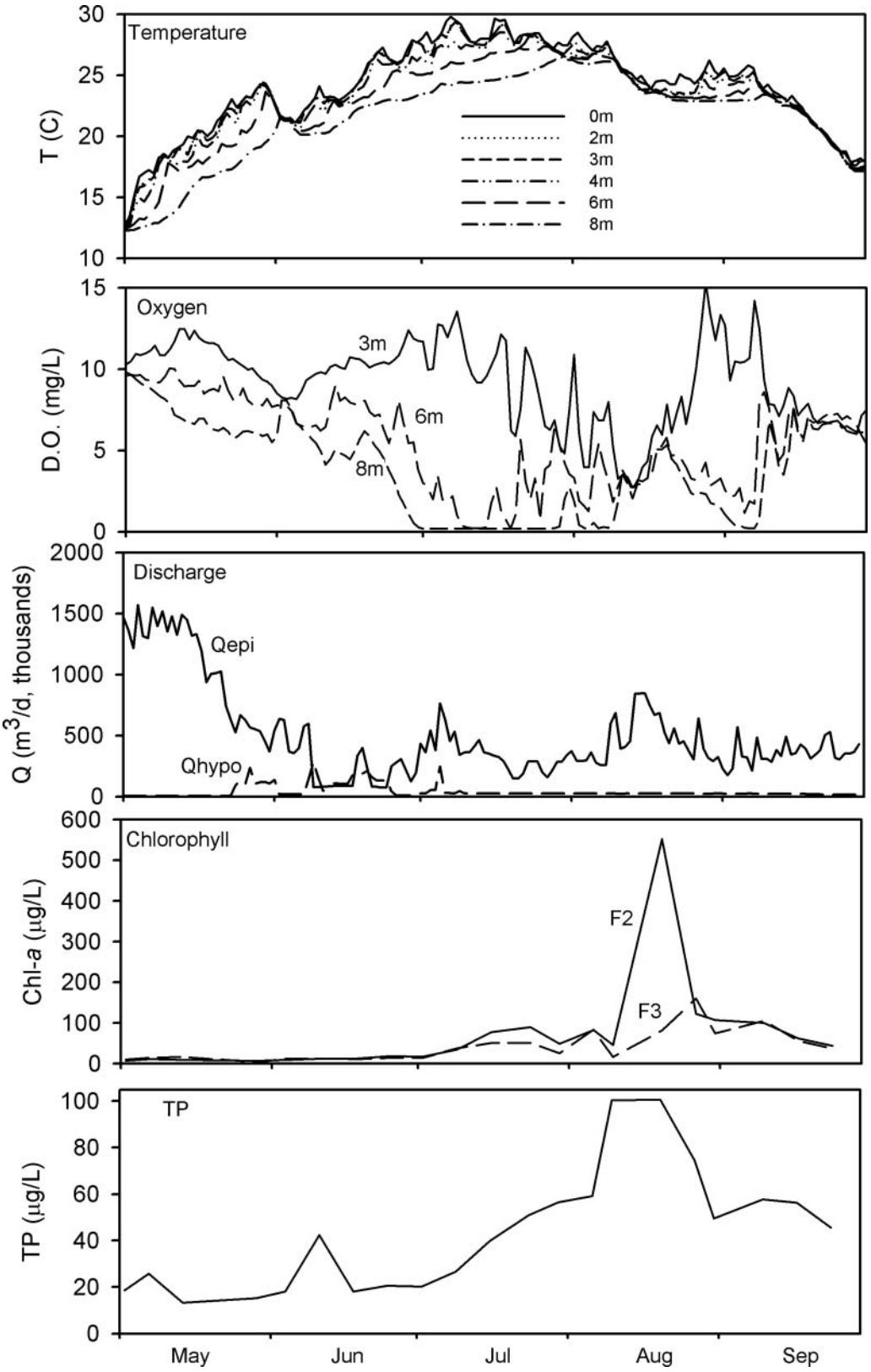

Figure 3.-Vertical distribution of temperature and dissolved oxygen (upper two panels) in Ford Lake from May to Sep 2012. Third panel: daily discharge at Ford Lake dam either from the surface (Qepi) or base (Qhypo). Fourth panel: surface Chl-*a* at stations F2 and F3. Lower panel: surface TP concentration at F3.

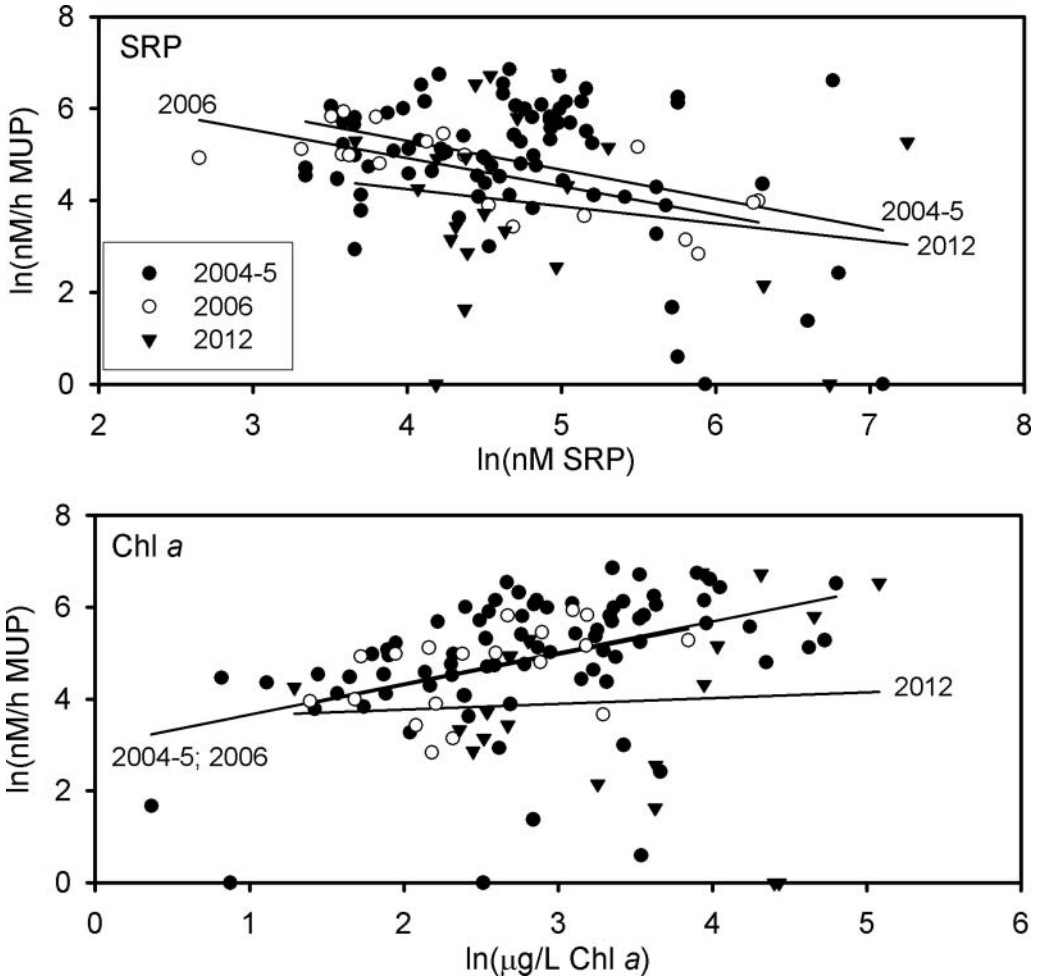

Figure 4.-Simultaneous ln-transformed concentrations of AP activity, SRP, and Chl-*a* at Ford Lake station F3 in 2004–2005, 2006, and 2012. Lines are least-square linear regression by year. In the chlorophyll panel, regression lines for 2004–2005 and 2006 are virtually indistinguishable from each other.

and Kalff 1981). Mean AP-specific activity in Ford Lake during summer 2012 was 4.7 (SE = 0.8, n = 42) nmol/h/μg Chl-*a*, comparable to Lake Memphremagog, where Smith and Kalff (1981) did not find phytoplankton to be strongly P limited. In Lake Champlain, Levine et al. (1997) cited the threshold activity level for severe P deficiency to be 0.2 nmol/min, or 12 nmol/h/μg Chl-*a*. For comparison, mean AP specific activity in Ford Lake during 2004–2005 and 2006 was 13.0 (SE = 1.2, n = 81) and 11.1 (SE = 1.7, n = 19) nmol/h/μg Chl-*a*, respectively.

In both 2004–2005 and 2012, the midsummer phytoplankton in Ford Lake was dominated by *Aphanizomenon*. The development of extended thermal stratification in 2012 precluded dominance by diatoms, which have been shown in this lake to sink quickly from the stratified epilimnion (Ferris and Lehman 2007). It seems that the intense anoxia in 2012 (Fig. 3) and high release rates of P from anoxic sediments (Lehman 2011) caused *Aphanizomenon* to be even less P limited that year than during 2004 and 2005.

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References

Chrost RJ, Overbeck J. 1987. Kinetics of alkaline phosphatase activity and phosphorus availability for phytoplankton and bacterioplankton in Lake Plußsee (North German eutrophic lake). Microbiol Ecol. 13:229–248.

Duhamel S, Gregori G, Van Wambeke F, Mauriac R, Nedoma J. 2008. A method for analysing phosphatase activity in aquatic bacteria at the single cell level using flow cytometry. J Microbiol Meth. 75:269–278.

Duhamel S, Gregori G, Van Wambeke F, Nedoma J. 2009. Detection of extracellular phosphatase activity at the single-cell level by enzyme-labeled fluorescence and flow cytometry: The importance of time kinetics in ELFA labeling. Cytom Part A. 75A:163–168.

Dyhrman S, Palenik B. 1999. Phosphate stress in cultures and field populations of the dinoflagellate *Prorocentrum minimum* detected by a single-cell alkaline phosphatase assay. Appl Environ Microbiol. 65:3205–3212.

Dyhrman S, Webb EA, Anderson DM, Moffett JW, Waterbury JB. 2002. Cell-specific detection of phosphorus stress in *Trichodesmium* from the Western North Atlantic. Limnol Oceanogr. 47:1832–1836.

Ferris JA, Lehman JT. 2007. Interannual variation in diatom bloom dynamics: roles of hydrology, nutrient limitation, sinking, and whole lake manipulation. Water Res. 41:2551–2562.

Gage MA, Gorham E. 1985. Alkaline phosphatase activity and cellular phosphorus as an index of the phosphorus status of phytoplankton in Minnesota lakes. Freshwater Biol. 15:227–233.

Goldenberg SZ, Lehman JT. 2012. Diatom response to the whole lake manipulation of a eutrophic urban impoundment. Hydrobiologia. 691:71–80.

Gonzalez-Gil S, Keafer BA, Jovine RVM, Aguilera A, Lu SH, Anderson DM. 1998. Detection and quantification of alkaline phosphatase in single cells of phosphorus-starved marine phytoplankton. Mar Ecol Prog Ser. 164:21–35.

Healey FP, Hendzel LL. 1979. Fluorometric measurement of alkaline phosphatase activity in algae. Freshwater Biol. 9:429–439.

Labry C, Delma D, Herbland A. 2005. Phytoplankton and bacterial alkaline phosphatase activities in relation to phosphate and DOP availability within the Gironde plume waters (Bay of Biscay). J Exp Mar Biol Ecol. 318:213–225.

Lehman EM, McDonald KE, Lehman JT. 2009. Whole lake selective withdrawal experiment to control harmful cyanobacteria in an urban impoundment. Water Res. 43:1187–1198.

Lehman JT. 2011. Nuisance cyanobacteria in an urbanized impoundment: interacting internal phosphorus loading, nitrogen metabolism, and polymixis. Hydrobiologia. 661:277–287.

Lehman JT, Bell DW, Doubek JP, McDonald KE. 2011. Reduced additions to river phosphorus for three years following implementation of a lawn fertilizer ordinance. Lake Reserv Manage. 27:390–397.

Levine SN, Shambaugh A, Pomeroy SE, Braner M. 1997. Phosphorus, nitrogen, and silica as controls on phytoplankton biomass and species composition in Lake Champlain (USA-Canada). J Great Lakes Res. 23:131–148.

Nedoma J, Strojsova A, Vrba J, Komarkova J, Simek K. 2003. Extracellular phosphatase activity of natural phytoplankton studied with ELF97 phosphate: fluorescence quantification and labelling kinetics. Environ Microbiol. 5:462–472.

Nriagu JO, Meier PM, Pascual DL, Lehman JT. 2001. *Nutrients in Ford and Belleville Lakes, Michigan, 1998-2000.* Report to Michigan Department of Environmental Quality Surface Water Quality Division;, August 2001. 99 p.

McDonald KE, Lehman JT. 2013. Dynamics of Aphanizomenon and Microcystis (cyanobacteria) during experimental manipulation of an urban impoundment. Lake Reserv Manage. 29: 103–115.

Rengefors K, Pettersson K, Blenckner T, Anderson D. 2001. Species-specific alkaline phosphatase activity in freshwater spring phytoplankton: Application of a novel method. J Plankton Res. 23:435–443.

Rengefors K, Ruttenberg K, Haupert C, Taylor CD, Howes BL, Anderson DM. 2003. Experimental investigation of taxon-specific response of alkaline phosphatase activity in natural freshwater phytoplankton. Limnol Oceanogr. 48:1167–1175.

Smith REH, Kalff J. 1981. The effect of phosphorus limitation on algal growth rates: evidence from alkaline phosphatase. Can J Fish Aquat Sci. 38:1421–1427.

Telford W, Cox W, Singer V. 2001. Detection of endogenous and antibody-conjugated alkaline phosphatase with ELF-97 phosphate in multicolor flow cytometry applications. Cytometry. 43:117–125.