New York State Energy Research and Development Authority

Chemical and Biological Monitoring of Adirondack Lakes to Examine Ecosystem Impacts and Recovery from Sulfur and Nitrogen Deposition

Final Report

April 2014

Report Number 14-20

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Chemical and Biological Monitoring of Adirondack Lakes to Examine Ecosystem Impacts and Recovery from Sulfur and Nitrogen Deposition

Final Report

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1 Introduction

1.1 Background

The Adirondack Region of New York State encompasses 2.6 million hectares (ha) of area that includes approximately 2,800 ponded waters (with a minimum size of about 0.4 ha), with a combined surface area of 99,715 ha (Colquhoun et al. 1984), and 9,400 kilometers (6,700 ha) of significant fishing streams (Colquhoun et al. 1982). Based upon historical surface water alkalinities, the Adirondacks was considered one of the largest regions susceptible to acidification in the eastern United States (Omernik and Powers 1982) since it received substantial inputs of acid deposition on an annual basis (Gibson and Linthurst 1982).

In view of its geographical sensitivity, there were projections of widespread destruction of water resources in the Adirondack region from acid deposition during the late 1970s and early 1980s. Although the New York State Department of Environmental Conservation (NYSDEC) had collected chemical and biological data since 1977 on waters that were believed to be sensitive to acidification, a review of these data gave an incomplete picture of both previous and existing conditions. It was apparent that a more standardized, detailed, and comprehensive survey was needed to examine the extent and magnitude of water resource acidification in the Adirondack region.

In 1983, the Adirondack Lakes Survey Corporation (ALSC), a NYS 501c3 not-for-profit corporation, was established by ESEERCO (Empire State Electric Energy Research Corporation) and the NYSDEC to conduct water chemistry and fisheries surveys to evaluate the status of water resources in the Adirondack Park. From 1984 to 1987, ALSC field investigations focused on collecting detailed chemical, physical, and biological data from 1,469 Adirondack lakes and ponds ranging in size from about 0.5 to 500 acres.

The data collected during the mid-1980s by the ALSC revealed that 352 Adirondack waters had pH values of 5.0 or less. Fish were not captured in 346 of the waters surveyed. The majority of acidified waters and those waters without fish captured were located in the western and southwestern Adirondack region, which is the geographical area most sensitive to acidification. Waters in which fish were not captured typically were small (<10 acres), shallow (mean depth <10 feet) and located at high elevation (>2,000 feet). In addition, fishless waters were characterized as having low pH (<5.0 s.u.), low acid neutralizing capacity (ANC, <0.0 μeq/L), low calcium concentrations and high aluminum values.

Historically, the most significant response from the general public and scientific community to the effects of acidification on aquatic populations was directed toward fish, primarily because of the historical importance of sport fishing in Adirondack lakes and ponds. No less important, however, are organisms such as bacterioplankton,

phytoplankton, zooplankton and macrophytes that have an integral role in regulating the energy and nutrient cycling within aquatic ecosystems. Independent studies and a study funded by NAPAP (National Acid Precipitation Assessment Program) in conjunction with the ALSC investigations were conducted during the 1980s and documented decreased species diversity, richness and biomass for phytoplankton (Siegfried et al. 1989), planktonic rotifers (Siegfried et al. 1984, Sutherland 1989), crustacean zooplankton (Sutherland et al. 1984, Sutherland 1989), and macrophytes (Singer and Boylen 1984, Jackson and Charles 1988, Lyons-Swift et al. 1989) to increasing acidification.

Although an extensive body of Adirondack acidification data resulted from the collective 1980s studies, uncertainties still remained regarding the extent and permanence of atmospheric deposition effects on aquatic community structure and function. The potential for these systems to recover in the face of present and future rates of deposition of atmospheric nitrogen and sulfur also was unclear.

The 1990 Clean Air Act Amendments (CAAA) mandated reductions in nitrogen (N) and sulfur (S) emissions nationwide in order to improve the quality of terrestrial and aquatic ecosystems. The CAAA also required an evaluation of the effects of emissions reductions through studies coordinated by the NAPAP. It was considered essential to initiate a program that was designed to provide comprehensive data of sufficient quantity and quality to evaluate directly the long-term response of biological communities to reduced emissions. Improvement in water chemistry can provide evidence of the first stage of recovery, but recovery of biological communities is dependent upon additional factors such as the ability of species to reinvade and/or populate restored habitats. Furthermore, biological recovery may be uncoupled from changes in water chemistry and respond more slowly to reduced emissions.

1.2 Adirondack Effects Assessment Program (AEAP)

The Adirondack Effects Assessment Program (AEAP) resulted from a 1992 federal appropriation attached to the 1990 CAAA and was appropriated specifically to support research on the effects of reduced air pollutant emissions in the Adirondack region. The fundamental issue expressed in reviews of the program was that it should provide data that can be used to assess recovery from acidification. Critical issues in design of the program were selection of study sites (lakes and ponds) and sampling protocols such that the data 1) would be representative of the Adirondack region, 2) could be used to make statistically valid conclusions, and 3) could be used to make conclusions on a regional scale. Furthermore, the data would be most valuable if they could be integrated with other databases and ongoing water chemistry monitoring programs. Because financial support of the program beyond the initial contract was uncertain, the program design had to prioritize site selection criteria (random vs. ongoing and previously-studied sites), with numbers of sites, frequency of sampling, and expected duration of the study.

1.2.1 Program History and Administration

The design of the AEAP was an outgrowth of discussions at a workshop of recognized experts held at Rensselaer Polytechnic Institute (RPI) in January 1993. Following the workshop, which was identified as AEAP Task 1, the U.S. Environmental Protection Agency (US EPA) contract with RPI was modified to include three additional tasks including 1) monitor the status of aquatic biological communities in Adirondack waters, 2) support continued monitoring of atmospheric deposition at the NADP sites in the Adirondack region (Huntington Forest and Bennett Bridge), and 3) perform research to determine the factors controlling the retention of atmospherically deposited nitrogen in Adirondack watersheds. Subsequent work plans were reviewed internally by the US EPA and by external peer reviewers.

The AEAP was implemented in 1994 to evaluate the effects of atmospheric deposition on the acidification of ponded waters and watersheds of the Adirondack Mountain region of New York State. The program was designed to both:

- Assess the current state of different biological trophic levels as an estimate of aquatic ecosystem health and a baseline upon which to evaluate future trends resulting from reduced emissions and deposition of acidic compounds.
- Assess the major factors controlling the watershed cycling and leaching of nitrogen, which will determine the effect of continued atmospheric deposition of nitrogen oxides on aquatic ecosystems.

The aquatic biota component was initiated in 1994 and the watershed nitrogen component was added in 1997. A federal appropriation provided support for the Program and was administered by the US EPA through a contract (EPA Contract 68D20171) to RPI.

1.2.2 Program Components and Schedule

The AEAP was conducted within the boundaries of the Adirondack Park in New York State (Figure 1-1) and consisted of three separate components: aquatic biota sampling, atmospheric deposition monitoring, and a study of watershed nitrogen cycling.

The Aquatic Biota Study included water chemistry and aquatic biota sampling, and was initiated in 1994. From 1994 through 1996, a total of 30 AEAP waters were sampled three times each summer. Starting in 1997, and continuing through 2006, the study waters were sampled twice each summer. In 2002, five additional sites were added to the 30 original study sites. Atmospheric deposition monitoring at the NADP network stations located at Huntington Forest and Bennett Bridge in upstate New York was supported by the AEAP between 1994 and 2006.

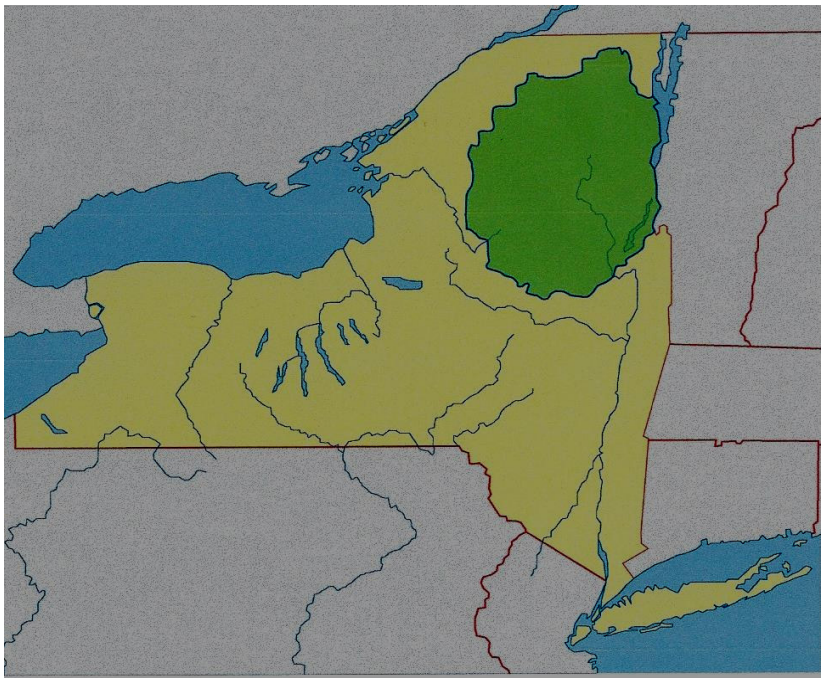
The Watershed Integrated Nitrogen Cycling Study was the third major component of the AEAP. Although watershed nitrogen research was proposed in 1994, the field studies portion of the AEAP were not implemented until 1997 in the Buck Creek watershed and these studies continued through 2006.

1.2.3 The Aquatic Biota Study

A major conclusion of the 1993 RPI workshop discussions was that significant knowledge gaps existed in understanding of the effects of acidification on aquatic organisms and that the lack of consistent biological data collection would make it difficult to assess whether lake and pond biological communities in the Adirondacks are changing over time in response to reduced emissions promulgated by the 1990 CAAA.

Figure 1-1. New York State map showing location of the Adirondack Park

The Adirondack Park is represented by the dark green polygon.



1.2.4 Study Goals

Accordingly, the goals of the AEAP Aquatic Biota Study were structured to:

- Provide long-term (temporal) benefits by collecting baseline information that could be used to evaluate the future recovery of lake communities.
- Provide short-term benefits in the increased understanding of the complex effects of acidification on community structure by simultaneously evaluating effects of acidification on multiple trophic levels in multiple types of lake systems.

1.2.5 Study Objectives

The objectives developed for the Aquatic Biota Study were:

1. Quantify the interactive relationships between environmental factors and species abundance within the bacterioplankton, phytoplankton, zooplankton, fish, and macrophyte communities.
2. Evaluate species within the bacterioplankton, phytoplankton, zooplankton, fish, and macrophyte communities as indicators of acidification to provide a basis for assessing recovery from acidification.
3. Categorize study waters into distinct types based on the abundance and assemblage of indicator species and physical/chemical ecosystem characteristics; compare categories based on indicator species and physical/chemical attributes and use categories to set target levels for recovery.
4. Document shifts of waters from categories typical of acidification to categories typical of unaffected lakes if recovery is occurring.
5. Detect association between trends of recovery (shifts of lakes between categories) and reductions in acidic atmospheric deposition.

1.2.1 Study Design

The Aquatic Biota Study was designed to provide 1) baseline data upon which to evaluate temporal changes, and 2) short-term gains in understanding the effects of acidification on ecosystem community structure. The specific challenges of designing the Study to meet these goals were as follows:

- Logistical constraints of sampling statistically significant numbers of ponded waters to provide adequate monitoring data,
- Providing an adequate diversity of study sites to cover the different lake types encountered in the Adirondacks and the wide geographic area encompassed by the Adirondack Mountain region,
- Providing a sufficient number of replicates that would allow analyzing trends in space and time, and
- Conducting adequate sampling of multiple trophic levels to allow the analysis of complex effects of acidification and to distinguish between the effects of acidification and other factors.

1.3 Study Region and Sites

To meet the objective of detecting temporal changes in biological community structure, the study region was limited to the southwest portion of the Adirondack Park since:

- This region of the Adirondack Park receives the highest deposition rates of air-borne pollutants originating in the Ohio Valley.
- Waters in this region are the most impacted, and may be most likely to demonstrate recovery.
- Restricting the area of the Study decreases geographic and climatic variability that may tend to increase variance and decrease the statistical power to detect temporal change.

To meet the third objective of the research and evaluation effort, the study sites (lakes and ponds) were selected to incorporate different hydrologic types including:

- TDL: thin till, drainage, low dissolved organic carbon (DOC).
- TDH: thin till, drainage, high DOC.
- MDL: medium till, drainage, low DOC.
- MDH: medium till, drainage, high DOC.
- MSL: mounded, seepage, low DOC.
- MSH: mounded, seepage, high DOC.
- C: carbonate waters.

This categorization of ponded waters is based on the classification scheme developed by Newton and Driscoll (1990) and is used by other researchers in the Adirondack Region.

Finally, to provide the highest degree of accuracy in relating spatial and temporal biological characteristics to lake and pond water chemistry, the study sites were selected to coincide with an on-going water chemistry monitoring program, the Adirondack Long-Term Monitoring (ALTM) Program, which was initiated in 1992 and sampled the water chemistry of 52 lakes and ponds monthly.

The focus of the Aquatic Biota Study on the southwest Adirondacks 1) assesses recovery where acidification is most prevalent and where fish communities are most affected, 2) results in decreased variability among study sites by restricting waters to a smaller region, and 3) increases the ratio of sampled to total waters in the region, allowing extrapolation of the results to the larger area affected by acidification.

The 35 ponded waters included in the Aquatic Biota Study are listed in Table 1-1 and located on a map in Figure 1-2. The listing includes the original 30 waters and five additional waters added to the Program in 2002. Some of the 35 waters have been included in other Adirondack research programs (Table 1-2).

1.3.1 Sampling Frequency

Budgetary constraints within the AEAP required balancing the sampling frequency with the total number of waters sampled. The waters were sampled three times during 1994-1996 to facilitate statistical analysis, and the three sampling visits were conducted during the summer period of thermal stratification (mid-June until mid-September) to provide the lowest variance within the ecosystem.

Although biological communities sampled during the mid-summer may not exhibit the direct impacts of episodic acidification associated with spring snowmelt and runoff, replication of sampling during the most stable part of the growing season increases the ability to detect temporal changes in the long-term effects of acidification. The Aquatic Biotic Study would not have been able to sample both spring and summer seasons adequately, and spring sampling is problematic (more variable, logistical problems associated with snowmelt and ice-out, unpredictable weather and hydrologic conditions). It was anticipated that by replicating sampling during summer, the lakes would be stratified, and biological communities would be relatively stable. The summer data can be used to assess inter-annual variability, rather than seasonal variability and, evaluate differences in biological community structure among lake types and detect changes over time.

Extension of the AEAP beyond 1996 required renegotiation of the AEAP work plan and approval by the US EPA and external peer reviewers. Following this process, US EPA mandated a reduction of the budget for the Aquatic Biotic Study. In order to accommodate the budget reductions and also minimize the impact of these reductions on the ability to detect temporal changes in aquatic biota, the frequency of sampling was reduced to twice each summer, rather than reduce the total number of lakes and ponds being studied.

Table 1-1. Study sites sampled during the Aquatic Biota Study, 1994-2006

Lake Name/ Study site	Code/ Abbreviation[1]	W# P# [3]	Latitude	Longitude	Hydrologic type[4]	Surface area (ha)	Maximum depth (m)
Hamilton County							
Brooktrout	26/btr	04874	433600	743945	TDL	28.7	23.2
Carry	27/car	05669	434054	742921	MSL	2.8	4.6
Cascade	17/cas	04747	434721	744846	MDL	40.0	6.1
Constable	14/con	04777	434950	744827	TDL	20.6	4.0
G	28/gla	07859	432505	743810	TDL	39.9	9.8
Helldiver*	33/hel	04877	434010	744200	MDH	6.5	3.4
Icehouse*	34/ice	04876	433858	744213	MSL	2.8	13.4
Indian	25/ind	04852	433724	744544	TDL	33.2	10.7
Jockeybush	29/joc	05259	431808	743509	TDL	17.3	11.3
Long	21/lon	05649	435015	742850	TDH	1.7	4.0
Queer	15/que	06329	434849	744825	TDL	54.5	21.3
Raquette	19/raq	06315a	434711	743912	MDH	1.5	3.0
Sagamore	20/sag	06313	434557	743743	MDH	68.0	22.9
Seventh*	32/sev	04787b	434447	744550	MDL	344.5	26.5
Squaw	24/sqw	04850	433810	744420	TDL	36.4	6.7
Willis	30/wil	05215	432217	741447	MDL	14.6	3.0
Herkimer County							
Big Moose	13/moo	04752	434902	745123	TDL	512.5	21.3
Dart	10/dar	04750	434736	745216	TDL	51.8	17.7
Grass	5/gr2	04706	434125	750354	MDL	5.3	5.2
Rondaxe	8/ron	04739	434523	745459	TDL	90.5	10.1
Limekiln	18/lim	04826	434248	744847	MDL	186.9	21.9
Loon Hollow	1/loo	04186	435741	750243	TDL	5.7	11.6
Moss	9/mos	04746	434652	745111	MDL	45.7	15.2
M Branch	3/bra	04707	434152	750608	TDL	17.0	5.2
M Settlement	4set	04704	434102	750600	TDL	15.8	11.0
North	22/nor	041007	433120	745655	TDL	176.8	17.7
Round	6/rou	04834	434412	745822	MSL	2.6	6.4
South	23/sou	041004	433034	745238	TDL	202.0	20.1
Squash	12/squ	04754	434932	745311	TDH	3.3	5.8
Sunday*	31/sun	04473	435140	750607	MDH	7.7	5.5
West	11/wes	04753	434841	745300	TDL	10.4	5.2
Wheeler	7/whe	04731	434424	745748	MSH	5.2	18.0
Willy's	2/wls	04210	435820	745720	TDL	24.3	13.7
Windfall	16/win	04750a	434818	744953	C	2.4	6.1
Warren County							
Trout*	35/tro	02379	433242	734147	MDL	95.8	22.9

[1] The code # locates the study site on Figure 1-2; the 3-letter abbreviation also is provided for future reference in the report.

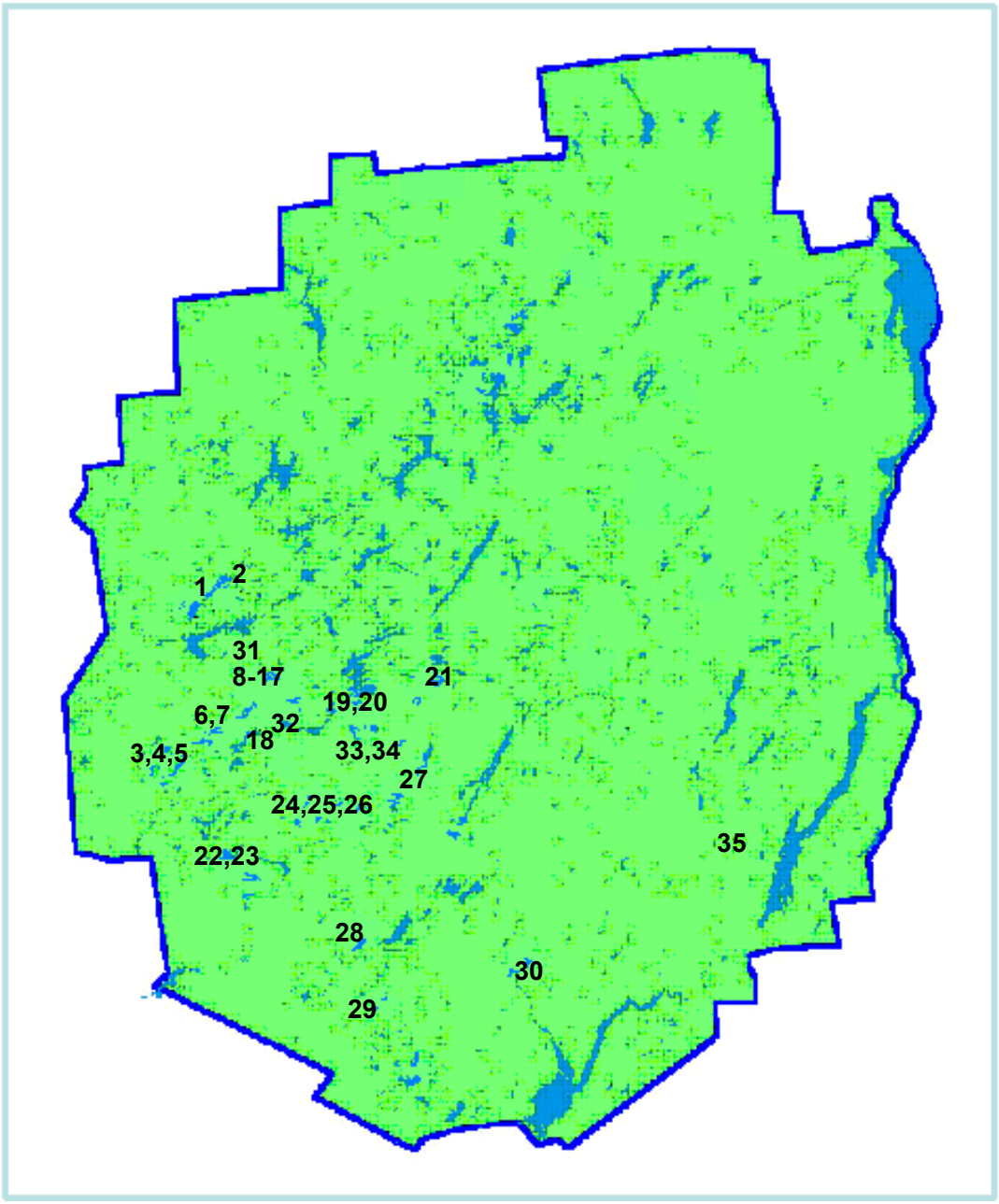
[2] W# = watershed number, i.e., the major drainage basin within which the ponded water is located. There are 17 major watersheds in New York State ("04" = Oswegatchie/Black, "05" = Upper Hudson, "06" = Raquette, "07" = Mohawk/Hudson).

[3] P# = pond number, i.e., the number designated for a specific ponded water by the NYSDEC in Part 800 of Codes, Rules and Regulations pertaining to Article 15 of the NYS Environmental Conservation Law.

[4] Hydrogeologic type is explained in the text of this document.

* Denotes lakes that were only sampled between 2002 and 2007.

Figure 1-2. Location in the Adirondack Park of the 35 Aquatic Biota Study sites



1.3.2 Sample Types

The Aquatic Biota Study collected samples for water chemistry, bacterioplankton, phytoplankton, zooplankton, fish and macrophytes, as well as depth profiles of temperature, dissolved oxygen and light (Table 1-3). Water samples were collected at the time of bacterioplankton, phytoplankton, and zooplankton sampling in order to provide contemporaneous water chemistry information. These different types of samples were collected during each mid-summer sampling period. Sampling for fish and macrophytes was more time consuming and labor intensive and, therefore, was conducted at different times.

Table 1-2 Aquatic Biota Study sites included in previous Adirondack studies

CODE	AEAP[1]	ALSC[2]	ALTM[3]	ABS[4]	DDRP[5]	PIRLA-I[6]	PIRLA-II[7]	RILWAS[8]	ILWAS[9]
MOO	X	X	X	X		X	X	X	
BTR	X	X	X	X					
CAR	X	X	X						
CAS	X	X	X	X					
CON	X	X	X	X	X		X		
DAR	X	X	X	X					
GLA	X	X	X	X					
GR2	X	X	X		X				
HEL				X					
ICE				X					
IND	X	X	X	X					
JOC	X	X	X						
LIM	X	X	X	X					
LON	X	X	X						
LOO	X	X	X						
BRA	X	X	X						
SET	X	X	X						
MOS	X	X	X	X					
NOR	X	X	X						
QUE	X	X	X			X			
RAQ	X	X	X						
RON	X	X	X						
ROU	X	X							
SAG	X	X	X	X				X	X
SEV									
SOU	X	X	X	X	X		X		
SQW	X	X	X	X			X		
SQU	X	X	X						
SUN									
TRO									
WES	X	X	X			X			
WHE	X	X							
WIL	X	X	X						
WLS	X	X	X						
WIN	X	X	X			X			

1 Adirondack Effects Assessment Program, Aquatic Biota Study

2 Adirondack Lakes Survey Corporation

3 Adirondack Long-Term Monitoring Program

4 Adirondack Biota Study

5 Delayed Direct Response Program

6 Paleoecological Integrated Regional Lake Assessment – Phase I

7 Paleoecological Integrated Regional Lake Assessment – Phase II

8 Regional Integrated Lake and Watershed Acidification Survey

9 Integrated Lake and Watershed Acidification Survey

Table 1-3. Physical, chemical, and biological parameters included in the Aquatic Biota Study, collection technique, and methodology, 1994-2006

Parameter	Collection Technique	Analytical Methodology
Physical Characteristics: (Light, Dissolved Oxygen, Secchi, Temperature)	Vertical profiles at 1m intervals (except Secchi) at deep site	Standard Secchi protocol; YSI dissolved oxygen-temperature meter; Licor light meter
Chemical Characteristics: (pH, ANC, conductivity, NO_3, NH_4, TN, TP, TFP, PO_4, DIC, DOC, Si, Ca, Mg, Na, K, Fe, SO_4, Cl, F,Tot Al, mono-Al, non-labile mono-Al)	Integrated epilimnetic sample; hypolimnetic grab sample at least 1 m above bottom sediment	Ion Chromatograph, Atomic Absorption, Autoanalyzer, Spectrophotometer, pH meter, DOC analyzer, Flow Injection Analysis, Inductively Coupled Plasma Emission
Biological Characteristics: Bacterioplankton	Integrated epilimnetic sample Hypolimnetic grab sample	16S ribosomal DNA, cloning, sequencing, phylogenetic analyses & 16S ribosomal RNA analysis (collected and stored for *in situ* hybridizations)
Biological Characteristics: Phytoplankton	Integrated photic zone sample (Integrated epilimnetic sample archived)	chlorophyll a, species identification and enumeration
Biological Characteristics: Zooplankton	Constant flow pump, 40 µm mesh net, integrated to 2 mg/L DO or within 1 m of bottom	Rotifers, crustaceans, Chaoborus
Biological Characteristics: Macrophytes	SCUBA transects	Species identification, density, diversity and dominance
Biological Characteristics: Fish	Trap-net, seine, SCUBA observations	Tags, identification, enumeration, size, gut contents, scale counts

The following sections briefly summarize sample collection activities that occurred during the course of AEAP.

1.3.2.1 Water Chemistry

Water samples were collected at each study site coincident with the collection of the mid-summer biological samples for bacterioplankton, phytoplankton and zooplankton. Two samples (epilimnetic and hypolimnetic) were collected from waters that exhibited thermal stratification; a single sample (epilimnetic) was collected from waters not thermally stratified. The water samples were placed on ice and transported to the field laboratory within a matter of hours following collection to be processed, preserved (when appropriate), and analyzed for the chemicals and nutrients presented in Table 1-4.

Table 1-4. Chemical parameters and analytical methods utilized in the Aquatic Biota Study, 1994 2006

Details found in Table 3-1.

Parameter	Analytical Method
pH	Electrometric (US EPA Method 150.1)
ANC	Gran Titration (US EPA Method 310.1)
Specific Conductance	Wheatstone Bridge type meter (US EPA Method 120.1)
Dissolved Oxygen	Membrane Electrode (US EPA Method 360.1)
Inorganic Anions (Cl, NO3, SO4)	Ion Chromatography (US EPA Method 300.0)
Total Nitrogen	Persulfate Oxidation
Phosphorus (total, total filterable)	Colorimetric (US EPA Method 365.2)
Dissolved Organic/Inorganic Carbon	IR Spectroscopy (US EPA Method 415.2)
Ammonium/Orthophosphorus	Flow Injection Analysis (Lachat)
Soluble Reactive Silica	Colorimetric (US EPA Method 370.1)
Total Metals	Atomic Absorption (US EPA Method 200)
Aluminum (total)	Inductively Coupled Plasma Emission
Aluminum (monomeric)	Flow Injection
Aluminum (non-labile monomeric)	Flow Injection
Chlorophyll	Fluorometric (Turner 1985)

1.3.2.2 Bacterioplankton

Sub-samples were taken from an integrated epilimnetic sample and a single depth hypolimnetic grab (1 m off the bottom) and approximately 900-1000 mL were filtered through a 0.22 um filter. The filters were preserved at -80 °C for subsequent DNA and RNA extractions to identify bacterial species from 16S ribosomal DNA and 16S ribosomal RNA sequences.

1.3.2.3 Phytoplankton

A single sub-sample (300 mL) collected from the water column down to the depth of 1% light penetration using the integrated hose technique was preserved and later examined by light microscopy for identification and enumeration.

1.3.2.4 Zooplankton

Samples were collected from the water column using a hose integration technique, a constant-flow pump, and a 64-µm mesh net. The samples were narcotized, preserved, and examined by light microscopy. Two zooplankton samples were collected during each site visit.

1.3.2.5 Macrophytes

Communities were observed and data recorded by SCUBA divers who followed transects from the shoreline to the extent of the littoral zone at each study site. During the 13-year duration of AEAP, a total 32 study sites were surveyed for macrophytes; this total included 28 of the 30 original sites and 3 of the 5 study sites added during 2002. One site, Brooktrout Lake, was sampled for macrophytes two times during the 13-year period.

1.3.2.6 Fish

These communities were sampled using trap nets and tag-and-recapture methods. Sampling typically occurred during the spring and fall each year on a regular subset of study waters (Moss and Dart Lakes were sampled from 1995 through 2006; Lake Rondaxe was sampled from 1996 through 2006). During the 13-year duration of AEAP, a total of 30 study sites were surveyed for fish; this total included 25 of the 30 original study sites.

Table 1-5 summarizes the scope of components included in the AEAP Aquatic Biota project during Phase I (1994-1996), Phase II (1997-2002), and Phase III (2003-2007).

Table 1-5. Modifications in major components of the Aquatic Biota Study, 1994-2006

Component	1994-1996 (Phase I)	1997-2002 (Phase II)	2003-2006 (Phase III)
Number of study sites	30	30	35
Water chemistry (anions, nutrients)	30 sites	30 sites	35 sites
Bacterioplankton	7 sites	25-30 sites	
Phytoplankton	30 sites	30 sites	35 sites
Zooplankton	30 sites	30 sites	35 sites
	14-15 sites	4-5 sites (total 20-25 sites)	all 30 original sites
	6 sites	4 additional sites (total=10); 8 sites fishless, 12 too large/inaccessible	all 30 original sites surveyed with nets or by snorkel/SCUBA

1.3.3 Sampling Schedule

Each year, the AEAP study sites were sampled synoptically during each mid-summer period to minimize variability and facilitate comparison of the biological data among waters. Each sampling period consisted of two 2-3 day intervals during consecutive weeks. One week was devoted to sampling remote waters by aircraft; waters accessible by land were sampled during the other week. A total of 4-7 sites usually were sampled each day.

1.3.4 Sampling Regime

Two sampling crews, consisting of 2 people in each crew, sampled the designated waters, returning to the field laboratory during the day with samples collected from 2-3 study sites for processing. At each site, the sampling crew positioned a boat or canoe at the location of maximum depth (using bathymetric maps and a depth sounder), and collected depth profiles of temperature, light, and oxygen.

Epilimnetic water samples were collected using a wide-diameter (2.54-cm ID) hose to provide a depth-integrated composite for chemistry; a hypolimnetic grab sample was collected from 1.0 m above the bottom using a horizontal Van Dorn sampler. Water also was collected from the surface to the depth of 1% light intensity using the integrate hose to sample phytoplankton. Zooplankton were collected by slowly lowering the integrate hose either from the surface to 1.0 m above the bottom or to a depth where the dissolved oxygen level was above 2.0 mg/L. The water was filtered through a 64-um zooplankton net as the hose traversed the water column. All collected water samples were stored on ice until delivery to the field laboratory. Phytoplankton and zooplankton samples were preserved in the field.

The laboratory crew, consisting of 3-4 people, worked in the field lab located at Eagle Bay, NY. All samples were immediately accessioned upon delivery to the field lab. The samples then were processed and either refrigerated and stored until delivery to the Keck Water Lab in Troy, NY, or immediately analyzed in the field lab (conductivity, chlorophyll a, conductivity, ammonium, and soluble phosphorus).

1.3.5 Methods

A detailed description of protocol and methodology for the Aquatic Biota Project was submitted to US EPA and approved prior to implementation of the Program (Rensselaer Polytechnic Institute 1994).

1.3.6 Data Entry, Handling, and Storage

A description of data entry, handling and storage for the Aquatic Biota Project is presented in the Quality Assurance (QA/QC) document that previously was submitted to US EPA and approved prior to implementation of the Program.

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2 The NYSERDA Project

2.1 Background

The AEAP resulted from a 1992 federal appropriation attached to the 1990 CAAA and was funded from 1994 through 2006. A final report for the AEAP was issued in 2008 and submitted to US EPA for review (Nierzwicki-Bauer et al. 2008). Following 13 years of continuous investigation on a group of waters in the Adirondack region and the extensive chemical and biological database that had accumulated during this period, the Darrin Fresh Water Institute (DFWI) was reluctant to terminate the Aquatic Biota Study (ABS). Realizing the importance of the long-term, contemporaneous chemical and biological data that were collected from the study sites in the Adirondack region between 1994 and 2006, the DFWI made a decision to extend the ABS beyond 2006, even though there was no outside funding to support this effort.

2.2 ABS Becomes the DFWI Project

The DFWI extended the sampling and the format of the ABS into 2007 and 2008. The DFWI commitment to continue sampling in the Adirondacks was based upon the desire to increase the 13-year record of important chemical and biological data and the philosophy of DFWI scientists that the real merit of the ABS would be recognized and sponsored for additional future funding from some outside entity.

Because funding was not immediately available to support this two-year effort, it was not possible to continue sampling 30 study sites. A subset of 17 sites was selected from the original group of 30. Several factors were considered during the site selection process including 1) the hydrologic type, 2) the net increase or decrease of major acidification analytes during the 13-year AEAP, 3) the availability of historical data for study sites prior to 1994, and 4) the ease of study site access for sampling since flight services to remote waters was uncertain. The 17 study sites listed in Table 2-1 were selected for continued sampling by DFWI. Figure 2-1 shows the location of the 17 study sites within the Adirondack region.

There were no changes in the structure and conduct of the ABS during 2007 and 2008. All of the ABS components described in Section 1 remained the same, and two additional years of data collection were successfully conducted. Following completion of the 2008 sample collection, continued funding of the ABS did not appear likely and DFWI decided to discontinue the sampling effort. Field and laboratory components of the ABS had placed a considerable drain on the DFWI resources during the previous two years.

In 2009, DFWI scientists responded to PON 1292 from the New York State Energy Research Development Agency (NYSERDA). The proposed work focused on a program interest area that included ecosystem impacts and recovery from sulfur and nitrogen deposition and specifically identified the establishment of a standardized monitoring network based on abiotic and biotic compartments of critical ecosystems in an area impacted by acid deposition. The DFWI application to NYSERDA also contained material related to a program interest area that addressed the synthesis of acid deposition data to better inform policy at state and federal levels.

Table 2-1. Study sites selected for ABS monitoring after completion of the AEAP in 2006

The (+) or (-) indicates the change in the relative value of the analyte during the period of the study.

Site #	Water	Hydro Type [1]	pH (s.u.)	ANC (µEq/L)	SO_4-S (µEq/L)	NO_3-N (µEq/L)
1	Big Moose [2]	TDL	5.97 (+0.71)	7.0 (+3.1)	49.6 (-13.0)	10.4 (-8.6)
2	Brooktrout [2,3]	TDL	5.60 (+0.46)	-1.3 (+1.3)	46.3 (-16.3)	9.9 (-2.2)
3	Cascade [2,3]	MDL	6.78 (+0.17)	62.1 (+11.0)	59.6 (-7.1)	15.0 (-6.4)
4	Darts [2]	TDL	6.31 (+0.67)	22.2 (+6.7)	53.2 (-9.4)	11.8 (-8.0)
5	G [2,3]	TDL	6.24 (+0.66)	14.6 (-2.1)	45.2 (-9.0)	4.2 (+0.6)
6	Indian [2,3]	TDL	5.10 (+0.05)	-0.7 (-8.4)	36.3 (-23.5)	0.9 (-3.6)
7	Jockeybush	TDL	5.79 (+0.63)	3.0 (+8.3)	49.6 (-19.9)	7.6 (-5.0)
8	Limekiln [2]	MDL	6.61 (+0.50)	33.2 (+16.7)	51.5 (-8.3)	10.6 (-4.4)
9	Middle Settlement [3]	TDL	5.70 (+0.62)	2.9 (+1.9)	43.6 (-13.4)	0.9 (-2.0)
10	Moss [2]	MDL	7.20 (+0.81)	89.7 (+31.7)	59.0 (-6.3)	9.0 (-8.4)
11	North	TDL	5.63 (+0.45)	6.9 (-6.8)	49.6 (-7.4)	13.2 (-3.7)
12	Rondaxe	TDL	6.94 (+0.38)	73.0 (+10.3)	51.7 (-9.5)	4.8 (-9.2)
13	Round	MSL	5.52 (+0.92)	-1.6 (-1.6)	23.1 (-25.6)	0.9 (-4.3)
14	Sagamore [2]	MDH	6.69 (+0.73)	61.5 (+29.0)	60.3 (-13.4)	6.6 (-5.1)
15	South [2]	TDL	5.87 (+0.57)	6.9 (-6.4)	47.5 (-8.1)	17.1 (-6.2)
16	Squaw [2,3]	TDL	6.06 (+0.19)	13.9 (-10.5)	47.7 (-19.0)	5.4 (-6.3)
17	Wheeler	MSH	6.66 (+0.28)	51.1 (+1.4)	44.2 (-8.6)	0.9 (-1.5)

[1] Hydrologic type (Newton and Driscoll, 1990); see Table 1 for description

[2] Study sites with 1980s baseline data available (Sutherland 1989 and unpublished)

[3] Remote study sites requiring NYS helicopter support for access

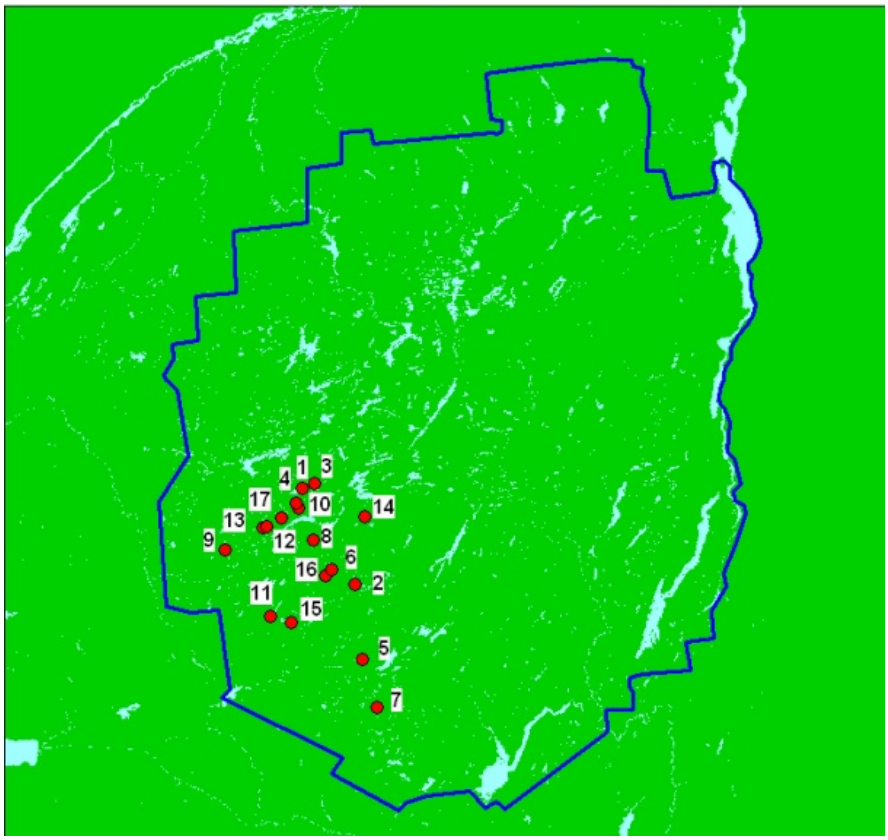
2.3 DFWI Project Becomes the NYSERDA Project

The NYSERDA project was funded for a three-year period, beginning in 2010 and continuing through 2012, and was defined by a contractual agreement between RPI and NYSERDA. The Statement of Work that described the Project identified five major tasks including:

- Sample Collection and Analysis.
- Statistical Analyses of the AEAP Database (Chemical, Physical, Biological).
- Evaluating Stakeholder Perceptions of Acid Deposition Impacts to Better Inform Policy.
- Data Dissemination via the Web.
- Project Management and Reporting.
- The NYSERDA Project continued the research-based biological and chemical assessment initiated by the AEAP during 1994 but was restricted to the subset of 17 study sites monitored by DFWI during 2007 and 2008. One study site (Middle Settlement) had to be dropped before the field sampling began due restricted helicopter flight time. 16 lakes were sampled after the lake was dropped for the remainder of the 3 year project.

The following sections provides a brief summary of the major tasks incorporated into the NYSERDA Project Statement of Work.

Figure 2-1. Location of the 17 study sites selected for post-2006 monitoring



2.3.1 Task 1: Sample Collection and Analysis

The NYSERDA Project used the same field and laboratory protocol defined previously for the ABS; there were two mid-summer sampling visits to each study site and the same chemical and biological parameters were collected each time. The site location for sample collection was originally determined using bathymetric maps provided by the New York State Department of Environmental Conservation prior to the AEAP. The same maps were utilized again during this project to replicate the original sampling location from the AEAP to provide homogeneity between the two programs. The point of maximum depth in the lake was chosen to best characterize the water body as a whole after chemical analysis was complete. Sampling protocols mirrored that of the AEAP project as described in Section 1.

Once complete on site, all samples were packed on ice in coolers and transported to the Keck Water Research Laboratory at the DFWI in Troy, New York for analysis. Phytoplankton and zooplankton samples were held at the lab until all sample collection was complete and were then shipped out to their respective collaborators for analysis. All samples had a field sheet associated with it along with a designated sample code unique to each sample collected. Chain of custody forms were also incorporated during transport to ensure proper quality control with the handling of samples.

There was a special study of the bacterioplankton incorporated into the work-plan that focused on a subset of three (3) study sites with contrasting pH values, including Indian (pH 5.1), Brooktrout (pH 5.6) and Moss (pH 7.2). Although this sample size did not guarantee that microbial diversity of Adirondack lakes would be described fully, preliminary data prior to 2010 suggested that this level of coverage would allow detection of meaningful differences between libraries correlated to physiochemical and biological properties of each lake sample.

The majority of analyses were performed at the RPI Keck Water Research Laboratory. Exceptions included aluminum samples that were performed by the U.S. Geological Survey in Troy, New York and bacterioplankton samples that were analyzed in DFWI's state-of-the-art molecular biology laboratory. The lab contains all of the necessary molecular biology instrumentation used for this project (including, gel electrophoresis units, DNA Fastprep system, thermocyclers, microcentrifuges, laminar flow hood, gel documentation system, fluorescent microscope, inverted microscope with image analysis system, FlowCAM, and other basic equipment). DNA clones for this project were sequenced at the MCLAB sequencing service (San Francisco, CA: www.mclab.com). A list of analytes run for the chemistry portion of the NYSERDA Project is provided in Table 3-2 along with the laboratory analytical methods.

2.3.2 Task 2: Statistical Analyses of AEAP Database (Chemical, Physical, and Biological)

The objective of this task was to identify the most important interactive relationships between environmental factors and the distribution and abundance of species of zooplankton, phytoplankton and bacterioplankton. Specific hypotheses to be tested and included in the work-plan were as follows:

- **H1**: Biotic recovery will occur more readily in low-lying drainage lakes than in high elevation or seepage lakes, in part due to geographic isolation.
- **H2**: Systems with the least buffering capacity (e.g. thin-till drainage lakes or lakes with greatest initial acidity levels) will require a longer period of time to show similar biological recovery collectively when compared to systems with greater initial buffering capacity or lower levels of acidity.
- **H3**: It will be possible to classify lakes according to plankton community assemblages, reflecting a composite of physical and chemical characteristics of these freshwater ecosystems.
- **H4**: In addition to pH, acid-neutralizing capacity, specific conductance, chlorophyll, dissolved organic carbon, sulfate, nitrate and monomeric aluminum (total and labile) will be the most important factors for predicting the distribution and abundance of plankton.

The work-plan described the use of Principal Component Analyses (PCA) of specific biota data, species richness regression, and Shannon-Weaver Diversity Index equations to correlate taxonomic groups with chemical, hydrological, and physical characteristics of each study site for all years with available data. Testing with multivariate analysis using the CANOCO program was described. Also, the SAS program was to be used for analysis of variance (ANOVA) to evaluate statistical significance of analyte values.

2.3.3 Task 3: Evaluating Stakeholder Perceptions of Acid Deposition Impacts to Better Inform Policy

The gradual development of a sizeable body of environmental data and ecosystem results associated with acid deposition research placed specific demands upon the efficient and effective dissemination of this information to policy makers. Even during current interactions between science and policy, questions still exist about the most appropriate way to design and target outreach materials and efforts to maximize the value of long-term monitoring programs and other environmental research efforts. More specifically, how can the collective research literature be used to better inform environmental policy, and how can gaps between ecological and social sciences research be reduced to better inform environmental policy? The NYSERDA Project was designed to address these questions using the four subtasks outlined in the following sections.

2.3.3.1 Identify and Interview Stakeholders

The objective of this sub-task was to assess the perceptions of various categories of stakeholders of the effects of acidic deposition in the Adirondacks and compare perceptions to the current scientific understanding. Identifying the gaps between perceptions and science would be helpful toward improving communication of scientific data and the formation of science-based policy at state and federal levels. The means of achieving this objective was a series of detailed interviews within different groups of stakeholders in New York State.

2.3.3.2 Integrate Qualitative and Quantitative Data to Improve Understanding of Impacts

The interview data would be categorized and analyzed to determine the best methods for dissemination of the scientific information. Key words and similarities in the answers provided by interviewees would be identified to group together and code similar responses and develop a qualitative class of answers to facilitate more in-depth analysis and labeling of key patterns. Once categories were developed, a "codebook" of important classification themes would be constructed. Information in the codebook could be used to obtain more quantitative data. These types of analyses are important to identify or verify hypotheses related to individual perceptions and reasons for these perceptions, as well as to identify any outlying biases of individuals (or groups of individuals).

2.3.3.3 Stakeholder Meeting

An examination of the dialogue from key stakeholders can provide a realistic view of what transpires when science and social sciences form partnerships to inform policy. One or more Stakeholder Workshops would be organized and implemented and include representatives from the interview categories to further examine the perceptions of each group. By stimulating and observing the discussion among the stakeholder groups, it would be possible to identify possible obstacles to the communication of scientific information and provide opportunity for improving communication strategies.

2.3.3.4 Development of a White Paper

Information collected from the stakeholder interviews would provide an understanding of stakeholders perceptions related to what is happening in Adirondack lakes as compared to the current scientific understanding. Through the observation of discourse among stakeholders, their perceptions, misconceptions, and/or knowledge gaps can be identified, along with a better understanding of the gaps between science and social science. This process will make it possible to develop strategies for improved communication of scientific data to varied stakeholders, allowing science to more effectively inform policy. Stakeholder perceptions and suggested communication strategies, along with the findings of the interviews and workshops, would be summarized into a white paper.

2.3.4 Task 4. Data Dissemination on the Web

All chemical, physical and biological datasets collected from 1994 through the end of the NYSERDA Project would be made web accessible to other scientists and the general public through the development and release of an interactive website. This website would serve as the organizational center for data management, quality control and dissemination of information.

2.3.5 Task 5. Project Management and Reporting

This task presented the reporting requirements for the NYSERDA Project including the quarterly progress eports, the final data report (this document), and a summary paper that translates scientific findings into language that is interesting, understandable, and appealing to a broad audience, including policy analysts, policymakers, and the interested general public. This task also describes peer-reviewed publications, Project coordination with NYSERDA's outreach contractors, meetings and presentations, and project metrics.

The remainder of this report contains sections that present and discuss the results collected during 2010 through 2012 as part of the NYSERDA Project as well as the interpretation of all chemical, physical and biological data collected during the entire duration of the AEAP beginning in 1994.

There were two noteworthy occurrences during the 2010-2012 NYSERDA Project. One occurrence involved an amendment to the original Project work-plan that increased funding by an amount that would cover the cost of the analysis of biological samples collected during 2007 and 2008. The other occurrence involved the removal of Middle Settlement Lake from the list of active study sites since access to remote waters was severely restricted during 2010 and there was no other way to gain access to that study site.

2.4 References

New York State Energy Research Development Agency. 2010. Chemical and Biological Monitoring of Adirondack Lakes to Examine Ecosystem Impacts and Recovery from Sulfur and Nitrogen Deposition. Exhibit A. Statement of Work, Agreement 16298. Attached to Contract with Rensselaer Polytechnic Institute.

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3 Water Chemistry Investigations

3.1 Background

While one of the goals of the NYSERDA Aquatic Biota Study (ABS) was to compile an additional three years of data to be added to the growing chemistry and biota database already established by the AEAP program, additional hypothesis testing was to be performed to identify specific interactive relationships between water chemistry and biota. Specific goals of the hypothesis testing were to better understand lake function, overall fitness, as defined by increased community richness and diversity, and how these dynamics affect change on a system currently recovering from the effects of acid deposition.

3.2 Methods

3.2.1 Site Selection

As discussed in Section 2, 16 lakes were sampled for chemistry. Table 2-1 provides a detailed description of each lake chosen along with its hydrologic characteristics.

3.2.2 Sampling and Analysis

Detailed methodology for chemistry sampling is provided in Section 2. The majority of analyses were performed at the RPI Keck Water Research Laboratory, except for aluminum samples which were performed by the U.S. Geological Survey in Troy, New York. A list of analytes run for the chemistry portion of the NYSERDA project is provided in Table 3-1 along with the laboratory analytical methods used.

3.3 Quality Assurance/Quality Control

The Quality Assurance/Quality Control (QA/QC) program utilized for the chemistry analysis was comprised of a set of standards and several checks to ensure the quality of data. For each analyte, a standard curve was performed at the beginning of analysis. For manual runs (such as total phosphorus), a set of standards was also run at the end of a given batch. Two replicates, spikes, blanks, and external certified check standards were run for every 20 samples analyzed (considered a “batch”). If the checks exceeded a 10% recovery range, then all samples for that batch were reanalyzed. The Keck Water Laboratory is state and nationally certified by the Environmental Laboratory Approval Program and the National Environmental Laboratory Accreditation Conference (ELAP/NELAC). Proficiency samples are analyzed every six months to assure quality control. The lab is audited on a biennial basis.

3.4 Statistical Analysis

The statistical design was established to answer the questions detailed in the project hypotheses in Section 3.5. To accomplish this analysis, a series of statistical Analysis of Variance (ANOVA), both one-way and two-way, Student's t-test, ordinary least squares regression, Pearson Product Moment Correlation, and Principal Component Analysis (PCA) tests were performed on the data. One key underlying assumption for these analyses is that the data are normally distributed. Therefore, prior to analysis, all data were subjected to normality testing. A Skewness/Kurtosis analysis was completed on each parameter. Data that failed normality testing were log transformed and tested again for normality. If the data again failed normality testing, an outlier analysis (Q-Q Plots, Box Plots, and others) was conducted and outliers removed. The data were once again subjected to normality testing. Data failing at this step were considered as a non-normal dataset and non-parametric analytical procedures were used. All of the parameters except NO_3^- were able to meet the normality standard. Non-parametric procedures (Wilcoxon Rank-Sum test and Spearman's Rank Correlation) were used for the NO_3^- analysis. The Regional Mann-Kendall trend analysis (Helsel et al. 2005) was utilized to determine regional trends for both biotic and abiotic parameters. Except as specifically described elsewhere in this report, significance was determined at $p \leq 0.05$. The strength of correlation analysis is determined based upon the categories described by Methe and Zehr (1999; Table 3-2). Unless specifically noted, all analyses were conducted with Stata/IC 11.2 (Stata Corp 2009).

Table 3-1. Listing of analytes and methods utilized for the chemical analysis of lake samples

Source: Methods for Chemical Analysis of Water and Wastes. United States Environmental

Parameter	Analysis	Analytical Method
Dissolved Oxygen	Membrane Electrode	US EPA Method 360.1
pH (Air Equilibrated pH)	Electrometric	US EPA Method 150.1
Acid Neutralizing Capacity (ANC)	Gran Titration	US EPA Method 310.1
Specific Conductance	Wheatstone Bridge type	US EPA Method 120.1
Dissolved Organic/Inorganic Carbon	IR Spectroscopy	US EPA Method 415.2
Ammonia as N (NH_4-N)*	Flow Injection	US EPA Method 350.1
Orthophosphorus (PO_4-P)*	Flow Injection	US EPA Method 365.1
Inorganic Anions (F^-, Cl^-, NO_3-N, SO_4-S)*	Ion Chromatography	US EPA Method 300.0
Phosphorus Total,Total filterable (TP/TFP)*	Colorimetric	US EPA Method 365.4
Total Nitrogen (TN)	Persulfate Oxidation	Langner & Hendrix 1982
Soluble Reactive Silica (SiO_2)*	Flow Injection	LACHAT 10-114-27-1-A
Total Sodium (Na^+)*	Atomic Absorption	SM 18-21-3111 B (99)
Total Potassium (K^+)*	Atomic Absorption	SM 18-21-3111 B (99)
Total Magnesium (Mg^{++})*	Atomic Absorption	SM 18-21-3111 B (99)
Total Calcium (Ca^{++})*	Atomic Absorption	SM 18-21-3111 B (99)
Aluminum, Total (Al)	Inductively Coupled	EPA 200.7 Rev. 4.4
Aluminum (monomeric, non-labile	Flow Injection	LATCHAT 10-113-33-A & 10-
Chlorophyll-a[a]	Fluorometric	Turner 1985[a]

* ELAP/NEELAC certified

[a] Turner, G.K. 1985. Fluorometry. Pages 43-78. *In* Bioluminescence and Chemiluminescence: Instruments and Applications. Vol 1 Edited.

Table 3-2. Strength of correlation analysis

Pearson Product Moment Correlation value is reported and the strength of the correlation is based upon Methe and Zehr (1999).

Strength of Correlation	Description	Significance (P-Value)
Strong	$\|r\| \geq 0.77$ ($r^2 \geq 0.60$)	0.05
Moderate	$\|r\| \geq 0.63$, $\|r\| < 0.77$ ($r^2 \geq 0.40, r^2 \leq -0.60$)	0.05
Weak	$\|r\| < 0.63$ ($r^2 < 0.40$)	0.05

3.5 Hypothesis Testing and Discussion

3.5.1 Introduction

A suite of 22 parameters were analyzed for the 16 lakes involved in the ABS during the 2010-2012 sample collection period. Seven primary analytes (pH, ANC, SO_4^{2-}, NO_3^-, DOC, Chl-a, Al) were proposed as influential factors affecting the interactive relationships of aquatic biota along with predicting their distribution and abundance in a lake system (Table 3-3). These seven analytes were incorporated into the hypothesis testing work for the project. Although this study only spanned three years, data collected for these seven parameters on the same 16 lakes during the previous AEAP project were viewed as imperative to properly address and answer questions regarding the long-term effects and interactions between water chemistry and aquatic biota in their environment. To provide a comprehensive analysis of potential changes in lake chemistry, the previous 12 years (1997-2008) of AEAP data for the 16 lakes, along with the data obtained from the three year NYSERDA study (2010-2012), including Middle Settlement for 2007, were integrated for the hypothesis testing. When necessary, data from the entire set of 30 lakes were incorporated into the analysis to provide additional insight into a specific issue being addressed.

A major hypothesis integral to the project design was that a significant difference in the concentration of the constituents on an individual basis existed between the epilimnetic and hypolimnetic layers. Following the assumption testing, analyses were conducted designed to support or reject this hypothesis. Upon completion of the assumption and stratification analysis, investigators set out to determine how these specific chemical parameters change as a function of time, as a function of lake type, and rate of change (if any), along with the interactions that occur with other lake parameters.

Table 3-3. Mean values of the seven primary analytes used for hypothesis testing

Lake Name	Sample Type	Sample Z (m)	Secchi (m)	pH air eq	ANC (ueq/L)	DOC (mg/L)	NO3-N (mg N/L)	NO3- (mg/L)	NO3- (ueq/L)	SO4-S (mg S/L)	SO4-- (mg/L)	SO4-- (ueq/L)	Chla (ug/L)	Total Al (ug/L)	Total mono Al (ug/L)	Nonlabile mono Al (ug/L)	Labile mono Ali (ug/L)	Hydrologic Type
Big Moose	INTEGRATE	4.5	3.52	6.05	20.10	3.79	0.13	0.59	9.41	0.88	2.65	53.07	4.41	160.79	41.39	18.22	23.17	TDL
Big Moose	GRAB	19.0	3.52	6.10	22.70	3.45	0.19	0.84	13.44	0.96	2.89	57.71	0.68	285.93	61.76	32.20	29.56	TDL
Brooktrout	INTEGRATE	5.0	5.91	5.91	11.62	2.16	0.06	0.28	4.48	0.86	2.57	51.35	1.69	119.78	22.13	0.98	21.14	TDL
Brooktrout	GRAB	20.2	5.91	5.93	15.45	1.91	0.12	0.53	8.49	0.91	2.73	54.54	0.68	220.36	44.16	10.55	33.61	TDL
Cascade	INTEGRATE	4.3	4.28	6.90	69.10	3.08	0.15	0.66	10.53	1.15	3.45	68.94	3.50	76.68	10.13	1.44	8.69	MDL
Cascade	GRAB	5.0	4.23	6.98	83.70	3.08	0.11	0.47	7.44	1.02	3.06	61.16	11.67	135.71	16.42	3.75	12.66	MDL
Dart	INTEGRATE	3.7	3.68	6.39	29.86	3.36	0.08	0.36	5.78	0.92	2.77	55.31	2.24	145.20	28.74	10.27	18.47	TDL
Dart	GRAB	13.8	3.68	6.75	59.42	3.14	0.10	0.45	12.40	1.14	3.42	68.40	9.40	195.66	7.04	0.00	7.04	TDL
G	INTEGRATE	3.8	4.59	6.41	24.94	2.51	0.06	0.25	3.95	0.79	2.37	47.33	5.03	104.12	10.78	0.67	10.11	TDL
G	GRAB	8.2	4.59	6.65	44.51	2.33	0.04	0.17	2.79	0.71	2.12	42.33	18.19	237.55	21.58	4.25	17.33	TDL
Indian	INTEGRATE	2.5	2.73	5.49	6.63	4.82	0.03	0.15	2.33	0.71	2.14	42.85	2.04	219.28	72.19	39.78	32.41	TDH
Indian	GRAB	9.3	2.73	5.87	16.37	3.58	0.07	0.30	4.79	0.74	2.21	44.14	1.23	305.04	99.14	61.88	37.26	TDH
Jockeybush	INTEGRATE	4.3	6.65	6.13	13.98	2.22	0.10	0.43	6.91	0.99	2.97	59.46	0.98	128.23	17.90	1.10	16.80	TDL
Jockeybush	GRAB	9.2	6.65	6.23	21.75	2.51	0.13	0.58	9.23	0.98	2.93	58.56	2.09	269.62	36.96	10.58	26.39	TDL
Limekiln	INTEGRATE	5.3	6.82	6.69	46.41	2.69	0.10	0.46	7.31	0.94	2.83	56.61	1.99	61.36	8.29	0.53	7.76	MDL
Limekiln	GRAB	18.7	6.82	6.65	42.50	2.38	0.17	0.73	11.73	0.93	2.79	55.89	1.08	133.80	20.62	4.51	16.11	MDL
Moss	INTEGRATE	2.7	3.85	7.06	104.64	3.58	0.08	0.34	5.44	1.27	3.82	76.46	2.88	79.82	13.50	2.51	10.99	MDL
Moss	GRAB	12.0	3.85	7.25	128.86	2.93	0.11	0.50	7.98	1.16	3.47	69.43	2.53	196.61	14.36	1.64	12.72	MDL
North	INTEGRATE	3.5	2.45	5.98	18.84	4.90	0.04	0.17	2.74	0.84	2.53	50.67	5.92	253.44	64.78	38.38	26.40	TDL
North	GRAB	12.8	2.45	6.06	25.44	3.50	0.17	0.74	11.92	0.87	2.62	52.40	0.61	464.85	92.85	59.65	33.20	TDL
Rondaxe	INTEGRATE	4.2	3.23	6.92	79.37	3.29	0.03	0.15	2.47	1.07	3.22	64.32	2.90	109.63	13.56	2.70	10.87	TDL
Rondaxe	GRAB	10.2	3.23	7.05	93.32	2.88	0.07	0.29	4.69	0.96	2.88	57.68	3.57	165.89	16.25	3.36	12.89	TDL
Round	INTEGRATE	4.3	4.70	5.47	2.22	2.34	0.02	0.08	1.29	0.63	1.88	37.63	5.43	56.59	6.56	0.14	6.42	MSL
Round	GRAB	5.9	4.10	6.34	26.38	1.07	0.02	0.08	1.29	0.50	1.49	29.70	11.99	64.05	14.41	5.72	8.69	MSL
Sagamore	INTEGRATE	2.8	2.23	6.62	58.42	7.46	0.08	0.35	5.57	1.15	3.46	69.10	4.04	276.20	61.96	45.80	16.17	MDH
Sagamore	GRAB	19.2	2.23	6.35	38.60	6.15	0.27	1.21	19.42	1.13	3.39	67.74	0.35	335.25	93.84	67.89	25.95	MDH
South	INTEGRATE	6.5	4.82	6.25	20.39	2.32	0.15	0.66	10.58	0.85	2.56	51.26	3.80	117.60	19.60	2.42	17.18	TDL
South	GRAB	18.0	4.82	6.26	23.08	2.08	0.15	0.69	10.97	0.80	2.39	47.84	1.91	192.37	34.47	8.12	26.34	TDL
Squaw	INTEGRATE	4.1	3.61	6.49	29.95	2.79	0.02	0.10	1.59	1.11	3.34	66.79	4.26	50.32	5.08	0.02	5.06	TDL
Squaw	GRAB	5.8	3.90	6.98	89.20	2.50	0.02	0.09	1.41	0.69	2.08	41.67	8.38	73.84	10.77	0.36	10.41	TDL
Wheeler	INTEGRATE	3.0	2.85	6.66	59.91	6.40	0.01	0.06	0.99	0.88	2.64	52.87	3.95	137.77	33.89	19.94	13.95	MSH
Wheeler	GRAB	14.1	2.85	6.95	105.36	6.54	0.09	0.39	6.28	0.75	2.25	44.94	2.53	247.24	48.83	35.29	13.54	MSH

3.6 Summary of Findings

This section summarizes the key findings from the project. Detailed analysis and supporting data can be found in each subsequent section of this report.

In general, the Mann-Kendall trend analysis demonstrated a significant ($p \leq 0.05$) trend for both biotic and abiotic parameters in the epilimnion and hypolimnion for the study region. However, the associated correlation (Tau) was weak. This value indicates that while trends do exist, there is high variability within the data. A deeper evaluation of the data indicates that the variation within the analyzed parameters is due to an oscillation of the data and not a random distribution of the values.

Overall, pH was the only parameter that showed significant variability when comparing data collected between the July and August sampling periods. Significant variability for a given depth or stratum within a site was found in a majority of the parameters analyzed.

Long term studies on Adirondack lakes and ponds have reported air equilibrated pH (pH) data displaying a positive linear trend over time (Waller et al. 2012). The AEAP/NYSERDA Project found that pH exhibits a non-linear relationship over time with periods of time with distinct increasing and decreasing trends. This trend was observed in the majority of the lakes sampled over the course of the study demonstrating that many of these lakes are highly dynamic and multifaceted in their response to inputs from acid deposition as well as changes in pH.

A negative linear regression was displayed for SO_4^{2-} from the data analyzed over the 15-year period of these two studies. The rate of change in lake SO_4^{2-} was found to be significantly slower than the atmospheric deposition rate of SO_4^{2-}.

With the increase in pH due to decreased acid deposition and decreasing concentrations of SO_4^{2-}, it was hypothesized that DOC levels would increase. This study found that DOC levels actually decreased over time on a regional basis. When ANOVA and regression analyses were performed on individual lakes, oscillating recurring patterns were observed in DOC without any observable long term trend in most lakes. This same pattern existed for chlorophyll *a*, but it was more amplified than DOC when lakes were grouped and viewed on an annual basis.

As one of the primary constituents associated with ecotoxicity of acid deposition, aluminum was analyzed in both the monomeric and labile forms. The analysis demonstrated that changing chemistry within the study lakes has reduced the risk of aluminum to the biota within them.

One of the most anticipated data analyses conducted was comparing these seven analytes to the different hydrologic lake types and how the lake types function and interact with these chemical parameters. Significant differences were found on multiple levels from lake type to lake type for each of the seven individual chemical constituents.

Thermal stratification of the lakes also had a direct impact on water chemistry. A significant difference in concentration between the epilimnion and hypolimnion occurred in 8 of 9 parameters analyzed (conductivity data were included Section 3.9.2). Furthermore, the data indicate that the onset and duration of thermal stratification has changed over the course of the project (see Section 8). This finding has implications for the study area from both an atmospheric deposition and climate change perspective.

The following discussion addresses the individual results for each analyte, how the different chemical parameters change as a function of time, how they change as a function of lake type, and their relationships to other lake parameters.

3.6.1 July/August comparison

One of the primary assumptions under the original AEAP design that carried through into the NYSERDA Project was samples collected during July and August represent replicate samples and are not significantly different from each other. The first step taken during the hypothesis testing portion of the project was to verify this assumption for each of the seven primary chemical analytes. Table 3-4 shows the breakdown of the comparison for the primary analytes for both the epilimnetic and hypolimnetic layers. Please note that conductivity was not included. Due to the differences in pH between July and August data, the pH data between July and August was not pooled but was independently analyzed.

Table 3-4. Percentage of lakes showing significant differences in mean analyte values between July and August sampling periods

These two tables represent mean values over the entire 15-year study.

Epilimnion							
	pH	**SO_4**	**NO_3**	**DOC**	**Chl-a**	**Total Mono Al**	**Labile Al**
Significant Difference (P≤ 0.05)	44%	3%	12%	0	13%	44%	53%
No Significant Difference (P> 0.05)	56%	97%	88%	100%	87%	56%	47%

Hypolimnion							
	pH	**SO_4**	**NO_3**	**DOC**	**Chl-a**	**Total Mono Al**	**Labile Al**
Significant Difference (P≤ 0.05)	68%	17%	21%	6%	3%	29%	20%
No Significant Difference (P> 0.05)	32%	83%	79%	94%	97%	71%	80%

3.7 pH

pH has often been associated with the word "recovery" in regard to the effects of acid deposition on the environment, specifically in the Adirondack region. To better identify how lakes change over time, *how* pH functions within the environment is equally important to understanding its long term effects on the aquatic system.

In regard to the layers themselves, 49% of the lakes showed a significant difference between the epilimnion versus the hypolimnion in July while 60% of the lakes showed a significant difference in the epilimnion versus the hypolimnion in August. When the data is evaluated in total, 71% of the lakes demonstrated a significant stratification effect with pH (Table 3-5).

Table 3-5. Significance of pH concentrations between epilimnetic and hypolimnetic layers on a month-to-month basis

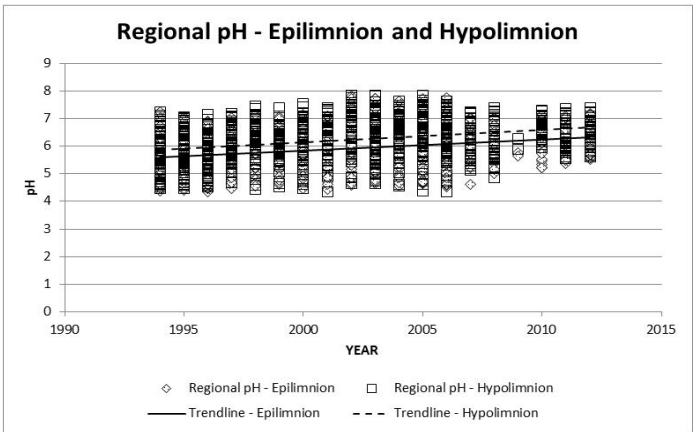
Percentage of lakes	Combined
Significant Difference	71%
No Significant Difference	29%

It clearly was identified that as the summer months progressed, thermal stratification continued to develop throughout the lake having a noticeable effect on pH, particularly in the hypolimnetic layer. This idea is discussed further in Section 3.9.2.

The regional Mann-Kendall trend analysis demonstrated a significant ($p \leq 0.05$) increasing trend in pH in both the epilimnion and the hypolimnion in the 16 study lakes (Figure 3-1). This overall trend is congruent with the hypotheses that the chemistry of the study lakes is changing as a result of decreased deposition of acid-related constituents.

Figure 3-1. Mann-Kendall trend analysis for pH

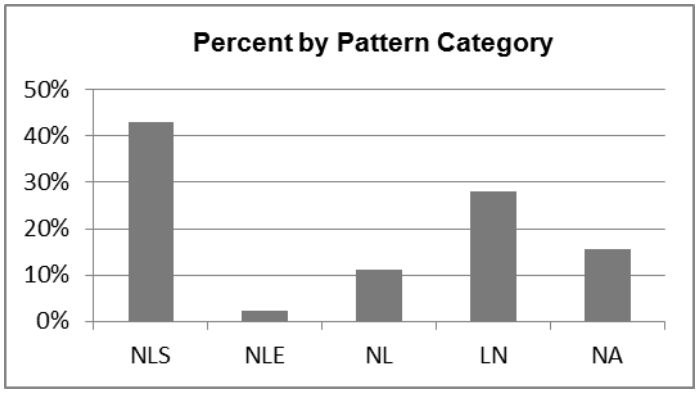
A scatter plot and trend line of pH data in both the epilimnion and hypolimnion of the 16 study lakes.



As demonstrated with the Mann-Kendall analysis, the data collected for the two projects (AEAP and NYSERDA) pH has clearly increased in value from 1997 through 2012 on a lake-by-lake as well as a regional basis. However, a more detailed evaluation of the data using regression analysis indicates a non-linear relationship between pH and time. The regression analysis for pH identified, five basic distribution patterns (Figure 3-2).

Figure 3-2. The five different distribution patterns found during regression analysis of pH along with their percent occurrence in the 35 AEAP/NYSERDA Project lakes

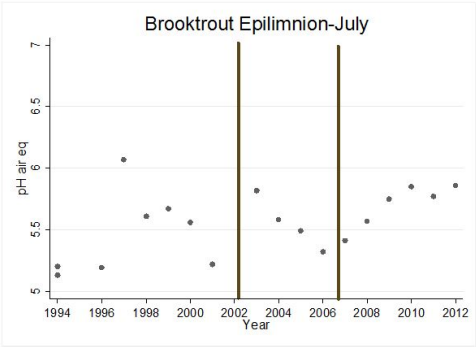
Curve definitions are – NLS: Non-linear with segments (3rd or 4th order polynomial), NLE: Non-linear with segments time shifted, NL: Non-linear without segments (Logistic), LN: Linear, NA: No relationship.



One of these patterns, non-linear with segments (NLS), was prevalent in the majority of the lakes sampled. The lakes that exhibited the NLS pattern showed a clear increase in pH from 1997-2001. Then from 2002-2006, a distinct drop in pH occurred. From 2006 through 2012, pH started to exhibit an increase again. The increase in pH during the first segment observed from 1997-2001 was possibly due to an initial response to the reduced levels of sulfur compounds being released into the atmosphere and deposited as acidic SO_4^{2-} ions over the Adirondack region. This initial increase in pH was followed by a reduction in pH with a subsequent upward trend as shown in the third segment observed from 2007-2012. It is hypothesized that this oscillating pattern represents the ecosystem modulating pH within the lakes themselves. Much like the population ecology "overshoot" concept, this pH pattern is representative of a system that is moving towards an equilibrium point. The oscillations are predicted to continue but with the amplitude of each oscillation becoming smaller. It is hypothesized that provided there is not some other anthropogenic influence on the system, the current pattern will continue until equilibrium is achieved.

Figure 3-3 uses Brooktrout Lake as an example of the NLS distribution pattern in pH. The data exhibiting the NLS pattern are consistent when looking at the epilimnion in July and August as well as the hypolimnion in July. The hypolimnion in August did not indicate a strong NLS distribution pattern. This could possibly be due to a stabilization of thermal stratification to a point of equilibrium. Alternatively, the lake may be in the initial stages of disruption of the thermocline.

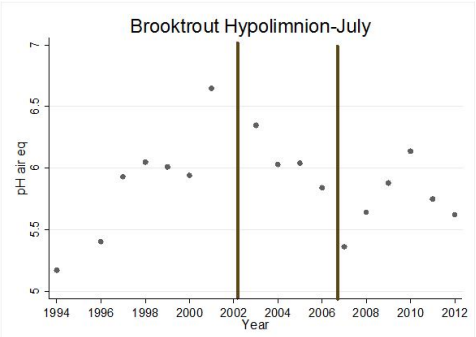
Figure 3-3. Example of non-linear with segments (NLS) distribution pattern of pH exhibited in Brooktrout Lake over time



Segment	R^2	Model	Slope
Pre-2002	0.43	NS	Positive (NS)
2002-2006	0.97	Sig	Negative (Sig)
Post 2006	0.79	Sig	Positive (Sig)

Note: Potential outliers in 1997 & 2001

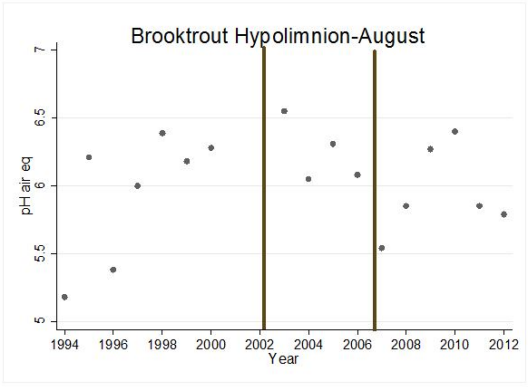
Overall Curve R^2 = 0.2234



Segment	R^2	Model	Slope
Pre-2002	0.48	NS	Positive (NS)
2002-2006	0.02	NS	Negative (NS)
Post 2006	0.57	NS	Positive (NS)

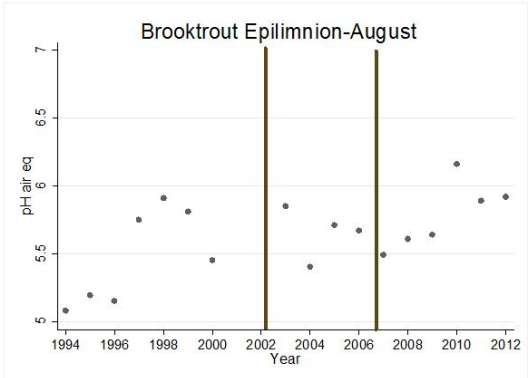
NOTE: Potential outlier in 2004

Overall Curve R^2 = 0.37



Segment	R^2	Model	Slope
Pre-2002	0.87	Sig	Positive (Sig)
2002-2006	0.86	NS	Negative (Sig)
Post 2006	0.14	NS	Non-Linear

Overall Curve R2 = 0.48



Segment	R^2	Model	Slope
Pre-2002			
2002-2006	0.41	NS	Negative (NS)
Post 2006			

Overall Curve R2 = NOT APPLICABLE

The oscillating pattern of the pH data raises numerous questions. First, can the pH response in the lakes be directly attributable to atmospheric deposition, specifically, SO_4^{2-}? Atmospheric deposition of SO_4^{2-} at DEC monitoring locations at Nick's Lake and Piseco Lake indicates a linear decrease in sulfur deposition following enactment of the CAAA (see Figure 3-10). This value was compared to the non-linear pH increase in the lakes. Although there is clearly a relationship, i.e. sulfur deposition was declining and the overall trend of pH was increasing, the cyclic pattern of pH change indicated that other factors are influencing this parameter.

Although it is unclear what is causing the oscillating pH pattern, three potential hypotheses were generated:

1. The watershed provides some type of modulation of pH change that results in periods of increasing and decreasing pH.
2. Variation in the timing and duration of stratification is acting as a confounding factor in interpreting the pH data.
3. Some other climate, geologic, chemical, or biotic parameters individually or in combination are influencing the pH dynamic in the lakes.

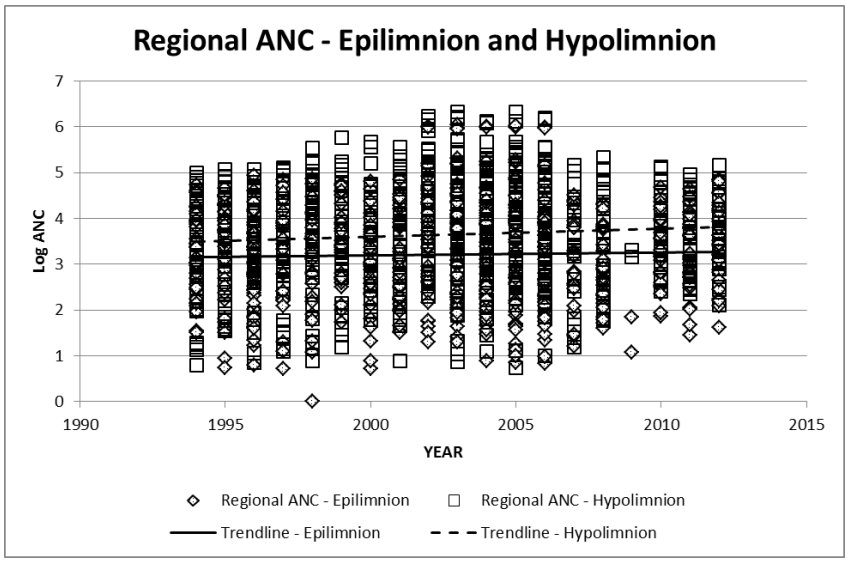
3.7.1 Acid Neutralizing Capacity (ANC)

When the individual layers were analyzed, significant differences in ANC were found in 17 out of 27 lakes for both July and August as well as the epilimnetic and hypolimnetic layers.

The regional Mann-Kendall trend analysis demonstrated a significant ($p \leq 0.05$) increasing trend in both the epilimnion and the hypolimnion ANC in the 16 study lakes (Figure 3-4).

Figure 3-4. Mann-Kendall trend analysis for ANC

A scatter plot and trend line of ANC data in both the epilimnion and hypolimnion of the 16 study lakes.

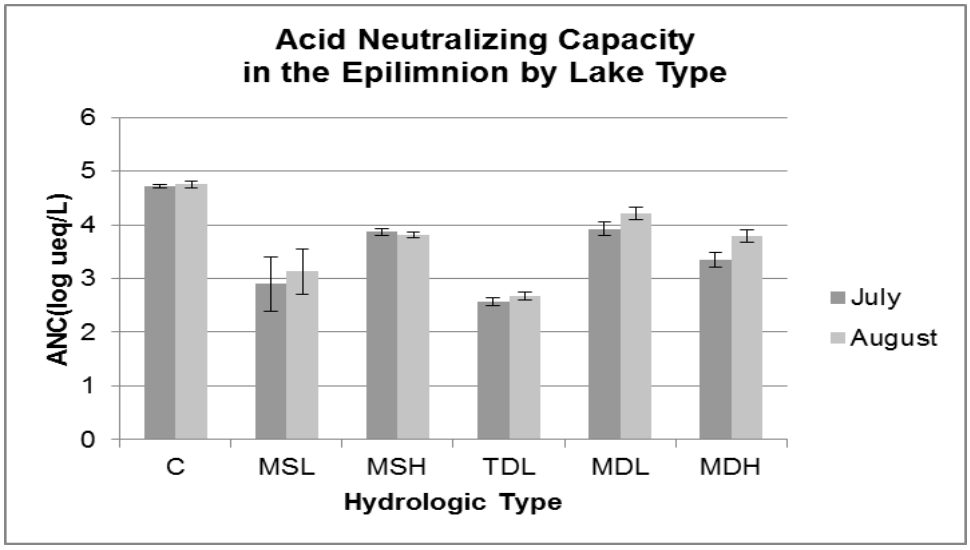


When ANC was evaluated by lake type, a two-way ANOVA indicated that there was no significant difference over time, yet there was a significant difference in ANC values between lake types (Figure 3-5). Significant differences were found for all time periods and layers.

As would be expected, ANC has a strong, significant correlation with pH (Figure 3-6).

Figure 3-5. Differences in mean ANC values by lake type

All results showed significant differences between each lake type for both epilimnetic and hypolimnetic layers.



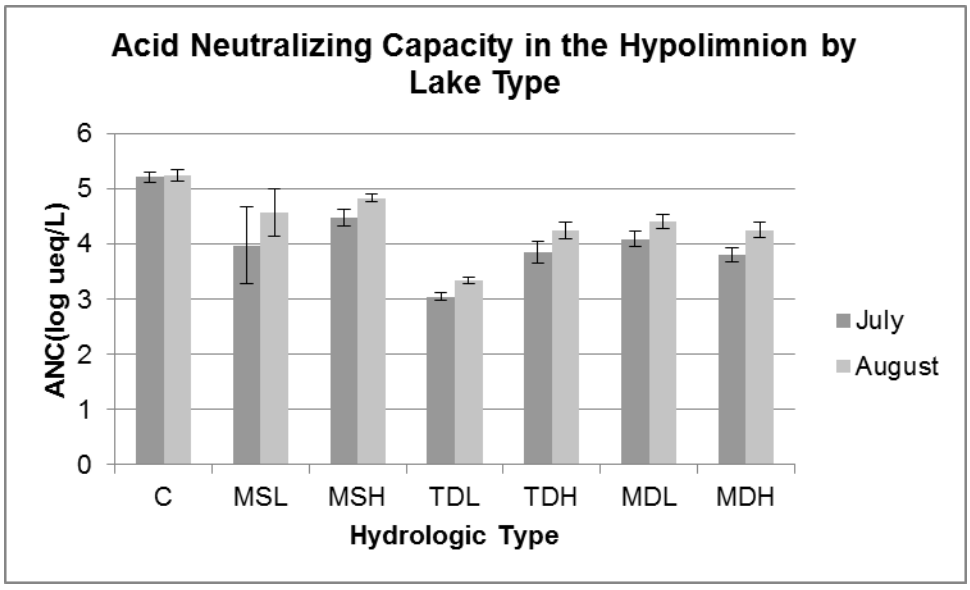
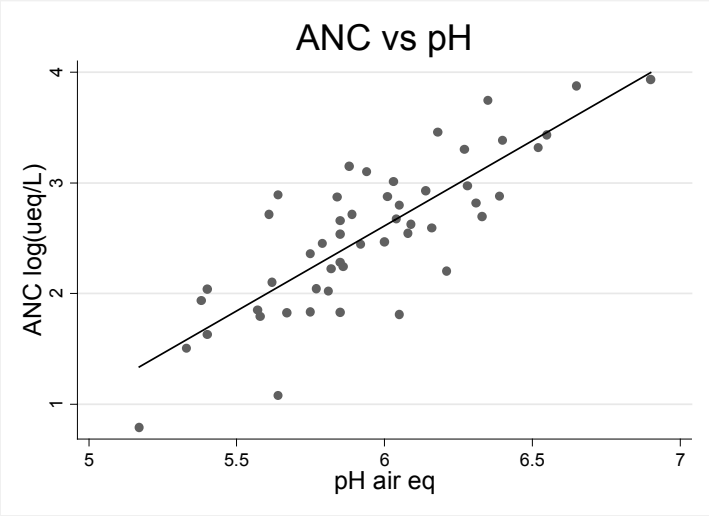


Figure 3-6. Scatterplot for ANC vs. pH for Brooktrout Lake (r = 0.89)

ANC and pH data are from 1994 – 2012. This result is categorized as a "Strong" correlation($r > 0.73$ and $p \leq 0.05$).



Future work includes a complete analysis of the base cations collected during the study (Na^+, K^+, Fe, Mg^{2+}, and Ca^{2+}) to determine how they interact with ANC as well as their overall role in relation to acid deposition.

3.7.2 Sulfate (SO_4^{2-})

Tests were performed to determine if there were significant differences in SO_4^{2-} between the epilimnetic and hypolimnetic layers on a month-to-month basis (Table 3-6).

Table 3-6. Significance of SO_4^{2-} between epilimnetic and hypolimnetic layers on a month-to-month basis

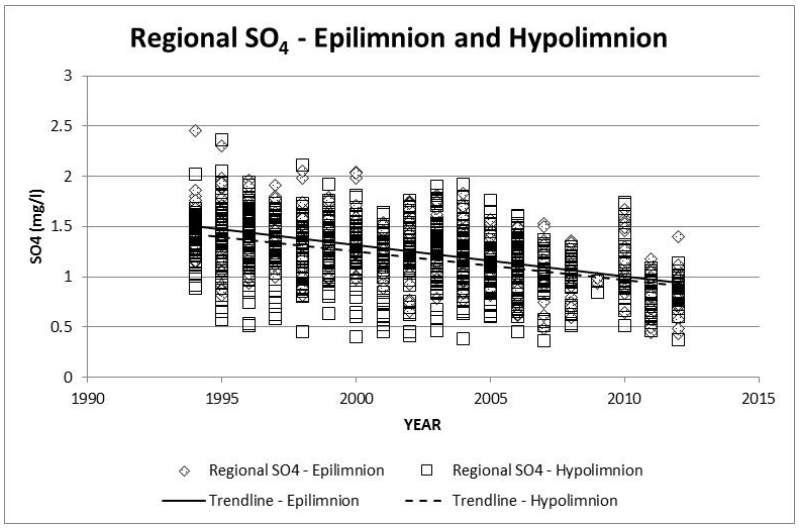
Month	Significant	Not Significant
July/August Combined	48%	52%

The layer test was done by month as well as with the months combined to provide additional information on proposed hypotheses relating to the timing, onset, and duration of stratification. The analysis showed that there were enough lakes with significant concentration differences in SO_4^{2-} to consider the epilimnion and hypolimnion as separate systems (from a regional standpoint).

The regional Mann-Kendall trend analysis demonstrated a significant ($p \leq 0.05$) decreasing trend in both the epilimnion and the hypolimnion for SO_4^{2-} in the 16 study lakes (Figure 3-7).

Figure 3-7. Mann-Kendall trend analysis for SO_4^{2-}

A scatter plot and trend line of SO_4^{2-} data in both the epilimnion and hypolimnion of the 16 study lakes.



One of the principal sources of SO_4^{2-} in aquatic systems is from atmospheric deposition and it is a key component in determining the effect of reduced acid deposition. Therefore, it is important to determine whether SO_4^{2-} concentrations have reduced in the lakes over time. The months of July and August were pooled together for a series of ANOVA tests. The results revealed that SO_4^{2-} was significantly lower in 2012 than in 1997 (Table 3-7).

Table 3-7. Reductions in SO_4^{2-} mean concentrations over time shown in both epilimnetic and hypolimnetic layers

This data demonstrates the net change in SO_4^{2-} concentrations from the beginning in the project to the conclusion. While the direction of change is the same in both the epilimnion and hypolimnion, the degree of change is different in the epilimnion and hypolimnion.

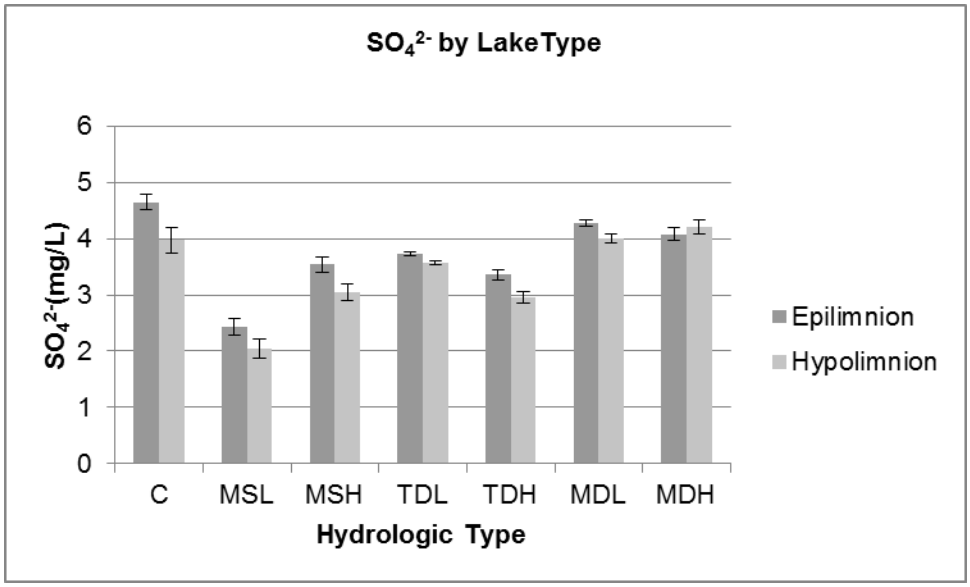
	1997	2012	Significant/Not Significant
Epilimnion	4.31 ppm	2.55 ppm	Significant
Hypolimnion	3.85 ppm	2.57 ppm	Significant

The epilimnion and hypolimnion were then analyzed individually on a year-by-year basis. Both layers also showed significantly lower levels of SO_4^{2-} between 1997-2012 (Figure 3-7).

Further investigations into whether SO_4^{2-} concentrations vary by lake type were made by performing another series of ANOVA tests. The results showed significant differences in SO_4^{2-} concentrations by lake type for both the epilimnion and hypolimnion (Figure 3-8).

Figure 3-8. Differences in SO_4^{2-} concentrations by hydrologic lake type

All results showed significant differences between each lake type for both epilimnetic and hypolimnetic layers.



The question was posed as to whether time and hydrologic lake type work together to create significant differences in SO_4^{2-} concentrations. Although year and hydrologic lake type have had significant effects on SO_4^{2-} concentrations, they do not appear to interact with each other to produce similar reductions in the epilimnion as or in lakes that do not stratify (Table 3-8). However, analysis showed there was a significant interaction between time and hydrologic type for SO_4^{2-} deposition in the hypolimnetic layer. At this time, it is not clear why the hypolimnion is acting different than the epilimnion with respect to SO_4^{2-}. One hypothesis that may be worthy of investigation is the relationship between hypolimnetic water chemistry and lake sediment. This hypothesis would hold that the sediment acts as a sink for SO_4^{2-} and changes in water chemistry of the hypolimnion, specifically reduced dissolved oxygen, in conjunction with sulfur reducing bacteria such as *Desulfovibrio vulgaris*, would enable a release of this sulfur. As the water chemistry of the hypolimnion differs from both the epilimnion and from non-stratified systems, these other systems do not have the same mechanism for release of sulfur over time.

Table 3-8. Interactive differences in SO_4^{2-} concentrations between time and hydrologic type

This two-way ANOVA demonstrates that both year and hydrologic type have a significant interaction. SO_4^{2-} is the only parameter that has this interaction.

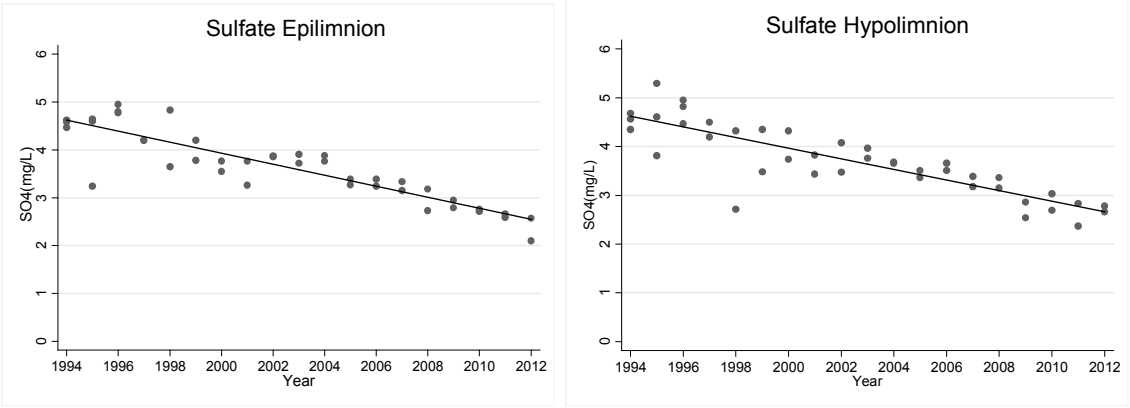
	Year	Hydro Type	Year * HT
Layer 1 (epilimnion)	Significant	Significant	Not Significant
Layer 2 (hypolimnion)	Significant	Significant	Significant
Layer 3 (non-stratified)	Significant	Significant	Not Significant

3.8 Rate of Change in SO_4^{2-} Concentrations

The suite of 30 lakes was analyzed on a lake by lake basis to determine if SO_4^{2-} concentrations vary at different rates as a function of lake and layer. Results of this analysis indicated a negative linear regression for all analyzed lakes in both epilimnion and hypolimnion indicating a reduced SO_4^{2-} loading into the system (Figure 3-9).

Figure 3-9. Regressions for Brooktrout Lake SO_4^{2-} concentrations over time in the epilimnion and hypolimnion.

R^2 for epilimnion regression is 0.80 and for the hypolimnion, R^2 is 0.73. Both regressions are significant ($p \leq 0.05$). It should be noted that the data is plotted from the beginning of the program (1994) but the statistical analysis is for data beginning in 1997 due to slight differences in collection and analytical protocols. All data is plotted for completeness.



Further investigation into the layers showed that all of the lakes except for five (Ice House, Middle Branch, Raquette, Sunday, and Trout) showed no significant difference in the rate of change of SO_4^{2-} concentrations between layers. When looking at the rate of change lake to lake, Moss was the only one that demonstrated a significant difference as compared to other lakes. South, North, and Lake Rondaxe also indicated differences in the rate of change compared to other lakes, but the results were not significant.

Finally, the rate of change in SO_4^{2-} for each lake was compared with the atmospheric deposition rate. The rate of change for atmospheric deposition was determined by using air monitoring stations at Nick's Lake and Piseco Lake (both NYSDEC stations in the SW quadrant of the Adirondacks). These lakes were chosen due to their close proximity to the lakes from the NYSERDA study area. A regression of each of these lakes can be seen in Figure 3-10. For each lake as well as lake layer (e.g., epilimnion, hypolimnion), there was a significant difference between the rate of change in lake SO_4^{2-} concentration and the rate of change in atmospheric deposition (Figure 3-11).

Figure 3-10. Scatter and fit plot of SO_4^{2-} (kg/ha) deposition at Nick's Lake and Piseco Lake's DEC air monitoring stations

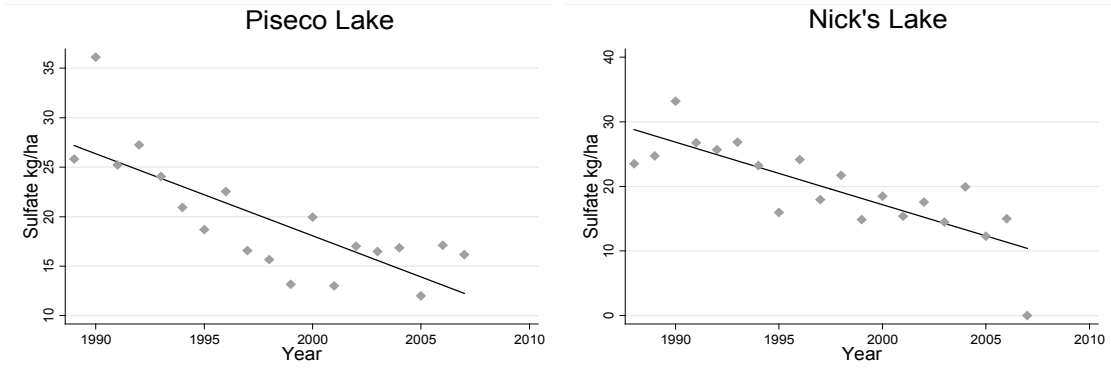
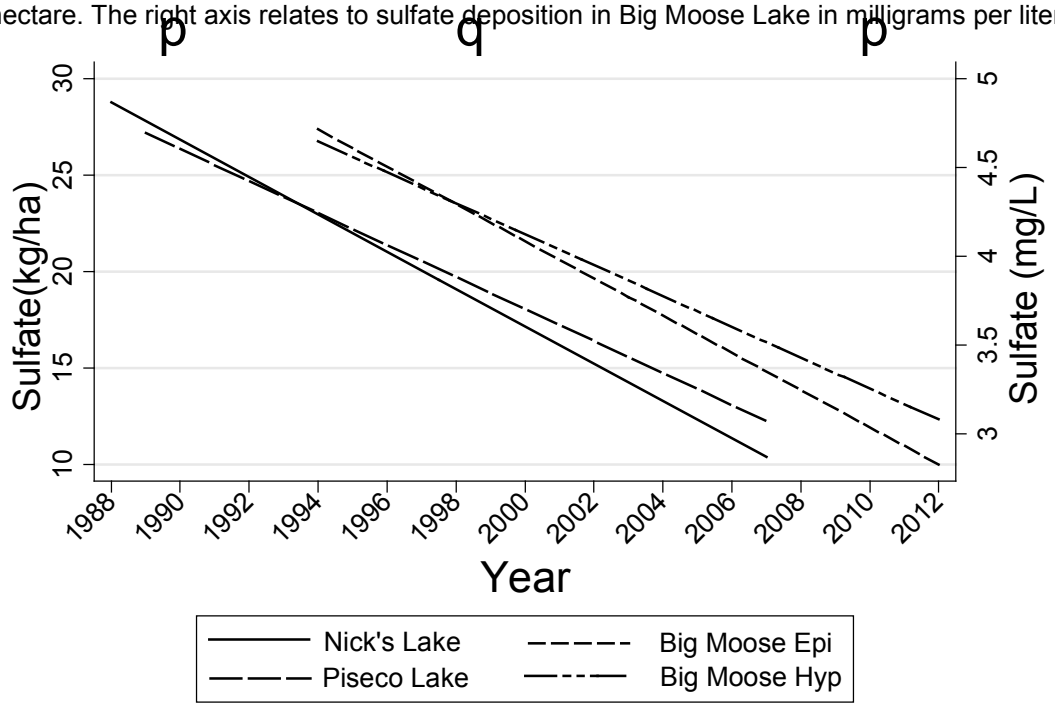


Figure 3-11. Comparison of Piseco and Nick's Lake rate of change in atmospheric SO_4^{2-} (left axis) plotted against the dissolved SO_4^{2-} (right axis) in the epilimnion and hypolimnion of Big Moose Lake

The left axis relates to atmospheric deposition over Nick's Lake and Piseco Lake in kilograms per hectare. The right axis relates to sulfate deposition in Big Moose Lake in milligrams per liter.



In summary, on a regional scale, there has been a clear linear reduction in dissolved SO_4^{2-} since the beginning of the AEAP/NYSERDA projects. This reduction occurs in both the epilimnion and the hypolimnion. The rate of change of sulfate in the lakes is significantly different than the rate of change in atmospheric deposition, the primary source of SO_4^{2-} inputs into the region. This difference in the rate of change between atmospheric inputs and dissolved sulfate would indicate that the watershed ecosystem is providing some form of modulation of sulfate concentrations.

The sulfate analysis also provides additional insight into the regional response of pH in the study lakes. As sulfate is one of the primary drivers for lake acidification, it was anticipated that changes in dissolved sulfate would strongly correlate with pH. However, while sulfate concentrations show a linear response to changes in atmospheric deposition, pH does not. As previously discussed, pH response indicates an oscillating pattern of change, not a linear change. This pattern results in low correlation between pH and sulfate deposition and indicates that factors other than dissolved SO_4^{2-} are regulating pH in these lakes.

3.8.1 Nitrate (NO_3^-)

A main focus of the Clean Air Act Amendments was a call for reductions in S and N emissions, the major contributors to atmospheric acid deposition. Reductions in SO_2 emissions were to begin in January 1995 while the majority of reductions in NO_x emissions were not to begin until 1997. The NO_3- data exhibited a bi-modal distribution with a heavily positive skew. Standard normalization techniques were unsuccessful; therefore, non-parametric analyses were employed. Data analyzed from the AEAP/NYSERDA Project for NO_3^- used two specific types of non-parametric analysis, the Wilcoxon Rank Test and the Spearman's Rank Correlation. There was no significant difference in NO_3^- concentrations between the epilimnetic and hypolimnetic layers when analyzed on a lake-by-lake basis (Table 3-9).

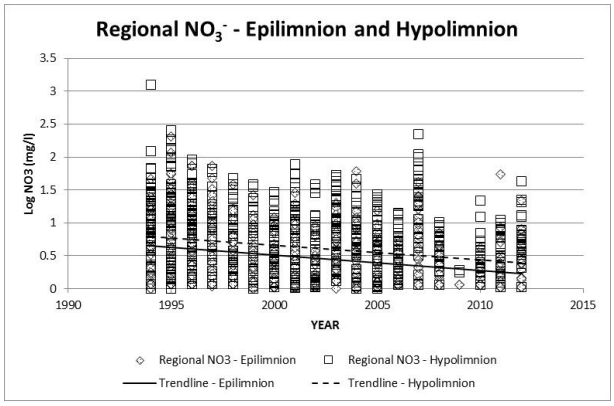
Table 3-9. Comparison of significance of NO_3^- between epilimnetic and hypolimnetic layers

	Significant	Not Significant
NO_3^- Differences	41%	59%

The regional Mann-Kendall trend analysis demonstrated a significant ($p \leq 0.05$) decreasing trend in both the epilimnion and the hypolimnion for NO_3^- for the 16 study lakes (Figure 3-12).

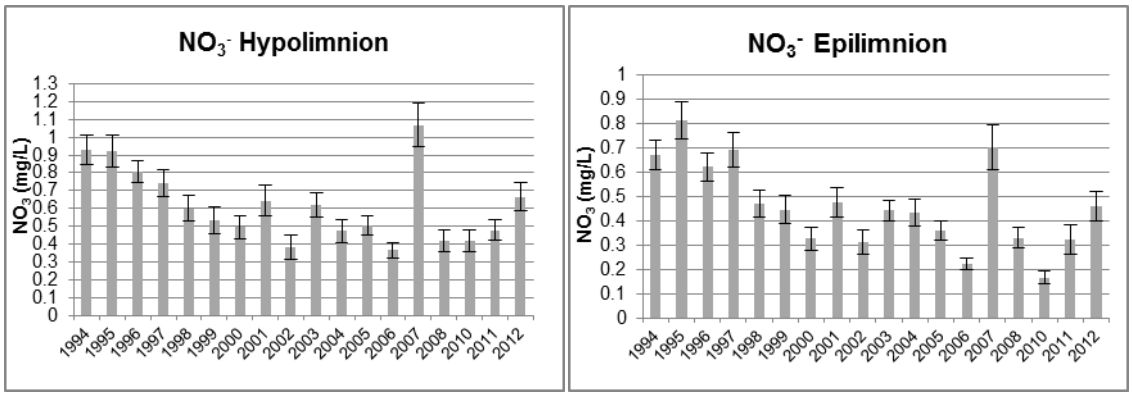
Figure 3-12. Mann-Kendall trend analysis for NO_3^-

A scatter plot and trend line of NO_3^- data in both the epilimnion and hypolimnion of the 16 study lakes.



When the data were evaluated as a function of time, there were significant differences in NO_3^- concentrations between layers exhibited on a year-to-year basis (Figure 3-13).

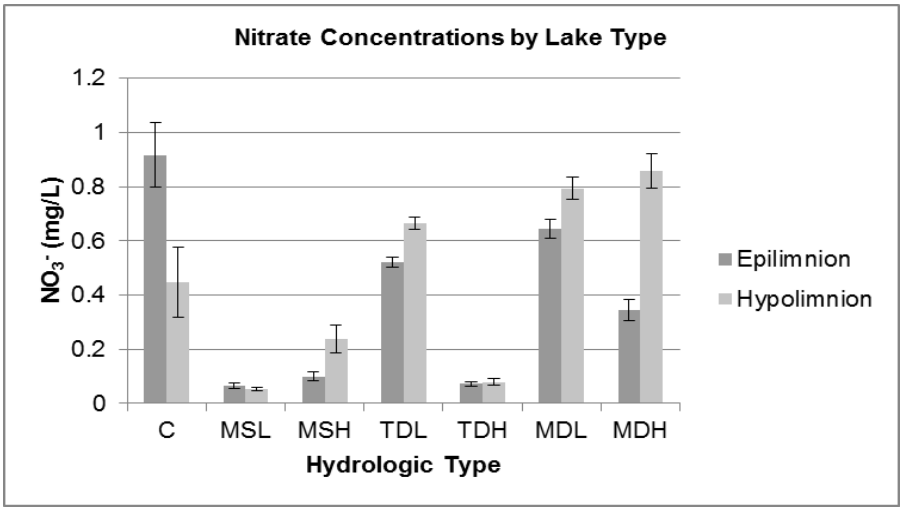
Figure 3-13. Mean NO_3^- concentrations in the epilimnetic and hypolimnetic layers on a year-by-year basis



In regards to hydrologic type, significant differences were observed in NO_3^- in both the epilimnetic and hypolimnetic layers (Figure 3-14). There was a negative correlation in NO_3^- indicating that concentrations were decreasing over time. However, the pattern appeared to be more cyclic in nature. As described in the sulfate discussion, it was determined that the correlation in SO_4^{2-} concentrations indicated a much more linear trend. While the main source of SO_4^{2-} in these lakes is from atmospheric acid deposition, there are many sources of NO_3^- in a forested lake ecosystem. These additional inputs of NO_3^- in the system could support a hypothesis that the repeated pattern of NO_3^- over time is a function of ecosystem input with an additive effect from atmospheric deposition. The concentration of NO_3^- in 2007 is clearly outside the normal pattern in the study lakes. The reason for this concentration is unclear. Other constituents did not show such an anomalous value for 2007. Further investigation is necessary to determine if this value is real and if so, what the cause may be.

Figure 3-14. Differences in mean NO_3^- concentrations by hydrologic lake type

This chart demonstrates a significant difference between lake types using a Wilcoxon non-parametric analysis ($p \leq 0.05$).



3.8.2 Dissolved Organic Carbon (DOC)

DOC concentration observed differed between the epilimnetic and hypolimnetic layers, but it was not nearly as pronounced as pH or SO_4^{2-} (Table 3-10). In combination with only small differences between the months of July and August (Table 3-4), it was concluded that DOC was well mixed in the lakes.

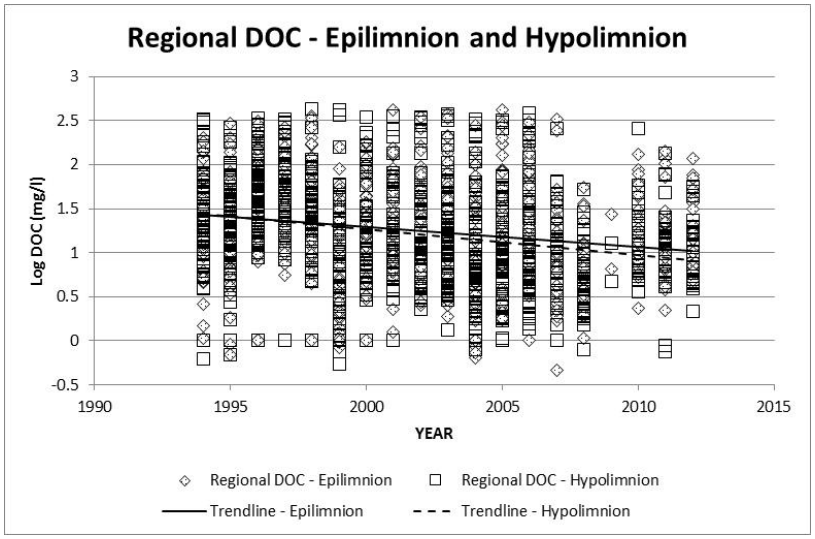
Table 3-10. Differences of DOC levels between epilimnetic and hypolimnetic layers

	Significant	Not Significant
DOC differences between Epilimnion	38%	62%

The regional Mann-Kendall trend analysis demonstrated a significant ($p \leq 0.05$) decreasing trend in both the epilimnion and the hypolimnion for DOC for the 16 study lakes (Figure 3-15).

Figure 3-15. Mann-Kendall trend analysis for DOC

A scatter plot and trend line of DOC data in both the epilimnion and hypolimnion of the 16 study lakes.



There are two sources of DOC in lake systems: allochthonous, which come from outside of the lake system and autochthonous, coming from inside the lake system. As acid deposition is reduced, DOC in lake systems may increase as a result of increased soil pH which would liberate more organic carbon making it more available to aquatic systems (Evans et al. 2005). Based on this idea, it was hypothesized that a clear rise in DOC levels of the study lakes over time should be correlated with a reduction in atmospheric deposition of SO_4^{2-}, as well as reduced concentrations of SO_4^{2-} within the water column. A series of ANOVA's were conducted to test this hypothesis against the NYSERDA/AEAP Project study sites. The results showed a significant difference in DOC values between 1997 and 2012 (Table 3-14). The results, however, were not due to an overall trend in concentration changes but only relative concentrations between the years (Figure 3-15).

Table 3-11. Differences of mean DOC concentrations between individual epilimnetic and hypolimnetic layers over time

DOC values are log transformed.

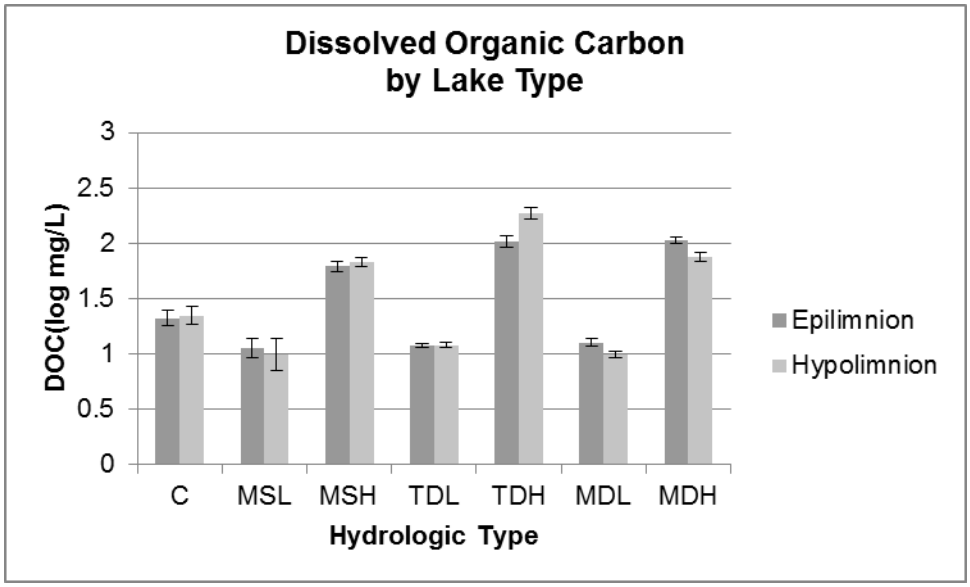
	1997	2012	Significant/Not Significant
Epilimnion	1.61	1.17	Significant
Hypolimnion	1.71	1.05	Significant

This inter-annual significance was primarily driven by low DOC concentrations in 1999 and 2008 which was evident in both the epilimnetic and hypolimnetic layers (Figure 3-15). The patterns exhibited in these figures appeared to be cyclic in nature. When the data are pooled on a regional basis, there does appear to be a slight negative trend, i.e., DOC concentrations are lower in 2012 than in 1997. When analyzed on a lake-by-lake basis, a rate of change regression analysis was unable to be conducted as any lake-specific change was masked by the variability within the annual sample values. However, the regional data indicate a significant decline in DOC concentration which is in contrast to what was has been published in the literature (Couture et al. 2011). One possibility is while there has been a significant reduction in SO_4^{2-} deposition resulting in increased pH levels in these lakes as proposed by Dillon and Molot (1997), Curtis (1998) and Schindler (1998); increased temperatures due to factors such as climate change can reduce soil moisture in eastern North America which can increase water replenishment times and result in a more rapid loss of DOC (Hudson, Dillon, and Somers 2003).

It should be noted that DOC is decreasing during the sampling time periods of this project (July and August only). Future work to address why this decrease is occurring would include more samplings of both the epilimnetic and hypolimnetic layers throughout the entire year to rule out whether this is a seasonal issue or another mechanism at work.

DOC variation was tested to see if there were significant differences when comparing hydrologic lake types. Results indicated that there were indeed significant differences in DOC as a function of lake type (Figure 3-16). Both an ANOVA and repeated measures ANOVA (with year as the repeated measure) were conducted and each yielded significant differences between lake types. As previously described, there was no significant difference in DOC between sampling months on a regional basis (Table 3-4). As shown in Figure 3-16, there is no significant difference in DOC between epilimnetic and hypolimnetic layers within the same lake type. This result indicates that DOC is well mixed within all the study lakes. Significant differences in DOC concentrations between lake types could be attributed to organic carbon entering into the system along with how it is transported. As an example, a drainage lake with medium till (MDL) would have significantly higher levels of DOC than a seepage lake with medium till (MSL) due to a tributary system that is most likely feeding higher levels of organic matter liberated from soils versus a seepage lake that does not have the same types of surface water inputs.

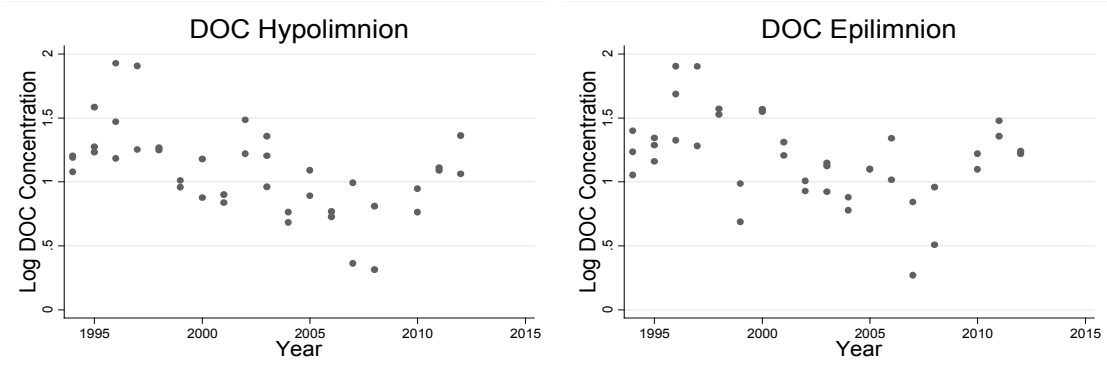
Figure 3-16. Differences in DOC concentrations when compared by hydrologic lake types in both the epilimnetic and hypolimnetic layers



Data reported by ALSC as well as data reported in the literature (Nierzwicki-Bauer et al. 2008, Couture et al. 2012) indicate an overall increasing trend for DOC in aquatic systems in areas of decreasing acid deposition. Data collected for this project indicates a different response. Scatterplots of each lake showed that an increasing linear trend did not exist within DOC for either the epilimnetic or the hypolimnetic layers. The scatterplots did, however, exhibit a form of increasing and decreasing concentrations in a cyclic type of pattern similar to those seen with pH (Figure 3-17).

Figure 3-17. DOC concentrations of Moss Lake over time

The plots of DOC concentration as a function of time in Moss Lake provide an example of the repeating pattern of increasing and decreasing DOC concentrations. This repeating pattern was exhibited across the study lakes.



Because DOC is driven by allochthonous and autochthonous inputs, it would seem logical to propose that something is moderating the organic carbon inputs into the lake. However, the chemical analysis from this study does not have the ability to distinguish DOC from terrestrial or aquatic sources so the exact mechanism is yet to be determined. It should also be noted that there was an interesting correlation between Secchi depth and DOC (Table 3-12). As the Secchi depth decreases (less transparent), DOC levels increase. Although the analysis of the data does not provide a clear mechanism, this increase could be attributed to levels of increasing biomass of phytoplankton and zooplankton in the water column over time along with increased particulate matter.

Table 3-12. Correlation between DOC vs. pH, SO_4^{2-} and Secchi depth

The values in this chart are correlation coefficients of the respective parameters.

	pH	SO_4^{2-}	Secchi Depth
Epilimnion DOC	-0.14*	0.02	-0.68*
Hypolimnion DOC	-0.10*	-0.08*	-0.57*

* denotes significant ($p \leq 0.05$) correlation.

The ALSC survey of 1,462 lakes utilized a 500-µmol/L criteria (which converts to 6.00 mg/L) to determine what was to be considered a high DOC lake category versus a low DOC lake category (Newton and Driscoll 1990). Any value below the 500 µmol/L threshold was in the low category while any value above it would be in the high category. The 500 µmol/L threshold was used as this was the median DOC value for all lakes in the ALSC study. While this was an acceptable means for classifying lakes at that point in time, the NYSERDA/AEAP Project has found that DOC shows a more dynamic DOC range. This results in many lakes switching between high and low DOC categories depending on the year the data was collected. For example, 50% of all the samples taken on Sagamore Lake (MDH category) from 1994 to 2012 had DOC concentrations that were lower than the 500 µmol/L threshold, which is contrary to the Driscoll-Newton classification system. Wheeler Pond (MSH category) showed similar results with 48% of all DOC samples analyzed had reported values below the threshold even though it is categorized in the ALSC and AEAP/NYSERDA studies as a high DOC lake. Based on this data, if the synoptic study on which the DOC categories were originally based was replicated, the changes in concentration demonstrated in the NYSERDA/AEAP Project would result in a median different than the 500µmole value which would change the definition of high and low DOC classification. The variability of DOC on an annual basis highlights the difficulty in establishing categories that are based upon non-biologically or hydrologically relevant metrics. The data collected in the NYSERDA/AEAP Project support a re-evaluation of the original DOC classification scheme.

3.8.3 Chlorophyll a (chl-a)

Chlorophyll *a* is a key parameter in understanding productivity in lakes. Higher concentrations of chlorophyll *a* represent greater primary productivity. Generally, lakes in the Adirondacks are considered oligotrophic. As lakes begin to change due to reduced acid deposition, it is anticipated that phytoplankton populations will increase resulting in greater primary productivity and higher chlorophyll *a* concentrations.

Layer tests were performed to look for chemical differences in chlorophyll *a* between the epilimnion and hypolimnion. Results showed a difference in chlorophyll as a function of time (Table 3-13). The regional Mann-Kendall trend analysis demonstrated a significant ($p \leq 0.05$) increasing trend in the epilimnion for chlorophyll *a*. A significant regional trend was not identified in the hypolimnion for the 16 study lakes (Figure 3-18).

Figure 3-18. Mann-Kendall trend analysis for chlorophyll-*a*

A scatter plot and trend line of Chlorophyll *a* data in both the epilimnion and hypolimnion of the 16 study lakes.

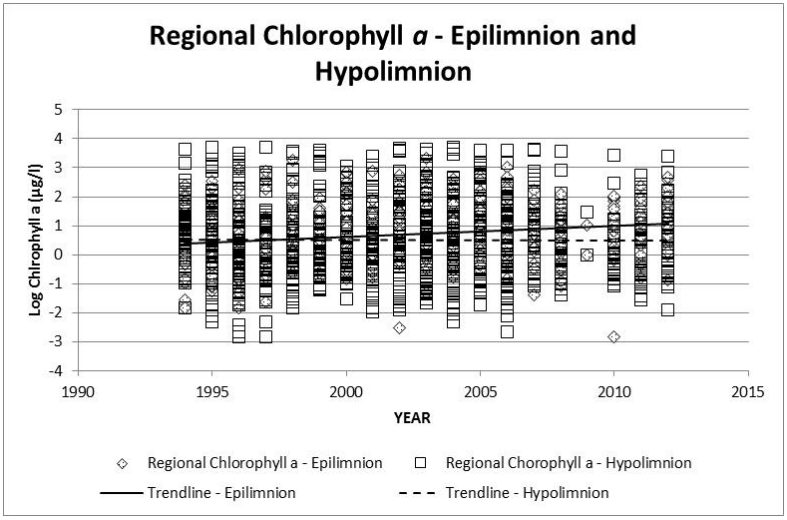


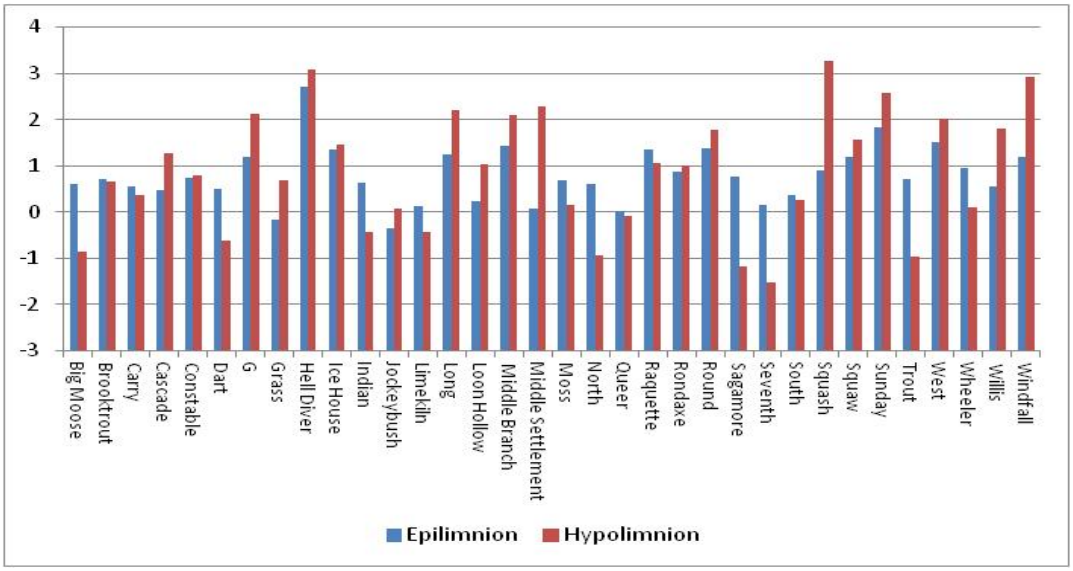
Table 3-13. Difference in chlorophyll *a* concentrations between the epilimnetic and hypolimnetic layers over time

	Significant	Not Significant
Chlorophyll-a differences between	59%	41%

Analysis of the data demonstrated an increased number of lakes with significant differences between the epilimnetic and hypolimnetic layers over the one-month period between July to August (Table 3-13). This analysis could be showing the effects of lake stratification on chlorophyll *a* levels when getting close to its seasonal peak. Based on the results, each layer was considered separately for the remainder of the analysis.

When the data are viewed on a regional basis (all lakes pooled), the mean concentration of chlorophyll *a* is greater in the epilimnion than in the hypolimnion. However, when the mean chlorophyll *a* value for each lake was compared between the epilimnion and hypolimnion, results showed the average chlorophyll *a* concentration was greater in the hypolimnion than in the epilimnion for 19 of the 35 lakes (Figure 3-19). There are several possible explanations for this result. First, as described in the AEAP report (Nierzwicki-Bauer et al. 2008), the hypolimnetic chlorophyll concentrations may be due to gravity-induced settling or sedimentation of phytoplankton (Abbott et al. 1984). An alternate hypothesis is that the phytoplankton grow in situ below the thermocline (Coon et al. 1987). This hypothesis could be the result of increased phosphorus availability or light limitation selecting for more chlorophyll rich phytoplankton at depth (Barbiero and Tuchman,2004, Felip and Catalan 2000) The overall chlorophyll levels could be the result of a combination of effects (Coon et al. 1987) along with differential grazing rates in different portions of the water by the zooplankton community (Fahnenstiel and Scavia 1987).

Figure 3-19. Log chlorophyll *a* (µg/L) as a function of lake layer



Further work is necessary to develop an understanding of the chlorophyll dynamics in these lakes and to determine which, if any, of these competing hypotheses may be correct (Shortreed and Stockner 1990, Wurtsbaugh et al. 2001).

An ANOVA with post hoc testing was conducted to determine if a difference exists in chlorophyll concentration between years. Each layer was analyzed independently. Results showed significant differences between 1997 and 2012 in the epilimnion, however not in the hypolimnion (Table 3-14).

Table 3-14. Comparison of mean chlorophyll a concentrations in different layers as a function of time (log mg/L)

Significance is determined at $p \leq 0.05$.

	1997	2012	Sig/NS
Epilimnion	0.38	1.05	Sig
Hypolimnion	0.23	0.52	Not Sig

Interannual differences were observed along with considerable variability in chlorophyll *a* concentrations between the years. On a regional basis, there appears to be a cycling of the chlorophyll *a* concentrations (Figure 3-20). This phenomenon is discussed in more detail in the Section below.

When chlorophyll *a* was analyzed to determine if significant differences existed as a function of hydrologic category, results showed significance in both epilimnetic and hypolimnetic layers (Figure 3-21).

Figure 3-20. Inter-annual pooled mean concentrations for chlorophyll *a* over time

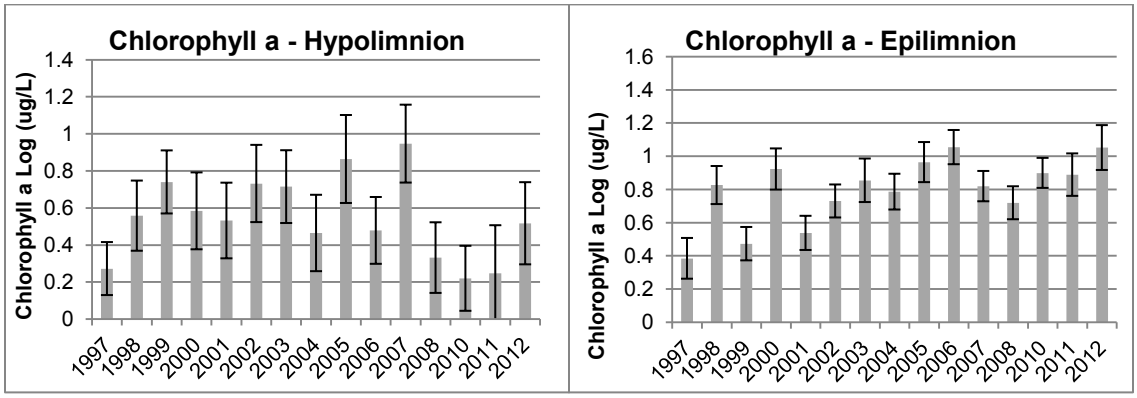
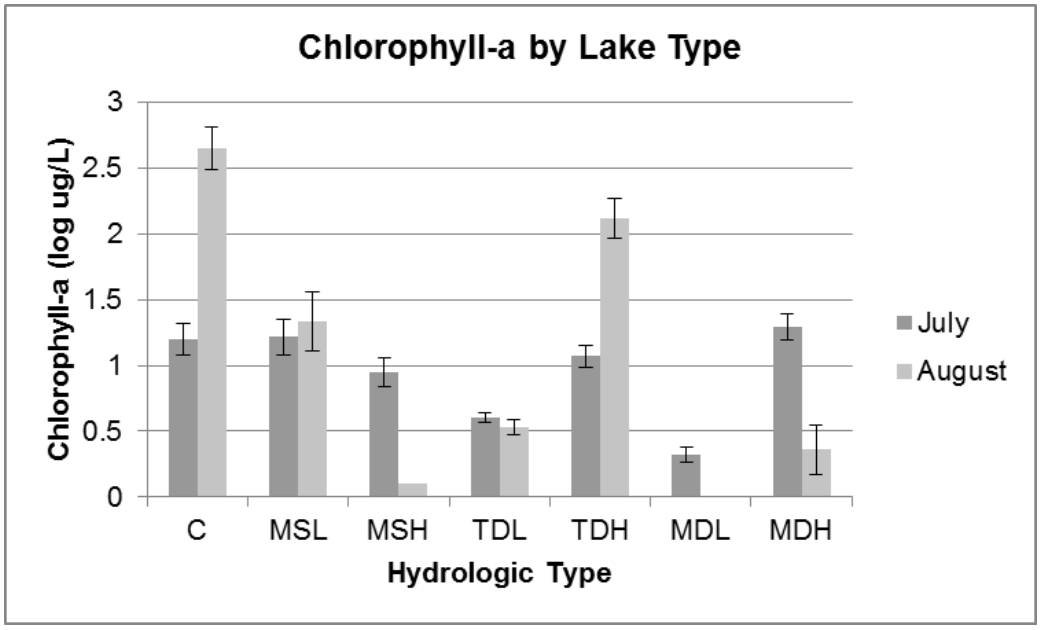


Figure 3-21. Chlorophyll *a* differences based on hydrologic category of lake

Please note different log scales on x-axis.



A two-way ANOVA was conducted to determine the interaction between year and lake type and found no significance. This result indicates that the two factors of year and lake type operate independently from one another without any significant effect upon either layer. These results were similar to results produced in the same analysis for DOC (Table 3-15).

Table 3-15. Comparison of 2-way ANOVA results between DOC and chlorophyll *a* concentrations in both the epilimnetic and hypolimnetic layers as a function of year and hydrologic lake type (HT)

Significance (sig) determined at $p \leq 0.05$.

Lake layer	Parameter	Year	HT	Year * HT
Epilimnion	Chlorophyll *a*	Sig	Sig	Not Sig
	DOC	Sig	Sig	Not Sig
Hypolimnion	Chlorophyll *a*	Not Sig	Sig	Not Sig
	DOC	Sig	Sig	Sig

3.9 Rate of Change in Chlorophyll a Concentrations

A regression analysis was performed on each lake to determine how chlorophyll varies over time. This was done by combining months but analyzing the layers separately. Results showed that there is a very weak predictive relationship between chlorophyll *a* and time. Table 3-16 shows that only 13% of the R^2 values in the epilimnion and 10% of the R^2 values of the hypolimnion account for a moderate or high degree of variation. The slope of the regression was significantly different from 0 only a small percentage of time (Table 3-16). Based upon these results, it is concluded that time is not an accurate predictor of chlorophyll *a* concentrations in Adirondack lakes.

Table 3-16. Predictive relationship of chlorophyll *a* over time in individual lake layers

These data demonstrate that time is a weak predictor of chlorophyll-*a* concentration in both the epilimnion and the hypolimnion.

	Epilimnion	Hypolimnion
Moderate/High variation	13%	10%
Slope Significant	35%	23%

On an individual lake basis, however, several interesting trends did appear in the epilimnetic and hypolimnetic layers. Six lakes including Dart*, Ice House, Queer, Raquette, South* and Squash (*represents lakes attached to the NYSERDA/AEAP study) showed trends in the epilimnion towards higher concentrations while the hypolimnion in the same lakes were trending towards lower concentrations. Three lakes that were not included in the three-year extension provided by NYSERDA but part of the previous long term AEAP program (Loon Hollow, Middle Branch, and Seventh) were showing the opposite trend. A possible explanation for these trends is that these lakes could be beginning to move to different trophic states. Further investigations into this phenomenon would include looking at chlorophyll a concentrations as a function of secchi depth, DOC and other parameters that impact the amount of visible light penetrating into the water column.

As detailed in Section 5, zooplankton has a direct impact upon phytoplankton populations. Clearly, the phytoplankton/zooplankton interaction will affect chlorophyll production; however, the exact relationship within the context of the Adirondack lakes is not clear. In part, this uncertainty is due to the role that hypolimnetic chlorophyll a plays in this system. The current project has not investigated the specific phytoplankton or zooplankton community in the hypolimnion. Until this investigation is done, a complete understanding of the zooplankton/phytoplankton/ primary production (as measured by chlorophyll) relationship in Adirondack lakes will not be possible.

3.9.1 Aluminum (Al)

Data from samples collected for aluminum analysis were reported as total monomeric aluminum and as non-labile inorganic aluminum. Concentrations of labile aluminum, which is the form of concern in aquatic systems, was calculated by subtracting non-labile inorganic aluminum from total monomeric aluminum. Total monomeric aluminum (referenced as monomeric aluminum), as it represents all monomeric forms, and labile aluminum, as it is the form of most concern from a toxicity perspective, were the forms of aluminum carried through the analysis.

Analyses were conducted to determine if a significant difference existed between the epilimnion and hypolimnion. A series of repeated measures ANOVAs were conducted to make this determination. When analyzed on a month-by-month basis, labile aluminum did not show a strong stratification difference. When the values were pooled, the stratification effect became more clear (Table 3-17). A stronger stratification effect was found with total monomeric aluminum, particularly in the hypolimnion.

Table 3-17. Percent of lakes that indicated significant difference in aluminum between epilimnetic and hypolimnetic layers over time

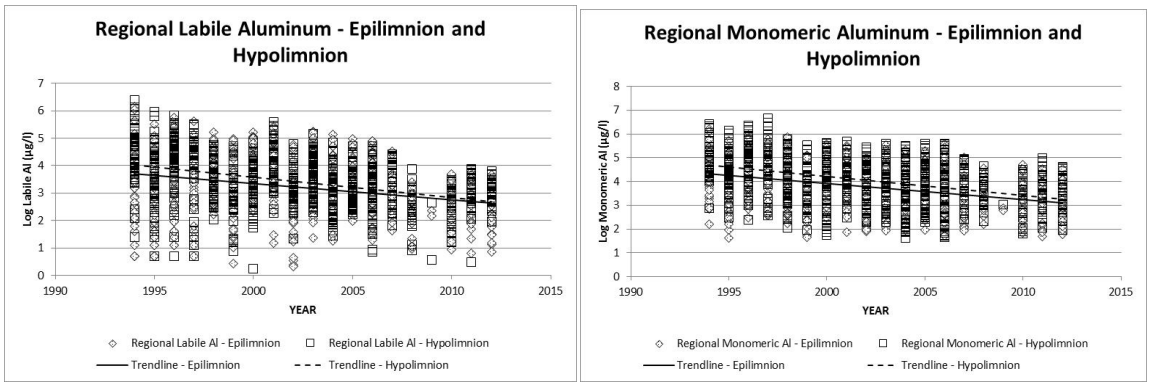
	Aluminum differences between Epilimnion and Hypolimnion
Labile Aluminum	50%
Total Monomeric Aluminum	59%

Interestingly, when labile aluminum data are pooled on a regional basis and statistically evaluated, there is a significant difference ($p \leq 0.05$) between the epilimnion and hypolimnion (see Section 3.9.2). Taken together, it is clear that there is a difference in aluminum concentrations between the epilimnion and hypolimnion in Adirondack lakes, but the biological significance of stratification must be evaluated on a lake-by-lake basis. This also provides an indication of the effect that the length of the stratification season has on aluminum, with a longer duration potentially resulting in greater amounts of aluminum being available in the system.

The regional Mann-Kendall trend analysis demonstrated a significant ($p \leq 0.05$) decreasing trend in both the epilimnion and hypolimnion for monomeric aluminum. A similar, statistically significant trend was found with labile aluminum (Figure 3-22).

Figure 3-22. Mann-Kendall trend analysis for monomeric and labile aluminum

A scatter plot and trend line of monomeric aluminum and labile aluminum data in both the epilimnion and hypolimnion of the 16 study lakes.



Both total monomeric aluminum and labile aluminum differed significantly ($P \leq 0.05$) as a function of year (Figure 3-23). The aluminum concentration was also significantly different ($P \leq 0.05$) when analyzed as a function of lake hydrologic type (Figure 3-24).

Aluminum varies significantly ($P \leq 0.05$) when analyzed as a function of hydrologic setting. This variation occurs in both the epilimnion and hypolimnion for July and August (Figure 3-24). However, when time (years) and hydrologic types are analyzed together, no significant interaction is found. Time and hydrologic type are completely independent from each other with respect to aluminum distributions. This independence is similar to the results found in the other individual constituent analyses.

Figure 3-23. Monomeric aluminum and labile aluminum by year or as a function of year

The analysis demonstrates significant variation on an annual basis of monomeric aluminum (A & B) and labile aluminum (C & D) in both the epilimnion and hypolimnion. The data in these charts are for the July sampling periods. A similar result is obtained for the August sampling period. Note the difference in scale between monomeric and labile aluminum.

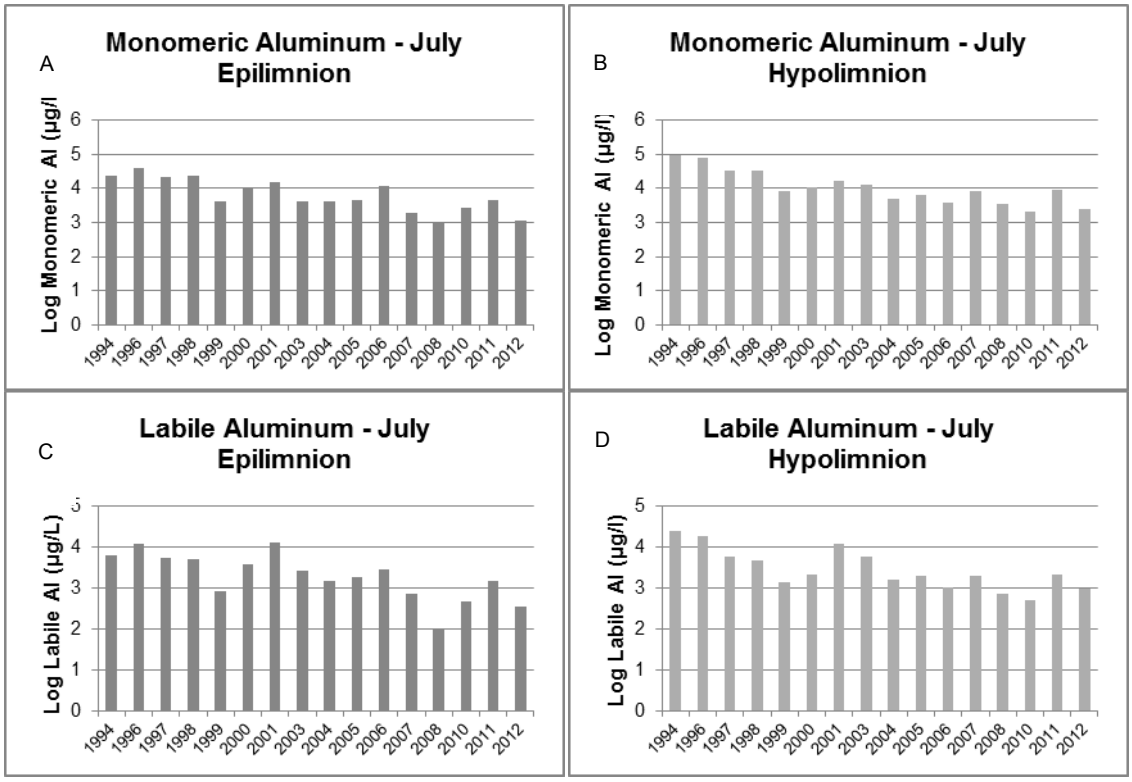
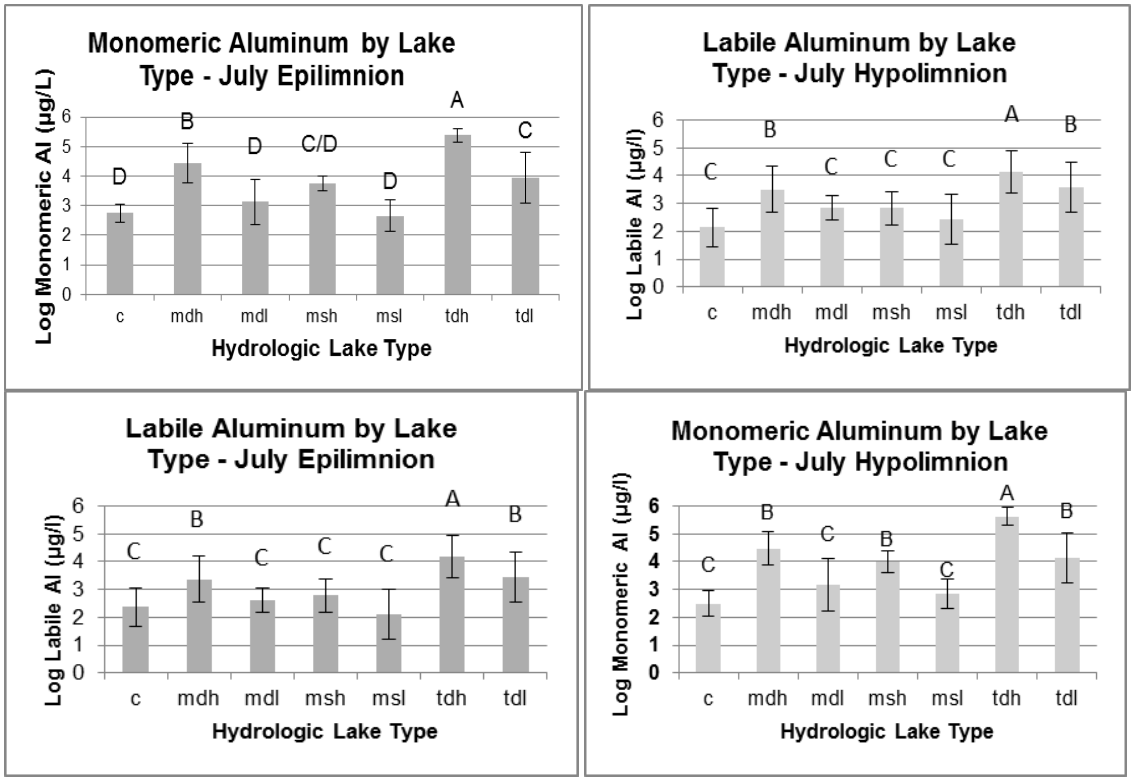


Figure 3-24. Aluminum as a function of hydrologic type

There is a significant difference in both monomeric aluminum (A and B) and labile aluminum (C and D) concentrations ($P \leq 0.05$) as a function of lake types for July. This difference in concentration appears in both the epilimnion (A and C) and the hypolimnion (B and D). The concentration differences appear in both July and August (August data not shown).



When aluminum (both monomeric and labile) concentrations are analyzed to determine rates of change over time, it becomes clear that time is not a good predictor of aluminum values. While there are a few notable exceptions (Big Moose), the overall predictive capability as elucidated by the r^2 value from an Ordinary Least Squares (OLS) regression indicates that time (in years) does not effectively predict aluminum concentrations (Table 3-18).

Table 3-18. Regression analysis summary for labile aluminum

The regression analysis provides an indication of the rate of change in labile aluminum over time as well as indicating the predictive capability of time on the concentration of aluminum. The data indicate that time is not an accurate predictor of labile aluminum concentration for the study lakes. The "Percent Significant r^2" row indicates the normalized number of regression slopes that have a significant dependent relationship between time and labile aluminum. The data for monomeric aluminum (not shown) are similar to that of labile aluminum.

	July Epilimnion	July Hypolimnion	August	August
Range r^2	0.00 - 0.68	0.00-0.52	0.00-0.82	0.00-0.77
Average r^2	0.26	0.35	0.16	0.25
Percent Significant r^2	27%	6%	33%	16%

The regression analysis result is not surprising as dissolved aluminum is a function of pH of the aqueous media. As discussed in the pH section, time is not a strong predictor of pH so it follows that dissolved aluminum would not be strongly linked to time. However, pH is a key to understanding aluminum in the study lakes.

Generally, above a pH of 6.0, aluminum is no longer soluble and ultimately precipitates out of solution to the sediment below. In and of itself, this phenomenon raises several interesting possibilities for understanding fish mortality in Adirondack lakes. Specifically, the lake bottom sediment could be viewed as a sink for future aluminum release that could occur should the pH of the surrounding pore water and lake water at the sediment/water interface begin to drop. Secondly, use of core samples may provide a record of historic pH/aluminum release events.

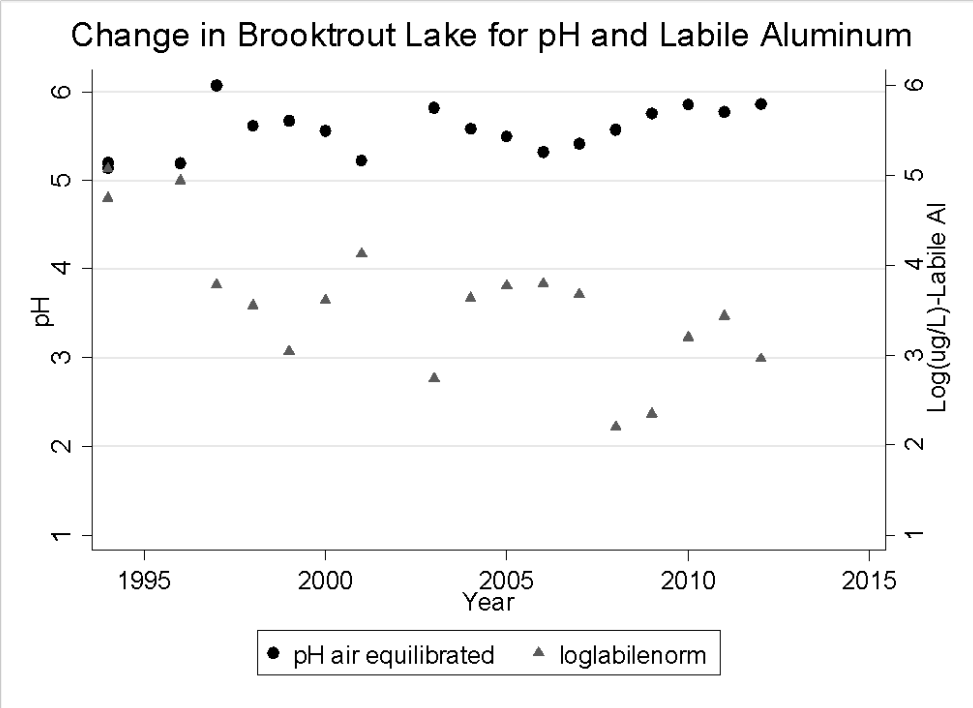
However, more importantly in developing an understanding of the impacts of acid deposition in Adirondack lakes, it was necessary to see how pH and aluminum interact. A correlation analysis was completed to determine if a relationship exists between pH and aluminum (monomeric and labile) within the study site. As seen in Table 3-19, moderate to strong correlations as defined by Methe and Zehr (1999) exist in both the epilimnion and hypolimnion. This relationship can be seen graphically in Figure 3-25 for pH and labile aluminum in Brooktrout Lake.

Table 3-19. Correlation coefficients (r) for monomeric and labile aluminum with pH in the epilimnetic and hypolimnetic layers. All correlation coefficients are significant ($p \leq 0.05$)

	Monomeric Aluminum	Labile Aluminum
Epilimnion	-0.75	-0.71
Hypolimnion	-0.69	-0.68

Figure 3-25. Relationship between pH and aluminum in Brooktrout Lake

These data represent the change in pH (circles) and labile aluminum (triangles) over time in the July epilimnion of Brooktrout lake. A negative correlation can be observed. Consistent with the region wide correlation analysis discussed in the text, the correlation between pH and labile aluminum in the July epilimnion of Brooktrout is -0.70 ($p \leq 0.05$).



With respect to the impacts of acid deposition, the correlation between pH and aluminum is important from a biotic perspective. Aluminum is one of the key constituents associated with biotic loss in acid-impacted lakes. Many organisms, including fish, have a low tolerance for aluminum. It is well established that the toxicity of aluminum is primarily related to labile aluminum. Further, it is understood that the toxicity of labile aluminum is greatest between a pH of 5.0 and 5.5 (ASTDR 2008).

While there are a number of constituents, such as calcium and magnesium that have the ability to reduce the toxicity of aluminum, the concentration of DOC in the system is key to evaluating the potential impact of pH driven aluminum toxicity (Environment Canada 2010). Research into the ameliorating effects of DOC on aluminum indicate that the higher the concentration of DOC, the lower the toxicity of aluminum. Concentrations of DOC greater than 10mg/l have been demonstrated to significantly reduce aluminum toxicity (Environment Canada 2010).

It should be noted that DOC has only a weak correlation with pH, labile aluminum, and monomeric aluminum (Table 3-20). In short, while there may be some relationship between them, DOC and pH/aluminum are independent of each other on a regional scale with a large contribution of DOC in the study lakes being allochthonous.

Table 3-20. Correlation coefficients (r) for pH, monomeric aluminum, and labile aluminum against DOC in the epilimnetic and hypolimnetic layers

All correlation coefficients are significant ($p \leq 0.05$).

	pH	Monomeric Aluminum	Labile Aluminum
Epilimnion	-0.14	0.50	0.29
Hypolimnion	-0.10	0.48	2506

Based upon the interactions of aluminum, pH, and DOC, an analysis was developed that evaluates when conditions occur that may have historically increased the likelihood of aluminum having negative effects on the aquatic systems of the study sites. A matrix was developed that categorizes the potential toxicity of aluminum in relation to pH and DOC (Table 3-21).

Table 3-21. Aluminum toxicity risk categories and parameters

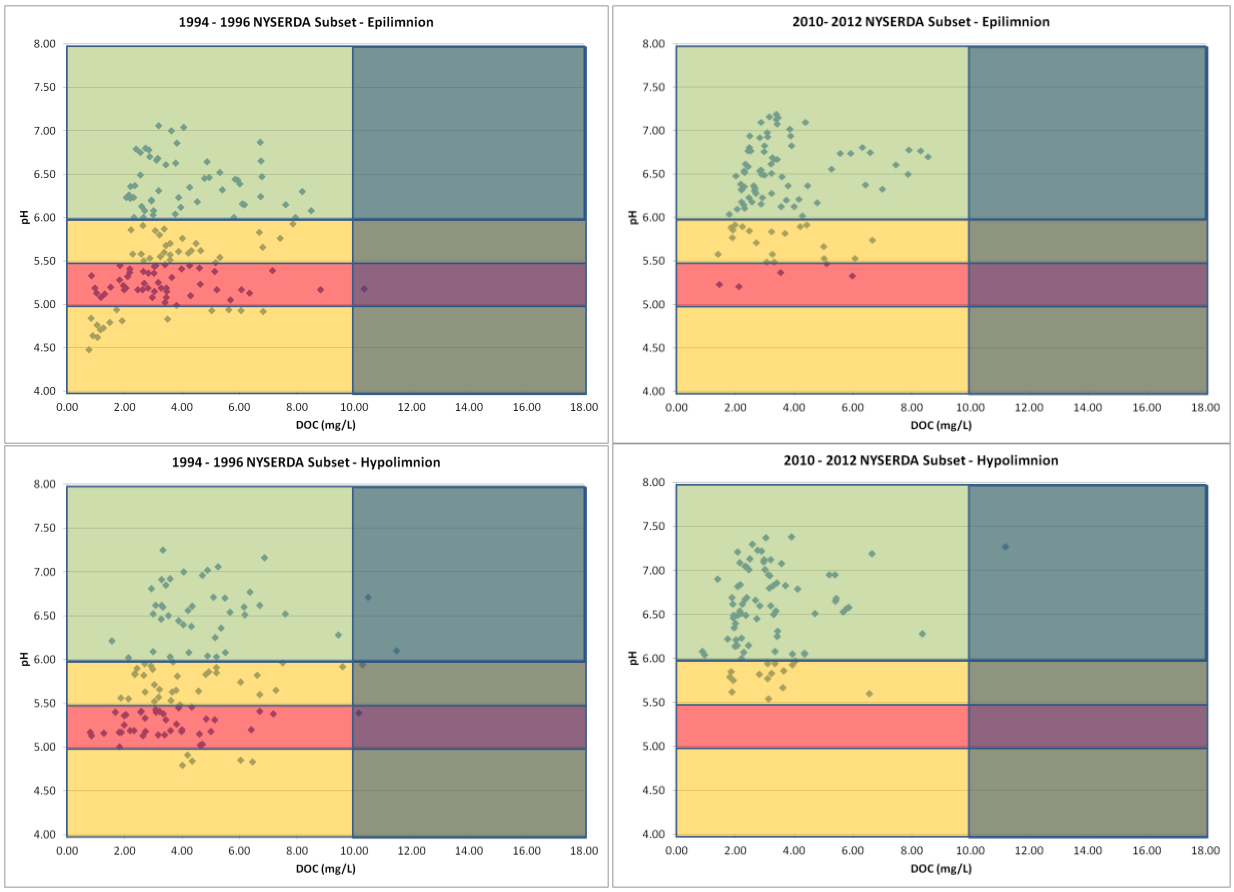
This table describes risk categories of aluminum based upon pH and DOC concentrations. The same concentration of aluminum in the High category is more likely to have an ecological impact than that concentration in the medium or low category.

Risk Category	Category Parameters
High	between 5.0 and 5.5 and DOC < 10mg/L
Moderate	pH < 5.0 and DOC < 10mg/L
Moderate	pH > 5.5 and pH < 6.0 with DOC < 10mg/L
Low	DOC > 10mg/L
Low	pH >6.0

The data from each sampling event were then analyzed and placed into the appropriate risk category. The data were then plotted within the risk matrix (Figure 3-26).

Figure 3-26. Labile aluminum concentrations and toxicity risk categories

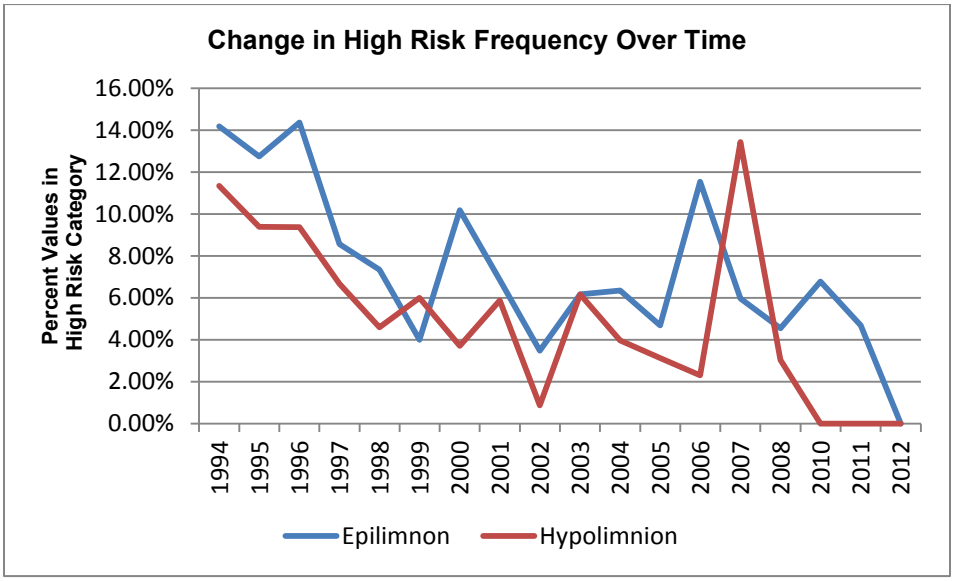
Aluminum data (µg/l) for the AEAP/NYSERDA Project lakes plotted within the risk category matrix. The top two charts are for the epilimnion and the bottom two charts represent the hypolimnion. The left column is for the time period 1994-1996 and the right column is for the time period 2010 – 2012. The risk matrix is categorized as follows: Red = High Risk, Orange = Moderate Risk and Green = Low Risk. The distribution indicates a shift away from the high and moderate risk categories.



Subsequently, the percentage of each category was determined. These values were plotted as a function of time (Figure 3-27) for both the epilimnion and hypolimnion. This provides a representation of the change in risk for aluminum toxicity over time within the study lakes.

Figure 3-27. Change in frequency of High Risk over time

The data indicate a general decline in the frequency of sample periods which fall within the High Risk category for aluminum toxicity.



Finally, to provide an indication of how the risk categories have changed over time in relation to acid deposition, a correlation analysis was conducted that compared the risk frequency values for both the epilimnion and the hypolimnion with sulfur deposition at the Nick's Lake and Piseco Lake monitoring stations. Table 3-22 provides the results.

Table 3-22. High Risk frequency correlation with SO_4^{2-} atmospheric deposition

The correlation coefficient (r) for SO_4^{2-} atmospheric deposition at Adirondack air monitoring stations with the frequency of Aluminum Toxicity High Risk Category events. R is significant ($P \leq 0.05$) for the both epilimnetic values but it is not significant for either hypolimnetic value.

	Epilimnion	Hypolimnion
Nick's Lake	0.56	0.46
Piseco Lake	0.80	0.35

Interestingly, the epilimnion has a higher correlation than does the hypolimnion. This correlation could be reflective of the real time impact atmospheric deposition has on the epilimnion compared to the hypolimnion, which is isolated from deposition during the stratification season due to the thermocline. The data reflect approximately one year lag in the impact of atmospheric deposition in the hypolimnion as compared to the epilimnion. The correlation data support this hypothesis exhibiting a much stronger correlation in the epilimnion than in the hypolimnion. Overall, these data support a conclusion that the risk of aluminum toxicity in the study lakes has been reduced and that this risk reduction is due to a decrease in atmospheric acid deposition within the region.

3.9.2 Effects of Stratification on Water Chemistry

3.9.2.1 Stratification

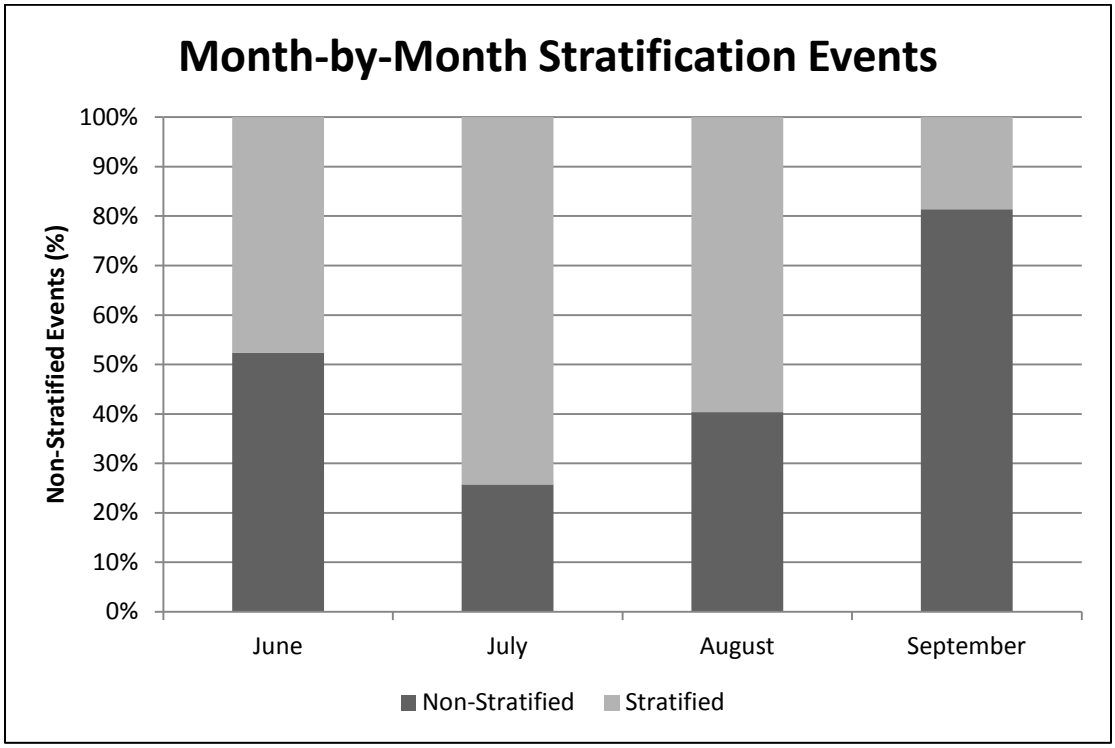
Many Adirondack lakes and ponds stratify, as is common in the temperate zone of the Northeast US. Of the 35 lakes in the AEAP/NYSERDA Project 60% stratified each year they were sampled and 40% periodically stratify. Every lake has been found to be stratified at least once during the project period although several lakes such as Round and Willis rarely stratify.

The AEAP/NYSERDA Project design focused sampling activities during July and August, with periodic samplings occurring in June and September. It is these expanded sampling dates that provide some additional information on the timing of stratification (Figure 3-28). While not definitive, the data clearly indicate that stratification begins in May or June, reaches its maximum in July and begins to disrupt as early as August in some lakes. Understanding the annual thermocline cycle in Adirondack lakes is important on several limnological levels. From an abiotic perspective, longer stratification seasons will potentially change the hypolimnetic chemistry to a degree that alters the redox potential at the sediment/water interface. Such alterations would change mobilization/adsorption dynamics of the system ultimately altering the chemical composition at the sediment/water interface. From a biological perspective, a change in the timing of the initiation of stratification could affect resource availability/utilization in both the epilimnion and hypolimnion as well as having phenological implications.

Sampling activities for the AEAP/NYSERDA Project were centered around July and August; consequently, samples were not consistently collected in the shoulder months of June and September. While stratification data are sufficient to describe a general annual pattern, there are insufficient datapoints (Figure 3-28) to determine if any changes to the annual pattern have occurred over the study period on an annual basis. An expanded dataset can be used to determine potential shifts in lake stratification in the Adirondack region.

Figure 3-28. Month-by-month stratified and non-stratification events

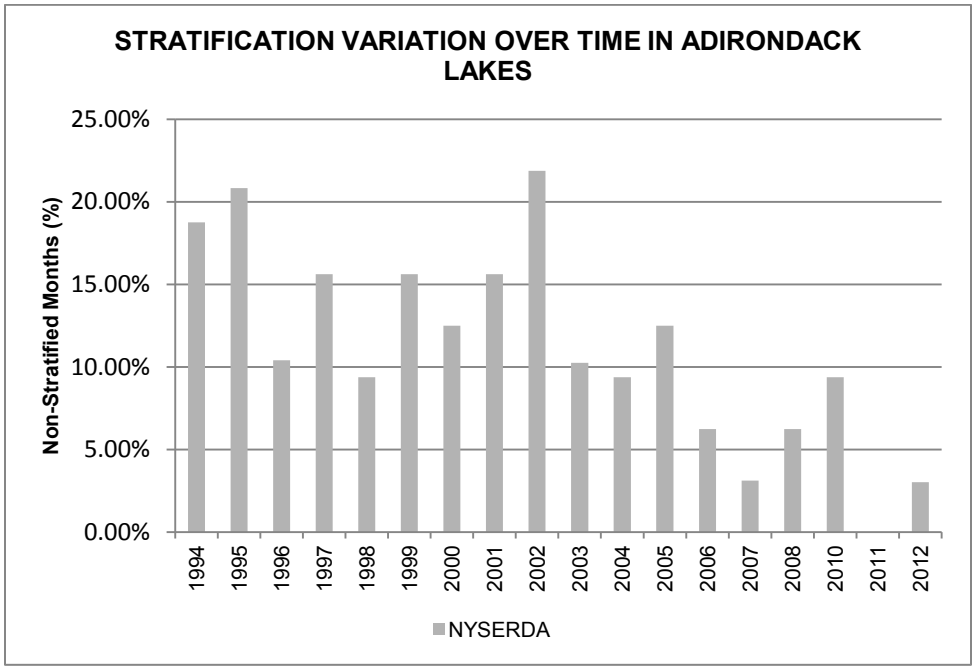
Measured as a function of sampling events, July is the peak stratification period in the study area with disruption of the thermocline beginning as early as August with most lakes destratified by September. N=102 for June, N=411 for July, N=455 for August, N=100 for September.



When stratification is viewed as a function of sampling events, the frequency of stratification relative to non-stratified periods can be evaluated. As the sampling schedule, frequency and design did not change throughout the course of the project, assessing change as a function of sampling event provides an alternative means to assess physical and chemical change within the lakes over time. Analyzing the data in this manner indicates that the frequency of non-stratified events, which is a surrogate measurement for the number of non-stratified lakes, has decreased over the time-period of the NYSERDA/AEAP project (Figure 3-29). While the specific cause of this change cannot currently be determined, it is evidence that the lakes are being subjected to different temperature (water and atmospheric) conditions. There are several potential causes, including in-lake changes in mixing regimes, changes in light penetration, wind and changes in regional temperature (climate change issues).

Figure 3-29. Stratification variation as a function of time

The number of non-stratification events has declined over the period of this study. This decline means that lakes and ponds that have historically not stratified are now developing a thermocline. On a regional basis, these data indicate a potential increase in atmospheric and/or water temperatures.

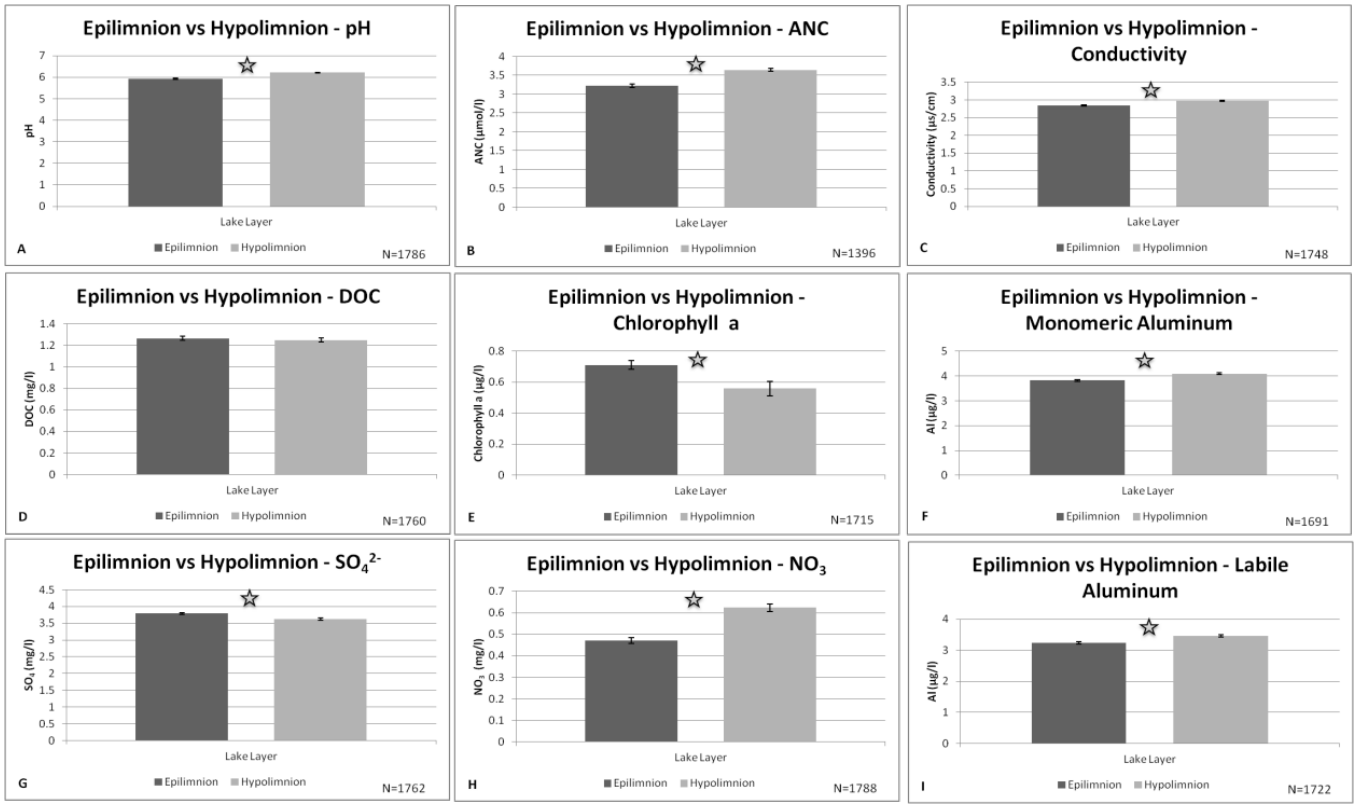


3.9.2.2 Epilimnion versus Hypolimnion

The sampling and analysis design of the NYSERDA/AEAP project has enabled an assessment of the partitioning of chemical parameters between layers. The data demonstrates that there is a clear difference in chemistry between the epilimnion and hypolimnion. Of the nine parameters included in this analysis, all but one (DOC) had significantly different concentrations between the epilimnion and hypolimnion when data were pooled over all lakes and sampling events (Figure 3-30). As demonstrated in individual chemical parameters previously discussed, this same pattern occurred on a lake-by-lake basis. In short, there is a significant difference between the epilimnion and hypolimnion in the water chemistry of stratified Adirondack lakes.

Figure 3-30. Chemical variation between the epilimnion and hypolimnion of Adirondack lakes

On a regional basis, all concentration differences are statistically significant ($p \leq 0.05$) with the exception of DOC. These data indicate that the epilimnion and hypolimnion are chemically different systems during the stratification season.



Consistent with the results reported in the AEAP Final Report (Nierzwicki-Bauer et al. 2008), the hypolimnion generally had higher concentrations than did the epilimnion. The exceptions were chlorophyll a and sulfate. The report hypothesized that lower hypolimnetic sulfate concentrations were due to lower dissolved oxygen and concomitant shifts toward anaerobic microbial respiration in the hypolimnion. This scenario is very likely and was not evaluated in this report, but will be analyzed at a later date.

More interesting is chlorophyll a. The AEAP report noted that a substantial difference in chlorophyll a between the layers with the hypolimnion having a“150% to 300%" greater concentration than the epilimnion. The conclusion in the AEAP report is that this relationship was “most likely the product of gravity-induced settling of epilimnetic-derived organics” (Nierzwicki-Bauer et al. 2008).

However, the current dataset indicates a much different result in chlorophyll a, with a significantly higher concentration in the epilimnion than in the hypolimnion (Figure 3-30) when data from all lakes and sampling periods are pooled. As discussed earlier, there are a number of individual lakes in which the chlorophyll concentrations remain higher in the hypolimnion than in the epilimnion.

It is not immediately clear as to the reason for this change in lake dynamics. Several other changes also have occurred during the intervening years. Most obvious is the change in phytoplankton populations. As detailed in Section 5 (Phytoplankton), there was a significant increase in phytoplankton between 2003 and 2004. This change, hypothesized to be due to a change in zooplankton populations, may also have altered the community structure and preferentially selected for phytoplankton species that tend to inhabit and remain in the epilimnion instead of sinking to the bottom. Other potential causes of this shift may be a change in the concentration and partitioning of nutrients such as phosphorus or changes in light penetration.

3.9.3 Effects of Precipitation on Chemistry

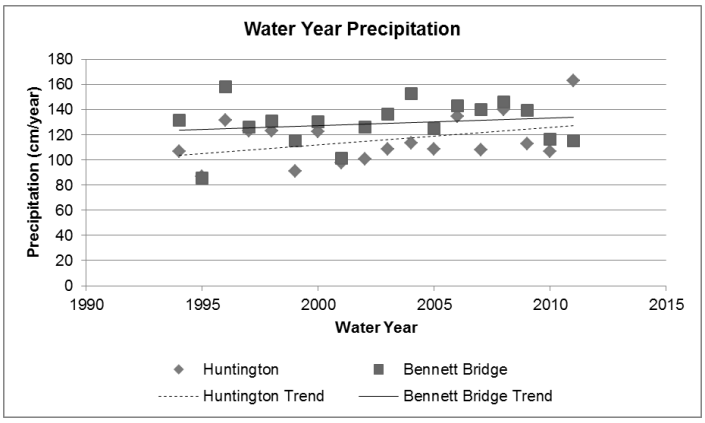
Climate data were not collected as part of the scope of this project. However, weather related phenomenon such as the variation in annual precipitation, ice-in/ice-out dates, wind and storm events all play a role in influencing the chemistry of lentic environments. Precipitation has been shown to be a particularly important influence in the chemistry of aquatic environments. Although not a part of this project, general precipitation patterns within the region were evaluated to determine if annual precipitation could explain the oscillating chemical patterns identified in previous sections.

Water year precipitation data was gathered from the NADP database for two locations located in the general vicinity of the study lakes. These locations (Huntington Forest and Bennett Bridge) are found on the east and west periphery of the study area respectively (Figure 3-31). While this data does not provide lake specific weather data, it does provide an indication of precipitation patterns across the study region. Two specific analyses were conducted for the collected precipitation data at each location. First, a Mann-Kendall trend analysis to determine if the annual precipitation amount changed over the course of the study. Second, a Pearson's Correlation analysis was conducted to determine to what extent the average concentration of parameters analyzed for this study changed with respect to the annual water year precipitation amounts.

The trend analysis indicates mixed results. There is a small, but significant increasing trend for the Huntington Forest location, but there is no trend found at Bennett Bridge (Figure 3-31). The Tau correlation coefficient calculated for both locations is low. These results indicate highly variable annual rainfall volumes with distinct geographic differences in precipitation.

Figure 3-31. Mann-Kendall trend analysis for precipitation

Scatterplot and trend lines for water year precipitation at NADP monitoring locations adjacent to the study area. Huntington trend is significant while Bennett Bridge is not significant ($p \leq 0.05$). Tau correlation for Huntington is 0.26 and 0.03 for Bennett Bridge.



The Pearson Correlation analysis has similar results. With no significant correlations and low r values (Table 3-23), the chemical parameter/precipitation correlation indicates that there is little association between precipitation and chemical concentrations within the study area when evaluated at the regional level. In conjunction with the trend analysis, these results suggest that while precipitation alone cannot account for the oscillating regional trends identified in the preceding sections.

Table 3-23. Precipitation/analyte correlation

Pearson Correlation for select analytes with annual water year precipitation values from the Bennett Bridge and Huntington NADP monitoring locations. None of these correlations are significant ($p \leq 0.05$).

Location	Layer	pH	DOC	ANC	SO4	Labile Al
Bennett Bridge	Epilimnion	-0.45	0.03	-0.32	0.31	0.28
	Hypolimnion	-0.16	0.09	-0.12	0.31	0.00
Huntington	Epilimnion	0.06	0.16	-0.21	-0.41	0.01
	Hypolimnion	0.12	-0.06	-0.20	-0.37	-0.12

3.10 Summary

This analysis clearly indicates, and reaffirms the findings in the AEAP report (Nierzwicki-Bauer et al. 2008), that the epilimnion and hypolimnion of Adirondack lakes are significantly different. While much work remains in order to determine the exact mechanisms associated with the individual parameters and further, how they interact with each other, the results demonstrate the necessity of evaluating each layer with respect to anthropogenic induced changes. Further, in order to understand the impacts of such changes, each lake layer must be considered its own system, each regulating the other, through the initial establishment of the thermocline and ultimate disruption.

When the abiotic differences are viewed within the context of changing the duration of stratification, the potential for significant changes in the ecosystem exists. These include changing chemical cycling within the lakes and lake sediments and phenological changes within the lake and supporting watersheds. The data used for the preceding analysis, while sound, are not robust enough to make definitive conclusions. However, this is not a trivial issue as the potential exists for changes in stratification initiation and duration to result in significant changes in the regional ecosystem. It warrants further study and analysis.

3.11 References

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4 Zooplankton Investigations

4.1 Background

The goals of the AEAP with respect to the zooplankton were: 1) To understand the biological and chemical interactions that occurs with acidification, 2) To generate a baseline to discover and assess chemical and biological recovery and 3) To quantify interactive environmental factors with species abundance. The first and third of these goals were adequately addressed in Chapter 6 of the AEAP Report (Adirondack Effects Assessment Program Final Report 2007). Although an adequate baseline was created, the report concluded that little chemical or biological improvement had occurred in the study sites through 2006. The state of chemical and biological recovery in Adirondacks was thought to be still incomplete at this time. Just three years before the conclusion of the AEAP, Driscoll et al. (2003) concluded that although decreases in acidic precipitation inputs have resulted in increases in acid neutralizing capacity (ANC) sufficient to shift levels of monomeric aluminum to less toxic forms in some lakes, many Adirondack lakes continue to exhibit low pH and concentrations of toxic forms of aluminum that are still harmful to biota. It was further reported just after the conclusion of the AEAP that ANC increased significantly in only about half of the lakes in a study in the Adirondacks (Burns et al. 2006) and another study (Driscoll et al. 2007) reported that five of eight Adirondack thin till drainage lakes showed significant declines in inorganic monomeric aluminum. Yan et al. (2003) echoed essentially the same argument for biological recovery from acidification. While surface waters are responding to reductions in atmospheric sulfur deposition in Europe and North America, recovery of biota is less consistent. It was felt at that time that there was not enough chemical recovery occurring by 2006 to have a reasonable expectation of biological recovery and that further monitoring was needed. Finally, it was thought that more robust statistical approaches could better quantify biological recovery if the monitoring program were continued. Another reason to justify continuation of monitoring was the concern that the rate of chemical recovery trend noted in 2006 might actually be in decline. We expected that a few more years of monitoring would address these concerns.

NYSERDA support for the examination of zooplankton field collections carried out by the Darrin Fresh Water Institute during 2007-2008 and the continuation of the AEAP monitoring and sample collection protocol for a subset of 16 lakes for 2010-2012 allowed a continuation of analyses begun on these lakes in 1994. These will subsequently be referred as the NYSERDA sites. This report will assess Goal #2 as it was attained by the AEAP sites by 2006 and by the NYSERDA sites by 2012.

Several metrics were analyzed to assess biotic recovery. Simple correlation analysis of community variables, species richness, Shannon-Weaver Diversity Index and community evenness with pH was applied to the AEAP sites individually for data generated during 1994-2006. These analyses were repeated for the NYSERDA sites. Species richness is here defined as the total number of species found at each site per year. Regression analysis of pH change over time was used to assess the rate of chemical recovery per site.

Since there was considerable variation in pH from year to year, the initial pH value for each site was taken as the average of all the values for the first three years (1994-1996) and the final pH values as the average of the last two years (2005-2006). Similarly, the pH values for the conclusion of the NYSERDA study were the average of the last two years (2011-2012). The community variables were computed by PC-ORD, version 4 and computed for the AEAP sites for 13 years and subsequently including the NYSERDA sites for 19 years. Community variables were averaged per annum.

In a recent overview of biological recovery from acidification, it was recommended that conclusions about biological recovery could be made more robust through the inclusion of multivariate approaches (Gray et.al, 2009). They stated that univariate approaches can identify recovery in progress, but have no benchmarks to indicate final recovery. However, multivariate approaches like correspondence analysis (Keller et al. 2002) and canonical correspondence analysis (Schautau et al. 2001) can compare zooplankton community composition of recovering lakes with non-acidified reference lakes to access the likelihood of final recovery occurring in those lakes. We applied canonical correspondence analysis to track recovery on the basis of the position of recovering lakes relative to reference lakes. We also used CCA to identify the species associated with the non-acidified sites for both crustaceans and rotifers. Because rare species complicate the application of CCA, they were excluded. The species included in the canonical correspondence are listed on Table 4-1.

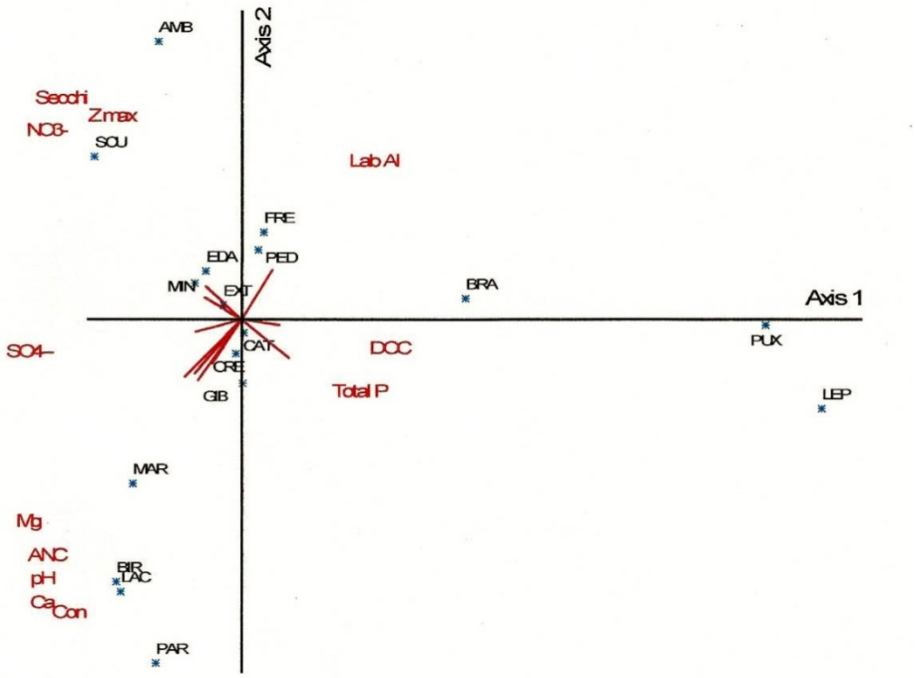
Table 4-1. The crustacean and rotifer species included in canonical correspondence analysis.

ABRV	Crustacean Species	ABRV	Rotifer Species
SCU	Cyclops scutifer Sars 1863	BOS	Kellicottia bostonensis Rousselet 1892
EXT	Tropocyclops extensus Kiefer 1931	LON	Kellicottia longispina Kelicott 1879
EDX	Mesocyclops edax Forbes 1891	COC	Keratella cochlearis Gosse 1851
MIN	Leptodiaptomus minutus Herrick 1893	HIE	Keratella hiemalis Carlin 1943
LEP	Aglaodiaptomus leptopus Lilljeborg 1889	TAU	Keratella taurocephala Meyers 1938
CAT	Daphnia catawba Coker 1926	CRA	Keratella crassa Ahlstrom 1943
AMB	Daphnia ambigua Scourfield 1947	CYC	Trichocerca cyclindrica Imhoff 1981
PUX	Daphnia pulex Leydig 1860	MUL	Trichocerca multicrinis Kelicott 1897
PAR	Daphnia parvula Fordyce 1901	ROU	Trichocerca rousseleti Voight 1901
FRE	Bosmina freyi De Melo 1994	ECU	Ascomorpha ecaudas Perty 1850
MAR	Eubosmina maratima De Melo 1994	STY	Gastropus stylifer Imhoff 1891
BRA	Diaphanosoma branchyurum Lieven 1848	PRI	Asplanchna priodonta Gosse 1850
BIR	Diaphanosoma birgei Korinek 1981	TRU	Ploesoma truncatum Levander 1894
GIB	Holopedium gibberum Zaddach 1855	MAJ	Polyarthra major Burckhardt 1900
PED	Polyphemus pediculus Linne 1761	VUL	Polyarthra vulgaris Carlin 1943
		PEC	Synchaeta pectinata Ehrbg. 1892
		DOS	Conochiloides dossaurius Hudson 1885
		UNI	Conochilus unicornis Rousselet 1892
		MUT	Collotheca mutabilis Hudson 1885

Four acid sensitive crustacean species, *Eubosmina maratima*, *Epischura lacustris, Daphnia parvula and Diaphanosoma bergei,* were identified in the lower left quarter of Axis I, which relates to the influence of recovery variables pH, ANC, Ca^{2+} and Mg^{2+} (Figure 4-1).

Figure 4-1. Crustacean species distribution in chemical space on CCA plots averaged for 1994-1996

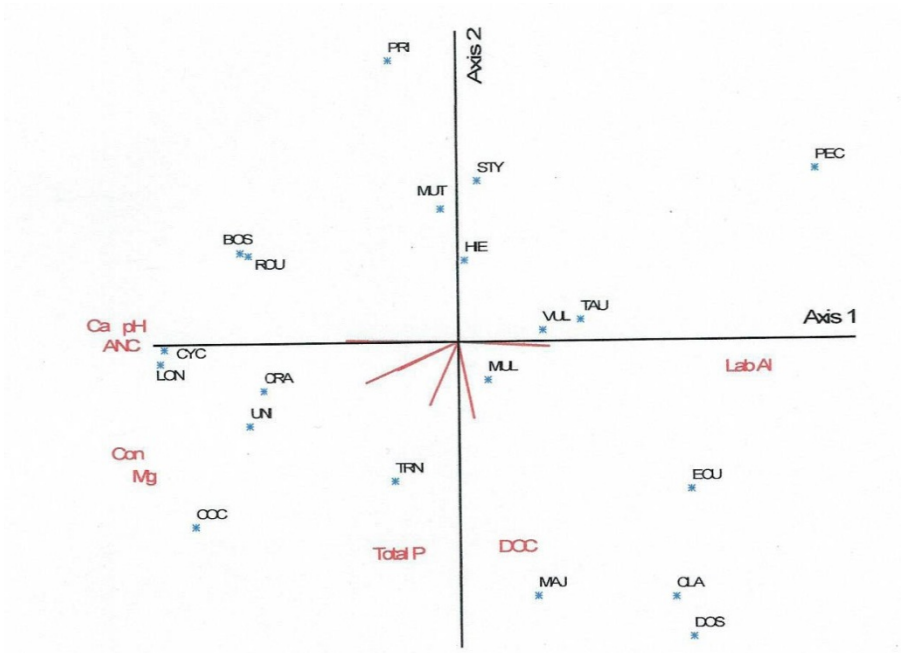
The species are indicated in Table 4-1.



Acid sensitive crustacean species were identified from the lower left-hand quadrant of Figure 4-1 that is consistent with increasing vectors of pH, ANC, and conductivity. Additionally, the zooplankton community composition of the non-acidified sites was examined to identify those that had the most diverse communities and considered these to be reference lakes. Some of the reference lakes also contained an abundance of species (*Holopedium gibberum, Daphnia catawba*, *Daphnia ambigua* and *Daphnia pulex*) also common in some acidic sites. Consequently these species were added to those identified by CCA to form the "crustacean reference community." Similarly, several acid sensitive rotifer species were identified from the central and lower left sections of the CCA plot, which was associated with the same acidification recovery variables as with the crustaceans (Figure 4-2). These species were *Kellicottia longirostris, Kellicottia bostonensis, Keratella cochlearis, Keratella crassa, Conochilus unicornis, Trichocerca rousseleti* and *Trichocerca cyclindrica.* Examination of reference lakes, as previously defined, indicated no abundant rotifers that were also abundant in some acidified sites.

Figure 4-2. Rotifer species distribution in chemical space on CCA plots averaged for 1994-1996

The species are indicated on Table 4-1.



An additional community variable (the percentage of reference species found) was created and added it to the community variables to be analyzed for each site in the AEAP and NYSERDA groups. The CCA plots were used to differentiate between sites that are in the process of recovering from those that have probably recovered and the amount of recovery that occurred in the AEAP and the NYSERDA sites respectively. We also applied regression analysis to track the rate of change of pH in both the AEAP the NYSERDA sites and to allow an estimation of the amount of time that may be required for final recovery of the Adirondack lakes.

4.2 Results and Discussion

4.2.1 Crustaceans

The correlation of community variables with pH in the 30 AEAP sites indicates that the recovery response in crustaceans in the AEAP sites is both variable and incomplete (Table 4-2).

Table 4-2. Significant (P<0.12) correlations crustacean community variables with pH on ANOVA tests for AEAP sites for 13 years and NYSERDA sites for 19 years.

Sites	% Reference		Species		Species		Community	
AEAP	p	r	p	r	p	r	p	r
Queer			0.05	0.37	0.02	0.42	0.12	0.29
South	0.02	0.43			0.00	0.65	0.00	0.68
G			0.11	0.31				
Dart			0.01	0.45	0.09	0.32		
Big Moose	0.10	0.31					0.08	0.33
Grass			0.05	0.37	0.07	0.34		
Jockeybush	0.07	0.34			0.08	0.34	0.08	0.33
Limekiln			0.08	0.33	0.11	0.30		
NYSERDA								
Limekiln			0.02	0.36	0.01	0.43	0.02	0.38
G			0.03	0.36	0.05	0.33		
Big Moose			0.08	0.28	0.02	0.38	0.04	0.33
South	0.00	0.46	0.05	0.31	0.00	0.69	0.00	0.64
Jockeybush	0.01	0.41			0.01	0.44	0.01	0.42
Dart			0.01	0.43	0.07	0.29		

Based upon a preliminary examination of the data, alpha was set at 0.12 in order to detect early zooplankton community changes. Seven sites had significant correlations within the community metrics. The most common were for species richness and diversity, often with evenness and only a few with increases in reference species composition. The correlation coefficients associated with these ranged from 0.25 to 0.68. There were fewer improvements in the percentage reference species that were mostly relatively weak. It was thought that changes in reference community composition were less likely during early stages of recovery than during later stages. Only South site had correlations that indicated improvements in all four community variables simultaneously, although the correlations were relatively weak (r=0.28 to r=0.42). Six of the seven recovering sites that were included in the NYSERDA subset continued to show correlations of similar magnitude in with species richness, diversity and evenness and in two of percentage reference species, indicating a continuation of recovery in these sites by 2012. No other sites showed significant improvement during this period.

Canonical correspondence analysis showed the extent to which the crustacean community began to resemble the communities of the non-acidified sites in the region. In all of the CCA ordination plots, as biological recovery occurs, lake position will progress in the direction indicated by the pH and ANC vectors. By 2006, five sites (Limekiln, Jockeybush, Big Moose, Dart and G) showed advancement in position on the CCA plot and although two sites (G and Jockeybush) revealed marginal positions, none overlapped the positions of the non-acidic group shown in the lower left quadrant of Figure 4-3. The non-acidic lakes are also identified on Table 4-5. The site abbreviations utilized for the CCA plot are indicated in Table 4.3.

Figure 4-3. The AEAP sites positions on CCA plots with respect to crustacean community composition distributed in chemical space averaged for initial study period 1994-1996 compared with positions obtained at the conclusion of the study averaged for 2005-2006

The initial position is represented by the site abbreviation (ABRV) and the final position with the site abbreviation (ABRV-2).

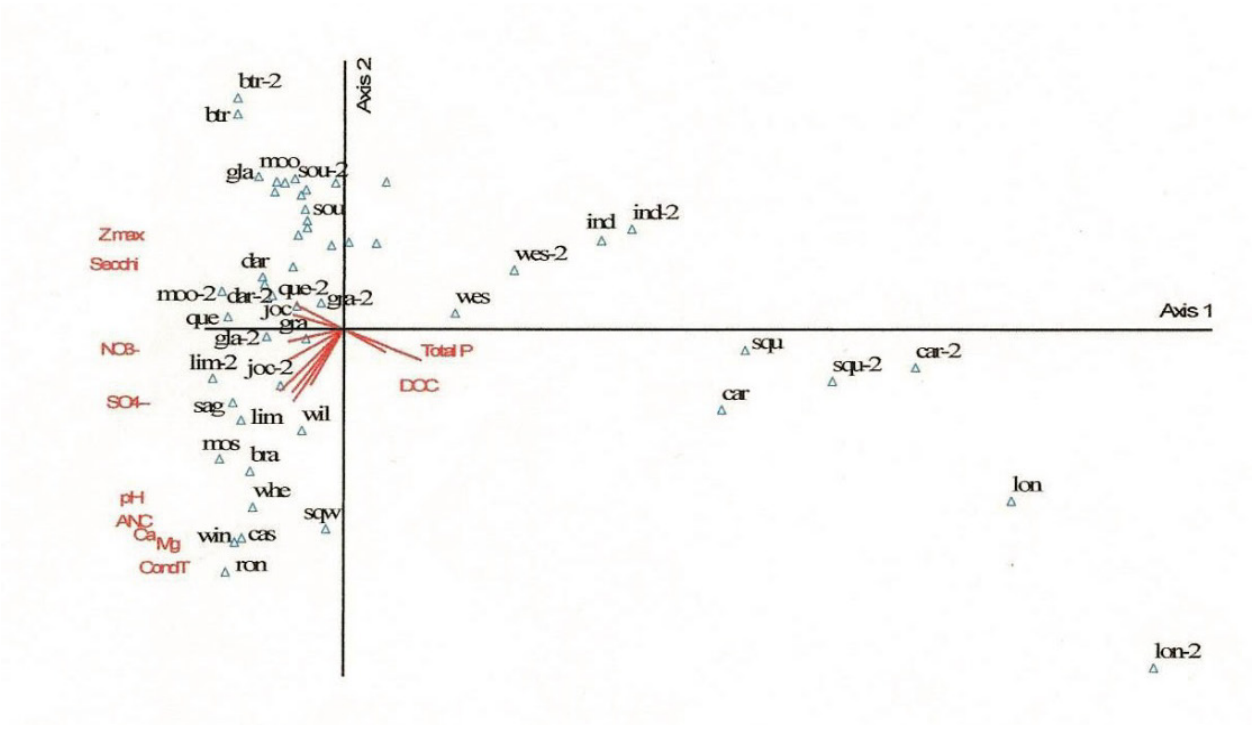


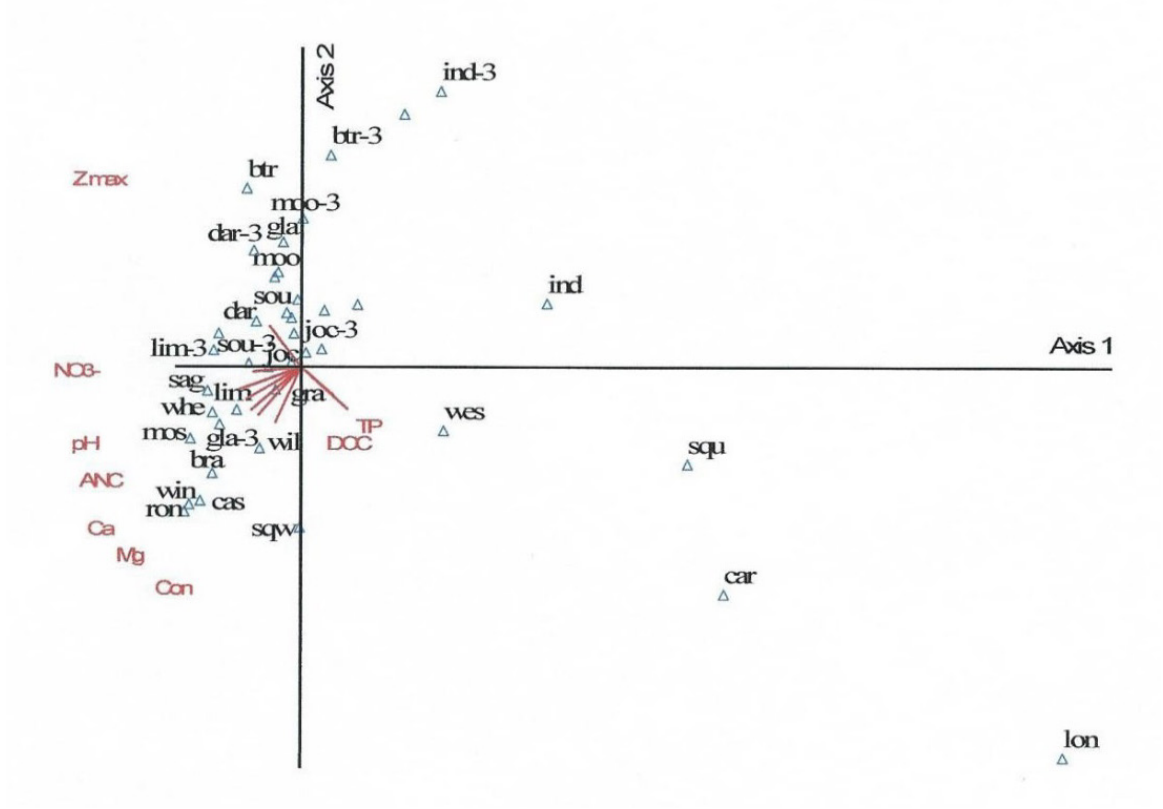
Table 4-3. Lake abbreviations utilized in the CCA Plots

Lake Name	Abbreviation	Lake Name	Abbreviation	Lake Name	Abbreviation
Big Moose	moo	Limekiln	lim	Round	rou
Brooktrout	btr	Long	lon	Sagamore	sag
Carry	car	Loon Hollow	loo	South	sou
Cascade	cas	Middle Branch	bra	Squash	squ
Constable	con	Middle Settlement	set	Squaw	sqw
Dart	dar	Moss	mos	West	wes
G	gla	North	nor	Wheeler	whe
Grass	gra	Queer	que	Willis	wil
Indian	ind	Raquette	raq	Willy's	wls
Jockeybush	joc	Rondaxe	ron	Windfall	win

By 2012, only two of the five sites (South and G) showed position advancement on CCA plots in which the crustacean community in G was similar and that of South, marginal in that the non-acidified sites (Figure 4-4). This indicates that in the period following the conclusion of the AEAP study, the zooplankton community of G had probably recovered and that of South was about to recover.

Figure 4-4. The AEAP site positions on CCA plots with respect to crustacean community composition distributed in chemical space averaged for initial study period 1994-1996 compared with positions obtained with the NYSERDA subset sites at the conclusion of the study averaged for 2011-2012

The initial position is represented by the site abbreviation (ABRV) and the final position with the site abbreviation (ABRV-3).



Three sites (Big Moose, Dart and Jockeybush) that showed improvement in 2006 (Figure 4-3) appear to occupy a retrograde position in 2012. This backslide is probably more apparent than real. Big Moose and Jockeybush had populations of *D. pulex* and Dart, *D. ambigua*, both of which were considered to be species in common distribution in some of the acidic sites in the CCA plots of the most acidic phase of the AEAP study (Figure 4-1). Even though

D. pulex and *D. ambigua* can and do occur in non-acidified sites, they are more common in some of the acidic sites. Therefore the presence of these species in recovering sites tends to bias their position toward that of the more acidic sites. This apparent retreat should be considered an artifact of this means of representation and not a return to acidic community composition.

4.3 Rotifers

The same community metrics were used for the rotifers as with the crustaceans plus an additional one, the percentage of *Keretella tauracephala* (%kt) composing the community. *Keratella taurocephala* is often the overwhelming community dominant in chronically acidified lakes and consequently a significant decline in its abundance can be considered a recovery response. The correlations of community variables with pH indicate that the recovery response in rotifers in the AEAP sites was less variable and more complete than for crustaceans (Table 4-4).

Table 4-4.Significant (P<0.12) correlations rotifer community variables with pH on ANOVA tests for AEAP sites for 13 years and NYSERDA sites for 19 years

Sites	% Reference Species		Species Richness		Species Diversity		Community Evenness		% *Keratella taurocephala*	
AEAP	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**	**p**	**r**
Limekiln	0.00	0.69	0.00	0.68	0.07	0.35			0.12	-0.29
Big Moose	0.04	0.39			0.11	0.30			0.02	-0.45
South	0.05	0.36	0.01	0.49						
Brooktrout			0.00	0.62	0.00	0.54	0.06	0.36	0.03	-0.40
Round			0.00	0.73	0.00	0.59	0.17	0.26	0.03	-0.12
Dart	0.06	0.33							0.03	-0.41
G			0.11	0.31	0.11	0.31				
Queer			0.01	0.46						
NYSERDA										
Limekiln	0.00	0.68	0.00	0.70	0.05	0.32			0.07	-0.30
G			0.03	0.36	0.05	0.32				
Dart	0.00	0.48	0.07	0.30	0.07	0.30			0.02	-0.38
Big Moose	0.00	0.48	0.00	0.52	0.00	0.53	0.00	0.44	0.00	-0.63
South	0.01	0.41	0.00	0.61	0.06	0.31				
Jockeybush			0.03	0.35					0.02	-0.39
Indian	0.02	0.38	0.07	0.29					0.01	-0.40
Brooktrout			0.00	0.58	0.02	0.37			0.04	-0.34
Round			0.00	0.64	0.00	0.57			0.04	-0.33

There were eight sites with significant correlations ($p<0.12$) with the correlation coefficients ranging from 0.264 to 0.735. As with crustaceans, the most frequent correlations were with species richness and diversity, often with evenness and only a few with increases in percentage reference species composition. The % Kt declined significantly in most of the recovering sites. Excepting Jockeybush, the same sites with significant correlations in crustaceans were also significant for rotifers. Two additional sites (Round and Brooktrout) were significant only for rotifers. As in the crustaceans, none of the sites had correlations signifying improvements in all four community variables simultaneously. However, unlike those of the crustaceans, the correlations in the rotifers were relatively strong. Excepting G, in which responses were weak, the r values ranged between 0.30 and 0.69. All six of these sites that were included in the NYSERDA subset continued to show correlations of similar magnitude in which most showed increases in species richness, diversity and evenness, and about half had strong improvements in percentage reference species. Taken together, these correlations indicate a strong continuation of recovery by 2012. One additional site (Indian) showed significant correlations, indicating early recovery progress during the NYSERDA study period.

Canonical correspondence analysis showed the extent to which the rotifer community began to resemble the communities of the non-acidified sites in the region. By 2006, five sites (Limekiln, Big Moose, Dart, Queer and G) showed advancement in position on the CCA plot. The positions of two sites (Queer and Dart) revealed a position similar to the non-acidified group of sites in the middle and upper left quadrant of the plot, indicating nearly or complete recovery (Figure 4-5).

By 2012, the sites showing advancement on the CCA plots in 2006 were joined by five additional sites (South, Jockeybush, Round, Brooktrout and Indian) (Figure 4-6).

Figure 4-5. The AEAP sites positions on CCA plots with respect to rotifer community composition distributed in chemical space averaged for initial study period 1994-1996 compared with positions obtained at the conclusion of the study averaged for 2005-2006

The initial position is represented by the site abbreviation (ABRV) and the final position with the site abbreviation (ABRV-2).

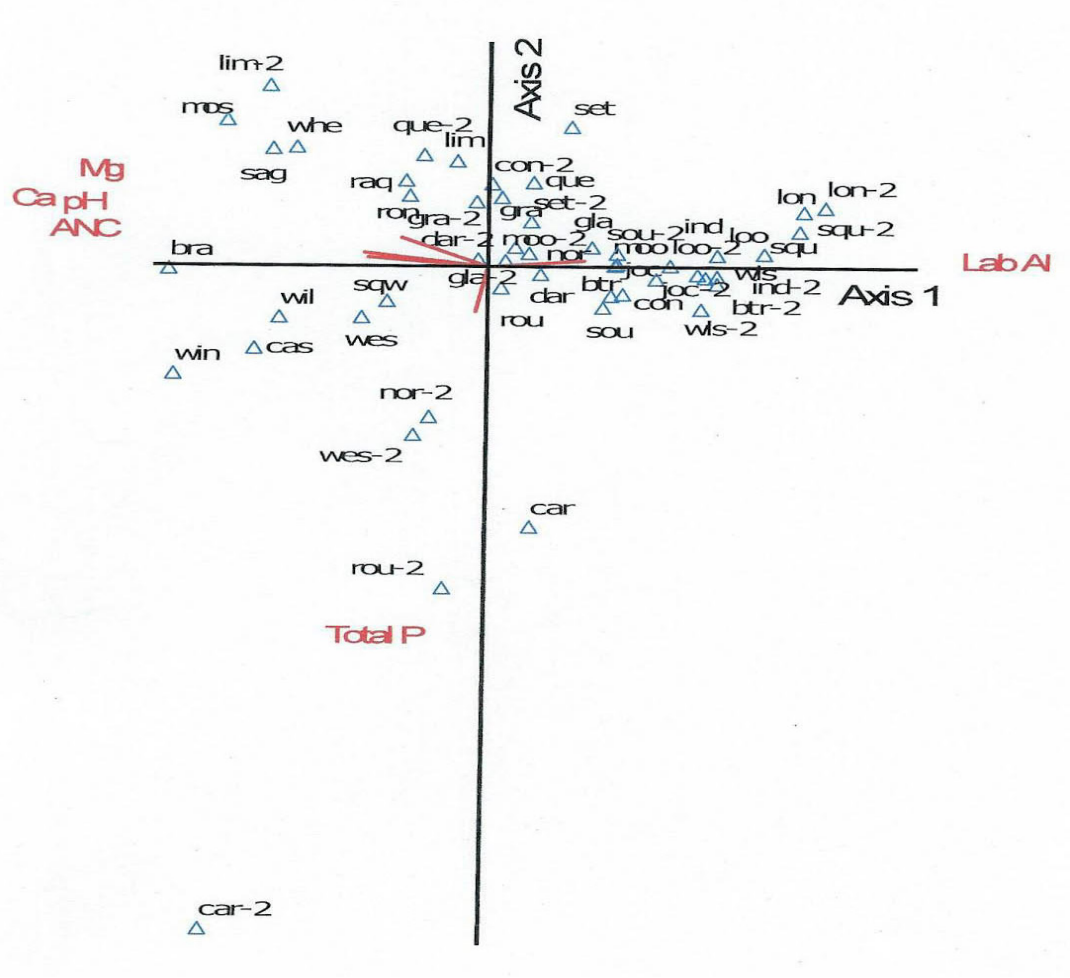
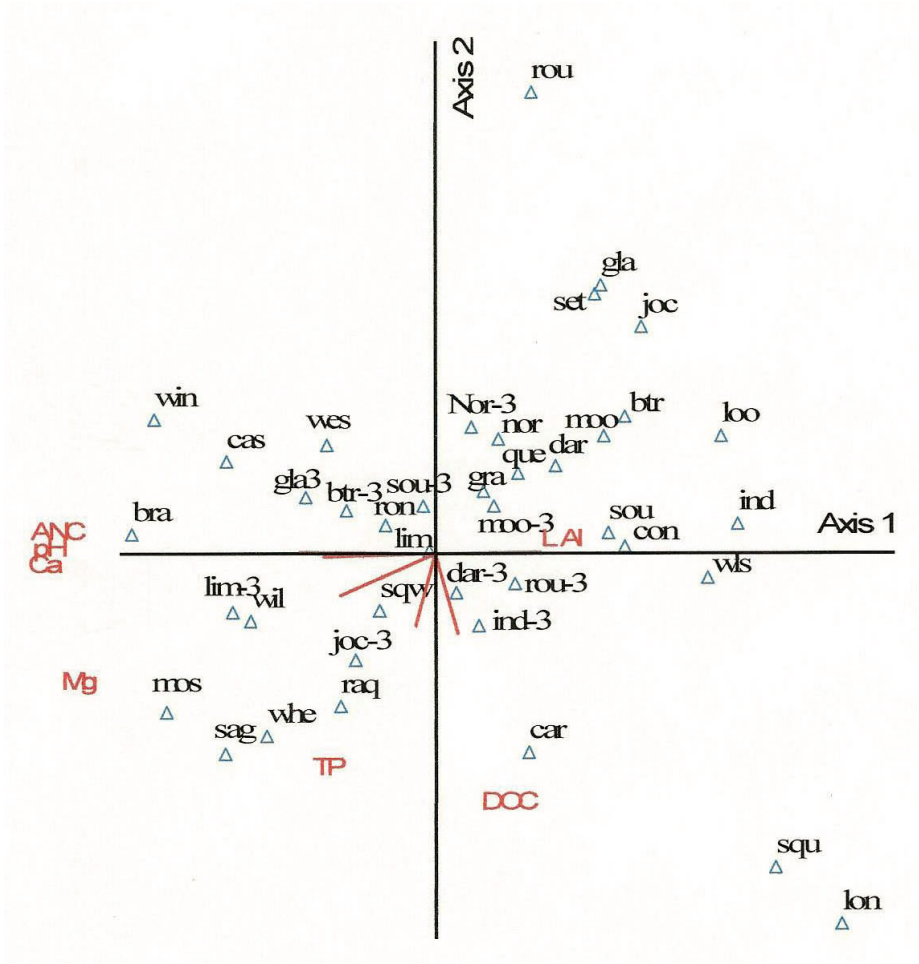


Figure 4-6. The AEAP sites positions on CCA plots with respect to rotifer community composition distributed in chemical space averaged for initial study period 1994-1996 compared with positions obtained with the NYSERDA subset sites at the conclusion of the study, averaged for 2011-2012

The initial position is represented by the site abbreviation (ABRV) and the final position with the site abbreviation (ABRV-3).



Four additional sites (South, Jockeybush, G and Brooktrout) joined Queer as having a rotifer community composition similar to the non-acidified sites. This clearly shows that several sites which displayed progress toward recovery, showed a more complete recovery by 2012. Comparison of the plots of the NYSERDA sites for crustaceans (Figure 4-4) with that of rotifers (Figure 4-6) indicates that community recovery was more advanced in rotifers than in crustaceans for the same period. Unlike with the crustaceans none of the sites appeared to occupy a retrograde position on the CCA plots. The strength of the p values of the regressions of the community variables was not predictive of the positions of the sites on any of the CCA plots, since many of the sites with the weakest p values

had the most change on the CCA plots. However, the regressions with $p<0.12$ were predictive of zooplankton community improvement since only one site (rotifers in Grass, 2006) without these correlations showed much position improvement.

There has been independent corroboration for the recovery in some Adirondacks evidenced here. Evidence for crustacean recovery in Big Moose is scant through 2006. This was corroborated by Arseneau et al. (2011), who found little or no increase in post abdominal claws of daphnids in paleolimnological samples taken during this period. However, the statistically significant response in community variables to pH change in Big Moose and the existence of substantial populations of daphnids in 2011-12 samples indicate that a crustacean (daphnia) recovery is presently in progress in Big Moose. Evidence for modest biological recovery underway in South and Queer comes from paleolimnological investigations of cores taken in 2009 and 2010, respectively (Arseneau 2013). The two lakes show a significant shift in chrysophyte species composition as indicated by multivariate assessment of differences in species composition. This shift is consistent with pH improvement in South and Queer and the improvements in community metrics of crustaceans and rotifers noted here.

4.3.1 Pace of Zooplankton Recovery in Adirondack Sites

The slopes of a regression of pH over time and the initial and final pH levels of the AEAP and NYSERDA sites divided into three groups based on pH levels existing at the beginning of the AEAP study are provided in Table 4-5.

The non-acidic group consists of 12 sites of nearly pH 6 and greater, most of which are medium till drainage lakes and one medium till seepage lake. These lakes tend to be resistant to acidification because of the thickness of the overlying soil. This group had the lowest average pH change (0.27 and 0.29 units/year) during the thirteen years of the AEAP study and 19 years of the NYSERDA continuation respectively. Grass, which showed some improvement in crustaceans and Limekiln, which had considerable improvements in both crustaceans and rotifers, were the only members of this group to show significant evidence of recovery. This indicates that some lakes in the low pH 6 range may be impacted by acidification. The seven slight to moderately acidified sites ranged in pH 5.3-5.9 and had the highest average rate of pH change (0.044 and 0.038 units/year) in the two site categories respectively. All of these sites were thin tilled drainage lakes. With exception of Limekiln and Grass, all of the sites giving evidence of crustacean and most of the rotifer recovery during the period of 1994-2006 came from this pH group. All evidence of improvement in community metrics in crustaceans was limited to sites that attained pH 5.8 by 2006 and further improvement occurred as pH continued to rise well above pH 6.0 by 2012. Eleven lakes belong to the acutely acidic group, in which pH ranged from 4.5-5.2. Two were medium till seepage sites and the rest were thin tilled drainage sites, five of which had moderate to high DOC (> 5.0 ppm) levels contributing to the acidity. This group had the second highest average rate of pH change (.035 & .031) in the two site categories respectively. Although there was no evidence of recovery with the crustaceans, three of these lakes showed evidence of rotifer recovery. Improvements in rotifer community metrics were first detected in sites, which had attained pH 5.5 (Brooktrout and Round, 2006) and Indian (2012).

Table 4-5. The pH trend regression values for AEAP and NYSERDA sites grouped into pH categories based on average pH for 1994-1996

The initial and final pH and the hydro-type classification of each site are included.

				AEAP SITES			NYSERDA SITES			
									TIME TO:	
GROUP	NAME	Abrv	HYDRO-TYPE	SLOPE ΔpH/YR	INIT. pH	FIN. pH	FIN. pH	SLOPE ΔpH/YR	pH 5.5	pH 5.8
NON-ACIDIC SITES	Windfall	win	c	0.02	6.90	7.18				
	Moss	mos	mdl	0.03	6.66	6.97	7.10	0.02	***	***
	Willis	wil	mdl	0.02	6.58	6.79			***	***
	Cascade	cas	mdl	0.03	6.47	6.81	6.89	0.03	***	***
	Middle Branch	bra	mdl	0.03	6.41	6.70				
	Rondaxe	ron	mdl	0.05	6.37	6.79	7.01	0.03	***	***
	Wheeler	whe	msh	0.02	6.27	6.59	7.67	0.04	***	***
	Raquette	raq	mdh	0.01	6.20	6.26				
	Limekiln	li	mdl	0.04	6.13	6.56	6.69	0.03	***	***
	Sagamore	sag	mdh	NS	6.10	6.57	6.65	0.03	***	***
	Squaw	sqw	tdl	0.00	5.97	6.06	6.52	0.02	***	***
	Grass	gra	mdh	0.06	5.94	6.46			***	***
			AVG.	0.03			AVG.	0.29		
SLIGHT TO MODERATE ACIDIC SITES	G'	gla	tdl	0.03	5.68	5.95	6.46	0.03	***	***
	Queer	que	tdl	0.04	5.55	5.95				
	Darts	dar	tdl	0.05	5.50	6.10	6.43	0.05	***	***
	North	nor	tdl	NS	5.35	5.45	6.16	0.01	***	***
	Big Moose	moo	tdl	0.04	5.33	5.80	6.07	0.04	***	***
	South	sou	tdl	0.06	5.27	5.85	6.24	0.05	***	***
	Jockeybush	joc	tdl	0.03	5.26	5.80	6.14	0.04	***	***
			AVG.	0.04			AVG.	0.04		
ACUTELY ACIDIC SITES	West	wes	tdl	0.03	5.23	5.48			0.00	10.22
	Indian	ind	tdl	-0.02	5.19	5.01	5.52	0.02	***	18.03
	Brooktrout	btr	tdl	0.04	5.17	5.55	5.86	0.03	***	6.63
	Middle Settlement	set	tdl	0.04	5.10	5.53			***	6.88
	Constable	con	tdl	0.03	4.95	5.18			12.67	24.36
	Carry	car	msl	0.04	4.89	5.48			***	8.97
	Willys	wls	tdl	0.02	4.82	4.98			26.00	41.00
	Round	wou	msl	0.07	4.71	5.48	5.59	0.05	***	4.59
	Loon Hollow	loo	tdl	0.02	4.66	4.76			37.13	52.13
	Long	lon	tdh	NS	4.61	4.63			N.A.	N.A.
	Squash	squ	tdh	NS	4.49	4.63			N.A.	N.A.
			AVG.	0.04			AVG.	0.03		

Mann-Kendall group trend analysis was applied to the NYSERDA sites for species richness, species diversity and percentage reference species for crustaceans and the same metrics plus % Kt for rotifers for each of the three pH groups to assess how recovery trends varied in each pH group. (Table 4-6).

Table 4-6. Statistics for Mann-Kendall non-parametric analysis of SR (species richness), SD (species diversity), % Ref (reference species), and % Kt (*Keratella taurocephala*) for pH groups 1 (Non-Acidic, 5.9-6.9), 2 (Slight-Moderately Acidic, 5.3-5.7), and 3 (Acutely-Acidic, 4.5-5.2).

Category	pH GRP	Metric	p-value	ΔYear	Tau
Crustaceans	1	SR	0.01	0.06	0.18
	2		0.01	0.03	0.17
	3		0.29	0.00	0.11
	1	SD	0.63	0.00	0.03
	2		0.00	0.02	0.27
	3		0.26	0.01	0.11
	1	% Ref	0.62	-0.09	-0.03
	2		0.03	0.53	0.15
	3		0.03	0.08	0.22
Rotifers	1	SR	0.00	0.17	0.31
	2		0.00	0.29	0.55
	3		0.00	0.30	0.46
	1	SD	0.28	0.01	0.07
	2		0.00	0.03	0.38
	3		0.00	0.04	0.31
	1	% Ref	0.07	0.61	0.12
	2		0.00	0.83	0.44
	3		0.00	0.10	0.40
	1	% Kt	0.18	0.27	0.08
	2		0.00	-1.67	-0.30
	3		0.01	-1.91	-0.28

In crustaceans, the p values are marginally significant for species richness in pH groups 1 and 2, species diversity in pH group 2 and percentage reference species in groups 2 and 3. The increasing trend in recovery in group 3 is probably related to the increase in acidophilic daphnids as previously noted. The low tau values indicate a significant variability in community responses in sites in both pH groups 1 and 2. In rotifers the p values are highly significant for increasing trends in species richness in all three pH groups and for species diversity, percentage reference species and %Kt in pH groups 2 and 3. Although the increasing trend in species richness wasn't visible in

the correlation results, the increasing trends in the other community variables in pH groups 2 and 3 were clearly predictable. The stronger tau values and the increasing recovery trends in pH group 3 corroborate the conclusions drawn from the regressions and ordination plots. The improvement of rotifer community metrics in three acutely acidic sites without corresponding improvement in these metrics in crustaceans and that the improvements were stronger with the rotifers than the crustaceans in the seven sites in which both improved indicates that rotifer recovery is initiated at lower pH levels than in crustaceans and is well under way before evidence of crustacean recovery is manifest. This would indicate that investigators interested in assessing early stages of biologic recovery should focus on rotifer community metrics and those interested in final zooplankton community recovery, on crustacean community metrics.

Squash and Long are bog-like and have very high humic acid levels and therefore are likely to maintain a naturally low pH value indefinitely. As a result, inclusion of these lakes in a predictive estimate of recovery would be unrealistic. Excluding these two sites and the 10 non-acidic sites not showing changes in their zooplankton community during the 19 years of study, there are 18 sites that could be expected to show signs of zooplankton recovery. By 2012, there were six sites showing recovery in progress and two sites with signs of final recovery for crustaceans and five sites with recovery in progress and five sites with evidence of final recovery for rotifers. That means that only about half of the sites that could be expected to recover have done so and that the recovery process is only about 25% completed in crustaceans and 50% completed in rotifers. It is clear that zooplankton recovery continued to occur in the NYSERDA sites after the conclusion of the AEAP and that it was more rapid and complete in the rotifers than in the crustaceans during the 19-year period of the total study. It is also clear that the additional six years of the NYSERDA adjunct to the AEAP made it possible to capture a zooplankton recovery response that was only hinted at by the AEAP in 2006.

Assuming that lakes are likely to show some evidence of recovery in rotifers if pH rises to 5.5 and in crustaceans if pH rises to 5.8, the lowest levels at which recovery was first detected in each group respectively, then the latest pH regression slope values (unit change/year) predict that in some of the most acidic sites the process of recovery could be delayed by about 10-40 years in rotifers and 10-50 years in crustaceans (Table 4-5). These estimates of course, are based on the assumption that pH improvement is linear for each site and continues to be over the time period of prediction. This may not be the case and therefore, the estimated time for zooplankton recovery generated for this analysis should be considered as worst case. Comparison of the pH regression slopes of the AEAP and the NYSERDA sites in Table 4-5 shows that most of the slopes of the AEAP and NYSERDA sites are reasonably similar, but do vary considerably in a few (North, Indian and Round). Extrapolating these results to the Adirondack Region, it is likely that zooplankton recovery in most previously acutely acidified lakes and some that were moderately acidified is still grossly incomplete and may still be many decades away. This extrapolation is in keeping with the prediction made by Driscoll et al. in 2007 that the time frame of chemical recovery will be many decades if current levels of acidification decreases are maintained.

4.4 References

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5 Phytoplankton Investigations

5.1 Background

Phytoplankton communities are an important indicator of aquatic ecosystem health (Xu et al. 2001). Their presence in a lake environment is indicative of the primary production of the system as well as the ecological performance of this trophic state. Phytoplankton diversity can be viewed as a one measure of ecological fitness (Litchman and Klausmeier 2008, Xu 1996). The goal of the phytoplankton analysis in this study is to determine how the phytoplankton community has changed over time and whether or not any relationship changes in community structure have occurred with decreased atmospheric deposition of acidifying compounds.

5.2 Methods

Phytoplankton was collected as a single integrated sample through the photic zone from each lake for each sampling period. The photic zone most frequently spanned the entire epilimnion as well as some portion of the hypolimnion. This contrasts to the methodology used for lake chemistry which collected an integrated sample for the epilimnion and a discrete sample for the hypolimnion. For the following phytoplankton analysis, unless it is specifically stated otherwise, the chemistry data are from the epilimnion. However, due to the described differences in the sampling protocol, interpretation of the data must be viewed cautiously.

While use of epilimnetic chemistry data can be justified by the fact that the majority of the phytoplankton sample came from the epilimnion, as was seen in the chlorophyll a analysis (Section 3), some primary production does occur in the hypolimnion of Adirondack lakes. Methodologies are under investigation by the authors for parsing phytoplankton populations into the epilimnion and hypolimnion; the analysis for this report did not incorporate such techniques.

Statistical analyses were completed using Shannon Index (Shannon and Weaver 1949), species richness, species biovolume and species abundance metrics.

The Shannon Index was calculated to measure phytoplankton biodiversity, the distributions for each genus in the ecosystem. Equation 5-1 describes the Shannon Index:

$$D = \sum_{i=1}^{R} p_i(\log p_i) \qquad \text{(Equation 5-1)}$$

where

- p_i is the proportion for the *i*th genus.
- R is the number of phytoplankton Genera within a lake for any given year.

Built into the Shannon Index are the evenness and richness metrics. Richness is a number of phytoplankton genus, and Equation 5-2 describes evenness and how uniformly distributed the genera are among each other:

$$E = \frac{D}{\log R} \qquad \text{(Equation 5-2)}$$

Evenness is a value between 0 and 1, where 1 represents an exactly even distribution with all genera having equal populations.

Biovolume is incorporated into the analyses as a metric for density. The average abundance per genus per year was also extracted which measured the density of all genera in one year divided by the total number of genera. This gave an indicator of average abundance change over time.

After these metrics were calculated, a one-way ANOVA (analysis of variance) was conducted to compare each metric by year, by hydrologic type, and by lake. A series of repeated measure ANOVAs was performed to determine whether the July sampling period showed significant difference from the August sampling period for these metrics. ANOVA does the same for dependent groups, and repeated measure analysis of variance test identifies overall differences between means for related groups.

Ordinary least squares (OLS) regression was completed determine change over time for each metric on each lake. The water chemistry parameters and phytoplankton are all measured variables; therefore, the values have sampling error associated with them. To account for this error, an error in variables regression (EIV) was conducted. The EIV method finds a reliability constant to amend such error which enables one dependent variable to be regressed upon another. Trend analysis (Helsel et al. 2005) was also conducted on all metrics.

As with the chemical parameters, lakes were compared between by July and August to validate the project assumption that July and August can be considered replicate data. Unlike the chemical parameter analyses, the data was not compared by layer, as the sampling methodology (previously discussed) did not allow for any parsing between epilimnion and hypolimnion.

5.3 Results and Discussion

While the raw phytoplankton database includes genus/species data by lake and year for all years and for all the lakes included in the AEAP/NYSERDA projects, this analysis addresses all the collected samples from the 16 lakes maintained through the NYSERDA project. The raw data received for analysis included the data separated into classes (i.e. greens, diatoms, etc.), genus and species. The phytoplankton community identified during this project includes 409 individual species from 126 genera contained within seven classes. Fifteen of the individual species are found in each of the 16 study lakes (Table 5-1) and 106 individual species were identified in only one lake. However, the current analysis combines the data to be analyzed as one group of total phytoplankton population data. Future analysis of these data will evaluate changes by class and functional groups.

Table 5-1. Ubiquitous algal species, by major group, that are found in each of the 16 study lakes for the NYSERDA project

Diatom	Chlorophyta	Cyanobacteria	Chrysophyta	Cryptophytes	Dinoflagellates
Aulacoseira ambigua	Dispora crucigenioides	Merismopedia tenuissima	Dinobryon sertularia	Cryptomonas ovata	Peridinium inconspicuum
Tabellaria fenestrata	Gonyostomum semen	Rhabdogloea smithii	Dinobryon bavaricum	Rhodomonas minuta	Peridinium wisconsinense
	Kirchneriella lunaris				
	Oocystis solitaria				
	Oocystis parva				

The raw phytoplankton data measured with the parameters Shannon Index, evenness, and richness are all normally distributed. Biovolume and average abundance data were log transformed, and outliers were removed. All data analysis was done with the normalized data set.

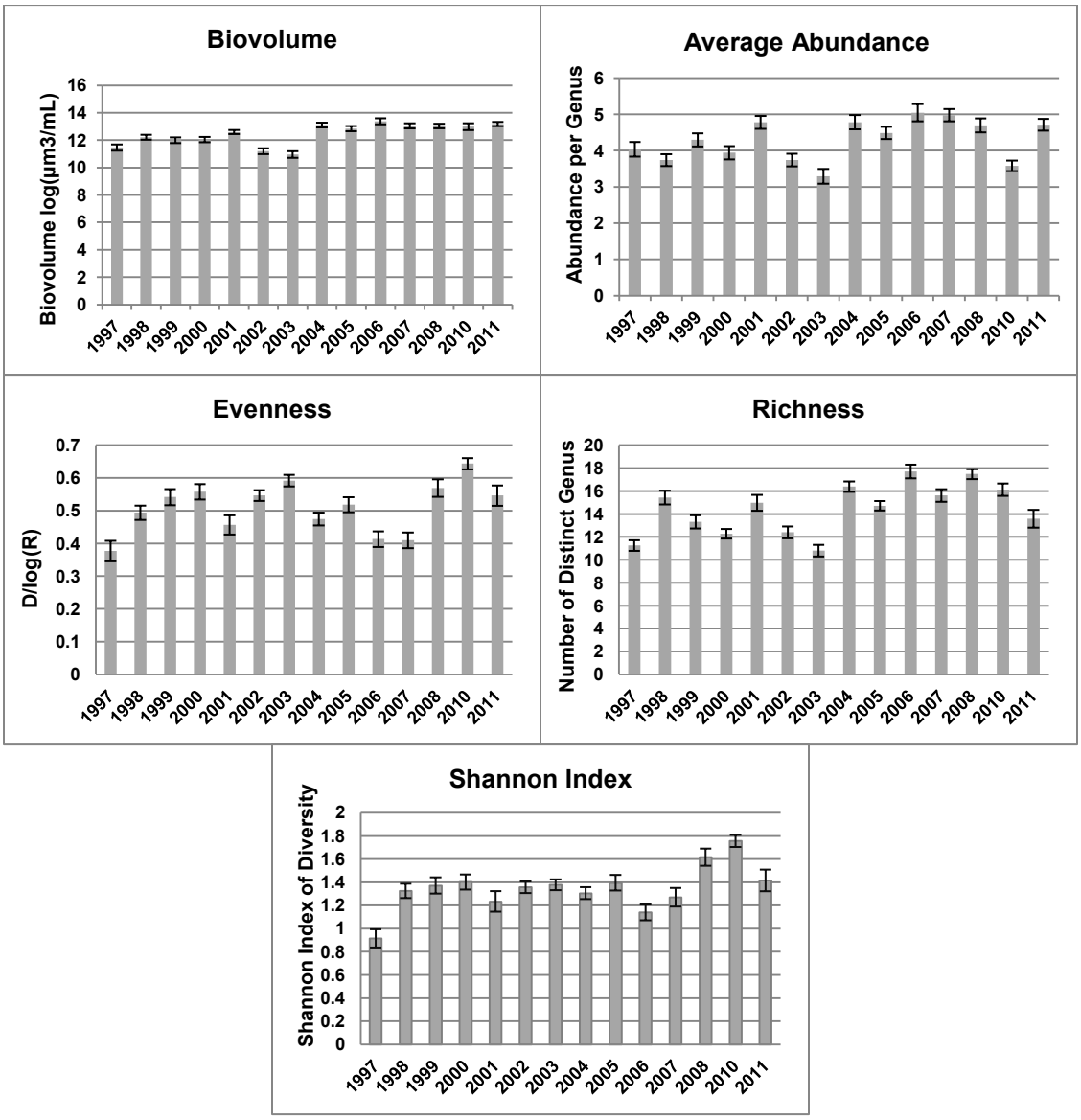
As with the chemistry and zooplankton, phytoplankton was collected in both July and August. A series of repeated measures analysis of variance tests were completed for each metric (Shannon Index, richness, evenness, biovolume, and average abundance) to determine if these samples could be considered replicate samples. A summary of the results are in Table 5-2. The analysis indicates very little difference between the months for any of the phytoplankton metrics. Therefore, unless specifically identified, all the analyses are done with data pooled between months.

Table 5-2. Percentage of lakes showing similarities between July and August

	Shannon	Evenness	Richness	Biovolume	Average
Significant difference	12.50%	12.50%	12.50%	0%	12.50%
No significant difference	87.50%	87.50%	87.50%	100%	87.50%

Data were compared across lakes and years. This analysis determined the effect that time and location had on each phytoplankton parameter. The annual mean showed a cyclic pattern (Figure 5-1).

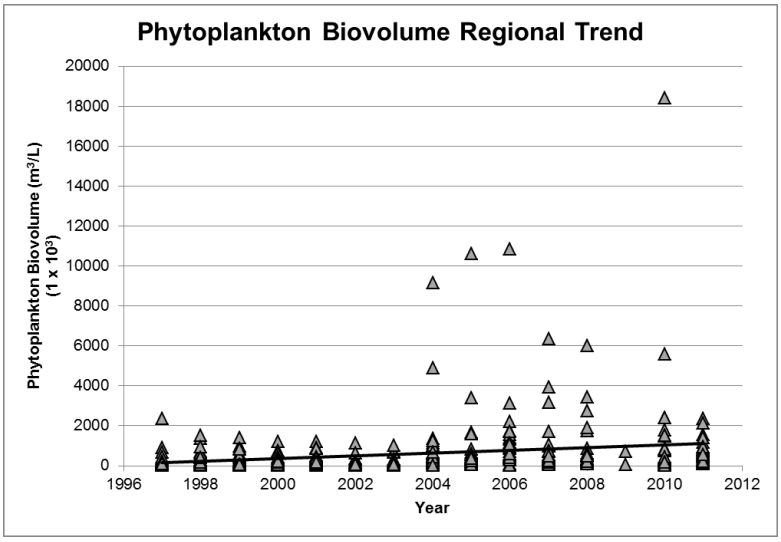
Figure 5-1. Mean values by year for each phytoplankton metric



It was anticipated that the annual patterns of the Shannon Index metrics would be similar. However, this was not the case. With respect to the Shannon Index, 1997 is significantly ($p \leq 0.05$) lower than all other years and 2010 is significantly ($p \leq 0.05$) higher from all but 2008. There is no significant difference between the remaining years. A similar pattern occurs with the Evenness metrics as 1997 significantly ($p \leq 0.05$) lower and 2010 is significantly ($p \leq 0.05$) higher than other years.

Figure 5-2. Mann-Kendall trend analysis for phytoplankton biovolume.

A scatter plot and trend line of phytoplankton biovolume data for the 16-study lakes.



As shown in Figure 5-1, the cyclic pattern of the annual mean for evenness and Shannon Index is similar. To determine actual similarities between the metrics, a regression analysis was completed for each of the 16 lakes. The regressions over time for both Shannon Index and evenness in each lake demonstrate similar patterns. In many of the scatterplots, Shannon Index and evenness increased between 2006 and 2010 followed by a sharp drop in 2011 (Figure 5-2). In most lakes, there also seemed to be a low point in 2004. Examples are seen with Dart Lake (Figure 5-3) and North Lake (Figure 5-4).

Figure 5-3. Scatterplots for phytoplankton parameters on Dart Lake

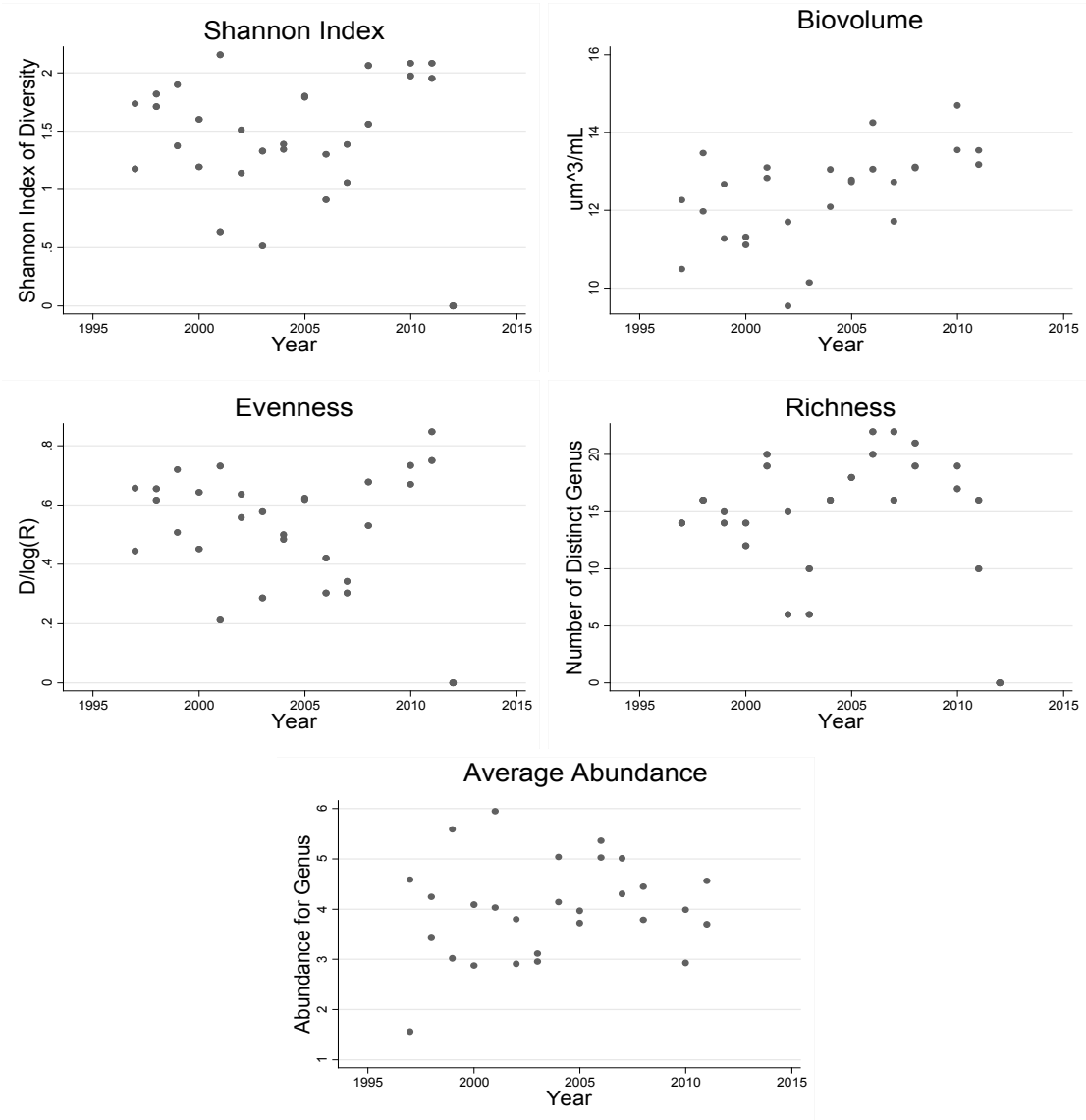
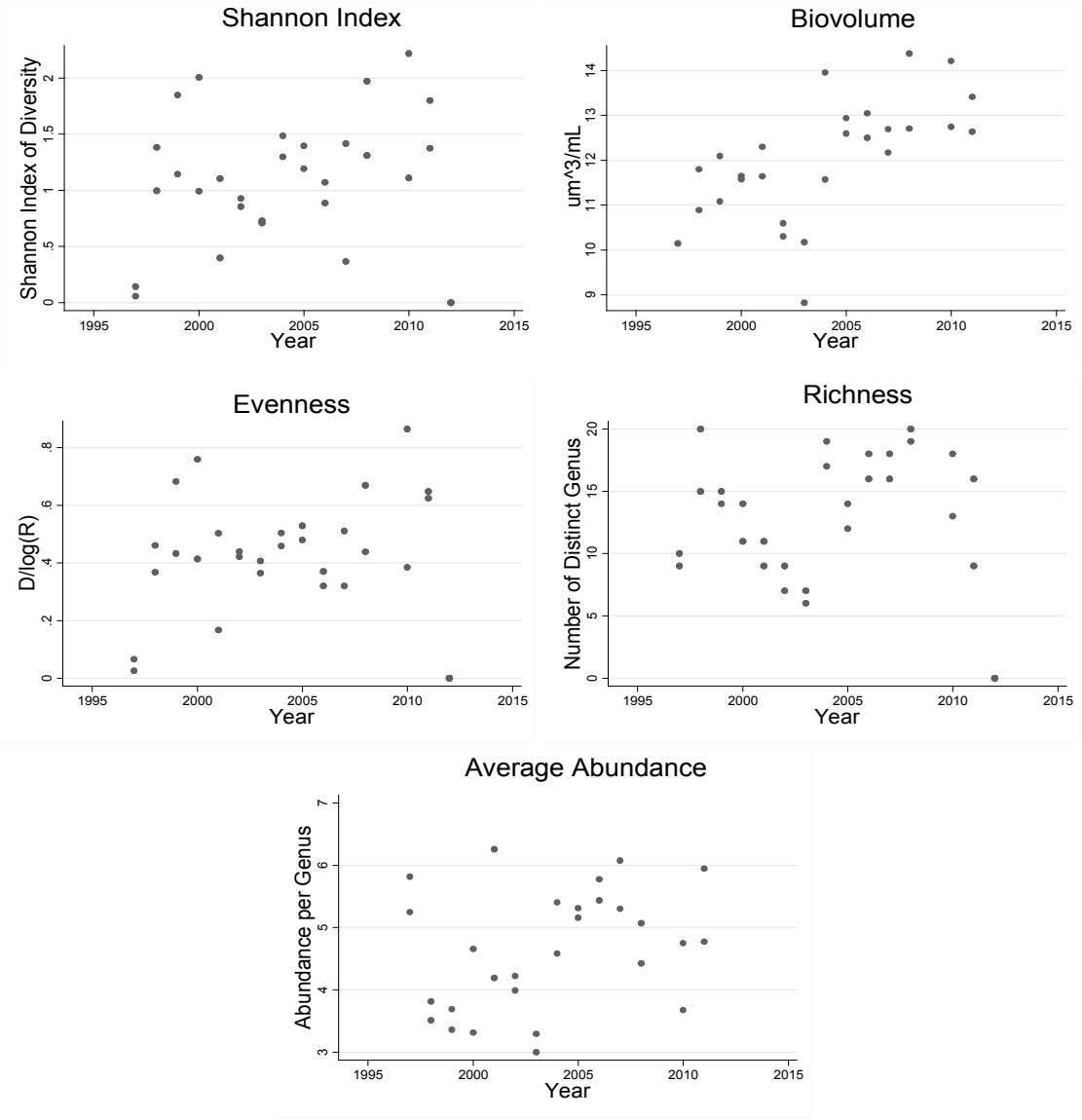


Figure 5-4. Scatterplots for phytoplankton parameters on North Lake



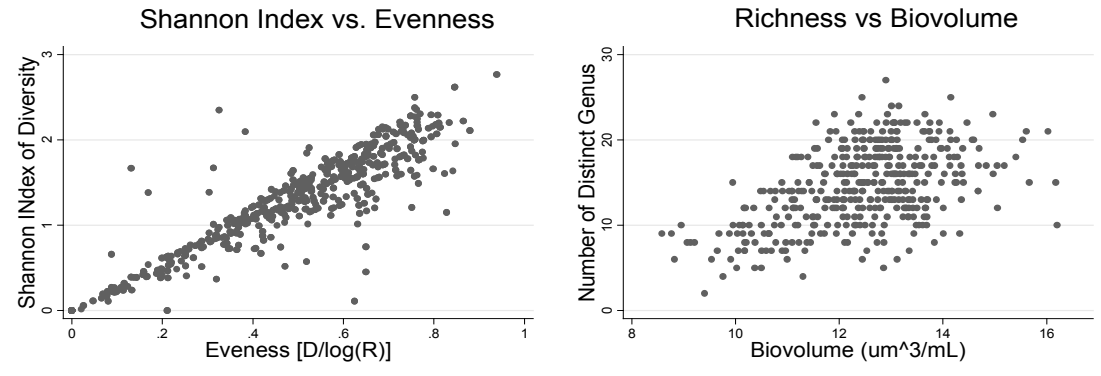
As expected, diversity in the 16 Adirondack lakes is closely related to the evenness. The link between Shannon Index and evenness shows that as the community becomes more even, the diversity index increases and when evenness decreases, the diversity also decreases (Figure 5-5). The correlation coefficient for Shannon Index and evenness is strong, r = 0.92.

However richness does not correlate as strongly with the Shannon Index as did evenness. The correlation coefficient for Shannon Index and richness is r = 0.61. Much of the strength of this correlation results from the intrinsic calculations of richness as it is embedded in the calculation for Shannon Index (see Equation 5-1). Even though the phytoplankton is becoming more diverse and more evenly distributed, this diversity is not necessarily correlated directly to the number of distinct genera that exist. The increase from 2006 to 2010 in Shannon Index and evenness does not occur in the richness scatterplots similar to the other two primary metrics. There is no specific year that stands out among the rest, as most years show values that are significantly different ($p \leq 0.05$) from other years.

Average abundance has a negative correlation with both Shannon Index and evenness, r = -0.42 and r = -0.56, respectively. As the population per genus grows or shrinks, the proportions of the genus become uneven. This means that some genus change at a greater rate than others.

There is an increase in richness from 2003 to 2004 that is not shown in evenness or Shannon Index, but is displayed in biovolume and average abundance. This shift is important to the discussion of change in community structure of lakes in the Adirondacks. Biovolume has a stronger positive correlation to richness (r =0.50) and average abundance (r = 0.53) than the two other phytoplankton metrics.

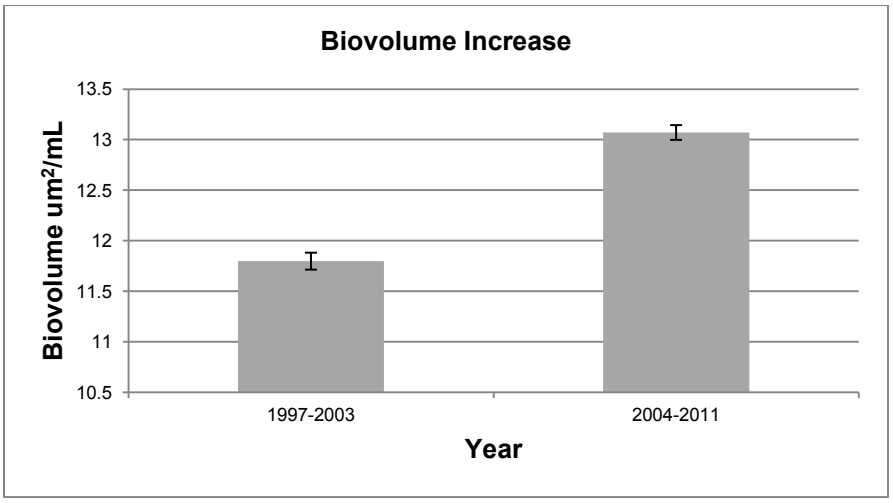
Figure 5-5. Scatterplots to display relationships between phytoplankton metrics



These results lead to a conclusion that for the Adirondack lakes analyzed, evenness plays a more dominant role in overall diversity than richness does and that richness is the key driver for biovolume.

With biovolume, the mean abruptly shifts to a higher range in 2004. A Student's t-test between the biovolume mean pre- and post-2004 (inclusive) was conducted. This analysis demonstrates a significant difference ($p \leq 0.05$) between these values (Figure 5-6).

Figure 5-6. Significant increase for Biovolume before 2004 and 2004 and after (p≤0.05).



These analyses clearly demonstrate that the phytoplankton community has changed since the initiation of this project. However, time does not seem to be a significant factor for change in the phytoplankton community. As seen in Table 5-3, regressing the diversity and biovolume metrics over time provides very little predictive capability. While a number of regression equations are significant ($p \leq 0.05$), the coefficient of determination (r^2) values are routinely low. Low values indicate that time is not the driving force for change in the Shannon Index, evenness, richness, biovolume, or average abundance metrics in phytoplankton communities.

Table 5-3. Time regressions for phytoplankton parameters over time

The green highlight shows a significant ($p \leq 0.05$) regression.

Lake name	Biovolume	Shannon	Richness	Evenness	Abundance
Big Moose	0.48	0.13	0.17	0.057	0.056
Brooktrout	0.0047	0.0052	0.060	0.0092	0.026
Cascade	0.17	0.51	0.24	0.32	0.021
Dart	0.28	0.065	0.080	0.020	0.029
G	0.037	0.053	0.10	0.12	0.20
Indian	0.0020	0.28	0.11	0.17	0.021
Jockeybush	0.056	0.019	0.0019	0.00	0.013
Limekiln	0.32	0.40	0.32	0.17	0.065
Moss	0.036	0.11	0.0014	0.17	0.037
North	0.43	0.18	0.10	0.21	0.095
Rondaxe	0.25	0.18	0.021	0.00	0.019
Round	0.40	0.65	0.24	0.18	0.027
Sagamore	0.16	0.18	0.14	0.20	0.052
South	0.30	0.026	0.33	0.013	0.14
Squaw	0.081	0.050	0.013	0.051	0.058
Wheeler	0.05	0.0033	0.030	0.0023	0.067
Significant	9	7	6	8	2
Not Significant	7	9	10	8	14

In order to better understand the cause(s) for variance within the phytoplankton community, pH, aluminum, and SO_4^{2-} were evaluated to determine their influence on biovolume and diversity. An error in variables regression method was used. The regression indicated that 80% of all lakes had no significant regression of pH on biovolume, shown in the first three columns of Table 5-4.

Table 5-4. pH regression with phytoplankton metrics

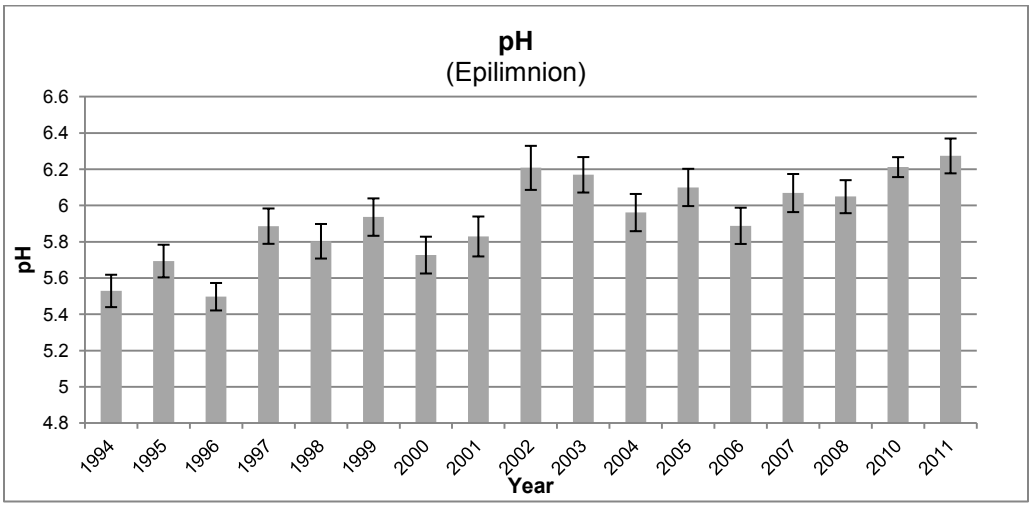
These metrics are Shannon Index, genus richness, genus evenness, organism abundance, and biovolume.

Lake name	Biovolume	Shannon	Richness	Evenness	Abundance
Big Moose	0.77	0.15	0.29	0.11	0.023
Brooktrout	0.026	0.27	N/A	0.17	0.026
Cascade	0.39	0.69	N/A	0.33	0.021
Dart	0.18	0.17	N/A	0.18	0.029
G	0.067	0.085	0.91	0.11	0.20
Indian	0.082	0.13	N/A	0.26	0.021
Jockeybush	0.048	0.11	0.67	0.12	0.013
Limekiln	0.63	0.017	0.26	0.16	0.065
Moss	0.078	0.032	N/A	0.0053	0.037
North	0.089	0.11	N/A	0.080	0.095
Rondaxe	0.087	0.45	0.67	0.10	0.019
Round	N/A	0.49	N/A	0.26	0.027
Sagamore	0.55	0.19	0.40	0.15	0.052
South	0.33	0.26	0.80	0.39	0.14
Squaw	0.50	0.050	N/A	0.0071	0.058
Wheeler	0.77	0.56	N/A	0.35	0.067
Total	4	2	2	2	2
Total Not	12	14	14	14	14

As discussed in the chemistry section, pH exhibits a repeating pattern (Figure 5-7). While phytoplankton also exhibit a repeating pattern, the regression analysis identified above, indicates that pH and phytoplankton are not dependent upon each other. Of particular interest is the decline in pH that occurs between 2002 and 2004. The mean biovolume (see previous discussion), richness and abundance values of phytoplankton also had demonstrable changes during this time period.

Figure 5-7. Change in pH over time

This chart provides the mean epilimnetic pH for all lakes from 1994 – 2011. The previously described oscillating pattern can be observed. Data are pooled for all epilimnetic pH data collected from the study lakes.

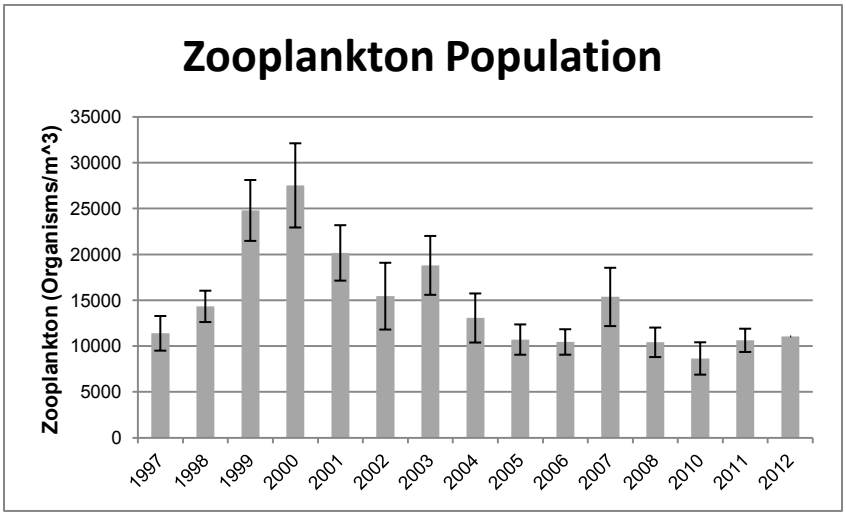


As demonstrated in the previous discussion, pH does not appear to directly affect biovolume or Shannon Index diversity which indicates some other factor is driving the phytoplankton population shift in 2003. The authors hypothesize that the phytoplankton shift is the result of biological interaction.

Zooplankton are the primary predator of phytoplankton, changes in zooplankton population would likely result in changes in the phytoplankton community (Lynch and Shapiro 1981). Zooplankton abundance (averaged across all lakes) declined during the 2000 to 2006 time period (Figure 5-8). As discussed in the zooplankton section, many zooplankton species are very acid sensitive. With the drop in pH during this time period, the lower pH may drive the zooplankton population lower. In effect, the oscillating reduction in pH suppressed the zooplankton population. Phytoplankton did not realize a similar pH effect. Based upon this observation, the authors hypothesize that as predation pressure was reduced, caused by the drop in pH, the overall density of phytoplankton increased. This increase can be seen in the stepwise increase in phytoplankton biovolume found between 2003 and 2004. As pH once again begins to increase, the zooplankton community (as measured by abundance) stabilizes. This stabilization results in a return to the oscillating phytoplankton biovolume found post 2004, but occurring at a higher density than the pre-2004 population (Figure 5-9).

Figure 5-8. Zooplankton population over time

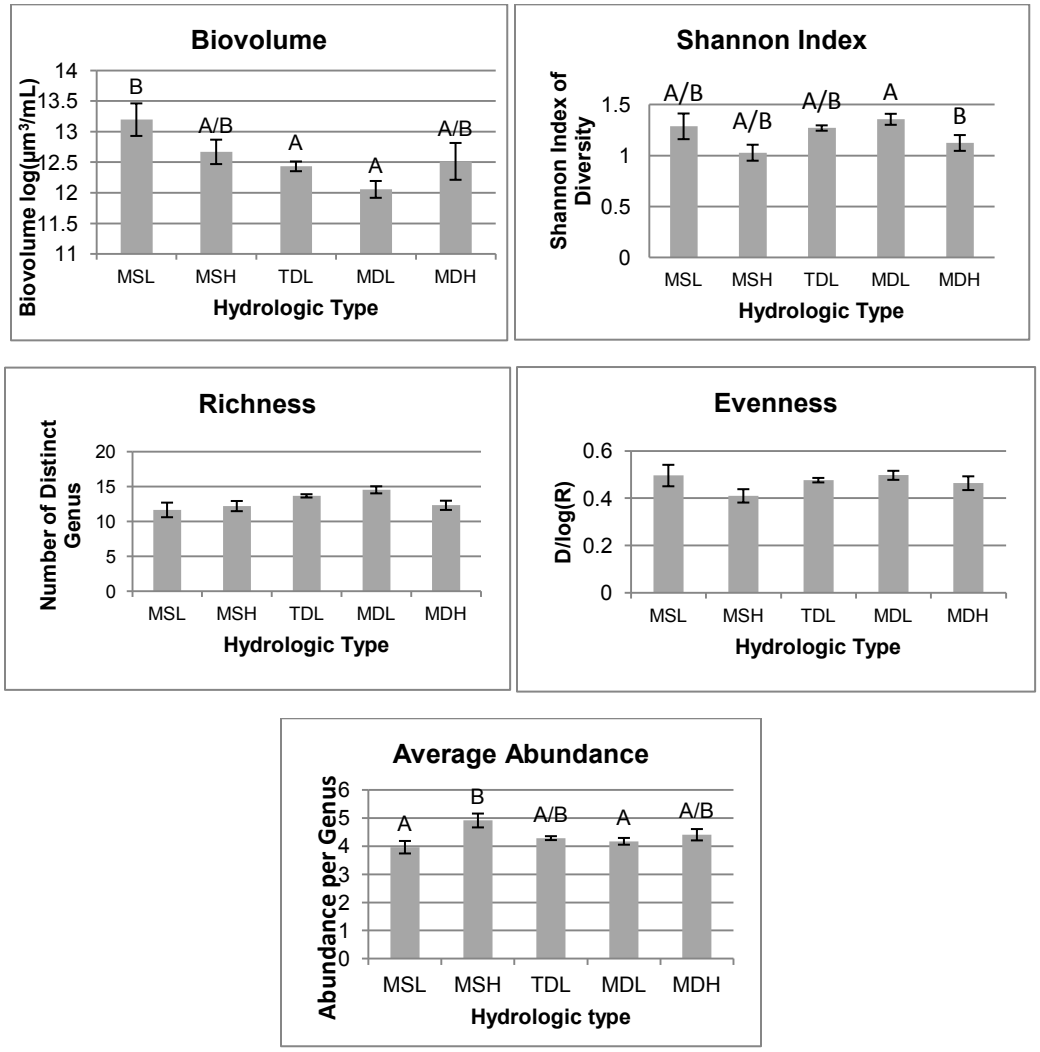
The density of zooplankton (number or organisms per cubic meter) averaged over the study sites.



When looking at the lakes by hydrologic type we see very little difference between the types (Figure 5-8). Overall, for these metrics, hydrologic type is not a clear indicator of phytoplankton community or population.

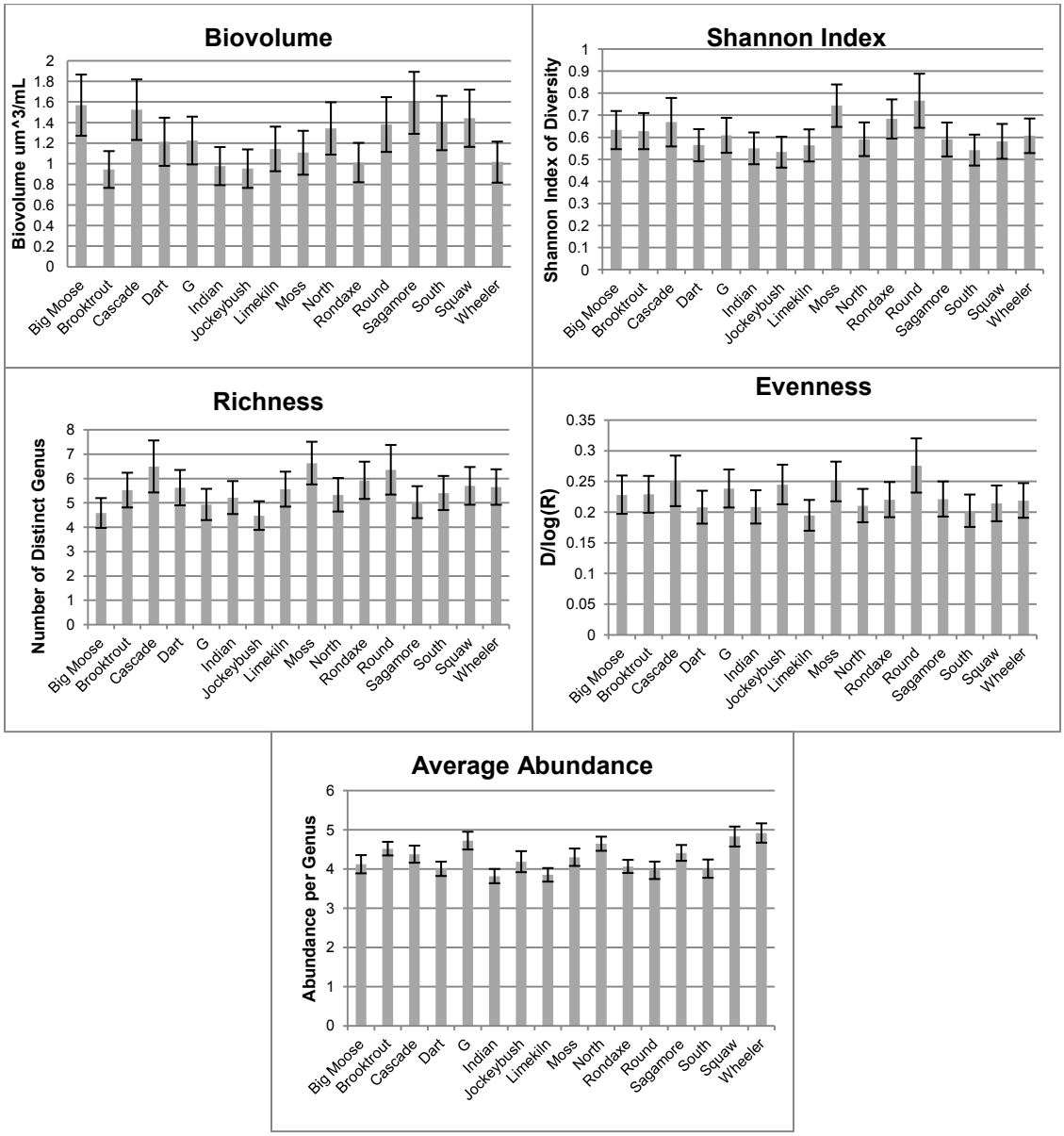
Figure 5-9. Phytoplankton metric by hydrologic type.

Different letter designation denotes significant difference (p≤0.05). There is no significant difference between hydrologic type for the Richness and Evenness metrics.



However, biovolume and richness show much variation among lakes with Jockeybush being significantly (p≤.05) lower than the other lakes (Figure 5-10). Preliminary work with phosphorus concentration shows that it may be the key factor for low biovolume in Jockeybush; however, more work is required before any final conclusions can be made.

Figure 5-10. Phytoplankton metrics by lake



All phytoplankton metrics were correlated with elevation, but there were few significant correlations. Those correlations that were significant had very low correlation coefficients, $r < 0.06$. More detailed analysis of the phytoplankton community structure will include a presence/absence and Bray-Curtis dissimilarity analysis. These analyses should provide a more refined view of changes in the community and enable development of more refined hypotheses.

5.3.1 Previously Undescribed/Unknown Species

As the AEAP/NYSERDA projects were designed to address biota, a consistent level of phytoplankton sampling occurred over the course of the projects. The consistency and intensity of sampling has resulted in additional data not originally envisioned for the project. Specifically, the taxonomic analysis has resulted in the identification of a number of unknown/unidentifiable taxa. This identification included taxa that likely have not previously been described. Table 5-5 provides a categorical breakdown of the unidentified algal taxa.

Table 5-5. Undefined phytoplankton categories

Likely Unique/Previously Undefined	Potentially Unique/Previously Undefined, further analysis required	Status Unknown, further analysis required	Likely Defined, Specimens need further analysis
Closterium	Unk Chlorophyte	Oscillatoria sp. 1	Unk Alga lobed
Cosmarium	Unk Chlor coccoid	Dinobryon sp. 1	Unk Alga oblong
Staurastrum	Unk Chlor colonial	Unk Crypto sp. 1	Unk Alga spindle
	Oocycstis		Unk Alga spherical
	Ankistrodesmus		Unk Alga elongate
	Dinobryon sp. 1		Unk Chryso sp. 1
			Unk Chryso sp. 2
			Unk Chryso sp. 3
			Unk Chryso sp. 4
			Unk Chryso loricate

At this time, the ecological significance of these organisms is unclear. Several of the species were extremely rare, occurring in only limited samples, others are more common. Currently, it is not known if these species are new to the Adirondack systems, if they have always been present or if they only are only present under specific environmental conditions (e.g., pH, DOC, Al, etc.). Further work will be required to understand the ecological implications, if any, of these organisms.

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6 Bacterioplankton Investigations

6.1 Background

Microbial communities associated with freshwater environments form the foundation of freshwater food webs and are the primary biogeochemical agents involved in nutrient cycling. During the past several decades, our appreciation of the diversity and complexity of microbial systems has dramatically increased, largely due to the development and application of new molecular genetic tools in environmental microbiology. The use of these molecular tools has focused largely on marine environments; consequently freshwater microbial populations have not been well studied (Zwart et al. 2002).

More recent examinations of bacterial communities in freshwater environments, primarily based on sequencing of small subunit ribosomal DNA, have suggested that they are distinguishable from marine communities, largely by the dominance of the β-proteobacteria and representatives of the *Actinobacteria* (Methe et al. 1998, Glöckner et al. 1999, Zwart et al. 2002, Warnecke et al. 2004, Wu and Hahn 2006a*)*. The representation of these groups appears, as predicted, to be intermediate in estuarine environments (Rappé et al. 2000).

During conceptualization of the AEAP, bacterioplankton in the Adirondack sites utilizing state-of-the-art, culture-independent, molecular-based techniques instead of classical plate culture techniques were examined. Initial investigations of bacterial communities in these sites were among the first to recognize unique freshwater bacterial assemblages and to identify what are now considered to be unique lineages of freshwater β-proteobacteria and *Actinobacteria* (Hiorns et al. 1997, Methe et al. 1998). Even less is known about freshwater *Archaea* communities, although studies (Pernthaler et al. 1998, Crump and Baross 2000, Jurgens et al. 2005, Urbach et al. 2007) have focused on them.

The importance and numerical abundance of these "typical" freshwater bacterial groups have been confirmed in studies employing Fluorescence in Situ Hybridization (FISH) approaches (Glöckner et al. 2000). Interestingly, the abundance and distribution of *Actinobacteria* and β-proteobacteria suggest that these two groups are influenced differently by lake hydrologic type, nutrient conditions, seasonality, and grazing pressures (Crump et al. 2003, Yannarell et al. 2003, Lindstrom and Bergstrom 2004). Hahn and others have succeeded in isolating representatives of some of the freshwater *Actinobacteria* and β-proteobacteria species, and ongoing investigations have begun to demonstrate the ecological significance of phylogenetically distinct groups of these major groups of freshwater bacteria (Hahn 2003, Hahn et al. 2003, Page et al. 2004, Simek et al. 2006, Wu and Hahn 2006a).

Relatively little is known about the response and, if any, recovery or community structure shifts (changes) of bacterial communities in association with acid deposition processes. Therefore, in the original AEAP study and now this NYSERDA study, the composition and diversity of bacterial communities in Adirondack sites recovering from

acidification were investigated. In this NYSERDA project, per our contract, we have focused on a subset of three of the original lakes in the AEAP program. Having completed the microbial community structure characterization from these lakes it will now be possible to examine the relationship between community structure and lake physiochemical and hydrological properties to elucidate some of the factors that influence overall microbial diversity.

6.2 Materials and Methods

6.2.1 Study Sites

A subset of three lakes with contrasting pH values, Indian (pH 5.1), Brooktrout (pH 5.6), and Moss (pH 7.2), were surveyed extensively for bacterioplankton. Although this sample size did not guarantee that microbial diversity of Adirondack lakes would be described fully, preliminary data suggested that this level of coverage would allow detection of meaningful differences between libraries correlated to physiochemical and biological properties of each lake sample.

6.2.2 Rational of Sampling and Analytical Approach

Bacterial classification of the study sites using molecular techniques does not lend itself easily to large sample analysis because of the scientific labor intensity associated with molecular extractions, cloning and gene sequencing. Often, studies are published dealing with only one or two lakes (Page et al. 2004). As such in the current NYSERDA project, detailed analyses of bacterioplankton were limited to three lakes (described below in study site selection section). Analyses of samples from these three lakes have been completed using cloning, RFLP analyses and sequencing.

6.2.3 Sample Collection

Water samples from these three lakes, which exhibit thermal stratification, was collected from the epilimnion (depth integrated) and the hypolimnion (one meter above the lake bottom) as described in Section 2. Two liters of water from each sample were transferred to sterile darkened containers, stored on ice, and transported to the lab. All samples underwent initial processing within 12 hours of collection by pre-filtration through a nylon mesh, filtration through 5-µm cellulose membrane filters to exclude larger eukaryotic plankton; the remaining plankton was collected onto 0.22-µm cellulose membrane filters and stored at -80 °C until further processing.

6.2.4 DNA Purification, PCR Amplification, Cloning and Sequencing

Total genomic DNA was purified using the soil DNA extraction kit (Q-Biogene) with a bead-beating step using filters cut into small pieces as the "soil." Purified DNA was quantified by spectrophotometry and analyzed for quality by visual assessment after agarose gel electrophoresis on 1% gels. Total genomic DNA was used for polymerase chain reaction (PCR) amplification of the 16S rDNA genes using universal forward 8F (5' aga gtt tga tcm tgg cttc ag) and reverse 1492R (5' ggt tac ctt gtt acg act t) primers (Balkwill et al. 1997). Amplification products were resolved by gel electrophoresis and subcloned into the pCR 2.1 vector using the TA Cloning® kit (Invitrogen). After cloning into the plasmid vector, the 16S rDNA insert was re-amplified with M13 cloning site targeted primers M13 Forward (-20) (5' gta aaa cga cgg cca gtg) and M13 Reverse (-27) (5' gga aac agc tat gac cat g). Amplified insert was verified visually by agarose gel electrophoresis (1% gels) and purified using the Qiagen MinElute Purification Kit following manufacturer's instruction (Qiagen). Purified PCR amplicon (typically 5-125 ng/µL) was eluted in 10 µL Buffer EB (10mM Tris.Cl, pH 8.5). Automated sequencing was performed by the MCLAB in San Francisco, CA. The identity of each clone sequence was determined by identifying its nearest neighbor using the Sequence Match and Classifier tools at the Ribosomal Database Project (http://rdp.cme.mse.edu/).

6.2.5 Clone Identification

Clones have partially been phylogenetically classified to the taxonomic level of class and subclass according to the hierarchical taxonomy proposed by Garrity et al. (2004) using the Ribosomal Data Base Project (RDP) Match and Classifier tool (http://rpd.cme.msu.edu/classifier/classifier.jsp). The taxonomic classification of Subclass is an intermediate taxonomic rank between class and order used by Garrity et al. (2004) and adopted by the RDP. Because comparison of such a wide diversity of bacteria in this study was attempted, and although the majority of sequences retrieved could be reliably classified to much lower taxonomic ranks, it was not possible to consistently assign all clone identities to less than a class designation, so the generic term subclass was used to define classifications below the rank of class but higher than order.

6.2.6 Database Organization

All clone information is maintained in an Excel spreadsheet and identified by a unique clone identification number consisting of an Adirondack (ADK) designation code, Lake Identifier, date (year) identifier, and clone number. Epilimnion and hypolimnion samples are designated by an 'e' or 'h', respectively, following the lake identifier. General lake identifier codes are shown in Table 6-1. For example, the clone identifier BTeJ11 is used for clones from Brooktrout Lake (BT), epiliminion (e), July (J), in 2011 (11). Sequences of all unique clones obtained in this study will be deposited in GenBank as required when publishing results.

Table 6-1. Summary of NYSERDA Microbial Community Analyses.

Lake	Layer	Sampling	# RFLP	# Clones
Indian Lake	Epilimnion	Jul-10	114	28
	Hypolimnion	Jul-10	106	14
	Epilimnion	Aug-10	144	80
	Hypolimnion	Aug-10	93	42
	Epilimnion	Jul-11	122	31
	Hypolimnion	Jul-11	91	25
	Epilimnion	Aug-11	96	34
	Hypolimnion	Aug-11	84	20
	Epilimnion	Jul-12	128	0
	Hypolimnion	Jul-12	128	0
	Epilimnion	Aug-12	96	21
	Hypolimnion	Aug-12	96	20
Brooktrout Lake	Epilimnion	Jul-10	62	24
	Hypolimnion	Jul-10	75	75
	Epilimnion	Aug-10	28	28
	Hypolimnion	Aug-10	133	56
	Epilimnion	Jul-11	114	32
	Hypolimnion	Jul-11	69	15
	Epilimnion	Aug-11	67	27
	Hypolimnion	Aug-11	58	25
	Epilimnion	Jul-12	125	21
	Hypolimnion	Jul-12	146	4
	Epilimnion	Aug-12	127	34
	Hypolimnion	Aug-12	128	21
Moss Lake	Epilimnion	Jul-10	49	44
	Hypolimnion	Jul-10	139	39
	Epilimnion	Aug-10	110	13
	Hypolimnion	Aug-10	18	8
	Epilimnion	Jul-11	76	21
	Hypolimnion	Jul-11	121	28
	Epilimnion	Aug-11	108	5
	Hypolimnion	Aug-11	52	0
	Epilimnion	Jul-12	126	0
	Hypolimnion	Jul-12	128	0
	Epilimnion	Aug-12	128	10
	Hypolimnion	Aug-12	128	7
Total			**3613**	**852**

6.2.7 Statistical Analyses

Prior to peer-reviewed publication richness at the taxonomic levels of class and subclass for clone libraries will be estimated by rarefaction analysis using the software package EstimateS v7.5.1 (Colwell 2005). The richness estimator Chao2 will be utilized without bias correction as recommended for samples with co-variances that exceed 0.5 (Chao 1987). Sampling sufficiency of each library will be independently determined as described by Kemp and Aller (2004) using the "Large Enough" estimator online at (http://www.aslo.org/lomethods/free/2004/0114a.html). Shannon Diveristy index and its component parts will be calculated on a lake and layer basis. Regression analysis will be conducted to determine rate of change in community diversity metrics over time. Change in community composition will be evaluated using the Bray-Curtis dissimilarity metric. The overall bacterioplankton community composition relationship with water chemistry and hydrologic characteristics will be explored by Principle Component Analysis and Cluster Analysis. All statistical routines will be conducted with statistical software packages such as SigmaStat© v3.0 (SPSS Inc.).

6.3 Results and Discussion

6.3.1 Clone Classification

A total of 3,613 clones were produced in clone libraries from the three NYSERDA study sites over three years. For the NYSERDA sites, the number of clones analyzed from each lake library (epilimnion and hypolimnion) in a single year (2010, 2011, or 2012) ranged from 298 (Brooktrout Lake 2010) to 526 (Brooktrout Lake 2012). The average number of clones analyzed per site per year based on ARDRA pattern analysis was 401 and by sequencing per site over the three-year period was 284. The exact number of clones analyzed for each site and layer by each method, for each year is presented in Table 6-2.

Table 6-2. Clone sequence identification at phylum, class and order levels

Domain	Phylum	Class	Order
Bacteria	Actinobacteria	Actinobacteria	Acidimicrobiales
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales
Bacteria	Bacteroidetes	Flavobacteria	Flavobacteriales
Bacteria	Bacteroidetes	Sphingobacteria	Sphingobacteriales
Bacteria	Elusimicrobia	Elusimicrobia	Elusimicrobiales
Bacteria	OP11	OP11_genera_incertae_sedis	
Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales
Bacteria	Proteobacteria	Alphaproteobacteria	Kiloniellales
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizomicrobium
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales
Bacteria	Proteobacteria	Betaproteobacteria	Hydrogenophilales
Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales
Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales
Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales
Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales
Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfovibrionales
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales
Bacteria	Proteobacteria	Deltaproteobacteria	Syntrophobacterales
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales
Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales
Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales
Bacteria	Verrucomicrobia	Opitutae	Opitutales
Bacteria	Verrucomicrobia	Opitutae	Puniceicoccales

Initially, amplified rDNA inserts from clones were verified visually by agarose gel electrophoresis (1% gels) to confirm the presence of full length rDNA inserts. Clone inserts were preliminarily identified based on restriction digestion profiling of amplified 16S rDNA gene fragments (Amplified Ribosomal DNA Restriction Analysis-ARDRA) essentially as previously described by Vergin et al. (2001) except that the isoschizomer of *Bsu*RI, *Hae*III was utilized. ARDRA patterns were assessed after agarose gel electrophoresis using 3% agarose gels. Restriction profiles were visually sorted into patterns differentiated by the number and size of fragments. Pattern recognition was further facilitated by digital image analysis using the Kodak 1D Image Analysis Software Package v3.6 (Eastman Kodak, Rochester, NY). Each unique ARDRA profile was assigned an identification code.

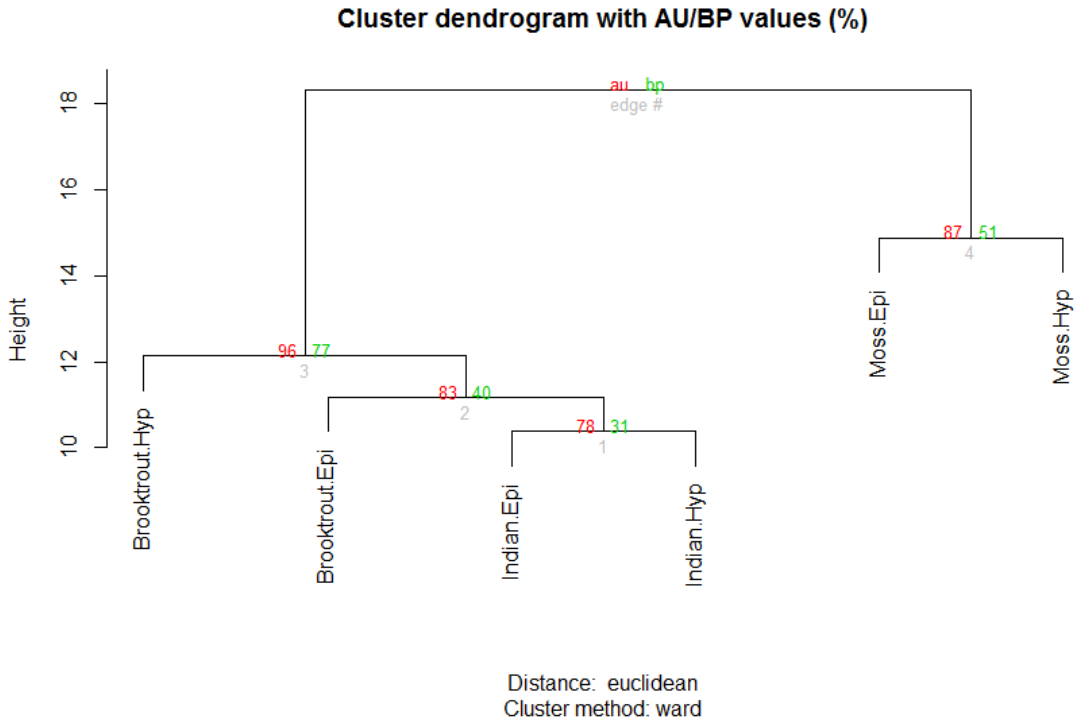
In an effort to elucidate significant differences in bacteria communities, a cluster analysis of the 2011 and 2012 bacterioplankton sequence data for Indian, Brooktrout, and Moss Lake was conducted. The cluster analysis was based on 16S rDNA sequence identification of bacterial clones to the level of genus, the number of clones identified

within that genus (that provided relative abundance), and separate grouping of the hypolimnion and epilimnion data. The results of the analysis are shown in Figure 6-1. The bacterioplankton community from Moss Lake (pH 2010-2012 average 7.2) was the most distant and distinct from the bacterial communities of Brooktrout (pH 2010-2012 average 5.9) and Indian Lake (pH 2010-2012 average 5.7). The epilimnion and hypolimnion bacteria from Indian Lake clustered together. Also, the bacterioplankton community from the epilimnion of Brooktrout Lake clustered more closely with the Indian Lake communities than did the Brooktrout hypolimnion community.

This report provides preliminary evidence that the bacterial communities in the more acidic lakes were more similar to each other than the neutral lake. These types of analyses may be useful in assessing recovery in ecological function as acidity lessens over time. Future work will include a larger data set of lakes with varying pH to expand on these interesting preliminary results.

Figure 6-1. Hierarchical cluster analysis based on abundances of types of identified bacteria from all 2011 and 2012 sequenced data to the genus level

All months and all years grouped to lake name and layer. Red and Green numbers provide two types of *p*-values: red-AU (Approximately Unbiased) computed by multiscale bootstrap re-sampling *p*-value and green BP (Bootstrap Probability) computed by normal bootstrap resampling.



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7 Ecosystem Analysis

7.1 Background

The individual parameter analyses previously discussed in this report provide insight on the effects of reduced sulfur deposition in the Adirondacks as related to lake chemistry and ecosystem functioning. However, while the individual parameters do provide significant information, it is also clear that none of these parameters act individually, and further, that no single parameter dominates these lakes. Instead, the lakes are complex systems in which the individual parameters interact with each other, with other abiotic factors, and with biotic factors.

The purpose of ecosystem analysis is to begin the process of evaluating Adirondack lakes as integrated units and their response to reduced atmospheric deposition. Although this analysis is not a requirement for completion of the project; this report provides the ideal forum to explore the development of new ecosystem-wide tools that enable the analysis and evaluation of the interaction of biotic and abiotic parameters on regional ecosystems. The Ecosystem Analysis is intended to begin to develop the analytical framework for a regional ecological approach to provide answers to questions such as:

- Can the lakes be evaluated and grouped based on chemical and biotic interactions i.e., can a functional grouping of lakes be developed?
- Does stratification of the study sites result in functional differences between the layers?
- Do biotic ecosystem components have an effect on the abiotic components with respect to lake functioning?

While the following analysis attempts to address the lakes in a holistic manner, it must be pointed out that only a limited number of chemical parameters are included in the analysis. Any interpretation of the results must be considered as preliminary. As additional analytical data are incorporated into the analysis, the results will become more fine-tuned, thereby allowing more definitive conclusions.

7.2 Methods

The ecosystem analysis used a stepwise process to evaluate how the lakes relate on a regional basis. The foundation for this analysis is the interaction of the individual abiotic parameters (pH, DOC, SO_4, aluminum, Secchi depth and conductivity) and biotic (chlorophyll a, phytoplankton diversity, phytoplankton biovolume, zooplankton diversity and zooplankton density) components of the lakes. ANC and NO_3^- were not included in this analysis due to data gaps and normalization issues.

These parameters are evaluated on a lake-by-lake basis to determine the relationship between each of these parameters. The individual lake results are then analyzed within a regional context. This regional analysis results in the development of groups based on how the constituents inter-relate, or more specifically, how the lakes function. In order to develop an understanding of how the biotic and abiotic parameters interact, the analysis is initially conducted with abiotic factors only. This is followed with a combined abiotic/biotic analysis. A comparison of these individual analyses provide a window into the overall role the biota plays in lake functioning.

The specific steps in the analysis are:

4. Principal Component Analysis (PCA) - A PCA was conducted on the data for each lake in order to determine how the parameters inter-relate within each lake. The analysis was conducted separately for the epilimnion and the hypolimnion. As the biotic components were collected primarily from the epilimnion, only the epilimnion is analyzed in conjunction with biota. Only the first four PCA components are carried through the analysis.
5. Component Similarity – Components 1-4 for each lake were evaluated to determine the similarity component construction. The dominant variables for each PCA component were compared between lakes which resulted in a similarity score for each lake pair.
6. Lake Groupings – Based upon the similarity indices generated for each lake, groups of lakes were assembled. Lake groupings were determined based upon epilimnetic chemistry, hypolimnetic chemistry and epilimnetic chemistry with biota (phytoplankton and zooplankton) included. As the biota were collected primarily in the epilimnion, a combined hypolimnion/biota analysis was not conducted.

7.3 Results

Based upon the PCA Component similarity analysis, a preliminary grouping of lakes was developed. The analysis indicates that lakes group differently when the epilimnion and hypolimnion are considered separately (Table 7-1). This analysis reinforces the differences between the layers described in the chemistry section in which the layers had significantly different concentrations of each constituent. The PCA-based functional groups are also different from the hydrologic based grouping of Newton and Driscoll (Table 7-2).

Table 7-1. Preliminary lake functional groupings

The lakes are grouped based on similarity of PCA component analysis. There are clear differences between the epilimnion and hypolimnion. Differences indicate that these layers function differently. Key to color coding is found in Table 7-2.

Hypolimnion						
Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**
Sagamore	Moss	South	Jockebush	Squaw	Cascade	Round
North	Indian	Wheeler	Limekiln	Big Moose	Brooktrout	Dart
		Rondaxe	G			

Epilimnion					
Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
Squaw	Jockeybush	Indian	Sagamore	Brooktrout	Round
Big Moose	Wheeler	Limekiln	Dart	Cascade	
North	Moss		G		
Rondaxe	South				

Epilimnion with Biota						
Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	**Group 7**
Wheeler	North	G	Big Moose	Squaw	Rondaxe	Round
	Dart	Cascade	Moss	Sagamore		
	Brooktrout		South	Limekiln		
	Indian			Jockeybush		

Table 7-2. NYSERDA study site hydrologic based groupings

The lakes are grouped based on the (Newton and Driscoll 1990) hydrologic classification system. Grouping lakes using this scheme provides different combinations than those groups based on biotic and abiotic functioning. Color coding by Lake Type is for use in Table 7-1 to make comparisons between the classification systems easier to visualize.

TDL	MDH	MDL	MSL	MSH
Big Moose	Sagamore	Cascade	Round	Wheeler
Brooktrout		Limekiln		
Dart		Moss		
G				
Indian				
Jockeybush				
North				
Rondaxe				
Squaw				
South				

The number of groups for each category (epilimnion, hypolimnion, etc.) is driven by the distance between lake similarities. A distinct break between similarity scores, such as a score of 7.5 to 6.5, is considered to be indicative of distinct groups. It is anticipated that as the number of groups will not change dramatically as the number of lakes included in the analysis increase.

As discussed in the following section, there are many factors that influence the PCA similarity score. However, it is interesting to note that Round Lake consistently had a markedly lower similarity score than any other lake. Round Lake is the site that stratifies the least.

When the individual component composition is evaluated, differences appear between the epilimnion and hypolimnion with respect to the constituents that drive the functional groups. Based upon the analysis, Component 1 (the component that accounts for the largest amount of variability within the system) of both the epilimnion and hypolimnion is dominated by aluminum and pH (Table 7-2). In the epilimnion, Component 2 is dominated by light penetration (as quantified by Secchi disk measurements) while the hypolimnion is driven by conductivity and chlorophyll a. Differences continue to appear in the dominant variables in components 3 and 4. When biota are added into the evaluation, the dynamics appear to change dramatically. No singular dominant species is identified in the first two components of the combined epilimnion/biota analysis. Instead, the variability in the system appears to be distributed relatively evenly across most of the constituents in the analysis. It is not until Component 3 and 4 that differences appear. In both of these components, the biotic measurements appear to be the driving factors.

Table 7-3. NYSERDA study site abiotic/biotic based groupings

	Component 1	Component 2	Component 3	Component 4
Epilimnion –	Monomeric Al	Secchi Depth	Conductivity	DOC
Hypolimnion –	Monomeric Al	Conductivity	Chlorophyll *a*	Secchi Depth
Epilimnion –			Zooplankton Density	Phytoplankton Diversity

While these functional groupings can only be considered preliminary due to the number of constituents included in the analysis, it is clear that there are differences between stratified layers and that biota has an effect on the functioning of the lake. What is not clear is how these groupings may change based upon changes in stratification initiation and duration, or how they are affected by mixing following dissolution of the thermocline. These lakes are dynamic systems and it will only be through more detailed analysis of lake functioning that a regional perspective will be attained.

As this work continues, the next step will be to incorporate the full suite of chemical and biotic constituents included in the project. That step would refine the functional groupings and enable a year-by-year analysis of the lakes. This refinement, in turn, will allow the data to be evaluated within the context of reduced atmospheric deposition as a function of time which is the ultimate goal of the NYSERDA/AEAP project.

8 Database Construction

One of the requirements of this contract was to construct an online database of all the components of the AEAP and NYSERDA programs making them available to other scientists and the general public through an interactive website maintained by the DFWI. The first installment of these data was to be uploaded to the web in the spring of 2011. Two more installments were made to the web, one after the 2011 NYSERDA sampling effort and the second after completion of the 2012 sampling effort.

The results for each major component of the project (Chemistry, Phytoplankton, and Zooplankton [Crustaceans and Rotifers]) were stored in separate Microsoft Excel tables and sent to QA/QC personnel. All data reported were subject to scrutiny based on conformity to a general template that specified data aligned vertically with each collection date and site (pond or lake) name. In the event of any inconsistencies the files were returned to the lead investigator of that component for a check against the original data sheets. All components were reported in a similar fashion. Each row in each excel table represented a separate lake sample with the lake name and collection date noted as well as all pertinent metadata. A total of 35 lakes are reported in this data set. Of the entire database, 16 lakes fall within the NYSERDA subset and have continuous data from 1994 through 2012 (except 2009 when lack of funding prevented sampling), 14 lakes have data continuous from 1994 to 2006 and 5 lakes have data from 2002 to 2006. One lake, Brooktrout, has continuous data for the entire 18-year effort.

For the chemistry database, a total of 24 measured analytes were reported, one per column in the spreadsheet. Data included all lake chemistry sampled on a given date in a given water layer (either epilimnion or hypolimnion). There are a total of 53 columns in the chemistry database that are reported. They include the chemistry data along with all of the pertinent metadata for the samples and the lakes including sample depth, sample type, hydrologic category, etc. Before being posted to the web, the chemistry data went through a rigorous data quality check to address outliers.

Data for each group of biota are reported in an identical way. The rows represent a distinct sample date from a site (lake). Each column represents an individual species and the number pertains to the abundance of that species. The data were set up in this fashion so that it would be easy to calculate species richness, species evenness and species diversity readily from this data set.

A total of 537 individual discrete phytoplankton species were reported throughout the duration of the project since 1994. Each of the phytoplankton species were reported in individual columns. A total of 59 individual discrete rotifer species were reported throughout the same duration as well as a total of 29 individual discrete crustacean species reported. However 46 columns are reported because sexual maturity of larger organisms were also reported, requiring their own columns in the Excel tables.

Data were disseminated through the Darrin Fresh Water Institute website. Each project component was made available as a separate Microsoft Excel data file. Files can be linked by generating a unique identifier based on lake name and date.

Additionally, an online interactive database was developed that linked all of the data files so that an analysis could be completed based on biota and chemistry. Included on this website are links to Microsoft security measures and a tutorial on how to use the data visualization program. This website was built using Microsoft Excel and Microsoft Access with the pivot table tool to create an interactive webpage employing component object model technologies to modify XML data. Drag-and-drop data are synthesized and expanded on any axis and filtered by any category making it possible to compare data across chemical, temporal, and spatial boundaries.

The database is located on the DFWI website at www.rpi.edu/dept/DFWI

This website serves as the organization center for DFWI for data management and quality control. It also serves to disseminate information about the Adirondack study sites. Data can be downloaded by individuals after a short registration process. A record is maintained of all users of the data set.

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Final Report
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