Water Resources Research Center

Annual Technical Report

FY 2000

Introduction

None

Research Program

Basic Information

Title:	Monitoring Demonstration at a Top-Soil Manufacturing Site in Hooksett, New Hampshire
Project Number:	B
Start Date:	3/1/2000
End Date:	2/28/2001
Research Category:	Water Quality
Focus Category:	Nitrate Contamination, Groundwater, Water Quality
Descriptors:	
Lead Institute:	University of New Hampshire
Principal Investigators:	William H. McDowell

Publication

Problem Statement

Beneficial re-use of residuals, such as biosolids and short paper fiber, has become an increasingly important topic in both environmental policy and science over the past decade. These materials, which are a natural by-product of waste management, are becoming a significant disposal problem. Reclamation activities, such as those at abandoned gravel pits, provide a way for these secondary products to be recycled back into the environment. However, the same attributes that make this material valuable as an organic material also may cause deleterious effects to groundwater without proper management and monitoring. Excessively high nitrogen content in biosolids may lead to nitrate concentrations in groundwater that exceed EPA allowable limits and lead to harmful environmental and human health effects.

The State of New Hampshire along with the entire New England region have been actively trying to enact policies dealing with the use of residuals specifically for reclamation activities. However, environmental policy and effective management practices are extremely difficult to develop without the aid of comprehensive scientific studies that examine the impacts of these activities on the ecosystem.

Project Purpose

The purpose of this project is to continue a groundwater monitoring demonstration project at a reclamation site utilizing residuals regulated by Env-Ws 800. Specifically, the project assesses the impact of residual application on nitrogen concentrations (nitrate, ammonium, and dissolved organic N) and dissolved organic carbon in groundwater at a topsoil manufacturing site in New Hampshire. This site uses biosolids and/or short paper fiber (SPF) to reclaim (revegetate) a former gravel pit and manufacture topsoil. The primary goal of the project is to demonstrate whether current management and application practices are sufficient to protect groundwater from contamination with NO_3-N and other forms of dissolved nitrogen. A secondary goal of the project is to assess levels of trace metals in groundwater at this reclamation site. In assessing these goals we aim to identify ways to improve best management practices (BMPs) and protect groundwater while continuing to provide a beneficial use for this nutrient-rich material.

Site Description

The site is Martin's Ferry located in Hooksett, NH and consists of a 5-acre topsoil manufacturing operation which has had reclamation activity with biosolids for the past ten years and an adjacent control field of approximately the same size with no history of biosolids activity. The site is currently permitted under Env-Ws 800 as a site appropriate for biosolids utilization. This site is being monitored primarily to examine the impact of long-term biosolids applications on groundwater. At the site, biosolids only have been applied approximately annually since 1989 with removal of the organic topsoil approximately every five years. An application of a biosolids and SPF mixture was applied in October of 1999 with a C:N ratio of 27:1 (Table 1). Approximately 1,000 cubic yards of topsoil was removed in 1996, returning soils to their native condition of excessively drained Windsor loamy sand.

Table 1: Residuals application rates from 1996 to 2000 at the Martin's Ferry Site.

Year	Residuals	Biosolids (yds^3)	Total Nitrogen (#/acre)
1996	Biosolids only	166	512
1997	Biosolids only	337	1040
1998	Biosolids only	460	826
1999	Biosolids & SPF	353	1600
2000	None	0	0

Sample Collection

Both treatment plots and control areas were identified, and groundwater wells were installed in both areas. Wells were also installed to monitor groundwater quality beneath the treatment plot, and at locations presumed to be hydrologically up- and down-gradient of the treatment plot. PVC wells (2 inch diameter) with 2' of slotted well screen were installed for groundwater collection. Samples of groundwater were collected every two weeks during the growing season (August 1 - November 1) and were continued on a monthly basis through the winter (December - April). Groundwater wells were evacuated with a Teflon bailer until three volumes of water within the well had been exchanged prior to sample collection, where feasible. If recharge rates were slow, less than three volumes were withdrawn. Samples from groundwater wells were placed in clean HCl-washed polyethylene bottles (HDPE) and remained on ice in coolers until delivery to UNH. Samples are filtered with a 0.7 µm GF/F ashed filter and frozen until analysis. Samples for trace metals analysis are filtered with a 0.45 µm membrane filter and treated with 3% nitric acid for storage until analysis.

Chemical Analysis

Samples were analyzed for NO_3^-, NH_4^+, DON, and DOC at the analytical laboratory (Ecosystems Analysis Laboratory) of Professor William H. McDowell, University of New Hampshire. Nitrate and NH_4^+ are analyzed using flow injection analysis colorimetry (Lachat) with cadmium reduction for NO_3^- and the phenol hypochlorite method with sodium nitroprusside enhancement for NH_4^+. Total dissolved nitrogen (TDN) is measured using high temperature catalytic oxidation (Shimadzu TOC 5000) with chemiluminescent nitrogen detection (Antek 720, Merriam et al. 1996); DON is then calculated as the difference between TDN and DIN (NO_3^- + NH_4^+). Dissolved organic carbon is measured using a total carbon analyzer (Shimadzu TOC 5000). Trace metals analysis will be analyzed using an Inductively coupled plasma (ICP) method at the Analytical Services Laboratory at the University of New Hampshire.

Conclusions

The monitoring demonstration at this biosolids application site in Hooksett, NH has produced several key findings. First, there are significant increases in average NO_3-N concentrations in groundwater when wells beneath biosolids treatment areas are compared to control and upgradient wells at the site. However, the concentrations of NO_3-N in groundwater both within and downgradient from the biosolids treatment area show high spatial variability. This suggests that NO_3-N contamination has not resulted from the relatively uniform biosolids applications, but rather has resulted from non-uniform stockpiling at the site. Based on the management history of the site and the

location of areas of high NO_3-N concentrations in groundwater we are fairly certain that this stockpiling activity has led to deleterious effects on groundwater.

We believe that gravel pit management practices can lead to nitrogen saturation of soils (Aber et al. 1989), a condition in which soil microbes and plants can no longer utilize available N in a predictable or effective way, resulting in contamination of groundwater with nitrogen. This was most likely the case at the Hooksett site where past stockpiling and application activity may have led to an inability of the soil to utilize the available N provided by the biomix application in October of 1999. Although available nitrogen appears to be high, the available carbon at the site appears to be quite low. This lack of available carbon as an energy source for microbial processing has led to increased levels of nitrification causing an increase in NO_3-N production and a subsequent leaching of NO_3-N to the groundwater.

In order to substantiate our conclusions that biosolids stockpiling has led to nitrogen saturation and leaching of NO_3-N to groundwater, soils analysis will be performed at the site. We have collected soil samples at various depths (0 to 20cm, 20 to 35 cm, and 35 to 50 cm) that will be analyzed for total nitrogen, total carbon, available NO_3-N and available NH_4-N. These soils analyses will provide insight into our overall understanding of the impacts of biosolids at the site.

Trace metal analysis indicates that there is no significant threat to groundwater due to trace metals. Concentrations of all trace metals analyzed were near detection limits and well below the EPA and NH DES Standards for these compounds in drinking water. Archived samples collected prior to and immediately after the biosolids application in October 1999 will be analyzed in the future to insure that contamination did not occur at that time. Soils samples will also be collected to examine the potential impact of biosolids stockpiling and application on trace metal concentrations in the soil at the site.

References

Aber, J.D., Nadelhoffer, K.J., Steudler, P., Melillo, J.M., 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39(6), 378 – 386.

Merriam, J.L., McDowell, W.H., Currie, W.S., 1996. A high-temperature catalytic oxidation technique for determining total dissolved nitrogen. Soil Science Society of American Journal 60(4) 1050-1055.

Basic Information

Title:	Stream Chemistry as an Index of Sustainability in the College Brook Watershed
Project Number:	
Start Date:	3/1/2000
End Date:	2/28/2001
Research Category:	Water Quality
Focus Category:	Surface Water, Nutrients, Water Quality
Descriptors:	
Lead Institute:	University of New Hampshire
Principal Investigators:	William H. McDowell

Publication

Background: Stream chemistry reflects the physical, chemical, and biological characteristics of a watershed. It is responsive to degradation or restoration of the watershed's biotic functions, reflecting the ecological health of the watershed. College Brook runs through the heart of the University of New Hampshire campus and monitoring of stream chemistry will provide an excellent means to assess the impacts and sustainability of landuse on the campus.

Previous work on College Brook in 1991 showed that the University was having a significant impact on water quality and the stream biota, suggesting that the campus could not be considered sustainable. The UNH incinerator was causing high organic matter loading, resulting in high biochemical oxygen demand (BOD) and low dissolved oxygen (DO) in the stream. Other practices, such as washing of waste art materials (slip, poster paint, etc.) into street drains near the Service Building, were also impacting College Brook.

With the closing of the UNH incinerator, and heightened awareness of College Brook on campus, water quality has likely improved. Sporadic tests of water quality and characterization of benthic invertebrates as part of class laboratory exercises suggested that it has. But there has been no attempt to systematically monitor water quality in College Brook, which is needed to establish that ecological conditions in the watershed have improved.

Methodology: A long-term water quality monitoring program has been established with sampling and analysis performed under the direction of the New Hampshire Water Resources Research Center. 7 sites were established within the University's area of impact. 6 of those sites are located along College Brook and 1 of the sites is at Pettee Brook (PB1), which is used as a baseline comparison. The sites along College Brook were chosen by the predicted impact that each would receive from the watershed. 2 of the sites are located upstream of the University (CB0 and CB1), 2 are located at the beginning of the campus (CB2 and CB3A), and 2 are located in the heart of the campus (CB4 and CB5). Samples from these sites are compared to samples that were collected in 1991 to determine the progress of the watershed. In 1991 there were 6 sites. 2 sites have been added in 2000 and 1 site, CB3, had to be removed due to placement of a building.

Monthly samples are taken at the 7 sites and analyzed in the Water Quality Analysis Laboratory. Duplicate BOD samples are analyzed with a 5-day incubation period and measured using an YSI DO Meter with YSI BOD probe. TSS samples in duplicate are filtered with pre-weighed 47mm glass fiber filters. Samples for chemical analyses are filtered (pre-combusted GF/F) and frozen until analysis. Waters brand ion chromatographs are used for anion and cation analysis, a Shimadzu TOC-5000 is used for carbon analysis, and an Antek Nitrogen detector is used for total nitrogen analysis. Flow injection analysis (FIA) of PO_4, NH_4, and SiO_2, using a Lachat QuikChem is also performed. Field analysis includes DO with an YSI DO Meter, temperature and specific conductance with an YSI Conductivity/Temperature Meter, and pH with a Beckman pH/Temp/mV Meter.

Progress Report: Analysis of the data from 2000 can be compared to the data from 1991 to establish if ecological restoration has occurred (sample analysis from 2000 is not

complete at this time, with anions, cations, total dissolved nitrogen, and SiO_2 analysis still to be performed). The completed analysis indicates that the water quality has improved at sites that were sampled in both 1991 and 2000. Yearly averages show overall improvement of water quality from 1991 to 2000 (Table1). DO has increased and BOD has decreased at every site, indicating that the closing of the incinerator has decreased organic matter loading. DOC has also decreased at every site.

There has been an overall improvement of NH_4, except at CB2 and CB4, where there have been increases. There has been an overall improvement in TSS as well, except at CB4, which has had a large increase. The increase in both of these parameters at CB4 may indicate that the placement of buildings over the stream has had a negative impact that needs to be considered.

Further analysis is needed to determine the sustainability of the UNH campus. Samples in 1991 were taken from February to September, while samples in 2000 were taken from May to December. While comparisons of samples between 1991 and 2000 taken at the same time of year show that there has been improvement in water quality, the picture is not as clear as the yearly averages indicate. There is much more variability in the data when the time of year is taken into account. A more complete sampling period is needed to determine the precise amount of improvement at all sites within the watershed and sampling has continued in 2001 to establish the ecological health of College Brook.

A web site has been created, which shows the progress of the restoration, a complete description of the project (with pictures of the sites), and completed data from the project (http://www.wrrc.unh.edu/collegebrook/college_brook.htm).

Table 1: Yearly Averages

Year: 2000

Site	DO (mg/L)	S. COND (uS/cm)	pH	BOD (mg/L)	DOC (mg C/L)	NH4 (ug N/L)	TSS (mg/L)
CB1	9.73	514	7.41	1.39	5.54	47.00	5.0
CB2	10.22	445	7.58	1.55	5.40	52.00	6.1
CB4	9.76	665	7.62	2.33	5.68	72.00	10.7
CB5	9.81	688	7.71	1.58	4.89	34.29	4.5
PB1	9.12	572	7.68	1.37	5.76	83.86	3.9
Total	9.73	576.91	7.60	1.64	5.46	57.83	6.03

Year: 1991

Site	DO (mg/L)	S. COND (uS/cm)	pH	BOD (mg/L)	DOC (mg C/L)	NH4 (ug N/L)	TSS (mg/L)
CB1	8.07	455	7.49	2.19	11.26	118.50	8.5
CB2	8.75	303	7.53	2.08	8.17	34.00	8.1
CB4	7.91	738	7.52	2.69	8.23	35.60	4.9
CB5	8.55	464	7.57	2.38	8.24	67.60	4.0
PB1	8.68	507	7.48	1.75	6.90	99.80	3.6
Total	8.39	493.28	7.52	2.22	8.56	71.10	5.80

Basic Information

Title:	Estimation of Flow-Duration Curves at Ungaged Stream Reaches in New Hampshire and Vermont
Project Number:	
Start Date:	6/1/1999
End Date:	8/31/2000
Research Category:	Climate and Hydrologic Processes
Focus Category:	Water Quantity, Surface Water, Hydrology
Descriptors:	Flow duration; Instream Flow; Water quality management; Water allocation; Streamflow depletion; Aquatic habitats; New Hampshire; Vermont
Lead Institute:	University of New Hampshire
Principal Investigators:	S. Lawrence Dingman

Publication

Problem and Research Objective

Magnitude and frequency of streamflow are two of the components of flow regime that together determine the integrity of aquatic ecosystems through their on impact water quality, energy sources for stream biota, the physical parameters of aquatic habitat, and biotic interactions. As all withdrawal uses of water affect the magnitude and frequency of downstream flows, the problem of instream flows has emerged as one of the region's major water-resource management issues. It has been a central issue in most hydropower dam licensing and relicensing proceedings for the last two decades. New developments such as snow-making threaten to alter stream regimes in pristine upland watersheds. The State of New Hampshire has recognized the severity and widespread nature of these impacts and has been developing an instream-flow program to develop guidelines and rules for protecting instream flows threatened by withdrawal uses and flow regulation.

Flow-duration curves (FDCs) are cumulative-frequency plots of mean daily discharge, hence they depict the magnitude and frequency of streamflow at a stream reach. They can be readily constructed for stream reaches which have been gaged continuously for a suitable period. However, magnitude-frequency information is usually required for reaches that have not been gaged.

The objective of the research proposed here is to develop improved techniques for estimating natural FDCs (FDCs unaffected by withdrawal or regulation) at ungaged stream reaches in New Hampshire and Vermont. Such curves would provide a baseline against which to evaluate the effects of proposed water-use, flow-regulation, or land-use developments on streamflow magnitude and frequency.

Methodology

The overall research plan involves the following steps:

1.   Identify a probability distribution that appears to describe FDCs for unregulated stream reaches in NH and VT.

2.   Establish quantitative relations between the parameters of the distribution and characteristics of drainage basins that can be determined a priori from maps, GIS data bases, or other sources.

3.   Use the relationships established in Step 2 to estimate FDCs at ungaged reaches.

4.   Determine the utility of this approach to estimating FDCs for ungaged unregulated stream reaches in NH and VT.

Results

Records from 44 gaging stations in NH and VT fit our criteria of having no significant regulation and at least 10 yr of daily streamflow records. To facilitate comparison among

drainage basins of varying size, we divided the actual measured flows at these stations by drainage area to give the specific discharge, q. We then constructed median-annual FDCs for the specific discharges at each station.

Using L-moment analysis, we determined that the Generalized Pareto Distribution (GPD) appeared to provide a good fit to the FDCs of the 44 stations. This distribution can be written as

$$EP(q) = [1 - \kappa \cdot (q - \xi)/\alpha]^{1/\kappa} \qquad (1)$$

where q is a particular value of specific discharge, $EP(q)$ is the probability that specific discharge equals or exceeds q, and κ, α, and ξ are parameters of the distribution. Basin-characteristic data were readily available for 31 of the 44 stations that fit our criteria. Of these, 9 were randomly selected and assigned to a validation data set, leaving 22 stations in the calibration data set. Using automated multiple-regression analysis, the best-fit relations between distribution parameters and basin characteristics were found to be:

$$\kappa = -0.635 - 0.000046 \cdot Z + 0.009 \cdot PDF \qquad (2)$$

$$\alpha = -0.604 + 0.000151 \cdot Z + 0.404 \cdot PDF \qquad (3)$$

$$\xi = 0.00022 \cdot Z - 0.0058 \cdot F + 0.0114 \cdot PDF - 0.003 \cdot L, \qquad (4)$$

where Z is mean basin elevation (ft), PDF is mean basin precipitation-delivery factor (in./yr), Z_{max} is maximum basin elevation (ft), S is main channel slope (ft/mi), F is percent of basin with forest cover, L is main channel length (mi).

Equations (2) – (4) were then used to estimate κ, α, and ξ for the 9 stations in the validation data set, the resulting values substituted into Equation (1), and the resulting estimated GPD-FDCs compared with the actual FDCs.

Comparisons of predicted with observed values of flow values associated with specified exceedence probabilities were disappointing. Table 1 gives the average deviations, the range of deviations, and the percentage of stations with deviations less than 15% for various values of exceedence probability.

Table 1.

Exceedence Probability (%)	Average Deviation (%)	Range of Deviations (%)	Number of Stations with < 15% Deviation
98	104	-45 to 415	0/9
95	95	-30 to 215	0/9
90	74	-5 to 157	1/9
75	44	-4 to 95	3/9
50	32	-4 to 67	3/9
25	20	-3 to 41	4/9
10	13	-1 to 25	5/9
5	12	-3 to 26	5/9

Basic Information

Title:	Development of Statewide Nutrient Loading Coefficients Through Geographic Information System Aided Analysis
Project Number:	
Start Date:	3/1/1999
End Date:	8/31/2000
Research Category:	Water Quality
Focus Category:	Non Point Pollution, Nutrients, Surface Water
Descriptors:	Eutrophication; Geographic Information Systems; Land use; Impervious surfaces; Land-water interactions; Nutrient loading coefficients; Phosphorous; Riparian vegetation; Spatial analysis; Water quality modeling; Watershed management
Lead Institute:	University of New Hampshire
Principal Investigators:	Jeffrey A. Schloss

Publication

Background and Problem Statement:

The waters of New Hampshire represent a valuable water resource contributing to the state's economic base through recreation, tourism, and real estate revenues. Some lakes and rivers serve as current or potential water supplies. For most residents (as indicated by boating and fishing registrations) our waters help to insure a high quality of life. New Hampshire currently leads all of the New England states in the rate of new development and redevelopment. The long-term consequences of the resulting pressure and demands on the state's precious water resources remain unknown. Of particular concern is the response of our waters to increasing non-point source pollutant loadings due to watershed development and land use activities. While watershed nutrient budget measurements and modeling have been attempted on a number of watersheds in the state, the recent cut in the Clean Lakes Program funding (Section 314) has limited the resources for current and future watershed diagnostic studies. No previous attempt has ever been made to review the existing data provided from previous studies and to investigate whether statewide nutrient loading coefficients can be developed using the powerful statistical and spatial analysis tools now available through GIS.

Current water quality models utilized for lake management and diagnostic purposes (when direct water nutrient budgets have not been measured) rely heavily on nutrient export coefficients derived primarily from out of state (Reckow et al) or limited, in terms of geographic area, New Hampshire data from Hubbard Brook. This research was initiated to finally make an effort to review and integrate together the existing data available from local, state, university, and federal watershed studies. Developing export coefficients from existing studies conducted over different areas of the state will allow for the estimation of watershed loadings with a greater confidence. Such coefficients would also allow for the efficient use of limited resources and provide baseline and benchmark data from which future studies can benefit.

Methods, Procedures and Results:

1) *Review, catalog and conduct a preliminary analysis of past and ongoing watershed studies done by the NH Department of Environmental Services, the University of New Hampshire, USGS, US Forest Service, NRCS and regional projects funded by State and Regional Planning Agencies*

Of 25 known watershed diagnostic studies performed by NH DES, UNH and other agencies, data from 24 studies were compiled. Dates of the studies ranged from June 1981 through to the present.

2) *Compile the digital data layers and information necessary to perform spatial and general statistical analyses. Care must be taken here in terms of completeness of data and time of study compared to what is available from the GIS archives.*

Of the 24 studies catalogued, 168 subwatersheds from 23 of the study watersheds were delineated and spatially digitized. Vector basemaps, raster USGS topography maps and Landsat derived generalized landcover maps were created for each of these

subwatersheds. Nitrogen data were severely limited, so Phosphorus was the nutrient of focus. Phosphorous loading data were available for only 19 of the studies as 6 studies were either still ongoing in terms of continued field measurements or data work-up. Digitized GIS soils coverage data and digital elevation model projections were expected to be complete before the end of this study by NH GRANIT (our state GIS data depository). However, delays in these independent projects resulted in missing soils coverage for 7 of the watersheds used in the study. See Table 1 for a catalogue of watershed studies available, data digitized (descriptive, summary and spatial), and those selected for analysis.

TABLE 1 - INVENTORY OF STUDIES SURVEYED AND STATUS

Watershed study	Town	Dates of Study	Study done by	Used in study	SubWS digitized	TP data summarized	Number of SubWS	Used in analysis	SubWS used in analysis	Total acres used in analysis
Baboosic	Amherst, Merrimack	4/83 – 3/84	DES	YES	YES	YES	8	no	-	-
Beaver	Derry	2/90 –1/91	DES	YES	YES	YES	8	YES	3	1,316.9
Bow	Northwood Strafford	Not completed	UNH	no	no	no	-	-	-	-
Crystal	Manchester	Not completed		no	no	no	-	-	-	-
Chocorua	Tamworth		UNH	YES	YES	YES	14	YES	14	7,812.3
Doors	Manchester	6/81 – 5/82	DES	YES	YES	YES	8	YES	4	1,062.8
Eastman	Enfield Grantham		DES	no	no	no	-	-	-	-
Flints	Hollis	10/92 – 10/93	UNH	YES	YES	YES	1	YES	1	1,224.0
Goose	Keene	Not completed	UNH	no	no	no	-	-	-	-
Great Pond	Kingston	11/94 – 10/95	DES	YES	YES	YES	4	no	-	-
Keyser	Henniker	12/90 – 11/91	DES	YES	YES	YES	4	YES	2	374.8
Kezar	Sutton	6/81 – 8/82	DES	YES	YES	YES	4	no	-	-
Mendums	Barrington	11/87 – 10/88	DES	YES	YES	YES	12	no	-	-
Newfound	Hebron, Bristol	1/92 – 12/92	DES	YES	YES	YES	8	YES	8	56,645.7
Northwood	Epsom Northwood	6/80 – 8/81	DES	YES	YES	YES	22	no	-	-
Ottarnic	Hudson	12/90 – 11/91	DES	YES	YES	YES	2	no	-	-
Pawtuckaway	Nottingham	2/90 – 1/91	DES	YES	YES	YES	12	no	-	-
Pleasant	Northwood	Not completed	DES	no	no	no	-	-	-	-
Robinson	Hudson	10/90 – 12/91	DES	YES	YES	YES	11	no	-	-
Silver	Harrisville	1998 – 1999	DES	YES	YES	YES	6	no	-	-
Squam	Ashland Center Harbor Holderness Moultonborough Sandwhich Newbury	12/98 – 6/00	UNH	YES	YES	YES	31	YES	24	16,964.2
Sunapee	New London Sunapee	3/89 – 12/89	UNH	YES	YES	YES	11	YES	11	20,281.5
Swains	Barrington	Not completed	UNH	no	no	no	-	-	-	-
Webster	Franklin	10/87 – 12/88	DES	YES	YES	YES	9	no	-	-
Wentworth	Wolfeboro	9/96 – 8/97	DES	YES	YES	YES	11	no	-	-
Totals				19	19	19	158	8	67	105,682.2

> 3) *Compare geographic areas studied, methods, extent, dates of study and select those watershed studies or subwatershed that will yield the best combination of variation and commonality of watershed characteristics.*

Figure 1 displays the locations of the studies. There was generally good representation in terms of range of productivity of the study lakes and land cover. The studies were dispersed within four of the six different Total Phosphorus regions of NH as defined by Rohm, Ommerick and Kiilsgaard (1995) based on lake phosphorus levels and a combination of landscape factors. The two phosphorus regions not represented are limited in terms of their spatial extent in New Hampshire, and thus, would include only a small number of lake watersheds. The studies surveyed also fall within all three New Hampshire " vegetative ecoregions" as defined by the NH Research Reserve Study and

both level II Ecoregions as defined by EPA BASINS (Lahlou et al 1998) from work by Ommerick.

FIGURE 1 - STUDY LOCATIONS

Project Study Coverage
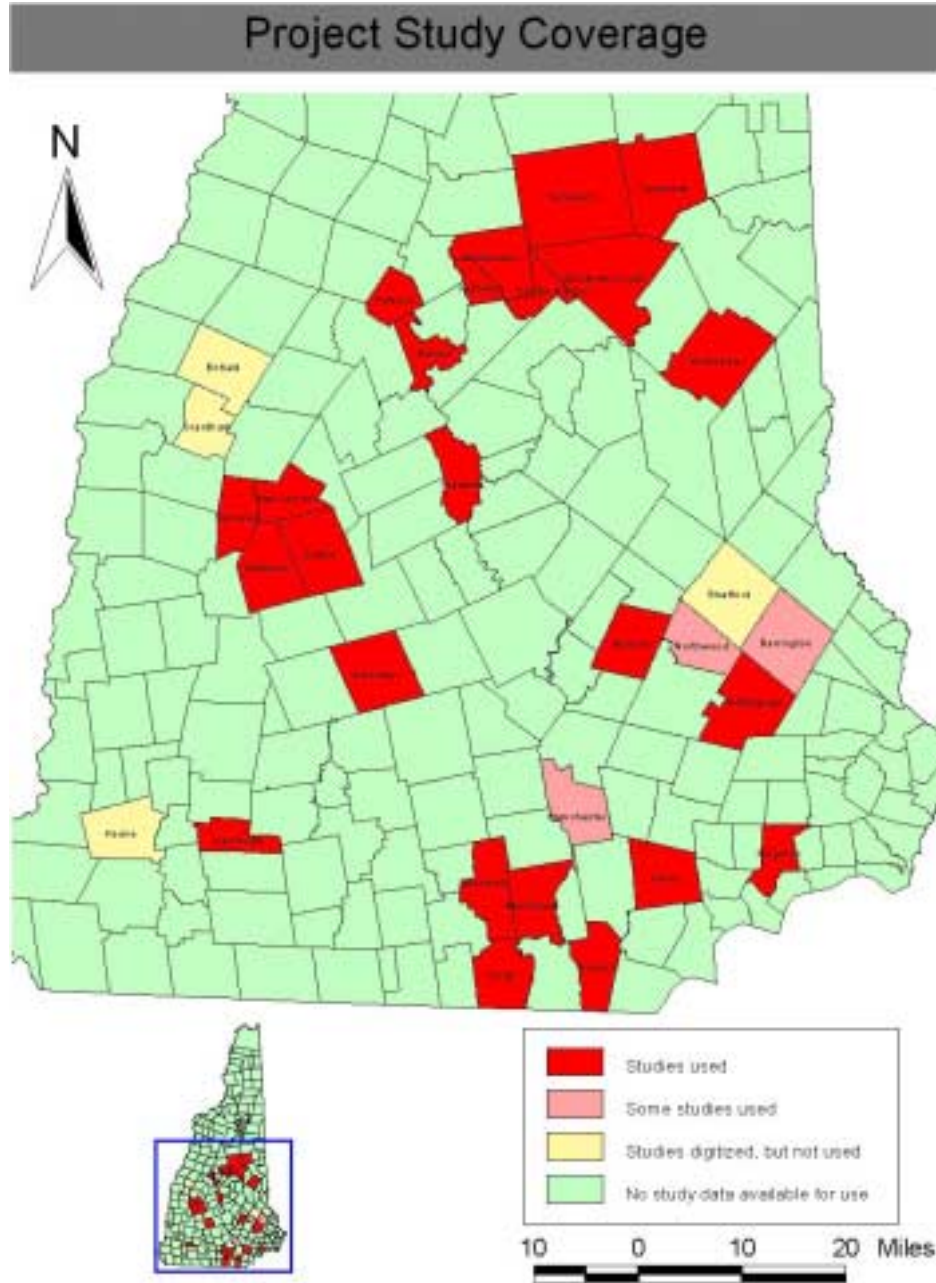
4) Perform watershed update visits to selected subwatersheds to ground truth existing GIS data, update land use activities and selectively re-sample streams to confirm historical data.

After study reviews, site visits to selected watersheds, review of more recently collected data, contact with local officials, and discussion with agency cooperators, the subwatersheds for combined analysis were chosen. The screening criteria included:
1- Subwatershed loading was determined through flow and nutrient monitoring as opposed to estimated from landcover or other means.
2- Completeness of data.
3- Acceptable accuracy of available generalized landcover data in GIS format.
4- No major subwatershed changes were known to occur (site visits or discussions).

In all, 67 subwatersheds were chosen for use in the analysis, covering over 105.6 thousand acres (See Table 1 for the breakdown of number of subwatersheds selected by lake for this study).

5) Conduct a preliminary analysis (applying "Occum's razor") of the data to determine export coefficient ranges, similarities and differences for a series of land-cover types and combinations.

Subwatersheds were categorized using cladistic analysis (SYSTAT Version 9, 1999, SPSS Inc., Chicago Il) into one of 8 subwatershed types depending on the breakdown of generalized land-cover dominance. Below is an overview of the cladistic results:
♦ Active Agriculture
♦ Highly Urban
♦ Mixed Urban/Agriculture/Cleared
♦ Highly Deciduous Forest Cover (>73% Deciduous)
♦ Deciduous Forest Cover (>58% Deciduous <73%)
♦ Mixed Forest Cover with dispersed wetlands
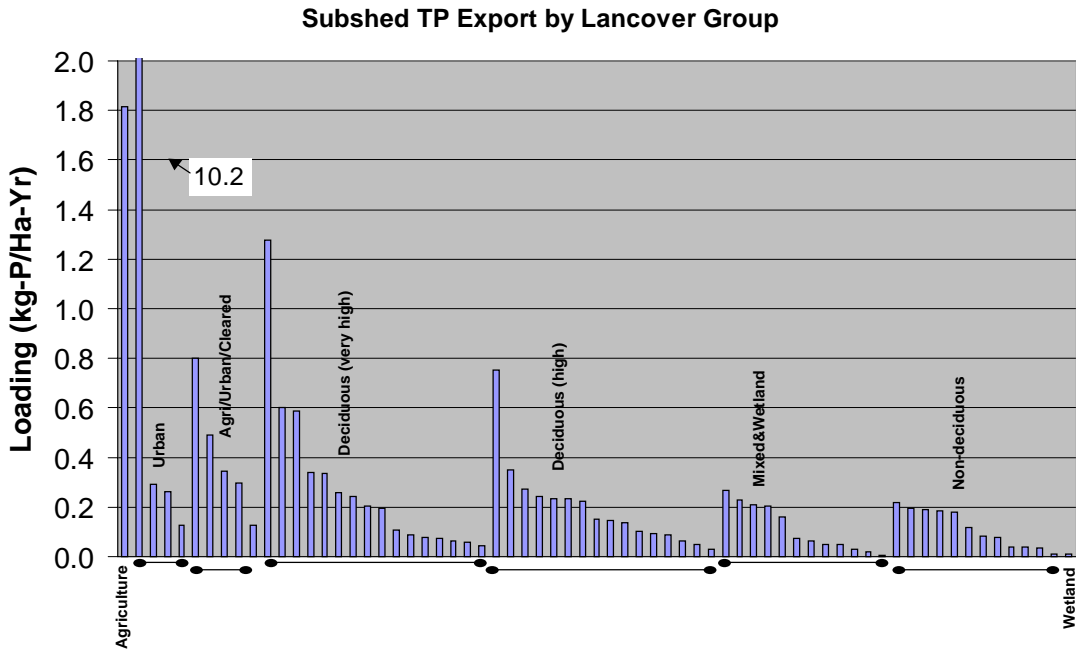♦ Non-deciduous Forest Cover (>55% Non-deciduous)
♦ Wetlands

Table 2 displays the resulting breakdown along with percent cover for the subwatersheds chosen for analysis. Figure 2 displays the subwatershed export coefficients by the subwatershed "typing" described above. Table 3 lists descriptive statistics on these data. Figure 3 displays the non-parametric box and whisker analysis of these results. Table 4 and Figure 4 contain similar descriptions and analyses on the subwatershed export coefficients after the data were normalized for yearly precipitation.
As can be seen, within each of these groupings there is a relatively wide range of export coefficients documented. Upon further examination using the available GIS coverages the average watershed slope was an important factor in many of the subwatersheds representing the higher loadings in their grouping. Also, subwatersheds consisting mostly of drainages that were derived from highway, roads, camp roads and low intensity

development were found to represent the higher end of loadings in the forested groupings. Thus a useful but unavailable GIS coverage would be an indication of development intensity within primarily forested areas. Available census block group data and the MRLC landcover interpretation (Multi-resolution Land Characteristics produced by Pacific Meridian Resources under contract to USGS) did not account very well for development density. As a result there is the wide variation in phosphorus export among forested subwatersheds. This may be better addressed using the expected update of state and local road data, or if the statewide 911-Emergency coverage is made available. Otherwise on-the-ground, or windshield surveys, or other means of data collection are necessary to characterize development intensity within the landscape since the GIS coverage is currently lacking for our predominately rural, low density developed watersheds.

On the lower range of export for each of the land cover groupings subwatersheds generally represented areas with more extensive riparian buffer or wetland complexes or relatively flat vegetated areas and undeveloped, protected forested areas.

FIGURE 2- SUBWATERSHED TP EXPORT BY LANDCOVER GROUPINGS

Subshed TP Export by Lancover Group

TABLE 2- STUDY SUBWATERSHEDS EXPORT BY LANDCOVER GROUPINGS

Lake Name	Sub-Watershed	Urban	AgClear	NonDec	Deciduous	Wetland	Group Type	Loading TP/Ha-Yr	Rainfall	Loading/Precip TP/Ha-Yr-m precip	Normalized Loading TP/Ha-NorYr
Squam	SheepIsland	0.0	42.0	2.9	55.1	0.0	Ag	1.816	1.254	1.448	1.385
Dorrs	T-5	70.9	21.0	2.1	0.1	5.9	Urban	10.153	1.203	8.438	8.416
Beaver	CatOBrk	36.5	8.0	45.0	8.3	1.7	Urban	0.292	1.277	0.229	0.215
Dorrs	T-4	44.0	5.8	18.2	23.7	4.8	Urban	0.263	1.203	0.219	0.218
Dorrs	T-2	70.1	0.0	8.7	19.7	1.6	Urban	0.127	1.203	0.106	0.105
Beaver	Rt102Inlet	12.3	32.7	31.2	16.4	6.3	Ag/Ur/Clear	0.800	1.277	0.626	0.588
Keyser	KeyserInlet	10.2	8.7	44.4	27.3	8.4	Ag/Ur/Clear	0.490	1.102	0.444	0.484
Squam	LSWachusts	0.0	16.8	6.5	76.5	0.3	Ag/Ur/Clear	0.344	1.254	0.274	0.262
Keyser	FrenchOut	7.0	26.3	13.9	29.7	4.2	Ag/Ur/Clear	0.295	1.102	0.268	0.292
Beaver	ComeauBrk	16.3	22.5	42.1	12.2	3.6	Ag/Ur/Clear	0.128	1.277	0.100	0.094
Squam	KentIsland	0.0	0.1	25.1	74.5	0.0	DecidVH	1.275	1.254	1.017	0.973
Squam	BelknapWd2	0.0	5.0	16.2	78.8	0.0	DecidVH	0.601	1.254	0.480	0.459
Squam	LiverCv2	0.0	4.2	19.7	76.1	0.0	DecidVH	0.587	1.254	0.468	0.447
Squam	BennetBrk2	0.0	2.4	24.9	72.7	0.0	DecidVH	0.341	1.254	0.272	0.260
Squam	EastmanBrk2	0.0	0.0	25.8	74.1	0.1	DecidVH	0.333	1.254	0.266	0.254
Squam	LSSW	0.0	1.1	23.1	75.6	0.0	DecidVH	0.256	1.254	0.204	0.195
Squam	CottonCv	0.0	0.0	11.6	88.4	0.0	DecidVH	0.240	1.254	0.192	0.183
Squam	LSEvansCv	0.0	3.1	17.6	79.1	0.2	DecidVH	0.203	1.254	0.162	0.155
Squam	TrueFarm	0.0	2.5	6.3	91.3	0.0	DecidVH	0.192	1.254	0.153	0.147
Squam	BennetBrk1	0.0	0.0	11.7	88.3	0.0	DecidVH	0.108	1.254	0.086	0.083
Squam	EastmanBrk1	0.0	0.1	17.9	81.5	0.0	DecidVH	0.086	1.254	0.068	0.065
Chocorua	LonelyLk	0.0	1.8	20.2	76.7	0.3	DecidVH	0.076	1.430	0.053	0.045
Newfound	CockerRiv	0.0	3.1	18.5	76.4	1.6	DecidVH	0.071	0.756	0.094	0.149
Chocorua	StonyBrk	0.0	0.2	25.2	74.4	0.2	DecidVH	0.065	1.430	0.046	0.038
Chocorua	BradgLdgWet	0.0	1.7	16.3	78.6	2.4	DecidVH	0.058	1.430	0.040	0.034
Dorrs	T-8	5.9	0.0	11.7	82.4	0.0	DecidVH	0.043	1.203	0.036	0.036
Squam	LiverCv1	0.0	0.3	29.8	70.0	0.0	DecidH	0.754	1.254	0.601	0.575
Newfound	GeorgesBrk	0.0	2.4	27.6	67.8	1.8	DecidH	0.348	0.756	0.461	0.731
Squam	OwlBrook	0.0	2.2	34.1	62.6	0.8	DecidH	0.273	1.254	0.218	0.209
Squam	CarinsCv	0.0	5.3	22.2	72.5	0.0	DecidH	0.244	1.254	0.195	0.186
Squam	ShadbshCv	0.0	0.9	28.6	67.9	0.1	DecidH	0.234	1.254	0.187	0.179
Newfound	WhitteBrk	0.0	3.4	26.3	68.6	0.9	DecidH	0.231	0.756	0.306	0.485
Squam	BelknapWd1	0.0	4.9	30.3	63.4	1.4	DecidH	0.223	1.254	0.178	0.170
Squam	SmithBrk	0.0	0.6	30.1	68.4	0.2	DecidH	0.150	1.254	0.120	0.115
Chocorua	Rt16-1	0.0	3.4	25.7	69.4	0.2	DecidH	0.147	1.430	0.103	0.086
Newfound	FowlerRiv	0.0	4.4	25.0	67.2	2.3	DecidH	0.137	0.756	0.181	0.287
Newfound	HemlockBrk	0.0	6.3	25.7	64.4	1.1	DecidH	0.100	0.756	0.132	0.210
Chocorua	NChocRiv	0.0	1.6	33.7	57.9	4.0	DecidH	0.091	1.430	0.064	0.053
Chocorua	NWDrainage	0.0	0.5	27.1	64.1	5.4	DecidH	0.085	1.430	0.060	0.050
Squam	BarvillePnd	0.0	1.4	25.2	68.9	1.9	DecidH	0.062	1.254	0.049	0.047
Newfound	WellingBrk	0.0	2.1	23.5	72.3	0.2	DecidH	0.048	0.756	0.064	0.101
Sunapee	JobsCrk	0.0	1.9	30.7	66.3	1.1	DecidH	0.031	1.130	0.027	0.029

**TABLE 2 (continued)- STUDY SUBWATERSHEDS EXPORT BY LANDCOVER GROUPINGS**

Lake Name	Sub-Watershed	Generalized Landcover					Group Type	Loading TP/Ha-Yr	Rainfall	Loading/Precip TP/Ha-Yr-m precip	Normalized Loading TP/Ha-NorYr
		Urban	AgClear	NonDec	Deciduous	Wetland					
Squam	NicolsCv	0.0	0.0	52.1	47.5	0.3	Mixed&Wet	0.268	1.254	0.214	0.204
Squam	EagleCliff	0.0	0.4	45.3	53.9	0.0	Mixed&Wet	0.229	1.254	0.182	0.174
Newfound	BlackBrk	0.0	5.6	36.7	52.7	3.8	Mixed&Wet	0.209	0.756	0.277	0.439
Squam	KesumpeBrk	0.0	0.2	27.1	51.4	3.9	Mixed&Wet	0.205	1.254	0.164	0.156
Chocorua	Rt16-2	0.0	3.1	41.5	50.6	0.6	Mixed&Wet	0.160	1.430	0.112	0.094
Sunapee	JohnsBrk	1.9	5.1	38.7	40.7	12.5	Mixed&Wet	0.074	1.130	0.066	0.070
Newfound	TitltonBrk	0.0	7.8	44.5	43.7	1.1	Mixed&Wet	0.063	0.756	0.084	0.133
Squam	WhiteOakPnd	0.0	4.2	36.1	43.1	6.7	Mixed&Wet	0.051	1.254	0.040	0.039
Chocorua	StrattonBrk	0.0	1.4	44.7	52.3	0.5	Mixed&Wet	0.050	1.430	0.035	0.030
Sunapee	HerrickMid	0.1	28.9	31.8	36.2	3.0	Mixed&Wet	0.030	1.130	0.026	0.028
Sunapee	OttPndBrk	0.0	6.7	44.6	35.6	3.0	Mixed&Wet	0.021	1.130	0.019	0.020
Sunapee	LdgPndBrk	0.0	3.6	41.6	45.3	0.3	Mixed&Wet	0.003	1.130	0.003	0.003
Chocorua	Rt16-4	0.0	0.3	67.8	29.0	0.3	Non-Decid.	0.218	1.430	0.152	0.128
Chocorua	Rt16-3	0.0	0.6	69.0	27.4	0.0	Non-Decid.	0.196	1.430	0.137	0.115
Chocorua	Rt16-5	0.0	2.9	75.5	15.8	0.3	Non-Decid.	0.189	1.430	0.132	0.111
Chocorua	LittleLk	0.0	2.7	66.9	19.8	1.5	Non-Decid.	0.186	1.430	0.130	0.109
Chocorua	SWDrainage	0.0	0.0	63.8	7.7	0.0	Non-Decid.	0.181	1.430	0.126	0.106
Flints	Inlet	0.0	10.9	54.8	17.7	10.9	Non-Decid.	0.115	0.998	0.115	0.138
Sunapee	KngHillBrk	0.0	14.1	63.6	18.5	3.6	Non-Decid.	0.084	1.130	0.075	0.079
Chocorua	BeaverPnd	0.0	0.2	56.7	28.8	0.2	Non-Decid.	0.077	1.430	0.054	0.045
Sunapee	BlodgBrk	3.1	7.7	58.9	18.8	10.0	Non-Decid.	0.040	1.130	0.035	0.037
Sunapee	PineCliff	0.4	2.5	66.1	29.0	1.0	Non-Decid.	0.037	1.130	0.033	0.035
Sunapee	ChandBrk	4.4	5.5	56.8	25.1	2.6	Non-Decid.	0.034	1.130	0.030	0.032
Sunapee	PikeBrk	0.3	3.8	81.4	10.8	3.6	Non-Decid.	0.008	1.130	0.007	0.007
Sunapee	HerrickBrk	0.0	7.4	25.4	13.1	53.7	Wetland	0.009	1.130	0.008	0.009

TABLE 3- SUBWATERSHED TP EXPORT SUMMARY STATISTICS BY GROUPING

VALUES are Kg TP per Hectare per Year

	Min	Max	Median	Q25	Q75	Mean	Sdev	N
Agriculture	-	-	1.816	-	-	1.816	-	1
Urban	0.127	10.153	0.278	0.229	2.757	2.709	4.963	4
Urb./Ag/Cleared	0.128	0.800	0.344	0.295	0.490	0.411	0.253	5
Deciduous-Very High	0.043	1.275	0.198	0.075	0.335	0.284	0.318	16
Deciduous	0.031	0.754	0.148	0.090	0.237	0.197	0.174	16
Mixed	0.003	0.268	0.069	0.045	0.206	0.114	0.094	12
Non-Deciduous	0.008	0.218	0.100	0.039	0.187	0.114	0.076	12
Wetland	-	-	0.009	-	-	0.009	-	1

FIGURE 3 - BOX AND WHISKER PLOT OF SUBWATERSHED TP EXPORT BY GROUPING


Box Plot (Quartiles) of Export Coefficients by Group
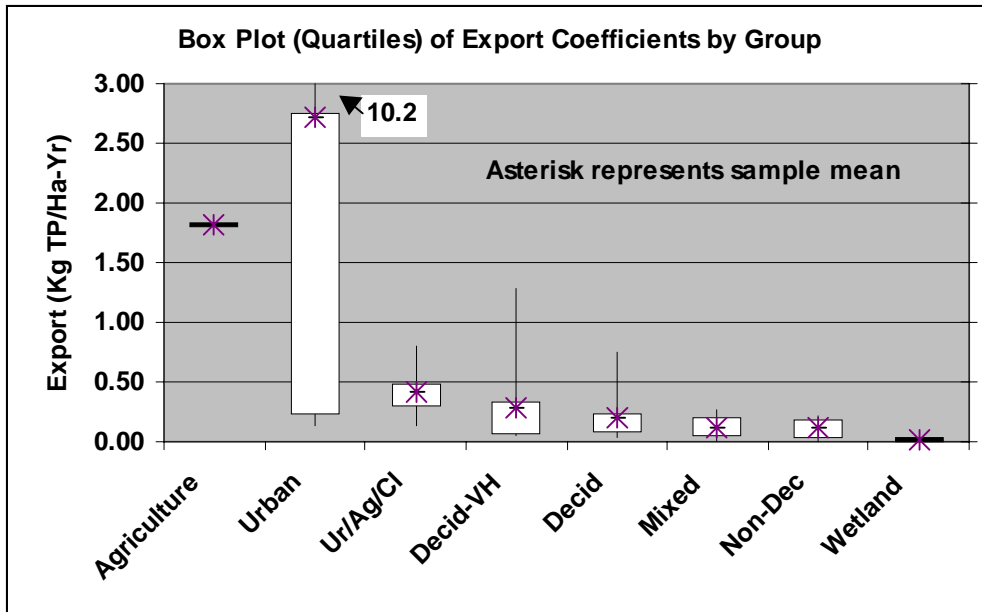
TABLE 4- SUBWATERSHED TP EXPORT SUMMARY STATISTICS BY GROUPING, NORMALIZED BY YEARLY PRECIPITATION

VALUES ARE Kg TP per Hectare per Year

	Min	Max	Median	Q25	Q75	Mean	Sdev	N
Agriculture	-	-	1.385	-	-	1.385	-	1
Urban	0.105	8.416	0.217	0.187	2.268	2.239	4.118	4
Urb./Ag/Cleared	0.094	0.588	0.292	0.262	0.484	0.344	0.194	5
Deciduous-Very High	0.034	0.973	0.152	0.060	0.256	0.220	0.241	16
Deciduous	0.029	0.731	0.174	0.078	0.229	0.220	0.206	16
Mixed	0.003	0.439	0.082	0.029	0.161	0.116	0.122	12
Non-Deciduous	0.007	0.138	0.093	0.037	0.112	0.079	0.045	12
Wetland	-	-	0.009	-	-	0.009	-	1

FIGURE 4 – BOX AND WHISKER PLOT OF SUBWATERSHED TP EXPORT BY GROUPING, NORMALIZED BY YEARLY PRECIPITATION

Box Plot (Quartiles) of Export Coefficients by Group Precipitation Normalized
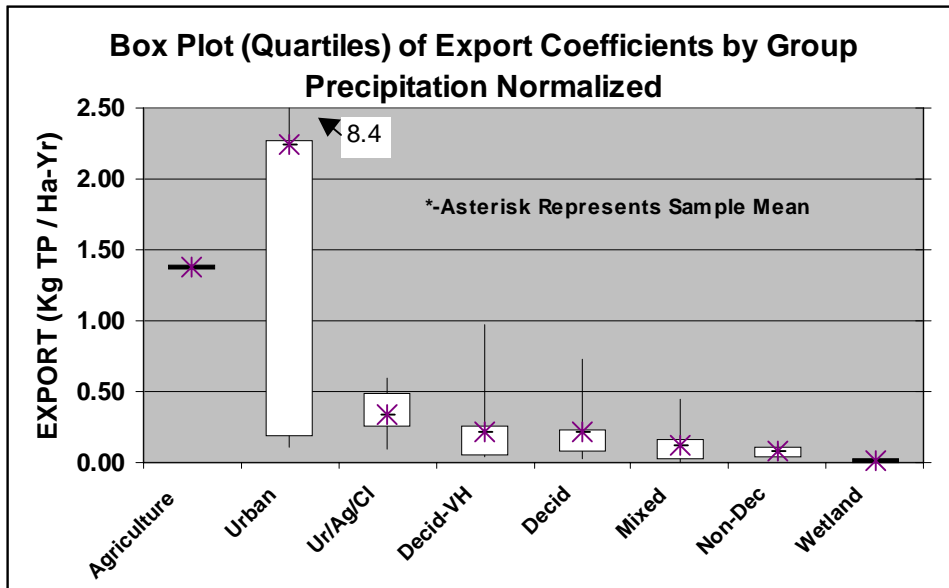
6) *Compile nutrient export coefficients that were previously recommended and/or used in earlier and ongoing NH studies to allow for the determination of under or over estimations based on the results of this study.*

Review of the NH watershed studies as well as relevant and commonly cited publications was completed and those coefficients were compared with the outcomes of this study. The survey of historical efforts indicated that coefficients used for similar land-cover / land-use categories varied between studies independent of ecoregion, date of study, and investigator. One study provided both chosen coefficients and the expected range for those coefficients. The expected range data allows for the generation of probability functions around the model estimates so they can be very important in model interpretation. Generally, only a limited range of land-cover or land-use classes were used in projects that involved measured loadings while a wider range of classes were employed for empirical based studies that involved no or limited field sampling. Table 5 displays a listing of export coefficient estimations used in the studies reviewed.

TABLE 5- TOTAL PHOSPHOROUS EXPORT COEFFICIENTS USED IN PREVIOUS STUDIES

Study	Done By	Land Type	Coefficient (Kg/Ha-Yr)	Lower	Upper
Newfound	DES 1992	Urban/Low Density Residential	0.90		
		Cleared Hay/Grass Fields	0.64		
		Forested/Wetlands	0.20		
		Forested	0.20		
Great Pond	DES 1994-95	Urban/Low Density Residential	0.50		
		Forested/Mixed	0.20		
		Wetland	0.25		
Wentworth	DES 1996-97	Urban/Low Density Residential	0.60		
		Forested/Mixed	0.25		
		Wetland/Surface Water	0.00		
		Cleared/Agriculture	0.20		
Baboosic	DES 1983-84	Forested	0.10		
		Rural/Agriculture	0.50		
Mendums	DES 1987-88	Urban/Low Density Residential	0.90		
		Forested/Mixed	0.20		
Sunapee	UNH 1989	Forested	0.05		
		Mixed Rural	0.11		
Chocorua	UNH 1997-98	Agriculture	0.80		
		Cleared	0.64		
		Low Density Development in Forest	0.20		
		Forest-No development	0.07		
		Wetlands	0.065	*Lower*	*Upper*
Flints	UNH 1992-93	Crops & Hay	0.60	0.20	1.00
		Orchards	0.30	0.05	0.30
		Pasture	0.80	0.50	1.30
		Forest	0.20	0.10	0.30
		Urban	0.90	0.35	2.70
		Wetland	0.05	0.05	0.05

Principal Findings and Significance

I. The range of export coefficients for various landcover combinations (types) found throughout New Hampshire were summarized for the first time.

II. Initial analyses of the data compiled disclosed that the differences between export coefficients could be better explained by incorporating other descriptive landscape level data available. This ranged through a series of spatial scales which ran from:
  a) Landcover classification and "typing".
  b) Generalized subwatershed slope.
  c) Location of potentially impacting land uses to channelized flow, tributaries or shorelines.

III. Disturbed land, shoreline development and riparian buffer and wetland complex extent were other factors that had influence on the loadings.

IV. The level of development intensity under forest cover oftentimes was a major factor that determined where in the range of loadings a subwatershed fell. GIS data available for analysis did not allow for accurate estimation of development extent.

V. Standardizing the export coefficients to a "normal" precipitation year decreased the variation within the data set analyzed and brought coefficients for similar land classes by different investigators using different techniques slightly closer. However, investigation into each separate study disclosed the importance of major storm events in the outcome of the loadings measured for each month when comparing multiyear studies. Thus, the utility of normalizing the data may be best for general management purposes or setting target levels while non-normalized data would be best for diagnostic lake response modeling for a given year and in management practice evaluations.

VI. Prediction improvements should occur through the use of these newly summarized coefficients and ranges over the existing coefficients used currently and in previous studies which might have overestimated forested landcover export and underestimated the range of urban and agricultural land cover export in light of these results.

Statement of Benefits:

The results of this investigation will allow for the improvement of predictive models used for watershed planning and management. The benefits of this are wide ranging from assisting watershed stewardship education efforts throughout the state and region to providing existing watershed based programs like the EPA Basins Model Initiative, the statewide Unified Watershed Assessment Initiative (under the federal Clean Water Action Program) as well as the regional initiative (US EPA Region 1 and NE states) to develop total daily maximum loading criteria (TMDLs). The information collected digitized and summarized will further our modeling efforts of New Hampshire's pristine lakes and rivers (and similar systems throughout our region). It will also serve to illustrate the importance (and justification) of proposed and existing regulations, and best management practices, to decision-makers and the public.

Relevance to Related Research:

No statewide or region-wide analysis has previously been made to develop nutrient export coefficients. Rohm, Omernik and Kiilsgard (1995), using existing nutrient data divided NH into six phosphorus "regions" based on historical in-lake phosphorous data, physiography, land-cover/land-use, vegetation, soil type and bedrock and surficial geology. However, this effort relied on in-lake phosphorus data and did not analyze actual watershed loadings at the watershed or lake tributary level. This project used more recent data from stream loading measurements and investigated relationships for characteristic watershed loading coefficients.

While much pioneering work on forest export coefficients and logging impacts on watershed water quality has been done in New Hampshire (Pierce et al 1970), these studies are limited to totally forested watersheds and logging impacts. This study includes these land cover/land use categories but also allow for the comparison to a wide range of watershed cover and use including urban, agriculture, wetlands, low intensity and high intensity development.

Reckow did develop a New England region-wide modification of his spreadsheet based lake response model EUTROMOD, called NELAKES (described in Draft Report to EPA 1990: Regional Lake Eutrophication Models). He has been very interested in seeing the development of GIS derived coefficients for model improvement (Dr. Ken Reckow, Duke University 1998-1999, personal communications). He has also communicated that work along these lines would further and improve techniques for the estimation of such coefficients as described in "Modeling Phosphorous Loading and Lake Response Under Uncertainty" (Reckow et al 1980) and improve the error estimations of existing models that are in use throughout the region.

In EPA's National Strategy for the Development of Regional Nutrient Criteria (EPA-822-F-98-002, Office of Water) it was announced that a technical guidance manual for assessing trophic state and developing region-specific nutrient criteria for lakes is under development. A draft of this document has been made available for review. Results from this project should enhance the review process in these efforts.

In addition, the results of this study should be quite useful in the development of any regional models as well as models currently used by cooperating agencies like QUAL2E (Brown and Barnswell 1987) and NPSM both of which are currently being used in the EPA BASINS GIS Watershed Analysis System (Lahlou et al 1998).

Information Transfer:

This project has already had impacts in terms of information transfer. The information is being shared between UNH and NH DES researchers and managers that deal with water quality monitoring. Particularly the Lakes Assessment Program and the Source Water Protection Program at NH DES have been involved and will incorporate the new findings in their current and future work. Initially, we will re-visit the current NE Lakes model used by both of our programs and adjust the coefficients and their ranges accordingly (also see Reckow discussion in *Relevance to Related Research* above).

There has also been interest from the New England Regional Assessment Program under the US Global Change Research Program to incorporate our findings to assist in global change projections and their modeling efforts. This is being done in conjunction with the NH DES Local Impact Assessment Project.

The watershed and subwatershed delineations created through this project as well as any of the processed GIS data will be made available to faculty, students, and cooperating agencies as these delineations go further than the current Hydrologic Unit delineations in terms of resolution (scale). They will be incorporated into a shared web site between UNH and NH DES that will allow Internet access to lake watershed data.

Much of the data compiled is also being used for an ongoing analysis of selected watersheds throughout New Hampshire for an EPA STAR program funded project looking into watershed characteristics that may influence the generation of cyanotoxins from blue green algae.

Future Research Potential

Initially, the scope of this project was to include additional landscape level analysis of impervious cover vs riparian extent to attempt to explain export coefficient variations. However, the expected improved impervious surface coverage for the state being produced by the Complex Systems Research Center under an unrelated grant was delayed. It is expected to be completed within the year though and would open up further analysis possibilities for the data generated from this project. Particularly of interest to this investigator would be the improved impervious cover coverage. Further work using this project's data may allow for the determination of the critical levels of impervious cover that most impact nutrient loadings. Imperviousness has recently become a "hot" topic in watershed management, planning and concern over "sprawl" (see discussions in Shueler (1994) and Arnold and Gibbons (1996)).

This study concluded that only limited agricultural lands export data was available in the previous watershed studies. Recent funding through the Non-point Source Program of NH DES to Cooperative Extension investigators will address this gap in data and investigate the efficiencies a range of buffer BMPs.

In addition, a new spatial model for Arcview GIS developed by the EPA, ATILA, has been made available to this researcher. We will be incorporating data generated from this effort into the new ATILA GIS model to test its usefulness at predicting phosphorous export coefficients. Finally, the SPARROW Model used on Chesapeake Bay (Smith et al 1997) is being considered by EPA New England Lake Nutrient Assessment Team for applications to lake watersheds (meetings facilitated by New England Interstate Water Pollution Control Commission 1/20/99 Lowell MA). This study's data may be of use in attempts to investigate the use of SPARROW in smaller sized watersheds.

Students Supported Directly from Project:

While only a few students were actually supported through the project's federal funding, many students participated in some aspect of the project through the use of other federal and local funds supporting the efforts of the NH Lakes Lay Monitoring Program. In general, each student worked between 10 and 20 hours a week during the fall and spring semesters and averaged 36 hours a week during the summer session. Students worked in all aspects of the project which included field monitoring of nutrients and flow, laboratory analysis, data input, data reduction and data analysis. In all, 4 undergraduate students and 1 graduate student were directly supported by the project while 12 additional undergraduate students worked on various aspects of the project.

Name	Academic Year	Major	Semester*
1999:			
Erin Clough	Senior	Marine & Freshwater Biology	Su, F
Enraku Sargent	Senior	Marine & Freshwater Biology	S,Su,F
Emese Simpter	Senior	Chemical Engineering	F

*- F= Fall, S=Spring and Su= Summer Semesters

Name	Academic Year	Major	Semester
2000:			
Jason Cabral	Junior	Forestry (Thompson School)	S, Su
Erin Clough	Senior	Marine & Freshwater Biology	S
Shane Brandt	Graduate	Zoology	Su

Additional Notes:

Emese Simpter was an exchange student from Hungary who ran nutrient samples to gain "hands on" analytical experience.

Enraku Sargent received two credit hours for his experience working on this project

Student Participation in Project/Additional Student Involvement:

Name	Academic Year	Major	Semester
1999:			
Christopher Clouser	Junior	Microbiology	F
Jennifer Drociak	Junior	Environmental Conservation	S su2000
Peggy Foss	Senior	Marine & Freshwater Biology	Su-s2000
Jennifer Lessard	Senior	Environmental Conservation	S,Su,F
Rachael Olszewski	Senior	Microbiology	S,Su,F
Stephanie Rizzolo	Junior	Environmental Conservation	S,F
Sara Sumner	Junior	Marine & Freshwater Biology	F
John Thompson	Senior	Marine & Freshwater Biology	Su-s2000
Jennifer Wishinski	Sophmore	Marine & Freshwater Biology	S,Su
2000:			
Christopher Clouser	Junior	Microbiology	S
Jennifer Drociak	Junior	Environmental Conservation	S, Su
Peggy Foss	Non-Degree	Marine & Freshwater Biology	S
Conan Flynn	Senior	Microbiology	Su
Melissa McCartney	Sophmore	Forestry	S,Su
Jeffery Oshea	Senior	Water Resource Management	S
Stephanie Rizzolo	Senior	Environmental Conservation	S
Sara Sumner	Junior	Marine & Freshwater Biology	S
John Thompson	Senior	Marine & Freshwater Biology	S

Additional Notes:

Erin Clough served as a shared intern between UNH where she worked during the Spring and Fall Semesters and the NH Department of Environmental Services (NH DES) where

she worked during the summer. While her major task was to inventory and compile the nutrient studies done by NH DES she also was given a chance to work on various programs and projects which included watershed and lake assessment work, bacteria sampling of beaches and inspection of boat holding tanks. UNH used her internship story in promotional materials for potential students. She currently holds a position at an environmental consulting firm in Boston and credits her experience gained from this project as a major factor in her acquiring this position.

Sara Sumner and Jennifer Drociak were part of the NH DES Volunteer Lake Assessment Program (VLAP) summer field team and gained additional experience as LLMP lab technicians on this project. Jen's experience helped her get subsequent jobs with NH Audubon as a summer naturalist, and more recently, employment with a component of the Merrimack River Watershed Project. Sara has been named the interim NH DES VLAP program coordinator.

Jen Lessard now works with the UNH Complex Systems Research Center following employment (and gaining GIS experience) through this project.

12 upper undergraduate and graduate students utilized the data from a subset of study watersheds as part of the requirements for completing the course: Multidisciplinary Lakes Management co-taught at UNH by the principal investigator during spring semester 2000.

<u>Theses or Dissertations Produced.</u>

None Completed – but Robert Craycraft a MS student in Natural Resources (Water Resources) utilized this project's data and additional time in the field to direct his thesis proposal. Additionally, this project may serve as the preliminary analysis to guide the Principal Investigator in further studies that will be used for his dissertation.

<u>Publications:</u>

We expect to continue some additional analyses and submit the results of this project to an appropriate journal sometime this year. We also will produce an internal publication: *Squam Lakes Watershed Project Report: Nutrient / Water Budgets* as part of a contract with the Squam Lakes Association in the spring of 2001 that will use data generated from this project. In addition as stated above the interpretation of some of our finding will be included in the final report of the final report of the New England regional Assessment Project, part of the US Global Change Research Program.

<u>Presentations:</u>

While no formal presentations involving the results of this project have been undertaken as yet, the project approach and preliminary results have been discussed in presentations and workshops given by the principal investigator:

The study was discussed as part of the presentation: "GIS Watershed Mapping: Developing and Implementing a Watershed Natural Resources Inventory". An invited 1 hour presentation for: Water Sensitive Ecological Planning & Design- An International Symposium to introduce, review and critically examine design options & planning

procedures for ensuring water sensitive development. February 25 - 26, 2000 Harvard University Graduate School of Design.

It was used as a successful model discussed in an invited lecture to University and Extension staff held during a conference on "Becoming and Engaged University": *Integration Of Undergraduate Students Into Research Projects And Community Service: The Lakes Lay Monitoring Model*. In March of 2000 at The New England Center, Durham, NH.

It was also used as an application example in an expanded three hour workshop - "GIS Watershed Mapping: Developing and Implementing a Watershed Natural Resources Inventory" which was presented at the New England Regional Lake Congress, Storrs, CT on June 3, 2000.

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Basic Information

Title:	A Survey of 50 NH Lakes for Microcystins
Project Number:	
Start Date:	9/1/1998
End Date:	8/31/2000
Research Category:	Water Quality
Focus Category:	Water Quality, Toxic Substances, Ecology
Descriptors:	biotoxins; microcystins; Microcystis; cyanobacteria; algae; eutrophication; lakes; water quality
Lead Institute:	University of New Hampshire
Principal Investigators:	James F. Haney

Publication

Title: **"A Survey of 50 NH Lakes for Microcystins (MCs)"**

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INTRODUCTION

Autotrophic microorganisms are the primary producers that comprise the basis of the food chain and energy budget in aquatic ecosystems. They utilize dissolved nutrients and photosynthesize new organic materials that support animal life at higher trophic levels. As nutrient levels and phytoplankton biomass increase with eutrophication, the dominant organisms may shift from eukaryotic protistan species to prokaryotic cyanobacteria, a.k.a. blue-green algae. The relationship between advanced eutrophication of freshwater ecosystems and the dominance of summer blooms of cyanobacteria has been demonstrated. Ordinarily, the primary productivity by algae is efficiently consumed and transformed into useable energy by primary consumers (i.e., zooplankton). The latter thrive, reproduce and provide food for higher trophic levels, e.g., larval fishes. Under optimal conditions, the water exhibits low turbidity, maintains suitable fish populations and generally has higher water quality for multiple uses.

Cyanobacterial blooms are often associated with secondary metabolites, or biotoxins, which harm animals and compromise the safe use of water for drinking or recreational purposes (Watanabe, *et al*., 1996; Chorus and Bartram, 1999). Toxic cyanobacteria blooms have been reported from at least 32 countries, including 16 in Europe, 5 Canadian Provinces and 27 of the United States. Biotoxins cause mortality of vertebrate animals (Carmichael, 1992, 1995) and may impact various planktonic, pelagic and benthic components of aquatic communities (Kotak, *et al* 1995). Dominant cosmopolitan cyanobacterial species may be sources of several types of neurotoxins and a family of liver toxins. The fast acting neurotoxins are (a) alkaloids called saxitoxins, or aphantoxins, which block sodium channels in nerve and muscle membranes (Adelman, *et al*., 1982; Sasner et al., 1984; Carmichael, 1995), (b) fast acting secondary amines called anatoxin-a, which depolarize postsynaptic nicotinic and muscarinic acetylcholine receptors, and (c) organic phosphates, called anatoxin-a(s), which inhibit acetlycholinesterase activity and enhance the effects of the transmitter acetylcholine (Carmichael, 1995; Watanabe, *et al* , 1996). Species that produce neurotoxins (*Aphanizomenon* and *Anabaena*) occur intermittently in New Hampshire, Vermont, and worldwide and cause infrequent problems. On the other hand, the slower-acting

hepatotoxins, called microcystins (MCs), produced by *Microcystis aeruginosa*, are particularly noteworthy and recognized as potent offenders around the world and commonly occur in New Hampshire lakes.

MCs comprise a family of slow acting, cyclic, heptapeptides that cause internal bleeding in hepatic tissues (Foxall and Sasner, 1982: Falconer, *et al*., 1991. The World Health Organization (WHO) in 1998 recommended safety guidelines of 1.0 µg MC L^{-1} (1.0 ppb) for drinking water (Chorus and Bartram, 1999). *Microcystis aeruginosa*, the most common producer of MCs, has been implicated in the poisoning of cattle, sheep, waterfowl, dogs, and trout (Galey, *et al*., 1987, Kotak, *et al*., 2001). The reported LD_{50} for MCs in mammals is 50 mg Kg^{-1} body weight (Carmichael, 1992). MCs have also been related to the mortality of sea-pen-reared Atlantic salmon, the anomaly called net-pen liver disease (NLD), in the northwest US and Canada (Anderson, *et al,* 1993) and the mass mortality of catfish in aquaculture ponds in the southeastern U.S. (Zimba, *et al*., 2001). MCs were also isolated from marine bivalves from the Western Pacific, the Canadian Maritimes and European waters (Chen, *et al*., 1993) although it is more commonly found in freshwater ecosystems. At the cellular level MCs inhibit protein phosphatase (PP1 and PP2a) activity (Yoshizawa, *et al,* 1990; Honkanen, *et al,* 1991) and promote tumor growth (Falconer, 1991). The impact of human exposure to *Microcystis* is noted in health reports from water supplies in Australia (Falconer, *et al*., 1993), from Harare, Zimbabwe, where blooms have been linked to seasonal outbreaks of gastroenteritis in children (Zilberg, 1986), and Brazil, where deaths occurred in numerous hemodialysis patients (Jochimsen, *et al.*, 1998). In the 1980s, toxic cyanobacterial blooms in New Hampshire were reported, intermittently, in at least 30 lakes, ponds and reservoirs, some with environmental, legal, and health-related implications. On two occasions, NH Public Health officials reported approximately 100 people with gastroenteritis and flu-like symptoms that occurred after contact with lake water at NH State Parks in which *Microcystis* blooms dominated the phytoplankton. Few studies have examined levels of MCs in lake compartments, since they are considered to be intracellular toxins that are released, sporadically, into the surrounding water when cells lyse. We have measured MC levels in Silver Lake, Hollis, NH and found significant concentrations in *Microcystis* cells (2500 µg MC g^{-1} dry wt), zooplankton (60 µg MC g^{-1} dry wt), benthic bivalves (7 µg MC g^{-1} dry wt.) and dissolved in lake water filtrates (0.4 - 20 µg MC L^{-1}) Capron, 1995, Nye, 1997). The latter result attracted our attention, since it exceeds the guideline of 1 µg L^{-1} suggested for safe exposure for recreation and drinking.

Most field studies of cyanobacteria toxins have examined nutrient-rich lakes with conspicuous surface blooms, with the assumption that biotoxin problems are confined to conditions of excessive phytoplankton growth and are directly linked to eutrophication. There are several lines of evidence that suggest this is not always the case, and should be tested. For example, Prepas et al (1997) used freshwater mussels as biomagnifiers of MC and demonstrated the presence of MCs in oligotrophic lakes in Canada. In addition, not all cyanobacteria species require high nutrient levels. For example, *Microcystis aeruginosa*, an important producer of MCs, is present in most of the lakes in New

Hampshire, and is the dominant phytoplankton in the meso-eutrophic Silver Lake as well as in Russell Pond, an ultra-oligotrophic lake. We have detected significant quantities of MCs in >30 NH lakes with varied trophic conditions. There is little known about the incidence of MCs in NH lakes, their effects on aquatic organisms, or how the toxins may be transferred or accumulated, although it appears that aquatic invertebrates pass these toxins through the food web by their grazing activities (Kotak et al. 1996). The presence of biotoxins, like MC, even in dilute systems may be problematic both as direct health threats to vertebrate animals, and as harmful secondary metabolites that may alter lake ecosystems. The recent development of MC ELISA methods increased assay sensitivity by three orders of magnitude. This increased sensitivity allows detection of MCs in lake water, phyto- and zooplankton components from a broader trophic range of lakes than was possible previously.

The project objective tests the hypothesis that proposes a direct relationship between nutrient concentrations, chlorophyll *a* and the presence of microcystins (MCs) in lakes. More specifically, we tested whether widely used eutrophication models describing a log-log relationship between the concentration of chlorophyll-a and total phosphorus (TP) concentration were also valid to predict the concentration of MCs in lakes. Statistically, the null hypothesis is that there is no difference in the slope of the linear regression between total phosphorous (TP) *vs.* chlorophyll *a* (Chl *a*), and TP *vs.* MC concentrations. A second objective aims to identify other variables that contribute to the MC toxicity found in the lakes, such as N: P ratio, species composition of the phytoplankton and zooplankton, lake basin characteristics, watershed features, and geographic region. The N: P ratio may influence the composition of the phytoplankton, as N-fixing cyanobacteria may be favored at low N: P ratios. *Microcystis*, one of the important producers of MCs, is not, however, considered to be effective at N-fixation in oxygenated waters since it lacks heterocysts. The wide range of lake basins and trophic conditions in the lakes of New Hampshire (Figure 1) offer an exceptional opportunity to test these hypotheses dealing with the nutrient control of biotoxins. To provide a representative data set for testing these hypotheses and constructing models, we selected 50 lakes from the databases of the UNH-Lakes Lay Monitoring Program (LLMP) and the NH Division of Environmental Services. These represent a broad range of lake basins, trophic conditions and watersheds characteristics. Study lakes represent the major regions in New Hampshire i.e., Northern Forest, White Mountains, Coastal Plain, and Southern In-land (urban and suburban) regions.

MATERIALS and METHODS

Study Sites

We selected study lakes from across New Hampshire to represent a range of trophic conditions and lake morphometry (Table 1-3). Field sampling was performed during the spring-summer seasons in 1999 and 2000.

Multiparameter Profiles and Light Measurements

Deep sites in each lake were located using a Humminbird 200DX sonar depth finder. A multi-parameter water quality probe (YSI-6600 Multiprobe), equipped with an YSI-610DM data logger, was used to take a single profile of the water column in each lake. The probe was lowered at a rate of approximately 0.5 m min^{-1} with the data logger recording eight parameters at 5 s intervals. The physical and chemical, parameters included depth (pressure), temperature, dissolved oxygen as concentration (DO), and percent saturation (% Sat), pH, oxidation-reduction potential (ORP), specific conductivity (corrected to 25° C), chlorophyll *a* (chl *a*), and turbidity. Maximum depth of the epilimnion was noted during the profiling. Light profiles were taken with a LICOR LI-1000 data logger equipped with a deck cell and an underwater 2π quantum photo sensor, designed to measure photosynthetically active radiation (PAR) at 400-700 nm. To compensate for immersion effects, separate air and underwater sensor calibration factors were used. The deck cell readings were used to correct for changes in the ambient light reaching the water's surface. Three replicate profiles were done by lowering the photo sensor through the water column on the sunny side of the boat and readings were recorded on the data logger at every 0.5 m interval until the light level was less than 0.1 μmol photons m^{-2} s^{-1}, or the sensor reached the lake bottom. Linear regressions of depth (m) versus ln light intensity were used to estimate the extinction coefficient (k_{ext}) for each lake. Water transparency was measured using a standard black-and-white Secchi disk (20 cm diam.) that was lowered into the water on the sunny side of the boat. A PVC view scope was used to eliminate effects from glare, wind, and wave action. The average of three readings depth (m) at which the Secchi disk disappeared from view was recorded as the Secchi disk depth (SDD).

Integrated Water Sampling and Analysis by the NH-LLMP

Integrated epilimnetic water samples were collected for the analyses of chlorophyll *a* (chl *a*), dissolved color, nitrate nitrogen (NN), total phosphorus (TP), total nitrogen (TN), acid-neutralizing capacity or alkalinity (ANC), and whole lake water (WLW) MC. One to several integrated epilimnetic water samples were collected and pooled to provide a 2 L composite reservoir of epilimnetic water for several analyses described below. To collect each integrated water sample, a weighted Tygon tube (2 cm O.D.) was lowered to the bottom of the epilimnion and clamped-off at the upper end to seal the water within the tube. The bottom end of the tube was retrieved using an attached line, the contents emptied into a 2-L plastic bottle, and stored on ice. The composite integrated epilimnetic samples were thoroughly mixed before being sub sampled for analysis of the components listed above. Collection of integrated water and sample preparation for chemical analyses are depicted in Figure 2.

Chemical analyses for chl *a*, dissolved color, NN, TP, and TN were performed by the Center for Freshwater Biology Analytical Laboratory at the University of New Hampshire following protocols listed in a generic limnological quality assurance project plan reviewed and on file with EPA New England (Region 1). Except where noted, standard methods (APHA 1998, EPA 1983) were employed.

Chlorophyll a

An integrated water sample (500 ml) was vacuum-filtered through a 0.45 µm cellulose membrane filter (Millipore Corp.) and the filters immediately stored in a darkened, portable desiccator before extraction for chl *a*. The filters were dissolved and the sample ground manually in a glass-to-glass tissue grinder (Kontes) containing acetone (15 ml of 95%) with $MgCO_3$ buffer (1%, vol/vol). The sample was mixed and stored (4° C; in dark; 4 h) to allow for chl *a* extraction. The sample was centrifuged (500 x g, 20 min.), the supernatant transferred to a near-UV cuvette (5 cm path length) and read at 663 and 750 nm using a Spectronic 1001+ spectrophotometer (Thermo Spectronic, Rochester NY) with a 2 nm bandwidth. Chl *a* concentrations were determined using the monochromatic chlorophyll equation (APHA method 10200H.2.b). A chl *a* absorption coefficient of 11.9 was used in the calculations based on research from Vollenweider (1969).

Dissolved Color
Fifty ml of filtrate of the composite, integrated water sample was transferred to a 60 ml high-density polyethylene (HDPE) bottle and stored on ice in the field then refrigerated in the laboratory. Samples were analyzed in quartz cuvettes in a Spectronic 1001+ spectrophotometer (2 nm bandwidth, 5 cm path length) at wavelengths of 390 nm, 440 nm, 750 nm and 880 nm (Thermo Spectronic, Rochester NY). Dissolved color in choroplatinate units (CPU) was calculated using a multiplication factor of 859 and the absorbance at 440 nm. The 750 nm measurement acts as a check for turbidity in the sample to insure that it was filtered correctly. The value of 859 was a color absorbance coefficient derived from a standard curve from fresh chloroplatinate standards (APHA 1998). The absorbencies at 750 nm and 880 nm were used to determine if there was significant particulate matter, or contamination, in the samples. The 390 nm measurement is used in other studies to determine a slope derivative for possible organic matter type identification.

Nitrate Nitrogen (NN)
Fifty ml of filtrate (0.45 µm) from the composite, integrated water sample was transferred to an acid-washed HDPE bottle (60-ml) and stored on ice in the field, and frozen (–40° C) in the laboratory before NN analysis. Samples were thawed and filtered again through Whatman 0.7 um 47 mm GF/F syringe filters. Sub samples (30 ml) were acidified (0.6 ml of 36 N H_2SO_4) and analyzed in quartz cuvettes in a Varian Cary 50 scanning spectrophotometer (5 cm path length). Samples were scanned at wavelengths between 190-250 nm and NN concentrations calculated from a standard curve using the sample's 2nd derivative peak between 226-229 nm following the methods of Crumpton *et al*, (1992) and Bachmann and Canfield, (1996).

Total Phosphorus (TP) and Total Nitrogen (TN)
Composite integrated epilimnetic water samples were placed in acid-washed, 500 ml HDPE bottles, stored on ice in the field, and frozen (–40° C) in the laboratory before analysis for total phosphorus (TP) and total nitrogen (TN). After thawing, H_2SO_4 (2 ml of 36 N) was added to the sample bottle to prevent any biological activity. The procedure for TP analysis followed Standard Method 4500-PB.3-E. Sub samples (50 ml) were acid

digested (1 ml of 11 N H_2SO_4 + 0.5 g of ammonium peroxydisulfate), autoclaved at 121° C for 0.5 h, pH neutralized with 10 N NaOH, and then reacted with a mixture of ammonium molybdate, ascorbic acid, and potassium antimonyl tartrate (together the color complex). Absorbance at 660 nm was measured in near-UV cuvettes (5 cm path length) in a Varian Cary 50 spectrophotometer and TP concentrations interpolated from a standard curve derived from a serial dilution of a phosphate standard. For TN analysis, sub samples (30 ml) were acid treated with a basic (2.0 N NaOH) potassium persulfate solution (4.5 ml of 6 g 100 ml^{-1}) and autoclaved (123° C, 0.5 h) to achieve complete digestion. Samples were pH neutralized with 2 N NaOH and analyzed in quartz cuvettes following the same method used in NN analysis, but with a different standard curve derived from a nitrate standard that has been digested as described above to better reflect post-digestion condition. (Crumpton et *al*, 1992; Bachmann and Canfield, 1996).

Acid Neutralizing Capacity (ANC)

Acid-neutralizing capacity (mg $CaCO_3$ L^{-1}) was determined from composite, integrated water samples (50 ml) transferred to white, HDPE sample cups (250 ml) to which 4 to 6 drops of a methyl-red-bromcresol-green indicator were added. Samples were titrated with H_2SO_4 (0.002 N) to a gray end-point (pH ~5.2; Lind, 1979).

Whole Lake water MC (WLW MC)

Composite, integrated whole lake water (WLW) samples were placed in HDPE sample cups (250 ml) and immediately placed on ice in the field and stored frozen (–40° C). The WLW samples were frozen/thawed (3X) to release intracellular MC. Samples (15 ml) were thoroughly mixed, transferred to borosilicate glass serum bottles (30 ml), and frozen on edge to maximize exposed surface area . The frozen samples were lyophilized in a freeze-dry system (LabConco Freezone 4.5) under vacuum (~30 X 10^{-3} mbar) at -50° C for 18-24 h. Dried material was then re-hydrated with 1.5 ml of water and mixed on a shaker table for 16-24 h. This procedure achieved a 10-fold concentration of materials and increased the sensitivity range of the ELISA methods used. About 1 ml of the re-hydrated sample was filtered through a 13 mm, 0.2 μm Whatman PTFE syringe filter into a 1.5 ml polypropylene centrifuge tube, which was stored frozen (–40° C) before ELISA analysis for MC.

Net (>50 mm) Phytoplankton and Zooplankton MC

Collection of net plankton samples and the preparation of materials for analyses are depicted in Figure 3. Integrated net plankton tows were taken from ~1 m above lake bottom to the surface using a 30 cm diam. plankton net with 50-μm Nitex mesh (Aquatic Research Instruments, ARI). Contents of the cod-end bucket were rinsed into a separatory bottle, composed of an inverted 2 L polycarbonate bottle with a clamped Tygon tube at the bottom and a hole at the top for adding the sample. The volume of sample was brought to 1L with filtered lake water. The bottle was then covered with black plastic to darken the interior, except for 2-3 cm at the bottom. After 15 min, the phototactic zooplankton migrated toward the light at the bottom of the bottle and into the Tygon tube, leaving the phytoplankton suspended (Capron, 1995). The first 100-125 ml of sample released from the Tygon tube contained most of the zooplankton that were collected on a

piece of 50 µm Nitex mesh. The mesh, containing the zooplankton, was blot-dried on a sponge and placed in a polypropylene sample vial (6 ml). The remaining contents of the separatory bottle (predominantly phytoplankton) were passed through a second piece of Nitex mesh and placed into another polypropylene vial (6 ml).   Zooplankton and phytoplankton vials were stored on ice and frozen (-40° C) in the laboratory.

        Plankton (phyto- and zooplankton) samples, concentrated on the Nitex meshes, were frozen/thawed (3X) and 0.02-0.045 g of each sample was extracted in a centrifuge tube (1.5 ml) with methanol (0.25 ml of 80% MeOH) (room temp., 18-24 h). Following MC extraction, 0.75 ml of phosphate buffer solution (PBS) was added and a 0.3 ml sample was filtered through a 13 mm, Whatman PTFE syringe filter (0.2 µm) into a new centrifuge tube (1.5 ml). Additional PBS (0.45 ml) was added to bring the final MeOH concentration to 8% (Honkanen, *et al*, 1995 and Metcalf, *et al*, 2000). The storage tubes were then frozen (–40° C) until ELISA analysis for MC.

Enzyme-Linked Immunosorbent Assay (ELISA) for MC
        All ELISA analyses for microcystins were performed using Microcystin 96-Well-Plate Kits (EnviroLogix Inc. Portland, ME). The three MC kit standards provided (160, 500, and 1600 pg ml$^{-1)}$ were further diluted with water to make 4 additional standards (15, 25, 53, and 100 pg ml^{-1}) to expand the standard curve and increase the sensitivity range of the assay. For MC analyses the 96-well-plates were warmed to room temperature, diluent (125 µl) was added to each well using a Titertek Digital Multichannel Pipette, and 20 µl of each sample or standard were added to separate wells. Each of the 7 standards (3 commercial + 4 prepared) was run in duplicate wells and compared before preparation of the final standard curve, against which all unknown samples were measured. The 96-well-plate was covered with parafilm and incubated (0.5 h, room temp.) on an orbital shaker (~200 rpm) to allow binding of MC-antibody to MC in the samples and standards. Following incubation, MC-enzyme conjugate (100 µl) was added to each well and incubated again (0.5 h, room temp.) to allow MC-enzyme conjugate binding to any free MC-antibody sites not occupied by the samples or MC standards. Well contents were removed and the plates were rinsed (3X) with a saline wash solution to remove unbound material. The plates were carefully emptied and chromogen-substrate (100 µl) was added to each well before a final incubation (0.5 h, room temp.) to allow chromogen-substrate, a colored indicator, to bind to the MC-enzyme-conjugate previously bound to MC-antibody. The binding of chromagen-substrate to the MC-enzyme conjugate produced a blue color in the wells. The intensity of the blue color was inversely proportional to the MC concentration, since the amount of MC-enzyme-conjugate binding in the wells was inversely proportional to the MC concentration in the original samples or standards. Finally, stop solution (100 µl of 1.0 N HCl) was added to each well to produce a yellow color. The OD of the 96-well-plate was read on a Bio-Tek EL800 Plate Reader (Winooski, VT) (sensitivity of ± 0.010 Abs) at a wavelength of 450 nm. MC concentrations were calculated based on a cubic log-log standard curve produced by the program KC Junior, a companion program to the Bio-Tek EL800 Plate Reader. The 96-well-plate was also read at a dual wavelength of 630 nm as a reference to remove any interference from bubbles in the sample or scratches on the plastic.

Net Plankton (>50 µm) Sampling for Enumeration

Integrated net plankton tows were taken with an ARI plankton net (30 cm diam., 50 µm Nitex mesh) following the same procedure described above for phyto- and zooplankton MC. Net contents were thoroughly rinsed into the cod-end bucket by pouring lake water onto the outside of the net so that no additional net plankton was added. Cod-end bucket contents were rinsed into sample cups (250 ml), preserved with 4% formalin-sucrose (3 ml per 50 ml sample), and stored at room temp. Before net phytoplankton and zooplankton counts were made, the samples were adjusted to a volume of 150 ml by either adding water or removing excess lake water with a syringe pipette (20 µm).

For phytoplankton enumeration, a Hensen-Stempel pipette was used to transfer thoroughly mixed sample (1 ml) into a Sedgewick-Rafter counting chamber. Some samples were diluted with water to provide the desired range of 150-500 total algal cells per chamber. One ml sub samples were counted and the phytoplankton identified to the genus level at 100X magnification using a compound microscope. For zooplankton enumeration, 1 ml of the thoroughly mixed, original sample was transferred into a Sedgewick-Rafter counting chamber using a Hensen-Stempel pipette. Cladocera and copepods were counted and identified to genus level at 40X magnification using a compound microscope. Samples were counted until at least 50 total organisms were recorded. Counts were used to estimate lake concentrations of each zooplankton genus, which were then multiplied by a factor of 2.18 to correct for the efficiency (~45%) of the 50 µm plankton net used to collect samples.

For enumeration of benthic *Microcystis*, samples fixed with 4% formalin-sucrose were thoroughly mixed, allowed to settle 24 h. In formalin-sucrose, *Microcystis* colonies float near the surface. The liquid was decanted and passed through Nitex mesh (50 µm) to collect the cyanobacterial colonies. The net contents were then rinsed with water into a Sedgewick-Rafter counting chamber. The total number of *Microcystis* colonies in each sample was counted at 100X magnification using a compound microscope.

Information from Databases

Watershed landcover analysis was performed using data provided by GRANIT NH, the centralized database for NH GIS data. Landcover information was produced by GRANIT from 1986-1990 LANDSAT Thematic Mapper data with an approximate resolution of 0.2 acres. Watershed boundaries were screen digitized in ArcView 3.2 based on 1996 1:24,000 USGS 7.5 minute topographic quadrangles. Final categories used for regression analysis were as follows: agriculture, cleared, forest, wetland, urban, water and other.

RESULTS AND DISCUSSION

The Study Lakes

Over 50 lakes in New Hampshire were surveyed. All of the lakes had detectable quantities of microcystins. For completeness and comparability, these results summarize

data collected from 44 different lakes plus multiple basins within three lakes (Squam Lakes, Swains Lake and Umbagog Lake) making a total of 47 lake basins (Table 1). Thirty of these lakes were sampled on two or three dates, with a total of 85 lake sampling dates for the two sampling years in 1999 and 2000. The lakes selected for this study were distributed throughout New Hampshire, including the contrasting southern (urban/suburban), coastal plain, western rural, White Mountains lakes region and the Northern Forest regions (Figure 1). The study lakes represented a wide range of sizes (5.8-2737 ha, 2.7-50 m maximum depth) and trophic conditions, from ultra-oligotrophic to eutrophic lakes (Figures 9-12, Tables 1-3). Based on mean TP (10.62 µg L^{-1}), TN (315.5 µg L^{-1}) Chl a (4.26 µg L^{-1}) and water transparency (5.03 m), the average lake in this study could be classified as oligo-mesotrophic. The study lakes closely match the mean values for the New England Highlands Ecoregion (TP 10.62 µg L^{-1}, TN 349.7, Chl *a* 4.46 µg L^{-1}, Secchi disk depth 4.36 m) as defined in the EPA Nutrient Ecoregions (NEIWPCC, 2000). Because of the contrasting weather conditions in 1999 (exceptionally dry and sunny) and 2000 (exceptionally wet and cloudy), we analyzed the combined data as well as for each year. Although TP was higher and TN was lower in the wet year (2000), these differences were not significant (t-test, $p < 0.05$, Table 2). Direct year comparisons are difficult, however, since not all lakes were sampled in each of the years. Based on our observations that cyanobacteria populations become abundant after mid-July, we have divided the data set into two seasons into spring (May – July 13) and summer (July 14 – October), similar to Jacoby et al. (2000) for their study of microcystins in a Washington State lake. There were no significant differences (t-test, $p < 0.05$) in the mean trophic parameters with season (Table 2). However, the range (max-min) of nutrient concentrations (TP, TN, NN) and chlorophyll *a* was much larger in the summer sampling period.

Toxin Concentrations
        Microcystins (MC) were found in lake water and plankton of all the lakes sampled. On only three of the 85 sampling dates for the 47 lakes were the levels of MC whole lake water (WLW) extrapolated from a standard curve, i.e. <1.5 ng L^{-1}. Mean MC concentration in the WLW for all sampling dates was 13.2 ng L^{-1}, ranging from 0.2-114.1 ng L^{-1} (Table 4, Figure 9). Mean summer concentrations of WLW MC were higher than spring WLW MC (10.2 vs 15.6 ng MC L^{-1}, although these were not significantly different (t-test, $p < 0.05$). MC concentrations in the net phytoplankton ranged over four orders of magnitude (Figure 10) from 0.8 (Newfound Lake) to 31472.0 ng g^{-1} w.wt (Silver Lake, Hollis) and were generally 4-5 X the concentration of MC in the zooplankton (0.9 - 8980.7 ng g w.wt^{-1}, Figure 11). Mean MC concentrations in the phytoplankton and zooplankton for all dates were 1577.4 and 330.1 ng g^{-1} w.wt., respectively. MC tended to be higher in the summer in the water and plankton. During the summer, mean phytoplankton MC was over four times the mean spring concentration (2375.5 vs 542.1 ng g^{-1} w.wt.) and median summer phytoplankton was approximately seven times the spring median concentration (242.4 vs 34.2 ng g^{-1} w.wt., Table 4, Figure 10). Average zooplankton MC was only slightly (not significant, test, $p < 0.05$) higher (293.8 vs 357.4 ng g^{-1} w.wt.) in the summer. Mean and median MC concentrations in the zooplankton were approximately 20 and 17%, respectively, of the phytoplankton MC.

MC concentrations were generally lower in the 2000-sampling season in all lake compartments measured (Table 3). This is quite likely related to the differences in weather during the two years, although this effect is difficult to isolate, due to other differences in the sampling years, e.g. 1999, sampling season was 2 June – 4 October, n = 65; 2000 sampling season 8 May – 24 August, n = 20.

Nutrient Control and the Eutrophication Hypothesis

The major hypothesis posed in this study was that the abundance of microcystin toxins in lakes is linked to the abundance of nutrients, comparable to the relationships established between nutrients and chlorophyll a. More specifically, we set out to (a) test whether there are correlations between nutrients (TP, TN, TN:TP) and MC and (b) compare these relationships with eutrophication models developed to predict chlorophyll a. TP and TN were significantly correlated with MC levels in the lake water and in the plankton for 1999 and the combined 1999-2000 data. The total phosphorus vs chlorophyll a relationship for the New Hampshire lakes in this study was highly significant ($p<0.001$) and predictive ($r^2=0.65$). The NH lakes model compared most closely with the OECD model of Vollenweider and Kerekas (1982) and Chow-Frazer (1991) (Figure 11).

Overall, log-log (power function) relationships were found between MC in the three lake compartments and both TP and TN, supporting the eutrophication hypothesis. No significant relationships were found with nutrient concentration and MC in the 2000 data set. Regression analysis thus focused on the 1999 and combined 1999-2000 data. Also, the ratio of TN:TP was not significantly ($p<0.05$) correlated with MC in the water or plankton for any of the years or seasons.

Total phosphorus was significantly correlated with the WLW MC concentration in both the spring and summer periods (Figure 12, Table 4). TP was most predictive of WLW MC during the summer ($r^2=0.31$, $p<0.001$), based on the 1999 samples. Total nitrogen was also highly correlated with WLW MC, explaining 44% of the variability in this toxin in 1999 (Figure 13, Table 4).

TP was also significantly correlated with the concentration of MC in the phytoplankton, accounting for 25% ($p<0.001$) of the summer phytoplankton MC variation and 16% ($p<0.05$) in the spring, 1999 (Figure 14, Table 5). Using the combined years, TP was still significantly correlated, but only slightly less predictive ($r^2=0.15$, $p=0.001$, Table 5, Figure 15). TN was the best predictor of phytoplankton MC in the 1999 spring ($r^2=0.36$, $p=0.01$, Table 5, Figure 16) and combined seasons for 1999 ($r^2=0.25$, $p<0.001$) and combined years ($r^2=0.22$, $p<0.001$, Table 5, Figure 17). Nitrate nitrogen (NN) was not correlated with the concentration of MC in the lake water or plankton.

Zooplankton MC was not so strongly linked with nutrients as the other two MC compartments. TP accounted for 15% ($p=0.005$, Table 6, Figure 18) of the zooplankton MC in the summer for the combined years and 13% ($p=0.008$) of the summer zooplankton MC in 1999 (Figure 19, Table 6). TN was a stronger predictor of zooplankton MC than TP in the spring for 1999 ($r^2=0.24$, $p<0.04$, Table 6, Figure 20) and combined years ($r^2=0.22$, $p<0.006$, Table 6, Figure 21).

Influence of Chlorophyll a, ANC and Water Transparency

Whereas TP was most strongly related to MC in the summer, chlorophyll *a* was highly correlated with WLW MC, phytoplankton and zooplankton MC in the spring, accounting for 35%, 30% and 21% of the MC variability, respectively (Figures 22-24, Tables 4-6). In the summer and combined seasons, chlorophyll a had significant, but lower correlations with MC concentrations.

Acid neutralizing capacity (ANC) was positively correlated with the WLW MC (r^2=0.12) when the seasons were combined (Table 4, Figure 25). Spring ANC was an even better predictor of the weight-specific MC concentration in the phyto- and zooplankton (combined years, r^2=0.27 and 0.26, respectively, Tables 5-6, Figures 26-27), as well as the MC concentrations in the phyto- and zooplankton per unit volume lake water (r^2=0.30 and 0.28, respectively, Figures 28-29).

Water transparency (SDD) was negatively correlated with MC in the lake water, phytoplankton and zooplankton, explaining the greatest percentage of the MC variability in the summer period (r^2=0.21, 0.21 and 0.14%, respectively, for the combined years, Tables 5-6, Figures 30-32).

Effects of Plankton Composition

The composition of the phytoplankton present in each lake was determined from microscopic examination. The percentage of cyanobacteria in the net phytoplankton was not correlated with the amount of MC in the lake water. This suggests that MC was not produced equally by all cyanobacteria in the lake or those smaller forms, such as the nanoplankton or picoplankton, also play an important role in microcystin production. The concentration of MC in the zooplankton was positively correlated with the % cladocerans in the spring zooplankton (p<0.01, r^2=0.21) and even more strongly correlated with the % *Daphnia* present in the spring (p<0.001, r^2=0.35). The percentage calanoid copepods was negatively correlated with the MC in the zooplankton during the spring period (p<0.01, r^2=0.24). Percentage of cyclopoid copepods was not correlated with the zooplankton MC concentration. Also, during the summer, there were no significant correlations between the zooplankton MC and composition of the zooplankton. These findings indicate the transfer of MC from the phytoplankton to the zooplankton is strongly influenced by the types of zooplankton grazers present.

Lake Morphometry and Watershed Features

Of the lake morphometric features examined (mean depth, maximum depth, area, volume), mean depth was most highly correlated with MC, i.e., MC concentrations generally decreased with increasing mean depth. Strongest relationships with mean depth were found with the spring period (p<0.01), with r^2 values of 0.18, 0.29 and 0.17 for the WLW MC, phytoplankton MC and zooplankton MC, respectively (Table Tables 4-6, Figures 33-35). The closer linkage between mean depth and phytoplankton MC concentration per unit biomass than with the MC concentration in the lake water, per unit volume, suggests the phytoplankton MC production is somehow enhanced by the greater contact with the bottom sediments, either through greater growth rates or by promoting specific cyanobacteria that produce microcystins.

We also determined watershed land cover features of 25 of the selected lakes using GIS. Watershed land cover types ranged from highly urbanized lakes (e.g., Horseshoe Pond, 49.2% urban) to lakes with largely forested watersheds (e.g., Lake Chocorua, 94.2%). Of the study lake watersheds none had agricultural use in excess of 10%, reflecting the general decline of farming in New Hampshire. At the watershed level, percentage of any land cover type was not significantly correlated ($p<0.05$) with the concentration of MC in any of the lake compartments.

GENERAL DISCUSSION

Evaluation of the Eutrophication Model

The phosphorus chlorophyll a models of Dillon and Rigler (1974) and Vollenweider and Kerekas (1982) have been widely used to implicate phosphorus as an important nutrient regulating the abundance of phytoplankton in lakes. These models have also been useful for predicting the impact of increasing or decreasing the phosphorus concentration. Data from the New Hampshire microcystin survey generally support the application of the eutrophication model to microcystin toxins. Best fits for both chlorophyll a and MC were with power function, i.e., log-log, regressions, suggesting similar patterns of change in chlorophyll *a* and MC occur with increasing total phosphorus concentration. As with any power function, slow responses to phosphorus increase occur at low phosphorus levels and increase rapidly at higher concentrations.

It is noteworthy that total nitrogen was, in fact, a better predictor of MC in all three MC compartments. This suggests that nitrogen may be a more useful tool for forcasting MC concentrations. The relatively high correlations between TN and WLW (0.57) MC as well as phytoplankton MC (0.64) also suggestive that nitrogen may play a key role in the promotion of microcystin production in these lakes. This is also supported by Lee et al. (2000), who demonstrated that the MC content of *Microcystis aeruginosa* in laboratory cultures was highly correlated with the TN content of the medium. Similarly, Codd and Poon (1988) and Watanabe and Oishi (1985) reported that the microcystin toxicity of M. *aeruginosa* decreased dramatically when nitrogen was decreased in the culture medium. Since heterocyst bearing cyanobacteria, such as *Anabaena*, might be expected to benefit competitively from a scarcity of available nitrogen, the correlative evidence with TN suggest non-heterocystous cyanobacteria, such as *Microcystis*, may be the important MC producers in the New Hampshire lakes and that MC production may be limited by the nitrogen content of the lake.

The ratio of total nitrogen to total phosphorus is often postulated as an important environmental parameter controlling the abundance of cyanobacteria, since low TN:TP would favor heterocystous, N-fixing cyanobacteria, such as *Anabaena* or *Aphanizomenon*. This hypothesis was supported by Kotak *et al*, (2000) who found that 70% of the variation in MC-LR was explained by the TN:TP in the lake water. This is especially interesting, considering that the MC-LR in their lakes was strongly correlated with the biomass of *Microcystis aeruginosa*, a non-heterocystous cyanobacterium. In contrast, we found no significant relationship between MC concentrations and the TN:TP ratios in New Hampshire lakes. There are several possible explanations for these differences. First, there was a very small range of TN:TP (max TN:TP approx. 25) in the

Canadian lakes, due to the high phosphorus concentrations in these eutrophic lakes, whereas the New Hampshire lakes were generally much higher (mean TN:TP = 37.6, max. 136), reflecting the more oligotrophic nature of these waters. At such high ratios one might expect phosphorus to be the primary limiting nutrient. In contrast to the Canadian lakes, very few of the New Hampshire lakes were dominated by a single cyanobacteria species, such as *M. aeruginosa* or other large colonial forms. Possibly, the MC-producing cyanobacteria in our study lakes were largely non-N-fixing species that derived no competitive advantage from a low TN:TP ratio. This idea is also supported by the positive correlation between nitrate nitrogen (NN) and the concentration of MC in the plankton.

Although TN and TP were highly correlated with MC, less than one-half of the variability in toxin concentration was explained by these nutrients. Clearly, for any given lake other factors play an important role in determining the MC content. For example, shallow lakes and relatively clear lakes (e.g. Silver Lake, Hollis tended to have relatively high concentrations of MC, considering the TP concentration. Even within the same lake, the shallow basins tended to have the highest concentrations of MC (Squam Lake, Squaw Cove cf. Squam Lake, Sandwich Bay). It appears that lakes with sediments exposed to sunlight promote the growth of cyanobacteria such as *Microcystis* when they are in the benthic phase of their life cycle. This could account for relatively high MC levels in the lake water and plankton in remote lakes, such as Little Diamond Pond, with median TP levels, but the 4th highest WLW MC and 3rd highest phytoplankton MC of the 47 lakes tested. Similarly, Russell Pond, one of the most oligotrophic lakes examined (Secchi disk depth of nearly 15 m, TP=2 ppb) had relatively toxic phytoplankton, compared to the other lakes. The great water clarity in Russell Pond exposes 100% of its sediments to 0.1% of surface light intensity and over 70% of its sediments to 1% surface light, despite a maximum depth of 23.7 m.

The differences in the data collected for the two years indicate climatic factors such as precipitation, temperature and solar light flux may also be important parameters that should be included in future models to predict toxin levels. In previous research on Silver Lake, Hollis, MC levels fell below detectable limits (using HPLC methods) during the 1992 with exceptionally low solar input and low water temperatures associated with major volcanic activity (Capron, 1995).

CONCLUSIONS AND RECOMMENDATIONS

Results from this survey indicate the presence of microcystin toxins in all of the lakes examined. These findings shift the emphasis from asking, "which lakes have toxic cyanobacteria?" to "what controls the level of cyanobacteria toxins in lakes?" By measuring the MC levels in the lake water as well as the weight-specific concentrations in the plankton we were able to demonstrate that some lakes have very small amounts of plankton that are relatively toxic and similarly, some lakes with large quantities of plankton with low specific toxicity.

Although eutrophication has been linked with problems of toxic cyanobacteria, the focus of most previous studies has been on "problem" lakes that exhibit blooms of cyanobacteria. We have demonstrated that microcystin toxicity parallels the relationship

between nutrients and phytoplankton biomass (chlorophyll *a*) and extends from ultra-oligotrophic to eutrophic lake conditions. This allows for a quantitative forecasting of the impact of nutrient enrichment on lake toxicity, which could be important for the management of surface water supplies for drinking water and recreation.

Because of the emphasis on phosphorus as a limiting factor for phytoplankton growth in lakes, we initially tested our microcystin-eutrophication hypothesis using total phosphorus as the driving nutrient. Surprisingly, nitrogen (total nitrogen) provided a better predictor of toxin concentration than phosphorus, suggesting future lake monitoring and research should also include the testing for total nitrogen.

The New Hampshire microcystin survey also demonstrated that microcystin toxin concentrations are correlated with other parameters commonly measured in lake monitoring programs, such as chlorophyll a, Secchi disk depth and acid neutralizing capacity. This is a significant finding in that it indicates the results for lake monitoring surveys can be applied to predict the likelihood of toxicity problems in a lake.

Microcystins were detected in an extremely broad range of concentrations in the net phytoplankton and zooplankton of all of the lakes tested. Overall, the zooplankton contained approximately 20% of the phytoplankton microcystin content, indicating considerable amounts of this toxin are passing into the lake food web and possibly being bioaccumulated by other lake biota, such as fish and benthic consumers. Some of the study lakes had high levels of microcystins in the zooplankton, compared to other lakes with comparable nutrient levels, raising questions concerning the roll of the composition of the lake food web in the transfer of toxins. The strong positive correlation between zooplankton MC and the % *Daphnia* in the lakes suggests the species composition of the zooplankton grazers may influence the transfer of microcystins. Likewise, it is likely that the degree of fish planktivory in the lake may indirectly impact the efficiency of movement of MC from the phytoplankton to the zooplankton grazer community. Clearly, investigations of microcystin transfer through lake food webs are needed to better understand these processes.

Our data support the model that, in general, nutrients promote the development of microcystin toxicity in lakes. The utility of these models, however, is limited by the high lake-to-lake variability. Other factors must clearly be included in future models to be useful for forecasting the effects of nutrient enrichment on lake toxicity. We have identified lake mean depth as an important variable, along with factors such as the buffering capacity or ANC of the water. Long-term studies should be undertaken on a subset of lakes to incorporate the influence of light and temperature. Such models would have greater predictive power for specific lakes and also permit long-range forecasting of the effects of global climate change on lake toxicity.

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Table 1. Summary of locations and morphometric features of theNew Hampshire microcystin survey lakes, 1999-2000.

Lake	Abbr.	Town	County	Latitude	Longitude	Mean Depth (m)	Max. Depth (m)	Lake Area (Ha)
Akers	AP	Errol	Coos	4448	7110	3.0	7.9	125.1
Arlington Mill Reservior	AMR	Salem	Rockingham	4249	7112	3.5	11.0	107.6
Armington Lake	AL	Piermont	Grafton	4357	7158	3.7	9.7	57.5
Baboosic Lake	BBL	Amherst	Hillsborough	4253	7135	4.1	8.8	89.8
Barbadoes Pond	BP	Madbury	Strafford	4311	7056	6.1	14.6	5.8
Bellamy Reservior	BR	Madbury	Strafford	4311	7057	2.2	8.2	154.7
Big Diamond Pond	BD	Stewartstown	Coos	4457	7119	10.1	35.6	72.6
Bow Lake	BL	Strafford	Strafford	4315	7111	8.0	28.3	470.1
Canobie Lake	CBL	Windham	Rockingham	4248	7115	4.6	16.8	151.1
Chocorua, Lake	LC	Tamworth	Carroll	4354	7114	3.6	8.8	90.1
Conway Lake	CWL	Conway	Carroll	4358	7104	6.0	15.2	525.9
Depot	DP	Milton	Strafford	4325	7059	5.5	16.8	72.8
Flint Pond	FP	Hollis	Hillsborough	4244	7133	1.5	2.7	19.4
Francis, Lake	LF	Clarksville	Coos	4503	7121	n.a.	25.9	681.3
Granite Lake	GL	Stoddard	Cheshire	4301	7209	10.4	32.9	984.2
Great Pond	GP	Kingston	Rockingham	4255	7104	4.5	14.6	82.6
Horseshoe Pond	HP	Merrimack	Hillsborough	4251	7129	2.3	6.1	15
Kezar Lake	KL	North Sutton	Merrimack	4322	7157	2.8	8.2	73.5
Leavitt Bay	LB	Ossipee	Carroll	4348	7105	3.5	12.8	71.4
Little Diamond Pond	LD	Stewartstown	Coos	4457	7120	1.9	4.6	21.5
Little Squam Lake	LSQ	Holderness	Grafton	4343	7136	10.1	21.9	165.2
Massabeic Lake	MBL	Auburn	Rockingham	4300	7123	5.2	18.3	1016.7
Medums Pond	MP	Barrington	Strafford	4311	7104	6.4	15.9	102.4
Merrymeeting Lake	ML	New Durham	Strafford	4329	7110	15.1	41.2	449.7
Newfound Lake	NL	Bristol	Grafton	4340	7146	22.5	55.5	1661.3
Northeast Pond	NEP	Milton	Strafford	4327	7058	2.7	14.9	228.5
Nubanusit Lake	NBL	Nelson	Cheshire	4259	7203	11.5	30.2	289.4
Ossipee Lake	OL	Ossipee	Carroll	4348	7108	8.5	15.2	1251.2
Otternic Pond	OP	Hudson	Hillsborough	4246	7125	2.3	4.8	13.8
Pleasant Lake	PL	Deerfield	Rockingham	4311	7116	7.0	19.8	199.7
Russell Pond	RP	Woodstock	Grafton	4401	7139	8.2	23.7	15.8
Second Connecticut Lake	2CL	Pittsburg	Coos	4509	7110	6.0	19.2	520.9
Silver Lake - Hollis	SLH	Hollis	Hillsborough	4245	7135	2.7	7.5	13.6
Silver Lake - Madison	SLM	Madison	Carroll	4352	7110	14.3	50.0	402.7
Squam Lake		Holderness	Grafton	4345	7131	7.4	30.2	2737.5
-Sandwich Bay	SQSB							
-Squaw Cove	SQSC							
Stonehouse Pond	SP	Barrington	Strafford	4312	7105	7.3	16.8	5.8
Sunapee Lake	SPL	New London	Merrimack	4323	7203	11.4	30.5	1653.1
Sunrise Lake	SRL	Middleton	Strafford	4327	7104	1.9	4.1	104
Swains Lake		Barrington	Strafford	4312	7103	3.0	8.8	170.2
-West Basin	SLW							
-East Basin	SLE							
Tarleton Lake	LT	Piermont	Grafton	4359	7158	8.0	18.3	127.5
Townhouse Pond	TP	Milton	Strafford	4326	7059	3.3	10.1	48
Umbagog Lake		Errol	Coos	4447	7103	3.8	14.6	3179.3
-Maine Side	ULME							
-New Hampshire Side	ULNH							
Upper Baker Pond	UB	Orford	Grafton	4355	7200	3.2	6.7	75.1
Wash Pond	WP	Hampsted	Rockingham	4253	7111	4.5	9.4	61.1

Table 2. Summary of trophic indicators for the New Hampshire microcystin survey lakes, 1999-2000.

All Dates

Trophic Indicator	units	n	Mean	SE	Median	Min	Max
Total Phosphorus	$\mu g\ L^{-1}$	84	10.62	0.78	9.15	1.50	52.90
Total Nitrogen	$\mu g\ L^{-1}$	76	315.5	19.6	284.7	63.0	1105.0
Nitrate Nitrogen	$\mu g\ L^{-1}$	84	50.5	5.3	36.5	1.3	337.7
Chlorophyll *a*	$\mu g\ L^{-1}$	85	4.26	0.39	3.18	0.80	22.17
Secchi Disk Depth	m	78	5.03	0.26	4.67	1.81	13.97

Spring

Trophic Indicator	units	n	Mean	SE	Median	Min	Max
Total Phosphorus	$\mu g\ L^{-1}$	37	11.83	0.88	10.60	3.70	25.40
Total Nitrogen	$\mu g\ L^{-1}$	31	289.35	19.89	272.30	117.70	512.30
Nitrate Nitrogen	$\mu g\ L^{-1}$	36	53.63	6.98	37.98	11.30	170.67
Chlorophyll *a*	$\mu g\ L^{-1}$	37	4.34	0.38	3.40	1.10	9.73
Secchi Disk Depth	m	35	4.67	0.38	4.02	2.00	13.97

Summer

Trophic Indicator	units	n	Mean	SE	Median	Min	Max
Total Phosphorus	$\mu g\ L^{-1}$	47	9.67	1.19	8.10	1.50	52.90
Total Nitrogen	$\mu g\ L^{-1}$	45	333.57	29.85	294.70	63.00	1105.00
Nitrate Nitrogen	$\mu g\ L^{-1}$	48	48.14	7.58	35.33	1.33	337.70
Chlorophyll *a*	$\mu g\ L^{-1}$	48	4.20	0.62	2.94	0.80	22.17
Secchi Disk Depth	m	43	5.32	0.35	4.85	1.81	11.44

1999

Trophic Indicator	units	n	Mean	SE	Median	Min	Max
Total Phosphorus	$\mu g\ L^{-1}$	65	10.52	0.95	9.00	1.50	52.90
Total Nitrogen	$\mu g\ L^{-1}$	57	336.25	24.71	303.30	63.00	1105.00
Nitrate Nitrogen	$\mu g\ L^{-1}$	64	48.89	6.03	37.15	6.70	337.70
Chlorophyll *a*	$\mu g\ L^{-1}$	65	4.39	0.48	3.21	1.10	22.17
Secchi Disk Depth	m	62	5.24	0.31	4.78	1.81	13.97

2000

Trophic Indicator	units	n	Mean	SE	Median	Min	Max
Total Phosphorus	$\mu g\ L^{-1}$	19	10.97	1.11	10.10	5.00	22.90
Total Nitrogen	$\mu g\ L^{-1}$	19	253.38	19.64	225.00	162.30	460.70
Nitrate Nitrogen	$\mu g\ L^{-1}$	20	55.62	10.81	36.50	1.33	170.67
Chlorophyll *a*	$\mu g\ L^{-1}$	20	3.85	0.52	2.93	0.80	9.73
Secchi Disk Depth	m	16	4.20	0.34	4.08	2.00	6.98

Table 3. Summary of microcystin concentrations in the lake water, phytoplankton and zooplankton in the New Hampshire microcystin survey lakes, 1999-2000.

All Dates

Compartment	units	n	Mean	SE	Median	Min	Max
Whole Lake Water	ng L^{-1}	85	13.22	1.98	7.10	0.20	114.10
Phytoplankton	ng g^{-1} w.w.	85	1577.41	491.11	69.80	0.80	31472.00
Zooplankton	ng g^{-1} w.w.	40	330.13	131.86	12.05	0.90	8980.70

Spring

Compartment	units	n	Mean	SE	Median	Min	Max
Whole Lake Water	ng L^{-1}	37	10.18	3.07	5.60	0.20	114.10
Phytoplankton	ng g^{-1} w.w.	37	542.07	399.82	34.20	0.80	15067.20
Zooplankton	ng g^{-1} w.w.	36	293.75	245.14	10.25	1.60	8980.70

Summer

Compartment	units	n	Mean	SE	Median	Min	Max
Whole Lake Water	ng L^{-1}	48	15.57	2.54	8.65	0.40	85.50
Phytoplankton	ng g^{-1} w.w.	48	2375.49	794.27	242.40	1.90	31472.00
Zooplankton	ng g^{-1} w.w.	48	357.41	139.31	17.45	0.90	5896.40

1999

Compartment	units	n	Mean	SE	Median	Min	Max
Whole Lake Water	ng L^{-1}	65	14.63	2.54	7.10	0.20	114.10
Phytoplankton	ng g^{-1} w.w.	65	2012.47	632.14	118.50	1.90	31472.00
Zooplankton	ng g^{-1} w.w.	65	417.62	168.79	17.30	0.90	8980.70

2000

Compartment	units	n	Mean	SE	Median	Min	Max
Whole Lake Water	ng L^{-1}	20	8.65	1.22	8.05	1.40	19.10
Phytoplankton	ng g^{-1} w.w.	19	163.50	70.33	29.00	0.80	1109.90
Zooplankton	ng g^{-1} w.w.	20	30.79	17.91	8.20	2.40	359.40

Table 4. Linear regressions of whole lake water microcystins (WLW MC) in the New Hampshire Lakes microcystin survey, 1999-2000.

Independent Factor	Units	Dependent Factor	Units	Year	n	r	Adj. r^2	p
				Spring (May - July 9)				
log(TP)	$\mu g\ L^{-1}$	log(WLW MC)	$\mu g\ L^{-1}$	1999 & 2000	37	0.424	0.156	0.009
log(TP)	$\mu g\ L^{-2}$	log(WLW MC)	$\mu g\ L^{-2}$	1999	20	0.498	0.206	0.026
log(TP)	$\mu g\ L^{-3}$	log(WLW MC)	$\mu g\ L^{-3}$	2000	17	0.278	0.016	n.s.
log(TN)	$\mu g\ L^{-4}$	log(WLW MC)	$\mu g\ L^{-4}$	1999 & 2000	31	0.547	0.276	0.001
log(TN)	$\mu g\ L^{-5}$	log(WLW MC)	$\mu g\ L^{-5}$	1999	14	0.696	0.441	0.006
log(TN)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-6}$	2000	17	0.343	0.059	n.s.
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-1}$	1999 & 2000	31	0.042	0.000	n.s.
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-2}$	1999	14	0.161	0.000	n.s.
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-3}$	2000	17	-0.007	0.000	n.s.
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-4}$	1999 & 2000	37	0.353	0.100	0.032
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-5}$	1999	20	0.616	0.345	0.004
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-6}$	2000	17	-0.16	0.000	n.s.
				Summer (July 13 – October)				
log(TP)	$\mu g\ L^{-1}$	log(WLW MC)	$\mu g\ L^{-1}$	1999 & 2000	47	0.56	0.298	<0.001
log(TP)	$\mu g\ L^{-2}$	log(WLW MC)	$\mu g\ L^{-2}$	1999	45	0.567	0.306	<0.001
log(TP)	$\mu g\ L^{-3}$	log(WLW MC)	$\mu g\ L^{-3}$	2000	2	-	-	-
log(TN)	$\mu g\ L^{-4}$	log(WLW MC)	$\mu g\ L^{-4}$	1999 & 2000	45	0.455	0.189	0.002
log(TN)	$\mu g\ L^{-5}$	log(WLW MC)	$\mu g\ L^{-5}$	1999	43	0.476	0.208	0.001
log(TN)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-6}$	2000	2	-	-	-
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-1}$	1999 & 2000	45	-0.263	0.048	n.s.
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-2}$	1999	43	-0.266	0.048	n.s.
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-3}$	2000	2	-	-	-
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-4}$	1999 & 2000	48	0.413	0.152	0.004
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-5}$	1999	45	0.460	0.194	0.001
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-6}$	2000	3	0.135	0.000	n.s.
				All Dates (May – October)				
log(TP)	$\mu g\ L^{-1}$	log(WLW MC)	$\mu g\ L^{-1}$	1999 & 2000	84	0.385	0.138	<0.001
log(TP)	$\mu g\ L^{-2}$	log(WLW MC)	$\mu g\ L^{-2}$	1999	65	0.428	0.170	<0.001
log(TP)	$\mu g\ L^{-3}$	log(WLW MC)	$\mu g\ L^{-3}$	2000	19	0.168	0.000	n.s.
log(TN)	$\mu g\ L^{-4}$	log(WLW MC)	$\mu g\ L^{-4}$	1999 & 2000	76	0.477	0.217	<0.001
log(TN)	$\mu g\ L^{-5}$	log(WLW MC)	$\mu g\ L^{-5}$	1999	57	0.506	0.243	<0.001
log(TN)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-6}$	2000	19	0.207	0.000	n.s.
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-1}$	1999 & 2000	76	-0.023	0.000	n.s.
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-2}$	1999	57	-0.070	0.000	n.s.
log(TN/TP)	-	log(WLW MC)	$\mu g\ L^{-3}$	2000	19	-0.008	0.000	n.s.
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-4}$	1999 & 2000	85	0.328	0.097	0.002
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-5}$	1999	65	0.453	0.192	<0.001
log(chloro a)	$\mu g\ L^{-6}$	log(WLW MC)	$\mu g\ L^{-6}$	2000	20	-0.295	0.036	n.s.

Table 5. . Linear regressions of phytoplankton microcystins (WLW MC) in the New Hampshire lakes microcystin survey, 1999-2000.

Independent		Dependent						
Factor	Units	Factor	Units	Year	n	r	Adj. r^2	p
Spring (May – July 9)								
log(TP)	µg L^{-1}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	37	0.365	0.109	0.026
log(TP)	µg L^{-2}	log(Phyto MC)	ng g^{-1} wet wt.	1999	20	0.454	0.162	0.045
log(TP)	µg L^{-3}	log(Phyto MC)	ng g^{-1} wet wt.	2000	17	0.19	0.000	n.s.
log(TN)	µg L^{-4}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	31	0.49	0.214	0.005
log(TN)	µg L^{-5}	log(Phyto MC)	ng g^{-1} wet wt.	1999	14	0.642	0.363	0.013
log(TN)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	2000	17	0.196	0.000	n.s.
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	31	0.035	0.000	n.s.
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	1999	14	0.073	0.000	n.s.
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	2000	17	-0.027	0.000	n.s.
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	37	0.515	0.244	0.001
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	1999	20	0.579	0.298	0.007
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	2000	17	0.41	0.112	n.s.
Summer (July 13 – October)								
log(TP)	µg L^{-1}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	47	0.522	0.256	<0.001
log(TP)	µg L^{-2}	log(Phyto MC)	ng g^{-1} wet wt.	1999	45	0.513	0.246	<0.001
log(TP)	µg L^{-3}	log(Phyto MC)	ng g^{-1} wet wt.	2000	2	-	-	-
log(TN)	µg L^{-4}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	45	0.475	0.207	0.001
log(TN)	µg L^{-5}	log(Phyto MC)	ng g^{-1} wet wt.	1999	43	0.488	0.219	0.001
log(TN)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	2000	2	-	-	-
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	45	-0.205	0.020	n.s.
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	1999	43	-0.189	0.012	n.s.
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	2000	2	-	-	-
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	48	0.427	0.164	0.002
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	1999	45	0.400	0.140	0.007
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	2000	3	-	-	-
All Dates (May – October)								
log(TP)	µg L^{-1}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	84	0.349	0.111	0.001
log(TP)	µg L^{-2}	log(Phyto MC)	ng g^{-1} wet wt.	1999	65	0.404	0.150	0.001
log(TP)	µg L^{-3}	log(Phyto MC)	ng g^{-1} wet wt.	2000	19	0.271	0.019	n.s.
log(TN)	µg L^{-4}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	76	0.477	0.217	<0.001
log(TN)	µg L^{-5}	log(Phyto MC)	ng g^{-1} wet wt.	1999	57	0.51	0.247	<0.001
log(TN)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	2000	19	0.075	0.000	n.s.
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	76	-0.008	0.000	n.s.
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	1999	57	-0.091	0.000	n.s.
log(TN/TP)	-	log(Phyto MC)	ng g^{-1} wet wt.	2000	19	-0.172	0.000	n.s.
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	1999 & 2000	85	0.392	0.143	<0.001
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	1999	65	0.398	0.145	0.001
log(chloro *a*)	µg L^{-6}	log(Phyto MC)	ng g^{-1} wet wt.	2000	20	0.385	0.101	n.s.

Table 6. . Linear regressions of whole zooplankton microcystins (WLW MC) in the New Hampshire lakes microcystin survey, 1999-2000.

Independent Factor	Units	Dependent Factor	Units	Year	n	r	Adj. r^2	p
				May - July 9				
log(TP)	$\mu g\ L^{-1}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	36	0.335	0.086	0.046
log(TP)	$\mu g\ L^{-2}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	20	0.379	0.096	n.s.
log(TP)	$\mu g\ L^{-3}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	16	0.236	0.000	n.s.
log(TN)	$\mu g\ L^{-4}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	30	0.493	0.216	0.006
log(TN)	$\mu g\ L^{-5}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	14	0.547	0.241	0.043
log(TN)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	16	0.178	0.000	n.s.
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	30	0.112	0.000	n.s.
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	1999	14	0.202	0.000	n.s.
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	2000	16	-0.077	0.000	n.s.
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	36	0.431	0.162	0.009
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	20	0.502	0.211	0.024
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	16	0.355	0.063	n.s.
				July 13 - October				
log(TP)	$\mu g\ L^{-1}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	47	0.406	0.146	0.005
log(TP)	$\mu g\ L^{-2}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	45	0.393	0.134	0.008
log(TP)	$\mu g\ L^{-3}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	2	-	-	-
log(TN)	$\mu g\ L^{-4}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	45	0.384	0.128	0.009
log(TN)	$\mu g\ L^{-5}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	43	0.412	0.149	0.006
log(TN)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	2	-	-	-
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	45	-0.162	0.004	n.s.
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	1999	43	-0.120	0.000	n.s.
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	2000	2	-	-	-
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	48	0.278	0.057	0.056
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	45	0.268	0.050	0.075
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	3	-	-	-
				May - October				
log(TP)	$\mu g\ L^{-1}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	83	0.329	0.097	0.002
log(TP)	$\mu g\ L^{-2}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	65	0.368	0.122	0.003
log(TP)	$\mu g\ L^{-3}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	18	0.248	0.003	n.s.
log(TN)	$\mu g\ L^{-4}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	75	0.417	0.162	0
log(TN)	$\mu g\ L^{-5}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	57	0.438	0.177	0.001
log(TN)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	18	-0.089	0.000	n.s.
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	75	-0.033	0.000	n.s.
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	1999	57	-0.088	0.000	n.s.
log(TN/TP)	-	log(Zoop MC)	ng g^{-1} wet wt.	2000	18	-0.258	0.008	n.s.
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	1999 & 2000	84	0.301	0.080	0.005
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	1999	65	0.322	0.090	0.009
log(chloro a)	$\mu g\ L^{-6}$	log(Zoop MC)	ng g^{-1} wet wt.	2000	19	0.195	0.000	n.s.

Figure 1. Map of New Hampshire showing the locations of the study lakes in the microcystin survey, 1999-2000.

Figure 2. Integrated epilimnetic water sampling and sub sampling methods for chemical analyses.

Sample bottle with 1.5-2.0 L integrated epilimnetic water collected with a weighted Tygon hose.

Sample (500 ml) filtered through Millipore filter (0.45 µm) with Millipore vacuum-filtration apparatus.

Chlorophyll α Sample:
Post filtration, Millipore filter (0.45 µm) stored in a darkened desiccator, dried, and stored in a vented zip-lock bag.

Dissolved Color Sample:
Filtrate (~50 ml) stored in bottle (60 ml) on ice. Refrigerated in the lab.

Nitrate Nitrogen Sample:
Filtrate (~50 ml) stored in acid-washed bottle (60-ml) and stored on ice. Frozen in the lab.

Total Phosphorus/Total Nitrogen Sample:
Water sample (~450 ml) stored in acid-washed bottle (500-ml) on ice. Frozen in the lab.

Acid-Neutralizing Capacity (Alkalinity):
Water sample transferred to white sample cup (250-ml). Methyl red-bromcresol green indicator (4-6 drops) added, and titrated with H_2SO_4 (0.002 N) from blue to grey color

Whole Lake Water Microcystin:
Water sample (~200 ml) stored in sample cup (250-ml) on ice. Frozen (-40° C) for ELISA analysis.

Figure 3. Net (a 50 pm) plankton sampling and separation of phyto- and zooplankton for MC analysis and enumeration.

Integrated plankton tows (6) taken from ~1 m above lake bottom to surface with 50-μm plankton net.

50-μm

3X

3X

Cod bucket contents rinsed into 2-L "separatory" bottle, dark at top, lit at bottom. Phototactic zooplankters migrate to light (bottom) & separate from suspended phytoplankton.

Zooplankton:
At 10-15 min, 100-125 ml (zooplankton), collected in 250-ml cup

Phytoplankton:
Rest of sample drained into a second 250-ml cup.

Plankton Enumeration:
Cod bucket contents rinsed into 250-ml cup and preserved with 4% formalin-sucrose

Sample passed through funnel with Nitex mesh attached, to collect net (≥ 50 μm) zooplankton.

Sample passed through funnel with Nitex mesh attached, to collect net (≥ 50 μm) phytoplankton.

Nitex mesh (50-μm) attached to the funnel necks with rubber rings.

Nitex mesh blotted on sponge to remove excess water (leaving plankton moist). Samples stored frozen in 6-ml polypropylene vials until processing for ELISA analysis.

Figure 4. Distribution of mean total phosphorus concentrations for the microcystin survey lakes, 1999-2000. Error bars = 1 SE.

Figure 5. Distribution of mean total nitrogen concentrations for the New Hampshire microcystin survey lakes, 1999-2000. Error bars = 1 SE.

Figure 6. Distribution of mean chlorophyll a concentrations for the New Hampshire microcystin survey lakes, 1999-2000. Error bars = 1 SE.
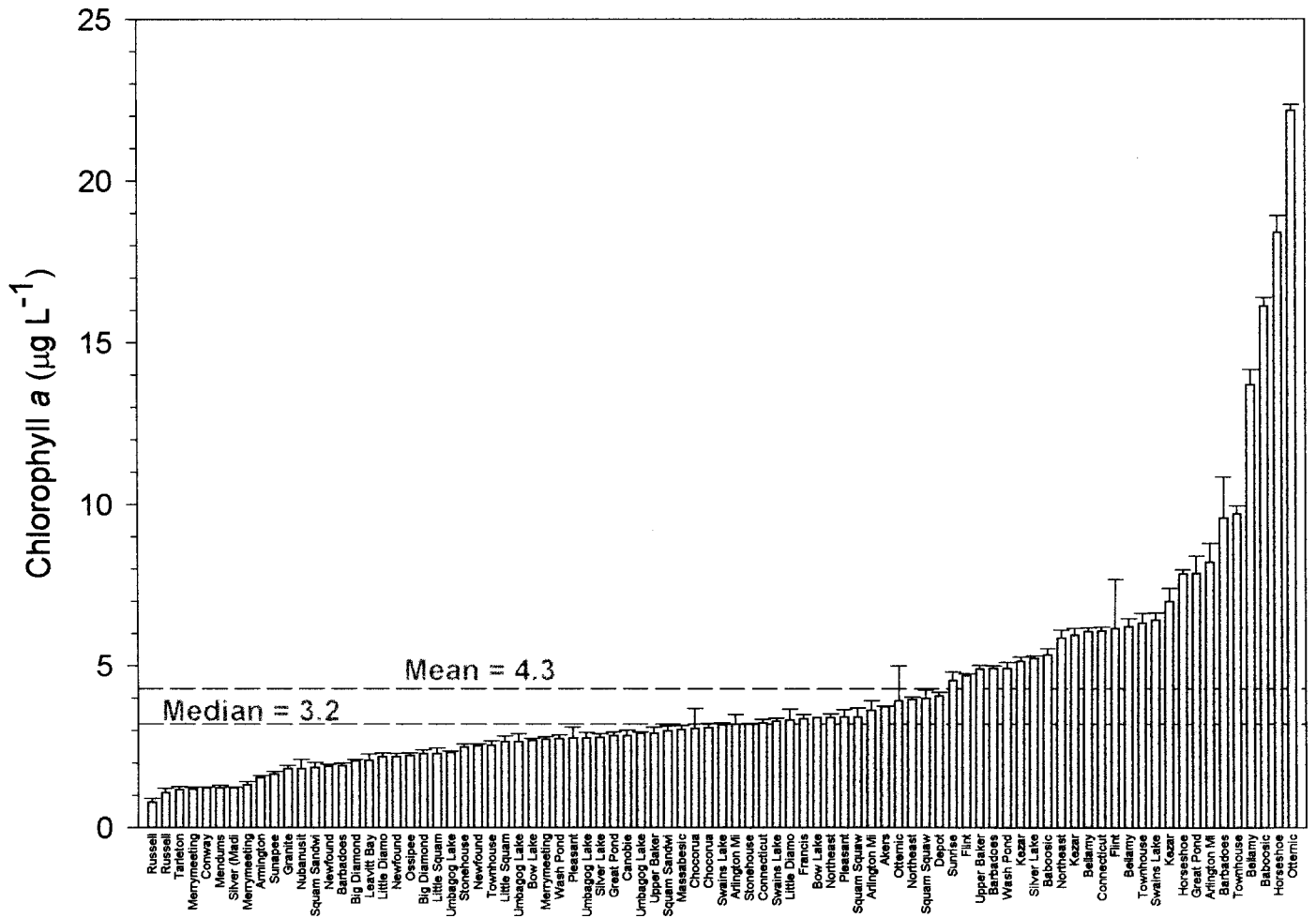
Figure 7. Distribution of mean Secchi disk depths for the microcystin survey lakes, 1999-2000. Error bars = 1 SE.
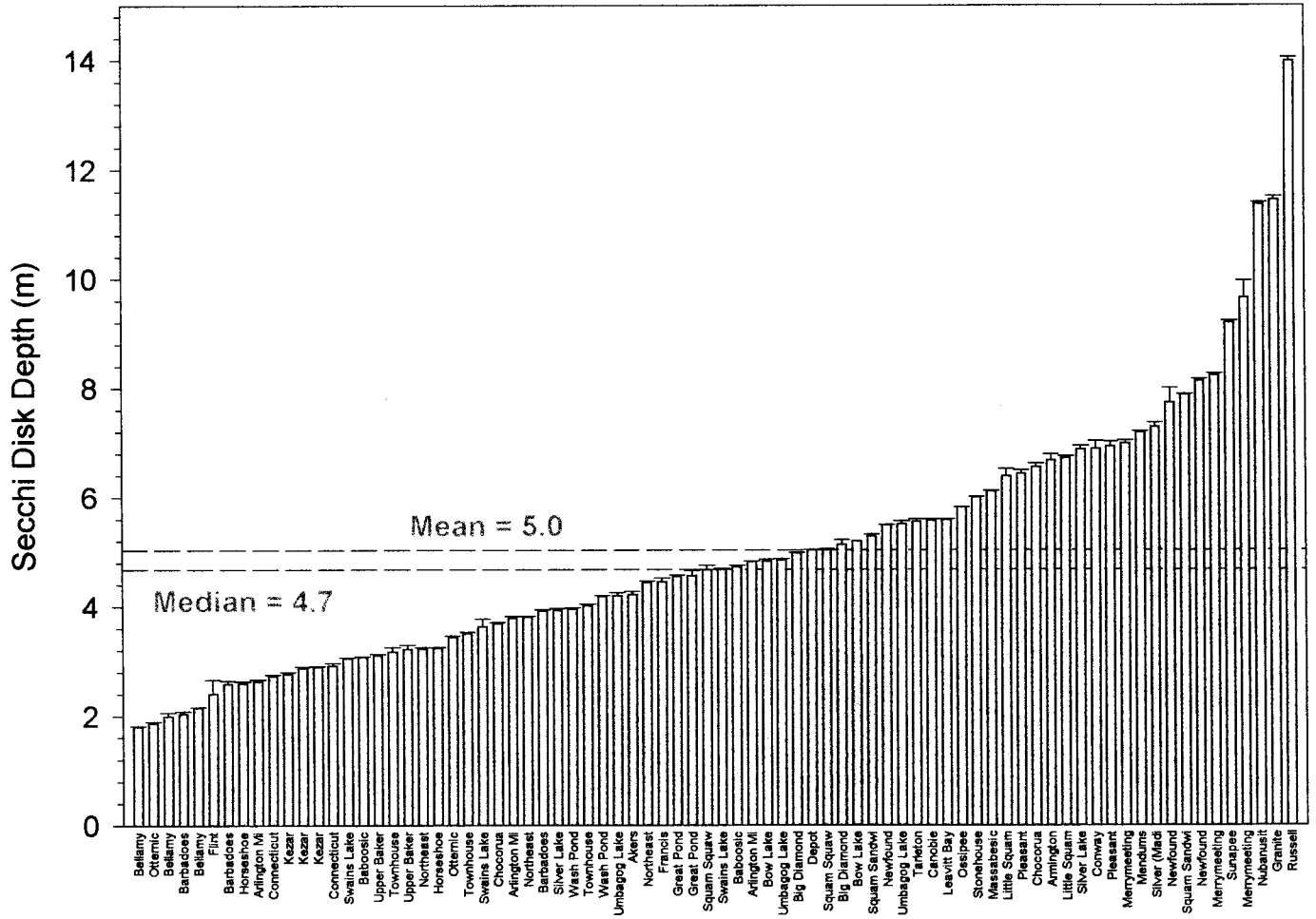
Figure 8. Distribution of mean whole lake water MC concentrations for the New Hampshire microcystin survey lakes, 1999-2000. Error bars = 1 SE.
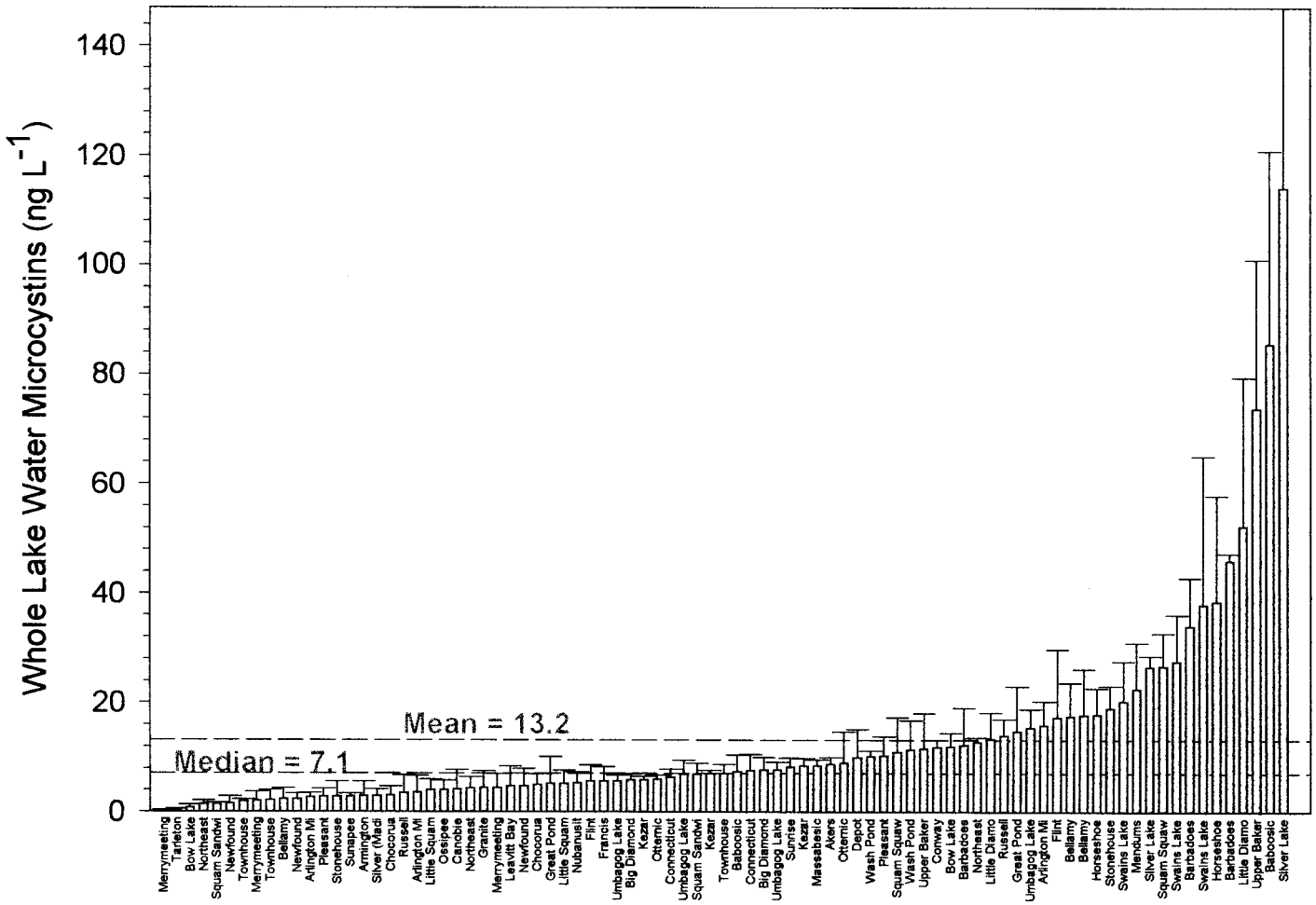
Figure 9. Distribution of mean phytoplankton MC concentrations for the New Hampshire microcystin survey lakes, 1999-2000. Error bars = 1 SE.
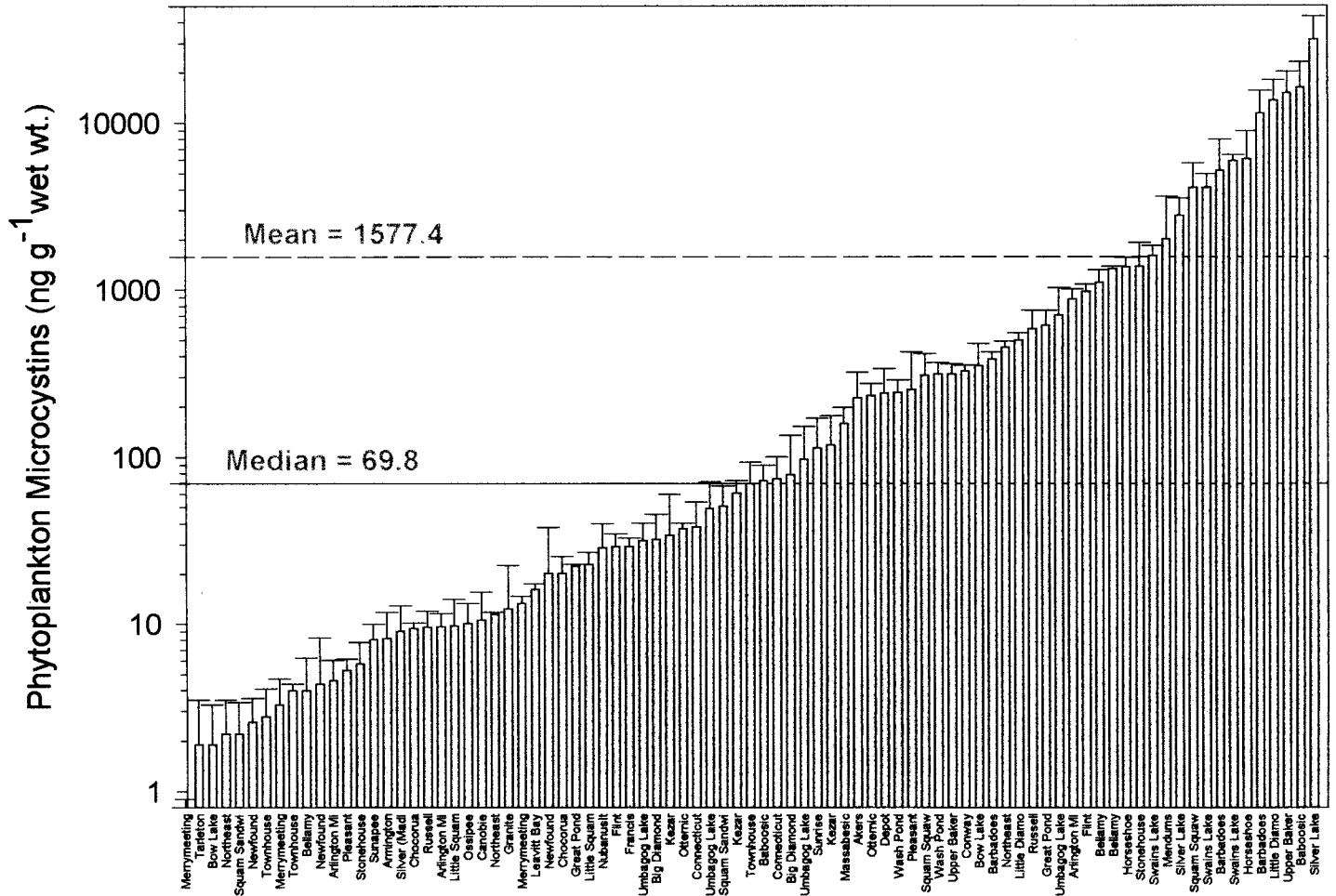
Figure 10. Distribution of mean zooplankton MC concentrations for the New Hampshire microcystin survey lakes, 1999-2000. Error bars = 1 SE.
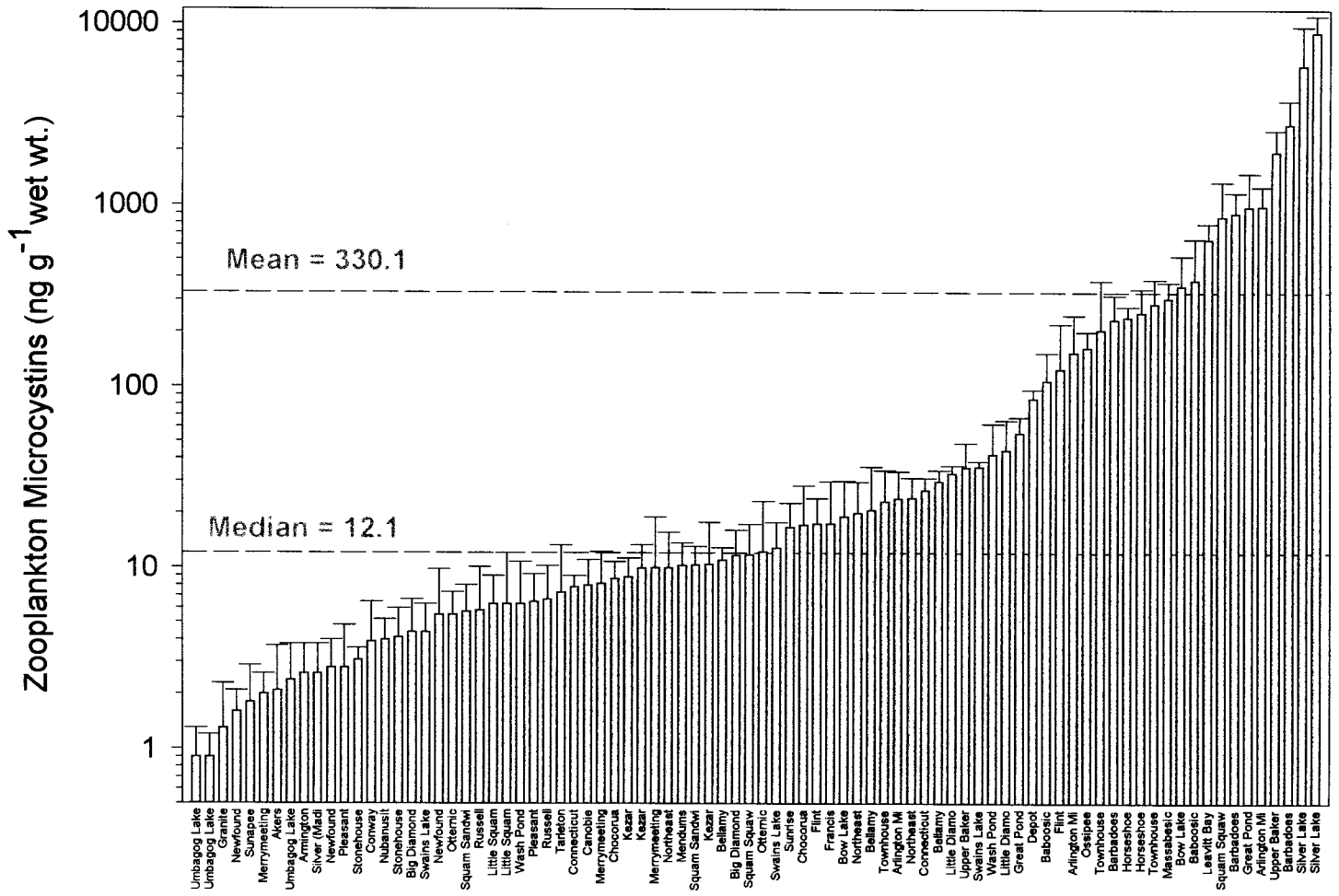
Figure 11. Comparison of total phosphorus vs. chlorophyll a relationships from the New Hampshire microcystins survey, 1999, with other studies.
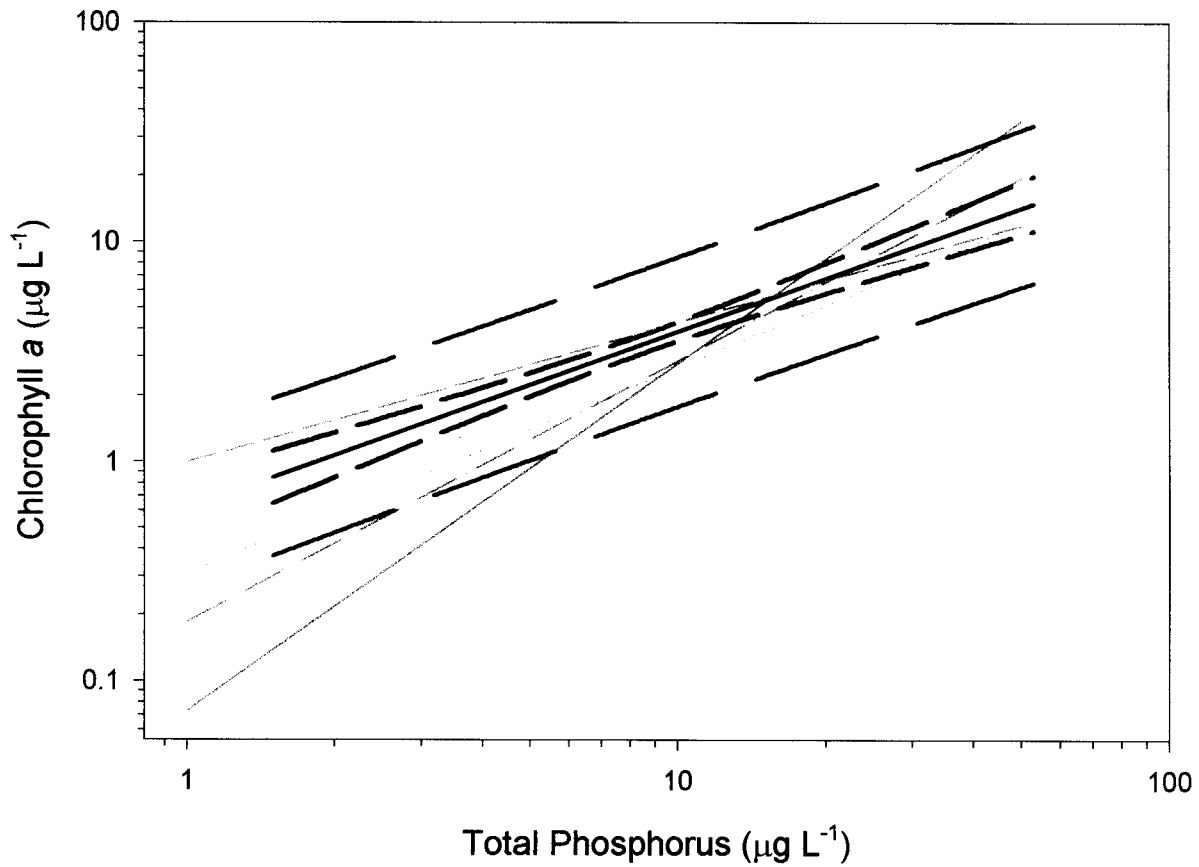
Figure 12. Linear regressions of total phosphorus and whole lake water microcystins (WLW MC). Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, gray=summer.
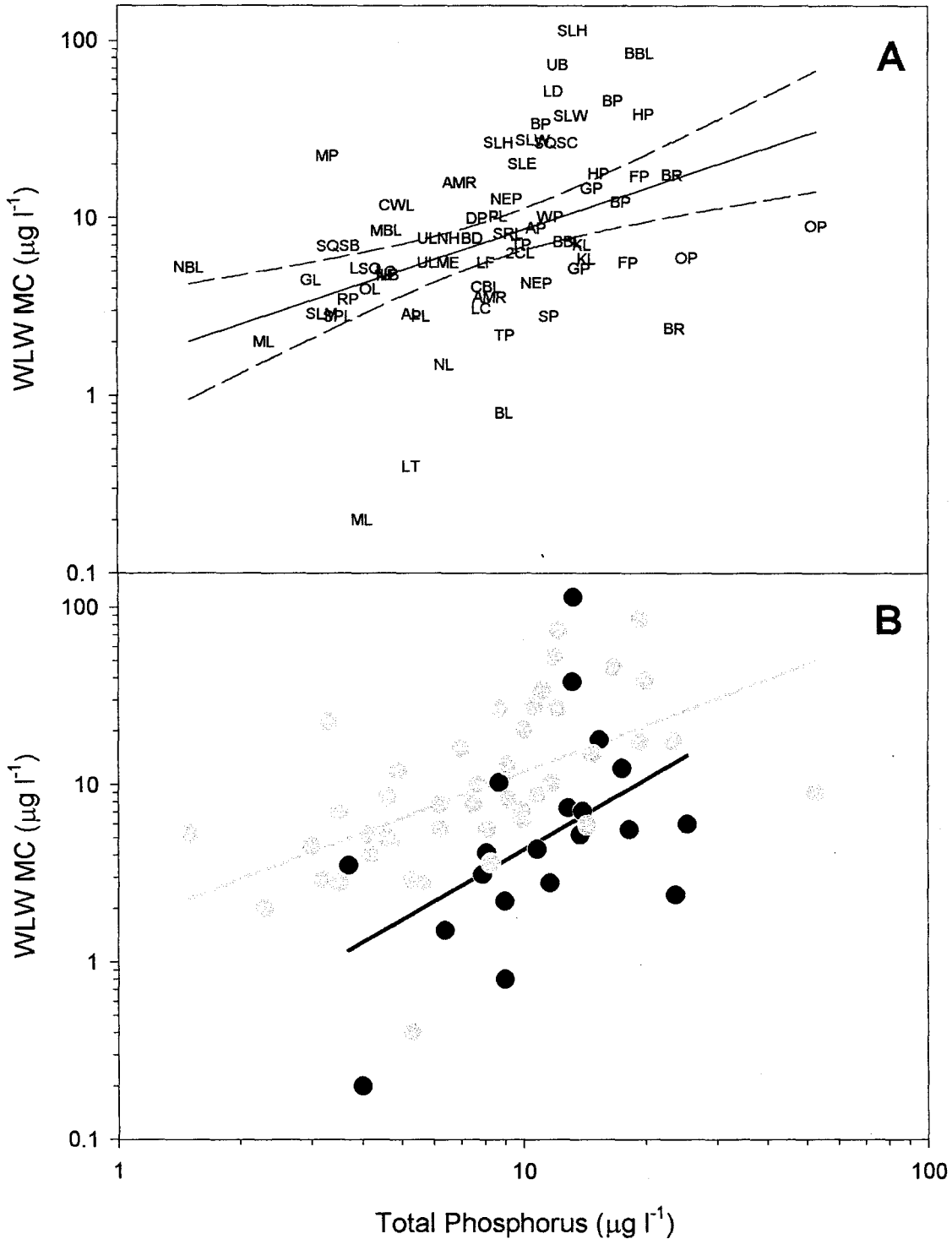
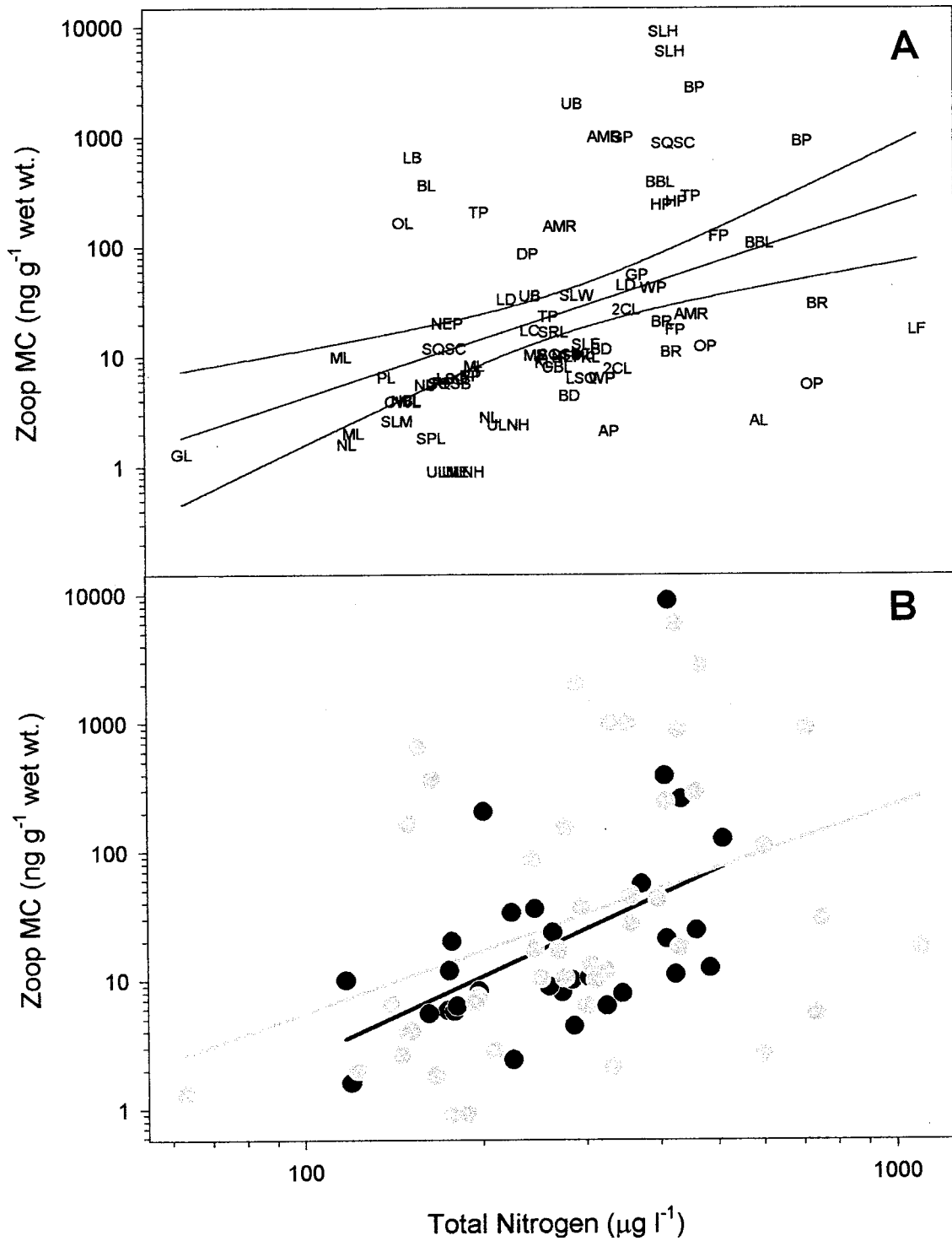Figure 13. Linear regressions of total nitrogen and whole lake water microcystins (WLW MC). Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, gray=summer.
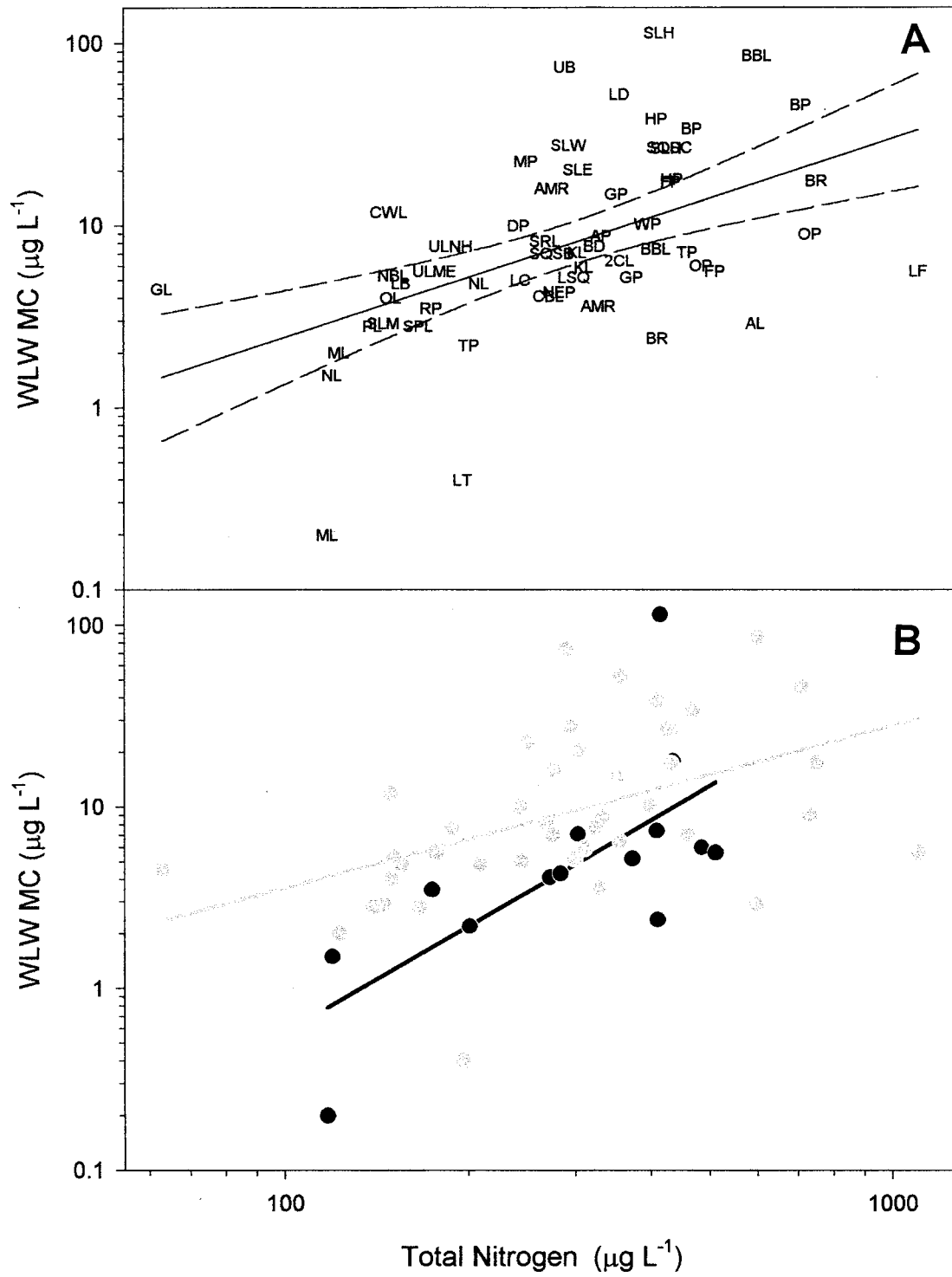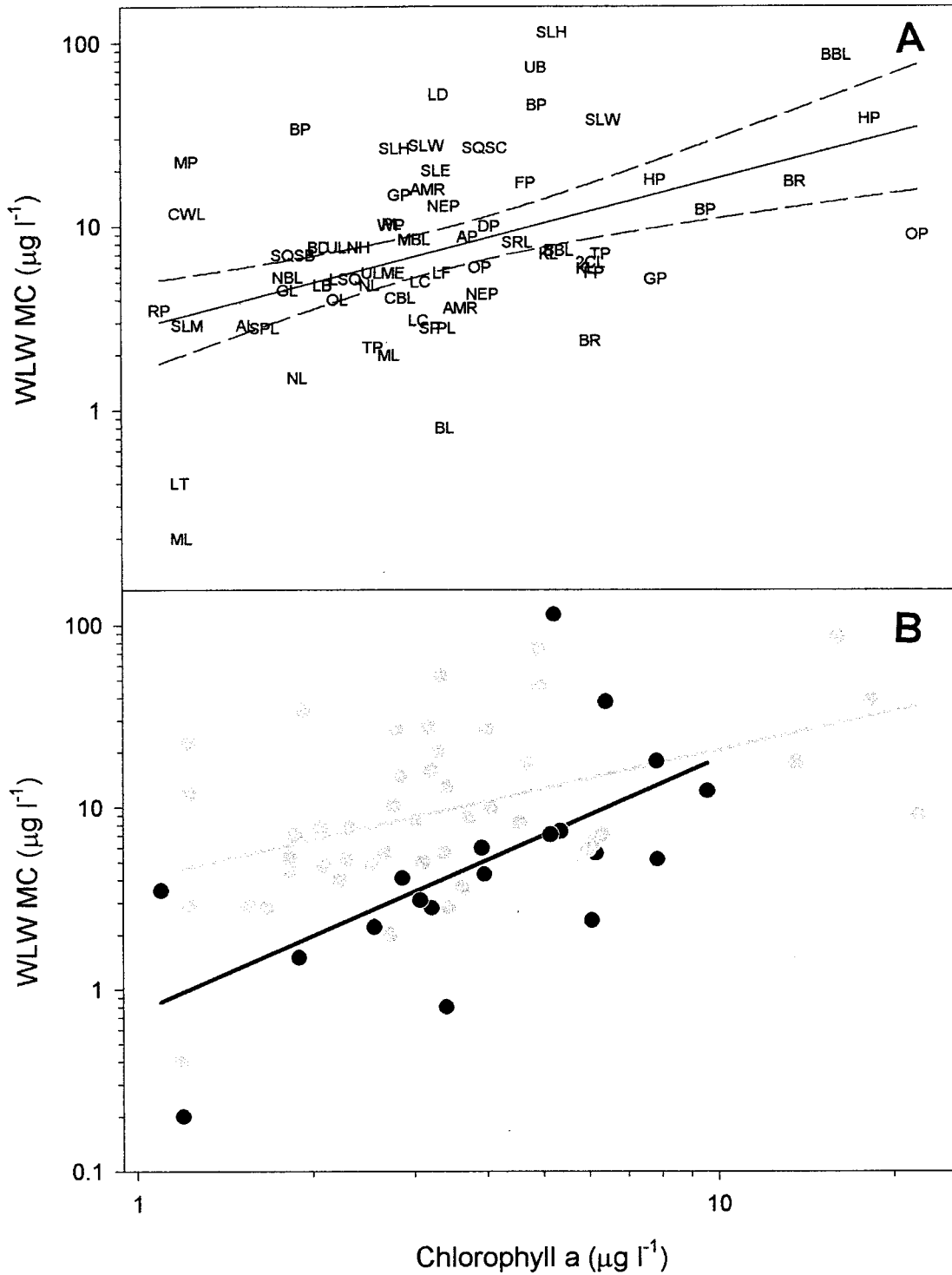
Figure 14. . Linear regressions of total phosphorus and net phytoplankton microcystins. Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, gray=summer.
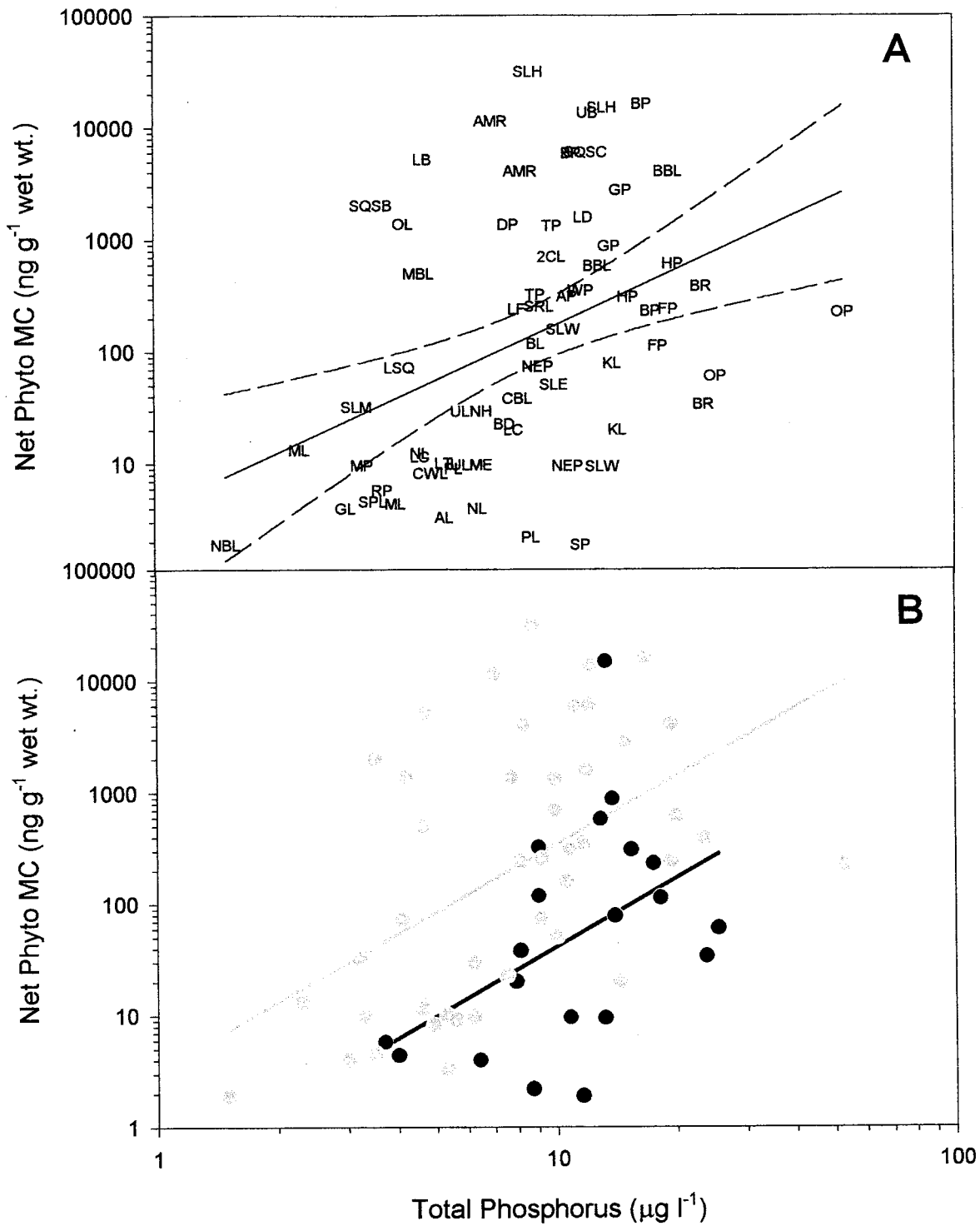
Figure 15. Linear regressions of total phosphorus and net phytoplankton microcystinse. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
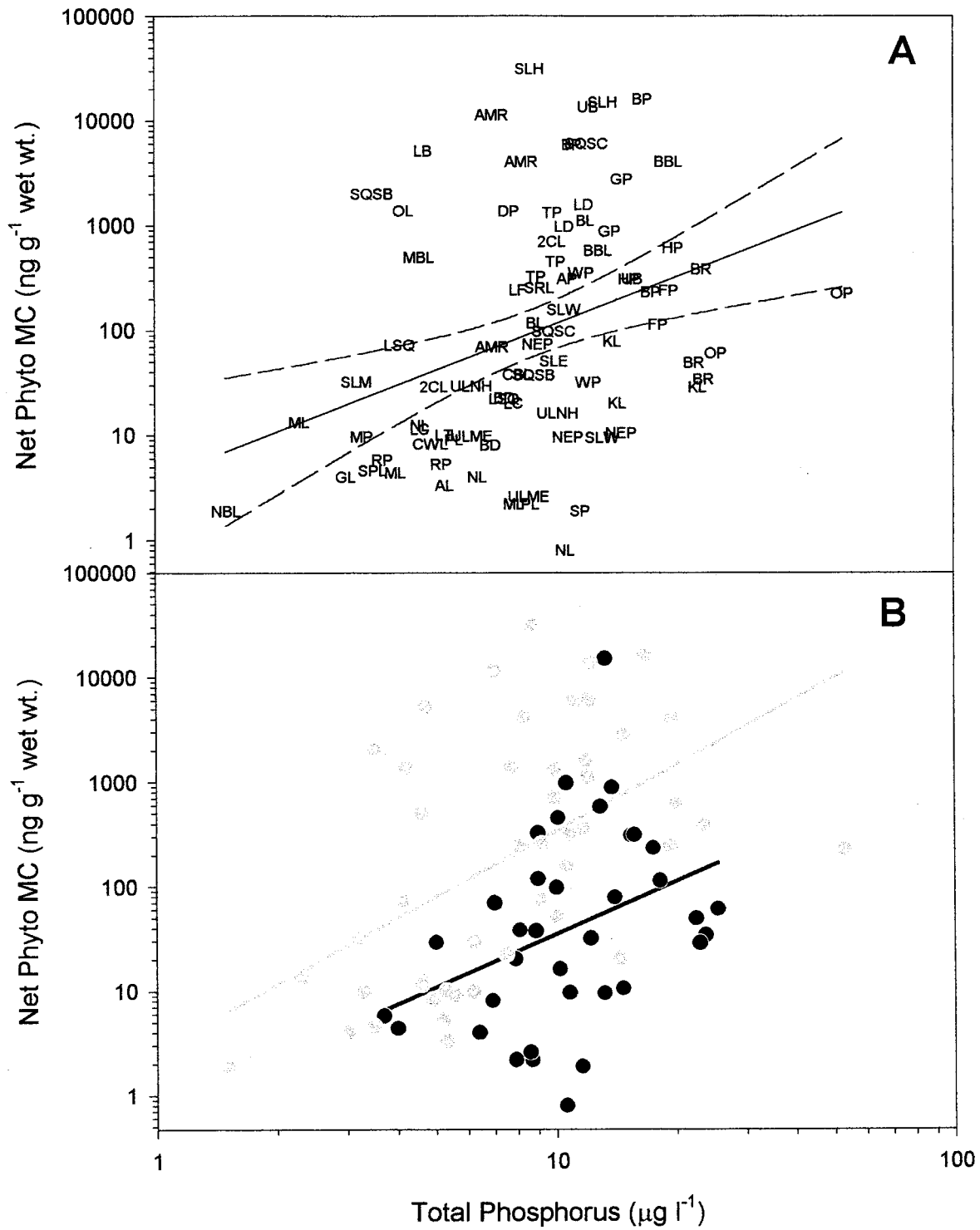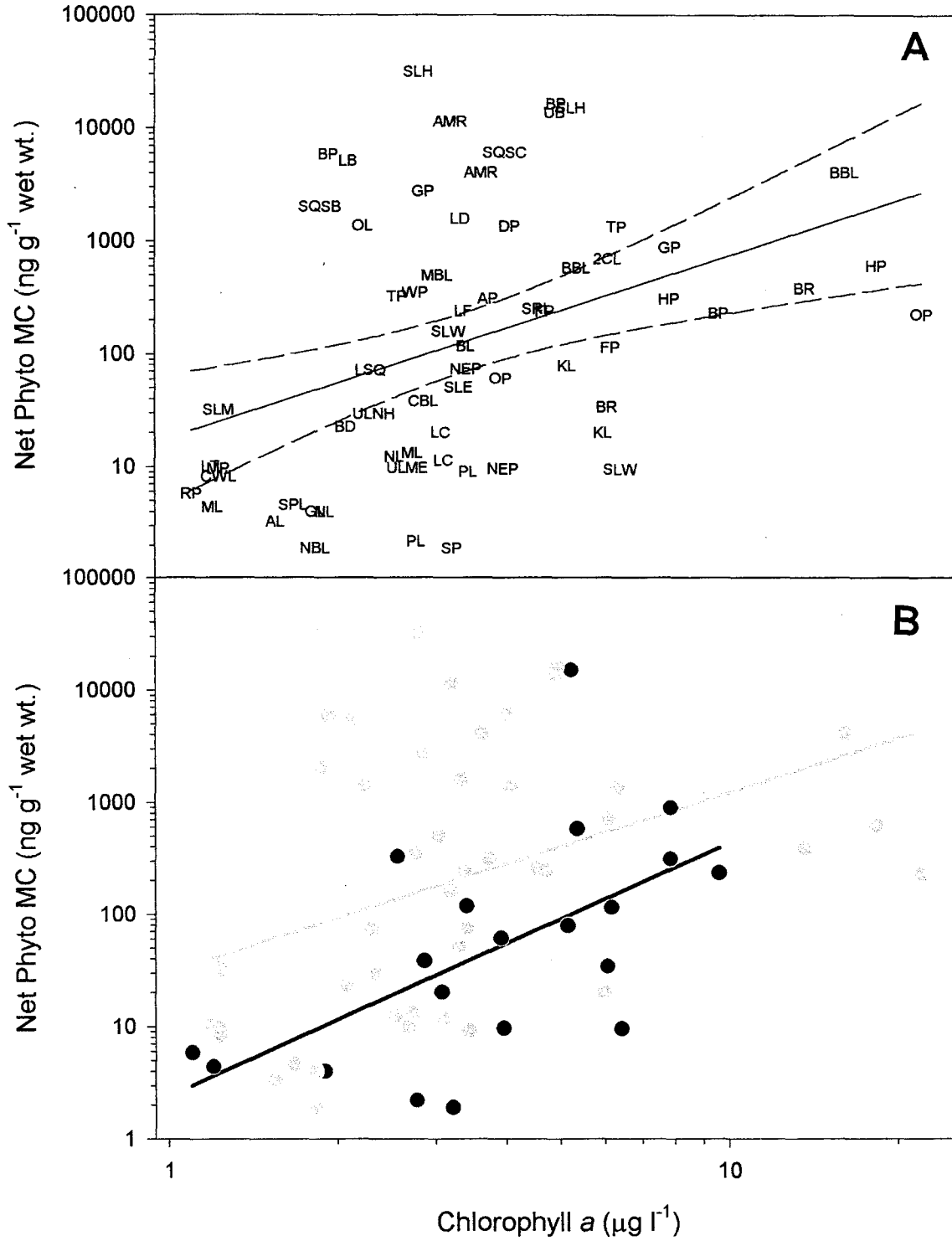
Figure 16. Linear regressions of total nitrogen and net phytoplankton microcystins. -- Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
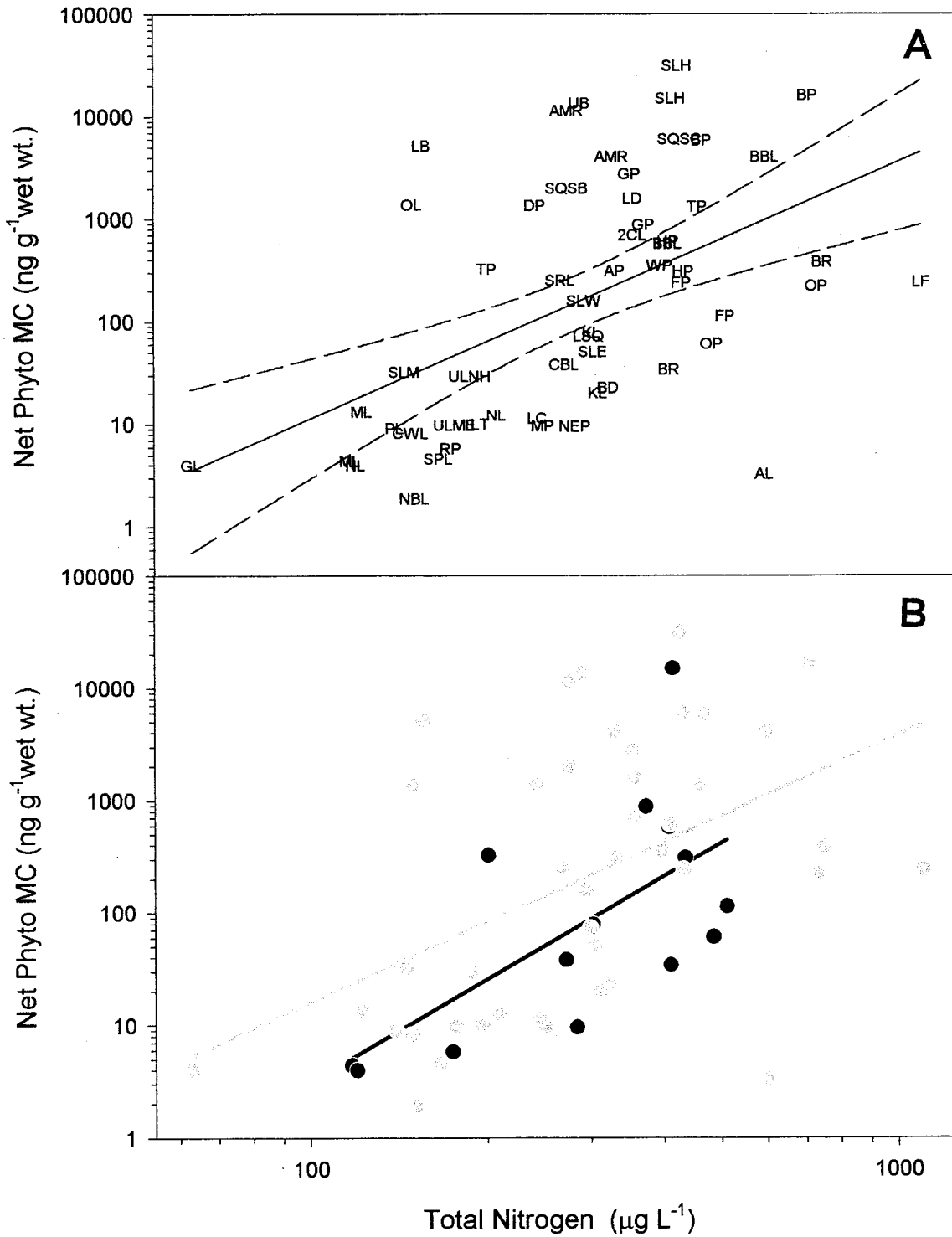
Figure 17. Linear regressions of total nitrogen and net phytoplankton microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
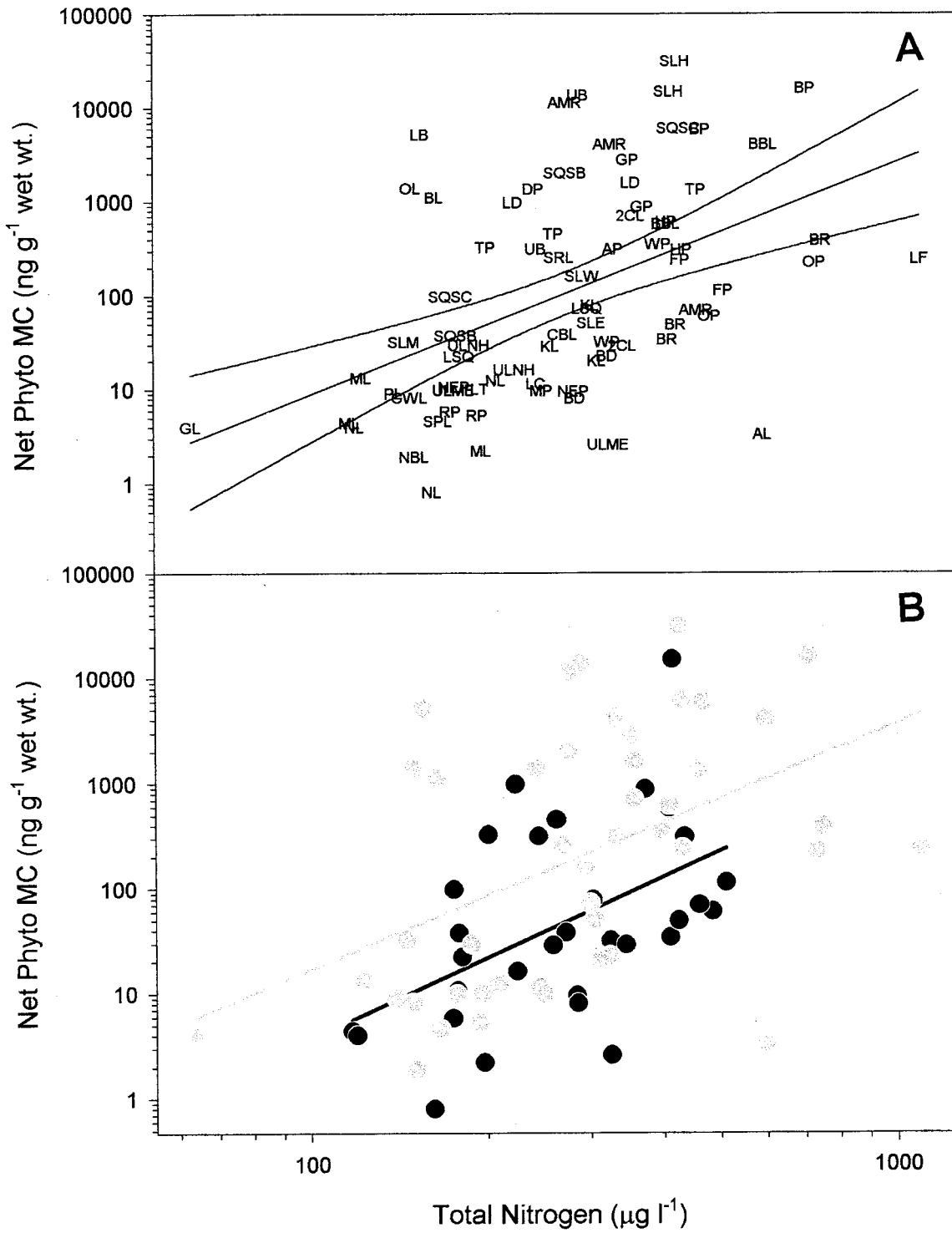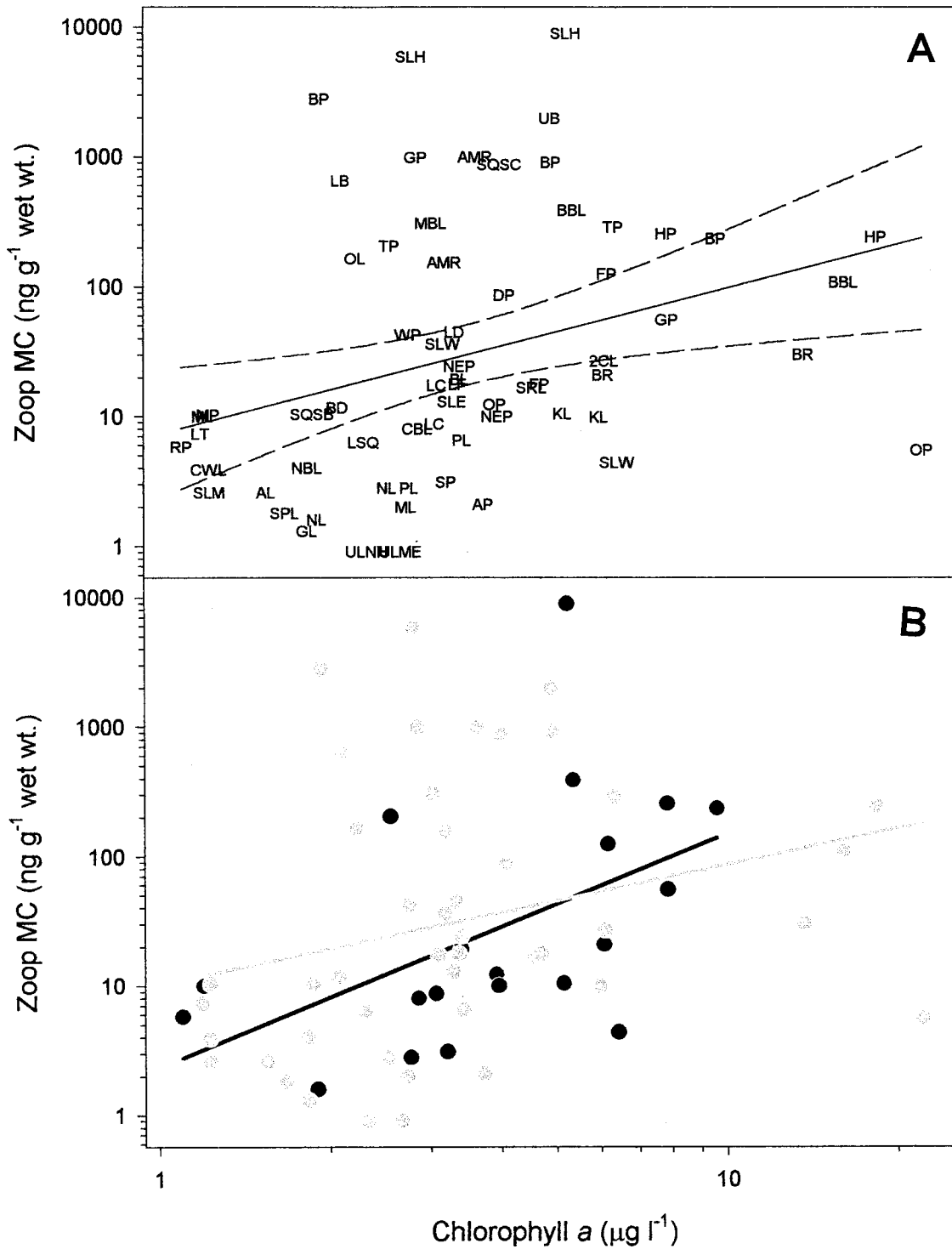
Figure 18. Linear regressions of total nitrogen and zooplankton microcystins Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
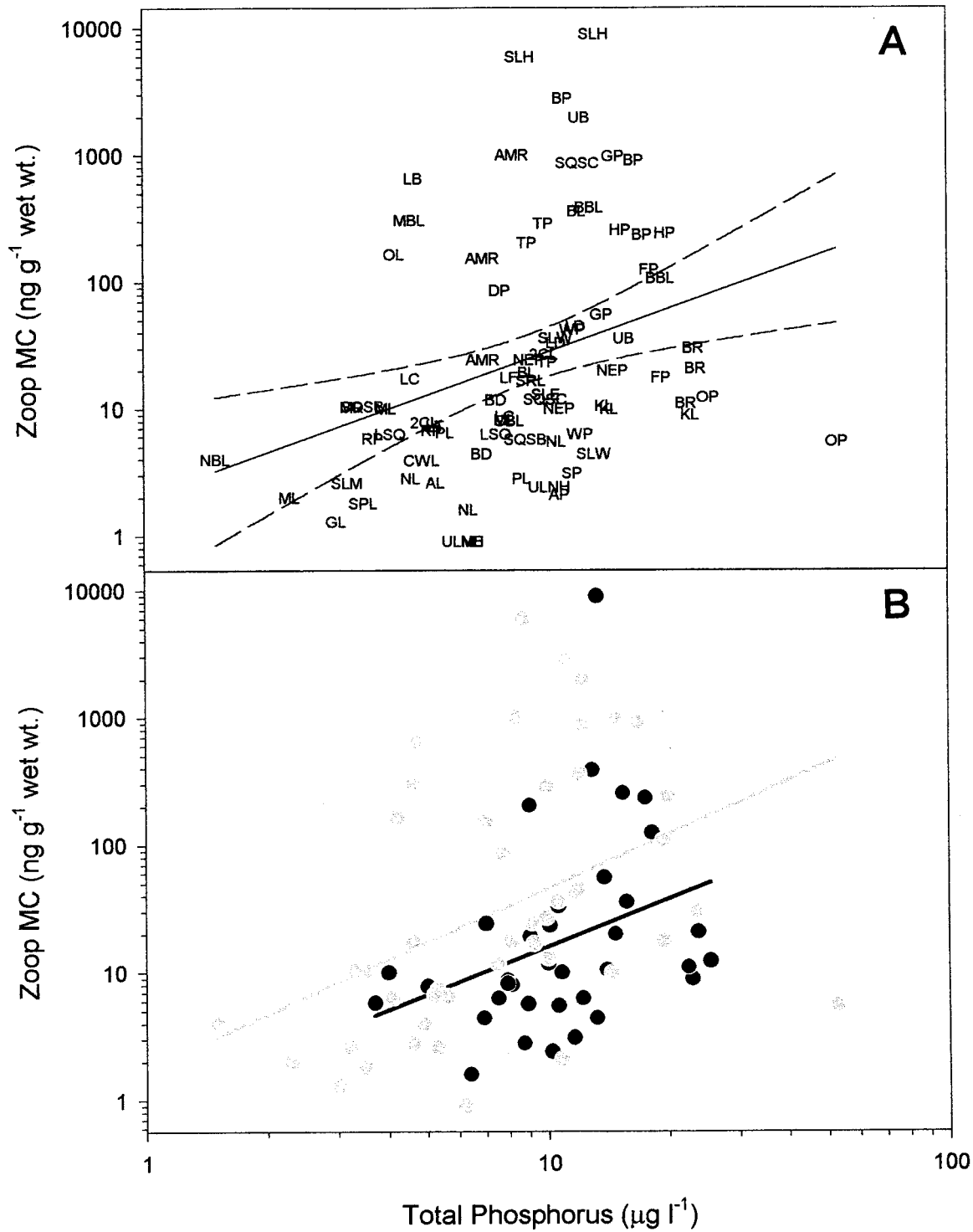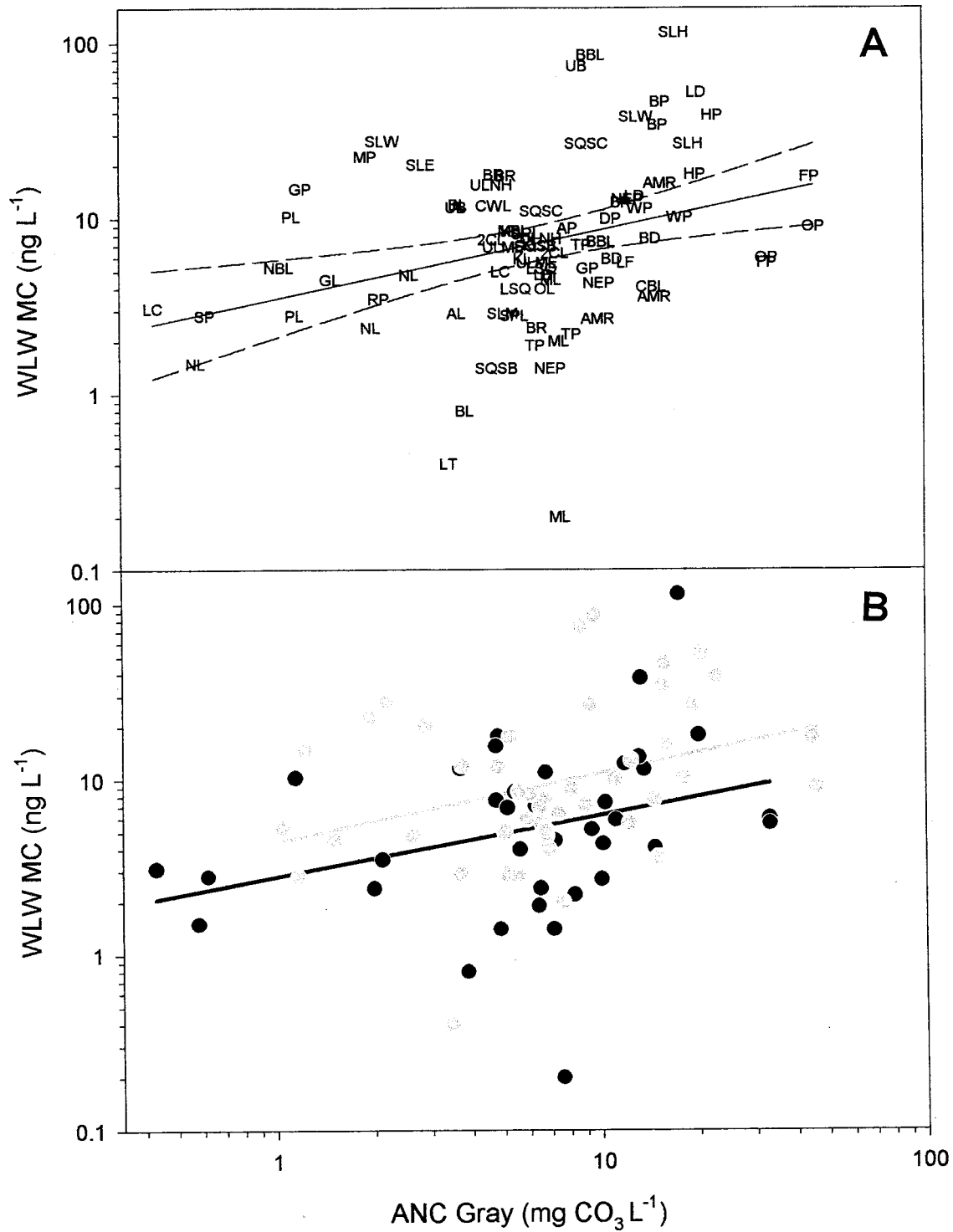
Figure 19. Linear regressions of total phosphorus and zooplankton microcystins. Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
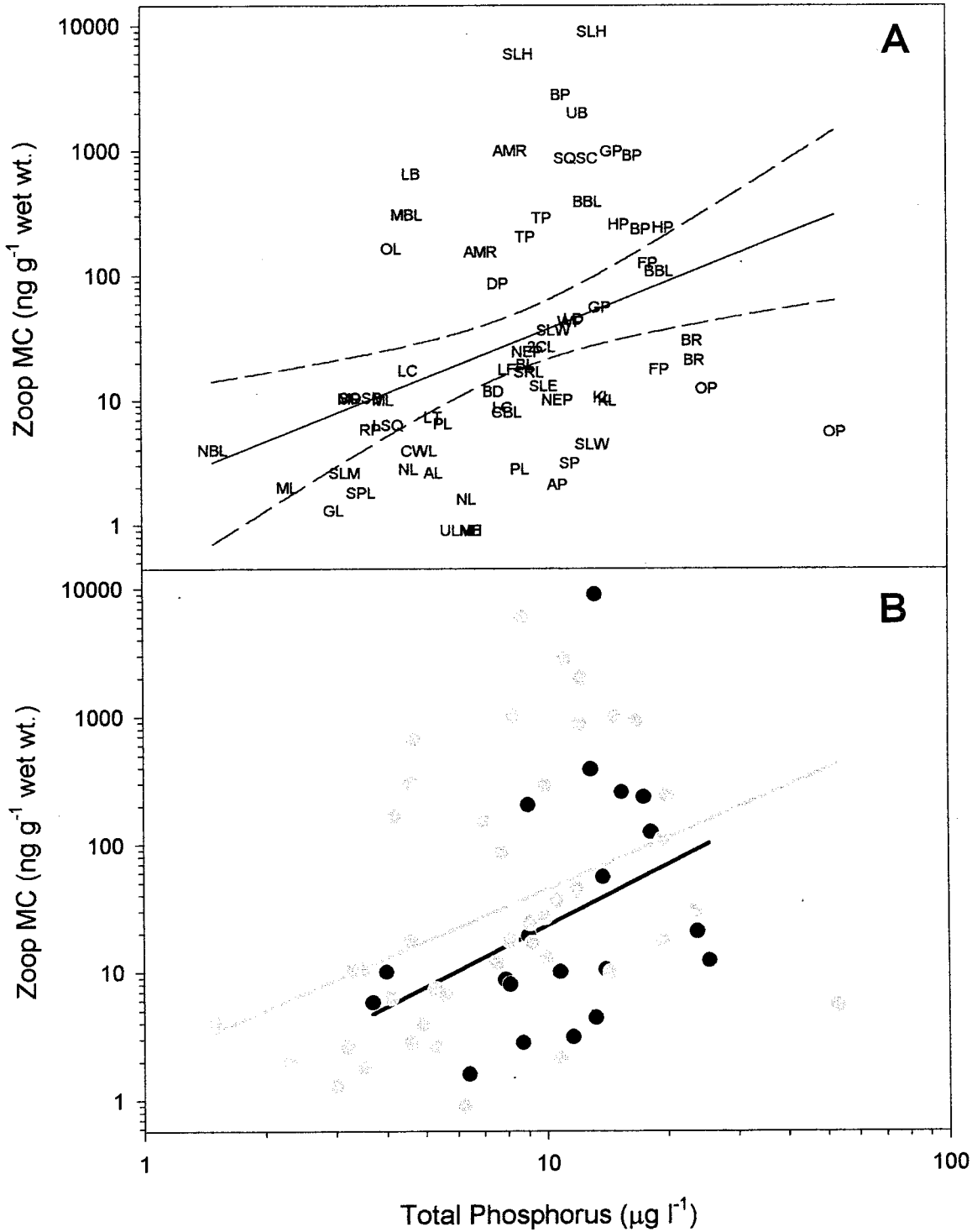
Figure 20. Linear regressions of total nitrogen and zooplankton microcystins. Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
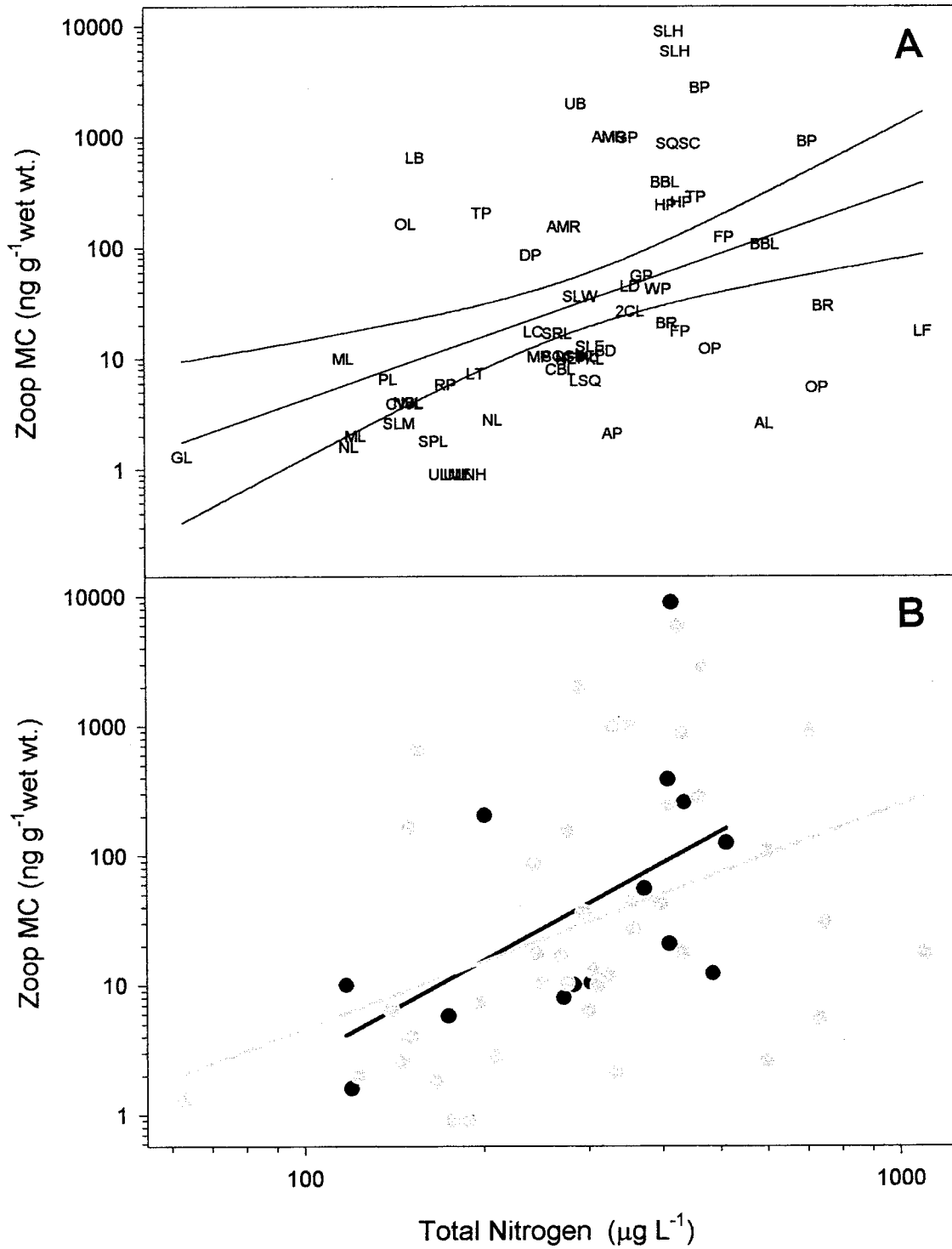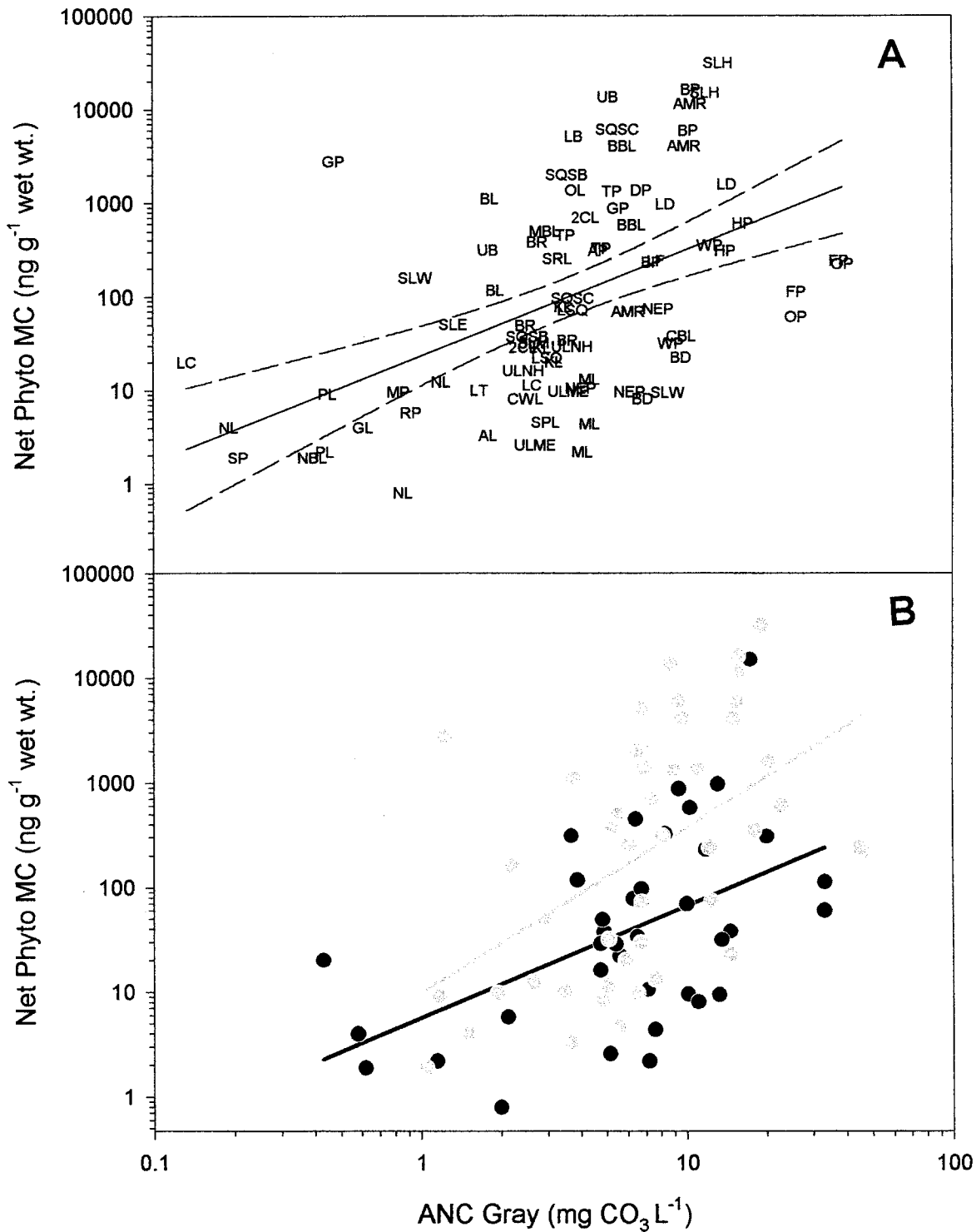
Figure 21. Linear regressions of total nitrogen and zooplankton microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
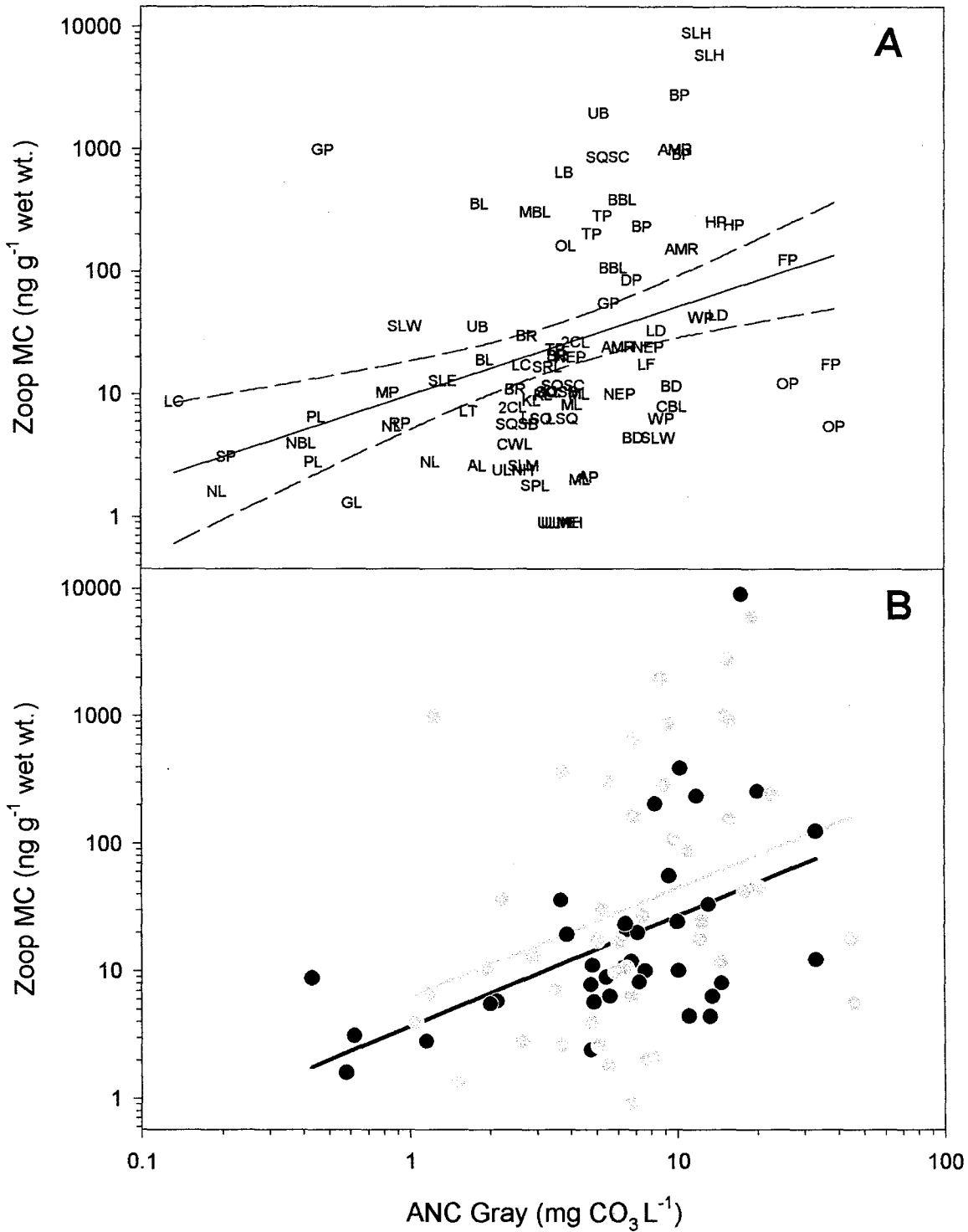
Figure 22. Linear regressions of chlorophyll a and whole lake water microcystins. Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.

Figure 23. Linear regressions of chlorophyll a and net phytoplankton microcystins. Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
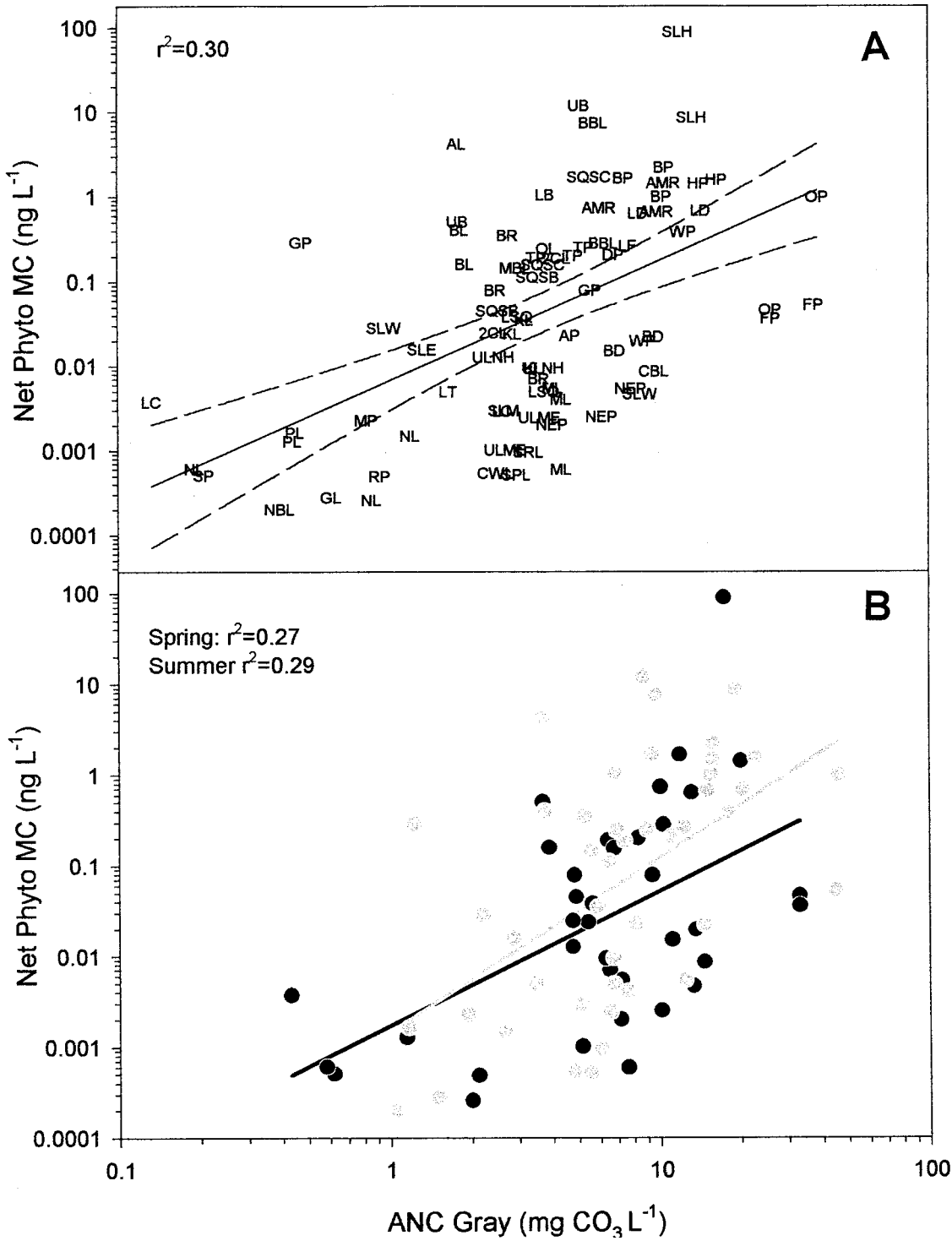
Figure 24. Linear regressions of chlorophyll a and zooplankton microcystins. Data from the New Hampshire microcystin survey, 1999. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.

Figure 25. Linear regressions of chlorophyll a and whole lake water microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
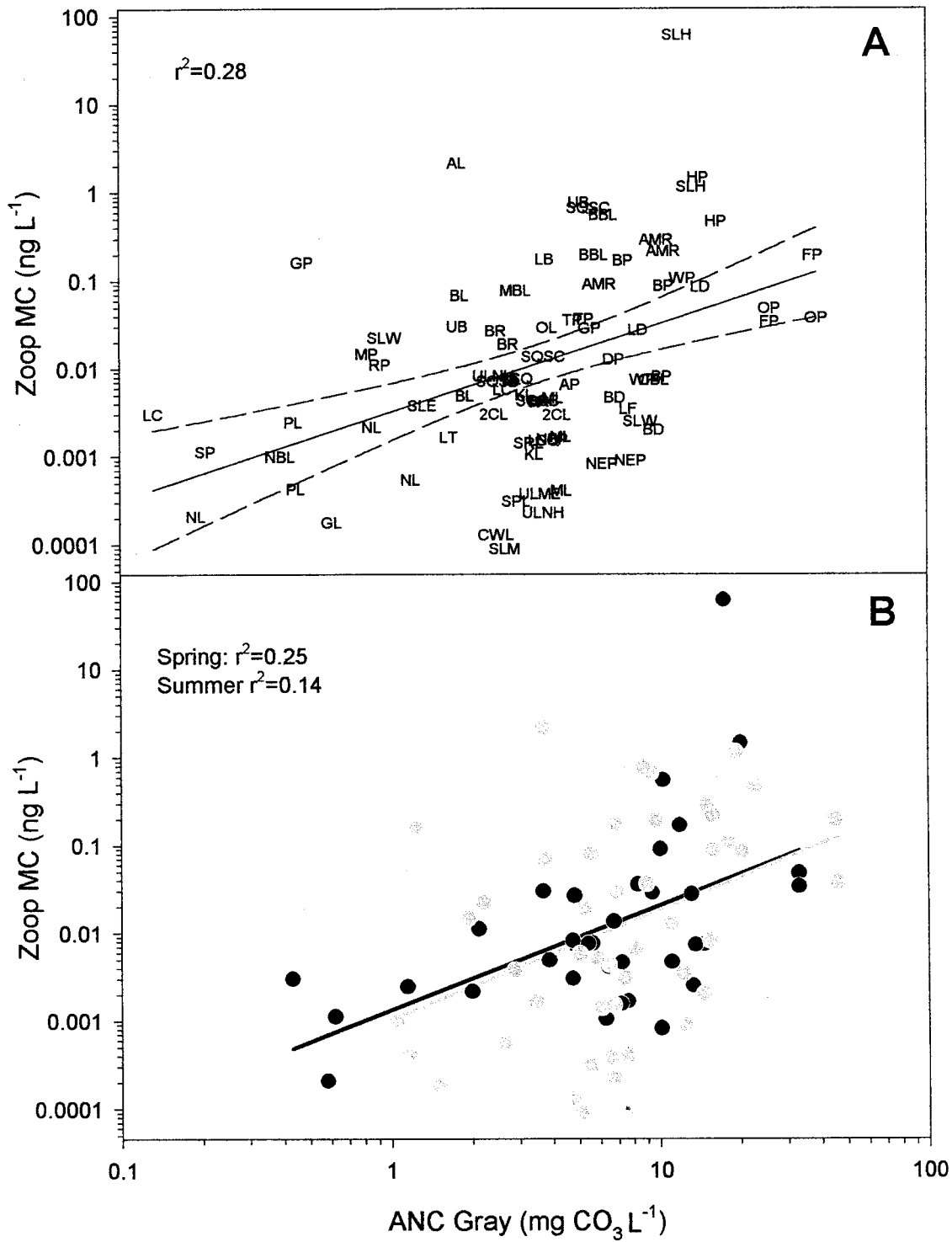
Figure 26. Linear regressions of ANC and net phytoplankton microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.

Figure 27. Linear regressions of ANC and zooplankton microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
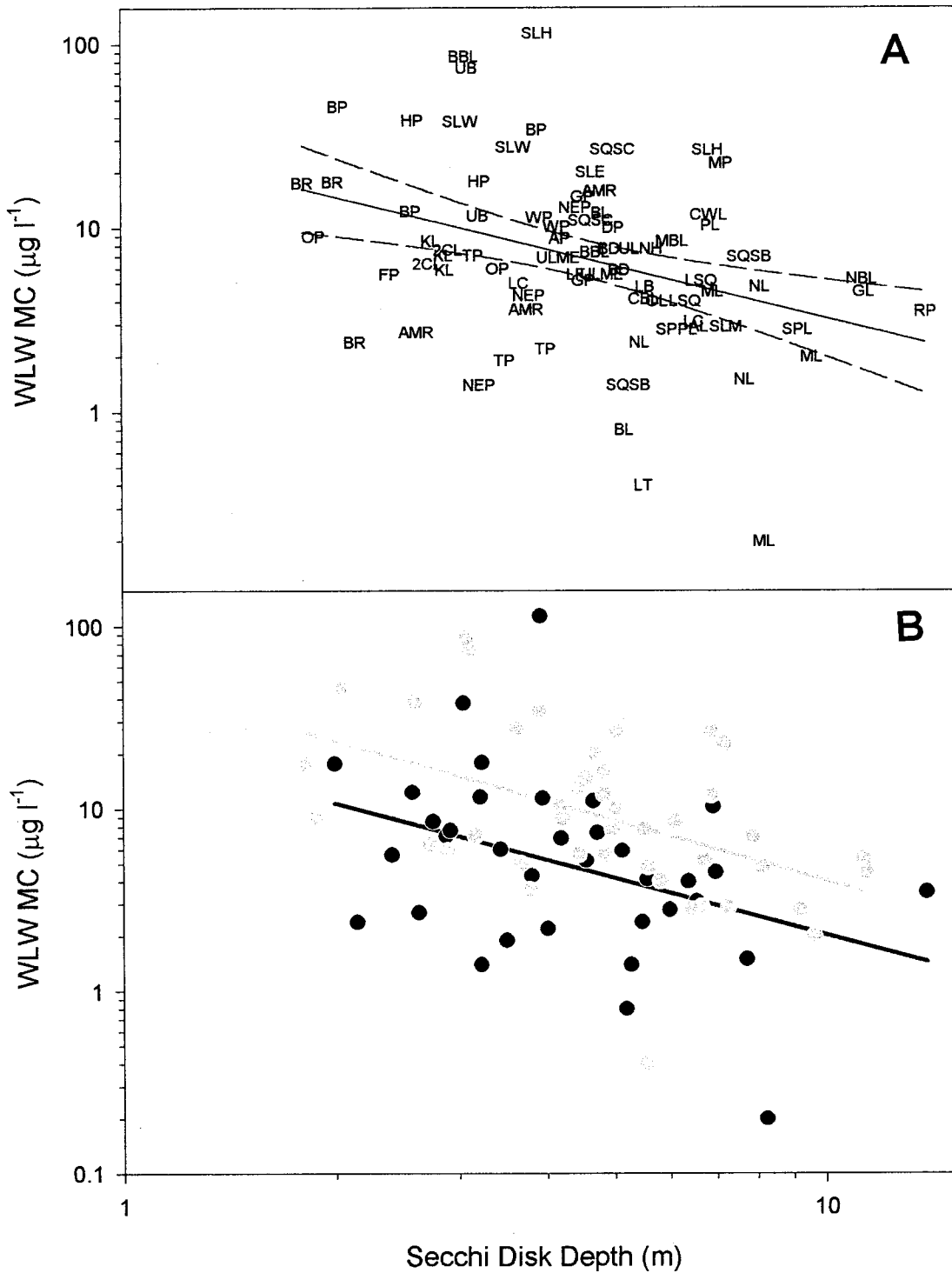
Figure 28. Linear regressions of ANC and net phytoplankton microcystins (lake conc.). Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
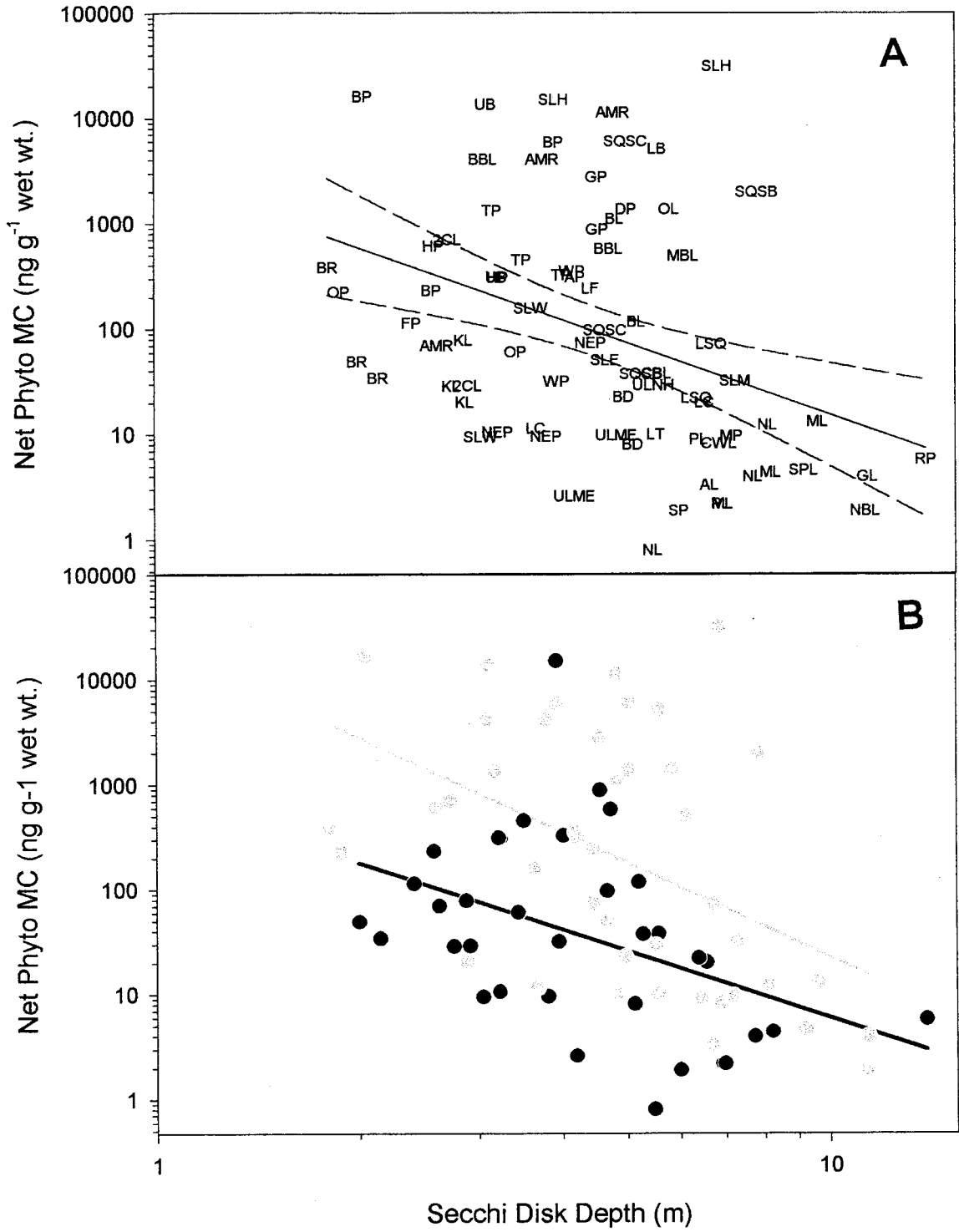
Figure 29. Linear regressions of ANC and zooplankton microcystins (lake conc.). Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.

Figure 30. Linear regressions of Secchi disk depth and whole lake water microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
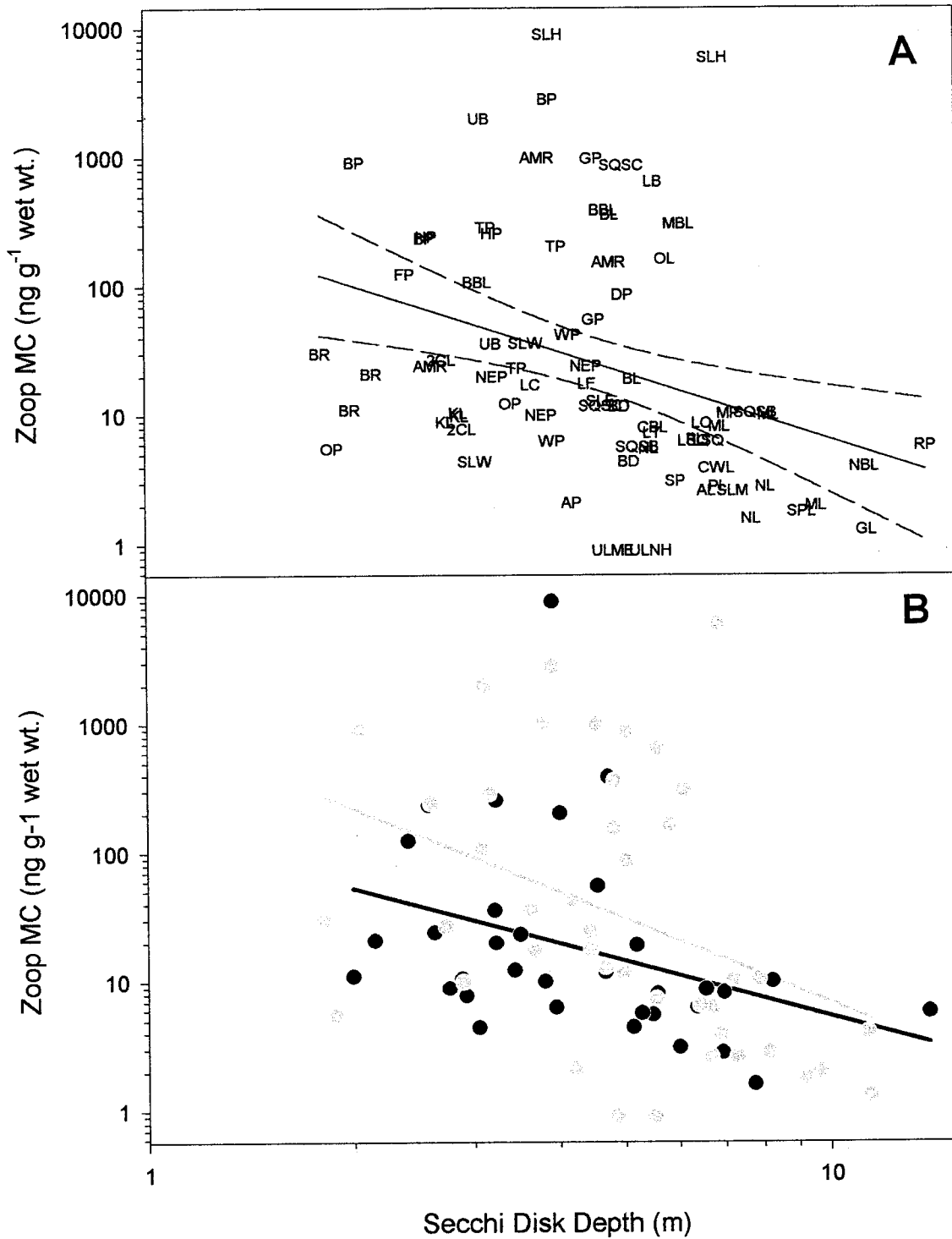
Figure 31. Linear regressions of Secchi disk depth and net phytoplankton microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.

Figure 32. Linear regressions of Secchi disk depth and zooplankton microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.

Figure 33. Linear regressions of mean depth and whole lake microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
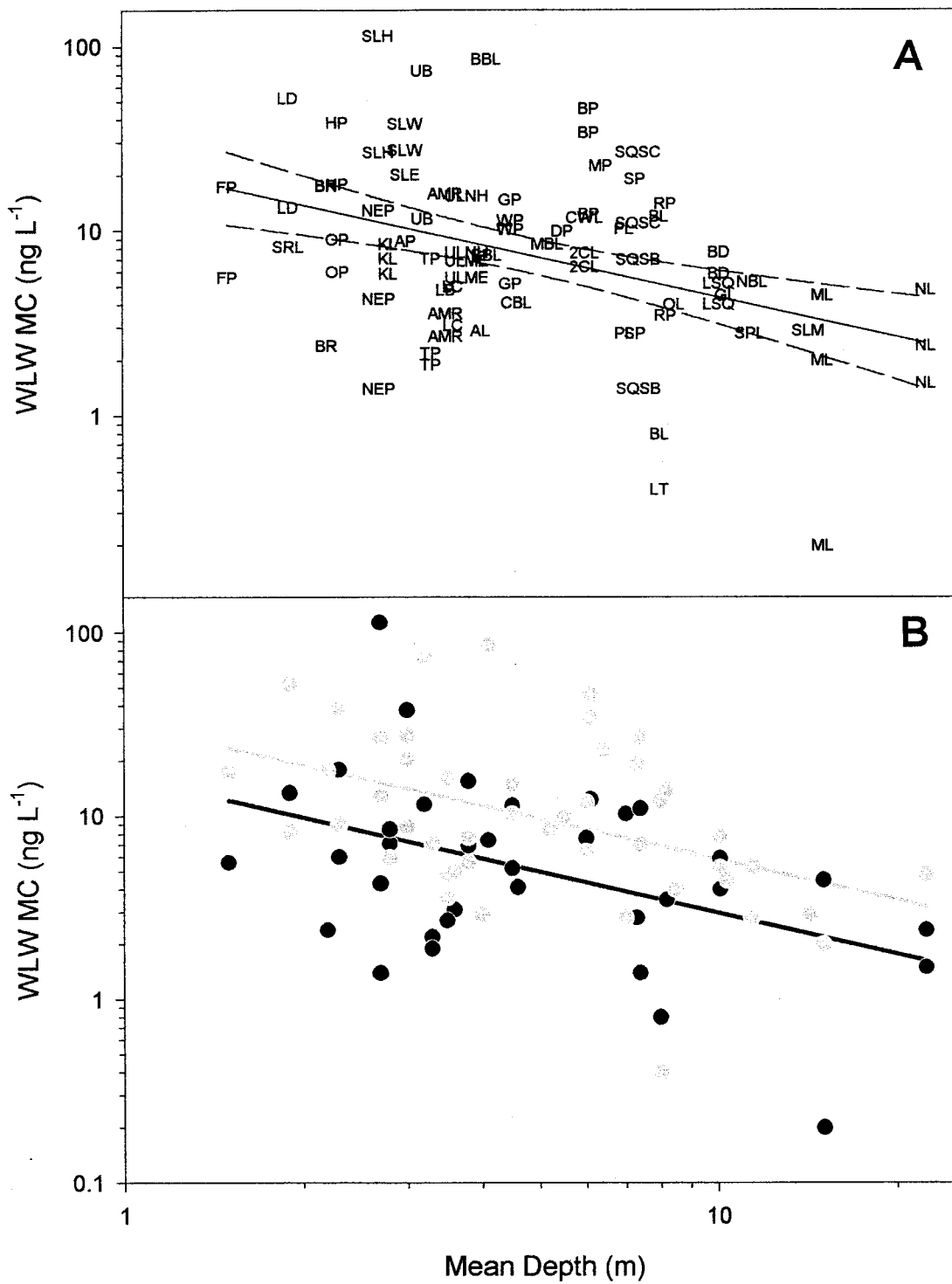
Figure 34. Linear regressions of mean depth and net phytoplankton microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.
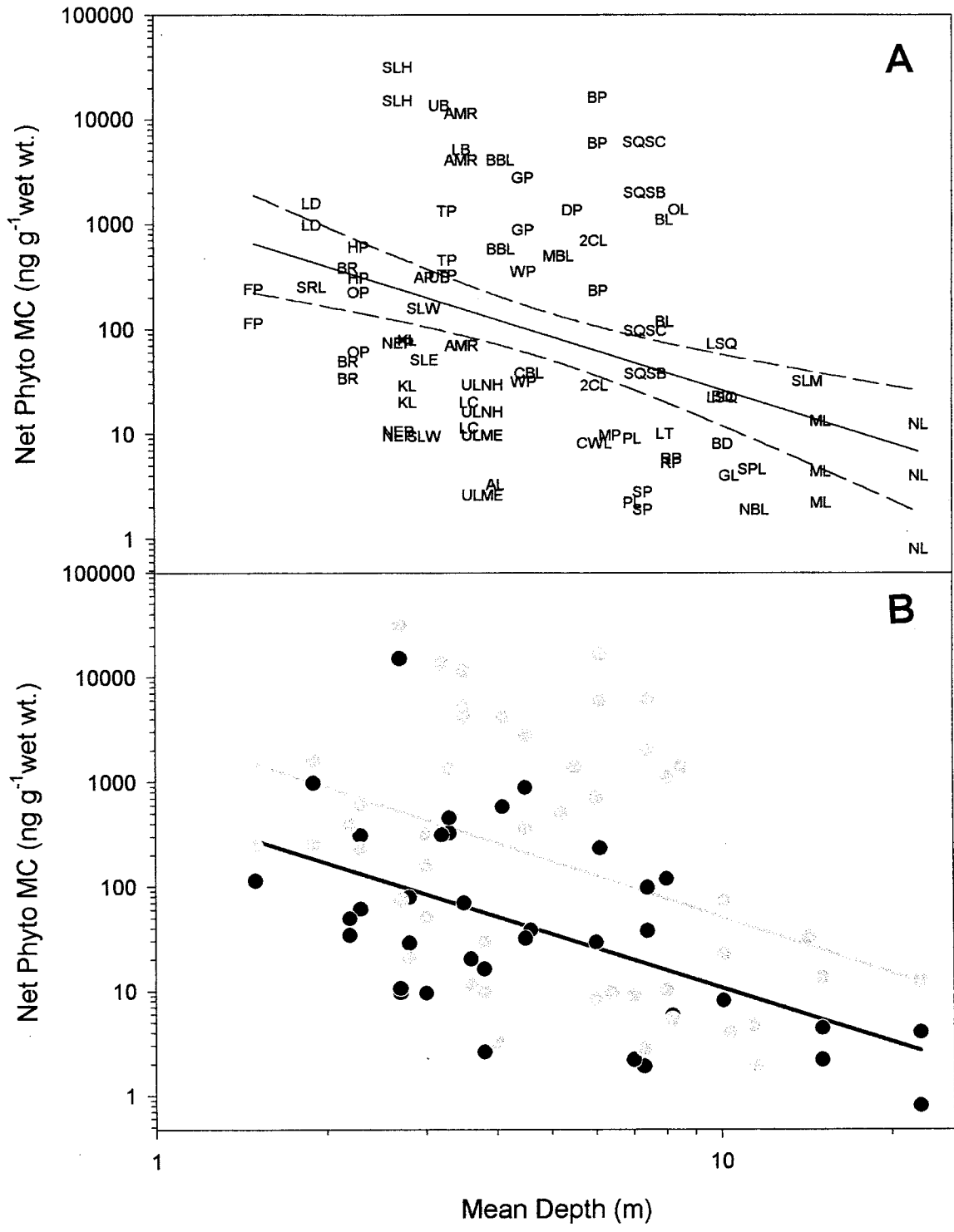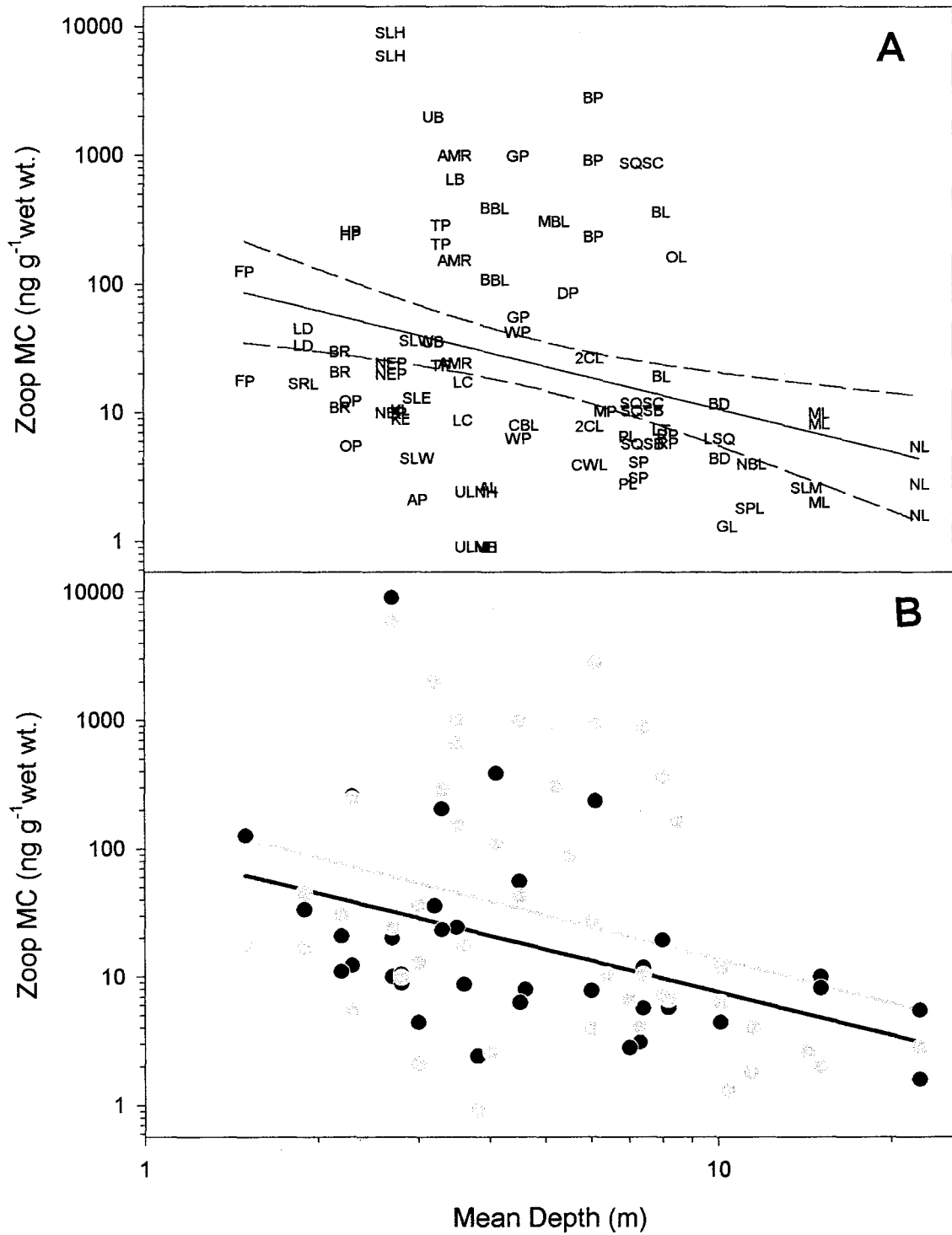
Figure 35. Linear regressions of mean depth and zooplankton microcystins. Data from the New Hampshire microcystin survey, 1999-2000. A. Combined seasons. Lake abbreviations in Table 1. Dashed lines are 95% conf. intervals. B. Symbols and lines: black=spring, grey=summer.

Information Transfer Program

USGS Summer Intern Program

Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	13	7	0	0	20
Masters	2	2	0	0	4
Ph.D.	1	0	0	0	1
Post-Doc.	0	0	0	0	0
Total	0	0	0	0	25

Notable Awards and Achievements

None

Publications from Prior Projects

None