

Water Resources Research Center Annual Technical Report FY 2002

Introduction

The Minnesota WRRP Program is administered by the University of Minnesota Water Resources Center (WRC), which is a collaborative enterprise involving several college-level units on the St. Paul campus: the College of Natural Resources (CNR), College of Agricultural, Food, and Environmental Sciences (COAFES), and the Minnesota Extension Service (MES), plus the University of Minnesota Graduate School. The latter unit provides funds to administer the Water Resources Science Graduate Program, which is housed administratively in the WRC. The WRC has co-directors who divide the overall responsibilities for Center operations between them. For FY 2002, the directors were Patrick Brezonik and James L. Anderson. Professor Brezonik reports to the dean of CNR and was responsible for administering the WRRP program. He also is Director of Graduate Studies for the WRS Program. Professor Anderson reports to the deans of COAFES and MES and is responsible for extension operations. The co-directors shared responsibilities for other outreach and research activities of the Center.

Research Program

Characterization of Nitrifying Bacterial Populations in Wastewater Treatment Bioreactors

Basic Information

Title:	Characterization of Nitrifying Bacterial Populations in Wastewater Treatment Bioreactors
Project Number:	2002MN1B
Start Date:	3/1/2002
End Date:	2/28/2004
Funding Source:	104B
Congressional District:	Fifth
Research Category:	None
Focus Category:	Treatment, Waste Water, Surface Water
Descriptors:	None
Principal Investigators:	Timothy Michael LaPara

Publication

Characterization of Nitrifying Bacterial Populations in Wastewater Treatment Bioreactors

Principal investigator

Timothy M. LaPara, Department of Civil Engineering, University of Minnesota.

Funding Source: USGS-WRRI 104B National Grants Competition

Reporting Period: March 1, 2002 through February 28, 2003

Summary

Excessive nitrogen loading to the Mississippi River basin has been recently linked to the development of a large hypoxic zone in the northern Gulf of Mexico. As a result, there is renewed interest in achieving complete nitrogen removal from municipal and industrial wastewater. One of the most critical challenges that must be addressed before complete nitrogen removal can be consistently achieved is to reduce and eliminate upsets in the nitrification process. Nitrifying bacteria are well known to be susceptible to numerous factors such as temperature, pH, and toxic compounds. One of the problems with eliminating nitrification upsets is that there is a virtually complete lack of knowledge regarding the community dynamics of nitrifying bacteria. The research proposed herein would track both nitrifier community structure and total nitrifier biomass at a municipal wastewater treatment facility for a period of one year. To date, we have collected samples from the aeration tanks of the Metropolitan Wastewater Treatment Facility twice per month from June 2002 through February 2003. We plan to continue collecting samples until May 2003. From these samples we will measure total microbial biomass (as particulate protein), total nitrifying bacterial biomass (as *amoA* gene copy number), and identify the physiologically relevant nitrifying bacteria using a nested PCR-DGGE approach. These results will be compared to influent and effluent biochemical oxygen demand concentrations, temperature, pH, dissolved oxygen concentrations, and ammonia concentrations at the treatment facility at the times of sample collection. This research will identify the specific nitrifying bacteria that are associated with excellent nitrification efficiency, thereby leading towards the development of specific operational strategies to promote the growth of these nitrifying bacteria. In conclusion, this research will be an important step in reducing the total nitrogen loading to the Mississippi River basin and the northern Gulf of Mexico.

Introduction

Nitrogenous pollutant removal from wastewater has received renewed interest recently due to a large hypoxic zone that has developed over the last several decades in the northern Gulf of Mexico. This large area ($> 8,000 \text{ km}^2$) has exhibited severely depleted dissolved oxygen concentrations ($\text{DO} < 2 \text{ mg L}^{-1}$) near the Mississippi River delta since the 1950s and 1960s (Rabalais *et al.*, 2001). Nitrogenous compounds, particularly nitrate, have long been suspected to be the cause of this hypoxic zone that has an adverse affect on aquatic life and commercial fisheries. River basins in southern Minnesota, Iowa, Illinois, Indiana, and Ohio have been identified as the primary N-sources (Goolsby *et al.*, 2001). Preliminary efforts are currently underway to reduce nutrient loading to the Mississippi River basin, including the construction of wetlands, riparian forests, and flood plains (Boesch and Brinsfield, 2000). These efforts will undoubtedly be extended to the regulation of municipal and industrial wastewater discharges.

Numerous process designs exist to adequately remove nitrogenous pollutants from wastewater. Operational control of these processes is hindered by inconsistent nitrification performance. Nitrifying bacteria are sensitive to pH, temperature, dissolved oxygen, and toxic compounds. Nitrifying bacteria are also slow-growing, so their recovery from perturbation is slow. One problem with controlling nitrification during wastewater treatment is an inadequate knowledge of the nitrifying bacterial population dynamics. Numerous cultivation-based techniques have been developed to quantify the biomass densities of nitrifying and denitrifying communities (e.g., the most-probable-number assay) (APHA, 1992), however these methods are infamous for underestimating the actual population density by an order of magnitude or more (Amann *et al.*, 1995). Furthermore, cultivation-based assays are time-consuming because nitrifying bacteria grow slowly and fail to provide relevant data on microbial community structure.

The goal of this research is to examine the nitrifying bacterial community at a full-scale municipal wastewater treatment plant over a period of one year. This objective will be achieved through the application of novel molecular-genetic techniques for the analysis of bacterial communities without cultivation. The total number of nitrifying bacteria will be determined using a quantitative real-time polymerase chain reaction (PCR). The types of nitrifying bacteria will be determined using denaturing gradient gel electrophoresis.

Methods

General Approach

The first phase of the proposed research is to optimize the quantitative competitive PCR approach for nitrifying bacteria. Concomitant to this laboratory work, samples are collected from the wastewater treatment facility will be used to characterize both the amount and type of nitrifying bacteria present in the wastewater treatment bioreactor. These samples are stored at -20°C until the DNA is extracted and then subjected to either quantitative PCR or denaturing gradient gel electrophoresis.

Sample Collection, DNA Extraction and Quantification

Bioreactor samples (10 mL) are collected in triplicate from the Metropolitan Wastewater Treatment Plant (St. Paul, MN), placed on ice, and immediately returned to the University of Minnesota. Samples are then centrifuged, the supernatant is removed, and the cell pellet is resuspended in 1 mL of lysis buffer (120 mM sodium phosphate buffer, pH 8.0, 5% sodium dodecyl sulfate). Samples in lysis buffer are then stored at -20°C until processed further. Samples will be collected once every two weeks from June 2002 through May 2003.

Total genomic DNA is purified from samples using the FastDNA Spin Kit per manufacturer's instructions (Qbiogene). Samples undergo three freeze-thaw cycles followed by bead-beating to lyse cells. Extracted DNA is quantified by staining with Hoescht dye 33258 and measuring fluorescence output on a TD-700 fluorometer (Turner Designs; Sunnyvale, CA); results are correlated to a standard curve of calf thymus DNA.

Total Biomass Measurement

Once the population densities of nitrifying bacterial populations have been quantified, it will be important to correlate these values to total biomass levels. Cellular protein is extracted (Herbert *et al.*, 1971) and measured according to the Lowry method (Lowry *et al.*, 1951) using bovine serum albumin (BSA) as a protein standard.

Quantification of Nitrifying Bacteria

Two different approaches may be undertaken to quantify the number of ammonia-oxidizing bacteria in wastewater treatment samples. The first method is quantitative competitive PCR (cPCR), which works by including a known amount of artificially synthesized DNA (i.e., competitor) in a PCR reaction; the concentration of an unknown sample is determined when the amplification of the unknown and competitor are equal. We will apply the cPCR technique described by Dionisi *et al.* (2002) to quantify *amoA* gene copy number. Quantitative real-time PCR will also be attempted. This method works by measuring the amount of PCR product generated over time during the thermal cycle program and comparing results to known standards. The advantage of real-time PCR compared to cPCR is that less labor intensive and a large number of samples can be processed simultaneously (instead of individually as with cPCR).

Nitrifying Community Structure

Bacterial community shifts will be detected by denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments specific for nitrifying bacteria. PCR-DGGE is a relatively simple method to detect qualitative shifts in bacterial community structure (Muyzer *et al.*, 1993). A detailed description of the PCR-DGGE approach that will be used has been presented elsewhere (LaPara *et al.*, 2000). The 16S rRNA gene of the known nitrifying bacteria will be amplified by PCR using the CTO189f and CTO654r primers (Kowalchuk *et al.*, 1993). A second PCR reaction will then be applied using primers PRBA338F and PRUN518R with a GC-clamp attached to the forward primer.

DGGE will be performed on a D-Code apparatus (BioRad; Hercules, CA) using a denaturing gradient ranging from 30 to 55% denaturant (100% denaturant contains 7 M urea and 40% formamide). Following electrophoresis, the gel will be stained with SYBR Green I (Molecular Probes; Eugene, OR), then visualized on a UV transilluminator and photographed with a digital CCD camera (BioChemi System; UVP, Inc; Upland, CA). Promiscuous bands will be excised, purified by repeated rounds of PCR-DGGE, and sequenced at the Advanced Genetic Analysis Center at the University of Minnesota.

Results to date

Sample Collection

Sample collection began in June 2002 and has continued to present (March 2003). Samples have been properly preserved and analysis will begin once the entire set of samples has been collected (anticipated: May 2003).

Total Biomass Measurement

Total biomass has been quantified from samples collected to date. Total biomass concentrations ranged from 400 mg/L to 1100 mg/L (as protein) (data not shown).

Quantification of Nitrifying Bacteria

Using samples from other projects, a competitive quantitative PCR technique has been optimized for quantification of *amoA* gene copy number (Figure 1). Band intensities shown in Figure 1 were quantified and compared to estimate the concentration at which target (Band A) and competitor (Band B) were equal (i.e., the number of *amoA* genes present in the sample).

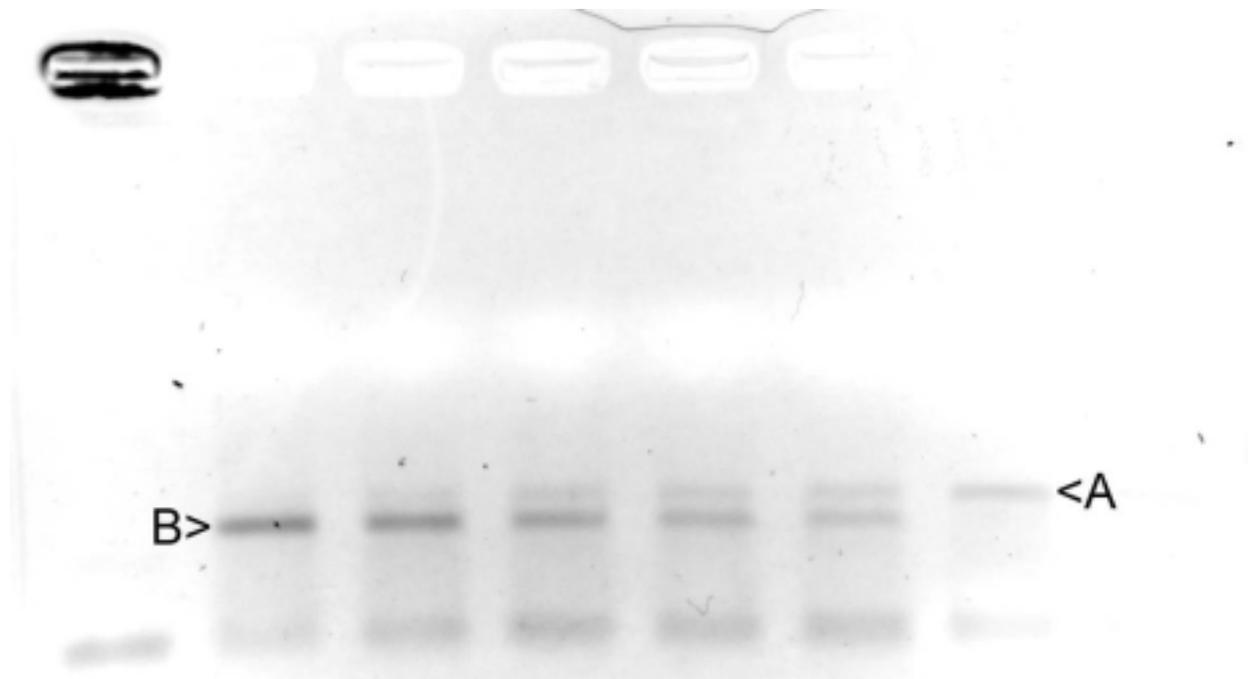


Figure 1: Competitive PCR of *amoA* genes.

Nitrifying Community Structure

Using samples from other projects, a nested PCR-DGGE technique has been developed for characterization of nitrifying bacterial communities (Figure 2). Of the five bands identified in Figure 2, DNA sequence analysis showed that three of these bands were phylogenetically related to known nitrifying bacteria. Two were *Nitrosomonas*-like (Bands A and B) and one was *Nitrospira*-like (Band E). Other bands were experimental artifacts not likely to be associated with nitrifying bacterial populations.

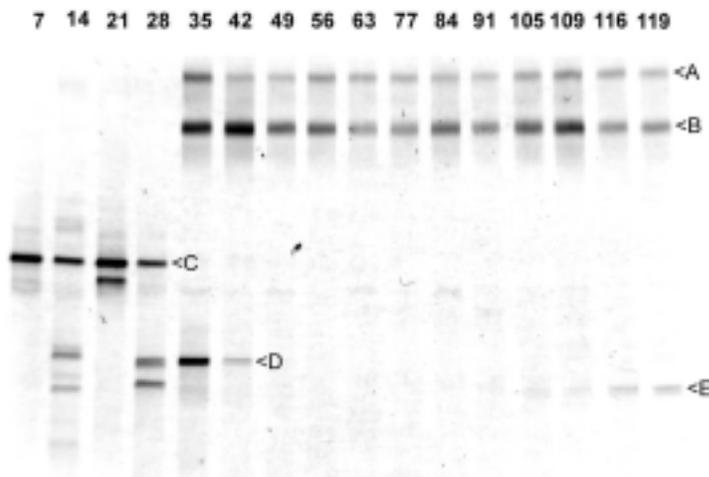


Figure 2: Nested PCR-DGGE for the analysis of nitrifying bacteria.

Summary of findings

The project is still on-going and sample collection is still underway. Methods to quantify nitrifying bacteria by competitive polymerase chain reaction have been successfully developed. A nested PCR-DGGE technique has also been developed to track nitrifying bacterial community structure. The principal investigator anticipates that substantive results will be rapidly generated once the sample collection period is complete.

References

1. Amann, R. I., W. Ludwig, and K.-H. Schleifer. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews* **59**:143-169.
2. American Public Health Association. 1992. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington D. C.
3. Boesch, D. F., and R. B. Brinsfield. 2000. Coastal eutrophication and agriculture: Contributions and solutions. In: *Biological Resource Management: Connecting Science and Policy*, pp 93-115.
4. Dionisi, H. M., A. C. Layton, G. Harms, I. R. Gregory, K. G. Robinson, G. S. Sayler. 2002. Quantification of *Nitrosomonas oligotropha*-like ammonia-oxidizing bacteria and *Nitrospira* spp. from full-scale wastewater treatment plants by competitive PCR. *Applied and Environmental Microbiology* **68**:245-253.
5. Goolsby, D. A., W. A. Battaglin, B. T. Aulenbach, and R. P. Hooper. 2001. Nitrogen input to the Gulf of Mexico. *Journal of Environmental Quality* **30**:329-336.
6. Herbert, D., P. J. Phipps, and R. E. Strange. 1971. Chemical analysis of microbial cells. *Methods of Microbiology* **5B**:209-234.
7. Kowalchuk, G. A., J. R. Stephen, W. De Boer, J. I. Prosser, T. M. Embley, and J. W. Woldendorp. 1997. Analysis of ammonia-oxidizing bacteria of the β -subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. *Applied and Environmental Microbiology* **63**:1489-1497.
8. LaPara, T. M., C. H. Nakatsu, L. Pantea, and J. E. Alleman. 2000. Phylogenetic analysis of bacterial communities in mesophilic and thermophilic bioreactors treating pharmaceutical wastewater. *Applied and Environmental Microbiology* **66**:3951-3959.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**:265-275.
10. Muyzer, G., E. C. Dewaal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* **59**:695-700.
11. Rabalais, N. N., R. E. Turner, and W. J. Wiseman. 2001. Hypoxia in the Gulf of Mexico. *Journal of Environmental Quality* **30**:320-329.

Publications associated with the project

None to date.

Students supported by the project -- graduate (MS, PhD) and undergraduate

Sudeshna Ghosh (January 1, 2003 – date)

Department of Civil Engineering, University of Minnesota
Degree being sought: Masters of Science

Shelby Stanek
Department of Civil Engineering, University of Minnesota
Undergraduate

Awards and achievements resulting from your project

None to date.

Seminar or poster presentations resulting from your project

None to date.

Effects of riparian forest harvest on instream habitat and fish and invertebrate communities

Basic Information

Title:	Effects of riparian forest harvest on instream habitat and fish and invertebrate communities
Project Number:	2002MN2B
Start Date:	3/1/2002
End Date:	2/28/2005
Funding Source:	104B
Congressional District:	7 and 8
Research Category:	None
Focus Category:	Water Quality, Management and Planning, None
Descriptors:	None
Principal Investigators:	Raymond Newman, Patrick Brezonik

Publication

Effects of riparian forest harvest on instream habitat and fish and invertebrate communities

Principal Investigators

R. M. Newman, B. Vondracek and J.A. Perry, Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota

Funding Source: USGS-WRRI 104B National Grants Competition

Project Duration: March 1, 2002 through February 28, 2003

Summary

Stream riparian zones are critical to the health of stream fish and invertebrate communities. Forest harvest within the riparian zone may thus impact stream fish and invertebrate communities and the determination of the level of acceptable harvest within the riparian zone is important to balance forestry needs with stream biotic integrity. We have designed a manipulative experiment to determine the effects of no, low and high levels of riparian harvest on stream habitat and fish and invertebrate communities. Sites have been selected, treatments have been planned and pre-harvest sampling will be initiated in summer 2003.

Introduction

Forest products are an important natural resource in the upper Midwest. In Minnesota, timber harvest has been increasing and will continue to increase in the near future (Anonymous 2001). Timber harvest activities have the potential to degrade water quality and aquatic resources and for this reason, best management practices (BMPs) or site-level forest management guidelines have been adopted to protect riparian and aquatic resources in Minnesota (MFRC 1999, Anonymous 2001). Although these best management practices are based on the best available scientific information, and implementation monitoring is being conducted (Anonymous 2001), they have not been evaluated for effectiveness at protecting aquatic resources. Most research on the effects of forest harvest on streams and the effectiveness of forest harvest BMPs has been conducted in more mountainous regions such as Tasmania (Davies and Nelson 1994), the Sierra Nevada's, the Pacific Northwest and the Appalachian East (e.g., Meehan 1991, Castelle and Johnson 2000). These results may not be directly applicable to the midwest (Perry et al. 1992).

Riparian zones provide many protective services to streams (Gregory et al. 1991, Castelle et al. 1994, Castelle and Johnson 2000). Determination of the necessary width of riparian buffers (e.g., Castelle and Johnson 2000) or the permissible level of harvest within a buffer is essential to adequately protect stream resources without removing a large portion of the basin from harvest. Most studies on the effectiveness of riparian buffers at protecting streams from upslope harvest have focused on the width of the buffer and have not considered harvest with the buffer zone (e.g., Barton et al. 1985, Castelle and Johnson 2000). Current Minnesota best management practices allow varying degrees of harvest within the riparian management zone (RMZ). Harvest within the zone may be used to promote regeneration of shade intolerant species and thus it is important to know what level of harvest within the zone reduces its effectiveness at maintaining stream quality.

The objective of this project is to experimentally determine the effectiveness of various levels of riparian harvest at protecting in-stream resources. We will examine site-based effects associated with high, low and no riparian harvest (30m Riparian Management Zone, upland clearcuts) on aquatic habitat, macroinvertebrates and fish. Specifically, we will evaluate effects on fish and invertebrate habitat (temperature, sediment composition and embeddedness, depth, width, cover, bank stability, canopy coverage, woody debris, etc.), benthic macroinvertebrates and stream fish communities.

Methodology

During 2002 we focused on site selection; good site selection is critical to the long-term success of this project. A full time US Forest Service employee and a retired DNR Forester were hired (with the LCMR matching funds) to make contacts and locate appropriate sites. Over 350 contacts were made with State, county, federal, Tribal, and private industry groups to introduce the project, solicit assistance, and to visit field sites.

By June 2002 it became apparent that we would not have enough sites to initiate sampling and it was also apparent that we would not find enough quality sites within the criteria of the initial design (4 replicate sites within each of 5 watersheds). After visits to 60 potential sites the investigators on the LCMR project decided to change the experimental design and criteria so we could find adequate sites while maintaining good control and site similarity. These design changes were approved by LCMR and resulted in 3 (control, low and high riparian harvest) rather than 4 treatments, and replication by treatment pairs rather than watersheds. Beaver activity and low riparian canopy densities limited our ability to find replicate sites within watersheds for four harvest treatments. Thus we established treatment sites within 8 pairs of stands in northern Minnesota. Within each pair we will establish a riparian control (upland clearcut) and one riparian management treatment to compare the effects of different residual basal area levels. Where possible we will also include a non-harvested control (both upland and riparian zone not harvested). We also dropped the need for harvest on both sides of the stream but increased the amount of stream length exposed to harvest (to 200m).

Results and on-going work

Based on the new design and examination of over 100 sites, eight pairs of treatment sites (riparian control and one harvest treatment) have been located, timber sales secured, and harvest plots have been marked. Sites are located in Beltrami, Carlton, Cook, Lake, and St. Louis counties. Pre-harvest sampling will be conducted in summer 2003 and the treatment sites will be harvested in winter 2003-2004. Post-harvest data will be collected in summer 2004.

We have accepted and hired two new graduate students who are well suited to the project; an additional PhD candidate with considerable experience on a related project will assist with setup and sampling during summer 2003. Funding for additional years of post-harvest assessment will be pursued from a variety of sources.

Literature cited

Anonymous. 2001. Minnesota's nonpoint source management program plan 2001. Minnesota Pollution Control Agency, St. Paul, MN.

- Barton, D. R., W. D. Taylor, and R. M. Biette. 1985. Dimensions of riparian buffer strips required to maintain trout habitat in southern Ontario streams. *North American Journal of Fisheries Management* 5:364-378.
- Castelle, A. J. and A. W. Johnson. 2000. Riparian vegetation effectiveness. National Council for Air and Stream Improvement, Technical Bulletin 799, Research Triangle Park, NC.
- Castelle, A. J., A. W. Johnson, and C. Conolly. 1994. Wetland and stream buffer size requirements-a review. *Journal of Environmental Quality* 23:878-882.
- Davies, P. E., and M. Nelson. 1994. Relationships between riparian buffer widths and the effects of logging on stream habitat, invertebrate community composition and fish abundance. *Australian Journal of Marine and Freshwater Research* 45: 1289-1305.
- Gregory, S. V., F. J. Swanson, W. A. McKee, and K. W. Cummins. 1991. An ecosystem perspective of riparian zones. *Bioscience* 41: 540-551.
- Meehan, W.R. 1991. Influences of forest and rangeland management on salmonid fishes and their habitats. Bethesda, MS: American Fisheries Society, Special Publication No. 19, 751 pp.
- Minnesota Forest Resources Council (MFRC). 1999. Sustaining Minnesota forest resources: voluntary site-level forest management guidelines for landowners, loggers and resource managers. Minnesota Forest Resources Council, St. Paul, MN.
- Perry, J., K. Brooks, W. Olsen, T. Geier, W. Johnson, R. Newman, L. Mizner and N. Troelstrup. 1992. Water quality and fisheries: a technical paper for a Generic Environmental Impact Statement on Timber Harvesting and Forest Management in Minnesota. Report to the Minnesota Environmental Quality Board by Jaakko Pöyry Consulting. 325 pgs.

Publications associated with the project

None

Students supported by the project

Starting in June 2003:

Dickson Atuke (PhD), Fisheries and Aquatic Biology Track in Conservation Biology

Nick Schlessler (MS), Fisheries and Aquatic Biology Track in Conservation Biology – additional support from fellowships and LCMR Grant

Nat Hemstad (PhD), Water Resources Science – 3 month field assistant supported on LCMR Grant

Awards and achievements resulting from your project

None

Seminar or poster presentations resulting from your project

None

Related grants submitted or funded as a result of this project

The Legislative Commission on Minnesota Resources is funding the manipulation, travel, supplies and field assistance

The Minnesota Forest Resources Council provided funds for some supplies and assistance

A proposal for longer-term continuation of this project has been requested by the Minnesota Department of Natural Resources and will be submitted in August 2003.

Paleohydrologic response of the Mississippi Headwaters watershed to Holocene climate change

Basic Information

Title:	Paleohydrologic response of the Mississippi Headwaters watershed to Holocene climate change
Project Number:	2002MN6B
Start Date:	1/1/2002
End Date:	6/30/2003
Funding Source:	104B
Congressional District:	
Research Category:	None
Focus Category:	None, None, None
Descriptors:	None
Principal Investigators:	Howard Mooers

Publication

Paleohydrologic Response of the Mississippi Headwaters Watershed to Holocene Climate Change

H.D. Mooers and **P.C. Larson**, Department of Geological Sciences, College of Science and Engineering, University of Minnesota, Duluth

Funding Source: USGS-WRRI 104B National Grants Competition

Reporting Period: March 1, 2002 through February 28, 2003

Summary

This study examines the sedimentary record of major changes in the hydrologic budget of three large lakes in the Mississippi Headwaters area (Cass, Leech, and Winnibigoshish) resulting from multiple diversions of the Mississippi River and its tributaries. Sediment cores collected from Cass and Leech Lakes, in combination with a core previously collected from Lake Winnibigoshish provide a record of the effects of regional-scale climate-driven changes on the hydrology of the Mississippi Headwaters watershed. The lakes' hydrologic budgets were affected by a series of diversion events that altered the main course of the Mississippi, and altered the watershed areas and throughflow rates of the three lakes.

One of the diversion events is recorded as a prominent, abrupt change in the nature of carbon sedimentation in Lake Winnibigoshish. However, this and other diversion events are not apparently discernable in Cass and Leech Lake sediments. The cause of the radically different response of Winnibigoshish to a diversion event is as yet enigmatic, however it implies the existence of an as yet unidentified control on the nature of lacustrine carbon sedimentation.

Ongoing geochemical, stable isotope, and diatom-nutrient calibration work will help distinguish the effects of climate-forced hydrologic variability from those of nutrient loading on sedimentation in the large Headwaters lakes.

Introduction

Most previous paleolimnological investigations in Minnesota have focused on interpreting the sedimentary records of relatively small lakes in small watersheds (Bradbury and Dean, 1993; Winter, 1997). While the high-resolution records found in these lakes are of undoubted value in interpreting past climatic and vegetation history, they are to some extent extremely sensitive to local conditions not reflective of the region as a whole (c.f. Schwalb and Dean, 2002). In contrast, the sedimentary records of the largest lakes in a drainage basin might be expected to record a regional-scale signal of climate change. This study examines the sedimentary record of major changes in the hydrologic budget of three large lakes in the Mississippi Headwaters area due to multiple diversions of the Mississippi River and its tributaries.

During the late Holocene, drainage patterns in the Mississippi Headwaters underwent a number of realignments. Prior to ca. 3 ka bp, the main stem of the Mississippi River flowed from Lake Bemidji to Leech Lake, thence down the present-day Leech Lake River. The outlet of Lake Winnibigoshish flowed southward to the Leech Lake River, and Cass Lake's outlet flowed south to Leech Lake. After this time, three stream piracy events led to diversion of major portions of the watershed into a new Mississippi River

channel. The Mississippi now flows from Lake Bemidji through Cass and Winnibigoshish, exiting Winnibigoshish by a new eastern outlet. Leech Lake lies in a much diminished watershed, and continues to drain toward the east.

During the mid-Holocene, Cass and Leech Lakes were characterized by large watershed:lake area ratios (W/L), while the corresponding W/L for Winnibigoshish was small. This condition led to evaporation-forced hydrologic closure of Winnibigoshish, lower lake levels, eolian erosion of bottom sediment, and development of a dunefield on the eastern shore of the lake. These geomorphic relationships, and their implications for the paleohydrologic budgets of the lakes, indicates strong linkages exist between climate, hydrology, landforms, and the sedimentary records of the lakes.

Methods

Coring. Cass and Leech Lakes were cored in August 2001 using the University of Minnesota Limnological Research Center's ETH-Kullenberg system, a raft-mounted piston corer designed for use in deep water. Recovered cores are 9-cm in diameter. On both lakes, the coring location was the deepest part of the lake.

A 640-cm core was recovered from 44-m water depth in Walker Bay of Leech Lake. Sediment in this portion of the lake has a relatively high clastic component and corresponding high density, resulting in incomplete penetration of the coring device. However, the key late Holocene portion of the sediment record was completely sampled.

A 351-cm core was recovered in 34-m water depth in the northern portion of Cass Lake. Although short, this core terminates in glaciofluvial gravel and likely represents a complete postglacial section.

Lake Winnibigoshish was previously cored using a modified Livingston corer. A 575-cm core was recovered in 20-m water depth. Similar to Leech Lake, sediment in the lower portion of the sequence has a relatively high clastic component and corresponding high density, resulting in the inability to recover a complete postglacial section.

However, the key late Holocene portion of the sediment record was completely sampled.

Sediment Dry Bulk Density. Sediment dry bulk density was determined by weighing 1-cm³ samples of water-saturated sediment, freeze-drying, and weighing of the dried sediment. Sediment water content (porosity) corresponds to water loss during drying, and dry bulk density to the mass of dried sediment divided by the original volume.

Magnetic Susceptibility. Whole-core magnetic susceptibility for all three cores was measured at the Limnological Research Center, University of Minnesota. Magnetic susceptibility is a proxy measurement for the relative amount of clastic material in the sediment.

Carbon Analyses. Inorganic carbon and total carbon content of sediment from the recovered core was determined by coulometry at the Large Lakes Observatory, University of Minnesota Duluth. The organic carbon content of sediment is calculated by subtracting the inorganic from the total carbon content.

CNS Analyses. Sediment total carbon, nitrogen, and sulfur contents of Lake Winnibigoshish sediments were analyzed using a LECO at the Large Lakes Observatory, University of Minnesota Duluth. Results of these analyses allow assessment of the origin of organic carbon stored in the Winnibigoshish's sediments.

Carbonate Mineralogy of Lake Winnibigoshish Sediment. The mineralogy of sediments below 265 cm core depth (the interval with an appreciable carbonate content) was

determined using a Philips X'Pert-MPD System x-ray diffractometer. The carbonate mineralogy of lacustrine sediments is sensitive to the salinity and Mg:Ca of the lake water in which the minerals formed (Kelts and Hsu, 1978). The Mg-content of calcite and dolomite (determined using the method of Goldsmith and Graf, 1958) and the presence or absence of aragonite will provide evidence of the presence and degree of hydrologic closure and evaporative concentration of lake water during the period of carbonate sedimentation in Winnibigoshish.

Results to Date

Lacustrine Stratigraphy and Sedimentology. The stratigraphy and sedimentology of sediment in each of the three lakes cored is unique, recording the unique history of each lake and its response river diversion events and

Lake Winnibigoshish. The Lake Winnibigoshish core consists of 300-cm of silty marl in the basal (pre-diversion) interval. The diversion event is recorded as the abrupt appearance of fine-grained sand in the sediments, marking the beginning of a 28-cm thick clastic-rich fining-upward sequence. The upper 247-cm of the core are diatom-rich black sapropel. Lake Winnibigoshish sediments have relatively high magnetic susceptibility in the pre-diversion interval, suggesting fluctuating lake levels may have resulted in a small, but steady, influx of silt from the littoral zone to the deep basin. These magnetic susceptibility peaks are absent in the post-diversion interval, suggesting lake level, and therefore the shoreline, was stable through this period.

The magnesium content of endogenic calcite in Lake Winnibigoshish varies between 4.5 and 7.6 mol% in the prediversion interval (Fig. 1). The Mg-content positively correlates with the amount of 'residual' material in the sediment. (The residual is the non-organic carbon and carbonate portion of the sediment, reflecting input of diatom silica clastic sediment eroded from the shoreline during periods of low or unstable lake levels.) This indicates that periods of lake level instability, and potentially hydrological closure, were characterized by a small degree of evaporative concentration and corresponding increase in Mg content of the lake's water.

In contrast to the pre-diversion interval, endogenic calcite deposited in the lake immediately after initiation of diversion of the Mississippi River into the lake is characterized by Mg mol% of 1.7 to 1.9. This suggests that the addition of the Mississippi's flow to the lake's hydrologic budget immediately relaxed the conditions of evaporative concentration and high Mg:Ca prevailing before the diversion.

The C:N ratio of organic material in lacustrine sediments is diagnostic of the organic material source; terrestrial plants are characterized by relatively high and aquatic plants by relatively low, C:N ratios (Meyers and Ishitwari, 1995). The organic C:N of Lake Winnibigoshish sediments ranged from 8 to 15 (Fig. 1); these low values are consistent with an aquatic plant source with little if any terrestrial input. C:N values rose slightly after the diversion event and have displayed a gradual decline since, however the relatively small magnitude of this change precludes attaching to it any great significance.

Organic carbon mass accumulation rates (MAR) were calculated between 0 and 247 cm depth in the core, based on dates of 1000 ± 32 ^{14}C yr bp at 248 cm core depth and 300 ± 32 ^{14}C yr bp at 32 cm core depth (Fig. 4). Organic carbon MAR ranged from 49 to

105 g·m⁻²·a⁻¹, averaging 70 g·m⁻²·a⁻¹, in the post-diversion interval; these values are higher by a factor of 2 to 5 with respect to late-Holocene organic carbon MAR in nearby Elk, Williams, and Shingobee Lakes (OC MAR of 36, 21, and 17 g·m⁻²·a⁻¹, respectively) (Dean, 1998). Essentially no carbonate was deposited during this interval, so the total carbon MAR is approximately organic carbon MAR.

Leech Lake. The Leech Lake core recovered 640-cm of silty marl occasionally interrupted by minor beds rich in detrital organic material, and thin sand lenses. The organic carbon content of Leech Lake sediments gradually increases from ~2% at the base of the core to ~8% immediately prior to initiation of agriculture and logging in the watershed (Fig. 2). Leech Lake sediments record the overall highest magnetic susceptibility values in the three lakes, reflecting relatively abundant influx of silt to Walker Bay (Fig. 2). Of particular interest are a single magnetic susceptibility peak at 260 cm, and numerous peaks between 434 and 501 cm core depth, corresponding to coarser-grained, clastic-rich intervals. The upper peak likely represents a mass flow event. Peaks in the interval 434-501 represent numerous mass flow events, perhaps recording a relatively long interval of shoreline erosion resulting from lake level instability.

Cass Lake. The Cass Lake core recovered 351-cm of organic-rich marl. The organic carbon content of Cass Lake sediments gradually increases from ~4% at the base of the core to ~8% at 40 cm core depth (Fig. 3). Carbonate shows a corresponding decrease from ~65% to ~45%. Cass Lake sediments have very low magnetic susceptibility, reflecting the very low input of clastic sediment to the northern basin of the lake through the Holocene. This is despite evidence that mid-Holocene lowstands may have resulted in significant sediment redistribution from the littoral zones of the lake.

Sedimentology of the Winnibigoshish dune field. While Grigal and others' (1976) radiocarbon dates have firmly established the presence of eolian activity adjacent to Lake Winniboshish during the mid-Holocene, their conjecture that 580 km² of dunefield was active during the mid-Holocene is debatable. The littoral zone exposed to wind erosion during periods of low lake level, and consequently the supply of sand available for export to the dunefield lying southeast of the lake, may have been as little as 10 km². It is difficult to conceive of covering an area of 580 km² with sand up to 10 m thick originating from a 10 km² source area.

Detail mapping of the dunefield as defined by Grigal and others (1976) and grain size analysis of dune sediment has defined two subareas within their dunefield. A large area (~570 km²) consists of sand with a wide range of mean grain size and no systematic variation. Dune forms are closely associated with older glacial landforms such as eskers and ice-walled lake plains. The orientation of dune features indicates a generally northwest to southeast sand transport direction. A relatively small area (~8 km²) immediately adjacent to the lakeshore (including the area of Grigal et al.'s radiocarbon dates) is characterized by dunes consisting of finer-grained sand displaying a systematic fining with distance from the lakeshore. Dune forms within this area are much more distinct than the other subarea. Dune crest orientations indicate a generally southwest to northeast sand transport direction.

These relationships suggest that only a small area of the dunefield was active during the mid-Holocene, and that most of the larger dunefield formed immediately after deglaciation. The close match in area of our mapped younger dunes and their potential source area in the littoral zone further supports this conclusion.

Description of Ongoing Work

Results of the diatom-nutrient calibration and carbonate carbon and oxygen isotope analyses are pending. In addition, some of the radiocarbon dates for the sediment cores are still pending. Final results will complete the geochemical picture of the evolution of these large lakes during the Holocene.

Diatom-nutrient calibration. Diatom analysis of sediment samples from all three lakes is being conducted by J. Kingston at the Natural Resources Research Institute, University of Minnesota Duluth. Diatom population concentration and taxa will be counted, and the resulting data integrated with existing and ongoing diatom-nutrient calibration data for the Minnesota region (Kingston et al. 1992, Ramstack 1999, Kingston, pers. comm. 2002). This diatom-nutrient calibration set references diatom abundance and taxa in modern bottom sediments to a variety of water-quality parameters, including water turbidity, Secchi transparency, color, and dissolved silica, chlorophyll-*a*, phosphorus, and nitrogen contents. The results of these analyses will be used to assess long-term changes in the nutrient status of the three Headwaters lakes. Particular emphasis is being placed on quantifying changes in nutrient status corresponding to transitions in the nature of sedimentation recognized in the cores.

Summary of Important Findings

In Lake Winnibigoshish, diversion of the Mississippi River into the basin and the corresponding increased throughflow rate is accompanied by a radical shift in the nature of carbon sedimentation in the lake, from entirely carbonate to entirely organic carbon. Pre-diversion sediments in the core have very little organic carbon (3-4%) and carbonate contents of 60%. Post-diversion sediments have organic carbon contents of ~20% ranging up to 37%, and carbonate contents generally <12%.

However, a similar diversion and increase in throughflow rate of Cass Lake is not reflected in its carbon sediment record. Similarly, complementary decreases in the throughflow rate of Leech Lake are not reflected in its carbon sediment record. Cass Lake sediments record a gradual increase in organic carbon content from 5-8%, and a complementary decrease in carbonate from 65-50%. In Leech Lake, sediments record a gradual, steady increase in organic carbon from 2-8%, while carbonate varies from 48-78%. This pattern is similar to those reported by Dean (1999) in numerous lakes in Minnesota.

Dean (1999) has suggested that the shift from carbonate to organic carbon preservation in lake sediments is driven by increasing primary productivity. The transition in Lake Winnibigoshish is the most rapid reported in any lake in Minnesota. A 1-cm thick layer of nearly pure organic material in the core immediately following the diversion event suggests diversion was accompanied by increased nutrient loading. However, while the instantaneous increase in watershed area may have delivered increased nutrients to the lake, the presence of Cass Lake serving as a nutrient sink just

upstream argues against this hypothesis. Alternately, pre-diversion carbonate sedimentation in Winnibigoshish may have been forced by evaporative concentration of lake water, a condition that was relieved with the initiation of increased throughflow. Results the diatom-nutrient calibration study will help distinguish between these two hypotheses.

Our results suggest that the nature of lacustrine carbon sedimentation is controlled in part by a threshold condition, one which has not been reached in most lakes in Minnesota, including Cass and Leech Lakes. The incremental increase and decreases in throughflow experienced by Cass and Leech during the late Holocene were apparently not of sufficient magnitude to trigger radical change in carbon sedimentation, while the increase experienced by Winnibigoshish was. This suggests that future climate-induced hydrologic changes in the Mississippi Headwaters basin, rather than being gradually manifested, may be characterized by rapid onset of perturbation of lacustrine ecosystems.

References

- Bradbury, J.P., and Dean, W.E., eds. 1993. Elk Lake, Minnesota: Evidence for Rapid Climate Change in the North-Central United States. *Geol. Soc. America Special Paper* 276.
- Dean, W.E. and Gorham, E. 1998. Magnitude and significance of carbon burial in lakes, reservoirs, and peatlands. *Geology* 26: 535-538.
- Dean, W.E. 1999. The carbon cycle and biogeochemical dynamics in lake sediments. *J. Paleolimnology* 21: 375-393.
- Goldsmith, J.R. and Graf, D.L. 1958. Relations between lattice constants and composition of the Ca-Mg carbonates. *American Mineralogist* 43: 84-101.
- Grigal, D.F., Severson, R.C., and Goltz, G.E. 1976. Evidence of eolian activity in north-central Minnesota 8,000 to 5,000 yr ago. *Geol. Soc. America Bull.* 87: 1251-1254.
- Kelts, K. and Hsu, K.J. 1978. Freshwater carbonate sedimentation. In: Lerman, A., ed. *Lakes: Chemistry, Geology, Physics*. New York: Springer-Verlag.
- Kingston, J.C., Cook, R.B., Kreis, R.G., Camburn, K.E., Norton, S.A., Sweets, P.R., Binford, M.W., Mitchell, M., Schindler, S.C., Shane, L.C.K., and others. 1990. Paleocological investigation of recent lake acidification in the northern Great Lakes states. *J. Paleolimnology* 4: 153-201.
- Meyers, P.A. and Ishiwatari, R. 1995. Organic matter accumulation records in lake sediments. In: Lerman, A., Imboden, D.M., and Gat, J.R., eds. *Physics and Chemistry of Lakes*. New York: Springer-Verlag.
- Ramstack, J.M. 1999. Pollution trends in Minnesota lakes: the use of fossil diatoms as paleoindicators of human impact. Bethlehem: Lehigh University, 72 p.

Schwalb, A. and Dean, W.E. 2002. Reconstruction of hydrological changes and response to effective moisture variations from North-Central USA lake sediments. *Quat. Sci. Reviews* 21: 1541-1554.

Winter, T.C., ed. 1997. Interdisciplinary Research Initiative: Hydrological and Biogeochemical Research in the Shingobee River Headwaters Area, North-Central Minnesota. U.S. Geological Survey, Water Resources Investigations Research 96-4215.

List of publications and presentations resulting from this project:

Larson, P.C. and H.D. Mooers. 2003. Stream piracy in the Mississippi Headwaters: Implications for mid-Holocene eolian activity in the Midcontinent. Submitted to Geological Society of America Annual Meeting, October 2003, Seattle, Washington.

Description of Student Training provided by the project:

Name: Phillip Larson

Program: Department of Geology and Geophysics, University of Minnesota

Degree being sought: Ph.D.

Name: Kristian Rosendahl

Program: College of Education and Human Service Professions, University of Minnesota Duluth

Degree being sought: B.A.S.

Independent research project.

Name: Ryan Smith

Program: Department of Geosciences, University of Minnesota Duluth

Degree being sought: B.S.

Independent research project.

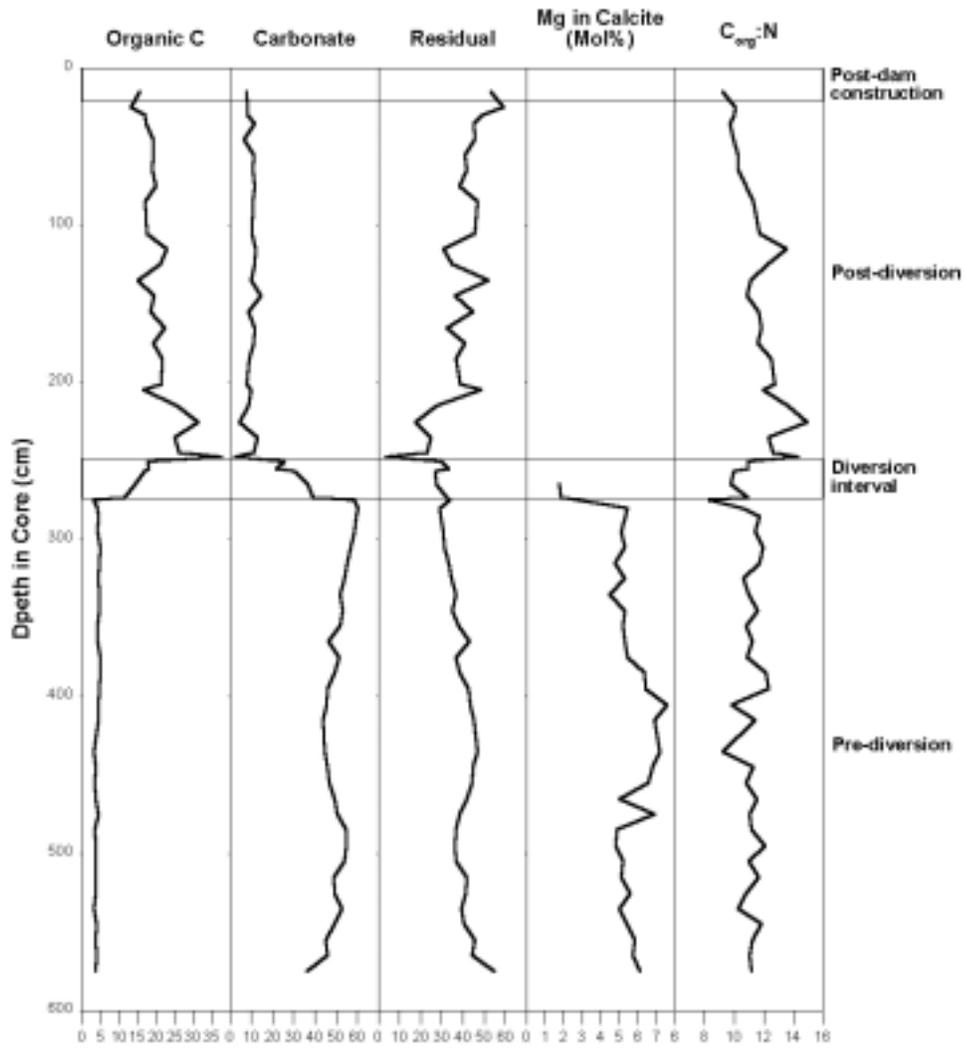


Figure 1.

Carbon content, Mg in calcite, and C:N profiles Lake Winnibigoshish.

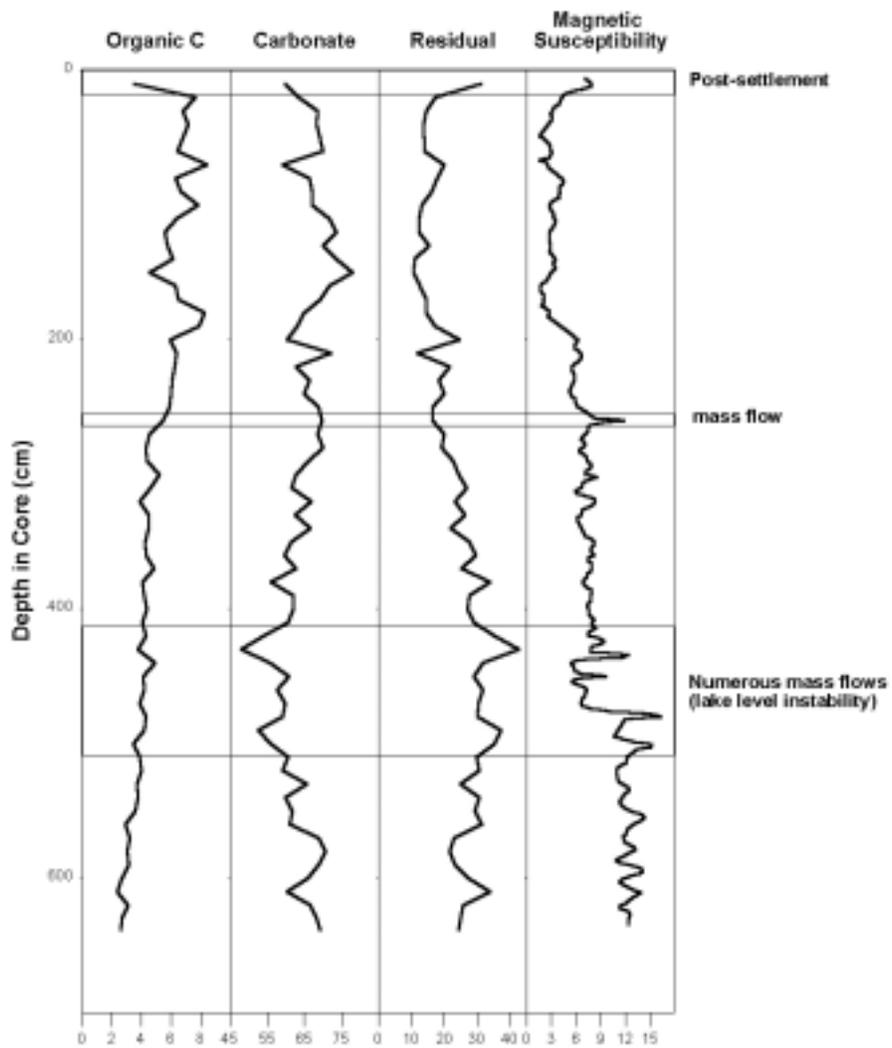


Figure 2.

Carbon content and magnetic susceptibility profiles Leech Lake.

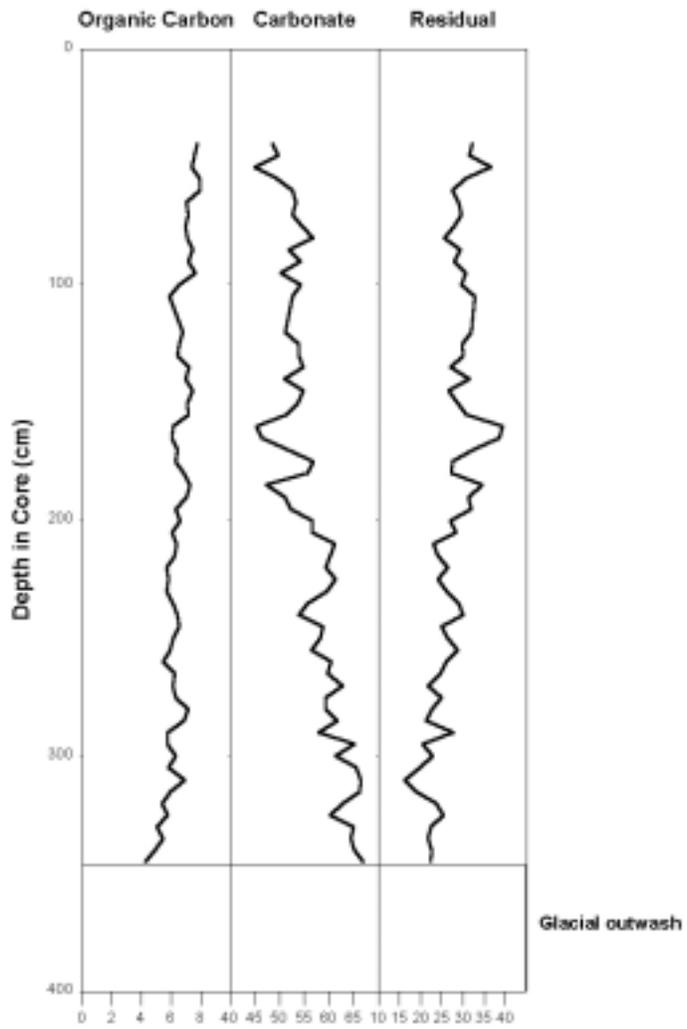


Figure 3.

Carbon content profiles for Cass Lake.

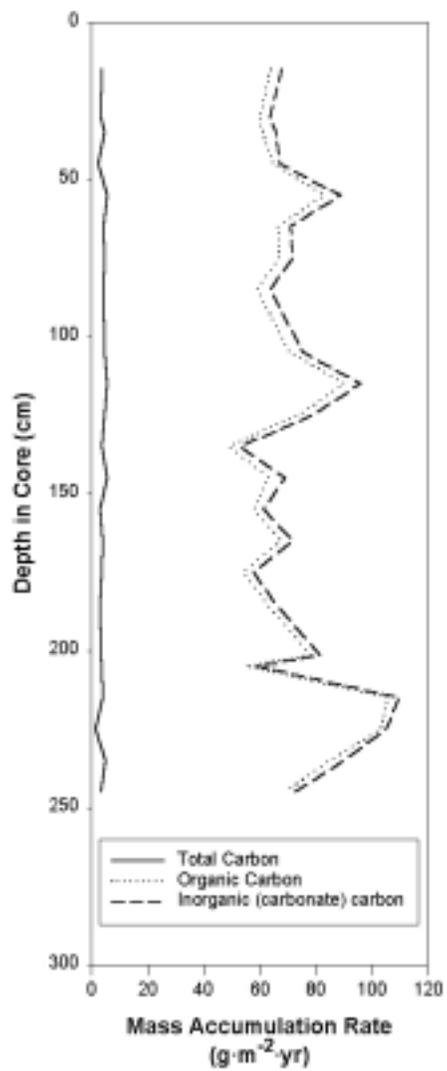


Figure 4.

Carbon mass accumulation rate (MAR) profile for Lake Winnibigoshish.

BIODIVERSITY IN URBAN PONDS AND LAKES: HUMAN EFFECTS ON PLANKTON POPULATIONS

Basic Information

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Publication

Biodiversity in urban ponds and lakes: human effects on plankton populations

Principal Investigator

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Research Assistants

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Summary

The objectives of this project are to explore the diversity of planktonic algae and zooplankton in small inland lakes and to describe how planktonic diversity is affected by urban development. We employed stratified random sampling using GIS as a tool and identified 100 sampling sites within the seven-county Twin Cities metropolitan area. We then sampled each of these sites three times during the 2002 growing season for phytoplankton and zooplankton communities, as well as basic limnological parameters including chlorophyll and phosphorus. To date, almost all plankton samples have been processed (only 1/3 of the phytoplankton samples remain to be enumerated under the microscope). We have found differences in biodiversity between urbanized and non-urbanized regions, with lower summertime zooplankton and lower spring and summer phytoplankton biodiversity in the urban regions than in the outer regions. Based as they are on a rigorous, random, site selection process, we believe these to be the first results documenting an effect of urbanization on these organisms.

Introduction

This project is addressing two sorely neglected aspects of water resources research: 1) urban habitats and 2) biodiversity of small, inconspicuous species. There is wide recognition that biodiversity is an important aspect of water quality, yet on several critical fronts, we lack sufficient scientific underpinning to incorporate biodiversity into most assessments of water quality. First, there is little scientific data on effects of different environmental factors on biodiversity of small aquatic organisms. Second, there has been little attention paid to the water resources closest to the large proportion of today's society that lives in urban environments. By undertaking serious study of biodiversity in urban ponds and lakes, this project seeks to establish whether the combined influences of urbanization are deleteriously affecting the majority of the biodiversity within those habitats.

The sustainability and integrity and of our water resources are threatened by many human-induced factors. Twenty six percent of total terrestrial evapotranspiration and fifty four percent of runoff that is geographically and temporally accessible are used by humans (Postel et al. 1996). Important contributing factors to reduced water quality due to human effects – well known to all who have an interest in water quality – include

increased nutrient loading and sedimentation, acid rain, and contamination by heavy metals and other toxicants. Though much is left to learn about these factors, significant scientific advances have been made on all of these fronts. However, it is becoming increasingly clear that reduced biodiversity ranks among the most critical problems in management of aquatic ecosystems (Naiman et al. 1995), and here our knowledge base is much weaker.

Most discussions of biodiversity center on terrestrial habitats such as rain forests. However, biodiversity is even more threatened in aquatic ecosystems than in terrestrial ecosystems (Naiman et al. 1995). Approximately 20% of the world's species of freshwater fish have declining abundance or are already extinct (Moyle and Leidy 1992). The Environmental Defense Fund has estimated that 30-70% of several major aquatic groups, such as mollusks and fishes, are threatened. Maintenance of aquatic biodiversity has been identified as a freshwater research priority second only to restoration and rehabilitation of aquatic habitats (Naiman et al. 1995). Local and global extinction of aquatic species may come about through overt habitat change or loss, such as river impoundment, excessive nutrient loading, and drainage of aquatic ecosystems. Although habitat loss and degradation are probably most damaging to aquatic biodiversity, other potentially important threats include exploitation of commercial species and introduction of exotic species. Biodiversity loss may also be caused by chronic introduction of substances such as sediment or nutrients that alter the habitat. Further, it may occur due to introduction of novel predators, such as the Nile Perch in Lake Victoria, or due to highly successful exotic species that capitalize resources, such as Eurasian Water Milfoil or the zebra mussel.

Shifts in human demographics affect water resource pressures. The human population is becoming increasingly urbanized. Approximately 41% of the world's human population now lives in urban areas. There are 411 cities worldwide with over 1 million human inhabitants. However, until recently ecologists have avoided urban areas for research (McDonnell and Pickett 1991), and only recently have they begun serious examination of urban habitats as unique, important ecosystems. Examining species relationships along rural to urban gradients can be extremely useful, because doing so address practical, applied questions while also providing insight on basic questions regarding the structure and function of ecosystems (McDonnell and Pickett 1991). Although limnologists have not shied away from addressing important practical problems, the bulk of their research has been conducted on non-urban sites. As the human population continues to encroach on and urbanize habitats, the importance of understanding the effects of human disturbance on aquatic species and ecosystems will increase.

Methods

For site selection previous to any sampling, we split the seven-county Twin Cities metro area into three zones: an urban core, a surrounding, less urban ring, and the nonurban outskirts; these zones were based on land use and percent impervious surface. For the present report, the two urban zones have been combined into a single zone, and we will contrast the inner urbanized zone with the surrounding zone (Fig. 1). To choose sites, we randomly distributed points within each zone and then we used a combination of GIS and ground searching to identify the nearest permanent (containing water year-

round) pond or lake to those random points. The urban area is centered between St. Paul and Minneapolis while the nonurban area contains some of the outer-ring suburbs and the agricultural land up to the seven-county boundary. Fifty ponds or lakes are located within both the urban habitat and the nonurban habitat (total = 100 sites). These lakes range from small to large (0.003 – 5667 ha) and from oligotrophic to hyper-eutrophic (0.126 – 21.4 μM TP), yet lake size and TP does not differ significantly with urbanization (Fig. 1B,C). Some of these lakes are surrounded by parking lots while others are in protected areas. During the 2002 ice-free season we sampled each lake 3 times: in the early spring, mid summer, and late fall. At each sampling we took standard limnological measurements such as chl a, total phosphorus, dissolved phosphorus, seston phosphorus, and seechi depth. We also preserved composite algal and zooplankton samples.

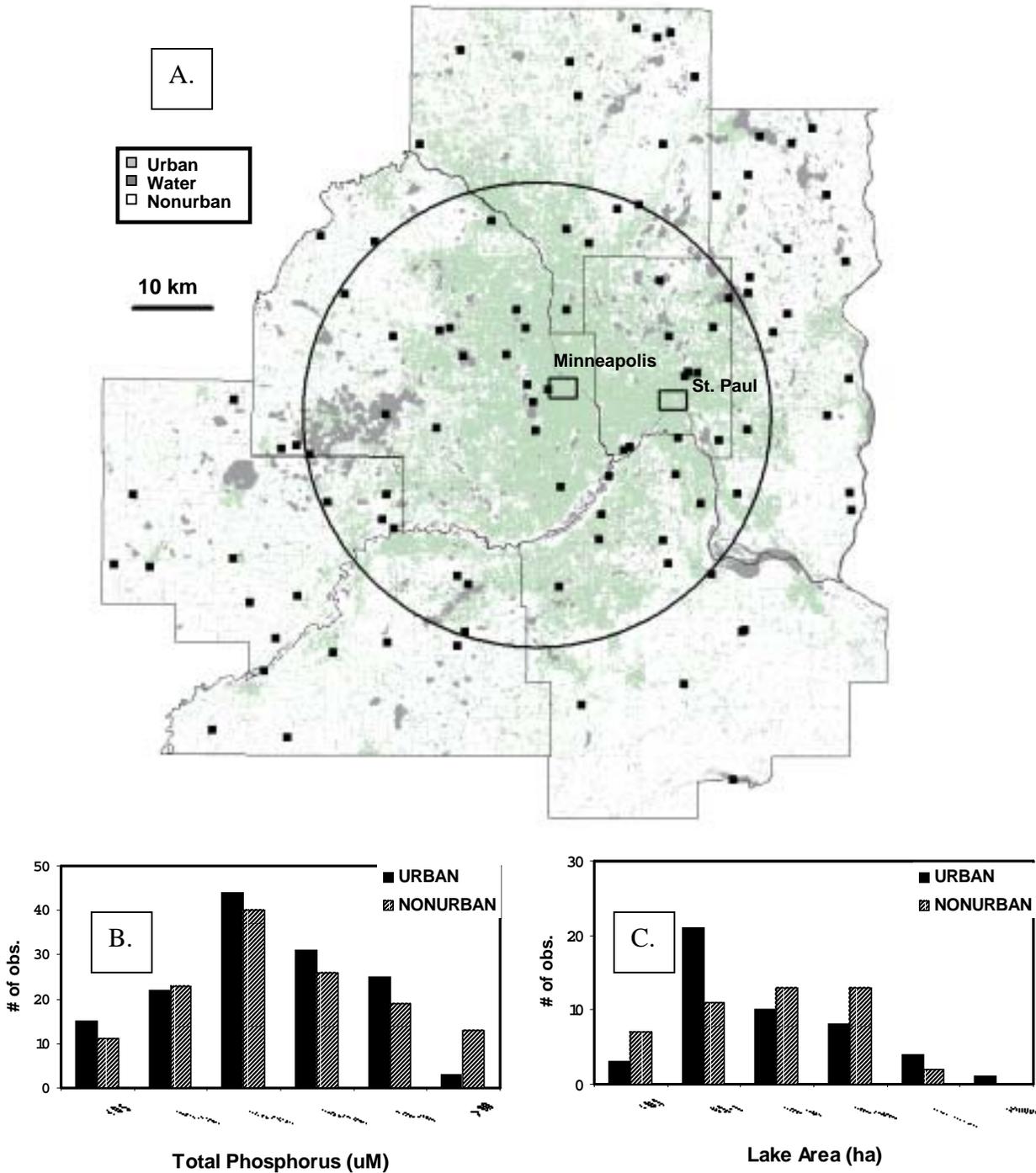


Fig. 1. A. Map of study sites. County boundaries are indicated. The heavy black circle demarks our division of “urban” and “non-urban” zones. This boundary does correspond to a fairly steep gradient in land use (“urban” land use is indicated by gray). There are fifty sites, selected randomly, within each of these two zones. B. Differences in lake fertility, as measured by total phosphorus, were minor between the two

regions. There were more lakes with very high phosphorus levels in the non-urban region. C. There were somewhat more sites in the 0.1-1 ha size classification in the urban region than the non urban. Other differences in lake size between the two zones were minor.

Phytoplankton samples were preserved with Lugol's iodine and stored at 4 °C in the laboratory. For species identification, we took a 10 ml subsample from each of the preserved samples and let the phytoplankton cells settle overnight in a 10 ml settling chamber. The samples were then observed under an inverted light microscope at x 400. Magnification of 1000x was also used when further details were needed for species identification. Phytoplankton cells larger than 5 microns in any dimensions were identified at least to the genus level and to the species whenever possible, and their counts were recorded for each of the six 250 µm x 250 µm fields of view, which were randomly selected. Filamentous algae, however, were counted if a trichome was longer than 5 µm, and the number of cells within a trichome was estimated by dividing the length of the trichome by the representative length of each cell. Some of the samples had a large number of cells and/or debris of algal or macrophytic origin, making accurate cell counts difficult. In those cases, the subsamples were diluted tenfold with deionized water before settling. We enumerated the zooplankton samples to species. To avoid bias associated with sampling effort we invested a similar quantity of effort in each sample. One 1 mL Sedgwick-grafter cell was counted per lake.

For both phytoplankton and zooplankton, we calculated the Shannon diversity index to evaluate species diversity for each sample.

To look for differences in diversity between regions, we tested frequency distributions using a Komolgorov-Smirnov test or using simple t-tests. For more detailed multivariate analyses, we used the Indicator test of Dufrene and Legendre (1997) to assess the influence of urban versus non urban watersheds on individual species. This test compares the frequency of occurrence and abundance of species between lakes in the urban and non urban categories, and identifies species that vary more between the two groups of lakes than would be expected by chance. We tested significance of each species as an Indicator of either lake group using permutation tests (1,000 permutations) (Dufrene and Legendre 1997). Because *P*-values associated with Indicator tests are based on data-dependent permutation procedures, there are no requirements for underlying data distributions. Indicator tests were performed using PC-ORD for Windows (McCune and Mefford 1997).

We also used indirect gradient analysis to summarize relationships among abundance and occurrence of individual species, urban and non urban lakes, lake size and depth, and species diversity and richness. We used correspondence analysis (CA) because preliminary ordinations with detrended correspondence analysis showed gradient lengths of 4 standard deviations, indicating a unimodal response model was most appropriate (ter Braak 1995). Following ordination of site and species scores, we developed a triplot using the ordination results and lake size, lake depth, species richness, and species diversity of individual lakes. Finally, we used canonical correspondence analysis (CCA) to test the statistical significance of relationships between community composition of zooplankton and urban/non urban watersheds, lake maximum depth, and lake surface area (ter Braak 1986). We used step-wise forward selection to determine whether watershed type, lake depth, and lake area (independent variables) were related to zooplankton community structure, and the significance of each variable was assessed

using permutation tests (1,000 permutations) (ter Braak and Verdonshot 1995). We maintained an overall error rate of $P < 0.05$ by applying sequential Bonferroni corrections to the results of the significance tests (Rice 1990), and restricted our final analysis to independent variables with significant marginal and conditional effects. We then developed a triplot using species scores, lakes scores, and vectors for significant independent variables. Vectors for species richness and species diversity were developed post CCA to assess relationships of these variables with the variables listed above. CA and CCA were performed using CANOCO (ter Braak and Smilauer 1998). So far, we have utilized multivariate analyses only on the zooplankton data.

Results to date

We have observed land-use effects on biodiversity both for phytoplankton and for zooplankton. For phytoplankton, both the spring and summer communities showed noticeably lower biodiversity in the urban zones (Figs. 2 and 3). Fall samples are not yet enumerated. We have not yet performed more detailed species-level analyses on the phytoplankton data.

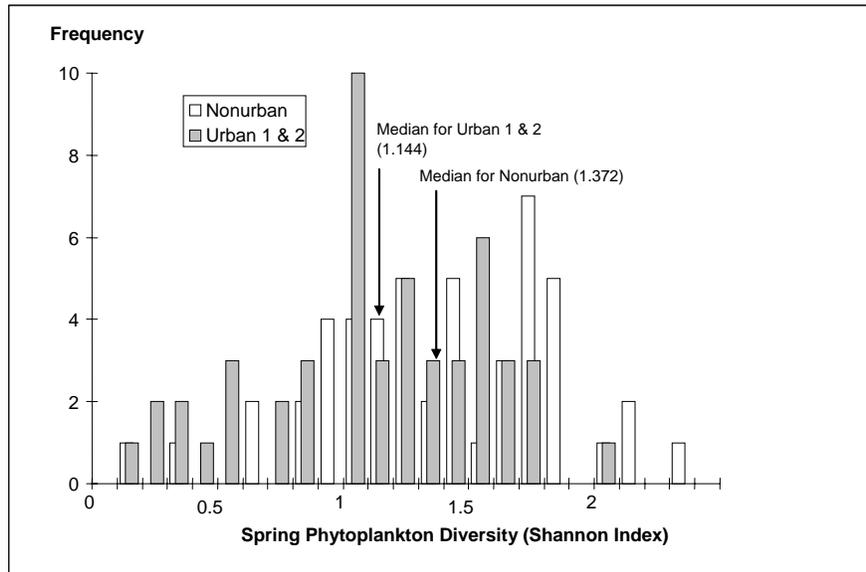


Figure 2. Spring phytoplankton biodiversity in the two regions. The differences were statistically significant.

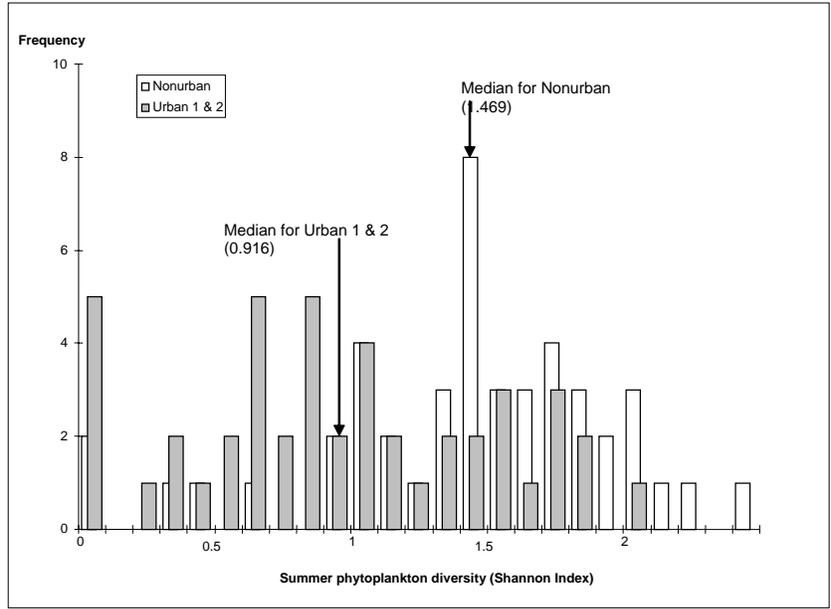


Figure 3. Summer phytoplankton biodiversity in the two regions. The differences were statistically significant.

For zooplankton, our results indicate that urbanization does decrease zooplankton diversity during the summer (K-S test: $p < 0.05$, Fig. 4). However, our urban and nonurban categories do not explain the variance in spring or fall zooplankton diversity.

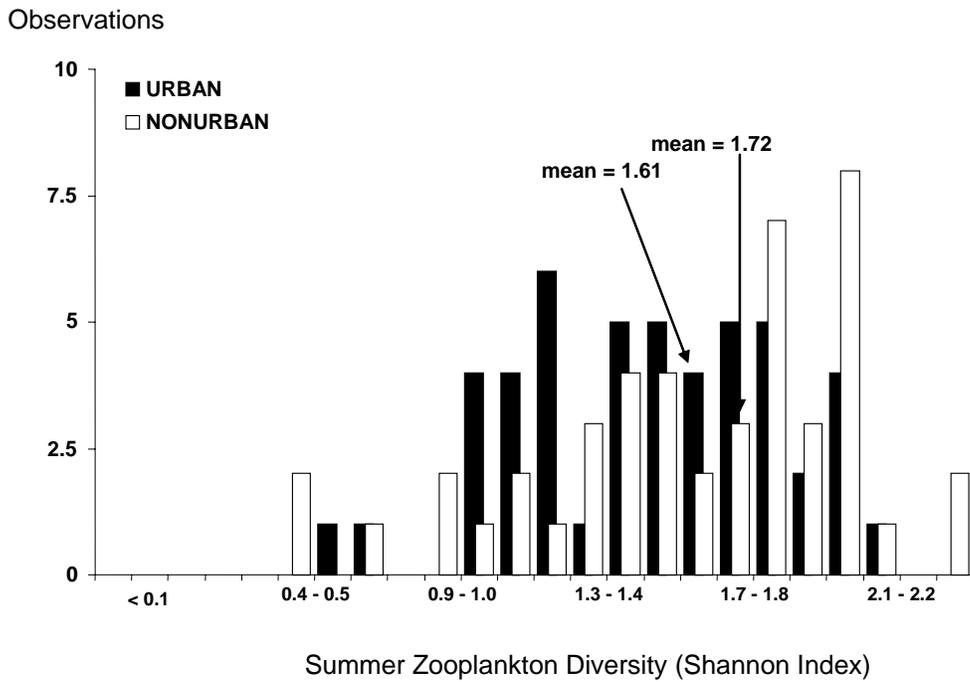


Figure 4. Summer zooplankton biodiversity in the two regions. The differences were statistically significant.

We have begun to explore whether lake or pond size or trophic status are predictors of biodiversity. For the zooplankton, we did not see any strong effects of size or productivity on biodiversity. We also performed a single preliminary comparison of zooplankton vs. phytoplankton diversity across sites and again did not see any relationships. These results are provisional and need to be repeated in more detail and with all the phytoplankton data once collected.

Indicator tests, which test whether certain species or groups of species are more sensitive to urbanization, showed that 7.5% of all species on the second sampling date were significant indicators for lakes with either an urban or non urban watershed (Table 1). Of the 12 significant species, 7 were more abundant and occurred more often in non urban sites, while 5 were observed in greater numbers and more frequently in urban sites.

CA results indicated variability in the average scores for urban and non urban lakes: non urban lakes scored higher on axis 1 and lower on axis 2, while urban lakes scored closer to the origin of both axes (Fig. 5). The difference in average lake scores reflects differing community composition between the two types of lakes; many species scored very high on axis 1 and very low on axis 2, indicating high affinity for non urban sites. In contrast, few species scored high on axis 2 and low on axis 1, which would have indicated high affinity for urban sites. Thus, the overall trend was a greater proportion of species showed higher abundance and occurrence in non urban sites than vice versa, while other species were equally common in both habitats. The vector for species diversity also indicated higher values in non urban lakes, while species richness showed a much weaker relationship with watershed type. Vectors for lake depth and area indicated a strong positive relationship between these two variables, but the opposing direction of these vectors with vectors for richness and diversity indicates strong negative relationships. A few species showed greater abundance and occurrence in larger, deeper lakes, but most species were more abundant and more common in shallower systems.

Forward selection in CCA indicated that urban/non urban watersheds ($P = 0.036$) and maximum depth ($P = 0.003$) both explained a significant amount of variation in the zooplankton data, while lake size did not ($P = 0.750$). The CCA triplot using watersheds and maximum depth supported the results of CA (Fig. 6). Some species scored between urban and non urban lake scores, indicating equal abundance and occurrence between lake types. However, more species had scores very close to site scores for non urban lakes compared to species scoring close to urban lakes, indicating many species are more abundant and more common in non urban sites. Scores for species diversity and richness (determined post CCA) were also closer to the scores for non urban sites, reflecting higher values compared to urban lakes. Finally, a few species showed a strong, positive relationship with lake depth, but the vast majority showed a strong negative relationship with depth. Overall, these results indicate that the abundance and occurrence of most species, as well as species richness and diversity, will be highest in shallow lakes in non urban watersheds.

Table 1. Significant indicator species for non-urban watershed (A) and urban watershed lakes (B). The difference in abundance and frequency of occurrence between urban and non urban lakes for these species is greater than expected by chance, and is higher in non urban and urban lakes for species in group A and B, respectively. P values represent the proportion of random permutations that generated differences in abundance and frequency of occurrence equal to or higher than the observed values.

Species	P value
(A) Indicator species for non-urban land use	
Belloid rotifer	0.004
<i>Euchlanis dilatata</i>	0.031
<i>Filinia longiseta</i>	0.013
<i>Lecane signifera</i>	0.023
<i>Monostyla lunaris</i>	0.011
<i>Monostyla quadridentata</i>	0.047
<i>Mytilinia ventalis</i>	0.004
(B) Indicator species for urban land use	
<i>Kellicottia bostonienses</i>	0.042
<i>Keratella cochlearis</i>	0.048
<i>Keratella cochlearis faluta</i>	0.020
<i>Brachionus quadridentatus</i>	0.046
<i>Trichocerca lata</i>	0.007

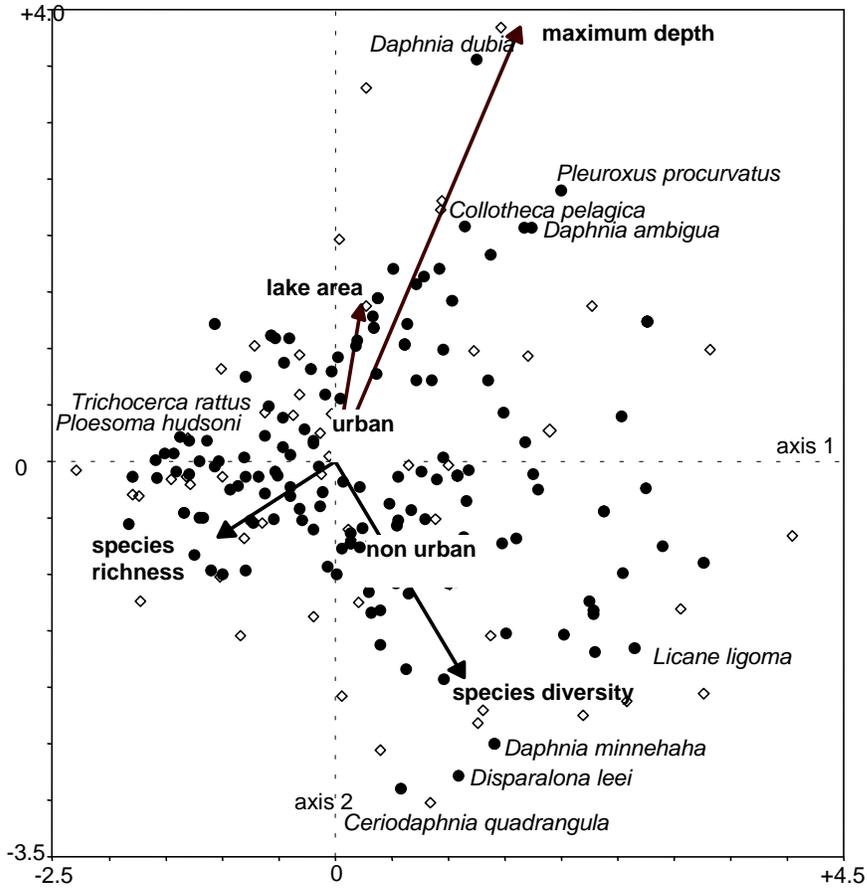


Figure 5. Triplot showing results of correspondence analysis of the second sampling date. Diamonds show scores for individual lakes and maximize the dispersion of the species scores. Average scores for urban and non urban lakes are indicated by text. Scores for individual species are shown as solid circles, and indicate the location of maximal abundance and frequency of occurrence across the lake scores. Arrows are vectors for the respective variables and point in the direction of increasing values; longer vectors indicate stronger relationships with the lake scores. Individual taxa represent examples of species associated with deep lakes with lower diversity (upper right), shallow lakes with lower diversity (middle left), and shallow lakes with higher diversity (lower right).

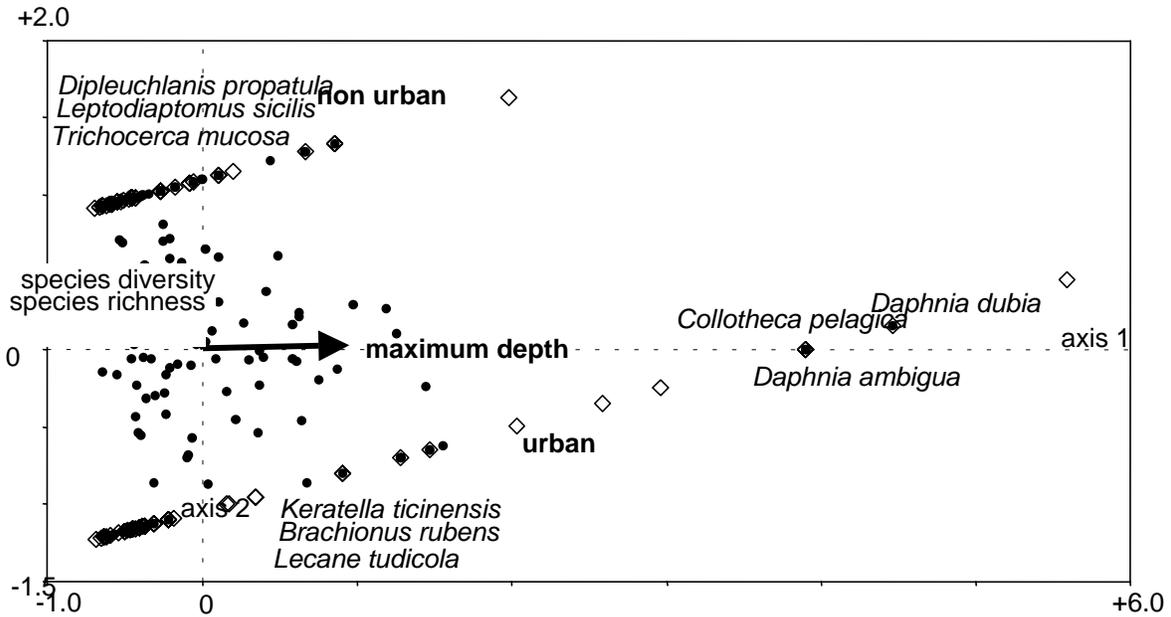


Figure 6. Triplot showing results of canonical correspondence analysis (CCA) of the second sampling date. Diamonds show scores for individual lakes and maximize the dispersion of the species scores, while also showing the relationships among species scores, lake type, and maximum depth. Scores for individual species are shown as solid circles, and indicate the location of maximal abundance and frequency of occurrence across the lake scores and in relationship to lake type and maximum depth. The arrow for maximum depth points in the direction of increasing values. Vectors for species richness and diversity were determined post CCA and also point in the direction of increasing values. Land use and maximum depth both explained a significant amount of variance in the zooplankton data.

Ongoing work

This project was designed to elucidate patterns in biodiversity in the Twin Cities metropolitan area. The patterns we've observed now naturally raise the question of what causes plankton biodiversity to tend to be lower in the central part of this region than in the outskirts?

The effects of human influence on diversity will very widely depending upon the type of human influence; while diversity also differs naturally according to factors such as large-deep lakes or small-shallow ponds. We have initiated two projects to tease apart the human effects on diversity from natural differences in diversity. First, we are determining the land use immediately surrounding each of the 100 study lakes, using GIS maps. With the land use surrounding each lake, we can investigate the effects of different types of human impact, such as urbanization and agriculture. In addition, land use information may help us understand high diversity urban lakes; for example, lakes protected in large urban parks. Second, we are collecting data on macrophyte abundance. This data will help us separate differences in diversity driven by human influence from those related to the habitat and cover provided by macrophytes.

Summary of findings

This study has demonstrated very rigorously that lakes and ponds with the central, more urbanized, portion of the Twin Cities metropolitan regions support a lower

planktonic biodiversity than sites within the more peripheral locations outside of the heavily urbanized core. Our preliminary analyses indicate that these differences cannot be ascribed to differences in habitat size (area) or productivity between the two regions.

References

- Dufrene, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs* 67:345-366.
- McCune, B., and M. J. Mefford. 1997. *Multivariate analysis of ecological data*. Version 3.0. MjM Software, Gleneden Beach, Oregon.
- McDonnell, M.J. and S.T.A. Pickett. 1991. Comparative analysis of ecosystems along gradients of urbanization: opportunities and limitations. Pp. 351-355 in J. Cole, G. Lovett and S. Findlay, eds. *Comparative Analysis of Ecosystems: Patterns, Mechanisms, and Theories*. Springer-Verlag, New York.
- Moyle, P.B. and R.A. Leidy. 1992. Loss of biodiversity in aquatic ecosystems: evidence from fish faunas. Pp. in P.L. Fiedler and S.K. Jain, eds. *Conservation Biology: The Theory and Practice of Nature Conservation*. Chapman and Hall, New York.
- Naiman, R.J., Magnuson, J.J., McKnight, D.M., and J.A. Sanford, eds. 1995. *The Freshwater Imperative*. Island Press, Washington.
- Postel, S.L., G.C. Daily and P.R. Ehrlich. 1996. Human appropriation of renewable fresh water. *Science* 271: 785-788.
- Rice, W.R. 1990. A consensus combined P-value test and the family-wide significance of component tests. *Biometrics* 46: 303-308.
- ter Braak, C.J.F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67:1167-1179.
- ter Braak, C.J.F. 1995. Ordination. In *Data analysis in community and landscape ecology*. Edited by R.H.G. Jongman, C.J.F. ter Braak, and O.F.R. van Tongeren. Cambridge University Press, Cambridge, England. pp. 91-173.
- ter Braak, C.J.F., and Verdonschot, P.F.M. 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences*. 57:255-289.
- ter Braak, C. J., and P. Smilauer. 1998. *Canoco Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination*. Version 4. Microcomputer Power, Ithaca, NY, USA.

Publications and Presentations

None to date.

Statement of related grants submitted or funded as a result of this project

A proposal to the NSF Ecological Diversity competition is being prepared for the July 15, 2003 deadline.

Description of student training provided by projects

This project has provided salary and research support for Kiyoko Yokota, a Ph.D. student in Ecology, Evolution and Behavior at the University of Minnesota.

Fluorochemicals in Minnesota Waters: An Emerging Environmental Issue

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Publication

Fluorochemicals in Minnesota Waters: An Emerging Environmental Issue

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Summary

Perfluorochemicals are an emerging class of global concern. To date the only established methods for their determination in environmental samples have been LC/MS/MS and ^{19}F NMR, requiring expensive equipment. In order to open the field of investigation to a broader range of environmental laboratories, we developed a single quadrupole LC/MS method for the determination of perfluorochemicals in environmental samples employing a fluorosilica gel column for the removal of chromatographic interference. This method has been validated for fish tissue samples by quadrupole time of flight mass spectrometry to insure that no other ions coelute with our PFCs and that all ion suppression/matrix effects have been removed. This clean-up method will enable users of HPLC coupled to any type of mass spectrometer to routinely analyze environmental samples for PFCs. Furthermore it will allow for the quantitation of ion suppression/matrix effects by manufacturing blanks that contain compounds from the matrix, but not PFCs. In developing this method, analyses indicate the presence of perfluorooctane sulfonate (PFOS), in livers from northern pike from three remote lakes, supporting our hypothesis that atmospheric deposition is responsible for transport of perfluorochemicals to the environment.

Introduction

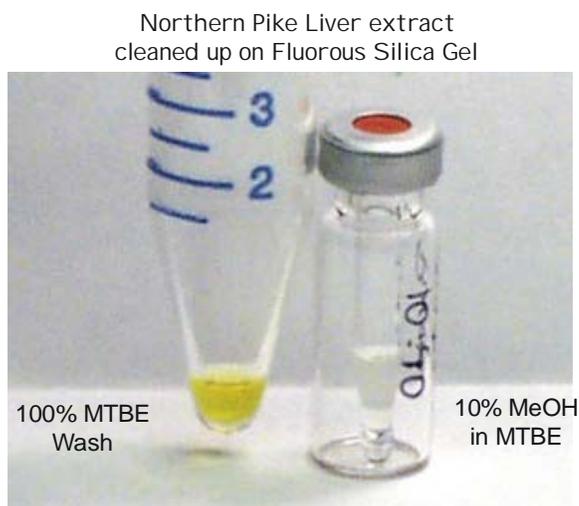
Perfluorochemicals (PFCs) represent an emerging issue of environmental concern due to their global distribution, persistence and bioaccumulation. It is the hypothesis of this project that their global distribution is a result of atmospheric transport. However, there are analytical barriers to testing this or any other hypothesis regarding the environmental fate and transport of perfluorochemicals. Unlike most of the well-studied hydrophobic organic contaminants (HOCs), perfluorochemicals are not lipophilic, but lipophobic and therefore do not accumulate in the fat stores of organisms. Rather they bind to proteins in the blood, liver and bile. The PFCs must first be liberated from the proteins using ion pair extraction in an established method. Once liberated, the PFCs must be detected. Contrary to most other HOCs, most PFCs are rather nonvolatile and cannot be determined by gas chromatography. The advent of liquid chromatography coupled with electrospray mass spectrometry has allowed for the analysis of PFCs. However, there are compounds that interfere with the determination of PFCs. To date this problem has been solved using LC/MS/MS where the transition from parent to daughter ion is monitored. LC/MS/MS is much more expensive than single quadrupole LC/MS, and not as available to most environmental chemistry researchers. Furthermore, other compounds present in the sample matrix can affect the initial ionization of analyte through what is often called ion

suppression or matrix effects. Other researchers have found these matrix effects/ion suppression by standard addition to environmental samples. This is time consuming and must be done to every sample if ion suppression/matrix effects are to be quantified, and knowledge of the presence of these effects still does not allow for the quantitation of the effect. Therefore what is needed is a robust clean-up technique for the analysis of a variety of environmental matrices for routine analysis of samples for PFCs. Once established, the clean-up technique will allow analysis by single quadrupole or triple quadrupole mass spectrometry for the investigation of environmental fate and transport of PFCs.

Results

Our inspiration for separating the interferences from the fluorochemicals comes from synthetic organic chemistry where fluorinated compounds are removed from nonfluorinated compounds using fluorous silica gel. The theory is that the fluorous silica gel will selectively retain fluorinated compounds, but release them with the appropriate solvent. Initial tests proved fruitful using a combination of methanol and water. Subsequent batches of fluorous silica gel and recleaned fluorous silica gel from the first batch did not behave the same. Therefore more extensive method development was performed on new and re-used fluorous silica gel from two different suppliers. The resulting method involves a glass column and either Fluoroflash (Hologent Technologies, Inc. Baldwin Park, CA) or Fluorochrom (Silicycle, Quebec Canada) fluorous silica gel as follows.

A slurry of methanol and approximately 10 g of FluoroFlash or Fluorochrom was added to a 1 cm diameter glass column to pack a 1 cm by 16 cm column. No less than 200 mL of methanol was eluted through the column to clean the fluorous silica gel. The column was then conditioned with 50 mL of MTBE. The fish tissue extract was loaded onto the column followed by three 50 μ L rinses of MTBE to quantitatively transfer the entire sample onto the column. The column was wash with 20 mL of MTBE and 20 mL of 5:95 methanol:MTBE. The column was eluted with 10:90 methanol MTBE to remove PFOS. The column was finally rinsed with 50 mL of methanol to remove any remaining extract components from the column. The 10:90 eluent was then reduced under a gentle stream of ultra-pure nitrogen to approximately 100 μ L. An internal standard (PFDoA) was added to the sample and sample delivered into an autovial for analysis. A depiction of the MTBE fraction and 10:90 fraction is given in Figure 1.



During standard tests, the PFCs were retained on the column until the final 100% methanol rinse, but it appears that the lipid components of the fish extracts act as a mobile phase to remove PFOS from the fluorosilica gel column.

Extracts of fish liver and whole fish homogenate were analyzed by single quadrupole LC/MS and PFOS was determined quantitatively without chromatographic interference. The extracts were then sent to Doug Kuehl of the US EPA's Mid-Continent Ecological Division in Duluth Minnesota for analysis by quadrupole time of flight mass spectrometry (LC/Q-TOF). Chromatograms run on the Q-TOF showed only PFOS in the extract, concluding that the fluorosilica gel clean-up method adequately removes all interfering compounds, eliminating ion suppression/matrix effects (Figure 2).

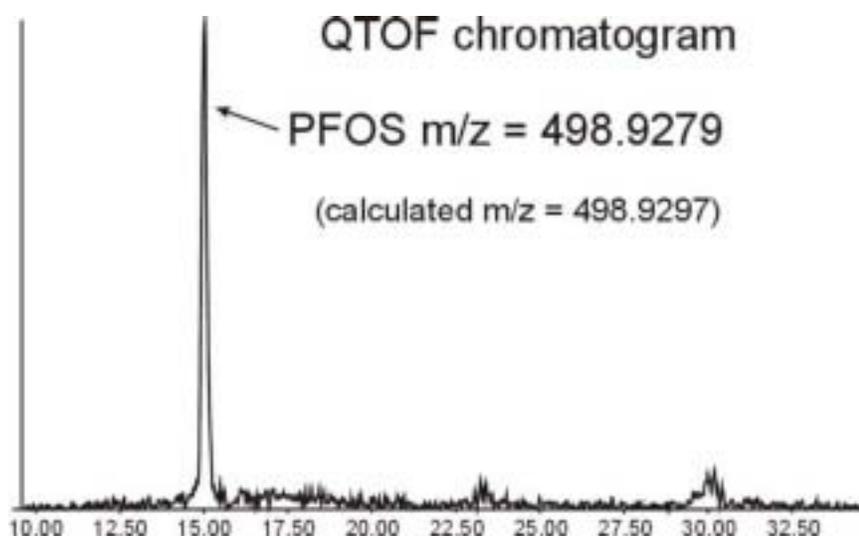


Figure 2. QTOF chromatogram showing PFOS in fish extract cleaned up by fluorosilica gel.

Another advantage to the fluorosilica gel clean-up method that we have developed comes about in the study of ion suppression/matrix effects. The traditional way of quantifying ion suppression/matrix effects is to add your analyte of interest between the HPLC and MS as a baseline, and then inject a blank into the HPLC and observe changes in the baseline at the retention time of your analyte. The reason that PFCs have become such an environmental concern is the detection of PFOS in humans and wildlife around the globe. As such there are no available blanks for analysis. Our fluorosilica gel clean-up method has the potential to manufacture blanks as seen by the MTBE fraction in Figure 1.

Samples used for the development of the fluorosilica gel clean-up method included livers from northern pike taken from three remote lakes in Voyageurs National Park on the northern border of Minnesota with Canada. The three lakes are Agnes, Locator and Little Trout. These lakes have no inlet, outlet or industry. The only access is by foot, therefore, it is felt that any PFCs present in these fish will represent atmospheric transport to these remote sites. Results of the fish liver samples are summarized in Table 2.

Table 2.

Lake	Tissue	PFOS Concentration (ng/g wet weight)
Locator	Liver	ND
Locator	Liver	12.2
Little Trout	Liver	0.81
Little Trout	Liver	ND
Little Trout	Liver	18.5
Little Trout	Liver	3.63
Agnes	Liver	0.57
Agnes	Muscle	0.08
Agnes	Liver	0.46
Agnes	Muscle	ND
Agnes	Liver	1.1
Agnes	Muscle	ND

Future work

The clean-up method is being prepared for publication and will make up the majority of Kelly J. Dorweiler's Masters Thesis. The method will also be tested for efficacy on water samples, and those samples collected by this study will eventually be cleaned and analyzed. These samples include:

Lake	County	Geographic Region	Media	Date Sampled
Locator	St. Louis	remote	water & fish	5/24/01
Loiten	St. Louis	remote	water	5/24/01
Shoepack	St. Louis	remote	water	5/23/01
Jorgens	St. Louis	remote	water	5/23/01
Agnes	St. Louis	remote	water & fish	5/23/01
Little Trout	St. Louis	remote	water & fish	5/24/01
Fish Trap	Morrison	agricultural	water	8/18/01
Itasca	Clearwater	remote	water	8/22/01
Minnetonka	Hennepin	suburban	water	6/3/01
			water	7/12/01
			water	8/16/01
			water	5/19/02
Mississippi River	Hennepin	urban	water	6/24/02
Lake Calhoun	Hennepin	urban	water	6/24/02
Lake of the Isles	Hennepin	urban	water	6/24/02
Lake Harriet	Hennepin	urban	water	6/24/02
Minnesota River	Scott	suburban/agricultural	water	6/24/02
White Bear Lake	Washington	suburban	water	7/11/02

Summary of Important Findings

The successful development of a method conducive to single quadrupole liquid chromatography mass spectrometry (LC/MS) will enable many more investigators to study the environmental chemistry of the emerging contaminant class of perfluorochemicals. It will also remove ion suppression/matrix effects for those investigators using LC/MS/MS and can be used to manufacture blanks for ion suppression/matrix effects experiments.

List of Publications and Presentations:

Kelly J. Dorweiler and Matt F. Simcik, "Detection and Quantitation of Perfluorinated Chemicals in Fish Samples Using Single Quadrupole LC/MS" submitted to *Environmental Science & Technology* **in review**

Matt F. Simcik, "Use of single quadropole LC/MS in perfluorochemical analysis." Presented at the Approaches to the analysis of PFOA and its salts in environmental matrices – problems and pitfalls Workshop in Hamburg, Germany. Sponsored by the Association of Polymer Manufacturers Europe May 1-2, 2003.

Matt F. Simcik and Kelly J. Dorweiler, "Detection and Quantification of Perfluorochemicals in Environmental Samples," Society of Environmental Toxicology and Chemistry 23rd Annual Meeting, Salt Lake City, UT, November 16-20, 2002

Kelly J. Dorweiler and Matt F. Simcik, Detection and Quantification of Perfluorinated Chemicals in Surface Waters, International Association for Great Lakes Research 45th Conference on Great Lakes Research, Winnipeg, MB June 2-6, 2002

Kelly J. Dorweiler and Matt F. Simcik, Analysis of Minnesota Surface Water Samples for Fluorinated Surfactants, Minnesota Water 2002 Conference, St. Cloud, MN. April 18, 2002.

Student Training:

Kelly J. Dorweiler, Environmental Health, M.S. expected Spring 2003.

Alison Wagner, Chemistry, undergraduate.

Antibiotic Losses in Runoff and Drainage from Manure-Applied Fields

Project 2002MN10G was not funded for FY2002.

Antibiotic Losses in Runoff and Drainage from Manure-Applied Fields

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Funding Source: USGS-WRRI 104G National Grants Competition

Reporting Period: March 1, 2002 through February 28, 2003

Summary

Antibiotics are commonly used as feed additive in animal production. Recently, there have been concerns regarding the effect of this practice on development of antimicrobial resistance in the environment. Most of the antibiotics fed to animals are excreted in urine or manure. Once excreted these antibiotics can enter surface and/or ground waters through non-point source pollution from manure-applied lands. Potentially this is one of the pathways for the spread of the antibiotics and the antimicrobial resistance into the wider environment. This study deals with assessing the effect of land application of antibiotic laced swine manure on antibiotic losses in surface runoff and tile drainage. Two antibiotics studied are chlortetracycline and tylosin. Field studies showed very little transport of chlortetracycline and tylosin through Webster clay loam soil into tile drainage. There was also no transport of dissolved chlortetracycline in surface runoff. However, 0.07% of the applied tylosin was transported as dissolved tylosin in surface runoff. Because of the difficulty of extracting soil-adsorbed antibiotics, the extent of antibiotic losses with sediment is unknown. Laboratory studies also showed that these two antibiotics are tightly adsorbed in high clay and high organic matter soils. However, there is some possibility that these antibiotics could move through coarse outwash soils and possibly through soils where preferential transport may be dominant. Research is underway to develop methods for quantifying the extent of adsorbed antibiotics on sediment and also to quantify the effect of temperature on antibiotic degradation.

Introduction

Since their discovery, antibiotics have been instrumental in treating infectious diseases that were previously known to kill humans and animals. However, it has now become clear that widespread use of antibiotics is not without problems (Halling-Sørensen et al., 1998; Jørgensen and Halling-Sørensen, 2000). The major concern is the development of antibiotic-resistant microorganisms, which are difficult to treat with existing antibiotics (Ford, 1994, Herron et al.,

1997). Increasingly more microorganisms are becoming resistant to multiple antibiotics (Goldburg, 1999).

According to one estimate, two million pounds of antibiotics were produced in the U. S. in 1954 compared to more than 50 million pounds being produced each year currently (Environmental Media Services, (EMS) 2000). Although most of these antibiotics are used for the treatment of infections in humans and animals, a significant portion is used as a supplement in animal feed to promote growth of food-producing animals. According to EMS (2000), more than 40% of the antibiotics produced in the U.S are used as feed supplements. The use of antibiotics in animal feed helps increase the animal's ability to absorb feed and thus reach market weight on time. In addition, supplementing antibiotics in animal feed helps counteract the effects of crowded living conditions and poor hygiene in intensive animal agriculture (EMS, 2000).

Antibiotics commonly used as feed additive for animals include aureomycin, bacitracin, bambarmycins, erythromycin, lincomycin, monensin, oleandomycin, oxytetracycline, penicillin, tylosin, and virginianmycin (Church and Bond, 1982). The antibiotic dose varies from 1 to 100 g per ton of feed depending upon type and size of the animal and the type of antibiotic. Most of the antibiotics added to animal feed are excreted in urine or manure. In some cases, as much as 80% of the antibiotic administered orally may pass through the animal unchanged (Levy, 1992).

Once excreted in urine and manure, these antibiotics may enter surface and/or ground waters through non-point source pollution from manure-applied lands. Land application of manure is a common practice in many parts of the U.S. In the northern tier of the country, manure is applied even during winter over snow. Manure is applied to land because of its value in supplying nutrients to crops as well as a means of disposing unwanted waste. Although it is strongly recommended that application rates of manure be based on the nutrient status of the soil and crop needs, this recommendation is not always followed and thus the manure applications have been higher than the recommended rate. The goal of this study is to determine whether or not there are losses of any antibiotics from manure-applied fields either in surface runoff or through subsurface drainage. Specifically, the objectives of this research are:

1. Quantify the effects of liquid swine manure application on antibiotic losses in surface runoff and subsurface drainage under a conventional (moldboard plowing) and a conservation (chisel plowing) tillage system.
2. Quantify the degree of antibiotic adsorption both in batch and flow through studies on a major soil in the upper Midwest.
3. Quantify the degree of antibiotic degradation at lower temperatures.

The field study will be conducted in the Minnesota River basin where artificial drainage is common. Besides subsurface tile drainage, farmers often install surface tile inlets that drain depressional areas in the field to subsurface tile drains. These tile inlets allow transport of sediment and surface-applied chemicals to subsurface tiles, which ultimately flow to surface waters including the Minnesota River. The manure applied at the site will be swine manure from a finishing pig operation.

Methods

Field Experiment.

Antibiotic losses were monitored in both surface runoff and tile drainage from a field experiment at Lamberton, MN. The drainage plots are 18.2 m long and 9.1 m wide (Fig. 1). Each plot is isolated to a depth of 1.8 m by trenching around plot borders and installing a 0.3 mm

plastic sheet (Zhao et al., 2001). A perforated plastic tile drain, 10 cm in diameter, is installed at 1 m depth and 1.5 m away from the plot boundary along its width. This arrangement drains 16.7 m (18.2 m minus 1.5 m) length of the plot, one-half side of tile drains that may be 33.4 m apart. Tile drains empty into a monitoring well. Surface inlets are located at the lowest point in the plots and also drain surface runoff into the monitoring well.

The tillage treatments are conventional (moldboard plowing) and conservation tillage (chisel plowing). Manure and urea application rates are based on the University of Minnesota recommendations corresponding to a yield goal of 150 bushel/acre. Both surface runoff and subsurface tile drainage are measured by tipping bucket devices that are connected to CR-10 data loggers. Volume- distributed (composite water sample over a certain number of tips) runoff samples from surface inlets are taken by automated ISCO[®] samplers. Time-distributed (composite water sample over a certain time interval) subsurface drainage samples are collected manually once a day. The other details of sampling set-up and protocol are given in Zhao et al. (2001) and Thoma (2003).

For the 2001-2002 crop year, primary tillage was done October 4, 2001 and subsequently liquid hog manure was injected on November 5, 2001 in half of the plots @ 45,794 L/ha. This corresponds to N application of 56 kg/ha. Two passes of secondary tillage were made on May 1, 2002. In the remaining half of the plots urea was applied at an equivalent of 161 kg-N/ha just before the secondary tillage. Corn was planted on May 1, 2002 right after secondary tillage.

For the 2002-2003 crop year tillage was done on 18 October 2002 and subsequently liquid hog manure was injected on the same day in half of the plots at 36,400 L/ha. Two passes of secondary tillage was done April 23, 2003 and corn planted the same day.

Currently, there are no standard methods for analysis of antibiotics in soil and water samples. Therefore, most of our effort this year has gone in the development of analytical methods for antibiotic in manure, water, and soil samples. The farmer supplying manure for our experiment mentioned that he is mixing aueromycin (chlortetracycline) and tylosin in swine feed. Therefore, our methods development was geared towards quantification of chlortetracycline and tylosin.

Development of ELISA Test

Subsequent analysis of runoff and tile line samples showed that both concentrations of chlortetracycline and tylosin were too low to be detected with HPLC. Therefore, a new method based on immuno assay (ELISA-Enzyme-Linked Immunosorbent Assay) was used to analyze runoff samples. Two commercially available enzyme-linked immunosorbent assay (ELISA) kits for tylosin or tetracycline residues in meat and milk were adapted for ultratrace analysis of these antibiotics in surface and ground waters. The ELISA test is based on solid phase immunoassay technology. Antibiotics containing standards or water samples are added to microtiter wells coated with high affinity capture antibody to tetracycline or tylosin. The antibiotic enzyme conjugate competes with antibiotic in the sample for binding sites on the capture antibody. After a wash step, a substrate is added which reacts to any bound enzyme, creating a different color. Antibiotic in samples blocks the binding of enzyme conjugate to the capture antibody, resulting in little or no color development depending on the amount of antibiotic in the sample. Results are quantified by measuring optical density values (450 nm) of both standards and samples after stopping the reaction with a stop solution in a microplate reader. The optical density is inversely proportional to antibiotic concentration in the sample.

Adsorption of antibiotic on soil and their subsequent extraction

Our other efforts in this project have gone in characterizing the adsorption characteristics of tetracycline, chlortetracycline and tylosin on two different soil types (Webster clay loam and Hubbard sandy loam). Adsorption studies were done both in batch (Fig. 2) and in flow through (Fig. 3) set-up. The surface samples of Webster clay loam soil were taken from urea plots of our field experiment at Lamberton. Hubbard sandy loam is a glacial outwash soil and represents a major soil group in Central Sands of Minnesota.

We have also been working on extraction procedures to quantify the amount of adsorbed antibiotics on soil/sediment. These procedures involve (1) using various extracting agents in trying to replace antibiotics on the exchange complex, and (2) testing the survival of microbes of a defined resistance level.

We used the following extractants in our desorption study: MeOH; MeOH-0.01M EDTA pH6.6; 1 N HCl; and 0.1 M Na₂ EDTA- McIlvaine buffer pH 4.0. The procedure included fortifying 1 g of soil sample with a given antibiotic of concentrations varying from 50-100 µg g⁻¹ soil, allowing the soil and antibiotic to equilibrate for 4hrs, and then using various extractants to evaluate antibiotic recovery.

Results and Discussion

Antibiotics concentrations in manure

Analysis of the 2001 hog manure from the supplier lagoon showed presence of chlortetracycline (5.0 mg/L of manure slurry) and tylosin (5.6 mg/L of manure slurry). At 45,794 L/ha, this is equivalent to 229 gm/ha of chlortetracycline and 256 gm/ha of tylosin. Analysis of the 2002 hog manure from the supplier lagoon showed presence of chlortetracycline (5.47 mg/L of manure slurry) and tylosin (4.52 mg/L of manure slurry) and oxytetracycline (1.31 mg/L of manure slurry). At 36,400 L/ha, this is equivalent to 199 g/ha of chlortetracycline and 165 g/ha of tylosin and 48 g/ha of oxytetracycline. Antibiotic analysis in manure sample was done on HPLC (High Performance Liquid Chromatography).

Antibiotic losses in runoff and tile line flow

We have completed the analysis of all 2002 surface runoff and tile line samples for presence of chlortetracycline using ELISA test. None of the samples showed any presence of chlortetracycline. Furthermore, there was no presence of tylosin in the tile line water. This is consistent with our laboratory batch adsorption and flow through studies that show strong tendency of chlortetracycline and tylosin to adsorb on the Webster clay loam soil. Tylosin losses in surface runoff for four storm events in 2002 amount to 168 mg/ha for the manure treatment compared to 41 mg/ha for the urea treatment (Table 1). These amounts translate to tylosin losses of about 0.07% of the tylosin applied in manure. Presence of tylosin in the urea treatment is possibly due to cross contamination of plots during tillage. Most of these losses are of dissolved tylosin. It is unknown how much of these antibiotics remain adsorbed on the soil and to what extent these antibiotics are transported with sediment losses.

So far, there has not been any major event in 2003 that has generated runoff from these plots. However, we have collected many tile line samples and we are in the process of analyzing those samples.

We also completed the analysis of nutrient and sediment losses from the field experiment. In 2002 there was a significant difference ($p=0.054$) by nutrient source treatment for surface losses of NO₃-N and combined NO₃+NH₄-N. Losses of surface NO₃-N and NO₃+NH₄-N from

urea treated plots were 0.58 kg/ha and 0.71 kg/ha respectively, while losses of surface $\text{NO}_3\text{-N}$ and $\text{NO}_3\text{+NH}_4\text{-N}$ from manure treated plots were 0.24 kg/ha and 0.34 kg/ha respectively (Table 2). However, these losses are relatively small compare to the losses by tile drainage. There was no difference in $\text{NO}_3\text{-N}$ and $\text{NO}_3\text{+NH}_4\text{-N}$ losses in tile drainage between the nutrient source treatments in 2002. The lower losses from manure compared to urea plots suggest slow but continuous release of manure organic N that is taken up by the crop more efficiently. Additionally, the inorganic fertilizer was not incorporated as deeply as the injected liquid hog manure. This may have left it more susceptible to surface transport, especially in a year like 2002, which had more intense storms than previous years as indicated by the greater surface runoff losses.

There was no significant difference between nutrient source (manure and fertilizer) or tillage (moldboard plowing, chisel plowing) treatments in terms of total P (TP), dissolved molybdate reactive phosphorus (DMRP), and total solids (TS) losses in surface runoff (Table 2). A complete report summarizing 4 years of data on sediment and nutrient losses from the field experiment is given in Thoma (2003).

Adsorption of antibiotics on soils:

Batch experiments showed that tetracycline and chlortetracycline are strongly adsorbed on both soils than tylosin. Among the soils, Webster clay loam has higher adsorption capacity than the Hubbard sandy loam. The differences in soil types are due to differences in clay and organic matter content of soils. Webster clay loam is higher in both clay and organic matter contents (34% & 4.4%) than the Hubbard sandy loam (10.4% & 2.2%). Flow through experiment with Hubbard sandy loam showed results consistent with the batch experiment i.e. chlortetracycline and tetracycline are more strongly adsorbed on the soil than tylosin.

Linear sorption coefficients (K_d) of chlortetracycline, tetracycline and tylosin on Webster clay loam were 2386, 2370, and 92 L/kg as compared to 1280, 1147, and 66 L/kg for Hubbard sandy loam. Thus at saturation, the retardation coefficient of chlortetracycline, tetracycline and tylosin in Webster clay loam will be 6083, 6042, 236 as compared to 4466, 4002, 231 for Hubbard sandy loam. The higher the retardation value, the greater is the adsorption potential of that chemical for a given soil. This number also reflects the quantity of water needed to displace a chemical through soil to the same distance as the non-adsorbing chemical. In other words, chlortetracycline will need 6083 times more water to displace than chloride or nitrate in a Webster clay loam at saturation. In other words, if it takes chloride or nitrate one year to reach a given depth then it will take 6083 years for chlortetracycline to reach the same depth. The variation in K_d values reduces when it was normalized with clay or organic carbon contents, thus suggesting that clay and organic may be the primary adsorption sites for these antibiotics.

Extraction of adsorbed antibiotics from soil particles:

Our best extraction was with 0.1 M Na_2 EDTA- McIlvaine buffer pH 4.0 but that recovery was only 41 to 67% (Table 3). The recovery was a bit higher for Hubbard sandy loam than Webster clay loam. This is expected because of high clay and high organic matter content of the Webster than Hubbard soil. At present there are no procedures available in the literature for full recovery of antibiotics from soil or sediments. The question at hand is whether the antibiotic on the soil is active or passive (in terms of its effect on microbial survival). We are testing an indirect method to assess the activity of soil-adsorbed antibiotics. The procedure involves use of

microbes of a defined resistance level and testing their survival when mixed with soil that contains adsorbed antibiotics.

Development of ELISA Tests

We have further completed the work on the enzyme-Linked immunosorbent assay (ELISA) method for ultratrace determination of antibiotics in aqueous samples. Two commercially available enzyme-linked immunosorbent assay (ELISA) kits that are commonly used for tylosin or tetracycline residues in meat and milk were adapted for ultratrace analysis of these antibiotics in surface and ground waters. Both ELISA techniques were found to be highly sensitive and selective for the respective antibiotics with detection limits of 0.10 and 0.05 $\mu\text{g L}^{-1}$ for tylosin and tetracycline, respectively. The recovery of both tylosin and tetracycline from spiked samples of lake waters, runoff samples, soil saturation extracts, and nano-pure water was close to 100%. Tetracycline ELISA was highly specific for tetracycline and chlortetracycline but not for other forms of tetracycline (oxytetracycline, demeclocycline, and doxycycline). These results indicate that both ELISA kits can be useful tools for low cost screening of tylosin, tetracycline and chlortetracycline in environmental waters. Furthermore, both ELISA procedures are rapid, portable, and easily adaptable to testing multiple samples simultaneously. The ELISA techniques reported here are inexpensive (approx. \$5 per sample for tylosin and \$15 per sample for tetracycline) and rapid, require a small sample volume ($< 100 \mu\text{l}$), are field portable, and work at very low but environmentally significant concentrations ($>0.10 \mu\text{g L}^{-1}$).

Ongoing work

We will continue characterizing antibiotic losses from our field site. Other questions include the effects of temperature on antibiotic losses in soil. We are planning to undertake incubation studies over the next month or so on temperature effects. One of the concerns in the incubation study is the recovery of soil-adsorbed antibiotic. We will continue with our test on the use of microbes to characterize the activity of soil-adsorbed antibiotics.

Summary of findings

Dissolved chlortetracycline and tylosin losses from manure-applied fields in the Minnesota River Basin will be relatively small both in surface runoff and in tile drainage. This is because of strong adsorption characteristics of these two antibiotics on high clay soil. However, it is unknown as to the extent of antibiotic losses with sediment. Since there is more potential for sediment losses from clay soils, this component could be important.

References

- Church, D.C., and W.G. Bond. 1982. Basic animal nutrition and feeding, 2nd Edition, John Wiley, Inc. NY. p238.
- Environmental Media Services (EMS). 2000. <http://www.ems.org/antibiotics/>
- Goldburg, B. 1999. Antibiotic resistance: Grave threat to Human health. Environmental Defense News Letter.
(http://www.environmentaldefense.org/pubs/Newsletter/1999/Jun/j_antibi.htm)
- Ford, Tim. 1994. Pollutant effects on the microbial ecosystem. Environ. Health Perspect., 102 (Suppl 12): 45-48.
- Halling-Sørensen, B., Nielsen, S.N., Lanzky, P.F., Ingerslev, F., Holten Lutzheft, H.C., and S.E. Jørgensen. 1998. Occurrence, fate and effects of pharmaceutical substances in the environment-A review. Chemosphere, 36: 357-393.

- Herron, P.R., Toth, I.K., Heiling, G.H.J., Akkermans, A.D.L., Karagouni, A., and E.M.H. Wellington. 1997. Selective effect of antibiotics on survival and gene transfer of streptomycetes in soil. *Soil Biol. Biochem.*, 30: 673-677.
- Jørgensen, S.E., and B. Halling-Sørensen. 2000. Editorial "Drugs in the Environment" *Chemosphere*, 40: 691-699.
- Levy, S.B. 1992. *The antibiotic paradox: How miracle drugs are destroying the miracle*. Plenum Publishing.
- Thoma, D.P. *Management Impacts and Remote Sensing Applications for Water Quality Assessment*. Ph. D. Thesis. University of Minnesota, St. Paul, MN.
- Zhao, S. L., S.C. Gupta, D.R. Huggins, and J.F. Moncrief. 2001. Tillage and nutrient source effects on surface and subsurface water quality at corn planting. *J. Environ. Qual.* 30: 998-1008.

List of publications & presentations resulting from this project

- Kumar, Kuldeep, A. Thompson, A.K. Singh, and S.C. Gupta. 2003. Enzyme-Linked Immunosorbent Assay for Ultratrace Determination of Antibiotics in Aqueous Samples. *J. Environ. Qual.* (in review).
- Gupta, S.C. and Y. Chander. Antibiotic losses from manure applied fields. 30 Minutes question answer show on KDHL Radio Station at Fairbault. 16 April 2003.
- Gupta, S., Kuldeep Kumar, Ashok Singh, Anita Thompson, and David Thoma. 2003. Antibiotic and Nutrient Losses in Runoff and Drainage from Swine Manure Application. Minnesota Pork Producers Association, Mankato. 27 June 2002.
- Gupta, S., Kuldeep Kumar, Ashok Singh, Anita Thompson, David Thoma, Yogesh Chander, and Wade Hammer. 2003. Antibiotic and Nutrient Losses in Runoff and Drainage from Swine Manure Application. Minnesota Pork Producers Association, New Ulm. 27 March 2003.
- Gupta, S., Kuldeep Kumar, Ashok Singh, Anita Thompson, David Thoma, Yogesh Chander, and Wade Hammer. 2003. Antibiotic and Nutrient Losses in Runoff and Drainage from Swine Manure Application. Minnesota Turkey Growers Association, Buffalo, MN. 13 May 2003.
- Kumar, Kuldeep, A. Thompson, A.K. Singh, and S.C. Gupta. 2002. Adsorption of antibiotics on soils. *Agronomy abstract*.

Statement of related grants submitted or funded as a result of this project

- USDA-Impact of swine manure application on phosphorus, nitrate-N, bacteria, and antibiotics concentration in surface runoff and subsurface drainage water. (Gupta, Singh, Kumar). \$12,346. 2002-2003.
- Minnesota Pork Producers Association. Antibiotic and nutrient losses in runoff and drainage from swine manure application. (Gupta, Singh, & Kumar). \$60,000. 2002-2004.

NCSARE- The occurrence of antibiotic resistant bacteria in manure, manure applied fields, and pets on the farm. (Gupta, Goyal, Singh, Kumar, Murray). \$99,835. 2002-2004.

U of M Grants-in-Aid. Antibiotic Losses in Runoff and Drainage from Manure-Applied Fields. Ashok Singh, Satish Gupta, and Kuldip Kumar. \$25,000. 2002-2004.

USDA-NRI- Antibiotic losses and development of antimicrobial resistance from manure application. (Gupta, Goyal, Singh, and Kumar). \$310,000. 2003-2006.

LCMR-The role of animal manure in spreading antibiotic resistance in the environment. (Goyal, Gupta, Singh, Kumar, and Murray) \$410,000 . NOT FUNDED.

Description of student training provided by project:

Name: David Thoma

Program: Department of Water Resource Science, University of Minnesota

Degree being sought: Ph.D.

Name: Erica Sherry

Program: Technical Writing

Degree being sought: B.S.

Name: Anne Marsdren

Program: Computer Science

Degree being sought: B.S.

Table 1: Tylosin losses via surface runoff in 2002.

Event	Manure (mg/ha)	Urea(mg/ha)
30 July	46.6	0
4 August	4.3	0.8
9 August	113.8	39.5
22 August	3.8	1.2
Total	168.5	41.4

Table 2. Average annual loads in runoff and tile drainage for the duration of the study.

Year	-----Surface runoff-----						-----Tile drainage-----				
	Flow (cm)	NH ₄	NO ₃	NO ₃ +NH ₄	TP	DMRP	TS	Flow (cm)	NH ₄	NO ₃	NO ₃ +NH ₄
	------(kg/ha)-----										
1999 [¶]	0.2	0.0	0.1	0.1	0.1	0.0	66.8	N [‡]	0.7	N [‡]	N [‡]
2000	2.8	0.0	0.6	0.6	0.8	0.1	691.7	3.7	0.1	6.3	6.4
2001	7.2	1.0	1.8	2.8	3.8	0.3	2615.5	T [‡]	0.6	27.1	27.3
2002 [¶]	3.7	0.1	N [‡]	N [‡]	0.9	0.1	1666.6	2.5	0.0	2.3	2.3

[¶]1999 includes precipitation events between 5 May and 31 December. 2002 includes precipitation events between 1 January and 22 August.

[‡](N) Nutrient source effect only or (T) tillage effect only

TP=Total phosphorus, DMRP=Dissolved molybdate reactive phosphorus, TS=Total solids

Mean recovery (%) of tylosin and tetracycline antibiotics from fortified soils.

Extractant	Hubbard				Webster			
	Tyl	OTC	TC	CTC	Tyl	OTC	TC	CTC
MeOH	62	22	27	21	49	11	17	15
MeOH-0.01 M EDTA, pH 6.6	69	33	47	35	55	21	23	19
1 N HCl	15	40	42	50	5	32	30	27
0.1 M Na ₂ EDTA- McIlvaine buffer pH 4.0	61	59	67	49	52	43	49	41

Tyl – Tylosin; OTC – Oxytetracycline; TC – Tetracycline; and CTC – Chlortetracycline.

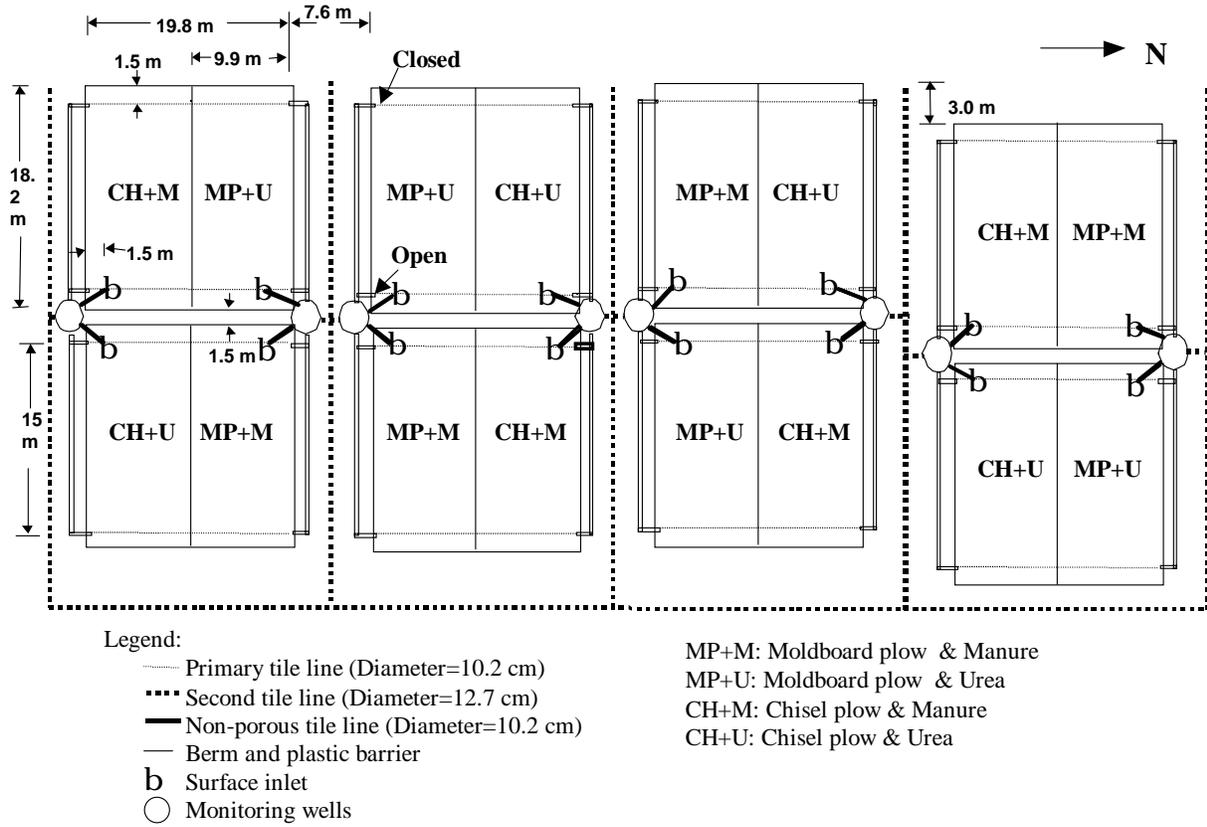


Fig. 1: Surf-n-sub plot lay out at the Southwest Research and Outreach Center in Lamberton, MN

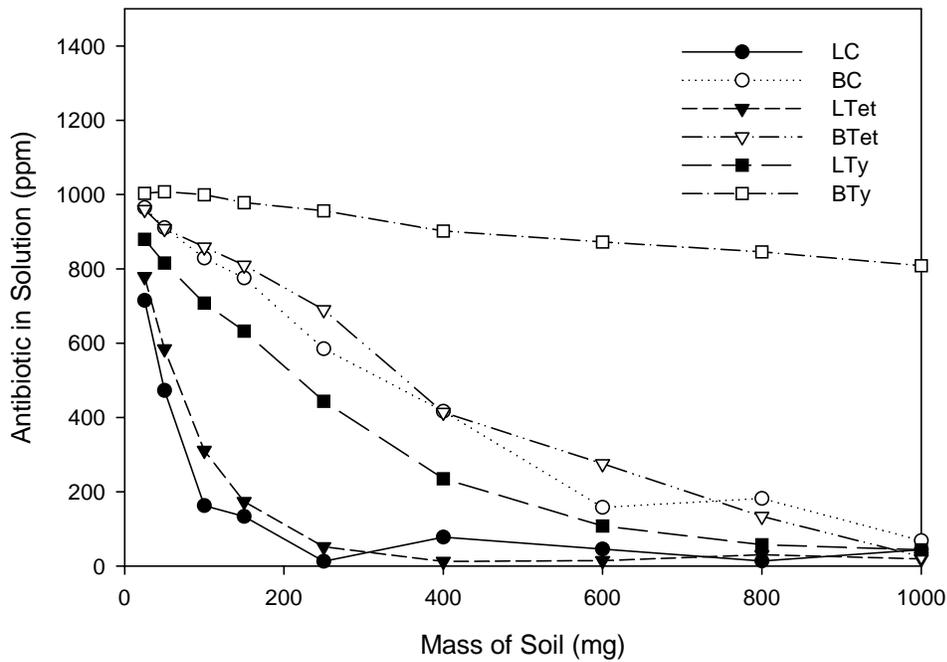


Figure 2. Antibiotic remaining in Solution after shaking 1000 ppm of antibiotic solution with various amounts of soil. L= Webster clay loam, B=Hubbard sandy loam, C=chlortetracycline, Tet=tetracycline, Ty=Tylosin. Top three curves are for Hubbard sandy loam soil whereas bottom three curves are for Webster clay loam soils. Tetracycline and chlortetracycline are strongly adsorbed on both soils than tylosin. Among the soils, Webster clay loam soil has higher adsorption capacity than the Hubbard sandy loam soil.

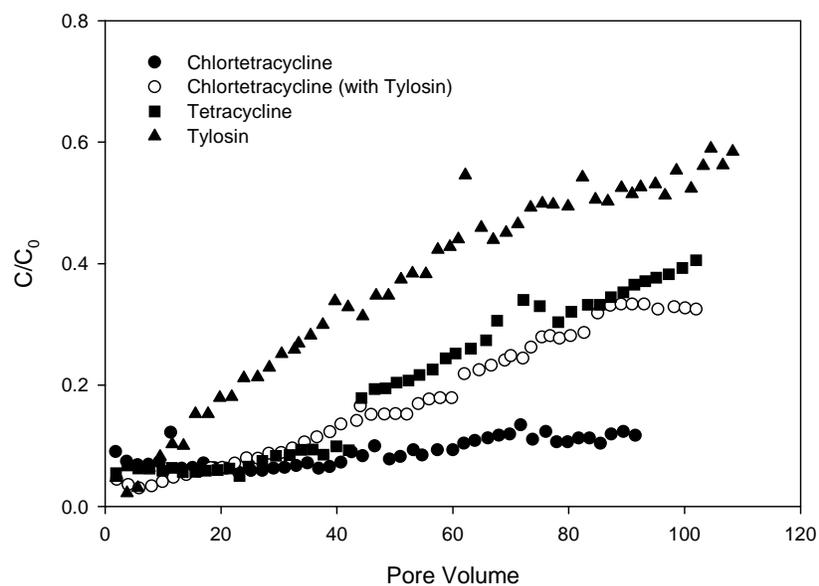


Figure 3. Breakthrough Curves for the Hubbard sandy loam soil for three antibiotics. Each Data Point is average of three replicates. As shown by batch adsorption studies, chlortetracycline and tetracycline are strongly adsorbed on the Hubbard sandy loam soil than tylosin.

In situ measurement of denitrification in agricultural streams

Project 2002MN11G was not funded for FY2002.

In situ measurement of denitrification in agricultural streams

Principal Investigators

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Funding Source: USGS-WRRI 104G National Grants Competition

Reporting Period: March 1, 2002 through February 28, 2003

Summary

Nitrate contamination of ground water and streams is common in landscapes dominated by agricultural activities. Associated impacts of this pollution range from local violations of drinking water standards designed to prevent methemoglobinemia to national concerns (e.g., Gulf of Mexico hypoxia). Significant quantities of nitrate are exported from agricultural lands through drainage ditches and low-order natural streams, but our understanding of nitrogen transport and transformation in these agricultural streams is far from complete. Denitrification may be an important mechanism for nitrate removal in these streams, and this would mitigate water quality and health hazards downstream. Several methods can be used to measure denitrification, but the most common ones involve laboratory experiments with sediment cores, where conditions are not conducive to obtaining *in situ* rates. Our research is comparing several methods used to assess *in situ* denitrification rates in agricultural streams and evaluating their accuracy. We also are evaluating how variations in key environmental factors may affect the importance of this process as a nitrogen sink.

Introduction

Contamination of ground water and streams by nitrate is a problem in many agricultural areas. Impacts associated with this pollution range from local (contamination of wells used for drinking water) to national (e.g., hypoxia in the Gulf of Mexico). Excess nitrogen loading, principally in the form of nitrate from the Mississippi River, is considered to be the cause of a large hypoxic zone in the nearshore Gulf of Mexico (Goolsby et al. 1999, Rabalais et al. 1999), and a large fraction of this nitrogen is thought to be derived from non-point agricultural sources in the Cornbelt region of the Upper Mississippi River Basin (UMRB). The UMRB generates roughly one-third of the nitrate loads reaching the Gulf of Mexico, while the Ohio River basin generates roughly one-fifth of the nitrate reaching the Gulf. The Midwest region thus is responsible for roughly half of the non-point source loads of nitrate entering the Gulf of Mexico. These loads have been attributed to heavy precipitation on intensively row-cropped soils that have extensive networks of surface ditches and subsurface tile drains, are high in organic matter content, and receive high rates of inorganic and organic nitrogen amendments (Randall and Mulla 1998). Nitrogen applied to the soil surface or mineralized from soil

organic matter can be delivered in the nitrate form to surface waters by leaching and drainage through subsurface tile drains after heavy precipitation.

The Minnesota River Basin (MNRB) is typical of tributary basins of the Upper Mississippi and Ohio River Basins that contribute to hypoxia in the Gulf of Mexico (Randall and Mulla 1998). The MNRB (Mulla and Mallawatantri 1997) has moderate to heavy annual precipitation (56-79 cm/yr), intensive row-cropping (92% of the land), extensive tile drainage (>40% of the land), extensive soils with high organic content (>80% of the land has an organic content exceeding 4%), and high rates of nitrogen application via fertilizer (county-wide averages up to 12.5 t km⁻² yr⁻¹) and manure (county-wide averages up to 4.5 t km⁻² yr⁻¹). As a result, the 4.0 million ha MNRB has a mean annual nitrate yield of 3.1 kg N km⁻² d⁻¹ (11.2 kg ha⁻¹ yr⁻¹), and its Le Sueur watershed has a mean annual nitrate yield of 6.4 kg N km⁻² d⁻¹ (Randall and Mulla 1998). These values are similar to the mean annual nitrate yields for other Midwest rivers; e.g., 5.5, 4.8, and 3.5, kg N km⁻² d⁻¹, respectively, for the Iowa, Illinois, and Wabash Rivers (Goolsby et al. 1999).

Much of the nitrate exported from agricultural lands in the Cornbelt is transported through drainage ditches, but we have only a poor understanding of the nitrogen transformation processes in these waterways. Depending on physical circumstances, denitrification could be a significant pathway for nitrate removal from these systems, enhancing water quality and reducing health hazards downstream. Several methods are available to measure denitrification, but they usually are conducted using sediment core experiments in the laboratory, where handling and incubation conditions are not conducive to obtaining accurate estimates of *in situ* rates. Even when rates are measured using field incubations, ambient hydrodynamic conditions are not replicated in the incubated samples, and correspondence between measured rates and *in situ* values is questionable.

The principal objectives of this project are to (i) assess *in situ* denitrification rates in agricultural streams and (ii) determine how these rates vary with stream stage, flow, and temperature. We are comparing *in situ* results obtained by ¹⁵N tracer, ¹⁵N natural abundance ratios, and mass balance methods. Part of our field work uses in-stream mesocosms for assessments. We are making measurements over a range of discharge and stage. Field work is being conducted in the eastern Minnesota River Basin, an agricultural area with high nitrate levels in its streams and rivers.

Methods

We selected two stream sites within the eastern region of the Minnesota River Basin: Waseca Stream in Waseca County and Beauford Stream in Blue Earth County. The Beauford site is ~ 20 miles west of the Waseca site (Figure 1). Beauford Stream has an older, downstream section with vegetation both on the banks and in the channel, as well as a recently ditched upstream section (constructed in 1999) that is devoid of vegetation. Comparison of the sections with respect to mass balance and stable isotope analysis is being done to assess effects of in-stream vegetation on nitrogen dynamics. We installed three piezometers adjacent to each of the three locations (Waseca, Beauford Unvegetated, Beauford Vegetated).

We found it necessary to apply different assessment methods for denitrification based on specific objectives. For example, we are using mass balance and stable isotopes

to assess large-scale denitrification rates and N-tracer and sediment core analyses to assess small-scale sediment/stream interactions. Details are given below.

(A) *Assessment of stream size, flow, and temperature effects on denitrification.* We measured denitrification rates for this component using mass balance (Bachmann et al. 1991) and stable isotope methods in the Beauford and Waseca systems. These two approaches allowed us to assess denitrification rates under differing flow and temperature regimes. During late summer (July/August, 2001 and 2002) we collected water samples in the Beauford and Waseca systems (first order, stations 400 m apart). Stream and ground water analyses (APHA 1998) included nitrate, ammonium, and total dissolved and total nitrogen for the mass balance method. The dual isotope method was employed concurrently to assess denitrification losses using stable isotopes, following the procedures of Revesz et al. (1997).

(B) *Assessment of sediment core-based denitrification measurement methods.* We are evaluating the importance of surface sediment nitrification as a nitrate source by measuring nitrate concentrations in pore water profiles of cores obtained across the stream cross-section at various locations along the Waseca Stream stretch. Cores were extruded into 1-2 cm thick segments in the laboratory, and pore water for chemical analysis was obtained by centrifugation (if the sediments were sufficiently unconsolidated) or dilution/extraction with deionized water (if the sediments were too firm for centrifugation). *In situ* mesocosms (clear plexiglass 14.6 cm diameter cylinders) with battery-powered stirrers were used in $^{15}\text{N}\text{-NO}_3^-$ addition experiments during the mass balance sampling periods (Waseca Stream only).

Results

Mass balance studies

The results of several upstream-downstream (400 m) mass balance studies during July and August of 2001 and 2002 are presented in Table 1. In general, nitrate concentrations were higher in the Waseca system than in the Beauford system, although elevated nitrate concentrations were present at Beauford during the August 28, 2002, sampling event.

Stable isotopes

During the mass balance studies, we collected composite water samples at the upstream and downstream ends of the 400-m stream reaches. These samples were sent to a laboratory in Canada where both the ^{15}N and ^{18}O isotope ratios were measured on the same nitrate ions (Table 2). Initial results indicate that N had a wide range of $\delta^{15}\text{N}$ values (from 7.82 to 15.23), while $\delta^{18}\text{O}$ values had a much smaller range (from 6.91 to 8.76). Results from the 2002 samples are still pending.

Sediment cores

In 2002 we collected stream bottom sediment cores after completing each mass balance study (Table 3). Initial results for porewater in the cores indicate that nitrate concentrations were relatively low (range: not detectable to 1.71 mg N/L) compared with ammonium concentrations (range: not detectable to 831 mg N/L). Additional porewater quality characteristics included soluble reactive P (range: 7.2 to 223.1 $\mu\text{g/L}$), loss on

ignition (range: not detectable to 5.66 %), chloride (range: not detectable to 9.4 mg/L), and sulfate (range: not detectable to 12.9 mg/L).

15N nitrate mesocosm additions

We added the equivalent of 1 mg/L $^{15}\text{N-NO}_3^-$ to three mesocosms and 2 mg/L to three other mesocosms. Although we have not completed analysis of the data, loss rates of total nitrate over time during the August 27, 2002, sampling period in Waseca Stream are summarized in Table 4. Initial results indicate loss rates of 0.06 and 0.04 mg NO_3^- -N/h over the 20-h period for the 1 and 2 mg/L additions, respectively.

Ongoing work and initial conclusions

We are analyzing our current data and waiting for results of stable isotope and mesocosm samples from a contract laboratory. We expect to receive this information in early 2003. Initial findings of the project will be presented at the American Society of Limnology and Oceanography meeting in February 2003 in Salt Lake City. Publications will be prepared after data analysis is completed.

Measurable differences in nutrient concentrations occur along the courses of our streams, and these differences are most noticeable during daylight. The loss of up to 1.64 mg/L TN along a 400-m stretch of stream (Table 1) indicates that in-stream loss processes are important at the stream-reach scale. The natural abundance of ^{15}N stable isotope in nitrate varied measurably in the stream samples suggesting this variable may be useful in estimating nitrate dynamics in the streams, but abundance of ^{18}O isotope showed only small variations suggesting that the dual isotope approach of Revesz et al. may not work in all streams.

References

- American Public Health Association. 1998. Standard methods for the examination of water and wastewater. 20th edition. APHA, New York, NY.
- Bachmann, R., W. Crumpton, and G. Hallberg. 1991. Nitrogen losses in an agricultural stream. *Verhandl. Internat. Verein Limnol.* 24: 1641-1643.
- Goolsby, D.A., W.A. Battaglin, G.B. Lawrence, R.S. Artz, B.T. Aulenbach, R.P. Hooper, D.R. Keeney, and G.J. Stensland. 1999. Flux and sources of nutrients in the Mississippi-Atchafalaya River Basin. Topic 3 Report for the Integrated Assessment on Hypoxia in the Gulf of Mexico. NOAA Coastal Ocean Program Decision Analysis Series No. 17, NOAA, Silver Spring MD, 130 p.
- Mulla, D.J. and A. Mallawatantri. 1997. Minnesota River Basin water quality overview. Bull. FO-7079-E, Minnesota Extension Service, Univ. of Minnesota, St. Paul, MN, 8 p.
- Rabalais, N., R. Turner, D. Justia, Q. Dortch, and W. Wiseman. 1999. Characterization of hypoxia. Topic 1 Report for the Integrated Assessment on Hypoxia in the Gulf of Mexico. NOAA Coastal Ocean Program Decision Analysis Series No. 15, NOAA, Silver Spring MD, 167 p.
- Randall, G. and D. Mulla. 1998. Nitrate-N in surface waters as influenced by climatic conditions and agricultural practices, in D.R. Keeney (ed.) *Hypoxia around the world: causes and solutions*. Madison, WI: Soil Science Society of America.
- Revesz, K., J. Bohlke, and T. Yoshinari. 1997. Determination of delta 18-O and delta 15-N in nitrate. *Anal. Chem.* 69: 4375-4380.

Photochemical Fate of Pharmaceutical Compounds Discharged and Detected in Natural Waters

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Photochemical Fate of Pharmaceutical Compounds Discharged and Detected in Natural Waters

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Funding Source: USGS-WRRI 104G National Grants Competition

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Summary

Recent studies have detected numerous pharmaceuticals and personal care products (PPCPs) in US surface waters. The potential environmental impact of these chemicals will be dictated by their persistence in the environment and the biological activity of any degradation products. One potential loss process for pharmaceuticals and personal care products is photodegradation. In this work, the direct photolysis and indirect photolysis (hydroxyl radical mediated and singlet oxygen mediated) of selected PPCPs was investigated. To date, the fate of antacids (cimetidine and ranitidine), non-steroidal anti-inflammatory drugs (naproxen, ibuprofen, and diclofenac), a lipid regulator (clofibrac acid), and antimicrobial compounds (triclosan and five members of the sulfa antibiotic class) have been studied. All the compounds studied react with hydroxyl radical at nearly diffusion limited rates, but given the low concentration of hydroxyl radical in natural waters, other processes appear to be more important for most of the compounds studied. The heterocyclic groups in cimetidine, ranitidine, and the sulfa drugs are susceptible to attack by singlet oxygen. Ranitidine and the sulfa drugs are subject to direct photolysis while cimetidine is not. Direct photolysis occurs rapidly for triclosan when present in the deprotonated, phenolate form. Naproxen and diclofenac are rapidly transformed via direct photolysis. Clofibrac acid reacts via a combination of direct photolysis and hydroxyl radical mediated indirect photolysis. Reaction with hydroxyl radical is the only photochemical loss process observed for ibuprofen. An important finding of this study is that the products of photolysis are not always benign. Specifically, photolysis of triclosan leads to the formation of 2,8-dichlorodibenzo-p-dioxin and 2,4-dichlorophenol, two priority pollutants.

Introduction

Pharmaceuticals and personal care products (PPCPs) are a class of chemicals that are continuously released into the environment through human activities, and, even though they have known biological effects, receive little attention (1,2). Examples of PPCPs include antibiotics, lipid regulators, psychiatric drugs, over the counter medications, and antimicrobial compounds. Most of these chemicals are introduced into the sewage system through their normal course of use. Once in the sewage system, many PPCPs are not completely removed at treatment plants (3) and thus, there is continuous introduction of these compounds to the environment. Numerous

PPCPs have been detected in both ground and surface waters throughout the United States and Europe (2,4-12).

The impacts of PPCPs on the environment are unknown. Undesirable effects on non-target aquatic organisms and damage to sensitive ecosystems are possible (2). Furthermore, antibiotic drugs and antimicrobial agents in the environment may aid in the development of resistant bacteria (2,13). The lifetimes of the PPCPs in aquatic systems will partially determine the magnitude of the effects and potential threats to drinking water supplies. Loss processes such as photolysis, therefore, will play an important role in the environmental impact of these compounds. This includes not only direct and indirect photolysis loss processes, but also where possible identifying intermediates and products that are formed through photolysis as transformation products may still have biological activity.

The research objective of this study is to determine the importance of both direct photolysis and indirect photolysis as loss processes for common PPCPs, including over-the-counter medications, antimicrobial compounds, and antibiotic medications. Additionally, the research aims to identify major products resulting from the photolysis experiments.

Methods

Direct and natural water photolysis experiments

Photolysis experiments were performed outdoors under natural sunlight or indoors under medium pressure Hg-vapor lamps. Sample solutions were contained in quartz test tubes (OD = 13 mm, ID = 11 mm, V = 10 mL) which were arranged on a turntable apparatus to ensure equal irradiation for all of the samples. For kinetic analyses ~ 0.5 mL samples were withdrawn from the quartz tubes at predetermined intervals and analyzed on an 1100 Series Hewlett Packard HPLC equipped with UV-absorbance detection and a computer driven data acquisition system. In experiments designed to probe for pH effects, various buffer solutions were employed to set the pH values. Quantum yields were calculated by comparing the rate constant for the disappearance of the PPCPs with the rate constant for the disappearance of a *p*-nitroanisole actinometer as described in ref. 14.

Natural water photolysis experiments were performed in 0.2 µm filtered Mississippi River (MRW) or Lake Josephine (LJW) water. To determine which pathways were responsible for the photodegradation, various quenchers were added to the water samples (sodium azide or DABCO for ¹O₂, isopropanol for radicals) and the substrate was also photolyzed in DI water in a separate tube.

Hydroxyl radical

The second-order rate constant for the reaction of PPCPs with hydroxyl radical was determined using Fenton's reagent. Serum bottle reactors contained a 100 µM solution of the PPCP of interest, 100 µM acetophenone, 0.2 mM Fe²⁺, and 5 mM hydrogen peroxide adjusted to pH 3 with sulfuric acid (15). Samples were withdrawn at predetermined intervals and mixed with an equivalent volume of methanol to quench the reactions (16). HPLC analysis for both the PPCPs and the acetophenone was performed.

The hydroxyl radical rate constant was determined using competition kinetics according to:

$$k_{OH}^S = \frac{\ln([S_t]/[S_0])}{\ln([R_t]/[R_0])} k_{OH}^R$$

where S is the substrate (the PPCP) and R is the reference compound with a known hydroxyl radical rate constant (acetophenone, $k_{OH} = 5.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, 17).

Singlet oxygen

Singlet oxygen reaction kinetics were measured in one of two ways, directly by laser flash photolysis (LFP) or indirectly by steady-state photolysis (SSP). In both types of experiment the substrate (typically at micromolar concentrations) and 40 μM Rose Bengal (RB), 100 μM Eosin Y, or 100 μM perinaphthenone, three well-defined singlet oxygen sensitizers, were dissolved in aqueous buffer solutions. In the LFP experiments, a pulse of laser light excites the sensitizer, which then produces singlet oxygen after the excited-state sensitizer is quenched by dissolved molecular oxygen. A sensitive Ge-photodiode detector then monitors the phosphorescence emission from singlet oxygen. The rate of disappearance of the singlet oxygen phosphorescence signal is a measure of a substrate's activity toward singlet oxygen. The resulting total quenching rate constant (k_{tot}) is the sum of the chemical reaction and physical quenching rate constants.

In SSP experiments, the samples are photolyzed continuously and small aliquots are removed for analysis by HPLC. In this case, the disappearance of the PPCP is monitored (as decreases in peak area), rather than the singlet oxygen signal. This allows for the determination of the chemical reaction rate constant (k_{rxn}) for the PPCP with singlet oxygen.

Product identification

To analyze the products of various photoreactions, GC-MS, LC-MS, and NMR spectroscopy were employed. An Agilent Technologies 6890 Gas Chromatograph with Mass Selective Detector was used to obtain mass spectra of various reaction mixtures. Photolysis samples run in organic solvents were analyzed by GC-MS to identify products. Product peaks were compared to mass spectral libraries to aid in their identification. Authentic samples of the likely products were then run under identical conditions to compare to the photolysis samples. Proper retention times and mass spectra indicated that a peak from the reaction mixture matched the standard solutions.

Results to date

Cimetidine and ranitidine

(Latch et al., 2003. Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine. *Environmental Science and Technology*, in press.)

The anti-ulcer drugs cimetidine and ranitidine both rapidly decayed in MRW, but with disparate mechanisms (see Figure 1). In MRW, cimetidine rapidly photodegraded, and the addition of 1 % isopropanol did not alter the rate. Addition of 10 mM sodium azide, a $^1\text{O}_2$ quencher, however, drastically diminished the photodegradation rate. The photodegradation rate of the azide spiked sample matched that of a dark control and a direct photolysis sample (in which cimetidine was dissolved in DI water with no sensitizer present). These results indicate that cimetidine does not undergo any direct photolysis, but is rapidly removed from natural waters due to its rapid reaction with $^1\text{O}_2$. Ranitidine, however, readily photodegrades in DI water, indicating that it undergoes direct photolysis. The photodegradation rate in MRW is slightly faster than it is in DI water, though, indicating a competing indirect photolysis mechanism. Quencher studies were used to determine that direct photolysis is the primary loss process in MRW, while reaction with $^1\text{O}_2$ causes a slight increase in the decay rate (as evidenced by the rate

retardation upon addition of the $^1\text{O}_2$ quencher DABCO and the lack of effect when isopropanol was added).

To better assess the environmental fate of ranitidine and cimetidine, studies were performed in aqueous samples buffered to different values. As a compound that is active toward direct photolysis, ranitidine ($\text{pK}_a = 8.2$) was photolyzed in DI water buffered to pH 6 and pH 10. At these values, ranitidine is > 99 % in its conjugate acid form and 98 % in its free base form, respectively. At these two end member pH values the degradation rates were nearly identical, with quantum yields (relative to a *p*-nitroanisole actinometer) of $5.3 \pm 0.1 \times 10^{-3}$ at pH 6 and $5.5 \pm 0.1 \times 10^{-3}$ at pH 10.

The pH dependence of reaction of cimetidine ($\text{pK}_a = 7.1$) with $^1\text{O}_2$ was determined using SSP. Photolysis experiments were performed using RB or perinaphthenone as sensitizer in buffered samples spanning from pH 4 to 10. The bimolecular reaction rate constants (k_{rxn}) were found to be highly pH dependent, with a value of $3.3 \pm 0.3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ at pH 6 and $250 \pm 20 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ at pH 10. At intermediate pH values, experimentally determined rate constants agreed well with those calculated from the end member rates and the speciation of the compound.

For ranitidine, the pH dependence of its reaction with $^1\text{O}_2$ was determined by LFP to minimize the competing direct photolysis reaction. Rose Bengal or Eosin Y were used as sensitizers for these experiments and D_2O buffer solutions were used as solvent due to the intrinsically longer lifetime of $^1\text{O}_2$ in D_2O than H_2O . The total quenching rate constant (k_{tot}) was determined at pD 6.4, 7.5, and 9.8. A fourfold difference in rate constants was observable across this range: $1.6 \pm 0.2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at pD 6.4, $2.65 \pm 0.07 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at pD 7.5, and $6.4 \pm 0.4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at pD 9.8.

The experiments performed with cimetidine and ranitidine allow for predictions regarding their fate in surface waters. The fate of cimetidine is expected to be highly variable and controlled primarily by the pH and $[\text{}^1\text{O}_2]_{\text{SS}}$ of the water body. Its environmental half-life is expected to vary between 53 min (in pH 8 water bodies with $[\text{}^1\text{O}_2]_{\text{SS}} = 10^{-12} \text{ M}$) and 900 h (in pH 6 water bodies and $[\text{}^1\text{O}_2]_{\text{SS}}$).

The loss of ranitidine from natural waters is expected to be due primarily to direct photodegradation. Based on calculated quantum yields and differences in seasonal and longitudinal solar irradiation, the half-life is expected to range from 65 min to 6 h and be insensitive to pH. In waters that contain a high $[\text{}^1\text{O}_2]_{\text{SS}}$, the half-life is expected to decrease slightly due to the competing reaction with $^1\text{O}_2$.

Triclosan

(Latch et al., 2003. Photochemical conversion of triclosan to 2,8-dichlorodibenzo-*p*-dioxin in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry*, v. 158(1), pp. 63-66.)

Triclosan was also found to decay rapidly in MRW. The primary mechanism by which it degraded, however, seemed to depend upon the season in which the MRW was collected. In MRW collected in the Spring or Summer, the degradation was found to arise from a mix of direct and indirect photolysis pathways. We were unable to determine what the active species was in the indirect photolysis pathway (though we could rule out $^1\text{O}_2$, OH and OOR, ^3DOC , and Fe/DOC interaction based on quenching and spiking studies). With MRW collected in Fall or LJW, however, the photodegradation in MRW matched that in DI water, indicating that there

was no indirect photolysis occurring. This finding is consistent with what other researchers have found (18, 19).

Acid drugs: naproxen, diclofenac, ibuprofen, and clofibric acid

(Packer et al., 2003. Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibric acid, and ibuprofen, *Aquatic Sciences*, in review.)

The photolysis of naproxen in Milli-Q water and in MRW by natural sunlight is shown in Figure 2. In Mississippi River water, naproxen was photodegraded slightly more slowly than in deionized water ($k_{\text{rel}} = k_{\text{MRW}}/k_{\text{DI}} = 0.78$). The rate constant for the interaction of singlet oxygen ($^1\Delta_g$) with naproxen was determined by LFP to be $1.1 \pm 0.1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, too slow to be of environmental significance for this compound. The radical inhibitor IPA reduced the photodegradation rate in Mississippi River water ($k_{\text{rel}} = k_{\text{IPA}}/k_{\text{MRW}} = 0.43$). Subsequent work with 1% IPA in Milli-Q water showed similar inhibition. The quenching of the reaction with IPA was not consistent with the reaction rate with hydroxyl radical determined with Fenton's reagent. Another possibility for the observed effect of IPA is the quenching of a radical form of the naproxen itself. Support for this possibility arises from previous phototoxicity work, in which the direct photolysis products of naproxen were identified (20-22). The first step is conversion of the carboxylate (COO^-) group to a carboxyl radical ($\text{COO}\cdot$). Decarboxylation, resulting in carbon dioxide and a benzylic radical, then occur giving a variety of products. If a portion of the carboxyl radical is quenched by IPA, then the resulting product would be naproxen and the overall degradation rate of naproxen would be slower in the presence of IPA. The overall effect on reaction rate would be dependent on the relative rates of quenching by IPA and decarboxylation. The participation of other radical species (e.g., peroxy radicals, DOM radicals), however, cannot be ruled out. Note that such a quenching effect by the DOC in the MRW (16 mg/L) would be minimal compared to that by 1 % IPA ($\approx 4700 \text{ mg/L DOC}$).

Sunlight photolysis of diclofenac in natural water proceeded at a rate that is equivalent to that in Milli-Q water ($k_{\text{rel}} = k_{\text{MRW}}/k_{\text{DI}} = 1.00$). The radical inhibitor IPA, however, increased the photodegradation rate in Mississippi River water ($k_{\text{rel}} = k_{\text{IPA}}/k_{\text{MRW}} = 1.68$). Further study revealed that IPA also increased the photodegradation rate of diclofenac in deionized water by a similar amount ($k_{\text{rel}} = k_{\text{IPA}}/k_{\text{DI}} = 1.45$). The quantum yield found in this study, 0.094, is very close to that reported in the literature (0.12-0.2) (12, 23). The acceleration of the reaction in the presence of IPA occurred in both Milli-Q water and in MRW, indicating that either a reaction with or mediated by IPA takes place. The results of this study and of previous researchers (12, 24, 25), however, indicate that direct photolysis is the dominant degradation mechanism for diclofenac.

Both clofibric acid and ibuprofen were transformed slowly in natural sunlight with < 5 % conversion over 90 minutes. The calculated quantum yield for clofibric acid was < 0.0012. For ibuprofen, the quantum yield was determined to be < 0.0006. Both compounds were transformed more rapidly in MRW than in Milli-Q water, and the introduction of IPA to the MRW slowed the reactions by ~50%. None of the observed changes (Milli-Q vs. MRW or MRW vs. MRW+IPA) were statistically significant at the 95% confidence level. The more rapid reaction observed in MRW vs. Milli-Q water and the quenching by IPA in MRW suggest a role indirect photochemical processes, but given the minimal transformation observed, quantification of the importance of these processes was not possible.

Because the limited transformation of clofibric acid and ibuprofen observed in sunlight made determination of quantum yields and comparison between experimental treatments difficult, further experiments were performed with artificial light. The photolysis kinetics of clofibric acid and ibuprofen are shown in Figures 3 and 4, respectively.

Clofibric acid was degraded in both Milli-Q water ($\Phi = 0.76$) and in MRW ($\Phi = 0.94$). Introduction of IPA decreased the observed rate constant by 10 % in both systems. We believe that the discrepancy in quantum yields between artificial and natural light is due to the incomplete filtering of the 289 nm Hg emission by the borosilicate well. These results suggest that for compounds such as clofibric acid that absorb light in the 280-290 nm range, but not in higher wavelength ranges, artificial light provided by an Hg-vapor lamp with a borosilicate filter may overestimate the importance of direct photolysis. The effect of IPA in the artificial light experiments, which is similar in both Milli-Q water and MRW, is again rationalized in terms of the quenching of a carboxyl radical formed from the carboxylate group of clofibric acid. Quenching of such a radical would lead back to the starting material, and thus lower the overall degradation rate. Although reaction with hydroxyl radical based on the experimentally determined values of $[\cdot\text{OH}]_{\text{ss}}$ and k_{OH} would account for >50% of the quenched rate in MRW, the fact that similar behavior was observed in MRW and Milli-Q water argues against this explanation.

Ibuprofen was negligibly degraded in Milli-Q water (with or without IPA) with a quantum yield of < 0.01 . In MRW, however, transformation did occur, and this reaction was quenched by the addition of IPA. Parallel experiments with acetophenone were used to determine the steady-state hydroxyl radical concentration in the irradiated MRW. From the observed first order rate constant for acetophenone disappearance ($4.4 \pm 0.9 \times 10^{-6} \text{ s}^{-1}$) and the known rate constant for the reaction of hydroxyl radical with acetophenone ($5.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$; 17), $[\cdot\text{OH}]_{\text{ss}}$ was determined to be $7.5 \pm 1.5 \times 10^{-16} \text{ M}$. The degradation of ibuprofen in the MRW and the quenching of this reaction by IPA argues for a radical mediated mechanism of degradation in MRW. Based on the k_{OH} value for ibuprofen and the measured $[\cdot\text{OH}]_{\text{ss}} = 7.5 \times 10^{-16} \text{ M}$, the first order rate constant for ibuprofen degradation in the MRW illuminated with the mercury vapor lamp should be $2.9 \times 10^{-4} \text{ min}^{-1}$. This is comparable to the observed degradation rate that can be attributed to radical processes ($k_{\text{MRW}} - k_{\text{MRW-IPA}} = 3.5 \times 10^{-4} \text{ min}^{-1}$).

Antibiotics: sulfa drugs

The sulfa drugs sulfamethoxazole, sulfamethizole, sulfathiazole, and sulfisoxazole were found to photodegrade in LJW at varying rates, and the degradation of all four matched that observed in DI H₂O, indicating the degradation is due solely to direct photodegradation. To further investigate the direct photolysis of the sulfa drugs, photolyses were performed at a range of pH values using buffered solutions. The direct photodegradation was found to be highly pH dependent for each of the sulfa drugs investigated.

The sulfa drugs are subject to reaction with hydroxyl radicals. The second order rate constants for these compounds are shown in Table 1. In addition to the reaction rate constants, environmental half-lives were calculated based on the range of hydroxyl radical concentrations expected in natural waters, which may range from 10^{-16} M in agriculturally impacted waters containing high nitrate levels to 10^{-18} M in pristine waters (26, 27).

The interaction of the sulfa drugs with $^1\text{O}_2$ was monitored in two stages. First, the total quenching rate constant (k_{tot}), the sum of the physical quenching and chemical reaction rate constants of substrate with $^1\text{O}_2$, for the interaction between the sulfa drugs and $^1\text{O}_2$ was

determined using laser flash photolysis. The values obtained were found to vary greatly within the class of sulfa drugs, and are shown in Table 2.

Table 1. Hydroxyl radical reaction rate constants and environmental half-lives for selected sulfa drugs.

Sulfa Drug	$k_{\text{rxn}, \cdot\text{OH}} (\text{M}^{-1}\text{s}^{-1})$	$t_{1/2}$	
		$[\cdot\text{OH}]_{\text{SS}}=10^{-16} \text{ M}$	$[\cdot\text{OH}]_{\text{SS}}=10^{-18} \text{ M}$
Sulfamethoxazole	$5.77 \pm 0.06 \times 10^9$	13.9 days	3.9 years
Sulfathiazole	$7.13 \pm 0.06 \times 10^9$	11.3 days	3.1 years
Sulfamethizole	$4.87 \pm 0.04 \times 10^9$	16.5 days	4.6 years
Sulfisoxazole	$6.59 \pm 0.06 \times 10^9$	12.2 days	3.4 years

Table 2. Total quenching rate constants for the interaction of selected sulfa drugs with $^1\text{O}_2$.

Sulfa Drug	$k_{\text{tot}} (\text{M}^{-1}\text{s}^{-1})$ pD ~ 9.5
Sulfamethoxazole	$2.3 \pm 0.4 \times 10^{4*}$
Sulfathiazole	$5.6 \pm 0.3 \times 10^7$
Sulfamethizole	$3.6 \pm 0.2 \times 10^6$
Sulfamoxole	$3.0 \pm 0.2 \times 10^8$
Sulfisoxazole	$6.5 \pm 0.2 \times 10^7$

* Measured in acetone.

The pH dependence on the total quenching rate constant was investigated for sulfamoxole and was found to exhibit a sigmoidal relationship, with elevated rate constants observed at more basic pH values.

Next, the specific chemical reaction rate constant was measured for both sulfathiazole and sulfisoxazole using steady-state photolysis. The reaction rate constants observed at pH 10 were $5.8 \pm 0.7 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ and $4.27 \pm 0.06 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$, respectively, indicating that the majority of the k_{tot} for both sulfa drugs is due to chemical reaction with $^1\text{O}_2$. The degradation was verified to be due to reaction with $^1\text{O}_2$ through the observed rate suppression upon addition of NaN_3 , a known $^1\text{O}_2$ quencher, as well as by the rate enhancement observed in D_2O due to the $^1\text{O}_2$ kinetic isotope effect.

Ongoing work

Current work is focusing on determining triclosan reaction products (other than 2,8-dichlorodibenzo-*p*-dioxin, which we have already characterized) using LC-MS or GC-MS after extracting the photolysate into organic solvent. We are also studying the photochemistry of mefanamic acid, a drug structurally similar to diclofenac. Additional work on the sulfa drugs is related to quantifying the pH dependence of the direct photolysis in addition to determining the products of the photolyses. Work with other antibiotic classes, including macrolides and tetracyclines, is also underway.

Summary of findings

The primary photodegradation mechanism for the antacid cimetidine is reaction with singlet oxygen while that for ranitidine is direct photolysis. These results reveal that minor structural changes can give rise to disparate environmental loss mechanisms. Direct photolysis occurs rapidly for triclosan when present in the deprotonated, phenolate form. For triclosan, an important finding of this study is that the products of photolysis are not always benign. Specifically, photolysis of triclosan leads to the formation of 2,8-dichlorodibenzo-*p*-dioxin and 2,4-dichlorophenol, two priority pollutants. Direct photolysis is the dominant loss process for naproxen and diclofenac. For clofibrac acid, direct photolysis and radical mediated indirect processes are of equal importance. Reaction with hydroxyl radical is the only photochemical loss process observed for ibuprofen. The sulfa drugs are susceptible to indirect photodegradation by hydroxyl radical and singlet oxygen and direct photolysis. Direct photolysis is the dominant pathway, and the rates are highly pH dependent, revealing the importance of environmental conditions on photodegradation.

References

1. Halling-Sorensen, B.; Nielsen, S. N.; Lanzky, P. F.; Ingerslev, F.; Lutzhoft, H. C. H.; Jorgensen, S. E. *Chemosphere* **1998**, *36*, 357-394
2. Daughton, C. G.; Ternes, T. A. *Environ. Health Perspect. Suppl.* **1999**, *107*, 907-938.
3. Richardson, M. L.; Bowron, J. M. *J. Pharm. Pharmacol.* **1985**, *37*, 1-12.
4. Buser, H.-R.; Poiger, T.; Mueller, M. D. *Environ. Sci. Technol.* **1999**, *33*, 2529-2535.
5. Stumpf, M.; Ternes, T. A.; Wilken, R.-D.; Rodrigues, S. V.; Baumann, W. *Sci. Total Environ.* **1999**, *225*, 135-141.
6. Ternes, T. A. *Water Res.* **1998**, *32*, 3245-3260.
7. Stan, H.-J.; Heberer, T.; Linkerhagner, M. *Vom Wasser* **1994**, *83*, 57-68.
8. Heberer, T.; Stan, H.-J. *Vom Wasser* **1996**, *86*, 19-31.
9. Heberer, T.; Dunnbier, U.; Reilich, C.; Stan, H.-J. *Fresenius Environmental Bulletin* **1997**, *6*, 438-443.
10. Sacher, F.; Lachow, E.; Bethmann, D., and Brauch, H.-J., *Vom Wasser.* **1998**, *90*, 233-243
11. Heberer, T.; Schmidt-Baeumler, K.; Stan, H.-J. *Acta Hydrochim. Hydrobiol.* **1998**, *26*, 272-278.
12. Buser, H.-R.; Poiger, T.; Mueller, M. D. *Environ. Sci. Technol.* **1998**, *32*, 3449-3456.
13. Hirsh, R., Ternes, T., Heberer, K., and Kratz, K.-L., *Sci. Total Environ.* **1999**, *225*, 109-118.
14. Leifer, A. *The Kinetics of Environmental Aquatic Photochemistry: Theory and Practice.* Chapter 6, 1988.
15. Tang, W. Z.; Huang, C. P. *Chemosphere* **1996**, *33*, 1621-1635.
16. Chen, R.; Pignatello, J. J. *Environ. Sci. Technol.* **1997**, *31*, 2399-2406.
17. Buxton, G. V., C. L. Greenstock, N. P. Helman and A. B. Ross, *J. Phys. Chem. Ref. Data*, **1988**, *17*: 513-886.
18. Tixier, C.; Singer, H.P.; Cononica S.; Mueller, S.R. *Environ. Sci. Technol.* **2002**, *36*, 3482.
19. Lindstrom, A.; Buerge, I.J.; Poiger, T.; Bergqvist, P.-A.; Mueller, M.D.; Buser, H.-R. *Environ. Sci. Technol.* **2002**, *36*, 2322.
20. Moore, D. E. and P. P. Chappuis, *Photochem. Photobiol.* **1988**, *47*(2), 173-180

21. Bosca, F., M. A. Miranda, L. Vano and F. Vargas, *J. Photochem. Photobiol. A: Chemistry* **1990**, *54*, 131-134
22. Jimenez, M. C., M. A. Miranda and R. Tormos, *J. Photochem. Photobiol. A: Chemistry* **1997**, *104*, 119-121.
23. Poiger, T., H.-R. Buser and M. D. Muller, *Env. Toxicol. Chem.* **2001**, *20*, 256-263.
24. Moore, D. E., S. Roberts-Thomson, D. Zhen and C. C. Duke, *Photochem. Photobiol.* **1990**, *52(4)*, 685-690
25. Andreozzi, R., M. Rafaele and N. Paxeus. *Chemosphere* **2003**, *50*, 1319-1330
26. Brezonik, P. L.; Fulkerson-Brekken, J. *Environ. Sci. Technol.* **1998**, *32*, 3004-3010.
27. Mill, T. *Chemosphere* **1999**, *38*, 1379-1390.

List of publications & presentations resulting from this project

Peer Reviewed Publications

Latch, D.E.; Stender, B.L.; Packer, J.L.; Arnold, W.A. ; McNeill, K., 2003. Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine. *Environmental Science and Technology*, in press.

Latch, D.E.; Packer, J.L.; Arnold, W.A.; McNeill, K., 2003. Photochemical conversion of triclosan to 2,8-dichlorodibenzo-*p*-dioxin in aqueous solution. *Journal of Photochemistry and Photobiology A: Chemistry*, v. 158(1), pp. 63-66.

Packer, J.L.; Werner, J.J.; Latch, D.E.; McNeill, K.; Arnold, W.A., 2003. Photochemical fate of pharmaceuticals in the environment: naproxen, diclofenac, clofibric acid, and ibuprofen, *Aquatic Sciences*, in review.

Boreen, A.E.; Arnold, W.A.; McNeill, K., 2003. Photodegradation of pharmaceuticals in the aquatic environment: A review, *Aquatic Sciences*, in review.

Invited Presentations

W.A. Arnold

Photochemical Fate of Pharmaceuticals in the Environment. Presented at the Water Resources Science seminar series, University of Minnesota, St. Paul, MN April 8, 2003.

Photochemical Fate of Pharmaceuticals in the Environment. Presented at the Department of Environmental Engineering Science, Washington University in St. Louis, St. Louis, MO, March 14, 2003.

K. McNeill

Environmental Fate of Pharmaceutical Pollutants. University of Wisconsin-Eau Claire, October 25, 2002.

Environmental Fate of Pharmaceutical Pollutants. Hamline University, October 16, 2002.

Environmental Fate of Pharmaceutical Pollutants. Macalester College, October 9, 2002.

Singlet Oxygen and the Fate of Pharmaceuticals in the Aquatic Environment. 34th American Chemical Society Great Lakes Regional Meeting, Minneapolis, MN, June 4, 2002.

Singlet Oxygen and the Fate of Pharmaceuticals in the Environment. Grinnell College, April 24, 2002.

Environmental Photochemistry of Pharmaceuticals, University of Minnesota Undergraduate Society for Lectures in Chemistry, November 28, 2001.

Singlet Oxygen and the Fate of Pharmaceuticals in the Environment. Portland State University, October 26, 2001.

Singlet Oxygen and the Fate of Pharmaceuticals in the Environment. Reed College, October 25, 2001.

Singlet Oxygen and Environmental Photooxidations. University of Minnesota, Duluth, September 21, 2001.

Featured Speaker: Singlet Oxygen and Environmental Photooxidations. American Chemical Society, Winona-La Crosse Section Meeting, September 18, 2001.

Conference Presentations

Boreen, Anne L.; Arnold, William A.; McNeill, Kristopher. Photochemical fate of pharmaceuticals in the environment: Sulfa drugs. 226th National Meeting of the American Chemical Society, New York, NY, September 2003.

Latch, D. E.; Packer, J. L.; Arnold, W. A.; McNeill, K. The Photochemical Fate of Cimetidine and Ranitidine Hydrochloride. Paper presented at 225th National Meeting of the American Chemical Society, New Orleans, LA, March 2003.

Packer, J.L.; Latch, D.E.; McNeill, K.; Arnold, W.A. Photochemical fate of pharmaceuticals in the environment: Naproxen, Ibuprofen, Diclofenac, and Clofibric Acid. Poster presented at American Chemical Society National Meeting, New Orleans, LA, March 23-27, 2003.

Packer, J.L.; Latch, D.E.; McNeill, K.; Arnold, W.A., 2002. Photochemical Fate of Triclosan in the Environment. Poster presented at the 17th Annual Conference on the Environment, Bloomington, MN, November 14, 2002.

Latch, D. E.; McNeill, K. The Photochemical Fate of Triclosan. Poster presented at 34th Great Lakes Regional Meeting of the American Chemical Society, Minneapolis, MN, June 2002.

Boreen, Anne L.; McNeill, Kristopher. Kinetics of the interaction between

singlet oxygen and sulfa drugs. Poster presented at 34th Great Lakes Regional Meeting of the American Chemical Society, Minneapolis, MN, June 2002.

Latch, D.E., W.A. Arnold, and K.McNeill (2002) *Singlet Oxygen and the Photochemical Fate of Triclosan*. Paper presentation at the 2002 national meeting of the American Chemical Society, April 7-11, 2002, Orlando, Florida.

Packer, J.L., K.McNeill, and W.A. Arnold (2002) *Direct and Indirect Photolysis of Triclosan*. Paper presentation at the 2002 national meeting of the American Chemical Society, April 7-11, 2002, Orlando, Florida.

K. McNeill, D. E. Latch, B. L. Stender and J. VanOverbeke, Singlet Oxygen and the Photochemical Fate of Triclosan. Paper presentation at 223rd ACS National Meeting, Orlando, FL, April 2002.

Latch, D. E.; McNeill, K. Singlet Oxygen and the Photochemical Fate of Ranitidine and Cimetidine. Poster presented at 24th Annual Midwest Environmental Chemistry Workshop, Minneapolis, MN, October 2001.

Latch, D. E.; Stender, B. L.; McNeill, K. Singlet Oxygen and the Photochemical Fate of Pharmaceuticals. Poster presented at 222nd National Meeting of the American Chemical Society, Chicago, IL, August 2001.

Statement of related grants submitted or funded as a result of this project

Dr. Arnold and Dr. McNeill have continued to apply for funding to continue this avenue of research. Dr. Arnold has received funding from the Center for Urban and Regional Affairs (University of Minnesota) to investigate the photodegradation of selected antibiotics in Minnesota waters in 2003-2004. Dr. Arnold is also a co-investigator on a United States Department of Agriculture grant (2003-2006) to investigate the loss of veterinary antibiotics in soil systems. Dr. McNeill and Dr. Arnold have also submitted a proposal to the USGS-WRRI 104G competition to study the degradation and biological activity of antibiotics and estrogen mimics.

Description of student training provided by project:

Name: Jennifer L. Packer

Program: Department of Civil Engineering, University of Minnesota

Degree earned: M.S. (2002)

Name: Douglas E. Latch

Program: Department of Chemistry, University of Minnesota

Degree being sought: Ph.D.

Name: Anne L. Boreen

Program: Department of Chemistry, University of Minnesota

Degree being sought: Ph.D.

Name: Jeffrey J. Werner

Program: Water Resources Science, University of Minnesota
Degree being sought: M.S./Ph.D.

Name: Jennifer L. VanOverbeke

Program: Dept. of Chemistry, Univ. of Minnesota Summer Undergraduate Research Program

Degree earned: B.S. (Northwestern University)

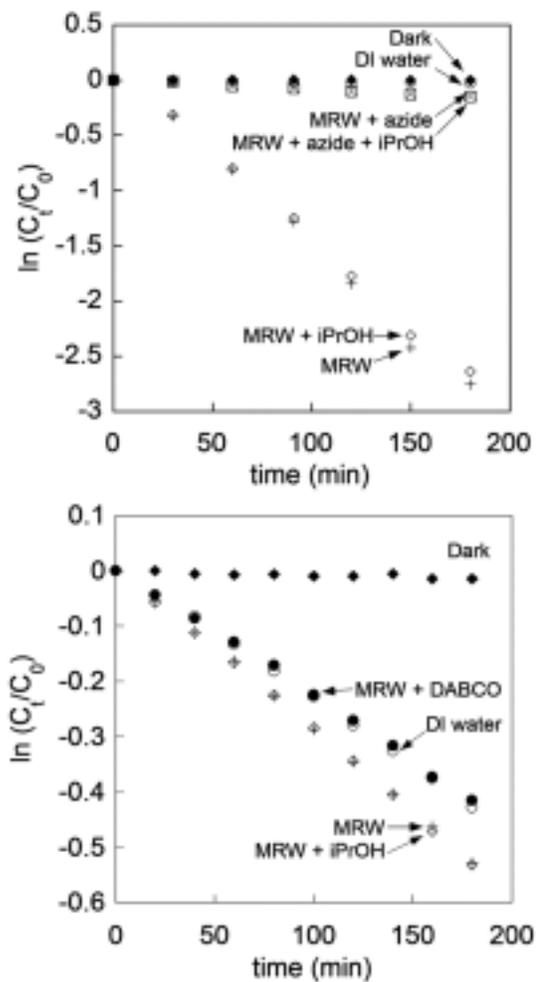


Figure 1. Degradation of cimetidine (top panel) and ranitidine (bottom panel) in various water samples.

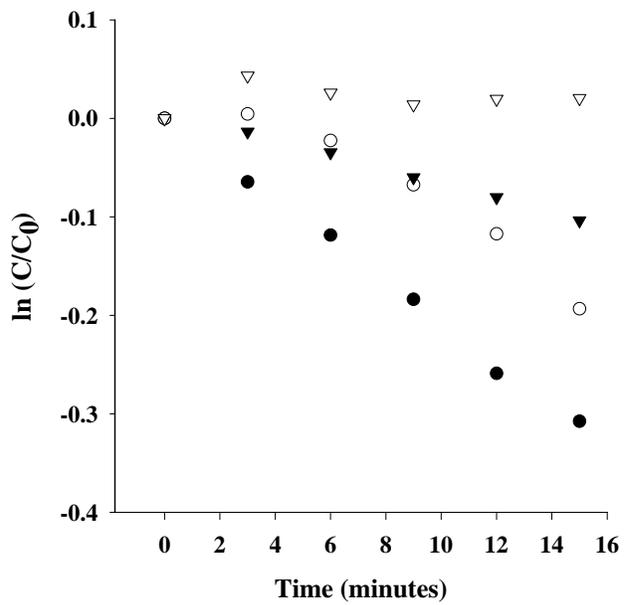


Figure 2. Direct photolysis of naproxen in H₂O in sunlight. Conditions are as follows: ● = substrate in Milli-Q water, ○ = Mississippi River water (MRW), ▼ = MRW with 1 % isopropanol, ▽ = dark control.

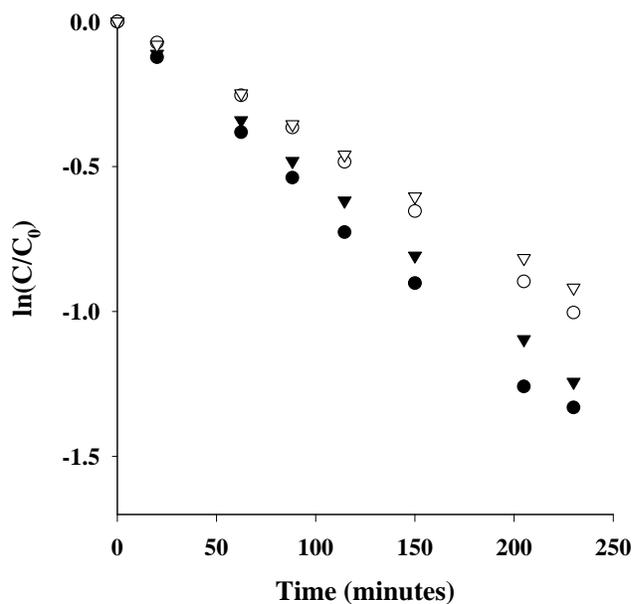


Figure 3. Direct photolysis of clofibric acid in H₂O by a Hg-vapor lamp. Conditions are as follows:
 ● = substrate in Milli-Q water, ○ = Mississippi River water (MRW), ▼ = Milli-Q water with 1 % isopropanol, ▽ = MRW with 1 % isopropanol.

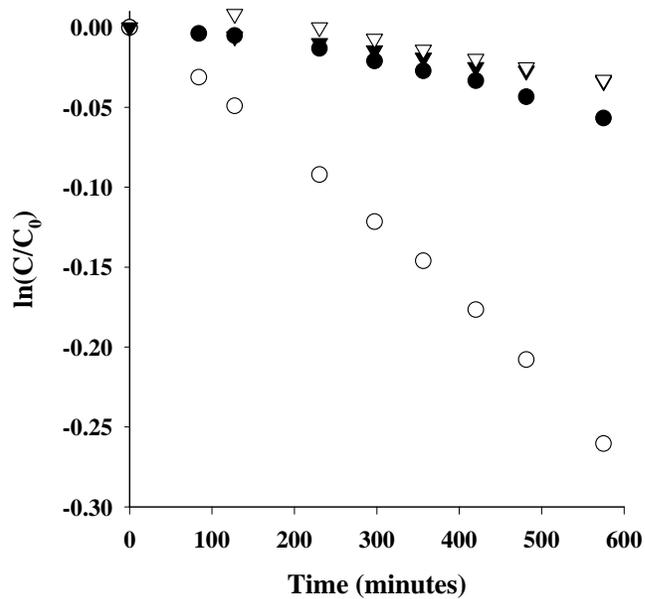


Figure 4. Direct photolysis of ibuprofen in H₂O by a Hg-vapor lamp. Conditions are as follows: ● = substrate in Milli-Q water, ○ = Mississippi River water (MRW), ▼ = Milli-Q water with 1 % isopropanol, ▽ = MRW with 1 % isopropanol.

Eutrophication and Remediation in Context: High-Resolution Study of the Past 200 Years in the Sedimentary Record of Lake McCarrons (Roseville, MN)

Basic Information

Title:	Eutrophication and Remediation in Context: High-Resolution Study of the Past 200 Years in the Sedimentary Record of Lake McCarrons (Roseville, MN)
Project Number:	2002MN13B
Start Date:	4/1/2001
End Date:	2/28/2003
Funding Source:	104B
Congressional District:	4
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	eutrophication, human impact, varved lake sediments, geochemistry, anoxia
Principal Investigators:	Emi Ito

Publication

Eutrophication and Remediation in Context: High-Resolution Study of the Past 200 Years in the Sedimentary Record of Lake McCarrons (Roseville, MN)

Principal Investigator

Emi Ito, Professor, Geology and Geophysics, University of Minnesota

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Funding source: USGS-WRRI 104B National Grants Competition

Reporting period: March 1, 2001-February 28, 2003

Summary

Lakes in urban settings receive major inputs of nutrients and salt from the lawns and roads that surround them. Increased biological productivity and salinization of lake bottom waters cause degradation of water quality in these valuable municipal resources. Monitoring efforts provide water quality information with high temporal resolution; however, due to logistical and budgetary constraints, monitoring rarely captures more than a few years' data; hence, these snapshots may not accurately represent the high interannual variability of physical and chemical parameters in lakes.

This study uses sediment cores and certain water chemistry analyses to generate a high-resolution record of historic and pre-European changes in the chemistry and productivity of Lake McCarrons, Roseville, Minnesota, with the goal of placing recent (1985-2001) monitoring data in a long-term context. Geochemical and isotopic parameters recorded in lake sedimentary components over time are used as proxies for processes and changes in the water column; a minimal number of water analyses are used to calibrate the system with respect to the isotopic proxies and to answer specific questions germane to the sediment study.

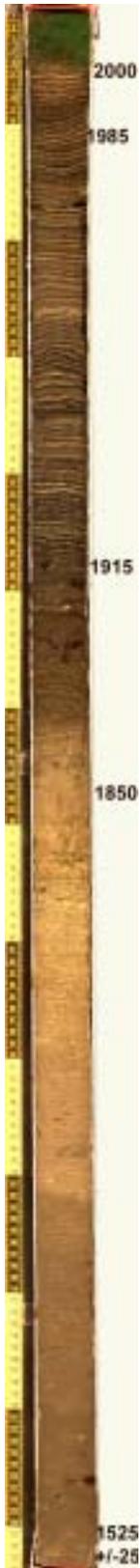
Results to date indicate that Lake McCarrons has undergone cycles of increased biological productivity in this century, and that productivity is as high now as it has been at any time during this period. Sediment data also suggest that the degree of bottom-water anoxia has increased over the past 40 years; this observation is further supported by water chemistry data indicating that the lake does not fully mix at fall and spring overturn, behavior which would feed back and support the development of greater anoxia. Sedimentation rates increase dramatically in the early 20th century as land-use changes add detrital material and nutrients to the lake; since about 1970, sedimentation rates have stabilized although eutrophication has continued. There is a strong relationship in the sedimentary record between moisture balance, lake stratification, and indices of biological productivity; the record of changes in the 20th century also demonstrate much higher variability than seen in the pre-human impact period. We hope that this information is useful to lake managers making decisions on remedial measures with reference to the "natural" state of the lake.

Introduction

Cultural eutrophication of urban lakes is a significant problem worldwide. The tension between shoreline development and the preservation of natural systems leads to expensive and sometimes divisive remediation efforts, the degree of success of which may not be known for years. Monitoring is a time- and labor-intensive effort, which can provide a high-resolution view of lake dynamics, but only on a short time scale. The historical record of lake level, productivity, anoxia, and chemistry, where it exists, is short and incomplete; water quality modelers then have only a few years of data with which models can be compared or validated. Long-term trends are easily masked by year-to-year variability in water quality; natural cycles are extremely hard to detect over the course of a few years, the typical duration of most monitoring projects. However, broad trends exert a significant influence on water quality assessments and remediation efforts.

The study of lakes through their sedimentary records (paleolimnology) provides a lower-resolution but much longer-term picture of lake evolution: geochemical and biological sediment components provide an array of information about past changes in chemistry, flora and fauna, and water level. In lakes whose sediments are varved (annually laminated), a history of the lake year by year may be constructed from this information. Where sediments are not laminated, we estimate deposition rates and produce a quasi-annual record.

This study has generated a high-resolution record in a number of geochemical proxies (stable isotopes, nutrients, minerals, etc.) for the past ~450 years (200 years presented here) in Lake McCarrons, a eutrophic Minnesota urban lake, based on a high-quality sediment core from 16 m water depth. This analysis will ultimately be supplemented by approximately 50 additional analyses performed on a 4.1-meter core taken in 1999 which is now curated at LacCore, the National Lacustrine Core Repository operated by the Limnological Research Center (LRC), University of Minnesota, Minneapolis. Combined, these studies will provide the context for long term (thousands of years) and medium-term (hundreds of years, including >300 years before European settlement) natural variability and rates of change in lake water quality, as well as illuminating trends which had their inception long before any human impact on the lake system.



Methods

Water column analyses

During the summers of 2001 and 2002, I collected water samples and basic limnological data every 2-4 weeks. Water samples were taken at three depths in the lake (2 m below the water surface; 1 m above the sediment surface, and ~1 m below the thermocline) for major ions and carbon stable isotopic composition ($\delta^{13}\text{C}$) of dissolved inorganic carbon. At the same time I measured Secchi depth, as well as dissolved oxygen, temperature, and pH at selected depths.

Sediment analyses

A sediment core from 16 m water depth was used for the study. Before sampling, digital images of the core were taken, and the boundaries between putative annual layers (cyclic packages of one dark [winter] and one light [summer] layer) were marked on these images using Photoshop. Later, a digital scan of the entire core was taken using polarized light to eliminate glare from reflected light (see figure 1). This image shows clearly the transition from poorly- to well-laminated sediments, and the variability in thickness and character of the laminations. For each layer, at least two smear slides (consisting of a small amount of sediment spread on a microscope slide and embedded in optical cement) were taken: one each from the dark and light layers, and one from any additional visible discrete layers. These slides are used to estimate abundance of sediment components such as algal organic matter, diatoms, and minerals such as calcite (precipitated in the water column) and quartz sand (washed in from the shoreline).

Based on the above determinations of how layers represent individual years' deposition, the laminated portion of the core (the top ~50 cm) was sectioned into 89 pieces, following the contacts between the dark (below) and light (above) layers. Numbered as "v" samples (for "varved," i.e. v01, v02, . . . , v89), these are taken to represent the 89 years of sedimentation preceding the year 2000, as the core was taken in the winter of 2000-2001. Below the top ~50 cm, the sediments are massive (i.e., not laminated or poorly laminated). 84 cm of this portion of the core was cut into 0.5 cm sections, and every other sample was analyzed (e.g., 1.0-1.5 cm and 2.0-2.5 cm but not 1.5-2.0 cm).

Many sediment studies present data in terms of component percentages at each analyzed level; accumulation rates provide a more realistic picture of changes in style of sedimentation, as percentage changes may be caused simply by dilution of one component by another. Sedimentation rates for the laminated section were determined based on varve thickness, water content (measured by freeze-drying a weighed volume of mud), and cross-sectional area of the core, to produce an index in terms of grams of material deposited per square meter per year. Comparison of varve counts and color changes farther downcore (discussed below) with ^{210}Pb dating of an earlier core analyzed by D.R. Engstrom (unpub. data) suggests that the

Figure 1. Image of core McC-01-1MP

laminations are indeed annual, and further provides an estimation of sedimentation rates for the non-varved

section of the core.

Sediment analyses conducted thus far include (1) carbon coulometry to determine weight percentage of carbonate, (2) elemental analysis to determine weight percent total carbon, nitrogen, and sulfur, (3) stable isotopic analysis to determine $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of calcium carbonate (cc). Since the continuation report (December 2002), additional analytical work has comprised: (4) biogenic silica measurements to determine weight percent diatom frustules, (5) stable isotopic analysis to determine $\delta^{13}\text{C}$ of organic matter, (6) scanning electron microscopy (SEM) to investigate size, preservation, and crystallinity of calcite grains over time, and (7) imaging of thin sections of core embedded with epoxy resin. The sedimentary phosphorus analysis budgeted in the original proposal has been put on hold because of the consideration that, due to its redox-sensitive mobility and biological importance, it is likely not a true measure of surface-water P concentrations (e.g., Engstrom and Wright 1984). As an alternative, this report includes an evaluation of the site for an additional study using fossil diatoms to infer P, Cl^- , and pH values.

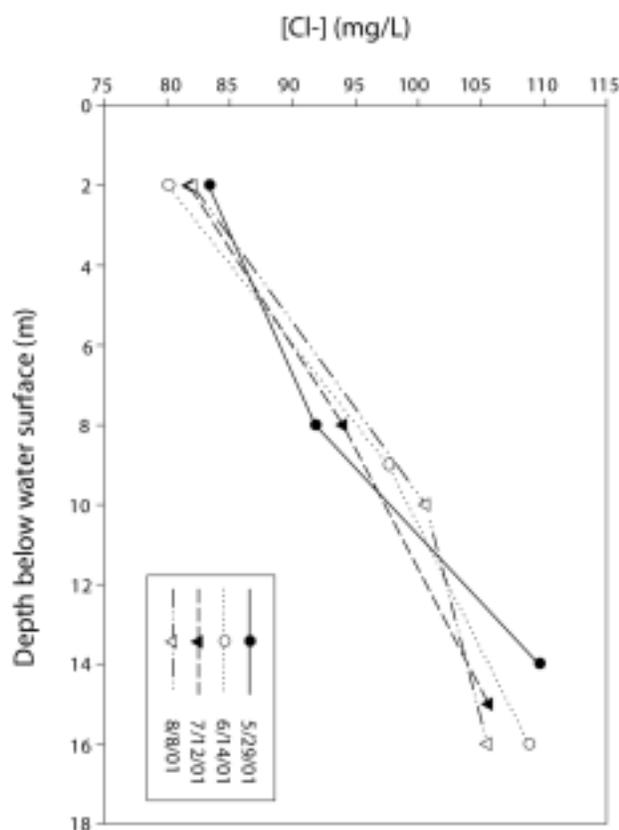


Figure 2. Chloride ion concentrations at sampled depths in Lake McCarrons during summer 2001.

quantity of dilute water enters preferentially into any given layer, the concentration of a conservative ion should be the same throughout the water column. As shown in figure 2, however, Cl^- concentrations are significantly higher in the hypolimnion than in the epilimnion throughout summer 2001 (data for 2002, and for Na^+ in 2001 and 2002, are similar). This

Results

Water chemistry

Major ions: The proposal for this study hypothesized that Lake McCarrons is meromictic or oligomictic (completely mixing never or only infrequently). It is well known from Metropolitan Council studies (Met Council 1988, 1997; West-Mack and Stefan 2000a, 2000b) that the lake is strongly thermally stratified during the summer; a thermister chain recording temperatures at 12 depths was deployed for several ice-free seasons. The Met Council reports do not demonstrate conclusively whether or not the lake does completely mix, in part because thermister measurements were sparse in the hypolimnion (on the order of 2-3.5 m spacing rather than the 1 m spacing of sensors in the epilimnion). Concentrations of conservative elements (i.e., those which do not participate in precipitation-dissolution reactions at concentrations found in the lake) such as Cl^- and Na^+ were used in this study as a measure of the degree of mixing of surface and deep waters. If the lake is mixed, and no great

suggests that lake bottom waters are not being entrained when the lake overturns in spring and fall. Concentrations of Cl^- throughout the lake are elevated to 20-50 times typical natural values for this region, presumably due to road salt inputs; it is possible that cold overland or wetland runoff high in dissolved road salt plunges into the hypolimnion in the spring, causing these higher hypolimnetic values. High levels of salt in the hypolimnion may also contribute to the density difference that retards mixing.

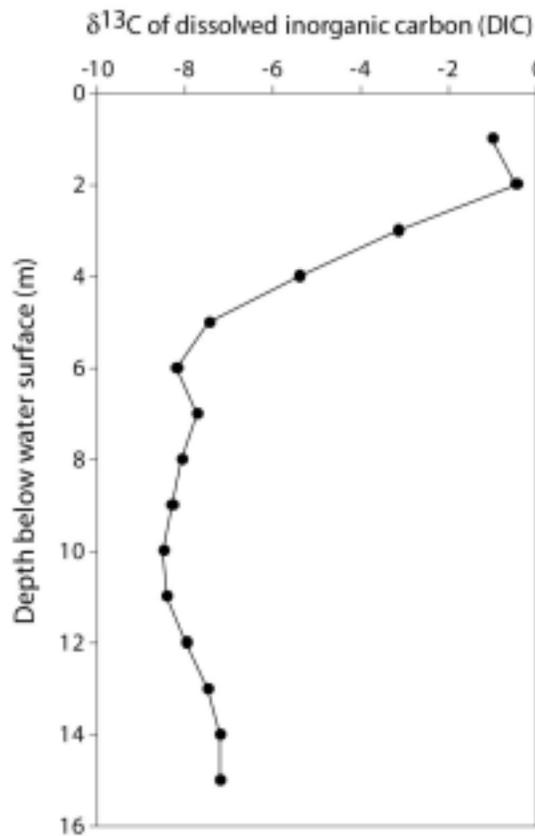


Figure 3. Carbon stable isotopic composition of DIC at sampled depths in Lake McCarrons on 8/9/2002.

δ¹³C of dissolved inorganic carbon (DIC): The systematics of carbon stable isotopes are too involved to discuss here. The salient points are that in general, δ¹³C values become more positive (“heavier”) under algal productivity (due to preferential uptake of ¹²C) and more negative (“lighter”) under degradation of organic matter (due to the release of the same light carbon); the δ¹³C value of DIC is also imparted to carbonate minerals and organic matter that become part of the sedimentary record. Figure 3 shows a profile of δ¹³C_{DIC} values in Lake McCarrons taken on 8/9/02. Note the increase of deep water values above intermediate (upper hypolimnion) values: this trend implies microbial methane production in the bottom waters, an indication of severe anoxia.

Sediment analysis

Figure 4 shows results of analysis of the entire laminated and part of the unlaminated portion of the sediment core. These data are compared with historical and climatological data for the area to

investigate the “fit” and utility of the sediment record for reconstructing prehistoric changes. From the data in hand, it is apparent that the lake has undergone cyclic swings in biological productivity over the past century (as shown by variations in the carbon isotopic signature of calcite), and that the δ¹³C_{cc} value is as positive in the most recent sediments as the highest values recorded during the century. Note that the passage of the Clean Water Act in 1972 (which in some other lakes is a significant event in the sedimentary record) appears to have more immediate effect (though still a minor one) on the state of the lake as recorded in the sedimentary components than the installation in 1985 of a detention pond to trap inflowing nutrients. Particular aspects of the sedimentary record are discussed with respect to the historical record below.

Discussion

Response to land use change

Land clearance, agriculture, shoreline development, population growth, and recreational use all contribute to eutrophication of lakes. These impacts are the subject of the considerable lake restoration/management literature (and, to a lesser extent, the paleolimnological literature). Sediment studies' contribution to the goals of management is to give an impression of the state of the lake before European settlement and the lake's subsequent response to known human and climatic factors.

Charles McCarrons was the first major human impact on the lake that bears his name. He arrived in what was then Rose Township in the mid-1850s and began dairy farming on 110 acres on the west end of the lake (Historical Society, 1998), a parcel of land through which the lake's inlet runs. The depth in the core corresponding to this event is estimated based on sedimentation rates; it could easily be pinned down by finding in the sediments dramatic increases in (1) *Ambrosia* (ragweed) pollen, an indicator of land disturbance, or (2) the biomarker molecule coprostanol, an indicator of (bovine) fecal input. The putative settlement horizon is marked by a

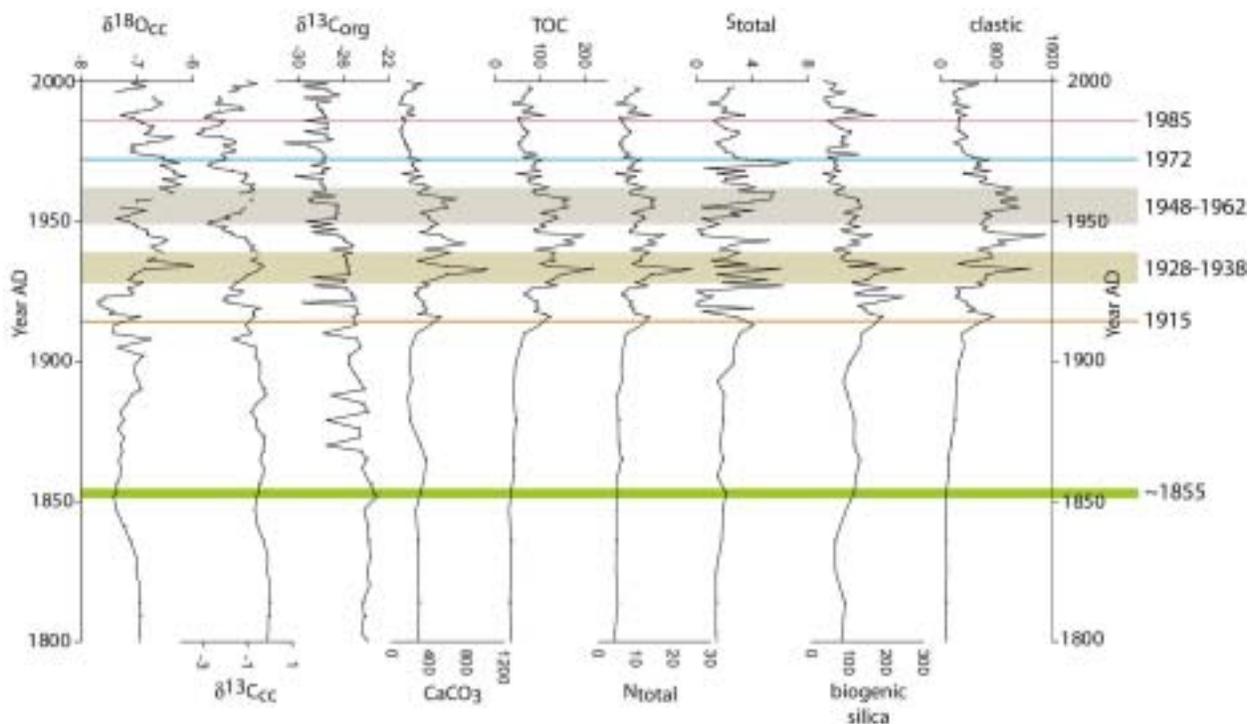


Figure 4. Sedimentary record of Lake McCarrons for the past 200 years. Isotopic values in per mil (VPDB); other components plotted as accumulation rates in grams per square meter per year.

color change (to darker sediments above) but by only minor increases in sediment accumulation rates. A steady increase in accumulation of clastic material (detrital clay and sand) occurs over the next 50 years, confirming that land clearance and development increased erosion in the watershed; local maxima in the accumulation rates of calcite and biogenic silica may be related to fertilization of surface waters by nutrient-rich runoff.

Faint laminations are visible throughout much of the Lake McCarrons core, indicating that bottom waters have been seasonally anoxic for some of the lake's past. By counting back, inception of persistent annual laminations, and by extension permanent bottom-water anoxia, occurred around 1915. Above this horizon, accumulation rate of material overall and of individual sedimentary components becomes much more variable. This phenomenon has been described by Cottingham et al (2000) in a study in a fertilized lake in the Experimental Lakes Area of northwestern Ontario, and is a general trend in many human-impact sites.

The cyclic nature of the calcite isotopic record ($\delta^{18}\text{O}_{\text{cc}}$, $\delta^{13}\text{C}_{\text{cc}}$) is correlated with the cycles of drought in the middle of the 20th century. Typically $\delta^{13}\text{C}_{\text{cc}}$ is interpreted in terms of biological productivity, but covariance between it and $\delta^{18}\text{O}_{\text{cc}}$ is a common feature of many lake sediment records (e.g., Talbot and Kelts 1986, Drummond et al 1995). The mechanism for covariance is not agreed upon, but in temperate lakes it may be that warmer summers and longer growing seasons favor both more biological productivity (increasing $\delta^{13}\text{C}_{\text{cc}}$) and dominance of summer rainfall (enriched in ^{18}O ; Drummond et al 1995) over winter precipitation. In this case, it is summer aridity rather than temperature that is correlated with enriched $\delta^{18}\text{O}_{\text{cc}}$ (see next section and figure 5); it could be that evaporation of lake water leads to enriched $\delta^{18}\text{O}$ and to more highly stratified lake conditions, which are associated with enrichment of calcite in ^{13}C . High accumulation rates of sulfur (indicating stratified, anoxic conditions) during the same periods support this hypothesis.

The $\delta^{13}\text{C}_{\text{org}}$ record presently suffers from an unresolved analytical problem in reproducibility which causes the sawtooth pattern in the core. If certain of the low duplicate values are removed, the record shows a gradual and relatively smooth decrease in $\delta^{13}\text{C}_{\text{org}}$ values over time. A trend toward more negative $\delta^{13}\text{C}_{\text{org}}$ would typically be interpreted in the sedimentary record as a decrease in biological productivity in the lake; however, with the knowledge that Lake McCarrons has become more eutrophic over time, the mechanism described by Hollander and Smith (2001) for Lake Mendota, in a study with some similarities to this one, is more likely. They conclude, based on $\delta^{13}\text{C}$ and biomarker studies, that the depletion in $\delta^{13}\text{C}_{\text{org}}$ over the past century is the result of an increase in the contribution to the organic matter pool of microbial biomass (which is ^{13}C -depleted) relative to phytoplankton. It is certainly true that cyanobacteria are the dominant algal type in Lake McCarrons (Met Council 1997; the strong decrease in accumulation rate of biogenic silica over the past ~60 years may be the result of a shift from diatoms to cyanobacteria as the main phytoplankton); however, these are photosynthetic and take their CO_2 from the same pool as do green algae, so they should have $\delta^{13}\text{C}$ values similar to those of green algae. The microbes that likely contribute ^{13}C -depleted biomass are chemoautotrophs such as sulfate reducers, methanogens, and methanotrophs, which use ^{13}C -depleted organic matter as a carbon source, and which all thrive under strongly stratified conditions with anoxic bottom waters.

The Lake McCarrons record shows a pattern typical of many human-impacted Minnesota lake sites: a broad peak in accumulation rates dating from the 1920s to 1970s (D.R. Engstrom, pers. comm.). This period of high accumulation rates is most pronounced in the records of calcite, organic carbon, and clastic material. The clastic (detrital) fraction especially has shown a stabilization since about 1970, which approximately marks the end of major development both directly surrounding the lake and in the greater watershed. The Clean Water Act could be

credited with the decrease in calcite and organic matter accumulation (as these both increase with productivity), but the similar quiescence in clastic accumulation suggests that local effects, rather than environmental legislation, are the main driver.

Remediation in the form of a detention pond complex installed in 1985 to trap inflowing nutrients shows up in the sediment record as a spike, rather than a low, in indicators of biological productivity. Biogenic silica, organic carbon and calcite all show local peaks after periods of decline or stability. The relationship between cyanobacteria and diatoms noted above is a clue. Nitrogen-fixing cyanobacteria are favored in high-phosphorus lakes; as phosphorus levels decline, as they did the first few years after wetland construction, diatoms can return to dominance. Indeed, in the first Metropolitan Council report (1988, figure 26), diatoms surge as a percentage of phytoplankton biovolume in 1986, 1987 (slightly lower), and 1988, matching very well the three points corresponding to those three years in the sedimentary record.

Response to wet/dry periods

Lakes respond to climate variability most immediately by changes in water level.

Paleolimnological studies routinely use $\delta^{18}\text{O}$ of carbonate minerals as a proxy for moisture balance: more positive values are characteristic of more arid or evaporative systems, while wetter environments typically carry more negative values. Changes within a given lake over time reflect the integration of changes in the source air mass of incident precipitation (which over ~100 years are minor) and its isotopic value plus changes in the ratio of precipitation and inflow to evaporation.

For Lake McCarrons, we are lucky to have both a long historical record of lake level and a nearby rainfall record (data courtesy of the Minnesota Department of Natural Resources and Midwestern Regional Climate Center, respectively) with which to compare sedimentary $\delta^{18}\text{O}$ variations. Figure 5 shows these records for about the past century (smoothed line is a 3-point running average; the scale is reversed for $\delta^{18}\text{O}$ relative to the normal representation in order to better visually compare it with the other records). The three records are generally quite coherent, although there appear to be lags between rainfall and lake level change (likely due to the mediation of lake level by groundwater). Clearly, the isotopic signature picks up the lake-level variations in terms of timing. The drought of the late 1920s-early 30s is a period of very low lake level (~1 m below present level, which would decrease the surface area of the lake by over 10%), and is marked by the most enriched $\delta^{18}\text{O}$ value (-5.8 ‰) of the past 500 years, although not the most enriched average values. The second major drought of the century, which is sustained through the 1950s, has a smaller effect on lake level but appears as a larger positive isotopic excursion. The nonlinear response of $\delta^{18}\text{O}$ is probably due to the aftereffects of the previous drought, which leave the “baseline” $\delta^{18}\text{O}$ (i.e., the value before the second drought) more enriched than the starting value before the first drought (-6.9 ‰ vs. -7.3 ‰). This type of comparison is useful for validating the $\delta^{18}\text{O}$ record for use further back in the sedimentary record, e.g., for understanding that the $\delta^{18}\text{O}$ system displays the cumulative effect of water balance variations, rather than bearing a one-to-one correspondence with lake level or incident precipitation.

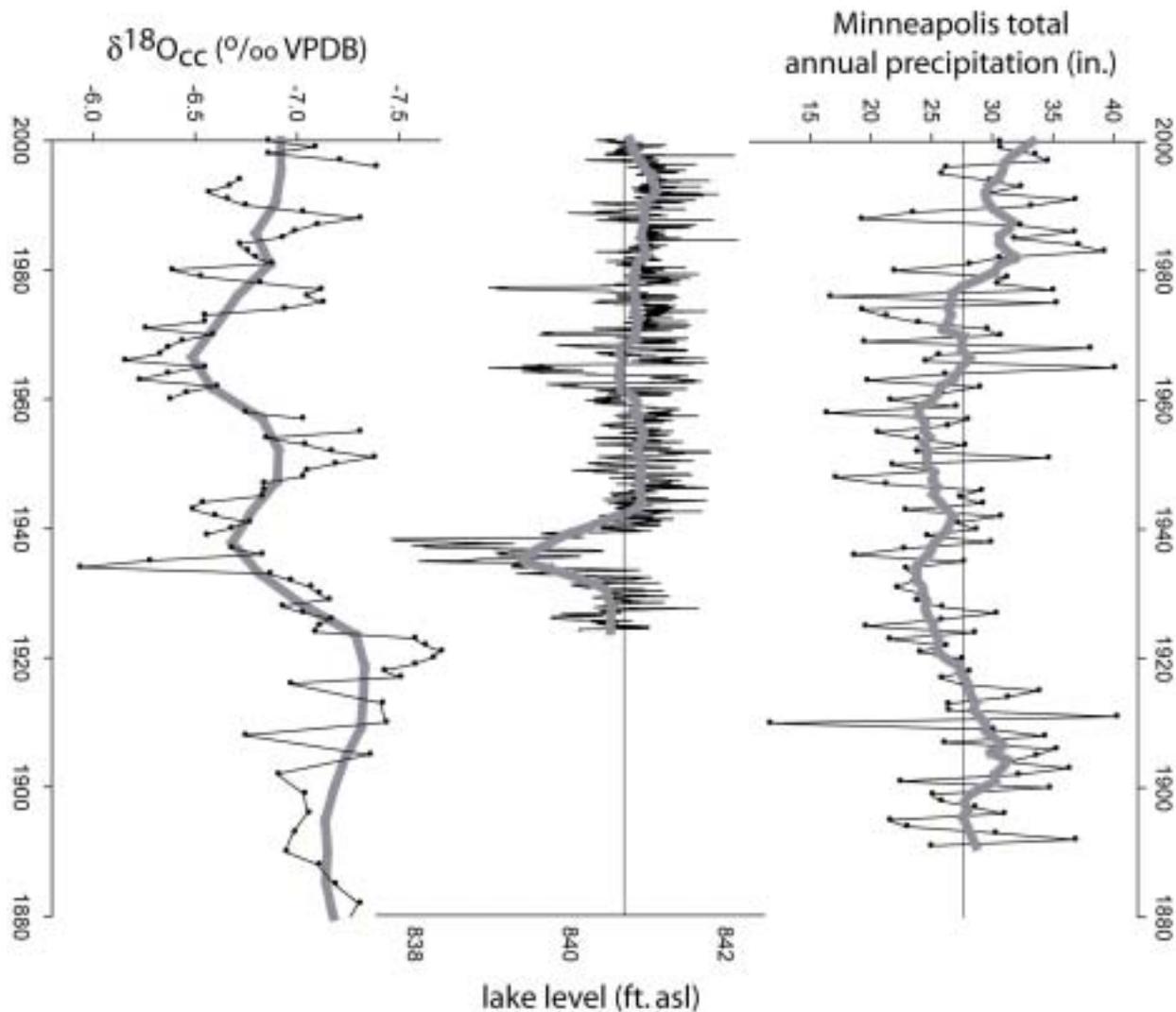


Figure 5. Comparison of oxygen stable isotopic values, lake level, and precipitation record.

Utility of sediment studies for urban lake history reconstruction

As this study makes apparent, it is easier to determine what caused the changes seen in the sedimentary record when one already knows what happened in the lake's history. The ambiguities inherent in isotopic responses and the significance of changes in chemistry and in dominance between algal groups require multiple lines of evidence to clarify. However, studies that have the luxury of drawing on historical records are also, conversely, valuable for determining what occurred in the lake watershed with finer resolution than is available from historical records. Comparison of the human-impact period of the lake's history with the longer record (in this report, 200 years; the poster presentation referenced below includes ~500 years of history) may help to clarify the goals of lake management, in order to determine what parameters have actually changed under cultural eutrophication.

Supplementary information

Related grant proposals

No additional funds have been requested for continuation or expansion of this project; however, I have discussed with personnel at the St. Croix Watershed Research Station (SCWRS) the possibility of conducting a diatom-inferred water quality study using the same core(s) as used in this project. Dr. Mark Edlund, a diatom expert at SCWRS, has looked at a number of smear slides from the laminated section of the Lake McCarrons core, and was impressed both by the preservation of diatoms in the sediment and by the dramatic floristic changes recorded over time. A diatom study would provide semiquantitative estimates of pH and concentrations of phosphorus and chloride in the lake over time; these data are difficult or impossible to obtain from any other type of analysis (and would replace the budgeted sediment P measurements). A grant funding this type of study might support a Master's student; alternately, the cost of preparation and counting of a sufficient number of samples (if simply handled as a contract by SCWRS, for example) is approximately \$6000. An evaluation of the value of a diatom study is underway.

Student training

Amy Myrbo, the Geology and Geophysics Ph.D. candidate who conceived of and wrote the original proposal, is solely responsible for managing and completing the project. Two undergraduates, Chris Merkes, a University of Minnesota genetics major, and Aimee Wendt, a St. Cloud State geology major who worked as an intern in the Limnological Research Center during the summer of 2002, have also been significantly involved in both field and laboratory work, and Aimee presented a poster on the project at an intern poster session in the geology department in August 2002.

Outreach

In addition to this WRRI final report, the primary researcher would like to give a presentation of results to lake residents and any other interested parties, and to prepare a "fact sheet," a one-page summary of the project for the public. Data from the project are also available to local agencies and researchers, such as the Metropolitan Council and Dr. Heinz Stefan of the University's Civil Engineering department, who have studied Lake McCarrons for some time.

Publications/presentations to date

LiMNology Retreat February 2001 presentation: "Sedimentary-Historical Context of Eutrophication and Remediation in Urban Lake McCarrons: would your granny care?";

Poster presentation at 3rd International Limnogeology Congress (ILIC3), Tucson, March 2003, "Sedimentary-Historical Context of Eutrophication and Remediation in Urban Lake McCarrons." Available as Adobe pdf at:

http://www.myrbo.com/amy/carbon/McC_ILIC3.pdf

Informal presentation on thesis research, of which this project is a major component, at evening Quaternary Paleoecology seminar, April 2001

A paper with a scope similar to this report is in preparation for submission to the journal *Lake and Watershed Management*.

References

- Cottingham, K. L., J. A. Rusak, and P. R. Leavitt 2000. Increased ecosystem variability and reduced predictability following fertilisation: Evidence from palaeolimnology. *Ecology Letters* 3: 340-348.
- Drummond C. N., Patterson W. P., and Walker J. C. G. (1995) Climatic forcing of carbon-oxygen covariance in temperate-region marl lakes. *Geology* 23, 1031-1034.
- Engstrom, D. R. and H. E. Wright 1984. Chemical stratigraphy of lake sediments as a record of environmental change. In Haworth, E. Y. and J. W. G. Lund (eds.), *Lake Sediments and Environmental History*. Leicester University Press, Leicester: 11-68.
- Hollander, D. J. and M. A. Smith 2001. Microbially mediated carbon cycling as a control on the $\delta^{13}\text{C}$ of sedimentary carbon in eutrophic Lake Mendota (USA): new models for interpreting isotopic excursions in the sedimentary record, *Geochimica et Cosmochimica Acta* 65(23): 4321-4337.
- Metropolitan Council of the Twin Cities Area. Lake McCarrons wetland treatment system: Final report on the function of the wetland treatment system and the impacts on Lake McCarrons. 1988. St. Paul, Minnesota.
- Metropolitan Council Environmental Services. Lake McCarrons wetland treatment system – Phase III study report. 1997. St. Paul, Minnesota.
- Roseville Historical Society. *Roseville, Minnesota: The Story of its Growth. Part Two*. Edinbrough Press, Roseville, MN, 1998.
- Schueler, T. R. 1999. Return to Lake McCarrons: does the performance of wetlands hold up over time? Technical Note 102. *Watershed Protection Techniques* 3(1), 597-600.
- Talbot, M. R. and K. R. Kelts 1986. Primary and diagenetic carbonates in the anoxic sediments of Lake Bosumtwi, Ghana. *Geology* 14, 912-916.
- West-Mack, D. E., and Heinz G. Stefan. Simulation of Water Quality and Primary Productivity Control Strategies for Lake McCarrons. University of Minnesota St Anthony Falls Laboratory Project Report No. 426. 2000a. Minneapolis, Minnesota.
- . Inflow Dynamics and Potential Water Quality Improvement in Lake McCarrons. University of Minnesota St Anthony Falls Laboratory Project Report No. 448. 2000b. Minneapolis, Minnesota.

Information Transfer Program

The WRC did not have any funded information transfer projects using WRI funds during 2002, but the Center has a very active outreach program. It produces a quarterly newsletter, Minnegram, that is widely distributed to water resources professionals throughout the state, it organizes numerous workshops and sponsored and organized its biennial state water conference, Minnesota Water 2002 during the reporting period. It publishes a variety of technical reports, as well as fact sheets and brochures, and the Center maintains four separate Web sites for its various programs.

Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	0	0	0	0	0
Masters	3	2	1	0	6
Ph.D.	2	0	0	0	2
Post-Doc.	0	0	0	0	0
Total	5	2	1	0	8

Notable Awards and Achievements

Publications from Prior Projects

- 2000MN5B ("Feasibility of Controlled Drainage for Mitigating Nutrient Loss from Tile Drainage Systems in South Central Minnesota") - Articles in Refereed Scientific Journals - Feyereisen, G. F., G. R. Sands, and B. Hansen. 2001. A Low Head, Low Power System For Continuous Flow Measurement. *Applied Engineering in Agriculture* 18(3): 307-310.
- 2000MN6B ("Assessing the Effects of Endocrine Disrupters (EDCs) from a St. Paul Sewage Treatment Plant on Sperm Viability and Testicular Development in Fish: Adding a New Dimension to an Existing Project") - Articles in Refereed Scientific Journals - Schoenfuss, H. L., J. T. Levitt, G. Van Der Kraak, and P. W. Sorensen. 2002. Ten week exposure to treated sewage effluent discharge has small, variable effects on reproductive behavior and sperm production in goldfish. *Environmental Toxicology & Chemistry* 21(10): 2185-2190.
- 2000MN6B ("Assessing the Effects of Endocrine Disrupters (EDCs) from a St. Paul Sewage Treatment Plant on Sperm Viability and Testicular Development in Fish: Adding a New Dimension to an Existing Project") - Water Resources Research Institute Reports - Sorensen, P.W., H.L. Schoenfuss, I.R. Adelman, D.L. Swackhamer. 2002. Assessing the effects of endocrine disrupters (EDCs) from a St. Paul sewage treatment plant on sperm viability and testicular development in fish: adding a new dimension to an existing project. *Minnesota Water Resources Center Technical Report* 142:35-37.
- 2000MN6B ("Assessing the Effects of Endocrine Disrupters (EDCs) from a St. Paul Sewage Treatment Plant on Sperm Viability and Testicular Development in Fish: Adding a New Dimension to an Existing Project") - Articles in Refereed Scientific Journals - Schoenfuss, H.L., Martinovic, D., and Sorensen, PW. 2001. Effects of exposure to low levels of water-borne 17b-estradiol on nest holding ability and sperm quality in fathead minnows. *Water Resources Update* 120: 49-55.
- 2000MN7B ("Investigation of Factors Affecting the Removal of *Cryptosporidium parvum* Oocysts in Porous Media Filters") - Articles in Refereed Scientific Journals - Dai, X. and Hozalski, R.M. (2003). Evaluation of Microspheres as Surrogates for *Cryptosporidium parvum* Oocysts in Filtration

- Experiments. *Environmental Science and Technology*, 37:5:1037-1042.
6. 2000MN7B ("Investigation of Factors Affecting the Removal of *Cryptosporidium parvum* Oocysts in Porous Media Filters") - Articles in Refereed Scientific Journals - Dai, X. and Hozalski, R.M. (2002). Effect of NOM and Biofilm on the Removal of *Cryptosporidium parvum* Oocysts in Rapid Filters. *Water Research*, 36:3523-3532
 7. 2000MN4B ("Investigation of the Abiotic Reduction of the Herbicides Trifluralin and Pendimethalin") - Articles in Refereed Scientific Journals - Wang, S. and Arnold, W.A., 2003. Abiotic reduction of dinitroaniline herbicides. *Water Research*, in press
 8. 2000MN3B ("Evaluation of Bank Erosion Inputs to the Blue Earth River with Airborne Laser Scanner") - Conference Proceedings - Thoma, D.P., S.C. Gupta, and M.E. Bauer. 2001. Quantifying riverbank erosion with scanning laser altimetry. In. *International Archives of Photogrammetry and Remote Sensing*, Vol. XXXIV-3/W4 Annapolis, MD, 22-24 October 2001