Occurrence and Distribution of Cyanobacteria and their Toxins in Silver Lake, New Hampshire

Françoise Morison

Dept. of Biological Sciences, University of New Hampshire, Durham, NH 03824

Abstract

A study of Silver Lake, NH was performed as part of a 5-lake assessment of cyanobacteria prevalence and distribution. Multi-parameter fluorescence probe measurements of chlorophyll a and cyanobacteria concentrations (PC, phycocyanin fluorescence) were evaluated in addition to physical and chemical characteristics of the lake. Silver Lake did not exhibit summer stratification suggesting a recent mixing event. It had oligotrophic levels of Chlorophyll a (1.93 ± 0.06 μg L^{-1}) and of TP (10.10 μg L^{-1}), yet PC levels were the highest of all the lakes studied (248691 ± 963 *Microcystis* cell equivalents mL^{-1}). The cyanobacteria *Microcystis* dominated the phytoplankton community. Microcystin levels varied from a mean 72.43 ± 21.21 pg mL^{-1} in transect water to 137.69 ± 46.9 pg mL^{-1} in sediment water. Chlorophyll distribution was rather homogeneous while cyanobacteria levels were highest towards the shallow, embayed NE part of the lake where a section of a State Park beach is located. Implications include potential increase in exposure to toxins by water users. Heterogeneous distribution of cyanobacteria emphasizes the importance of extensive sampling beyond pelagic sampling sites to more accurately inform decision-making regarding health and safety of water bodies.

Introduction

The production of toxins by cyanobacteria (Chorus et al., 2000) has magnified the problems blooms create, consequently raising concern about the quality of freshwater resources (Reynolds, 2006). Thorough assessments of the prevalence and spatial distribution of cyanobacteria within bodies of freshwater are needed in order to make well-informed decisions regarding the management and use of water resources. Cyanobacterial blooms in ponds, lakes, and rivers are not a new occurrence, yet in the past 50 years they have become more common (Reynolds, 2006; Carmichael, 2007). Pelagic photosynthetic communities tend to become dominated by cyanobacteria in nutrient enriched systems (Reynolds, 2006). Harmful

bloom events and episodes of related fatal intoxication of cattle (Mez et al., 1997) and dogs (Edwards et al.1992; Hoff et al., 2007; Cadel-Six et al., 2007) have been increasingly documented worldwide. Similar events have happened throughout North America (Carmichael, 2007). Good reviews of the various cyanobacterial toxins exist in the literature (Falconer, 1999; Briand et al., 2003).

Microcystis is the most common genus of cyanobacteria to form blooms (Chorus et al., 2000; de Figueiredo et al., 2004). Along with other genera, it produces the hepatotoxin microcystin (MC). Several cases of illnesses have been attributed to exposure to the toxin both in drinking and recreational waters (Chorus et al., 2000). *M. aeruginosa* commonly occurs in New Hampshire lakes. A statewide study detected the presence of the cyanobacteria and of its MC toxin in the 50 lakes surveyed in New Hampshire (Haney and Ikawa, 2001).

Risk of exposure to potentially harmful levels of MC may be increased by the tendency of certain *Microcystis* to form surface scums (Chorus and Bartram, 1999). Surface accumulations may occur as a result of the cells' ability to regulate their buoyancy by the formation of gas vesicles

(Reynolds, 2006). The detection of any heterogeneous distribution of cyanobacteria or their toxins would mandate sampling lakes more extensively, so that collected data can accurately inform local decision-making about the management of a lake and its use.

The objective of the present study was to collect data to assess the current physical, chemical, and biological conditions of Silver Lake, a southern New Hampshire body of water popular for summer recreation. We sought to gain an understanding of the distribution of cyanobacteria and of MC in the lake, their relationship with each other, with the lake's trophic status, and with wind and water currents. We expanded upon traditional lake assessments, whose protocols limit measurements of parameters at a deep sampling site. We sampled along horizontal transects in order to detect potential localized surface accumulations of cyanobacteria using a fluorescent probe, and investigated the effect of wind and currents using wind drogues.

Methods and Materials

The field investigation of Silver Lake was performed on 20 September 2007, as part of a broader assessment of New Hampshire lakes, each under various development-pressure and of different trophic status, including Barbadoes Pond (BP), Lower Sawyer Pond (LSP), Mirror Lake (ML), and Willand Pond (WP) (App. I, table 1).

Study Site - Silver Lake is located in Hollis, Hillsborough County, New Hampshire (Lat. 42° 45' 25" – Long. 71° 35' 54") (App. I, fig. 1). Silver Lake is a small natural lake with a dam, situated at an elevation of 274 ft, and covering an area of 13.64 ha. It is relatively shallow with a maximum depth of 7.4 m and a mean depth of 2.8 m. The lake's eastern shore is bordered by NH route 122, and the lake's eastern riparian zone is reduced as a consequence of a dense development of houses between the road and the lake. Silver Lake State Park is situated at the lake's northern end, near the lake's outlet. The park's 300-meter sandy beach runs along most of the North end and approximately 1/3 of its western side and is popular during the summer, when water activities

include swimming and paddle boat rentals (NHStateparks.com). Motorboat usage is restricted for speed but permitted (NH department of safety, bureau of marine patrol). There is another smaller beach at the southeastern end, and several houses are built on its southern shore.

Physical and Chemical Profiles

Vertical Profile - A vertical multi-parameter profile of the lake was obtained using a Yellow Spring Instrument (YSI) 6600 V2 multi-parameter probe outfitted with a YSI-650 data logger. The probe was lowered into the water column at an approximate rate of 0.5 m min^{-1}, at a site (Fig. 1) determined to approximate the lake's maximum depth using a Speedtech Instruments 400 kHz Depthmate Portable Sounder. Every 3 s the logger recorded: depth (m); temperature (C); specific conductivity (μS cm^{-1}); pH; oxidation-reduction potential (ORP; mV); chlorophyll a (μg L^{-1}); dissolved oxygen as percent saturation (% Sat), and concentration (DO; mg L^{-1}); cyanobacteria concentration (phycocyanin fluorescence calibrated as microcystic (Mic) cells mL^{-1}).
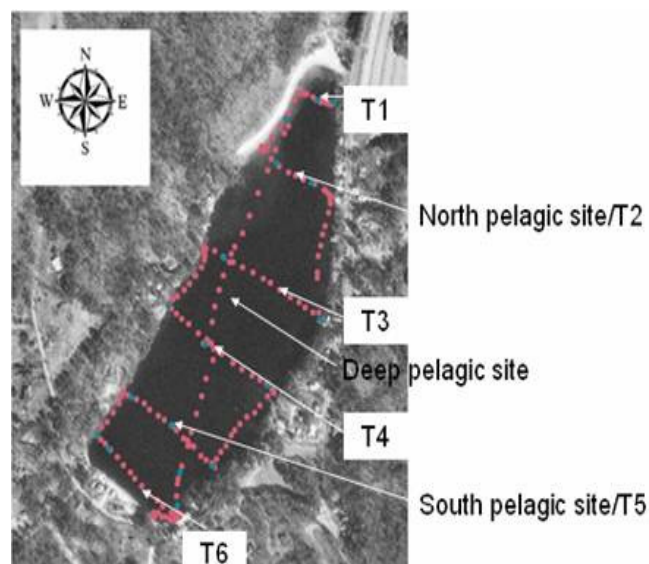

Fig. 1- Silver Lake, Hollis NH – Location of transects and pelagic sites where data were collected on 20 September 2007.

Light Profile - A light profile was taken using a LI-COR light meter with 1400 data logger. The

light probe was lowered through the water column on the sunny side of the sampling boat. Readings were recorded every 0.5 m down to the measured bottom depth. Water transparency was measured using a standard 20 cm diameter Secchi disk. Measurement and recording of Secchi disk depth (SDD) were performed following the standard protocol (Lind, 1974). In order to eliminate interference of surface glare and wind-action, readings were taken through a view scope.

Horizontal Profile - A horizontal multi-parameter profile was obtained for each of six linear transects conducted across the lake at even intervals (Fig. 1). Multi-parameter data collection was performed using the YSI multi-parameter probe and data logger used in the vertical profile. Transects were run at a rate of ~3 mph while maintaining the YSI multi-parameter probe at a depth of approx. 0.80 m for data collection. The logger recorded data at 3 s intervals. Time was noted and GPS waypoints were marked to accurately determine the track of each transect. Simultaneously lake-bottom topography and plankton spatial distribution were charted using a sonar system consisting of a high frequency (200-kHz) Lowrance LCX 25 echosounder and a Loran-C navigation receiver. All multi-parameter data and light meter data were uploaded into Microsoft® Office Excel and transferred to Systat SigmaPlot. Sonar files were uploaded and viewed in Sonar Viewer (Lowrance LCX-25C).

Water Currents - Water currents were measured using 4 GPS-tracked drogues made of four nylon panels attached to a PVC frame at right angle to each other. Each drogue was pre-set at a different depth (0.5 m, 1.5 m, 2.5 m, or 3.5 m) and connected to a Garmin® Geko 201GPS unit placed in a floating waterproof housing. Drogues were deployed at the same deep pelagic station where the vertical profiling was performed (Fig. 1). Time was recorded and GPS waypoint was marked at drogues release and retrieval. Wind velocity and air temperature were measured using an Extech Instrument mini thermo-anemometer. Wind direction was measured using a hand-held compass.

Water Samples for MC and Total Phosphorus Analyses - Whole lake water (WLW) samples were collected for analysis of both horizontal and vertical MC concentrations. For horizontal MC, one 500 ml WLW sample per transect was obtained using a portable Masterflex® peristaltic water sampling pump. These samples were collected concurrently with and from the same depth (approx. 0.80 m) as the multi-parameters gathered during the horizontal profiling. For vertical MC, three 500 ml integrated (0-3 m) water samples (IT WLW) were collected from each of the three pelagic sites (Fig. 1) lowering an integrated sampler (weighted ~2 cm diameter Tygon tube) to a depth of 3 m. Three other integrated (0-3 m) WLW samples were collected from the deep pelagic station for total phosphorus (TP) analysis. All water samples were kept on ice until they were brought to the laboratory, where they were kept frozen until analysis.

Benthic Sediment Samples Collection for MC Analysis - Three sediment samples were obtained from the benthos at each of the three pelagic stations using a "sediment skimmer" (App. I, fig. 2; Will Young, Aquatic Instruments Inc. new design, 2007). The skimmer is designed to collect sediment from the upper 2-3 cm layer. Samples were transferred into 500 ml opaque bottles that were stored on ice in a dark cooler until further processing in the laboratory. The sediments in each bottle were allowed to settle and transferred to 90 ml histology jars. These and 20-ml samples of excess sediment water were frozen until analysis.

Water and Sediment Samples Analyses - In preparation for MC analysis, the frozen WLW integrated samples underwent three freeze-thaw cycles to lyse the cyanobacterial cells and release the endotoxins. Three 10-mL subsamples were then transferred to borosilicate glass serum bottles, which were then frozen at a 30° angle to maximize the exposed surface area. Samples were then lyophilized (LabConco Freezone 4.5) under a vacuum (~30 x 10-3 mbar) for 18-24 h at -50°C. In order to achieve a 10-fold concentration of materials and increase the sensitivity range of the performed assay, the dried samples were rehydrated

with 1.0 mL of double distilled Milli-Q water and placed on an orbital shaker for 60 min and the vials were rotated every 15 min. Approx. 1.0 mL of each sample was then filtered through a 13-mm diam, 0.2 μm Whatman PTFE syringe filter into a 1.5 mL centrifuge tube, and frozen. MC analyses were performed on all frozen water samples using Microcystin 96-Well-Plate Kits (EnviroLogix Inc. Portland, ME) Enzyme-Linked Immunosorbent Assay (ELISA). Sediment and sediment water samples for MC analysis underwent a similar preparation excluding the lyophylization, and were also assayed for MC using ELISA. The New Hampshire Lakes Lay Monitoring Program performed the TP analyses.

Graphics and Statistics - Statistic analyses were performed (p<0.05) and graphics were generated using SigmaStat and SigmaPlot softwares respectively. ORP was corrected for pH (Lampert and Sommer, 1997). Averages of the vertical and horizontal profiles' various parameters were calculated from the data recorded by the multi-parameter probe, and are reported ± standard error. To allow for comparison of triplicate values using ANOVA, North (N), Middle (MNS), and South (S) regions were assigned data as follows: N: T1 to T3; MNS: T3 to T5; S: T4-T6 (Fig. 2).
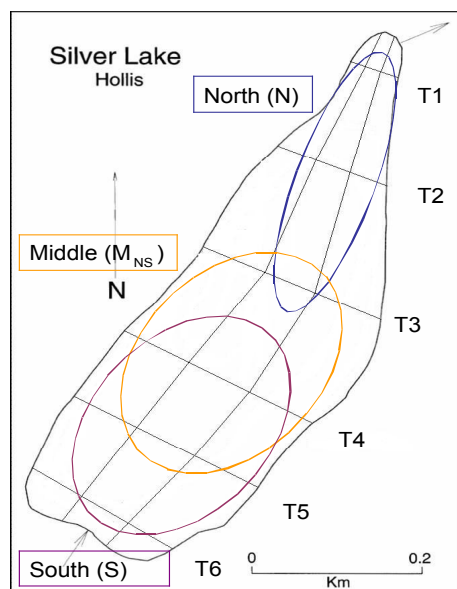

Fig. 2- Diagram of Silver Lake, Hollis NH Showing arbitrary division into 3 lakes zones (N, M$_{NS}$, S) for analysis of data collected on 20 September 2007.

West (W), central (MEW), and East (E) regions of each transect refer to the corresponding 1/3 of the multi-parametric data of the specific transect (Fig. 3).
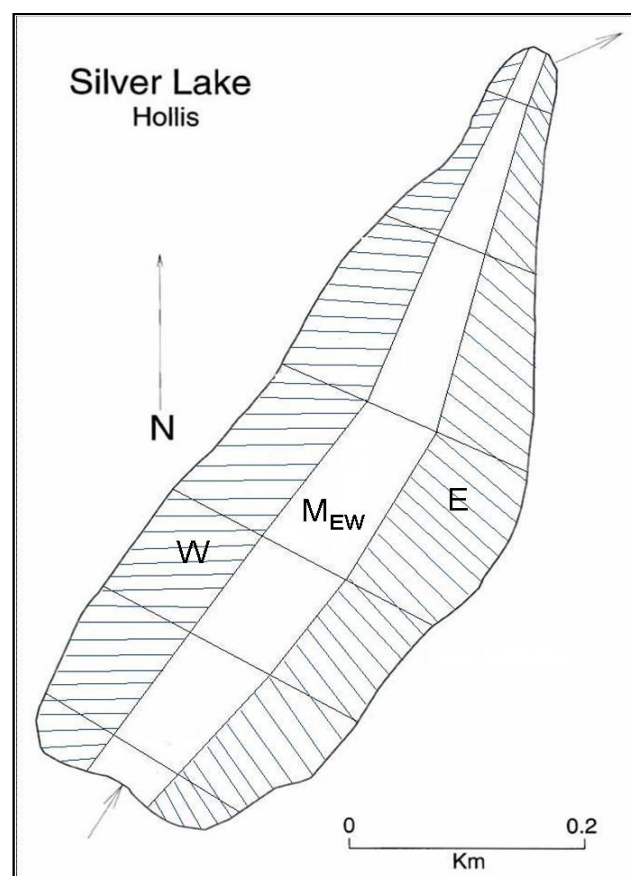

Fig. 3- Diagram of Silver Lake, Hollis, NH, showing how the lake was longitudinally divided into three regions from East to West (E, M$_{EW}$, W) to evaluate horizontal distribution of phytoplankton using pigment surface concentration data collected with a fluorescent probe on September 20, 2007).

Results

Wind Direction and Movement of Drogues- On the day of the sampling, a breeze was blowing from the SW at 0.6 kn. Drogue movement at all depths was limited. The overall trajectory of the surface drogue (0.5 m) was N, the drogue first moving to the NW and changing course towards the NE to return at the same longitude. The 1.5 m drogue main direction was SE and the 3.5 m drogue started out to the NW to return almost exactly over its track to its starting point (Fig. 4).
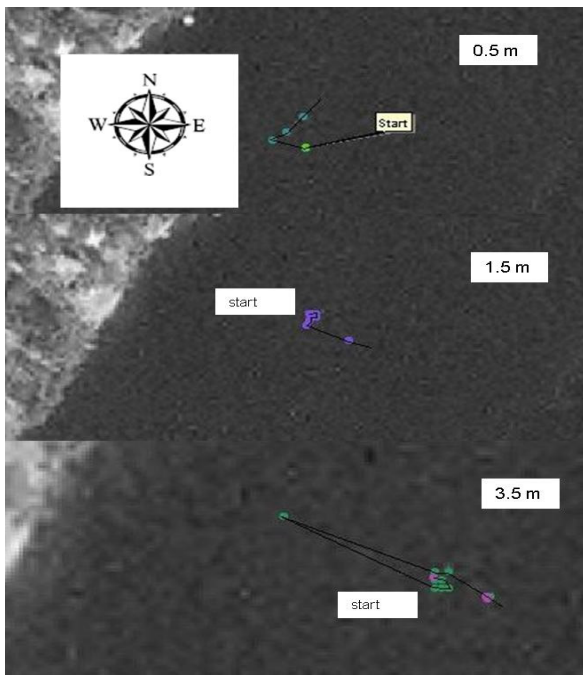
Fig. 4 – Silver Lake Hollis NH current directions at various depths as measured on 20 September 2007 using drogues that were released at the deep site and tracked for approx 1 h using attached GPS units.

Horizontal Distribution of Phytoplankton - The six transects (T1-T6) were compared for chlorophyll a (chl a) (Fig. 5a) and phycocyanin (PC) (Fig. 5b). Pigment means varied significantly among transects, T1 having both lowest chl a (2.00 µg L^{-1}) and highest PC (1.82 x 10^4 Mic cells mL^{-1}), and inversely T6 having highest chl a (2.68 µg L^{-1}) and lowest PC (1.56 x 10^4 Mic cells mL^{-1}). Calculated ratios of PC:chl a per transect had median values that varied significantly (P<0.001) from a highest value of 9149 (T1) to a lowest value of 6055 (T6) (Fig. 5c). Except for T2, there was a gradual increase in the ratio of PC:chl a from SW (T6) to NE (T1), (Fig. 6; adj. r2=0.55, P=0.0570). N, MNS, and S lake zones were compared for both chl a and PC using combined raw data of three transects for each zone (Fig. 3). Median chl a in zone N (2.3 µg L^{-1}) was lower than in both S (2.5 µg L^{-1}) and MNS (2.4 µg L^{-1}) (n = 306, 369, 361, df = 2, P<0.001). There was no significant difference between regions in PC median concentration.
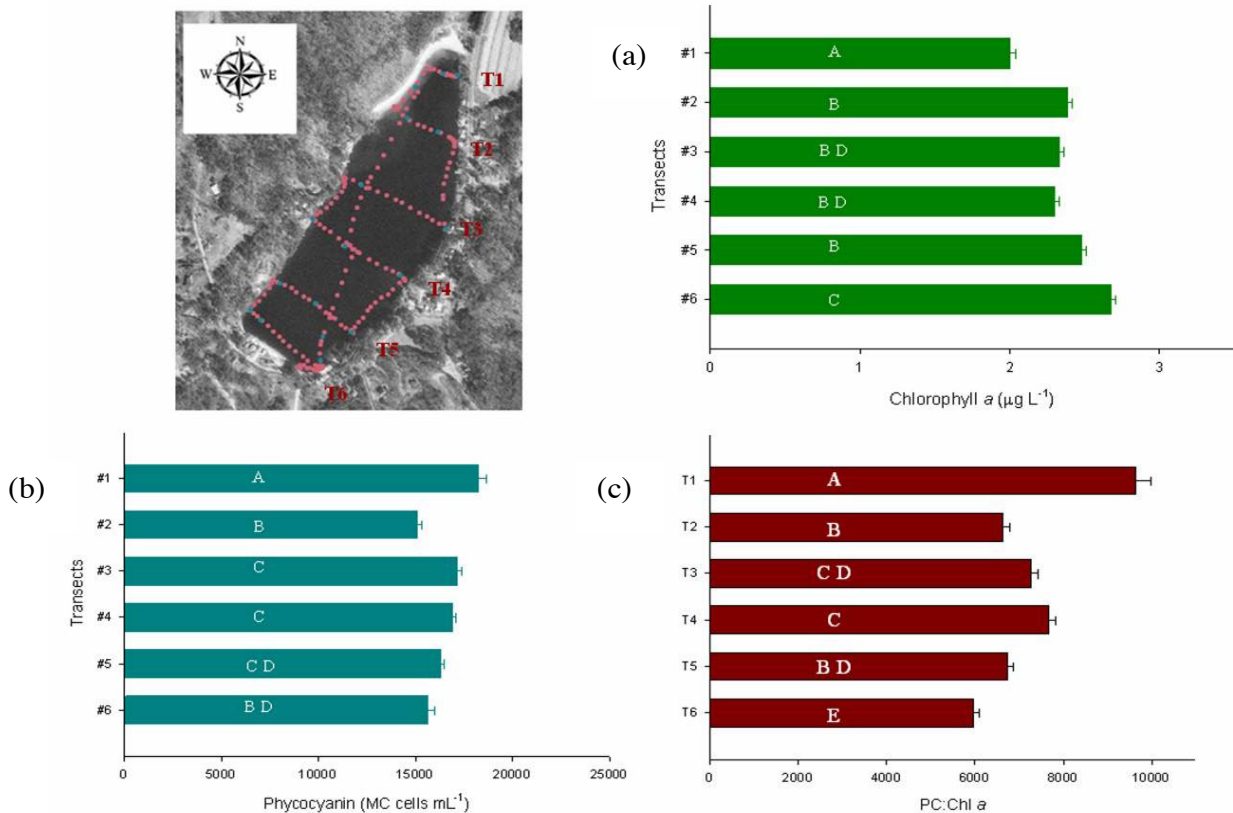

Fig. 5- Horizontal distribution of phytoplankton (Chl a (a) and PC (b) in Silver Lake, Hollis NH, on 20 September 2007. Transects values were compared and found to be significantly different (P<0.05). There was a significantly higher proportion of cyanobacteria (c) in T1 (P<0.05).
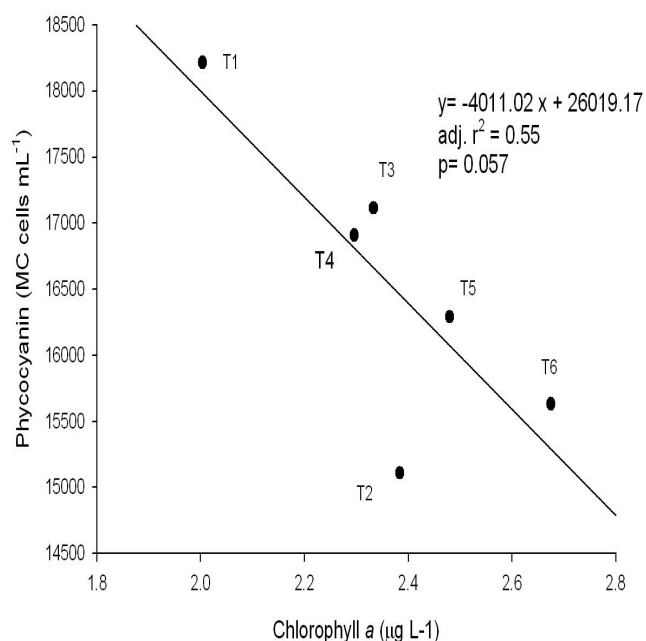
Fig. 6. Silver Lake, Hollis NH. Regression analysis of Chl *a* vs. PC (transect means). Proportions of cyanobacteria in the phytoplankton increased from SW (T6) to NE (T1).

The same lake zones' pigment concentrations did not vary significantly when compared based on transect means (n = 3, df = 2, power = 0.050; Table 1). More PC cells were measured in the eastern region (E) of the lake than in the West (W) (Table 2): when the lake was divided into three longitudinal zones E, MEW, and W, (Fig. 4) the chl a horizontal distribution was found to be fairly homogeneous, while E concentration of PC was higher

(median = 1.78 x 104 Mic cells mL^{-1}) than MEW (median = 1.61 x 104 Mic cells mL^{-1}) and than W (15744 Mic cells mL^{-1}) (n = 223, 221, 223; P<0.05). Pigment concentrations were also measured along regions of the shore separating transects: their E median values, especially PC, were higher than W median values (n = 229, 262; chl a medians, W = 2.1 µg L^{-1}, E = 2.2 µg L^{-1}; P = 0.005; PC medians, W = 1.57 x 104 Mic cells L^{-1}, E = 1.79 x 104 Mic cells L^{-1}, P<0.001). Further investigation comparing E, MEW, and W regions per transect revealed relative lowest concentrations of PC in the MEW regions of T6-T4, and increased concentrations in the MEW and especially E regions of T3-T1 (Table 3).

Table 2. Mean transect concentrations of Chl a varied little between longitudinal zones (West, Middle, and East) in Silver lake, Hollis NH, on 20 September 2007, while PC concentrations were significantly higher in the East.

	Chl a (µg L^{-1})	PC (Mic cells mL^{-1})
West	2.38 ± 0.03	15487 ± 160
Middle	2.35 ± 0.03	16006 ± 191
East	2.37 ± 0.03	17936 ± 188*

*significantly higher than West or middle

Table 1. Horizontal distribution of pigments and MC concentrations (transect water) per zone (North, middle, and South) in Silver Lake, NH, on 20 September 2007. Chl a mean concentrations slightly increased from North to South. There was no difference between zones in either Chl a, PC, or MC concentrations*.

	Chl a (µg L^{-1})	PC (Mic cells mL^{-1})	MC (pg mL^{-1})
North	2.27 ± 0.02	16646 ± 184	51.86 ± 6.90
(T1-T3)			
Middle	2.38 ± 0.02	16759 ± 115	96.30 ± 40.59
(T3-T5)			
South	2.45 ± 0.02	16336 ± 135	93.00 ± 42.18
(T4-T6)			

*Statistical Power <0.800

Table 3. Comparison of Silver Lake NH PC concentration per transect region, 20 Sept. 2007. Bold numbers indicate significant difference. From South to North, up to T4 the middle values are lower, and from T3 North, middle and especially East values increase, suggesting that surface cells might have been displaced in a S-N/NE fashion, resulting in accumulations in the NE corner.

Transect #	West	Middle	East
1	13961	18782	21898
2	13819*	15508*	15634*
3	14754	17819	18755
4	17018	16426	17269
5	16275	15688	16908
6	16357	12533	17915

*Median values were compared

Vertical Profile of Physical and Chemical Parameters - The water column at the deep site (7.2 m) was physically and chemically well mixed (Fig. 7). Silver Lake thermal profile exhibited a secondary stratification that lacked an epilimnion (Fig 7a). The average temperature was 19.4 °C (± 0.05). Temperature varied within a 2.5 °C range. It decreased in the upper 2 m from 21.2 °C at the surface to 19.4 °C, and then remained fairly constant to the bottom (18.7 °C).

DO concentration was steadily high to the bottom (mean: $8.3 ± 0.01$ mg L^{-1}), at ~90% saturation, and varied very little (0.6 mg L^{-1} range) (Fig. 7a). The mean value was a constant between approx. 1-3.5 m. Highest values occurred at approx. 3.5-4.3 m of depth, and lowest values occurred both at the surface (0-1 m) and at the bottom (5.3-7.2 m).

The Secchi disk was visible on the bottom (VOB, SDD = 7.1 m). Light intensity at the surface was approx. 600 µM m^{-2} s^{-1} and decreased rapidly by 500 µM m^{-2} s^{-1} in the upper 4 m. The vertical light attenuation had a mean coefficient of 0.462 (± 0.03).

Vertical Biological Profile - The average chl a concentration for the entire water column was 1.93 (± 0.06 µg L^{-1}) (Fig. 7a). The highest value for the profile (6.9 µg L^{-1}) was found in a thin layer just above the bottom sediments (6.8 m). The median chl a concentration value of 2.0 µg L^{-1} for the hypolimnion was significantly greater than in the metalimnion (1.7 µg L^{-1}) (n = 82, 43; P<0.001).

The mean concentration of PC was 248691 ± 963 Mic cells mL^{-1} (Fig 7a). An increase in the PC vertical curve occurred between approx 4-5.5 m of depth (peak at ~ 2.6 x 10^4 Mic cells mL^{-1}); it was accompanied by a parallel, moderate increase
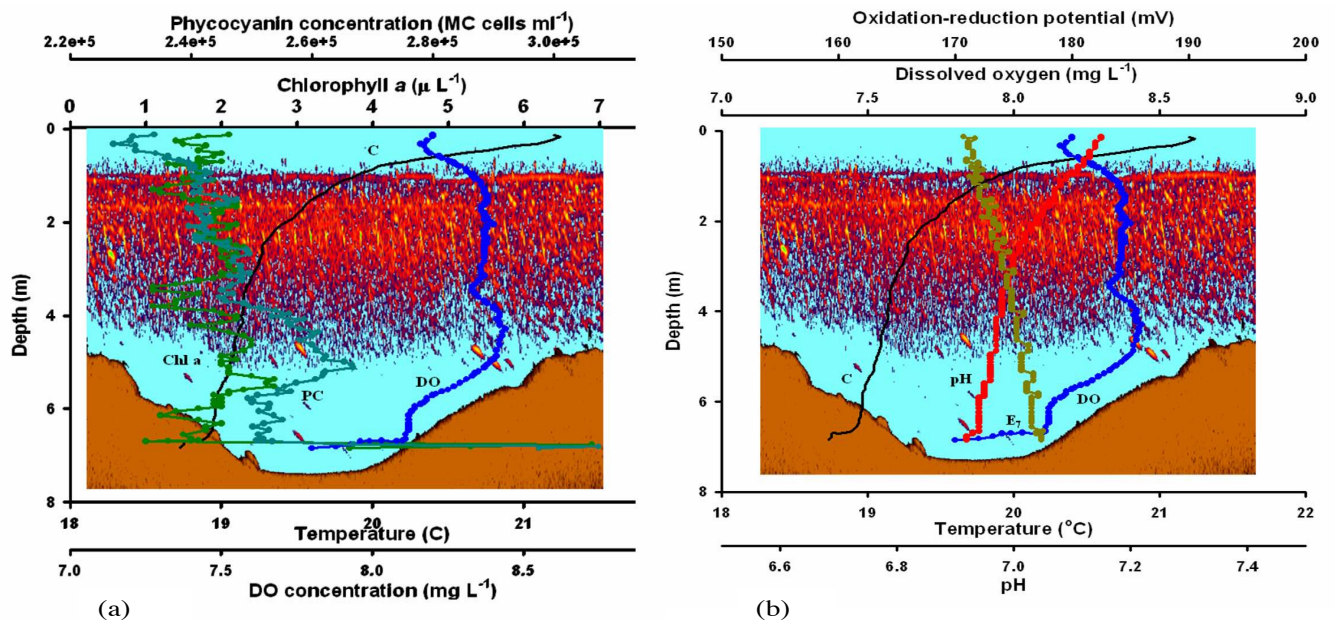

Fig. 7- Silver Lake, Hollis NH 20 September 2007 vertical profile of physical and chemical parameters measured using a YSI 6600 V2 multi-parameter probe. The curves show a lack of stratification and as a result, a greatly homogeneous physical and chemical nature of the water column, indicating a recent mixing event. The sonar image represents the deepest point of all transects studies, situated on T4 (7.4 m) and shows noteworthy fish activity.

in DO. A major peak (max. concentration: 307348 Mic cells mL^{-1}) inflated the calculated average for the water column; it occurred right above the bottom sediments in unison with the bottom chl a peak. The difference between the median PC concentrations of the metalimnion (2.41 x 104 Mic cells mL^{-1}) and the hypolimnion (2.51 x 104 Mic cells mL^{-1}) was significant (n = 43, 82; P<0.001).

The phytoplankton deep-site samples were dominated by cyanobacteria (~45%), *Microcystis* being the most abundant representative (36%; 133.8 cells/colonies L^{-1}). The majority of their total count was located at 1-m depth. Other well represented genera were Peridinium (20%; 74.4 cells L^{-1}), 75% of which were concentrated at 0.5 m, Ceratium (15.2%; 56.3 cells L^{-1}), and Pediastrum (12.7%; 47.1 cells L^{-1}). Other genera occurred sparingly. Both Ceratium and Pediastrum were found at all depths sampled (App. I, fig. 3). Cyanobacterial dominance was also confirmed by the fact that they were the most active members of the phytoplankton on the sampling day (Fig. 8: T2 treated as outlier; P = 0.008, r2 = 0.90).

Concentrations of TP and MC - The TP average value for Silver Lake was 10.10 ± 0.51 µg L^{-1}. Transects WLW MC mean concentration was 72.43 ± 21.21 pg mL^{-1}, with the highest value found in transect 5 (177.35 pg mL^{-1}) (Fig. 9). The highest mean MC concentration for the lake was found in sediment water samples (137.69 ± 46.9 pg mL^{-1}) (Table 4). No significant differences were found between the three pelagic sites for IT WLW, sediment or sediment water samples.Nor were there any when transects WLW MC concentrations were compared grouping transects into lake zones.
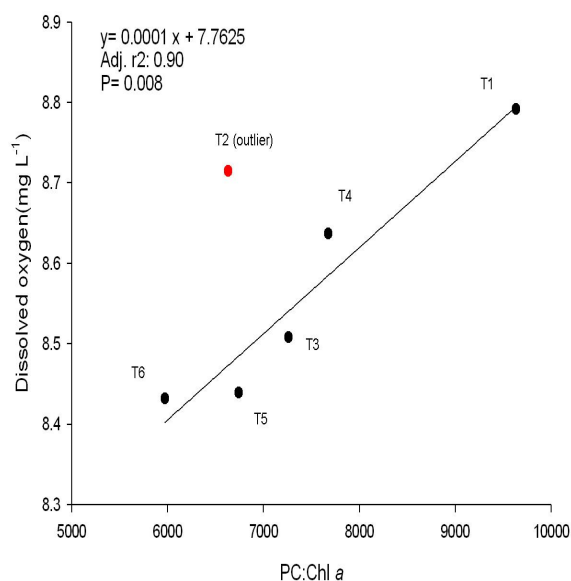

Fig. 8- Positive correlation between ratios of PC:Chl *a* and dissolved oxygen indicates that cyanobacteria were the most active oxygen producing members of the phytoplankton in Silver Lake on 20 September 2007.
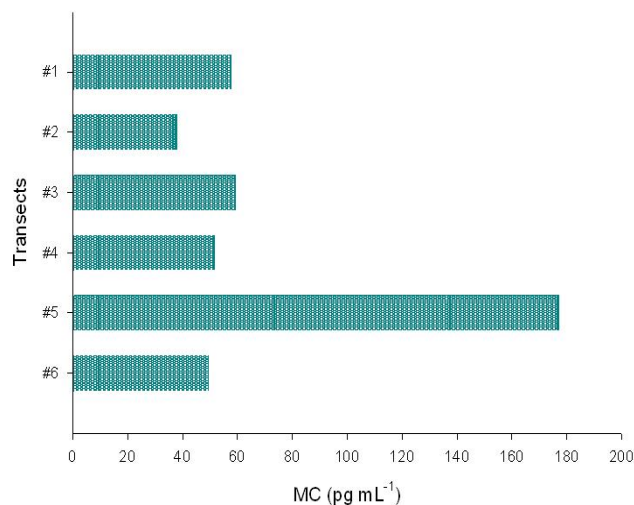
Fig. 9- Silver Lake, Hollis NH, 20 September 2007 MC concentration per transect (mean = 72.43 ± 21.21 pg mL^{-1}). Highest concentration was measured in T5 (177.35 pg mL^{-1}).

Table 4. MC concentrations (pg mL^{-1}) of water, sediment, and sediment water samples collected at Silver Lake on 20 September 2007 varied between the three pelagic sampling sites, yet not significantly*. Numbers indicate a trend for higher MC at the North site. Highest values occurred in sediments and sediment water.

	IT MC	SED. H$_2$O MC	SED. MC
North	61.83 ± 17.61	235.99 ± 124.25	113.76 ± 68.13
Middle	42.49 ± 13.04	34.05 ± 16.49	29.96 ± 10.44
South	15.04 ± 3.36	143.02 ± 21.84	123.15 ± 44.21

*Statistical power <0.800

Except for T5, which was treated as an outlier in the regression analysis, there was a relatively strong (Adj. r2 = 0.66) positive relationship between PC and MC, yet the trend was not significant (P = 0.060) (Fig. 10).
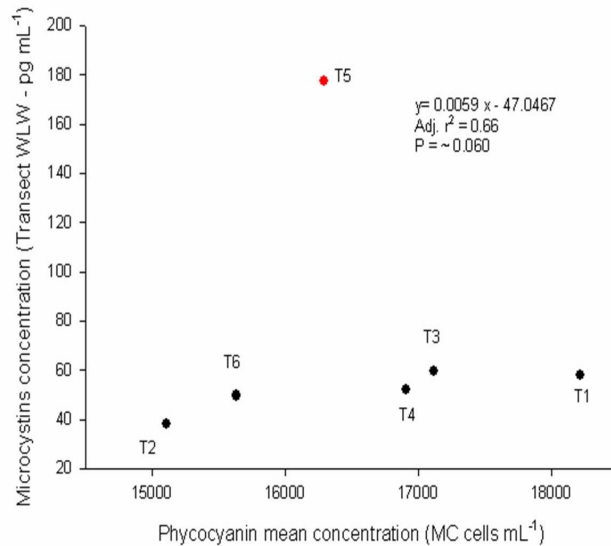

Fig. 10- Silver Lake, Hollis NH. Except for T5 which was treated as an outlier in the regression analysis, there was a reasonably strong (y = 0.0059 x - 47.0467, adj. r2 = 0.66) positive relationship between PC and MC, yet the trend was not significant (P = ~0.060)

Discussion

Horizontal Distribution of Cyanobacteria and toxins - With the increase in the occurrence of potentially toxic cyanobacterial blooms (Carmichael, 2007), freshwater resource managers are increasingly confronted with the need to make important decisions regarding the safe use of bodies of water. Sampling is traditionally performed at a lake's deepest point. Results of such sampling can be used as an informative tool in the management of lakes and ponds. Data provided by a deep-water sampling may however misrepresent the true state of a lake when distribution of cyanobacteria is patchy. Detection of heterogeneous distribution of both cyanobacteria and their toxins is essential for sound lake management.

Our analysis of cyanobacteria distribution across Silver Lake both in a North/South and an East/West direction pointed to their accumulation towards the eastern shore, and in particular in the northeastern end of T1. Mixing and the calm weather on and around the day of sampling suggest that accumulations may have formed at the surface. This phenomenon can occur when buoyant cells lose their entrainment in the water column in calm weather following a period of turbulence; it has been recognized for cyanobacterial species that produce gas vesicles to regulate their vertical position in the water column (Reynolds, 2006). Cells that are brought to the surface are then driven to the downwind shores and bays where they form scums (Chorus and Bartram, 1999).

The overall NE direction of the surface drogue (depth 0.5 m) suggests that the accumulation of cyanobacterial cells was induced by wind. So does the finding that surface levels of PC in the middle regions of T6-T4 were low in comparison to E and W, and were highest in the middle and E regions of T3-T1. Several studies have demonstrated patchiness of phytoplankton, indicating wind as the essential factor (George and Edwards, 1976; Baker and Baker, 1976). *Microcystis* appear to be particularly susceptible to accumulation downwind (Yuwei et al., 2003). Velocities of surface currents are highest at moderate wind speed and patchy accumulations generally do not form at wind speed above 400 cm sec^{-1} (George and Edwards, 1976). This corresponds to a wind-speed of 0.78 knots. The 0.6 knots SW wind velocity recorded on the sampling day could be representative of recent wind conditions, under which localized cell aggregations could have developed.

Wind data collected over a longer period of time preceding the sample date, together with longer deployment of the wind drogues, would have greatly improved our ability to evaluate the effect of wind and currents on cyanobacterial cell accumulations. Long-term wind data were not available for the precise location. Regular monitoring of wind conditions may enhance the ability to predict when accumulations may occur.

The observed accumulation could have important implications, especially if accompanied by high concentrations of MC, because part of the State Park beach is located at the lake's end where high concentrations of PC were found. Exposure to MC has been linked to human illnesses in many countries. The toxin was responsible for the death

of 76 dialysis patients in Brazil, who received hospital treatment that used a toxin-contaminated water source (Carmichael et al., 2001). MC have also been found to have a cumulative damaging effect on the liver (Chorus et al., 2000) and chronic exposure has been correlated with greater incidence of cancer (Falconer, 1999). The main route of exposure to MC is through ingestion of drinking water, but recreational use of bodies of water presents health hazards as well, through direct contact, accidental ingestion, or inhalation (Chorus et al., 2000).

Our study found that horizontal distribution of MC was also patchy; however MC concentrations did not directly correlate with distribution of PC. The highest MC concentration in WLW from horizontal transects was recorded at T5. The lack of correlation between PC and MC distribution may be due to a mixed *Microcystis* population, with interspecific variation in toxin production. For example, a study of the spatial and temporal variations of *Microcystis* species and MC concentration in lake Biwa, Japan, found that MC concentrations depends less on the total number of *Microcystis* cells than on the proportion of toxic M. aeruginosa (Ozawa et al., 2005). Our method does not discriminate between cyanobacterial species and this may contribute to the lack of correlation we found between PC and MC. It is possible that vertical mixing increased the surface MC concentration at T5. While it is usually assumed that the return flow in shallow lakes consists of surface currents along the lake's periphery rather than in a deeper return current, areas of downwelling and upwelling have also been observed (George and Edwards, 1976). Movement of the deeper drogues gave no evidence of a "cycloidal path" (Baker and Baker, 1976). A returning bottom current could presumably re-suspend benthic MC from senescing cells if an upwelling was created by its encounter with a shallower depth. The change in depth from ~7.4 m at T4 to ~4.8 m at T5 as observed by sonar recordings may be sufficient to generate such an upwelling. Vertical concentrations of PC at the sediment interface, accompanied by a decrease in DO, suggest that such cells might have been present. So do the MC values for sediment and sediment water.

MC-related human illnesses in many countries prompted the World Health Organization (WHO) to establish a guideline of 1.0 µg L^{-1} as the maximum allowable concentration of MC in drinking water (WHO, 1998). Overall, MC values for Silver Lake were well below the WHO recommended guidelines. However there have been past reports of children with stomach ailments, ear infections and skin rashes associated with scum-forming M. aeruginosa (Hollis conservation commission, 2006). No data of MC concentrations at the time of the reports were available, however occurrences of illness suggest that either MC concentrations can vary, or be sufficient to have harmful effects.

Lake Trophic State - Cyanobacteria tend to dominate in eutrophic systems (Reynolds, 2006). Hence a lake's trophic status presumably could help predict the occurrence of cyanobacteria. The results of the present study however show that determining a lake's trophic status based on chl a and nutrient parameters should not exclude an extensive sampling of PC. Indeed, based on criteria of SDD, Chl a, and TP used in Carlson's trophic classification of lakes, on the day of the sampling, Silver Lake rated as oligotrophic (App. I, Table 2). The various measured physical, chemical, and biological parameters showed great stability throughout the water column and the lake was not thermally stratified, suggesting a recent mixing event. Previous assessments of the lake had found a similar situation (EPA trophic report; September 1998, February 1999). Because of its shallow depth it is possible that Silver Lake is polymictic as shallow lakes often are, allowing frequent mixing of the water column while periods of thermal stratification may occur during the summer (Scheffer, 1998). Supporting the latter idea, the temperature curve indicated a secondary stratification with a shallow metalimnion with a rapid decrease in temperature occurring within the upper 2 m before stabilizing to the bottom.

Silver Lake vertical profile contrasted with the other lakes assessed (App. I, Fig. 4). Its 2.5 °C temperature range was much smaller than the average temperature range for other lakes (12.3 °C ± 1.72). All other lakes exhibited stratification into

an epilimnetic and a hypolimnetic layers separated by a metalimnion with an average thermocline at 8m. The chl a concentration was the lowest in the lakes group, less than the group's average (4.45 µg L^{-1} ± 1.05). Both BP and WP had higher TP levels than Silver Lake (39.13 µg L^{-1} ±1.46 and 23.40 µg L^{-1} ±0.51 respectively). Paradoxically, despite Silver Lake's apparent oligotrophic status and its condition in relation to other lakes, its mean concentration of PC not only was the highest of all lakes, it also was three times (compared to ML) to 65 times (compared to LSP) their concentration, almost seven times their average. A shift in the structure and composition of a lake's phytoplankton community to one where cyanobacteria are dominant has been attributed to the addition of nutrients, phosphorus in particular, resulting in an increase in the total phytoplankton biomass (Havens, 2007; Reynolds, 2006). In the present study, the results confound the assumption that high levels of PC are associated with high levels of chl a.

Two reasons can be suggested to explain the contrasting levels of PC and chl a in Silver Lake. The first one concerns the method used to measure the lake's pigment concentrations. The system used by the probe to measure pigments is based on fluorescence. Fluorescence depends on the fact that when a photon excites a pigment, the pigment returns to its ground state by emitting a fluorescence photon of longer wavelength than the photon absorbed, which is measured by the probe. All phytoplankton groups contain chl a, which has two absorption maxima at 425 nm and 675 nm. Cyanobacteria and cryptomonads also contain PC. The phycobilin pigment provides them with a species-variable additional absorption spectrum found in deeper water that peaks at a wavelength of approx. 620 nm, and at which chl a has lower absorption capacity (MacColl, 1998). In the presence of cyanobacteria, some of the wavelengths emitted by the excitation of chl a may be absorbed by cyanobacterial cells, resulting in readings of chl a that are negatively affected by the interference.

Contrasting levels of PC and chl a can also be explained by a phytoplankton community consisting mostly of cyanobacteria, as our calculated ratios of PC:chl a suggest, and which our own

observations in the field, and the phytoplankton counts identified as *Microcystis*. This agreed with a previous study (1998-2000) that found it to be the lake's dominant phytoplankton (Haney and Ikawa, 2001). While the genus constituted only 36% of the total counted phytoplankton, microscopic enumeration of cyanobacterial cells is often challenged by the presence of large colonies consisting of thousands of cells that are difficult to quantify. Our own enumeration did not discriminate between cells and colonies.

On the day of the sampling, Silver Lake's measured parameters classified it as oligotrophic, however PC cell counts were high. Our findings that cyanobacteria were not homogeneously distributed throughout the lake, emphasizes the importance of extending sampling beyond the traditional deep site where sampling usually occurs, in order to determine safe water usage. With extensive sampling, patterns of distribution may be revealed, which in turn can help understand the intricacies of a lake's system and contribute to better management practices.

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Appendix I
Tables

Appendix I, table 1. Morphometric data of the lakes sampled during the fall of 2007. When available, data were compiled from the Environmental Protection Agency lake trophic reports. Otherwise, they were obtained by internet search.

Lake	Abbr.	Sampling Date	Town	Lat.	Long.	Elev. (ft)	Mean Depth (m)	Max. depth (m)	Lake Area (A_0) (Ha)	Watershed Area (A_w) (Ha)	$A_w:A_0$ ratio	Vol. (m³)	Flush rate (year⁻¹)
Barbadoes Pond	BP	9/13/07	Madbury	43°11'25"	70°56'00"	132	6.1	14.6	5.7	38.8	6.81	348500	0.5
Lower Sawyer Pond	LSP	10/4/07	Livermore	44°04'95"	71°38'05"	1937	12.2	30.5	19	310.8	16.36	1584000	1
Mirror Lake	ML	10/11/07	Tuftonborough	43°37'18	71°15'50"	509	*	13.4	134.76	*	*	*	*
Silver Lake	SL	9/20/07	Hollis	42°45'25"	71°35'54"	274	2.8	7.3	13.64	248.6	18.23	387000	3.2
Willand Pond	WP	9/27/07	Sommersworth	43°14' N	70°53' W	182	4.7	11.2	34.8	116.5	3.35	1627000	0.3

* no data found

App. I, table 2. Carlson's trophic state index (TSI). Carlson, R.E. and J. Simpson. 1996. Adapted from Coordinator's Guide to Volunteer Lake Monitoring Methods. North American Lake Management Society.

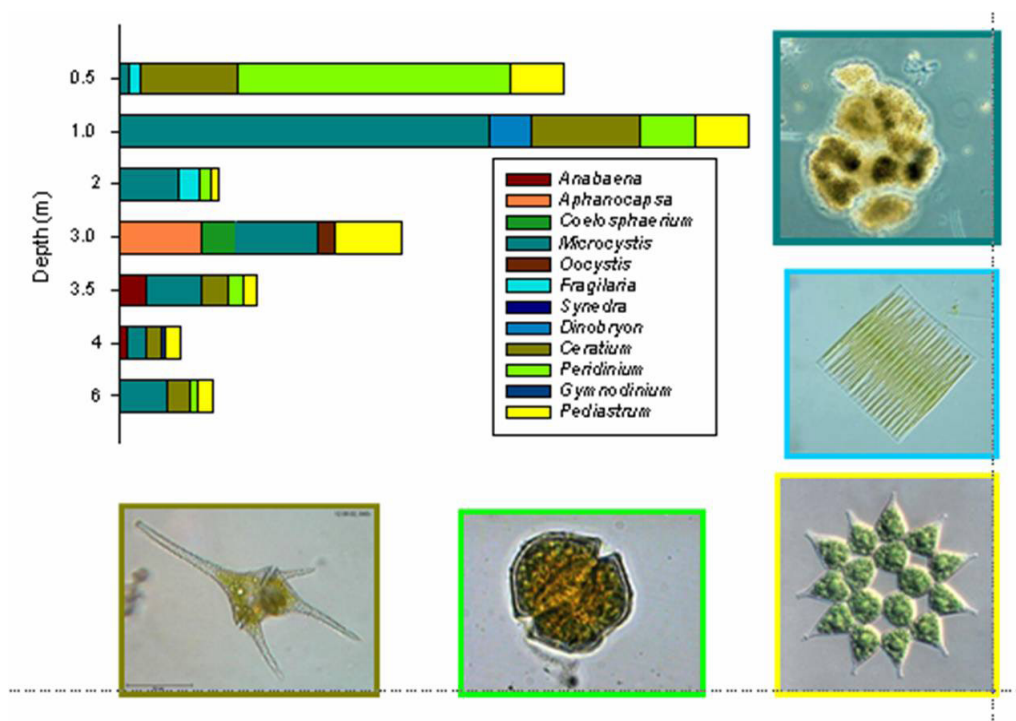| TSI	Chl (ug/L)	SD (m)	TP (ug/L)	Trophic state
<30-40	<2.6	>8-4	<12	Oligotrophy
40-50	2.6-7.3	2-Apr	24-Dec	Mesotrophy
50-70	7.3-56	2-0.5	24-96	Eutrophy
>70	>56	<0.5	>96	Hypereutrophy

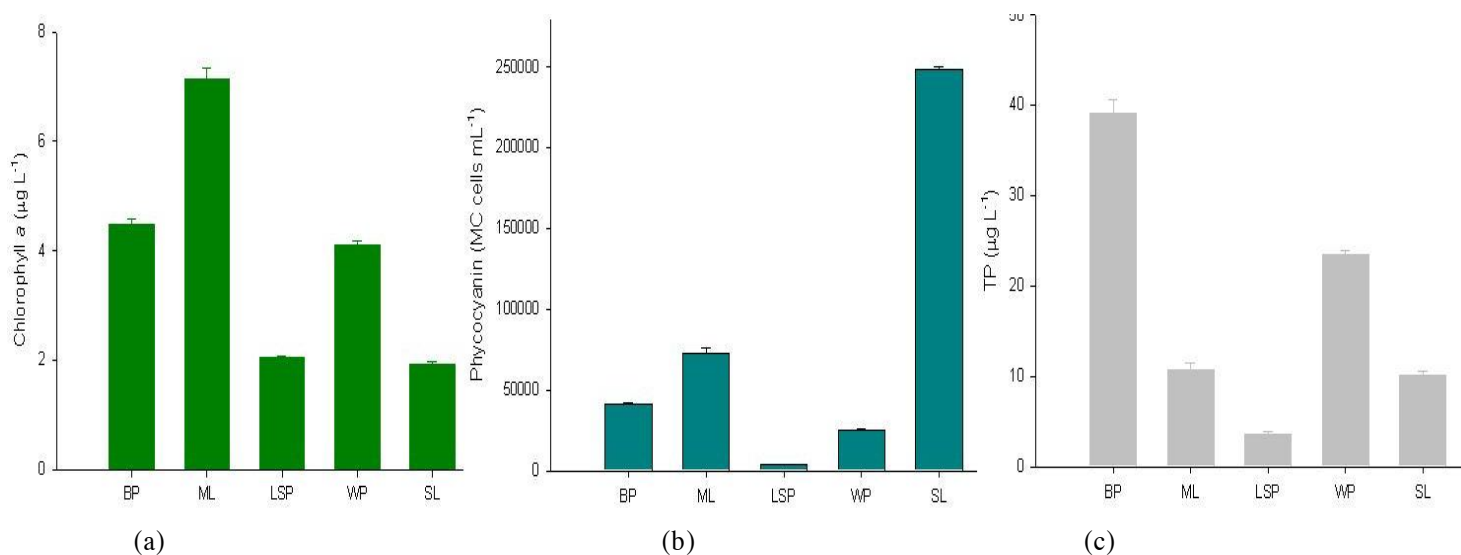<http://dipin.kent.edu/tsi.htm#Relating%20Trophic%20State%20to%20the%20State%20of%20the%20Waterbody>
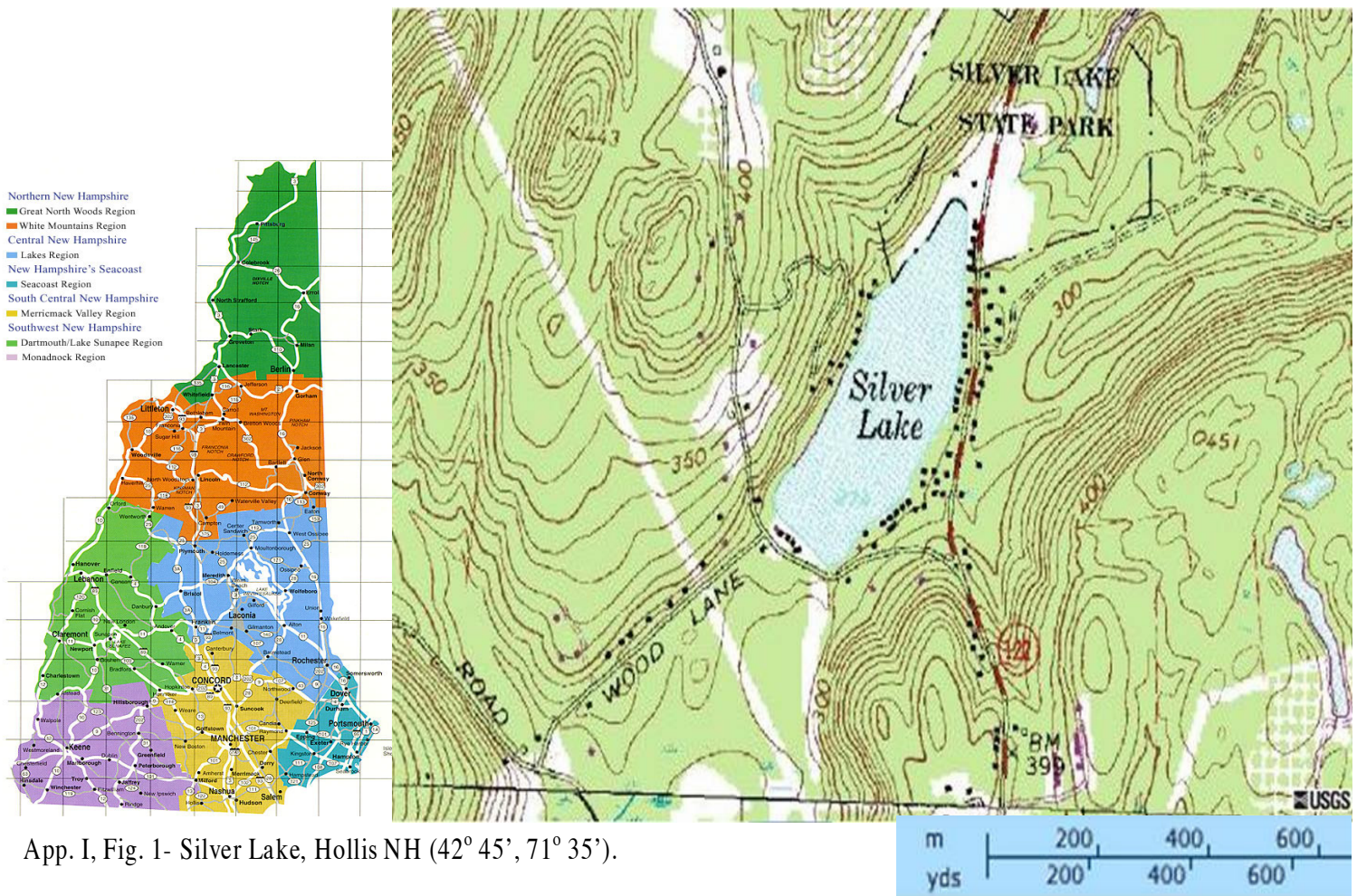
App. I, Fig. 1- Silver Lake, Hollis NH (42° 45', 71° 35').

App. I, Fig. 2- Sediment "skimmer" used to collect benthic samples at Silver Lake, Hollis NH, on September 20, 2007

App. I, Fig. 3- Vertical profile of phytoplankton distribution on 20 September 2007 at Silver Lake, Hollis NH, deep site: Cyanobacteria (45%) were dominant, especially *Microcystis* (36%), followed by dinoflagellates (35.2%).

| (a) | (b) | (c) |

App. I, Fig. 4- Comparison of mean concentrations of Chl *a* (a), PC (b) as calculated from the vertical multi-parameter measurements, and TP (measured in IT WLW of pelagic site; (c)) of the various lakes surveyed during the Fall 2007 UNH Field limnology class. Silver Lake, Hollis NH had the highest concentration of PC (248691 ± 963 MC cells mL^{-1}), 3 to 65 times that of other lakes. This was not replicated for Chl *a* (1.93 ± 0.06 _g L^{-1-}) or TP (10.10 ± 0.51 _g L^{-1}) concentrations.