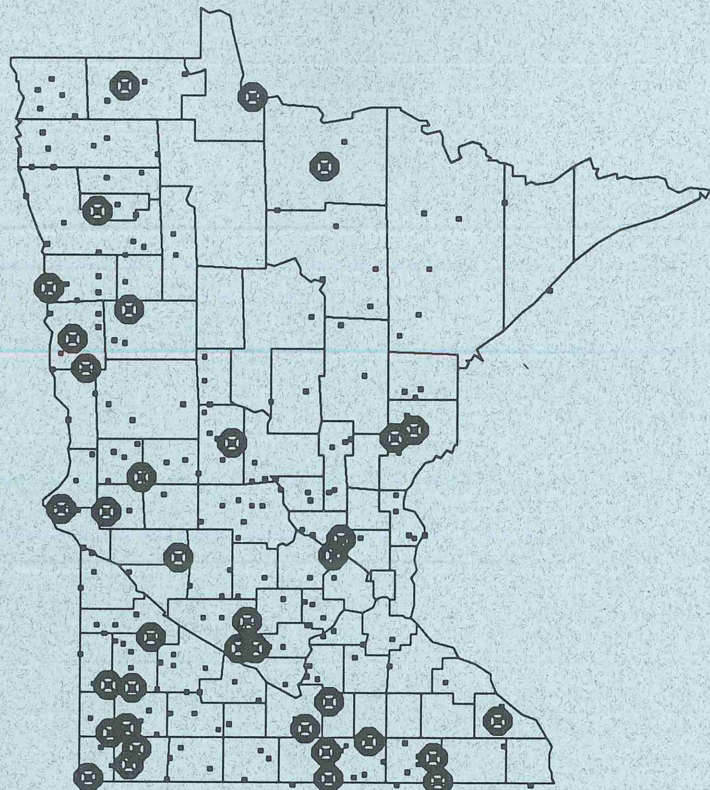


The Biology and Chemistry of Waste Stabilization Ponds in Minnesota

Minnesota Pollution Control Agency
Water Quality Division

Pursuant to 1989 Laws, Chap 335
Article 1, Section 29, subd 4(d)

The Biology and Chemistry of Waste Stabilization Ponds in Minnesota

A final technical report
to the Minnesota State Legislature
1992

Dr. Judy Helgen
Assessment and Planning Section
Water Quality Division
Minnesota Pollution Control Agency
520 Lafayette Road
St. Paul, MN 55155

with contributions by

Dr. Patrick Brezonik
Department of Civil and Mineral Engineering
University of Minnesota

The cover:
The map displays the locations of the 253 waste stabilization pond systems in the state of Minnesota. Sites indicated with the large dark circular symbol had a total suspended solids (TSS) reading over 60 mg/L in the effluent water during 1987 - 1990. In the vast majority of the communities, there were no solids violations.

Acknowledgements

1. This project was made possible with funding approved by the Minnesota Legislature (ML 1989, Chapter 335, Sec. 29, Subd. 4 (d)), as recommended by the Legislative Commission on Minnesota Resources from the Minnesota Future Resources Fund. The funding was to the MPCA, which formed a contract with Dr. Pat Brezonik, University of Minnesota, for the water chemistry analysis. Dr. Heinz Stefan of the University of Minnesota was a cooperator on this project.

2. The water chemistry analysis for this project was performed principally by Lakshmi Buddhavarapu and Karl Rockne of Dr. Brezonik's laboratory. Some of the analyses were done by the Minnesota Department of Health.

3. Dr. Stefan's students assisted and cooperated in the field effort, especially Charles Hathaway and Fred Luck. Their good work was much appreciated.

4. Excellent assistance to Dr. Helgen for the field and laboratory biological work and analysis was provided by Ann Palmer, a student at the University of Wisconsin River Falls and Tom Wilcox, a student at Macalester College who did several of the graphs in this report. Excellent field assistance was also provided by Quentin Kramer and Karma Dunlop, both students at the University of Minnesota.

5. Many staff members at MPCA assisted in this project: Harold Weigner, Jerry Flom, Gene Erickson, Carol Sinden, Gary Rott, Jeanne Soban, Gerry Blaha, Bennett Davis, Linda Brooks, Gene Soderbeck, Greg Gross, and especially Dr. Ed Swain, who conceived and initiated this project, and helped along the way.

6. Thanks to Dale Miller of Harris, MN and Pete Moulton of St. Peter, MN and the other wastewater treatment operators who were also helpful in the cities of Balaton, Bellingham, Bird Island, Bricelyn, Browerville, Chandler, Clarks Grove, Cosmos, Currie, Edgerton, Fulda, Janesville, Kiester, Mapleton, Murdock, and Sanborn, MN.

7. Thanks to Dr. Daniel Hornbach at Macalester College for sharing his digitizing equipment, and to Dr. Bob Megard of the University of Minnesota for sharing the zooplankton pumping setup until we made our own.

8. Thanks to biologist colleague Pat Bailey for support and encouragement.

Contents

Executive Summary 7
Introduction 9
Seasonal Daphnia populations in waste stabilization ponds
    Field methods for quantitative zooplankton analysis 12
    Results of field zooplankton analysis
        Resting egg densities in sediments 13
        Field densities of Daphnia 15
        Reproduction and size measurements 16
        Stratified and day-night zooplankton 17
        Zooplankton densities and water quality factors 17
    Overall conclusions from field zooplankton data 18

Bioassays with waste stabilization pond water
    Methods for bioassays 30
    Results of bioassays
        Mortality of Daphnia magna 31
        Reproduction of Daphnia magna 32
        Fed versus unfed bioassays 33
    Statistical results of reproduction and water quality factors 33
    Overall conclusions from bioassay data 33

Analysis of waste stabilization pond algae
    Methods of algae analysis 38
    Results of algae analysis
        Seasonal succession in ponds 38
        Chlorophyll in relation to water quality factors 40
            Total suspended solids 40
            Secchi disc depth 40
            Dissolved oxygen 40
            pH 41
            Soluble reactive phosphate 41
            Ammonia 41
            Alkalinity 41
            Bluegreen algae 41

Contents (continued)

Overall conclusions from the algae data 42

Outstate survey of waste stabilization ponds
Outstate survey methods 51
Statistical results of survey 51
Overall conclusions from the outstate survey 52

Overnight respiration and dissolved oxygen in waste stabilization ponds
Method for measuring overnight respiration 59
Results of overnight respiration measurements 59
Daytime surface oxygen data 60

Chemical and physical data from waste stabilization ponds
Chemical and physical methods (Dr. Brezonik's lab) 66
Quality control and assurance procedures 67
Chemical and physical data
Ammonia 67
Phosphorus 68
pH 68
Nitrogen and carbon budgets (Dr. Brezonik) 69

References 80

Appendices
Appendix 1.HPLC analysis of waste stabilization pond algae pigments 82
Appendix 2.Data for ammonia, nitrate, soluble reactive phosphate and total phosphate 84
Appendix 3.Data on dissolved oxygen, alkalinity, conductivity, pH, TSS, chlorophyll and secchi disc depth 88
Appendix 4.Data on calcium, magnesium, sodium, potassium, chloride, and sulfate 94

Tables

1. Densities of ephippia of Daphnia in sediments 14
2. Field densities of Daphnia for Harris, Janesville and St. Peter 19
3. Field densities of Simocephalus and Moina 20
4. Regressions of density of cladocerans and water chemistry 29
5. Regressions of Daphnia reproduction in bioassays and water chemistry 37
6. Types of algae dominant in waste stabilization ponds 43
7. Regressions of chlorophyll a and water chemistry 45
8. Outstate survey data 53
9. Regressions of outstate survey data with chlorophyll and bluegreen algae 56
10. Regressions of outstate survey data with TSS and TSS violations 57
11. Whole pond respiration rate of secondary ponds 60
12. Mean values for phosphorus and nitrogen compounds 72

Figures

1. Seasonal changes in cladocerans, TSS and chlorophyll at Harris, MN 21
2. Seasonal changes in cladocerans, TSS and chlorophyll at Janesville, MN 22
3. Seasonal changes in cladocerans and chlorophyll at St. Peter, MN 23
4. Seasonal changes in cladocerans and TSS at St. Peter, MN........................... 24
5. Reproduction of cladocerans in bioassays at Harris secondary pond 25
6. Reproduction of cladocerans in bioassays at Janesville secondary pond 26
7. Reproduction of cladocerans in bioassays at St. Peter secondary pond 27
8. Vertical distribution of Daphnia at Harris in March 28
9. Vertical distribution of Daphnia and Chaoborus at Harris in July 28
10. Daphnia reproduction in bioassays with Harris water 35
11. Daphnia bioassays with Janesville and St. Peter water 36
12. Chlorophyll a in secondary ponds at Harris, Janesville and St. Peter 46
13. Total suspended solids and chlorophyll a 47
14. TSS and secchi disc depth 48
15. Secchi depth in secondary ponds in 1990 49

Figures (continued)

16. Chlorophyll a and soluble reactive phosphate 50
17. Cumulative TSS on pond water alkalinity 58
18. Overnight respiration at Janesville and St. Peter 61
19. Overnight respiration at Harris 62
20. Surface dissolved oxygen at Harris in 1990 63
21. Surface dissolved oxygen at Janesville in 1990 64
22. Surface dissolved oxygen at St. Peter in 1990 65
23. Ammonia data for Harris, 1990 71
24. Soluble reactive phosphorus data for Harris, 1990 73
25. Surface pH at Harris 1989 - 1990 74
26. Surface pH at Janesville,1990 75
27. Surface pH at St. Peter, 1990 76
28. Nitrogen mass flux through Harris ponds 77
29. Carbon mass flux through Harris ponds 78
30. Carbon and nitrogen mass flux through Harris ponds 79

Executive Summary

Waste stabilization pond systems are being used for treatment of wastewater in 253 communities in Minnesota. Without any mechanical mixing or aeration devices, the majority of these sites produce a discharge that meets the standards for water quality as given in Minnesota Ch. 7050. For example, from 1987-1990, 187 of the 253 communities had no violations of the standard for total suspended solids.

The purpose of this project was to measure and analyze the biological, chemical and physical factors that affect the performance of stabilization ponds, particularly in the processing of the total suspended solids. The original hypothesis for the study was that ponds with good Daphnia populations have lower solids levels than ponds without Daphnia.

During 1989-1990, the zooplankton, algae, water chemistry and other elements of waste stabilization ponds were analyzed. In cooperation with Dr. Heinz Stefan and Dr. Pat Brezonik at the University of Minnesota, the research effort was focussed intensively on the stabilization ponds at Harris, MN. To get a broader perspective on the solids violations problem, MPCA added two additional sites for frequent biological and chemical sampling, Janesville and St. Peter, MN. Fifteen other pond systems were surveyed once in fall of 1990, and the data, referred to as the 'outstate survey', was related to pond performance factors.

One important goal of the work was understanding the factors that affect total suspended solids (TSS) and TSS violations in waste stabilization ponds. Chlorophyll, representing the amount of algae, was very significantly related to TSS levels, both in the seasonal study of the three intensive sites and in the outstate survey. The algae perform the important service of providing oxygen, but uncontrolled algae growth could cause increases in the solids levels.

Cumulative TSS violations of the 18 sites in the study were significantly related to the pond alkalinity. Data on public water supply alkalinity of 216 communities in MN with stabilization ponds was related to the records at MPCA on discharge TSS levels. Both the number of times TSS was over 45 mg/L and the number of times it was over 60 mg/L were significantly related to the water supply's alkalinity levels.

It may be that sites with higher well water alkalinity have more algal productivity and therefore more tendency to have higher solids levels. The amount of solids in the secondary ponds may be the productivity of the algae. Well water alkalinity was significantly related to the chlorophyll levels measured at the 18 sites in the study.

Chlorophyll a and algae

From the combined seasonal data from all the ponds at Harris, Janesville and St. Peter, chlorophyll levels were most significantly related, in a positive way, to the level of dissolved oxygen in the ponds, to the TSS values and the pH of the pond water. All three factors can be the result of activity by the algae: oxygen and elevated pH caused by photosynthesis, and increased TSS by carbon fixation and growth of the algae. The chlorophyll was significantly related, in a negative way, to the measure of the water clarity, the secchi disk depth, and the soluble reactive phosphate (SRP) in the water. Of the three more intensive study sites, the secondary pond at Harris had the lowest amount of chlorophyll, the clearest water, and the highest level of soluble phosphate.

Another important focus of this project was to find out which factors were significantly related to the amount and the kinds of algae in the ponds, especially to the relative abundance of the bluegreen algae. Much of the time, the ponds were dominated by a variety of green algae. When chlorophyll levels were higher, however, the percentage of bluegreen algae present was greater. There were significant relationships between percent bluegreen algae and turbidity of the water and the total organic carbon.

Daphnia

Another goal of the work was to understand the factors that affected the Daphnia populations in waste stabilization ponds. The project developed around the idea that the sites with low solids levels in the secondary ponds had good Daphnia populations, but sites with high solids lacked Daphnia. This was based on anecdotal reports from wastewater treatment operators. In the ideal waste stabilization pond, the algae grow fast after ice-out, and produce oxygen that is used in the respiratory breakdown of the influent solids accumulated over the winter. Solids are broken down to $C0_2$ and water, and the Daphnia keep the algae populations from overshooting by eating them.

Daphnia were analyzed in two ways: by bioassays of waste pond water to observe Daphnia reproduction, and by quantitative measurements of Daphnia densities in the ponds from ice-out through November, 1990. In the bioassays, Daphnia reproduction had a significant, positive relationship with chlorophyll levels at Harris, and a significantly negative relationship to chlorophyll levels at Janesville. The type of algae at Janesville was a filamentous bluegreen alga, inedible to Daphnia, so the negative relationship is not surprising. Pond water pH was significantly related to Daphnia reproduction only with St. Peter pond water.

During the spring, the Daphnia population in the secondary pond at Harris developed closely after the algae populations did, and the algae subsequently declined as the Daphnia increased. Densities of Daphnia were significantly related, in a negative way, to the level of chlorophyll and the TSS. This is consistent with the knowledge that Daphnia can reduce algae by consuming them. Daphnia densities were significantly related to the water clarity. Higher densities of Daphnia occur in clearer water. This is consistent with the hypothesis that a major function of Daphnia in the stabilization ponds is reducing algae levels and clarifying the water. Daphnia populations were significantly related to other parameters: calcium, magnesium, sulfate, sodium, conductivity and iron, the latter relationship had a negative slope.

Water chemistry

Many physical and chemical parameters in the ponds were measured, and the data are presented in the report.

Ammonia did not have a major toxic effect in the ponds. Ammonia levels were highest in the spring, lower in summer and early fall, and increased again in November. Unionized ammonia was occasionally over 1 mg/L, but it did not relate significantly to Daphnia reproduction in bioassays or Daphnia densities in the field data. Ammonia itself, however, was significantly related to the chlorophyll levels, in a negative way, probably because the algae are taking up the ammonia as a preferred nutrient. About 70% of the nitrogen was removed from the ponds in the summer, or twice the efficiency observed in the spring. Nitrate levels were typically very low in the ponds, indicating low denitrification activity.

Carbon removal efficiencies were on the order of 90-95% at the Harris site, which compares well with an 85 - 90 percent carbon removal efficiency in secondary activated sludge.

Oxygen levels varied widely through the 24 hour cycle. The renewal of surface water oxygen is very dependent on the algal activity. The respiratory use of oxygen will depend on the amount of degradable organic matter in the pond. Continuous oxygen data overnight at the three sites allowed estimation of the overnight respiration rates of oxygen removal at St. Peter and Janesville, but not at Harris where there was almost no change in high levels of oxygen overnight in the secondary pond in summer. In contrast, oxygen went to zero in the night at St. Peter, and was at 2.5 mg/L at Janesville at dawn. Only at St. Peter was low oxygen significantly related to Daphnia reproduction in bioassays. This approach gives an excellent comparison of oxygen demand in the different ponds.

This project has produced data that is now available for additional analysis of waste stabilization pond performance, for instance, the effects of water supply alkalinity could be explored further. We can conclude that a proper balance of Daphnia and algae are important for best pond performance, that Daphnia reduce chlorophyll and solids, and clarify the water. The significant relationship between the cumulative TSS violations and number of discharges and the ratio of daily flow/design flow reported here reinforces the importance of removing sources of extraneous water infiltration such as sump pumps, tiles connected to sewer lines, and stormwater drains to improve stabilization pond performance.

Introduction

There are 253 waste stabilization pond sites in Minnesota, most of which serve very small communities. The majority of these systems work efficiently in converting wastewater to an effluent that complies with the State's standards for effluent discharge, listed in the Minnesota Water Quality Rule Ch. 7050. The standards are for discharge water from waste stabilization ponds are: 5 day CBOD, 25 mg/L, total suspended solids (TSS) 45 mg/L, pH 6 - 9, fecal coliforms 200/100ml and phosphate 1 mg/L.

The purpose of this project was to understand the biological and chemical factors in stabilization ponds that affect pond performance, particularly in the processing of the total suspended solids (TSS) and production of algae. In addition, the hypothesis was formed that Daphnia play a major role in clarifying waste pond water by filtering out the algae that grow in the ponds. This idea came from the observations by treatment operators that when Daphnia were present, the water in their secondary ponds was clearer, and therefore lower in solids, and vice versa. It is also known that the spring "clear water phase" in natural ponds and wetlands is often the result of filter-feeding activity by Daphnia.

A high TSS value in a secondary pond is most likely caused by an algae bloom in the enriched water. This study describes a strong relationship between concentrations of algae and the solids levels in the secondary ponds. Algae growth is a normal and essential part of pond functioning, especially in the first two ponds of a typical three-pond facility, where the algae produce oxygen that drives the breakdown of the influent waste material. In a good pond system, the water in the secondary pond becomes clearer as it warms up in spring. After ice-out, the wastewater treatment operator has to move water from the primary pond into the middle pond, and from the middle pond into the secondary, or third pond in the sequence. There are often two discharges in late spring, after which the ponds are stable until the two or three discharges in the fall to prepare room for the winter influent.

To understand the role of Daphnia in promoting good water quality in secondary waste ponds, and the factors that would stimulate the massive algae blooms seen in some sites, we sampled the algae, the Daphnia and the water chemistry in sewage ponds in a cooperative and parallel effort with Dr. Heinz Stefan and Dr. Pat Brezonik at the University of Minnesota. The sampling effort was intense through the season at the Harris site, where Dr. Stefan's group worked on physical and climatological parameters in the ponds for their models. The Harris site was selected because of the desire to study and model a site with an excellent record in meeting the water quality standards for discharge.

Additionally, MPCA decided to include sites that had histories of problems in meeting the TSS standard. Therefore we studied the ponds at Janesville and St. Peter on a regular basis through 1989-1990, as well as those at Harris. In the fall of 1990, we surveyed 15 other waste stabilization pond systems, and the data from this "outstate survey" has produced valuable results on pond performance. When alkalinity showed up in this data set as significantly related to the amount of algae in the secondary ponds, data on the well-water alkalinity of the town's water supply was related to the TSS violations histories of all the sites in Minnesota statistically, and found significant.

The chemical and biological data have been merged using EXCEL 3.0 and analyzed statistically and graphed with SYSTAT, McDraw II and McDrawPro on the Macintosh 2ci computer. Most of the chemical data will be included in appendices to this report. The statistical results are reported in tables of the regressions. Non-significant results are included, so the reader can see regressions that were run on the data. In general, the methods are given at the beginning of each section. Some detailed data from this project, such as the analysis of other invertebrates in the stabilization ponds and details of the HPLC pigment analysis on the waste pond algae will not be reported here. Papers will be submitted to scientific journals.

This report contains the following sections: seasonal Daphnia populations in waste stabilization ponds, bioassays with waste stabilization pond water, analysis of waste pond algae, outstate survey of waste stabilization ponds, overnight respiration and dissolved oxygen in waste stabilization ponds, and chemical and physical data from waste stabilization ponds.

The study sites

Except where indicated, the study sites were non-mechanical, non-aerated stabilization pond systems. Most of the sites were three cell or pond systems.

Harris, MN.

This waste stabilization pond system, constructed in 1980, is located in Chisago County, MN in the Anoka sand plain and was the focus of the intensive study part of this project. These ponds were sampled frequently for biological and chemical parameters, twice a week in spring, and weekly thereafter from November,1989 through November, 1990. The site was also the focus of the project on physical modelling of waste ponds by Dr. Heinz Stefan and graduate students who have reported on the physical limnology (Luck and Stefan, 1990) and stratification dynamics and mixing (Gu and Stefan, 1991).

The Harris site was selected because of its consistent compliance with MPCA discharge standards for total suspended solids (TSS) Typical TSS values at discharge, in mg/L were 1.5 - 14 (1987), 6 - 22.5 (1988), 14.5 (1989), and 5.7 - 15 (1990). With a total pond acreage of 4.8 acres and population of 813 (1988 census), the Harris site has 0.005904 acres of pond per capita, or .59 acres/100 people. Over a four-year period (1987 - 1990), the site has been operating at 72.4% of design capacity (see Table 8).

Janesville, MN.
Janesville is located in south central Minnesota, east of the city of Mankato in Waseca County, MN, in a predominantly agricultural area. The site was sampled less frequently than the Harris site, every two weeks in spring and usually every four in summer and fall.

The Janesville site was selected because of its history of high solids levels in the secondary pond, with TSS levels in mg/L of 71 - 158 (1987), 67 - 131 (1988), 54 - 190 (1989), 54 - 140 (1990). With a total of 21 pond acres, and a population of 1968 (1988 census), there are .0107 acres/capita, or 1.02 per 100 people. Over the period of 1987 - 1990, this site has been operating at 1.08 design capacity. The secondary pond has had to be discharged much more frequently than most stabilization ponds, 27 discharges in 1987 - 1990 compared with 10 for Harris.

St. Peter, MN.
Located by the Minnesota River north of the city of Mankato in Le Sueur County in south-central Minnesota, St. Peter's waste stabilization pond system is one of the largest non-mechanical wastewater treatment systems in the state. Built in 1960, a total of 201.8 pond acres serves a population of 9257 (1988 census), or .0218 acres/capita or 2.18 acres/100 people.

This site was selected because of its occurrences of algae blooms in the secondary pond and subsequent treatments with copper sulfate to reduce suspended solids before discharge. The TSS values for the secondary pond at St. Peter are, in mg/L, 18 - 42 (spring 1987), 4 - 12 (fall 1987), 6 - 30 (1988), 22 - 42 (1989), and 10 - 30 (1990). The ponds were sampled every two weeks in spring, and every four weeks in summer and fall.

The outstate survey sites
These sites were sampled only once, in the fall of 1990. The sites included the communities of Balaton, Bellingham, Bird Island, Bricelyn, Browerville, Chandler, Clarks Grove, Cosmos, Currie, Edgerton, Elko-New Market, Fulda, Kiester, Long Prairie, Mapleton, Murdock, and Sanborn, as well as Harris, Janesville and St. Peter. The sites were selected to include some with a history of high and some with low solids in the discharge water.

Seasonal Daphnia populations in waste stabilization ponds

Daphnia populations were sampled quantitatively in all three ponds at Harris, Janesville and St. Peter from ice-out in early April through November, 1990. Triplicate samples were taken for each sampling date and pond, and these data were related to physical and chemical parameters in the ponds through the season. Harris ponds were sampled quantitatively for zooplankton on 22 dates in 1990, for a total of 261 samples from the three ponds. Janesville ponds were sampled on 10 dates for 75 samples, and St. Peter ponds on 11 dates for 83 samples. On most dates all three ponds, including the primary pond, were sampled, on just a few dates, only the secondary pond was sampled. The samples were taken from a small boat anchored at three different locations in the ponds. Small vinyl-coated mushroom anchors were used, so the pond liner would not be penetrated.

The hypothesis of the project was that the Daphnia, as filter-feeders, play an important role in producing good water quality in the discharge ponds by consuming the algae and reducing the solids loads in the discharge water. In this section, the methods for the field analysis of the zooplankton or Daphnia populations are described, followed by the results and analysis of Daphnia densities in relation to several water quality factors.

Field methods for quantitative zooplankton analysis

The Daphnia populations were assessed quantitatively with a filtering-pumping system which solves the problem of sampling for zooplankton in shallow water areas. The system was modelled after the one devised by Tom Berman and Bob Megard for use in Israel. In our system, an in-line digital flowmeter was added, and couplings were inserted to allow disassembly of the components.

The self-priming diaphragm bilge pump (ITT JABSCO 36680-0000, 315 GPH at 5 ft head), was coupled to 3/4" ID hoses, and powered with a 12V deep cycle marine battery. It was mounted on a board for use in the duckboat, and performed extremely well through the sampling season. Water passed through the pre-filter, then through the pump to an in-line irrigation filter cartridge system (Amiad 3/4" No. 39-0 Y type) holding a 130 μm filter, then through the flowmeter, and out the exit hose. The bilge pump moved water at about 10 - 15 L/minute. The irrigation filter cartridge worked remarkably well, even during swarms of Daphnia. During the dense bloom of Microcystis at St. Peter on one date, it was necessary to brush the filter cartridge free of the green gelatinous coating to prevent clogging.

The system has a bypass flow pipe with closing ball valves. After the flow is going, the ball valves are switched, allowing flow only through the filter cartridge, while the flow meter is activated. The nylon electronic digital turbine flowmeter from Great Plains Industries (model 6N12LM) operated best at 10-100 L/min. The meter units were calibrated by measuring pumped volumes of water.

The metered pumping method was used for all quantitative seasonal assessments of Daphnia at Harris, Janesville and St. Peter ponds. For the single-visit field trips to several 'outstate' wastewater treatment sites, only qualitative samples were taken using an 83 μm plankton tow net thrown from the edge of the ponds three times per sample.

Triplicate samples, each 25 - 40 liters in volume,were taken on each of the three ponds on each sampling date, at 30 - 50 cm. Samples were concentrated with a plankton bucket and preserved with 5% sucrose-formalin. Zooplankton were analyzed in the laboratory at MPCA with an Olympus SZH microscope and a Wards plankton counting wheel. Usually at least 20% of the sample, or 100 organisms were counted. Subsampling was done with a one to two ml Hensel-Stempfl pipet. Primarily the cladocerans, i.e. Daphnia and related species, such as Simocephalus and Moina sp., were counted for most dates. Cladoceran eggs were counted.

Daphnia lengths

Daphnia body lengths were measured with a digitizer and Kurta pad with an SZH Olympus microscope and a camera lucida drawing attachment. The digitizer was supported by Sigma Scan software (Jandel Scientific) in an IBM 286 computer (in the laboratory of Dr. Dan Hornbach, Macalester College). Measurements of 25 - 100 Daphnia per sample were made from the base of the posterior spine to the anterior edge of the head. For plotting of data,15 size classes of 0.3 mm were designated, and the proportion of the sample in each size class was plotted for each sample by date. This data will not be shown in this report.

Resting eggs in sediments

Analysis of the resting egg bank of Daphnia overwintering eggs, or ephippia, in the pond sediments involved coring the waste pond sediments through the ice with a Wildco brass hand core sampler with extension handle 2.5" clear core liner tubes. This was done to establish whether there were resting eggs with the potential to form Daphnia populations in the ponds. Pond sediments were seived in the lab through a 500mm seive to retain the ephippia where were counted under the microscope. Viability of these eggs was not tested. Data is reported as number of ephippia/m^2, each of which typically contains two eggs. On the 'outstate' site visits, we attempted to collect surface sediment from the secondary pond with a seiving long-handled grab sampler to establish presence or absence of Daphnia resting eggs.

Day-night survey methods

The diel, or day and night surveys, were carried out with all cooperators involved on 4/23/90 and 7/19 at Harris, and on 8/16-8/18 at St. Peter and Janesville. The purpose of the overnight survey was to understand any vertical migration patterns of the zooplankton and physical and chemical changes that occur overnight in the ponds. For the diel study, zooplankton sampling was done at four depths, (10, 50, 90, 140 cm) at three sites in each pond at different times of day and night. Physical data was taken by Dr. Stefan's group every four hours. Daphnia were sampled late in the afternoon, then at one to two hours after sunset, at dawn and midday.

Results of field zooplankton analysis

Ephippia (resting egg) densities in sediments

This may be the first report on the densities of Daphnia resting eggs in waste stabilization ponds. Herzig (1985) reported a maximum mean value of Daphnia resting eggs in the profundal zone of a lake of 82,000 ephippia/m^2, with a maximum single value of 359,000/m^2. In two German lakes, maximum mean Daphnia egg densities of 72,000 and 120,000/m^2 were observed (Carvalho and Wolf, 1989).

The maximum single value for Daphnia resting eggs for the 31 pond sediment cores analyzed in this study was 1,290,616/m^2, and the maximum mean value was 1,128,184/m^2 (n=4 cores), both at St. Peter pond 1. The secondary ponds at Harris, Janesville and St. Peter had densities ranging from 421,000 to 509,000 (see Table 3 below). The numbers of ephippia are reported here. The actual number of resting eggs would be double these numbers, since there are usually two eggs in each ephippium.

Table 1. Densities of ephippia or resting eggs of Daphnia magna plus Daphnia pulex in sediments of waste stabilization ponds in Minnesota. Means of the number of resting eggs or ephippia per meter^2 of pond bottom are given, with SD = standard deviation, n = number of cores analyzed.

Site	Pond	Ephippia/m^2	SD	n
Harris	2	272,378	97,959	3
Harris	3	420,936	189,068	9
Janesville	1	98,159	14,111	3
Janesville	2	121,548		1
Janesville	3	670,539	54,979	3
St. Peter	1	1,128,184	102,223	4
St. Peter	3	509,258	49,549	4

These results indicate that the waste stabilization ponds have extremely high densities of Daphnia resting eggs in their sediments, considerably higher than values reported for lake sediments. Assuming normal vialibity of the resting eggs, these results would suggest a high potential for development of Daphnia populations in all the ponds analyzed, when conditions permit.

The viability of the resting eggs is unknown. Carvalho and Wolf (1989) tested Daphnia resting egg viability and observed a maximum of 14.4% viability. One problem with testing viability of resting eggs is the difficulty of reproducing in the lab the conditions for hatching. It was our intention to test viability of ephippia collected from Harris ponds in wastewater from all three sites, to test for any effects of the different pond waters on egg hatch. The resting eggs were collected in March when massive quantities of ephippia caused a meter-wide black band on the surface at the open edge of the ice-covered pond at Harris. Unexpectedly, the eggs proceeded to hatch in the dark in the refrigerator, at 4°C, thereby ending the experiment before it began.

Carvalho and Wolf (1989) reported maximal hatching of Daphnia resting eggs at 12°C, but the Daphnia pulex eggs from Harris were hatching in the lab at 4°C, and the first juvenile Daphnia collected in April at Harris were present in 6°C water. It appears that this species, at least, is adapted to the climate of Minnesota, by its ability to hatch at near-freezing temperatures.

The extremely high density of resting eggs in the sediments of all three sites provides a "bank" from which Daphnia can recruit and develop. The strategy for Daphnia may be to periodically form massive blooms and produce resting eggs to deposit in the "bank". This egg bank allows the populations to continue developing when the water is not supporting reproduction, or when predation pressure is high.

Species of cladocerans in the ponds

For the data given in this report, Daphnia includes both Daphnia pulex and Daphnia magna, and total cladocerans includes species of Daphnia, Simocephalus and Moina. The rotifers were not counted, but they could be important in waste stabilization ponds as algae consumers. Janesville pond 2 had a thick bloom of the herbivorous rotifer Brachionus in May, so thick it colored the water an orange-brown.

Harris ponds were dominated by Daphnia pulex until late summer (1990) when the cladoceran Simocephalus sp. developed. Daphnia pulex is found in open water in shallow wetlands and deep ponds and lakes, whereas Simocephalus is more often associated with vegetated areas. It occurs in the littoral zone of lakes, in wetlands and vegetated ponds. Harris pond 3 developed a submersed bed of macrophytes late in August into fall of 1990, and this is where the Simocephalus population developed.

The dominant species in St. Peter pond 3 was Daphnia magna, but all three ponds had dense populations of Moina sp. in May, and Daphnia pulex had been present in all three ponds, based on resting egg analysis. Janesville ponds were dominated by Daphnia magna. In the monograph by Brooks (1958), the range of Daphnia magna did not extend into Minnesota, but obviously it is present in the state, at least in waste stabilization ponds.

Field densities of Daphnia

The mean field densities of Daphnia and other cladocerans for the Harris, Janesville and St. Peter ponds are given in Tables 2 and 3. The maximum mean Daphnia density was 322/L, and the maximum single sample value was 1226/L in St. Peter pond 1. Sewage ponds are known to support Daphnia at densities far greater than occur in natural waters. In lakes, 30 Daphnia/liter would be a high peak density.

The seasonal changes in Daphnia and Simocephalus populations combined are plotted against chlorophyll and total suspended solids (TSS) for Harris in Figure 1, for Janesville in Figure 2, and for St. Peter in Figures 3 and 4. Of the three sites studied, Harris had the largest Daphnia population in April and May during the critical time before the first discharge. In St. Peter, there was a Daphnia population in May before the discharge, but at Janesville, the Daphnia did not develop until June and July, well after several discharges.

At Harris, the Daphnia pulex and Simocephalus sp. populations increased after the chlorophyll increased, especially in the spring, but also in fall (Figure 1). This is the expected effect of Daphnia populations in a well-functionning waste stabilization pond. As algae are increasing rapidly in spring, so do the Daphnia, and before the first major discharge from the pond, the Daphnia have consumed the algae, thereby reducing the solids levels in the ponds. See the algae section of this report for information on the relationship between chlorophyll levels and total suspended solids and other factors in the ponds.

At Harris, Daphnia populations did not really develop in pond 1, the primary pond. Pond 2 also had almost no Daphnia, with low densities April 23 and July 5. Pond 3 had a large population, peaking in May and June (201 - 183/L), and present through August. When submersed vegetation developed late in August, the cladoceran Simocephalus appeared from August 30 into fall, when no adult Daphnia were observed.

At Janesville, none of the ponds developed Daphnia populations in the spring. In pond 3, chlorophyll levels showed a definite increase in the discharge pond through the spring to extremely high values in the summer (Figure 2). Pond 3 had some Daphnia magna in June and July, when the filamentous bluegreen alga Oscillatoria was blooming (99-95% of algae), but the Daphnia could not control the massive bloom, nor could they develop large populations under these conditions. Oscillatoria is not considered an edible food for Daphnia.

The primary pond at Janesville was hydraulically overloaded, as evidenced by the frequent discharges through the year from the secondary pond (see outstate survey section). The facility had 27 discharges in four years (6.75 average/yr), compared with 10 for Harris (2.5/yr) and 16 (4/yr) for St. Peter. The frequent water transfers may have caused the massive algae blooms by providing renewed sources of nutrients to the algae.

At St. Peter pond 1, a large Daphnia magna population was present in June and October. Pond 1 was not serving as the primary pond in the winter of 1989-1990, as the study was beginning, but it began receiving the influent in May of 1990. Pond 2 had been the primary pond through the winter, and had no Daphnia in early spring. By summer of 1990, Daphnia developed in pond 2, and were present there in the fall.

Daphnia at St. Peter pond 3 were up and down. (Figures 3 and 4). The lower chlorophyll concentration on May 8 could be the result of the Daphnia magna and Moina populations present then, or the copper sulfate treatments in April. The lower densities of Daphnia in pond 3 in June and July are not caused by inedible algae: the types of algae present through spring into July were edible green algae. It is not clear from this data whether the low Daphnia densities in pond 3 were the result of low oxygen, low TSS and low chlorophyll, or some other factor such as predation.

Reproduction and size measurement

Reproduction data for Daphnia in the secondary ponds at the three sites are given in Figures 5, which shows the percent gravid females, or the percent of total females that were egg-bearing, and the mean number of eggs per gravid female. The Harris secondary pond shows a pattern typical for Daphnia in ponds: a spring and a fall peak in gravidity and in fecundity.

Harris pond 3 had a daytime peak of 9.3% of females bearing eggs on 4/23. During the rest of the spring and into summer, the gravidity is otherwise very low, from 0 - 0.6%. This is reflected in the high percentage of juvenile-sized Daphnia on most dates. The mean eggs per gravid female peaked on April 26 and 30 at 22-23 eggs/female. The large Daphnia pulex population that developed in May resulted from the late April egg production, but also could have originated from continuing recruitment from resting eggs in the sediments. The increase in gravidity and in eggs per female seen at Harris in the fall represents reproduction by Simocephalus. Only juvenile Daphnia were present in fall.

In Janesville pond 3, there is a definite lag in Daphnia magna reproduction in spring (Figure 6). Something appears to be inhibiting the April bloom of Daphnia in this pond. When Daphnia do appear at Janesville, the means of eggs/female in June and July are less than half those in the other two sites. Like St. Peter, Janesville Daphnia reproduction declines going into fall. It is as if the spring and fall peaks of reproduction are compressed into the summer at this site. The consequence is that the Daphnia are simply not present at the right time for clearing the water before the spring discharges. A major contributing factor must be the early presence of the bluegreen alga, Oscillatoria, which is not used by Daphnia.

In St. Peter gravidity of Daphnia magna was high in early May (over 30 eggs/f), and lower thereafter (Figure 7). The number of females that were egg-bearing increased from spring into fall. In contrast to Harris, the number of eggs/female at St. Peter declined in late fall, possibly because of the bluegreen algae bloom in pond 3.

Stratified and day-night zooplankton sampling.

Mid-summer analysis of Daphnia densities sampled at four depths showed no significant difference by depth at three times of day: afternoon, night and early morning (Figures 8, 9). At all four depths, there were higher densities of Daphnia at night than during the afternoon or morning. On 7/19, Harris pond 3 was 168 cm deep, with the secchi depth to the bottom. At the maximum depth sampled, the funnel for the pumping system was at 140 cm, but this presumably drew water from a few cm below the funnel opening. Placing the funnel deeper than this would pull up bottom sediment, and not Daphnia. If the Daphnia were right on the bottom during the day, one would expect to see more Daphnia at the 140 cm depth than the upper levels, which was not the case. It is doubtful that the Daphnia could avoid the funnel during the daylight and not in the dark, because Chaoborus, a much more active swimmer, was not sampled at higher densities at night. For the analyses in this study, the same sampling method was used at all three sights, but we may have underestimated the Daphnia populations because most of the sampling was done in the daytime.

Zooplankton densities in relation to water quality factors

For the statistical analyses, the combined densities of the filter-feeding cladocerans, or Daphnia, Simocephalus and Moina, were used. Results of regressions of cladoceran densities and several parameters measured in the ponds are given in Table (). Densities were very significantly related to pond water concentrations of calcium and magnesium, which are measures of the hardness of the water, and sulfate ($p<.001$). One can only conjecture whether greater water hardness might protect Daphnia from toxicants in the wastewater, or whether exoskeleton development and growth of these crustaceans are better at greater hardness.

Cladoceran densities were significantly related to the chlorophyll a ($p = .038$) and to the total suspended solids (TSS, $p = .002$), each with a negative slope. This means that when Daphnia populations are greater, the chlorophyll and solids are lower. This is consistent with the knowledge that Daphnia consume algae. See the algae section of this report for the significant relationship between TSS and chlorophyll concentrations. In the bioassays, the reproduction of test Daphnia was significantly related to chlorophyll for Harris with a positive slope, and for Janesville with a negative slope. In bioassays, the test Daphnia were added as juveniles and the chlorophyll level was the field measurement at the time the water was taken for the assays. For Harris, more young were produced when the water had more algae, but for Janesville, having higher chlorophyll had a negative effect.

The relationship between Daphnia densities and water clarity as log of the secchi depth is also highly significant ($p<.001$), that is, higher Daphnia densities occur in clearer water. Daphnia are often reported as causing the "clear water phase" in ponds in spring because of their feeding activity that filters out particulate matter, especially the algae from the water. The relationship here is consistent with the hypothesis that Daphnia clarify the water in stabilization ponds.

Daphnia field densities were not significantly related to the alkalinity of the ponds or to the pH (Table ()). There was a significant overall relationship between pH and Daphnia reproduction as young/female/day in the bioassays of St. Peter (see Bioassay section, and Table ()). The regression of the field densities and pH for the same dates as the bioassay was not significant. Daphnia densities were also significantly related to sodium, conductivity and iron, the latter a negative relationship.

Daphnia densities were not significantly related to other parameters: temperature, nitrate, unionized ammonia, phosphate (SRP). Unionized ammonia was present in the spring at toxic levels on some dates (see chemistry section), but this was not enough, apparently, to have a significant overall impact on the Daphnia populations. In the bioassays, ammonia was not significant for the acute and chronic Daphnia test (see Table () in the section on bioassays).

Overall conclusions from field zooplankton data

1. Densities of Daphnia ephippia in waste pond sediments are extremely high, and provide an 'egg bank' for recruitment of Daphnia populations when conditions are favorable.

2. Stabilization ponds were most often dominated by the cladocerans Daphnia magna and Daphnia pulex, but sometimes by Simocephalus sp. or Moina sp. Diaphanosoma sp. dominated one of the outstate sites.

3. Densities of Daphnia ranged seasonally and by site and pond. At Harris pond 3, the spring increase in algae was followed by a Daphnia increase, after which the algae declined. At Janesville, there was a major increase in algae, but Daphnia did not increase until early summer. At St. Peter, Daphnia appeared to fluctuate more widely, and surface swarms were sometimes present.

4. Daphnia reproduction was lower at Janesville than at St. Peter and Harris.

5. Daphnia did not appear to migrate vertically at Harris, as the distribution of densities was even at each depth. However, the fact that the nighttime densities were greater at all depths is unexplained.

An exploration of migration patterns under overnight oxygen stress could be interesting. At St. Peter, oxygen went to zero overnight, and surface swarms did occur, especially in the morning in calm water. Under these conditions, Daphnia might be migrating upwards as the night progresses and the oxygen continues to decline. At Harris, oxygen levels were high overnight.

6. Daphnia densities were significantly related to several water quality factors: significant positive relationships were with calcium, magnesium, conductivity, secchi depth, sulfate and sodium. Significant negative relationships were with TSS, iron and chlorophyll a. There was no significant relationship with pH.

7. Only a Harris was the relationship to chlorophyll significant and negative, consistent with the removal of the algae by the Daphnia. At Janesville and St. Peter, Daphnia densities and chlorophyll a levels were not significantly related.

8. Only at St. Peter was there a significant negative relation between Daphnia densities and dissolved oxygen. In the overnight respiration study, only St. Peter pond 3 went to zero oxygen overnight. Probably below 2 mg/L oxygen, Daphnia reproduction is inhibited.

Table 2. Field densities of Daphnia spp. for Harris, Janesville and St. Peter wastewater treatment ponds in 1990. Means are for triplicate samples at each pond, ± one standard deviation. Value of 0 means the sample was counted and there were no Daphnia. No sample is indicated by -.

Harris Daphnia/L

Date	Pond 1	Pond 2	Pond 3	Date	Pond 1	Pond 2	Pond 3
Apr-9	0	0	0.25 ± 0.23	Jun-22	0	0.01 ± 0.02	30.0 ± 18.93
Apr-12	0.04 ± 0.04	0.01 ± 0.02	0.50 ± 0.44	Jul-5	0.1 ± 0.08	0.3 ± 0.44	60.4 ± 10.50
Apr-16	0	0	0.34 ± 0.10	Jul-19	0.1 ± 0.08	0	12.1 ± 6.48
Apr-19	0.01 ± 0.02	0.03 ± 0.02	1.11 ± 0.59	Jul-20	-	-	5.5 ± 8.10
Apr-23	0	0.3 ± 0.06	0.54 ± 0.31	Aug-4	0.01 ± 0.02	0	17.0 ± 5.71
Apr-30	0	0.01 ± 0.02	17.26 ± 1.16	Aug-30	0	0	3.4 ± 5.68
May-7	0	0.05 ± 0.02	21.51 ± 8.05	Sep-13	0	0	0
May-14	0.03 ± 0.02	0.01 ± 0.02	149.13 ± 34.81	Sep-25	–	–	0
May-21	0.01 ± 0.02	0.01 ± 0.02	54.72 ± 30.26	Oct-9	0	0	0
May-29	0.07 ± 0.05	0.01 ± 0.02	201.45 ± 42.41	Oct-25	0.01 ± 0.02	0	0
Jun-6	0	0	183.48 ± 79.57	Nov-6	0	0.1 ± 0.07	0

Janesville Daphnia/L

Date	Pond 1	Pond 2	Pond 3
Apr-17	0	0	0
May-1	0.3 ± 0.247	0.1 ± 0.037	0.05 ± 0.062
May-15	1.1 ± 1.169	0.18 ± 0.249	0.04 ± 0.039
Jun-15	5.1 ± 2.206	0.1 ± 0.149	14.0 ± 9.95
Jul-12	0.5 ± 0.227	1.4 ± 0.695	12.2 ± 3.0
Aug-18	21.4 ± 18.134	0.2 ± 0.06	0.1 ± 0.046
Sep-18	–	–	0.2 ± 0.1
Oct-16	134 ± 32.006	2.26 ± 2.395	4.8 ± 3.763
Nov-13	–	–	1.2 ± 0.28

St. Peter Daphnia/L

Date	Pond 1	Pond 2	Pond 3
Apr-10	0.5 ± 0.60	–	0.05 ± 0.02
Apr-26	5.3 ± 2.31	0.2 ± 0.06	1.3 ± 0.12
May-08	3.9 ± 2.17	–	31.8 ± 25.33
Jun-28	322 ± 602.19	0.1 ± 0.04	0.1 ± 0.10
Jul-28	0	5.9 ± 0.69	5.4 ± 3.60
Aug-17	0.04 ± 0.00	3.4 ± 1.31	213 ± 356.49
Sep-18	–	–	0.03 ± 0.03
Oct-02	7.5 ± 2.21	31.8 ± 3.79	0.5 ± 0.45
Oct-31	49.4 ± 35.99	21.3 ± 10.66	15.5 ± 13.81
Nov-13	–	–	18.2 ± 8.70

Table 3. Field densities of Simocephalus sp. at Harris and Moina sp. at St. Peter and Janesville wastewater treatment ponds, 1990. Means are for triplicate samples, ± sd. May 8 St. Peter had one sample only.

Simocephalus/L at Harris ponds.

Date	Pond 1	Pond 2	Pond 3
Aug-30	0	0	3.7 ± 5.48
Sep-13	0.3 ± 0.14	0.01 ± 0.02	6.6 ± 10.54
Sep-25	-	-	5.0 ± 5.17
Oct-9	0	0	0
Oct-25	0	0	53.0 ± 44.72
Nov-6	0	0	24.5 ± 0.65

Moina/L at St. Peter

Date	Pond 1	Pond 2	Pond 3
Apr-10	0	-	0
Apr-26	31.0 ± 5.47	0	0
May-08	253.8	-	66.1
Jun-28	0	0	0
Jul-28	41.1 ± 27.02	2.1 ± 0.13	0
Aug-17	2.6 ± 3.30	0.06 ± 0.04	0.04 ± 0.07
Sep-18	-	-	0
Oct-02	0	0	0
Oct-31	0	0	0
Nov-13	-	-	0

Moina/L at Janesville ponds.

Date	Pond 1	Pond 2	Pond 3
Apr-17	0	0	0
May-01	0	0	0.01 ± 0.02
May-15	0	0	0.4 ± 0.42
Jun-15	21.0 ± 6.73	0.1 ± 0.15	1.8 ± 3.10
Jul-12	0	0	0
Aug-18	0	0	0
Sep-18	-	-	0
Oct-16	0	0	0
Nov-13	–	-	0

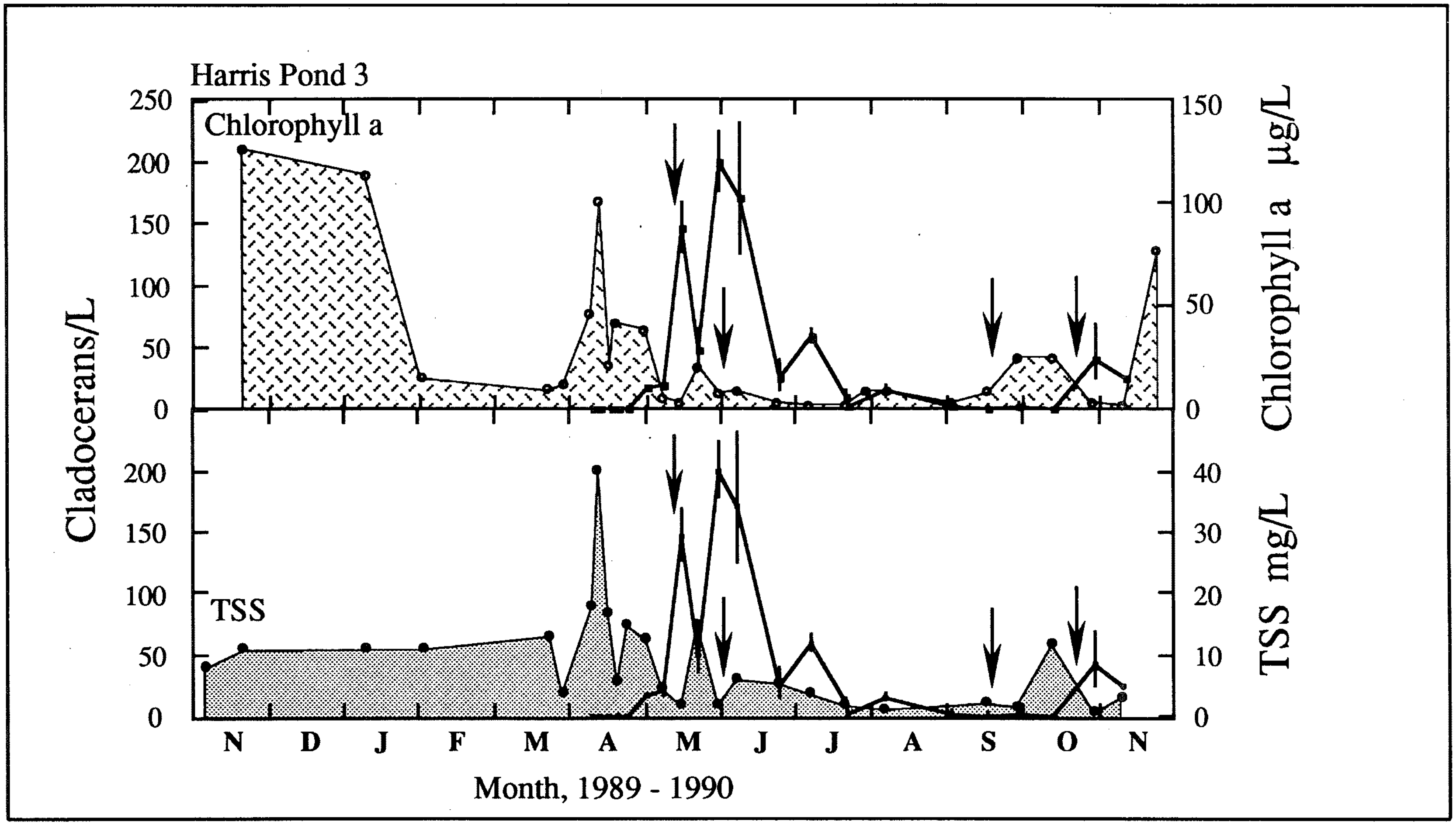


Figure 1. Seasonal changes in cladoceran (Daphnia plus Simocephalus), densities from April through November, 1990, and total suspended solids (TSS) from November, 1989 through November, 1990 in Harris pond 3. [TSS symbol] = TSS, [chlorophyll symbol] = chlorophyll a, µg/L, ——➤ = beginning of discharges, —■— = cladocerans/L.

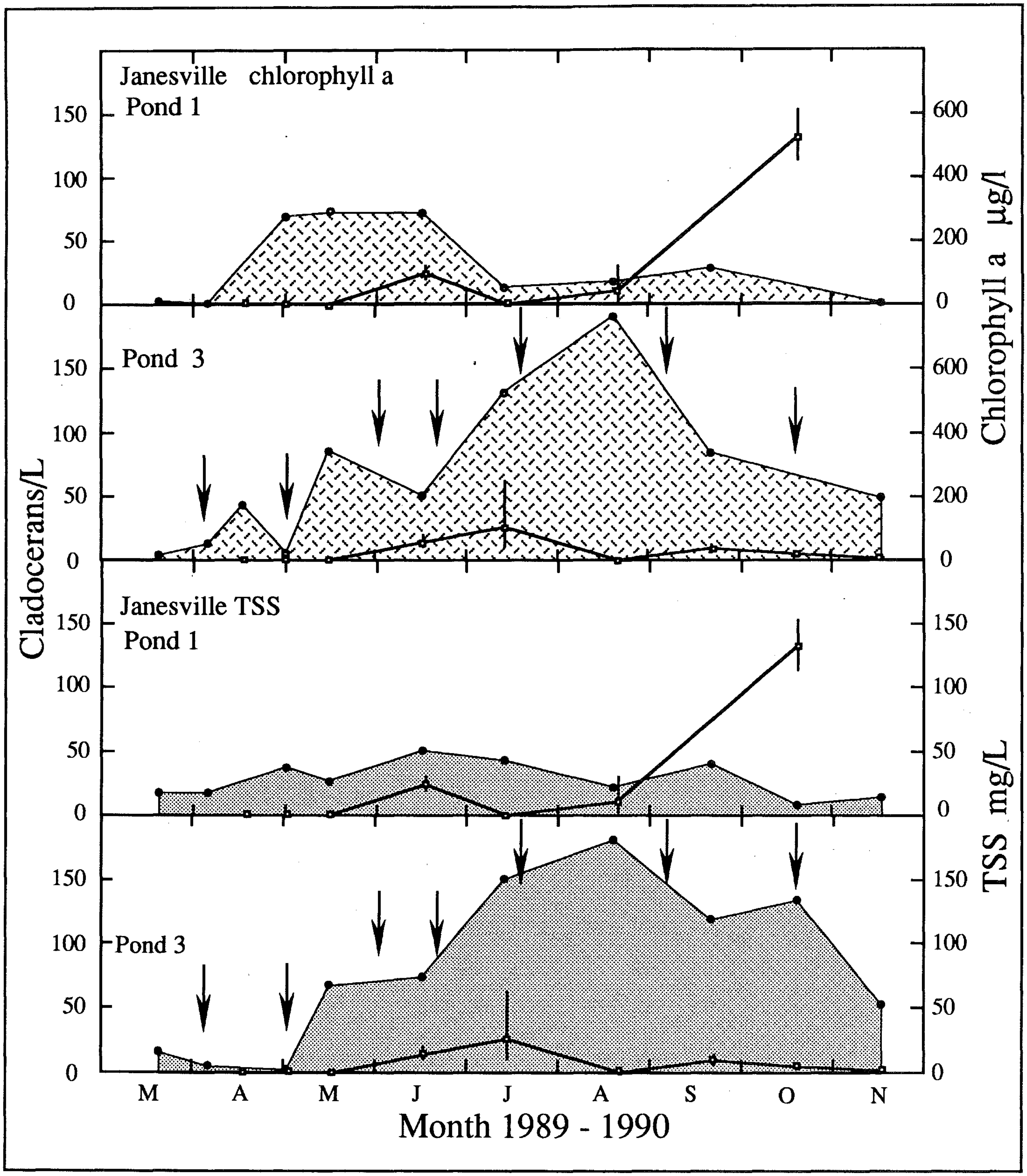


Figure 2. Seasonal changes in cladoceran, or Daphnia plus Moina, densities from April through November 1990, with chlorophyll a (top) and total suspended solids (bottom) in Janesville stabilization ponds 1 and 3 from November, 1989 through November, 1990. [chlorophyll a symbol] = chlorophyll a, [TSS symbol] = TSS, ▫ = cladocerans/L, ⟶ = discharges.

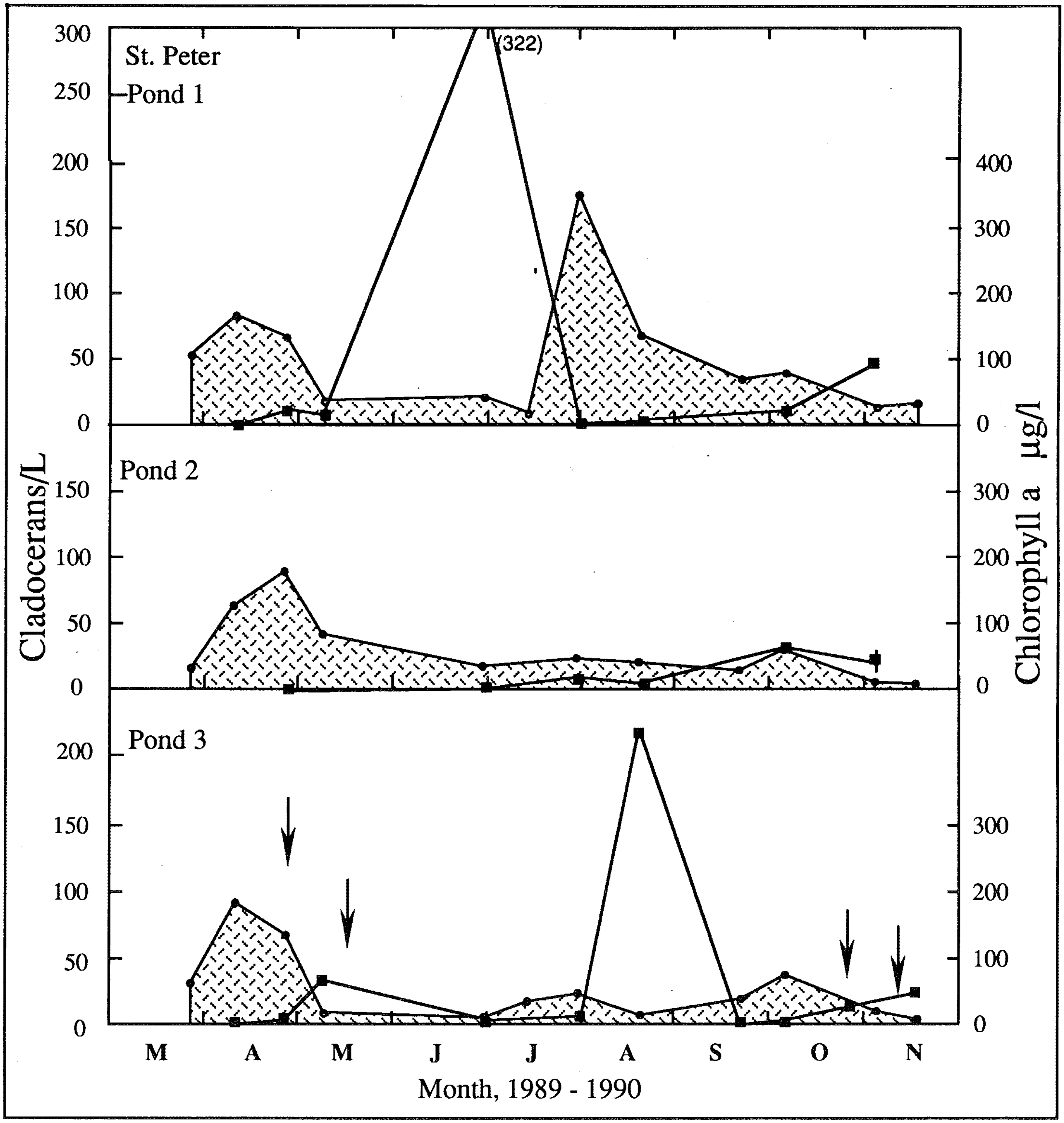


Figure 3. Seasonal changes in cladoceran, or Daphnia plus Moina, densities and chlorophyll a from April through November, 1990 in the three stabilization ponds at St. Peter, MN. [legend symbol] = chlorophyll a, ■ = cladocerans/L, ⟶ = beginning of discharge events.

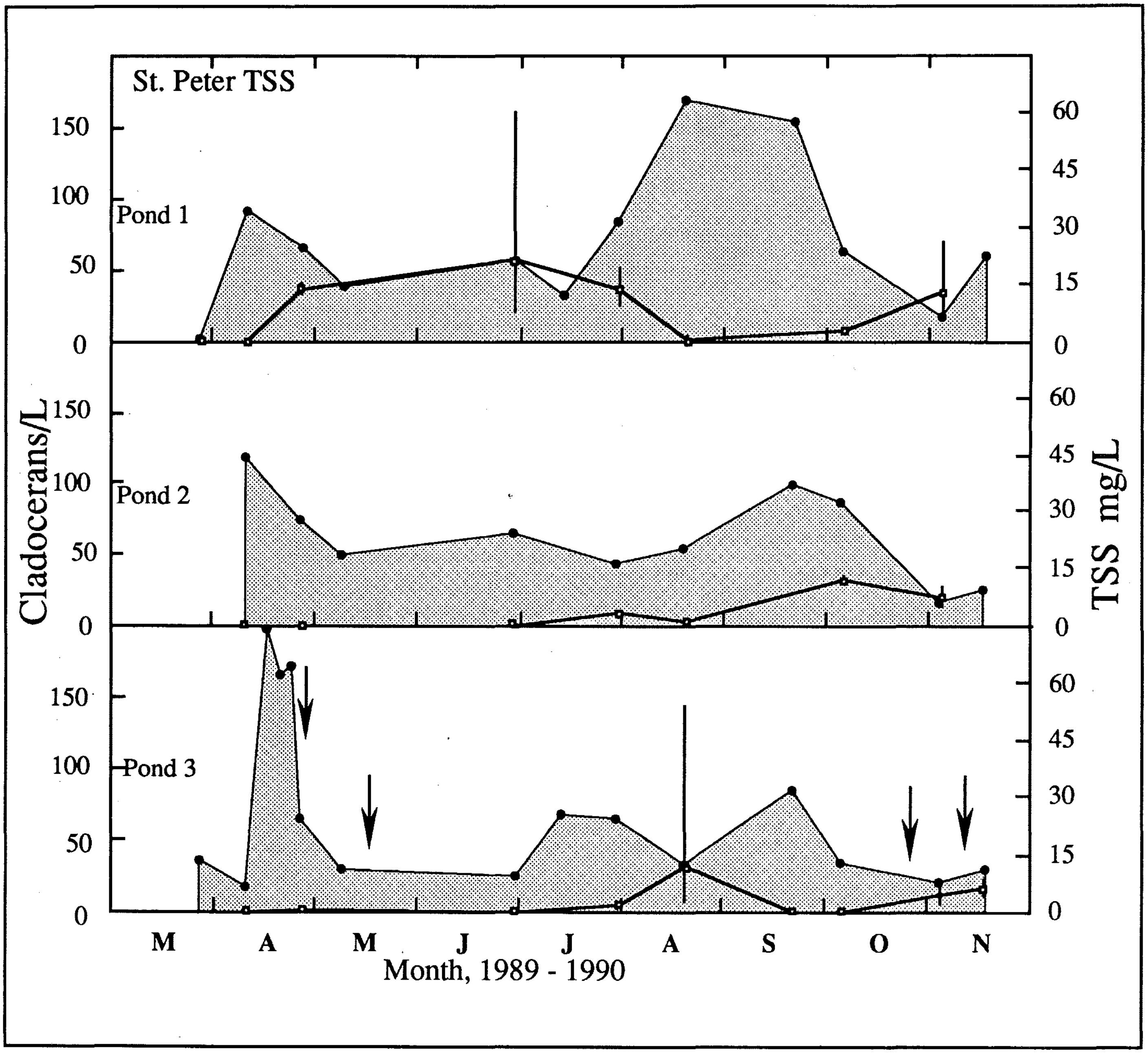


Figure 4. Seasonal changes in cladoceran (Daphnia plus Moina) densities from April through November 1990, with total suspended solids (TSS) in St. Peter stabilization ponds 1, 2 and 3. ⟶ = beginning of discharges, [legend symbol] = TSS mg/L, ▫ = cladocerans/L.

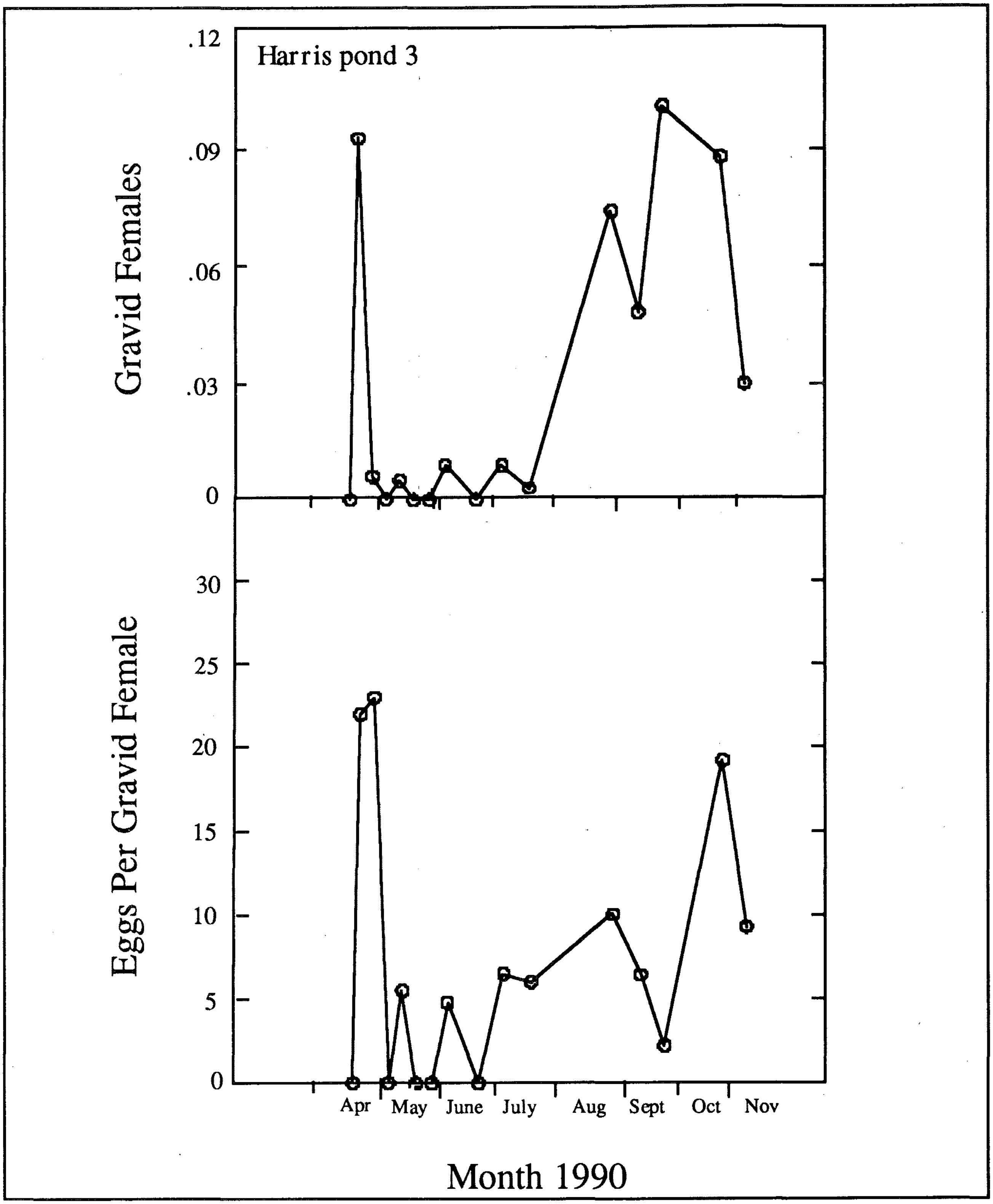


Figure 5. Reproduction of Daphnia plus Simocephalus in Harris pond 3, percent of all females that are egg bearing, or gravid, and mean number of eggs per gravid female.

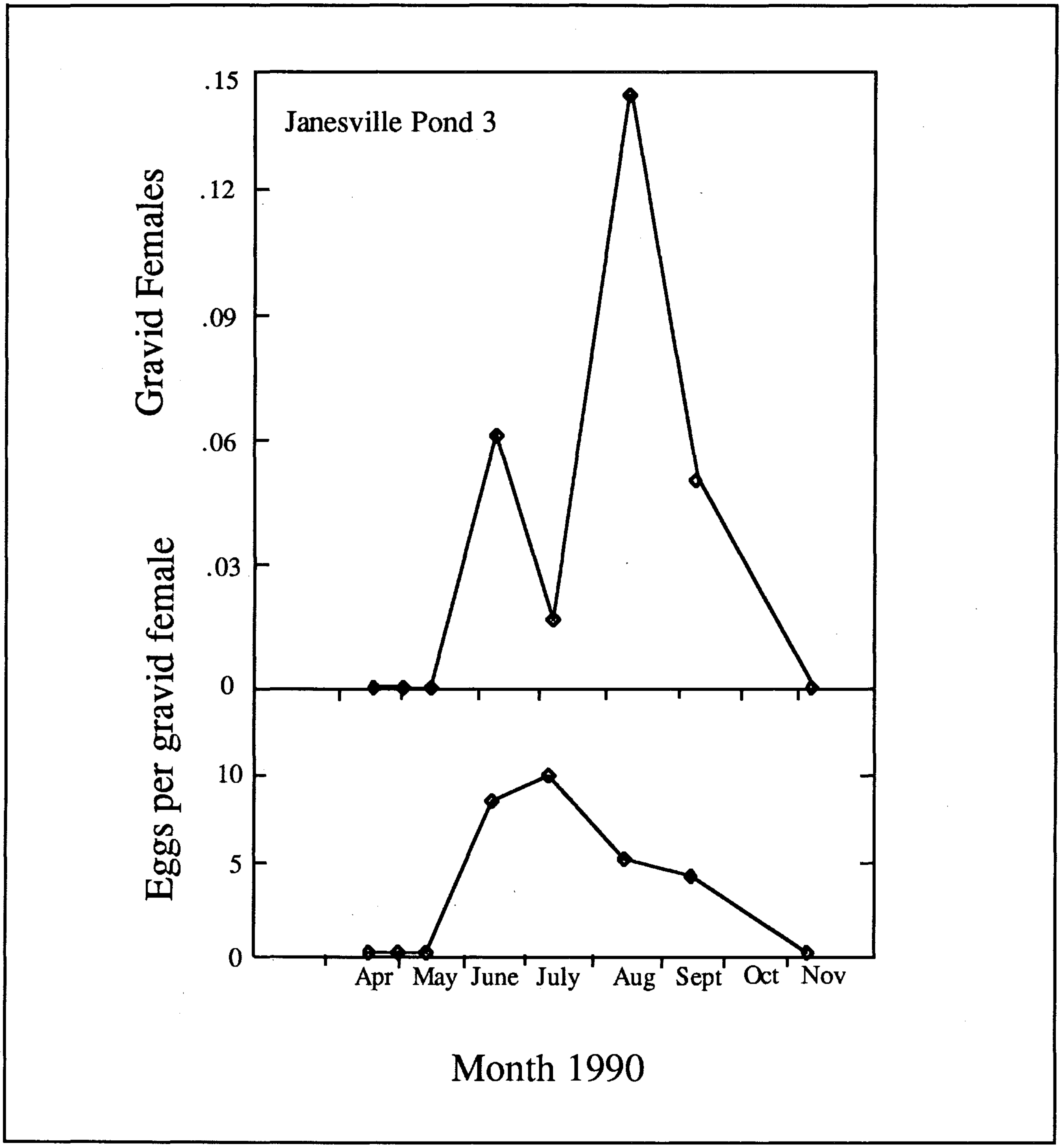


Figure 6. Reproduction of Daphnia in Janesville pond 3 by date, given as percent of all females that are gravid, and eggs per gravid female.

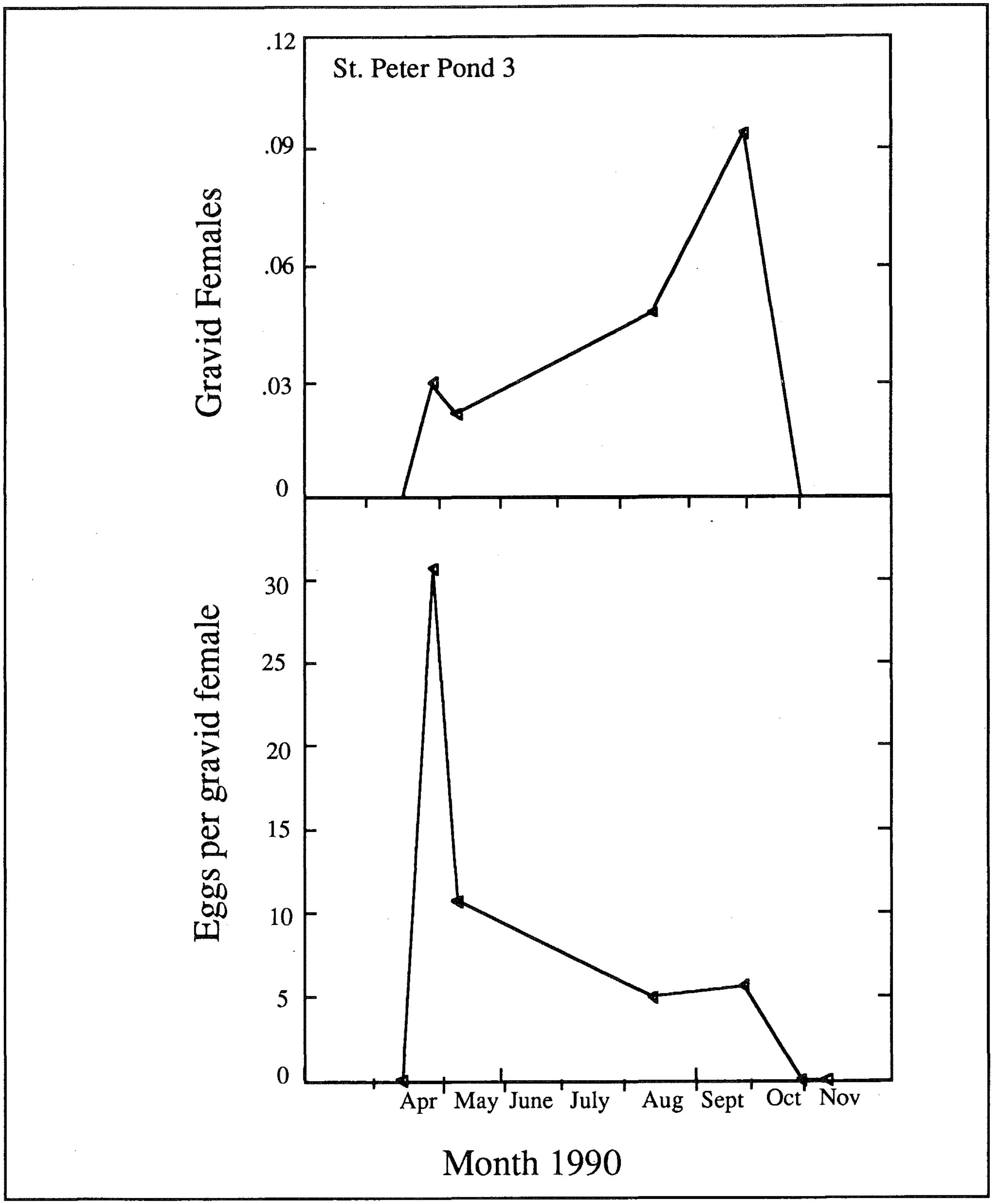


Figure 7. Reproduction of Daphnia in St. Peter pond 3, given as percentage of females that are egg-bearing and eggs per gravid female.

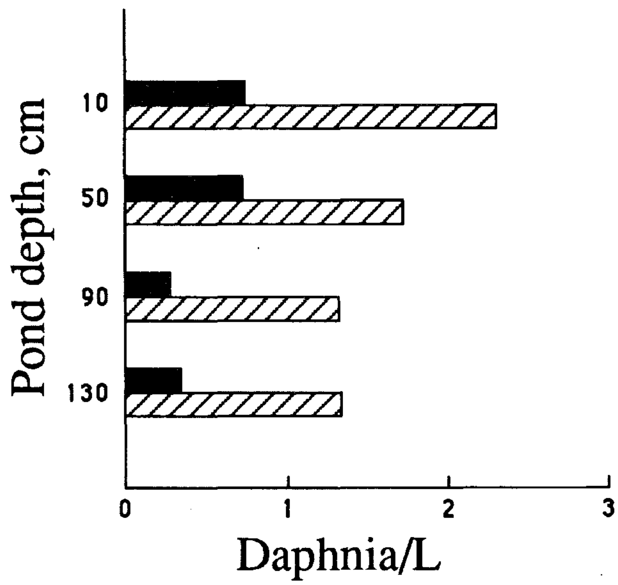


Figure 8 Daphnia distributions by depth in Harris pond 3 on March 23, 1990.
▨ = afternoon density ■ = evening density

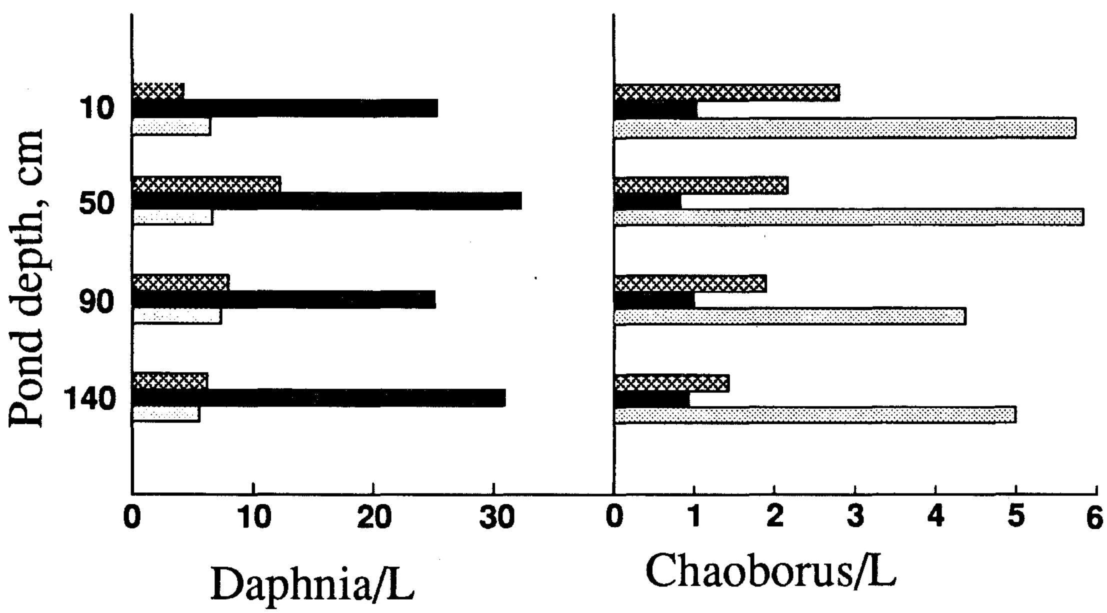


Figure 9. Vertical distribution of Daphnia and Chaoborus in Harris pond 3 on July 19 - July 20, 1990. ▩ = 7/19 afternoon, ■ = 7/19 late evening, ░ = 7/20 morning.

Table 4. Regressions of number of cladocerans/L (log transformed) against several parameters of waste stabilization ponds. 'All data' is from all three ponds at Harris, Janesville and St. Peter through 1990. Analyses by site are shown also. P values with * are statistically significant, r = regression coefficient, n = number of sampling dates.

	p	r	n	slope
All data				
Calcium	<.001*	.742	21	.051
Log conductivity	<.001*	.867	21	5.316
Magnesium	<.001*	.747	21	.258
Log secchi depth	<.001*	.348	105	.718
Sulfate	<.001*	.789	15	.023
Sodium	.001*	.656	21	.010
Log TSS	.002*	.298	102	-.890
Iron	.009*	.470	30	-.006
Log chlorophyll a	.038*	.250	69	-.544
Temperature	.155	.140	105	.028
Total organic carbon	.169	.161	75	-.030
Potassium	.364	.209	21	.020
Nitrate	.416	.092	80	-.003
Alkalinity	.459	.088	73	-.002
Log unionized NH3	.503	.065	108	-.116
SRP phosphate	.594	.062	77	-.091
pH	.526	.062	108	.143
Harris (all data)				
Log Chlorophyll a	<.001*	.565	41	-1.071
DO	.741	.044	58	.008
Log unionized NH3	.473	.094	60	-.150
pH	.130	.198	60	.420
Janesville (all data)				
Log Chlorophyll a	.110	.509	11	1.146
DO	.120	.359	20	.044
Log unionized NH3	.194	.281	23	-.510
pH	.287	.232	23	.471
St. Peter (all data)				
Log Chlorophyll a	.984	.032	17	.090
DO	.001*	.623	24	-.158
Log unionized NH3	.995	.001	25	.002
pH	.199	.266	25	-.631

Bioassays with waste stabilization pond water

These tests were designed to try to determine if survivorship and reproduction of the cladoceran Daphnia magna were affected by exposure to waters from waste stabilization ponds.

Methods for bioassays

The method used was modified from the US EPA short-term methods for estimating chronic toxicity of effluents (see Horning and Weber, Dec.1985; Weber, Reltier and Norberg-King, 1989). For the tests reported here, Daphnia magna was the test organism used, instead of Ceriodaphnia dubia. Bioassays were conducted by MPCA personnel and by an assistant supported by this project.

In the tests reported below, supplemental food was not added except in the tests of summer waste pond waters where "fed and unfed" Daphnia were analyzed. The tests were done by static renewal of waste pond water, rather than continuous flow. Renewals were usually at 24 and sometimes at 48 hour intervals. Tests were initiated within 24 hours of collection of the samples by grab sampler from ponds. Water samples were stored in 4 liter plastic cubitainers at or below $4^oC \pm 2$ between renewals, and warmed to room temperature ($24^oC \pm 3$) by placing the vessels in a warm water bath. The wastewater samples were pre-filtered through a 150 μm^3 nylon net screen to remove any native Daphnia while allowing passage of the algae. The tests were conducted under fluorescent lights at a photoperiod of 16L:8D at $24^oC \pm 2$. Each assay consisted of two 250 ml glass beakers each containing 200 ml of test water and five Daphnia magna, <24 h old, or a total of ten Daphnia per test. Daphnia magna were originally supplied by the U.S. EPA Environmental Research Lab, Duluth, Minnesota in 1978.

Because it is a chronic test covering the development from newborn to reproducing adult, the endpoint of the test is reproduction. The Daphnia magna generally produced their first brood of young after seven to eight days in the test water, and usually three to four broods were produced within the 10 - 12 days exposure time of the test. Young were counted and removed each day, and mortality of the original test females was recorded. An effect would be a significant reduction in young production in the wastewater when compared to a control or other water source.

The daily reproductive rate, or fecundity of the Daphnia as young/female/day (yfd^{-1}) was calculated and tested statistically. Tests varied in number of days run, so yfd^{-1} was considered a better measure to compare results than total young produced per female during the test. Yfd^{-1} is calculated as shown below:

$$yfd^{-1} = \text{Sum } [y_1/f_1 + y_2/f_2 + \ldots.. y_n/f_n]/\text{days*}$$

* = days means days of production of young, i.e., counted from the first day young were observed through the day the last count of young was made.

Waste pond water was taken from the Harris stabilization ponds and tested on 11/6/89, 1/10, 2/2, 5/1, 5/29, and 7/6/90, from Janesville on 11/14/89 and 5/2, 5/10 and 7/13/90, from St. Peter on 11/14/89, and 4/27, 5/10, 7/13, and 7/29/90 and from Browerville, Long Prairie, Kiester and Mapleton in November, 1989.

Both pH and ammonia were measured at the beginning of each bioassay. pH was tested with a calibrated Electronic pH Paper ± 0.1, model 5841-00 from Cole-Parmer. Ammonia was measured with an Orion 407A meter with a 95-12 probe +-0.1 ionalyzer calibrated at each use. These parameters were related to fecundity of Daphnia by analysis of variance (ANOVA) and multiple regression techniques.

Method for 'fed' and 'unfed' test

It became apparent as the project progressed that there was wide variation in the amount and potential quality of the food, or algae, in the waste pond water, and that this might affect the resulting reproduction by Daphnia in bioassays. For example, the water of a pond that is low in algae may appear to be toxic by not supporting Daphnia reproduction, if the Daphnia were not given supplemental food.

The supplemental food for the "fed" tests was a daily one ml feeding of the EPA Ceriodaphnia food solution (TCY) of yeast, commercial fish food and the dehydrated green plant material, Cerophyll, the same food used to support the stock Daphnia cultures.

Statistical analyses such as Student's t tests and ANOVA's were performed on the fecundity data, and two-way ANOVA's were run on pH and ammonia in relation to young production results. All analyses were run on the Macintosh 2ci computer with SYSTAT 5.0 for statistical and plotting programs, and EXCEL 3.0 for data handling.

Results of bioassays and discussion

Mortality of Daphnia magna in bioassays

The main difference in pond response was an absence of Daphnia mortality in water from Harris pond 3, and the presence of frequent mortalities seen in St. Peter pond 3 water. The lack of mortality of test Daphnia in bioassays in Harris pond 3 water is consistent with the other measurements of water quality in this secondary pond: high dissolved oxygen (DO) levels, good water clarity, low suspended solids and low ammonia.

Of the 80 bioassays (counting replicates) performed on Harris waste pond water, two bioassays of pond 1 and one of pond 2 had 100 percent mortality. Mortality in pond 1 occurred in 1/10/90 water, and is not surprising since the water under the ice was accumulating influent material. Field measured dissolved oxygen (DO) was zero, lab measured was 0.5 on that date. There were no mortalities in bioassays of pond 3 at Harris.

Of the 26 bioassays on Janesville water, there was some mortality with 7/13/90 water from pond 3, 100 percent in the unfed test, 20 percent in the fed test. At this time, the filamentous bluegreen alga, Oscillatoria, was 95 percent of the algae in pond 3 water, and this may explain the mortality in the unfed test, since this algae is not considered edible food for Daphnia (see section on fed and unfed test results, and section on algae composition of the ponds).

Of the 30 bioassays on St. Peter waste water, half of the pond 3 'fed' assays had mortalities, from 20 - 60 percent, and half of the 'unfed' assays had mortalities, from 20 - 80 percent. Mortality in pond 3 seems independent of the laboratory test food regime, and does not seem to relate to the algal composition of the St. Peter ponds at the time. In pond 3, the algae on 7/13 were predominantly edible green algae, and on 7/29 a colonial blue-green alga, Merismopedia, dominated pond 3 (60%), but 40 percent of the algae consisted of edible greens. It is not known if Merismopedia has any toxicity against Daphnia. Pond 2 water caused no mortalities, and in one out of six assays on pond 1 water, there was 20 percent mortality.

The mortalities in St. Peter pond 3 water are unexplained on the basis of algae or food regime. This pond was treated with copper sulfate for algae control in the spring, but it is considered unlikely that copper toxicity could be released from the carbonate-bound form in the sediments.

Bioassays of water from Browerville, Kiester, Mapleton and Long Prairie secondary ponds resulted in mortalities of Daphnia only in the water from Kiester from both the primary and the secondary ponds. Both ponds had substantial populations of edible green algae (90% greens, and high chlorophyll values), so food was abundant. These mortalities are unexplained on the basis of food availability. pH was fairly high (9.35, 9.37), but ammonia was low compared to other sites, unusually low in the primary pond (0.5 mg/L). Oxygen was high in Kiester pond water (ca 13 mg/L), indicating algae activity. Reproduction was also strongly inhibited (see below).

Reproduction (yfd^{-1}) of Daphnia magna in bioassays

Harris bioassays

The data on Daphnia fecundity, given as young per female per day (yfd^{-1}) is summarized for Harris pond waters in Figure (). This seasonal plot shows the reduced reproduction during the winter especially in water from ponds 1 and 2. As noted above, there was almost zero oxygen in pond 1 Harris test water in January, and only 1.2 mg/L DO in February. Pond 2 was almost as low in oxygen in these two months. In addition, algae were not seen in Harris pond 1 and two samples from these two winter months, and chlorophyll levels were low. Bacteria, which could be a food source for Daphnia, were not measured.

Pond 3 water at Harris was extremely clear in May and June, and chlorophyll dropped sharply in pond 3 in May through July, meaning the concentrations of food or algae were very low in this pond. Since Daphnia reproduction is strongly food-dependent, the reproduction seen in pond 3 is very low in bioassays, especially in May and July.

Other bioassays

In Janesville, reproduction was low in November in all 3 ponds. The first pond lacked any chlorophyll, so food was low, and the third pond was dominated by inedible bluegreen algae. There is no explanation for the lower reproduction observed in the bioassays with water from the second pond, since green algae dominated, and pH, DO and ammonia weren't extreme. Reproduction in Pond 3 was high in May and low in July. The algae in Pond 3 were predominantly edible greens in May, and nonedible filamentous bluegreen algae in July.

Reproduction in bioassays of St. Peter wastewater was better in the first and second ponds in May than in the secondary pond (pond 3). Edible green algae dominated all three ponds in May. Chlorophyll was lower (14.7 µg/L) in the third pond than in ponds 1 and 2 (32, 80 µg/L), but not so low that reproduction would be so sharply reduced (mean yfd^{-1} 0.58). It would appear that there is some other source of toxicity to the Daphnia in St. Peter pond 3 water such that reproduction is inhibited. Water from this pond taken on 4/26 supported excellent reproduction in bioassay tests, and had dissolved oxygen at 10 mg/L. By 5/8, dissolved oxygen was very low (1.2 mg/L) in pond 3 bioassay water.

The treatment of algae with copper sulfate could cause a loss of photosynthetic output of oxygen in the pond. The pond had been treated with copper sulfate on April 4 and again on April 24th. It was discharged beginning April 27 and again May 16. The operator noticed a decline in oxygen levels in the secondary pond from May 11 to May 16, after pond 2 water was moved overland to pond 3. By July 12, St. Peter pond 3 had Daphnia obviously present, and the bioassay shows better reproduction occurring in pond 3 water.

Waste water taken in November from Browerville, Long Prairie, Mapleton and Kiester supported low reproduction in assays. Reproduction was lowest in Kiester primary and secondary water (0.4 - 1.1 yfd^{-1}), and Long Prairie pond 1. The low reproduction in Long Prairie pond 1 is not surprising: the oxygen was very low, 1.5 mg/L. In spite of the high ammonia (42 mg/L NH_3N), there was no mortality in test females, probably because of the low pH (7.86).

Fed versus unfed bioassays

The low reproduction by Daphnia in Harris pond 3 bioassays led to a study where the assays were carried out with the Daphnia given supplemental food ("fed" test), or just the wastewater ("unfed" test) as described above in the methods section. In this experiment, the pond 3 water from Harris was not tested.

The results of the unfed and fed bioassays are displayed in Figure (). In Student's t tests and single factor ANOVA analysis, there was no significant difference of fed vs unfed when all ponds and sites were pooled and tested. Tests within St. Peter and Harris (pond 2 only, pond 3 was not tested) showed no significant difference between fed and unfed Daphnia reproduction. In Janesville, however, the ANOVA showed significance between fed Daphnia (pond 1 and 3) and unfed (pond 1 and 3), and between all pond 3 and all pond 1 reproduction. Note that reproduction was higher in pond 1 (fed and unfed) than in pond 3 (fed and unfed). Chlorophyll was adequate (near 50 µg/L) in pond 1 and extremely high in pond 3 (over 500 µg/L). On 7/12 pond 1 was dominated by edible green algae (Pandorina, 70%), and pond 3 by inedible filamentous bluegreens (Oscillatoria, 95%). Separate t tests on the Janesville and St. Peter fed and unfed tests.

In the U.S. EPA's standard test procedure, it is recognized that the amount of food present in the test water may affect the results of the bioassay, and that any food added during the test may confound results by sequestering metals or other toxic substances (Horning and Weber, 1985, p. 59). It is preferable to run toxicity tests without added organic matter (i.e. food) that would absorb or otherwise affect the toxicants being tested. However, in a chronic test of Daphnia reproduction, the results may be affected greatly by the type and concentration of available algae contained with the water sample being tested.

Statistical test of chemical parameters and bioassay reproduction results

The simple linear regression tests for all bioassay reproduction data are given in Table (). The data used was from the bioassays other than the fed vs unfed test, that is, the Daphnia were not given supplemental food, nor was the algae filtered out of the water. The effect of pH on reproduction was significant for St. Peter water only. Chlorophyll was a significant factor in Harris pond water where chlorophyll, and a significant negative factor at Janesville where the high chlorophyll was from inedible algae. Ammonia was not quite significantly related to Daphnia reproduction ($p = .094$).

Overall conclusions from bioassay data

1. Statistical tests relating Daphnia reproduction to water quality showed a significant relationship ($P = .033$) overall between reproduction (yfd or young/female/day) and dissolved

oxygen (DO). This relationship was significant for Harris bioassays (P=.018), but not for Janesville or St. Peter bioassays alone. At Harris, depressed oxygen levels occurred in the winter water samples, especially in ponds 1 and 2 in January and February

2. Overall the relationship of pH and reproduction in bioassays is not significant at the .05 level (P=.071), but it was significant at St. Peter alone (p=.03). The relationship is such that reproduction is increasing with pH. What causes this relationship is unclear. Higher pH occurs during active photosynthesis, and lower pH is known to inhibit feeding rates in Daphnia (Hebert, 1978).

3. The fed and unfed bioassays showed a significant difference only in the tests on Janesville water, where supplemental feeding improved reproduction in Daphnia tested in pond 3 water. This was corroborated by the significant negative relationship of chlorophyll and reproduction in Janesville bioassays when the inedible bluegreen alga Oscillatoria was not filtered out. As Oscillatoria concentrations increased, Daphnia reproduction decreased. In spite of very high levels of chlorophyll at Janesville, the reproduction of the Daphnia was impaired.

4. When testing for the presence of a toxic substance, supplemental feeding of test Daphnia in bioassays of waste water is necessary, because there could be an inedible food present in the water.

5. Ammonia was not quite a significant factor in toxicity to Daphnia in these assays (P = .094 overall). Unionized ammonia can be toxic to Daphnia in the 1 - 3 mg/L range, although data is limited (U.S. EPA, 1985, Ambient Water Quality Criteria for Ammonia). Levels of unionized ammonia in test water were over 1.0 mg/L for all three ponds at Harris (5/29), for Janesville ponds 1 and 2 on 11/13/89 and 5/1, and St. Peter ponds in November, April and May. In spite of several tests with St. Peter water at high initial unionized NH_3 levels, reproduction was not significantly reduced, and mortalities that occurred did not relate to ammonia levels. We did not measure ammonia at the end of the test, however. In the field situation, one would expect some mortalities when ammonia is high and a rise in pH from photosynthesis occurs.

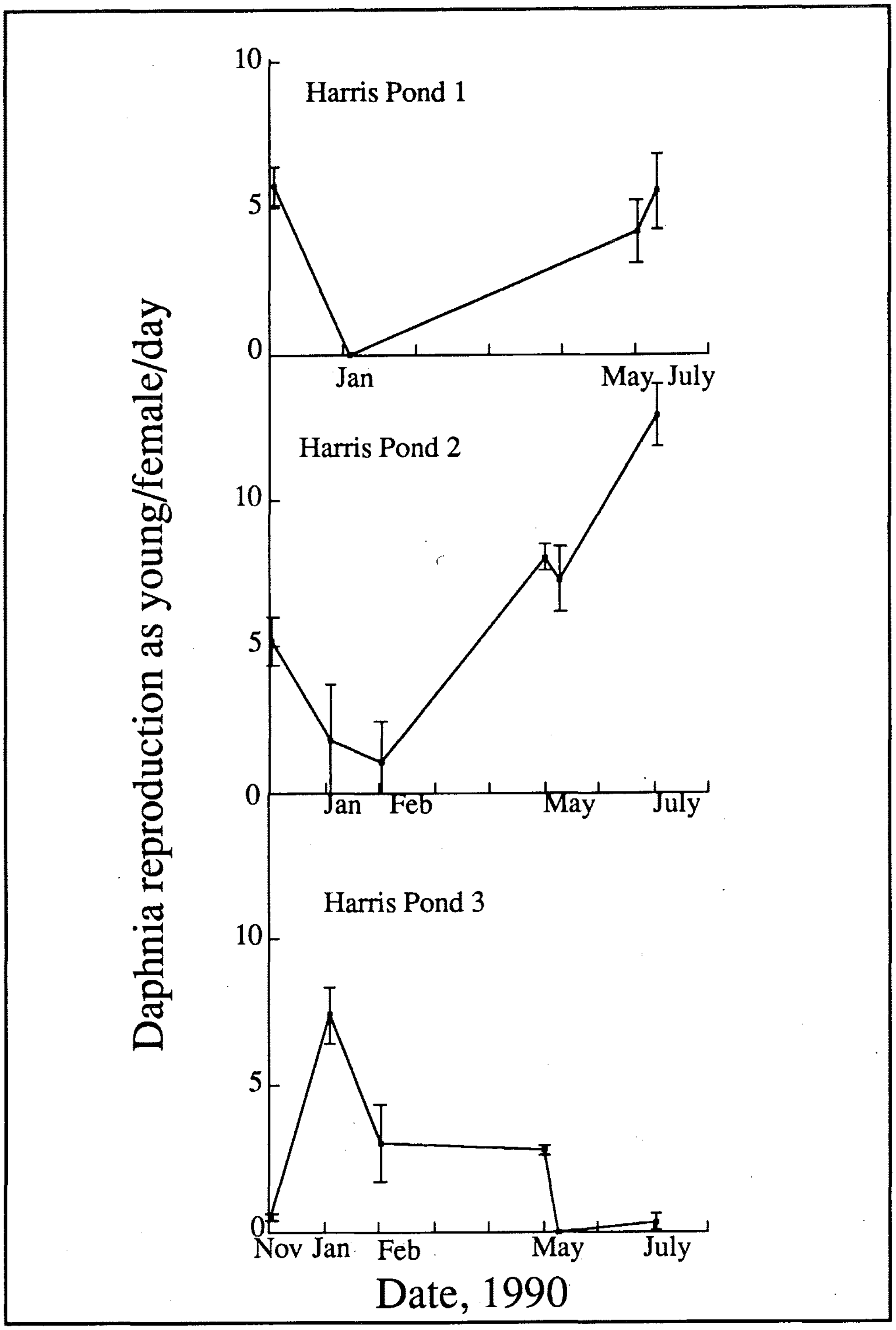


Figure 10. Daphnia reproduction in bioassays with waste stabilization pond water from Harris ponds on different dates in 1989-1990. Reproduction is the number of young produced per adult female per day. Bars are one SD.

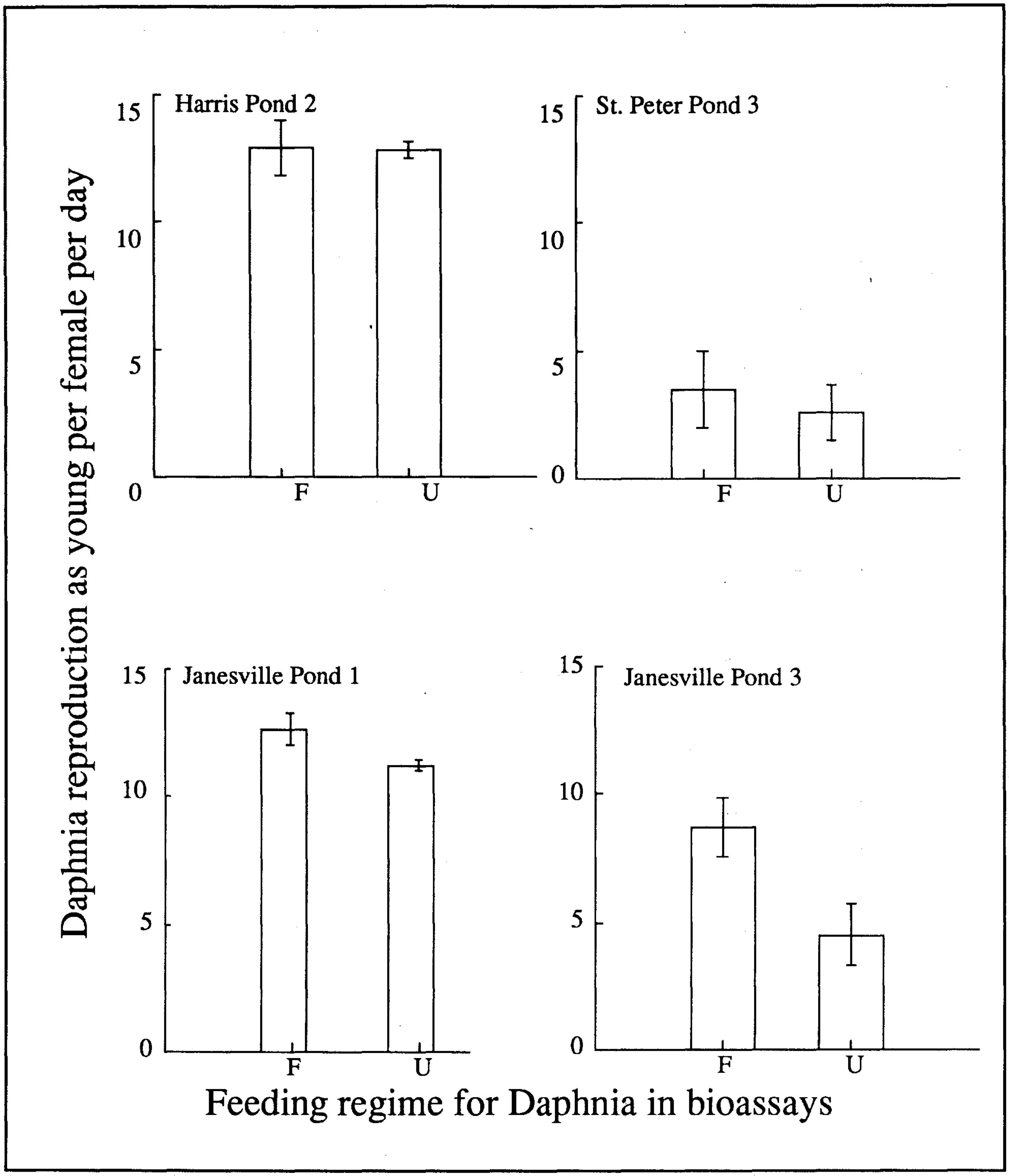


Figure 11. Daphnia reproduction in bioassays of waste stabilization pond water on Daphnia magna with two feeding regimes. F = fed supplemental food, U = unfed, in unfiltered pond water. Bars are one SD.

Table 5. Regressions of Daphnia reproduction in bioassays (yfd = young/female/day) with chlorophyll a (log transformed), dissolved oxygen (DO), ammonia and pH. 'All sites' includes Harris, Janesville, St. Peter plus Browerville, Long Prairie, Mapleton and Kiester. Supplemental food was not added, and native algae were not filtered out of the pond water. * = significant result (p<.05), r = regression coefficient.

<u>All sites bioassay reproduction data</u>

	p	r	n	slope
Chlorophyll a	.262	.130	77	.698
DO	.033*	.227	89	.195
NH3	.094	.170	98	.752
pH	.071	.182	99	1.535

<u>Harris bioassay reproduction data</u>

	p	r	n	slope
Chlorophyll a	<.001*	.668	24	3.358
DO	.018*	.417	32	.277
NH3	.157	.256	32	-.086
pH	.124	.278	32	1.583

<u>Janesville bioassay reproduction data</u>

	p	r	n	slope
Chlorophyll a	<.001*	.820	14	-7.343
DO	.286	.284	16	.369
NH3	.411	.200	19	.918
pH	.623	.117	20	2.059

<u>St. Peter bioassay reproduction data</u>

	p	r	n	slope
Chlorophyll a	.220	.254	25	-1.579
DO	.962	.011	23	.009
NH3	.489	.134	29	.448
pH	.030*	.403	29	3.538

Analysis of waste pond algae

Methods of algae analysis

In this section, the methods for analyzing the algae will be described first. Results summarizing the seasonal succession of the dominant types of algae and the percent of the total that were bluegreen algae will be given. Finally, the algae, as represented by chlorophyll measurements, will be related to the various water quality chemical measurements.

It was important to estimate the relative abundances of different kinds of algae, because we wanted to know whether there were algae present of the type that Daphnia could eat, or whether the "inedible" algae were predominating. The bluegreen algae are generally considered to be inedible for Daphnia, and not a nutritious food for good reproduction. The green algae are good food for Daphnia, and frequently dominated the ponds. The relative abundance of bluegreen algae, or the percentage of the total algae, was related to the water chemistry of the ponds in the outstate survey sites, and to parameters of pond performance, such as the number of TSS violations.

Method for chlorophyll a analysis

Routine chlorophyll a analyses used for much of the analysis in this report were performed in Dr. Brezonik's laboratory at the University of Minnesota. Pond water was filtered within four hours of sample collection through glass fiber GF/C filters, and the filters were stored frozen in small petri dishes wrapped to exclude light. Chlorophyll a was determined after 90% acetone extraction of chlorophyll from the glass fiber filters. Chlorophyll and phaeophytin were determined as described in Standard Methods (APHA, 1989).

Method for rapid assessment of algae relative abundances

Algae were collected on each sampling date from the pooled grab samples used for chemical analysis for each pond at a site, and preserved in Lugol's solution (Standard Methods, 1989) in glass vials with plastic-lined caps. The grab samples were usually taken from three to four locations on one side of the pond, and pooled in a large jug. Algae were analyzed by a rapid assessment technique, where subjective estimates of relative abundances of major algal groups or genera were recorded from wetmounts on slides viewed at 400x and 1,000x. The intention was to describe in a general way the major differences among the ponds by season, and to understand if algae edible by Daphnia were present.

Results of algae analysis

Seasonal succession in waste ponds

A narrative overview of the successional changes in each pond is given below. This data is based on the rapid assessment procedure with the microscope and confirmed with the HPLC pigment analysis method described above and in Appendix (). The genera and groups of algae in the ponds are summarized in Table () with the number of sampling dates given when the alga dominated. Dominance means either over 50% abundance, or adding to over 50%. The percentages of bluegreen algae during fall of 1990 are listed in Table () with the 'outstate survey' data.

The green algae are important in the waste stabilization ponds as producers of oxygen for aerobic decomposition. Green algae grow better than bluegreen algae when conditions that foster

bluegreen algae, such as high pH and low light, are not present. Some of the green algae common in
waste ponds (Euglena, Chlamydomonas, Chlorella) are able to take up organic nutrients from the water, and essentially feed and grow in the dark as well as perform photosynthesis (Pearson et al, 1987). In addition, Chlamydomonas is more tolerant of sulfide, able to grow over anaerobic water, and Chlorella is more resistant to ammonia inhibition than other algae like Euglena.

Many of the bluegreen algae, such as Anabaena, can fix atmospheric nitrogen and have a competitive advantage over the green algae which must get their nitrogenous nutrients from the water. Anabaena, however, was dominant only in two sites of the outstate survey,

Summary of Harris pond algae seasonal changes

Overall, the Harris ponds were often dominated from late fall and through the winter by the flagellate protozoan Cryptomonas, which disappeared in late winter from ponds 1 and 2. In the transition time around ice-out, pond 2 was dominated by what appeared to be fine filamentous bacteria. After ice-out, the ponds were dominated by flagellated and non-flagellated green algae, on most dates. In pond 3, Chlamydomonas was the first to develop, followed by Chlorella and a diverse succession of green algae. As the water in pond 3 clarified, from July into fall of 1990, there were almost no algae in the samples.

Most of the species of algae present at Harris through the season, are considered edible food for Daphnia, with the exception of the bluegreen algae and the filamentous green alga present once. Bluegreen algae rarely dominated in these ponds: Microcystis (5/21, pond 1) and Oscillatoria (8/30, pond 3) each dominated once. Oscillatoria was present in lower concentrations in ponds 1 (6 dates) and 2 (7 dates), and in pond 3 only on 8/30.

Summary of Janesville algae

During April of 1990, the algae in each pond at Janesville were different: the primary pond 1 had no algae, as reflected in the low chlorophyll values, and in pond 2 the bluegreen algae Oscillatoria was present before and after ice-out, dominating over Chlamydomonas (4/17). It was not until May that diverse green algae bloomed in pond 2. Pond 3 was dominated by Oscillatoria before ice-out (3/20), but edible flagellated green algae were present, and Chlorella and Chlamydomonas prevailed at the ice-out time.

Ponds 2 and 3 were dominated by Oscillatoria from 6/15 into fall of 1990. Pond 1, interestingly, maintained green algae dominance through the summer, and never had the high densities of Oscillatoria seen in ponds 2 and 3. Chlorophyll levels in 2 and 3 were very high in July and August, over 500 mg/m^3, and lower in pond 1. This is also reflected in the greatly increased solids in ponds 2 and 3 compared to pond 1 and in the sharply reduced transparency of the water. Most species of this genus do not fix nitrogen. The Oscillatoria at Janesville was probably not a nitrogen fixing strain, because there was high nitrogen in the ponds and because the daytime surface oxygen levels were high, which could inhibit nitrogen fixation (see section on overnight respiration and oxygen).

Summary of St. Peter algae

The dominant algae in all three ponds after ice-out was Chlamydomonas, followed later in April by Chlorella. In May, pond 1 green algae maintained dominance, but ponds 2 and 3 had a mix of bluegreen (Oscillatoria) and green algae. The ponds developed full bluegreen algae populations at some point in the summer: pond 1 on 8/17 (Oscillatoria), pond 2 on 8/17 (Microcystis), and pond 3 on 7/28 (Merismopedia 7/28, then Microcystis 8/17).

Results of algae analysis

Chlorophyll in relation to physical and chemical factors

The changes in chlorophyll for the secondary ponds of the three study sites are plotted together in Figure 12, where the extraordinarily high values for the Janesville secondary pond can be seen. The seasonal changes in chlorophyll a for Harris, Janesville and St. Peter secondary ponds can be seen in Figures 1-3 in the zooplankton section, which show the seasonal changes in the Daphnia populations. The results of statistical analysis showing significant relationships between the chlorophyll a data, as log (value+.01) transformation, and several parameters are given in Table 7.

Of the three sites studied more intensely, Janesville had the greatest summer increase in chlorophyll a and TSS, and decrease in water clarity in its secondary pond (see figures). Pond 3 was dominated by the filamentous bluegreen alga Oscillatoria from June 15th into November. Extremely high chlorophyll levels occurred, 343 µg/L in May in Janesville pond 3, 519 µg/Lin July and 334 µg/L on 9/19. Harris pond 3 maintained low chlorophyll levels after the spring increase.

Total suspended solids (TSS)

Noteworthy is the relationship between chlorophyll and total suspended solids (TSS). This data is plotted in Figure 13, where the tight relationship is seen. This means that as the chlorophyll, or algae, increases, the solids can increase, or vice versa. In Janesville (see Figure 2), the solids and chlorophyll curves through the season are very similar in shape, and it appears that in Janesville, there is actually an increase in solids in the secondary pond, in sharp contrast to the very low chlorophyll and TSS values in the secondary pond at Harris. It appears that high solids in the secondary pond can be the result of algae overgrowth.

Secchi depth

The significant relationship between chlorophyll and secchi disc depth has a negative slope, meaning the more algae there are in the pond, the less clear the water is. Likewise, TSS and secchi measurements were significantly negatively related (Figure 14).

One hypothesis to explain development of bluegreen algae blooms is that the bluegreen algae tolerate lower light than the green algae (see Shapiro, 1990; Paerl and Ustach, 1982). Light penetration, as indirectly measured by secchi depth, certainly was reduced at Janesville pond 3, even in May (secchi reading, 20cm) and June (14cm), compared with the clear water at Harris pond 3 in spring (143cm, 59cm, May and June, see Figure 15). Reduced light penetration may have contributed to the major bloom of the bluegreen alga Oscillatoria that occurred in Janesville in spring and summer. At St. Peter, the lowest secchi depths were seen on 7/12 (12cm), and on 9/19 (10cm), but in both cases, green algae were dominating. The lowest secchi depth at Harris was 32cm (on 7/29), when green algae were dominant but not plentiful.

Dissolved oxygen

There is a significant relationship also between the chlorophyll and the oxygen concentrations in the pond. The sharp increase in dissolved oxygen in the ponds after ice-out comes from liberation of 0_2 by the algae during the process of photosynthesis. The demand for oxygen in the water is greater in the primary than in the secondary pond, so it takes longer for the algae to overcome the oxygen deficit of winter. Harris pond 1, for instance, had 0_2 of zero through 4/19 in spring, whereas pond 3 had oxygen at 5 mg/L right after ice-out (4/6) so by 4/23 oxygen was over 15 mg/L. Essentially, the algae are aerating the ponds in spring.

spring and summer. At St. Peter, the lowest secchi depths were seen on 7/12 (12cm), and on 9/19 (10cm), but in both cases, green algae were dominating. The lowest secchi depth at Harris was 32cm (on 7/29), when green algae were dominant but not plentiful.

pH

The significant relationship between chlorophyll and pH means that when there is more algae in the pond, the pH in surface waters in the day will be higher. This is because the process of photosynthesis by the algae causes the pH in the water to rise. Bluegreen algae may be more successful than the green algae in more basic waters. In the outstate study, however, the relationship of the percent bluegreen algae to pond pH was not significantly related to chlorophyll at the .05 level (P = .087).

Soluble Reactive Phosphate

The amount of chlorophyll is significantly related to the soluble reactive phosphate, or phosphate in the water (see Figure ()). Note that the slope of this relationship is negative. This means that when the chlorophyll is higher and there is more algae in the pond, the amount of dissolved phosphate in the water itself is less. Presumably, the phosphate has been taken up by the algae. For SRP analysis, the algae are filtered out of the water in the field. Looking at it another way, there may be a higher soluble phosphate level in secondary pond water that lacks an algae bloom. This appears to be true for the secondary pond at Harris, where SRP levels of 3.9 and 4.5 mg/L were present in late October and early November, when chlorophyll was very low, right around fall discharge time. The secondary pond at Janesville around this time had 0.7 and 1.4 mg SRP/L, and St. Peter had 0.9 and 1.1 mg/L. The seasonal mean value for SRP at Harris pond 3 was almost double that of Janesville and St. Peter (see Table ()).

Unionized ammonia and ammonia

The relationship of chlorophyll to unionized ammonia was not significant for this study overall, but there could have been inhibition of algae periodically, during times of high unionized toxic ammonia. The primary ponds at all three sites had higher levels of unionized ammonia (Harris pond 1, 23 mg/L on 4/20; St. Peter pond 1, 2.8 mg/L on 4/27, Janesville 2.5 mg/L on 5/16, (see water chemistry section). Ammonia is actually a nutrient for algae, their preferred nitrogen source, but at higher concentrations and high pH, it can be toxic enough to 'wash out' the algae and cause an oxygen crash in high-rate oxidation ponds (Abeliovich and Azov, 1976). The relationship of chlorophyll to ammonia itself, however, was significant (p=.03), with a negative slope. The algae use ammonia as a nutrient, so when there are more algae, there would be less ammonia in the water as it is taken up by the cells.

Alkalinity

The relationship between chlorophyll and alkalinity in the water at Harris, Janesville and St. Peter is also significant (Table ()). The slope of the relationship is positive, meaning there is more chlorophyll in water where there is more alkalinity, or vice versa. Pond water alkalinity was also significantly related to the number of TSS violations over four years in the outstate sites, and the town's well water alkalinity was significantly related to the amount of chlorophyll in the secondary ponds (see outstate section, Table ()). Some analysis still in progress in Dr. Brezonik's group suggests the ponds may be undersaturated for CO_2. Higher alkalinity as carbonates could provide the algae more carbon when pH shifts are reducing the relative available carbon.

Bluegreen algae

reasons bluegreens didn't develop. For data on nitrogen in the ponds, see Dr. Brezonik's discussion of nitrogen budgets and the water chemistry section.

The relationship between percent bluegreen algae in this study and the ratio of total N to total P was not significant ($p = .23$), based on the outstate data set (see outstate section). Changes in nutrient (N/P) ratios have been suggested as one possible cause of the shift towards bluegreen algae dominance.

In the outstate survey data, there was no significance in the regression of per cent bluegreen algae vs cumulative TSS violations over a four year period for 20 towns in the study ($p = .52$), nor was the relation of percent bluegreen algae and TSS values significant ($p = .111$). Percent bluegreen algae was, unsurprisingly, significantly related to chlorophyll levels ($p = .008$). There was no significance with pond alkalinity ($p = .801$), dissolved oxygen ($p = .284$), or alkalinity of the town's public water supply ($p = .284$). Also, none of the other pond features averaged for a four year period, such as number of discharges, daily flow/design ratio, MGD per person, acres of secondary pond/person was significant in relation to percent bluegreen algae.

Overall conclusions from the algae data

1. The first two ponds at Harris maintained high chlorophyll levels, often over 100 µg/L while the secondary pond at Harris had relatively low levels of chlorophyll, less than 10 µg/L through the summer. At Janesville, the secondary pond maintained chlorophyll over 200 µg/L through the summer, with a maximum of 519 µg/L. The secondary pond at St. Peter had chlorophyll levels aroung 40 µg/L through the summer.

2. Chlorophyll was significantly related to the total suspended solids levels, and the increase in TSS at Janesville pond 3 was accompanied by an increase in chlorophyll.

3. Chlorophyll was significantly related to the levels of dissolved oxygen.

4. Chlorophyll was significantly related in a negative manner to the secchi depths. The more algae, the less clear was the water in the ponds.

5. Chlorophyll was significantly related to pH levels as expected because of the pH elevation caused by photosynththesis.

6. Chlorophyll was significantly related to soluble reactive phosphate, with a negative slope. When more algae were present, there was less dissolved phosphate in the water.

7. The ponds were frequently dominated by a variety of green algae, especially the flagellated green algae such as Chlamydomonas, Phacus and Trachelomonas, but non-flagellates such as Micratinium and Scenedesmus also were common. Nitrogen-fixing bluegreen algae, Anabaena spiroides, dominated only in two of the 18 study sites. Otherwise, when bluegreen algae were present or dominant, they were of the non-nitrogen fixing types. This suggests nitrogen was not a limiting nutrient in the ponds.

8. See the outstate survey section for relationships with percent bluegreen algae in the ponds.

Table 6. Types of algae dominant in waste stabilization ponds. Data reported as the number of dates the alga was dominant. Dominant means over 50%, or among the groups adding to over 50% abundance. Harris (HA), Janesville (JA), and St. Peter (SP) were sampled through 1990, the rest of the sites in fall, 1990 only. Site codes are BAL, Balaton; BEL, Bellingham; BIR, Bird Island; BRI, Bricelyn; BRO, Browerville; CHA, Chandler; CLA, Clarks Grove; COS, Cosmos; CUR, Currie; EDG, Edgerton; ELK, Elko-New Market; KIE, Kiester; LON, Long Prairie; MAP, Mapleton; MUR, Murdock; 1,2,3 is pond number. All others are secondary ponds.

GREEN ALGAE	HA 1	HA2	HA3	JA1	JA2	JA3	SP1	SP2	SP3	BAL	BEL	BIR
Actinastrum								1	1			
Ankistrodesmus			1	2				1	1			
Chlamydomonas	7	7	4		1	2	2	3	1			
Chlorella			1	1	2	2	1	1	2			
Chrococcus	1											
Closterium			1				2	1	4			
Dictyosphaerium			1							1		
Eudorina	1	1								1		1
Euglenophyceae	1	2	3									
Filamentous green			1									
Gloeocystis	1											
Micratinium	2	1					1					
Pandorina		2		1								
Phacus			1									
Scenedesmus		1			2	1	1	1	2	1	1	
Sphaerocystis				1	1	1						
Trachelomonas	1	6	1									
BLUEGREEN ALGAE												
Anabaena spiroides												
Dactylococcopsis							2					
Merismopedia									1			
Microcystis	1							3	1			
Oscillatoria		1		2	10	9	3	2	2		1	
Phormidium								3				
CRYPTOPHYCEAE												
Cryptomonas	5	5	5									

Table 6 (continued from previous page). Types of algae dominant in waste stabilization ponds. Site codes are BAL, Balation; BEL, Bellingham; BIR, Bird Island, BRI, Bricelyn; BRO,Browerville; CHA, Chandler; CLA = Clarks Grove; COS, Cosmos; CUR, Currie; EDG, Edgerton; ELK, Elko-New Market; KIE, Kiester; LON, Long Prairie; MAP,Mapleton; MUR, Murdock. Sanborn sample had no algae. Sites other than Harris, Janesville and St. Peter were sampled only in fall, 1990.

GREEN ALGAE	BRI	BRO	CHA	CLA	COS	CUR	EDG	ELK	KIE	LON	MAP	MUR
Actinastrum												
Ankistrodesmus												
Chlamydomonas				1	1							
Chlorella										1		
Chrococcus												
Closterium												
Dictyosphaerium		1										
Eudorina												
Euglenophyceae												
Filamentous green												
Gloeocystis												
Micratinium												
Pandorina		1										
Phacus						1						
Scenedesmus												
Sphaerocystis												
Trachelomonas									1			
BLUEGREEN ALGAE												
Anabaena spiroides	1											1
Dactylococcopsis												
Merismopedia												
Microcystis					1							
Oscillatoria			1		1		1	1			1	
Phormidium												
CRYPTOPHYCEAE												
Cryptomonas												

Table 7. Regressions of log chlorophyll a on various factors in pond water. Data from all three ponds at Harris, Janesville and St. Peter from November 1989 through November 1990. Results listed in order of p (probability) values. Parameters with * are significantly related to the chlorophyll ($p<.05$). TSS = total suspended solids, SRP = soluble reactive phospate, r = regression coefficient, n = number of samples.

Factor	p	r	n	slope
Secchi depth	<.001*	.61	77	-.011
Dissolved oxygen	<.001*	.40	85	.037
TSS, mg/L	<.001*	.50	85	.014
pH	<.001*	.41	89	.558
SRP, mg/L	.001*	.36	82	-.308
CBOD, mg/L	.010*	.74	11	.072
Total phosphorus	.022*	.28	65	-.219
Alkalinity, mg/L	.028*	.24	83	-.003
Ammonia, mg/L	.030*	.23	89	-.026
Iron, mg/L	.047*	.39	27	-.003
Total organic C (mg/L)	.068	.21	80	.025
Turbidity (mnu/cm)	.586	.12	22	
Chloride (mg/L)	.727	.09	18	
Conductivity (log)	.758	.04	78	
Total nitrogen (mg/L)	.841	.02	82	
Nitrate (mg/L)	.916	.01	88	

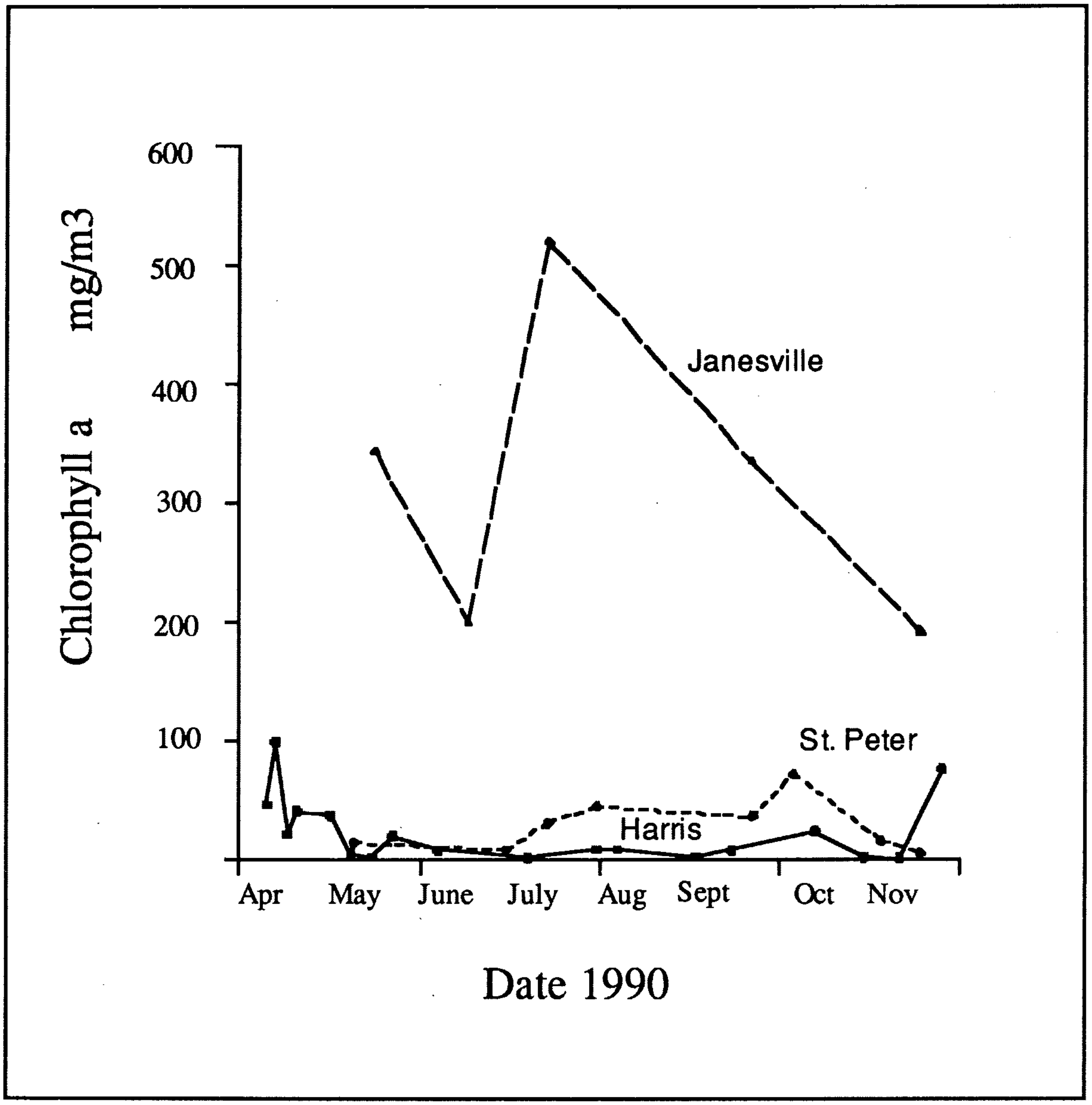


Figure 12. Chlorophyll a (mg/m3) in the secondary, or discharge ponds at Harris, Janesville and St. Peter through 1990.

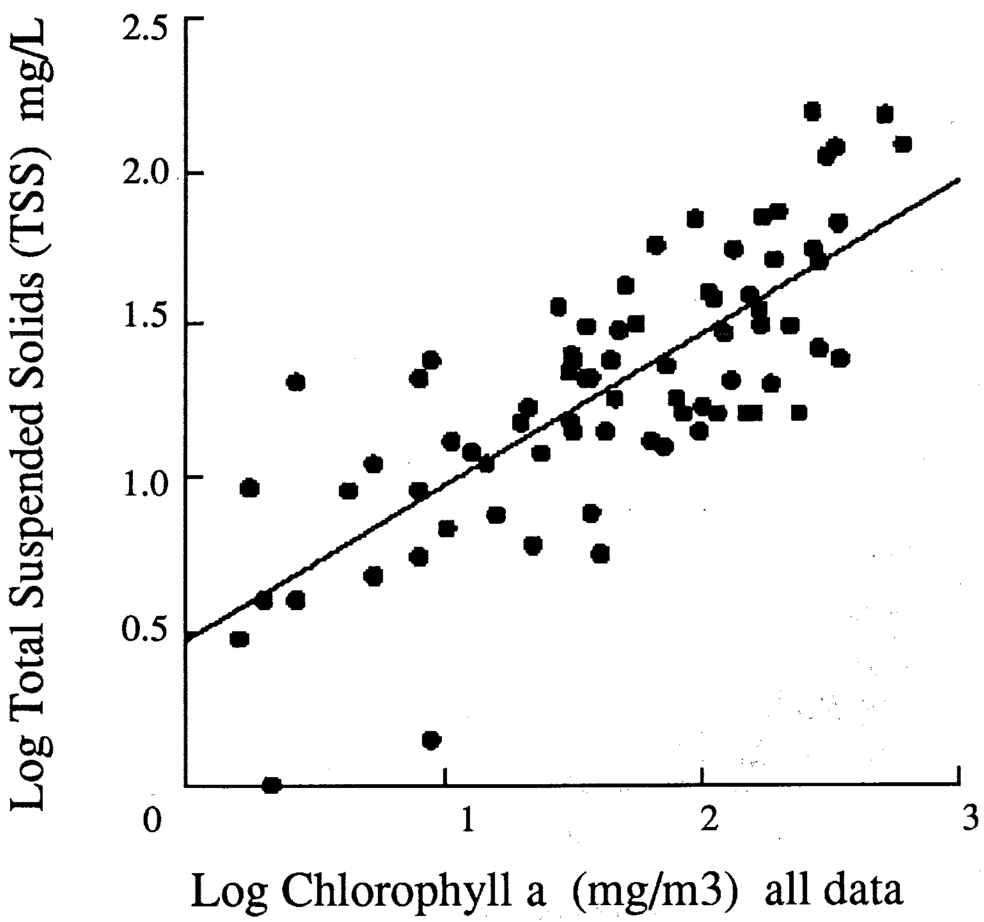


Figure 13. Regression of log chlorophyll and log total suspended solids (TSS) for all data from Harris, Janesville and St. Peter through 1990. The relationship of the amount of chlorophyll and the amount of solids is highly significant ($p<.001$).

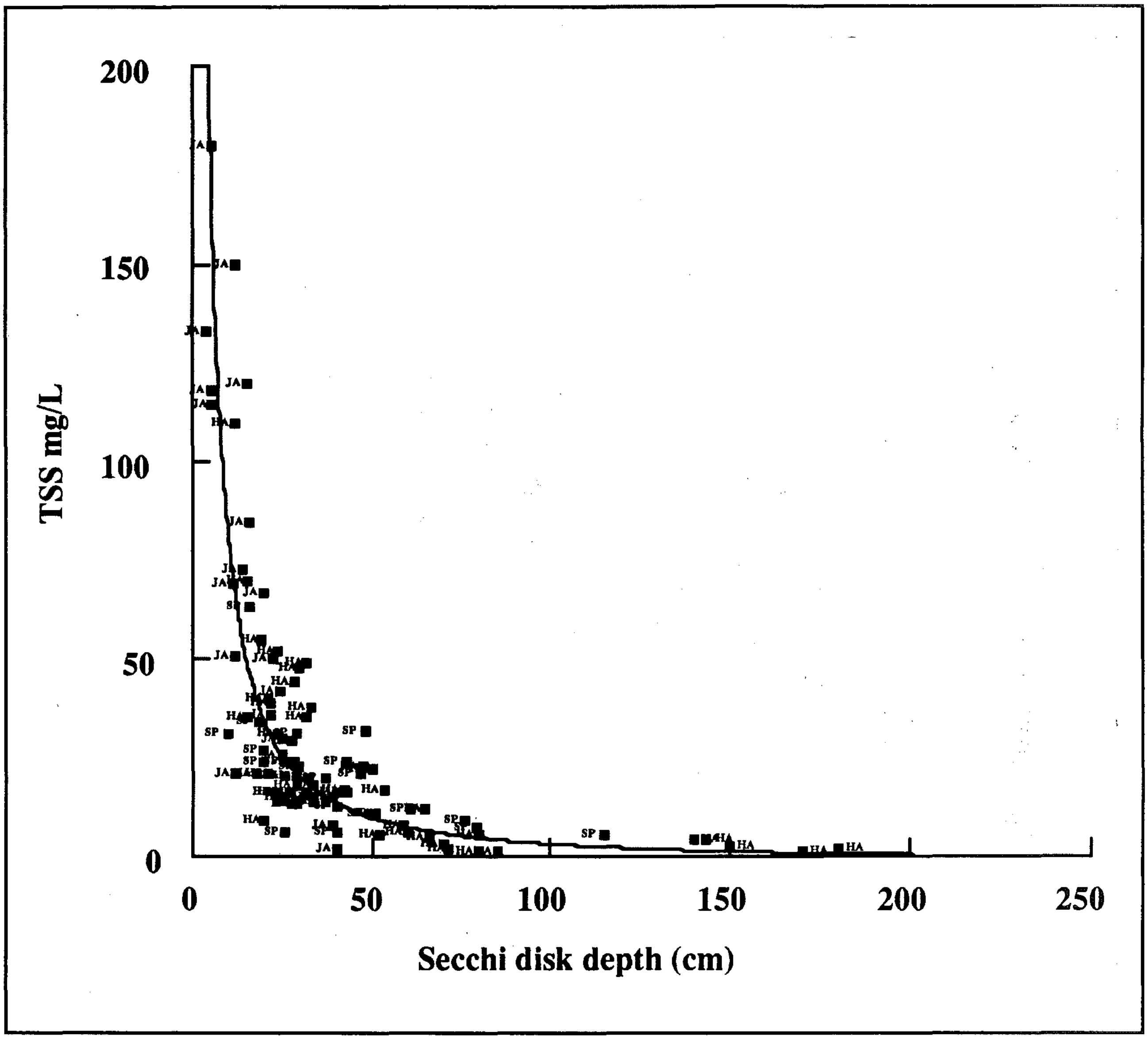


Figure 14. Relationship between total suspended solids (TSS) in mg/L and secchi disk depth in cm for 1990 in all three stabilization ponds at Harris, Janesville and St. Peter, MN. The relationship was not plotted against the inverse of the secchi depth, so the reader can see the range of the secchi depths. This relationship is very highly significant ($p < .0001$, $r = .85$).

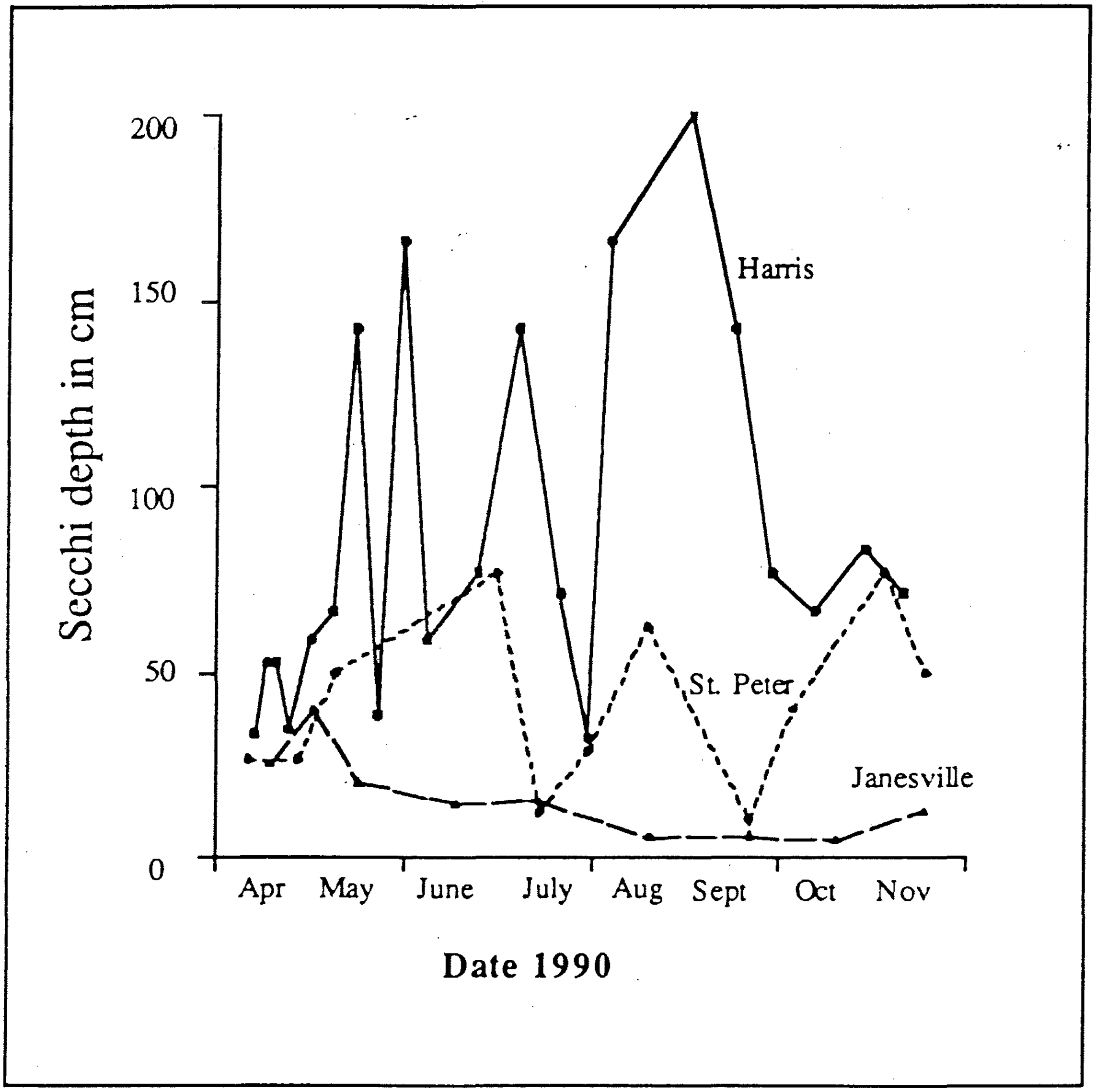


Figure 15. Secchi depths in the secondary ponds at Harris, Janesville and St. Peter, MN during 1990.

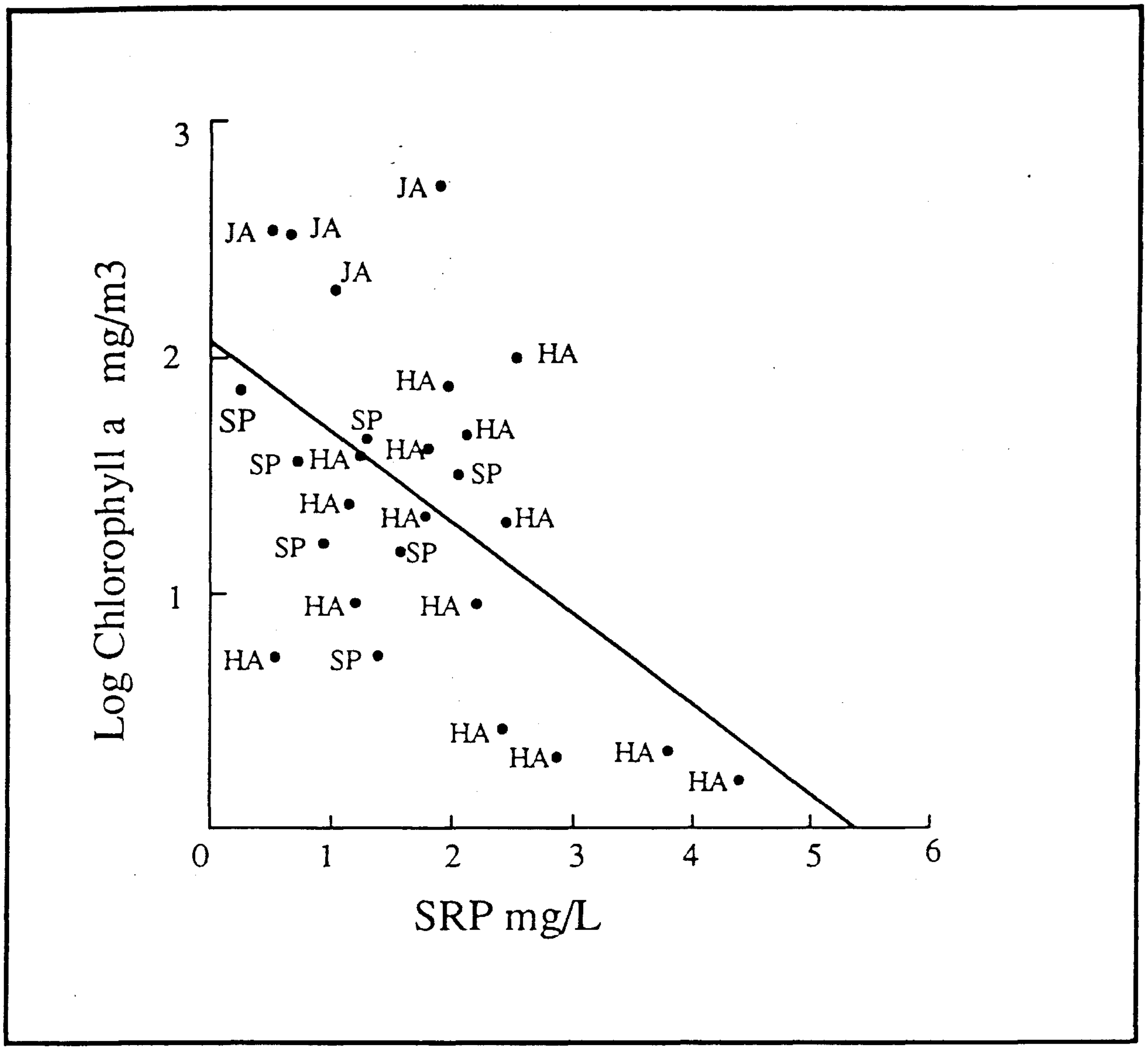


Figure 16. Regression of log chlorophyll a and soluble reactive phosphate (SRP, mg/L) for Harris (HA), Janesville (JA) and St. Peter (SP) waste stabilization ponds in 1990, p = .004, r = .544, slope = -.379.

Outstate survey of waste stabilization ponds

Eighteen sites were sampled in fall of 1990 to relate water chemistry data and pond operation parameters to algae composition, solids levels, solids violations histories and community water supply alkalinity. Data from Harris, Janesville and St. Peter from the same time period are included in this analysis. The data is summarized in Table 8. The data elements in this table are explained below.

Outstate survey methods

The data sources for the pond design and operational factors are listed below:

Total system acres is the total acreage in the ponds for each system.
Design flow is the flow capacity in millions of gallons of influent per day.
Mean daily flow is taken from DMR reports at MPCA for influent volume averaged per day for the period 1987-1990.
Daily flow/design flow is the ratio of the actual mean daily flow/design influent flow, to show if the system is operating below or above the design capacity.
Population is taken from the 1988 census data.
Acres/person is the total pond acres divided by the population.
Gallons influent/day/capita is the average daily influent volume for 1987-1990 divided by the population.
Number of discharges was taken from the monthly DMR reports at MPCA for the years 1987-1990.
Number of solids violations was the total number of TSS violations for the period 1987-1990.
Mean discharge TSS (mg/L) was the total discharge TSS reported to MPCA, divided by the number of discharges.

The water chemistry data reported in Table () was from single visits to the sites in the fall of 1990. See chemistry section for methods.

The methods for the rapid assessment procedure for the percent bluegreen algae and for chlorophyll measurements are given in the section on algae. The presence of Daphnia and other cladocerans was determined from samples taken from shore with three throws of a 62mm plankton net. Ephippia were seived from shore into a grab sampler with net-covered bottom.

Results of statistical analyses

Results of regressions on the outstate survey data are given in Tables () and (). In Table (), significant relationships ($p<.05$) are seen between chlorophyll a and total suspended solids (TSS), well water alkalinity, percent bluegreen algae, and cumulative TSS violations. Table () shows TSS was significantly related to chlorophyll, turbidity of the water, percent bluegreen algae, and cumulative TSS violations. For the algae, the most significant relation for chlorophyll was to TSS, well water alkalinity, and percent bluegreen algae.

The alkalinity of the town's wellwater was investigated when the significant relation was seen between the cumulative TSS violations history (Table () and Figure ()) and pond alkalinity as measured in fall of 1990. Well water alkalinity came up as a significant factor in relation to chlorophyll levels and TSS violations histories. We compiled data on 216 stabilization systems in Minnesota, the TSS measurements at discharge, and the well water alkalinity of the communities. A regression was run on the number of times the site had TSS over 45 mg/L from 1987-1990 vs the mean well water alkalinity of the town with a significant result, $p = .003$. A similar regression of the number of times TSS was over 60 mg/L was significant, $p = .013$. It appears that alkalinity is an important factor in relation to higher solids in secondary stabilization ponds.

The cumulative TSS violations, the focus of this study, were most significantly related to the number of discharges by the system for 1987 - 1990 (Table ()). A site with more frequent discharges is hydraulically overloaded, its water would have less residence time in stabilization ponds, and therefore, less time to be processed. The significant relation between cumulative TSS violations and the ratio of daily influent flow to design flow also relates to overloading of the system. This suggests that reduction of the volume of influent, by reducing storm water infiltration and removing sump pumps and house tiles, could help sites with TSS violations problems.

Overall conclusions from the outstate survey

1. Chlorophyll a levels were significantly related to solids levels (TSS), to well water alkalinity, to the percent bluegreen algae, and to the cumulative TSS violations. When the algae are more productive, there tends to be more bluegreen algae in the ponds and solids are greater. The higher chlorophyll with higher well water alkalinity for the community could relate to greater productivity by the algae that otherwise might be carbon limited. The Harris ponds were sometimes undersaturated for $C0_2$.

2. Percent bluegreen algae was significantly related to the turbidity of the pond water and to the total organic carbon. Bluegreen algae are known to outcompete green algae in low light conditions.

3. Cumulative TSS violations were significantly related to the mean number of discharges per year, the alkalinity of the pond water, the amount of chlorophyll a present, and to the ratio of the daily flow to the design flow. Sites with uncontrolled algae blooms will have more solids in the effluent water. Sites with more frequent discharges have more problems with high solids levels in discharge waters. It appears that sites with more alkalinity in the pond water have higher solids, which presumably is from algae growth.

Table 8. Design and operational parameters for 1987-1990, with chemical and biological data for fall, 1990 from 18 stabilization pond systems in Minnesota. Population data from 1988 census. Mean discharge TSS (total suspended solids) averaged from monthly reports for 1987-1990 at MPCA. Table continues next page.

Town name	Balaton	Bellingham	Bird Island	Bricelyn	Browerville	Chandler
Design and Operational Factors						
Total system acres	15.60	5.48	25.15	11.74	49.10	9.04
Design flow (MGD)	0.070	0.030	0.240	0.067	0.215	0.055
Mean daily flow (MGD)	0.084	0.025	0.194	0.029	0.213	0.050
Daily flow/design flow	1.172	0.848	0.807	0.438	1.312	0.918
Population	728	262	1349	432	712	280
Acres/person	0.021	0.021	0.019	0.027	0.069	0.032
Gallons influent/day/capita	115	95	144	67	299	179
Number of discharges††	15	8	17	5	7	10
Number of solids violations†	10	12	17	6	18	16
Violations/discharge	0.67	1.50	1.00	1.20	2.57	1.60
Mean discharge TSS (mg/L)	31.5	84.0	90.6	42.6	158.3	53.5
Water chemistry data from fall of 1990						
Alkalinity (µg/L)	250	190	270	180	-	310
Ammonia (mg/L)	0.07	0.06	2.32	0.07	-	0.04
Conductivity (µhmos)	2222	4500	1020	816	-	2222
Nitrate (µg/L)	14.7	8.3	30.0	14.4	-	11.4
pH	7.3	9.2	8.7	9.9	-	8.4
Phosphate (SRP, mg/L)	0.66	0.89	1.35	0.30	-	0.66
TSS fall, 1990	26.9	33.4	3.7	54.0	-	10.4
Biological Data						
Bluegreen algae, %	0	80	0	100	0	95
Chlorophyll a, mg/m3	32.0	162.9	18.7	265.5	-	534.0
Daphnia present	-	+	+	-	-	-
Cladocera, other	+	+	+	+	-	+
Ephippia presence	-	+	+	+	-	+

†† total for 1987-1990

Table 8 (con). Design and operational parameters, chemical and biological data for 18 waste stabilization ponds in Minnesota. Table continues on next page.

Town name	Clarks Grove	Cosmos	Currie	Edgerton	Fulda	Harris
Design and Operational Factors						
Total system acres	22.00	10.75	5.69	21.00	24.60	4.80
Design flow (MGD)	0.116	0.09	0.045	0.119	0.178	0.038
Mean daily flow (MGD)	0.057	0.07	0.027	0.086	0.089	0.027
Daily flow/design flow	0.491	0.767	0.768	0.742	0.496	0.724
Population	645	559	325	1088	1294	813
Acres/person	0.034	0.019	0.018	0.019	0.019	0.006
Gallons influent/day/capita	88	125	83	79	69	33
Number of discharges††	8	9	7	10	10	10
Number of solids violations††	0	0	0	2	0	0
Violations/discharge	0	0	0	0.2	0	0
Mean discharge TSS (mg/L)	10.58	16.63	8.53	30.19	8.83	8.16
Water chemistry data						
Alkalinity (µg/L)	240	360	190	260	150	250
Ammonia (mg/L)	0.04	0.06	0.33	0.08	0.04	0.05
Conductivity (µhmos)	923	1450	3190	2300	4470	830
Nitrates (µg/L)	5.3	0	8.3	17.5	5.3	20.3
pH	9.58	9.07	7.26	8.13	8.51	9.1
Phosphate (SRP, mg/L)	0.51	0.84	0.03	0.13	0.27	1.41
TSS fall, 1990	10.4	3.6	2.2	131	2.2	3
Biological Data						
Bluegreen algae, %	0	60	0	83	0	5
Chlorophyll a mg/m3	-	28.3	3.3	384.5	10.7	1.6
Daphnia spp.	-	-	-	-	+	+
Cladocera, other	+	+	+	-	+	+
Ephippia presence	+	-	+	-	+	+

†† total for 1987-1990

Table 8 (con). Design and operational parameters, chemical and biological data for 18 waste stabilization ponds in Minnesota.

	Janesville	Kiester	Mapleton	Murdock	Sanborn	St. Peter
Design and Operational Factors						
Total system acres	21.00	12.60	56.40	6.60	9.80	201.00
Design flow (MGD)	0.153	0.090	0.170	0.042	0.056	1.4
Mean daily flow (MGD)	0.174	0.079	0.209	0.039	0.038	0.966
Daily flow/design flow	1.083	0.750	1.228	0.917	0.693	0.706
Population	1968	621	1489	330	483	9257
Acres/person	0.011	0.020	0.038	0.020	0.020	0.022
Gallons influent/day/capita	88	127	140	118	79	104
Number of discharges††	27	22	8	12	2	16
Number of solids violations††	32	26	7	24	0	6
Violations/discharge	1.19	1.18	0.88	2.00	0.00	0.38
Mean discharge TSS (mg/L)	73.7	44.3	56.8	60.6	32.0	24.1
Water chemistry data						
Alkalinity (µg/L)	400	450	240	280	220	250
Ammonia (mg/L)	0.05	0.09	N/A	0.23	0.08	0.07
Conductivity (µhmos)	400	1665	1628	1960	3050	2160
Nitrate (µg/L)	25.3	77.7	8.3	0.0	8.0	4.9
pH	8.9	8.9	9.0	9.4	8.1	9.2
Phosphate (SRP, mg/L)	0.73	1.34	0.61	0.40	0.43	0.66
TSS fall, 1990	51.0	120.0	19.4	50.5	0.9	11.0
Biological Data						
Bluegreen algae, %	94	11	70	95	0	30
Chlorophyll a, mg/m3	192.2	546.8	117.0	293.7	1.6	5.3
Daphnia spp.	+	-	-	-	-	+
Cladocera, other	-	-	+	+	+	-
Ephippia presence	+	+	+	-	+	+

†† total for 1987-1990

Table 9. Regressions of outstate survey data for chlorophyll a and percent bluegreen algae on chemical and operational design parameters of 18 waste stabilization pond systems. Statistically significant p values have p>.05. Chemical data from single site visits in fall, 1990, discharge data from monthly reports for 1987-1990, well water alkalinity from MN Department of Health records. r = regression coefficient, n= number of sites.

Log Chlorophyll a	p	r	n
TSS	<.001	0.793	18
Well alkalinity	0.013	0.589	18
% Bluegreen algae	0.018	0.551	18
Cum TSS violations	0.021	0.54	18
Pond alkalinity	0.144	0.358	18
Phosphate (SRP)	0.207	0.312	18
Acres secondary	0.213	0.308	18
Total phosphorus	0.222	0.035	18
Mean # discharges	0.372	0.224	18
Ammonia	0.478	0.179	18
MGD per person	0.574	0.142	18
Daily flow/design flow	0.897	0.033	18
Percent bluegreen algae			
Turbidity	0.027	0.53	
Total organic carbon	0.037	0.5	
pH	0.087	0.47	
Total nitrogen	0.089	0.41	

Table 10. Outstate survey regressions of total suspended solids (TSS, mg/L) measured in the fall of 1990 and cumulative TSS violations on chemical and operational design parameters of 18 waste stabilization pond systems in Minnesota. Statistical significance is $p < .05$. Chemical data from single site visits in fall, 1990, discharge data from monthly reports for 1987-1990, well water alkalinity from MN Department of Health records, r = regression coefficient, cum TSS violations = cumulative violations 1987-1990. Some of the slopes are shown.

Log TSS	p	r	n	Slope
Log chlorophyll	<.001	0.893	18	0.674
Turbidity	<.001	0.758	18	0.029
% Bluegreen algae	0.006	0.617	18	0.981
Cum TSS violations	0.019	0.547	18	0.035
Log total organic carbon	0.087	0.415	18	0.816
Wellwater alkalinity	0.108	0.404	18	
Pond water alkalinity	0.133	0.083	18	
Total phosphorus	0.158	0.347	18	
Cumulative TSS violations				
Number of discharges	0.001	0.73	18	1.226
Pond alkalinity	0.009	0.598	18	0.083
Daily flow/design flow	0.01	0.56	18	
Log chlorophyll a	0.021	0.54	18	6.296
MGD influent per person	0.093	0.385	18	

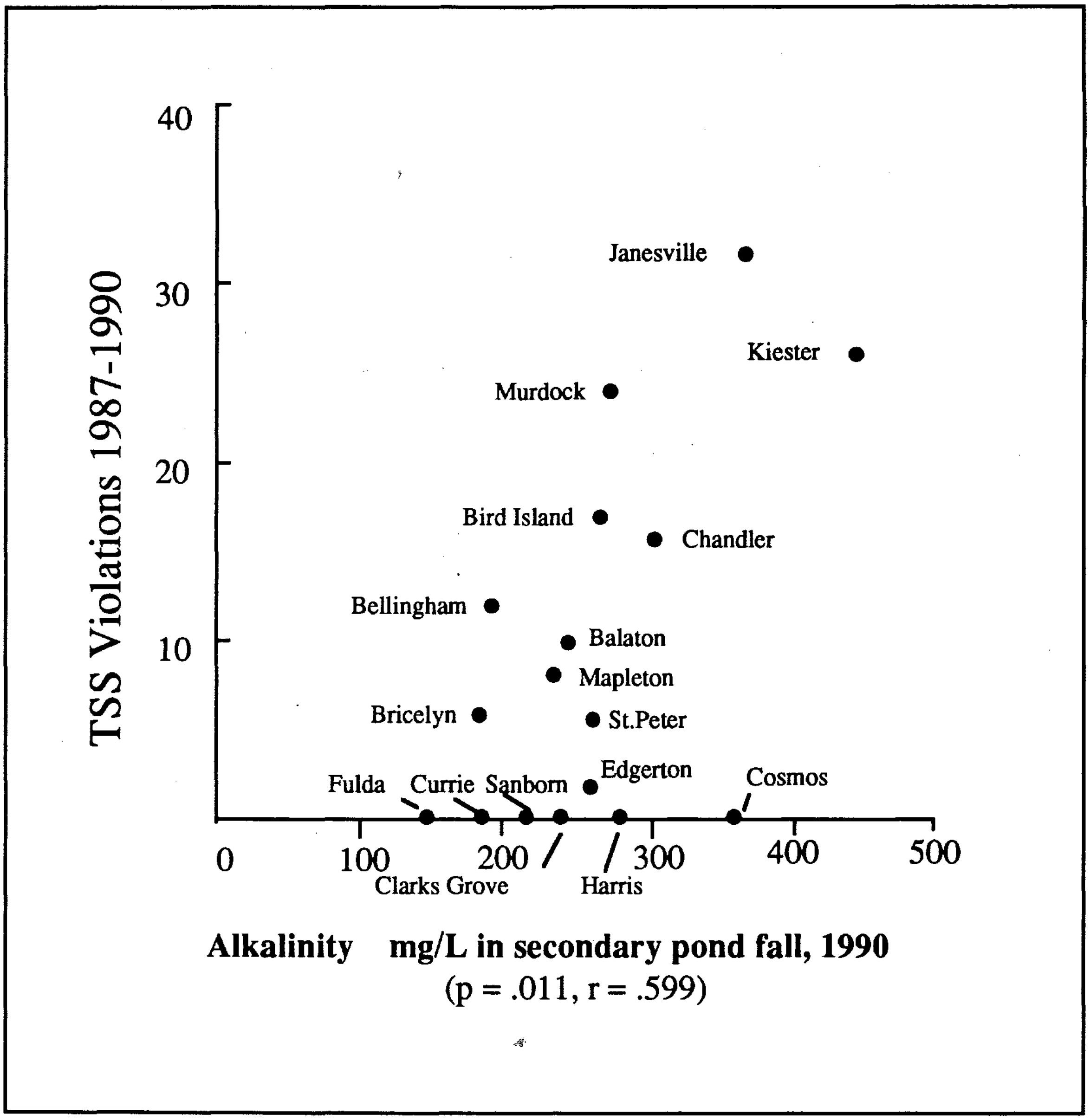


Figure 17. Cumulative total suspended solids standard violations for effluent for 1987 - 1990 related to alkalinity measured in the secondary ponds in the fall of 1990.

Overnight respiration and dissolved oxygen in waste stabilization ponds

This section describes the results of measurements of overnight respiration and the seasonal changes in surface water dissolved oxygen in the ponds. Additional data on pond oxygen, particularly on the stratification of the ponds for oxygen, can be found in Luck and Stefan (1990).

Measurement of changes in dissolved oxygen overnight in ponds indicates the amount of respiration, or use of the oxygen in metabolism by the microorganisms, plants, and animals in the ponds. A pond that has a higher 'oxygen demand' will experience a greater oxygen reduction through the night than a pond with little metabolic activity. The length of time the oxygen is at zero may limit the kinds of organisms that can survive there. During the day, as photosynthesis occurs, the oxygen levels recover and can become very supersaturated in the late afternoon.

Method for measuring overnight respiration

Overnight changes in oxygen levels were recorded at 50 cm depth with a YSI model 58 dissolved oxygen (DO) meter with stirring probe. The probe was calibrated in equilibrated tap water with the Winkler method at the beginning and end of the recording sessions. For the data obtained August 16-18 at St. Peter and Janesville, and Harris July 16/17, a YSI model 58 DO meter with a four-channel Rustrak Ranger datalogger and playback system was used. Readings were recorded every 650ms into the datalogger which played back the readings into an IBM PC computer supported with the Pronto application software. Readings were averaged every 15 minutes, and this data was used for graphing and analysis. For the data for Harris pond 3 on August 30-31, 1990, a stripchart recorder was used with the YSI model 58 DO meter. The stripchart was point digitized with Jandel Scientific's quantitative digitizing program interfaced with a digitizing tablet.

The whole pond respiration rate (WPR) was calculated by fitting the quadratic equation to the oxygen readings, then calculating the slope of the tangent to the function at the point where the water is 100% saturated with dissolved oxygen. At this point, oxygen exchange with the air is minimal, and all changes in DO are attributable to respiration (Madenjian et al, 1990). Thus the slope of the tangent at this point describes the instantaneous rate of decrease in pond DO (or the instantaneous WPR).

Results of overnight respiration measurements

The averaged oxygen readings are plotted in Figures 18 and 19, along with the quadratic fit lines, and the tangential lines for Janesville and St. Peter. The method of estimating the overnight respiration rate could not be used for Harris pond 3. Because there was so little respiration in this pond that it never became undersaturated for oxygen through the night. The tangent line is fitted to the point where oxygen goes from 100% to less than 100% saturation, based on the calculated saturation concentrations for the temperatures taken with the oxygen readings. St. Peter had the sharpest drop in oxygen through the night. During the next day, the oxygen did not come back up as it had in Janesville, perhaps because of a tremendous storm that occurred at dawn.

The estimated whole pond respiration rates (WPR) are given in Table 11. Again, rates of oxygen use are not given for Harris because the tangent could not be applied. The sharper drop in oxygen at St. Peter pond 3 is evident, compared with Janesville pond 3. Some of the oxygen readings from the overnight respiration data are given in Table 11. Late in the day at all three ponds, the oxygen readings were high, over 11 mg/L, the result of algal photosynthesis during the day. By midnight, oxygen was still high at Harris, but Janesville had dropped to 6 mg/L, and St. Peter to 3.6. By dawn, Harris pond 3 was as high as the evening before, whereas Janesville was down to 2.5, and St. Peter was near zero mg/L.

Daytime surface oxygen data

Surface water oxygen levels for the ponds at Harris are plotted in Figure 20, for Janesville in Figure 21, and for St. Peter in Figure 22. Winter oxygen levels were measured only at the Harris ponds, where ponds 1 and 2 became depleted of oxygen in January and February, and pond 3 went close to zero in the end of March, just before ice-out. The sharp rise in oxygen in spring after ice-out in April is seen in all three ponds. Pond 3 recovered its oxygen ahead of the first two ponds, which had the winter influent load. In Janesville, the oxygen in the secondary pond frequently became supersaturated, reaching a maximum of 41 mg/L in June. In St. Peter, oxygen levels were low in May after the copper sulfate treatments and also in July and August.

As a review of statistical tests presented earlier in this report, dissolved oxygen had a significant relationship with the densities of cladocerans for St. Peter but not for Harris and Janesville (Table 4). With the highest rate of overnight respiration, St. Peter had oxygen near zero at dawn in August. Below 2mg/L, Daphnia have difficulty reproducing. Low oxygen does appear to affect the Daphnia populations in the secondary pond at St. Peter.

Table 11. Whole pond respiration rate (WPR) of the secondary ponds at Harris, Janesville and St. Peter, MN, and dissolved oxygen levels in mg/L at different times of day in August, 1990. Dissolved oxygen was measured at 50 cm depth continuously (see text). WPR, in mg oxygen/L/h used, is calculated as the tangent to the point when pond oxygen was at 100% saturation. No rate is given for Harris pond 3 because oxygen did not become undersatured. Night only WPR was from 10:00 PM to 5:00 AM, 20 hr WPR was from around 5:00 PM to 1:00 PM the next day.

	Harris	Janesville	St. Peter
Date	30-Aug-90	17-Aug-90	16-Aug-90
WPR, 20 hr		1.3	1.56
WPR, night only		0.86	2.01
Oxygen, 5:30 PM	11.8	11.1	12.4
Oxygen, 12:00 PM	11.8	6.1	3.6
Oxygen, 5:00 AM	11.7	2.5	0.2

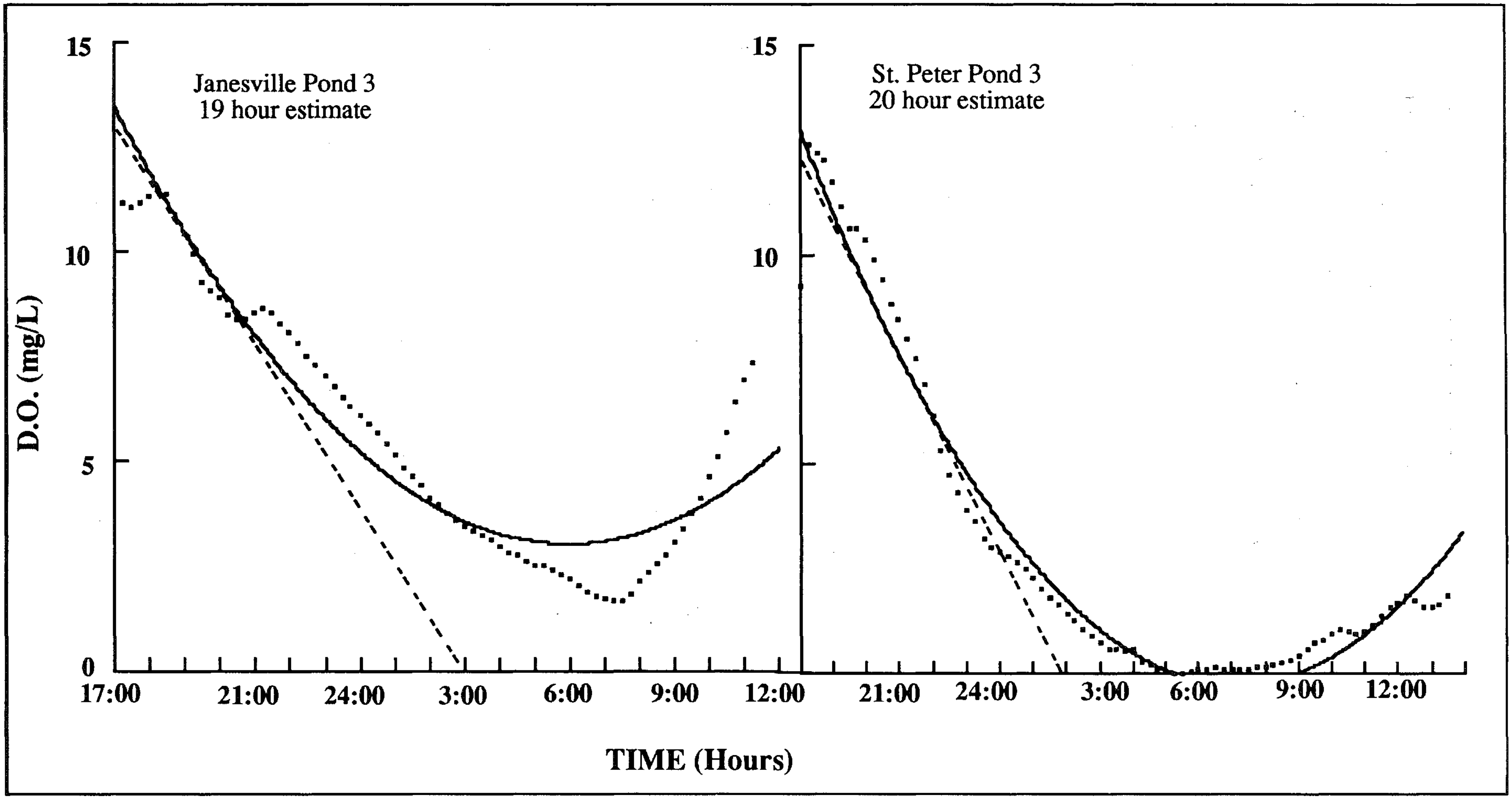


Figure 18. Dissolved oxygen readings from August 16-17 at St. Peter and August 17-18 at Janesville secondary waste stabilization ponds. Dotted line represents actual readings averaged for each 15 minute period through the night to midday. The solid line represents the quadratic curve fit to the data. The dashed line is the tangent to the quadratic function at the point where there is 100% saturation of dissolved oxygen. At this point, oxygen exchange with the air is minimal, and changes in oxygen are attributable to respiration.

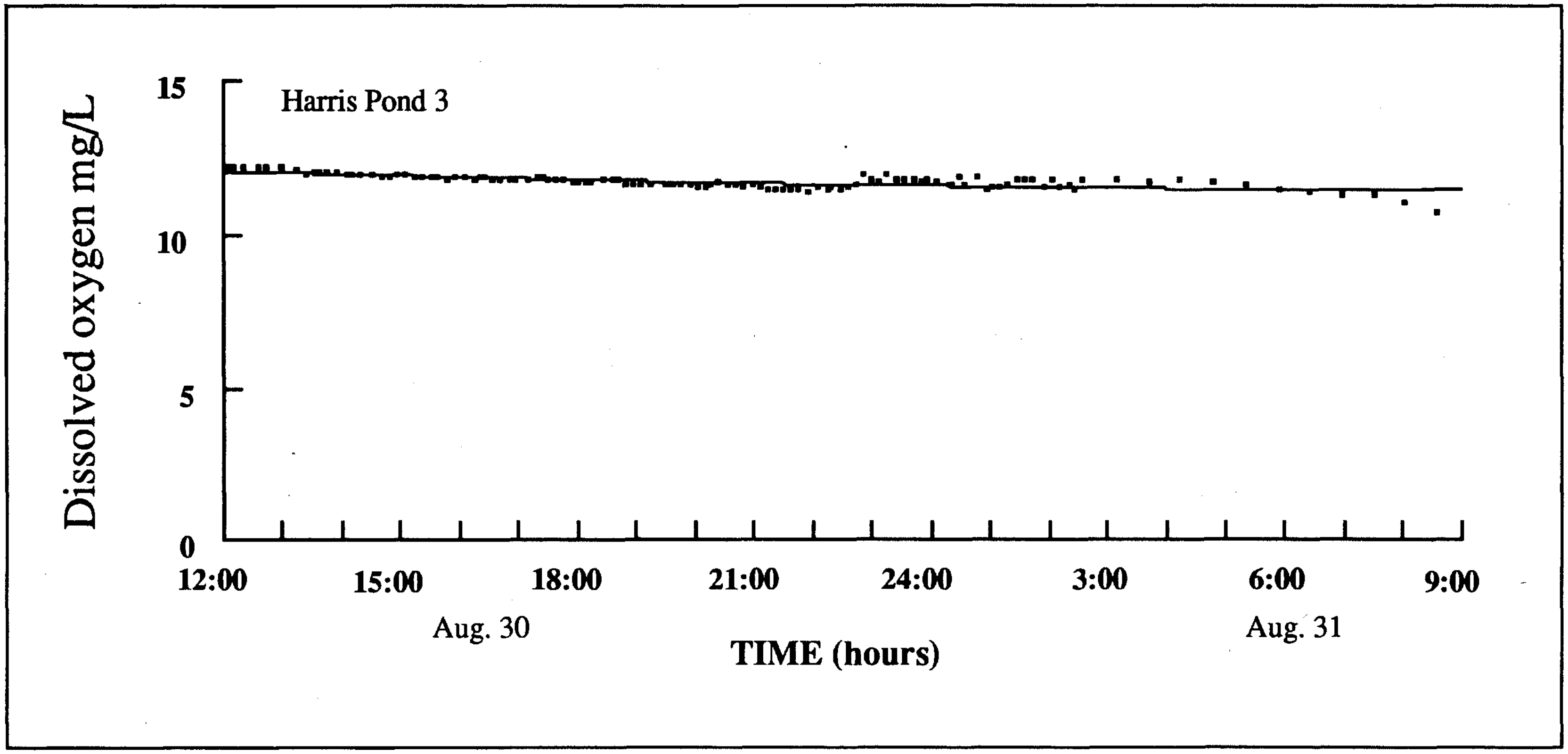


Figure 19. Dissolved oxygen readings from August 30-31 at the Harris secondary waste stabilization pond. Dotted line represents actual readings averaged for each 15 minute interval. The solid line represents a quadratic fit line. The method for estimating whole pond respiration rate (WPR) with a tangent draw could not be used, because the oxygen did not become under-saturated overnight. This means that very little respiration was occurring in this pond.

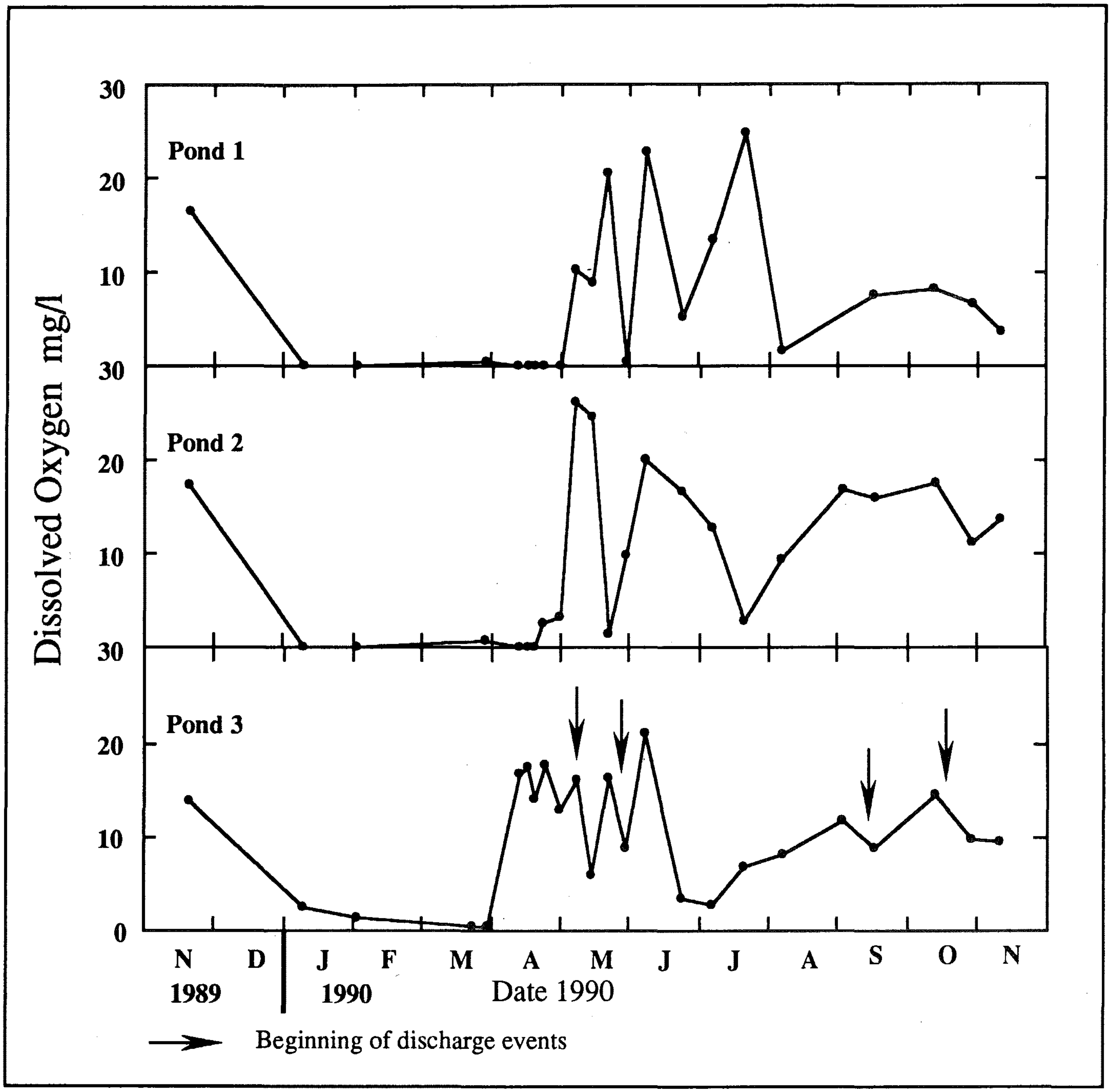


Figure 20. Concentrations of dissolved oxygen in the surface waters of waste stabilization ponds at Harris, MN from November, 1989 - November 1990. Azide modification of the Winkler method was used.

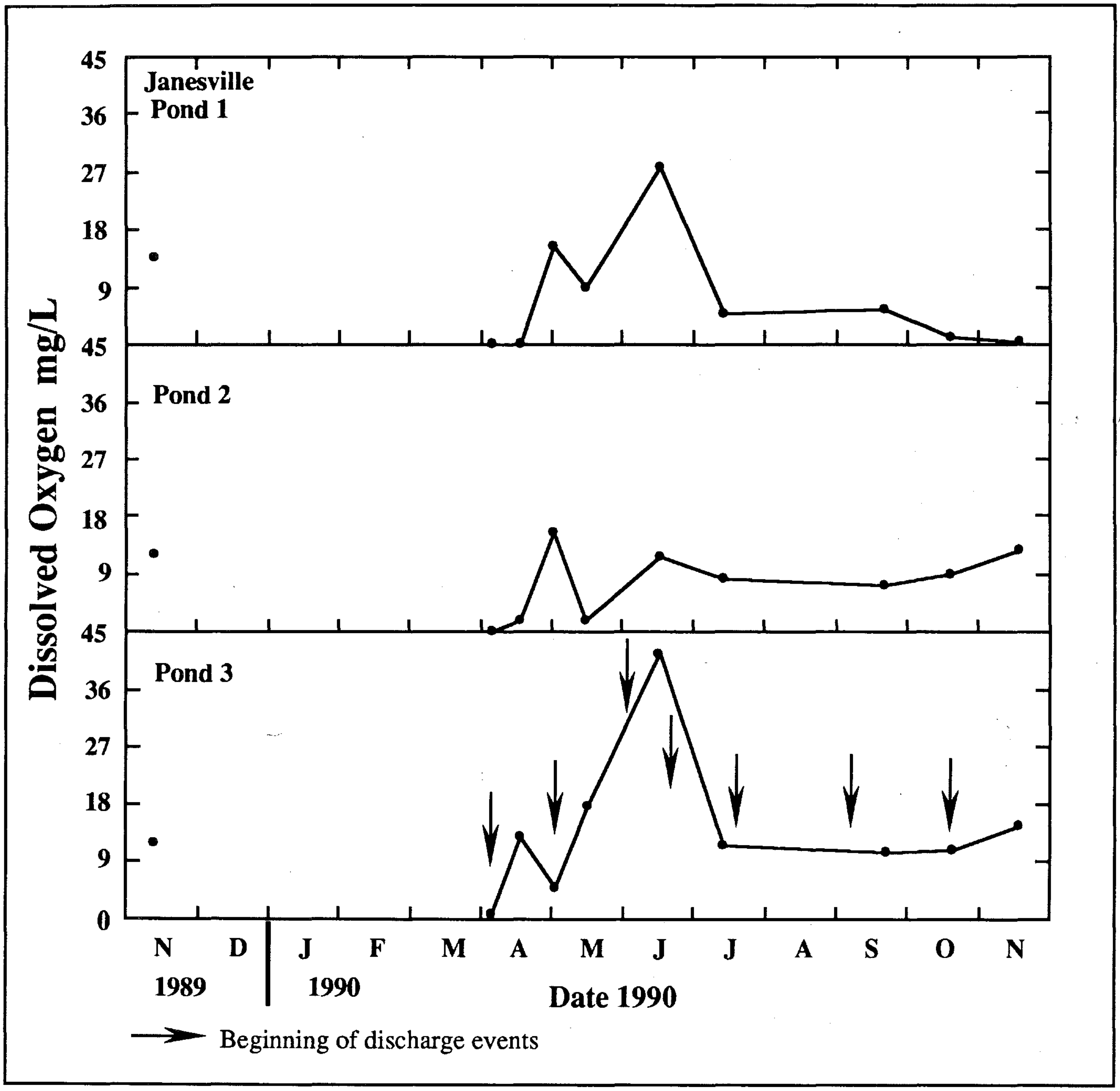


Figure 21. Concentrations of dissolved oxygen in the surface water of waste stabilization ponds at Janesville, MN in November 1989 and from spring 1990 - November,1990. Azide modification of the Winkler method was used.

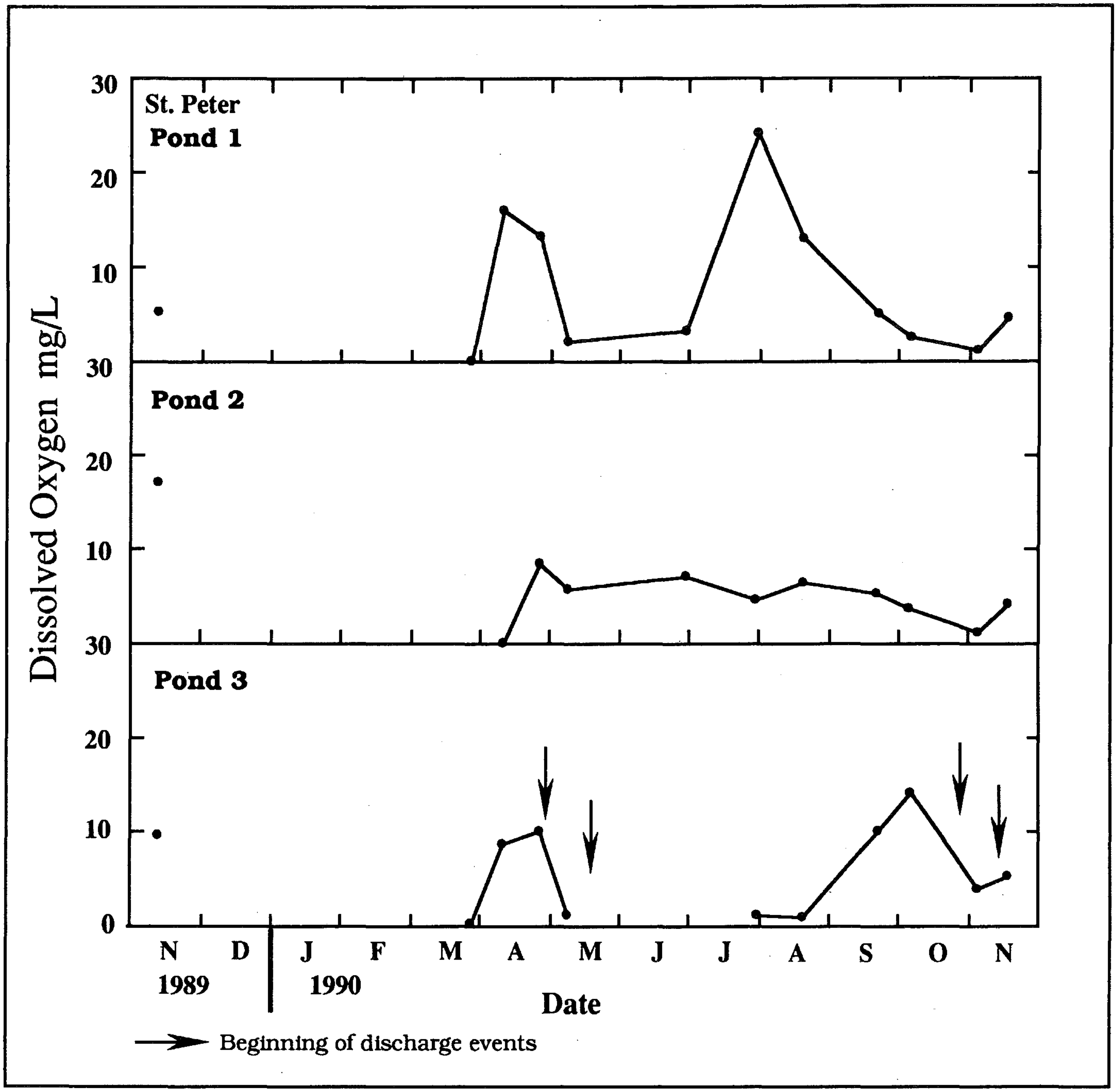


Figure 22. Concentrations of dissolved oxygen in the surface waters of waste stabilization ponds at St. Peter, MN in November 1989 and from spring, 1990 - November 1990. Azide modification of the Winkler method was used. Copper sulfate treatments occurred in April, 1990 in pond 3.

Chemical and physical data from waste stabilization ponds

Chemical and physical methods

Alkalinity, or acid neutralizing capacity, ANC, was performed by the end point titration method as stated in Standard Methods (APHA, 1989). Standardized 0.020 N sulfuric acid was added to 25 ml of sample to an endpoint of pH 4.6.

Ammonia was analyzed on samples that were filtered and preserved in the field with concentrated sulfuric acid (0.8 ml/L of sample). Ammonium ($NH4^+$) was analyzed manually by the Berthelot reaction (phenol-hypochloride method), as given in Standard Methods.

Anions (NO_3^-, NO_2^-, SO_4^{2-}, Cl^-) were determined by ion chromatography (Dionex Model 10) after filtering the samples through 0.4 mm Nuclepore membrane filters. Cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) were measured by flame atomic absorption spectrophotometry (AAS), using instrument settings recommended by the instrument manufacturer. Prior to AAS, the samples were filtered through 0.4 mm Nuclepore membrane filters and acidified with Ultrex nitric acid.

Biochemical oxygen demand (BOD_5) analyses were performed by the Minnesota Department of Health. Samples were delivered to that laboratory within 48 h of sample collection. Nitrification-inhibited 5 day BOD was performed according the Standard Methods (APHA, 1989).

Chlorophyll and phaeophytin were determined by the spectrophotometric method as described in Standard Methods. Chlorophyll was extracted with 90% acetone from samples preserved on glass fiber filters wrapped in foil to exclude light and frozen until analysis.

Conductivity was measured with a YSI model 32 conductance meter.

Phosphate as soluble reactive phosphate (SRP) was analyzed on samples that were filtered and preserved in the field with concentrated sulfuric acid (0.8 ml/L of sample). SRP was analyzed manually by the ascorbic acid-molybdate method as given in Standard Methods.

Physical parameters such as temperature and pH were measured on unfiltered, unpreserved water samples.

Total nitrogen was measured on unfiltered samples by converting all nitrogen forms to nitrate by alkaline persulfate oxidation and subsequent analysis of nitrate by the automated cadmium reduction method.

Total organic carbon (TOC) was measured by converting the organic matter to CO_2 by persulfate/uv digestion and subsequent analysis by a Dohrmann IR gas analyzer.

Total phosphorus was determined by the ascorbic acid method after persulfate digestion of unfiltered samples.

Total suspended solids (TSS) was measured according the the procedure in Standard Methods (APHA, 1989). A known volume of the sample was filtered through a pre-weighed 0.45 mm standard glass-fiber filter. The residue on the filter was dried to a constant weight at 103 to 105^{o}C in an oven. The increase in weight of the filter represents the TSS.

Turbidity was measured on a Hach 2100A turbidimeter.

At the beginning of the project in spring of 1990, analysis of TSS and ammonia was done by the Minnesota Department of Health using the standard methods described above. The rest of the analyses were performed in the laboratory of Dr. Patrick Brezonik at the University of Minnesota.

Field measurements by MPCA

Dissolved oxygen (DO) was measured by the modified Winkler method as described in Standard Methods (1989). This method was used not only to calibrate the YSI model 58 DO meter, but to provide information on supersaturated oxygen levels over 20 mg/L.

pH was measured with a Beckman model 11 pH meter with gel-filled electrode.

Secchi depth was measured with a standard secchi disc as the depth in centimeters when the disc reappears after disappearing from view.

Quality Control and Assurance Procedures

1. Analytical measurements followed the procedures from the 17th edition of Standard Methods (APHA< 1989) except where more appropriate and better procedures exist, e.g. for total nitrogen.
2. Analyses on duplicate samples (randomly determined) are done on at least 10% of the samples.
3. Certified EPA standards are analyzed routinely every analytical run, to check on analyst performance.
4. The laboratory is audited by the US EPA QA supervisory personnel approximately once a year, and any recommendations they provide for changes in QA/QC practices are put into effect.
5. Technicians are trained by experienced individuals before conducting analyses themselves, and their performance is reviewed routinely by their supervisors.
6. Instruments are maintained according to manufacturer specifications.
7. Where possible and appropriate, a variety of post-analytical checks are performed to determine data consistency and reliability. For example, ion balances and computed vs. measured conductance are evaluated routinely on samples analyzed for major ions.

Chemical and physical data

The reader is referred to the sections on the outstate survey and Tables 4, 5, 7, 9 and 10 and the section on algae for information on the statistical relationships between the biological and the chemical data for this project. A few of the relationships will be mentioned here. See also the subpart on nitrogen and carbon budgets below.

Ammonia and nitrate

The data for ammonia and nitrate by date and pond are given in Appendix 2. Unionized ammonia values were taken from the U.S. E.P.A. table of aqueous ammonia values (1979) for the concentration of ammonia, temperature and pH. The mean values for ammonia over all dates are given in Table 12. This shows the expected higher ammonia in the primary ponds of the three sites. For an analysis of the mass balance on nitrogen in the ponds, see the section by Dr. Brezonik on nitrogen and carbon budgets.

Plots of the ammonia data for all dates and ponds at Harris (Figure 23) show that spring ammonia values were the highest, and that ammonia increased again in the fall in all ponds. Maximum spring ammonia in Harris pond 3 was 6.6 mg/L on 4/9 right after ice-out. From the time of ice-out on, ammonia levels dropped until the minimum value in pond 3 was reached, 0.03 mg/L in the fall (10/9). In Janesville pond 3, the maximum spring values were 19 (4/17) , the minimum was 0.05 (9/18). For St. Peter pond 3, the maximum was 20.1 mg/L (4/10), the minimum was 0.04 (10/2). At all three sites, the ammonia levels increased again in November (see Appendix 2). Unionized ammonia levels in the secondary ponds at the three sites was over 1 mg/L on only a few dates.

In the outstate sites sampled in fall of 1990, ammonia values ranged from .04 mg/L at Clark's Grove and Fulda to 2.3 at Bird Island, the only site with ammonia over 1 mg/L in September/October (see Table 8.

Ammonia was not statistically significant in relation to Daphnia reproduction (bioassays) or field densities of Daphnia. It was significantly related to chlorophyll (p=.03, Table 7 in algae) with a negative slope and low r value (.23). Ammonia, a nutrient for algae, can be toxic to algae at higher pH.

Harris pond 3 nitrate ranged from 18.1 to 175 µg/L, in Janesville pond 3 the range was 2.2 to 191.1, and in St. Peter pond 3, from 2 to 121.8 µg/L (Appendix 2). Nitrate values for the outstate sites ranged from 0 at Cosmos to 30 at Bird Island (Table 8).

Phosphorus

The data for soluble reactive phosphate (SRP) and total phosphorus are given in Appendix 2. Maximum phosphate occurred in Harris pond 3 on 10/25 (3.86 mg/L), in pond 2 it occurred on 4/19 (4.18 mg/L), and in the primary pond on 4/30 (4.84). SRP values for Harris are plotted against Julian day in Figure 24. Mean phosphorus values for all dates are given in Table 12. In Janesville and St. Peter, the phosphate level was lower overall in the secondary ponds, but at Harris it was actually higher in pond 3 than in pond 2. Around the time of fall discharge, SRP was well over 1 mg/L in the secondary pond at Harris. This can be explained by the low concentration of chlorophyll at Harris pond 3. See Table 7 in the section on algae for the significant relationship (p<.001) between chlorophyll and SRP for all data from all sites with the slope negative. This means that when the chlorophyll level or algae populations increases, the phosphate in the water decreases, as it is taken up by the algae. What is not explained is the higher mean total phosphorus in Harris pond 3 (Table 12).

pH

Daily surface pH values are listed in Appendix 3 and plotted in Figures 25, 26, and 27 for Harris, Janesville and St. Peter ponds. For data on pH through the water column and through the diurnal cycle, see Luck and Stefan (1990). The maximum pH at Harris was 9.84 in pond 2 on 8/4/90. Pond 3 had pH values well over 9.0 in spring through June. At Janesville, the maximum pH was 9.74 in pond 3 on 6/15, at St. Peter it was 9.51 in the primary pond on 4/26.

In the bioassays, Daphnia reproduction was significantly related to pH only for St. Peter pond 3 water (see Table 5 in the bioassay section), but the relation of pH to field densities of Daphnia was not significant (Table 4). Chlorophyll levels were significantly related to pH ($p<.001$, slope positive Table 7, as would be expected because the elevated pH is caused by the algal photosynthesis. The pH rises recorded in the ponds spring are the results of photosynthesis by the algae.

Nitrogen and carbon budgets

Nitrogen budgets for the Harris pond system are shown in Figures 28 and 30. The ponds are twice as efficient at nitrogen removal in the summer than in spring. During the summer, they are removing ~70 percent of the total N from the system. This is attributed to the higher levels of biological activity and subsequent use of nitrogen in cell mass production during summer. In spring, a larger percentage of nitrogen removal is ammonia volatilization. This is suggested by the fact that total ammonium concentrations are much higher in the spring (0.10-23 mg N/L in ponds 1 and 2). Denitrification is not an important removal mechanism for nitrogen because nitrate concentrations found in the ponds are very low (typically < 20 ppb as N).

The summer nitrogen removal efficiency compares favorable with secondary activated sludge treatment. Typical removal efficiencies range between 10 - 20 percent removal for conventional treatment and 70 - 95 percent removal for systems with nitrification and denitrification treatment (U.S. EPA, 1975). The Harris system falls in the high end of the removal efficiency range reported by the EPA for oxidations ponds (0-90% removal).

Given the fact that the Janesville and St. Peter ponds have much different operating efficiencies for suspended solids, it was expected that they also would have different removal rates for nitrogen. Total nitrogen concentrations were somewhat higher in these ponds, but influent data were not taken and a complete nitrogen mass balance therefore could not be done. Ammonium concentrations in both the St. Peter and Janesville systems were similar to those of the Harris system and they tended to increase from pond 1 to ponds 2 and 3.

The total organic carbon (TOC) budget for the Harris system is shown in Figures 29 and 30. In contrast to the mass balances for nitrogen, there is little difference between the spring and summer carbon removal efficiencies. This probably reflects the fact that there is less dependence on algal growth for removal efficiency; sedimentation of volatile suspended solids and microbial oxidations is the primary removal mechanism for the pond system. In both seasons, about 80 percent of the influent carbon was removed in the primary pond. The main difference between the carbon and nitrogen mass balances is the increase in carbon mass in pond 2 in the summer period. This is attributed to high algal growth, which depleted the dissolved CO_2, allowing CO_2 gas transfer from the atmosphere to occur. This is indicated by the occurrence of CO_2 undersaturation in pond 2 during periods for high algal growth.

The carbon removal efficiency of the Harris system compares well with other treatment systems. The removal efficiency for BOD_5 is in the 90 - 95 percent range (influent BOD_5 averaged 200 mg/L; effluent ~10 mg/L). This compares well with secondary activated sludge (85-90% removal efficiency (2)). TOC removal is in the 85 percent range, which again compares favorably to activated sludge (70-90% removal, Metcalf & Eddy 1990).

Because influent concentrations were not measured at Janesville and St. Peter, quantitative mass balances for carbon cannot be computed. However, carbon removal for the St. Peter pond system should be similar to that at Harris because the TOC and BOD_5 concentrations were similar in the two systems. The Janesville system exhibited elevated TOC levels in the second pond, however. In fact, Janesville pond 2 typically had higher TOC concentrations than the primary pond. The range for the second pond (17-36 mg C/L) is higher than that of any other pond studied except for Bellingham, Minnesota in September 1990. The explanation for this is uncertain.

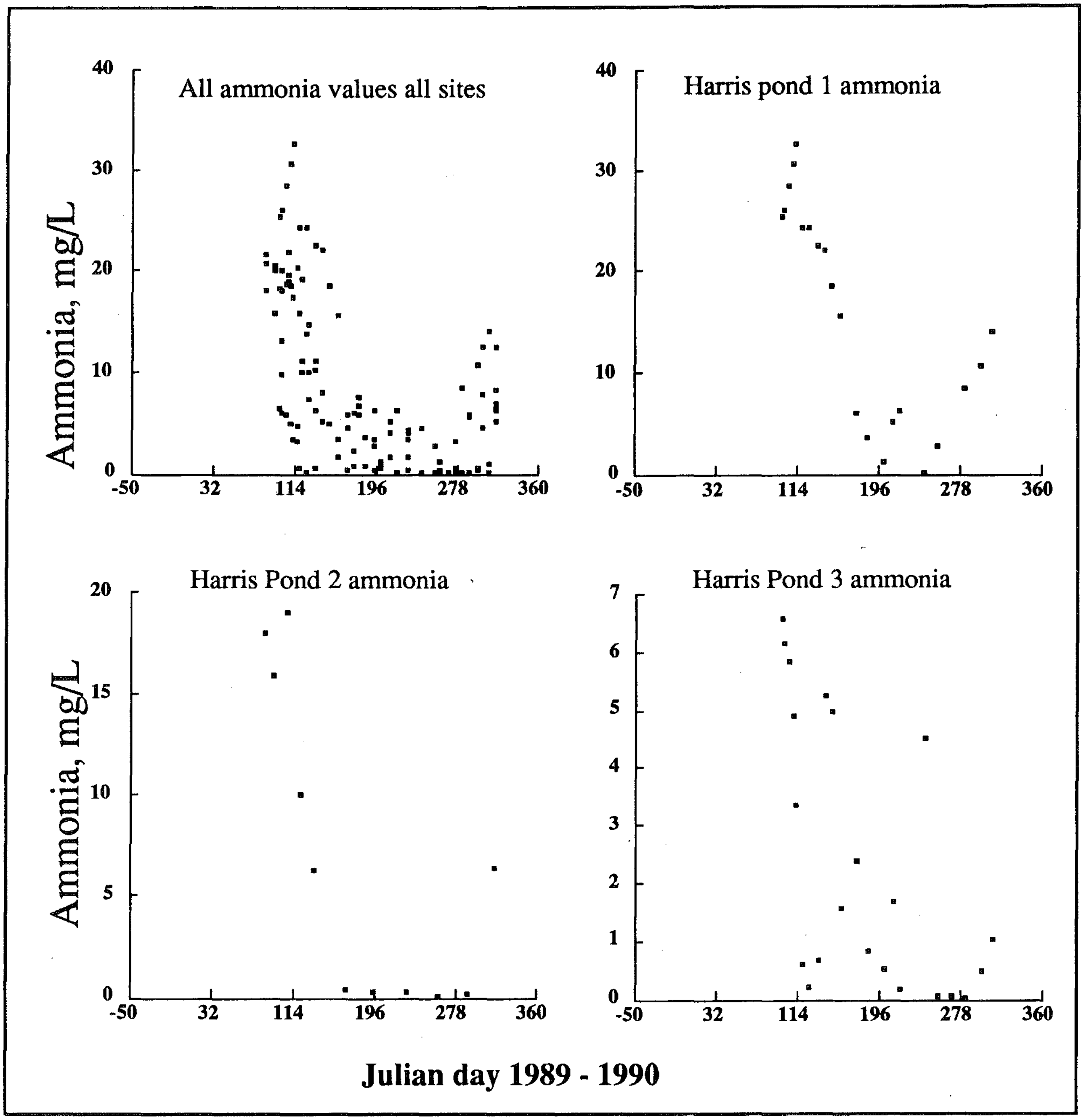


Figure 23. Ammonia data for Harris, Janesville and St. Peter from November,1989 through November 1990 (A.), and for Harris ponds 1, 2, and 3 with julian dates (0 = January 1, 1990). Note the different scales for ammonia on the lower two graphs.

Table 12. Mean values for phosphorus and nitrogen compounds in waste stabilization ponds April through November, 1990. Means in mg/L except nitrate (NO_3^-), which is µg/L. SRP = soluble reactive phosphorus, TP = total phosphorus, n = number of samples, HA = Harris, JA = Janesville, SP = St. Peter, # is pond number, SD is one standard deviation.

		Phosphorus						Nitrogen					
Site	#	SRP	SD	n	TP	SD	n	NH_3	SD	n	NO_3^-	SD	n
HA	1	2.55	.966	20	3.32	.936	14	15.66	10.583	17	48.99	47.69	17
HA	2	1.87	.929	16	2.42	1.146	14	7.37	7.707	18	60.14	64.667	20
HA	3	2.60	1.481	16	3.21	1.161	14	2.36	2.34	19	54.78	51.202	20
JA	1	2.77	.935	4	2.77	.812	4	11.4	8.066	6	29	32.718	5
JA	2	1.44	.346	4	2.42	1.02	4	9.42	7.75	5	31.52	29.895	5
JA	3	1.04	.609	4	1.67	.402	4	6.96	7.639	6	40.6	36.364	5
SP	1	2.62	.954	7	3.08	.819	6	6.58	3.775	7	115.41	254.83	7
SP	2	1.92	.669	7	1.99	.068	6	7.57	6.216	7	14.18	9.376	7
SP	3	1.17	.583	7	1.30	.508	7	5.08	5.751	7	31.03	41.658	9

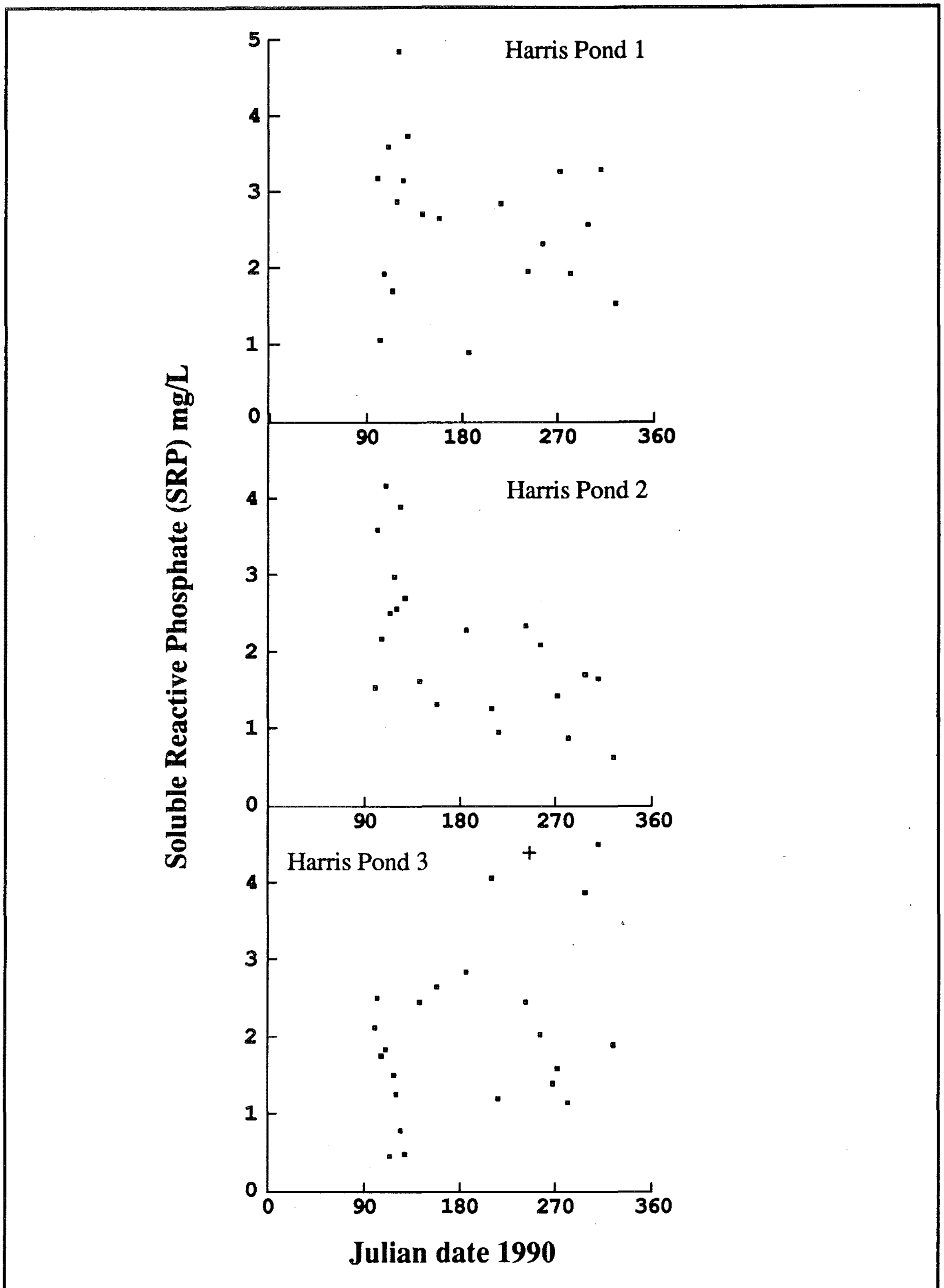


Figure 24. Plots of SRP phosphate values for Harris ponds by Julian date in 1990 (see text).

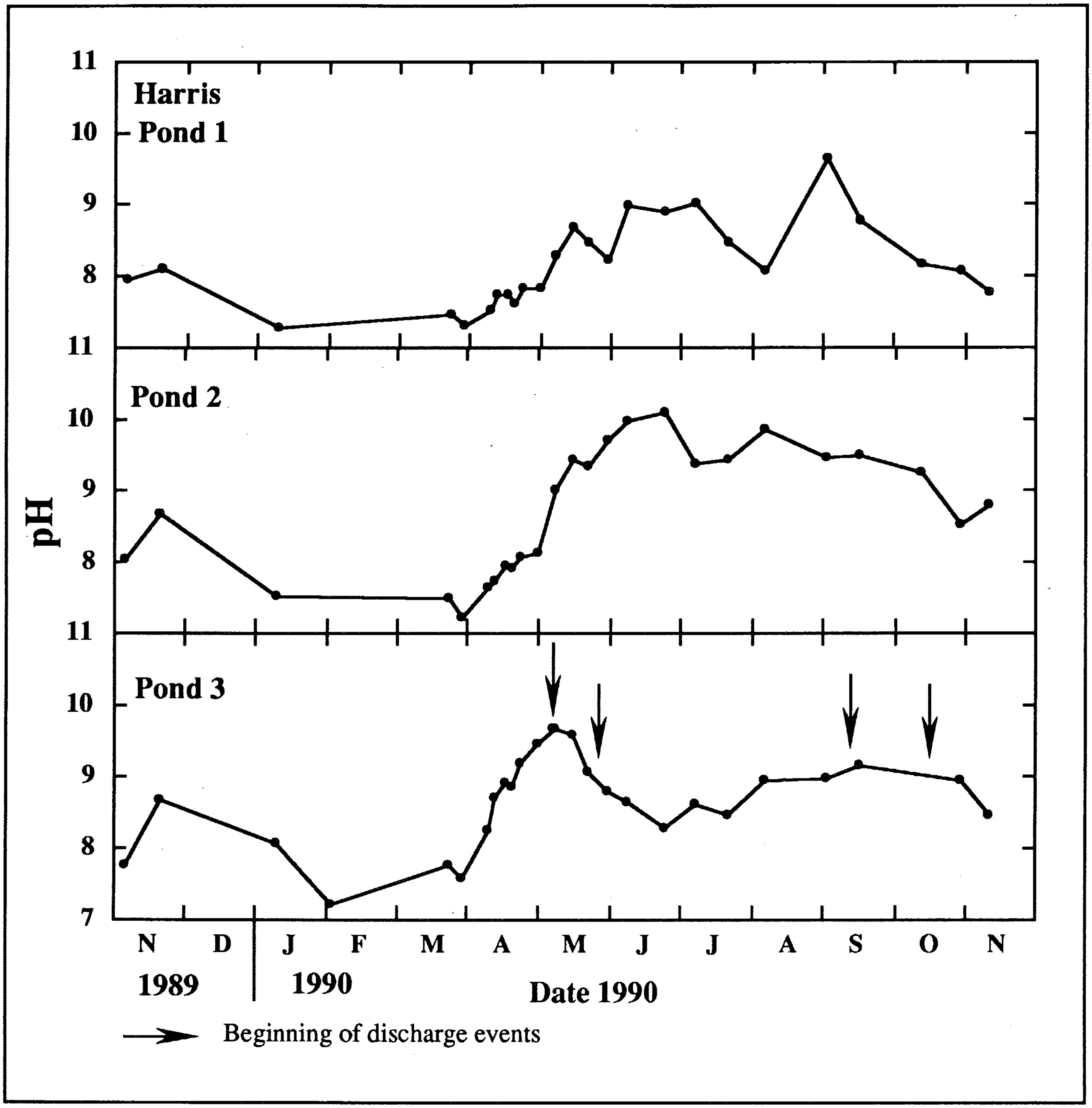


Figure 25. Surface pH at Harris ponds from November 1989 through November 1990. Samples were taken during the zooplankton sampling.

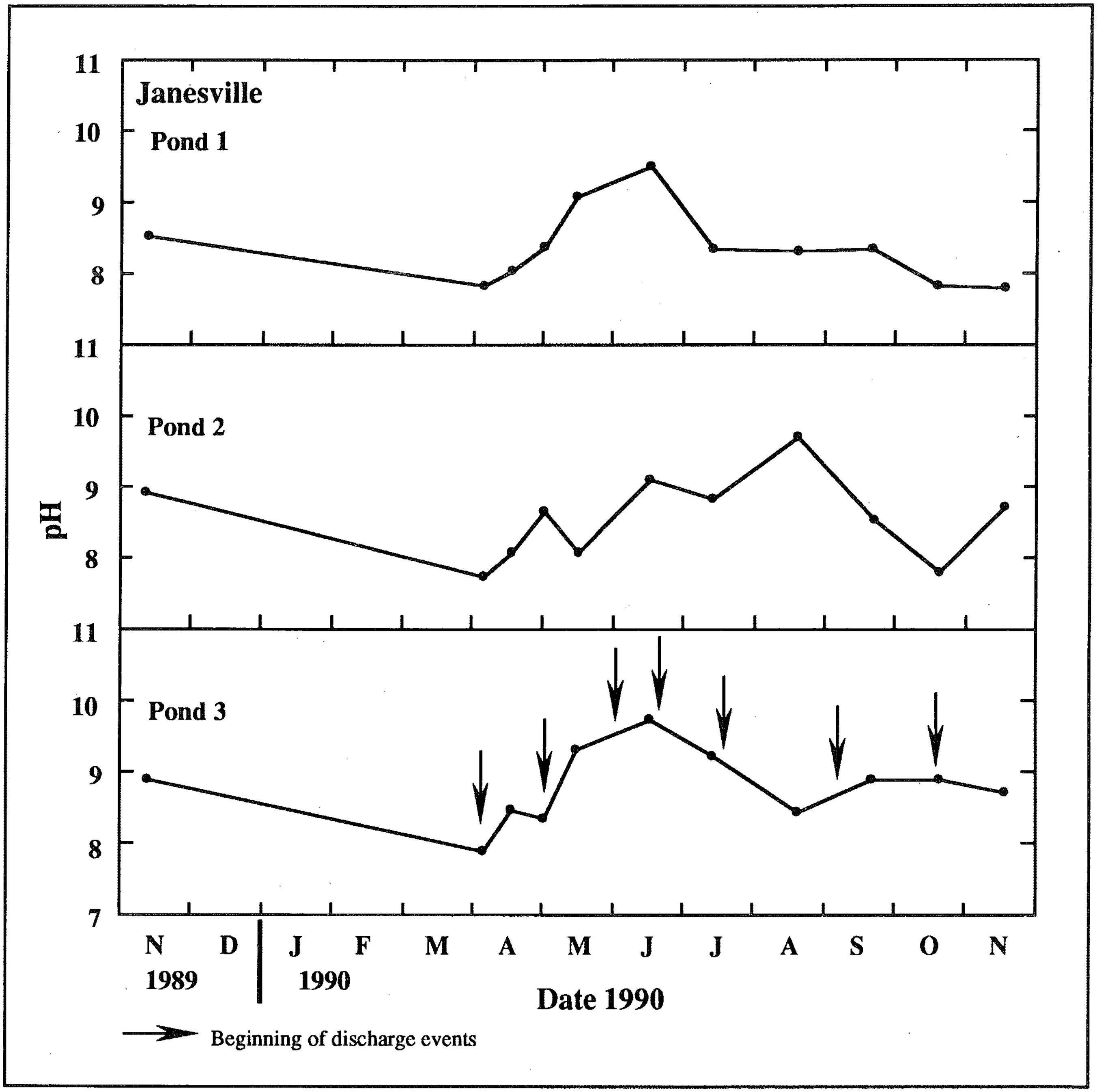


Figure 26. Surface pH at Janesville ponds from November 1989 through November, 1990. Samples were taken during the zooplankton sampling.

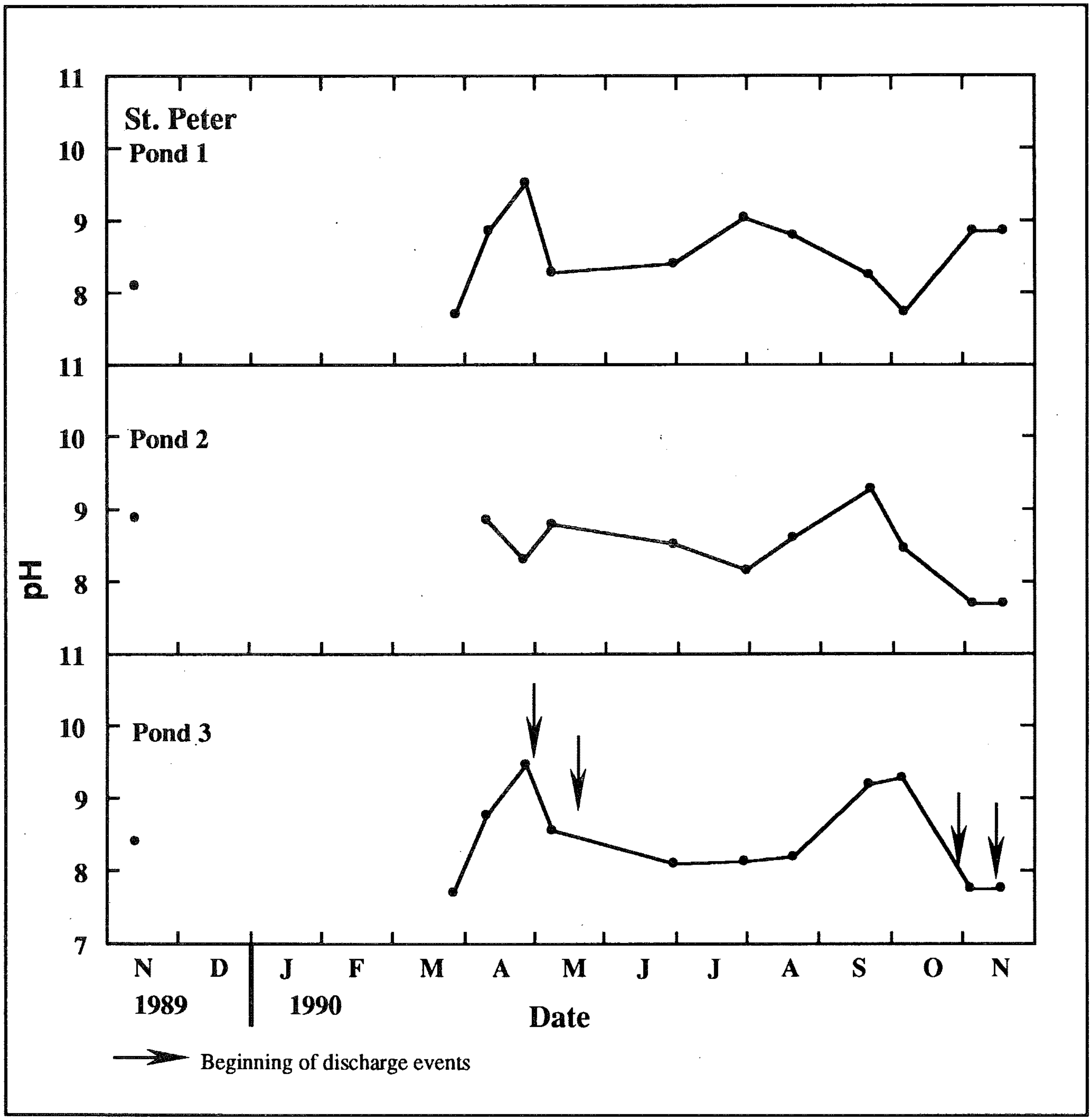
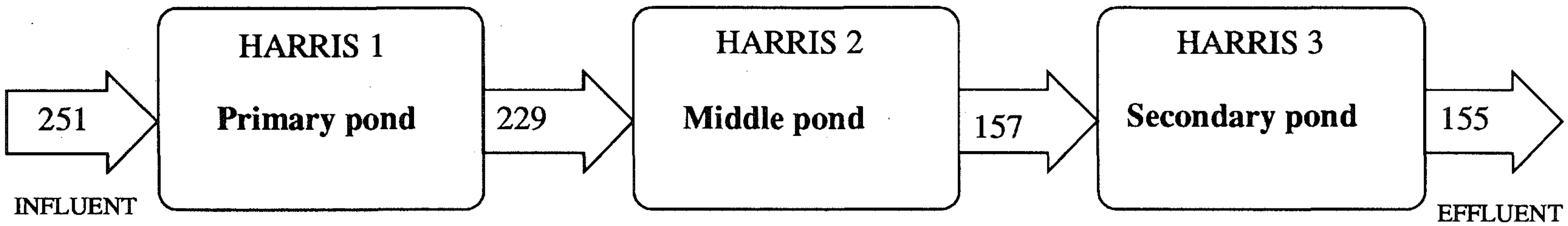


Figure 27. Surface pH at St. Peter ponds from November 1989 through November 1990. Samples were taken during the zooplankton sampling.

Nitrogen flux in spring, 1990



Nitrogen flux in summer, 1990

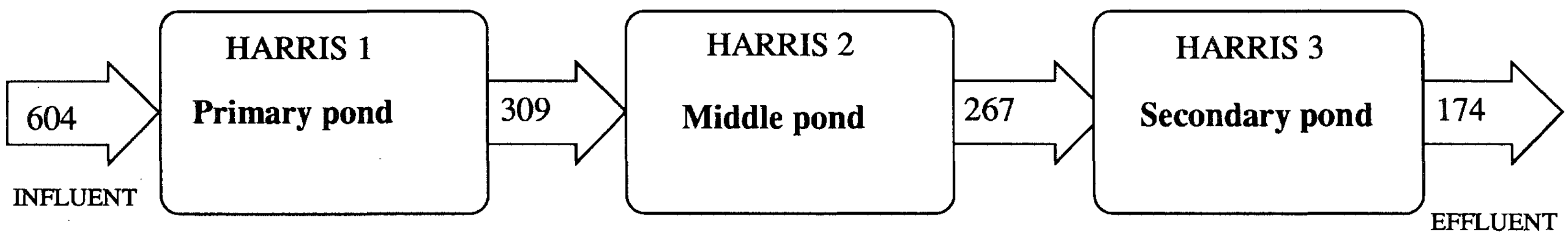


Figure 28. Total nitrogen mass flux through the Harris pond system during the spring (March - June) and summer (June - October) seasons in 1990. Values shown are nitrogen estimates in kg. Squares symbolize the three stabilization ponds, the third pond is the secondary from which the effluent is discharged.

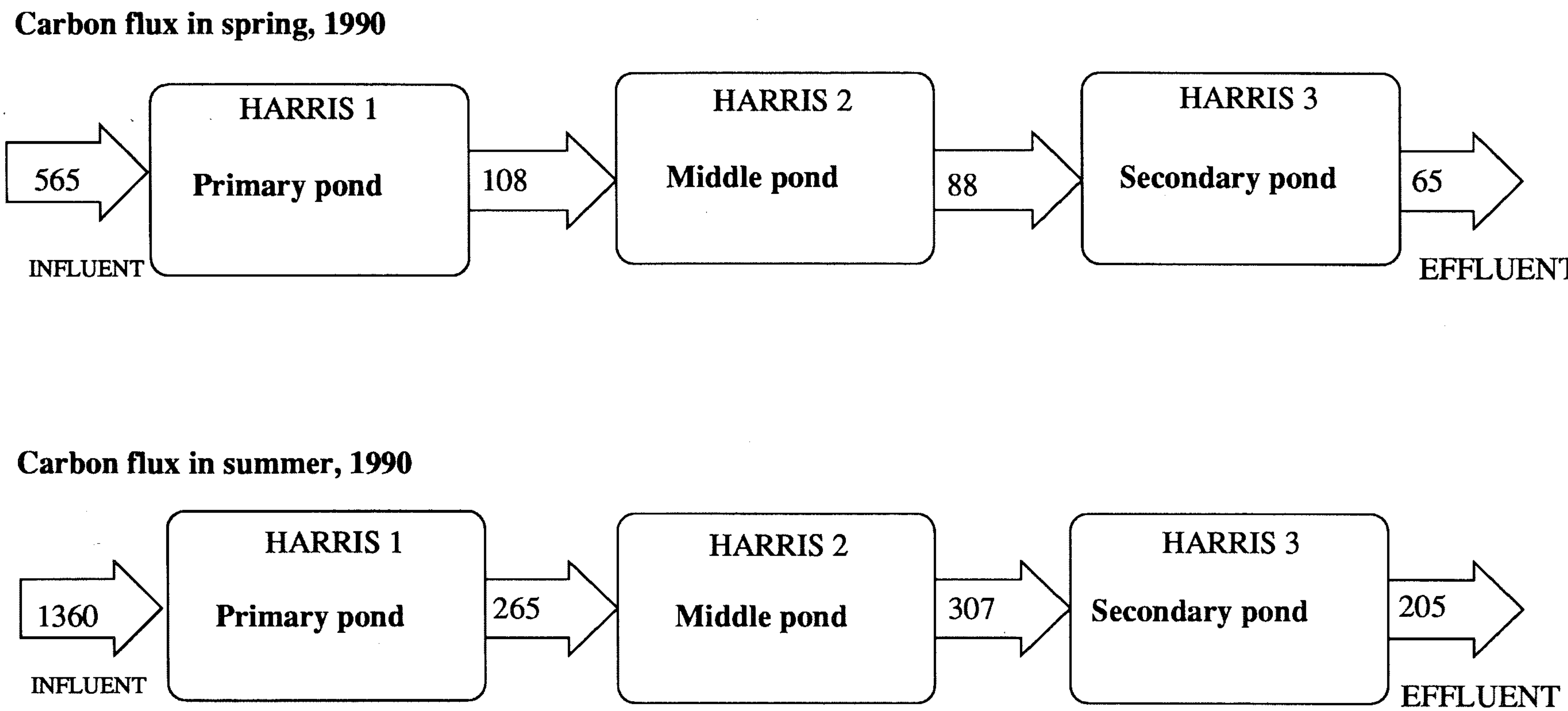


Figure 29. Total carbon mass flux through the Harris pond system during the spring (March through June) and and summer (June - October) seasons in 1990. Values shown are carbon estimates in kg. Squares symbolize the three stabilization ponds, the third pond is the secondary from which the effluent is discharged.

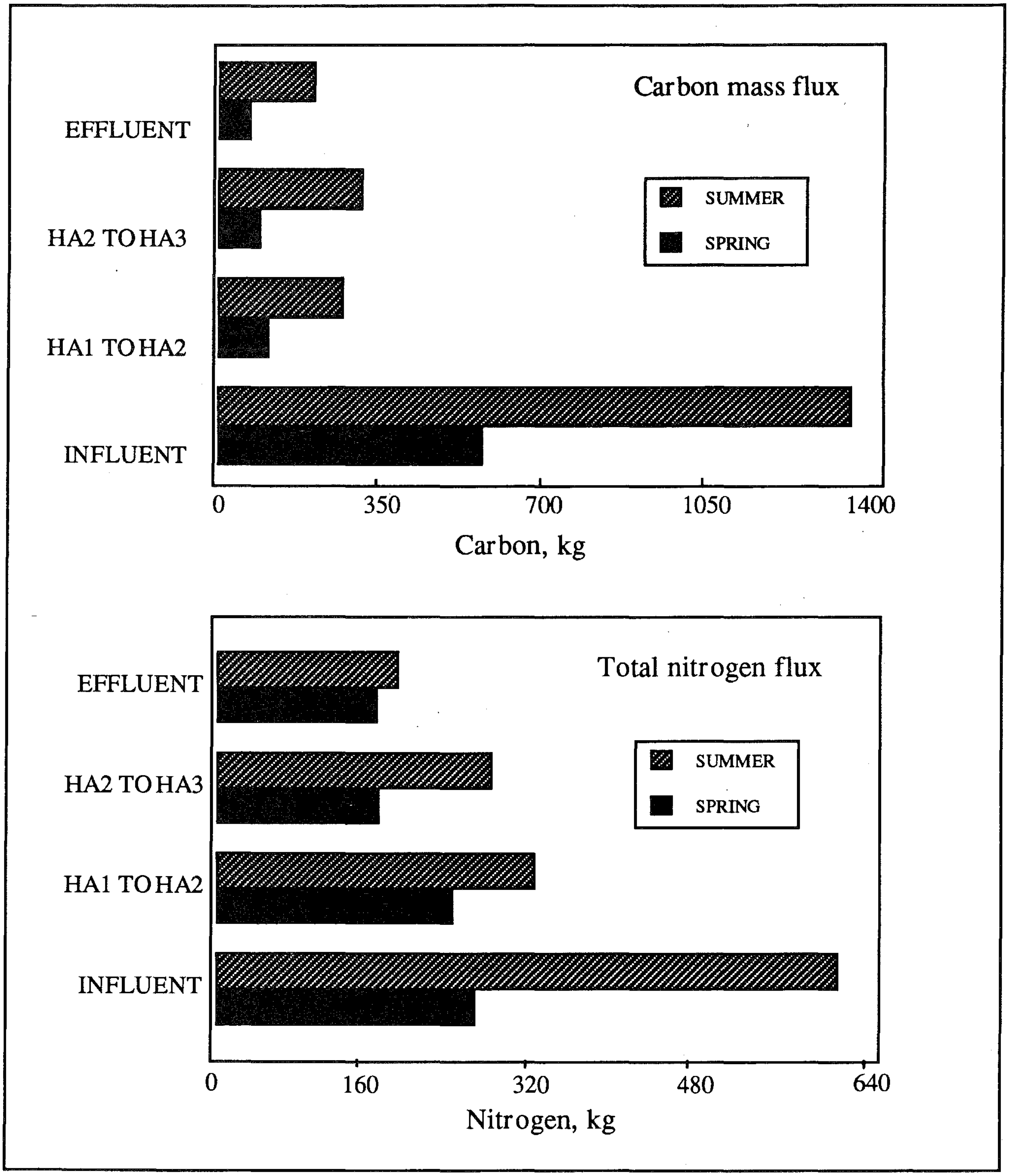


Figure 30. Carbon and nitrogen mass flux (kg) through Harris waste stabilization ponds in the spring and summer seasons in 1990. HA1 = primary pond, HA2 = middle pond, HA3 = secondary or discharge pond.

References

Abeliovich, A. 1986. Algae in wastewater oxidation ponds. In A. Richmond ed, CRC Handbook of Microalgal Mass Culture. CRC Press. Boca Raton, Florida.

Clesceri, L.S., A.E. Greenberg, R.R. Trussell. 1989. Standard Methods For the Examination of Water and Wastewater. 17th edition. American Public Health Association.

Gu, R. and H.G. Stefan. 1991. Numerical simulation of stratification dynamics and mixing in wastewater stabilization ponds. Project Report No. 316. St. Anthony Falls Hydraulic Laboratory, University of Minnesota. A report prepared for the Legislative Commission on Minnesota Resources. Minneapolis, MN.

Carvalho, G.R. and H.G. Wolf. 1989. Resting eggs of lake -- Daphnia I. Distribution, abundance and hatching of eggs collected from various depths in lake sediments. Freshwater Biology 22: 459-470.

Davies, B.H. 1976. Carotenoids. In T.W. Goodwin (ed), Chemistry and Biochemistry of Plant Pigments, Vol. 2. Academic Press, London, pp. 38-165.

Hathaway, C. 1992. Modelling Daphnia populations in wastewater stabilization ponds in Minnesota. Masters thesis in preparation. St. Anthony Falls Hydraulic Laboratory. University of Minnesota.

Hebert, P.D.N. 1978. The population biology of Daphnia. Biol. Rev. Vol. 53: 387-426.

Herzig, A. 1985. Resting eggs -- a significant stage in the life cycle of crustaceans Leptodora kindti and Bythotrephes longimanus. Verh. Internat. Verein. Limnol. 22: 3088-3098.

Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. U.S. Environmental Protection Agency. EPA/600/4-85/-14. Dec.1985. Environmental Monitoring and Support Laboratory. Cincinnati, Ohio 45268.

Konig, A., H.W. Pearson and S.A. Silva. 1987. Ammonia toxicity to algal growth in waste stabilization ponds. Wat. Sci. Tech. Vol. 19 (12): 115-122.

Lee, R.E. 1980. Phycology. Cambridge University Press. New York.

Luck, F.N. and H.G. Stefan. 1990. Physical limnology of the Harris wastewater stabilization ponds: July 1989 to October 1990. Project Report No. 309. St. Anthony Falls Hydraulic Laboratory, University of Minnesota. A report prepared for the Legislative Commission on Minnesota Resources. Minneapolis, MN.

Madenjian, C.P. 1990. Nighttime pond respiration rate: oxygen or temperature dependent? Can. J. Fish. Aquat. Sci. Vol. 47:180-183.

Madenjian, C.P., G.L. Rogers, A.W. Fast. 1990. Estimation of whole pond respiration rate. Can. J. Fish. Aquat. Sci. Vol. 47: 682-686.

Mantoura, R.F.C. and C.A. Llewellyn, 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high performance liquid chromatography. Anal. Chim. Acta 151: 297-314.

Metcalf and Eddy. 1990. Wastewater Engineering. McGraw-Hill, New York.

Minnesota Department of Health, Public Water Supply Data. 1989. Volume 1 - Volume 2.

Paerl, H.W. and J.F. Ustach. 1982. Blue-green algal scums: an explanation for their occurrence during freshwater blooms. Limnol. Oceanogr. 27: 212-217.

Pearson, H.W., D.D. Mara, S.W. Mills, D.J. Smallman. 1987. Factors determining algal populations in waste stabilization ponds and the influence of algae on pond performance. Wat. Sci. Tech. 19: 131-140.

Shapiro, J. 1990. Current beliefs regarding dominance by blue-greens: the case for the importance of CO_2 and pH. Verh. Internat. Verein. Limnol. 24: 38-54.

U.S. E.P.A. 1975. Process Design Manual for Nitrogen Control. Washington D.C.

U.S. E.P.A. 1985. Ambient Water Quality Criteria for Ammonia -- 1984. U.S. Environmental Protection Agency. EPA 440/5-85-001. Duluth, MN. 217 pp.

Walmsley, R.D. 1984. A chlorophyll a trophic status classification system for South African impoundments. J. Environ. Qual. Vol. 13 (1): 97-104.

Weber, C.I. et al. 1989. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. 2nd ed. U.S. Environmental Protection Agency. EPA/600/4-89/-001. March, 1989. Environmental Monitoring Systems Laboratory. Cincinnati, Ohio 45268.

Wrigley, T.J. and D.F. Toerien. 1990. Limnological aspects of small sewage ponds. Wat. Res. Vol. 24 (1): 83-90.

Appendix 1. HPLC analysis of waste stabilization pond algae pigments.

Early in the project, filters of algae from Harris, Janesville and St. Peter from November 1989 - January 1991 were analyzed by Dr. Dan Engstrom at the U. of MN. by HPLC analysis (high pressure liquid chromatography) for the various chlorophyll and carotenoid pigments found in algae. The intention of this work was to see if the composition of algal populations could be detected by HPLC analysis alone. The method used for HPLC pigment analysis of algae by Dr. Engstrom is given below, followed by a summary of the results.

For the HPLC analysis, pigments were extracted from filters by soaking them for 24 h at 4°C in a mixture of acetone, methanol and water (80:15:5 by volume), followed by centrifugation and filtration through 0.2mm Gelman Nylaflow membrane filters. Carotenoid and chlorophyll concentrations were quantified by RP-HPLC, reverse phase, high-pressure liquid chromatography, modified from Mantoura and Llewellyn, 1983. A Hewlett Packard 1090 HPLC mainframe equipped with a diode-array spectrophotometric detector and HP-310 microcomputer workstation. Analytical separation was achieved by isocratic delivery (1.0 ml min^{-1}) of mobile phase A (10% IPR-ion paring reagent, 10% water, 80% methanol) for one minute, followed by a 9-minute linear gradient to 100 percent phase B (20% acetone, 80% methanol), and an isocratic hold at 100% B for 13 minutes. The column was re-equilibrated by a 2-minute linear gradient to 100% A and a 5 minute hold. The IPR contained 1.5 g tetrabutyl ammonium acetate and 7.7 g ammonium acetate in 100 ml water. Samples were scanned for absorbance at 409, 435, 473, and 492 nm, relative to a reference at 545 nm.

The HPLC system was calibrated by measuring sample absorbance at 435 nm on a Beckman model-24 spectrophotometer, and calculating pigment concentration from the portion of absorbance due to the pigment of interest (base on the 435-nm HPLC chromatogram), and specific extinction coefficients from Namtoura and Llewellyn (1983) and Daview (1976). HPLC calibrations represent mean values from a selection of samples and standards with strong, chromatographically pure HPLC spectra. Pigments were identified by comparing retention times (relative to chlorophyll a) and spectral characteristics with values obtained from commercial standards, pigments isolated from algal cultures, and published absorbance maxima.

For analysis of algae types based on HPLC pigment analysis, the pigment composition as described in Lee (1980) was used.

Results of HPLC analysis

Filters from samples taken in fall of 1989 and early winter from Harris, Janesville, St. Peter, and four other sites were analyzed chromatographically by HPLC. The goal of this work was to test whether pigment analysis could clarify the algal composition of the ponds, and whether it could take the place of microscopic identifications of algae (the rapid assessment method). The major groupings of algae are different, for instance in the types of chlorophyll. Bluegreen algae have chlorophyll a only, green algae have both chlorophyll a and b. Other groups have chlorophylls a and c. The algae also differ in many of their carotenoid pigments, although all algae have b-carotene (Lee, 1980). Often called 'accessory pigments,' carotenoids assist in trapping light in photosynthesis.

Overall, the criteria used here for pigment analysis was correct for identifying the presence of Cryptomonas 7/7 times, with some question about the presence of alloxanthin at three sites, when Cryptomonas was not observed microscopically. Pigment analysis identified the presence of green algae every time they were present. On two occasions, (St. Peter Pond 3 and Kiester pond 2) there was no violaxanthin detected when green algae were present. Prediction of bluegreen algae by pigment analysis was correct for 6/9 of the samples. For 3 samples, bluegreens were present but not detected by pigment analysis. One of these samples, JA2, had high concentrations of Oscillatoria as well as green algae. It is possible the spectra for the green algae may have covered the oscillaxanthin peak (Dr. Engstrom, pers comm).

Appendix 2. Values for ammonia-N and unionized ammonia (mg/L), nitrate (mg/L), soluble reactive phosphate (SRP, mg/L) and total phosphorus (mg/L) for Harris (HA), Janesville (JA), and St. Peter (SP) waste stabilization ponds from November 1989 through November 1990. Unionized ammonia (NH3-un) values are from the U.S. E.P.A.,1979.

Date	Site	Pond	NH3	NH3-un	NO3	SRP	TP
4/9/90	HA	1	25.4	0.13	0	3.17	.
4/9/90	HA	2	18.2	0.14	20.5	1.53	.
4/9/90	HA	3	6.56	0.01	123.3	2.11	.
4/12/90	HA	1	26	0.18	6.8	1.07	.
4/12/90	HA	2	18.1	0.13	3.6	3.6	.
4/12/90	HA	3	6.14	0.05	107.4	2.52	.
4/16/90	HA	1	28.4	0.4	31.9	1.93	3.08
4/16/90	HA	2	18.8	2.35	35.8	2.17	2.82
4/16/90	HA	3	5.84	0.73	29.1	1.75	4.14
4/19/90	HA	1	30.6	23.04	0	3.58	.
4/19/90	HA	2	18.6	0.28	0	4.18	.
4/19/90	HA	3	4.9	0.58	34.4	1.83	.
4/23/90	HA	1	32.6	0.78	2.2	1.71	2.98
4/23/90	HA	2	17.4	0.7	49	2.52	4.44
4/23/90	HA	3	3.34	0.84	18.1	0.45	3.81
4/30/90	HA	1	24.2	0.3	21.1	4.84	.
4/30/90	HA	2	15.8	0.17	23.6	2.57	.
4/30/90	HA	3	0.6	0.22	175.3	1.27	.
5/4/90	HA	1	.	.	12.9	3.14	4.62
5/4/90	HA	2	.	.	20.5	3.9	4.96
5/4/90	HA	3	.	.	14.7	0.79	1.55
5/7/90	HA	1	24.2	1.84	191.1	3.73	.
5/7/90	HA	2	13.8	3.66	92.4	2.69	.
5/7/90	HA	3	0.2	0.13	35.8	0.48	.
5/14/90	HA	1	22.6	3.12	35.2	.	.
5/14/90	HA	2	10.2	4.59	148.1	.	.
5/14/90	HA	3	0.66	0.34	84.5	.	.
5/21/90	HA	1	22	2.27	89.7	2.71	.
5/21/90	HA	2	8.2	3.62	279	1.61	.
5/21/90	HA	3	5.26	1.41	.	2.44	.
5/29/90	HA	1	18.6	0.97	.	.	.
5/29/90	HA	2	5.04	3.1	.	.	.
5/29/90	HA	3	4.98	0.83	.	.	.
6/6/90	HA	1	15.6	3.85	24.8	2.65	3.55
6/6/90	HA	2	3.54	2.73	84.5	1.32	2.36
6/6/90	HA	3	1.57	0.24	175.4	2.64	4.22

Appendix 2(con).

Date	Site	Pond	NH3	NH3-un	NO3	SRP	TP
7/5/90	HA	1	3.6	1.39	17.7	0.9	4.22
7/5/90	HA	2	0.68	0.39	22.9	2.28	2.73
7/5/90	HA	3	0.84	0.17	27.4	2.84	3.64
7/28/90	HA	1	5.26	2.2	.	1.99	5.43
7/28/90	HA	2	4.2	0.32	32.5	0.995	1.5
7/28/90	HA	3	1.68	0.12	.	2.23	3.81
8/4/90	HA	1	6.3	0.35	83.2	2.83	2.81
8/4/90	HA	2	0.14	0.11	26.8	0.95	1.06
8/4/90	HA	3	0.18	0.06	27.4	1.21	3.08
8/30/90	HA	1	0.19	0.14	37.8	1.96	2.24
8/30/90	HA	2	0.03	0.02	47.5	2.35	3.13
8/30/90	HA	3	4.53	1.71	25.4	2.44	2.52
9/13/90	HA	1	2.88	0.63	50.8	2.31	3.06
9/13/90	HA	2	0.06	0.04	23.5	2.1	2.28
9/13/90	HA	3	0.05	0.02	18.3	2.03	2.41
9/25/90	HA	3	0.05	.	18.3	1.41	.
9/29/90	HA	1	.	.	31.3	3.25	2.51
9/29/90	HA	2	.	.	24.8	1.44	1.48
9/29/90	HA	3	.	.	20.3	1.59	1.38
10/9/90	HA	1	8.56	0.25	52.1	1.93	2.55
10/9/90	HA	2	0.17	0.05	102.7	0.87	1.17
10/9/90	HA	3	0.03	0.01	19.6	1.16	1.38
10/25/90	HA	1	10.72	0.02	76.7	2.55	2.94
10/25/90	HA	2	0.3	0	108.5	1.7	1.92
10/25/90	HA	3	0.49	0	31.3	3.86	3.88
11/6/90	HA	1	14.04	0.1	96.2	3.29	3.81
11/6/90	HA	2	0.19	0.01	56.6	1.65	1.95
11/6/90	HA	3	1.01	0	54	4.5	4.69
11/20/90	HA	1	.	.	2.7	1.53	2.72
11/20/90	HA	2	.	.	0	0.61	2.05
11/20/90	HA	3	.	.	55.5	1.91	4.44
3/27/90	JA	1	21.6	.	.	.	.
3/27/90	JA	2	20.7	.	.	.	.
3/27/90	JA	3	18	.	.	.	.
4/5/90	JA	1	20.4	0.18	.	.	.
4/5/90	JA	2	20	0.16	.	.	.
4/5/90	JA	3	15.9	0.16	.	.	.
4/17/90	JA	1	21.8	0.35	.	.	.
4/17/90	JA	2	19.6	0.33	.	.	.
4/17/90	JA	3	19	0.78	.	.	.
5/1/90	JA	1	19.2	0.84	.	.	.
5/1/90	JA	2	11.3	0.81	.	.	.

Appendix 2 (con).

Date	Site	Pond	NH3	NH3-un	NO3	SRP	TP
5/1/90	JA	3	9.98	0.37	.	.	.
5/15/90	JA	1	10.2	2.48	10.5	1.05	2
5/15/90	JA	2	11.2	0.34	2.7	0.92	1.66
5/15/90	JA	3	6.22	2.23	72.3	0.5	1.6
6/15/90	JA	1	5.88	3.72	.	.	.
6/15/90	JA	2	4.54	1.65	.	.	.
6/15/90	JA	3	0.36	0.28	.	.	.
7/12/90	JA	1	2.84	0.24	5.5	1.93	2.28
7/12/90	JA	2	3.46	0.69	3.4	1.55	2.17
7/12/90	JA	3	0.29	0.12	2.7	1.88	2.12
8/17/90	JA	1	4.1	0.48	.	.	.
8/17/90	JA	2	0.25	0.19	.	.	.
8/17/90	JA	3	0.25	0.04	.	.	.
9/18/90	JA	1	1.22	0.07	86.2	2.12	.
9/18/90	JA	2	0.06	0	27.5	0.89	.
9/18/90	JA	3	0.05	0.01	4.9	0.66	.
10/16/90	JA	1	5.63	0.07	21.3	3	2.98
10/16/90	JA	2	5.87	0.07	68.3	1.64	3.92
10/16/90	JA	3	0.18	0.04	42.9	0.7	1.8
11/13/90	JA	1	12.55	0.09	21.5	2.97	3.82
11/13/90	JA	2	6.66	0.37	55.7	1.63	1.94
11/13/90	JA	3	6.36	0.35	80.2	1.06	1.16
4/10/90	SP	1	13.1	1.59	.	.	.
4/10/90	SP	2	9.76	1.16	.	.	.
4/10/90	SP	3	20.1	1.89	.	.	.
4/26/90	SP	1	4.82	2.8	18.9	2.86	.
4/26/90	SP	2	20.2	1.62	9	2.97	.
4/26/90	SP	3	3.12	1.68	79.5	1.5	.
5/8/90	SP	1	7.36	0.46	26.4	2.52	2.68
5/8/90	SP	2	14.8	2.89	21.5	2.36	2.81
5/8/90	SP	3	10	1.21	21.5	1.55	1.61
6/28/90	SP	1	6.8	0.9	.	.	.
6/28/90	SP	2	7.64	1.26	0	.	.
6/28/90	SP	3	5.98	0.45	2.1	.	.
7/12/90	SP	1	6.24	2.55	.	1.74	.
7/12/90	SP	2	.	0.12	0.6	1.81	1.29
7/12/90	SP	3	3.34	0.12	5.2	2.03	1.57
7/28/90	SP	1	5.26	0.12	2	1.29	2.54
7/28/90	SP	2	4.2	0.12	.	1.87	.
7/28/90	SP	3	1.68	0.12	2	1.34	1.18
8/16/90	SP	1	4.34	1.27	.	.	.
8/16/90	SP	2	3.42	0.72	.	.	.

Appendix 2 (con).

Date	Site	Pond	NH3	NH3-un	NO3	SRP	TP
8/16/90	SP	3	1.77	0.17	.	.	.
9/18/90	SP	1	0.32	0.02	693	2.55	2.6
9/18/90	SP	2	0.19	0.07	9.5	2.15	2.36
9/18/90	SP	3	0.07	0.02	25.3	0.73	0.9
10/2/90	SP	1	3.21	0.05	20.7	1.64	2.45
10/2/90	SP	2	0.66	0.05	18.2	0.94	1.06
10/2/90	SP	3	0.04	0.02	8	0.26	0.71
10/31/90	SP	1	12.56	1.18	18.8	3.42	3.83
10/31/90	SP	2	7.79	0.06	23.1	1.86	2.06
10/31/90	SP	3	4.57	0.04	13.9	0.92	0.98
11/13/90	SP	1	8.32	0.78	28.1	4.04	4.39
11/13/90	SP	2	7.02	0.06	24.7	1.32	2.37
11/13/90	SP	3	5.16	0.04	121.8	1.38	2.17

Appendix 3. Data on dissolved oxygen (DO), total suspended solids (TSS), chlorophyll a (mg/L), alkalinity (meq/L), conductivity (mnu/cm), temperature (C), and secchi depth (cm) from waste stabilization ponds at Harris (HA), Janesville (JA), and St. Peter (SP), MN from November 1989 - November 1990.

Date	Site	Pond	Alk	Cond	Temp	pH	DO	TSS	Chlorophyll	Secchi
11/21/89	HA	1	.	.	.	.	.	.	242	.
11/21/89	HA	2	.	.	.	.	.	.	149.5	.
11/21/89	HA	3	.	.	.	.	.	.	125.5	.
2/1/90	HA	1	.	.	.	.	.	.	13.35	.
2/1/90	HA	2	.	.	.	.	.	.	10.34	.
2/1/90	HA	3	.	.	.	.	.	.	13.61	.
3/23/90	HA	1	.	795	.	.	.	.	20.21	.
3/23/90	HA	2	.	640	.	.	.	.	4.01	.
3/23/90	HA	3	.	840	.	.	.	.	8.5	.
4/6/90	HA	1	310	763	.	.	.	.	.	.
4/6/90	HA	2	360	695	.	.	.	.	.	.
4/6/90	HA	3	330	675	.	.	.	.	.	.
4/9/90	HA	1	430	780	.	7.49	.	34	0	.
4/9/90	HA	2	310	740	.	7.65	.	6.8	10.27	.
4/9/90	HA	3	320	720	.	8.24	.	18	46.28	.
4/12/90	HA	1	480	830	6.4	7.71	0	21	8.01	17.86
4/12/90	HA	2	440	805	6.2	7.73	0	13	10.68	29.41
4/12/90	HA	3	330	795	7.2	8.71	16.6	14	98.79	33.33
4/16/90	HA	1	370	815	9	7.71	0	20.4	2.67	25.64
4/16/90	HA	2	380	785	9	7.93	0	.	16.02	30.3
4/16/90	HA	3	300	740	9	8.92	17.5	16.8	21.36	52.63
4/19/90	HA	1	.	.	10	7.61	0	9.2	1.76	20
4/19/90	HA	2	.	.	10	7.91	0	15	31.41	26.32
4/19/90	HA	3	.	.	10	8.86	14.1	5.6	40.82	52.63
4/23/90	HA	1	390	1021	19	7.82	0	16	.	23.26
4/23/90	HA	2	390	960	19	8.05	2.57	18	.	29.41
4/23/90	HA	3	320	880	18	9.18	17.6	15	.	34.48

Appendix 3(con).

Date	Site	Pond	Alk	Cond	Temp	pH	DO	TSS	Chlorophyll	Secchi
4/27/90	HA	1	450	874	.	.	.	.	.	.
4/27/90	HA	2	350	805	.	.	.	.	.	.
4/27/90	HA	3	300	748	.	.	.	.	.	.
4/30/90	HA	1	340	880	11	7.8	0	21	35.6	20.83
4/30/90	HA	2	320	852	11	8.11	3.05	13	64.08	27.78
4/30/90	HA	3	280	775	11	9.45	12.8	7.6	37.38	58.82
5/4/90	HA	1	450	910	.	.	.	.	.	.
5/4/90	HA	2	360	815	.	.	.	.	.	.
5/4/90	HA	3	230	710	.	.	.	.	.	.
5/7/90	HA	1	345	921	21	8.25	10.3	35	168.2	15.38
5/7/90	HA	2	325	829	19	8.99	26.1	39	154.9	22.22
5/7/90	HA	3	256	655	19	9.66	16	4.8	5.33	66.67
5/14/90	HA	1	240	890	18	8.67	8.8	110	307.1	12.05
5/14/90	HA	2	300	801	17	9.41	24.4	55	273.7	19.61
5/14/90	HA	3	268	796	16	9.58	5.7	4	2.67	142.86
5/21/90	HA	1	364	860	20.5	8.45	20.5	55	133.5	19.61
5/21/90	HA	2	252	865	19	9.33	1.25	70	173.5	15.38
5/21/90	HA	3	242	908	17	9.06	16.2	15	20.03	38.46
5/29/90	HA	1	.	.	18	8.2	0.4	22	.	50
5/29/90	HA	2	.	.	17	9.7	9.7	48	.	30.3
5/29/90	HA	3	.	.	17	8.8	8.8	2	.	166.67
6/5/90	HA	1	.	.	.	.	.	.	193.6	.
6/5/90	HA	2	.	.	.	.	.	.	206.9	.
6/5/90	HA	3	.	.	.	.	.	.	8.01	.
6/6/90	HA	1	280	743	19	8.95	22.7	52	.	23.81
6/6/90	HA	2	240	628	19.5	9.96	20.1	40	.	20.83
6/6/90	HA	3	320	705	21	8.63	21	6	.	58.82
6/22/90	HA	1	.	.	22	8.86	5.26	23	.	47.62
6/22/90	HA	2	.	.	22	10.1	16.5	35	.	32.26
6/22/90	HA	3	.	.	23	8.27	3.39	5.4	.	76.92

Appendix 3(con).

Date	Site	Pond	Alk	Cond	Temp	pH	DO	TSS	Chlorophyll	Secchi
7/5/90	HA	1	240	580	27	8.98	13.4	20	186.9	32.26
7/5/90	HA	2	280	591	26	9.35	12.6	14	42.72	37.04
7/5/90	HA	3	380	650	26.5	8.61	2.7	4	2	142.86
7/19/90	HA	1	.	.	28	8.44	24.8	44	.	28.57
7/19/90	HA	2	.	.	26.5	9.43	2.66	18	.	34.48
7/19/90	HA	3	.	.	27	8.44	6.68	2	.	71.43
7/28/90	HA	1	.	.	27.5	9.03	24.2	31	222.5	29.41
7/28/90	HA	2	.	.	25.3	8.15	4.38	16	240.3	29.41
7/28/90	HA	3	.	.	25	8.14	1.2	24	8.9	32.26
8/4/90	HA	1	290	674	24	8.05	1.48	16	115.7	27.03
8/4/90	HA	2	250	608	24	9.84	9.33	16	160.2	43.48
8/4/90	HA	3	270	634	25	8.95	8.18	1.4	8.9	166.67
8/30/90	HA	1	270	610	27	9.61	34.6	.	729.8	15.87
8/30/90	HA	2	260	585	27	9.45	16.9	.	46.21	28.57
8/30/90	HA	3	240	640	27	8.97	11.6	.	2.67	200
9/11/90	HA	1	.	.	.	.	.	.	210.3	.
9/11/90	HA	2	.	.	.	.	.	.	114.8	.
9/11/90	HA	3	.	.	.	.	.	.	8.01	.
9/13/90	HA	1	270	738	23	8.75	7.55	48.9	.	32.26
9/13/90	HA	2	260	693	24	9.48	15.9	37.8	.	33.33
9/13/90	HA	3	250	733	24	9.14	8.86	2.5	.	142.86
9/25/90	HA	3	.	830	17	.	14.4	1.5	.	76.92
9/29/90	HA	1	370	700	.	.	.	.	.	.
9/29/90	HA	2	300	580	.	.	.	.	.	.
9/29/90	HA	3	290	680	.	.	.	.	.	.
10/9/90	HA	1	310	1027	12	8.14	8.05	29.3	122.8	27.78
10/9/90	HA	2	240	840	13	9.25	17.5	20.5	130.8	29.41
10/9/90	HA	3	240	834	12.2	8.94	9.6	11.9	24	66.67
10/25/90	HA	1	315	1185	5.9	8.04	6.45	31.3	170.9	23.81
10/25/90	HA	2	240	953	5.5	8.5	11.2	16.9	101.5	41.67

Appendix 3 (con).

Date	Site	Pond	Alk	Cond	Temp	pH	DO	TSS	Chlorophyll	Secchi
10/25/90	HA	3	240	958	5.5	8.45	9.43	1	2.14	83.33
11/6/90	HA	1	330	1138	5.3	7.75	3.5	16	149.5	23.81
11/6/90	HA	2	240	920	4.6	8.79	13.5	16	85.4	40
11/6/90	HA	3	270	962	4.6	7.96	8.55	3	1.6	71.43
11/20/90	HA	1	330	1048	.	.	.	.	181.6	.
11/20/90	HA	2	240	869	.	.	.	.	106.8	.
11/20/90	HA	3	260	915	.	.	.	.	75.8	.
3/27/90	JA	1	.	1991	.	.	.	17	.	.
3/27/90	JA	2	.	1777	.	.	.	15	.	.
3/27/90	JA	3	.	1856	.	.	.	16	.	.
4/5/90	JA	1	.	.	7.5	7.8	.	.	.	.
4/5/90	JA	2	.	.	7	7.73	.	.	.	.
4/5/90	JA	3	.	.	6	7.88	.	.	.	.
4/17/90	JA	1	.	.	7.5	8.02	0	.	.	20
4/17/90	JA	2	.	.	7.5	8.06	1.7	.	.	29.41
4/17/90	JA	3	.	.	7	8.47	12.8	.	.	25
5/1/90	JA	1	.	.	11	8.36	15.2	36	.	22.22
5/1/90	JA	2	.	.	9.5	8.64	15.5	85	.	15.87
5/1/90	JA	3	.	.	9.5	8.34	4.85	1.6	.	40
5/15/90	JA	1	.	.	15	9.07	8.9	26	287	25
5/15/90	JA	2	.	.	15	8.05	1.9	30	48.06	25
5/15/90	JA	3	.	.	15	9.31	17.5	67	342.7	20
6/15/90	JA	1	320	.	25.4	9.47	27.6	50	287.5	22.73
6/15/90	JA	2	340	.	25	9.09	11.8	69	94.74	10.99
6/15/90	JA	3	300	.	25.7	9.74	41.6	73	200	14.08
7/12/90	JA	1	290	.	21	8.34	4.85	42	50.86	24.39
7/12/90	JA	2	380	.	20	8.8	8.1	120	610.3	24.39
7/12/90	JA	3	330	.	21	9.21	11.4	150	519	14.93
8/17/90	JA	1	.	.	27	8.3	.	21	.	12.05
8/17/90	JA	2	.	.	27.1	9.7	.	210	.	9.01

Appendix 3 (con).

Date	Site	Pond	Alk	Cond	Temp	pH	DO	TSS	Chlorophyll	Secchi
8/17/90	JA	3	.	.	26.6	8.45	.	180	.	5
9/18/90	JA	1	380	1644	15	8.33	5.45	40	106.8	.
9/18/90	JA	2	320	1658	13.2	8.5	7.15	154	271.9	.
9/18/90	JA	3	400	1584	15	8.9	10.5	118	333.8	5.41
10/16/90	JA	1	.	1810	10.3	7.82	1.13	7.8	.	38.46
10/16/90	JA	2	.	.	9.6	7.77	9.1	114.6	.	5.59
10/16/90	JA	3	.	1708	9.5	8.88	10.6	133	.	4.2
11/13/90	JA	1	485	2280	5	7.77	0.17	14	0	.
11/13/90	JA	2	460	2420	4	8.7	12.8	38	112.1	.
11/13/90	JA	3	450	.	4	8.7	14.5	51	192.2	12.05
3/27/90	SP	1	.	.	9.3	.	.	.	.	29.07
3/27/90	SP	3	.	.	7.7	.	.	.	.	38.02
4/10/90	SP	1	.	.	10.5	8.85	15.9	34	.	18.52
4/10/90	SP	2	.	.	.	8.86	0	44	.	.
4/10/90	SP	3	.	.	9.5	8.76	8.76	6	.	26.32
4/26/90	SP	1	.	.	21	9.51	13.2	24	.	20
4/26/90	SP	2	.	.	21	8.31	8.35	27	.	20
4/26/90	SP	3	.	.	20.5	9.47	10	24	.	26.32
5/8/90	SP	1	192	1640	18.5	8.28	2.05	14	32.04	34.48
5/8/90	SP	2	430	1795	.	8.81	5.7	18	80.1	.
5/8/90	SP	3	360	1765	20	8.54	1.2	11	14.69	50
6/28/90	SP	1	250	1310	26	8.4	3.3	21	37.38	47.62
6/28/90	SP	2	230	1437	26	8.51	6.87	24	32.04	43.48
6/28/90	SP	3	260	1520	27	8.09	.	9	8.01	76.92
7/12/90	SP	1	260	2060	21	9.21	11.4	12	12.86	14.93
7/12/90	SP	2	.	.	21	9.21	11.4	.	.	12.05
7/12/90	SP	3	250	2110	21	9.21	11.4	25	31.55	12.05
7/28/90	SP	1	250	1715	25	8.14	1.2	24	347.1	32.26
7/28/90	SP	2	270	1951	25	8.14	1.2	24	44.5	28.57
7/28/90	SP	3	150	1146	25	8.14	1.2	24	44.5	28.57

Appendix 3 (con).

Date	Site	Pond	Alk	Cond	Temp	pH	DO	TSS	Chlorophyll	Secchi
8/16/90	SP	1	.	.	27	8.8	13	63	.	15.87
8/16/90	SP	2	.	.	27.5	8.6	6.34	20	.	37.04
8/16/90	SP	3	.	.	27	8.2	1.01	12	.	62.5
9/18/90	SP	1	290	2310	16.5	8.26	5.1	57	66.75	.
9/18/90	SP	2	270	2210	16.7	9.29	5.25	36	28.03	.
9/18/90	SP	3	250	2160	18	9.19	10	31	36.05	10
10/2/90	SP	1	330	2400	15	7.73	2.6	23	73.42	30.3
10/2/90	SP	2	290	2290	16	8.46	3.57	31.6	56.07	47.62
10/2/90	SP	3	240	2170	16	9.29	14.2	12.5	72.09	40
10/31/90	SP	1	370	3014	7	8.85	1.25	6	22.25	40
10/31/90	SP	2	320	2726	8	7.7	1.05	5.5	8.01	111.11
10/31/90	SP	3	290	2652	7	7.77	3.85	7.5	16.02	76.92
11/13/90	SP	1	390	2520	7	8.85	4.55	22	30.71	.
11/13/90	SP	2	340	2340	8	7.7	3.95	9	4.27	.
11/13/90	SP	3	320	.	7	7.77	5.2	11	5.34	50

Appendix 4. Values for ions from waste stabilization ponds at Harris (HA), Janesville (JA), and St. Peter (SP), MN. Calcium, magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl) are mg/L, sulfate (S04) is mg/L.

Date	Site	Pond	Calcium	Mg	Na	K	Cl	SO4
4/9/90	HA	1	48	21	106	15	143	20
4/9/90	HA	2	58	25	126	13	110	14
4/9/90	HA	3	53	23	121	14	118	23
5/4/90	HA	1	52	22	128	12	111	17
5/4/90	HA	2	55	24	117	12	.	.
5/4/90	HA	3	38	24	117	12	113	16
8/4/90	HA	1	49	20	89	10	83	15
8/4/90	HA	2	40	19	107	10	90	11
8/4/90	HA	3	49	20	102	10	92	9
10/9/90	HA	1	55	22	113	13	95	18
10/9/90	HA	2	51	21	97	10	87	22
10/9/90	HA	3	46	20	103	13	86	12
5/15/90	JA	1	95	27	380	13	239	.
5/15/90	JA	2	88	25	297	13	80	.
5/15/90	JA	3	78	26	342	12	203	.
10/16/90	JA	1	113	32	261	9	52	.
10/16/90	JA	2	106	31	342	11	490	.
10/16/90	JA	3	83	29	260	89	339	.
5/8/90	SP	1	90	29	331	18	504	167
5/8/90	SP	2	93	31	337	19	528	121
5/8/90	SP	3	95	31	335	19	537	119
10/2/90	SP	1	107	34	341	19	492	131
10/2/90	SP	2	91	30	324	18	469	147
10/2/90	SP	3	68	28	326	19	475	122