Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2013 Minnesota Aquatic Invasive Species Research Center
Sub-Project Work Plan

Date of Report: July 31, 2017

Date of Next Status Update Report: July 31, 2017

Date of Work Plan Approval: February 1, 2017

Sub-Project Completion Date: June 30, 2019

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No

SUB-PROJECT TITLE: MAISRC Sub-Project 7-2: Developing eradication tools for invasive species Phase II: Virus Discovery and evaluation for use as potential biocontrol agents

Sub-Project Manager: Dr. Nicholas Phelps

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Location: Statewide

Total ENRTF Sub-Project Budget:	**Sub-Project Budget:**	**$445,210**
	Amount Spent:	**$1,904**
	Balance:	**$443,306**

Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

Appropriation Language:
$4,350,000 the first year and $4,350,000 the second year are from the trust fund to the Board of Regents of the University of Minnesota to develop and support an aquatic invasive species (AIS) research center at the University of Minnesota that will develop new techniques to control aquatic invasive species including Asian carp, zebra mussels, and plant species. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. SUB-PROJECT TITLE: MAISRC Sub-Project 7-2: Developing eradication tools for invasive species: Phase II: Virus Discovery and evaluation for use as potential biocontrol agents

II. SUB-PROJECT STATEMENT:

Rationale: Although ambitious, eradication of aquatic invasive species is the ultimate goal of many aquatic invasive species. One possible approach would be through the introduction or promotion of species-specific pathogens. This high-risk, high-reward approach must be carefully assessed with thorough investigation and scientifically justified risk assessment. Phase I (Years 1-2.5) of the long-term project provided initial baseline data on viruses of carp species in the region. Phase II (Years 2.5-6) will build upon this work for carp species and now include zebra mussels to utilize newly developed techniques to more strategically identify viral biocontrol candidates for control of invasive carp and zebra mussels. More specifically, Phase II will 1a) Collect apparently healthy invasive carp and mussel species in the Midwest region; 1b) Collect samples from mortality events of native and invasive fish and mussel populations in the Midwest region; 2) Conduct virus discovery by next generation sequencing and culture potential pathogens; 3) Determine the disease causing potential of two selected viruses, one for native and invasive fish and the other for native and invasive mussels; and 4) Communicate findings to scientific, management, and public stakeholders. This will provide the scientific foundation to begin to evaluate specific pathogens for invasive species control. Furthermore, understanding the virome of invasive species will serve as a potential early indicator for the movement and distribution of pathogens that may threaten native species. Phase II will largely be basic research (60%) generating baseline data on the virome diversity of invasive and native species. Significant effort will also be in applied research (40%), whereby diagnostic and disease challenge findings will be used to inform the health management of fish populations.

III. SUB-PROJECT STATUS UPDATES:

Sub-Project Status as of July 31, 2017:
During the first part of the project, we have focused our efforts on sample collection. We have collected samples from six fish kill events of invasive and native fish. Koi Herpes Virus (KHV) was identified from a large common carp mortality event in Lake Elysian. This is a significant finding since this is the first report of KHV in wild fish in Minnesota and the candidate biocontrol agent for common carp in Australia. We are working with the MN DNR and hope to conduct follow up surveys in the coming months to estimate viral persistence, mortality rates and prevalence in surrounding lakes. Sampling of healthy and sick/dead fish and mussels will continue in the coming months.

We have made changes within our personnel category due to the promotion of Dr. Sunil Kumar Mor. Dr. Mor is now an Assistant Professor with the Minnesota Veterinary Diagnostic Laboratory and head of the Molecular Development section. Although his percent effort will be lower, the capacity and value he brings with this new position will be highly beneficial to the project. In addition, the official start dates of Dr. Mor and Dr. Alex Primus has been delayed to 7/1/17. With the cost savings we have hired Dr. Soumesh Kumar Padhi to be a full time post doctoral associate starting in August 2017. We have also hired Dr. Todd Knutson, a bioinformatics specialist to assist part-time with the project. Lastly, we have added Isaiah Tolo to the team. Isaiah received the competitive University of Minnesota Diversity Scholars Fellowship for the 2017-2018 academic year and will be at no cost to the project until Year 2. The descriptions in Column A of the budget spreadsheet have been updated accordingly. These changes in personnel do not affect the overall budget, but have delayed spending, hence a full balance on this budget line. In the meantime, Meg Thompson has provided assistance on the project by collecting and processing samples. She is currently being paid from a non-ENRTF source of funds.

We learned from Phase I of this project (MAISRC SubProject 7-1) that an increased communication effort was needed to generate collaboration on sample collection. We have presented at the joint meeting of the American Fisheries Society – Fish Health Section, Eastern Fish Health Workshop and the Great Lakes Fish Health Committee to present on this project. The presentations were titled: "Investigating fish kills: Looking back, looking deep and looking forward" and "Understanding the virome of invasive carp: What it could mean for biocontrol". These presentations resulted in an active discussion on the potential use of viruses for biocontrol, interest to submit samples for the project and potential collaborations for future research efforts related to this project. In addition, we have invited a world leader on the use of viruses for biocontrol, Dr. Ken McColl (Commonwealth Scientific and Industrial Research Organization, Australia), to present at the 2018 iCOMOS meeting to be held at the University of Minnesota, more information: http://icomos.umn.edu. We expect that as part of Dr. McColl's visit, we will host meetings with members of state and federal agencies to socialize this approach and generate ideas for future research needs.

Sub-Project Status as of January 31, 2018

Sub-Project Status as of July 31, 2018:

Sub-Project Status as of January 31, 2019:

Sub-Project Status as of July 31, 2019:

Overall Sub-Project Outcomes and Results:

IV. SUB-PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Sample collection – Collect apparently healthy invasive carp and mussel species and collect samples from native and invasive fish and mussel mortality events in the Midwest region.

Description: Investigation into the viral communities of apparently healthy animals, in particular invasive species, is rare, yet critically important to the long-term goals of this project. In addition, the opportunistic collection of animals found moribund or freshly dead as a result of a mortality event will provide diagnostic evidence of pathogenic viruses (or other microbes). While these events are occasionally investigated, communication is limited and diagnostic quality and approaches are highly variable. These limitations will be overcome in this project by conducing a standardized and thorough diagnostic exam.

The Project Team will opportunistically collect fish in coordination with local DNRs, commercial fishermen, and researchers to maximize collection success and minimize costs. The collaborators will determine the specific sites and collection times; however, the goal is to collect common carp from ten Minnesota lakes during the project period. In addition, silver carp will be collected from four locations in the Illinois and Mississippi River systems. At each collection, 60 fish will be collected to ensure a statistically valid sample, assuming a 5% pathogen prevalence and 95% confidence in the diagnostic assays. In total, the sample collection goals are 600 common carp and 240 silver carp. The collection of zebra mussels from 20 locations has/will occur as part of an ongoing MAISRC project (Project Manager: Dr. Michael Sadowsky) and shared with this project.

Opportunistic sampling of naturally occurring mortality events of native fish and mussels, as well as invasive carp and zebra mussels, will also be included in this activity. All species of fish and mussels will be included from wild, farm-raised, and laboratory sources. This is important to evaluate disease causing potential and species specificity of novel viruses. Samples from mortality events across the Midwest region will be accepted, but focus will be given to invasive carp and mussel populations in Minnesota. Aquatic animal health experts from the region will be provided with a collection protocol, submission form, and other information related to this

project offering a no-cost diagnostic exam. This will help ensure consistent and appropriate samples and observational data are collected. A minimum of five fish or mussels with clinical lesions consistent with the mortality event will be targeted from each event. The number of fish mortality events in Minnesota alone is estimated at approximately 500; however, the number that will be reported and have quality samples collected is unknown. Our goal is to include 20 events per year in this project.

Summary Budget Information for Activity 1:

ENRTF Budget: $69,500
Amount Spent: $ 0
Balance: $69,500

Activity Completion Date:

Outcome	Completion Date
1. *Collect 600 common carp from 10 locations in Minnesota*	December 2018
2. *Collect 240 silver carp from 4 locations in the Illinois and Mississippi Rivers*	December 2018
3. *Collect 1,200 zebra mussels from collaborating researchers*	December 2018
4. *Collect samples from 40 fish or mussel mortality events in the Midwest region*	December 2018

Activity Status as of July 31, 2017:
The initial focus of this project has been to collect samples from healthy and sick/dead fish to identify viruses in the fish populations. We have collected samples from two carp mortality events: Lake Jonathan (6/13/17) and Lake Elysian (6/28/17). Standard water quality and diagnostic testing, including bacteriology, parasitology, and virus isolation did not yield significant findings for either case. However, Lake Elysian was confirmed positive for Cyprinid Herpes Virus-3 (aka Koi Herpes Virus, KHV) by molecular diagnostics. The population was re-sampled (7/10/17) and confirmed the diagnosis. This is a significant finding since this is the first report of KHV in wild fish in Minnesota and the candidate biocontrol agent for common carp in Australia. We are working with the MN DNR and hope to conduct follow up surveys in the coming months to estimate viral persistence, mortality rates and prevalence in surrounding lakes. Samples have also been collected from four mortality events of native species (i.e., bluegill, black crappie, muskellunge): Schwanz Lake (5/13/17), Lake Nokomis (6/7/17), Forrest Lake (6/13/17) and Maple Lake (7/14/17). Standard diagnostic and water quality testing did not yield significant findings. Tissue samples (e.g., gill, spleen, kidney) for all mortality events have been stored for next generation sequencing (Activity 2).

Sampling of healthy and sick/dead populations will continue in the coming months. An increased effort, in collaboration with other MAISRC researchers, to sample invasive zebra mussels and native mussels will be a priority as this has proved challenging so far.

Activity Status as of January 31, 2018

Activity Status as of July 31, 2018:

Activity Status as of January 31, 2019:

Activity Status as of July 31, 2019:

Final Report Summary:

ACTIVITY 2: Characterize virome – Conduct virus discovery by next generation sequencing (NSG) and culture potential pathogens.

Description: Characterizing the virome of invasive carp and mussel species is an initial step towards risk assessment and evaluation of viruses as biological control agents. Phase I of this project was the first virus discovery effort in common carp and no similar survey has been done for zebra mussels. The methods developed and refined in Phase I proved the utility of next generation sequencing techniques, having identified 10+ novel viruses. There is little doubt important new viruses will be found in Phase II.

A necropsy will be performed on all fish and mussels collected as part of Activity 1. Various tissues (i.e. kidney, spleen, gills, etc) will be collected by aseptic techniques for virus discovery. If the fish or mussels were collected from a fish kill (Objective 1b), a thorough diagnostic exam will be performed to determine the cause of death and, if present, understand the pathology of viral agents. All samples will be processed for NGS for the unbiased amplification of viral RNA and DNA viruses following our methods developed in Phase I. Samples will be pooled in groups of five for a total goal of 168 pools of invasive carps, 240 pools of zebra mussel and 80 pools from mortality events. We will multiplex (barcode) 50 samples in a single lane of HiSeq 250 paired end cycle run to reduce sequencing cost while maintaining critical sequence depth. Bioinformatics methods for virome analyses are already in place and in routine use by the Project Team and colleagues at the Minnesota Veterinary Diagnostic Laboratory (MVDL). The pipeline consists of two steps, sequence quality screening and sequence analysis. Quality sequence reads will be compared to the most closely related sequences available in GenBank. Specific primers will be designed for standardization of PCR for each virus detected by NGS. These specific assays will be used for screening of individual samples and future diagnostic needs.

If novel viruses are identified by NGS, the original sample will be subjected to virus isolation for further characterization. A variety of immortal fish cell lines (i.e. EPC, FHM, CHSE-214, etc) are currently in use by the Project Team and will be available for this project. No permanent cell lines derived from bivalves are available for isolation of viruses and the only permanent cell line from any mollusk is from the snail, *Biomphalaria glabrata* (Hansen 1976; American Type Culture Collection number CRL-1494). Ongoing work by the USGS and US FWS towards the development of a zebra mussel cell line looks promising and should be available for this project. However, the Project Team has experience developing primary cell lines and will pursue this approach if needed or use the CRL-1494 snail cell line as a last resort. To visualize viral particles, infected cell culture fluids will be freeze-thawed thrice followed by light centrifugation. The supernatant will be examined by negative contrast electron microscopy. Morphological classification of the viral isolate will support findings of NGS.

Summary Budget Information for Activity 2:

ENRTF Budget: $260,110
Amount Spent: $ 0
Balance: $260,110

Activity Completion Date:

Outcome	Completion Date
1. *Database and isolate archive of viruses of fish*	June 2019
2. *Database and isolate archive of viruses of mussels*	June 2019

Activity Status as of July 31, 2017:
Samples from six mortality events have been archived in preparation for Activity 2. These samples will be processed and analyzed in the coming weeks. Given the important finding of KHV, we aim to sequence the entire genome of this virus to determine the virus subtype (e.g., European vs. Asian strain). Knowing the strain is important for biocontrol considerations and identifying the introduction source.

Activity Status as of January 31, 2018

Activity Status as of July 31, 2018:

Activity Status as of January 31, 2019:

Activity Status as of July 31, 2019:

Final Report Summary:

ACTIVITY 3: Disease challenge – Determine the disease causing potential of two selected viruses, one for native and invasive fish and the other for native and invasive mussels.

Description: The potential use of viral agents as a means of biological control for invasive carp and zebra mussels is dependent on the identification of a virus that is capable of negatively impacting the health of the invasive species while at the same time having little or no impact on native species.

Two novel viral agents (one from fish and one from mussels) identified in Activity 2 will be selected based on the pathogenic potential to the target species (i.e. common carp and zebra mussels) and ability to propagate in cell culture. These viruses will be used to challenge the target species as well as a native fish species (fathead minnow) and a native mussel species (three ridge mussel). These fathead minnow was chosen as a representative native fish species because it is closely related to common carp and suitable for laboratory confinement. The three ridge mussel was chosen as a good representative native mussel species because of its accessibility and suitability for captive culture.

Fish and mussels will be held at the Minnesota Aquatic Invasive Species Research Center's Containment Laboratory in in 30-gallon flow-through aquaria and monitored at least once daily throughout the experiment. Flow-through rates will be adjusted to maintain water quality, while minimizing the need for costly effluent treatment. Prior to initiating a viral challenge, animals will be acclimated for a period of at least 14 days and a subsample of each species involved will undergo a full diagnostic examination to ensure suitability for participation in the study. Each species will be challenged at two temperatures (10-15°C and 25-30°C) and two viral inoculum concentrations; each challenge will be conducted in duplicate, and one negative control per species per temperature will be used. Each species will therefore require the use of ten tanks. Each tank will contain thirty individuals. Animals will be assigned to challenge tanks (and therefore treatment groups) without any intentional bias.

Fish will be challenged by intraperitoneal (IP) injection of viral inoculum. Briefly, fish will be anesthetized with buffered tricaine methanesulfonate (TMS). Once fish reach stage 3 anesthesia, each fish will be injected with 0.1ml of viral inoculum or cell culture growth medium (negative control). Two different viral concentrations will be used for the inoculum of challenged fish: ~ 10^8 pfu/ml and ~10^5 pfu/ml (which, when administered in a 0.1 ml dose will result in administration of ~10^7 and ~10^4 pfu per fish, respectively).

Mussels will be challenged using a static bath challenge method whereby water flow is terminated and a standardized amount of viral inoculum/infectious material is added to the tank water; water flow may be turned back on after a fixed amount of time (e.g. 24 hours post inoculation). If the mussel virus that is used is amenable to cell culture, preparation of viral inoculum stock solution and quantification of virus in stock solution will follow the procedures outlined above for the fish virus; final concentrations of ~10^3 and ~10^1 pfu/ml would be ideal for bath challenge. If the mussel virus selected is not amenable to *in vitro* amplification, infected tissues will be used to create a stock solution; the stock solution will then be used for the higher concentration viral challenge treatment groups, and a 100-fold dilution will be used for the lower concentration viral challenge. In the later scenario, quantitative PCR would be used to quantify the dose.

Animals will be closely monitored for 60 days to evaluate host response to the virus, at which time all surviving animals will be humanely euthanized. Clinically ill individuals will be euthanized prior to the end of the study if necessary. A complete diagnostic exam will be completed on all organisms in the study to determine health status, as well as persistence and replication of the virus within the individual. All fish studies will run simultaneously and will require 4 months' continuous time in the infectious disease challenge room. All mussel studies will run simultaneously and will require an additional 4 months' continuous time in the infectious disease challenge room.

Summary Budget Information for Activity 3:

ENRTF Budget: $94,600
Amount Spent: $ 0
Balance: $94,600

Activity Completion Date:

Outcome	Completion Date
1. *Determine disease causing potential of selected fish virus*	December 2018
2. *Determine disease causing potential of selected mussel virus*	June 2019

Activity Status as of July 31, 2017:
Potential viral biocontrol candidates are still being considered. This Activity will begin in mid-2018.

Activity Status as of January 31, 2018

Activity Status as of July 31, 2018:

Activity Status as of January 31, 2019:

Activity Status as of July 31, 2019:

Final Report Summary:

ACTIVITY 4: Communication – Communicate findings to scientific, management, and public stakeholders

Description: Disseminating research findings to relevant stakeholders and framing the future conversation on the use of pathogens for biocontrol of invasive species is an important part of this project. In so doing, we will provide stakeholders with the information to fill key knowledge gaps, guide future research and inform long-term approaches to manage AIS.

A diverse communication approach will be used to ensure appropriate delivery and detail to each relevant stakeholder group. The data generated as part of this project will result in the publication of three peer-reviewed articles in high-impact journals. Efforts will be made to provide open access availability to all published articles. The results will be presented at the American Fisheries Society – Fish Health Section annual meeting, or similar meeting each year. In addition, all research findings will be disseminated through the MAISRC newsletter, social media, and annual Research Showcase. Publication and presentation topics will be determined based on the project progress and findings. Through active and ongoing collaborations with MN DNR fish health and AIS specialists, important project findings will be shared directly.

Given the acknowledgement that the use of pathogens for biocontrol is a high-risk, high-reward research endeavor, we must look forward to the potential implementation of such a strategy. We will prepare a thorough review paper for journal submission to frame the conversation on the use of this strategy for aquatic invasive species control in inland waters of United States.

Summary Budget Information for Activity 4:

ENRTF Budget: $ 21,000
Amount Spent: $ 1,904
Balance: $ 19,096

Activity Completion Date:

Outcome	Completion Date
1. *Three peer-reviewed manuscripts submitted*	June 2019
2. *Three scientific conference presentations*	June 2019
3. *Dissemination of research findings via MAISRC communications*	June 2019

Activity Status as of July 31, 2017:
We learned from Phase I of this project (MAISRC SubProject 7-1) that an increased communication effort was needed to generate collaboration on sample collection. To that end, the Project Manager (Dr. Nick Phelps) and Co-Investigator (Dr. Sunil Kumar Mor) attended the joint meetings of the American Fisheries Society – Fish Health Section, Eastern Fish Health Workshop and the Great Lakes Fish Health Committee to present on this project. The presentations were titled: "Investigating fish kills: Looking back, looking deep and looking forward" and "Understanding the virome of invasive carp: What it could mean for biocontrol". These presentations resulted in an active discussion on the potential use of viruses for biocontrol, interest to submit samples for the project and potential collaborations for future research efforts related to this project. Funding was only provided to Dr. Mor for attendance, Dr. Phelps used other funds.

To discuss the applications of viral biocontrol, we have invited a world leader, Dr. Ken McColl (Commonwealth Scientific and Industrial Research Organization, Australia), to present at the 2018 iCOMOS meeting to be held at the University of Minnesota, more information: http://icomos.umn.edu. We expect that as part of Dr. McColl's visit, we will host meetings with members of state and federal agencies to socialize this approach and generate ideas for future research needs. Dr. Nick Phelps is on the iCOMOS organizing committee and has arranged all costs to be covered from other sources.

Activity Status as of January 31, 2018

Activity Status as of July 31, 2018:

Activity Status as of January 31, 2019:

Activity Status as of July 31, 2019:

Final Report Summary:

V. DISSEMINATION:

Description: Dissemination of this project is critical to advancing the broader scientific understanding, achieving our goals, and successful implementation of the findings. To that end, communications have been explicitly included as Activity 4 to account for the associated budget and identify outcomes.

Status as of July 31, 2017:
We learned from Phase I of this project (MAISRC SubProject 7-1) that an increased effort was needed to communicate the need to collaborate on sample collection. To that end, the Project Manager (Dr. Nick Phelps) and Co-Investigator (Dr. Sunil Kumar Mor) attended the joint meetings of the American Fisheries Society – Fish Health Section, Eastern Fish Health Workshop and the Great Lakes Fish Health Committee to present on this

project. The presentations were titled: “Investigating fish kills: Looking back, looking deep and looking forward” and “Understanding the virome of invasive carp: What it could mean for biocontrol”. These presentations resulted in an active discussion on the potential use of viruses for biocontrol, interest to submit samples for the project and potential collaborations for future research efforts related to this project. Funding was only provided to Dr. Mor for attendance, Dr. Phelps used other funds.

To discuss the applications of viral biocontrol, we have invited a world leader, Dr. Ken McColl (Commonwealth Scientific and Industrial Research Organization, Australia), to present at the 2018 iCOMOS meeting to be held at the University of Minnesota, more information: http://icomos.umn.edu. We expect that as part of Dr. McColl’s visit, we will host meetings with members of state and federal agencies to socialize this approach and generate ideas for future research needs. Dr. Nick Phelps is on the iCOMOS organizing committee and has arranged all costs to be covered from other sources.

Status as of January 31, 2018

Status as of July 31, 2018:

Status as of January 31, 2019:

Status as of July 31, 2019:

Final Report Summary:

VI. SUB-PROJECT BUDGET SUMMARY:

A. Preliminary ENRTF Budget Overview:

*This section represents an overview of the preliminary budget at the start of the project. It will be reconciled with actual expenditures at the time of the final report. See the Sub-Project Budget document for an up-to-date project budget, including any changes resulting from amendments.

Budget Category	$ Amount	Explanation
Personnel:	$238,650	Dr. Sunil Kumar Mor (1.0FTE total), Dr. Alex Primus (0.25 FTE total), TBD Graduate student (1.0FTE total), and student support (0.5FTE total). All positions will be supported for a total of two years.
Professional/Technical Services and Contracts:	$125,720	Diagnostic services (i.e. bacteriology, electron microscopy, histology, virology) will be provided by the Minnesota Veterinary Diagnostic Laboratory (MVDL) on a fee-for-service basis for a total budget of $27,350. Sequencing services (Ilumina MiSeq) will be provided by the University of Minnesota Genomics Center (UMGC) on a fee-for-service bases for a total of $77,370. Service contracts with the MVDL and UMGC are assuming sample numbers and conditions are as proposed. Disease challenge experiments will be performed in the MAISRC Containment Laboratory for eight months for a total of $15,000. Publication costs for three

		manuscripts for a total of $6,000.
Equipment/Tools/Supplies:	$56,840	A variety of consumable supplies will be purchased for this project, including molecular kits, diagnostic supplies, anesthesia, fish and food, disinfectant, etc for a total of $54,840. In addition, one laptop ($2,000) will be purchased for data management by the graduate student.
Capital Expenditures over $5,000:	$8,000	One biosafety cabinet will be purchased to prepare samples for diagnostic testing at a cost of $8,000.
Travel:	$16,000	Travel within Minnesota to collect samples and meet in-state stakeholders for a total of $3,000. Domestic travel to collect samples from river systems where invasive carp are present and travel for an investigator to attend three scientific conferences and present findings for a total of $13,000.
Other:	$0	
TOTAL ENRTF BUDGET:	**$445,210**	

Explanation of Use of Classified Staff:

Explanation of Capital Expenditures Greater Than $5,000: One biosafety cabinet will be purchased to prepare samples for diagnostic testing at a cost of $8,000. This cabinet was budgeted in Phase I of this project, but was not purchased at that time because the project team had access to a shared biosafety cabinet, which is no longer available.

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 2.75 FTE

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0 FTE

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
	$	$	
State			
	$	$	
TOTAL OTHER FUNDS:	**$**	**$**	

VII. SUB-PROJECT STRATEGY:

A. Sub-Project Team/Partners: The project team for this project has a track record of collaborative and successful research efforts. The Project Manager, Dr. Nicholas Phelps, will provide overall guideance for the project. Dr. Sunil Kumar Mor's effort is critical to the success of the project. He will contribute to the sample collection and processing, diagnostics, sequence analysis, report preparation, and manuscript publication. Dr. Alex Primus will contribute diagnostic support and lead the disease challenges. One graduate student will be supported on this project and contribute to all activiites. Local, national, and international stakeholders will be encouraged to participate in the project to help guide implementation and maximize impact.

B. Sub-Project Impact and Long-term Strategy: There will be a variety of potential benefits from this research. Most importantly, the results will help to inform science-based risk assessments to pursue the use viruses for invasive species control. In addition, the results will benefit fish health management decisions that impact the short and long-term sustainability of native and invasive fish populations in Minnesota by identifying emerging threats.

This is the second phase of a long-term effort to evaluate the use of viruses as biological control agents for aquatic invasive species. Understanding the virome is a critical first step, followed by infection trials, environmental and risk assessments, development of release strategies, and implementation. The current Project Team is well suited for the initial stages and aims to build collaborations for future work in the areas of risk assessment and release strategies. These conversations have already begun with researchers at the Commonwealth Scientific and Industrial Research Organization in Australia and the United States Department of Agriculture, among others. Meanwhile, it is critical to engage stakeholders at all levels to evaluate/ensure a viable path forward should the research prove promising.

C. Spending History:

Funding Source	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or FY12-13	M.L. 2013 or FY14

(add or remove rows and columns as needed)

VIII. ACQUISITION/RESTORATION LIST: N/A

IX. VISUAL ELEMENT or MAP(S): N/A

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH PROPOSAL: See attached research proposal.

XII. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than July 31, 2017, January 31, 2018, July 31, 2018, January 31, 2019, and July 31, 2019. A final report and associated products will be submitted within two months of the anticipated sub-project completion of June 30, 2019.