



Environment and Natural Resources Trust Fund (ENRTF)

M.L. 2013 Minnesota Aquatic Invasive Species Research Center

Sub-Project Work Plan

Date of Report: January 30, 2017

Date of Next Status Update Report: July 31, 2017

Date of Work Plan Approval: June 20, 2016

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 [#12]: Characterizing spiny water flea impacts using sediment records

Sub-Project Manager: Donn Branstrator

Organization: University of Minnesota Duluth

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Location: Statewide

| | | |
|--|----------------------------|------------------|
| Total ENRTF Sub-Project Budget: | Sub-Project Budget: | \$207,766 |
| | Amount Spent: | \$12,372 |
| | Balance: | \$195,394 |

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: Characterizing spiny water flea impacts using sediment records

II. SUB-PROJECT STATEMENT: The non-native species of freshwater zooplankton called spiny water flea represents a potential threat to Minnesota lakes but little is known about how this invader changes a lake's ecology and whether time delays occur before potential changes manifest in an invaded ecosystem. Spiny water flea is a member of the Crustacea, a large taxonomic group that has freshwater and marine representatives

including crayfish, shrimp, and crabs. Spiny water flea grows to a length of about one centimeter as an adult and can mature and reproduce in about one week. As a result of its prolific rate of growth, invading individuals can quickly increase in number and colonize a lake.

A major potential risk for the health of Minnesota lakes is that spiny water flea is a carnivore that feeds aggressively on native herbivorous zooplankton, a food resource that is shared as prey by many species of young fish including walleye, northern pike, and yellow perch. This potential competitive interaction with young fish could slow the growth and health of many native fish species in Minnesota. A second potential risk for the health of Minnesota lakes is that herbivorous zooplankton play key roles as grazers on algae, the microscopic plants that form the base of aquatic food webs. Higher concentrations of algae are directly related to lower water clarity. Thus, through removal of herbivorous zooplankton, spiny water flea threatens to reduce the health of fish through competition and to reduce water clarity through eliminating native grazers. These impacts could bring changes to Minnesota lakes that have serious implications for recreation and wildlife. At present, about 40 Minnesota lakes are infested with spiny water flea including some of the state's largest and most recreationally important aquatic ecosystems that include Lakes Basswood, Burntside, Gunflint, Island, Kabetogama, Lake of the Woods, Mille Lacs, Rainy, and Saganaga.

The goal of this project is to determine the types, magnitude, and timing of changes that occur to lakes after spiny water flea invades by describing and comparing long-term historical trends in key components of the food webs of two invaded lakes (Kabetogama Lake and Mille Lacs Lake) and two non-invaded, control lakes (Leech Lake and Winnibigoshish Lake). The results will document the changes occurring in populations of spiny water flea, herbivorous zooplankton prey species, and algae during the 48-year period from 1970 to 2017. This period spans the date of initial detection of spiny water flea in Kabetogama Lake (2007) and Mille Lacs Lake (2009). The two control lakes allow for an evaluation of the presence and strength of potential external-driven impacts (e.g., by climate) and internal-driven impacts (e.g., by zebra mussel) on zooplankton and algae that may be driving ecosystem change independent of spiny water flea invasion. Documenting not only the types and magnitude of changes, but also the chronology of changes that occur post-invasion, including the duration of potential time delays (known as lag times), is a key contribution of this project. The methods will enable assessment of the chronology of ecosystem change with a temporal resolution of 1-2 years.

The goal of this project will be achieved through the collection and analysis of sediments that accumulate on the bottoms of lakes. Lake sediments contain rich archives of information about the composition and abundance of biota that were once living in a lake. This information can be dated and reconstructed with considerable precision. A piston corer will be used to collect sediment material at four locations in each invaded lake and two locations in each control lake. Sediment from each piston core will be sectioned in the lab in 0.5-cm increments. The age of sediment sections will be determined by dating with naturally occurring lead and cesium isotopes by staff at the St. Croix Watershed Research Station (Science Museum of Minnesota) who will perform the analytical and interpretive work. Zooplankton populations will be reconstructed through microscopic analysis of diagnostic body parts that are preserved in the sediment record. Algae populations will be reconstructed through the analysis of photosynthetic pigments that are preserved in the sediment record. This will be done at the Environmental Quality Analysis Laboratory (University of Regina, Canada) directed by Dr. Peter Leavitt. Dr. Leavitt is the world's top expert in analysis of pigments in lake sediments. His laboratory is fully equipped to perform these analyses, and due to this infrastructure Dr. Leavitt is able to provide the lowest price per sample analysis. He will also provide input on interpretation (appropriateness, quality assurance/quality control) of the results after project results are developed. Domestic laboratories that perform pigment analysis are available, but from them we will not receive the price, experience, and follow-up input that we will receive from Dr. Leavitt.

Part of the data analysis will include the development of mathematical functions that relate the number of spiny water flea and herbivorous zooplankton in the water column to the number that are recovered in the sediments. This calibration step, as it is referred to by ecologists, is necessary in order to make direct

comparisons among lakes of the densities of organisms in the sediments. Zooplankton collected from the water column by research scientists at the Minnesota Department of Natural Resources and Voyageurs National Park will be used to develop the calibrations. Letters of commitment for this collaboration are included in the research proposal.

This project will help define threats of spiny water flea to the health of Minnesota lakes, including potential threats to ecosystem services such as sport fishing and water quality. The results will build capacity to characterize these threats through a detailed understanding of the types, magnitude, and timing of changes that take place in aquatic food webs in response to invasion. The results will benefit scientists at the Minnesota Department of Natural Resources and Voyageurs National Park in their efforts to evaluate their own, long-term data sets on fish populations in response to spiny water flea invasion in Kabetogama Lake and Mille Lacs Lake. Quantifying the impacts and timelines of response to invasion will benefit academic, management, and non-management organizations in Minnesota in their efforts to build broader understanding of cause and effect between spiny water flea and the environment. This information can be applied to achieve better awareness and action surrounding spiny water flea, including more effective management and education efforts to reduce its spread.

III. SUB-PROJECT STATUS UPDATES:

Sub-Project Status as of January 31, 2017:

We have been preparing for the field season (February and March, 2017) when we will collect sediment cores from the 4 study lakes (Kabetogama, Leech, Mille Lacs, and Winnibigoshish) on this project. This preparation has included the hiring of an undergraduate research assistant (Mr. Ben Block), application for a permit to remove lake bottom sediment from Lake Kabetogama in Voyageurs National Park (a federally protected area), ordering of additional supplies for the field work, and the collection and interpretation of information from the MNDNR and Voyageurs National Park on suitable coring locations (latitude, longitude) in the study lakes based on historical work that these organizations have done related to spiny water flea presence. During an upcoming meeting of the research team (Branstrator, Reavie, Kennedy), final coring locations will be chosen. Preliminary coring locations in two of the lakes are indicated in the table below under Activity 1.

We have also made progress on outreach goals. Branstrator gave two 50-minute presentations at the MAISRC Annual Showcase (September 12, 2016) in St. Paul and conducted four 10-minute laboratory demonstrations during an afternoon workshop at the Annual Showcase. During the presentations, the goals and general methods of this project were described.

Sub-Project Status as of July 31, 2017:

We completed a successful field season during February and March when we collected 13 sediment cores including 7 cores from Lake Mille Lacs and 6 cores from Lake Kabetogama. Their coordinates are given below under the activity status section. We also began laboratory preparation and examination of core contents. All 13 cores were sectioned. Water and organic content was done on 3 cores from Lake Mille Lacs and subsamples from one of the cores was prepped (freeze dried) and sent to the St. Croix Watershed Research Station for Lead-210 and Cesium-137 dating. We recruited a graduate student, Nichole DeWeese, into the Water Resources Science Graduate Program. She will assist with fossil analysis of spiny water flea and other zooplankton in the core material, and use this project as the centerpiece of the MS degree.

We met methodological challenges that prevented us from collecting sediment cores from all of the field sites this winter. On Lake Mille Lacs we encountered problems locating firm sediment at times and had to abandon one of the four sites. We will return to Lake Mille Lacs this coming winter (2017-2018) to complete the field work. Due to an early spring thaw and poor, thinning ice conditions, we were unable to collect sediment cores

from all four sites in Lake Kabetogama. We will return to Lake Kabetogama this coming winter to complete field work. Due to an early spring thaw, we were also unable to collect sediment cores from Leech Lake and Winnibigoshish Lake, and we will return to both lakes this coming winter to conduct field work. These delays will not affect the pace of data collection on the project because there is plenty of work to be done on the 13 cores that were collected. Funds remain in the budget for the remaining field work.

Sub-Project Status as of January 31, 2018:

Sub-Project Status as of July 31, 2018:

Sub-Project Status as of January 31, 2019:

Overall Sub-Project Outcomes and Results:

IV. SUB-PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Sediment Core Location Selection and Retrieval

Description: Sediment coring locations will be identified and sediment cores will be collected from four locations in each of two lakes that are invaded by spiny water flea (Kabetogama Lake and Mille Lacs Lake) and from two locations in each of two non-invaded lakes (Leech Lake and Winnibigoshish Lake). The selection of coring locations will be guided by lake depth, known site-specific variation in sedimentation rates, and historical/contemporary collections of zooplankton and fish made by research scientists at the Minnesota Department of Natural Resources and Voyageurs National Park. Sediment cores will be retrieved using a piston corer with a 7-cm diameter polycarbonate core barrel and operated by rigid drive rods. The sediment cores will be returned to the laboratory for analysis. The sediment cores will be collected through the ice during the winter months when the lakes are ice-covered. This activity represents the field work part of the project.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 41,013

Amount Spent: \$ 12,118

Balance: \$ 28,895

Activity Completion Date:

| Outcome | Completion Date |
|---|-------------------|
| 1. Lists of suitable coring locations, given as latitude and longitude, identified and justified for each of the four study lakes. | December 31, 2016 |
| 2. Retrieval of sediment cores from each of the four study lakes and their return to, and storage at, the Natural Resources Research Institute. No fewer than 12 sediment cores will be collected for analysis. | March 31, 2017 |

Activity Status as of January 31, 2017:

Our preliminary coring locations are noted below. These locations were chosen based on a number of factors including proximity to previous and ongoing studies on zooplankton including spiny water flea. Locations marked TBD (to be determined) will be chosen at an upcoming meeting of the research team. Locations are subject to change based on accessibility to these locations and other factors.

| Site # | Latitude | Longitude | Justification |
|--------------|-----------|-----------|---|
| Kabetogama 1 | 48.4582°N | 92.9651°W | Voyageurs long-term monitoring site |
| Kabetogama 2 | 48.4525°N | 92.8965°W | Site of Kerfoot et al. (2016) collections |
| Kabetogama 3 | TBD | TBD | |
| Kabetogama 4 | TBD | TBD | |

| | | | |
|------------------|-----------|-----------|-------------------------------------|
| Leech 1 | TBD | TBD | |
| Leech 2 | TBD | TBD | |
| Mille Lacs 1 | 46.2576°N | 93.7607°W | DNR zooplankton collection site ZO2 |
| Mille Lacs 2 | 46.2493°N | 93.6794°W | DNR zooplankton collection site ZO3 |
| Mille Lacs 3 | 46.3336°N | 93.5999°W | DNR zooplankton collection site ZO8 |
| Mille Lacs 4 | 46.1873°N | 93.6159°W | DNR zooplankton collection site ZO5 |
| Winnibigoshish 1 | TBD | TBD | |
| Winnibigoshish 2 | TBD | TBD | |

Activity Status as of July 31, 2017:

Outcomes 1 and 2 are partially complete. Below are the locations and dates of the 13 sediment cores collected thus far on the project.

| Lake Name | Date | Core Code # | Location |
|------------|-----------|-------------|----------------------|
| Mille Lacs | 2/16/2017 | DKB3A (#1) | 46.2494 N, 93.6794 W |
| Mille Lacs | 2/16/2017 | DKB3B (#2) | 46.2494 N, 93.6794 W |
| Mille Lacs | 2/16/2017 | DKB3C (#3) | 46.2494 N, 93.6794 W |
| Mille Lacs | 2/17/2017 | DKB5A (#4) | 46.2249 N, 93.6113 W |
| Mille Lacs | 2/17/2017 | DKB5B (#5) | 46.2249 N, 93.6113 W |
| Mille Lacs | 2/17/2017 | DKB8A (#6) | 46.3143 N, 93.5963 W |
| Mille Lacs | 2/17/2017 | DKB8B (#7) | 46.3143 N, 93.5963 W |
| Kabetogama | 3/19/2017 | KAB1A (#1) | 48.4958 N, 93.0727 W |
| Kabetogama | 3/19/2017 | KAB1B (#2) | 48.4958 N, 93.0727 W |
| Kabetogama | 3/19/2017 | KAB2A (#3) | 48.4842 N, 93.0359 W |
| Kabetogama | 3/19/2017 | KAB2B (#4) | 48.4842 N, 93.0359 W |
| Kabetogama | 3/19/2017 | KAB3A (#5) | 48.4608 N, 93.0089 W |
| Kabetogama | 3/19/2017 | KAB3B (#6) | 48.4608 N, 93.0089 W |

Activity Status as of January 31, 2018:

Activity Status as of July 31, 2018:

Activity Status as of January 31, 2019:

Final Report Summary:

ACTIVITY 2: Sediment Core Processing

Description: Sediment material will be extruded and sectioned in the laboratory in 0.5-cm increments and analyzed for water content and organic content (Natural Resources Research Institute), isotopic age (Science Museum of Minnesota), abundances of body remains of spiny water flea and other zooplankton species (University of Minnesota Duluth), and algae pigment composition and concentration (University of Regina). This activity represents the laboratory work part of the project.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 160,837

Amount Spent: \$ 254

Balance: \$ 160,583

Activity Completion Date:

| Outcome | Completion Date |
|--|------------------------|
| 1. Sectioned sediment cores in 0.5-cm increments. | June 30, 2017 |
| 2. Results of water and organic content of core sections in all 12 cores. | June 30, 2017 |
| 3. Results of isotopic aging of core sections in all 12 cores. | December 31, 2017 |
| 4. Results of composition and abundance of zooplankton remains in all 12 cores for sections dating from 1970-2017. | March 31, 2019 |
| 5. Results of algae pigment composition and concentration in 6 of 12 cores for sections dating from 1970-2017. | March 31, 2019 |

Activity Status as of January 31, 2017:

No activity this period.

Activity Status as of July 31, 2017:

All cores collected to date have been sectioned, so Outcome 1 is complete. Outcome 2 is partly complete. Water and organic content (LOI) has been completed on 3 cores from Mille Lacs, and one of those cores has had subsamples sent for Pb-210 and Cs-137 processing. It is expected that LOI analyses will be complete on these cores in October.

Activity Status as of January 31, 2018:

Activity Status as of July 31, 2018:

Activity Status as of January 31, 2019:

Final Report Summary:

ACTIVITY 3: Analysis and Dissemination of Results

Description: The results will be analyzed using a variety of statistical approaches including linear and non-linear model-fitting, time-series, and multivariate methods. Calibrations will be constructed that relate the number of spiny water flea and herbivorous zooplankton in the water column of a lake to the number that are recovered in the sediments. The project manager and PhD graduate student will present the results at a science conference. This activity represents the data analysis and dissemination parts of the project.

Summary Budget Information for Activity 3:

ENRTF Budget: \$ 5,916

Amount Spent: \$ 0

Balance: \$ 5,916

Activity Completion Date:

| Outcome | Completion Date |
|--|------------------------|
| 1. Results of statistical analysis and interpretation, including the construction of calibrations, for data collected in activity 2. | June 30, 2019 |
| 2. Two oral and/or poster presentations of the results given at a science conference. | June 30, 2019 |

Activity Status as of January 31, 2017:

No activity this period.

Activity Status as of July 31, 2017:

No activity this period.

Activity Status as of January 31, 2018:

Activity Status as of July 31, 2018:

Activity Status as of January 31, 2019:

Final Report Summary:

V. DISSEMINATION:

Description:

The project manager will work closely with the MAISRC to share the results of the project and make them available and interpretable in a timely fashion. The project manager will participate in MAISRC service by participating on 1-2 committees, MAISRC outreach including participation at the annual Showcase event, and MAISRC extension on media efforts and communications via the AIS Spotlight e-newsletter, www.maisrc.umn.edu, and Facebook and Twitter accounts. A PhD dissertation will be one product of this project. All data will be submitted to the MAISRC at the completion of the project.

The selection of the study lakes was done with careful consideration through consultation with research scientists at the Minnesota Department of Natural Resources and Voyageurs National Park in order to identify lakes with substantial historical and contemporary data on fish and zooplankton populations that will maximize opportunities for those organizations to test impacts of spiny water flea invasion on fish. In assembling this proposal, Jodie Hirsch and David Staples (Minnesota Department of Natural Resources) and Ryan Maki (Voyageurs National Park), were consulted extensively. These people will be important contacts for sharing the results of this project.

Status as of January 31, 2017:

Branstrator participated in the MAISRC Annual Showcase event (September 12, 2016) where he gave two lectures and four laboratory presentations. He also sat on the MAISRC Showcase Planning Committee for 2016, and the MAISRC Research Needs Assessment Team for 2016.

Status as of July 31, 2017:

Branstrator and other team members were featured on ABC Eyewitness News (KSTP in Minneapolis and St. Paul) on February 17, 2017, describing field work on Lake Mille Lacs and the objectives of the project. Find link at <http://kstp.com/news/spiny-water-flea-potential-clue-to-walleye-decline-on-mille-lacs-minnesota-dnr/4403483/>. The project was featured in a newspaper article in the Brainerd Dispatch on February 18, 2017. Find link at <http://www.brainerddispatch.com/outdoors/4220896-core-samples-mille-lacs-lake-may-explain-walleye-woes>. Branstrator was featured in a live, 20-minute radio interview with host Ann Possis of WTIP North Shore Community Radio (Grand Marais) on March 31, 2017, (posted April 3, 2017) describing the biology of spiny water flea and the objectives of the project. Find link at <http://www.wtip.org/u-ms-donn-branstrator-spiny-water-flea>. Branstrator, who is a member of the Mille Lacs Fishery Advisory Committee, presented a 10-minute overview of the project and answered questions at a public meeting in Isle, Minnesota, on February 15, 2017. Branstrator gave a 50-minute invited seminar entitled "Invasion Biology in the BWCAW and Quetico" to a group of 25 college students and 4 faculty at the Coe College Wilderness Field Station (Ely, Minnesota) on July 22, 2017 where he discussed this MAISRC project and the invasion status of spiny water flea in Minnesota.

Status as of January 31, 2018:

Status as of July 31, 2018:

Status as of January 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: | \$148,766 | Branstrator/Associate Professor: \$25,651 salary, \$8,645 benefits (33.7% fringe rate), 0.23 FTE total for 3 years; Reavie/Sr. Research Associate: \$26,317 salary, \$8,868 benefits (33.7% fringe rate), 0.23 FTE total for 3 years; TBN/Ph.D. Graduate Research Assistant: \$34,285 salary, \$20,298 benefits (17.6% fringe rate), 0.69 FTE total for 3 years; TBN/Undergraduate Research Assistant: \$5,339 salary, \$0 benefits, 0.27 FTE total for 3 years; Kennedy/Principle Lab Tech: \$15,199 salary, \$4,164 benefits (27.4% fringe rate), 0.35 FTE total for 3 years. |
| Professional/Technical Services and Contracts: | \$48,200 | Sample shipping for dating and pigment analyses (\$200); services provided by St. Croix Watershed Research Station of the Science Museum of Minnesota for sediment core dating (\$36,000); services provided by Environmental Quality Analysis Laboratory at the University of Regina, Canada, for sediment core pigment analyses (\$12,000). |
| Equipment/Tools/Supplies: | \$1,300 | Field and lab supplies including core tubes, plastic vials, supplies for sectioning, freeze-drying, loss on ignition analyses, and microscopy (\$1300). |
| Capital Expenditures over \$5,000: | \$ | |
| Travel: | \$9,500 | Travel in MN to field sites (Kabetogama, Leech, Mille Lacs, and Winnibigoshish Lakes) to conduct project activities including mileage, lodging, and meals (\$6000); travel to a professional conference to present results including conference registration, transportation, lodging, and meals (\$3,500) for project manager and PhD student. |
| Other: | \$ | |
| TOTAL ENRTF BUDGET: | \$207,766 | |

Explanation of Use of Classified Staff: None

Explanation of Capital Expenditures Greater Than \$5,000: None

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 1.77 total FTEs

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: None

B. Other Funds: None

| 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:

- 1) Staff at the Minnesota Department of Natural Resources will contribute data on crustacean zooplankton and will collaborate on the development of calibration functions. A letter of commitment from Jodie Hirsch for this part of the project is included in the proposal.
- 2) Staff at Voyageurs National Park will contribute data on crustacean zooplankton and will collaborate on the development of calibration functions. A letter of commitment from Ryan Maki for this part of the project is included in the proposal.

B. Sub-Project Impact and Long-term Strategy:

A recent study concluded that 41% of lakes in northern Minnesota provide suitable habitat for spiny water flea. This is concerning given the potential impact that this invader may have on the environment. Further, it is believed that recreational boating and angling equipment are the primary land transportation routes by which spiny water flea spreads to new lakes. Therefore, human choices about behavior and equipment are major keys to preventing further range expansion of this invader. Research that demonstrates the short-term and long-term impacts of spiny water flea on the quality of aquatic natural resources should therefore have substantial utility in supporting the continued development and defensibility of best management practices (e.g., education materials and policies) to reduce spread of this invader.

Kabetogama Lake and Mille Lacs Lake are recognized by the Minnesota Department of Natural Resources and Voyageurs National Park as high priority lakes for this study owing to the availability of pre-existing food-web data. For both lakes there are extensive fish data collected by these organizations from as early as the 1980s. Both organizations are highly supportive of this research and will use it to test hypotheses about the impacts of spiny water flea on fish with outcomes that could help shape future management decisions.

There are plans to continue this research by expanding the methods to evaluate the impacts of spiny water flea in the Minnesota waters of Lake Superior and the Duluth-Superior Harbor as well as other infested inland Minnesota lakes (e.g., Rainy Lake) for which long-term fish data are available or eventually become available.

C. Spending History:

| Funding Source | M.L. 2008 or | M.L. 2009 or | M.L. 2010 or | M.L. 2011 or | M.L. 2013 or |
|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | | | | |

| | FY09 | FY10 | FY11 | FY12-13 | FY14 |
|--|------|------|------|---------|------|
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| | | | | | |
| | | | | | |

VIII. ACQUISITION/RESTORATION LIST: N/A

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

X. ACQUISITION/RESTORATION REQUIREMENTS WORKSHEET: N/A

XI. RESEARCH PROPOSAL:

XII. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than [DATE], [DATE], and [DATE]. A final report and associated products will be submitted within two months of the anticipated sub-project completion of [date of sub-project completion].