2018 Project Abstract
For the Period Ending June 30, 2022

PROJECT TITLE: Evaluate Emerging Pathogens in Lakes, Rivers, and Tap Water to Keep Drinking Water Safe
PROJECT MANAGER: Timothy M. LaPara
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 04f as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

APPROPRIATION AMOUNT: $325,000
AMOUNT SPENT: $313,8054
AMOUNT REMAINING: $11,196

Sound bite of Project Outcomes and Results

Seven full-scale drinking water systems were investigated for the presence of *Legionella* and *Mycobacteria*, opportunistic bacterial pathogens of health concern. This research demonstrates these organisms are commonly found in drinking water during the late summer/early fall; water utilities are encouraged to sustain a residual disinfectant to help suppress these pathogens.

Overall Project Outcome and Results

The goal of this project was to investigate the presence of opportunistic pathogens at seven public water utilities within the State of Minnesota that treat surface water (i.e., lakes and rivers), as those water sources are expected to be at greater risk of pathogen contamination than deep groundwater wells. Samples were collected from the water prior to treatment (i.e., the water supply), the water immediately after treatment (i.e., finished water), and at two locations from within each drinking water distribution system. Each of the seven utilities was sampled from one to five times at the four locations for a total of 94 unique sample events.

This project demonstrated that known opportunistic pathogens (e.g., *Legionella* species) can be routinely detected throughout the year in surface water supplies in Minnesota and that water treatment is effective at removing them by 99% or more in most cases. The most concerning opportunistic pathogens that we tested for, *Legionella pneumophila* and *Mycobacterium avium* complex (MAC), were rarely detected and all the observed concentrations in tap water were well below the levels whereby these organisms would be of direct concern (i.e., none of our research results suggest a direct concern with respect to human health). Our results, however, are of indirect concern because these organisms could multiply within a drinking water distribution system should the conditions become favorable for their growth. Hence, our recommendation is that water utilities meticulously maintain a residual disinfectant throughout their distribution systems, particularly in the late summer/early fall when the warmer water creates conditions where *Legionella* species and MAC are most likely to multiply in the system and to be detected.

Project Results Use and Dissemination

Dissemination activities related to this project were severely hindered by the COVID-19 pandemic. We were, however, able to make two different presentations of our preliminary results to the Minnesota Section of the American Water Works association; these presentations were titled “Emerging Pathogens in Lakes, Rivers, and

Tap Water" (September 24, 2020) and "Opportunistic Pathogens in Lakes, Rivers, and Tap Water" (September 16, 2021). We are currently writing three different manuscripts for publication in the peer-reviewed literature that will include the results from this study. We will also share our results with Minnesota Department of Health personnel.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2018 ENRTF Final Report

Today's Date: August 3, 2022

Final Report

Date of Work Plan Approval: 06/05/2018

Project Completion Date: June 30, 2022

Does this submission include an amendment request? Yes

PROJECT TITLE: Evaluate Emerging Pathogens in Lakes, Rivers, and Tap Water to Keep Drinking Water Safe

Project Manager: Timothy M. LaPara

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Location: Statewide

Total Project Budget: $325,000

Amount Spent: $313,804

Balance: $11,196

Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 04f as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

Appropriation Language: $325,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to evaluate emerging pathogens including Legionella and mycobacteria to ensure that surface water used for drinking water and tap water is safe to drink. This appropriation is available until June 30, 2021, by which time the project must be completed and final products delivered.

M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2022]

I. PROJECT STATEMENT:

Minnesota's surface waters serve as the source for drinking water for 25% of Minnesota's population. Water utilities in the State of Minnesota generally do an excellent job of treating this surface water so that it meets all regulatory requirements, suggesting that it is safe for all Minnesotans to drink. From regulatory and microbiological perspectives, drinking water safety is usually evaluated based on the presence of enteric organisms (i.e., organisms found in the digestive tracts of warm-blooded animals), such as total coliforms or *E. coli*. Recent research has suggested, however, that drinking water may also serve as the source for two opportunistic pathogens (i.e., non-enteric organisms), *Legionella pneumophila* and *Mycobacterium avium*. Unlike enteric pathogens, where exposure is via the so-called fecal-oral route, these opportunists are environmental bacteria that tend to occur naturally in water and the exposure route is through inhalation of droplets (aerosols) containing the organisms. This exposure can occur through showering, use of hot tubs, and other means.

Legionella pneumophila is the causative agent of Legionnaires' disease, a deadly form of pneumonia. There was a recent outbreak of Legionnaires' disease in Hopkins, MN in the Fall of 2016, affecting more than 20 people, including one fatality. The elderly and heavy smokers are typically the most likely to develop Legionnaires' disease. Similarly, *Mycobacterium avium* is an organism that causes a deadly pneumonia in immunocompromised people. Both organisms are relatively common in the environment, including surface waters, but little is known about how the abundance of these organisms varies with location (i.e., land use) and over time (i.e., season). Similarly, we know relatively little about the presence of *Legionella* and *Mycobacteria* in surface waters or in tap water because they are not enteric pathogens, which are historically the primary organisms of concern in terms of public health and hence the ones that are targeted by monitoring programs (i.e., fecal indicators).

There is growing evidence and concern that public water supplies are pertinent carriers of *Legionella* and *Mycobacteria*. Bacteria in drinking water are literally distributed to every home, business, and industry connected to a water distribution system. We have observed *Legionella* in tap water in our previous research, even in systems with a substantial disinfectant residual. Thus, we suspect that most public water supplies can serve as the inoculum for *Legionella* to grow in so-called premise plumbing inside of buildings. For example, *Legionella* are known to grow in hot water pipe loops in apartment buildings and hospitals and in hot water heaters operated at less than 125°F. Similarly, we have detected non-pathogenic strains of *Mycobacteria* in drinking water. Although we have not specifically detected *M. avium* in public water supplies, other researchers have detected *M. avium*, suggesting that this opportunistic pathogen could be present in some public water supplies in Minnesota. Fortunately, there is also evidence that a residual of monochloramine (resulting from the reaction of free chlorine and ammonia) in the water can be effective at controlling *Legionella* spp. in building hot water systems.

This project will characterize and quantify both *L. pneumophila* and *M. avium* from source water to tap in four or five water systems in the State of Minnesota. We hypothesize that the majority of surface water samples will have low but measurable levels of both *L. pneumophila* and *M. avium.* We hypothesize that drinking water treatment technologies will substantially reduce the quantity of all microorganisms in the water, including (but not specifically) *L. pneumophila* and *M. avium.* We hypothesize that the maintenance of a chlorine or chloramine disinfectant will further suppress *L. pneumophila* but select for *Mycobacteria* species (these organisms are known to be resistant to disinfectants used in drinking water). We finally hypothesize that the maintenance of a strong residual will select against *M. avium* compared to other species within the genus of *Mycobacteria*.

II. OVERALL PROJECT STATUS UPDATES:

First Update January 31, 2019

With the assistance from personnel from the Minnesota Department of Health, a communication plan has been developed to present our results once they are obtained. This communication will be further developed in future project periods. In addition, four public water utilities have agreed to participate in our study. To date, eighteen different water samples have been collected from these four different public water supplies. Metagenomic DNA has been extracted and purified from these samples and is awaiting analysis.

Amendment Request (2/12/2019)

We are requesting an amendment to our budget. In our original budget, we budgeted for a post-doctoral researcher who was to be hired on July 1, 2019. Instead, we have hired a graduate research assistant who began school at the University of Minnesota in Fall 2018. This student would like to begin working on this project during the Spring 2019 semester. In addition, we are requesting a shift of $1500 from the category of Equipment/Tools/Supplies to Professional/Technical/Service Contracts. This budget adjustment is needed because a superspeed centrifuge in our laboratory broke during the course of our work and is now in need of repair (current repair estimate = $2,000). This superspeed centrifuge is critically important to this project because it is used to concentrate bacterial cells during Activity 1. The remaining repair costs will be paid by other projects that also use the superspeed centrifuge but to a lesser extent (i.e., the cost of repair is being apportioned according to use, past and future).

Amendment Approved by LCCMR 3/8/2019

Second Update June 30, 2019

We initially contacted five water treatment facilities and four of those agreed to participate in the study. To date, the four facilities have been sampled on two different occasions (summer/fall of 2018 and spring/summer of 2019). From these sampling trips, 29 different water samples have been collected. Three more facilities have been contacted as possible additions to the first four facilities. One of the three facilities has agreed to participate, and we are waiting for the responses from the other two facilities. Metagenomic DNA has been extracted and purified from most of these samples and is awaiting analysis (qPCR and sequencing). The Legiolert test, a commercially available culture-based assay for *Legionella pneumophila* – the causative agent in most cases of Legionnaires disease, has been validated in our laboratory using standard bacterial samples. Legiolert testing of water was performed on the 12 samples that were recently collected (spring/summer 2019) from three different facilities. Colilert testing for 19 of the water samples has also been completed. Colilert is a commercially available culture-based assay for fecal coliform bacteria, an indicator of water that has been contaminated with fecal matter. Finally, the water samples have been analyzed for conventional water quality parameters including pH, temperature, and chlorine (both free and total; on treated water samples only).

Third Update January 31, 2020

Currently, in total 7 facilities have agreed to participate in the study, including the initial four facilities and three more facilities that were contacted. To date, all these facilities have been sampled, and except for one which agreed to participate in November 2019, all other 6 facilities have been sampled on at least two different occasions (summer and fall of 2019). One of these facilities has been sampled for all four seasons and the sampling of 5 of the others are expected to be completed by the end of May 2020. The one facility that participated last will be sampled until November 2020. From these sampling trips, 82 different water samples have been collected, adding 53 more to what we had by the last update. Among these samples, recent 65 samples have been applied with PMA dye to prepare for viable qPCR and tested with commercial kit Legiolert as well. Colilert testing for 71 of the water samples has also been completed. Same as last update, all the water

samples have also been analyzed for conventional water quality parameters including pH, temperature, and chlorine (both free and total; on treated water samples only). Metagenomic DNA extraction is in progress and extracted DNA is awaiting analysis (qPCR and sequencing, and probably ddPCR).

Fourth Update June 30, 2020

To date, samples have been collected from all 7 facilities that volunteered to participate in our project. We have completed seasonal sampling (Spring, Summer, Fall, Winter) for one of the 7 facilities; 5 of the other facilities have been sampled for only Summer, Fall and Winter. The planned spring sample collection events at these facilities had to be cancelled due to safety concerns related to the COVID-19 pandemic. For all of the facilities, the seasonal sampling is expected to be finished no later than May 2021. Since the last update, 12 additional samples were collected from 3 different facilities, which are or will be analyzed for genetic targets, fecal coliforms, and cultivable Legionella spp. All water samples have also been analyzed for conventional water quality parameters including pH, temperature, and chlorine (both free and total; on treated water samples only). We are hoping to resume laboratory activities and sample collecting, but this will depend on the status of the COVID-19 pandemic.

Fifth Update January 31, 2021

Progress during the most recent 6-month period has been severely hindered by the COVID-19 pandemic. Due to safety issues, we have not collected any additional samples. We have continued to process and analyze the samples that have been previously collected. Our preliminary results suggest that the surface water supplies used by Minnesota water utilities naturally contain less than 100 million bacteria per liter and of those bacteria, a small fraction (0.001 to 0.1%) are *Legionella* spp. Preliminary results indicate that our drinking water treatment systems reduce the quantities of all bacteria, including *Legionella* spp., in the source water (by 99% to 99.9%), but there is some evidence of the re-growth of *Legionella* within the distribution system and/or in building plumbing systems. The increased risk of *Legionella* within the distribution system appears to primarily occur in the summer and the fall, when water temperatures are higher. A presentation on our preliminary results was given at the annual meeting of the Minnesota section of the American Water Works Association on September 24, 2020.

Amendment Request (1/28/2021) - Amendment Approved by LCCMR 02/05/2021.

We are requesting an amendment to our budget. In our original budget, we budgeted for a post-doctoral researcher who was to be hired on July 1, 2019. Instead, we have hired a graduate research assistant who began school at the University of Minnesota in Fall 2018. This student began working on this project during the Spring 2019 semester and has made excellent progress since then. Unfortunately, this graduate student recently left our program and is no longer working on this project. We have the opportunity to hire a post-doctoral researcher to finish this project, but we will need to again re-budget the project to allow the hiring of a post-doctoral researcher rather than a graduate student.

Project extended to June 30, 2022 by LCCMR 7/1/21 as a result of M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18, legislative extension criteria being met.

Sixth Update June 30, 2021:

Progress during the most recent 6-month period focused on finishing analysis of previously collected samples and analysis of the data generated from those samples. DNA from all samples has now been extracted and purified and used as template to quantify specific organisms (e.g., all Bacteria, *Legionella pneumophila*, *Mycobacteria avium*, *etc*). We also performed more detailed analysis of the *Legionella* spp. detected in our

samples by sequencing PCR-amplified 16S rRNA genes specific to the genus *Legionella*. The results are similar to those reported in the previous work plan update, although the datasets are larger and allow for more robust statistical analysis. Data analysis is on-going.

Amendment Request (8/15/2021) - Amendment approved by LCCMR 8/24/21

We are requesting an amendment to our budget. In our original budget, we budgeted more than $34,000 for undergraduate researchers to work on this project. This prediction substantially exceeded our actual need for undergraduate researchers. Simultaneously, we budgeted $26,000 for expendable laboratory supplies, which we now know is an insufficient amount of funds for the work that we needed to perform; this project category is currently overspent by $7,185.20. As such, we request an amendment to our budget in which we reduce the money budgeted for undergraduate researchers by $8,000 and transfer this money to cover the costs of laboratory supplies.

Seventh Update January 31, 2022:

During this most recent project period, we have attempted to complete all the laboratory analyses associated with this project. Unfortunately, our real time PCR machine started performing poorly and it needs to be replaced (see below for an amendment request). At this point, we have a nearly complete dataset but we are concerned that some of our data was adversely affected by a poorly performing instrument (i.e., the one that needs to be replaced), so we need to re-run our samples.

Otherwise, we are currently attempt to author manuscripts for the peer-reviewed literature to publish our results. Generally speaking, we were able to demonstrate that substantial concentrations of both *Legionella* spp. and *Mycobacteria* spp. are found in the source water. Drinking water treatment technologies generally reduce the quantities of these organisms. Following treatment and distribution, however, *Mycobacteria* spp. usually increase in abundance. This is somewhat expected as *Mycobacteria* spp. are well-known to be resistant to low levels of chlorine and chloramine disinfectant. Most importantly, the opportunistic pathogen, *Mycobacterium avium*, was rarely detected in our samples and only at very low cell concentrations. Alternatively, *Legionella* spp. were sporadically detected, primarily in the late summer months, when water temperatures are the highest and residual disinfectant concentrations are usually at their lowest.

Amendment Request (2/24/2022) - Amendment approved by LCCMR 3/24/22

We are requesting an amendment to our budget. The principal data generated as part of this study comes from quantifying microorganisms via their DNA using a technique known as real time polymerase chain reaction (PCR). We have performed this analysis in the past using a CFX Connect Real Time PCR system by Bio-Rad. Unfortunately, in October/November of 2021, our machine started malfunctioning. We attempted to repair this instrument, but the cost of the repairs ($15,000) was the same as purchasing a new instrument ($15,000). We are requesting an amendment to our budget, therefore, to allow us to spend $2,500 of funds allocated to this project to help purchase a new instrument. The remaining funds for the purchase of this instrument will hopefully come from other three on-going projects (two funded by the MN ENTF; one funded by another agency – the other agency has already agreed to this purchase) and from the discretionary funds from two different University of Minnesota faculty members in the Department of Civil, Environmental, and Geo- Engineering ($5,000 total). The request of $2,500 from this project reflects our projection of the amount of work that will be needed to complete this project using this instrument. From this project, we plan to test ~600 samples for 6 different gene targets representing specific microorganisms. This would, therefore, involve ~50 runs of the real time PCR machine that we propose to purchase. The UMGC would allow us to "rent" one of their instruments, at a cost of $43 per run (i.e., estimated cost for machine time from UMGC would be about $2000, not including increased labor costs and increased difficulty do to working in a second laboratory on the University of Minnesota campus). In our original budget, we had allocated $15,000 to lab/medical services, of which $8,080

remains unspent and we do not anticipate spending. We propose to shift $2,500 from this budget category to a capital equipment category.

Final Update June 30, 2022

The goal of this project was to investigate the presence of opportunistic pathogens at 7 public water utilities within the State of Minnesota that treat surface water (i.e., lakes and rivers), as those water sources are expected to be at greater risk of pathogen contamination than deep groundwater wells. Samples were collected from the water prior to treatment (i.e., the water supply), the water immediately after treatment (i.e., finished water), and at two locations from within each drinking water distribution system. Each of the 7 utilities was sampled from 1 to 5 times at the four locations for a total of 94 unique sample events.

This project demonstrated that known opportunistic pathogens (e.g., *Legionella* species) can be routinely detected throughout the year in surface water supplies in Minnesota and that water treatment is effective at removing them by 99% or more in most cases. The most concerning opportunistic pathogens that we tested for, *Legionella pneumophila* and *Mycobacterium avium* complex (MAC), were rarely detected and all the observed concentrations in tap water were well below the levels whereby these organisms would be of direct concern (i.e., none of our research results suggest a direct concern with respect to human health). Our results, however, are of indirect concern because these organisms could multiply within a drinking water distribution system should the conditions become favorable for their growth. Hence, our recommendation is that water utilities meticulously maintain a residual disinfectant throughout their distribution systems, particularly in the late summer/early fall when the warmer water creates conditions where *Legionella* species and MAC are most likely to multiply in the system and to be detected.

Amendment Request (8/15/2022)

We are requesting an amendment to our budget. In our original budget, we budgeted $264,500 for personnel working on this project but we actually spent $264,542 on personnel. Similarly, we budgeted $38,000 for Equipment/Tools/Supplies but we actually spent $38,466. In contrast, we budgeted $5,000 for travel, of which we only spent $3,876. We therefore request to amend our project budget to increase the budget for personnel from $264,500 to $264,542, increase the Equipment/Tools/Supplies budget from $38,000 to $38,466, and concomitantly decrease our travel budget from $5,000 to $4,492.

Amendment approved by LCCMR 10/11/22

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Sample collection, DNA extraction and preservation
Description: Water samples will be collected from the major surface water supplies in the State (the Mississippi River, the Red River, and Lake Superior) and from four or five public water utility systems that use these water sources. Participating water utilities will remain anonymous and will be identified only by their source water type (lake or river) and the data that we generate. We will collect the "raw" or source water (i.e., lake or river water) from near the intake point for each utility. We will also collect treated water prior to disinfection and following disinfection (i.e., finished water). Finally, we will collect tap water from a publically accessible location (city hall, fire department, etc) within the distribution system of each participating City. We will collect five replicate samples from each utility and from each sample location within the first 16 months of the project period (i.e., 5 utilities $\times$ 4 sample locations $\times$ 5 replicates = 100 samples).

ENRTF BUDGET: $ 94,450

Outcome	Completion Date
1. Sample Collection	October 31, 2019
2. DNA extraction and purification	January 31, 2020

First Update January 31, 2019

Seventeen different water samples have been collected from four different water utilities. The DNA has been extracted and purified from these samples and is awaiting analysis (Activity 2).

Second Update June 30, 2019

Twelve more water samples (29 total) have been collected from four different water utilities. The DNA has been extracted and purified from these samples and is awaiting analysis (Activity 2). Legiolert testing of water was performed on the 12 samples that were recently collected (spring/summer 2019) from three different facilities. Colilert testing for 19 of the water samples has also been completed. Finally, the water samples have been analyzed for conventional water quality parameters including pH, temperature, and chlorine (both free and total; on treated water samples only). Regarding the Colilert testing, raw water samples (i.e., from the surface water supplies) were uniformly positive for fecal coliform bacteria while samples of treated or "finished" water leaving the treatment plants as well as water from taps in the distribution systems were uniformly negative for fecal coliform bacteria, demonstrating the effectiveness of the treatment plants at removing or inactivating harmful pathogens. Regarding Legiolert testing, 3/3 raw water samples were negative, 1/2 finished water samples were positive, and 2/4 of the tap water samples were positive for *L. pneumophila*. Three of the finished/tap water results had to be discarded because the chlorine was not adequately neutralized prior to the test. These extremely limited results suggest that *L. pneumophila* are not always present in surface waters but they may be residing in the water distribution systems or plumbing systems of the buildings where the samples were collected. It is premature to make any firm conclusions about these Legiolert results because they are based on only one complete set of data from only two facilities. More results are needed from follow up testing at these two facilities as well as from the other facilities in the study.

Third Update January 31, 2020

Fifty-three more water samples (82 total) have been collected from seven different water utilities with different features. The pH of samples taken ranged from 6.1 to 9.5 and the temperature measured ranged from 7℃ to 25.3℃. DNA extraction is in progress and extracted DNA is awaiting analysis (Activity 2). In total, Legiolert testing of water was performed on the 65 samples that were recently collected (summer, fall and winter 2019) from these seven different facilities. Colilert testing for 71 of the water samples has also been completed. Finally, the water samples have been analyzed for conventional water quality parameters including pH, temperature, and chlorine (both free and total; on treated water samples only). Regarding the Colilert testing, consistent with data presented in last update, raw water samples were uniformly positive for fecal coliform bacteria while samples of treated or "finished" water leaving the treatment plants as well as water from taps in the distribution systems were uniformly negative for fecal coliform bacteria, demonstrating the effectiveness of the treatment plants at removing or inactivating harmful pathogens. Regarding Legiolert testing, 16/18 raw water samples were negative, 4/16 finished water samples were positive, and 9/31 of the tap water samples were positive for *L. pneumophila*. These results support the idea in our last update that *L. pneumophila* are not always present in surface waters but they may be residing in the water distribution systems or plumbing systems of the buildings where the samples were collected. Additionally, obvious seasonal variations have been observed - except for 2 fall samples with low detection, all other *L. pneumophila* positive samples were summer samples. Nevertheless, Legiolert testing alone may have its restrictions, thus further confirmation by qPCR and ddPCR results will be needed and could provide us with more comprehensive understandings. Besides, most detection of *L. pneumophila* were at very low level, yet the highest concentration of *L. pneumophila* was found in tap water with as high as 939.8 MPN/100mL given by Legiolert, indicating that although judging from these

preliminary data, these facilities have been performing well in controlling the growth of *L. pneumophila*, attentions are still needed for Legionella issue in our drink water systems.

Fourth Update June 30, 2020

Twelve more samples have been collected from three different water utilities on the list. The pH of samples taken ranged from 8.2 to 9.5 and the temperature measured ranged from 7.5°C to 10.3°C. Legiolert and colilert testing of the water was performed on all the 12 samples (winter 2019). Finally, the water samples were analyzed for conventional water quality parameters including pH, temperature, and chlorine (both free and total; on treated water samples only). Raw (untreated) water samples were uniformly positive for fecal coliform bacteria, while samples of treated or "finished" water leaving the treatment plants as well as water from taps in the distribution systems were uniformly negative for fecal coliform bacteria, demonstrating the effectiveness of the treatment plants at removing or inactivating harmful pathogens. Regarding Legiolert testing, all 3 raw water samples were negative and none of the 3 finished water samples were positive. In contrast, 1 of 6 of the tap water samples was positive for *L. pneumophila*.

Fifth Update January 31, 2021

No additional samples were collected during this time period due to safety concerns related to the COVID-19 pandemic. The DNA from all samples collected to date have been extracted and purified.

Sixth Update June 30, 2021

After careful consideration of the *a priori* project goals as well as the safety limitations imposed by the COVID-19 pandemic, we decided that value added by the collection of additional samples was not sufficient to offset the additional risk of collecting those samples. That is, we have concluded all sample collection efforts as part of Activity 1.

Seventh Update January 31, 2022

All work for this activity was completed prior to this project period.

Final Update June 30, 2022

We were able to collect samples from 94 different locations/times from seven different drinking water distribution systems. These efforts exceeded our goals with respect to the number of participating water utilities. We were unable to collect as many total samples as we had initially planned (i.e., at least four sampling events at every utility), which was primarily caused by the COVID 19 pandemic that completely halted sampling activity on this project beginning in Spring 2020.

ACTIVITY 2: Quantification and characterization of microorganisms (especially *Legionella* and *Mycobacteria*)
Description: We will use quantitative polymerase chain reaction (qPCR) to quantify total bacteria (as 16S rRNA genes), total *Legionella* spp. (*ssrA* genes), *Legionella pneumophila* (*mip* genes), and *Legionella pneumophila* serogroup 1 (*wzm* genes), total *Mycobacteria* spp. (*atpE*), and *M. avium* (ITS region) in the samples collected from Activity 1. The composition of the bacterial communities in the water samples from Activity 1 also will be determined by using the polymerase chain reaction (PCR) to amplify the V3-region of the 16S rRNA gene, which will then be sequenced using an Illumina MiSeq instrument at the University of Minnesota Genomics Center. The *Mycobacteria* in the samples will be characterized to the strain level using PCR to amplify the gene encoding a 65-kDa heat-shock protein (*hsp65*) and then sequencing the PCR products by Illumina MiSeq.

ENRTF BUDGET: $ 230,550

Outcome	Completion Date
1. qPCR targeting *Legionella* and *Mycobacteria*	May 31, 2020
2. PCR and Illumina MiSeq analysis of 16S rRNA genes	May 31, 2020
3. PCR and Illumina MiSeq analysis of *hsp65* genes	August 31, 2020
4. Data Analysis	December 31, 2020

First Update January 31, 2019

No progress has been made with this Activity. Samples are currently accumulating so that many samples can be processed simultaneously, which is more efficient and economical.

Second Update June 30, 2019

No progress has been made with this Activity. Samples are currently accumulating so that many samples can be processed simultaneously, which is more efficient and economical.

Third Update January 31, 2020

No progress has been made with this Activity. Samples are currently accumulating so that many samples can be processed simultaneously, which is more efficient and economical.

Fourth Update June 30, 2020

No progress has been made with this Activity due to safety reasons stemming from the COVID-19 pandemic. The samples that we have been collecting, however, have been preserved properly and we will begin quantifying these samples as soon as it is deemed safe to do so by the University of Minnesota.

Fifth Update January 31, 2021

A substantial fraction of the samples collected to date (> 75%) have been analyzed for the presence of all bacteria, *Legionella* spp., and *Mycobacteria* spp. Analysis of these data is on-going.

Sixth Update June 30, 2021

We have analyzed all of the collected samples using quantitative polymerase chain reaction (qPCR) to quantify total bacteria (as 16S rRNA genes), total *Legionella* spp. (*ssrA* genes), *Legionella pneumophila* (*mip* genes), and *Legionella pneumophila* serogroup 1 (*wzm* genes), total *Mycobacteria* spp. (*atpE*), and *M. avium* (ITS region). Legionella spp. (*ssrA* genes) were detected in a substantial fraction of the samples (~80%), most commonly during the warmer weather periods. *Legionella pneumophila* (*mip* genes) were rarely detected (~1% of samples); *Legionella pneumophila* serogroup 1 (*wzm* genes) were not detected in any of the samples that we collected.

We have also determined the composition of the bacterial communities in the water samples by sequencing PCR-amplified 16S rRNA genes. These results suggested that bacterial communities were unique to specific public water systems and they also varied as a function of the time of year. We were also able to detect *Legionella* spp. in these microbiome profiles, consistent with the qPCR results targeting the *ssrA* gene.

Although we had proposed to further characterize the *Mycobacteria* in the samples by sequencing PCR-amplified *hsp65* genes, we decided to not pursue this analysis because *Mycobacteria* were not commonly detected in our samples at substantial concentrations. In addition, we directly analyzed for the *Mycobacteria*

species of greatest concern in water systems, *M. avium*, and did not detect it. Instead, we identified an analogous DNA sequencing method that could more precisely identify the *Legionella* spp. in our samples. The purpose of this sequencing work was to determine if any of the *Legionella* species present are among those that have been identified as the causative agents of disease in humans. *L. pneumophila*, for example, is well known for its ability to cause disease in humans, but there are several other disease-causing species such as *Legionella longbeachae*. Although this analysis is on-going, it seems that most of the *Legionella* spp. in our samples have not been previously cultured or characterized, so their potential to cause disease in humans is unknown but unlikely to be significant.

Seventh Update January 31, 2022

Our primary goal for this project period was to analyze our results and begin authoring manuscripts for publication in the peer-reviewed literature. During the process of authoring these manuscripts, we identified a few potential irregularities with our data and we attempted to re-analyze some of the samples (i.e., we had collected and preserved the DNA from these samples, thus allowing for re-analysis). Unfortunately, our machine that performs the PCR analysis started malfunctioning so we were unable to complete this work. We are hoping to quick re-analyze these samples during the final project period and author manuscripts describing our novel results and conclusions.

Final Update June 30, 2022

We were able to perform quantitative PCR on all of our samples for all bacteria, *Legionella* species, and *Mycobacteria* species, as initially envisioned. We decided not to perform detailed sequence analysis of the *hsp65* gene from *Mycobacteria* species because the qPCR assay targeting MAC allowed us to directly quantify the most concerning *Mycobacteria* species from a public health perspective. We were able apply Illumina MiSeq technology to PCR-amplified 16S rRNA genes to provide a detailed cataloging of the organisms in our samples; these results (while not explicitly discussed above) were used to validate our qPCR methods targeting *Legionella* species and *Mycobacteria* species.

IV. DISSEMINATION:

Description:

Findings will be disseminated directly to each of the sampled utilities as a written report and an in-person presentation (if desired by the utility). Findings will also be disseminated and archived via reports to LCCMR, peer-reviewed publications (published open-access), and presentations at conferences (particularly the annual meeting of the State's American Water Works contingency in Duluth). We will also, when appropriate, disseminate results via press releases to the media and via the MDH website. 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. We will preserve the anonymity of participating utilities in our press releases and publications upon their request and work with them and the MDH to determine how best to communicate the results from their individual systems to their customers.

First Update January 31, 2019

There has been no dissemination activity during this project period.

Second Update June 30, 2019

There has been no dissemination activity during this project period.

Third Update January 31, 2020

There has been no dissemination activity during this project period.

Fourth Update June 30, 2020

There has been no dissemination activity during this project period.

Fifth Update January 31, 2021

We gave a presentation on our preliminary results at the annual meeting of the Minnesota Section of the American Water Works Association on September 24, 2020. The title of the presentation was "Emerging Pathogens in Lakes, Rivers, and Tap Water."

Sixth Update June 30, 2021

There has been no dissemination activity during this project period.

Seventh Update January 31, 2022

We gave a presentation on our preliminary results at the annual meeting of the Minnesota Section of the American Water Works Association on September 16, 2021. The title of the presentation was "Opportunistic Pathogens in Lakes, Rivers and Tap Water."

Final Update June 30, 2022

Dissemination activities related to this project were severely hindered by the COVID-19 pandemic. We were, however, able to make two different presentations of our preliminary results to the Minnesota Section of the American Water Works association; these presentations were titled "Emerging Pathogens in Lakes, Rivers, and Tap Water" (September 24, 2020) and "Opportunistic Pathogens in Lakes, Rivers, and Tap Water" (September 16, 2021). We are currently writing three different manuscripts for publication in the peer-reviewed literature that will include the results from this study.

V. PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview: See attached budget spreadsheet

Explanation of Capital Expenditures Greater Than $5,000: NA

Explanation of Use of Classified Staff: NA

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours: 8090	Divide by 2,080 = TOTAL FTE: 3.9

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours:	Divide by 2,080 = TOTAL FTE:

B. Other Funds:

SOURCE OF AND USE OF OTHER FUNDS	Amount Proposed	Amount Spent	Status and Timeframe
Other Non-State $ To Be Applied To Project During Project Period:			
	$	$	
Other State $ To Be Applied To Project During Project Period:			
	$	$	
Past and Current ENRTF Appropriation:			
M. L. 2016, Chp 186, Sec. 2, Subd. 04f	$299,000	$287,463	Project is completed.
Other Funding History:			
	$	$	

VI. PROJECT PARTNERS:

A. Partners receiving ENRTF funding

Name	Title	Affiliation	Role

B. Partners NOT receiving ENRTF funding

Name	Title	Affiliation	Role

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

The implications of this project could be substantial with respect to the operation and management of public water supplies in the State of Minnesota. We will provide specific technical support to the participating utilities regarding strategies to minimize the risk posed by *Legionella* and *Mycobacteria* to public health; we will provide analogous general technical support to non-participating utilities within the State of Minnesota also (i.e., the quality of our technical support will depend, to a certain extent, on our knowledge of a public water supplier). It is also possible that this project will uncover new questions that require further research; in that case, we will apply to the Minnesota Environment and Natural Resources Trust Fund or other sources (e.g., National Science Foundation) for financial support.

VIII. REPORTING REQUIREMENTS:

- **The project is for 4 years, will begin on July 1, 2018, and end on June 30, 2022.**
- **Periodic project status update reports will be submitted January 31 and June 30 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2022.**

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet - NA

D. Acquisition, Easements, and Restoration Requirements - NA
E. Research Addendum – Separate document

Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2018 Final Budget Spreadsheet

Project Title: Evaluate Emerging Pathogens in Lakes, Rivers, and Tap Water to Keep Drinking Water Safe
Legal Citation: M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 04f
Project Manager: Timothy M. LaPara
Organization: University of Minnesota
College/Department/Division: Department of Civil, Environmental, and Geo- Engineering
M.L. 2018 ENRTF Appropriation: $325,000
Project Length and Completion Date: 4 years; June 30, 2022
Date of Report: August 3, 2022

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Amended Budget 8/15/22	Amount Spent	Balance	
BUDGET ITEM				
Personnel (Wages and Benefits) - Overall	$264,542	$264,542	$0	
Project Management, Timothy LaPara ($53,877: $17,431 in year 1, $17,954 in year 2, $18492 in year 3;8.3% FTE 75% to salary, 25% to fringe benefits)				
Project Management, Raymond Hozalski ($59,761: $19334 in year 1, $19,914 in year 2, $20512 in year 3;8.3% FTE 75% to salary, 25% to fringe benefits)				
Post-doctoral researcher ($23,404, 100% FTE, 82% to salary, 18% to benefits)				
Research Assistant at U of M ($100,722 ; 50% FTE; 57% to salary, 43% to benefits)				
Undergraduate Researchers ($34,734; 1.4 FTE; 100% salary, 0% to benefits)				
Professional/Technical/Service Contracts	$12,500	$6,920	$5,580	
UMGC for PCR and Illumina Sequencing				
Service and repair of Superspeed Centrifuge				
Equipment/Tools/Supplies - Overall (estimated below)	$38,466	$38,466	$0	
General lab supplies ($4500)				
IDEXX Supplies for measuring E coli and coliforms ($3000)				
Peristaltic pumps for sample collection (3; each = $2500)				
Membrane filters ($2500)				
Portable power generators (3; each = $1000)				
qPCR Supplies ($5000)				
DNA Extraction Kits ($1200)				
PCR purification kits ($800)				
Real Time Machine (partial purchase; $2500)				
Travel expenses in Minnesota				
Travel to water utilities in Minnesota to collect water samples; travel to MN water conferences to present research; travel to water utilities to disseminate results	$4,492	$3,876	$616	
Other				
Open-access Publication Fees	$5,000	$0	$5,000	
COLUMN TOTAL	**$325,000**	**$313,804**	$11,196	$11,196