M.L. 2019 Project Abstract
For the Period Ending June 30, 2021

PROJECT TITLE: Restoring Native Mussels in Streams and Lakes – Continuation
PROJECT MANAGER: Mike Davis
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03b

APPROPRIATION AMOUNT: $500,000
AMOUNT SPENT: $500,000
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results
Reestablishing historical mussel assemblages through laboratory propagation began in 2016 at the MNDNR Center for Aquatic Mollusk Programs (CAMP). Since then, CAMP has released 9,541 sub-adult mussels from five species in three watersheds; restoring ecosystem services and enhancing Minnesota rivers with each mussel.

Overall Project Outcome and Results
Minnesota's native mussels are critically important to aquatic ecosystems but have been lost or diminished in many water bodies. Harvest for pearls and buttons, pollution, dams, and destabilized waterways have caused mussel populations to decline dramatically, 80% of Minnesota's species are affected. Improvements from Clean Water Act implementation, stream restoration work, and protective laws are creating opportunities to reverse this trend. However, dams that limit fish movement are still hindering mussel recolonization, because mussels rely on fish as hosts to complete their life cycle. Thus, conservation methods such as laboratory propagation and reintroduction are needed to help mussel populations recover, and ultimately, restore ecosystem benefits. CAMP has implemented this work for three watersheds in Minnesota, which were chosen based on historical records, habitat, and fish communities. We constructed several propagation systems specifically designed for juvenile recovery and culture over time, improving our success along the way. Since 2016, CAMP has produced more than 1.5 million juvenile mussels. Due to the challenges of culture, survivorship varies between species and years. Juvenile survival after 90-days ranged from 0 – 84%. Newly metamorphosed juveniles were placed into various culture containers including a recirculating system, static system, or a flow-through system. Survival rates vary between systems, and within systems. Factors such as dissolved oxygen, ammonia, pH, and conductivity are monitored throughout growing period. Overall, survival is highest in the flow-through system, however, the system requires the most person-hours per juvenile. From July 2019 until June 2021, CAMP has released 7,038 sub-adult mussels from five species in three watersheds. Since our first ENRTF grant CAMP has released more than 9,500 sub-adult mussels. Mussels will enhance water clarity and improve habitat in the Cannon, Cedar, and Mississippi Rivers for years to come.

Project Results Use and Dissemination
CAMP's efforts to restore native freshwater mussels were featured in several news articles, including an Episode 1 of Season 3 on MN DNR Prairie Podcast. The Star Tribune and Cedar Watershed District discussed our efforts to reclaim stretches of the river with mussel populations. Moreover, CAMPs newsletters reach more than 5,000 users. Lastly, with the upcoming launch of Clam Counter App for IOS and Android platforms, a digital field guide and general information regarding mussels will be available to all smartphone users.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2019 ENRTF Final Report (Main Document)

Date of Status Update: October 11, 2021

Final Report

Date of Work Plan Approval: June 5, 2019

Project Completion Date: June 30, 2021

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Project Manager: Mike Davis

Organization: MN DNR

College/Department/Division: EWR

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Location: Statewide

Total Project Budget: $500,000

Amount Spent: $ 500,000

Balance: $ 0

Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03b

Appropriation Language: $500,000 the first year is from the trust fund to the commissioner of natural resources to restore native freshwater mussel assemblages, and the ecosystem services they provide, in the Mississippi, Cedar, and Cannon Rivers and to inform the public on mussels and mussel conservation. This appropriation is available until June 30, 2021, by which time the project must be completed and final products delivered.

I. PROJECT STATEMENT:

Minnesota's native mussels are a diverse and critically important component of aquatic ecosystems. Mussels function as ecosystem engineers by filtering and cleaning vast volumes of water, cycling nutrients, and forming a basis for aquatic food webs by capturing and depositing organic matter on which other organisms depend. As sentinels of ecosystem health, mussel populations have declined dramatically in North America including Minnesota, where 80% of our species are affected. Improvements from Clean Water Act implementation and watershed and stream restoration work are creating opportunities to reverse this trend. However, the complex life cycle of native mussels prevents some populations from recovering naturally. Captive culture and reintroduction provides an established alternative to restore native mussel populations that have been lost. Reestablishing mussel assemblages will improve habitat and water quality, and will help to restore biotic communities to their natural state. Furthermore, increasing populations of threatened and endangered mussels will help delist species in conservation need. This proposal will continue and build upon previous mussel conservation efforts by restoring historical native mussel assemblages and their ecosystem services.

II. OVERALL PROJECT STATUS UPDATES:

First Update December 1, 2019

Late in summer of 2019, the first release of propagated mussels into the Cedar River took place! Black Sandshell and Mucket mussels have likely been absent in the river below Austin Minnesota for over a century, and have finally returned due to efforts by the MN DNR with funding from the ENRTF. This milestone officially starts the restoration of species in the Cedar River.

Additionally, the 2019 propagation efforts yielded 164,582 juvenile mussels of species of greatest conservation need. Eight species were propagated from three watersheds: one species from the Cannon River, four species from the Cedar River, and three species from the Mississippi River. All juvenile mussels continue to thrive at CAMP with plans to overwinter 100% of the surviving juveniles.

Second Update June 1, 2020

Beginning in December, juvenile mussels were transitioned into raceway culture systems, where immediate growth proceeded. A total of six raceways were set-up to accommodate all mussels. Their growth and survival was monitored every 35 to 45 days. In all raceways, growth was linear and survival was strong. In March, CAMP biologists estimated production of over 605,000 juvenile mussels' in- and ex-situ. However, the reality of the past 10-weeks of Covid-19 concerns has dramatically reduced staffing and restricted our ability to collect broodstock mussels. Propagation efforts for 2020 will diverge from the plan and remain unknown, however, staff are committed to maintaining the health of all juvenile mussels currently at CAMP.

AMENDMENT Request as of June 29, 2020

We propose to change the completion date for Activity 3, Outcome #2 (create and publish mussel ID app for phones) from June 30, 2020 to June 30, 2021. This change is requested because of a delay in establishing the app developers as a vendor with the state of Minnesota. Foreign Vendors require additional paperwork that has been postponed due to Covid-19. Amendment Approved by LCCMR 8/20/2020

Third Update December 1, 2020

In July, 13,689 juvenile mussels were moved out of the laboratory and into secondary culture systems. Secondary culture systems include: Lake Byllesby (Cannon River), East Side Lake (Cedar River), St. Croix River, Minnesota Zoo, and Waterville Fish Hatchery (MN DNR). The Minnesota Zoo and Waterville Fish Hatchery allow for multiple watersheds to be placed in the same location. The juveniles were divided into 40 groups, and placed into either totes, submerged baskets, or floating baskets (depending on the secondary culture location). If growing conditions are ideal, these juvenile mussels should be released into their native rivers in 2021, boosting the population by thousands!

Moreover, 5,513 mussels were released into the Cannon and Cedar Rivers in late summer 2020. This year makes the first release of Mucket into the Cannon River system, and the second consecutive year of Black Sandshell into the Cedar River. Mussels were tagged with either PIT (Passive Integrated Transponder) tag, Hallprint, or black-dotted superglue to signify they were propagated mussels. These reintroduction efforts will help improve water quality locally by increasing filtration and nutrient processing. Additionally, they will help to build robust macroinvertebrates habitats, which will enhance the native fish population. Freshwater mussels are ecosystem engineers that will continuously help the integrity of Minnesota's waterways.

Final Update Summary
Host fish inoculations began in May, 2021, and 91,937 juvenile Mucket, Snuffbox, Higgins Eye, and Spectaclecase mussels were produced through the grant period. Juveniles are being reared in three types of culture systems in the laboratory, and are assessed every 3 – 4 weeks to learn about each species' survival and growth responses to these systems. Additionally, 10,850 Spectaclecase were transferred to the Minnesota Zoo in June to learn about the utility of a natural water and food source for this species.

Mucket, Snuffbox, and Higgins Eye mussels from the 2018 cohorts were reintroduced in June at two locations on the Mississippi River. This marks the second consecutive year for the Hidden Falls site and the first year for the Lock and Dam site.

Minnesota's native mussels are critically important to aquatic ecosystems but have been lost or diminished in many water bodies. Harvest for pearls and buttons, pollution, dams, and destabilized waterways have caused mussel populations to decline dramatically, 80% of Minnesota's species are affected. Improvements from Clean Water Act implementation, stream restoration work, and protective laws are creating opportunities to reverse this trend. However, dams that limit fish movement are still hindering mussel recolonization, because mussels rely on fish as hosts to complete their life cycle. Thus, conservation methods such as laboratory propagation and reintroduction are needed to help mussel populations recover, and ultimately, restore ecosystem benefits. CAMP has implemented this work for three watersheds in Minnesota, which were chosen based on historical records, habitat, and fish communities. We constructed several propagation systems specifically designed for juvenile recovery and culture over time, improving our success along the way. Since 2016, CAMP has produced more than 1.5 million juvenile mussels. Due to the challenges of culture, survivorship varies between species and years. Juvenile survival after 90-days ranged from 0 – 84%. Newly metamorphosed juveniles were placed into various culture containers including a recirculating system, static system, or a flow-through system. Survival rates vary between systems, and within systems. Factors such as dissolved oxygen, ammonia, pH, and conductivity are monitored throughout growing period. Overall, survival is highest in the flow-through system, however, the system requires the most person-hours per juvenile. From July 2019 until June 2021, CAMP has released 7,038 sub-adult mussels from five species in three watersheds. Since our first ENRTF grant CAMP has released more than 9,500 sub-adult mussels. Mussels will enhance water clarity and improve habitat in the Cannon, Cedar, and Mississippi Rivers for years to come.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1 Title: Restore historic mussel populations of threatened and endangered species in select streams to improve stream health through restoration of their unique provisioning of ecosystem services.
Description: Use established field and laboratory methods to propagate and reintroduce up to six threatened or endangered (T&E) mussel species in the Cedar River, up to three T&E species in the Cannon River, and up to six T&E species in the Upper Pool 2 of the Mississippi River in St. Paul.

ACTIVITY 1 ENRTF BUDGET: $375,000

Outcome	Completion Date

1. Yearly field collection of female broodstock and host fishes for each mussel species. 2-10 mussels per species; 10-200 host fish per mussel species.	June 30, 2021
2. Propagate and rear juvenile mussels to releasable size for each species and river system. 10-1000 juvenile mussels to releasable size per species.	June 30, 2021
3. Reintroduce cultured juvenile mussels to selected restoration sites. Release mussels at 1-3 sites in each river system.	June 30, 2021

First Update December 1, 2019

Broodstock (gravid female mussels)

Field collection of broodstock occurred before this reporting period. Long-term brooders are typically collected in early spring, April – May, with some species collected in late winter. However, short-term brooders are typically collected in late-spring to summer. For the 2019 propagation effort, a total of 79 gravid female mussels of 10 species were collected. Only 24 were used for propagation after July 1, 2019 (Table 1). Gravid females, except short-term brooders, were placed into a two-door laboratory refrigerator designed to hold multiple 9L tanks in which a submersible pump recirculates water. The cold temperature prolongs the period over which the mussels will retain their glochidia, and allows for controlled propagation efforts.

Host fish

Common host fish include Walleye (*Sander vitreus*, WAE), Largemouth Bass (*Micropterus salmoides*, LMB), Logperch (*Percina caprodes*), and Goldeye (*Hiodon alosoides*). The majority of WAE used for production are provided by Waterville Fish Hatchery (50317 Fish Hatchery Road Waterville, MN 56096, waterville.fisheries@state.mn.us), and LMB were purchase from by Ron Rademacher's Fish Farm in Waconia, MN. Other fishes are collected by various methods, such as seine, dip net, and electrofishing. Prior to propagation, host fish are housed primarily in recirculating raceways setup with adequate bio-filtration. Goldeye are kept in a circular tank to promote swimming and reduce the injury to fish caused by collision with sidewalls.

There were 187 host fish used for propagation related efforts in 2019; fish hosts had over 85% survival rate. The mortality associated with host fishes were a result of system failure or ich (white spot disease). White spot disease was treated by exchanging the fish back and forth between tanks on a daily basis. Each tank was wiped down with a 1:10 bleach solution, then refilled the following day when fish were added back to the tank. Tank exchanging is very labor intensive, however, it is the most effective way to treat for white spot disease. The system failure was a result of loss of power; an alarm system was installed in the following weeks to alert staff on any system malfunctions in the future.

Propagation of Native Mussels

All species

Cohort Year 2019

The 2019 propagation season yielded 164,582 juvenile mussels of species of greatest conservation need (SGCN) (Table 2). Eight species were propagated from three watersheds: one species in the Cannon River, four species in the Cedar River, and three species in the Mississippi River. Cedar River propagation was dominated by Black Sandshell and Spike, 30% and 23%, respectively.

Juveniles were placed into static culture tanks; static culture tanks (herein referred to as ST's) are 9L tanks or 5-gallon buckets filled with 5µm filtered Lake Pepin River water and a small amount of sieved river sediment which provides the juvenile mussels with substrate to burrow. River sediment was collected at the headspring of Gorman Creek in Wabasha County, MN, fine silica sand was also used as substrate.

Survival of juveniles was reduced in 2019 due to water quality occurrences in August and September, limiting overall survival and growth. The limited growth and survival could be a result of an unknown compound within

the source river water or sediment. We determined that juvenile mussels were exposed to potentially lethal levels of ammonia during this time. New water quality measurements were adopted in August when high rates of mortality began to occur. Future plans for supplemental nitrifying bacteria will be tested throughout the winter.

A newly designed pulse flow system (PF, Figure 1) was operational in August, and several hundred juveniles were moved into the system. Only two species of newly metamorphoses juveniles were placed directly into the PF system: Snuffbox and Threeridge. Survival of Threeridge was 67% after 4 ½ months (Figure 2). Threeridge had moderate growth rates in the PF system, and poor survival and growth in ST; ST experienced 100% mortality after 3 months. Survival of Snuffbox was low in PF (Figure 3), with zero surviving in the ST. This is unusually low survival for Snuffbox juveniles, survival in the previous year exceeded 73% after 3 months.

Rearing of Native Mussels in Secondary Culture Systems

Waterville Fish Hatchery

Cohort Year 2018

In an attempt to diversify juvenile grow-out methods, juveniles that overwintered at CAMP were placed in natural waterbodies within their watershed, and several thousand were placed into floating totes at the Waterville Fish Hatchery. In September 2019, all totes were removed from Waterville. Unfortunately, only 10% of the juveniles survived. This low survival could have been due to increased water temperatures inside the totes, limited water movement, or limited algal supply. These variables will be addressed for 2019 efforts.

Reintroduction of Native Mussels

CAMP was able to release propagated mussels for the first time in late summer of 2019; 2,502 sub-adult mussels were released into Minnesota's rivers in 2019.

Cedar River

Cohort Year 2016 and 2017

An historical moment occurred this summer, as propagated Black Sandshell mussels were reintroduced to the Cedar River below Austin, MN. The Black Sandshell have likely been absent from the MN portion of the Cedar River for over 100 years. In September, 1,467 Black Sandshell were released at three sites along the river; Lafayette Park, Riverwood Canoe Access, and Orchard Creek confluence. All released mussels were tagged (Figure 4) prior to release, and about 600 were placed inside a grid for future monitoring. In addition, 966 – propagated Muckets were released in the Cedar River at Riverwood Canoe Access and Orchard Creek Confluence in late September. Similarly, about 200 Muckets were placed inside a grid downstream of the Black Sandshell grids for future monitoring.

Mississippi River

Cohort Year 2016 and 2017

In September, 30 totes were removed from the St. Croix River at Lakeland. Throughout the field season of 2018, we aimed to examine the different stages in which juvenile mussels can be placed into cages in a natural setting. Several species were placed into benthic totes (Figure 5) at various life stages; inoculated host fishes, newly metamorphosed juveniles, 1 – 2mm juveniles, and 7+ mm juveniles. In all species, the highest (and sometimes only) survival occurred for juveniles that were placed into totes after approximately 1 year in the laboratory and were greater than 7mm in length. Solidifying the need to overwinter all juvenile mussels at CAMP.

In total, 619 juvenile mussels were released into the Mississippi River near Hidden Falls. Hidden Falls has been a mussel reintroduction area for the DNR since 2001. All released mussels were uniquely tagged with either green hull-print tag, or black super glue. A diver hand placed Higgin's Eye (n=17) and Snuffbox (n=43) along a metal cable on the river bottom, and Muckets (n=559) were spread from the boat within

the area. Additionally, nine Spike were translocated to Hidden Falls. The Spike were found inside the totes upon removal, but were not propagated.

Second Update June 1, 2020

Covid-19 has severely impacted CAMP; beginning March 23, only one worker was allowed at CAMP for the basic maintenance and upkeep of the native mussels and fish. Due to these restraints, no brooding females for the 2020 propagation season have been collected, all 2019 cohorts remain at CAMP, and the 2018 cohorts remain undisturbed in the natural grow-out systems in East Side Lake, Lake Byllesby, and St. Croix River at the Hudson Narrows, and the MN Zoo. Species impacted (as of June 1, 2020) by the restrictions for broodstock collection include: Actinonaias ligamentina (all watersheds), Cumberlandia monodonta, Epioblasma triquetra, and Lampsilis higginsii.

Propagation of Native Mussels

All species

Cohort Year 2020

Propagation plans for 2020 can be found in Table 3; CAMP hopes to produce 315,000 juvenile mussels in-house, and aims to produce an additional 290,000 juvenile mussels ex-situ in floating baskets or benthic totes. The central focus of 2020 propagation efforts is to continue to work with species and locations that were previously successful and have been released into natural systems. In 2019, a YSI Pro Plus Multi-Parameter Water Quality Meter (pH, Temperature, DO, Conductivity, and Chloride) and Hanna Low Range Ammonia Colorimeter allowed for better water quality monitoring of juvenile systems. To expand data collection, a series of experiments will be conducted to allow for comparisons across different juvenile culture systems. This knowledge will aid in future management actions at CAMP.

Propagation of Native Mussels

All species

Cohort Year 2019

Beginning in December, juvenile mussels were transitioned into raceway systems at CAMP. Each raceway system is fitted with a small submersible pump that recirculates water from the sump to the trough above. The nominal flow rate for the pump is 700 gallons per hour, flow is adjusted with a ball valve for each system. Plastic trays (13" x 10-7/8" x 1-7/8" HDPE Developing Trays) with approximately 6-cups of rinsed silica sand and fine sediment contain the juvenile mussels. Each raceway system is fed daily a food mixture of commercially available algae from Reed Mariculture (Shellfish Diet, Nanno 1800, and TW 1800). Food is delivered by a peristaltic pump at a rate of 125mL per hour, additional food is added by hand to ensure adequate food per individual mussel.

Juvenile mussels placed into trays by species, and visually examined daily for behavior. Growth rates and survival were checked at 35 to 45 day intervals during system cleaning. Growth rates discussed were sampled from December to June 2020. In each species, the shell length over time was linear. Growth rates have been continuing to increase since December (Figure 6). Growth rates from April to May have ranged among species from 10.6 – 27.7 $\mu m \cdot d^{-1}$. Growth rates were fastest in Black Sandshell and slowest in Snuffbox, a smaller species so not unexpected. Some Black Sandshell and Mucket juveniles are greater than 20mm (Figure 7). Additionally, survival from December to June has been robust, as of 18 May 2020 13,854 juvenile mussels remain at CAMP (Figure 8).

Rearing of Native Mussels in Secondary Culture Systems

All species

Cohort Year 2018

All species remain undisturbed inside benthic totes at East Side Lake, Lake Byllesby, and St. Croix River and at the Hudson Narrows. We anticipate to recover all 2018 benthic totes this summer, in hopes to release mussels into the Cannon, Cedar, and Mississippi rivers. Additionally, the MN Zoo has 2018 species growing inside their facility.

Third Update December 1, 2020

Covid-19 severely impacted CAMP; beginning March 23, 2020, only one worker was allowed at CAMP for the basic maintenance and upkeep of the native mussels and fish. Due to these restraints, no new propagation efforts took place in 2020.

Host fish

Host fish were maintained in various aquariums and large recirculating raceways. Unfortunately, at the beginning of July, Ich (*Ichthyophthirius multifiliis*) was discovered in a very late stage inside the large Walleye raceway. Ich is a ciliated protozoan and obligate parasite that is extremely difficult to control due to its lifecycle and limited susceptibility to chemical treatments. The feeding protozoan, Trophont, matures under the scales and skin of fishes, during which it is protected it from chemical treatment. When it matures, the Tomont leaves its host fish surrounded by a gelatinous cyst wall. The Tomont divides into immature daughter Tomites. When the Tomites are released, they are considered to be ineffective Theronts, which must find a host fish to survive. Only at this stage is Ich susceptible to chemical treatment (Figure 10). Unfortunately, when the Ich was discovered on the Walleye, each fish was infected with hundreds of Trophonts, and thus had been thriving undetected for weeks. Within 3-days of its discovery, all Walleye had succumbed to Ich. Effective management of Ich includes early detection and rotating infected fishes into new tanks daily or chemical treatment. Tank swapping is highly effective in the early stages of Ich, as fish are removed from the tank with Theronts. Rotating tanks does not allow for Theronts to find their host, therefore, removing this stage of development. Our facility has successfully defeated Ich in small aquariums, however, large raceways are extremely difficult. Additionally, the infection rate at discovery impacts overall survival. Our reduced staffing due to Covid-19 restriction undoubtedly contributed to this situation.

Logperch (*Percina caprodes*) were collected in preparation for 2021 Propagation Efforts in late September 2020. Two seining events took place to collect 216 Logperch. Logperch were divided into 6 aquariums and, thus far, minimal mortality has occurred. In total, CAMP has greater than 275 native fish. Routine aquarium maintenance is key to the continued survival and health of these fish.

Rearing of Native Mussels in Secondary Culture Systems

All species

Cohort Year 2019

Juveniles were maintained in raceways systems until July 2020. All species were moved into raceway systems around 8 months of age (250 days), and a noticeable increase in their growth rates occurred (Figure 11). Growth rates from June 2 to June 30 increased for nearly all species, ranging from 11.4 – 28.9 $\mu m \cdot d^{-1}$. (Figure 12). Black Sandshell average growth increased in June to 28.9 $\mu m \cdot d^{-1}$ and Snuffbox increased to 12.0 $\mu m \cdot d^{-1}$. Threeridge had the lowest growth rate at this time, 11.4 $\mu m \cdot d^{-1}$.

At the beginning of July all Cohort 2019 juveniles were placed into a secondary culture system. Secondary culture systems include: Lake Byllesby (Cannon River), East Side Lake (Cedar River), St. Croix River, Minnesota Zoo, and Waterville Fish Hatchery (MN DNR). The Minnesota Zoo and Waterville Fish Hatchery allow for mussels native to multiple watersheds to be placed in the same location because they are not connected to any river system.

In the summer 2019, the Waterville Fish Hatchery pond did not yield the results we had anticipated, we believe that the initial floating tote design created an unsuitable environment for mussel growth. To correct this improper design, we used modified fish baskets that allow for better water circulation (Figure 13). To compare juvenile growth and survival, mussels were placed into two neighboring ponds. The first pond is drained seasonally, and contains Muskie fingerlings. Some benthic vegetation was noted in July. The second pond was noted as less clear, had some floating vegetation, and contained Channel Catfish. This pond is only drained as needed; it was last drained in 2017/2018. In October, baskets from pond 1 were moved into pond 2 due to harvesting and draining the pond in November. Upon our return, pond 2 had become densely populated with floating vegetation. Baskets were handed-checked during the move, and pond 1 mussels were generally more robust than pond 2 (Figure 14).

No shell was detected in either pond. The temperatures in the two ponds were significantly different (t-test, p=0.0004) (Figure 15).

Below is the secondary culture breakdown for all species by watershed.

Cannon River

Lake Byllesby

Two totes were placed into the lake, each holding 277 juvenile Mucket ($\overline{x}$ =10.6mm). Totes were placed on 17 July 2020 using SCUBA. Lake Byllesby has nearly zero visibility in the summer, so a leadline was placed from shore to the totes, this will help staff to locate the totes more easily next summer.

Waterville Fish Hatchery

Juveniles were divided into the two ponds and placed into submerged fish baskets, 271 and 270 Mucket, respectively ($\overline{x}$ =9.9 and 9.7 mm).

Cedar River

East Side Lake

On 2 July 2020, six totes (Figure 16) were placed into East Side Lake, Austin, MN. 1,504 Black Sandshell ($\overline{x} = 10.8$mm) and 1,578 Mucket ($\overline{x}$ =9.3mm) were placed into 3 totes per species. Additionally, at the end of July, 635 Threeridge ($\overline{x} = 4.1$mm) were placed into two totes, along with nine Spike ($\overline{x}$ = 8.2mm).

On 29 September 2020, we pulled totes that were placed in 2019 with newly metamorphosed juveniles: two Black Sandshell totes and one Spike tote. No live juveniles or shell were recovered.

Minnesota Zoo

355 Black Sandshell ($\overline{x} = 14.1$mm) were moved to the Minnesota Zoo on 9 July 2020. These juveniles were placed into the pan system (Figure 17, Figure 18) for additional grow-out. Black Sandshell juveniles remained in the pan system until October, when they were moved into floating baskets in A Lake.

Waterville Fish Hatchery

One fish basket of each species, Black Sandshell and Mucket, were placed into each pond on 7 July 2020. Juveniles placed into submerged fish baskets in pond 1 include 218 Black Sandshell ($\overline{x} = 9.2$ mm), and 268 Mucket ($\overline{x} = 9.4$ mm). Juveniles placed into submerged fish baskets in pond 2 include 253 Black Sandshell ($\overline{x} = 9.9$ mm), and 268 Mucket ($\overline{x} = 9.4$ mm).

Mississippi River

St. Croix River at Lakeland

Ten totes were placed on 28 July 2020 into the St. Croix Rive at Lakeland, MN. All totes were grouped together in a grid form at the upstream site, which consists of 24 totes of juveniles or host fish placed in 2019 and 2020. Comparatively, the downstream site consists of 24 totes for juveniles or host fish placed in 2018.

Juveniles placed into totes include: Higgins Eye, Mucket, and Snuffbox. Higgins Eye were divided into two totes, containing 381 ($\overline{x} = 8.9$ mm) and 383 juveniles ($\overline{x} = 10.5$ mm). Mucket were placed into seven totes, containing an average of 377 per totes, size range from 9.7 – 11.7mm. Lastly, 97 Snuffbox ($\overline{x} = 4.5$ mm) were placed into a single tote. The Snuffbox were placed centrally in the grid to reduce the amount of sedimentation that collects inside each tote. The most upstream totes have been known to collect the most sediment.

Minnesota Zoo

Greater than 2,500 juveniles from the Mississippi Watershed were transferred to the MN Zoo for secondary culture: 971 Higgins Eye ($\overline{x} = 8.0$ mm) and 1,605 Mucket ($\overline{x} = 7.6 – 12.7$ mm). Juveniles were first placed into the Pan System, then eventually moved into 8 floating baskets in the fall.

Waterville Fish Hatchery

One fish basket of each species, Higgins Eye and Mucket, were placed into each pond on 7 July 2020. Juveniles placed into submerged fish baskets in pond 1 include 265 Higgins Eye ($\overline{x} = 8.7$ mm), and 260 Mucket ($\overline{x} = 8.5$ mm). Juveniles placed into submerged fish baskets in pond 2 include 265 Higgins Eye ($\overline{x} = 8.7$ mm), and 260 Mucket ($\overline{x} = 8.5$ mm).

All Species

Cohort Year 2018

Mississippi River

All secondary culture locations were inspected during the summer of 2020, there were 26 totes from the Cohort Year 2018 at Lakeland, MN. On 28 July 2020, 23 totes were hand-checked using SCUBA, two were not checked, and one tote was missing. No juvenile Snuffbox were found in totes containing inoculated Logperch (0 of 12), additionally, no Snuffbox juveniles were found in the two totes placed with newly metamorphosed juveniles. In contrast, a few small Higgins Eye were found in one of three totes placed with newly metamorphosed juveniles in June 2018. Comparatively, Higgins Eye juveniles placed in July 2019 had more growth for the same cohort. Juvenile Mucket and Snuffbox placed in July 2019 had varied growth. It was determined to leave all totes in place for another growing season to ensure adequate size at the time of release.

Reintroduction of Native Mussels into Native Rivers

We were able to release 5,513 mussels into the Cannon and Cedar Rivers in late summer 2020. This year makes the first release of Mucket into the Cannon River, and the second consecutive year of Black Sandshell into the Cedar River. Mussels were tagged with either PIT (Passive Integrated Transponder) tag, Hallprint, or black-dotted superglue to signify they were propagated mussels.

During the first release in the Cedar River in 2019, a grid system was used to place known mussels in a specific location. Therefore, the recovery is dependent on visual encounters and manual excavation, as well as, placing the grid in its original location. Due to its limited recovery rate, we abandoned this method in 2020 and released mussels throughout a known distance at each site. We believe the PIT tags will be an effective tool for tracking released mussels and increase the accuracy of survival and growth estimates. Additionally, PIT tags should enhance our ability to collect tagged mussels when they are burrowed into the sediment or in low-visibility sites.

Below is release information for the Cannon and Cedar Rivers for multiple cohort years.

Mucket

Cannon River

Cohort Year 2016

During a search for the tote location in Lake Byllesby, a CAMP biologist using SCUBA disoriented themselves away from the target location and came across a single tote nearly 50 feet from the others. This recovered tote had been missing from its platform since June 2018, and 3 attempts were made to locate it prior to this recovery. The Mucket juveniles inside were originally from inoculated Largemouth Bass placed in eight metal cages in the lake on 8 June 2016. The metal cages were recovered via SCUBA on 27 September 2017, and 138 Mucket juveniles were found and replaced into a single tote for additional growth. Lost for over 2 years, the Mucket continued to grow trapped inside. Upon its discovery, the 126 Mucket had grown substantially and were between 30 – 83mm ($\overline{x} = 63.4$ mm) (Figure

19). The 2016 Cohort of Mucket were released into the Straight River (Site 3), a tributary of the Cannon River, on 25 August 2020 (Figure 20).

Mucket and Black Sandshell
Cannon and Cedar Rivers
Cohort Year 2018

Cannon River

All three totes containing 2018 Cohort of Mucket were removed from Lake Byllesby, counted, measured, tagged for release. The tote survival rate was 91%, 82%, and 85%, yielding 1,656 Mucket juveniles. The Mucket were individually tagged: PIT tag (18%, 300), Hallprint (53%, 874), and black-dot (29%, 482). Then were divided and released into 3 sites along the Cannon and Straight Rivers (Figure 20). Mucket size ranged from 21 – 61mm, average was 33.2mm.

Cedar River

Ten totes of Black Sandshell placed in 2019 were recovered from East Side Lake in October. The average tote survival in East Side Lake was 81% (58 – 95%). Total number of Black Sandshell tagged from totes in East Side Lake was 2,628: the average length was 42.9 mm (25 – 70mm). Additionally, 1,103 Black Sandshell were reared in A Lake at the MN Zoo for secondary grow-out. These juveniles had an average length of 44.1 mm (range of 15 – 59mm), and were statistically significant (t-test, p=0.00005). Black Sandshell were divided among three relocation sites in the Cedar River (Figure 21) and released on 6 October 2020.

Final Report Summary

Covid-19 severely impacted CAMP; beginning March 23, 2020, staff restrictions resulted in no new propagation efforts in 2020. Restrictions eased in April 2021, allowing for out-of-state travel and field collection with multiple staff. In May 2021, full-time staff returned the office with some constraints. Operations for propagation were minimally impacted from May 2021 – June 30, 2021.

Broodstock (gravid female mussels)

Similar to years past, broodstock collection largely occurred from April through June. One exception, Mucket from the Cannon River were collected in November 2020. Wading and SCUBA were the primary collection methods. All broodstock collected were placed directly in the recirculating refrigerator for long-term holding.

Four hours of search time in the Cedar River near Osage, Iowa yielded 23 mussels of which only 9 were gravid. Black Sandshell gravidity rate was 83%, in contrast, Mucket gravidity was 23%. Warmer spring temperatures may have resulted in early natural production of Mucket. This trend continued during Mucket collection in the St. Croix River in May. Only 5 of 26 Muckets were gravid. Although mussel sex was not determined on all individuals, several were noted to have flat gills indicating the mussel had previously held larvae. Due to increased difficultly collecting viable broodstock in the spring, plans for intensive fall collection is planned for 2021. Collected broodstock will be held at CAMP or in A Lake at the MN Zoo. Holding broodstock in a natural environment, such as the MN Zoo, should decrease the stress on the mussel and increase our overall propagation efforts.

Throughout this project 150 gravid females were collected, and greater than 70% of broodstock were used for propagation efforts for this grant period. The Spectaclecase mussel accounted for 45% of broodstock collected. They were collected in high number due to an inability to determine gravidity in the field. All broodstock were returned to their collection location within three months of use.

Host Fish

Host fish health is imperative for the longevity of propagation at CAMP. For the grant period over 450 host fish were used for production of juvenile mussels. Maintaining high volumes of fish in recirculating systems and various aquaria has proven to be difficult with larger predator fish entering our facility from a hatchery or other source. Walleye were collected from Genoa National Fish Hatchery (GNFH) on April 5, 2021. These fingerling Walleye were held in concrete raceways from October to April. Upon collection, the Walleye were roughly 4 – 6 inches, therefore, too small for immediate mussel production. Larger Walleye (9 – 13 inches) were delivered from the DNR East Metro Fish Hatchery on April 22, 2021. Unfortunately, during the 2-week quarantine period Ich was discovered. The smaller Walleye were heavily infected, and the larger fish were infected by shared water. One week post formalin treatment all large Walleye perished. In contrast, the smaller Walleye were moved into a new tank every 4-days were thriving and had a noticeable reduction of white spots/Ich. It was later determined Ich was present on the Walleye from GNFH, therefore entering our facility at the beginning of April. The flow-through systems at GNFH have a high flow-rate which limits the impact Ich and other bacterial outbreaks. More information regarding Ich, see December 2020 updated and Figure 10.

Moreover, non-predator fish, Logperch, collected in nearby Lake Pepin have a high rate of survival. Logperch are maintained in multiple 50+ gallon aquaria with routine tank maintenance and daily feeding. Logperch are fed frozen bloodworms which minimizes the risk of disease transfers from other fishes.

Goldeye were collected via electrofishing boat on May 25, 2021. Goldeye were transported in a 300-gallon insulated hauling tank, fitted with pure oxygen for aeration. A total of 34 Goldeye were placed into a 1,000-gallon recirculating system upon arrival to CAMP. Goldeye slowly adapted to eating night crawlers and were held in culture until propagation efforts began.

Propagation of Native Mussels

Two systems were developed for placement of newly metamorphosed juvenile mussels. The Pulsed Flow (Figure 1) was adapted from Dr. Chris Barnhart at Missouri State University and an earlier rendition of large scale system. Another system was constructed in 2021 - The Sprinkler System is a recirculating system consisting of a group of 9-liter containers with a centralized sump filled with bio-media for ammonia control. A submersible pump directs water to a multiport manifold, then into each container. Water exits through the opposite end of the container and is gravity fed back to the sump. The systems are fed by a peristaltic pump that delivers food to the sump at 30-minute intervals (Figure 22).

All Species

Cohort Year 2021

The planned propagation efforts in 2021 were focused on 5 main species (Table 5), producing a 4th year-class cohort for Mucket, Higgin's Eye, and Black Sandshell. Each species will have a minimum of 5 year-class cohorts released into native rivers before concluding production.

Prior to the end of the reporting period, almost 92,000 juveniles had been collected from 4 species, including Mucket (Cannon River), Snuffbox, Higgin's Eye, and Spectaclecase (St. Croix River) (Table 6).

All juvenile Snuffbox and Higgin's Eye were placed into a Sprinkler System, along with 90% of Muckets. Other Mucket juveniles were placed into static 5-gallon buckets. Most juveniles were collected at the end of June, and on 24 June 2021, over 10,800 Higgin's Eye were transferred to the Minnesota Zoo. The Higgin's Eye were 0.32mm in length and 0 – 3 days old.

Rearing of Native Mussels in Secondary Culture Systems

All species

Cohort Year 2019

All 2019 cohort mussels remained in their secondary culture containers for another growing season, with plans for an early summer release in 2022. Juveniles in the St. Croix River at the Hudson Narrows were checked using SCUBA, and due to minimal sedimentation were left undisturbed. Juveniles in Eastside Lake and Waterville were removed, examined, and reset into new culture containers. Lake Byllesby was checked in August 2021.

Cedar River

Eastside Lake Tote Reset

Mucket and Black Sandshell were removed from the original totes and reset with new substrate for an additional year's growth. Muckets, thus far, had doubled in size; average length 25.0mm. Black Sandshell also doubled in size in two (of three) of totes; average length 31.2mm. One Black Sandshell tote was slightly removed from the group, likely due to a fisherman, had the largest increase in size; average length 38.4mm. Totes had minimal mortality overall. However, on 21 June 2021, a Black Sandshell tote was discovered ashore completly dry. All juveniles present in the tote were dead and desiccated; it is estimated that more than 300 Black Sandshell were lost due to this act of vandalism.

All Watersheds

Waterville Fish Hatchery Submerged Basket Reset

Juveniles were placed into two separate hatchery ponds from July to October 2020. Due to seasonal draining of the fish pond [1], all submerged baskets were moved into the same pond for the winter and subsequent summer 2021. Although mussels were in the same pond for 8-months, little growth occurs at temperatures below 10°C. During the basket reset, it was noted that the group originally in Pond 1 were more robust. It is suspected that the variation in growth is a result of ponds productivity from June to October 2020 (Figure 23). Each basket was reset with new substrate for an additional year's growth (Figure 24).

Reintroduction of Native Mussels into Native Rivers

Release at Hidden Falls

Cohort Year 2018

Juvenile mussels (Cohort 2018) were inside benthic totes on the St. Croix River near Lakeland, MN. In total, 26 totes were removed via SCUBA from the river and examined for growth and survival. Overall, juvenile mussels that were held greater than one year at CAMP had the highest survival, compared to inoculated fishes or recently metamorphosed juveniles. For example, twelve totes containing inoculated Logperch only produced four Snuffbox. Only eight Snuffbox were found in the totes containing 30-day-old juveniles (survival rate 0.005%). However, Snuffbox that overwintered at CAMP then placed into totes one year post metamorphoses had a survival rate of 23%.

Inoculated Largemouth Bass were similar to Logperch, only yielding ten Higgin's Eye. While Higgin's Eye juveniles placed into totes with an average length of 7.2mm had a survival rate of 66%; Higgin's Eye juveniles placed at 20.1mm had a 90% survival rate. Similarly Mucket survival was 66% and 96%, respectively.

Propagated mussels were individually marked or tagged with a Black Dot, Hallprint, or PIT tag (Figure 25), then released into the Mississippi River near Hidden Falls on 17 June 2021. Three species, totaling 1,525 juveniles were released at two sites (Figure 26). At the previous release site, 783 mussels were gently placed along shoreline where there is an established mussel bed. 100 of each Higgin's Eye and Mucket were PIT tagged as part of a 3-year release and recapture study. Moreover, a second release site was established downstream of an island below Lock and Dam 1 for 742 mussels. Only Hallprint and Black Dot mussels were released at the second site. A total of 2,426 mussels have been released at these locations during the grant period, and monitoring of the Hidden Falls site will begin in summer 2022 to assess survival and growth.

Release Summary

Throughout the reporting period 7,038 laboratory raised sub-adult mussels have been released into their native rivers. Each release marking a new milestone for our program. Including both LCCMR grant periods, CAMP has released 9,541 sub-adult mussels from five species in three watersheds. Each release helps to increase water quality in the Cannon, Cedar, and Mississippi River. Monitoring of each release location will begin in the summer of 2021, and continue yearly for a minimum of 3-years.

Activity 2: Support research into contaminants of emerging concern
Provide glochidia, propagated juvenile mussels, and adult mussels to supply research projects at University of St. Thomas, University of MN St. Anthony Falls Lab, and the MN Zoo.
ACTIVITY 2 ENRTF BUDGET: $50,000

Outcome	Completion Date
1. Collect or produce the necessary number of glochidia, juvenile mussels, and adult mussels for research projects. 5-20 gravid females; 1000-5000 newly metamorphosed; 50-500 juveniles 2-10mm; 5-100 adults (>30mm).	June 30, 2021

First Update December 1, 2019
As part of collaborative host suitability research, we provided glochidia from *Lampsilis sietmani,* Canary Kingshell to Mark Hove, a University of Minnesota researcher. Plans to provide juvenile mussels to the University of Minnesota for genetic and morphometric studies are in development.

Second Update June 1, 2020
CAMP biologist provided juvenile Giant Floater (*Pyganodon grandis*) mussels for a project examining the effect of habitat on morphological features. A gravid mussel was collected by University of Minnesota researcher, Sean Keogh, and using Yellow Perch for a host fish, a small inoculation occurred. All fish were held at CAMP in a recirculating propagation system (RPS) until glochidia had completely metamorphosed into juveniles (Figure 9). Over 14,000 juveniles were collected and transported to the MN Zoo, where they will grow until reaching the size needed for the project to proceed.

Third Update December 1, 2020
Nearly 7,000 Giant Floaters Floater (*P. grandis*) were tagged and placed into nine sites within the Cannon River drainage. CAMP biologists assisted with the placement of 420 marked juveniles into Lake Byllesby on 19 August 2020. The released juveniles were placed along leadline connected to a cinder block; roughly 40 animals were placed per meter of the leadline.

Final Report Summary

University of Minnesota
Giant Floaters will remain undisturbed for one additional growing season. Plans to examine growth and survival will occur in August 2022.

Hormel Nature Center: Dobbins Creek
As continuation to a survey completed in 2020, the Hormel Nature Center (Austin, MN) sought to determine if Dobbins Creek was suitable for reestablishing a native mussel population. Dobbins Creek has been a central focus for the Cedar River Watershed District for over a decade, and several improvements have been made within the watershed. A previous survey found a significant difference between mussel populations above and below the dam at Eastside Lake. The current dam was built in 1934, and thus has prevented fish movement for 85 years. Additionally, an earlier stone dam might have impeded fish movement for decades more. Above the dam, only two species were found live, in comparison to the 8 species below the dam.

In May, staff assisted 4H and FFA students with a study to examine growth and survival of Black Sandshell and Mucket (Cohort 2019) in Dobbins Creek. Propagated mussels were removed from Eastside Lake (secondary culture containers), tagged with unique ID numbers, and placed into concrete mussel silos (Figure 27) at three sites: North Branch, South Branch, and a control site below the dam at Eastside Lake. Four concrete silos were placed per site, each containing 10 mussels (5 Black Sandshell, 5 Mucket). Silos were placed within each site based on bottom substrate, flow, and water depth. Starting lengths of Black Sandshell and Mucket were $\overline{x} =$ 29.0mm $\overline{x} = 22.0$mm respectively. Measurements of growth and survival were taken once a month. Additionally, water quality data was recorded on a biweekly basis. The study will conclude in September 2021.

Minnesota Zoo

The Minnesota Zoo received over 10,000 newly metamorphosed Higgin's Eye juveniles in June 2021. Although the MN Zoo has previously received several thousand one-year-old juveniles, this was the first attempt to culture juveniles of this age. Future culture plans will include examining all species as newly metamorphosed juveniles transferred directly to MN Zoo culture.

Activity 3: Engage and inform the public about freshwater mussels

Develop and produce interpretive displays in high traffic areas to illustrate the importance of mussels to aquatic ecosystems and their benefits to people.

ENRTF BUDGET: $75,000

Outcome	Completion Date
1. Interpretive video for MNDNR website and YouTube	June 30, 2021
2. Create and publish mussel ID app for phones	June 30, 2021
3. Added content for the Minnesota DNR and MN Zoo's website on mussel importance and ongoing conservation activities within the state.	Ongoing
4. Create public displays at Minnesota State Parks and boat launches that serve rivers and lakes with native mussel populations.	June 30, 2021

First Update December 1, 2019

MN DNR staff have been in contact with Clam Counter App developers. In January, staff will discuss data sharing and specific development of the App for completion sometime during 2020.

We have been mailing the Mussels of Minnesota posters and the MN Volunteer Magazine article about CAMP to various agencies, watershed groups, and individuals.

Second Update June 1, 2020

MN DNR staff have been in contact with Clam Counter App developers. Due to Covid-19, progress has been reduced. Currently, we are awaiting to establish the developers as a vendor through the state of Minnesota.

Third Update December 1, 2020

Private contractors for developing the MN Clam Counter application were established as vendors through the State of Minnesota in November 2020. Immediately following, the app developers and CAMP staff created an outline for the project. The current Clam Counter application will be customized to MN DNR. Species data will be displayed by watershed. And all species information text and pictures will be supplied by CAMP staff prior to April 2021. The project will be completed by June 2021.

Final Report Summary

The Clam Counter application is near completion. Interface updates from the previous IOS can be found on Figure 28. The application will contain information on all Minnesota's native and invasive mussels. Moreover, it will serve as a field guide for civilians, and allows for reporting of species outside of their known range. General information

about mussels will open the minds of users to their ecological importance and role they play in Minnesota waters. All text and photographs were edited and supplied by MN DNR.

Updates to MN DNR Webpage are delayed due to staffing and editing restrictions for DNR Webpages. With new staff aboard, we hope to update the website over the next year with new videos, and project outlines. Throughout the last two field seasons, a significant push for photographs and videos of mussels was initiated. Video shorts were created for presentations, including the Natural Heritage Society, Freshwater Mollusk Conservation Society, and several outreach programs. Eventually this content will be available on our webpage, and all content will be ADA compliant.

Furthermore, we have revised our efforts for signage at MN State Parks and Trails. In the future we intend to create an informational pamphlet containing mussel lifecycle and their importance. This document will be shared with park managers for display inside their facilities. If possible, we plan to explore the use of existing signage space to include general information about freshwater mussels. Creating a mussel only kiosk is unlikely due to cost, signage protocols and committee groups necessary for placement and context. Additionally, kiosk costs restrict our ability to reach multiple parks.

Although Covid-19 restrictions were in place throughout the reporting period, staff were still able to perform virtual PowerPoints and presentations about our program, including: Natural Heritage Society, Mussel Coordination Team Annual Meeting, Freshwater Mollusk Conservation Society Virtual Symposium, and DNR Ecological Resources Division update. Moreover, several in-person presentations were given as well, including, Hormel Nature Center, IJ Holton Middle School, and Whitewater State Park. Other in-person events were postponed until late 2021 and 2022.

IV. DISSEMINATION:

Description:
Progress updates will be disseminated via additions to the DNR website, our quarterly mussel newsletter, DNR Facebook, MN Conservation Volunteer, at scientific conferences, symposia and during interagency meetings. A finished video will be published to YouTube.

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement Guidelines.

Update December 1, 2020
CAMPs newsletter is reaching more users than ever. The last newsletter, published 17 November 2020, reached 3,144 recipients. Subscribe to the newsletter here: http://tinyurl.com/gd-mussels

Additionally, the CAMPs release of propagated mussels received attention in 2019 and 2020 from several news sources:

2019

- Trying to build up some mussels in the Cedar River (KIMT News 3)
- Black Sandshell Mussels Reintroduced to Cedar River State Water Trail in Mower County (ABC 6 News)
- Painstaking efforts to restore Minnesota's freshwater paying off (Post Bulletin)

2020

- Mussel Propagation in the Cedar River, Post Bulletin
- Adding some "mussel" to the clean the Cedar River, ABC 6 News

- DNR transplants more native mussels into the Cedar River, Cedar Watershed District
- MN Zoo Facebook Post
- Mussel Mania Center for Aquatic Mollusk Programs staff were featured in Episode 1 of Season 3 of the MN DNR Podcast, Prairie Pod
- Mussel restoration project could lure wildlife back to barren stretch of Mississippi River in Minneapolis Star Tribune

Final Report Summary
CAMP's efforts to restore native freshwater mussels were featured in several news articles, including an episode on MN DNR Prairie Podcast. Digital prints of the articles are bulleted above. Moreover, CAMPs newsletters reach more than 5,000 users. Lastly, with the upcoming launch of Clam Counter App for IOS and Android platforms, a digital field guide and general information regarding mussels with be available to all smartphone users.

Additionally, our mussel efforts in the Cedar River included working with Austin's High School FFA students on a project in Dobbins Creek.:

> The Cedar Reader - May 2021 e-news for CRWD: Students with Austin High School's FFA Club place mussel silos May 19 in Dobbins Creek's south branch at the Jay C. Hormel Nature Center as part of a new project with the Minnesota Department of Natural Resources' mussel team out of Lake City. Students will check on native, juvenile mussels growing in the silos to study how they do in different parts of Dobbins Creek.
>
> More information at: https://www.facebook.com/CedarRiverWD/: www.cedarriverwd.org

V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

Explanation of Capital Expenditures Greater Than $5,000: N/A

Explanation of Use of Classified Staff:
Classified staff salaries will be backfilled from funds received from Federal grants matched with ENRTF funds and from contract work with federal and state agencies.
Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours for entire duration of project: 13,520	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE: 6.5

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

Enter Total Estimated Contract Personnel Hours for entire duration of project: N/A	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE: N/A

VI. PROJECT PARTNERS:

A. Partners outside of project manager's organization receiving ENRTF funding

Name	Title	Affiliation	Role
Ben Minerich	Mussel Conservation Specialist	MN Zoo	Fish culture support, mussel rearing and contaminants
Dalma Martinovic	Associate Professor	St. Thomas University	Contaminant research

B. Partners outside of project manager's organization NOT receiving ENRTF funding

Name	Title	Affiliation	Role
Dan Kelner	Fish Biologist	U.S. Army Corps of Engineers	Support monitoring of federally endangered mussels
Tamara Smith	Endangered Spp. Biologist	USFWS	Planning and permitting
Nathan Eckert	Mussel Biologist	USFWS	Mussel propagation support
Byron Karns	Ecologist	National Park Service	Permitting support in NPS areas
Jessica Kozarek	Research Associate	University of Minnesota St. Anthony Falls Lab	Contaminant research

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

Native mussel restoration in Minnesota is a long-term strategy to rebuild our aquatic ecosystem infrastructure and a natural progression of efforts that build on prior public investments that have made this feasible. Successful restoration can take a decade or more of effort. Indications of success can be demonstrated within 3-6 years of ENRTF expenditures. Demonstrating success is intended to lead to permanent non-ENRTF funding of this work. This project is a natural progression from 1999 when a LCCMR grant began mussel surveys of the state's rivers. Surveys continued with other funds resulting in data from nearly 4,000 sites covering all 81 major watersheds in the state. This information is used to identify streams for mussel reintroductions. Our research identified host fish relationships needed to propagate most of Minnesota's mussel species. This proposal builds on these investments. Expertise in accomplishing mussel restoration began in 2000 with the USFWS Jeopardy Decision for the Higgin's eye pearly mussel. The first propagated Higgin's eye were produced by the MNDNR in Lake Pepin in 2001. Funding from the USACE expanded that effort for 10 years resulting in the release of over 40,000 propagated Higgins' eye in the Mississippi River. New recruits from this population were found in 2012, a first ever milestone for a reintroduced federally endangered mussel. This project expands those efforts to other species and other river systems.

This is a long-term project, mussels that are released need one to five years to become sexually mature and begin reproducing. Their offspring will take two to five years to grow to an easily detectable size. It is evidence for self-perpetuating populations that ultimately defines the success of these reintroduction efforts.

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted March 1 and December 1 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2021

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet N/A
D. Acquisition, Easements, and Restoration Requirements N/A
E. Research Addendum N/A

Table 1. 2019 Broodstock Collection Information.

Species	Common Name	River Drainage	No. Collected	Date Collected
Actinonaias ligamentina	Mucket	Cannon River	2	16-May-19
		Cedar River	8	31-Oct-18
		St. Croix River	6	4-Oct-18
			3	17-May-19
Amblema plicata	Threeridge	Cedar River	5	10-Jul-19
Alasmidonta marginata	Elktoe	Cedar River	2	31-Oct-18
Cumberlandia monodonta	Spectaclecase	St. Croix River	25	3-Jun-19
Elliptio dilatata	Spike	Cedar River	4	21-Jun-19
Epioblasma triquetra	Snuffbox	St. Croix River	2	17-May-19
Fusconaia flava	Pigtoe	Cedar River	9	10-Jul-19
Lampsilis higginsii	Higgins' eye	Mississippi River	3	6-Jun-19
Ligumia recta	Black sandshell	Cedar River	7	11-Jun-19
Quadrula fragosa	Winged mapleleaf	St. Croix River	3	11-Sep-19
			79	

Table 2. Propagation Effort 2019.

Species	Common Name	River Drainage	Host Fish (location)	No. Host Fish Survived	No. of Juveniles Produced
Amblema plicata	Threeridge	Cedar River	Walleye (hatchery)	15	13,270
Cumberlandia monodonta	Spectaclecase	St. Croix River	Goldeye (Red River)	3	20,270
Elliptio dilatata	Spike	Cedar River	Walleye (hatchery)	17	37,945
Epioblasma triquetra	Snuffbox	St. Croix River	Logperch (Lake Pepin)	68	12,516
Fusconaia flava	Pigtoe	Cedar River	Mix species	13	4,661
Lampsilis higginsii	Higgins' eye	Mississippi River	Walleye (hatchery)	25	12,132
Ligumia recta	Black sandshell	Cedar River	Walleye (hatchery)	13	49541
Quadrula fragosa	Winged mapleleaf	St. Croix RIver	Channel Catfish (hatchery)	4	14,247
			Total	158	164,582

Table 3. Planned propagation effort for 2020, created in March 2020.

Watershed	Species	Estimated No. of Gravid Females	Estimated No. Host Fish	# of Host Fish	Propagation Plan		
					In-House	Cage-Culture	Free-Release
Cannon	*Actinonaias ligamentina*	5+	Walleye	60	30,000	20,000	
Cedar	*Actinonaias ligamentina*	7+	Walleye	80	50,000		
	Ligumia recta	8+	Walleye	20	50,000		
Mississippi	*Actinonaias ligamentina*	6+	Walleye	50	50,000	20,000	
	Cumberlandia monodonta	3+	Goldeye	10+	50,000		
	Epioblasma triquetra	1+	Logperch	100	10,000	20,000	
	Lampsilis higginsii	6+	Largemouth Bass	60	25,000	30,000	
	Quadrula fragosa	1+	Channel Catfish	Pending		50,000	Yes
					315,000	290,000	
					Total	605,000	

***Covid-19 has severely impacted the propagation plan, propagation efforts for 2020 remain unknown*
***December 2020 update: Propagation of species for 2020 did not occur due to Covid-19 restrictions during brooding period.*

Tables

Table 4. 2021 Broodstock Collection Information.

Species	Common Name	River Drainage	No. Collected	Date Collected
Actinonaias ligamentina	Mucket	Cannon River	3	4-Nov-20
		Cedar River	4	22-Apr-21
		St. Croix River	6	5-May-21
Cumberlandia monodonta	Spectaclecase	St. Croix River	25	25-May-21
			18	8-Jun-21
Epioblasma triquetra	Snuffbox	St. Croix River	4	5-May-21
Lampsilis higginsii	Higgins' eye	Mississippi River	7	2-Jun-21
Ligumia recta	Black sandshell	Cedar River	4	22-Apr-21
			71	

Table 5. 2021 Planned Propagation Efforts.

Watershed	Species	Estimated No. of Gravid Females	Estimated No. Host Fish	# of Host Fish	Propagation Plan	
					In-House	Cage-Culture
Cannon	*Actinonaias ligamentina*	5+	Walleye	25	15,000	15,000
Cedar	*Actinonaias ligamentina*	5+	Walleye	25	15,000	15,000
	Ligumia recta	5+	Walleye	25	15,000	15,000
Mississippi	*Actinonaias ligamentina*	5+	Walleye	25	15,000	15,000
	Cumberlandia monodonta	3+	Goldeye	10+	20,000	40,000
	Epioblasma triquetra	1+	Logperch	100	5,000	5,00
	Lampsilis higginsii	5+	Walleye	25	15,000	15,000
					100,000	115,000
					Total	215,000

Table 6. Propagation Effort 2021 (through June 30, 2021).

Species	Common Name	River Drainage	Host Fish (location)	No. Host Fish Survived	No. of Juveniles Produced
Actinonaias ligamentina	Mucket	Cannon River	Walleye (Genoa NFH)	23	40,980
Epioblasma triquetra	Snuffbox	St. Croix	Logperch (Lake Pepin)	46	4,899
Lampsilis higginsii	Higgins' eye	Mississippi	Walleye (East Metro)	22	30,517
Cumberlandia monodonta	Spectaclecase	St. Croix	Goldeye (Red River)	2	15,541
					91,937

Figure 1. (Top) Pulse Flow system; constructed in August 2019. River water from the footer tank is pumped up into the header tank, where it fill the food tank. Once the food tank is full, the water recirculates from the header tank to the footer tank. At 30-min intervals, food is added to the food tank, and the ball opens to release the water from the food tank into each of the beakers (bottom). Each beaker contains juvenile mussels. When the displaced from the beakers, the water empties into the waste tank.

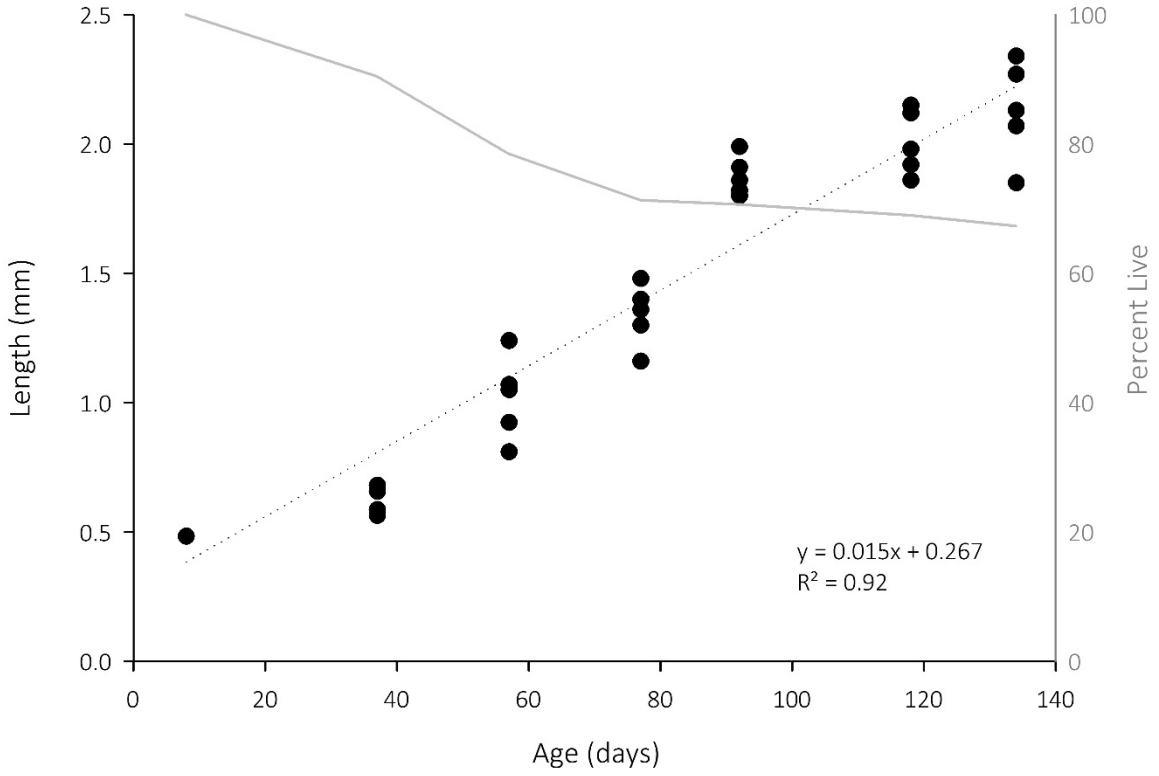


Figure 2. Growth and survival of Threeridge juveniles in the Pulse Flow system.

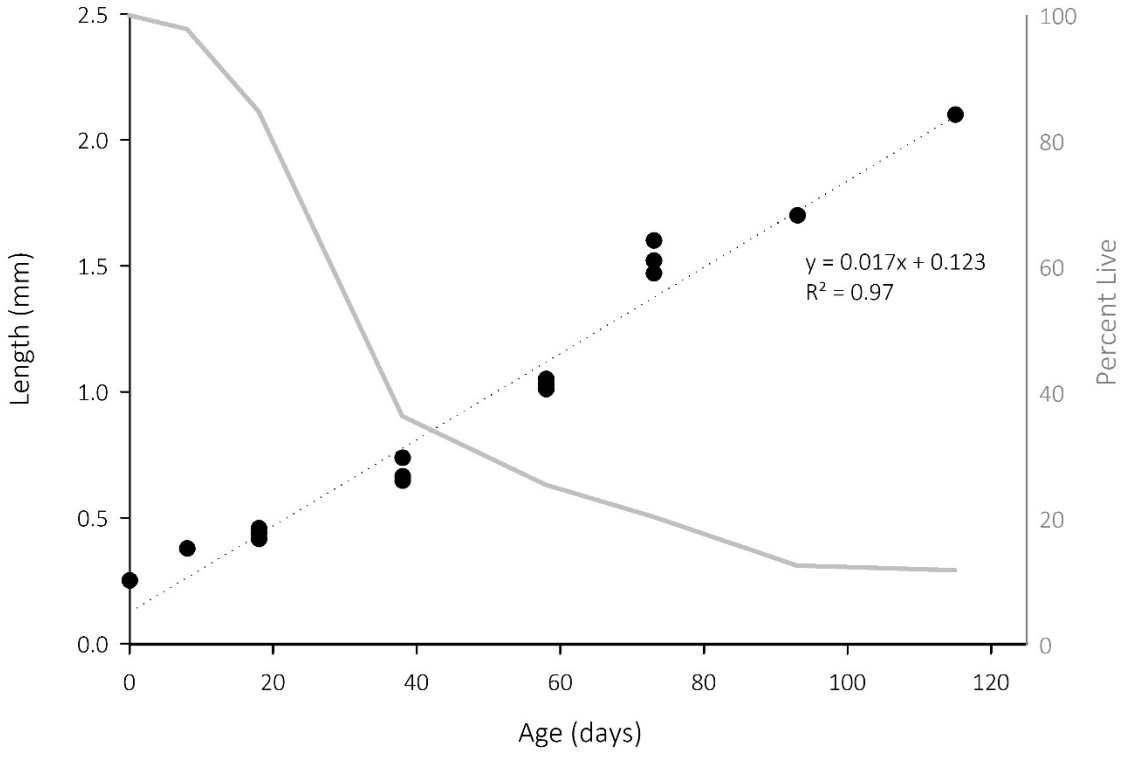
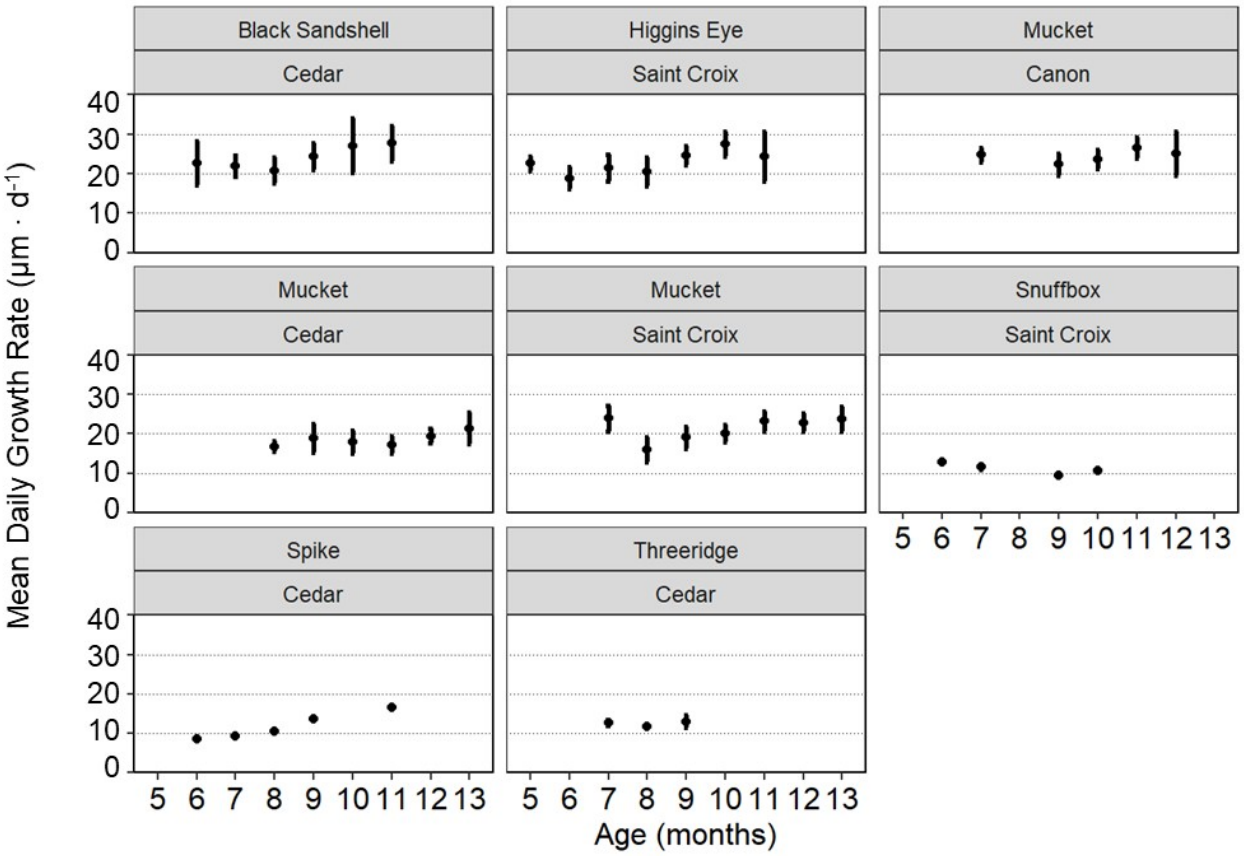


Figure 3. Growth and survival of Snuffbox juveniles in the Pulse Flow system.

Figure 4. Yellow Hull-print tags on Black Sandshell juveniles prior to release into the Cedar River in 2019. Hull-print tags provide a unique ID number to each mussels. Tags are attached with Loctite super glue gel. Each watershed is designated a color; Cedar = Yellow, Cannon = Pink, Mississippi = Green.

Figure 5. Underwater view of totes at Lakeland in the St. Croix River. Juvenile mussels are placed into plastics totes on platforms underwater for continued growth. Juvenile mussels will grow for up to three growing seasons in the plastic totes prior to release.



Figures

Figure 6. Mean absolute daily growth rate (µm · d^{-1}) for each species by watershed. Means included all system types: static, pulsed flow, and raceway. All means are weighted for number of mussels in each system. (updated in December 2020, see Figure 11).

Figure 7. Mucket (*Actinonaias ligamentina)* juveniles from 2019 propagation efforts in the Mississippi River. Juveniles are 11 months old, and have almost doubled in length in past 5-months. The largest juvenile pictured is about 20mm.

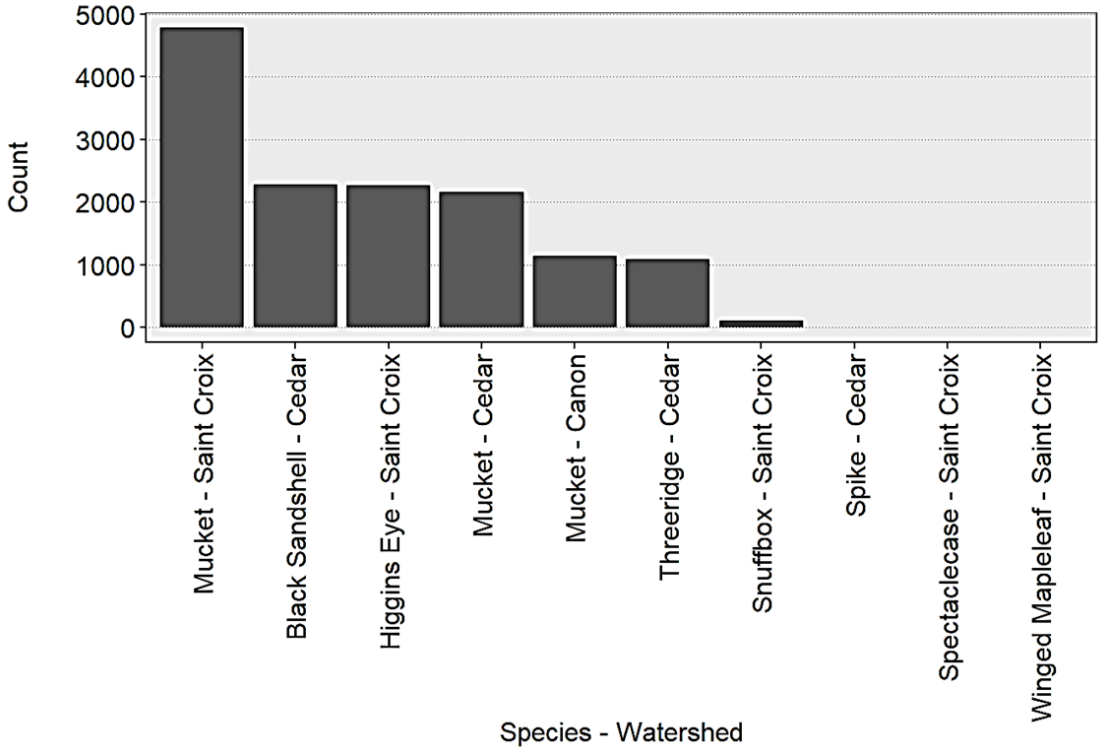


Figure 8. Number of each species in lab-culture at CAMP as of May 18, 2020.

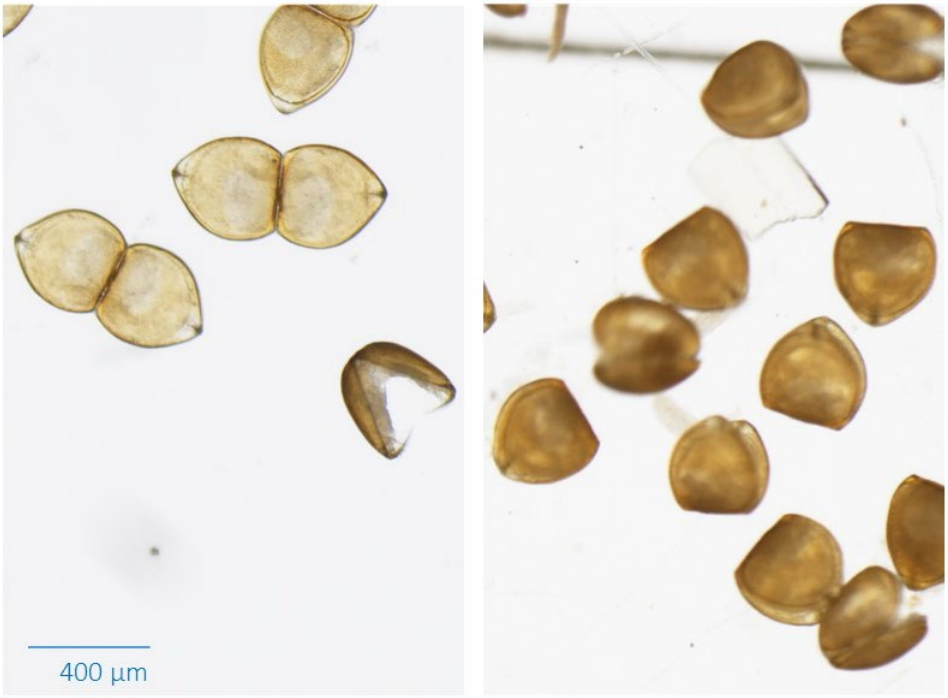


Figure 9. (Left) Hooked glochidium of the Giant Floater (*Pyganodon grandis*) that attach to the gills and fins of their host fish. After attachment, the glochidia are encapsulated as part of the fish's immune response, and over a short period it undergoes a metamorphosis to a juvenile mussel. Pictured on the right, 16-days post inoculation, are free-living juvenile Giant Floaters.

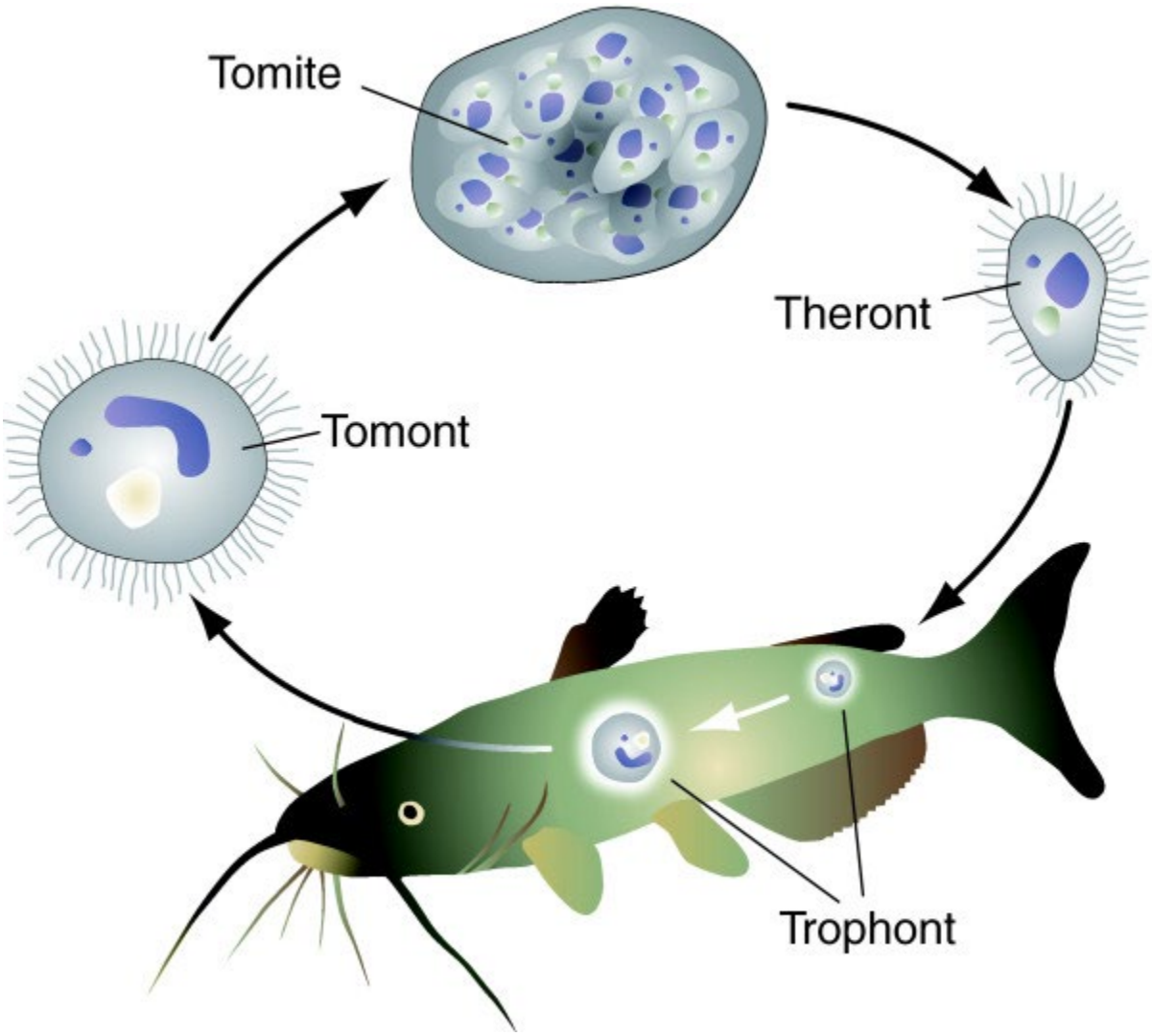


Figure 10. Lifecycle to Ich (https://www.researchgate.net/figure/Life-cycle-of-Ich-Infective-theronts-bore-through-the-surface-mucus-and-take-up_fig1_51720908).

Coyne, Robert & Hannick, Linda & Shanmugam, Dhanasekaran & Hostetler, Jessica & Brami, Daniel & Joardar, Vinita & Johnson, Justin & Radune, Diana & Singh, Irtisha & Badger, Jonathan & Kumar, Ujjwal & Saier, Milton & Wang, Yufeng & Cai, Hong & Gu, Jianying & Mather, Michael & Vaidya, Akhil & Wilkes, David & Rajagopalan, Vidyalakshmi & Clark, Theodore. (2011). Comparative genomics of the pathogenic ciliate Ichthyophthirius multifiliis, its free-living relatives and a host species provide insights into adoption of a parasitic lifestyle and prospects for disease control. Genome biology. 12. R100. 10.1186/gb-2011-12-10-r100.

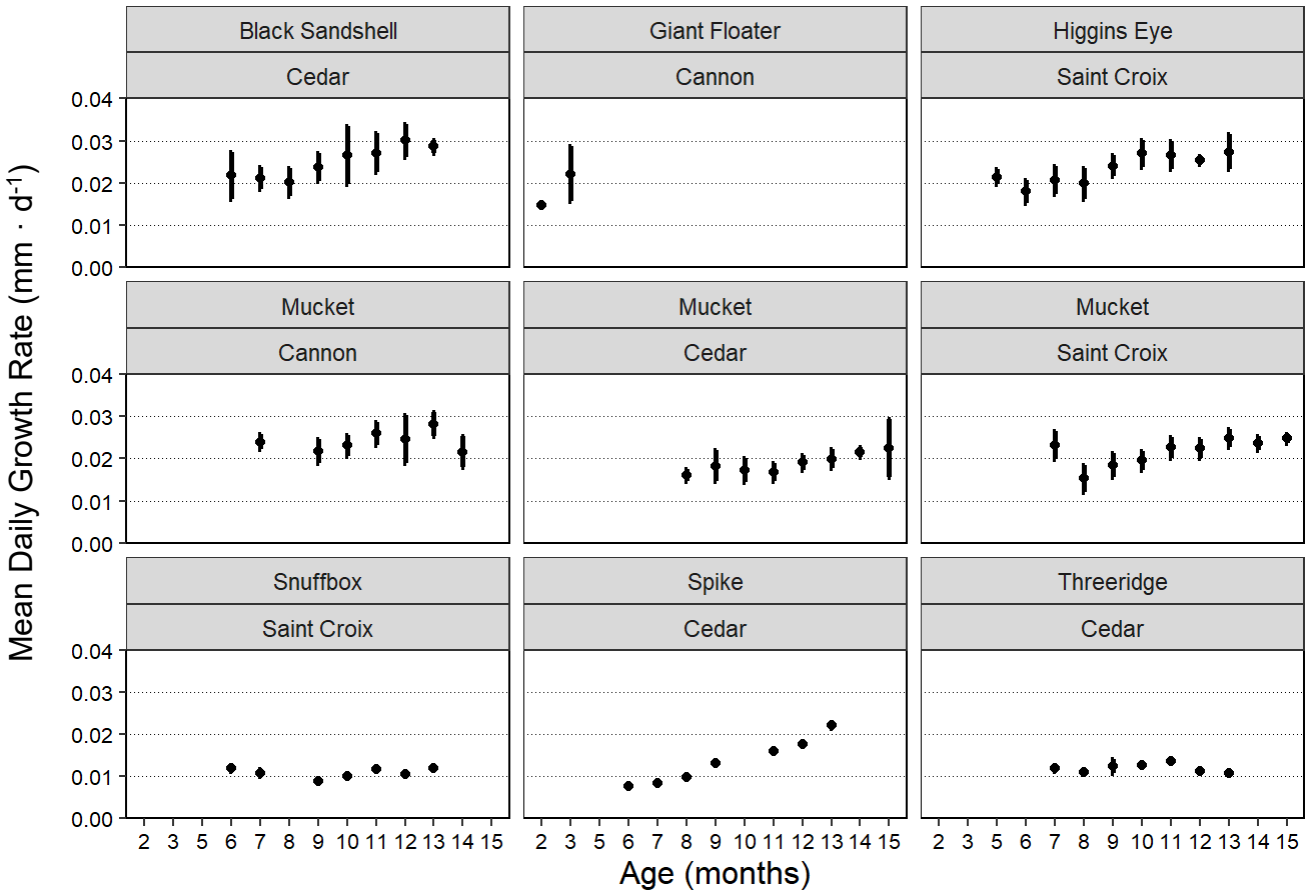


Figure 11 (updated Figure 6). Mean absolute daily growth rate (mm · d^{-1}) for each species by watershed. Means included all system types: static, pulsed flow, and raceway. All means are weighted for number of mussels in each system. Most species were moved into Raceway Systems around 8 months (250 days)

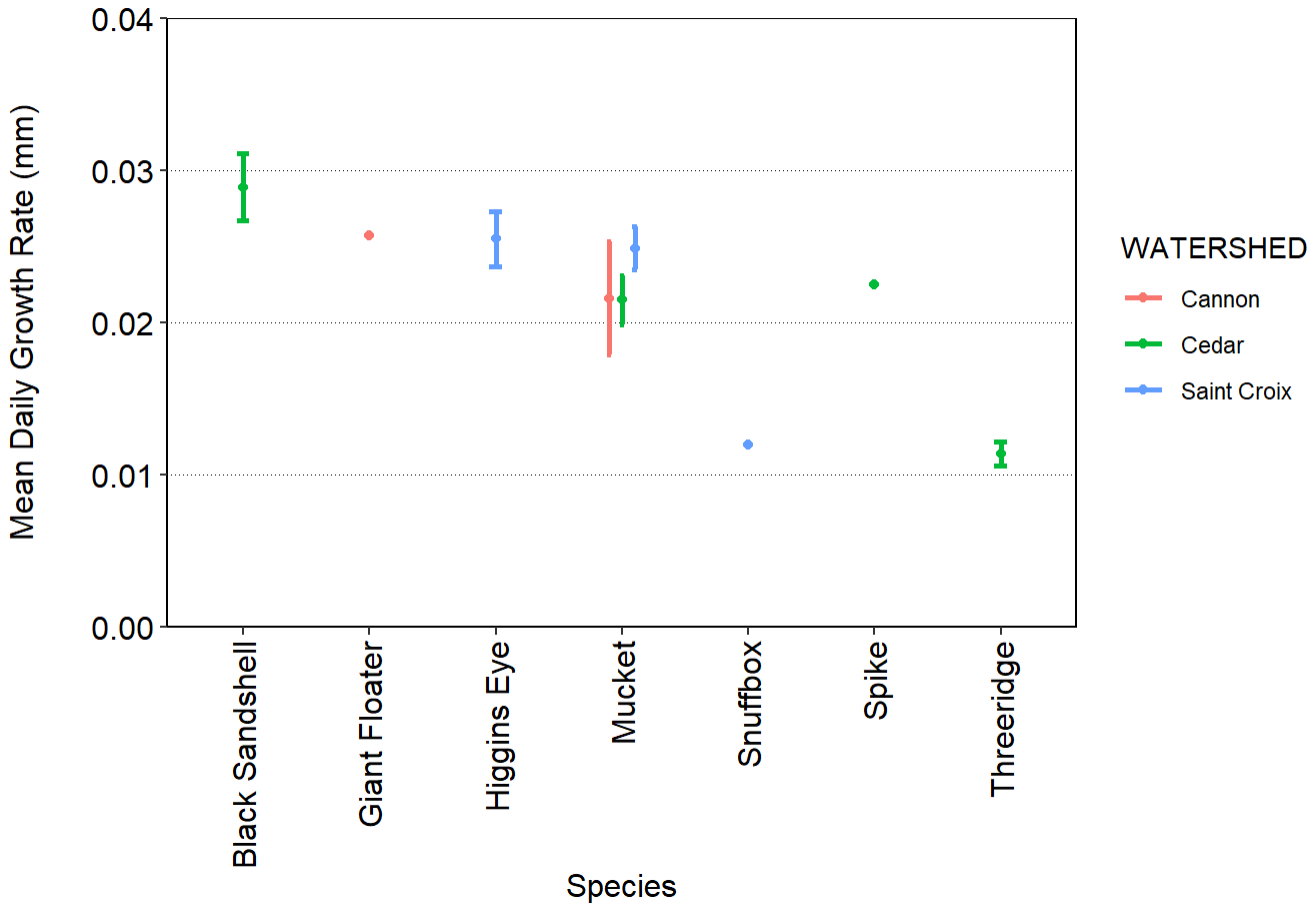


Figure 12. Estimated growth rates above that incorporate all data points, these growth rates are based solely on the most recent size estimate at age for mussels cleaned 7/2/20 to 7/30/20. Means are weighted for number of mussels in each container.

Figure 13. Baskets used to contain juvenile mussels placed at secondary (grow-out) culture locations. Mesh screen (400 micron) or rubber pond liner covers the bottom of the basket. Substrate is added to allow mussels to burrow and orient themselves. One basket design includes using a cement anchor with a foam lid, which keeps the basket upright and suspended in the water. Another design uses a mesh lid to limit predators, and a rope secures the basket to a platform.

Figure 14. Mucket juveniles at Waterville Fish Hatchery, hand-checked during the move from Pond 1 to Pond 2. Mucket size varied within baskets.

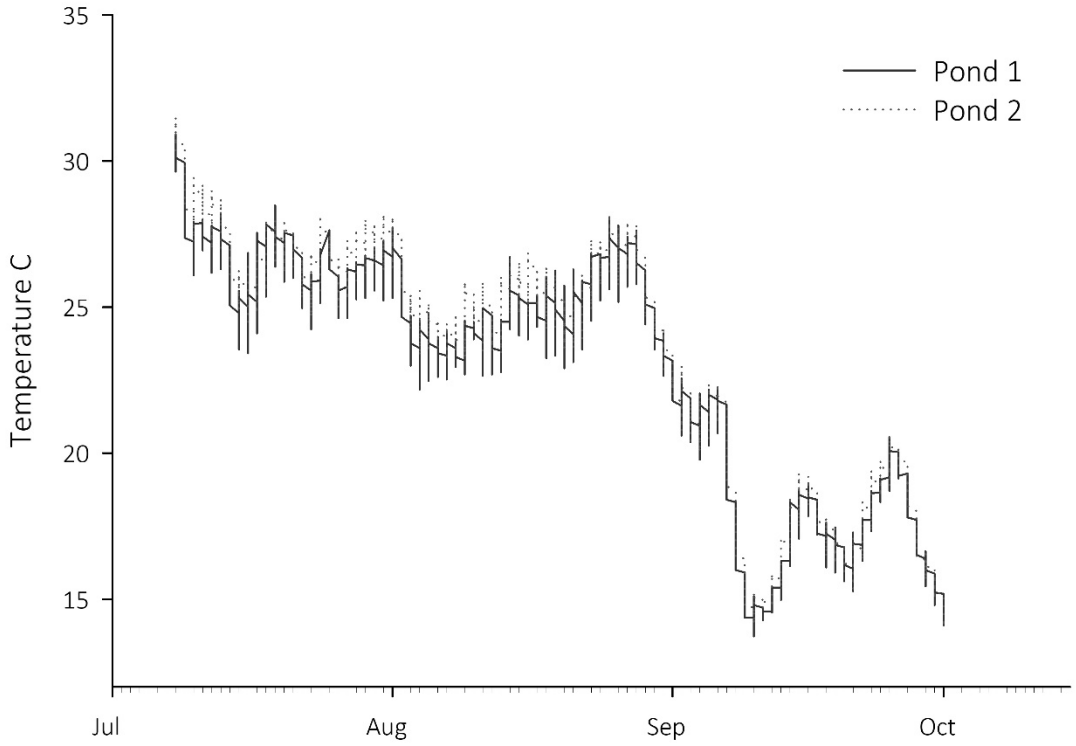


Figure 15. Waterville Fish Hatchery ponds. The temperatures in the two pond were significantly different (t-test, p=0.0004)

Figure 16. Totes used to contain juvenile mussels placed at secondary (grow-out) culture locations. A generic storage tote, approximately 2' x 3' x 1.5', is secured to a metal and wood platform base using bungee cords. Small holes drilled into the sides of the tote allows water exchange, while the lid limits predators. Substrate allows mussels to burrow and orient themselves. The unit can be placed at any depth by wading or diving.

Figure 17. Pan system located at the Minnesota Zoo used to rear juvenile mussels larger than ~ 2 mm. This system was adapted from designs by biologists at the Department of Game and Inland Fisheries, Aquatic Wildlife Conservation Center in Marion, Virginia (see Hua et al. 2013), and the DuPage Forest Preserve District's Urban Stream Research Center. Each system has 12 round, 7-quart drain pans with a center standpipe to maintain water level. Water from the zoo's "A Lake" is pumped through filter media into a sump where it is aerated and supplied to each pan. Effluent from each pan is directed through a filter and a nitrifying biotower before returning to the lake. This system can be temperature controlled.

Figure 18. Black Sandshell juveniles (cohort 2019) moved to Minnesota Zoo in July, 2020.

Figure 19. Mucket tagged for release into the Cannon River (Cohort Year 2016 from missing tote). Hallprint tags, PIT tags, or black dot were placed on all mussels prior to release.

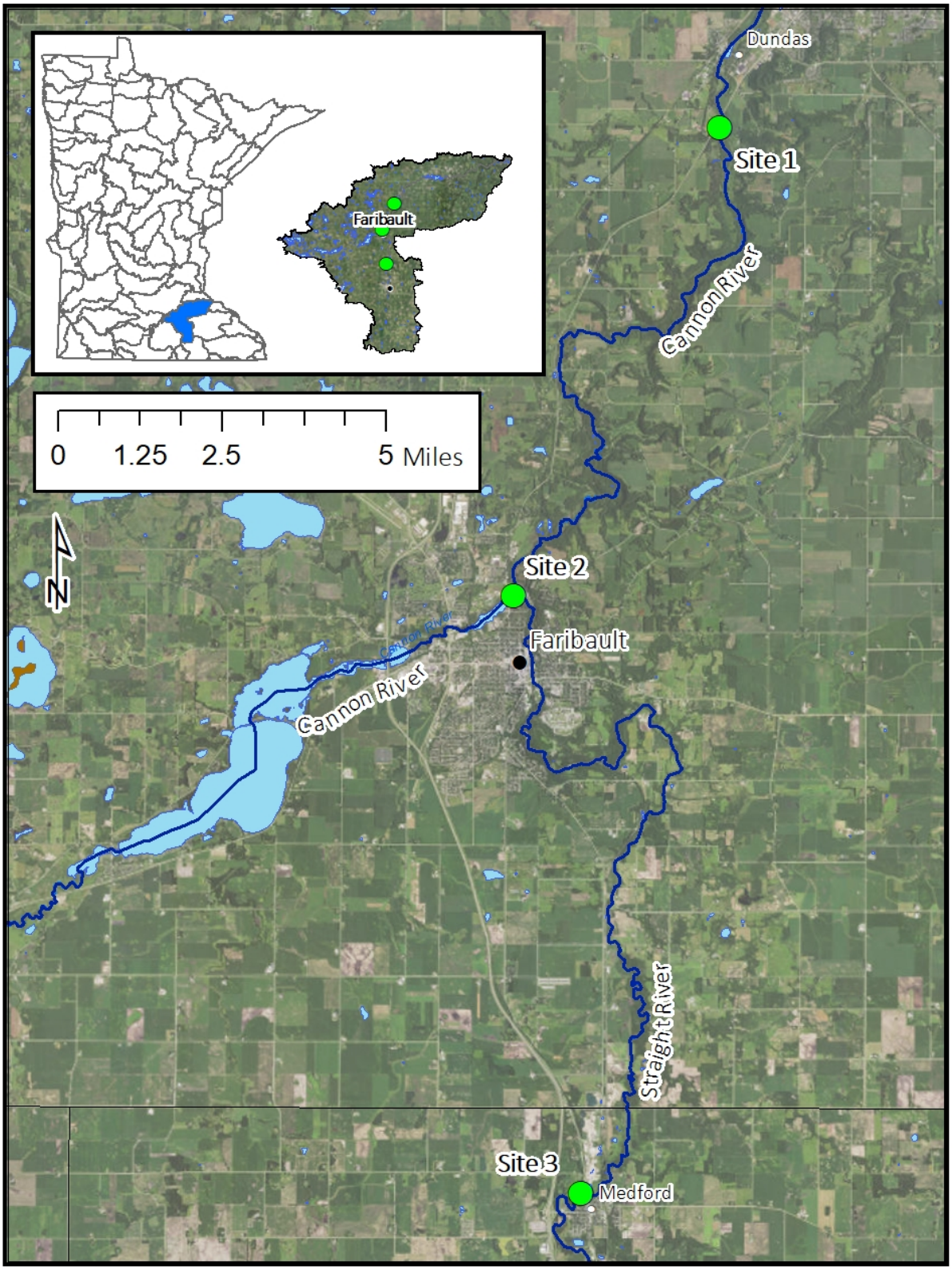


Figure 20. Release locations for Mucket in the Cannon River.

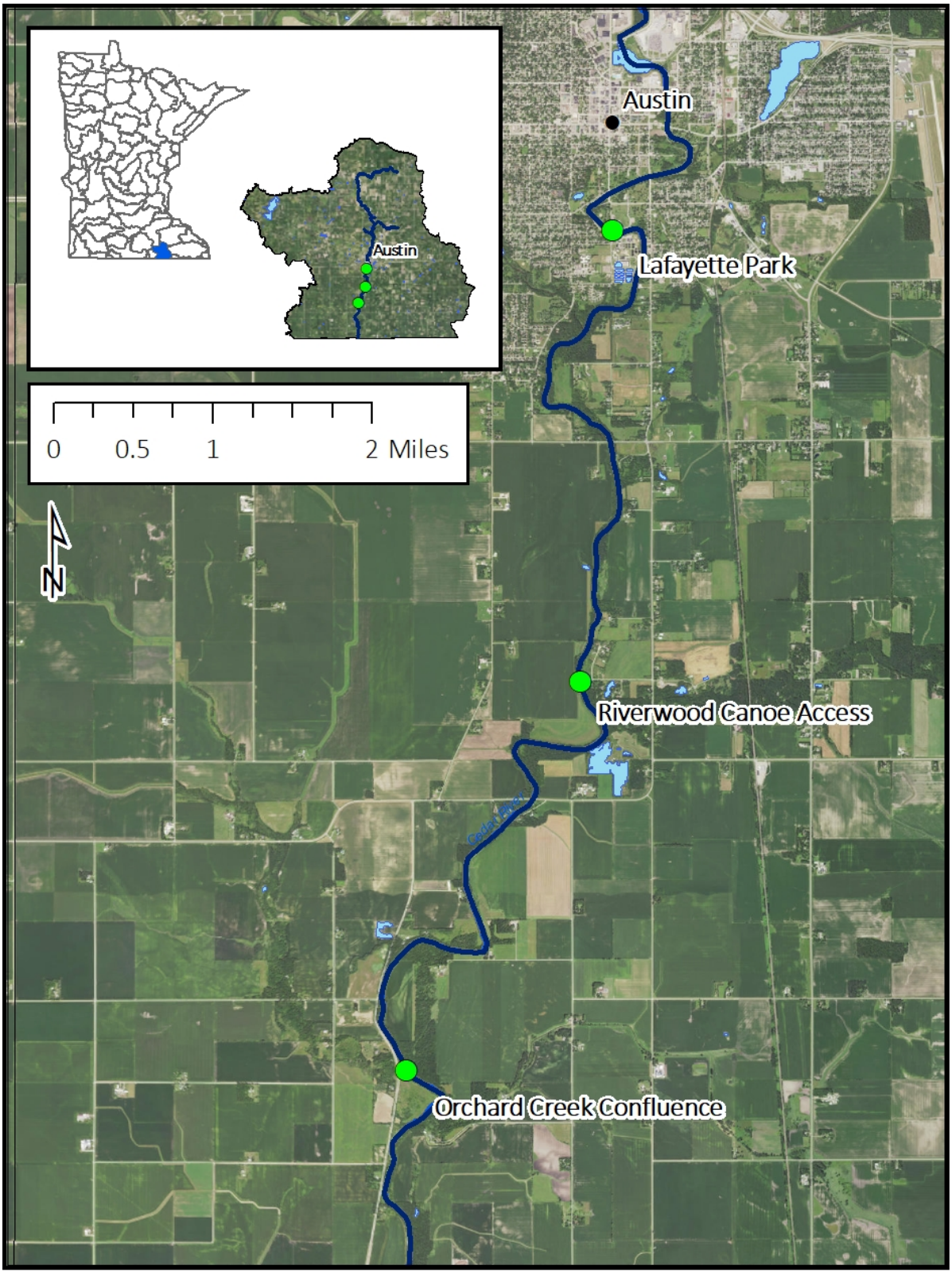


Figure 21. Release locations in the Cedar River (release dates include 2019 and 2020).

Figure 22. The Sprinkler System is a recirculating system consisting of a group of AHAB containers with a centralized sump filled with bio-media for ammonia control. A submersible pump directs water to a multiport manifold, then into each container. Water exits through the opposite end of the container and is gravity fed back to the sump. The systems are fed by a peristaltic pump that delivers food to the sump at 30-minute intervals.

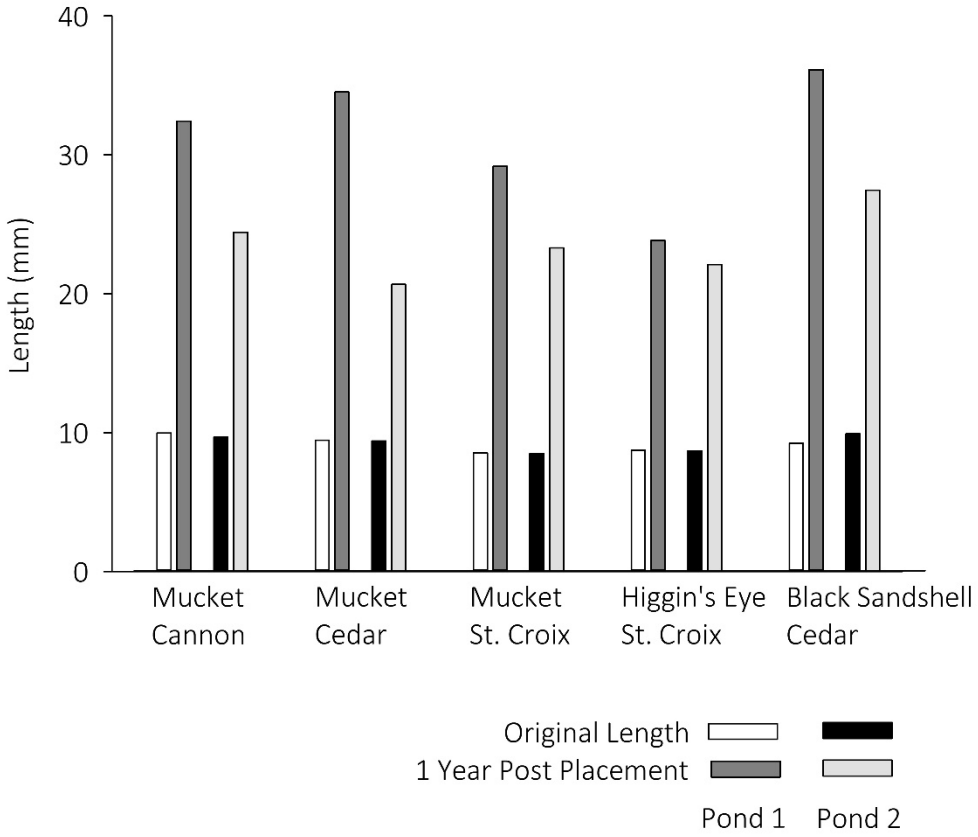


Figure 23. Length comparison of multiple species (Cohort 2019) in two hatchery ponds used to secondary culture. Juvenile mussels were placed into separate ponds from July – October 2020. It was noted in 2020 that Pond 1 juveniles were more robust, measurements were taken in June 2021.

Figure 24. 2019 Cohort submerged basket reset and measurement at Waterville Fish Hatchery.

Figure 25. Tagging and reintroduction of St. Croix mussels (Cohort 2018) for release into the Mississippi River near Hidden Falls.

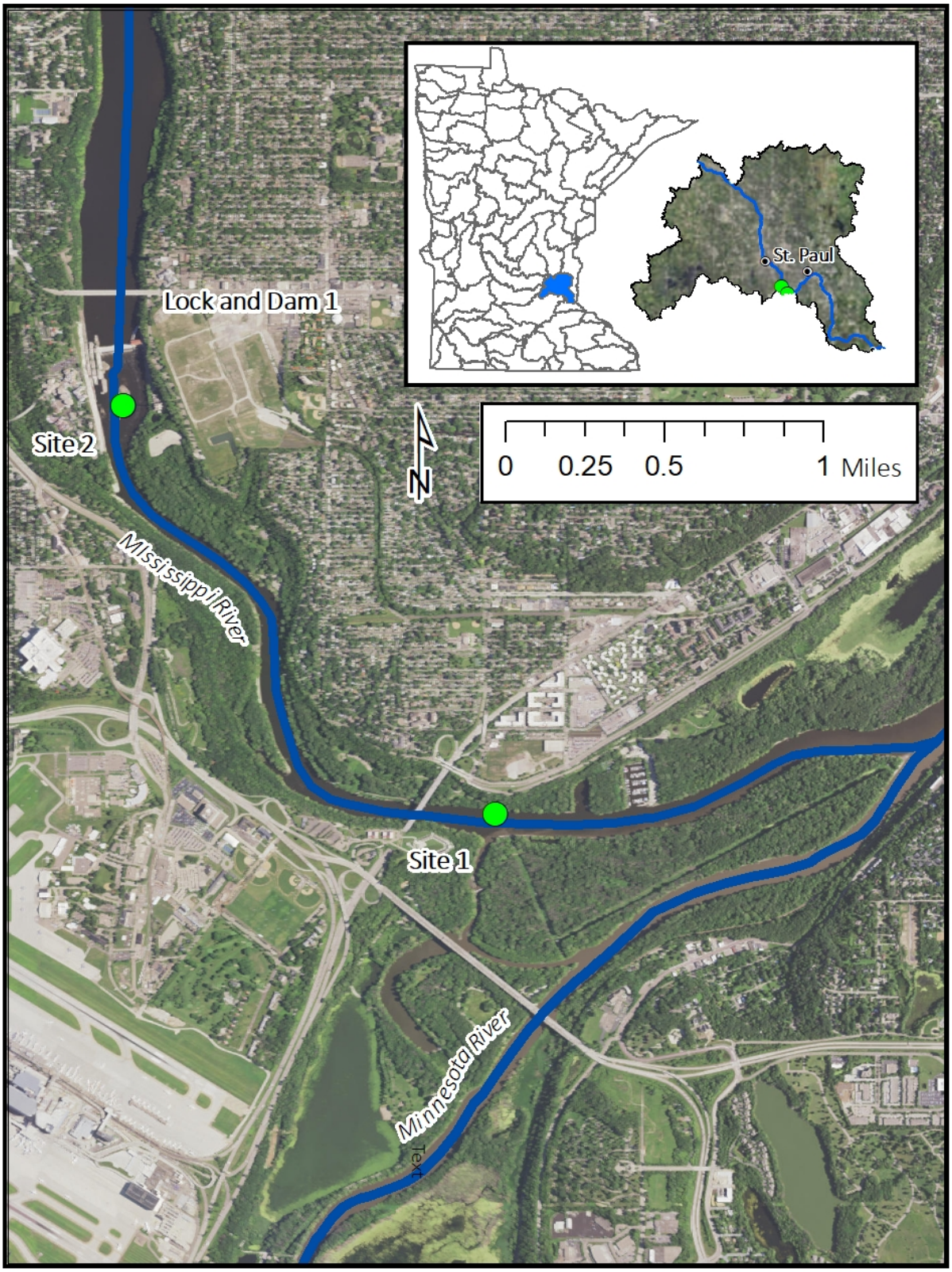


Figure 26. Release locations in the Mississippi River.

Figure 27. Dobbins Creek, Austin MN, growth and survival study. Custom built mussel silos were placed at 3 sites along Dobbins Creek to examine its potential for native mussel reintroduction. A survey completed by the DNR in August 2020 found a stable and diverse population of mussels below the dam at Eastside. Above the dam, only two species were found alive. The current dam was built in 1934, and a previous stone dam likely impacted fish movement upstream too. This study is the first part to examine the likelihood of reestablishing a native mussel population in Dobbins Creek.

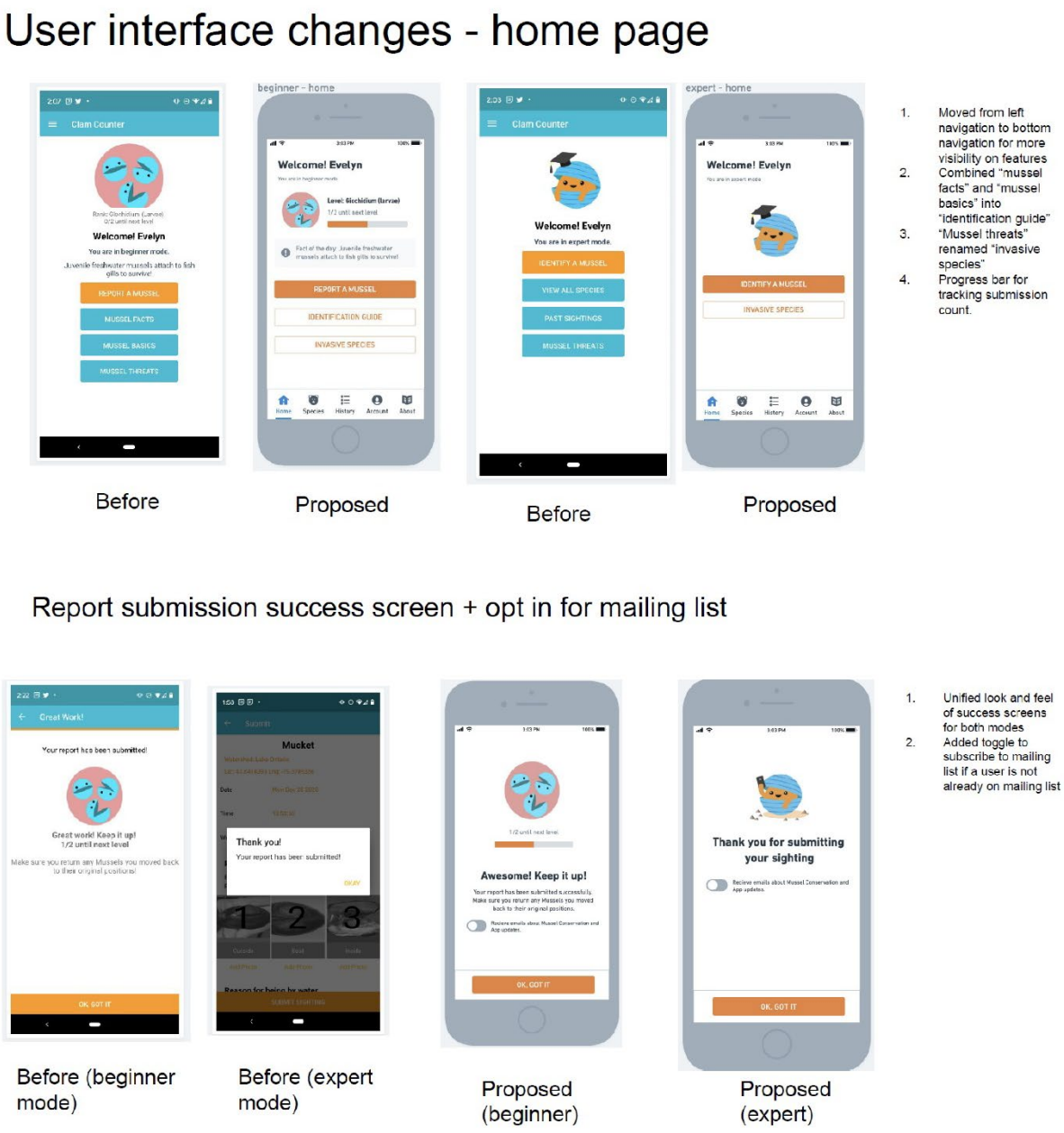


Figure 28. Proposed updates for Clam Counter 2021. Images provided by App Developers Evelyn Liu (Designer and Scientific Illustrator, eliubc@hotmail.com).

Final Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2019 Final Budget Spreadsheet
Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03b
Project Manager: Mike Davis
Project Title: Restoring Native Mussels in Streams and Lakes
Organization: MNDNR
Project Budget: $500,000
Project Length and Completion Date: 2-years, July 1, 2019 - June 30, 2021
Today's Date: August 15, 2021

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Budget	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	**$ 436,001**	$ 436,001	**$ -**
Mussel Culture Biologist-1FTE (72% salary, 28% benefits) $130,469			$ -
Aquarist/survey diver, 1 FTE (72% salary, 28% benefits) $105,448			$ -
Malacologist, .5 FTE (72% salary, 28% benefits) $86,982			$ -
Lab/Database/survey diver, .5 FTE (72% salary, 28% benefits) $60,071			$ -
Project Manager, .25 FTE (72% salary, 28% benefits) $53,031			$ -
Professional/Technical/Service Contracts			
Mussel Phone App. - contract with Evelyn Liu, University of British Columbia	$ 10,000	$ 10,000	$ -
Equipment/Tools/Supplies			
Host fish purchases, mussel food.	$ 6,469	$ 6,469	$ -
Capital Expenditures Over $5,000			
	$ -	$ -	$ -
Fee Title Acquisition			
	$ -	$ -	$ -
Easement Acquisition			
	$ -	$ -	$ -
Professional Services for Acquisition			
	$ -	$ -	$ -
Printing			
	$ -	$ -	$ -
Travel expenses in Minnesota			
Travel to and from donor mussel sites and reintroduction monitoring sites	$ 5,997	$ 5,997	$ -
Other			
Direct and Neccesary DNR charges: HR Support (~$9,604), Safety Support (~$1,989), Financial Support (~$5,879), Communications Support (~$1,251), IT Support ($21,751), Planning Support (~$1,059) necessary to accomplish funded project.	$ 41,533	$ 41,533	$ -
COLUMN TOTAL	$ 500,000	$ 500,000	$ -

OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State: C-SWG federal funds	Secured	$ 254,512	$ 254,512	$ -
State: Match funds from LCCMR at 35% to 65% federal funds		$ 113,625	$ 113,625	$ -
In kind:		$ -	$ -	$ -

PAST AND CURRENT ENRTF APPROPRIATIONS	Amount legally obligated but not yet spent	Budget	Spent	Balance
Current appropriation:		$ -	$ -	$ -
Past appropriations:		$ 600,000	$ 600,000	$ -

PROJECT TITLE: Restoring Native Mussels in Streams and Lakes – Continuation

Project Manager: Mike Davis

Organization: MN DNR

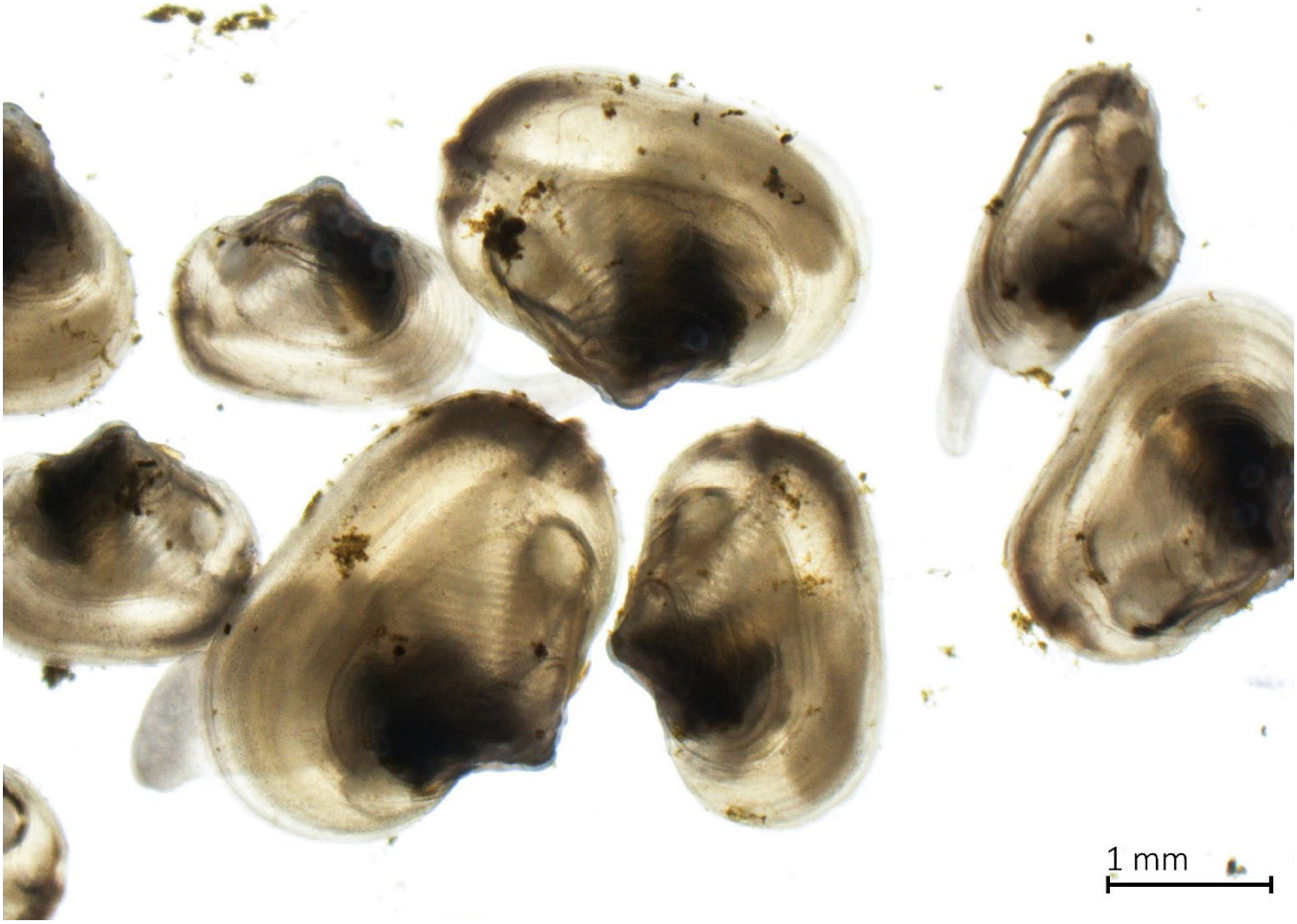
Sub-adult Mucket (*Actinonaias ligamentina*) tagged with unique ID's and ready to be released into the Mississippi River near Hidden Falls. These mussels were raised inside the MN DNR Center for Aquatic Mollusk Programs for almost a year, then placed into a culture container on the river bottom. The DNR has released 1,432 Muckets into the Mississippi River thus far, and has released over 9,500 of 5 species into 3 rivers in Minnesota.

Sub-adult Mucket (*Actinonaias ligamentina*) and Higgins Eye (*Lampsilis higginsii*) tagged with unique ID's or PIT Tags (Passive Integrated Transponder). These mussels are about to be released into the Mississippi River near Hidden Falls as part of an ongoing reintroduction effort funded by ENRTF. These mussels were raised inside the MN DNR Center for Aquatic Mollusk Programs for almost a year, then placed into culture containers on the river bottom. The DNR has released 1,432 Mucket and 593 Higgins Eye into the Mississippi River thus far, and has released over 9,500 of 5 species into 3 rivers in Minnesota.

Lindsay Ohlman (MN DNR), propagation biologist, releases Muckets (*Actinonaias ligamentina*) into the Straight River near Medford. A Mucket mussel population still exists below the dam in Northfield and upstream of the Byllesby Dam near Cannon Falls, however, they were extirpated upstream and downstream of this reach sometime prior to our first survey in 1987. The release of propagated Muckets is a historical milestone, marking the beginning of the DNR reintroduction efforts of mussels into the Cannon River system. Mussels will provide habitat for fish and other invertebrates, as well as improving water quality by filtering out bacteria and other particles.

Hundreds of juvenile Higgins Eye (*Lampsilis higginsii*) were transferred to the MN Zoo in July 2020. These mussels were raised at the Center for Aquatic Mollusk Programs for nearly one year. They will continue to grow and mature at the MN Zoo until they are about 2-inches in size when they will be tagged and released. Higgins Eye, a federally endangered species, is one of nine species the MN DNR is raising for reintroduction efforts in Minnesota.

Madeline Pletta (MN DNR), kicks off Cedar River Watershed Day at IJ Holton Intermediate School with a presentation about a valuable resource in Minnesota, its Freshwater Mussels. Nearly 400 student listened to the presentation, afterwards, student played several mussel related games in small groups.



4-month old Higgins Eye (*Lampsilis higginsii*) propagated at CAMP. These young mussels have not developed their periostracum, the outermost layer of the shell of mussels, and appear somewhat translucent. Through the shell the stomach, gills, and muscular foot are visible.