Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

Project Manager Name: William Arnold

Project Manager Email address: arnol032@umn.edu

Project Title: Water: Antibiotics and Antibiotic Resistance Genes in Minnesota Lakes

Project number: 030-B

1. Abstract

Pharmaceuticals are found in water bodies all across Minnesota. These compounds are biologically active and can disrupt the function of ecological communities or have other adverse effects. Of particular concern are antibiotics, one of the greatest inventions of the 20th century. The utility of antibiotics is at risk, however, due to resistance in clinical settings. The release of antibiotics and antibiotic resistance genes into the environment may also pose a threat to human health by encouraging broader development of antibiotic resistance or by leading to the harboring of elevated levels antibiotic resistance genes in environmental matrices. There is also potential for antibiotics to disrupt the proper functioning of ecosystems. While there is a background level of naturally occurring antibiotic resistance, elevated or persistent levels due to human activities have the potential to cause harm to human, veterinary, or ecosystem health. The overall goal of this project is to improve water quality and to protect human and ecosystem health by 1) quantifying the current and historical levels of selected human and veterinary antibiotic compounds in lake sediments, and 2) determining the current and historical levels of genes that code for resistance to the selected human and veterinary antibiotics in lake sediments. The results of this work will reveal if the environmental presence of human and veterinary antibiotics in Minnesota lake sediments leads to the retention of resistance genes.

2. Background

Antibiotics are compounds that specifically target a unique feature of bacterial cell physiology to kill or to prevent the growth of bacteria. These drugs are very effective from a medical perspective, because they are capable of attacking bacterial pathogens that cause illnesses without directly affecting the human patient. Since the beginning of the antibiotic era (early 1940s), however, bacteria have evolved numerous mechanisms of resistance to antibiotics, thereby threatening the efficacy of virtually all applications of antibiotic chemotherapy (Levy, 2005). Indeed, many scientists fear that the “antibiotic era” will soon end.

Historically, concerns about antibiotic resistance were almost entirely ignored, for it was assumed that new antibiotics would be discovered. The discovery of new antibiotics, however, has almost completely stopped (Jabes, 2011). Presently, in the United States, one type of antibiotic resistant infection (methicillin-resistant Staphylococcus aureus – MRSA) leads to more fatalities than the combination of HIV/AIDS, Parkinson’s disease, and homicides (Spellberg et al., 2011). The estimated economic cost of antibiotic resistance ranges from $21 to 34 billion dollars per year (Spellberg et al., 2011).

The primary focus of the medical community to thwart the development of antibiotic resistance has been to limit inappropriate use and to improve hygiene within the hospital setting. The latter

efforts are intended to limit nosocomial infections – secondary infections, which are often resistant to antibiotic treatment, that develop during hospital visits (hospitals are viewed as hotspots of antibiotic resistance). The effort to reduce inappropriate use has been much more challenging (and sadly, less effective), but includes initiatives to: (1) reduce inappropriate antibiotic prescriptions (i.e., viral infections, like the common cold, are unaffected by antibiotics), (2) eliminate antibiotic use in agriculture for growth promotion and prophylaxis, and (3) reduce the superfluous use of antibacterial use in soaps and other personal care products (antibacterials in most of these cases are redundant and unnecessary; this practice also continues). While each of these initiatives by the medical community is an excellent idea, they are difficult to implement (politically or technically) and they are likely to be insufficient to indefinitely extend the antibiotic era. New and complementary initiatives are therefore needed to help resolve this critically important problem.

Over the past decade or so, there has been a growing body of evidence that the environment plays a large role in the proliferation of antibiotic resistant bacteria. Research has shown that antibiotic resistant bacteria are common in the environment but that pathogenic bacteria, which live inside the human body, are typically antibiotic-sensitive (D'Costa et al. 2007). The proliferation of antibiotic resistant bacteria, therefore, stems from the genetic exchange that inevitably occurs when these two types of organisms are intermixed as well as the selective pressure imposed by the heavy antibiotic use. Indeed, some researchers have posited that environmental bacteria are the most prominent source of the genes that are observed among medically-relevant pathogens (Allen et al. 2010).

The goal of the research proposed herein is to better understand the importance of various sources of antibiotics and of antibiotic resistant bacteria (which will be tracked as antibiotic resistance genes (ARGs)) in Minnesota. The two most prominent sources of antibiotics and ARGs are agricultural runoff (up to 80% of all antibiotics are used for prophylaxis and growth promotion in agriculture; US FDA, 2009) and municipal wastewater discharges (LaPara et al. 2011; Storteboom et al. 2011). Our overall hypothesis is that sediment cores collected from lakes and rivers in Minnesota can be used as a record of antibiotic use and of antibiotic resistance as functions of both location and of time (i.e., sediments can be "dated" to reflect historical activity).

Several studies have sought to link antibiotic levels with the presence of resistance genes. In water samples of a river, Jiang et al. (2013) observed positive relationships between tetracycline and sulfanoamide concentrations and the presence of tetracycline and sulfonamide resistance genes, respectively. This is likely due to the simultaneous discharge of both from wastewater treatment plants rather than development of resistance in the river. Pei et al. (2006) found higher levels of resistance genes and antibiotics (tetracyclines and sulfonamides) in Cache La Poudre River (Colorado) sediments impacted by urban/agricultural activity than at unimpacted control sites; a recent modeling effort, however, attributed elevated levels of ARGs to heavy metal contamination (heavy metals are also known to select for ARGs due to cross resistance) (Hellweger, 2013). Sediments in the Haihe River in China also showed positive correlation between the levels of sulfonamide antibiotics and resistance genes (Luo et al., 2010). While detecting both antibiotic compounds and resistance genes in water and sediment samples from three water supply reservoirs, Huerta et al. (2013) did not find clear associations between the occurrence of antibiotics and resistance genes. To our knowledge, the only study to investigate the abundance of antibiotic resistant bacteria and resistance genes as a function of depth (i.e., time) in a sediment core is the study by Thevenon et al. (2012), which showed that the effect of wastewater effluent and changes in trophic state led to elevated levels of resistance in the sediment over time. In this study, the concentrations of antibiotics were not measured, so it is not clear if the presence of antibiotics is the pressure leading to the increased resistance levels.

To our knowledge, there has only been a single study (Tamtam et al., 2011) that investigated temporal trends of antibiotic loading via analysis of dated sediment cores (and to our knowledge, no study has investigated ARGs within dated sediment cores). (There have been several studies, including our own, that have quantified temporal trends for the antibacterials triclosan and triclocarban (Buth et al., 2009; Anger et al., 2013). In the Seine River, Tamtam et al. (2011) detected 1-30 μg/kg of flumequine, oxolinic acid, sulfamethoxazole, and nalidixic acid in dated sediment cores. While the concentrations varied over time, compounds were not detected prior to their date of commercialization. This study indicates that sediments do preserve antibiotics and that temporal trends are discernible. Although it is unclear if antibiotic resistant bacteria will be preserved over time in sediment cores, the selection for and maintenance of ARGs is certainly more likely when antibiotic compounds are also present.

The proposed research will be novel, therefore, as previous studies of sediments have primarily focused on spatial distributions of antibiotics and ARGs, with a common theme being that wastewater effluents and/or agricultural activity leads to higher levels of antibiotic compounds in sediments (Pei et al. 2006, Storteboom et al. 2011).

3. Hypothesis

Our hypothesis is that the levels of resistance to particular antibiotics will be recorded in sediments and will correlate with the presence and/or usage trends of particular antibiotics. If our hypothesis is confirmed, this would suggest that the presence of the antibiotics allows for resistance to be maintained in the environment and that additional measures should be taken to reduce the discharge of antibiotics and of antibiotic resistance genes to the environment from municipal, hospital, or agricultural sources. If the data do not support the hypothesis, the study would still provide the critical information that discharge of antibiotics and antibiotic resistance genes from municipal, hospital, or agricultural sources does not lead to persistence of resistance genes in aquatic systems.

4. Methodology

Sediments serve as integrators of the chemicals introduced into a lake over time. By analyzing the antibiotic concentrations as a function of depth, it will be possible to assess the “dosage” each lake received as a function of time. Based on our previous ENTRF sponsored work, we have identified three wastewater impacted sites (Lake Pepin, Duluth Harbor, and Lake Winona) for study. Both Duluth Harbor and Lake Winona directly receive wastewater effluent. Lake Pepin (a natural “lake” within the Mississippi River) receives some effluent directly, but its watershed covers two-thirds of the state of Minnesota, so it serves as an integrative site. To complement the samples from these sites, we will also collect surface sediment samples behind Ford Dam in St. Paul (just upstream of the confluence of the Minnesota and Mississippi Rivers) and from Rice Lake in Brainerd. These latter two samples will help us parse out the effects of large fractions of the State’s watershed. The control site will be Little Wilson Lake, which has no wastewater input.

Cores will be collected by a piston or box-type corer. Riverine surface sediment samples will be collected with a dredge or scoop, depending on the depth. The cores will be extruded in the field in 1 to 4 cm sections with subsamples being taken for dating and determination of resistance gene levels. The remainder of the sample will be dedicated to chemical analyses. The Lake Pepin core will be dated via magnetic susceptibility (Engstrom and Rose, 2013), and it will be sectioned in the laboratory after dating is performed. The other cores will be dated using lead-210 and cesium-137 methods and other chemical markers as described in Dr. Engstrom’s recent work (Engstrom et al., 2009). Dr. Engstrom has all of the required equipment and

facilities to collect and date sediment cores. Briefly, ^{210}Pb will be analyzed by polonium isotope-dilution, alpha spectrometry following high-temperature distillation; dates will be calculated from the stratigraphic profile of excess ^{210}Pb according to the constant rate of supply (c.r.s.) model. Cesium-137 will be analyzed by low-background gamma spectrometry, and the peak and onset of ^{137}Cs will be used as dating markers (1963 and c. 1950, respectively) for atmospheric radiocesium release by above-ground nuclear testing. We will collect cores that are deep enough (i.e., go back it time far enough) such that we will have core sections that date to prior to the deployment of the antibiotic classes (1930-1960 depending on the class). The water and organic matter content will be determined as a function of depth via loss on ignition analysis. Because antibiotic resistance levels may be related to heavy metal content (Hellweger, 2013), all sediment samples will be analyzed via inductively coupled plasma-mass spectrometry (ICP-MS; Department of Earth Sciences, U of MN) to determine the metal concentrations. Sediment deposition rates as a function of time will be calculated based on the mass of sediment contained between dated points in the core section.

For analysis of the pharmaceuticals, we will follow literature protocols of Gibs et al. (2013) and Massey et al. (2010). Wet samples with a mass corresponding to ~10 g dry weight will be freeze dried. The freeze-dried sample will be spiked with ^{13}C-labelled compounds (one for each antibiotic compound class to be studied: sulfonamides, macrolides, fluoroquinolones, and tetracyclines) as isotope dilution internal standards. A single un-spiked blank sample of clean sand will be processed and analyzed to ensure that there is no triclosan contamination. A recovery standard (a sediment from depth great enough that it should have minimal antibiotics present) will be spiked with $^{13}C_{12}$-labeled and unlabelled antibiotics to test recovery. The samples will be extracted using an accelerated solvent extraction system. The exact protocol will need to be optimized, but two options are a 50:50 mixture of pH 6 phosphate buffer and methanol (reported apparent recoveries 40-60% for sulfonamides, and 53-107% for macrolides, fluroroqunionlones, and tetracyclines; Gibs et al. (2013)) or a 75:25 ratio of acetonitrile and water (50-75% recovery in initial tests). The extract is then evaporated to remove the organic solvent, and the water portion is cleaned up and concentrated using pre-washed Oasis HLB solid phase extraction cartridges. After elution in acetonitrile/methanol, the eluate is then concentrated, and solvent exchanged into the appropriate eluent matrix with a volume of 100-200 μL. Detection limits will be on the order of 1 ng antibiotic per gram of sediment (Gibs et al., 2013). Note that both the pore water and sediment are extracted, but given the high solid to water ratios, the pollutant levels are attributed to the sediment phase. Analysis of the samples will be performed according to US Geological Survey protocols (Meyer et al., 2007)) using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with electrospray ionization (available in the U of MN Cancer Center on an hourly basis). From the data derived from analyses above, the concentrations (mass per mass) and accumulation rates (mass per area per time) of the antibiotics will be calculated. Because clinical use of antibiotics began in the 1930s, sediments deposited prior to this date will serve to reveal and natural background concentrations for those compounds that can be produced naturally (i.e., macrolides and tetracyclines).

Genomic DNA will be extracted and purified from sediment samples as described previously (LaPara et al. 2011). Briefly, about 500 mg of sediment (wet weight) will be processed using a bead beater to lyse cells. Genomic DNA will be then extracted and purified from sediment samples using a FastDNA Spin Kit for soil (MP Biomedicals; Solon, OH). All genomic DNA extractions will be performed in triplicate and stored at -20ºC until needed. Quantitative real-time PCR (qPCR) will be used to quantify 16S rRNA genes (a measure of total bacterial biomass) as well as three genes encoding tetracycline resistance (*tet*(A), *tet*(W) and *tet*(X)), the integrase gene of class 1 integrons (*intI1*), one gene encoding sulfonamide resistance (*sul1*), and one gene encoding resistance to macrolides (*erm* (B)) as previously described (Burch et al. 2013). These genes will be targeted in this study because these genes encompass a variety of

resistance mechanisms as well as resistance genes encoding proteins that act against different classes of antibiotics. The qPCR analysis will be conducted using an Eppendorf Mastercycler ep realplex thermal cycler (Eppendorf; Westbury, NY). Each qPCR run will consist of initial denaturation for 10 min at 95°C, followed by forty cycles of denaturation at 95°C for 15 s, and anneal and extension at 60°C (most targets) or at 56°C (human-specific Bacteroides) for 1 min. A 25 μL reaction mixture contained 12.5 μL of iTaq SYBR Green Supermix with ROX (Bio-Rad; Hercules, Calif.), 25 μg bovine serum albumin (Roche Applied Science; Indianapolis, Ind.), optimized quantities of forward and reverse primers, and a specified volume of template DNA (usually 0.5 μL). The precise volume and concentration of template DNA will be empirically optimized for each sample to generate the lowest detection limit while minimizing inhibition of PCR. The quantity of target DNA in unknown samples will be calculated based on a standard curve generated using known quantities of template DNA. Standards for qPCR have already been prepared by PCR amplification of genes from positive controls, followed by ligation into pGEM-T Easy (Promega; Madison, Wisc.) as described previously (Diehl and LaPara, 2010; LaPara et al. 2011; Burch et al. 2013). Ten-fold serial dilutions of plasmid DNA will be prepared and run on the thermal cycler to generate standard curves ($r^2 > 0.99$).

5. **Results and Deliverables**

This research is broken into three tasks/results: 1) the collection and dating of sediment cores; 2) the determination of antibiotic concentrations; and 3) the quantification of resistance genes For the first result, the deliverable is the set of dated sediment cores for three wastewater impacted lakes and a control site. For the second task, the deliverables are the optimized method for sediment extraction and the concentrations of antibiotics in the sediment cores. We expect that antibiotic concentrations in the sediments will be minimal prior to 1930, and will generally increase with time but that variation will exist based on prescription rates over time (for example, tetracyclines are not as commonly used in human medicine as they were in the past). For the third task, the result is a quantification of antibiotic resistance genes as a function of depth in the cores. We expect that the genes will be present throughout the core, but that the levels will increase in sections where the residual antibiotic concentration is higher.

Overall, this project will provide an understanding of the historical levels of antibiotics used in human and veterinary medicine that have entered Minnesota lakes. Additionally, this will be the first study to investigate how the discharge of these chemicals has or is affecting the levels of resistance genes in the environment. This is information critical to protecting human and ecological health and may provide information relevant to antibiotic use and development. This study will reveal if additional treatment to remove antibiotics from wastewater or runoff is necessary or unnecessary in terms of proliferation of resistance genes.

6. Timetable

The research tasks outlined above will be accomplished according to the following schedule. Shaded regions are continuous efforts and X's mark discreet events.

	Year 1				Year 2							
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Result 1: Core collection and dating												
Core collection												
Core dating and determination of sediment deposition rates.												
Data analysis												
Product deilvery					X							
Result 2: Measurement of sulfa, tetracycline, macrolide, and fluoroquinoline antibiotics in sediment cores												
Extraction and analytical method optimization												
Antibiotic conc. measurments												
Metal conc. measurements												
Data analysis												
Product deilvery			X			X						X
Result 3: Measurement of antibiotic resistance genes												
DNA extraction and purification												
Quantify resistance genes												
Data Analysis												
Product delivery												X
Reporting		X		X		X		X		X		X

7. **Budget**

The requested funds from LCCMR total $300,000. See attached sheets for cost breakdown. The justificaiton is as follows.

Staff or Contract Services. Over the three year project, Dr. Arnold will devote 6% (3 weeks per year) of time in Y1 and Y2 and 4% of time in Y3 to the project (total salary and fringe benefits of $36,000). Prof. LaPara will devote 2% time in Y1 and Y2 and 1% in Y3 ($9,600). The responsibilities of the principal investigator include experimental design, product coordination, data analysis, student guidance, and report/product preparation. Two additional personnel will be employed by the project. Either this will be two graduate students (working 25-50% time on the project; 50% time is considered full time for a graduate student) or one graduate student (50% time) and one postdoctoral researcher (~75% time in Y1 and Y2). The remaining $204,400 of personnel funds will be used for salary, fringe benefits, and tuition (for graduate students) of these workers. We will decide how to appropriate the funding based on the availability of the best qualified individuals.

A subcontract to the Science of Museum of Minnesota for $16,000 is budgeted for assistance with the collection and dating of sediment cores. This is for 2% of Dr. Engstrom's time in Y1, travel expenses associated with core collection for Dr. Engstrom, and laboratory analyses.

Supplies and Maintenance. A total of $25,000 is requested for laboratory supplies (chemical standards, isotope standards, microbiologial/DNA extraction kits, instrument/analytical time for antibiotic and DNA analysis, solvents, consumable supplies, notebooks, software licenses). Additionally, $4,000 is requested for maintenance and repair of laboratory equipment that will be used by the project.

Travel expenses: A total of $5,000 is requested for in-state travel to collect sediment samples .

Other. In-kind contributions total $125,000. These funds include in-kind effort of the Drs. Arnold and LaPara; $10,700) and the facilities/administrative costs that are waived on State of Minnesota funded research (52% of direct costs excluding permanent equipment and graduate student academic year fringe benefits; approximately $114,000).

8. Credentials

Dr. Arnold is a nationally-known researcher with expertise on the fate and transport of anthropognic organic chemicals (including solvents, pesticides, and pharmaceuticals). He has been studying the fate of pharmaceutical and pesticide compounds in aquatic environments for fourteen years. His recent work has quantified triclosan and its photochemically produced dioxins in dated sediment cores. He has published over twenty peer-reviewed papers on pharmaceutical fate since 2003, and he is the co-author of a textbook on water chemistry published in 2011. Dr. Arnold will co-supervise the postdoctoral scientist and/or graduate student(s) with Dr. LaPara, and he will focus on the quantification of antibiotic compounds in the sediments.

Dr. LaPara is also a nationally-known researcher with substantial research experience in the areas of environmental biotechnology, microbial ecology, and environmental microbiology. He is one of the leading researchers on municipal wastewater treatment and its potential role in thwarting the spread of antibiotic resistance. He has substantial expertise in quantifying ARGs in the environment, from both aquatic and sediment samples, and he will lead this portion of the project. He has co-authored more than 50 manuscripts in the peer-reviewed archival literature that have been cited more than 1000 times according to Web of Science.

Dr. Engstrom is a leading expert on the use of lake sediment records to understand long-term environmental change, particularly the effects of human activities on water quality, atmospheric chemistry, and biogeochemical processes on a global scale, respectively. Dr. Engstrom will focus on collection and dating of the sediment cores.

WILLIAM A. ARNOLD, Ph.D., P.E.
University of Minnesota, Department of Civil Engineering, 122 Civil Engineering Building, 500 Pillsbury Dr., SE, Minneapolis, MN 55455, Phone: (612) 625-8582; Fax: (612) 626-7750; Email: arnol032@umn.edu

Professional Preparation

Massachusetts Institute of Technology, Chemical Engineering, Minor in Chemistry, S.B., 1994
Yale University, Chemical Engineering, M.S., 1995
The Johns Hopkins University, Environmental Engineering, Ph.D., 1999

Appointments

2010-present	**Joseph T. and Rose S. Ling Professor**, Department of Civil Engineering, University of Minnesota and member of the graduate faculty in Water Resources Science and Stream Restoration Science and Engineering
2013-2014	**Visiting Researcher**, Woods Hole Oceanographic Institution, Department of Marine Chemistry & Geochemistry
2005-2010	**Associate Professor**, Department of Civil Engineering, University of Minnesota and member of the graduate faculty in Water Resources Science and Stream Restoration Science and Engineering
2006-2007	**Visiting Researcher**, Eawag, The Swiss Federal Institute of Aquatic Science and Technology
1999-2005	**Assistant Professor**, Department of Civil Engineering, University of Minnesota and member of the graduate faculty in Water Resources Science

Selected Honors and Awards

Super Reviewer Award from the journal *Environmental Science and Technology*, 2013
Arcadis/AEESP Frontier in Research Award 2012
George W. Taylor Award for Distinguished Research, 2011
Joseph T. and Rose S. Ling Professorship in Civil Engineering, 2009-present
CH2M Hill/AEESP Outstanding Doctoral Dissertation Award, 2000

Professional Societies
American Chemical Society, Environmental Chemistry Division (ACS), American Geophysical Union, Hydrology Section (AGU), American Society of Civil Engineers (ASCE), Association for the Sciences of Limnology and Oceanography (ASLO), Association of Environmental Engineering and Science Professors (AEESP)

Selected Peer-Reviewed Publications
Five most closely related to the proposed project:
1. Anger, C.T.; Sueper, C.; Blumentritt, D.J.; McNeill, K.; Engstrom, D.R.; **Arnold, W.A.** 2013. Quantification of Triclosan, Chlorinated Triclosan Derivatives, and their Dioxin Photoproducts in Lacustrine Sediment Cores. *Environmental Science and Technology,* 47, 1833-1843.
2. Steen, P.O.; Grandbois, M.; McNeill, K.; **Arnold, W.A.** 2009. Photochemical formation of halogenated dioxins from hydroxylated polybrominated diphenyl ethers (OH-PBDEs) and chlorinated derivatives (OH-PBCDEs). *Environmental Science and Technology,* 43, 4405-4411.
3. Zeng, T.; **Arnold**, **W.A.** 2013. Pesticide photolysis in prairie potholes: Probing photosensitized processes. *Environmental Science and Technology,* 47, 6735-6745.
4. Kelly, M.M.; **Arnold, W.A.**, 2012. Direct and Indirect Photolysis of the Phytoestrogens Genistein and Daidzein. *Environmental Science and Technology*, 46, 5396-5403.
5. Page, S.E.; **Arnold, W.A.**; McNeill, K. 2011. Assessing the contribution of free hydroxyl radical in organic matter-sensitized photo-hydroxylation reactions. *Environmental Science and Technology*, 45(11) 2818-2825.

Five other products:
1. Ryan, C.R.; Tan, D.T.; **Arnold, W.A.** 2010. Direct and Indirect Photolysis of Sulfamethoxazole and Trimethoprim in Wastewater Treatment Plant Effluent. *Water Research*, 45, 1280-1286.
2. Page, S.E.; **Arnold, W.A.**; McNeill, K. 2010. Terephthalate as a probe for photochemically generated hydroxyl radical. *Journal of Environmental Monitoring* 12, 1658-1665.
3. Surdo, E.M.; Cussler, E.L.; Novak, P.J.; **Arnold, W.A.** 2009. Geomembranes containing powdered activated carbon have the potential to improve containment of chlorinated aromatic contaminants. *Environmental Science and Technology*, 43(23) 8916-8922.
4. Boreen, A.L.; **Arnold, W.A.**; McNeill, K., 2005. Triplet-sensitized photodegradation of sulfa drugs containing six-memebered heterocyclic groups: identification of an SO_2 extrusion photoproduct, *Environmental Science and Technology,* 39, 3630-3638.
5. Latch, D. E.; Packer, J. L.; Stender, B. L.; VanOverbeke, J; **Arnold, W.A.**, McNeill, K., 2005. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-*p*-dioxin and oligomerization products, *Environmental Toxicology and Chemistry* 24, 517-525.

Synergistic Activities
1. National Research Council's Committee on Future Options for Management in the Nation's Subsurface Remediation Effort (Water Science and Technology Board; 2010-2012)
2. Multicultural Center for Academic Excellence, Office of Equity and Diversity President's

Distinguished Faculty Mentor Program Participant (named an Outstanding Mentor)
3. President (2011-2012) and Member of the Board of Directors, Minnesota Section of ASCE
4. Resident Fellow of University of Minnesota Institute on the Environment and Associate Fellow of the Minnesota Supercomputing Institute
5. Associate Editor of *Environmental Science and Technology Letters* (2013-)

TIMOTHY M. LAPARA, Ph.D., P.E.
University of Minnesota, Department of Civil Engineering, 122 Civil Engineering Building, 500 Pillsbury Dr., SE, Minneapolis, MN 55455, Phone: (612) 624-6028; Fax: (612) 626-7750; Email: lapar001@umn.edu

Professional Preparation
Massachusetts Institute of Technology, Chemical Engineering, Minor in Chemistry, S.B., 1994
Yale University, Chemical Engineering, M.S., 1995
The Johns Hopkins University, Environmental Engineering, Ph.D., 1999

Appointments
2013-present **Professor**, Department of Civil Engineering, University of Minnesota
2006-2013 **Associate Professor**, Department of Civil Engineering, University of Minnesota
2000-2006 **Assistant Professor**, Department of Civil Engineering, University of Minnesota

Professional Societies
International Water Assocation (IWA), Water Environment Federation (WEF), International Society for Microbial Ecology (ISME), American Society for Microbiology (ASM), American Society of Civil Engineers (ASCE)

Selected Peer-Reviewed Publications
Five most closely related to the proposed project:
1. Burch TR, MJ Sadowsky, and TM LaPara. 2013. Air-drying beds reduce the quantities of antibiotic resistance genes and class 1 integrons in residual municipal wastewater solids. *Environmental Science and Technology* **47**(17):9965-9971.
2. Ling AL, N Pace, MT Hernandez, and TM LaPara. 2013. Tetracycline resistance and Class 1 integron genes associated with indoor and outdoor aerosols. *Environmental Science and Technology* **47**(9):4046-4052.
3. Burch TR, MJ Sadowsky, and TM LaPara. 2013. Aerobic digestion reduces the quantity of antibiotic resistance genes in residual municipal wastewater solids. *Frontiers in Microbiology – Antimicrobials, Resistance and Chemotherapy* **4**:17. doi: 10.3389/fmicb.2013.00017.
4. LaPara TM, TR Burch, PJ McNamara, DT Tan, M Yan, and JJ Eichmiller. 2011. Tertiary-treated municipal wastewater is a significant point-source of antibiotic resistance genes into Duluth-Superior Harbor. *Environmental Science and Technology* **45**(22):9543-9549.
5. Diehl DL, and TM LaPara. 2010. Effect of temperature on the fate of genes encoding tetracycline resistance and the integrase of class 1 integrons within anaerobic and aerobic digesters treating municipal wastewater solids. *Environmental Science and Technology* **44**(23):9128-9133.

Five other products:
1. Wunder DB, DT Tan, TM LaPara, and RM Hozalski. 2013. The effects of antibiotic cocktails at environmentally relevant concentrations on the community composition and acetate biodegradation kinetics of bacterial biofilms. *Chemosphere* **90**(8):2261-2266.

2. Nelson DK, TM LaPara, and PJ Novak. 2012. Structure and function of assemblages of Bacteria and Archaea in model anaerobic aquifer columns: Can functional instability be practically beneficial? *Environmental Science and Technology* **46**(18):10137-10144.
3. Nelson DK, TM LaPara, and PN Novak. 2010. Effects of ethanol-based fuel contamination: Microbial community changes, production of regulated compounds, and methane generation. *Environmental Science and Technology* **44**(12):4525-4530.
4. Ghosh S, SJ Ramsden, and TM LaPara. 2009. The role of anaerobic digestion in controlling the release of tetracycline resistance genes and class 1 integrons from municipal wastewater treatment plants. *Applied Microbiology and Biotechnology* **84**(4):791-796.
5. Zhang P, TM LaPara, EH Goslan, Y Xie, SA Parsons, and RM Hozalski. 2009. Biodegradation of haloacetic acids by bacterial isolates and enrichment cultures from drinking water systems. *Environmental Science and Technology* **43**(9):3169-3175.

Synergistic Activities

- Editorial Board, *FEMS Microbiology Ecology* (2013-date)
- Editorial Board, *Applied and Environmental Microbiology* (2007-date)
- Associate Editor, *Journal of Environmental Engineering* (2008-date)
- Faculty Mentor, Minnesota Environmental Engineers, Scientists and Enthusiasts (2001-date)
- Undergraduate Research Mentor (22 students total; 2001-date)

DANIEL R. ENGSTROM, PH.D.
St. Croix Watershed Research Station — Tel: 651-433-5953
Science Museum of Minnesota — Fax: 651-433-5924
Marine on St. Croix, MN 55047 — E-mail: dre@smm.org
http://www.smm.org/scwrs/

(a) Expertise as Related to Proposed Research

My research centers on the use of lake sediment records to understand long-term environmental change, particularly the effects of human activities on water quality, atmospheric chemistry, and biogeochemical processes. I'm particularly interested in approaches that quantify the magnitude and rates of change and establish mechanistic linkages to modern-day systems. My specific expertise is in environmental chemistry, geochemistry, and radiometric dating.

Areas of Current Research:

1. Atmospheric mercury deposition and cycling in temperate, tropical, and arctic regions
2. Agricultural impacts on nutrient and sediment loading to the upper Mississippi River
3. The effects of climate change on boreal lake ecosystems

(b) Professional Preparation

B.A.	1971	University of Minn., Duluth (Zoology, minor: chemistry) Magna cum Laude
	1971-73	University of Wisconsin, Madison (Zoology: Limnology)
M.S.	1975	University of Minnesota, Duluth (Zoology, minor: Botany)
Ph.D.	1983	University of Minnesota, Minneapolis (Ecology)

(c) Appointments

1999-	Director, St. Croix Watershed Research Station, Science Museum of Minnesota
1995-99	Sr. Scientist, St. Croix Watershed Research Station, Science Museum of Minnesota
1990-	Adjunct Professor, Department of Earth Sciences, University of Minnesota
2004-	Adjunct Professor, Water Resources Science, Univ. of Minnesota

1983-95 Research Associate, Limnological Research Center, Univ. of Minnesota

(d) Professional Affiliations

American Quaternary Association
American Geophysical Union
American Society of Limnology and Oceanography
Ecological Society of America
North American Lake Management Society
International Society of Limnology

(e) *Publications (130 Total)*

(i) Five Project Related

Anger, C.T., C. Sueper, D.J. Blumentritt, K. McNeill, **D.R. Engstrom**, and W.A. Arnold. 2013. Quantification of triclosan, chlorinated triclosan derivatives, and their dioxin photoproducts in lacustrine sediment cores. *Environmental Science & Technology* DOI 10.1021/es3045289

Engstrom, D.R. and N.L. Rose. 2013. A whole-basin, mass-balance approach to paleolimnology. *Journal of Paleolimnology* 49: 333-347.

Hobbs, W.O., **D.R. Engstrom**, S.P. Schottler, K.D. Zimmer, and J.B. Cotner. 2013. Estimating modern carbon burial rates in lakes using a single sediment sample. *Limnology and Oceanography Methods* 11: 316-326.

Anderson, N.J., R.D. Dietz, and **D.R. Engstrom**. 2013. Land-use change, not climate, controls organic carbon burial in lakes. *Proceedings of the Royal Society B* 280: 20131278.

Blumentritt, D.J., **D.R. Engstrom**, and S.J. Balogh. 2013 A novel repeat-coring approach to reconstruct recent sediment, phosphorus, and mercury loading from the upper Mississippi River to Lake Pepin, USA. *Journal of Paleolimnology* DOI 10.1007/s10933-013-9724-8.

(ii) Five Other Significant

McLauchlan, K.K., J.J. Williams, and **D.R. Engstrom**. 2013. Nutrient cycling in the palaeorecord: fluxes from terrestrial to aquatic ecosystems. *The Holocene* 23: 1635-1643.

Lamborg, C.H., **D.R. Engstrom**, W.F. Fitzgerald, and P.H. Balcom. 2013. Apportioning global and non-global components of mercury deposition through ^{210}Pb indexing. *Science of the Total Environment* 448: 132-140.

Schottler, S.P., J. Ulrich, P. Belmont, R. Moore, J.W. Lauer, **D.R. Engstrom**, and J.E. Almendinger. 2013. Twentieth century agricultural drainage creates more erosive rivers. *Hydrological Processes* DOI: 10.1002/hyp.9738.

Cooke, C.A., H. Hintelmann, J.J. Ague, R. Burger, H. Biester, Sachs, J.P., and **D.R. Engstrom**. 2013. Use and legacy of mercury in the Andes. *Environmental Science & Technology* DOI 10.1021/es3048027.

Nagorski, S.A., **D.R. Engstrom**, J.P. Hudson, D.P. Krabbenhoft, E. Hood., J.F. DeWild, and G.R. Aiken. 2013. Spatial distribution of mercury in southeastern Alaskan streams influenced by glaciers, wetlands, and salmon. *Environmental Pollution* 184: 62-72.

(f) Synergistic Activities

Elected Member: *Academy of Science and Engineering,* Swenson College of Science and Engineering, University of Minnesota – Duluth, 2011-

Executive Board and Advisory Committee: *International Paleolimnology Association*, 2006-present

Recipient (on behalf of the SCWRS): *Gulf Guardian Award*, 2010, US Environmental Protection Agency, Gulf of Mexico Program.

Co-chair: *10th International Paleolimnology Symposium*, Minneapolis, MN 2006

Member: *Lake Pepin-Mississippi River TMDL Science Advisory Panel,* 2006-present

Radiometric Dating: Internationally recognized program in ^{210}Pb and ^{137}Cs dating of lake sediments and peat cores; collaboratively dated sediments for research projects involving 150 institutions and university departments; more than 1000 cores dated since 1985.

9. Dissemination and Use

Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. We will also, when appropriate, disseminate results via the media. The audience is not only the scientific community, but also the public, policymakers, and practitioners. The work will also be of interest to the medical community, and we will seek avenues to share the results with this community.

Sediment samples will be freeze-dried for potential future analyses. Extracts will also be labeled and archived (frozen) for potential future analyses.

10. References

Allen, H.K.; Donato, J.; Wang, H.H.; Cloud-Hanse, K.A.; Davies, J.; Handelsman, J. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology*, 8, 251-259.

Anger, C.T.; Sueper, C.; Blumentritt, D.J.; McNeill, K.; Engstrom, D.R.; Arnold, W.A**.** 2013. Quantification of triclosan, chlorinated triclosan derivatives, and their dioxin photoproducts in lacustrine sediment cores. *Environmental Science and Technology,* 47, 1833-1843.

Burch, T.R.; Sadowsky, M.J.; LaPara, T.M. 2013. Air-drying beds reduce the quantities of antibiotic resistance genes and class 1 integrons in residual municipal wastewater solids. *Environmental Science and Technology*, 47, 9965-9971.

Buth, J.M; Steen, P.O.; Sueper, C.; Blumentritt, D.; Vikesland, P.J.; Arnold, W.A; McNeill, K. 2010. Record of Dioxin Photoproducts of Triclosan and its Chlorinated Derivatives in Riverine Sediment Cores. *Environmental Science and Technology,* 44, 4545–4551.

D'Costa, V.M.; Griffiths, E.; Wright, G.D. 2007. Expanding the soil antibiotic resistome: Exploring environmental diversity. *Current Opinion in Microbiology*, 10,481-489.

Diehl, D.L.; LaPara, T.M. 2010. Effect of temperature on the fate of genes encoding tetracycline resistance and the integrase of Class 1 integrons within anaerobic and aerobic digesters treating municipal wastewater solids. *Environmental Science and Technology*, 44,9128–9133.

Engstrom, D. R.; Almendinger, J. E.; Wolin, J. A., 2009. Historical changes in sediment and phosphorus loading to the upper Mississippi River: mass-balance reconstructions from the sediments of Lake Pepin. *Journal of Paleolimnology***,** 41, 563-588.

Engstrom, D.R.; Rose, N.L. 2013. A whole-basin, mass-balance approach to paleolimnology. *Journal of Paleolimnology*, 49, 333-347.

Gibs, J.; Heckathorn, H.A.' Meyer, M.T.; Klapinski, F.R.; Alebus, M.; Lippincott, R.L. 2013. Occurrence and partitioning of antibiotic compounds found in the water column and bottom sediments from a stream receiving two wastewater treatment plant effluents in Northern New Jersey, 2008. *Science of the Total Environment*, 458-460, 107-116.

Hellweger, F. 2013. Simple model of tetracycline antibiotic resistance in aquatic environment: accounting for metal coselection. *Journal of Environmental Engineering,* 139, 913-921.

Huerta, B.; Marti, E.; Gros, M;, Lopez, P.; Pompeo, M.; Armengol, J.; Barcelo, D.; Balcazar, J.L.; Rodriguez-Mozaz, S.; Marce, R. 2013. Exploring the links between antibiotic occurrence, antibiotic resistance, and bacterial communities in water supply reservoirs. *Science of the Total Environment*, 456-457, 161-170.

Jabes, D. 2011. The antibiotic R and D pipeline: an update. *Current Opinion in Microbiology* 14, 564–569.

Jiang, L.; Hu, X.; Xu, T.; Zhang, H.; Sheng, D.; Yin, D. 2013. Prevalence of antibiotic resistance genes and their relationship with antibiotics in the Huangpu River and the drinking water sources, Shanghai, China. *Science of the Total Environment*, 458-460, 267-272.

LaPara, T.M.; Burch, T.R.; McNamara, P.J.; Tan, D.T.; Eichmiller, J.J. 2011. Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *Environmental Science and Technology*, 45, 9543–9549.

Levy, S.B. 2005. Antibiotic resistance – the problem intensifies. *Advanced Drug Delivery Reviews*, 57, 1446-1450.

Luo, Y.; Mao, D.; Rysz, M.; Zhou, Q.; Zhang, H.; Xu, L.; Alvarez, P.J. 2010. Trends in antibiotic resistance genes occurrence in the Haihe River, China. *Environmental Science and Technology,* 44, 7220-7225.

Massey, L.B.; Haggard, B.E.; Galloway, J.M.; Loftin, K.A.; Meyer, M.T.; Green, W.R. 2010. Antibiotic fate and transport in three effluent-dominated Ozark streams. *Ecological Engineering,* 36, 930-938.

Meyer, M.T.; Lee, E.A.; Ferrel, G.F.; Bumgarner, J.E.; Varns, J. 2007. Evaluation of offline tandem and online solid-phase extraction with liquid chlromatography/electrospray ionization-mass spectrometry for analysis of antibiotics in ambient water and comparision to an independent method: U.S. Geologycial Survey Scientific Investigations Report 2007-5021, 28 p.

Pei, R.; Kim, S.-C.; Carlson, K.H.; Pruden, A. 2006. Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARGs). *Water Research*, 40, 2427-2435.

Spellberg, B.; Blaser, M.; Guidos, R.J.; Boucher, H.W.; Bradley, J.S.; Eisenstein, B.I.; Gerding, D.; Lynfield, R.; Reller, L.B.; Rex, J.; Schwartz, D.; Septimus, E.; Tenover, F.C.; Gilbert, D.N. 2011. Combating antimicrobial resistance: policy recommendations to save lives. *Clinical Infectious Diseases*, 52(S5), S397–S428.

Storteboom, H.N.; Arabi, M.; Davis, J.G.; Crimi, B.; Pruden, A. 2010. Tracking antibiotic resistance genes in the South Platte River Basin using molecular signatures of urban, agricultural, and pristine sources. *Environmental Science and Technology*, 44, 7397–7404.

Tamtam, F.; Le Bot, B.; Dinh, T.; Mompelat, S.; Eurin, J.; Chevreuil, M.; Bonte, P., Mouchel, J.-M.; Ayrault, S. 2011. A 50-year record of quinolone and sulphonamide antimicrobial agens in Seine River sediments. *Sediments, Section 1: Sediment Quality and Impact Assessment,* 11, 852-859.

Thevenon, F.; Adate, T.; Wildi, W.; Pote, J. 2012. Antibiotic resistant bacteria/genes dissemination in lacustrine sediments highly increased following cultural eutrophication of Lake Geneva (Switzerland). *Chemosphere*, 86, 468-476.

US Food and Drug Administration. 2009 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals. Available online: http://www.fda.gov/downloads/ForIndustry/UserFees/AnimalDrugUserFeeActADUFA/UCM231851.pdf

2014 Detailed Project Budget

Project Title: Water: Solar driven destruction of pesticides, pharmaceuticals, contaminants

IV. TOTAL ENRTF REQUEST BUDGET *3* years

BUDGET ITEM *(See "Guidance on Allowable Expenses", p. 13)*	**AMOUNT**
Personnel: Arnold (PI, 8% time per year, salary 74.8% of cost, fringe benefits 25.2% of cost). Project supervision, supervision of graduate and undergraduate students and project reporting. Development of tool to predict microcontaminant photodestruction potential.	$ 54,300
Personnel: Graduate student (50% time during academic year, 50% time in summer; 56% salary, 33% tuition, 11% fringe benefits). Conducting solar pesticide removal evalutions, water collection and characterization. Quantify links between organic matter quality and quantity with reactivity.	$ 131,700
Personnel: Undergraduate student #1 (100% time during summer, 12.5% time in academic year; 93.3% salary, 6.7% fringe benefits). Water sample collection and characterization.	$ 18,000
Personnel: Undergraduate student #2 (100% time during summer, 12.5% time in academic year; 93.3% salary, 6.7% fringe benefits). Water sample collection, assist graduate student with photolysis experiments.	$ 18,000
Equipment: Horiba Aqualog benchtop fluorometer for organic matter characterization. Equipment is not available at UMN and critical to fast processing of hundreds of samples.	$ 40,000
Supplies: Supplies (chemical standards, instrument/analytical time, solvents, consumable supplies, notebooks, software licenses; $16,000 total).Maintenance and repair of liquid and gas chromatographs and solar simulator required for analyses and experiments ($6,000 total)	$ 22,000
Travel: Mileage charges and univeristy vehicle rental charges for trips to collect water samples. Hotel/meal charges if overnight stay required.	$ 5,000
Additional Budget Items: Shipping costs for samples collected by others.	$ 2,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 291,000

V. OTHER FUNDS

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ Being Applied to Project During Project Period: none	$ -	
Other State $ Being Applied to Project During Project Period: none	$ -	
In-kind Services During Project Period: Arnold will also devote 1% time per year in kind ($6900). Because the project is overhead free, laboratory space, electricty, and other facilities/adminstrative costs (52% of direct costs excluding permanent equipment and graduate student academic year fringe benefits) are provided in-kind ($103,100)	$ 110,000	secured
Remaining $ from Current ENRTF Appropriation (if applicable): no current project directly applicable	$ -	
Funding History: Proposed project is a result of unexpected findngs of FY 2007 ENTRF funding and past National Science Foundation project	$~200,000	past funding