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SEASONAL MERCURY LEVELS IN PHYTOPLANKTON AND THEIR RELATIONSHIP WITH ALGAL BIOMASS IN TWO DYSTROPHIC SHIELD LAKES

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Abstract—Our study focused on the seasonal dynamics of total Hg in the phytoplankton (living and dead) of two dystrophic shield lakes (Mouse and Ranger). Phytoplankton samples were taken from metalimnetic and hypolimnetic depths in the euphotic zone and were collected and analyzed using ultraclean techniques. In both lakes, phytoplankton Hg (PHYTO-Hg) levels (pg/L) in the metalimnion did not significantly change among dates over the season, although Ranger Lake exhibited significant differences between Hg values measured at the beginning and end of the season. In contrast, PHYTO-Hg significantly increased in the hypolimnia of both lakes by the end of the season. Combined influences of external Hg inputs, remineralization, phytoplankton sedimentation, and increased methylmercury production in the hypolimnia over the season may have contributed to these trends. A highly significant positive relationship existed between PHYTO-Hg levels and whole-water Hg levels ($r^2 = 0.90$), and the mean bioconcentration factor for Hg between the water column and phytoplankton was significantly higher in the hypolimnion compared to the metalimnion for both lakes. In most cases, parameters associated with algal biomass had significant positive correlations with PHYTO-Hg levels. Weight-specific PHYTO-Hg (pg/mg dry weight) varied significantly over the season, and there were interlake differences with respect to seasonal trends. On the basis of these results, we recommend that future sampling regimes include collection of phytoplankton at different limnetic depths throughout the season to account for spatial and temporal variations. Weight-specific Hg levels in phytoplankton could not be explained well by the parameters tested, and the only significant regressions were with parameters reflecting algal biomass. This study provides in situ evidence of Hg accumulation in lake phytoplankton as a function of algal biomass on a seasonal basis (as opposed to biodilution) and stresses the need to confirm these trends in other lake systems.

Keywords—Mercury Phytoplankton Biomass Dystrophic Seasonality

INTRODUCTION

During the last decade, advancements in ''ultratrace'' Hg detection in remote aquatic systems has fostered our understanding of Hg as a global contaminant. It has been established that increasing Hg burdens in freshwaters are the result of anthropogenic inputs [1,2]. More than 50% of Hg entering lakes originates from atmospheric deposition and is mostly in the form of inorganic Hg [3–6]. Once inorganic Hg has entered the water column, it has the potential to be either directly accumulated by lake phytoplankton or transformed into organic Hg before accumulation by phytoplankton. Accumulation in this sense includes adsorption/absorption and uptake into algal cells.

To date, the majority of research on Hg accumulation in aquatic biota has focused on the higher trophic levels. Few data exist for lake phytoplankton, in part because of problems in ultratrace Hg detection. Phytoplankton are the free-floating algae in lakes that serve as a carbon and nutrient source for both herbivorous zooplankton and the microbial community [7,8]. Although methylmercury, a relatively toxic compound, has been implicated as the predominant mercurial species of concern in trophic biomagnification studies, techniques were not established at the time of our study to measure the extremely low levels of methylmercury in the phytoplankton from these pristine lakes. What we can infer is that generally a smaller proportion (13–30%) of total Hg in lake phytoplankton is methylmercury as compared to concentrations in fish tissues (>90%) [9]. This supports conventional knowledge that algae are capable of accumulating various species of Hg [10–15].

Algae have been found to exhibit a number of metabolism-dependent and -independent mechanisms for heavy metal uptake and accumulation [10–15]. Both dead and living algal cells have been found to accumulate heavy metals, although mechanistic differences probably exist [13]. At this point, little is known about the ecological factors that regulate total Hg accumulation in phytoplankton (both living and dead). In this study, we specifically collected the phytoplankton fraction of lake water and measured Hg levels over the season at discrete limnetic depths to assess the effects of various physical and biological parameters.

The phytoplankton communities from two dystrophic lakes (Mouse and Ranger) on the Precambrian Shield near Dorset, Ontario, Canada, were assessed monthly in parallel from June to September 1995 for the analyses of phytoplankton Hg (PHYTO-Hg), algal biomass, algal community structure, and associated physicochemical data. Although samples were collected from all limnetic depths in the euphotic zone, we decided to limit our analyses to metalimnetic and hypolimnetic samples only. This decision was based on the extremely low phytoplankton dry weight, chlorophyll *a*, and algal biovolume

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Table 1. 1995 Seasonal means and SEs of physicochemical and biological parameters for Mouse and Ranger Lakes[a]

	Mouse Lake	Ranger Lake	
Parameter[b]	Mean (SE)	Mean (SE)	*p*
Secchi depth (m)	3.88 (0.09)	2.98 (0.23)	0.03
DOC (mg/L)	5.43 (0.16)	6.19 (0.15)	0.001
DO (mg/L)	6.18 (0.74)	6.56 (0.73)	0.72
Temperature (°C)	14.07 (1.12)	12.39 (1.14)	0.30
Total phosphorus (μg/L)	8.58 (3.45)	5.56 (1.40)	0.46
Chlorophyll *a* (μg/L)			
Total (μg/L)	46.22 (10.89)	27.28 (3.39)	0.10
Corrected	6.64 (1.22)	7.22 (1.12)	0.73
Algal biovolume (μg/L)	578.46 (213.87)	980.50 (502.20)	0.47
PHYTO-DW (mg/L)	3.62 (1.06)	2.40 (0.63)	0.34
PHYTO-Hg (pg/L)	854.82 (318.20)	462.03 (122.70)	0.27
PHYTO-Hg (pg/mg DW)	212.17 (37.64)	208.54 (32.82)	0.94

[a] A Student's *t* test was performed to detect significant differences between lakes. All samples were collected in parallel from discrete depths in the euphotic zone monthly from June to September. Dissolved organic carbon was included to verify the dystrophic nature of each lake and is represented by seasonal mean values taken from a 4-year data-set (1991–1994) of dates matching the 1995 sampling regime.

[b] DO = dissolved oxygen; DOC = dissolved organic carbon; DW = dry weight; PHYTO-DW = phytoplankton dry weight; PHYTO-Hg = phytoplankton mercury.

estimates determined for the epilimnion over the season. We were concerned that Hg in epilimnetic phytoplankton could not be adequately resolved because of the very low Hg signals in the analysis. Therefore, we focused on the prevalent plankton layers (as determined by biomass estimates) of the lower depths, where sample Hg measured was always more than twice the background Hg. This approach is in accordance with the results of Watras and Bloom's [16] study, in which high Hg concentrations in lake water were associated mainly with plankton layers found in the metalimnia and hypolimnia.

METHODS

The two study lakes, Mouse and Ranger, are located on Precambrian Shield granite/sand basins in south-central Ontario (45°11′N, 78°51′W and 45°09′N, 78°51′W, respectively) with minimal shoreline development. Mouse and Ranger are small (~11 ha) dystrophic lakes with similar edaphic and morphological characteristics (Table 1). Mouse Lake is shallower than Ranger Lake in both maximum depth (9 and 13 m, respectively) and mean depth (4.88 and 5.62 m, respectively). Each lake consists of a single basin with high estimated flushing rates (more than twice per year). Most of this flow is derived from snow melt in early spring, whereas during summer months, flow volume is low [17]. Although the lakes are located in different drainage basins, neither watershed experiences direct impacts from human development (i.e., forestry, industry, or agriculture).

Equipment preparation, Hg extractions, and analyses were all performed within the Ontario Ministry of the Environment and Energy's (OMEE) ''Mercury Clean Lab,'' Dorset Research Centre, Dorset. Great care was taken to minimize Hg contamination by using ultraclean techniques throughout sampling, extraction, and analysis protocols. Most equipment was washed with detergent and treated overnight with a strong acid extractant called BES (20% nitric acid, 2% hydrochloric acid, and 0.05% potassium dichromate). Exceptions included Nitex® mesh (detergent wash only) and GF/C® filters (20% nitric acid and 5% hydrochloric acid treatment). Lint-free suits and vinyl gloves were worn during cleaning and equipment preparation.

Using a closed water collection system consisting of a peristaltic pump with Teflon® in-line filters and C-Flex® silicone tubing, monthly parallel water samples were collected from the metalimnia and hypolimnia at the deep station of each lake from early June to September 1995. Nitex-filtered (<63 μm) replicate 1-L water samples were collected for PHYTO-Hg, chlorophyll *a*, and phytoplankton dry-weight (PHYTO-DW) measurements. A mesh size of 63 μm was chosen to exclude zooplankton from sample water while allowing phytoplankton to pass through. Independent one-way analysis of variance (ANOVA) confirmed that for both chlorophyll *a* and biovolume estimates, there were no significant differences between parallel whole-lake water samples and filtered water. Therefore, we were confident that phytoplankton collected for analysis was free of zooplankton but reflected whole-lake phytoplankton concentrations.

In a preliminary study, we attempted to trap the edible size fraction of phytoplankton by using 20- and 30-μm mesh. However, clogging caused back-flow problems during water collection. Rapid clogging of these small pores was believed to be a combination of trapped suspended particulates and algal exudates in the water sampled. Filtered water for biovolume estimates and algal community composition were collected in 4-oz glass bottles and preserved with acid Lugol's iodine in the field. Replicate water samples (125 ml) were collected in acid-washed plastic vials for total phosphorus (TP) analysis. Temperature and dissolved oxygen (DO) parameters were measured using a YSI 5700 probe, and euphotic zones were estimated as twice the Secchi depth transparency (empirically determined in previous years) (P. Chow-Fraser, unpublished data).

All water samples were transported in ice-packed coolers and taken to the laboratory for processing within 3 h of collection. Laboratory processing involved the collection of phytoplankton onto GF/C filters (0.45 μm) using a vacuum pump system of acid-washed Teflon in-line filters, Teflon tubing, and a glass waste flask. Parallel water samples were filtered onto GF/C filters, wrapped in aluminum foil, and then frozen for future chlorophyll *a* and PHYTO-DW analyses. Individually

preweighed filters used for PHYTO-DW analyses were initially placed in filtering units and treated with distilled water to remove loose fibers. Gravimetric experiments confirmed that filter weights did not differ significantly (Student's *t* test, $p > 0.1$) between filters with water run through them once and those with water run through twice (to mimic the filtering effect of lake water). Therefore, this ensured that preweights of filters would not be affected by lake-water filtration during sample collection. Total phosphorus samples were processed according to standard protocols established by the OMEE (i.e., modified molybdenum blue method of Murphy and Riley [18]).

Phytoplankton samples on GF/C filters collected for Hg analysis were carefully transferred to Pyrex® petri dishes and injected with 8 ml of BES. This potassium dichromate acid solution destroyed reducing matter in the phytoplankton samples and maintained Hg in solution with its high oxidizing potential. After each injection of acid, Pyrex lids were placed on top of each sample. An extraction period of 24 h was used because there were no significant differences (ANOVA, $p > 0.1$) in Hg extracted after 24, 72, and 120 h. After the 24-h extraction period, extractant from each sample was transferred via a small volume pipette to acid-washed borosilicate vials with Teflon-lined screw caps until analysis. To account for background contamination, replicate blanks were prepared with each set of phytoplankton samples. Blanks consisted of G/FC filters run through the same cleaning and processing regimen as sample G/FC filters and then treated with the same lot of BES extractant. Along with PHYTO-Hg samples, 1-ml subsamples of extractant from these blanks were analyzed for Hg.

Atomic fluorescence spectroscopy with the aid of a Gilson model 222 encased automatic sampler (Gilson Medical Electronics, Milwaukee, WI, USA) was used to determine Hg levels in each sample. The method is based on a purge-and-trap procedure for isolating and preconcentrating Hg from the sample and on detection by AFS. Using acid-washed polyethylene centrifuge tubes, 1-ml subsamples of extractant from each sample was injected into 40 ml of reverse-osmosis water. Each centrifuge tube with aqueous sample was placed in the automatic sampler rack, where a Teflon tube could draw the sample into c-flex tubing, which transported the sample to a purge vessel. In the purge vessel, a solution of sodium borohydride in NaOH was added to both decompose Hg compounds and reduce free Hg (II) to Hg (0). The sample was first purged with Hg-free argon gas and then flushed into and trapped by amalgamation onto gold-coated sand. After preconcentration, the Hg was thermally absorbed and flushed into a detector. The instrumental detection limit for Hg in aqueous media was 10 pg, and sample viability was dependent on background contamination always being less than 50% of Hg measured. The OMEE quality-assurance protocol was implemented for all sample runs. Correction curves were used to account for sensitivity drift throughout each run of samples analyzed.

Phytoplankton biomass was estimated using three parameters: dry weight, chlorophyll *a* concentration, and biovolume measurement. The PHYTO-DW samples on GF/C filters were desiccated in a food dehydrator for 24 h and then transferred to a Nalgene desiccator with Dry-Rite anhydrous calcium sulfate pellets for 1 h. The phytoplankton filters were weighed in an Ohaus enclosed microbalance (0.0-mg detection limit) with a petri dish of anhydrous calcium sulfate pellets set inside the housing chamber. The PHYTO-DW varied from 0.70 to 7.95 mgL and is accurate to the first decimal place. Chlorophyll

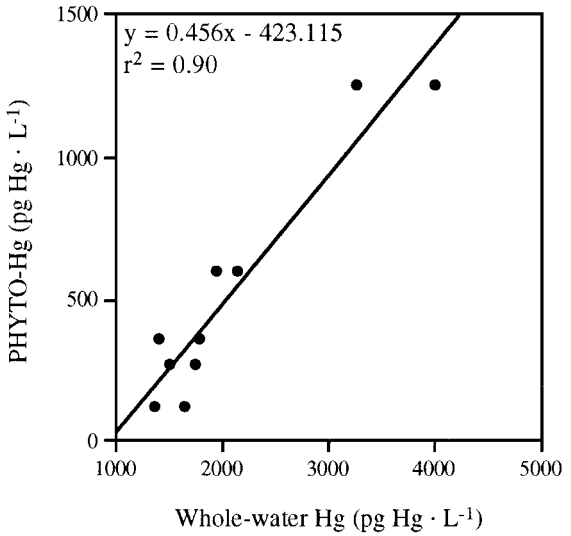

Fig. 1. Relationship between Hg in phytoplankton (PHYTO-Hg) and whole-water Hg.

a was extracted with 90% reagent-grade acetone for 1 h in a freezer, a protocol confirmed by extraction time trials (P. Chow-Fraser, unpublished data). A Milton Roy 301 spectrophotometer (Milton Roy, Ivyland, PA, USA) was used to determine absorbence readings. Both total (viable and degraded algae) and corrected (pheopigment-corrected for viable algae only) chlorophyll *a* concentrations were calculated.

For the determination of viable algal biovolume and composition, 5-ml subsamples of preserved lake water were settled for 24 h in algal settling chambers. Using 200× magnification, algal cells and colonies were counted and taxonomically identified along one full transect. The entire slide was scanned for large cells and colonies to ensure that their proportion in the sample was accurately recorded. Algal biovolumes were calculated by approximation to geometric shapes and converted to mass units by assuming a specific gravity of 1. Average dimensions of the algae were determined with the aid of an eyepiece micrometer at 400× magnification.

RESULTS

The physical and chemical characteristics of Mouse and Ranger Lakes were analyzed for statistical differences between lakes (Table 1). All parameter values were statistically similar between lakes, except for Secchi depth and dissolved organic carbon (DOC) (Secchi depth was significantly higher and DOC was significantly lower in Mouse Lake than in Ranger Lake. Because of the time and labor intensiveness of this study, we could include only a relatively small sample size. Thus, regression analyses were performed using a pooled data set from both lakes. A comparison of parallel samples of PHYTO-Hg and whole-water Hg that were collected from July to September (Fig. 1) indicated a strong positive correlation beween the two variables ($r^2 = 0.90$). Although a clear relationship existed between Hg levels in phytoplankton and whole-lake water, the ratio between these two variables differed between depths (Fig. 2). This ratio, or bioconcentration factor, appears to be higher in the hypolimnion than in the metalimnion. However, it should be noted that the majority of Hg (>50%) is bound in the lake-water phase.

Phytoplankton Hg trends in each lake show that both the metalimnion and hypolimnion in each lake exhibit similar Hg

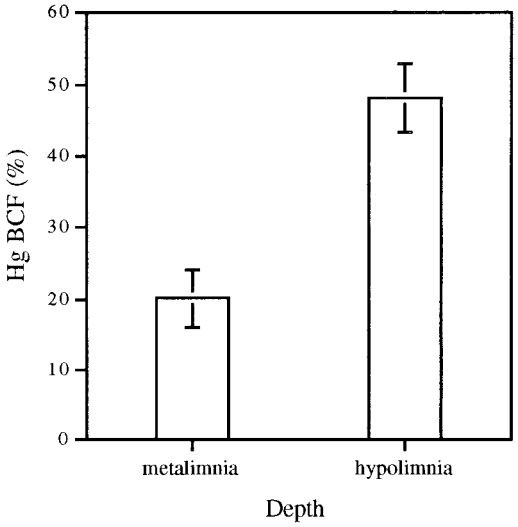

Fig. 2. Comparison of mean bioconcentration factor (BCF) values for Hg in phytoplankton between the metalimnia and hypolimnia.

levels at the beginning of the season, when spring turnover has occurred and stratification has been initiated (Fig. 3). For both lakes, there was a gradual decrease of Hg in the metalimnia and a marked increase of Hg in the hypolimnia. One-way ANOVA indicated that there were no significant differences among dates in the metalimnion of each lake. However, there was a significant decrease in PHYTO-Hg between the first and last dates of the season in Ranger Lake (Student's *t* test, $p < 0.05$). The hypolimnion of each lake showed significant differences among dates (ANOVA, $p < 0.05$), and PHYTO-Hg significantly increased from the beginning of the season to the end of the season (Student's *t* test, $P < 0.05$). Therefore, significant increases of PHYTO-Hg in the hypolimnion over the season do not appear to be the result of only phytoplankton accumulation from the metalimnion.

In Fig. 4, we standardized the data to present changes in weight-specific PHYTO-Hg (pg Hg/mg DW) over the season. These data have a larger margin of error because they are compounded by the inherent variation found in both of the original parameters (pg Hg/L and mg PHYTO-DW/L) used to calculate weight-specific values. In Mouse Lake, metalimnetic concentrations were highest in July, whereas hypolimnetic concentrations were highest in August (Fig. 4a). In comparison, in Ranger Lake, weight-specific Hg in metalimnetic samples was highest in June and continued to decline throughout the season, although values for the last three dates were not significantly different from one another ($p > 0.05$). In the hypolimnion of Ranger Lake, decreasing Hg levels occurred throughout the season until September, when PHYTO-Hg concentrations peaked (Fig. 4b).

It is evident that seasonal variations in phytoplankton community structure exist for all limnetic depths in each lake (Fig. 5). In the metalimnion of both lakes, where highest viable biomass levels occurred, there were greater proportions of obligate photoautotrophs (blue-greens and greens) in Mouse Lake than in Ranger Lake. With the exception of a diatom peak on day 187 in Ranger Lake, both lakes were seasonally dominated by chrysophytes and cryptophytes. These groups have many genera which can grow both autotrophically and heterotrophically (i.e., mixotrophy). In the hypolimnion, Mouse Lake had proportionately more photoautotrophs than Ranger Lake throughout the season until the last date, when there was a peak in photoautotrophs in Ranger Lake. Generally, chrysophytes and cryptophytes dominate the hypolimnetic community in Mouse Lake, whereas cryptophytes and diatoms dominate in Ranger Lake.

Both PHYTO-Hg per 1 L of lake water and weight-specific Hg were regressed against various limnological parameters for the two lakes to reveal significant relationships (Tables 2 and 3). The independent variables representing physical effects included Julian day and water temperature. Julian day reflected both a seasonal effect and, indirectly, sedimentation effects over time. Temperature was regressed against PHYTO-Hg to screen its potential effects on Hg reaction and uptake kinetics in phytoplankton. Total chlorophyll *a*, corrected chlorophyll *a*, and algal biovolume were all regressed against PHYTO-Hg to detect possible relationships with algal biomass. Phytoplankton dry weight represented the combined mass of viable and detrital material in the phytoplankton and tested the effect

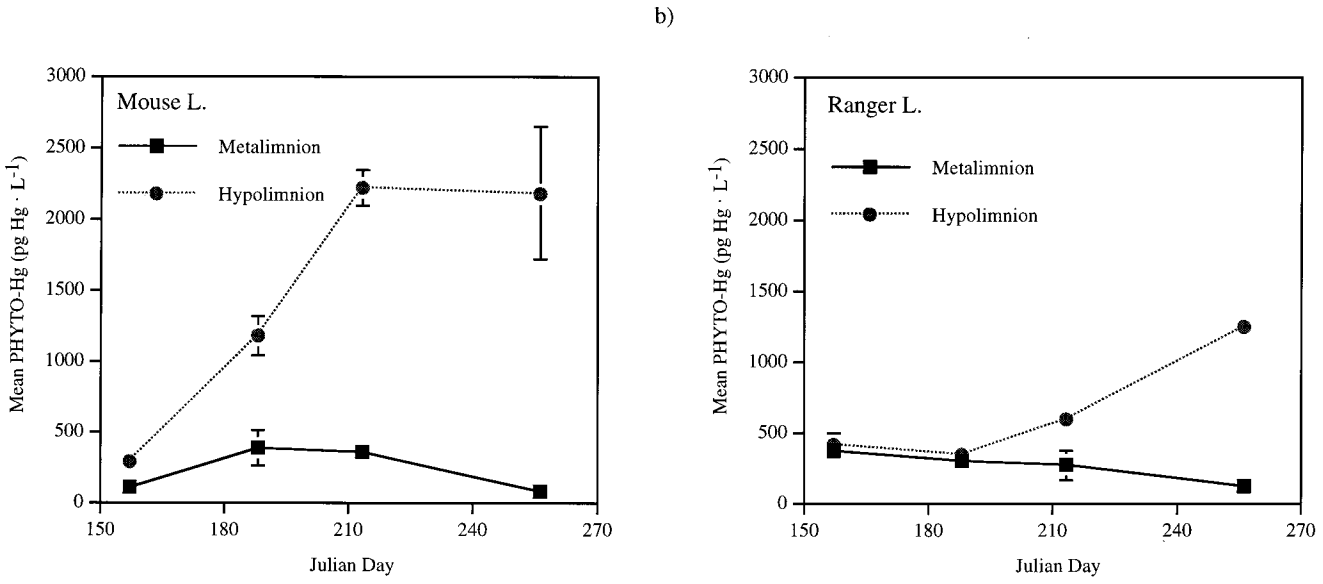

Fig. 3. Seasonal distribution of Hg in phytoplankton (PHYTO-Hg) in the metalimnia and hypolimnia of (**a**) Mouse Lake and (**b**) Ranger Lake.

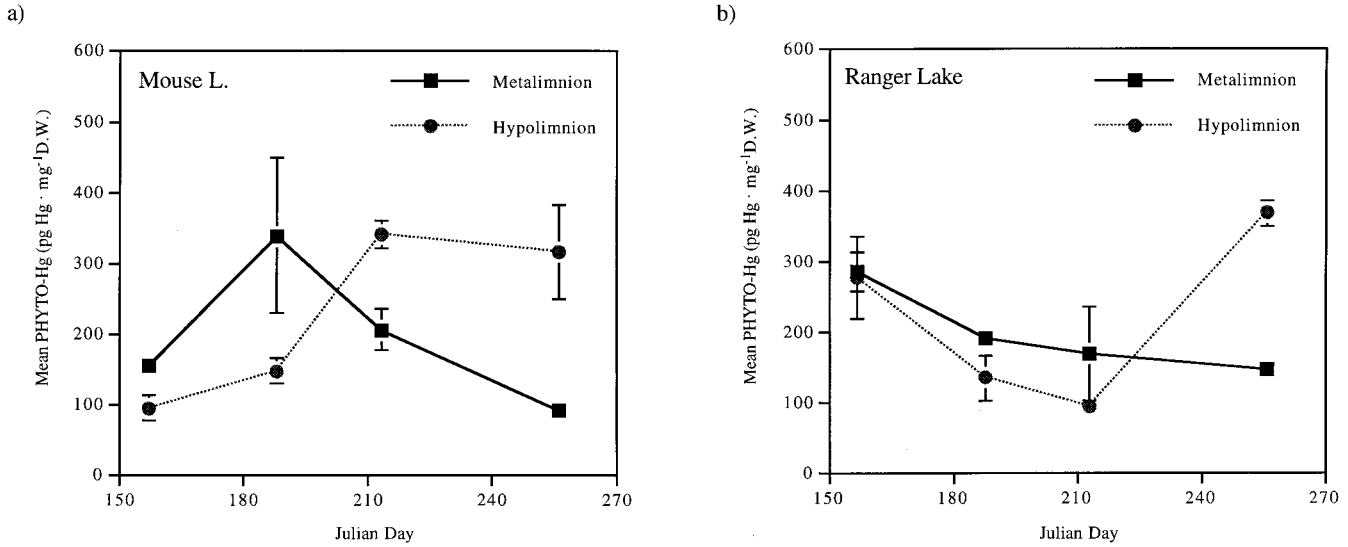

Fig. 4. Seasonal distribution of weight-specific Hg in phytoplankton (PHYTO-Hg) in the metalimnia and hypolimnia of (**a**) Mouse Lake and (**b**) Ranger Lake.

of mass on PHYTO-Hg levels. The effect of changing proportions of viable and detrital material in phytoplankton on PHYTO-Hg were tested using biovolume:PHYTO-DW and corrected chlorophyll:PHYTO-DW ratios. Because TP can also reflect algal biomass, it was regressed against PHYTO-Hg as a possible controlling variable. Finally, DO measurements were tested as a reflection of the changing redox environment and net photosynthetic activity.

Of these parameters, mainly those associated with algal biomass yielded statistically significant regression coefficients (Tables 2 and 3). In Table 2, the best predictor of PHYTO-Hg for the pooled data set was total chlorophyll, which explained 85% of the variation in Hg levels. Also, TP explained 83% of the variation in PHYTO-Hg levels. Because TP is a conventional predictor of algal biomass, it is not surprising that TP and total chlorophyll were highly correlated ($r^2 = 0.82$, $p < 0.05$). It is important to note that other parameters (including PHYTO-DW, corrected chlorophyll *a*, biovolume:PHYTO-DW, DO, and temperature) all yielded significant results but did not explain as much of the variation. Taking into account the physicochemical differences between the metalimnion and hypolimnion, we divided the pooled data set into each respective stratum. The only significant regression in the metalimnion was PHYTO-DW. This indicates that as phytoplankton mass increased, the amount of Hg measured also increased. No other parameter significantly predicted PHYTO-Hg, although it is important to note that the power of these relationships was low ($n = 8$). In contrast, the pooled hypolimnetic data provided a number of significant regressions. Total phosphorus and total chlorophyll *a* explained 91 and 90% of PHYTO-Hg, respectively. The significant regression with DO explained 54% of the variation in PHYTO-Hg.

Regression analyses of weight-specific PHYTO-Hg concentrations against the same parameters yielded fewer significant results than PHYTO-Hg (pg Hg/L). For the pooled data set, the only significant regression was total chlorophyll *a*, which explained 27% of the variation in Hg concentration (Table 4). No significant relationships were found when pooled data from the metalimnion were analyzed (Table 5). However, hypolimnetic data show that the ratio between chlorophyll *a* and PHYTO-DW significantly explained 49% of the variation in PHYTO-Hg concentration.

DISCUSSION

By comparing the Hg values measured in phytoplankton to those measured in whole water, it is apparent that the majority of Hg is in the dissolved phase (Figs. 1 and 2). Considering the dystrophic nature of these two lakes (Table 1), much of the dissolved Hg is most likely complexed by DOC (mainly humic material) in lake water. The role of humic material as an important ligand in Hg complexation has been well established, and some authors suggest that Hg bioavailability is reduced with increasing DOC, particularly in fish [19,20]. However, it is important to acknowledge that up to 50% of whole-water Hg in these dystrophic systems is tied up in the phytoplankton community. Our results seem to suggest, however, that Hg is bioavailable to the phytoplankton to a certain extent in these lakes, especially in the hypolimnia.

It would seem that lake mixing had created a homogeneous Hg distribution in the water column at the beginning of the season (Fig. 3). With the onset of stable stratification over the summer, PHYTO-Hg levels in the respective strata diverged. With respect to the hypolimnetic Hg results, our PHYTO-Hg data concur nicely with the lake-water Hg trends found in pristine Little Rock Lake, Wisconsin, USA, and urban Onondaga Lake, New York, USA, where Hg levels peaked at the end of summer in these stratified systems [21,22]. If samples had been collected during fall mixes, we may have seen similar Hg values again between limnetic depths. However, compared to spring values, there may have been a net increase in PHYTO-Hg based on the contributions of hypolimnetic Hg concentrations witnessed on the last sampling date.

These data show that PHYTO-Hg levels do not significantly change over the season in the metalimnia, regardless of the amount of phytoplankton present on each date. Watras and Bloom [16] proposed that the settling of epilimnetic particulates that have scavenged atmospherically introduced Hg can remineralize in lower water layers. If this were true for the lakes in this study, there was perhaps a constant level of Hg being maintained in the metalimnion despite losses from sink-

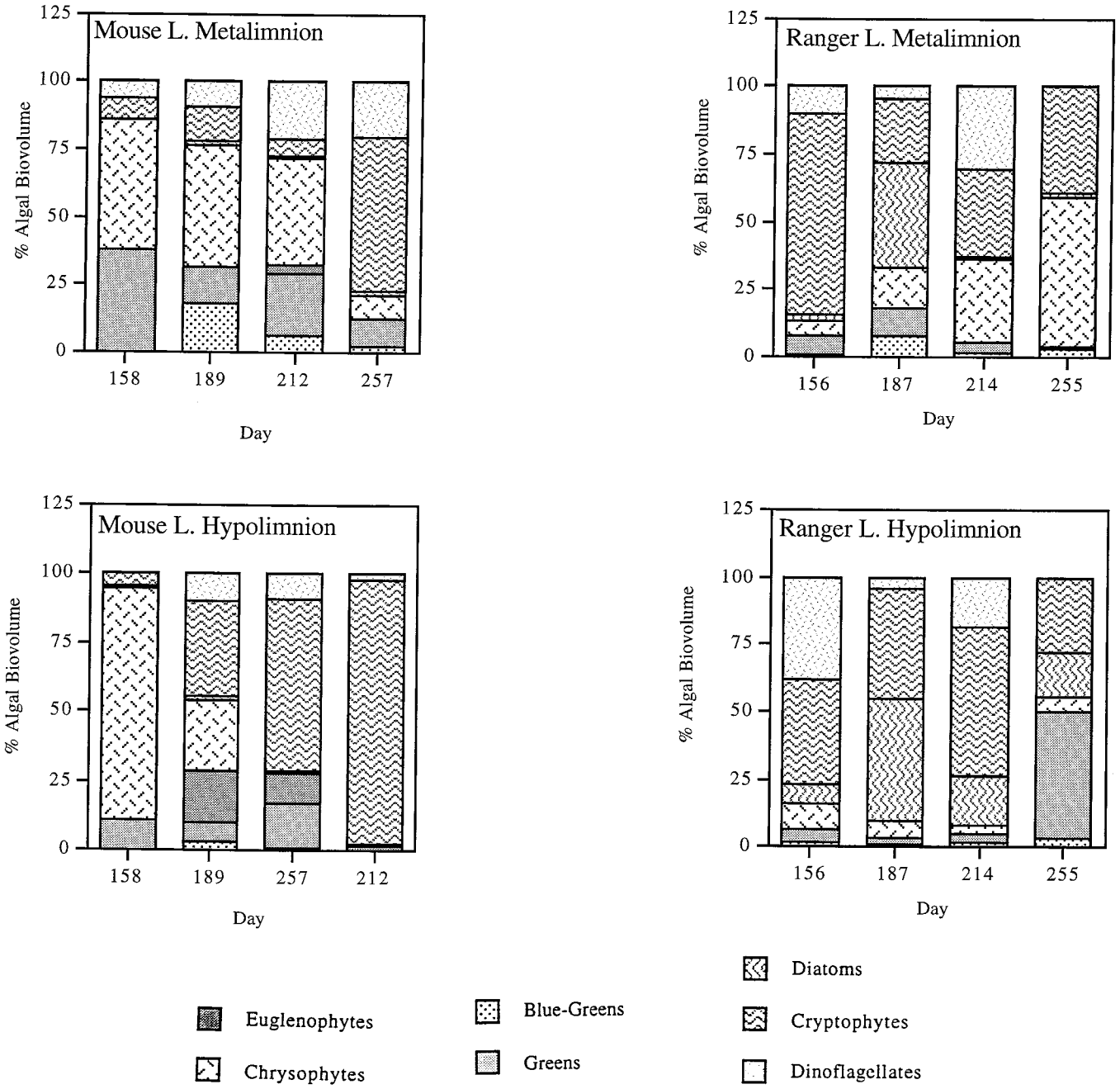

Fig. 5. Seasonal distribution of algal group proportions in the metalimnia and hypolimnia of Mouse Lake and Ranger Lake.

ing metalimnetic phytoplankton. Another explanation for the apparently low net loss of Hg from the metalimnia over the season involves the relatively high algal biomass that occurred there. Mercury may be incorporated into the phytoplankton via adsorption and/or accumulation by the physical and metabolic activities of the phytoplankton community. The oxidative mineralization or respiration activities of bacteria and other heterotrophs may be responsible for the partial destruction of phytoplankton matter, which in turn would release bound Hg and make it available for uptake by viable and dead algal cells in the metalimnion. The concurrent processes of Hg accumulation and remineralization in the metalimnion may partially explain why net loss of Hg was low there. As such, the microbial association, or ''loop,'' between algae and bacteria may be an important process in maintaining Hg levels in the metalimnia as it is in carbon and nutrient cycling [7].

In the hypolimnion, stable lake stratification would promote the sedimentation of remaining Hg-laden phytoplankton from upper waters to the lower depths. Considering that water density was highest in the hypolimnia, settling phytoplankton would have been trapped there, resulting in a net increase of Hg by the end of the season. This physical explanation may only partially explain the increasing Hg levels in hypolimnetic phytoplankton over the season. Although a larger mass of dead phytoplankton was found in the hypolimnion compared to the metalimnion, there still existed a significant, viable community of organisms. The hypolimnion is typically enriched with nutrients over the season in stratified lakes and, in the case of

Table 2. Summary statistics pertaining to the least squares linear regression analysis of mean phytoplankton Hg levels (PHYTO-Hg, pg Hg/L) against various mean lake parameters

Data set	Parameter[a]	Slope	Intercept	r^2	p[b]
Pooled data for both lakes over the season (n = 16)	Julian day	6.42	−648.51	0.12	0.19
	PHYTO-DW (mg/L)	223.70	−15.49	0.64	<0.001
	Algal biovolume (μg/L)	−0.28	805.57	0.02	0.57
	Chlorophyll *a* (μg/L)				
	Total	13.13	128.23	0.85	<0.001
	Corrected	66.94	154.15	0.46	0.004
	Biovolume: PHYTO-DW	−1.37	1,079.40	0.28	0.03
	Chlorophyll: PHYTO-DW	−31.06	774.45	0.04	0.45
	Total phosphorus (μg/L)	68.45	−10.15	0.83	<0.001
	DO (mg/L)	−99.11	1,329.87	0.49	0.003
	Temperature (°C)	−59.53	1,393.26	0.24	0.05

[a] DO = dissolved oxygen; PHYTO-DW = phytoplankton dry weight.
[b] p is the probability that the slope is significantly different from zero at $\alpha = 0.05$. Significance is denoted by $p \leq 0.05$.

these lakes, becomes virtually anoxic by midsummer. Promoted by the physicochemical characteristics of the hypolimnion, methylation of Hg is more prevalent here than in the metalimnion [23–27].

Thus, an interesting scenario develops that promotes the accumulation of Hg in lake phytoplankton, even on a weight-specific basis. Both living and dead phytoplankton in the hypolimnia are exposed to increasing levels of settled particulate-bound Hg over the season. The anoxic and high nutrient conditions encourage the production of methylmercury, a chemical species with a higher affinity for uptake by algae than inorganic Hg [28–30]. As such, Hg concentrates more in the phytoplankton of the hypolimnion (Fig. 2) and accumulates over the season in the hypolimnion rather than the metalimnion (Figs. 3 and 4).

The differences in weight-specific Hg dynamics in phytoplankton between lakes demonstrated the difficulty in generalizing about the forces that control Hg loss and accumulation. It is clear that in addition to physically accumulating a greater quantity of phytoplankton in the hypolimnion over the season, there is a disproportionately higher concentration of Hg in a given unit of phytoplankton by the end of the summer. Conversely, there appears to be a reciprocal relationship for metalimnetic samples, in which there is a net reduction in Hg per unit of phytoplankton at the end of the season compared to early summer. In addition to the potential effects of physicochemical parameters, the mixed and successional structure of the phytoplankton community combined with the detrital components of lake phytoplankton suggest that PHYTO-Hg concentrations could be influenced by a number of variables.

In Table 2, PHYTO-DW explained 64% of PHYTO-Hg variation, which indicated that the amount or mass of phyto-

Table 3. Summary statistics pertaining to the least squares linear regression analysis of mean phytoplankton Hg levels (PHYTO-Hg, pg Hg/L) against various mean lake parameters for each depth

Data set	Parameter[a]	Slope	Intercept	r^2	p[b]
Pooled data for metalimnion (n = 8)	Julian day	−1.54	566.78	0.21	0.24
	PHYTO-DW (mg/L)	235.37	−39.10	0.52	0.04
	Algal biovolume (μg/L)	0.14	159.59	0.28	0.18
	Chlorophyll *a* (ηg/L)				
	Total	0.87	236.05	0.01	0.83
	Corrected	0.52	250.67	0.00	0.96
	Biovolume PHYTO-DW	0.13	187.74	0.06	0.56
	Chlorophyll: PHYTO-DW	−5.09	283.78	0.05	0.59
	Total phosphorus (μg/L)	4.10	289.39	0.02	0.82
	DO (mg/L)	19.47	45.30	0.20	0.26
	Temperature (°C)	9.61	86.60	0.04	0.62
Pooled data for hypolimnion n = 8)	Julian day	14.38	−1,863.80	0.49	0.05
	PHYTO-DW (mg/L)	217.09	25.17	0.44	0.07
	Algal biovolume (μg/L)	−0.03	1,074.48	0.00	0.98
	Chlorophyll *a* (μg/L)				
	Total	12.02	340.89	0.90	<0.001
	Corrected	81.76	374.95	0.80	0.003
	Biovolume: PHYTO-DW	−5.20	1,615.38	0.27	0.18
	Chlorophyll: PHYTO-DW	448.78	324.71	0.54	0.03
	Total phosphorus (μg/L)	77.31	−108.08	0.91	0.003
	DO (mg/L)	−239.35	1,738.37	0.59	0.02
	Temperature (°C)	446.67	−2,175.62	0.41	0.09

[a] DO = dissolved oxygen; PHYTO-DW = phytoplankton dry weight.
[b] p is the probability that the slope is significantly different from zero at $\alpha = 0.05$. Significance is denoted by $p \leq 0.05$.

Table 4. Summary statistics pertaining to the least squares linear regression analysis of weight-specific phytoplankton Hg levels (PHYTO-Hg, pg Hg/mg DW) against various mean lake parameters

Data set	Parameter[a]	Slope	Intercept	r^2	p[b]
Pooled data for both lakes over season (n = 16)	Julian Day	0.25	159.82	0.01	0.72
	Algal biovolume (μg/L)	−0.01	213.73	0.00	0.93
	Chlorophyll *a* (μg/L)				
	Total	1.04	168.35	0.27	0.04
	Corrected	6.32	162.74	0.21	0.07
	Biovolume: PHYTO-DW	−0.04	222.89	0.01	0.67
	Chlorophyll: PHYTO-DW	0.38	208.92	0.00	0.95
	Total phosphorus (μg/L)	2.20	186.81	0.04	0.57
	DO (mg/L)	−1.82	222.69	0.00	0.73
	Temperature (°C)	−0.79	220.19	0.00	0.86

[a] DO = dissolved oxygen; DW = dry weight; PHYTO-DW = phytoplankton DW.
[b] p is the probability that the slope is significantly different from zero at $\alpha = 0.05$. Significance is denoted by $p \leq 0.05$.

plankton influenced the amount of PHYTO-Hg measured. This is an intuitive observation, whereby an increase in phytoplankton signifies more cells with the associated high surface area: volume available to accumulate Hg. Most interesting is that algal biomass, as measured by total chlorophyll *a*, explained most of the variation in PHYTO-Hg for pooled lake data (Table 2). Verta and Matilainen [31] suggest, on the basis of their results from Finnish forest lakes, that pigmented particles may control most of the methylmercury in those lakes, although no correlation analyses were performed to support this assessment.

With respect to individual depths, phytoplankton mass was the only significant regression in the metalimnia, explaining 52% of the variation in PHYTO-Hg. These results suggest that in general, increasing Hg levels measured from the mixed dead and living phytoplankton samples are associated with algal biomass based on the positive relationship. The analysis of hypolimnetic data resulted in a number of significant regressions explaining PHYTO-Hg. Julian day significantly regressed with PHYTO-Hg, explaining 49% of the variation. From this result, one can infer that seasonality and cumulative sedimentation may play a more important role in hypolimnetic Hg dynamics than seen in the metalimnia. Although low oxygen levels in the hypolimnia accompanied high Hg concentrations, it is difficult to ascertain whether this is a causal relationship because the settling of Hg-laden phytoplankton over the season into the hypolimnion would occur concurrently with the development of anoxia. Because methylation occurs optimally under low-oxygen conditions, perhaps what we are witnessing is an indirect relationship between oxygen and PHYTO-Hg.

Regression analyses performed for weight-specific PHYTO-Hg produced fewer significant relationships than those found for PHYTO-Hg (pg Hg/L). By standardizing the influence of phytoplankton mass on PHYTO-Hg levels, it becomes more difficult to distinguish parameters that have an effect on Hg accumulation, although there is a greater margin of error with weight-specific data. The only parameters found to be

Table 5. Summary statistics pertaining to the least squares linear regression analysis of weight-specific phytoplankton Hg levels (PHYTO-Hg, pg Hg/mg DW) against various mean lakes parameters for each depth

Data set	Parameter[a]	Slope	Intercept	r^2	p[b]
Pooled data for metalimnion (n = 8)	Julian day	−1.21	445.27	0.35	0.12
	Algal biovolume (μg/L)	0.03	176.81	0.04	0.65
	Chlorophyll *a* (μg/L)				
	Total	0.35	191.28	0.00	0.89
	Corrected	0.84	192.83	0.00	0.89
	Biovolume: PHYTO-DW	0.03	180.22	0.01	0.80
	Chlorophyll: PHYTO-DW	−0.99	204.26	0.00	0.87
	Total phosphorus (μg/L)	3.40	219.25	0.03	0.76
	DO (mg/L)	15.02	37.37	0.31	0.15
	Temperature (°C)	1.03	180.41	0.00	0.93
Pooled data for hypolimnion (n = 8)	Julian Day	1.71	−125.62	0.33	0.14
	Algal biovolume (μg/L)	−0.09	258.46	0.04	0.64
	Chlorophyll *a* (μg/L)				
	Total	1.17	152.04	0.40	0.09
	Corrected	8.13	153.87	0.38	0.11
	Biovolume: PHYTO-DW	−0.45	269.75	0.09	0.46
	Chlorophyll: PHYTO-DW	62.32	119.78	0.49	0.05
	Total phosphorus (μg/L)	5.33	116.43	0.23	0.33
	DO (mg/L)	−17.71	272.28	0.15	0.34
	Temperature (°C)	59.43	−208.58	0.34	0.13

[a] DO = dissolved oxygen; DW = dry weight; PHYTO-DW = phytoplankton DW.
[b] p is the probability that the slope is significantly different from zero at $\alpha = 0.05$. Significance is denoted by $p \leq 0.05$.

significant reflected algal biomass. Pooled-data analyses yielded a significant relationship between total chlorophyll *a* and PHYTO-Hg concentration. However, only 27% of the variation in PHYTO-Hg concentration could be explained. When metalimnetic data for both lakes were analyzed, no significant relationships were evident. This result indicates that either there are different controlling variables in the metalimnion of each lake, and thus pooling the data masks these effects, or none of the parameters tested have a controlling effect on PHYTO-Hg concentrations in the metalimnia. Perhaps the concurrent processes of uptake and remineralization, which are not accounted for in our analyses, ultimately control weight-specific Hg concentrations in the metalimnia.

When data from the hypolimnia were analyzed, only the changing proportion of viable algae in phytoplankton had a significant effect on weight-specific Hg concentrations. This result indicates that as the viable proportion of lake phytoplankton increased, the concentration of Hg in the phytoplankton increased. Why this relationship occurred in the hypolimnia and not the metalimnia of these lakes is not clear, but the reason may be linked to differences in Hg speciation rates and remineralization kinetics that exist between limnetic depths. Also, contrasts between the phytoplankton communities (including algal genera and size-distribution differences) could account for this discrepancy (Fig. 5).

CONCLUSIONS

The findings of this study not only shed light on the seasonal dynamics of PHYTO-Hg but also emphasize the differences in Hg levels between the metalimnia and hypolimnia over the season. As such, future investigations of Hg in phytoplankton should account for these temporal and spatial variations, particularly in the hypolimnia. In general, these data support the results of past in vivo studies, which demonstrated the ability of algae to accumulate heavy metals [12–15]. Regardless of variations in phytoplankton mass (i.e., dry weight), algal biomass parameters such as total chlorophyll *a* and TP appear to influence Hg levels in the phytoplankton community.

On the basis of the positive slopes found between significant biomass parameters and PHYTO-Hg, it is clear that Hg accumulation and concentration, rather than biodilution, are occurring in lake phytoplankton. Although the importance of algal biomass is evident, the link between productivity or growth with PHYTO-Hg levels is not discernible. Glooschenko [32] found that dead algal cells accumulated twice as much Hg as living (nondividing) cells. Dividing cells in light accumulated Hg longer than nondividing cells, suggesting that active uptake of Hg was also important. Our results do not negate these findings but are rather in general agreement that increasing amounts of viable and dead phytoplankton result in increased levels of particle-bound Hg in lake water.

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