

**Water Resources Center
Annual Technical Report
FY 2006**

Introduction

The Minnesota WRRI program is a component of the University of Minnesota's Water Resources Center (WRC). The WRC is a collaborative enterprise involving several colleges across the University, including the College of Food, Agriculture and Natural Resource Sciences (CFANS), University of Minnesota Extension, Minnesota Agricultural Experiment Station (MAES) and the University of Minnesota Graduate School. The WRC reports to the Dean of CFANS. In addition to its research and outreach programs, the WRC is also home to the Water Resources Sciences graduate major. The WRC has three co-directors, Professor Deborah Swackhamer, Professor James Anderson, and Faye Sleeper who share the activities and responsibilities of administering its programs. Faye Sleeper was hired in April and comes from the State of Minnesota's Pollution Control Agency with extensive experience in surface water management.

The WRC funds 3-4 research projects each year, and the summaries of the current projects are found in the rest of this report.

Research Program

Assessing the Ecotoxicology of Alkylphenol Mixtures Across the Aquatic Food Chain

Basic Information

Title:	Assessing the Ecotoxicology of Alkylphenol Mixtures Across the Aquatic Food Chain
Project Number:	2005MN147G
Start Date:	9/1/2005
End Date:	8/31/2007
Funding Source:	104G
Congressional District:	6
Research Category:	Not Applicable
Focus Category:	Water Quality, Toxic Substances, Ecology
Descriptors:	
Principal Investigators:	Heiko L Schoenfuss, Larry B Barber, Matthew Julius

Publication

1. Bistodeau, T.J., L.B. Barber, S.E. Bartell, R.A. Cede, K.J. Grove, J. Klaustermeier, J.C. Woodard, K.E. Lee and H.L. Schoenfuss. 2006. Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. *Aquatic Toxicology* 79: 268-277.
2. Julius, M.L., J. Stepanek, O. Tedrow, C. Gamble and H.L. Schoenfuss. Estrogen -receptor independent effects of two ubiquitous environmental estrogens on *Melosira varians* Agardh, a common component of the aquatic primary producer community. In preparation.

Assessing the Ecotoxicology of Alkylphenol Mixtures Across the Aquatic Food Chain

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Start Date: 9/1/2005

End Date: 8/31/2007

Abstract

Among estrogenic endocrine disrupting compounds, alkylphenolic surfactants stand out due to their ubiquitous presence in anthropogenically altered surface waters and their occurrence in complex mixtures. Although the parent compounds (nonylphenol and octylphenol) of most alkylphenol polyethoxylates are orders of magnitude less estrogenic than 17 β -estradiol, they are also found in concentrations orders of magnitude greater than the natural estrogen in many treated wastewater effluents and receiving streams and rivers. In addition, the longer-chained alkylphenol polyethoxylates are altering the bioavailability of nonylphenol and octylphenol, thus potentially facilitating the uptake of the more potent parent compounds by aquatic organisms exposed to the alkylphenol mixtures. Furthermore, the chemical nature of surfactant raises the specter that organisms at different levels of the trophic cascade may experience differential effects that may be estrogen receptor independent (diatoms) or estrogen receptor mediated (daphnia and fathead minnow). As a consequence, we proposed to test the effects of an alkylphenol polyethoxylate mixture, realistic in composition and concentration, on three tiers of an abbreviated aquatic food chain: the primary producer community (diatoms); a primary consumer (*Daphnia magna*); and a secondary consumer (fathead minnow, *Pimephales*

promelas). Once we have documented the effects of alkylphenolic mixtures on each level of this aquatic food-chain, we proposed to test food-chain effects of exposure by feeding exposed diatoms to daphnia or larval fathead minnows. We have completed our diatom and fathead minnow exposures to the alkylphenolic mixture and are in the progress of exposing daphnia magna to alkylphenols. We have also developed and tested food-chain experimental protocols that will allow us to produce food pellets of exposed diatoms that can be fed to unexposed *D. magna* or larval fathead minnows. Our findings from the single organism mixture exposure experiments indicate a degrading effects of alkylphenols on the primary producer community, especially on diatoms which represent the preferred food source of many larval and fingerling fishes. In addition, we have found that diatoms are more sensitive to alkylphenolic compounds than they are to the potent estrogen 17 β -estradiol suggesting that the effects of alkylphenolic compounds might disrupt receptor independent pathways at subsequent tiers of the trophic cascade (Julius et al. In preparation). As a consequence of the diatom exposure, the nutritional value of diatoms for *D. magna* and larval fathead minnows was greatly diminished. We have also established that alkylphenol mixtures have a more potent effect on fathead minnows than the parent compound nonylphenol alone (Bistodeau et al. 2006). We are currently finalizing the food chain exposure experiments and are well underway to complete all objectives stated in the original grant proposal.

Introduction & Research Objectives

Endocrine disrupting compounds have been detected in many anthropogenically altered surface waters in North America (Kolpin et al. 2002, EST 36: 1202-1211), and Europe (Desbrow 1998, EST 32: 1549-1558). Several classes of endocrine disrupting compounds are usually recognized, including natural/synthetic hormones (estrone, estradiol, ethynylestradiol), personal care products (i.e., the antimicrobial soap ingredient Triclosan) and alkylphenolic surfactants. The latter have been found almost ubiquitously in anthropogenically altered surface waters in part because their use is inherently water related. Alkylphenols are a group of compounds used in large quantities as industrial and household surfactants and have been found to be estrogenic (Hemmer et al. 2001, ETC 20:336-343). Alkylphenols are known to bind to the estrogen receptor of mammalian cells and disrupt the homeostasis of the internal milieu of the organism. Environmental estrogens such as alkylphenols are known to disrupt normal endocrine hormone that are central to maturation and reproduction in fishes, and the ubiquitous presence of these biologically active compounds in surface waters should be of environmental and human health concern. To date, alkylphenol studies have focused on 4-nonylphenol, the metabolic product of both aerobic and anaerobic microbial degradation of higher-chained alkylphenols and the US EPA has recently proposed effluent emissions criteria for this compound. However, mixtures of nonylphenol and higher chained alkylphenols are found routinely in effluents and their combined action is entirely unknown. In this study, we propose to examine the effects of alkylphenol mixtures on three tiers of the aquatic food chain: the primary producer community (diatoms), a primary consumer (*Daphnia magna*), and a vertebrate near the top of the food chain (the fathead minnow). We furthermore will link all three tiers through feeding trials to examine the effects of alkylphenol mixtures on the aquatic food chain. The present study proposes three objectives to

determine the relationship between alkylphenol contamination of surface waters and adverse organismal effects. These are (1) determine the effects of alkylphenol exposure on the reproductive success of three tiers of the aquatic food chain; (2) determine the impacts of alkylphenol mixtures across the food chain; and (3) to test the three assays at a field site known to discharge alkylphenols.

To date we have completed the fish and diatom exposures with alkylphenol mixtures. We have been able to demonstrate that the combined effects of alkylphenols exceeds that of individual alkylphenols in the fathead minnow (Bistodeau et al. 2006) and have also established that diatoms serve as sensitive indicators of biological disruption caused by the presence of alkylphenol mixtures (Julius et al. In preparation). Furthermore, we have established protocols for the *Daphnia magna* exposure and are currently exposing these organisms as an intermediate level in the trophic cascade. Finally, we have developed an experimental design to link all three tiers of our abbreviated food chain through pelleting large quantities of alkylphenol exposed diatoms for larval fathead minnow feeding. In summary, we are well underway to complete all proposed components of the National Institute of Water Resources funded study.

Methodology & Preliminary Findings

We have completed several rounds of diatom exposures (*M. varians*) to graded concentrations of 4-nonylphenol (NP) singularly and to mixtures of alkylphenolic compounds (Table 1) including NP, nonylphenol-1-ethoxylate (NP1EO), nonylphenol-2-ethoxylate (NP2EO), nonylphenol-1-carboxylate (NP1EC), and nonylphenol-2-carboxylate (NP2EC). In addition, we exposed diatoms to 17 β -estradiol, a compound with known endocrine disrupting activity that served as a reference exposure for this study.

Table 1: Concentrations used in *M. varians* exposures for 4-nonylphenol (4-NP), 17 β -estradiol (E2) and the alkylphenol mixture (AP).

Treatment	Low Exposure Concentration (mg/L)	Medium Exposure Concentration (mg/L)	High Exposure Concentration (mg/L)
17 β estradiol	3	30	300
4-nonylphenol	2	20	200
Alkylphenol mixture*	74.5	373	746

Table 2: Alkylphenol compounds detected in the Metropolitan treated wastewater effluent (St. Paul, MN) and their environmental concentrations, used for determining experimental dose values.

Compound	Concentration (mg/L)
NP	2.11
NP1EO	3.536
NP2EO	6.987
NP1EC	25.201
NP2EC	33.618
SUM	71.5

For the diatom exposures, monocultures of *Melosira varians* were grown in sterile WC media, then exposed to pre-determined test chemical concentrations and incubated in diurnal growth chambers with a 12:12 light:dark cycle for a period of ten days. Procedures were as follows:

100 ml of homogenized culture aliquots were added to 900 ml of sterile media and allowed a period > 24 hours to acclimate. Due to its affinity for binding to glass, sterile polystyrene cell tissue rollers were used in the case of the 4-nonylphenol exposures, and 2 liter glass Florence flasks were used for the estradiol trials. Treatments consisted of control, low,

medium and high exposure concentrations. Once treated, samples were taken on day one for cell count and chlorophyll-a analysis. Exposed cultures were then allowed to grow for a period of ten days, after which samples were once again obtained for chlorophyll-a and cell count. The chlorophyll-a content of the cells was measured using a fluorometer and averages for each treatment group were determined in order to quantitatively assess diatom health. Elevated chlorophyll A tissue concentration indicate a stress-effect caused by the exposure. In three exposure experiments using graded series of 17 β -estradiol (experiment 1: 2 μ g/L, 20 μ g/L, 200 μ g/L; experiment 2: 4 μ g/L, 40 μ g/L, 400 μ g/L; experiment 3: 8 μ g/L, 80 μ g/L, 800 μ g/L) treatments at or above 80 μ g/L consistently found statistically significant (one way ANOVA, $p < 0.05$) increases in chlorophyll A: lipid ratio indicating a reduced nutritional value of diatoms for higher levels of the trophic cascade. Clearly these 17 β -estradiol concentrations are well beyond environmentally relevant concentrations and indicate that 17 β -estradiol does not adversely affect exposed diatoms. In contrast, three exposure experiments using series of alkylphenols (experiment 1: 2 μ g/L, 20 μ g/L, 200 μ g/L; experiment 2: 4 μ g/L, 40 μ g/L, 400 μ g/L; experiment 3: 8 μ g/L, 80 μ g/L, 800 μ g/L) found statistically significant (one-way ANOVA, $p < 0.05$) increases in the Chlorophyll A ; lipid ratio at and above 40 μ g/L. This concentration of total alkylphenols has been exceeded in many treated wastewater effluents and indicates that the primary producer community is likely adversely affected by environmental concentrations of alkylphenols.

In addition to the diatom exposures, we have also completed the fathead minnow exposures to a mixture of several alkylphenolic compounds (NP, NP1EO, NP2EO, NP1EC, NP2EC) in mixture or to nonylphenol alone at the effluent measured concentration (similar to the concentration in the alkylphenol mixture - Table 2). Our results indicate that reproductive competence is impaired in male fathead minnows that were exposed to the mixture for 28 days at

realistic concentrations (Fisher's Exact test; $p < 0.05$). In addition, secondary sexual characters and the gonadosomatic index are significantly reduced when compared to control males (Student t-test, $p < 0.05$). Even at a mixture concentration representing 50% the mixture concentration measured in the treated effluent, reproductive competence was significantly reduced. In contrast, nonylphenol alone had an excitatory effect on nest holding ability (Fisher's Exact test, $p < 0.05$) that is likely the result of a priming effect of the low-concentration estrogenic compound. Detail results of the fathead minnow exposures are published in Bistodeau et al. (2006).

Publications, Presentations, or Published Abstracts:

Publications

Bistodeau, T.J., L.B. Barber, S.E. Bartell, R.A. Cedie, K.J. Grove, J. Klaustermeier, J.C. Woodard, K.E. Lee and H.L. Schoenfuss. 2006. Larval exposure to environmentally relevant mixtures of alkylphenolethoxylates reduces reproductive competence in male fathead minnows. *Aquatic Toxicology* 79: 268-277.

Julius, M.L., Stepanek, J., Tedrow, O., Gamble, C. and H.L. Schoenfuss. Estrogen -receptor independent effects of two ubiquitous environmental estrogens on *Melosira varians* Agardh, a common component of the aquatic primary producer community. In preparation.

Presentations (* indicates student presentation)

Schoenfuss, HL and TJ Bistodeau. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Gable, C*, A. Gikineh and ML Julius. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Allen, AK*, T Loes and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Grove, KJ*, RA Cediell and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Koch, JK*, M Minger and HL Schoenfuss. 2006 Midwest SETAC Meeting, St. Cloud, MN March 20-22, 2006.

Schoenfuss, HL, Bistodeau, TJ 2006 Minnesota Water, Brooklyn Park, MN, October.

Schoenfuss, HL, Bistodeau, TJ, Society for Environmental Toxicology and Chemistry, Montreal, Canada, November 2006.

Student(s) supported by this project:

Kent Grove (MS expected Fall 2007)

Jason Koch (MS expected Spring 2007)

Nathan Jahns (MS expected Spring 2008)

Roberto Cediell (MS expected Summer 2007)

Carolyn Gamble (MS expected Fall 2007)

Angela Allen (undergraduate project Summer 2006)

Tim Loes (undergraduate project Summer 2006)

Bradley Sivanich (undergraduate project Summer 2007)

Josh Stepanek (undergraduate project academic year 2006-07)

Awards

None to date

Factors Affecting Revegetation Success in Lakeshore Restorations

Basic Information

Title:	Factors Affecting Revegetation Success in Lakeshore Restorations
Project Number:	2006MN153B
Start Date:	3/1/2006
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Funding Source:	104B
Congressional District:	4
Research Category:	Biological Sciences
Focus Category:	Ecology, Management and Planning, Wetlands
Descriptors:	
Principal Investigators:	Susan M. Galatowitsch

Publication

Factors Affecting Revegetation Success in Lakeshore Restorations

Principal Investigator

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Research Assistant

Dana Vanderbosch, Graduate Program in Water Resources Science, UMN

Start Date: 3/1/2006

End Date: 2/29/2008

Abstract

Revegetating aquatic zones is crucial to the overall success of lakeshore restoration since improving fish habitat and reducing shoreline wave impacts depend on the development of emergent beds. Currently, there is little understanding of why most aquatic plantings fail. A lack of predictability stems, in part, from a lack of knowledge of the effects of planting time, water depth, root/rhizome development on the survival of emergent aquatic transplants. We are investigating the factors affecting the establishment of *Scirpus validus* (soft-stem bulrush), the most commonly used species in lakeshore restorations. This study will provide the essential data needed to determine:

1) which factors are most critical to maximizing bulrush transplanting survival and post-planting expansion; 2) which combinations of factors are likely to result in a very high chance of revegetation failure; 3) the relative importance of planting techniques and lake site choice to transplant survival and post-planting expansion, and 4) the quantitative differences in survival that will likely result from different restoration decisions (e.g., what is the probability in survival for May vs. July transplants). After the first of two field seasons of study, our preliminary observations indicate several factors are particularly important for limiting bulrush stand establishment. In general, initial bulrush mortality is highest in shallow water because of direct and indirect effects of wave

impacts. Plants installed as mats are more prone to wave impacts in these shallow areas than are those installed in pots, perhaps because of differences in root development. Establishment success is greatest in mid-summer, most likely stemming from differences in the size and quality of plants received from the nursery rather differences encountered at planting sites. In spite of protecting all plantings from herbivores, muskrat herbivory also limited bulrush establish at every site. In 2007, these experimental beds will continue to be monitored to determine over-winter mortality and first-year stand establishment. This information will be used by Minnesota Department of Natural Resources, who oversees the statewide lakeshore restoration program, watershed management districts who frequently partner with DNR to pursue these restorations, and private landowners (including sportsmen's groups) who are increasingly interested in improving lakeshore quality.

Introduction

Removing native vegetation mechanically or chemically when lakeshores are developed can have adverse consequences for entire lake ecosystems. These consequences include loss of fish and wildlife habitat, shoreline erosion and water quality degradation. Many states, including Wisconsin and Minnesota, have been actively advocating shoreland restoration for the past decade; however, there has been no formal evaluation of sites that have been restored. Further, very little has been published on restoration that incorporates the upland, shoreline and shallow water areas of the shoreland area, which adds to the difficulty of proper planning. Some of the most problematic issues seem to be selection and availability of appropriate plant species,

selection of appropriate planting practices (i.e., water depth and planting season), and maintenance of the restoration to ensure control of invasive plant species.

A 2005 survey of 24 shoreland restorations in Minnesota's Twin Cities Metropolitan Area showed that the success rates of plantings in the aquatic zone are much lower than those above the ordinary high water line, in the riparian zone (Vanderbosch and Galatowitsch, unpublished report). Revegetating aquatic zones is crucial to the overall success of lakeshore restoration since improving fish habitat and reducing shoreline wave impacts depend on the development of emergent beds. Currently, there is little understanding of why most aquatic plantings fail while a few succeed. This lack of predictability stems, in part, from a lack of knowledge of the effects of planting time, water depth, root/rhizome development on the survival of emergent aquatic transplants. Information on each of these factors and how they interact to either hinder or facilitate plant establishment is needed.

In January 2006, we initiated a research study designed to determine factors that affect transplanting of *Scirpus validus* (soft-stem bulrush, hereafter bulrush). Our goals are to investigate: 1) if survival and establishment are affected by time of transplanting, 2) whether or not the depth and overall development of the rhizomes/roots affect transplant survival and spread, 3) the effect of water depth on transplant survival and initial vegetative spread, and 4) whether or not there is an optimal combination of factors including time, water depth and rhizome/root development for establishment of bulrush.

Study Site Selection

We began the process of site selection by approaching local governmental units who had implemented shoreland restoration projects in public parks in past years, and were located within the 7-county Twin Cities Metropolitan area (e.g. includes Ramsey, Hennepin, Dakota, Washington, Scott, Carver and Anoka counties). Through this interviewing process, we identified twenty-six lakes with public parkland that had potential as research sites. From this initial pool, five lakes were ultimately included in our study. All are located on public land; two are located in Ramsey County and three are located in Hennepin County. The sites are listed in Table 1.

Table 1. Soft-stemmed bulrush (*Scirpus validus*) was planted on research sites on each of the five lakes shown below to determine factors that affect the establishment of emergent vegetation.

Site Locations	County
Snail Lake	Ramsey
Island Lake	Ramsey
Lake Sweeney	Hennepin
Lake Calhoun	Hennepin
Lake Harriet	Hennepin

Plants to be used for the experiment were germinated from seed collected by Minnesota Department of Natural resources staff from bulrush on lakes and streams within Minnesota, and grown at the Ramsey County Correctional Facility. The plants used for this study were grown to a 1-gallon pot size and also grown into an 11" x 11" coconut fiber degradable mat. Three to four seedlings were grown into each 1-gallon pot. The mats contained five seedlings. Plants were at least eight weeks old upon planting.

Experimental Design

Each research site was split into two adjacent plots running parallel to the shore; each stratified by lake bottom elevation. The shallow water zone was defined as 0-32 cm below the ordinary high water level (OHWL); the deep-water zone was 33-70 cm below OHWL. We used the water level in May 2006 as a baseline. Treatments within each block consisted of ten combinations of two root zone sizes (1-gallon-pot and 11" x 11" pre-vegetated mat) and five planting dates.

Seasonal plantings occurred once per month between May and September 2006. Each plot was split into five smaller subplots, one of which was randomly selected each month. Within a given subplot, the two root zone sizes were randomly planted 18 inches apart in rows; rows were staggered. The 1-gallon pots were planted in the lake substrate. The pre-vegetated mats were placed on the lake bottom and staked securely (Figures 1 and 2).

Four feet high steel fencing with a 3" by 3" mesh size was installed around each site immediately after planting to discourage muskrats (*Ondatra zibethicus*). A trapper was also hired to remove muskrats after herbivory damage was observed on selected plots.

Data Collection

The condition of each plant was assessed and classified at planting time using three different measurements to estimate its robustness: shoot count, the height of the three tallest stems of the clump, and a rating of the overall condition of the clump. After recording the assessment of each plant, it was randomly placed within the appropriate seasonal subplot and its position was mapped.

We estimated survivorship and classified the emergent growth twice since planting: once 30 days after planting, and a second time just prior to winter. A third data collection period is planned for July and August 2006 to estimate winter survival of plants.

Results

The following results represent our qualitative assessment of the study. A statistical analysis of the data collected will be pursued in the coming year.

Our field observations indicate that bulrush planted in the deeper water zone (33-70 cm) established more reliably than did bulrush planted in shallow water. Often, bulrush planted in less than 32 cm of water was completely covered by sand and aquatic vegetation (*Myriophyllum spicatum* and *Potamogeton crispus*) washing onto the shore. Also, plants in the shallow water zone were more frequently physically damaged by wave impacts than plants further from the shore, in deeper water. In this shallow water zone, prevegetated mats appeared more vulnerable than potted plants to wave impacts because they were more readily stripped from the lake substrate despite being staked to anchor them to the lake bottom.

Overall, potted bulrush seemed to establish more reliably than did bulrush grown in prevegetated mats. The most prevalent problem was that wave action pulled even staked mats from the lake bottom, as mentioned previously. This was less of a problem in deeper water, but some mats did disappear even from the deeper water zone indicating that wave action broke the mats apart. In addition, the root systems of plants grown into the mats were often not robust. The mats often broke apart in our hands as we removed

them from planting containers and it was easy to see that the small plants were only loosely adhered to the mat. Potted plants often lacked robust root systems, too, but it was possible to anchor them into the lake substrate since roots had more vertical development. Conversely, plants that were loosely attached to the mat could not be easily adhered to the lake bottom, even on mucky soils.

Initial impressions of the study plots indicate that June and July plantings established best and were more robust than plots that were planted in May, August and September. Reasons why those plantings likely established best include lower water levels and a decreased chance of inundation, maximum hours of sunlight for growth, and robust stock. Stock received from the supplier in May seemed underdeveloped, leading us to conjecture that greenhouse growing conditions in early spring (cool outdoor temperatures and relatively short daylight hours) limit a grower's ability to produce high quality stock with robust root systems for May plantings. Similarly, stock produced for August and September plantings is subject to less than optimal growing conditions. The stock intended for the August planting for this project experienced a severe setback when the growers watering system malfunctioned leaving plants unwatered for three days during a period when outdoor temperatures rose well over 90 degrees Fahrenheit. The September stock, though in fairly good condition, did not seem very robust. The grower believes that by late summer, decreasing daylight impacts greenhouse growing conditions.

In spite of metal fencing enclosing each subplot, muskrat herbivory is a significant mortality factor for aquatic plantings (Figure 3). Herbivory occurred on plots on two lakes in early summer. Trapping slowed the herbivory and no further damage was

observed on the untouched subplots or on other lakes until September 2006, after a cold snap that lasted for two weeks. By November 2006, muskrat herbivory was observed on ~75% of all subplots and on every lake included in the study (Figure 4). In some cases, the damage was minor; in other cases, entire subplots were sheared off at the water level. An examination of the fencing revealed that muskrats had bitten through the fencing on subplots on two lakes. Trapping will continue throughout the winter months to attempt to protect remaining dormant bulrush.

Publications, Presentations, or Published Abstracts:

None to date

Student(s) supported by this project:

Name: Dana Vanderbosch

Program: Water Resources Science

Degree being sought: Master of Science

Awards

None to date



Figure 1. Planting *Scirpus validus* at Lake Sweeney, Hennepin County. Each plant received a unique identification number. The position of each plant was randomized and its location within the plot was recorded on the wooden lathe stakes and on a master plan so that each plant's mortality can be tracked.



Figure 2. A one-gallon pot of *Scirpus validus* about to be planted at Lake Calhoun, Hennepin County.



Figure 3. Muskrats typically nipped bulrush stems off at water level and ate some stems while leaving a majority of the clipped stems floating on the lake surface.



Figure 4. A plot of *Scirpus validus* planted in July 2006 at Island Lake, Ramsey County. Stakes marking bulrush that no longer exist are a common site at Island Lake, which suffered heavy losses from muskrat herbivory.

Ecological Stoichiometry and Microbial Biodiversity Effects on Water Quality in Minnesota Lakes

Basic Information

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Project Number:	2006MN155B
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End Date:	2/29/2008
Funding Source:	104B
Congressional District:	5
Research Category:	Water Quality
Focus Category:	Surface Water, Ecology, Water Quality
Descriptors:	
Principal Investigators:	James B Cotner, Timothy Michael LaPara

Publication

Ecological Stoichiometry and the Relevance of Prokaryotic Heterotroph Biodiversity

Principal Investigator

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Timothy M. LaPara, Associate Professor and co-PI, Department of Civil Engineering, UMN

Research Assistants

Audrey Wiley and Kara Holtzmilller, Undergraduates Students, UMN

Start Date: 3/1/2006

End Date: 2/29/2008

Abstract

Prokaryotic heterotrophs are extremely abundant and have large impacts on global biogeochemistry and ecosystem processes such as nutrient regeneration and productivity (Cotner and Biddanda 2002). Ecological stoichiometry examines the balance of energy and chemical elements in living systems (Sterner and Elser 2002). In the work discussed here, the importance of microbial diversity and ecological stoichiometry to biogeochemical processes is being examined in aquatic systems.

Microbial stoichiometry and diversity interact to affect nutrient regeneration; stable interactions are promoted when decomposers are limited by organic carbon and the stoichiometry of decomposers is similar to that of autotrophs. Furthermore, biodiversity promotes redundancy and reliability in ecosystem function. However, the relationships among microbial stoichiometry, diversity, and ecosystem function have not been explored. This study is determining whether microbial diversity promotes stability in ecosystem function by providing increased stoichiometric diversity with subsequent effects on nutrient regeneration and productivity.

Hypotheses being tested are that (a) individual strains of bacteria are strongly homeostatic and (b) variable microbial community stoichiometry is achieved through variability in community composition. It is further hypothesized that (c) the efficiency at which nutrients are remineralized by the microbial community is directly dependent on the diversity present in a given lake/ecosystem.

Bacterial strains have been isolated from several lakes and characterized with respect to their stoichiometry under conditions of varying nutrients, resource ratios, and growth rates. Bacterial diversity is being ascertained using various PCR techniques and most of the previous year was spent developing those methods. We feel comfortable that we have some good measurements of microbial diversity now and the next year we will apply these methods using more field collected samples. Mathematical models are examining the importance of homeostasis at multiple levels, i.e., strains vs. communities.

Results to date:

Sampling

Last summer, ca. 10 lakes in Itasca State Park and surrounding areas were sampled twice (May and August) and 8 lakes in the Twin Cities Metro area were sampled multiple times (4-5). Water samples were collected from the mixed layer of the lakes. Samples were collected with a Van Dorn water sampler into acid-cleaned, sterilized containers.

Isolation and characterization of microbial strains

We have isolated over 50 strains of Bacteria from the lakes that we have sampled using the streak plate technique. Strains were isolated using several different types of media to increase the potential for greater metabolic and genetic diversity. Most of these isolates have been

screened initially by rep-PCR, which is a genomic fingerprinting technique that distinguishes bacteria to the sub-species or strain level (Rademaker and De Bruijn 1997) and are being sequenced in the Advanced Genetic Analysis Center at the University of Minnesota.

Phylogenetic identification of nucleotide sequences will be determined by comparison with sequences available in the GenBank database (Benson et al. 1999) and the Ribosomal Database Project (RDP) database (Maidak et al. 1999).

In the coming year, we will manipulate substrate nutrient ratios and dilution/growth rates in culture to characterize strain stoichiometry and address some of the key components of our hypotheses. In mid-March 2007, we will add a post-doctoral fellow to our team (Thad Scott, from Baylor University) and he will be responsible for a large proportion of this culture work.

Community analysis and biodiversity quantification

To measure bacterial diversity in the lakes that we are studying, we have attempted the following techniques: (1) denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA gene fragments (PCR-DGGE), (2) terminal restriction fragment length polymorphism of nearly complete 16S rRNA genes (tRFLP), and (3) automated ribosomal intergenic spacer analysis (ARISA). We have determined that ARISA is best-suited to our purposes for the following reasons. PCR-DGGE is laborious, not well-liked by the scientific community, and it can detect only a limited number of taxa simultaneously (~15-20). tRFLP is less laborious and it has the potential to detect numerous taxa simultaneously. In contrast, ARISA is very simple to perform, which is of substantial importance because it would allow us to process many more samples (i.e., it can be very high throughput). It also targets a more variable region (the genetic material between the 16S rRNA gene and the 23S rRNA gene) than either PCR-DGGE or tRFLP. In theory, it is also biased against cyanobacteria, because they have a very large ITS region.

Another task of importance in the following year will be to further develop this method and to make comparisons among lakes and seasons to see if we can get an idea of how diversity varies. We anticipate that use of ARISA for making diversity measurements among lakes is sufficiently novel that it will merit publication (possibly Applied and Environmental Microbiology).

Chemostats and nutrient measurements

As mentioned above, we have sampled ca. 18 different lakes and all of the lakes have been sampled multiple times. We have collected samples for measurements of both seston and bacterial elemental composition. Currently, we have processed nearly 500 samples for particulate P content and should be obtaining results for the carbon and nitrogen content this winter from analyses performed elsewhere. In the coming year, chemostat experiments will be performed on individual strains that have been isolated. We will manipulate the elemental composition of their media and observe the effects on strain homeostasis and elemental composition.

We will also assemble 10 microbial communities by randomly selecting 2-5 strains and running them in chemostats at constant dilution rates with varying C:P ratios. However, we will focus primarily on high C:P ratios as this is the region where we expect diversity to matter most. We will measure biomass stoichiometry, dissolved nutrient content, DOC, dissolve inorganic C (DIC) to examine growth and re-generation efficiencies.

Mathematical modeling of biodiversity, homeostasis, and ecological stoichiometry

Both presence and absence of homeostasis of individual strains can result in bacterial communities that are not homeostatic. Whether or not bacterial strains are homeostatic can only be determined empirically. We can, however, use models to predict under which environmental conditions homeostasis at the strain level is favored. Building on standard chemostat models (Monod model and Droop model, as described in (Thingstad and Pengerud 1985) for bacterial

growth, investigated the growth of mixed bacterial communities under various nutrient-limited conditions (C or P; Neuhauser, submitted). The Monod model assumes fixed cell quota, which imposes the constraint that individual strains cannot modulate the ratio at which they consume other nutrients (e.g., nitrogen or phosphorus). The Droop model assumes flexible cell quota and can be extended to allow the maximum nutrient uptake rate to vary with cell quota ((Morel et al. 1987)). Under constant nutrient supply the number of coexisting bacterial strains cannot exceed the number of resources according to resource competition theory (Tilman 1981). However, it has been shown under the fixed cell quota assumption that the number of strains coexisting at variable supply stoichiometry may be higher. Few theoretical studies have been conducted to investigate the effects of variable supply stoichiometry on diverse communities under the variable cell quota assumption (Grover 1991). The two proposed models will allow us to compare the ecological consequences of fixed and variable cell quota in the two models, and to determine which environmental conditions (amplitude and frequency of nutrient supply) favor one mode versus the other. Both analytical tools and numerical simulations (Matlab) will be used to investigate the proposed models.

Publications, Presentations, or Published Abstracts:

Presentation

Cotner, J.B.; Tim LaPara; Andre Amado; Meghan Funke, and Audrey Wiley. Bacterial diversity and its effects on nutrient and carbon cycling in lakes. American Museum of Natural History Conference on Microbial Conservation, To be held April 26-27, NY, NY.

Student(s) supported by this project:

Name: Audrey Wiley
Program: Biochemistry
Class: Undergraduate

Name: Kara Holtzmilller
Program: Environmental Science
Class: Undergraduate

Awards

None to date

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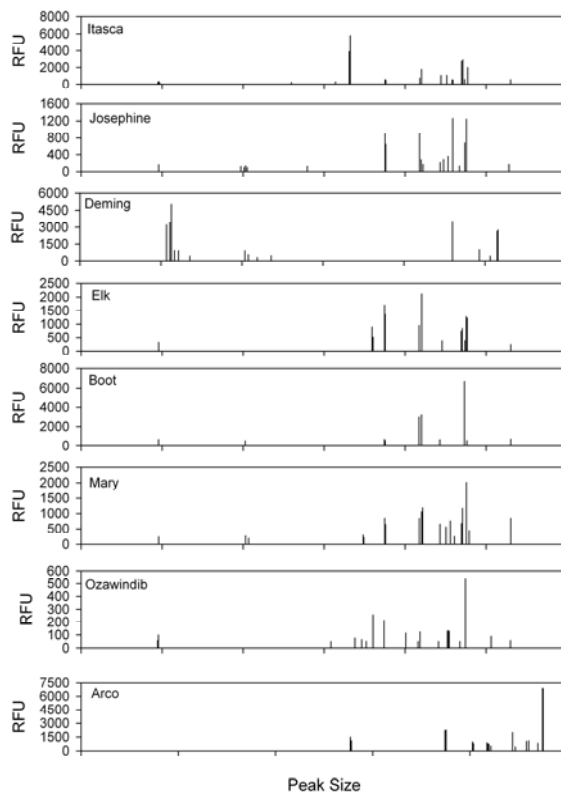
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Table 1. Diversity measures in Itasca Lakes, May and Aug 2006.

Sample Description	Number of Populations	Total Peak Area	Shannon Index	Simpson Index
Arco (Aug)	156	462303	1.6	16
Boot (Aug)	143	366615	1.46	8.66
Josephine ISP May 20	142	304144	1.6	11.18
Itasca (aug 1)	141	463052	1.5	14.2
Itasca (May)	123	389754	1.43	11.92
Long (august)	113	336276	1.46	13.13
Arco (May)	108	311849	1.16	3.87
Mary (Aug)	101	243999	1.52	17
Elk (Aug)	100	226923	1.59	21.3
Deming (May)	94	250894	1.31	8.21
Mary (May)	90	145444	1.56	14.1
Josephine ISP Aug	67	144963	1.39	12.61
Elk (May)	48	74872	1.25	6.5
Long May 20	41	130172	1.15	6.52
East Twin May 20	38	60001	1.42	16.1
Deming (Aug)	25	188680	1.02	6.97
Ozawindib (Aug)	19	49178	1.05	7.35
West Twin May 20	14	25858	1.05	9.93

Figure 1. ARISA results from Itasca State Park lakes, Aug 2006.



Development of a DNA Marker Gene System to Determine Sources of Fecal E. coli in Watersheds

Basic Information

Title:	Development of a DNA Marker Gene System to Determine Sources of Fecal E. coli in Watersheds
Project Number:	2006MN161B
Start Date:	3/1/2006
End Date:	2/29/2008
Funding Source:	104B
Congressional District:	5th
Research Category:	Water Quality
Focus Category:	Water Quality, Surface Water, Methods
Descriptors:	None
Principal Investigators:	Michael Jay Sadowsky

Publication

1. Yan, T., M. Hamilton, and M. J. Sadowsky. 2007. High throughput and quantitative procedure for determining sources of *Escherichia coli* in waterways using host-specific DNA marker genes. *Appl. Environ. Microbiol.* 73:890-896

Development of a DNA Marker Gene System to Determine Sources of Human and Cow Fecal *E. coli* in Watersheds

Principal Investigator

Michael J. Sadowsky, Professor, Department of Soil, Water, and Climate, UMN

Research Assistants

Matt Hamilton, Department of Microbiology, and Charlie Sawdey, Graduate Program in Water Resources Science, UMN

Progress Report

Many of Minnesota's rivers, lakes, and streams do not meet the Clean Water Act's "swimmable" goal due to elevated numbers of fecal coliform bacteria. Sources of fecal coliform bacteria include runoff from feedlots and manure-amended agricultural land, wildlife, inadequate septic systems, urban runoff, and discharge from sewage systems. Moreover, high levels of fecal bacteria in Minnesota's watersheds threaten the use of these water resources for recreational use and drinking. In this study, we used pooled genomic tester and driver DNAs in suppression subtractive hybridizations to enrich for host source-specific DNA markers for *Escherichia coli* originating from cows and humans. Thus far in the project, we have concentrated our efforts on isolating human specific marker DNAs. Three separate subtractive hybridizations were done using 5-60 human *E. coli* strains as tester DNAs and 20-50 *E. coli* from other animals as driver DNAs. This generated 576 potential marker genes specific for human *E. coli*. Of these, 160 were screened by dot-blot Southern hybridization for reactivity to *E. coli* from humans, and 146 reacted with human *E. coli* control DNA. All the probes were tested for specificity in hybridization reactions with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. Results of these analyses indicated that 21 cloned DNA fragments showed some hybridization specificity to DNA from *E. coli* isolated from humans. However, while our best probes identified greater than 50% of the 210 human *E. coli* strains tested, they also cross hybridized to a significant numbers of

non-human strains. Current studies are being done to increase the number of human *E. coli* strains identified with these probes and to increase hybridization specificity.

Over the past decade, several microbial source tracking (MST) methods have been intensively investigated, leading to the development of a wide variety of potential methods. Most methods to date, however, have suffered from low discriminatory power. In contrast, several genotypic-based methods have been found to be highly efficient in discriminating amongst bacteria originating from different animal hosts. We have developed a genetic marker based detection system (using DNA probes) for host-specific traits that are ecologically meaningful with respect to the microorganism studied. We have been using a multi-strain, genomic comparison approach to identify DNA fragments unique to *E. coli* strains isolated from a particular type of host source. Using this approach we have successfully developed DNA probes specific for *E. coli* strains originating from Canadian geese and ducks.

In our current studies we focused our efforts on the development of marker probes for *E. coli* strains originating from cows and humans. The prioritization of these two types of host sources was mainly due to their predominance as contributors to agricultural- and urban-derived fecal contamination in watersheds. To achieve our goals, we used the technique of subtraction suppressive hybridization (SSH) to identify DNAs that are specific for *E. coli* originating from humans and cows.

We used a multi-strain genomic comparison approach for the identification of host-specific DNA markers. The suppression subtractive hybridization (SSH) technique was used to enrich for DNA fragments unique to *E. coli* from each type of host sources. The *E. coli* strains used in SSH and subsequent specificity analyses were obtained from a library of unique isolates previously isolated from the feces of 12 known animal host sources (cats, chickens, cows, deer,

dogs, ducks, geese, goats, horses, pigs, sheep, and turkeys), and humans. Suppressive subtractive hybridizations were done using the CLONTECH PCR-Select™ Bacterial Genome Subtraction kit (BD Biosciences CLONTECH, Mountain View, CA). In initially, three different subtraction hybridizations were done; Human subtraction 1 used 5 human *E. coli* strains as tester DNA , and 5 goose *E. coli* strains as driver DNAs. Following transformed of subtraction products, 192 clones were picked. These were screened by dot-blot hybridization and 11 probes were found to be tester specific (all 11 were confirmed as specific by Southern Hybridization). All 11 probes were tested for specificity by robot arrayed colony hybridization with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. However, none of the probes reacted with a large number of human strains and cross hybridization with strains from other hosts was pronounced. This suggested that a larger number of tester and driver DNAs were needed.

In subsequent analyses, 20 human *E. coli* strains were used as tester DNAs and 20 *E. coli* from animals (5 cows, 5 geese, 5 pigs, 1 chicken, 1 dog, 1 cat, 1 horse, 1 sheep) were used as driver DNAs. Following transformed with the subtraction mixture, we picked 480 clones and screened 75 of these by dot blot Southern hybridization. All 75 clones had strong hybridization signal when probed with pooled DNAs from the tester strains (Figure 1) and only weakly hybridized when probed with driver strains. Sixty-four of these clones were confirmed as tester specific by Southern hybridization and restriction enzyme digestion analysis showed there were 41 unique probes. Of these 15 were tested for specificity by robot arrayed colony hybridization with 48 cat, 96 chicken, 96 cow, 96 deer, 96 dog, 81 duck, 135 goose, 42 goat, 78 horse, 210 human, 96 pig, 60 sheep, and 96 turkey *E. coli* isolates. Nine probes were shown to react predominately with human strains, but only about 10% of the human strains reacted and the same

human strains reacted with probes (Figure 2). One probe reacted with 26 of 210 human strains and only 2 chickens, 2 horse, and 1 sheep strain. No further colony hybridizations were attempted because the same human strains were identified with almost all 15 probes.

Consequently, we tried an additional subtraction using 60 human strains as tester DNAs and same 20 animal strains as driver DNAs as discussed above. Following transformation of the subtraction products, we picked 576 clones and screened these by dot blot Southern hybridization. Of these clones, 74 were selected as being tester specific and 71 of the 74 were confirmed as being tester DNA specific by Southern hybridization. Twelve of these were tested by colony hybridization with same strains as discussed above, and all 12 tested hybridized with greater numbers of human strains than probes from the first or second human subtractions. Some of these 12 clones identified greater than 50% of the 210 human strains. Unfortunately, they also cross hybridized with significantly greater numbers of non-human strains, compared to probes from the first human subtraction (10-30% for several hosts). In one case, nearly 60% of cat strains cross reacted with the tested probes.

We are currently doing additional subtraction hybridizations to increase the number of human *E. coli* strains identified with these probes and to increase hybridization specificity. To achieve this goal, we will use 60 human *E. coli* strains as tester DNAs and approximately 50 animal strains as driver. The driver strains will be picked according to the results obtained from colony hybridizations done in the third subtraction above. We will also use more cat *E. coli* strains in the driver mix to reduce cross hybridizations. We are also in the process of determining the hybridization specificity of probes directed against *E. coli* from cows.

Publications, Presentations, or Published Abstracts:

Publication

Yan, T., M. Hamilton, and M. J. Sadowsky. 2007. High throughput and quantitative procedure for determining sources of *Escherichia coli* in waterways using host-specific DNA marker genes. *Appl. Environ. Microbiol.* 73:890–896.

Presentations

Sadowsky, M. J. 2006. Alternate source and sinks of Pathogens in the Environment. Annual Meetings of the American Society of Agronomy (ASA), Crop Science Society of America (CSSA), and Soil Science Society of America (SSSA), Indianapolis, IN.

Sadowsky, M. J. 2006. Development and Use of a High-Throughput Robotic Method to Determine Sources of *E. coli* in the Environment, University of South Florida, Tampa, FL.

Sadowsky, M. J. 2006. Has Human Activity Outstripped the Environments Ability to Rid Itself of Fecal Bacteria? Albrecht Lecture, Earth Day, University of Missouri, Columbia, MO.

Student(s) supported by this project:

Name: Matt Hamilton

Program: Microbiology

Degree being sought: Ph.D.

Name: Charlie Sawdey

Program: Graduate Program in Water Resources Science

Degree being sought: Master of Science

Application of Wireless and Sensor Technologies for Urban Water Quality Management

Basic Information

Title:	Application of Wireless and Sensor Technologies for Urban Water Quality Management
Project Number:	2006MN187G
Start Date:	9/1/2006
End Date:	8/31/2008
Funding Source:	104G
Congressional District:	MN 5
Research Category:	Water Quality
Focus Category:	Nutrients, Surface Water, Non Point Pollution
Descriptors:	
Principal Investigators:	William Alan Arnold, Miki Hondzo, Raymond M Hozalski, Paige J Novak

Publication

Application of Wireless and Sensor Technologies for Urban Water Quality Management

Principal Investigators

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Paige J. Novak, Associate Professor and co-PI, Department of Civil Engineering, UMN

Start Date: 9/01/2006

End Date: 8/31/2008

Report Duration: 9/01/2006-1/15/2007

Abstract

The water quality of streams draining watersheds has been degraded by increasing urbanization. The general symptoms of this degradation include more frequent large flow events, reduction in channel complexity, reduced retention of natural organic matter, and elevated concentrations of nutrients. Newly emerging urban water quality threats, including insecticides, herbicides, pharmaceuticals, and estrogens, are known or suspected to damage the health of humans and ecosystems. The restoration and management of streams have traditionally attempted to improve the hydrological and water quality conditions in-stream or in riparian zones. Recent studies have indicated the portion of a watershed covered by impervious surfaces and connected to the stream by stormwater drainage is the primary degrading process of stream ecology and health. These findings suggest that the sustainable restoration and management of stream water quality require quantification of hydrological, chemical, biological, and geomorphological processes, and that these processes must be assessed across a range of scales. Furthermore, interactions among biogeochemical processes across watersheds are either non-linear processes or linear processes dependent on non-linear drivers. The monitoring of such a system inherently requires a change in traditional field sampling strategies. We propose to transform traditional and very limited (in terms of spatial and temporal resolution) field

measurements through the integration of multi-scale, spatially-dense, high frequency, real-time, and event-driven observations by a wireless network with embedded networked sensing.

The goals of the proposed research are to assess the benefits of stormwater best management practices in mitigating the pollutant loads from urban and peri-urban sources, to evaluate the effectiveness of traditional grab sampling in calculating pollutant loads, and to develop correlations to predict the concentrations of non-sensed chemical or biological pollutants. These goals will be achieved by establishing a wireless sensor network capable of monitoring fundamental water quality parameters at high spatial and temporal resolution. It is hypothesized that sensed fundamental water quality parameters can be used for predicting the presence of emerging chemical contaminants in urban streams. It is also hypothesized that the water quality in streams draining similar impervious urban areas is controlled by the mean and variance of effective stormwater residence time. The mean and variance of water residence time, the time it takes urban runoff to travel between the impervious urban land and a receiving aquatic body, will be characterized by radio frequency identification technology (RFID), which will augment the proposed wireless network. Ultimately, data generated from such a monitoring network will enable mechanistically-based scaling and forecasting of water quality in urban streams and rivers. This will transform urban planning practices and management of water quality in streams draining urban land.

Progress

The project is in the initial stages, so progress to date is limited. Sensor packages, including data loggers, communication equipment, Hydrolab sensor units (pH, specific conductivity, temperature, pressure, turbidity, dissolved oxygen, photosynthetically active

radiation), ISCO water samplers, and a nutrient analyzer (nitrate, phosphate) were purchased.

Trial deployments were conducted in October and November 2006 before the weather required the sensor systems to be removed from the field.

Current work is focused on implementing extraction and gas chromatography-mass spectrometry methods for the simultaneous detection of the target organic analytes (atrazine and prometon). Additionally, the programming of the sensors is being optimized, and the sensor packages will be tested in a flume at St. Anthony's Falls Laboratory in February and March 2007.

Once the weather allows redeployment in the Spring, the network will be deployed in Shingle Creek. Grab samples for the target pesticides, fecal coliforms, and chloride will be taken, as well as samples for sensed variables for verification. These data will be used to determine relationships between sensed parameters and those measured via grab samples. Efforts will be coordinated with activities of the USGS and local watershed district.

In the summer/fall, the network will be redeployed to test a stormwater BMP along the creek.

Publications, Presentations, or Published Abstracts:

Presentations

Kim, S.-C.; Hondzo, M.; Hozalski, R.M.; Novak, P.; Arnold, W.; Jazdzewski, J.D.; Jindal, N.; Capel, P.D., 2006. Integrated urban water quality management: wireless technologies and embedded networked sensing. Poster presented at the American Geophysical Union National Meeting, San Francisco, CA. December 2006.

Presentations will also be given at the May 2007 American Society of Civil Engineers Environmental and Water Resources Institute Conference in Tampa, FL and at the July 2007 Association of Environmental Engineering and Science Professors Education and Research Conference in Blacksburg, VA.

Student(s) supported by this project:

None to date. A new student is currently being sought.

Awards

None to date

Information Transfer Program

Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	2	4	0	0	6
Masters	4	5	1	0	10
Ph.D.	1	1	0	0	2
Post-Doc.	0	0	0	0	0
Total	7	10	1	0	18

Notable Awards and Achievements

Gelsey, Giana 2006-2007 NSF GK12 Fellowship National Science Foundation. Ms Gelsey is a MS student in the Water Resources Science program. She was supported by the Water Resources Center.

Schram, Erica, 2006-2007 NSF GK12 Fellowship National Science Foundation. Ms Schram is a MS student in the Water Resources Science program. She was supported by the Water Resources Center.

Sealock, Adam, 2006 Best Oral Presentation by a Graduate Student, 1st Runner-Up VXI International Symposium on Chironomidae, Portugal, July 2006. Mr Sealock is a MS student in the Water Resources Science program. Mr. Sealock was supported by Dr. Ferrington's grant.

Serieyssol, Claire, 2006 Bell Museum Dayton Wilkie Grant, University of Minnesota. Ms Serieyssol is a PhD student in the Water Resources Science program. She was supported with Dr. Ferrington's grant.

Streets, Summer, 2006 Runner-up Best Student Platform Presentation Midwest Environmental Society of Toxicology and Chemistry Conference. Ms Streets is supported by Prof. Deb Swackhamer, Co-Director of the Water Resources Center

Werner, Jeffrey, 2006 Graduate Student Paper Award American Chemical Society, Division of Environmental Chemistry. Mr. Werner was a PhD student in the Water Resources Science program. Mr. Wernes was supported with Drs. William Arnold and Kris McNeill's grants.

Werner, Jeffrey, 2006 Graduate Student Award American Chemical Society, Division of Environmental Chemistry. Mr. Werner was a PhD student in the Water Resources Science program. Mr. Wernes was supported with Drs. William Arnold and Kris McNeill's grants.

Publications from Prior Projects

1. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Werner, J.J., K.H. Wammer, M. Chintapalli, W.A. Arnold, and K. McNeill. Environmental photochemistry of tylosin: efficient, reversible photoisomerization to a less-active isomer, followed by photolysis, *J. Ag. Food Chem.* Submitted.
2. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Werner, J.J.; W.A. Arnold.; K. McNeill. 2006. Water Hardness as a Photochemical Parameter: Tetracycline Photolysis as a Function of Calcium Concentration, Magnesium Concentration, and pH, *Environ. Sci. Technol.* 40: 7236-7241.
3. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Edlund, B. L., W. A. Arnold, K. McNeill. 2006. Aquatic Photochemistry of Nitrofurantoin Antibiotics, *Environ. Sci. Technol.* 40: 5422-5427.
4. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Wammer, K.H.; T.M. LaPara, K. McNeill, W. A. Arnold, D.L. Swackhamer. 2006. Changes in Antibacterial Activity of Triclosan and Sulfa Drugs due to Photochemical Transformations. *Environ. Toxicol. Chem.*, 25: 1480-1486.
5. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Boreen, A.L., W. A. Arnold, K. McNeill. 2005. Triplet-sensitized photodegradation of sulfa drugs containing six-membered heterocyclic groups: Identification of an SO₂ extrusion photoproduct, *Environ. Sci. Technol.* 39: 3630-3638.
6. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Werner, J.J., K. McNeill, W.A. Arnold. 2005. Environmental photodegradation of mefenamic acid. *Chemosphere* 58: 1339-1346.
7. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Latch, D.E., J.L. Packer, B.L. Stender, J. VanOverbeke, W.A. Arnold and K. McNeill. 2005. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin and oligomerization products, *Environ. Toxicol. Chem.* 24 (3): 517-525.
8. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Articles in Refereed Scientific Journals - Boreen, A.L., W.A. Arnold and K. McNeill. 2004. Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups, *Environ. Sci. Technol.*, 38: 3933-3940.
9. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Book Chapters - Arnold, W.A., and K. McNeill. 2007. Abiotic Degradation of Pharmaceuticals: Photolysis and Other Processes to appear in *Analysis, Fate And Removal Of Pharmaceuticals In The Water Cycle* Eds. M. Petrovic and D. Barcelo. Elsevier Science & Technology Books.
10. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Dissertations - Latch D. E. 2005. Environmental photochemistry: Studies on the degradation of pharmaceutical pollutants and the microheterogeneous distribution of singlet oxygen. Ph.D. Dissertation, Department of Chemistry, University of Minnesota, Minneapolis, MN, 2005, 256 pp.
11. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Dissertations - Boreen, A.L. 2006. Enhanced photolysis in natural waters: naturally

- occurring sensitizers and substrates and application to the fate of aquatic pollutants. Ph.D. Dissertation, Department of Chemistry, University of Minnesota, Minneapolis, MN, 263 pp.
12. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Dissertations - Werner, J.J. 2006. The environmental photochemistry of pharmaceutical compounds in aqueous solution and on a clay surface. Ph.D. Dissertation, Graduate Program in Water Resources Science, University of Minnesota, Minneapolis, MN, 119 pp.
 13. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Werner, J.J., W. A. Arnold, K. McNeill. 2006. Environmental photochemistry of the antibiotic compound tetracycline: dependence on acid-base and metal binding speciation. Paper presented at the Minnesota Water 2005 and Annual Water Resources Joint Conference, Brooklyn Center, MN, October 25-26, 2006.
 14. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Werner, J.J., W.A. Arnold, K. McNeill. 2006. The environmental photochemical kinetics of tetracycline as a function of pH and water hardness. Presented in Environmental Chemistry Awards, Environmental Chemistry Division, American Chemical Society National Meeting, San Francisco, CA, September, 2006.
 15. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Edhlund, B.L., W.A. Arnold, K. McNeill. 2006. Aquatic Photochemistry of Nitrofurantoin Antibiotics. Poster Presentation. Environmental Sciences: Water Gordon Research Conference, Plymouth, NH, June 25-June 30, 2006.
 16. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Werner, J.J., and K. McNeill. 2005. Water Hardness as a Critical Photochemical Parameter: The Case of Tetracycline Antibiotics, Pacificchem 2005 Congress, Symposium on Environmental Contaminants of Emerging Concern: Anticipating, Understanding and Intercepting Future Environmental Crises, Honolulu, HI, December 15, 2005.
 17. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Edhlund B.L., W.A. Arnold, K. McNeill. 2005. Aquatic Photochemistry of Nitrofurantoin Antibiotics. Poster Presentation. Minnesota ACS Meeting, St. Paul, MN, November 2005.
 18. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Edhlund, B.L., W.A. Arnold, K. McNeill. 2005. Aquatic Photochemistry of Nitrofurantoin Antibiotics. Poster Presentation. Minnesota Water 2005 and Annual Water Resources Joint Conference, Brooklyn Center, MN, October 2005.
 19. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Edhlund, B.L., W.A. Arnold, K. McNeill. 2005. Aquatic Photochemistry of Nitrofurantoin Antibiotics. Poster Presentation. ENVR, 230th ACS National Meeting, Washington, DC, August 2005.
 20. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - McNeill, K. 2005. Photochemical approaches to environmental pharmaceutical pollutants, American Chemical Society (ACS) National Meeting Symposium: Strategies and Molecular Mechanisms of Contaminant Degradation Chemistry, Washington, D.C., August 28-Sept. 1, 2005.
 21. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Werner, J.J., K. McNeill, W.A. Arnold. 2004. Speciation-dependent photochemistry of tetracycline antibiotics: acid-base speciation and metal-binding effects. Oral Presentation. Midwest Environmental Chemistry Workshop, Madison, WI, October 16-17, 2004.

22. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - McNeill, K., W.A. Arnold. 2004. Contribution of photochemistry to the fate of pharmaceuticals and personal care products in surface waters. Oral Presentation. ENVR, Presented at the special symposium on Environmental aspects of pharmaceuticals and personal care products at the 228th ACS National Meeting, Philadelphia, PA, August 2004.
23. 2003MN32G ("Photochemistry of Antibiotics and Estrogens in Surface Waters: Persistence and Potency") - Conference Proceedings - Boreen, A.L., W.A. Arnold, K. McNeill. 2004. Photochemical fate of sulfa drugs in the aquatic environment. Oral Presentation. ENVR, Presented at the special symposium on Environmental aspects of pharmaceuticals and personal care products at the 228th ACS National Meeting, Philadelphia, PA, August 2004.
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