METAGENOMIC ANALYSIS OF MICROBIAL POPULATIONS IN AQUAPONICS SYSTEMS

Chairperson: Matthew Rogge, University of Wisconsin-Stevens Point

Industry Advisory Council Liaison: Rebecca Nelson, Nelson and Pade Aquaponics, Inc.

Extension Liaison: Nicholas Phelps, University of Minnesota

Funding Request: $24,596

Duration: 1 year (July 1, 2016 to June 30, 2017)

Objectives:

1. Collect microbial samples from multiple sites within an aquaponics system and from various systems across a geographic range and extract bacterial DNA using commercially-available kits.
2. Submit DNA samples to the UW-Madison Biotechnology Center for PCR amplification, Illumina next-generation sequencing, and bioinformatics analyses to compare microbial populations among sampling sites within a system, among systems at a single production site, and among production sites in the North-Central Region (NCR).
3. Develop a fact sheet explaining the importance of microbial populations in an aquaponics system, including the risk that certain organisms can cause (e.g., plant, fish, and human pathogens), develop a recorded presentation for dissemination using the NCRAC website, and produce oral presentations and a peer-reviewed manuscript.

Proposed Budget

Institution	Principal Investigators	Objective(s)	Year 1	Total
University of Wisconsin-Stevens Point	Matthew Rogge	1, 2, 3	$18,596	$18,596
University of Minnesota	Nicholas Phelps	1, 3	$4,500	$4,500
University of Wisconsin-Stevens Point	Daniel Graf	2, 3	$1,500	$1,500
		Total	$24,596	$24,596

Non-funded Collaborator

Rebecca Nelson	Nelson and Pade Aquaponics, Inc.

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PROJECT SUMMARY

Microbial populations are vital to the success of an aquaponics system; however, the community composition and functional diversity of microbial populations in a system are largely unknown. We propose to use metagenomic DNA sequencing techniques to characterize populations of microbes in aquaponics systems in the Midwest. Metagenomic analyses use DNA sequences to identify the microbes, as opposed to traditional culture-based techniques. It is estimated that less than 1% of microbes are culturable in a laboratory setting; therefore, identifying the microbial community composition based on DNA is a more robust method of studying microbial populations. These techniques are used extensively to study the microbial populations of the human body (e.g., the human microbiome) and have been used to identify communities of microbes in soil, wastewater treatment facilities, and more recently, those found in biofilters of recirculating aquaculture systems. Metagenomic analysis offers a cutting-edge, robust method of studying and characterizing the "aquaponics microbiome," which may lead to enhanced fish and plant production in aquaponics systems. A better understanding of the microbial populations involved in removing chemicals toxic to fish while producing necessary plant nutrients will likely result in an optimized, more efficient, and likely more profitable production system.

JUSTIFICATION

Aquaponics is an agricultural practice that combines techniques used in aquaculture with those used in hydroponics (Rakocy et al. 2006). In this type of system, the physiological activities of the fish and plants complement each other. In modern, commercially-viable aquaponic systems, fish are fed a quality fish food, and the waste generated by the fish is processed through a biofilter, which contains substrate for the growth of nitrifying bacteria. The populations of bacteria in the biofilter, as well as those associated with other surfaces in the system, convert the toxic nitrogenous wastes of fish into nitrate, which is much less toxic. The nitrate produced serves as fertilizer for the plants growing in the system. Other amendments also can be required, depending on the mineral content of the water and fish feed in order to provide optimum plant growth (Rakocy et al. 2006). Because fish waste biomass is converted into plant tissue, the water can be reused, resulting in a sustainable source of food production. Although there is a rudimentary understanding that microbes are involved in the nutrient cycling within an aquaponics system, a better understanding of the species present and the functions they perform will lead to improved production of a fresh, safe, and local food supply.

There are likely many important populations of microbes present in an aquaponics system playing a variety of roles in the success of the system. As described above, there are populations that are necessary for the efficient breakdown of fish food and fish wastes into materials available for uptake by plant roots. An aquaponics system is an ideal place for microbes to inhabit due to the warm, moist environment and high nutrient content of the water. Initial studies in our lab indicated that the microbial load of total aerobic microbes over a 12-month period was approximately $10^{5.5}$ colony forming units per ml (CFUs/ml) of water (unpublished results). The microbial populations on the plant roots, however, were significantly greater at $10^{7.2}$ CFU/g during the same period, suggesting that surfaces within an aquaponics system may harbor larger microbial populations or microbial diversity. It is unknown what these microbes are, which are beneficial or detrimental to an aquaponic system, or if the growth of beneficial organisms can be enhanced or the growth of detrimental organisms can be inhibited. Plants and fish have a normal microbiome that aids in digestion, immune function, and other factors related to health and growth (Mendes et al. 2013; Oulas et al. 2015; Perez et al. 2010; Tan et al. 2015), and characterizing the microbiome of an aquaponics system can allow producers to colonize systems with beneficial microbes, support those beneficial populations, and ultimately increase efficiency and production in their systems, thereby increasing profits and economic viability.

Due to the vast numbers and types of microbes that can inhabit an aquaponics system, it is unreasonable to attempt traditional culture techniques to identify common or uncommon microbes in a system. Recent advances in next generation sequencing and metagenomic analyses to study microbial population structure (Aguiar-Pulido et al. 2016; Oulas et al. 2015) eliminate the need to culture individual organisms and identify them through biochemical, physiological, or other culture-based strategies. Furthermore, because many microbes cannot be culture in laboratory settings, the information collected through traditional methods provides an incomplete view of the microbial populations. By studying the DNA that is present in an environment and how abundant that DNA is, scientists can assess microbial diversity and abundance without having to culture any of the microbes. With these analyses, the

microbial populations present in different parts of an aquaponics system, among different systems within a facility, or among different aquaponics facilities can be identified, analyzed, and compared to determine risks associated with aquaponic growing, as well as identify microbes that are required or beneficial for rapid waste conversion and production in a system.

There have been no published studies that describe the study of an aquaponics microbiome. The potential benefits of metagenomic analyses in aquaculture and aquaponics, however, have recently been reviewed (Martínez-Porchas and Vargas-Albores 2015; Munguia-Fragozo et al. 2015). Although this technology has not yet been used to study the microbial populations within an aquaponics system, a recent publication described the use of metagenomics to characterize populations of microbes in recirculating marine aquaculture biofilters (Huang et al. 2016). Metagenomic analyses have been used to study the gut microbes of many fish species to determine beneficial microbes involved in digestion (Wong et al. 2013; Wu et al. 2012) . Metagenomics has also been used to study microbial populations involved in nutrient recycling from wastewater (Beale et al. 2016; Marshall et al. 2013; Nielsen et al. 2012; Sales and Lee 2015). Finally, many studies have analyzed the microbiome of plant roots, which have significant interaction with soil microbes (Reinhold-Hurek et al. 2015; Tkacz and Poole 2015)

There is a great wealth of information currently available regarding the role of microbes in the various functions that occur in an aquaponics system. It is necessary to begin identifying the microbes present in aquaponics systems and compare those to populations of microbes demonstrated to be important in nutrient cycling, plant health, and fish health in order to optimize production within the system. This proposal aims to begin that process by identifying microbial populations present on surfaces in several parts of aquaponic systems in greenhouses and warehouses. Those populations will be compared among regions of the system, systems at a facility, and among different facilities to compare and contrast microbial populations. Once successful, further analyses can be done at additional facilities nationally and internationally to better understand the roles that microbes play in an aquaponics system. A better understanding of these microbes will allow for colonization of aquaponics systems with the beneficial microbes and maintenance of those microbes to increase efficiency and productivity of the systems.

RELATED CURRENT AND PREVIOUS WORK

Extensive literature searches did not locate any studies that used metagenomic analyses to characterize microbial populations in aquaponics systems. A recent review (Munguia-Fragozo et al. 2015), however, evaluated the potential benefits that "-*omic*" techniques, including metagenomics, can have for aquaponic production, but to date, there are no studies published that describe metagenomic analyses.

Studies have been conducted that evaluate food safety aspects of aquaponics produce, specifically evaluating the risk of foodborne pathogens in an aquaponics system. Although these studies exist, they tend to be small-scale studies and not peer-reviewed. Aquaponics facilities, both commercial and backyard-scale, were analyzed for the presence of generic *Escherichia coli*, *E. coli* O157:H7, and *Salmonella* (Fox et al. 2012). Although low levels of generic *E. coli* and fecal coliforms were detected, neither *E. coli* nor *Salmonella* were detected by PCR. A second study compared aquaponically-grown lettuce to conventionally- and organically-grown lettuce for the presence of total aerobic bacteria, coliforms, *E. coli*, mold and yeasts (Sirsat and Neal 2013), but did not evaluate microbial loads in the various production systems.

Recently, our lab completed a 12-month study assessing microbial loads in a greenhouse production-scale aquaponics system. Water, lettuce leaf, and lettuce root samples were analyzed for total aerobic bacteria, coliforms, *E. coli*, *Salmonella*, and *Shigella*. Lettuce roots had significantly greater ($p < 0.05$) total aerobic bacteria than leaf or water samples, suggesting that surfaces within the system carry greater microbial loads than the system water. Coliform levels averaged between 1,000 and 10,000 per 100 mls or per gram for all samples. Neither *Salmonella* nor *Shigella* were detected during the sampling period. Biochemical and genetic analyses of the coliform isolates indicated that 95% (445 out of 468) of the coliforms isolated were from the genus *Citrobacter*. Many of these *Citrobacter* isolates exhibited characteristics that could lead to false-positive identification for *E. coli*, such as vigorous fermentation of lactose and occasional production of β-glucoronidase, which is used by many approved food and water testing protocols to specifically identify *E. coli*. Other *Citrobacter* isolates also resembled *Salmonella* on Hektoen enteric agar, because they produced hydrogen sulfide gas and did not ferment lactose,

resulting in a black colony. These findings indicate that non-pathogenic organisms in an aquaponics system may result in false-positive results when routine water and food safety tests are being conducted.

Although these studies have preliminarily characterized limited populations of microbes in an aquaponics system, the total aerobic counts indicate significantly greater total populations of microbes. The total aerobic counts, however, do not allow the growth of anaerobic microbes that are likely present in the system, so it is likely that the total microbial load is greater than estimated by using total aerobic counts. Furthermore, it is estimated that less than 1% of microbes can be cultured in laboratory settings. All of this suggests there is a significant abundance and diversity of microbes in an aquaponics system. Thus, it is vital to the aquaponics industry to be aware of and understand the microbes present in their systems.

The following databases were examined to verify that the research proposed here is original and does not duplicate previously funded projects:

- http://cris.nifa.usda.gov
- http://reeis.usda.gov
- National Sea Grant Office Funding Page: http://seagrant.noaa.gov/fudning/rfp.html
- State Sea Grant Program: http://www.seagrant.noaa.gov/other/programsdirectors.html
- NOAA Office of Aquaculture Funding Opportunities Page: http://www.nmfs.noaa.gov/aquaculture/funding/funding.html

There were no current or previously funded projects found in any of the databases that were similar to this project.

ANTICIPATED BENEFITS

Aquaponic production is not a new practice (Rakocy et al. 2006), but the industry has not yet proven to be economically viable on a large-scale production scale. While there can be numerous variables involved, e.g., biosecurity, utility costs, feed prices, water sources, etc., if microbial productivity can be enhanced leading to optimized production of healthier plants and fish, the practice may prove to be economically viable. This study aims to characterize bacterial communities in various aquaponics systems and will allow for comparisons between location of the system (greenhouse versus warehouse), types of fish in the systems, fish stocking densities, different types of surfaces in a system (e.g., foam, plastic, netting, etc.). The effect that surface structure plays in the types and abundance of microbes in a system is unknown. Although aquaponics producers presume that heterotrophic bacteria are present to convert solids wastes into minerals that plants can use, and nitrifying bacteria convert nitrogenous fish waste into nitrate that plants use, it is not known where these populations occur in a system and if their growth can be enhanced. Furthermore, because the systems offer space and resources for microbes to grow, there is a risk that harmful microbes can be introduced and thrive. These analyses may better identify where those harmful microbes can flourish, and allow targeted treatment (cleaning, UV sterilization, etc.) of those areas to eliminate the harmful populations. Ultimately, this work will provide a baseline from which to generate additional studies nationally and internationally to characterize the "aquaponics microbiome." From that, further studies to manipulate microbial populations, such as prebiotic or probiotic treatment, can be conducted to enhance production in a system. Once a better understanding of important microbes is available, further research into different fish species, stocking densities, plants, water temperatures or any other production variable can also incorporate the study of the microbial populations to determine if those production variables positively or negatively affect microbial populations.

OBJECTIVES

The objectives of this work are to

1. Collect microbial samples from multiple sites within an aquaponics system and from various systems across a geographic range and extract bacterial DNA using commercially-available kits.
2. Submit DNA samples to the UW-Madison Biotechnology Center for PCR amplification, Illumina next-generation sequencing, and bioinformatics analyses to compare microbial populations among sampling sites within a system, among systems at a single production site, and among production sites in the North-Central Region (NCR).

3. Develop a fact sheet explaining the importance of microbial populations in an aquaponics system, including the risk that certain organisms can cause (e.g., plant, fish, and human pathogens), develop a recorded presentation for dissemination using the NCRAC website, and produce oral presentations and a peer-reviewed manuscript.

PROCEDURES

Objective 1: Collect microbial samples from various parts of an aquaponics systems and from various systems across a geographic range and extract DNA using commercially-available kits.

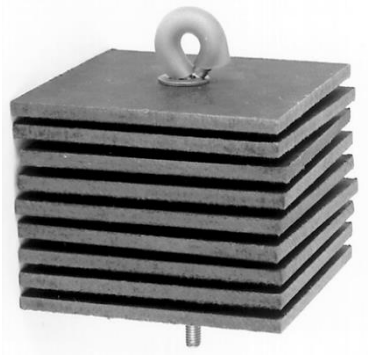

Figure 1. Hester-Dendy benthic sampler. (http://www.rickly.com/as/images/hestersq.jpg)

Microbial samples will be collected from aquaponics systems using samplers that mimic surfaces within that system. The samplers will resemble Hester-Dendy benthic samplers (Fig. 1), but will be constructed using materials used in construction of the aquaponics system. For example, microbial samplers used in fish tanks will be made of material from which the tanks are constructed. We feel that providing the specific substrate available for colonization will result in the best representation of actual microbial populations found at that site. Samplers will be placed at each location and allowed to develop local microbial populations for three months before sampling. This sampler design will allow for multiple, repeated sampling of the microbial populations over time. Due to the limited time allowed for the Rapid Response Program, however, only one sample in time will be analyzed. Future studies resulting from this preliminary work will incorporate the analysis of microbial populations over time. In the event that sampling sites allow for the removal of representative substrates (e.g., gravel from gravel filters), a sample of colonized media will be removed and processed. Finally, the roots of the plants provide surface area for microbial growth. Root samples will be aseptically removed from the plants, sliced using sterile scalpels, and used for DNA extraction.

Samples will be collected in duplicate and processed for DNA extraction. The duplicated samples are necessary to ensure overlapping populations of microbes between the two samples, which indicate that the sampling techniques consistently represent the entire microbial population present in the samples. Sampler plates will be placed into sterile Whirl-Paks containing 50 ml of sterile phosphate-buffered saline (PBS), and root cuttings and media samples will be deposited into centrifuge tubes. All samples will be stored on ice and transported to the lab for further processing. Organic material and biofilm found on the samplers will be removed using sterile tissue culture scrapers and rinsing with the sterile PBS. The liquid containing organic debris will be aseptically transferred to a centrifuge tube and centrifuged to pellet the debris and bacterial cells. The pellet will be raised to an approximate volume of 250 µl using sterile PBS. The media samples and root cuttings will also be resuspended in 250 µl of sterile PBS. All samples will be subjected to DNA extraction using the PowerSoil DNA® Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA), which uses bead-beating techniques to pulverize cells. This soil kit effectively removes PCR inhibitors commonly found in DNA purified from organically rich substrates. Removal of these contaminants is necessary to prevent inhibition of downstream applications, such as PCR and DNA sequencing. An extensive list of relevant publications that have successfully used this kit to prepare samples for metagenomic analysis can be found at the product website (https://mobio.com/powersoil-dna-isolation-kit.html). Concentrations of purified DNA will be determined using a Qubit 2.0 Fluorimeter (Thermo Fisher Scientific, Waltham, MA) per the manufacturer's instructions.

Sampling will occur at the University of Wisconsin-Stevens Point Aquaponics Innovation Center (UWSP AIC), the University of Minnesota Aquaponics Facilities, and at commercial aquaponics facilities in Minnesota. The UWSP AIC has six replicate greenhouse aquaponics systems that will be available for sampling. At the time of this proposed work, Chris Hartleb will be performing a study in the systems to compare low, medium, and high stocking densities of walleyes in aquaponics systems. Chris has agreed to allow us to sample the microbial populations simultaneously with his study. Samplers will be placed in the fish tanks, mineralization tanks, and raft tanks of each of the systems. The materials used to make the samplers for the mineralization tank will feature orchard netting, which is used to provide abundant surface area in the mineralization tanks. The samplers in the fish and raft tanks will incorporate pieces of the plastic used to form those tanks. Also in the raft tanks, samplers will be constructed using the foam material used to construct the floating rafts. Finally, duplicate root cuttings from each raft tank will

be collected from each system, and colonized beads from the biofiltration unit will be collected. Sampling at the UWSP AIC site will allow for comparison between different locations within the same system, between different systems at the same facility, and to evaluate the effects of different fish stocking densities on the populations of microbes within the system.

Sampling at the Minnesota sites will include eight replicated greenhouse systems raising yellow perch and using gravel filters; a warehouse aquaponics system raising koi and using a mineralization tank; and a greenhouse system with two replicated systems raising koi that use gravel filters. Samplers will be placed in the mineralization tank of the warehouse system. Alternatively, at sites that incorporate a gravel filter, media from the filter will be collected for analyses. From each system, root cuttings will also be taken for analyses. Thus, sampling in Minnesota will also allow for within-system, among-system, and among-location analyses of microbial populations in aquaponics systems, as well as the potential for comparisons between Wisconsin and Minnesota samples. Samples collected from Minnesota sites will be shipped to UWSP for DNA extraction.

Objective 2: Submit DNA samples to the UW-Madison Biotechnology Center for PCR amplification, Illumina next-generation sequencing, and bioinformatics analyses to compare microbial populations among sampling sites within a system, among systems at a single production site, and among production sites in the North-Central Region.

Metagenomics, which uses next-generation sequencing techniques, has traditionally been used for medical and other human health-related studies, thereby justifying high per-sample costs. The popularity and widespread use of these technologies, however, has resulted in decreased costs, making them more accessible to non-medical labs. The potential benefits of metagenomics analyses in aquaculture and aquaponics have recently been reviewed (Martínez-Porchas and Vargas-Albores 2015; Munguia-Fragozo et al. 2015). Although this technology has not yet been used to study the microbial populations within an aquaponics system, a recent publication described the use of metagenomics to characterize populations of microbes in recirculating marine aquaculture biofilters (Huang et al. 2016). Various types of biofilters, including moving bed bioreactors, submerged biofilters, and fluidized sand biofilters at different aquaculture facilities were analyzed and compared. Notable results included the discovery of greater populations in static beds, likely due to less shearing forces from water, and that the sampled biofilter types had relatively little overlap in the types of microbes present. The analyses provided valuable insight into the populations of nitrifying bacteria colonizing the filters.

Metagenomic analyses generate millions of base pair reads, resulting very large data sets produced. The enormity of these sets necessitates that a facility not only have the sequencing machines, but also the computing power to handle and analyze that much data. As such, it is common to have sequencing labs provide the sequencing and analysis services. University of Wisconsin-Madison has agreed to work with our group to perform the sequencing and bioinformatics analyses necessary for this project. Our group will submit purified DNA samples to the UW-Madison Biotechnology Center for next-generation Illumina sequencing. The DNA will first be used as template in PCR using v3-v4 primers, which span a variable region in the prokaryotic 16S DNA sequence. This PCR is standard for metagenomics analysis of microbial populations, and was the primer set used by Huang et al. (Huang et al. 2016). Theoretically, most bacterial species have a slightly different sequence in this region, and sequencing of that region will allow us to distinguish different types of bacteria. Each PCR product is processed to add a terminal barcode sequence that will identify that sequence from all others in the reaction. The individual DNA pieces are then sequenced to determine the ATGC code. The sequences are compared to genetic databases to determine the organism from which the specific DNA sequence originated. These identifications are compiled and analyzed using bioinformatics analyses (described below) to determine microbial community populations in the samples. From this data, populations in different parts of the system, or from different systems across a geographic range, can be compared.

Microbial α- and β-diversity (within and among samples, respectively) in aquaponics samples will be estimated from ultra-high-throughput metagenomic analyses of 16S DNA. This approach applies cutting-edge technology to provide a snapshot of microbial community composition. The QIIME 1.9.0 (Quantitative Insights Into Microbial Ecology) bioinformatics pipeline (Caporaso et al. 2010) (http://qiime.org; as implemented by the UW-Madison Bioinformatics Resource Center) provides standard estimates of OTU (Operational Taxonomic Unit, roughly equivalent to "species") composition and richness. These analyses are routinely applied to identify organisms (phyla, classes, genera) and quantify the microbial diversity from environmental samples (Costello et al. 2009; Huang et al. 2016). Rarefaction curves based on sampling of sequencing reads will verify the efficacy of

metagenomic analysis to accurately sample the microbial community composition. Changes in OTUs among samples (i.e., turnover) will be determined through UniFrac analysis, which quantifies the genetic dissimilarity among microbial communities. Various clustering analyses (i.e., Principle Component Analysis, UPGMA) of the UniFrac distance matrix will be used to summarize the extent to which microbial lineages are shared among samples. In addition, pairwise similarity indices (i.e., Jaccard, Raup-Crick) will be applied to estimate similarity of OTU composition among samples (Gülay and Smets 2015), and each community will be characterized by functional groups (nitrite- and ammonia-oxidizing bacteria, denitrification, mineralization, pathogens, etc.). Initial analyses will be provided by UW-Madison, and additional data analysis will be conducted by UWSP personnel.

Objective 3: Develop a fact sheet explaining the importance of microbial populations in an aquaponics system, including the risk that certain organisms can cause (e.g., plant, fish, and human pathogens), develop a recorded presentation for dissemination using the NCRAC website, and produce oral presentations and a peer-reviewed manuscript.

The information collected from these analyses will set the foundation for future microbial population studies in aquaponics and potentially aquaculture. The functional microbial populations present in both aquaponics and RAS systems (e.g., ammonia- and nitrite-oxidizing microbes) are vital for the efficient operation and optimized production of the systems. Beyond the populations involved in the nitrogen cycle, there lies the potential for identifying and monitoring other important groups of microbes in the system, such as fish pathogens, plant pathogens, human pathogens, or other undesirable microbes. As conditions change in an aquaponics system (e.g., temperature, fish species, plant species, stocking densities, etc.), there is likely to be an effect on microbial populations, too. While previous types of research analyses justifiably focus on fish yield, plant yield, water chemistry parameters, and other important measurables, little attention has been paid to truly understanding the populations of organisms that have significant roles in making a production system optimized and efficient. Metagenomic analyses now allow us to pull away the curtain from these "black box" processes occurring in production systems to potentially cultivate and manipulate beneficial microbial populations while monitoring and inhibiting the growth of undesired microbes. The ONE question that needs to be answered before any of that can occur, however, is, "What microbes are there, and what are they doing?" That is what we do not know. As such, an important part of this proposal is to share with the producers a breakdown of the microbes present in their systems, how some microbes are benefiting the system, how some may be harming it, and how producers can get the right microbes to help increase efficiency and profitability in their systems.

A fact sheet will be produced that will explain the populations of microbes in the systems, how to implement good biosecurity practices that reduce the risk of introducing harmful microbes, risks that certain microbes and certain practices create, and signs to watch for in an aquaponics system that can signal a producer that the microbial populations are becoming unbalanced. In addition, a recorded presentation will be created by the investigators to provide audio/visual information and instructions regarding microbial populations in an aquaponics system. The presentation will focus on identifying and describing areas that are rich in microbial life; describing the useful microbes in the system, as well as ways that microbes can be problematic; and providing methods to improve the system environment to encourage the growth of useful microbes while inhibiting the growth of unwanted organisms. UWSP and UM personnel will be involved in preparing these deliverables.

Oral presentations will be prepared for delivery to other scientists in aquaponics and aquaculture to build the knowledge base and foster further research into the microbial populations and their roles in aquaponics and aquaculture. A manuscript will be prepared and submitted to a relevant peer-reviewed journal for worldwide dissemination of the information. Ultimately, the hope is that this work will provide the basis for future, larger, federally-funded studies that collect samples from across the region, nation, or world, and incorporate the effects of time to advance our knowledge of the microbial processes at play in aquaponics and aquaculture settings. UWSP and UM personnel will be involved in preparing these deliverables.

LOGIC MODEL

Name of Program: Metagenomic Analysis of Microbial Populations in Aquaponic Systems

Situation: Aquaponics is a sustainable agricultural practice that allows for the culture of both plant and fish for human consumption. The conversion of fish waste into materials that can be consumed by plants for growth depends upon populations of microbes to convert fish waste and excess feed into nutrients for plant uptake. While this system is effective, the populations of microbes have not been characterized. Furthermore, the system properties, e.g., warm, moist, and nutrient-rich, may allow for the colonization of undesirable microbes, such as plant, fish, or human pathogens. This study proposes to use metagenomic techniques to identify microbes in an aquaponics system and determine if the populations differ among locations within a system, among systems at a production facility, or among production facilities in different geographic locations.

Goal: Identify beneficial and detrimental microbial populations in aquaponics systems.

Objective: Sample biofilms on different surfaces within mature aquaponics systems, and characterize those populations using metagenomic techniques to estimate microbial abundance and diversity within and among systems.

INPUTS	OUTPUTS		OUTCOMES – IMPACT		
	Activities	Participation	*Short term*	*Medium term*	*Longer term*
a) Faculty at University of Wisconsin-Stevens Point and University of Minnesota **b)** NCRAC Rapid Response Program funds **c)** Current aquaponic production facilities in Wisconsin and Minnesota	**What we do:** **a)** Sample surfaces that provide potential for microbial colonization in an aquaponics system **b)** Isolate DNA from the microbes and use metagenomic analyses to identify microbes and estimate microbial community structure **c)** Disseminate findings to interested parties through fact sheets and voice over presentations available on the NCRAC website, presentations at professional meetings, and peer-reviewed articles	**Who is reached:** **a)** Aquaponic producers **b)** Extension educators and specialists **c)** Researchers **d)** Food safety inspectors	**a)** Identification of major microbial populations in various portions of an aquaponics system **b)** Comparison of populations among different portions of a single system, among systems at a facility, and among facilities to determine if there is an aquaponic "microbial fingerprint" **c)** Determine if different fish species result in different microbial populations in a system	**a)** Strategies to enhance the growth of desirable organisms and inhibit or control the growth of undesirable organisms **b)** Colonization strategies for starting up new systems **c)** Identifying and manipulating surfaces in a system for better colonization of desirable microbes **d)** Better understanding of tailoring microbial populations to production of particular species of fish or plants	**a)** Producers understand the role of beneficial microbes to enhance both plant and fish production **b)** Producers have strategies to colonize and maintain beneficial microbes to enhance both plant and fish production **c)** Due to enhanced production from a better understanding of the biological interactions, aquaponics profitability increases, allowing for the sustainable production of a safe protein and produce source

ASSUMPTIONS

1) Metagenomic analysis provides enough taxonomic resolution for meaningful interpretations of the data
2) The DNA from all microbial taxa can be purified using one purification kit

EXTERNAL FACTORS

1) Minimal microbial overlap among systems separated geographically
2) Differences in system startup and pre- or probiotics used

FACILITIES

The UWSP AIC is in Montello, WI, approximately one hour from the UWSP campus. The AIC has six replicate, integrated aquaponics systems consisting of two 250-gallon, round, plastic, self-cleaning fish tanks, a cylindro-conical clarifier (solids), a filter tank with orchard netting for trapping fine solids, a biofilter for nitrification, UV sterilizer, a sump tank, four hydroponics revised agriculture float technology (raft) tanks and a ½ hp in-line water pump. The hydroponic tanks are 4 ft long and 6 ft wide and contain a total growing space of 8 x 12 ft. The microbiology laboratory at the UWSP campus contains the necessary equipment to process the samples to extract DNA, including water baths, heat blocks, microcentrifuges, vortexing platforms, micropipettors, and -20°C and -80°C freezers. The UW-Madison Biotechnology Center's facilities for amplification, sequencing, and bioinformatics analyses are described in their letter of support.

The facilities in Minnesota include a variety of greenhouse and warehouse aquaponics systems are available for use on this project at the University of Minnesota. This includes eight 200-gallon floating raft systems, two 200-gallon ebb-and-flow systems, two 200-gallon A-Frame systems, and a 120-gallon Nelson and Pade system. The systems have been operational for two years and will be stocked with new fish and plants for this project.

STATEMENT OF DUPLICATION OF RESEARCH

The following databases were examined to verify that the research proposed here is original and does not duplicate previously funded projects:

- http://cris.nifa.usda.gov
- http://reeis.usda.gov
- National Sea Grant Office Funding Page: http://seagrant.noaa.gov/fudning/rfp.html
- State Sea Grant Program: http://www.seagrant.noaa.gov/other/programsdirectors.html
- NOAA Office of Aquaculture Funding Opportunities Page: http://www.nmfs.noaa.gov/aquaculture/funding/funding.html

There were no current or previously funded projects found in any of the databases that were similar to this project.

REFERENCES

Aguiar-Pulido, V., and coauthors. 2016. Metagenomics, Metatranscriptomics, and Metabolomics Approaches for Microbiome Analysis. Evolutionary Bioinformatics (5619-EBO-Metagenomics,-Metatranscriptomics,-and-Metabolomics-Approaches-for-Mic.pdf):5-16.

Beale, D. J., and coauthors. 2016. An 'omics' approach towards the characterisation of laboratory scale anaerobic digesters treating municipal sewage sludge. Water Research 88:346-357.

Caporaso, J. G., and coauthors. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Meth 7(5):335-336.

Costello, E. K., and coauthors. 2009. Bacterial community variation in human body habitats across space and time. Science 326(5960):1694-1697.

Fox, B. K., and coauthors. 2012. A preliminary study of microbial water quality related to food safety in recirculating aquaponic fish and vegetable production systems. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, 11 pp.

Gülay, A., and B. F. Smets. 2015. An improved method to set significance thresholds for β diversity testing in microbial community comparisons. Environmental Microbiology 17(9):3154-3167.

Huang, Z., and coauthors. 2016. Metagenomic analysis shows diverse, distinct bacterial communities in biofilters among different marine recirculating aquaculture systems. Aquaculture International:1-16.

Marshall, C. W., E. V. LaBelle, and H. D. May. 2013. Production of fuels and chemicals from waste by microbiomes. Current Opinion in Biotechnology 24(3):391-397.

Martínez-Porchas, M., and F. Vargas-Albores. 2015. Microbial metagenomics in aquaculture: a potential tool for a deeper insight into the activity. Reviews in Aquaculture 7:1-15.

Mendes, R., P. Garbeva, and J. M. Raaijmakers. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiology Reviews 37(5):634-663.

Munguia-Fragozo, P., and coauthors. 2015. Perspective for aquaponic systems: "Omic" technologies for microbial community analysis. BioMed Research International 2015:10.

Nielsen, P. H., A. M. Saunders, A. A. Hansen, P. Larsen, and J. L. Nielsen. 2012. Microbial communities involved in enhanced biological phosphorus removal from wastewater — a model system in environmental biotechnology. Current Opinion in Biotechnology 23(3):452-459.

Oulas, A., and coauthors. 2015. Metagenomics: Tools and Insights for Analyzing Next-Generation Sequencing Data Derived from Biodiversity Studies. Bioinformatics and Biology Insights 9(4809-BBI-Metagenomics:-Tools-and-Insights-for-Analyzing-Next-Generation-Sequenc.pdf):75-88.

Perez, T., and coauthors. 2010. Host-microbiota interactions within the fish intestinal ecosystem. Mucosal Immunology 3(4):355-360.

Rakocy, J. E., M. P. Masser, and T. M. Losordo. 2006. Recirculating aquaculture tank production systems: aquaponics-integrating fish and plant culture. SRAC Publication No. 454. 16 pp.

Reinhold-Hurek, B., W. Bünger, C. S. Burbano, M. Sabale, and T. Hurek. 2015. Roots Shaping Their Microbiome: Global Hotspots for Microbial Activity. Annual Review of Phytopathology 53(1):403-424.

Sales, C. M., and P. K. H. Lee. 2015. Resource recovery from wastewater: application of meta-omics to phosphorus and carbon management. Current Opinion in Biotechnology 33:260-267.

Sirsat, S., and J. Neal. 2013. Microbial Profile of Soil-Free versus In-Soil Grown Lettuce and Intervention Methodologies to Combat Pathogen Surrogates and Spoilage Microorganisms on Lettuce. Foods 2(4):488.

Tan, J., C. Zuniga, and K. Zengler. 2015. Unraveling interactions in microbial communities - from co-cultures to microbiomes. Journal of Microbiology 53(5):295-305.

Tkacz, A., and P. Poole. 2015. Role of root microbiota in plant productivity. Journal of Experimental Botany 66(8):2167-2175.

Wong, S., and coauthors. 2013. Aquacultured Rainbow Trout (Oncorhynchus mykiss) Possess a Large Core Intestinal Microbiota That Is Resistant to Variation in Diet and Rearing Density. Applied and Environmental Microbiology 79(16):4974-4984.

Wu, S., and coauthors. 2012. Composition, Diversity, and Origin of the Bacterial Community in Grass Carp Intestine. PloS One 7(2):e30440.

PROJECT LEADERS

Name, State	**Institution**	**Areas of Specialization**
Matthew Rogge, Wisconsin	University of Wisconsin-Stevens Point	Aquatic animal disease, molecular biology, microbiology
Nicholas Phelps, Minnesota	University of Minnesota	Aquatic animal disease, molecular biology, microbiology
Daniel Graf, Wisconsin	University of Wisconsin-Stevens Point	Bioinformatics, ecological community structure analysis

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

ORGANIZATION AND ADDRESS University: University of Wisconsin-Stevens Point Address: 2100 Main St City, State, ZIP: Stevens Point, WI 54481 PROJECT DIRECTOR(S) PI Name Matt Rogge				**USDA AWARD NO.** Year 1: Objectives 1, 2, 3 Duration Proposed Months: 11 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS** Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates . . . b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				$0			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$5,500			
F. Travel				$4,500			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$14,596			
K. Total Direct Costs (C through I)				$24,596			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K)				$24,596			
N. Other							
O. Total Amount of This Request				$24,596			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) Non-Cash Contributions (both Applicant and Third Party)						Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director		
Authorized Organizational Representative		

BUDGET JUSTIFICATION FOR UNIVERSITY OF WISCONSIN-STEVENS POINT
(Rogge)

OBJECTIVES 1, 2, and 3

A. Salaries and Wages – None requested

E. Materials and supplies – A total of $5,500 is requested for materials and supplies. These funds will be used to construct microbial biofilm samplers, purchase materials related to sampling, including gloves, plasticware (pipet tips, tubes, plates, etc.), DNA purification kits, DNA quantification kits, and related reagents. A total of $1500 will be subcontracted to University of Minnesota for Nicholas Phelps to conduct sampling and ship samples to UW-Stevens Point.

F. Travel – A total of $4,500 is requested, which will be divided between the three investigators. Travel money will be used to travel to aquaponics facilities for sampling and used for investigators to offset costs related to attendance professional meetings to disseminate information gathered from the studies.

J. Other direct costs – A total of $14,596 is requested for other direct costs. These funds will be used to pay for metagenomic analyses (PCR, Illumina sequencing, and basic bioinformatics analyses – see attached quote). The remaining money will be used in preparation of deliverables (fact sheet and voice-over presentation).

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

ORGANIZATION AND ADDRESS University: University of Wisconsin-Stevens Point Address: 2100 Main St City, State, ZIP: Stevens Point, WI 54481 PROJECT DIRECTOR(S) PI Name: Nicholas Phelps				**USDA AWARD NO.** Year 1: Objectives 1, 2, 3 Duration Proposed Months: 11 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS** Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. __ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. __ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				$0			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$1,500			
F. Travel				$1,500			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$1,500			
K. Total Direct Costs (C through I)				$4,500			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K)				$4,500			
N. Other							
O. Total Amount of This Request				$4,500			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) Non-Cash Contributions (both Applicant and Third Party)						Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director Nicholas Phelps, Assistant Professor		JUNE 3, 2016
Authorized Organizational Representative Andrea Marshall, Principal Grant and Contract Administrator		JUNE 3, 2016

BUDGET JUSTIFICATION

Nicholas Phelps, University of Minnesota

Objectives 1 and 3

A. Salaries and Wages – None requested

E. Materials and supplies – A total of $1,500 is requested for supplies for sample collection and shipping to University of Wisconsin-Stevens Point for DNA purification, quantification, and submission for metagenomic analyses.

F. Travel – A total of $1,500 is requested for travel costs to the aquaponics facility for sampling and used for the investigator to offset costs related to attendance professional meetings to disseminate information gathered from the studies.

J. Other direct costs – A total of $1,500 is requested for other direct costs related to preparation and distribution of outreach materials, including fact sheet and presentation.

TOTAL REQUEST: $4,500

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

ORGANIZATION AND ADDRESS University: University of Wisconsin-Stevens Point Address: 2100 Main St City, State, ZIP: Stevens Point, WI 54481 PROJECT DIRECTOR(S) PI Name Daniel Graf				**USDA AWARD NO.** Year 1: Objectives 1, 2, 3 Duration Proposed Months: 11 **Funds Requested by Proposer**	Duration Proposed Months: ____ **Funds Approved by CSREES** (If different)	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
A. Salaries and Wages 1. No. of Senior Personnel	**CSREES FUNDED WORK MONTHS** Calendar	Academic	Summer				
a. ___ (Co)-PD(s)							
b. ___ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ___ Research Associates-Postdoctorates . . .							
b. ___ Other Professionals							
c. ___ Paraprofessionals							
d. ___ Graduate Students							
e. ___ Prebaccalaureate Students							
f. ___ Secretarial-Clerical							
g. ___ Technical, Shop and Other							
Total Salaries and Wages							
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B)				$0			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)							
E. Materials and Supplies				$0			
F. Travel				$1,500			
G. Publication Costs/Page Charges							
H. Computer (ADPE) Costs							
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)							
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)				$0			
K. Total Direct Costs (C through I)				$1,500			
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)							
M. Total Direct and F&A/Indirect Costs (J plus K)				$1,500			
N. Other							
O. Total Amount of This Request				$1,500			
P. Carryover -- (If Applicable) . . . . . **Federal Funds: $**				**Non-Federal funds: $**		**Total $**	
Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O) Cash (both Applicant and Third Party) Non-Cash Contributions (both Applicant and Third Party)						Leave Blank	

NAME AND TITLE (Type or print)	**SIGNATURE** (required for revised budget only)	**DATE**
Project Director Daniel Graf, Assistant Professor		JUNE 1, 2016
Authorized Organizational Representative Katherine P. Jore, Associate Vice Chancellor		JUNE 1, 2016
Signature (for optional use)		

BUDGET JUSTIFICATION

Daniel Graf, University of Wisconsin-Stevens Point

Objectives 2 and 3

A. Salaries and Wages – None requested.

E. Materials and supplies – None requested.

F. Travel – A total of $1,500 is requested to defray costs related to attendance professional meetings to disseminate information gathered from the studies.

J. Other direct costs – None requested.

TOTAL REQUEST: $1,500

BUDGET SUMMARY FOR EACH PARTICIPATING INSTITUTION

	Year 1				
	Objective #	UWSP (Rogge)	UM (Phelps)	UWSP (Graf)	Project Total
Salaries and Wages	N/A	N/A	N/A	N/A	N/A
Fringe Benefits	N/A	N/A	N/A	N/A	N/A
Total Salaries, Wages, and Fringe Benefits	N/A	N/A	N/A	N/A	N/A
Nonexpendable Equipment	N/A	N/A	N/A	N/A	N/A
Materials and Supplies	1, 2	$4000	$1500	N/A	$5,500
Travel	3	$1500	$1500	$1500	$4,500
All Other Direct Costs	2, 3	$13,096	$1500	N/A	$14,596
Total		$18,596	$4,500	$1,500	$24,596

SCHEDULE FOR COMPLETION OF OBJECTIVES

Start date: August 1, 2016
Completion date: June 30, 2017

Objectives and Tasks	Year 1									
	Aug	Sept	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Objective 1										
Construction of samplers										
Colonize samplers										
Remove samplers, cut root samples, collect filtration media										
Purify and quantify DNA										
Objective 2										
Submit samples to UW Biotechnology Center for analysis										
Analyze bioinformatics data										
Objective 3										
Develop fact sheet										
Develop voice-over presentation										

PARTICIPATING INSTITUTIONS AND PRINCIPAL INVESTIGATORS

University of Wisconsin-Stevens Point
Graf, Daniel
Rogge, Matthew

University of Minnesota
Phelps, Nicholas

VITA

Matthew L. Rogge
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EDUCATION

B.S.	University of Wisconsin-Stevens Point, 2000, Biology
M.S.	Iowa State University, 2002, Fisheries Biology
Ph.D.	Louisiana State University School of Veterinary Medicine, 2009, Veterinary Medical Sciences

POSITIONS

2014 – Present	Assistant Professor of Biology, UWSP Dept. of Biology
2012 – 2014	Research Program Manager, UWSP Northern Aquaculture Demonstration Facility
2009 – 2012	Postdoctoral Researcher, Department of Pathobiological Sciences, LSU SVM

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
AFS – Fish Health Section
AFS – Fish Culture Section
American Society for Microbiology

SELECTED PUBLICATIONS

Dubytska, L. P., M. L. Rogge, and R. Thune. Changes in pH of the *Edwardsiella ictaluri* vacuole are required to initiate translocation of T3SS effectors to the host cell cytoplasm. mSphere DOI: 10.1128/mSphere.00039-16.

W. A. Baumgartner, L. Dubytska, M. L. Rogge, P. J. Mottram, and R. L. Thune. 2014. Modulation of vacuolar pH is required for replication of *Edwardsiella ictaluri* in channel catfish (*Ictalurus punctatus*) macrophages. Infection and Immunity 82:2329-2336.

Suanyuk, N., M. Rogge, R. Thune, M. Watthanaphiromsakul, N. Jampat, and W. Wiangkum. 2014. Mortality and pathology of hybrid catfish, *Clarias macrocephalus* x *Clarias gariepinus* associated with *Edwardsiella ictaluri* infection in southern Thailand. Journal of Fish Diseases 37:385-395.

Rogge, M. L., L. Dubytska, T. S. Jung, J. Wiles, A. A. Elkamel, A. Rennhoff, and R. L. Thune. 2013. Comparison of Vietnamese and United States Isolates of *Edwardsiella ictaluri*. Diseases of Aquatic Organisms 106:17-29.

Hawke, J. P., M. Kent, M. Rogge, W. Baumgartner, J. Wiles, J. Shelley, L. C. Savolainen, R. Wagner, K. Murray, and T. S. Peterson. 2013. Edwardsiellosis caused by *Edwardsiella ictaluri* in Laboratory Populations of Zebrafish *Danio rerio*. Journal of Aquatic Animal Health 25:171-183
.

Rogge, M. L., and R. L. Thune. 2011. Regulation of the *Edwardsiella ictaluri* Type III Secretion System by pH and Phosphate Concentration through EsrA, EsrB, and EsrC. Applied and Environmental Microbiology 77:4293-4302.

Thune, R. L., D. H. Fernandez, J. L. Benoit, M. Kelly-Smith, M. L. Rogge, N. J. Booth, C. A. Landry, and R. A. Bologna. 2007. Signature-Tagged Mutagenesis of *Edwardsiella ictaluri* Identifies Virulence-Related Genes, Including a *Salmonella* Pathogenicity Island 2 Class of Type III Secretion Systems. Applied and Environmental Microbiology 73:7934-7946.

VITA

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E-mail: phelp083@umn.edu

EDUCATION

B.A.	Bemidji State University, 2005, Aquatic Biology
M.S.	University of Arkansas – Pine Bluff, 2007, Aquaculture/Fisheries
Ph.D.	University of Minnesota, 2012, Veterinary Medicine

POSITIONS

2013-Present	Assistant professor, College of Veterinary Medicine, University of Minnesota
2009-Present	Aquaculture specialist, Extension, University of Minnesota
2009-2013	Instructor, College of Veterinary Medicine, University of Minnesota
2007-Present	Lead, Aquatic Diagnostic Program, Veterinary Diagnostic Laboratory, University of Minnesota
2007-2009	Scientist, Veterinary Diagnostic Laboratory, University of Minnesota

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society – Fish Health Section (Chair, Technical Standards Committee)
North Central Regional Aquaculture Center (Technical Committee – Extension, Executive Committee)

SELECTED PUBLICATIONS

Escobar, L. E., G. Kurath, J. Escobar-Dodero, M. E. Craft, N. B. D. Phelps. 2016. Potential distribution of the viral hemorrhagic septicemia virus (VHSV) in the Great Lakes region. Journal of Fish Disease doi:10.1111/jfd/12490.
Mor, S. K., N. B. D. Phelps. 2016. Detection and molecular characterization of a novel piscine myocarditis-like virus from baitfish in the USA. Archives of Virology doi:10.1007/s00705-016-2873-0.

Phelps, N. B. D., S. K. Mor, A. Armién, K. Pelican, S. M. Goyal. 2015. Description of the microsporidian parasite, *Heterosporis sutherlandae* n. sp., infecting fish in the Great Lakes region, USA. PLOS One 10(8):e0132027.

Papenfuss, J. T., N. B. D. Phelps, D. Fullton, P. Venturelli. 2015. Smartphones reveal angler behavior: A case-study of a popular mobile fishing application in Alberta, Canada. Fisheries 40:318-327.

Phelps, N. B. D., M. E. Craft, D. Travis, K. Pelican, S. M. Goyal. 2014. Risk-based management of viral hemorrhagic septicemia virus (VHSV-IVb) in Minnesota. North American Journal of Fisheries Management 34:373-379.

Phelps, N. B. D., S. K. Mor, A. G. Armien, W. Batts, A. E. Goodwin, L. Hopper, R. McCann, T. F. F. Ng, C. Puzach, T. B. Waltzek, E. Delwart, J. Winton, S. Goyal. 2014. Characterization of the novel fathead minnow picornavirus. PLOS One 9:e87593.

Rodger, H. D., N. B. D. Phelps. 2015. Percid fish health and disease. In: P. Kestemont, K. Dabrowski, R. Summerfelt, editors. Biology and Culture of Percid Fishes – Principles and Practices. Springer.

Mor, S. K., and N. B. D. Phelps. 2016. Picornaviruses of fish. Aquaculture Virology. Chapter 7.9 *in* Kibenge, F. and M. Godoy, editors. Aquaculture Virology, 1st Edition.

VITA

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EDUCATION

B.S.	University of Minnesota, 1993, Biology
M.S.	Northeastern University, 1997, Biology
Ph.D.	University of Michigan, 2001, Biology

POSITIONS

2012 – Present	Assistant Professor, UWSP Dept. of Biology
2008 – 2012	Assistant Professor, Assistant Curator of Invertebrates, and Full Member of the Graduate Faculty, Dept. of Biological Sciences, University of Alabama
2002 – 2008	Assistant Curator of Malacology, Academy of Natural Sciences of Philadelphia

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Malacological Society
Freshwater Mollusk Conservation Society

SELECTED PUBLICATIONS

Pfeiffer, J.M. & D.L. Graf. 2015. Evolution of bilaterally asymmetrical larvae in freshwater mussels (Bivalvia: Unionoida: Unionidae). *Zoological Journal of the Linnean Society* 175: 307-318. doi:10.1111/zoj.12282

Graf, D.L., H. Jones, A.J. Geneva, J.M. Pfeiffer & Michael W. Klunzinger. 2015. Molecular phylogenetic analysis supports a Gondwanan origin of the Hyriidae (Mollusca: Bivalvia: Unionida) and the paraphyly of Australasian taxa. Molecular Phylogenetics & Evolution 85: 1-9. doi:10.1016/j.ympev.2015.01.012

Graf, D.L., A.J. Geneva, J.M. Pfeiffer & A.D. Chilala. 2014. Phylogenetic analysis of *Prisodontopsis* Tomlin, 1928 and *Mweruella* Haas, 1936 (Bivalvia: Unionidae) from Lake Mweru (Congo Basin) reveals endemic Quaternary radiation in the Zambian Congo. Journal of Molluscan Studies 80: 303-314. doi: 10.1093/mollus/eyu012

Pfeiffer, J.M. & D.L. Graf. 2013. Re-analysis confirms the polyphyly of *Lamprotula* Simpson, 1900 (Bivalvia: Unionidae). Journal of Molluscan Studies 79: 249-256. doi:10.1093/mollus/eyt022

Graf, D.L. 2013. Global diversity of freshwater bivalves. American Malacological Bulletin 31: 135-153. doi:10.4003/006.031.0106