M.L. 2013 Minnesota Aquatic Invasive Species Research Center Subproject Abstract
For the Period Ending June 30, 2019

SUBPROJECT TITLE: MAISRC Subproject 12: Characterizing spiny water flea impacts using sediment records
SUBPROJECT MANAGER: Donn Branstrator
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FUNDING SOURCE: Environment and Natural Resources Trust Fund (ENRTF)
LEGAL CITATION: M.L. 2013, Chp. 52, Sec. 2, Subd. 06a

SUBPROJECT BUDGET AMOUNT: $212,266
AMOUNT SPENT: $211,708
AMOUNT REMAINING: $558

Overall Subproject Outcome and Results
Although aquatic invasive species threaten Minnesota’s environment, economy, and recreation, we still know little about the colonization histories and ecosystem impacts of some of the state’s invaders such as spiny water flea. This project made large advances in understanding the colonization and impact of spiny water flea in Lake Mille Lacs, Lake Kabetogama, Lake Winnibigoshish, and Leech Lake through the collection and analysis of organism remains in lake bottom sediments over about a 120 year period from present (2017 or 2018) back to the year 1900. The results provide replicated evidence that spiny water flea was resident continuously in Lake Mille Lacs and Lake Kabetogama since the 1930s, or about 80 years before it was first detected in the open waters of either lake. Evidence demonstrates that spiny water flea had a prolonged history of low abundance in both lakes before about the year 2000 at which time it began to increase rapidly. Zooplankton that are prey and competitors of spiny water flea often declined in abundance after spiny water flea increased in abundance. There was no evidence of spiny water flea in the sediments of Lake Winnibigoshish. There was evidence of a small population of spiny water flea in the sediments of Leech Lake that dated to the year 2001, possibly representing a failed invasion. To date, Leech Lake has never been known to contain this organism. The data allow us to test hypotheses about the timing and impact of spiny water flea on the food webs of Minnesota lakes. The results re-cast our understanding of the timeline of spiny water flea invasion in Minnesota and underscore the value of lake sediments to study invasive species. The results suggest that traditional methods of spiny water flea detection with nets, as carried out by academic units and management agencies in Minnesota, may be inadequate to detect spiny water flea when it is low or transient in abundance.

Subproject Results Use and Dissemination
We have disseminated our project results at a variety of conferences and meetings as summarized below.

1) MAISRC Research & Management Showcase (St. Paul, MN) – two platform presentations (September 12, 2016)
2) MAISRC Research & Management Showcase (St. Paul, MN) – four laboratory presentations (September 12, 2016)
3) Coe College Wilderness Field Station (Ely, MN) – platform presentation (July 22, 2017)
4) MAISRC Research & Management Showcase (St. Paul, MN) – two platform presentations (September 13, 2017)
5) MAISRC All Members meeting (St. Paul, MN) – platform presentation (November 28, 2017)
6) MAISRC Science-In-Seconds competition (St. Paul, MN) – platform presentation (May 30, 2018)
7) MAISRC Research & Management Showcase (St. Paul, MN) – poster presentation (September 12, 2018)
8) Upper Midwest Invasive Species Conference (Rochester, MN) – poster presentation (October 15-18, 2018)
9) Association for the Sciences of Limnology and Oceanography Conference (San Juan, Puerto Rico) – poster presentation (Feb 23 – Mar 2, 2019)
11) Minnesota Department of Natural Resources meeting (St. Paul, MN) – skype presentation (May 14, 2019)

We have included images of two poster presentations that were displayed at science conferences.
SUBPROJECT TITLE: MAISRC Subproject 12: Characterizing spiny water flea impacts using sediment records

Subproject Manager: Donn Branstrator
Organization: University of Minnesota Duluth
Mailing Address: Department of Biology, 1035 Kirby Drive, 207 SSB
City/State/Zip Code: Duluth, MN 55812
Telephone Number: (218) 726-8134
Email Address: dbranstr@d.umn.edu
Web Address: http://www.d.umn.edu/biology/faculty/branstrator.html

Location: Statewide

Total ENRTF Subproject Budget: $4,350,000
Subproject Budget: $212,266
Amount Spent: $211,708
Balance: $558

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. SUBPROJECT TITLE: Characterizing spiny water flea impacts using sediment records

II. SUBPROJECT 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. Sediment samples will be predigested in Potassium Hydroxide (KOH) to remove unwanted organic material and help concentrate the subfossil remains. 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. SUBPROJECT STATUS UPDATES:

Subproject 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.

Subproject 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.

**Subproject Status as of January 31, 2018:**
We completed a successful start to the laboratory analyses. Two of the sediment cores were processed for dates (measured for age by depth) based on Pb-210 at the St. Croix Watershed Research Station. One of these two cores was also processed for algae pigments (measured as concentrations and types of pigments by depth) at the University of Regina. We processed this same core for zooplankton remains (measured as subfossil numbers and types by depth) in our lab at UMD. We worked out a variety of sample preparation methods prior to processing the sediment for zooplankton remains. The Minnesota Department of Natural Resources staff shared some of their data with us on zooplankton abundance in Lake Mille Lacs that we will use to construct calibrations to help us infer abundances of zooplankton remains in the sediment samples from that lake.

**Subproject Status as of July 31, 2018:**
During this period we collected the final sediment cores for the project. All 25 sediment cores have now been collected, bringing Activity 1 to a close. We continued to process the sediment cores for water and organic content, isotopic aging, zooplankton subfossils, and algae pigments, all under Activity 2. We adopted a technique to help predigest unwanted organic material in the sediments before we search them for subfossils. This necessitated an amendment to the proposal that will allow us to purchase enough of the chemical to complete the work. We hired three undergraduate students at UMD who are assisting us in the laboratory this summer. We are making good progress and we are on schedule to meet our outcome deadlines.

**Amendment Request July 31, 2018:**
In the course of our project we learned that Potassium Hydroxide (KOH) will digest away unwanted organic material in the sediment samples and lessen the time required to search and recover zooplankton subfossils. It is a desirable technique and we wish to use it here but the cost of KOH was not budgeted in the original proposal. Each sediment core requires about $80 of KOH. Given that we are analyzing 12 cores for zooplankton subfossils, we underbudgeted $960 (12 x $80) for lab supplies under Activity 2. In the course of our project we also learned that we overbudgeted for travel associated with the retrieval of sediment cores under Activity 1. Therefore, we propose to amend the budget by shifting $1000 from Travel under Activity 1 to Equipment/Tools/Supplies under Activity 2.

Amendment Approved: [07/31/2018]

**Subproject Status as of January 31, 2019:**
During this period we worked mainly on the outcomes under Activity 2. We continued to analyze sediment cores for age and have completed that Outcome 3 for 11 of 12 cores. We continued to analyze sediment cores for zooplankton subfossils back to 1970 and have completed that Outcome 4 for 6 of 12 cores. We continued to analyze sediment cores for algae pigments back to 1970 and have completed that Outcome 5 for 2 of 6 cores. We are generally on or near schedule to meet our outcome deadlines for Activity 2 and 3 as specified in the work plan. The only exception is Activity 2 (Outcome 3, sediment dating, deadline December 31, 2017) but this outcome should be completed in the next month. Under Activity 3 (Outcome 2), we gave a poster presentation at the Upper Midwest Invasive Species Conference (Rochester, Minnesota) on this project.

**Amendment Request January 31, 2019:**
As the project nears end, we have come close to meeting our intended budget allocations but some adjustments need to be made. To complete the project, we are asking to re-allocate some of the remaining funds.
Amendment 1
We request an amendment to re-budget funds in order to align our remaining resources to meet project needs leading up to the completion of the project. Requested budget amendment is as follows:

Activity 1 – decrease total budget from $40,013 to $38,955; Net +$1,058

- **Travel – MN** – decrease from $5,000 to $3,942 to zero out the budget. Travel for field work in Activity 1 is complete.

Activity 2 – decrease total budget from $161,837 to $161,391; Net +$446

- **Personnel** – increase from $112,137 to $114,603 for undergraduate students to continue to work on helping us with subfossil recovery from the sediment cores.
- **Services - Office and General Operations** – increase from $200 to $250 to cover additional shipping costs for isotope analysis.
- **Professional Services and Contracts** – decrease from $36,000 to $33,000. The Pb-210 dating profiles were very clean on many of the cores. This allowed us to forego purchasing Cs-137 dating on those cores for an overall savings of $3,000.
- **Supplies - Lab and Field** – increase from $1,500 to $1,538 to cover costs for supplies needed to complete Activity 2.

Activity 3 – increase from $5,916 to $7,420; Net -$1,504

- **Personnel** – increase from $2,416 to $6,416 for increased graduate student assistance (Nichole DeWeese) to do statistical analyses on the project results.
- **Travel – Domestic** – decrease from $3,500 to $1,004. Some of the travel expenses for Nichole DeWeese to the upcoming conference (ASLO in Puerto Rico, February 2019) are being covered by the UMD Graduate School and the WRS Graduate Program. Also, most of the travel expenses for Nichole DeWeese to the UMISC conference in Rochester, Minnesota, in October 2018, where she presented on this project, were covered by Minnesota Sea Grant.

Amendment 2
Activity 2 – we request an amendment to add an additional $4,500 to **Personnel** funds in Activity 2, bringing the total **Personnel** funds in Activity 2 to $119,103. These funds would be an addition to the existing project budget and would increase the overall project total to $212,266. Additional personnel funds will allow us to hire two undergraduate researchers (40 hrs/week at $10.26/hr) who will help extend our search for subfossil evidence of spiny water flea to earlier time periods, with the objective of finding the transition between presence and absence of spiny water flea subfossils in the sediments. MAISRC approved an additional $4,500 in funding on 1/30/2019.

Amendment 3
We request approval to send our graduate student, Nichole DeWeese, to a meeting of the Association for the Sciences of Limnology and Oceanography in Puerto Rico in February 2019 to present our results. Our results have revealed fascinating trends that suggest spiny water flea were present in Lake Mille Lacs and Lake Kabetogama since the 1970s, if not longer. This meeting attracts a large group of international experts on invasive species and paleolimnology and we believe it will be an influential venue for presentation. The funds we would use for attending this conference are already allocated in the **Travel-Domestic** budget.

Amendments Approved: 03/11/2019
Overall Subproject Outcomes and Results:
Although aquatic invasive species threaten Minnesota’s environment, economy, and recreation, we still know little about the colonization histories and ecosystem impacts of some of the state’s invaders such as spiny water flea. This project made large advances in understanding the colonization and impact of spiny water flea in Lake Mille Lacs, Lake Kabetogama, Lake Winnibigoshish, and Leech Lake through the collection and analysis of organism remains in lake bottom sediments over about a 120 year period from present (2017 or 2018) back to the year 1900. The results provide replicated evidence that spiny water flea was resident continuously in Lake Mille Lacs and Lake Kabetogama since the 1930s, or about 80 years before it was first detected in the open waters of either lake. Evidence demonstrates that spiny water flea had a prolonged history of low abundance in both lakes before about the year 2000 at which time it began to increase rapidly. Zooplankton that are prey and competitors of spiny water flea often declined in abundance after spiny water flea increased in abundance. There was no evidence of spiny water flea in the sediments of Lake Winnibigoshish. There was evidence of a small population of spiny water flea in the sediments of Leech Lake that dated to the year 2001, possibly representing a failed invasion. To date, Leech Lake has never been known to contain this organism. The data allow us to test hypotheses about the timing and impact of spiny water flea on the food webs of Minnesota lakes. The results re-cast our understanding of the timeline of spiny water flea invasion in Minnesota and underscore the value of lake sediments to study invasive species. The results suggest that traditional methods of spiny water flea detection with nets, as carried out by academic units and management agencies in Minnesota, may be inadequate to detect spiny water flea when it is low or transient in abundance.

IV. SUBPROJECT 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:          | $38,955 |
| Amount Spent:          | $38,955 |
| Balance:               | $0      |

Activity Completion Date:

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lists of suitable coring locations, given as latitude and longitude, identified and justified for each of the four study lakes.</td>
<td>December 31, 2016</td>
</tr>
<tr>
<td>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.</td>
<td>March 31, 2017</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Lake Name</th>
<th>Date</th>
<th>Core Code #</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mille Lacs</td>
<td>2/16/2017</td>
<td>DKB3A (#1)</td>
<td>46.2494 N, 93.6794 W</td>
</tr>
<tr>
<td>Mille Lacs</td>
<td>2/16/2017</td>
<td>DKB3B (#2)</td>
<td>46.2494 N, 93.6794 W</td>
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<tr>
<td>Mille Lacs</td>
<td>2/16/2017</td>
<td>DKB3C (#3)</td>
<td>46.2494 N, 93.6794 W</td>
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<tr>
<td>Mille Lacs</td>
<td>2/17/2017</td>
<td>DKB5A (#4)</td>
<td>46.2249 N, 93.6113 W</td>
</tr>
<tr>
<td>Mille Lacs</td>
<td>2/17/2017</td>
<td>DKB5B (#5)</td>
<td>46.2249 N, 93.6113 W</td>
</tr>
<tr>
<td>Mille Lacs</td>
<td>2/17/2017</td>
<td>DKB8A (#6)</td>
<td>46.3143 N, 93.5963 W</td>
</tr>
<tr>
<td>Mille Lacs</td>
<td>2/17/2017</td>
<td>DKB8B (#7)</td>
<td>46.3143 N, 93.5963 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/19/2017</td>
<td>KAB1A (#1)</td>
<td>48.4958 N, 93.0727 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/19/2017</td>
<td>KAB1B (#2)</td>
<td>48.4958 N, 93.0727 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/19/2017</td>
<td>KAB2A (#3)</td>
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<td>48.4608 N, 93.0089 W</td>
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<td>3/19/2017</td>
<td>KAB3B (#6)</td>
<td>48.4608 N, 93.0089 W</td>
</tr>
</tbody>
</table>

Activity Status as of January 31, 2018:
Outcomes 1 and 2 remain partially complete. We will collect the remaining sediment cores during February-March, 2018. They will include 2 cores (Mille Lacs), 2 cores (Kabetogama), 4 cores (Leech), and 4 cores (Winnibigoshish). The photo below (taken 2/16/2017) shows some of our team members preparing a sediment core apparatus on Lake Mille Lacs. Pictured are left Dr. Andrew Bramburger (researcher), center Mr. Matthew Bambach (MS student), and right Dr. Donn Branstrator (PI on the project).
### Activity Status as of July 31, 2018:

Outcomes 1 and 2 for this activity are now complete. The table below lists the locations and the collection dates of the 25 sediment cores that we collected on this project.

<table>
<thead>
<tr>
<th>Lake Name</th>
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<td>2/16/2017</td>
<td>DKB3B (#2)</td>
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<tr>
<td>Mille Lacs</td>
<td>2/16/2017</td>
<td>DKB3C (#3)</td>
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<td>46.3143 N, 93.5963 W</td>
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<tr>
<td>Mille Lacs</td>
<td>2/23/2018</td>
<td>DKB10A (#8)</td>
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<td>DKB10B (#9)</td>
<td>46.3156 N, 93.7470 W</td>
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<td>Kabetogama</td>
<td>3/19/2017</td>
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</tr>
<tr>
<td>Kabetogama</td>
<td>3/19/2017</td>
<td>KAB1B (#2)</td>
<td>48.4958 N, 93.0727 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/19/2017</td>
<td>KAB2A (#3)</td>
<td>48.4842 N, 93.0359 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/19/2017</td>
<td>KAB2B (#4)</td>
<td>48.4842 N, 93.0359 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/19/2017</td>
<td>KAB3A (#5)</td>
<td>48.4608 N, 93.0089 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/19/2017</td>
<td>KAB3B (#6)</td>
<td>48.4608 N, 93.0089 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/18/2018</td>
<td>KAB4A (#7)</td>
<td>48.4543 N, 92.9550 W</td>
</tr>
<tr>
<td>Kabetogama</td>
<td>3/18/2018</td>
<td>KAB4B (#8)</td>
<td>48.4543 N, 92.9550 W</td>
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<tr>
<td>Big Winnibigoshish</td>
<td>3/2/2018</td>
<td>WIN1A (#1)</td>
<td>47.4656 N, 94.1669 W</td>
</tr>
<tr>
<td>Big Winnibigoshish</td>
<td>3/2/2018</td>
<td>WIN1B (#2)</td>
<td>47.4656 N, 94.1669 W</td>
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<tr>
<td>Big Winnibigoshish</td>
<td>3/2/2018</td>
<td>WIN2A (#3)</td>
<td>47.4105 N, 94.1676 W</td>
</tr>
<tr>
<td>Big Winnibigoshish</td>
<td>3/2/2018</td>
<td>WIN2B (#4)</td>
<td>47.4105 N, 94.1676 W</td>
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<tr>
<td>Leech</td>
<td>3/3/2018</td>
<td>LEC1A (#1)</td>
<td>47.0977 N, 94.4489 W</td>
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<tr>
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<td>3/3/2018</td>
<td>LEC1B (#2)</td>
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<tr>
<td>Leech</td>
<td>3/3/2018</td>
<td>LEC2A (#3)</td>
<td>47.1281 N, 94.4390 W</td>
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<tr>
<td>Leech</td>
<td>3/3/2018</td>
<td>LEC2B (#4)</td>
<td>47.1281 N, 94.4390 W</td>
</tr>
</tbody>
</table>

Although this activity is ostensibly finished, we are going to leave some money in the travel budget (post amendment) in case we need to return to any of the field sites or we have other travel associated with this activity.

### Activity Status as of January 31, 2019:

All outcomes for Activity 1 were complete as of July 31, 2018.
**Final Report Summary:**

A total of 25 sediment cores were successfully retrieved under this project. Coring was done with a piston corer with a 7-cm diameter polycarbonate core barrel operated by rigid drive rods. The sediment cores were returned to the NRRI laboratory at UMD for analysis. Of the 25 sediment cores collected, 9 cores came from Mille Lacs, 8 came from Kabetogama, 4 came from Winnibigoshish, and 4 came from Leech. The cores were collected from 4 widely spaced locations in Mille Lacs, 4 widely spaced locations in Kabetogama, 2 widely spaced locations in Winnibigoshish, and 2 widely spaced locations in Leech. The cores were all collected during ice-covered periods in 2017 or 2018. They provided the material that we needed to successfully carryout Activities 2 and 3.

**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: | $165,891 |
| Amount Spent: | $165,803 |
| Balance: | $88 |

**Activity Completion Date:**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sectioned sediment cores in 0.5-cm increments.</td>
<td>June 30, 2017</td>
</tr>
<tr>
<td>2. Results of water and organic content of core sections in all 12 cores.</td>
<td>June 30, 2017</td>
</tr>
<tr>
<td>3. Results of isotopic aging of core sections in all 12 cores.</td>
<td>December 31, 2017</td>
</tr>
<tr>
<td>4. Results of composition and abundance of zooplankton remains in all 12 cores for sections dating from 1970-2017.</td>
<td>March 31, 2019</td>
</tr>
<tr>
<td>6. Results of composition and abundance of zooplankton remains in all 12 cores for sections dating pre-1970.</td>
<td>June 30, 2019</td>
</tr>
</tbody>
</table>

**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:**

Outcome 2: Processing for water and organic content (LOI) are still being completed on one core from Lake Mille Lacs and three cores from Lake Kabetogama. Those analyses should be done by the end of March, 2018.

Outcome 3: Core DKB5B and Core KAB1A have been processed for isotopic aging. The aging results from the St. Croix lab were sent to us in Excel format. Our initial analysis of the results indicate excellent preservation of the temporal record, ensuring that the dating is highly reliable. This is an important first step in the analysis of the fossil pigment and zooplankton remains because it ensures that the pigments we measure and the fossils we recover will be assignable to specific dates (years) in the sediment record. We have prepped four additional
cores that we collected in 2017 (two from Lake Mille Lacs and two from Lake Kabetogama) for dating. We expect all isotopic analyses for this project to be done by October, 2018.

Outcome 4: Before the core samples were processed for zooplankton remains, we worked out a reliable protocol for the removal of unwanted organic material from the samples. Our protocol uses 10% KOH as a solvent, heat (85°C) for 1 hour, and post-filtration (53 micron Nitex mesh). We processed core DKB5B to 1970 for bosminid zooplankton remains and to 1990 for spiny water flea zooplankton remains and daphniid zooplankton remains. We partially processed core DKB5A for zooplankton remains. Our results indicate spiny water flea were present in Lake Mille Lacs as early as the late 1990s, or about a decade before they were first detected in the water column in 2009. Our results also indicate that the abundance of bosminid zooplankton began to decline after 2010 in Lake Mille Lacs, a pattern that is consistent with the zooplankton records collected by the Minnesota DNR. These two assessments are preliminary and will need to be confirmed and refined by additional work on the other sediment cores we are collecting from the lake.

Outcome 5: Core DKB5B has been processed for algae pigments at the University of Regina. The data were sent to us in Excel format. We are beginning to analyze and interpret the data.

Activity Status as of July 31, 2018:
Outcome 1: All 25 cores have been sectioned. This outcome is now complete.

Outcome 2: All 25 cores have been processed for water content and organic content. This outcome is now complete.

Outcome 3: Seven of the 12 cores have undergone isotopic aging. The initial results indicate excellent preservation of the temporal record, ensuring that the dating is highly reliable. The remaining five cores that will be dated are being prepped. They are scheduled for isotopic aging later this summer. This outcome is behind schedule because we had to delay the collection of some cores by one year due to an early spring thaw in 2017. Nonetheless, we are catching up, and this delay is not impeding our overall progress on the project objectives.

Outcome 4: One Mille Lacs core (DKB5B) and two Kabetogama cores (KAB1A, KAB2A) have been processed for the composition and abundance of zooplankton subfossil remains in strata dating from 1970-2017. A second Mille Lacs core (DKB5A) has been processed in a few select strata in order to compliment the data from DKB5B.

Our results indicate that spiny water flea was clearly present in Mille Lacs and Kabetogama for decades before it was first detected in the water column. Our results also hint that density changes occurred in several prey and competitor species of the spiny water flea coincident with their increased density during invasion. These assessments are preliminary and will need to be confirmed and refined by additional work on replicate sediment cores from each lake.

During the last 6 months we hired and trained three undergraduate students to help us process sediments for zooplankton subfossil remains. Two of them are each working for us at about 20 hrs/week this summer. With their help, and our commitment, we expect to complete this outcome by January 31, 2019, ahead of schedule.

Outcome 5: One Mille Lacs core (DKB5B) and one Kabetogama core (KAB1A) have been processed for algae pigments at the University of Regina. The data were sent to us in Excel format. We are beginning to analyze and interpret the data. Four additional cores (one from each of the four study lakes) will be sent for pigment analysis later this summer.

Activity Status as of January 31, 2019:
Outcome 1: All 25 cores have been sectioned. This outcome was complete as of July 31, 2018.
Outcome 2: All 25 cores have been processed for water content and organic content. This outcome was complete as of July 31, 2018.

Outcome 3: Eleven of the 12 cores have undergone isotopic aging. The results indicate excellent preservation of the temporal record, ensuring that the dating is highly reliable. The remaining single core that has not been dated has been sent to the St. Croix Laboratory and we expect to see results soon. At this point, the St. Croix Laboratory has encumbered only $4,800 of the budgeted $36,000 for this work. They will invoice the balance ($28,200) of what they intend to charge for the entire project ($33,000 total) once the final core has been dated and the final report has been delivered to us.

Outcome 4: Three Mille Lacs cores (DKB5B, DKB3B, DKB8A), and three Kabetogama cores (KAB1A, KAB2A, KAB3A) have been searched entirely for the composition and abundance of zooplankton subfossil remains in strata dating from 1970-2017. One core from Leech (LEC1A) has been partially searched for subfossil remains. A fourth Mille Lacs core (DKB5A) has been searched in a few select strata in order to compliment the data from DKB5B.

The results indicate that spiny water flea was present in Lake Mille Lacs and Lake Kabetogama for decades before it was first detected in the water column (first water column detection was in 2009 in Mille Lacs, and in 2007 in Kabetogama). All three cores searched from Mille Lacs and all three cores searched from Kabetogama provide evidence that spiny water flea was present in both lakes during the 1970s. The figure below shows the change in spiny water flea accumulation rate at two coring locations in Kabetogama based on the subfossil evidence. Additional results (not shown) indicate that several prey species of spiny water flea increased in density at about the time spiny water flea began to increase in density (between 1990-2010). These assessments are preliminary and will need to be confirmed and refined with additional analyses.

During the last 12 months we have hired and trained nine undergraduate students to help us search sediments for zooplankton subfossil remains. We expect to complete this outcome by March 31, 2019.

Outcome 5: One Mille Lacs core (DKB5B) and one Kabetogama core (KAB1A) have been processed for algae pigments at the University of Regina. The data were sent to us in Excel format. We are beginning to analyze and interpret the data. Four additional cores (one from each of the four study lakes) have been sent for algae pigment analysis. We anticipate receiving the results by April, 2019, slightly behind schedule.

Final Report Summary:
Outcome 1: Each of the 25 sediment cores collected was sectioned, using an extruder device in the laboratory, in 0.5-cm thick increments from the surface to a depth of 20 cm, and then sectioned in 1.0-cm thick increments.
thereafter from 20 cm to the bottom of the core. Upon extrusion, material at the edge of each increment in contact with the core tube was discarded in order to minimize artifacts that could result from smearing of sediment in contact with the core tube. Each sediment increment was packaged in a Whirl-Pak bag designed to store lake sediment material. All data are organized and stored in Excel worksheets.

Outcome 2: All sediment increments were analyzed for water content and organic content at the NRRI at UMD using the loss-on-ignition (LOI) method. All data are organized and stored in Excel worksheets.

Outcome 3: Sectioned sediment from 12 of the 25 cores was freeze-dried and sent to Dan Engstrom at the St. Croix Watershed Research Station of the Science Museum of Minnesota for dating. Of the 12 cores, 4 were from Mille Lacs, 4 were from Kabetogama, 2 were from Winnibigoshish, and 2 were from Leech. Lead-210 ($^{210}\text{Pb}$) or Lead-214 ($^{214}\text{Pb}$) analyses were used to assign age in all 12 cores. Cesium-137 ($^{137}\text{Cs}$) was additionally used to clarify sediment age in 1 core from Winnibigoshish and 1 core from Leech. Near the tops of the cores, where the sediment has been most recently deposited and is youngest, a 0.5-cm thick increment typically represented several months of time. Near the bottoms of the cores, where the sediment has been compacted and is oldest, a 1-cm increment typically represented 2-3 years of time. The only exception to this chronology was in Core DKB10B from Lake Mille Lacs. At that location in Lake Mille Lacs, each 0.5-cm increment of sediment typically represented about 7-9 years of time which indicates that the sedimentation rates there were much slower than at the other locations in Lake Mille Lacs and at the locations in the other lakes we studied.

There is statistical uncertainty in the dating on the order of 1-3 years for each date. For example, for an increment dated to 1990, a statistical uncertainty of 3 years indicates that we are fairly confident that the actual age of the sediment increment is somewhere between 1987 and 1993. This level of uncertainty is important to understand. It indicates that we are not able to determine the exact year that a subfossil entered the sediment record. However, we can constrain the date fairly narrowly within a band of 2 to 6 years of time. All data are organized and stored in Excel worksheets.

Outcome 4: Sectioned sediment from 12 of the 25 cores was processed and analyzed for zooplankton subfossil remains. Before recovery of *Bosmina* subfossils, raw sediment was weighed (Sartorius microbalance), heated (85°C for 1 hour) in 10% KOH as a solvent to remove unwanted organic debris, then stained (Safranin O) to enhance the visibility of the subfossils, and filtered (53 micron Nitex mesh). Volumetric subsamples of the material retained on the mesh filter was plated onto 0.1-milliliter Palmer counting cells and searched by microscopy. Before recovery of spiny water flea and other zooplankton subfossils, raw sediment was weighed, stained (Eosin Y), and filtered (210 micron Nitex mesh). Material retained on the mesh filter was plated onto petri dishes and searched by microscopy. Subfossils diagnostic of the genera *Bythotrephes*, *Bosmina*, *Eubosmina*, *Daphnia*, *Ceriodaphnia*, *Leptodora*, and *Simocephalus*, were removed and in some cases measured for length. Subfossils included portions of tailspines, mandibles, carapace extensions (mucros, antennules), caudal rami, and dormant eggs. Material dated from 1970 to date of core collection (2017 or 2018) was searched for crustacean zooplankton subfossils. All data are organized and stored in paper notebooks and Excel worksheets.

Outcome 5: Sectioned and freeze-dried sediment from 6 of the 25 cores was sent to Peter Leavitt (University of Regina) for algae pigment analyses. Of the 6 cores, 2 were from Mille Lacs, 2 were from Kabetogama, 1 was from Winnibigoshish, and 1 was from Leech. In total, 21 different algal pigments were measured in the increments for each of the 6 sediment cores back to at least 1970. All data are organized and stored in Excel worksheets.

Outcome 6: This outcome was added to the project as an amendment beginning in January 2019. For the 12 sediment cores that we searched back to 1970 for crustacean zooplankton subfossils under Outcome 4, we extended the search back to 1900 for all taxa other than *Bosmina* using the methods described above under Outcome 4. All data are organized and stored in Excel worksheets.
Overall Summary: The outcomes of this activity offer a large, rich data set on the relative abundances of spiny water flea, other species of crustacean zooplankton, and algae pigments through time from 1900 to present (2017-2018) for four large, recreationally important Minnesota lakes – Mille Lacs, Kabetogama, Winnibigoshish, and Leech. The results allow for numerous hypotheses to be tested about the invasion dynamics of spiny water flea and the cause-and-effect relationships between invasion by spiny water flea and changes in the relative abundances of other trophic levels in the pelagic food webs of these lakes. This will be done in the next 6-12 months as the graduate student on the project (Ms. Nichole DeWeese) writes her Master’s thesis and as we prepare the results for publication.

The most surprising result was the abundant, replicated evidence that spiny water flea was consistently present in sediments of Lake Mille Lacs and Lake Kabetogama beginning in the 1930s. The figures below show the timeline of occurrence of spiny water flea subfossils (translated into accumulation rates) in the sediments of the two lakes. Accumulation rates are a proxy for production. They indicate the number of spiny water flea that settled onto the sediments across a square meter of lake bottom each year. For Lake Mille Lacs we show average results from the three coring sites where sedimentation rates were on the order of 1-3 years per increment. For Lake Kabetogama we show average results from the four coring sites where sedimentation rates were on the order of 1-3 years per increment. As shown, there was evidence in both lakes for presence of spiny water flea in the sediments as early as the 1900s. However, continuous evidence (from time of first occurrence until present day) in all of the core sites in each lake was not apparent until the 1930s.

As written in our subproject statement “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).” Work on this goal will continue beyond the duration of this final report as we continue to analyze data and write a master’s degree thesis and several peer-reviewed publications.

Below, we share the interpretation of some of the research results that have particular implications for policy and management of aquatic invasive species in Minnesota.

As noted and shown above, the results of this project demonstrate that spiny water flea (Bythotrephes longimanus) was consistently present in the sediments of Lake Mille Lacs and Lake Kabetogama beginning in the 1930s. This conclusion is corroborated by evidence from all four sediment cores from Lake Mille Lacs and all four
sedi
ment cores from Lake Kabetogama for which spiny water flea was analyzed. The results place spiny water flea in these two lakes beginning about 80 years before they were initially detected by traditional sampling methods which was in 2007 (Kabetogama) and 2009 (Mille Lacs). The results demonstrate that population abundances of spiny water flea were initially low for the first 80 years (approximately 1930s to 2000) of the species presence in both lakes. Thereafter, spiny water flea abundances began to increase exponentially in both lakes, and their abundances have remained high ever since.

These results carry two significant management implications. First, they re-cast our understanding of the timeline of spiny water flea invasion in Minnesota. Prior to this study, the first known occurrence of spiny water flea in Minnesota was in 1982 in Island Lake Reservoir, Duluth. Our study pushes the replicated timeline of first occurrence of spiny water flea in Minnesota lakes back about 50-60 years to the 1930s. In doing so, our study underscores the value of using sediment subfossil reconstruction techniques to detect invasive species presence and describe their timelines of invasion.

Second, the results of this project suggest that traditional methods of spiny water flea sampling and detection carried out by academic units and wildlife management agencies in Minnesota, including the Minnesota Department of Natural Resources, may be inadequate to detect spiny water flea when it is low or transient in abundance. Traditional methods of spiny water flea detection employ vertical net tows of the water column conducted during daylight hours. From 2006 to 2009, samples collected by the Minnesota Department of Natural Resources in this fashion in Lake Mille Lacs failed to detect spiny water flea despite the use of a spatially and temporally rigorous sampling plan. Long periods of undetected presence may enhance risk of spread and reduce control opportunities. The results of this project thus support consideration of sediment core analysis as an alternative or supplemental method of detection for spiny water flea in Minnesota waters.

We also point out that we discovered a very small amount of subfossil evidence of spiny water flea in Leech Lake. The evidence is a single fragment of a spiny water flea tailspine from a sediment interval dating to the year 2001 in one of the two Leech Lake cores. While the finding may indicate a failed invasion, secure confirmation of a failed invasion would require additional work on the lake. If confirmed as a failed invasion, however, this type of evidence would enhance our understanding of the invasion dynamics of spiny water flea and could benefit management by indicating where (and possibly when) spiny water flea dispersal has occurred.

**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: | $7,420 |
| Amount Spent: | $6,950 |
| Balance: | $470 |

**Activity Completion Date:**

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<tr>
<th>Outcome</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Results of statistical analysis and interpretation, including the construction of calibrations, for data collected in activity 2.</td>
<td>June 30, 2019</td>
</tr>
<tr>
<td>2. Two oral and/or poster presentations of the results given at a science conference.</td>
<td>June 30, 2019</td>
</tr>
</tbody>
</table>

**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:
We have begun to plot and analyze the results from core DKB5B. We have also been working with Jodie Hirsch (Minnesota DNR) to analyze zooplankton data from Lake Mille Lacs. Those data were collected by net tows as part of the Minnesota DNR annual surveys of the lake. We are using those data to construct our calibrations (Outcome 1).

Activity Status as of July 31, 2018:
We continue to plot and analyze the results from cores DKB5B, DKB5A, KAB1A, and KAB1B.

Activity Status as of January 31, 2019:
Outcome 1: We continue to plot and analyze the results.
Outcome 2: Nichole DeWeese gave a poster presentation on our results at the Upper Midwest Invasive Species Conference in Rochester, Minnesota (October 15-18, 2018).

Final Report Summary:
Outcome 1: At this time, we have not completed all of our intended statistical analyses and interpretations as outlined in Outcome 1 because of a re-allocation of our time. Beginning in January, 2019, we began work on Outcome 6 (a new outcome added in January 2019) under Activity 2. This re-direction of effort reduced our time available to complete Outcome 1 under Activity 3. We believe this was warranted for two reasons. First, discovery that spiny water flea subfossils were present in lakes Mille Lacs and Kabetogama as early as 1970 was unanticipated and prompted questions about its actual first date of presence. Second, MAISRC was nimble to this discovery and was willing to grant us additional funding to search the sediment core material back in time to 1900 with the goal of identifying the pre-invasion period in both lakes. Because we had a new objective that was very relevant to the project goals, and funding available to pursue it, we decided to act on it and add Outcome 6 under Activity 2 to the project.

We will complete the statistical analyses in Outcome 1 under a new MAISRC project “MAISRC Subproject 12.2: Historical analyses of spiny water flea invasion patterns” beginning July 2019. Under that subproject we will be returning to Lake Mille Lacs and Lake Kabetogama to study sediment mixing in the near surface sediment region. The results will improve our interpretations of the time sequence of subfossil presence in the cores, and thereby help guide our statistical analyses.

That said, we have made some progress on the statistical analyses and interpretation of the data. Thus far, we have used the following statistical approaches to analyze data:

- Non-metric multidimensional scaling (NMDS) was used to compare similarities in the assemblages of taxa among cores and through time
- Depth-constrained cluster analysis (broken stick method) was used to identify distinct temporal zones in the zooplankton assemblages over time within a core
- Simple linear regression was used to compare water column densities to sediment core abundances through time for all taxa within each core
- Piece-wise linear and non-linear modelling was used to quantify lag phases and population growth patterns of spiny water flea through time within each core
- Generalized additive models (GAMs) were used to determine population dynamics of all taxa through time within each core
As an example of the results that we have generated, below we provide a figure that conveys the trends of a depth-constrained cluster analysis that we conducted on the zooplankton assemblage from Lake Mille Lacs. The data set represents the composite (averages) of the analyzed cores from the lake. The data set includes information from the period 1970 to 2015. On this figure, the vertical axis (y-axis) is year, as determined by radiometric dating, and the horizontal axes (x-axes) indicate abundance for that taxa as the number of individuals that settled on a square meter of lake bottom per year. Five taxa are represented. These include spiny water flea (Bytho), three genera of herbivorous crustacean zooplankton that are prey of spiny water flea (Daphnia, Ceriodaphnia, and Bosmina), and a native crustacean zooplankton predator (Leptodora) that is a competitor of spiny water flea. The branching tree diagram in the right-hand panel is the pathway of the cluster analysis which identified four distinct temporal zones in the data, labeled with dashed horizontal lines. These zones are roughly 1970 to 1980, 1981 to 1994, 1995 to 2009, and 2010 to present. Cluster analysis determined these four zones based on a statistical procedure that considered the variation in the data. Cluster analysis is a useful tool for visualizing temporal structure in taxa abundance within and among core sequences.

The data in this figure also serve to demonstrate the long-term temporal trends in the abundance of spiny water flea in the lake as shown in the left-hand panel. There, it is clear that spiny water flea subfossils have been present annually since 1970. Additional data shown above demonstrate that this trend continues back to the 1930s in all of the cores from Lake Mille Lacs and Lake Kabetogama.

Outcome 2: We gave two poster presentations of the results at science conferences. This included a poster presentation at the Upper Midwest Invasive Species Conference in Rochester, Minnesota (October 15-18, 2018), and a poster presentation at the Association for the Sciences of Limnology and Oceanography Conference in San Juan, Puerto Rico (February 23-March 2, 2019).
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:
Branstrator participated in the MAISRC Annual Showcase event (September 13, 2017) where he gave two lectures on this project. He also gave a lecture on this project at the MAISRC all-members meeting on November 28, 2017, at the University of Minnesota.

Status as of July 31, 2018:
Nichole DeWeese participated in MAISRC’s Science in Seconds event (May 30, 2018) in St. Paul, Minnesota, in which she gave a 3-minute mini-lecture on the methods and objectives of this project. Her presentation won third place out of 11 graduate student presenters.

Status as of January 31, 2019:
Nichole DeWeese gave a poster presentation on our results at the MAISRC Annual Showcase event (September 12, 2018) and she gave a poster presentation on our results at the Upper Midwest Invasive Species Conference
in Rochester, Minnesota (October 15-18, 2018). Donn Branstrator gave a seminar in the Department of Biology, University of Minnesota Duluth, on our results (November 2, 2018).

**Final Report Summary:**
As detailed below, we have disseminated our project results at a variety of conferences and meetings, and the project manager (Branstrator) has participated in a variety of MAISRC service.

*Presentations at conferences and meetings*
1) MAISRC Research & Management Showcase (St. Paul, MN) – two platform presentations (September 12, 2016)
2) MAISRC Research & Management Showcase (St. Paul, MN) – four laboratory presentations (September 12, 2016)
3) Coe College Wilderness Field Station (Ely, MN) – platform presentation (July 22, 2017)
4) MAISRC Research & Management Showcase (St. Paul, MN) – two platform presentations (September 13, 2017)
5) MAISRC All Members meeting (St. Paul, MN) – platform presentation (November 28, 2017)
6) MAISRC Science-In-Seconds competition (St. Paul, MN) – platform presentation (May 30, 2018)
7) MAISRC Research & Management Showcase (St. Paul, MN) – poster presentation (September 12, 2018)
8) Upper Midwest Invasive Species Conference (Rochester, MN) – poster presentation (October 15-18, 2018)
9) Association for the Sciences of Limnology and Oceanography Conference (San Juan, Puerto Rico) – poster presentation (Feb 23 – Mar 2, 2019)
11) Minnesota Department of Natural Resources meeting (St. Paul, MN) – skype presentation (May 14, 2019)

*MAISRC Service done by Branstrator (year)*
1) MAISRC Showcase Planning Committee (2016)
2) MAISRC Research Needs Assessment Team (2016)
3) MAISRC Technical Committee (2018)
4) MAISRC Research Needs Assessment Committee (2018)

**VI. SUBPROJECT 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.*

<table>
<thead>
<tr>
<th>Budget Category</th>
<th>$ Amount</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personnel:</td>
<td>$159,271</td>
<td>Branstrator/Associate Professor: $22,890 salary, $7,680 benefits (33.7% fringe rate), 0.23 FTE total for 3 years; Reavie/Sr. Research Associate: $24,316 salary, $8,192 benefits (33.7% fringe rate), 0.23 FTE total for 3 years; DeWeese/M.S. Graduate Research Assistant: $25,273 salary, $19,882 benefits (17.6% fringe rate), 0.69 FTE total for 3 years; Undergraduate Research Assistants: $10,925 salary, $0 benefits, 0.55 FTE total for 3 years; Lab Techs: $31,376 salary,</td>
</tr>
</tbody>
</table>

18
### Professional/Technical Services and Contracts:

- **$8,737 benefits (27.4% fringe rate), 0.72 FTE total for 3 years.**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Amount</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample shipping for dating and pigment analyses</td>
<td>$45,248</td>
<td>Services provided by St. Croix Watershed Research Station of the Science Museum of Minnesota for sediment core dating ($33,000); services provided by Environmental Quality Analysis Laboratory at the University of Regina, Canada, for sediment core pigment analyses ($12,000).</td>
</tr>
</tbody>
</table>

### Equipment/Tools/Supplies:

- **$2,252 Field and lab supplies including core tubes, plastic vials, supplies for sectioning, freeze-drying, loss on ignition analyses, and microscopy ($2,252).**

### Capital Expenditures over $5,000:

- **$0**

### Travel:

- **$4,937 Travel in MN to field sites (Kabetogama, Leech, Mille Lacs, and Winnibigoshish Lakes) to conduct project activities including mileage, lodging, and meals; travel to a professional conference to present results including conference registration, transportation, lodging, and meals for project manager and PhD student ($4,937).**

### Other:

- **$0**

**TOTAL ENRTF BUDGET:** **$211,708**

### 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: 2.42 total FTEs

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

### B. Other Funds: None

<table>
<thead>
<tr>
<th>Source of Funds</th>
<th>$ Amount Proposed</th>
<th>$ Amount Spent</th>
<th>Use of Other Funds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-state</td>
<td>$</td>
<td>$</td>
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<tr>
<td>State</td>
<td>$</td>
<td>$</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL OTHER FUNDS:</strong></td>
<td><strong>$</strong></td>
<td><strong>$</strong></td>
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### VII. SUBPROJECT STRATEGY:

#### A. Subproject 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. Subproject 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:

<table>
<thead>
<tr>
<th>Funding Source</th>
<th>M.L. 2008 or FY09</th>
<th>M.L. 2009 or FY10</th>
<th>M.L. 2010 or FY11</th>
<th>M.L. 2011 or FY12-13</th>
<th>M.L. 2013 or FY14</th>
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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].
**ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET**

**Activity 1 Budget**

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Activity 1 Budget</th>
<th>Activity 1 Spent</th>
<th>Activity 1 Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment Core Location Selection and Retrieval (08/2016 - 03/2017)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sediment Core Processing (04/2017 - 03/2019)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis and Dissemination of Results (06/2019)</td>
<td></td>
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</tr>
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</table>

**TOTAL BUDGET**

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**Activity 2 Budget**

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Activity 2 Budget</th>
<th>Activity 2 Spent</th>
<th>Activity 2 Balance</th>
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</thead>
<tbody>
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**TOTAL BUDGET**

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**Activity 3 Budget**

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Activity 3 Budget</th>
<th>Activity 3 Spent</th>
<th>Activity 3 Balance</th>
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<tbody>
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**TOTAL BUDGET**

| | | | |
| | | | |

**TOTAL SPENT**

| | | | |
| | | | |

**Personnel (Wages and Benefits) - Total**

<table>
<thead>
<tr>
<th>Position</th>
<th>Salary</th>
<th>Benefits</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Research Associate</td>
<td>$25,651</td>
<td>$8,645</td>
<td>$34,296</td>
</tr>
<tr>
<td>Senior Research Associate</td>
<td>$26,317</td>
<td>$8,868</td>
<td>$35,185</td>
</tr>
<tr>
<td>Graduate Research Assistant</td>
<td>$34,285</td>
<td>$20,298</td>
<td>$54,583</td>
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<tr>
<td>Undergraduate Research Assistant</td>
<td>$5,339</td>
<td>$0</td>
<td>$5,339</td>
</tr>
<tr>
<td>Principle Lab Tech</td>
<td>$15,199</td>
<td>$4,164</td>
<td>$19,363</td>
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</table>

**Professional Services and Contracts - Total**

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Budget</th>
<th>Spent</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Croix Watershed Research Station</td>
<td>$30,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environmental Quality Analysis Laboratory, University of Regina, Canada</td>
<td>$12,000</td>
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<td></td>
</tr>
</tbody>
</table>

**Equipment/Tools/Supplies - Total**

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Budget</th>
<th>Spent</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplies - Lab and Field</td>
<td></td>
<td></td>
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</table>

**Travel - Total**

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Budget</th>
<th>Spent</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN: Round trip vehicle travel from Duluth to field sites (Kabetogama, Leech, Mille Lacs, Winnibigoshish Lakes)</td>
<td></td>
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<tr>
<td>Domestic: Registration, transportation, lodging, and meals for project manager and graduate student to present results at a professional conference</td>
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</tbody>
</table>
Paleolimnological method for finding spiny water flea

1) In the field, lake bottom sediment was collected into a long tube through a hole in the ice.

2) In the lab, the sediment was extruded from the tube in ½ - cm thick fractions.

3) Each sediment fraction was dated with Lead-210 methods, and searched by microscope for spiny water flea remains.

4) Pieces of the tailspine (left) allowed us to count the number of spiny water flea in each sediment fraction and reconstruct past abundances through time.