APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1983, p. 1538–1544
0099-2240/83/051538-07$02.00/0

Vol. 45, No. 5

Microbiological Survey of Adirondack Lakes with Various pH Values

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Received 3 January 1983/Accepted 10 March 1983

Nine high-altitude oligotrophic Adirondack lakes in upstate New York having water of pH 4.3 to 7.0 were surveyed for total bacterial numbers and possible adaptation of the microbial communities to environmental pH. The number of heterotrophic bacteria from water samples recoverable on standard plate count agar were low (10^1 to 10^3 per ml) for most of the lakes. Acridine orange direct counts were approximately two orders of magnitude higher than plate counts for each lake. Sediment aerobic heterotrophs recovered on standard plate count agar ranged from 1.4×10^4 to 1.3×10^6 per g of sediment. Direct epifluorescence counts of bacteria in sediment samples ranged from 3.0×10^6 to 1.4×10^7 per g. Low density values were consistent with the oligotrophic nature of all the lakes surveyed. There were no apparent differences in numbers of bacteria originally isolated at pH 5.0 and pH 7.0 between circumneutral lakes (pH > 6.0) and acidic lakes (pH < 5.0). Approximately 1,200 isolates were recultured over a range of pH from 3.0 to 7.0. Regardless of the original isolation pH (pH 5.0 or pH 7.0), less than 10% of the isolates grew at pH < 5.0. Those originally isolated at pH 5.0 also grew at pH 6.0 and 7.0. Those originally isolated at pH 7.0 preferred pH 7.0, with 98% able to grow at pH 6.0 and 44% able to grow at pH 5.0. A chi-square contingency test clearly showed ($P < 0.005$) that two distinct heterotrophic populations had been originally isolated at pH 5.0 and pH 7.0, although there is undoubtedly some overlap between the two populations.

Atmospheric acidic deposition, which has captured considerable public interest, has not been well described in terms of its effects on aquatic microbial populations. The emphasis of recent research on bacteria in acid-stressed oligotrophic lakes has been placed primarily on decomposition processes in these environments. Grahn et al. (4) and Hultberg and Grahn (10) described the accelerated accumulation of organic detritus in acidic Swedish lakes. They concluded that detrital accumulation resulted from reduced bacterial decomposition concomitant with increased fungal growth (as they called it) on the sediment surface. Hendrey et al. (6) and Hendrey and Barvenik (7) also reported the displacement of bacterial decomposers by supposed fungi. Restoration of neutral pH via liming brought a rapid recovery of normal decomposition rates (3, 16). Hendrey and Vertucci (8) and Stokes (21) reported that earlier observations of so-called fungal mat development were incorrect and that the mats were actually composed primarily of filamentous algae.

Schindler *et al.* (17) found no evidence that decomposition was reduced after experimental lake acidification. Gahnstrom et al. (3) found no difference in glucose turnover time or oxygen consumption between the profundal sediments of lakes with pH < 5 and those of lakes with pH > 6.5. Scheider and Dillon (16) found significant differences in the planktonic bacterial populations of acidified and nonacidified Canadian lakes, but Traaen (24, 25) found no major differences in similar studies in Scandinavian lakes. Clearly, a review of the recent literature reveals many discrepancies.

There are over 2,800 lakes and ponds within the Adirondack region of New York State comprising over 114,000 ha (13). Many of these lakes have low alkalinities, primarily because of a carbonate-poor geology. These waters are particularly sensitive to high H^+ inputs from acidic deposition. Since 1975, pH measurements have been made on 849 lakes and ponds throughout the Adirondack region (14). Some 25% of these waters, encompassing 4,230 ha, had pH readings below 5.0. Schofield (18) surveyed 214 high-elevation lakes (above 600 m [2,000 feet]) and found that 52% exhibited surface water pH levels below 5.0. Most of these lakes were pristine, oligotrophic, and low in buffering capacity. Clearly, these sensitive ecosystems may

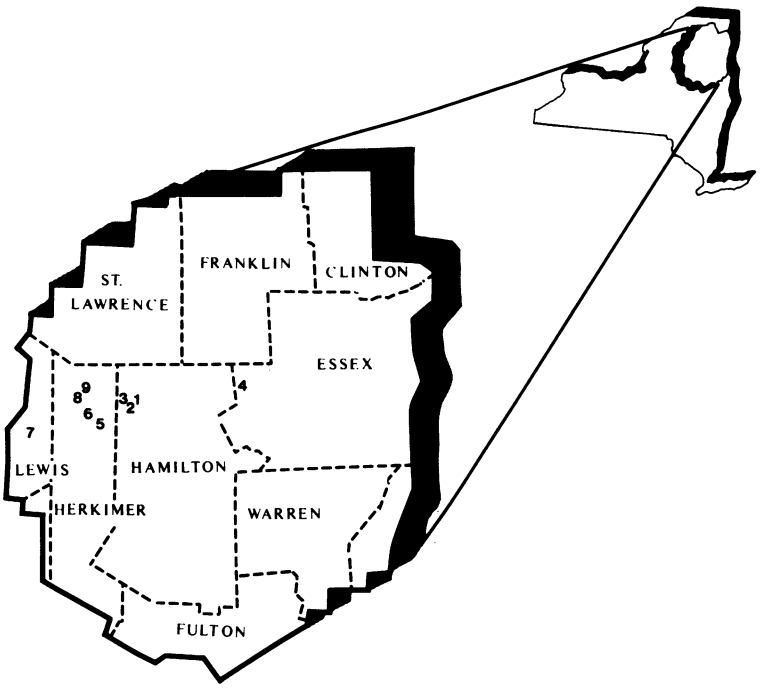

FIG. 1. Locations of nine surveyed Adirondack lakes with different pH values. The inset shows the boundary of the Adirondack Park of New York State.

be in a significant state of transition, and yet their ecology has been poorly documented (5). Information on their basic microbiology is totally lacking.

It is important that these ecosystems be examined so that future changes can be appraised. Major differences in precipitation chemistry and local geological variations make it imprudent to extrapolate from investigations done in Europe and Canada. We present here our initial characterization of the bacterial populations of nine Adirondack lakes and an investigation of bacterial isolates from these lakes with regard to their adaptation to their environmental pH.

MATERIALS AND METHODS

Site description. Nine small (<40 ha) remote Adirondack lakes were selected for study (Fig. 1). The lakes varied in summer subsurface water pH from ca. pH 7.0 to pH 4.0 (Table 1). They were all similar in size, elevation, and latitude, with minimum to no human habitation. Water column oxygen profiles showed oxic conditions existing to the sediment. They were all sampled in a 12-day period during August 1981.

Culture techniques. Water samples for microbiological determinations were collected at 0.5 m from midlake. Duplicate samples were taken at least 10 m apart. Dilutions were made in 0.03 M potassium phosphate buffer adjusted to pH 7.0, and 0.1-ml samples were plated. All bacterial enumerations were made on standard plate count agar that had been adjusted to pH 7.0 or pH 5.0 with 1 N HCl before autoclaving. After plates were poured, the pH of the media was reconfirmed by a surface pH electrode.

Triplicate sediment cores (diameter, 5 cm) at least 3 m apart were collected by a diver from a depth of 2 m. The top 2 cm of the cores was examined for microbial populations. Slurries were made from the sediments in filter-sterilized lake water, diluted, and plated as described for the water samples. All water and sediment plates were inoculated at lakeside and were incubated at 20 to 25°C until they were returned to the laboratory, where incubation continued at 22°C. Sediment bacterial counts were expressed per gram (dry weight).

To determine whether any of the bacteria had adapted to low pH, the water and sediment isolates from each lake were first recultured at the same pH used for the initial isolation. These colonies were subsequently transferred to standard plate count agar that had been adjusted to pH 3.0, 4.0, 5.0, 6.0, and 7.0. The colonies were next incubated at 22°C for 14 days to determine whether growth would occur at the various pH values.

Epifluorescence microscopy. Water and sediment samples were preserved with buffered Formalin (final concentration, 4%). Direct counts were made by a modification of the method of Hobbie et al. (9). Nuclepore polycarbonate filters (pore size 0.1 μm; diameter, 25 mm) were stained for 24 h in a solution of Sudan Black B in 50% ethanol (1:15,000). A preserved water sample (1 ml) was mixed with 1.5 ml of acridine orange solution (1:10,000 in 6.6 mM phosphate buffer, pH 6.7). After 5 min, the sample was filtered through the prestained filter. The mounted filter was examined and counted with a Leitz Dialux 2 microscope equipped with an HBO-100 W mercury lamp that

TABLE 1. Physical and chemical characteristics of study lakes in the Adirondack region of New York State

Lake	Water pH	Site no.	Latitude N–longitude W	Elevation (m)	Surface area (ha)	Shoreline (km)
Deer Pond 2	7.0	4	44° 02′ 04″–74° 15′ 06″	509	36.3	2.74
Dear Pond 1	6.7	3	43° 56′ 00″–74° 47′ 01″	601	18.1	1.93
East Pond	6.4	1	43° 56′ 13″–74° 41′ 25″	598	23.3	2.09
Stony Lake	6.0	7	43° 46′ 10″–75° 13′ 10″	407	31.1	2.74
South Pond	4.9	2	43° 53′ 31″–74° 43′ 50″	610	10.4	2.74
Woods Lake	4.8	6	43° 51′ 56″–74° 57′ 20″	607	25.9	2.74
Silver Lake	4.8	5	43° 50′ 30″–74° 54′ 32″	641	23.3	1.93
Shallow Pond	4.4	8	43° 55′ 13″–75° 02′ 24″	587	5.2	1.62
Lyon Pond	4.3	9	43° 56′ 30″–75° 01′ 30″	610	33.7	3.06

[a] NR, Not recorded.

provided vertical UV illumination through a Leitz B-Q excitation-emission filter under an oil immersion objective. At least 20 fields were examined per filter. A scored grid was used to facilitate counting.

Sterile sodium pyrophosphate (final concentration, 1%) was added to sediment slurries before staining to facilitate separation of cells from sediment particles. The slurry was then agitated on a rotary shaker for 1 h at 22°C. After an additional 30 min, 1 ml was withdrawn from the upper phase and treated as described for the water samples.

Chemical analyses. Water chemistry analyses were performed on triplicate samples collected from midlake at 0.5 m. Metals were determined by atomic absorption spectrophotometry on samples that were acidified and stored until analysis. Water for chlorophyll determinations was filtered in the field, and the filters were frozen for subsequent acetone extractions and analysis by standard methods (1). Sulfate, nitrate, ammonium, and chloride were determined in the laboratory with a Technicon Auto Analyzer from samples filtered in the field. Phosphorus determinations were performed on samples which were frozen on dry ice in the field. Careful technique in using a molybdate blue single reagent method (22) and 10-cm spectrophotometer cells in a double-beam instrument allowed a limit of detection and precision of 1 μg/liter. Aluminum was measured colorimetrically in an *n*-butyl acetate extract after interferences were removed with phenathroline and the aluminum was reacted with 8-hydroxyquinolone (C. T. Driscoll, personal communication).

Alkalinity was determined on the site by a Gran titration plot (23). pH was measured in the field with a Photovolt portable pH meter after the sample was equilibrated with air by stirring for 5 min.

Representative sediment cores 15 to 20 cm long were taken in triplicate at a depth of 2 m by a diver with a plastic corer (diameter, 5 cm). The top 2 cm of each core were separated from the rest of the core. These materials were emptied into plastic bags. The pH was measured, and the contents were frozen for subsequent chemical analyses. The cores were thawed in the laboratory. One core was centrifuged to extract the interstitial water for measurement of available phosphorus. The other cores were dried, ignited at 550°C to determine volatile residue, and extracted in concentrated HNO_3 (1 ml/g of dry sediment). The acid slurry was diluted and centrifuged; the supernatant was treated like the water samples for metal determinations and total phosphorus. The phosphorus and aluminum subsamples were brought up to pH 3 with NaOH before determination.

Oxygen and temperature profiles were measured at midlake from a boat with a YSI model 57 field meter. Specific conductance was measured with a YSI model 31 conductivity bridge immediately upon returning to the laboratory. Conductance was corrected for temperature and is reported at 25°C. This conductance was used to verify field readings taken with a YSI model 33 conductivity-salinity meter.

RESULTS

Enumeration of bacterial populations. The numbers of heterotrophic bacteria recovered from water and sediment samples from the nine lakes surveyed are shown in Fig. 2 and 3, respectively. Densities ranged from 10 to 10^3 bacterial CFU/ml of water and from 10^4 to 10^6 CFU/g (dry weight) of sediment. No trends in bacterial numbers relative to decreasing lakewater pH were evident. Almost all the values were within an order of magnitude in the two groups (water and sediment), irrespective of environmental pH. Bacteria were usually recovered in slightly higher numbers on the pH 7.0 medium than on the pH 5.0 medium.

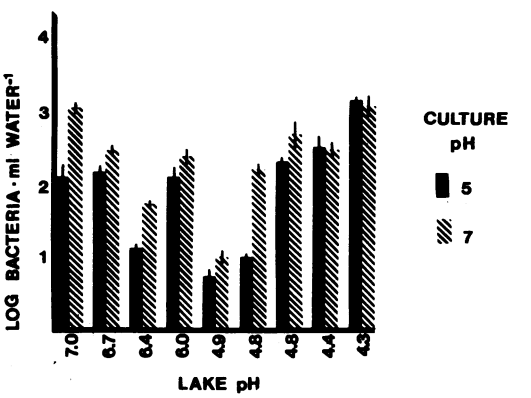

FIG. 2. Heterotrophic bacterial plate counts from water samples taken from the nine lakes determined at pH 5 and 7. Bars represent ranges of triplicate determinations.

TABLE 1—*Continued*

Alkalinity (μeq)	Conductivity (μS)	Total phosphorus (μg/liter)	Orthophosphate (μg/liter)	Ammonia (mg/liter)	Nitrate (mg/liter)	Sulfate (mg/liter)	Chloride (mg/liter)
105	35	8.0	3.0	0.01	0.02	2.2	0.7
42	30	5.0	3.0	0.03	0.42	2.2	2.2
34	37	4.0	3.0	0.01	0.46	2.3	1.2
16.8	27	10.0	8.0	0.01	0.17	1.6	0.6
0.0	29	NR[a]	NR	0.10	0.14	2.2	2.4
−8.4	23	8.0	4.0	0.04	0.16	1.9	0.5
−8.5	27	1.0	1.0	0.09	0.47	2.0	0.4
−50	31	3.0	1.0	0.03	0.45	1.9	0.4
−44	34	NR	NR	0.03	0.56	1.9	0.5

Acridine orange direct counts on water and sediment samples from each lake are presented in Table 2. Counts ranged from 1.3×10^4 to 7.1×10^4 cells per ml of water and from 2.9×10^8 to 9.9×10^8 cells per g (dry weight) of sediment. Again, no trends in these numbers relative to decreasing water pH were evident. Direct counts were remarkably similar in all samples analyzed.

Nutrient chemistry. Water and sediment chemistry data are shown in Tables 1 and 3, respectively. Phosphorus and nitrogen levels were approximately similar for all the lakes and characteristic of ultra-oligotrophic waters (11). In the circumneutral lakes, the pH of the sediment was usually lower than that of the overlying water; in the acidic lakes, on the other hand, it was always higher. The total volatile residue of the sediments varied approximately threefold among the lakes studied. All the sediments had over 15% volatile material. Phosphorus levels in the interstitial water were usually an order of magnitude higher than concentrations found in the water column.

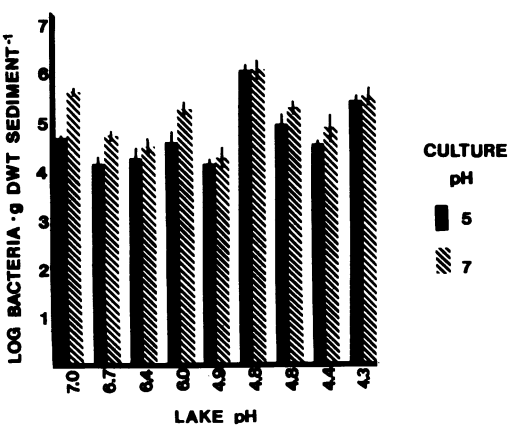

FIG. 3. Heterotrophic bacterial plate counts from sediment samples taken from the nine lakes determined at pH 5 and 7. Bars represent ranges of triplicate determinations.

pH adaptation. From the original platings, 649 colonies were isolated at pH 7.0. Of these, 210 were isolated from circumneutral lakes. Of the 210, 98% (206 colonies) would grow on pH 6.0 media, 41% (87) on pH 5.0 media, and 4% (8) on pH 4.0 and pH 3.0 media. Of the 439 colonies originally isolated at pH 7.0 from the acidic lakes, 98% (429 colonies) would grow on pH 6.0 media, 45% (196) on pH 5.0 media, 3% (15) on pH 4.0 media, and 1% (6) on pH 3.0 media (Fig. 4).

From the original platings, 529 colonies were isolated at pH 5.0. Of these, 132 were isolated from circumneutral lakes. Of the 132, 100% would grow on pH 7.0, 6.0, and 5.0 media, 8% (10 colonies) would grow on pH 4.0 media, and 4% (5) would grow on pH 3.0 media. Of the 397 colonies originally isolated at pH 5.0 from the acidic lakes, 100% would grow on pH 7.0, 6.0, and 5.0 media, 10% (39 colonies) would grow on pH 4.0 media and 6% (24) would grow on pH 3.0 media (Fig. 5).

In each case, whether originally isolated at pH 7.0 or 5.0, those bacteria subsequently recultured at pH 3.0 were always part of the group recultured at pH 4.0; those recultured at pH 4.0 were always part of the group recultured at 5.0; and so on.

The recovery of isolates from the water col-

TABLE 2. Total bacterial counts by epifluorescence microscopy of Adirondack lakes

Lake	pH	Cell count	
		Water (per ml, $\times 10^4$)	Sediment (per g [dry weight], $\times 10^8$)
Deer 2	7.0	5.2	6.5
Deer 1	6.7	5.3	4.2
East	6.4	2.7	2.9
Stony	6.0	7.1	5.6
South	4.9	3.1	5.1
Silver	4.8	1.3	4.7
Woods	4.8	3.6	7.5
Shallow	4.4	5.0	9.9
Lyon	4.3	3.2	9.5

TABLE 3. Chemical analyses of sediment samples from Adirondack lakes

Lake	Sediment pH	% Total volatile residue	Total interstitial phosphorus (μg/liter)	Total phosphorus (mg/g [dry wt])	Calcium (mg/g [dry wt])	Iron (mg/g [dry wt])
Deer 2	6.9	33.2	200	1.34	1.04	10.72
Deer 1	6.2	20.2	25	0.65	0.41	15.94
East	6.0	15.5	125	0.90	0.22	7.93
Stony	6.1	29.3	50	0.49	0.65	6.40
South	5.8	48.0	75	0.97	0.89	25.11
Woods	5.8	23.8	50	1.69	0.25	62.80
Silver	5.7	34.0	<25	0.87	0.16	15.47
Shallow	5.9	41.1	50	1.13	0.22	13.13
Lyon	4.6	32.3	50	0.59	0.13	32.90

umn and the sediments when recultured at pH 3.0, 4.0, 5.0, 6.0, and 7.0 was analyzed by pooling the data from the circumneutral lakes (pH > 6.0) and those from the acidic lakes (pH < 5.0). Two-way analyses of variance were performed with the lake pH and the pH at which isolates were recultured as variables for the isolates originally cultured on pH 7.0 media and for those originally on pH 5.0 media. All the colonies grew best on the media of higher pH ($P < 0.01$), irrespective of the pH of the lake from which they were collected.

A chi-square contingency table analysis was performed to determine whether the colonies isolated at pH 5.0 represented a different part of the microbial community from the colonies isolated at pH 7.0. The numbers of recoverable isolates from the different lakes were pooled, and the contingency table (5 by 2) was used to test the total number of isolates from each reculture pH versus the original isolation pH. This sort of analysis is not affected by differences in the absolute number of colonies in any of the treatments since the expectations are derived from the numbers of colonies counted, not from any a priori assumptions (20). Results of the analysis prove that the success of reculturing on different media is contingent on the pH of the original isolation ($P < 0.005$). Most of the discrepancy between the actual reculture success and the expected success based on chance alone ($\Sigma\chi^2$ = ca. 60% total $\Sigma\chi^2$) was due to the difference in the ability of the original isolates to grow at pH 5.0. This pH level inhibited the colonies originally from pH 7.0 but had no effect on the pH 5.0 isolates. These data suggest that

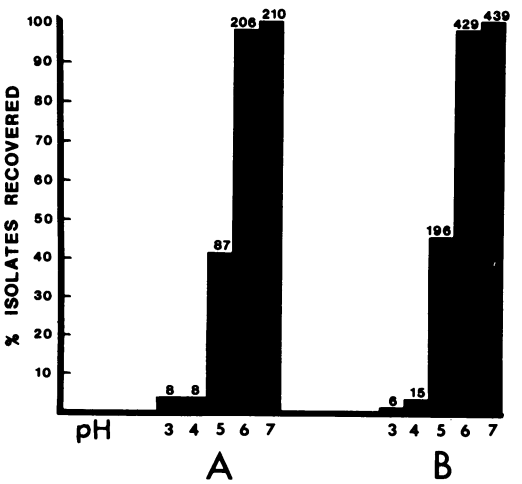

FIG. 4. Percentage of reculturing success at various pH values of colonies originally isolated at pH 7.0. Results are pooled for water and sediment isolates from circumneutral lakes (A) and acidic lakes (B).

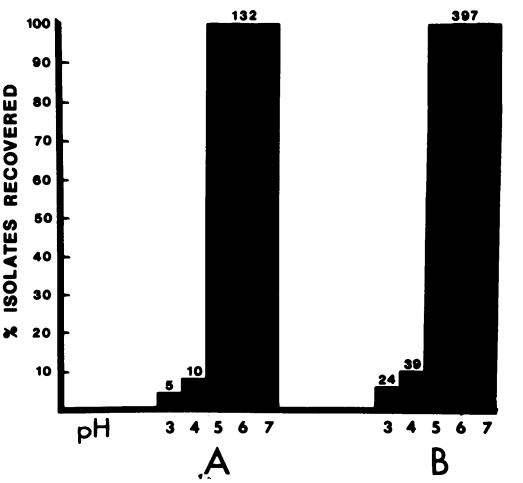

FIG. 5. Percentage of reculturing success at various pH values of colonies originally isolated at pH 5.0. Results are pooled for water and sediment isolates from circumneutral lakes (A) and acidic lakes (B).

the methods used in the original isolations selected distinct subpopulations, although some overlap between the two populations would be expected.

DISCUSSION

Bacterial enumerations from the nine lakes studied show no large differences in population densities with respect to their environmental pH values. The bacterial heterotrophic community in each lake reflects the oligotrophic nature of these lakes more than the different pH values. This is confirmed by the chemical quality and low nutrient concentrations of the waters themselves (Table 1). Our observations agree with those of Traaen (24, 25), who found no differences in the numbers of planktonic bacteria in acidic and nonacidic lakes. He concluded that the concentration of dissolved organic matter was the major factor determining open-water bacterial densities. Muller (12) acidified enclosures placed in a Canadian lake and found no change in bacterial numbers before and after acidification. However, Scheider et al. (15) reported that the planktonic microbial populations of aerobic heterotrophs in acidified lakes near Sudbury, Ontario, Canada, were markedly lower for those in similar nonacidified lakes. Sediment microbial populations, however, were not different. When the acidified lakes were chemically neutralized, they found that the planktonic bacterial densities quickly responded and soon rose to resemble those in the nonacidic lakes.

To date there has been no proof from studies in acid-stressed lakes that microbial communities are dominated by bacterial species that have become adapted to a lowered environmental pH. The initial dilutions of sediment slurries were made with pH 7.0 buffer, which might have adversely affected the recovery of acidophilic bacteria. Nevertheless, no true acidophilic bacteria were isolated from undiluted water samples taken from the acidic lakes studied. True acidophilic bacteria exist in nature, but they are usually restricted to very acidic environments (pH < 3.0), such as thermal acidic waters (2) and acidic mine drainage streams (19).

Our study did not isolate any truly acidophilic heterotrophs. The original colony isolations done at pH 7.0 and the subsequent reculturing on media of various pH values demonstrate the existence of neutrophilic bacterial populations in all nine of the lakes studied. Results of reculturing the pH 5.0 isolates indicate the presence in all the lakes of bacterial populations that are tolerant of lower pH. The presence of neutrophilic bacterial populations in the acidic lakes included in this study suggests that lake acidification may be a fairly recent occurrence; on the other hand, the subpopulation tolerant of low pH indicates a possible adaptation of some bacteria to the lower pH conditions found in these lakes.

ACKNOWLEDGMENTS

This study was supported by the Fresh Water Institute of Rensselaer Polytechnic Institute.

Grateful appreciation is expressed to Karen Smith for help in the field and laboratory. Technical suggestions were generously offered by Denise Taber. Lake access was provided by the Lewis family (Silver Lake), the Binghams and the Brandreth Lake Association (South Pond, East Pond, and Deer Pond 1), Dick Sage and the State University of New York, School of Environmental Science and Forestry (Deer Pond 2), and Creative Forest Products (Shallow and Lyon Ponds).

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