2019 Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Restoring Native Mussels in Streams and Lakes
PROJECT MANAGER: Mike Davis
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04c

APPROPRIATION AMOUNT: $600,000
AMOUNT SPENT: $600,000
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 produced 1,332,592 juvenile mussels from 11 species in three watersheds. Now thousands of sub-adult mussels are awaiting their release to restore and enhance our native rivers.

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 effected. Improvements from Clean Water Act implementation and stream restoration work are creating opportunities to reverse this trend. Mussel dependence on fish hosting their larval stage, and dams blocking fish movement can prevent populations from recovering limiting ecosystem recovery. Laboratory propagation began in 2016 as a means to reestablish mussel assemblages and the ecosystem services mussels provide. Three watersheds were selected for reintroduction efforts based on historical mussel records, habitat type and fish communities. We constructed several propagation systems specifically designed for juvenile recovery and culture over this period. In total, the Center for Aquatic Mollusk Programs (CAMP) has produced 1,332,592 juveniles of eleven species. In addition, almost 1,000 inoculated fishes were placed in benthic plastic totes or metal cages. The range of survival in the laboratory after 90 days ranges from 0 – 84%; Mucket and Black Sandshell have been our most successful species. Almost 75,000 juvenile mussels were raised to 2mm in length and moved into secondary culture systems for continued growth for release within two years. By restoring mussels, we hope to recover mussel species and improve water clarity. Recovering mussels will improve habitat for fish communities. Minnesotan's value clean water and fish; this project intends to enhance both.

Project Results Use and Dissemination

The results of this project has been featured in the Minnesota Conversation Volunteer in the September – October 2019 edition. Our Mussels of Minnesota poster was updated with all new photographs and text, and published in June 2019. CAMP's annual mussel newsletter generated attention, and our subscriber numbers have increased to over 2,300.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan

Date of Report: November 30, 2019

Date of Next Status Update Report: completed as of June 30, 2019

Date of Work Plan Approval: 6/07/2016

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No.

PROJECT TITLE: Restoring Native Mussels in Streams and Lakes

Project Manager: Mike Davis

Organization: MNDNR

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Location: Statewide

Total ENRTF Project Budget:	**ENRTF Appropriation:**	**$600,000**
	Amount Spent:	**$600,00**
	Balance:	**$0**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04c

Appropriation Language:

Appropriation Language: $600,000 the second year is from the trust fund to the commissioner of natural resources in cooperation with the Minnesota Zoological Garden for a statewide mussel program to rear, restore, and re-establish native mussel species in streams and rivers. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

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I. PROJECT TITLE: RESTORING NATIVE MUSSELS FOR CLEANER STREAMS AND LAKES

II. PROJECT STATEMENT:

1. Minnesota's native mussels are a critically important component of aquatic ecosystems, but have been lost or diminished in many Minnesota water bodies. Harvest for pearls and buttons, pollution, dams and destabilized waterways has caused mussel populations to decline dramatically in North America including Minnesota where 80% of our species have been affected (see graphics). 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' (see graphic) prevents some populations from recovering naturally. This leaves an ecological gap in our stream restoration efforts that will limit ecosystem recovery. Reestablishing the water cleansing, and nutrient processing capacity that mussel populations provide will improve water quality and restore the biotic communities that mussels support and that fish and wildlife depend on while helping delist species in trouble.

2. Goals for this project are:

A. Restore historic mussel species aggregations in select streams to improve stream health through restoration of their unique provisioning of ecosystem services
    a. Reintroduce up to six species of mussels historically present in the Cedar River between Austin, MN and the Iowa border.
    b. Reintroduce up to three species of mussels historically present in the Cannon River system of SE Minnesota.
    c. Reintroduce up to six species historically present in the Mississippi River to Upper Pool 2 in St. Paul.

B. Reestablish mussel populations that contribute to delisting state endangered and threatened species
    a. Four state threatened mussel species (Mucket, Elktoe, Monkeyface and Fluted Shell) will be reintroduced to the Cedar River between the Iowa border and Austin, MN. Females for propagation will be obtained from the Cedar River in Iowa in cooperation with the IA DNR.
    b. Juveniles of one state threatened mussel species, Mucket, now limited to the Cannon River between Lake Byllesby and Northfield, MN will be produced and raised for reintroduction to two other reaches of the Cannon River system; one that is now missing entirely, Elktoe, will be obtained from the nearby Zumbro River drainage and juveniles produced and raised for reintroduction. Expanding the range of these species contributes towards eventual delisting.
    c. Propagation and reintroduction of three federally endangered mussel species (Higgins' eye, Snuffbox, and Winged Mapleleaf) and one state threatened species (Mucket) will continue in the Mississippi River between the Ford Dam and the Mississippi/Minnesota River confluence. Additional species may be added to this effort.

C. Engage and inform the public about the importance of aquatic ecosystems and the unique role that mussels play in benefiting people by rearing juvenile mussels in full view of MN Zoo visitors.
    a. A cooperatively developed interpretive display will be placed inside the building next to the bridge over the Zoo's lake where passersby can see young mussels being grown for reintroductions and learn about the important ecological role they play in freshwater.

b. The MN Zoo website will be further developed to feature mussel reintroduction work and the importance of mussels to freshwater ecosystems.

3. Female mussels will be collected from a population within the target river system where possible, or from the nearest river system when not. Mussel larvae will be non-lethally harvested and host fish inoculated. Fish will be held in the DNR's Center for Aquatic Mollusk Programs lab, held in cages in the river, or released into the area of the stream targeted for reintroduction. In the lab, transformed juvenile mussels (see graphic) will be collected and placed into rearing systems where filtered river water augmented with commercial food will feed them until they have grown to 2-3 mm (see graphic). Juveniles not retained in the lab will be transferred to the MN Zoo where they will be grown using the zoo's lake water until they are 30-50 mm in length (see graphic), then released into the river segment targeted for restoration. Fish placed in cages will be removed after mussel transformation is complete and any resulting juveniles allowed to grow within the cage to protect them from predation and to facilitate collection later. Inoculated fish will be released and presumably scatter the transformed juvenile mussels along a continuum of the stream that is traversed by the fish. Annual monitoring of released mussels and searching for those resulting from fish releases will allow us to document growth and survival and to determine when mussels have been successfully restored.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of December 1, 2016

A mussel culture biologist was appointed to lead propagation activities for the Center of Aquatic Mollusk Programs (CAMP). Beginning 1 July 2016, a total of 85,842 juvenile mussels have been collected from their host fish. Juvenile mussels were propagated from 41 gravid mussels. Gravid mussels included four species that were collected in three rivers across the state. Juvenile mussels were raised in the laboratory and in propagation cages, with the expectation of release into reintroduction sites beginning in fall 2017.

Project Status as of May 1, 2017

During the winter months, we built and designed several systems. Most markedly, we collaborated with the Minnesota Zoo to construct and build a recirculating propagation system. The four tank system will be the main system used for the collection of excysted juveniles from their host fish. Also, we built a new system for holding gravid mussels. The new system allows for species and watersheds to easily be kept separate from one another. Also, allows for more visual inspection and accessibility. In addition to the systems, several species of juvenile mussels were held at the facility overwinter. Each species were examined weekly, growth and survivorship were measured. Fresh river water was collected multiple times throughout the winter. An ice auger was used to gain access to freshwater, and water was held for several days to warm before use in propagation. Additionally, two species of mussels were further examined to determine grow-out culture system preference. It was concluded that sediment culture tanks yielded a higher growth rate, as well as, higher survival rate compared to pulse flow tanks. Lastly, in May, all juvenile mussels from the facility were placed into cages or floating totes. Cages containing 3,001 *Ligumia recta* (Black Sandshell) were placed into East Side Lake in Austin, MN. Then five species containing nearly 3,400 juveniles were placed into experimental floating totes at the Lake City Marina in Lake City, MN. Species include: *Actinonaias ligamentina, Epioblasma triquetra* (Snuffbox)*, Lampsilis higginsii* (Higgin's Eye), and *Quadrula fragosa* (Winged Mapleleaf).

Project Status as of December 1, 2017

CAMP's laboratory propagation efforts produced 319,507 juvenile mussels in 2017. Production of juveniles was largely a result of the increased capacity to hold host fishes. Our new recirculating propagation system (RPS) maintained a healthy environment for fishes, thus reducing mortality from bacteria, fungus, and protozoans. All

juvenile mussels were placed in sediment tanks (ST) for initial growth. The federally endangered Snuffbox mussel had the highest overall survival; survival ranged from 79 – 84%. Throughout the summer, over 100,000 juveniles were placed into totes and cages in natural systems for final grow out. Nearly half of these juvenile mussels were greater than 60 days old, and ranged in size from 1 – 3mm. Additionally, Mucket (average size 2.6mm) propagated during the winter from a host trial were placed into an experimental floating tote in Lake Pepin, Lake City, MN for four months (May – August). These juveniles grew greater than four times their starting length, some attaining lengths of 20-30mm. In contrast, newly metamorphosed juveniles did not survive in the floating totes, while newly metamorphosed juveniles showed growth in totes placed on river and lake bottoms. Juveniles from the Cedar River and St. Croix River propagated in 2016 and 2017 will be tagged for release in summer 2018.

We are pleased to announce our funds from the ENRTF funds allowed us to match a federal Cooperative State Wildlife Grant for expanding the Cedar River mussel work in collaboration with the Iowa DNR and purchasing a new microscope system for our lab. These funds also enabled our program to make a nationally significant discovery in identifying the host fish for the federally endangered Spectaclecase mussel (*Cumberlandia monodonta*), an enigma for mussel scientists for more than 20 years. Mooneye and Goldeye fish are the earth's only two surviving species in the fish Family Hiodontidae. They are migratory, pelagic river dwellers with a wide distribution within the Mississippi River Basin. They are particularly difficult to transport and support in a laboratory setting. Our staff worked with the MN Zoo aquarists to develop a holding system that kept them alive throughout the metamorphosis of mussel larvae into juveniles. Over 300,000 juvenile Spectaclecase were recovered and placed into various rearing systems in our lab, the Genoa National Fish Hatchery lab, and Missouri State University mussel lab. Currently, we have juveniles that range in size up to 3mm, a first in the world of mussel propagation.

Project Status as of May 1, 2018

Amidst the routine maintenance, spring brings a new tempo in the laboratory as we prepare for incoming student interns, last minute construction, broodstock collection, and orchestrating host fish deliveries and collections. Over the past few months, we have maintained over 500 host fish in the facility, continued to raise three species of juvenile mussels, and completed an experiment testing growth and survival in different substrates. Additionally, system modifications and improvements are never ending.

The 2018 propagation plan includes two new species from the Cedar River; *Alasimdonta marginata* (Elktoe) and *Lasmigona costata* (Fluted Shell). Both species are from the mussel tribe Anodontini. This expands our propagation efforts to three of five mussel tribes found in Minnesota. We plan to examine the growth and survival in up to three culture systems for both species. Overall, we have planned to propagate nine species from three watersheds this season. We also plan to release reared mussels into the Cannon River, Cedar River and the Mississippi River in the late summer. Each mussel released will be marked with an identifying tag that correlates to the release date.

Project Status as of December 1, 2018

The summer of 2018 brought new challenges and successes within the program. Late ice-melt delayed the growth of host fishes resulting in later inoculations. In addition, collection of broodstock was difficult due to high water conditions during brooding period. Despite these circumstances, CAMP's propagation efforts resulted in the production of 780,318 juveniles from three watersheds and the numbers of species expanded to ten species: New species include; *Amblema plicata* (Threeridge, Cedar River), *Fusconaia flava* (Wabash Pigtoe, Cedar River), and *Lasmigona costata* (Fluted Shell, Cedar River). Additionally, Higgin's Eye was successfully propagated in a laboratory for the first time.

As of November, almost 80,000 juveniles of six species were alive and thriving (not including the federally endangered Winged Mapleleaf). The high number of juveniles are a result of a low rate of survival of small juveniles placed into cage culture in the fall months. For example, in October 2017, over 100,000 juveniles were placed into cages for grow-out during the 2018 season, however, upon removal in November 2018, less than 1% survived. In

contrast, juveniles placed after 10+ months in culture showed a survival rate between 38 – 68%. Because of holding high numbers of juvenile mussels, new systems specific for juveniles greater than four millimeters were developed and are currently being tested. New systems include a recirculating trough with sand and sediment trays, also, fish hatchery heath trays are in operation. These systems are designed for middle life-stages through adults. It is our hopes that juveniles mussels will continue to grow throughout the winter months in preparation for cage placement in the early spring.

AMENDMENT REQUEST JUNE 5, 2019

We are requesting funds be shifted from the Poster budget line to personnel.

Poster budget would be reduced by $5,331 to a revised budget of $2,839

Personnel budget would increase by $5,331 to a revised budget of $549,272

These changes are being requested because the bid for producing the poster was less than the budgeted amount and we can better use the funds for personnel salary.

Project Status as of June 30, 2019

Propagated juveniles from cohorts produced in 2018 overwintered at CAMP and were placed into secondary culture systems in natural systems during the spring. A total of 14,098 juveniles of seven species were placed into holding locations, such as, Lake Byllesby, East Side Lake, and the St. Croix River. Additionally, juveniles were placed in floating totes at Waterville Fish Hatchery, and the MN Zoo. The range of juvenile sizes were from 5 – 15mm. We anticipate greater than half of these mussels should survive and be released in during the summer of 2020.

The 2019 propagation season began in June. Due to the high survival rate of 2018, it was decided to reduce the overall number to juvenile mussels to ensure mussel containers received proper maintenance and care. For the reporting time, only two species, Mucket (3x watersheds) and Elktoe, had completed juvenile production, and three additional species, Black Sandshell, Spectaclecase and Spike, were inoculated on its respected host fish.

Overall Project Outcomes and Results:

Minnesota's native mussels are a 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 effected. Improvements from Clean Water Act implementation and stream restoration work are creating opportunities to reverse this trend. Mussel dependence on fish hosting their larval stage, and dams blocking fish movement can prevent populations from recovering limiting ecosystem recovery. Laboratory propagation began in 2016 as a means to reestablish mussel assemblages and the ecosystem services mussels provide. Three watersheds were selected for reintroduction efforts based on historical mussel records, habitat type and fish communities. We constructed several propagation systems specifically designed for juvenile recovery and culture over this period. In total, CAMP has produced 1,332,592 juveniles of eleven species. In addition, almost 1,000 inoculated fishes were placed in benthic plastic totes or metal cages. The range of survival in the laboratory after 90 days ranges from 0 – 84%; Mucket and Black Sandshell have been our most successful species. Almost 75,000 juvenile mussels were raised to 2mm in length and moved into secondary culture systems for continued growth for release within two years. By restoring mussels, we hope to recover mussel species and improve water clarity. Recovering mussels will improve habitat for fish communities. Minnesotan's value clean water and fish; this project intends to enhance both.

The inception of the laboratory propagation at the Center for Aquatic Mollusk Program has been successful in its first years of operation. We have come to realize the ebb and flow of juvenile mussel culture, relating to newly metamorphosed juveniles, rearing juveniles, and moving juveniles to a secondary culture system. Each step in the process of propagation was a new quest to reach our goal of restoration. Our facility is effectively rearing mussels from three watersheds, and working with 11 species. For each species, we are given one opportunity per year to

collect broodstock, and host fishes in order to produce juveniles. Both objectives must have been met in numbers for a successful propagation effort to be made. Eight of the 11 species have yielded greater than 10,000 juveniles produced in three season. In total, CAMP has produced 1,332,592 juveniles (Table 9). In addition to metamorphosed juveniles in the laboratory, we have caged almost 1,000 inoculated fishes in benthic plastic totes or metal cages. The majority of the fishes used for propagation are collected in the fall prior to use. Our facility holds greater than 500 native fishes at any given time, and increases during summer production. Aquarium husbandry and maintenance is as important as juvenile culture.

Rearing of juveniles has proven its triumphs and failures. We have experimented with several systems, including; a large-scale pulse flow, static sand, static sediment, mucket buckets, and placement of juveniles directly in secondary culture systems. A system that was in use originally, often has been replaced as new methods are developed. Additionally, every species has a different culture requirement (flow, substrate, food, etc.), such that, one system may not work for another. The range of survival in culture 90-days ranges from 0 – 84%; Mucket and Black Sandshell have been out most successful species thus far.

Moreover, we have raised almost 75,000 juvenile mussels to at least 2mm in length. When juveniles reach 2mm, they are moved in secondary culture systems for continued growth. These culture systems include: benthic metal cages, benthic plastic totes, floating plastic totes, and a flow-through pan system at the MN Zoo. In the fall of 2016 and 2017, a large effort was put forth to place juvenile mussels into natural systems. However, only 0.01% of these juveniles survived. Due to the poor outcome, in 2018, all juvenile mussels overwintered at CAMP. In the spring of 2019, over 14,000 juveniles were moved out of the facility and placed into secondary culture systems.

The final step in propagation is to release reared mussels into their native rivers. This step, like the very first step of broodstock collection, is dependent on water conditions for an accurate release location. During floods, base flows are hard to determine and one site may be completely dewatered on normal flows. For this reason, a release of propagated mussels did not occur until after June 30, 2019. However, hundreds of mussels are of a releasable size are being held in various systems.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Juvenile mussel propagation
Description:

Up to ten female mussels of each subject species holding larvae will be collected by wading, snorkel or SCUBA. This activity will occur from early spring to late fall depending on the targeted species life history that we are working with. Larvae will be harvested from each female and placed into containers holding the proper host fish species and allowed time for attachment to the hosts to complete. Fish are then placed into tanks set aside for each species from each river in an artificial aquatic habitat system, placed in cages in a river or released at targeted mussel restoration sites. For the next 2-12 weeks juvenile mussels held in our lab will be collected from the artificial aquatic habitat system by flushing them out of each fish tank and onto a fine mesh screen.

Summary Budget Information for Activity 1 as of Dec 1, 2016:	**ENRTF Budget:**	**$ 190,880**
	Amount Spent:	**$ 190,880**
	Balance:	**$ 0**

Outcome	Completion Date
1. Number of gravid females of each species collected each year from each river to assure adequate genetic variation of propagules.	April – October each year depending on mussel species
2. Number of host fish inoculated with each mussel species from each river and the estimated number of larvae attached to each.	Usually within 30 days of collection

	unless being held overwinter in chilled water
3. Number of juveniles collected from host fish of each mussel species and transferred to growing chambers.	6-12 weeks post inoculation unless fish are held overwinter in chilled water

Activity Status as of December 1, 2016

ACTIVITY 1:
Description: Juvenile mussel propagation

In 2016, MN DNR collected 41 gravid females for the Environmental and Natural Resources Trust Fund work project (ENTRF, Table 1). Mussels were collected via SCUBA or wading, then inspected for gravidity. Host fish for 2016 propagation season included; channel catfish (*Ictalurus punctatus*), common shiner (*Luxilus cornutus*), largemouth bass (*Micropterus salmoides*, LMB), smallmouth bass (*Micropterus dolomieu*), spotfin shiner (*Cyprinella spiloptera*), and walleye (*Sander vitreus*).

Channel catfish were collected via eletrofishing from the Mississippi River in the discharge canal of Excel Energy at Prairie Island and from Mississippi River upper pool 4. Fishes collected were pretreated with a salt and formalin bath to reduce stress and combat bacteria/fungus growth. This effort was somewhat successful, however, 1/3 of collected channel catfish succumbed to ich (*Ichthyophthirius multifiliis*) before juvenile production.

In early August, 600 LMB were delivered by Ron Rademacher's Fish Farm in Waconia, MN. The LMB were immediately used for *Actinonaias ligamentina* (Mucket) inoculation. Unfortunately, the stress of summer heat, transportation, and immediate inoculation lead to marginal success for propagation due to disease and mortality at our facility.

The 2016 propagation season for the ENRTF work project closed with eight inoculations, producing a total of 85,842 juveniles mussels (Table 2). Four species of native Minnesota mussels were propagated using nearly 1,000 host fishes. Propagation was dominated by Mucket, in spite of high mortality of host fish, Mucket yielded almost 63,000 juveniles (73.2%).

Activity Status as of May 1, 2017

ACTIVITY 1:
Description: Juvenile mussel propagation

The main portion of the 2017 propagation season will likely begin in early June. High water levels have inhibited collection of gravid females. Water levels must be a safe, workable condition for staff. Thus far, we have collected 21 *Actinonaias ligamentina* (Mucket) from two watersheds, and two *Epioblasma triquetra* (Snuffbox) from the St. Croix River (Table 3). To date, we have completed one inoculation. A small inoculation of Mucket concluded 20 January 2017, producing a total of 8,791 juveniles. Juveniles were placed into two types of culture systems, sediment tanks and pulse flows tank. The goal was to determine which type of systems yields a high growth and survival rate.

We retrieved three cages of channel catfish inoculated with *Quadrula fragosa* (Winged Mapleleaf) on 24 April 2017. Cages were placed in late fall of 2016. Caged channel catfish had a 92% survival rate throughout the winter. Channel catfish were placed into a recirculating system that allows for juvenile collection. Only 27 juveniles were recovered,

and then placed into a sediment tank for grow-out. Due to the marginal results, in the future, catfish will not overwinter in cages for spring propagation.

Lastly, hatchery raised largemouth bass (LMB) were delivered on 10 May 2017 from Ron Rademacher's Fish Farm in Waconia, MN. LMB are being held in a flow-through system to prevent water quality concerns. Other host fish include log perch, which overwintered in our facility. Log perch is the host fish for Snuffbox, which will be one of the first propagation efforts.

Description: Mussel propagation systems

Working cooperatively with the Minnesota Zoo, we designed a 500-gallon recirculating propagation system (RPS, Figure 1). Four round tanks are situated around a 135-gallon sump and bead filter. Water is pumped through 100µm melt-blown cartridge filter, activated carbon filter, and 240 watt UV sterilizing filter. Plus a drop in chiller (1/2 HP) will maintain water temperatures around 21°C. Water flows from the sump, through the filter system, and into each round tank. A plankton net is hung at the outflow of each round tank to capture juvenile mussels as they are released. The RPS will be the main propagation system used for large batches of fishes, size range 2 – 5 inches.

Moreover, we are in the process of constructing a chilled system for gravid females. A recirculating system will be held within a two-door, laboratory grade refrigerator. Each side of the refrigerator will be fashioned with a submersible pump that moves water thru plastic aquaria. Gravid mussels will be separated in aquaria by rivers, species, and individuals (Figure 2).

Activity Status as of December 1, 2017

ACTIVITY 1:
Description: Juvenile mussel propagation

CAMP biologists collected 62 mussels for propagation in 2017 (Table 3, Activity Status as of May 1, 2017). Mussels were collected from three river systems; Cedar River, Cannon River, and St. Croix River. Host fishes included; Channel Catfish (*Ictalurus punctatus*, CCF), Logperch (*Percina caprodes*), Common Shiner (*Luxilus cornutus*), Largemouth Bass (*Micropterus salmoides*, LMB), and Walleye (*Sander vitreus*, WAE).

Log Perch were collected in Lake Pepin, Lake City, MN in the fall of 2016 and overwintered at our facility.

CCF, LMB and WAE were purchased and delivered by Ron Rademacher's Fish Farm in Waconia, MN. LMB were delivered in the spring, and then held in a flow-through raceway. The flow-through system removed potential contaminants, allowing a larger volume of fishes to be held in a small system. CCF and WAE were delivered mid-summer prior to the inoculation of glochidia. Common Shiners were collected via electrofishing in the Zumbro River by DNR Area Fisheries staff (Lake City Area Office).

In 2017, the propagation season closed with ten inoculations, producing a total of 319,507 juvenile mussels (Table 4). Six different Species in Greatest Conservation Need were propagated, using more than 1,000 host fishes. Additionally, 16-plastic totes and 5-wire cages containing inoculated fishes of three species were placed in two river systems where the natural food sources result in faster growth. Plastic totes are modified by drilling a series of holes for water movement, fine particle silica sand provides suitable substrate, and the units are secured to a platform on the river bottom (Figure 4).

We were given a surplus electrofishing boat by Mankato State University in October that will help us in obtaining host fish species for propagation work in 2018.

Activity Status as of May 1, 2018

ACTIVITY 1:
Description: Juvenile mussel propagation

We anticipate the 2018 propagation season to begin in mid-May. The LCCMR propagation plan includes three watersheds, and nine species of freshwater mussels. In total, we plan to produce as many as 400,000 juveniles in the laboratory with additional juveniles propagated through cage-culture and free-release (Table 5). The success of the propagation plan will be determined by our ability to acquire broodstock and host fish species.

In November, we collected broodstock of three mussel species in the Cedar River (Table 6). Thus far, spring collections have been limited due to river conditions. In early April, two attempts were made to collect broodstock in the Cannon River and Zumbro River. One female from the Cannon River was collected; none from the Zumbro River. As of May 1, 2018, river conditions have been unsafe for collection.

Last fall, CAMP received YOY walleye and channel catfish from MN DNR operated fish hatcheries. Most of the fish received were less than 1 inch upon arrival; both species of fish have more than quintupled in length and are now an acceptable size for mussel inoculation. Moreover, Logperch collected in the fall had the highest long-term survival rate ever in our facility. The growth and health of all fishes at CAMP indicates adequate food supply and exceptional water quality. Maintaining greater than 500 native fishes is no a small feat; many staff hours focus on the health and success of the host fish.

Description: Mussel propagation systems and fish holding systems

To increase our holding capacity, CAMP is building another recirculating propagation system (RPS) for propagation. In addition, a 30-foot fiberglass raceway was donated to our program from a closing fish hatchery in Duluth, MN. Staff have modified the raceway to hold multiple species of fish for the upcoming propagation season (Figure 11).

Activity Status as of December 1, 2018

ACTIVITY 1:
Description: Juvenile mussel propagation

The continued success of the mussel propagation program was evident, as 780, 318 juveniles from three watersheds were produced this summer (Table 7). Thirty-eight female donor mussels were used for propagation; many were not collected until mid-May or later due to high water and unsafe conditions (Table 6). Species propagation efforts increased from seven species propagated in 2017 to ten species in 2018. New species include *Amblema plicata* (Threeridge, Cedar River), *Fusconaia flava* (Wabash Pigtoe, Cedar River), and *Lasmigona costata* (Fluted Shell, Cedar River).

The prolonged cold weather also influenced host fish acquisition. Largemouth Bass, a primary host fish for several species produced at CAMP, were delivered on June 14, 2018 (size range 2 – 4”). Other species of host fishes were kept over winter at our facility. This includes Logperch, Walleye, and Channel Catfish. It was our primary effort to maintain the health of these fishes. The majority of our propagation success was a result of this effort.

Below summarizes CAMP’s efforts in each watershed:

Cannon River

Only one species, the Mucket, from the Cannon River was propagated in 2018, and production was split between laboratory culture and cage culture. In 2016, cage culture was the only method of propagation in the Cannon River. We split our efforts due to the limited success of the cage culture. One brooding female

was collected in mid-April when water conditions were suitable for searching. In June four totes of inoculated fishes were placed into Lake Byllesby. In the laboratory, 6,620 juveniles were recovered from 40 Largemouth Bass (Length: 2 – 3")

Cedar River

The Cedar River was a large focus of this year's propagation, as three new species were added in the laboratory. These species include Threeridge, Wabash Pigtoe, and Fluted Shell; Threeridge and Wabash Pigtoe were a part of host suitability trials at our facility. Black Sandshell, Monkeyface, and Muckets were propagated as well. Black Sandshell yielded 10% of the propagation totals using Walleye raised at CAMP as host fish; each fish produced over 1,500 juveniles.

Mississippi River

Propagation efforts in the Mississippi River accounted for 83% of the total number of juveniles produced. This is largely due to a high metamorphosis rate of the federally endangered Spectaclecase mussel and Winged Mapleleaf mussel, 50.4% and 22.8% respectively. Additionally, the federally endangered Higgin's Eye mussel were successfully propagated in the laboratory for the first time. More than 50,000 Higgin's Eye mussels were produced in the laboratory, and, three totes containing inoculated fishes were placed in the St. Croix River near Lakeland, MN.

Project Status as of June 30, 2019

ACTIVITY 1:
Description: Juvenile mussel propagation

The 2019 propagation season began in June, making strides to follow the 2019 Propagation Plan (Table 8). Due to the high survival rate of 2018, it was decided to reduce the overall number to juvenile mussels to ensure mussel containers received proper maintenance and care. For the reporting time, only two species, Mucket (3x watersheds) and Elktoe, had completed juvenile production, and three additional species, Black Sandshell, Spectaclecase and Spike, were inoculated on its respected host fish.

Below summarizes CAMP's efforts in each watershed:

Cannon River

Dangerous water conditions prevented the collection of broodstock until 16 May 2019, when divers were able to enter the water near the stockpile site along the Cannon River; a stockpile site of Mucket (*Actinonaias ligamentina*) was created in August of 2018. All stockpiled Muckets were found during spring collection, however only two remained gravid. Glochidia was extracted from both mussels, and 71 fishes were inoculated (40 Largemouth Bass, and 31 Walleye). All Largemouth Bass were placed into totes in Lake Byllesby, while the Walleye remained in captive culture, yielding a total of 26,712 juvenile mussels.

Cedar River

Three species of broodstock were collected in late fall of 2018, then placed into the laboratory refrigerator to overwinter. In mid-March, two Elktoe (*Alasidonta marginata*) released their brood, resulting in an immediate inoculation. The sudden inoculation, marginal host fish, and old brood were contributing factors in the poor metamorphosis rate. A mere 130 juveniles were recovered, and survival was poor; 6 juveniles survived until June, however, a change in water chemistry likely resulted in their mortality. Moreover, the

survival of the host fish was poor, and strides are being made to accommodate for more sensitive host fishes for future propagation.

The Mucket accounted for 23% of the total juvenile mussels propagated. All mucket juveniles were metamorphosed at CAMP, and remain in culture for continued growth. Other Cedar River propagation efforts includes Black Sandshell (*Ligumia recta*), Threeridge (*Amblema plicata*)and Spike (*Elliptio dilatata*); Spike and Threeridge are a collaborative effort with the Iowa State University for restoration work in Iowa.

Mississippi River

Three of four species of mussels from the Mississippi Watershed were propagated starting after June 30; the propagation of Mucket ended 11 June 2019, producing over 60,000 juveniles. These juveniles were from larvae of female mussels that were collected in October of 2018; four of six mussels were recorded to have 95%+ viable glochidia.

Description: Host fish and aquariums

Greater than 1,000 host fishes are held in the laboratory overwinter; predominantly consisting of Channel Catfish, Largemouth Bass, Logperch, and Walleye; including 20+ aquariums with an assortment of native fishes. Fish health remained top priority throughout the winter to ensure suitable numbers for propagation beginning in the spring.

Final Report Summary:

ACTIVITY 1:
Description: Juvenile mussel propagation

Juvenile mussel propagation has grown dramatically at CAMP since its commencement. Starting with system development; CAMP has designed and construction two system specifically designed to collect recently metamorphosed juvenile mussels, a recirculating aquatic habitat unit inside of a laboratory grade refrigerator to hold brooding female mussels at 5°C, and several raceways to hold host fishes.

Through the system advances, juvenile mussel metamorphosis has increased on a yearly bases. In total, CAMP has produced 1,332,592 juvenile mussels of 11 different species, in three watersheds, using 204 brooding female mussels (Table 9).

ACTIVITY 2: Rearing juvenile mussels
Description:
Transformed juvenile mussels will be collected from fish holding tanks and placed into containers with filtered river water augmented with commercial foods flowing past them to provide food and oxygen. Survival and growth will be monitored. When 2-3 mm in length (one growing season) surviving juveniles will be either placed into cages submerged in a river or lake or moved to the MN Zoo and grown in public view in the Zoo's lake or indoor display for another 2-4 growing seasons.

Summary Budget Information for Activity 2:	**ENRTF Budget:**	**$ 272,355**
	Amount Spent:	**$ 272,355**
	Balance:	**$ 0**

Outcome	**Completion Date**

1. Number of juvenile mussels of each species that survive and grow to 2-3 mm in length	Varies by species 100-400 days
2. Number of juvenile mussels of each species transferred to MN Zoo	30 days from transfer
3. Number of mussels of each species raised at the zoo and released into rivers.	July 1, 2018

Activity Status as of December 1, 2016

ACTIVITY 2
Description: Rearing juvenile mussels

Post metamorphosis, juveniles are placed in three types of grow-out systems. The systems include mucket buckets (Barnhart 2006), sediment AHAB tanks (ST's), and pulse flow-through tanks (PF's). Mucket buckets, are a recirculating downwelling system, with screened end chambers in which the juvenile mussels reside. Chambers are sprayed off daily to prevent screen obstruction that may impede water flow. Additionally, water is replaced weekly with 10µm filtered Lake Pepin River water. ST's and PF's are 9L or 3L tanks, with aeration and bottom sediment for juveniles to borrow. ST's contain about 50 mL of sieved (250µm) river sediment. River sediment was collected at the headspring of Gorman Creek in Wabasha County, MN. St. Peter Sandstone was collected and sieved (200µm) for use in PF's. ST's are a static system, while PF's are refreshed with at least 70% of river water every 2-hours. Additional commercial food (Reed Mariculture Shellfish Diet 1800, TW 1200, and Nanno 3600) is added to each type of system. Approximately 250 ml of concentrated commercial algae is delivered to PF system with a Stenner peristaltic pump (Model 45M5) during water refreshment. While individual peristaltic pumps are used to feed a dilute concentration of commercial algae to mucket buckets. Moreover, ST's received 2ml of concentrated of commercial algae manually each day.

An unforeseen water quality issue occurred in August, when Lake City Public Utilities added chlorine to public water lines as part of an annual cleaning. During the treatment period, river water was collected at the outflow site near Roschen Park and Landing. It is suspected that the increased chlorine levels resulted in high mortality rates of juvenile mussels. A decline in juvenile health was noted on 15 August 2016 for all juveniles, by 12 September 2016, most species experienced 100% mortality. The surviving juveniles were the eldest, and most were greater than 0.5mm. The surviving juveniles were propagated with other funding sources; juveniles include: mucket (St. Croix River), snuffbox (*Epioblasma triquetra*, St. Croix River), and black sandshell (*Liguma recta*, Cedar River). However, juvenile culture was transitioned into ENRTF funding after July 1, 2016.

Juvenile Black Sandshell were collected from their host fish 1 July – 13 July 2016; juveniles were placed into mucket buckets (25%) and PF's (75%). After two months in culture, juvenile survival was 0.0% in mucket buckets, and 18% in PF's. Mortality in mucket buckets remains unknown, majority of the mortality occurred within the 14 days of collection. Mortality in PF's was attributed to overcrowding, however, high numbers were placed into each unit due to space limitations. It was concluded a maximum of 3,000 juveniles can be added to a 9L PF tank.

In early October, 9,956 Black Sandshell (2 – 7 mm) were moved into propagation cages at East Side Lake, in Austin, MN. Juveniles were divided into five propagation cages. The cages will be remain in East Side Lake until next fall, when they will be retrieved and mussels released into the Cedar River. An additional, 3,616 juveniles will remain in the laboratory overwinter. As of 21 November 2016, laboratory Black Sandshell have an average size of 5.4mm, and range from 1.1 – 8.9 mm.

Juvenile mussels were not transferred to MN Zoo during the propagation season due to renovations to the mussel program at the MN Zoo.

**Barnhart, M. C. 2006. Buckets of muckets: a compact system for rearing juvenile freshwater mussels. Aquaculture 254:227-233.

Activity Status as of May 1, 2017

ACTIVITY 2
Description: Rearing juvenile mussels

Several species of juvenile mussels overwintered in the laboratory. Most notably, black sandshell (*Liguma recta*) from the Cedar River. Overall survival from Oct – May exceeded 82%, with an average size 7.6mm, and range from 2.5 – 16.4mm. The average growth rate was slow, 0.3mm/month. The slow growth rate is attributed to cooler lab temperatures and less available food in the collected river water. River water was collected once or twice a month; collection of river water was dependent on daily temperatures and ice thickness (Figure 3). On 8 May 2017, all Black Sandshell juveniles were placed into propagation cages in East Side Lake in Austin, MN. Two cage types were used; metal ½" mesh design, and plastic totes with around 40 - ½" holes drilled for water circulation. (Figure 4).

MN DNR and U.S. Fish and Wildlife Service collectively propagated Winged Mapleleaf mussels (*Quadrula fragosa*) from the St. Croix River. In total, we collected 6,723 juveniles and FWS provided an additional 12,000 juveniles to be raised at our facility. Juveniles were placed into sediment containers and pulse flow, and were counted weekly. WML in the pulse flow system experienced 100% mortality within one month. Sediment containers experienced a steady decrease in survival, then 75-days post excystment, juvenile mussel survival plummeted (Figure 5). In total, nine WML juveniles survived, two juveniles provided by FWS and seven propagated from DNR (Figure 6). FWS also made an effort to raise juveniles WML, and although they started with many more juveniles, they experienced similar results with 100% mortality within 45 days from excysting from channel catfish.

The small propagation effort of Mucket (*A. ligamentina*) in January allowed for investigation of different culture systems. Juveniles were placed into sediment and pulse flow tanks. Sediment tanks were noted to have a much higher survival rate compared to its counterpart. In addition, sediment tank Muckets were 1.5x the size compared to the pulse flow, 3.4mm and 2.2mm respectively. Due to this result, we plan to utilize sediment tanks more than pulse flow for the upcoming propagation efforts. Lastly, all 2017 muckets (count 2,137) were also combined and placed into a floating tote at the Lake Pepin Marina, Lake City MN for additional summer growth.

Activity Status as of December 1, 2017

ACTIVITY 2
Description: Rearing juvenile mussels

The 2017 propagation season more than doubled the 2016 propagation totals, making it the largest production effort thus far. Juvenile metamorphoses yielded just under 320,000 individuals. Three species; *L. higginsii*, *L. recta*, and *Q. metanevera* experienced low metamorphosis and no juveniles collected in the laboratory; species accounts are not reported below. The following is an overview of the 2017 propagation effort:

*Actinonaias ligamentina (*ALIG, Mucket)
Cedar River

Gravid females were collected at Idlewild State Park, Floyd, IA on 17 May 2017; average size 116mm. A total of 76,125 juveniles were collected from three batches of fish. The batches were spread out from early May to June. All juveniles recovered were placed into sediment tanks (ST's). Overall juvenile survival 60 days post metamorphoses was highest in the earliest batch; 33 – 84%, average of 58%. ALIG juveniles propagated after 15 June 2017, had a lower rate of survival 60 days post metamorphoses; 0 – 33% survival, average of 6% (excluding one ST with 100% survival). Difference in survival was thought to be a result of old glochidia, warmer water temperatures, and increased competition due to an influx of zooplankton into tanks around August. In addition to laboratory culture, three totes and one wire cages containing inoculated LMB were placed into East Side Lake, Austin, MN (Figure 4, May 01, 2017 status update).

In October, four totes containing 12,544 juveniles were placed into East Side Lake for grow-out. 1,590 juveniles (Figure 7, average size 3.7mm) will be held at CAMP throughout the winter.

St. Croix River

Gravid females were collected at Interstate Park, St. Croix Falls, WI on 16 May 2017; average size 92.8mm. A total of 124,540 juveniles were collected from one inoculation. All juveniles recovered were placed into ST's. Juvenile survival 30 – 60 days post metamorphoses ranged from 0 – 78%; three tanks experienced 0% survival, five tanks ranged from 1 – 8% survival, and the remaining eight tanks survival ranged from 19 – 78%. Due to space limitations, ST's contained greater than 8,000 juveniles, exceeding desired maximum per tank. Juveniles were reared in the laboratory until the end of October. Totes were placed into Lake Frontenac and the St. Croix River at Lakeland, MN: five totes, containing 16,538 juveniles and four totes, containing 10,483 juveniles, respectively (average size 1.09mm). Totes in Lake Frontenac will be checked in the spring for survival. St. Croix River totes will be removed in the fall 2018, and pending size, will be prepped for release.

Epioblasma triquetra (Snuffbox, ETRI)
St. Croix River

In 2017, two methods of propagation were used; caged and captive culture. A total of 94 logperch were inoculated; 60 fishes were placed into two plastic totes, and one wire cage in the St. Croix River at Lakeland, MN. The remaining fishes yielded a total of 3,255 ETRI juveniles. Captive culture juveniles were placed into ST's and into a floating tote in Lake Pepin, Lake City, MN. The rate of survival 90-days post metamorphoses in ST's was 73% and 84% (average size 2.9mm). In late October, 1,237 ETRI juveniles were placed into one tote in the St. Croix River at Lakeland, MN. As of 11 December 17, 450 ETRI juveniles (average size 3.0mm) remain in the lab. There were no juveniles recovered from the floating tote in Lake Pepin.

Quadrula fragosa (Winged Mapleleaf, WML)
St. Croix River

Glochidia were manually extracted from six mussels for the propagation of WML in 2017 (Figure 8). Fishes were inoculated and placed into the RPS unit for juvenile collection. An additional 107 fishes were placed directly into ten totes in the St. Croix River at Lakeland, MN for the winter. Channel catfish will be released in the spring from totes, totes will remain in St. Croix River for at least two growing seasons. Juveniles collected in the laboratory totaled 114,956. One week post metamorphosis, greater than 50,000 juveniles (average size 0.35 mm) were placed into totes in the St. Croix River at Lakeland, MN. Within 6 weeks of metamorphosis the remaining laboratory WML, experienced 100% mortality. In January 2018, US FWS will provide us with several thousand newly metamorphosed juveniles with hopes of collaboratively testing different methodologies for survival.

Juvenile mussels were not transferred to MN Zoo during the propagation season due to unfinished renovations to the mussel program at the MN Zoo.

Activity Status as of May 1, 2018

ACTIVITY 2
Description: Rearing juvenile mussels

Three species have overwintered in the laboratory: *Actinonaias ligamentina* (Mucket, Cedar River), *Cumberlandia monodonta* (Spectaclecase, St. Croix River), and *Epioblasma triquetra* (Snuffbox, St. Croix River).

As part of a SWG project, our laboratory determined the host fish for the elusive Spectaclecase. This provided us with a unique opportunity to be the first lab in the world to rear these federally endangered juvenile mussels. Over 300,000 juveniles were recovered during the host trial; however, the majority did not survive. There was no indication why large die offs occurred, however, most mortality occurred a few months post metamorphoses. The containers that survived greater than two months are prospering.

We tracked one container since its origin. Similar to nearby containers, 45% mortality occurred around two months of age. At this point, the surviving juveniles were split into multiple containers. To date, this container has increased in size by great than 10X. Juveniles collected post metamorphoses ranged from 0.25 – 0.4mm; after two months in culture the juveniles were almost 1.0mm; currently, juveniles are an average size of 4.0mm (Figure 12). Since September, this container's survival rate was greater than 32% (Figure 13). The slow decline in survival has remained constant since the original collection date. Juvenile survival is another benchmark for this species and our facility. Currently, the U.S. Fish and Wildlife Service is working on a propagation and reintroduction plan for Spectaclecase.

Description: Pilot Study: Growth and survival of newly metamorphosed juveniles in different substrate types

The objective of the pilot study was to determine the best substrate type for rearing newly metamorphosed juveniles to a size at which survival in a tote in the river or a basket at the zoo is likely.

In the winter of 2017, *Actinonaias ligamentina* (Mucket) from a host trial were placed into two-types of rearing tanks; pulse-flow sand and static sediment. The static sediment container had a much better rate of survival and growth compared to the pulse-flow sand (See May 2017 update). To expand on this knowledge, another pilot study using plastic sandwich boxes examined static silt-sediment and static sand sediment boxes.

Newly excysted juveniles were collected and randomly assigned to boxes; five boxes per substrate type. Sediment types included: sterile silica sand (<250µm), live silt (<250µm, collected from Gorman Creek headspring), and no substrate. Each box contained 500mL of filter river water. Sand and silt-sediment boxes contained 12mL of substrate type, each sieved to <250µm. Boxes received up to 10mL of commercial food mixture daily (Reed Mariculture Shellfish Diet and Nanno 1800). Experimental setup can be seen on Figure 14.

The survival rate over six weeks was highest in the sand boxes (Figure 15). Sand boxes had an overall survival of 43%; however, two boxes yielded 90% and 61% survival. Silt sediment and no substrate resulted in 18.5% and 1.3% survival respectively. The growth rate was highest in the sand boxes (0.82mm or 278%) compared to silt sediment (0.40mm or 86%) and no substrate boxes (0.62mm or 185%) (Figure 16 and 17). The poor survival and lack of growth of the silt sediment mussels was not expected. Previous experience with 9L- aerated silt sediment boxes had higher growth rates compared with the pilot study. Disturbance or attachment of Vorticella, a protozoan that has stalks to attach themselves to substrate or to the shell of mussels, may have negatively affected the growth and survival on silt-sediment boxes.

Additional studies to determine the ideal algal mixture and feeding amount for optimum growth of Minnesota mussels is needed in the future. Primary food sources for native mussels may change within the first two weeks to two months post metamorphosis. When a juvenile drops off a fish, it may begin pedal feeding immediately. The transition from pedal feeding to filter feeding is a critical period in juvenile development, and can directly affect the success of a captive culture mussel facility.

Lastly, the surviving juveniles will be used to determine if the Minnesota Zoo's A Lake can effectively support mussels. A two-sample study will be conducted between A Lake and Lake Pepin. Groups of juveniles will be placed into floating baskets with substrate in each system. The baskets will be undisturbed for 60 days, upon which, each basket will be examined for survival and growth.

Activity Status as of December 1, 2018
ACTIVITY 2
Description: Rearing juvenile mussels

As our techniques are developing, our ability to successfully inoculate host fishes and recover juvenile mussels have improved. This season is a tribute to our previous accomplishments, as well as, a testament to the potential of our program. This year yielded over 780,000 juveniles from 10 species in three watersheds. Additionally, juvenile mussel survival for almost all species has improved; the following summarizes the 2018 propagation effort in each watershed.

Cannon River

The Mucket was the only species propagated from the Cannon River watershed. All Mucket from laboratory culture were placed into six sediment tanks, each tank containing ~1,500 juveniles. The overall survival of the Muckets after 160 days in culture was nearly 80%, and had an average growth rate of 28µm a day (Figure 18).

Cedar River

The Cedar River had the most number of species attempted for propagation. Six species were used for propagation related activities: Mucket, Threeridge, Wasbash Pigtoe, Fluted Shell, Black Sandshell, and Monkeyface. The Wabash Pigtoe and Threeridge were a result of host suitability trials. These two species have never been raised in culture; roughly, 1,400 Threeridge mussels remain in culture today. Moreover, three inoculations were performed for the production of Fluted Shell. Unfortunately, fish health declined rapidly in all batches resulting in only a few hundred juveniles collected. All Fluted Shell juveniles experienced 100% mortality within 60 days of metamorphosis. Another species, the Mucket, did not fair well in the laboratory this summer. Due to space limitations and host fish availability, brooding Muckets from the Cedar River were held for nearly 7 months prior to inoculation. We believe this resulted in poor juvenile health; most juvenile Muckets did not survive 90 days. Monkeyface is another species of great difficulty. Almost 200 Spotfin Shiners were collected and used for Monkeyface propagation, however, only 685 juveniles were recovered. The low transformation rate has left us perplexed, next summer we plan to use multiple species of host fishes on Monkeyface.

Black Sandshell showed greater than 50% survival after three months in culture and have an average growth rate of 29µm a day (Figure 19). Due to the high rate of survival, Black Sandshell juveniles have been placed in multiple different culture systems. Currently, we are testing the survival of Black Sandshell in vertical Heath tray incubation systems that were donated by a fisheries program within the DNR (Figure 20). Heath trays have been used for the production of Tar River Spinymussels in North Carolina. Each tray can hold about 1,500 juvenile mussels. In addition, large recirculating troughs with sand and sediment containers are being used to mimic a river (Figure 21). Similarly, each container can hold between 1,500 – 3,000 juveniles.

Mississippi River

Species propagated from the Mississippi River accounted for greater than 83% of total. However, the highest yielding mussel species, Spectaclecase, did not survive well in culture. Less than .01% remain alive in culture as of December 15, 2018. This is believed to be a result of overcrowding due to the lack of adequate space in the laboratory at the time. We have since expanded our capacity for housing transformed mussels. In comparison, Higgin's Eye and Mucket juveniles have a survival rate after 90 days

in culture of 31% and 42% respectively. This is the first successful laboratory based propagation effort for Higgin's Eye; in the previous year, no juveniles survived in the lab. The majority of Higgin's Eye (size 2.1 – 2.4mm) were moved into sand and sediment containers in a recirculating trough (Figure 21). Moreover, another milestone for CAMP is the survival of thousands of Winged Mapleleaf. Winged Mapleleaf juveniles are currently thriving in culture: in the previous year the majority of juveniles experience high rates of mortality within 30 days of metamorphosis.

Description: Growth examination of Muckets in A-Lake

To examine the growth potential of propagated mussels in A Lake at the MN Zoo, both agencies completed a small-scale pilot study. The study compared growth and survival of 1,200 Muckets in A Lake and Lake Pepin (control). Six-hundred mussels were placed into floating baskets and left undisturbed for 60 days, and then examined for growth and survival. Lake Pepin and A Lake had similar outcomes. Therefore, A Lake has adequate food and water quality for juvenile freshwater mussels, and the plans to build a permanent mussel facility may commence.

Project Status as of June 30, 2019

ACTIVITY 2

Description: Rearing juvenile mussels

Winter brought new challenges, as record snowfall prevented us from collected the quantity of river water needed to support the juvenile mussels at our facility. Juvenile mussels were divided into four recirculating troughs, which mimics water flow in a natural system. Also, one experimental heath tray was tested with Black Sandshell. Systems were fed commercial algae to support healthy growth, however, two unexpected high mortality events occurred in February and again in March. The die-offs occurred in two raceway systems. One was attributed to the change in water temperature for the bacteria in the bio-filter, likely killing the bacteria and therefore leading to increased ammonia. The second die-off occurred when water was scare, and the system had not been cleaned thoroughly in 5-weeks. The system experiencing the high mortality was the most populated raceway system.

Cannon River:

Total survival (310 days) in the laboratory was 35%, with an average growth rate of 28µm per day. In May 2019, three totes containing 1,930 juvenile Mucket were placed in Lake Byllesby for continued growth. We anticipate a release in fall 2020 (Figure 24).

Cedar River:

Black Sandshell were divided into three troughs, as well as a heath tray system. One trough, unfortunately, experienced a high rate of mortality during March; decreasing the number of live Black Sandshell by greater than 75%, resulting in 8,783 Black Sandshell live in April 2019 (average length 7.2mm). At the end of April, the Minnesota Zoo obtained 2,000 Black Sandshell to be raised at their facility. The remaining Black Sandshell were split between totes to East Side Lake (~3,000 juveniles) and Waterville Fish Hatchery in Waterville, MN. In conjunction with the hatchery, juvenile mussels were placed in one of the ponds for continued growth (Figure 25). Success is to be determine in fall 2019.

Mississippi River

Higgin's Eye, the second most abundant juvenile mussel in the laboratory in October 2018 experience a large die-off in late February. In March, only 15% of the Higgin's Eye remained in culture, with dwindling numbers as the summer approached. Higgin's eye were placed into totes at Waterville Fish Hatchery and St. Croix River near Lakeland, 500 and 681, respectively. Additionally, Mucket and Snuffbox had a higher

rate of survival in comparison to other species, 68% and 54% survival from January to May. These juveniles were placed at Waterville Fish Hatchery, as well as, placed into totes in the St. Croix River.

Final Report Summary:
ACTIVITY 2
Description: Rearing juvenile mussels

Improvements to our propagation efforts have been evident for three years. When the CAMP facility opened in 2014, their goal was to create a juvenile mussel propagation facility to restore native mussels across Minnesota. In 2016, the vision started to take hold as the first juvenile mussels were collected from fish. However, it takes more than mussels and fish for a successful program. Many ideas, efforts, and knowledge have changed and grown throughout the years. Reminiscing to the first mussels were placed into juvenile culture systems, and knowing that we have continuously refined our techniques as time progresses. A system that was in use originally, often has been replaced as new methods are developed. Additionally, every species has a different culture requirement (flow, substrate, food, etc.), such that, one system may not work for another. The LCCMR grant provided CAMP biologists with the expertise and fluidity to expand our program to meet the needs of several species of native freshwater mussels.

Juvenile mussel culture of animals greater than 2mm have consistently moved into secondary culture systems. These culture systems include: benthic metal cages, benthic plastic totes, floating plastic totes, and a flow-through pan system at the MN Zoo. In the fall of 2016 and 2017, a large effort was conducted to move mussels greater than 2mm to a natural system to overwinter. Between the three watersheds, over 55,000 juveniles were placed into benthic metal cages and plastic totes. Upon removal of the secondary culture system in the fall of 2018 and 2019, only 760 juveniles has survived. In comparison, juvenile mussels placed into secondary culture systems in the spring (May/June) of 2017 and 2018 (approximately 4,200 juveniles), had a recovery percentage of 46%, 1,961 juveniles recovered. Due to poor results of juvenile placement in the fall, all juvenile mussels have overwintered in our facility since fall 2018.

Tracking one cohort of Black Sandshell:

> Juvenile culture systems include mucket buckets, static sediment tanks, and pulse flow units. The first operating season, 2016, resulted in high mortality due to an unforeseen water quality concern. However, new protocols were installed to prevent from reoccurring in the future. Only 18% of Black Sandshell collected as newly metamorphosed juveniles were raised beyond 6 months; at this time, greater than 6,000 were placed into East Side Lake, while 3,600 overwintered at CAMP. Overwintering proved to beneficial, 83% overall survival rate from November 2016 to May 2017; comparatively, juveniles placed into East Side Lake in the fall barely exceeded 0.01% survival. Furthermore, after one year of captive culture 3,000 Black Sandshell, the remaining laboratory juveniles were placed into metal cages or totes in East Side Lake. Overall metal cage survival was 12%, while plastic totes was 75% survival. Although the overall survival from metamorphosis was 0.02%, several factors of experimentation occurred to determine how to be successful in the future.

Lastly, during the summer of 2019, various secondary culture methods were tested again. We continued to place benthic plastic totes according to the juvenile watershed. In addition, we placed floating totes at Waterville Fish Hatchery (MNDNR, 50317 Fish Hatchery Rd, Waterville, MN 56096). Waterville Fish Hatchery provided a unique opportunity to culture mussels in a protected hatchery pond. A pond was fashioned with a small hand-built aerator to provide was circulation through the totes. The juveniles were placed in June of 2019, results are pending until fall 2019.

For detailed information of secondary culture methods, see the appendices A-C.

ACTIVITY 3: Releasing and monitoring juvenile mussels

Description:

Juvenile mussels 30-40 mm or larger will be gathered at the MN Zoo or from growing cages, counted, measured for length, and released at reintroduction sites in rivers. These mussels will be permanently marked to identify them as captive raised individuals and monitored annually to determine survival and growth.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 80,706
Amount Spent: $ 80,706
Balance: $ 0

Outcome	Completion Date
1. Number of juvenile mussels of each species that survive and grow to 2-3 mm in length	Varies by species 100-400 days
2. Number of juvenile mussels of each species transferred to MN Zoo	30 days from transfer
3. Number of mussels of each species raised at the zoo and released into rivers.	July 1, 2018

Activity Status as of December 1, 2016

ACTIVITY 3
Description: Releasing and monitoring juvenile mussels.

No sub-adult or adult mussels were released into reintroduction sites. Plans to release marked mussels will occur in fall 2017.

Activity Status as of May 1, 2017

ACTIVITY 3
Description: Releasing and monitoring juvenile mussels.

No sub-adult or adult mussels were released into reintroduction sites. Plans to release marked mussels will occur in fall 2017.

Activity Status as of December 1, 2017

ACTIVITY 3
Description: Releasing and monitoring juvenile mussels.

Two stream-side inoculations and immediate release of host fish were completed in 2017. An estimated 300 lbs. of WAE (100+ fish), ages 1 – 3, were inoculated with LREC and released into the Cedar River south of Austin, MN. Another 300 lbs. of WAE were inoculated with Mucket glochidia and released into the Cannon River upstream of Dundas, MN (Figure 10). WAE were supplied by MN DNR Waterville Fish Hatchery, Waterville, MN.

No sub-adult or adult mussels were released into reintroduction sites in 2017. However, in late September, Mucket totes placed in early spring with inoculated LMB were checked for survival. Upon a quick examination, the totes seemed to be prosperous and had several 10mm+ juveniles. Therefore, totes placed spring 2017, are planned to be retrieved and prepped for release in late summer 2018.

Black Sandshell totes and cages from East Side Lake, Austin, MN were removed and examined. Five wire cages containing almost 10,000 juveniles were placed in October 2016, average size 2- 7mm. A sample of these were rinsed and counted this fall. A total of 97 Black Sandshell were recovered, average size 29.5mm (range 20.4 – 40.3mm). Also, one tote, which was placed in May, was examined; it was estimated that juvenile survival was greater than 50% (Figure 9). All totes and cages will be removed and examined in the summer of 2018, it is

presumed juveniles will be large enough to tag and, thus, release. Based on our observations of production this year, we hope to release over 1,000 Black Sandshell into the Cedar River next fall.

Activity Status as of May 1, 2018

ACTIVITY 3
Description: Releasing and monitoring juvenile mussels.

No sub-adult or adult mussels were released into reintroduction sites during the winter. Hall-print tags, unique identifying tags, were purchased to mark each mussel to be released in 2018. Release of marked mussels will occur in summer/fall 2018.

Activity Status as of December 1, 2018

ACTIVITY 3
Description: Releasing and monitoring juvenile mussels.

No releases took place this year due to high water events and unsafe conditions. Rather, totes in East Side Lake, Austin, MN were removed, checked, and reset. Moreover, totes in St. Croix River at Lakeland were examined via SCUBA diving in anticipation for the spring.

Cedar River Watershed; East Side Lake, Austin MN

In November, totes and metal cages were removed from East Side Lake and rinsed thoroughly to determine the survival of juvenile Black Sandshell and Muckets from 2016 and 2017 propagation years (Figure 22). For both species three different methods were tested:

1. Inoculated fishes were placed in totes,
2. Juvenile mussels were placed into totes in fall (October), and
3. Juvenile mussels were placed into totes in spring (May-June).

Survival only occurred in totes of juveniles that were placed in the spring. Mucket survival ranged from 38 – 68% after almost five months in East Side Lake. Moreover, Black Sandshell had nearly 50% survival since its placement into East Side Lake in May 2016.

To prepare for a release into the Cedar River, (prior to June 30, 2019) relocation sites for tagged mussels must be determined. With guidance from the Mower Soil and Water Conservation District, and small reconnaissance surveys, two relocation sites have been decided: one within Austin Township, the second south of Austin. The total number of relocations sites will be based on the number of mussels to be released.

Mississippi Watershed: St. Croix River, Lakeland MN.

Totes at Lakeland, MN are only accessible by SCUBA; 30 totes from the 2016 and 2017 propagation years were hand checked underwater. Similar to East Side Lake, totes were placed with inoculated fish, juveniles placed in fall, and juveniles placed in spring. Since totes were not removed, only presence or absence was noted. There was no detection of Winged Mapleleaf placed with host fish or as juveniles in the fall; however, Mucket totes with inoculated fish had presence of juveniles in two of three. Likewise, Snuffbox had presence of juveniles from inoculated fishes in one of four. There was an absence of all juvenile mussel species placed in the fall. The two totes with a spring placement had a positive detection of juveniles, including Spectaclecase.

Activity Status as of June 30, 2019

ACTIVITY 3
Description: Releasing and monitoring juvenile mussels.

The Minnesota Zoo obtained 2,000 juveniles Black Sandshell in May to place into their new facility. The Black Sandshell were held in quarantine for 30 days, before being moved into secondary culture systems. Black Sandshell were 5.8mm upon arrival at the Zoo, after 2 months, their average length was 15.9mm. This growth rate is result of a natural system, and indicates the Zoo has high potential for all secondary growth systems of freshwater mussels.

Also, no releases took place this spring due to high water events and unsafe conditions. [*July 18, 2019, after reporting time, a release of Black Sandshell occurred on the Cedar River; 1,500 mussels (average length 56 mm)*].
Final Report Summary:

The final step in propagation is the release of mussels that are large enough to prevent most fish predation, and almost sexually mature. Mussels released at this size have the higher chance of survival in a natural system. In addition, will likely start producing offspring within a few years. This final step, like the very first step of broodstock collection, is dependent on water conditions for an accurate release location. During floods, base flows are hard to determine and one site may be completely dewatered on normal flows. For this reason, a release of propagated mussels did not occurs until June 30, 2019. However, hundreds of mussels of a releasable size are being held in various culture systems.

For detailed information of secondary culture methods, see the appendices A-C.

V. DISSEMINATION:
Description:
Project progress will be disseminated via annual or semiannual MNDNR news releases and at least one full article in the MN Conservation Volunteer. An updated "Mussels of Minnesota" poster showing the present status of all mussel species on the front with QR codes linking smart phones to more information on the MN DNR website such as life history descriptions and current efforts funded by this grant. Posters will be provided free of charge to the public. Annual updates of our progress will be presented as either PowerPoint or poster presentations at annual mussel conservation meetings, workshops and symposia. An updated MNDNR webpage will feature this proposal and present progress reports annually.

Status as of: May 1, 2017

We are in the process of photographing native mussels to be used on the new "Mussels of Minnesota" poster.

Status as of: May 1, 2018

New photographs of all native and invasive species will be show cased on the updated mussel poster. We are working with the creative services on the design. Currently, a mock design is in place. Final poster and printing plans should follow in the coming months.

Status as of: December 1, 2018

Mussels of Minnesota Poster: All photographs and artistry is complete; Figure 23 is a draft version of the poster. It was recently determined that Slough Sandshell or Yellow Sandshell were two separate species. In order to have the

most up-to-date species naming information on the mussel poster, we are awaiting the final publication prior to sending the poster to print.

The MN Conservation Volunteer began an article on CAMP this November. They accompanied us while checking Black Sandshell totes in East Side Lake, Austin MN (Figure 22). This is the beginning stages of the article; a fall 2019 publication date is expected.

Status as of: June 30, 2019

CAMP staff attended the semi-annual Freshwater Mollusk Conservation Society meeting in San Antonio, Texas in April 2019. Several new propagation ideas were discussed; the number of propagation facilities continues to increase in the US. Moreover, Madeline Pletta was nominated as Co-Chair of the Propagation Committee of the society. Abstracts and information about the conference can be found at: https://molluskconservation.org/Events.html

Our lab was featured in the Minnesota Conservation Volunteer (September – October 2019). The article was an informative spread, titled "Mussel Building; a team of DNR scientists is bringing back beautiful, beneficial native mussels to Minnesota Rivers."
https://www.dnr.state.mn.us/mcvmagazine/issues/2019/sep-oct/mussels.html

The Mussels of Minnesota Poster was printed and distributed around the state in June. https://www.dnr.state.mn.us/mussels/index.html

Final Report Summary:

Joel Sartore (National Geographic Photographer and Speaker, and founder of Photo Ark) made his second visit to CAMP to photographer several species of native fishes, and juvenile mussels. See all the species he has photographed at: https://www.joelsartore.com/keyword/minnesota-department-of-natural-resources-center-for-aquatic-mollusk-programs/

Additionally, a semiannual newsletter continues to be sent to subscribers; subscribe to our Mussel Newsletter at: http://tinyurl.com/gd-mussels. And mussel staff continue to provide severl tours, presentations, and middle-school guest education speaker. Most notably in Austin, MN at the IJ Holton Middle School, and Hormel Nature Center.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:		
	$543,941	Project manager, .75 FTE yr 1, 2 - $144,348; Malacologist, .75 FTE yr1, 2 - $116,500; Aquarist/Malacologist, .75 FTE yr 1, 2 – $77,502; Database Manager/Malacologist, .75 FTE yr1, 2 - $79,869; Mussel Culture Biologist, 1 FTE yr 1, 2 - $125,722.
Printing:	~~$8,170~~ $3,049	Updating and reprinting Mussels of MN poster
Travel Expenses in MN:	~~$1,108~~ $6,229	Travel to collect gravid female mussels, transporting juveniles to MN Zoo, hauling river water for mussel food, travel to mussel release sites to monitor mussels.
		People support – $11,640

		Safety support - $2,744
		Financial support - $7,745
		Communication support - $1,236
		IT support - $22,352
		Planning support - $829
		Procurement support - $235
Total Direct and Necessary cost	$46,781	
TOTAL ENRTF BUDGET:	**$600,000**	

Explanation of Use of Classified Staff: Two .75 FTE classified staff positions will be supported, these positions do not have a permanent dedicated funding base and so the MN DNR cannot backfill the ENRTF portion of their salaries. Classified staff manage this program but they may not be retained to work on mussels without the support of this ENTRF grant. Retaining both of these positions is essential for implementing this project.

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

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 8.0

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

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
State Wildlife Grant, cash, $40,000/year	$80,000	$40,000	Facility operations, rent ($40,000/year)
US Army Corps of Engineers IPAs, cash reimbursement	$70,000	$70,0000	Staff Time - monitoring data analysis, reports
State			
Mussel Mitigation, cash	$20,000	$20,000	Staff time and expenses
TOTAL OTHER FUNDS:	**$170,000**	**$170,000**	

VII. PROJECT STRATEGY:

A. Project Partners: Dan Kelner, U. S. Army Corps of Engineers, funding and planning partner in support of monitoring reintroduction sites of federally listed mussel species; U. S. Fish and Wildlife Service, Tamara Smith, planning and permitting support and Nathan Eckert Genoa National Fish Hatchery, host fish supply and some juvenile mussels; National Park Service, Byron Karns and Alison Holdhusen, planning and staff support for monitoring in the St. Croix and Mississippi Rivers; Iowa DNR, Kelly Poole, Jen Kirth and Scot Gritters, permitting and logistical support for acquiring donor female mussels; MN Dept of Transportation, Jason Alcot, mitigation funds per project requirements; MN Zoo, Tara Harris, Alan Maguire, Ben Minerich, and Matt McLaughlin, providing facility and staff time for mussel rearing and public information.

B. Project Impact and Long-term Strategy: 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 LCMR grant began mussel surveys of the state's rivers. Surveys continued with other funds resulting in data from over 3,200 sites covering all 81 major watersheds in the state. This information is used to identify streams with potential for reintroduction. Our research has established host fish relationships needed to propagate most of Minnesota's mussel species. This proposal builds on these investments.

Reestablishing the water cleansing, and nutrient processing capacity that mussel populations provide will restore the biotic communities that mussels support and that fish and wildlife depend on while helping delist species in trouble. Expertise in accomplishing mussel restoration began in 2000 with the USFWS Jeopardy Decision for *Lampsilis higginsii* (Higgin's eye pearly mussel). This decision held that the US Army Corps of Engineers was responsible for the jeopardy by virtue of operating the navigation system on the Mississippi River that transported zebra mussels from the Illinois River upstream as far as St Paul, MN. The first propagated Higgin's eye were produced by the MNDNR in Lake Pepin in 2001, funding from the Corps expanded that effort and over the next 10 years over 40,000 propagated Higgins' eye were released at sites in the Mississippi River by a team of state and federal biologists and facilities. This project expands those efforts to other species and other river systems. As we develop additional expertise and capacity we hope to reintroduce mussels as water quality and aquatic food web engineers throughout Minnesota, eventually delisting rare species as well.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
US Army Corps of Engineers, propagation, release, monitoring of Higginsii and winged mapleleaf mussels	2010-2015	$30,000
State Wildlife Grant, surveys, host research, propagation and release of state and federally listed mussel species.	2010-2015	$150,000
MNDNR mussel mitigation fund, propagation, rent.	2012-2015	$100,000

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S):

X. RESEARCH ADDENDUM: N/A

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted no later than December 1, 2016, May 1, 2017, December 1, 2017, May 1, 2018 and December 1, 2018. A final report and associated products will be submitted between June 30 and August 15, 2019.

Activity Status as of December 1, 2016

Table 1. 2016 Broodstock collection information (Outcome 1).

Species	River	Number Collected	Date Collected
Actinonaias ligamentina	Cedar River	16	11-May
Lampsilis higginsii	Mississippi River	1	19-Jul
	St. Croix River	6	20-Apr
Quadrula fragosa	St. Croix River	1	16-Sep
		2	21-Sep
		2	23-Sep
Quadrula metanevra	Cedar River	13	5-Jul
	Total	**41**	

Table 2. Propagation Information for 2016 season (Outcome 2 & 3).

Species	Host Fish	River	Number of Juveniles Produced
Actinonaias ligamentina	LMB/SMB	Cedar River	62,907
Lampsilis higginsii	LMB/SMB	Mississippi River	4,383
		St. Croix River	11,587
Quadrula fragosa	Channel Catfish	St. Croix River	6,723
Quadrula metanevra	Spotfin/Common Shiner	Cedar River	242
		Total	**85,842**

Activity Status as of May 1, 2017

Table 3. 2017 Broodstock collection information (Outcome 1, *updated December 2017*)

River	Species	Number Collected	Date Collected
Cannon River	*Actinonaias ligamentina* - Mucket	2	10/18/2017
Cedar River	*Actinonaias ligamentina* - Mucket	15	5/17/207
	Ligumia recta – Black Sandshell	7	11/28/2017
		7	6/17/2017
		3	09/28/2017
	Quadrula metanevra - Monkeyface	8	7/5/2017
St. Croix River	*Actinonaias ligamentina* – Mucket	4	11/2/2016
		7	5/11/2017
	Epioblasma triquetra – Snuffbox	2	5/16/2017
	Lampsilis higginsii – Higgins' Eye	1	11/2/2016
	Quadrula fragosa – Winged Mapleleaf	6	10/5/2017
	Total	62	

Activity Status as of December 1, 2017

Table 4. Propagation Information for 2017 season (Outcome 2 & 3). **indicate tote and cage culture used.*

Species	Common Name	River	Number of Juveniles
Actinonaias ligamentina	Mucket	Cedar River	76,125*
		St. Croix	124,540
Epioblasma triquetra	Snuffbox	St. Croix	3,255*
Lampsilis higginsii	Higgins' Eye	Mississippi	416
Ligumia recta	Black Sandshell	Cedar River	*
Quadrula fragosa	Winged Mapleleaf	Cedar River	114,956*
Quadrula metanevera	Monkeyface	Cedar River	215
		Total	319,507

Activity Status as of May 1, 2018

Table 5. 2018 native mussel propagation plan

River	Species	# of Females	Host Fish	# of Host Fish	Propagation Plan		
					In-House	Cage-Culture	Free-Release
Cannon	*Actinonaias ligamentina*	5+	LMB/WAE	200+	50,000	50,000	Yes
	Alasidonta marginata	3+	Suckers	50	5,000		
Cedar River	*Actinonaias ligamentina*	7+	LMB/WAE	200+	75,000	50,000	Yes
	Alasidonta marginata	3+	Suckers	50	5,000		
	Lasigona costata	5+	Suckers	50	10,000		
	Ligumia recta	5-Feb	WAE	50	50,000	50,000	
	Quadrula metanevra	10+	Spotfin Shiner	50-200	5,000		
Mississippi River	*Actinonaias ligamentina*	6+	LMB/WAE	200	?	100,000	
	Cumberlandia monodonta		Goldeye	15	100,000		
	Epioblasma triquetra	6+	Logperch	250	10,000	5,000	
	Lampsilis higginsii	3+	LMB	200	25,000	100,000	
	Quadrula fragosa	AMAP	CCF	Pending	50,000	50,000	
					385,000	**405,000**	
					Total	**790,000**	

Activity Status as of December 1, 2018

Table 6. 2018 Broodstock collection information, not all mussels collected were used for propagation (Outcome 1, updated December 2018)

River	Species	Common Name	Number Collected	Date Collected
Cannon River	*Actinonaias ligamentina*	Mucket	1	4/12/2018
Cedar River	*Actinonaias ligamentina*	Mucket	7	11/28/2017
	Amblema plicata	Threeridge	12	8/2/2018
	Fusconaia flava	Pigtoe	2	8/2/2018
	Lasmigona costata	Fluted Shell	4	11/28/2018
	Ligumia recta	Black Sandshell	6	6/7/2018
	Quadrula metanevera	Monkeyface	8	7/31/2018
St. Croix	*Cumberlandia monodonta*	Spectaclecase	25	5/17/2018
	Epioblasma triquetra	Snuffbox	7	5/17/2018
	Lampsilis higginsii	Higgins' Eye	6	5/16/2018
			3	5/17/2018
	Quadrula fragosa	Winged Mapleleaf	1	8/28/2018
			1	9/20/2018
		Total	**83**	

Table 7. Propagation Information for 2018 season (Outcome 2 & 3). *indicate cage culture used in addition.

Species	Common Name	River	Number of Juveniles Produced
Actinonaias ligamentina	Mucket	Cannon River	6,620*
		Cedar River	16,853
		St. Croix	25,364
Amblema plicata	Threeridge	Cedar River	11,045
Cumberlandia monodonta	Spectaclecase	St. Croix	393,477
Epioblasma triquetra	Snuffbox	St. Croix	14,942*
Fusconaia flava	Pigtoe	Cedar River	812
Lampsilis higginsii	Higgins' Eye	Mississippi	53,479*
Lasmigona costata	Fluted Shell	Cedar River	494
Ligumia recta	Black Sandshell	Cedar River	78,925
Quadrula fragosa	Winged Mapleleaf	St. Croix	177,622
Quadrula metanevera	Monkeyface	Cedar River	685
		Total	**780,318**

Activity Status as of June 30, 2019

Table 8. 2019 native mussel propagation plan.

					Propagation Plan		
River	Species	# of Females	Host Fish	# of Host Fish	In-House	Cage-Culture	Free-Release
Cannon	*Actinonaias ligamentina*	5+	LMB/WAE	60	15,000	20,000	
	Alasidonta marginata	3+	Suckers	10+	5,000		
Cedar River	*Actinonaias ligamentina*	7+	LMB/WAE	80	25,000		Yes
	Alasidonta marginata	3+	Suckers	10+	5,000		
	Amblema plicata	5+	WAE	5+	20,000		
	Elliptio dilatata	5+	WAE	5+	20,000		
	Lasigona costata	3+	Suckers	10+	5,000		
	Ligumia recta	8+	WAE	20	25,000		
	Quadrula metanevra	6+	Spotfin Shiner	5-200	1,000		
Mississippi River	*Actinonaias ligamentina*	6+	LMB/WAE	50	15,000	20,000	
	Cumberlandia monodonta	3+	Goldeye	10+	50,000		
	Epioblasma triquetra	AMAP	Logperch	100	10,000	20,000	
	Lampsilis higginsii	6+	LMB	60	30,000	30,000	
	Quadrula fragosa	AMAP	CCF	Pending		50,000	Yes
					226,000	140,000	
					Total	366,000	

Table 9. 2016 – 2019 Propagation Totals

		Number of Juvenile Mussels Produced					
Species	Common Name	River	2016	2017	2018	2019	Total
Actinonaias ligamentina	Mucket	Cannon River			6,620*	26,712*	**33,332**
		Cedar River	62,907	76,125*	16,853	69,714	**225,599**
		St. Croix		124,540	25,364	61,408	**211,312**
Amblema plicata	Threeridge	Cedar River			11,045		
Alasmidonta marginata	Elktoe	Cedar River				136	**136**
Cumberlandia monodonta	Spectaclecase	St. Croix			393,477		**393,477**
Epioblasma triquetra	Snuffbox	St. Croix		3,255*	14,942*		**18,197**
Fusconaia flava	Pigtoe	Cedar River			812		**812**
Lampsilis higginsii	Higgins' Eye	Mississippi	15,970	416	53,479*		**69,865**
Lasmigona costata	Fluted Shell	Cedar River			494		**494**
Ligumia recta	Black Sandshell	Cedar River		*	78,925		**78,925**
Quadrula fragosa	Winged Mapleleaf	St. Croix	6,723	114,956*	177,622*		**299,301**
Quadrula metanevera	Monkeyface	Cedar River	242	215	685		**1,142**
			85,842	**319,507**	**780,318**	**157,970**	1,332,592

Figure 1. RPS Unit used for propagation. Each tank will typically hold 150 inoculated host fish. System was constructed March 2017 with assistance from the MN Zoo.

Figure 2. Laboratory refrigerator for mussel broodstock. Collected gravid females are held at 6ºC to prolong the period over which the mussels will retain their glochidia. System was constructed in June 2017.

Figure 3. Collection of Mississippi River water in January at Hok Si La boat ramp, Lake City, MN.

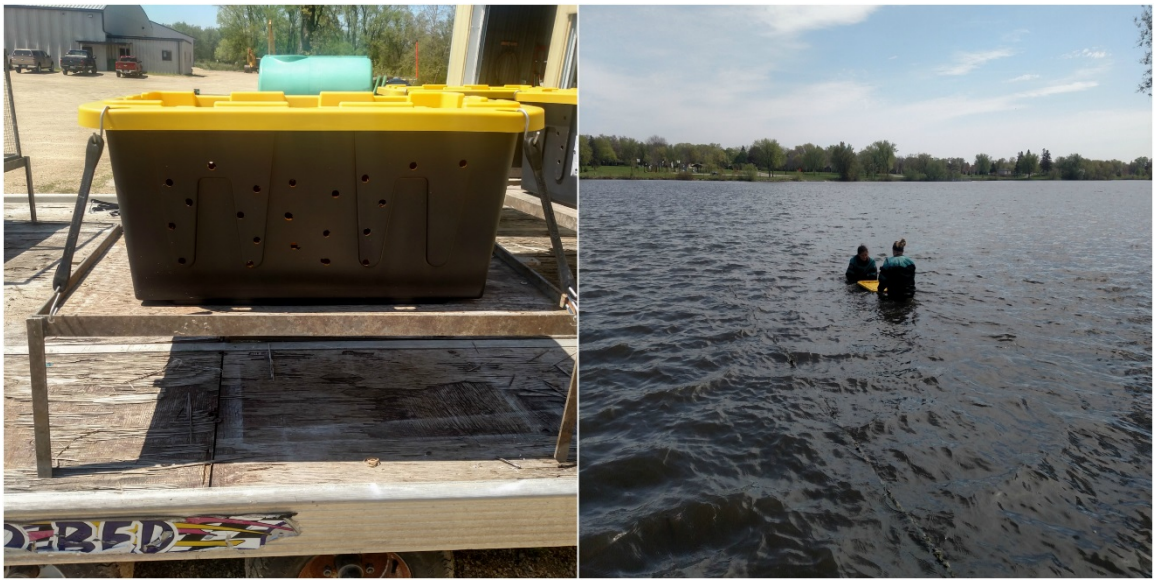


Figure 4. Left: Experimental propagation cage using plastic totes. Right: Divers carrying experimental cage into East Side Lake, Austin MN.

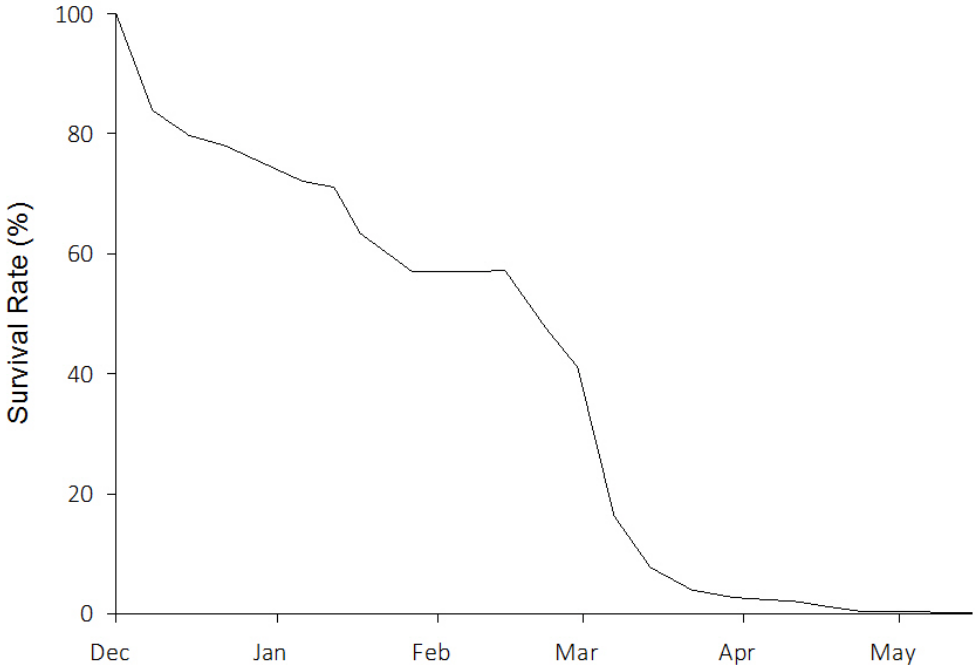


Figure 5. Survival rate of U.S. FWS Winged Mapleleaf juvenile mussels maintained at CAMP (December n=12,000, May n=2).

Figure 6. Seven Winged Mapleleaf juveniles propagated at CAMP from DNR propagation efforts.

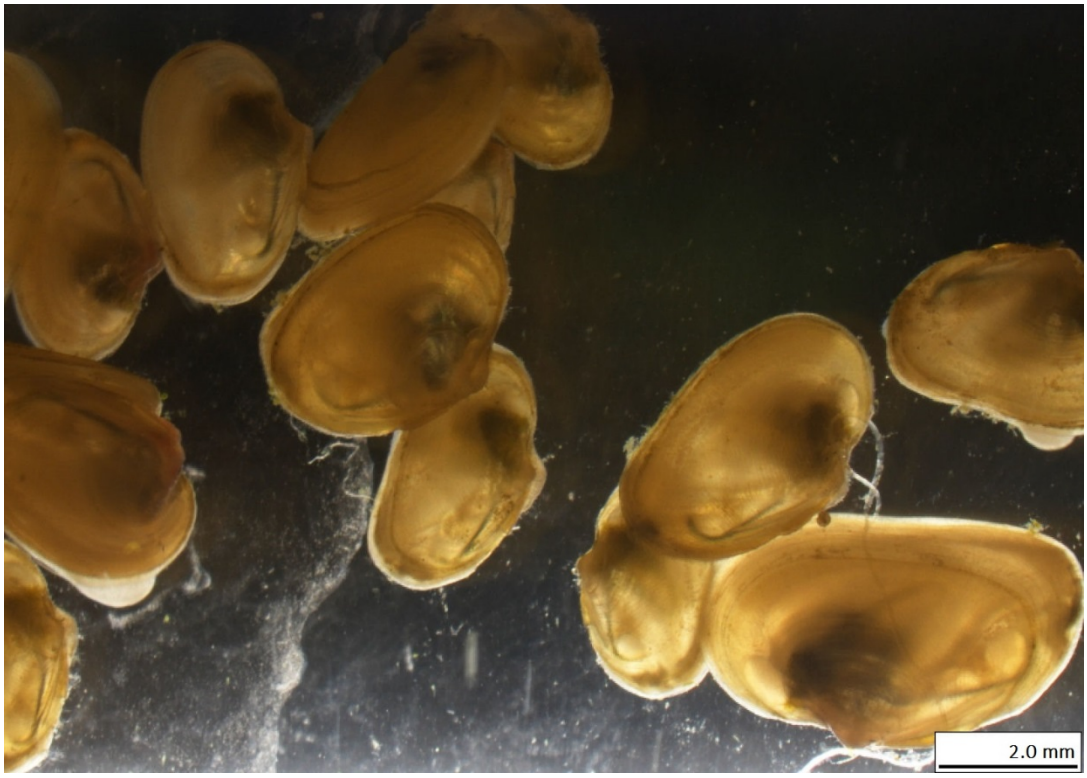


Figure 7. Mucket juveniles from the Cedar River, greater than 6 months old, average size 3.7mm.

Figure 8. Manual extraction of Winged Mapleleaf for propagation. Pasteur pipette was used to pull glochidia from the tower of each brooding mussel.

Figure 9. Black Sandshell juveniles recovered in October 2017, totes originally placed in May 2017. It was estimated that greater than 50% had survived inside the tote, and had more than doubled in size.

Figure 10. Stream side inoculation along the Cannon River. Mucket glochidia were placed into a water bath with 150 lbs. of WAE, age 1- 3 years. WAE were held in aerated coolers with the glochidia for ~10 minutes, then released into the Cannon River.

Figure 11. 30-foot flow through raceway, modified with compartments, used to hold host fishes such as Channel Catfish, Largemouth Bass, and Walleye.

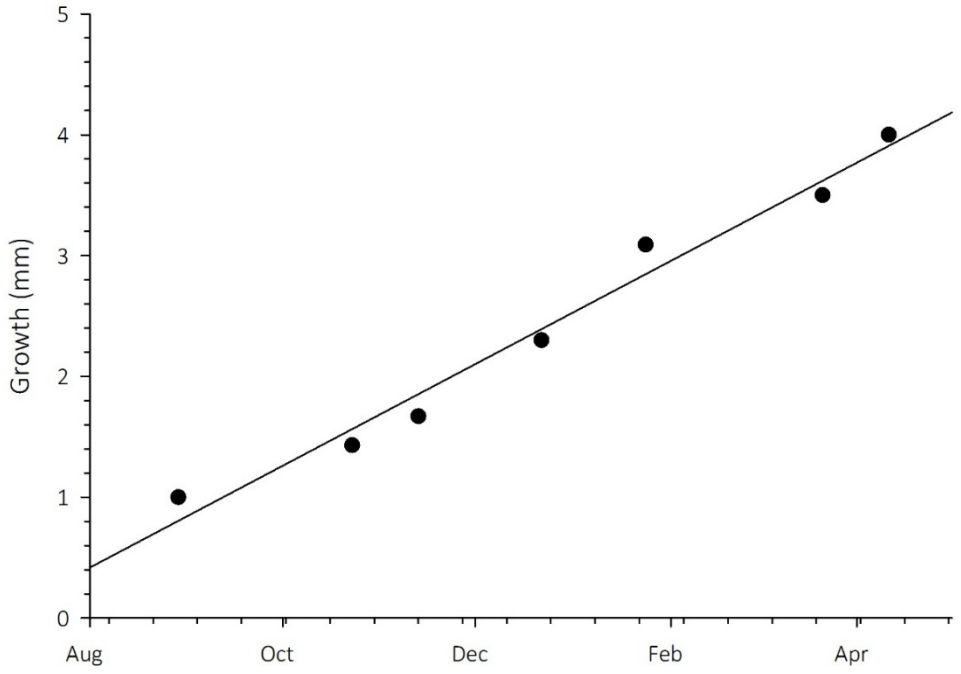


Figure 12. Growth of Spectaclecase (DOB: 26 June 17) in the laboraroty from September 2017 – April 2018.

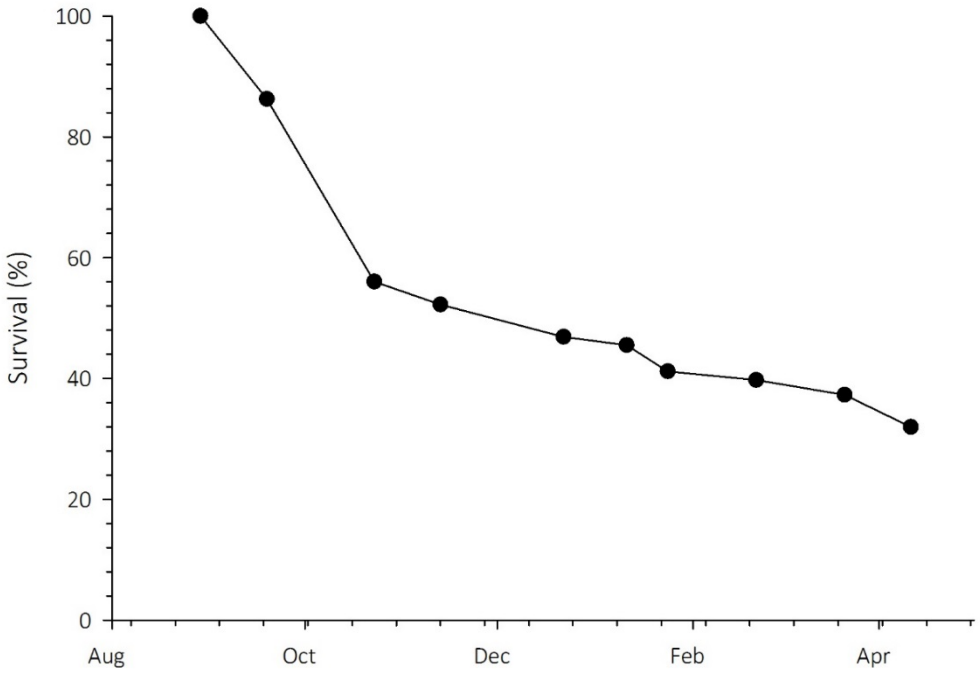


Figure 13. Survival of Spectaclecase (DOB: 26 June 17) in the laboraroty from September 2017 – April 2018. Survival was measured after initial die-off of juvenile mussels.

Figure 14. Pilot Study: Experiemental setup. Juveniles were randomly assigned to treatment group; 200 juveniles per replicate.

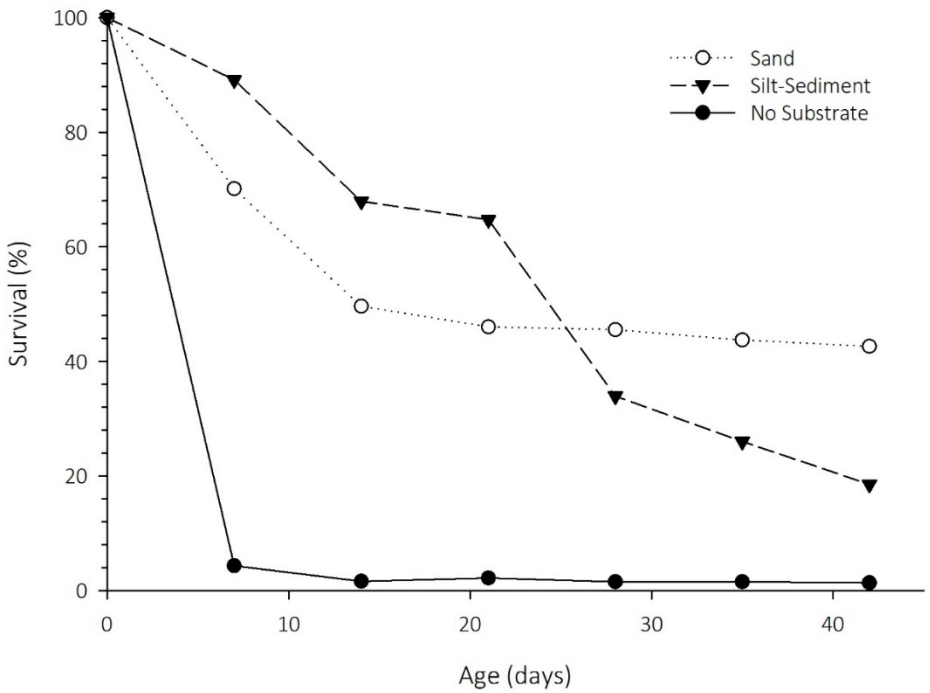


Figure 15. Pilot Study: Percent survival of newly metamorphosed juveniles in different substrate types.

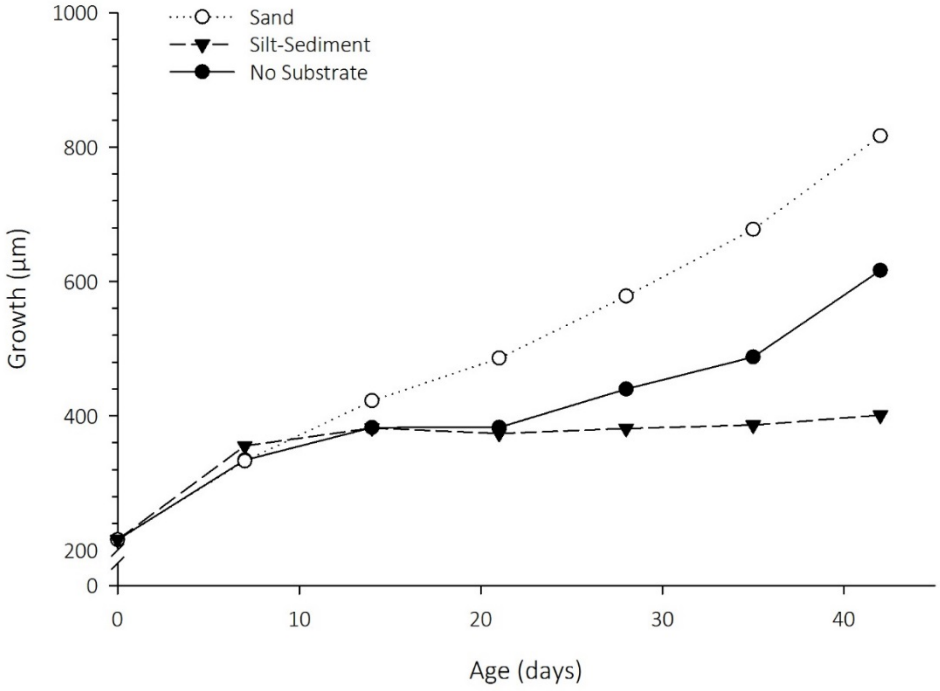


Figure 16. Pilot Study: Growth of newly metamorphosed juveniles in different substrate types.

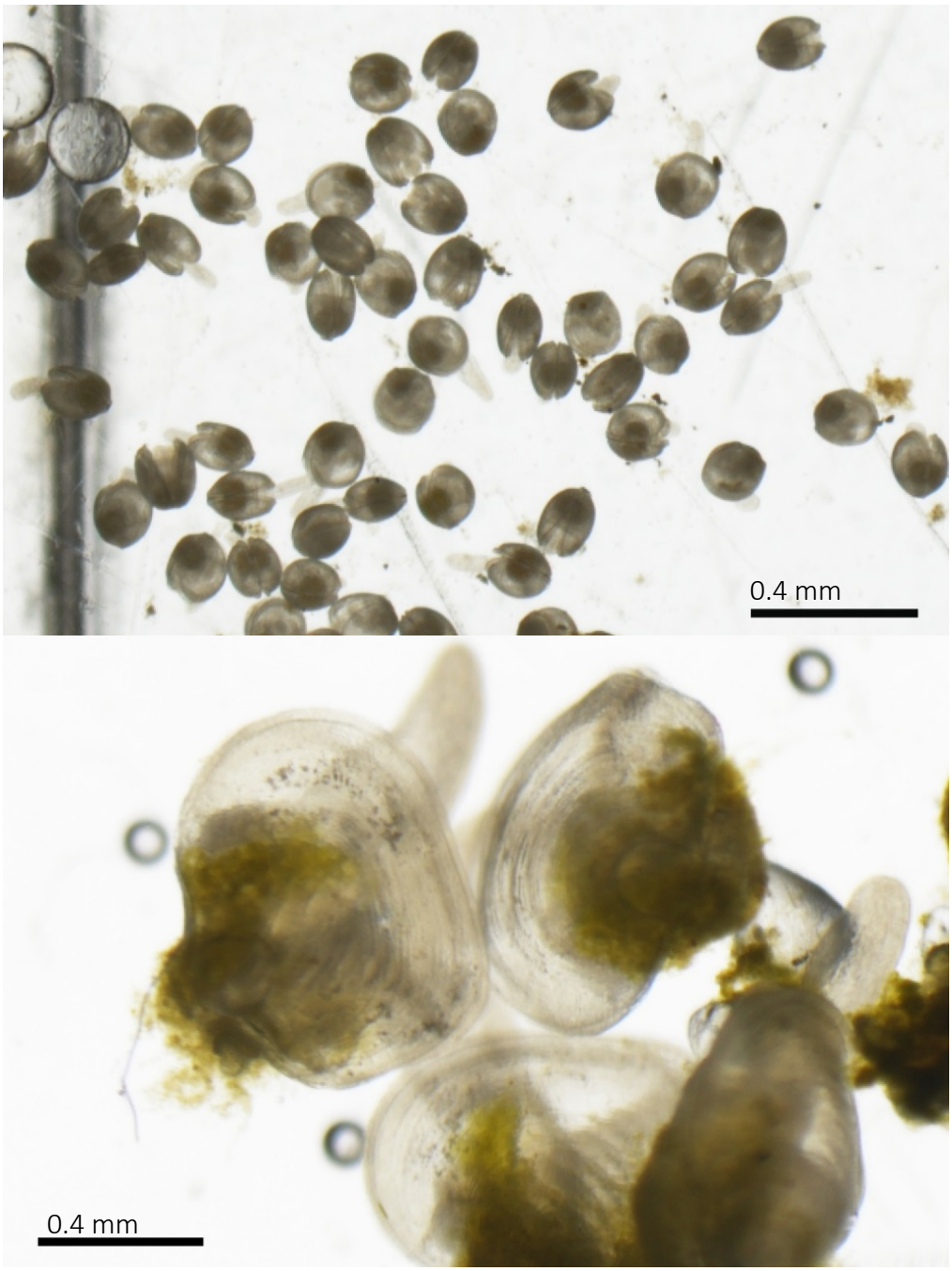


Figure 17. Pilot Study. Top; Mucket juveniles post metamorphoses (day 0): Bottom; Mucket juveniles from sand substrate on day 45.

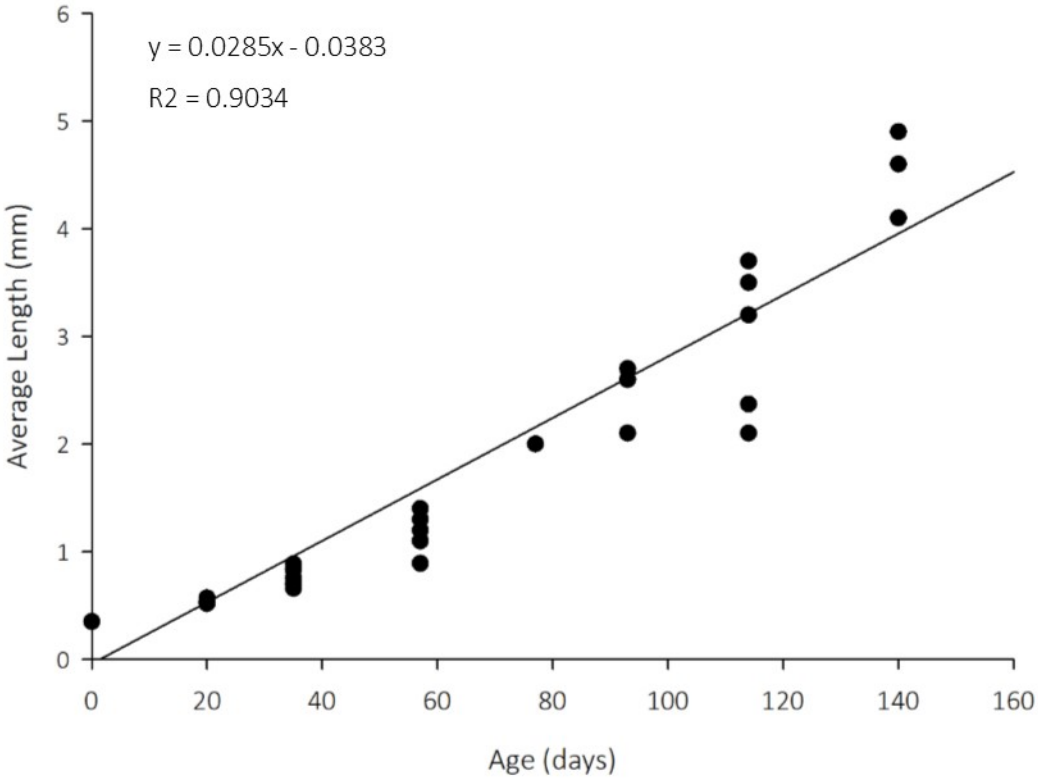


Figure 18. Growth of Cannon River Muckets verses time.

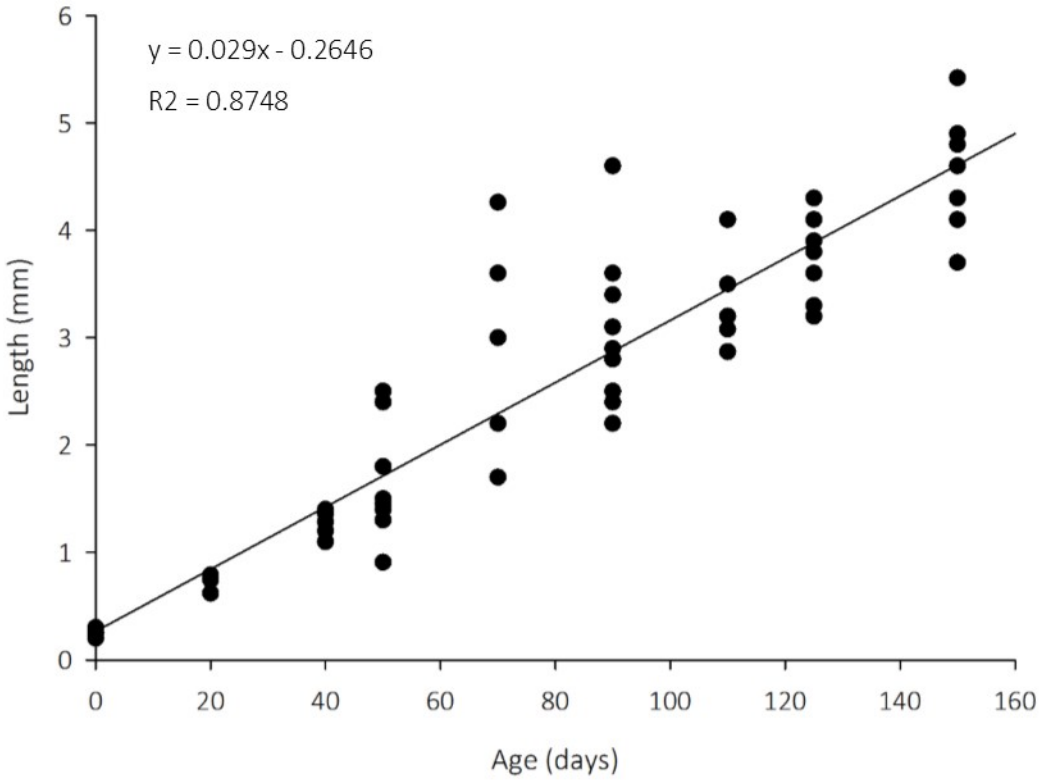


Figure 19. Growth of Cedar River Black Sandshell verses time.

Figure 20. Fish Heath Tray system for juvenile mussel growth.

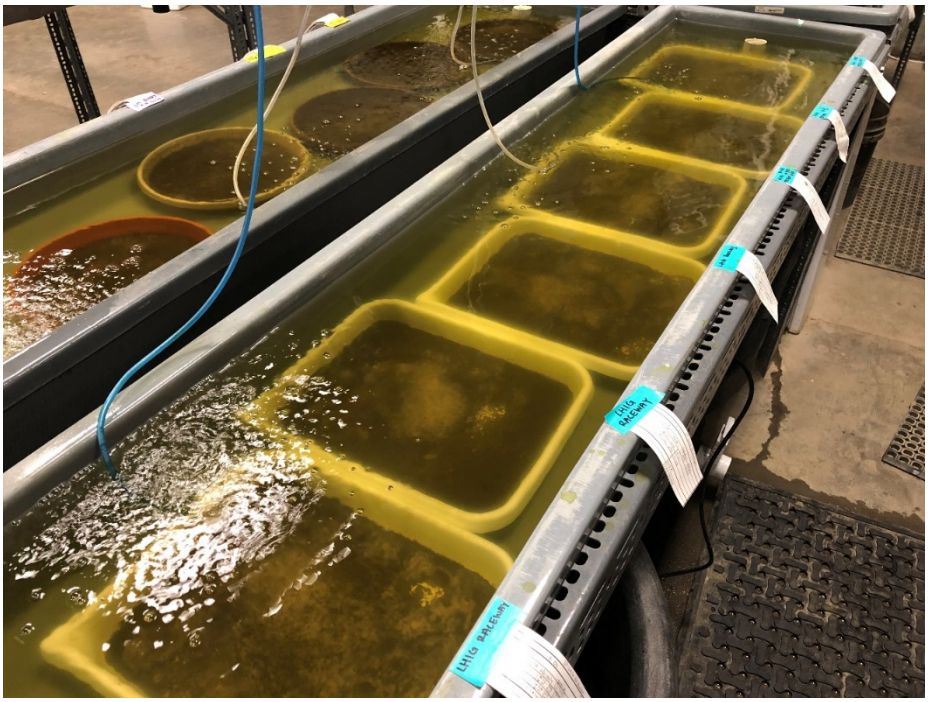
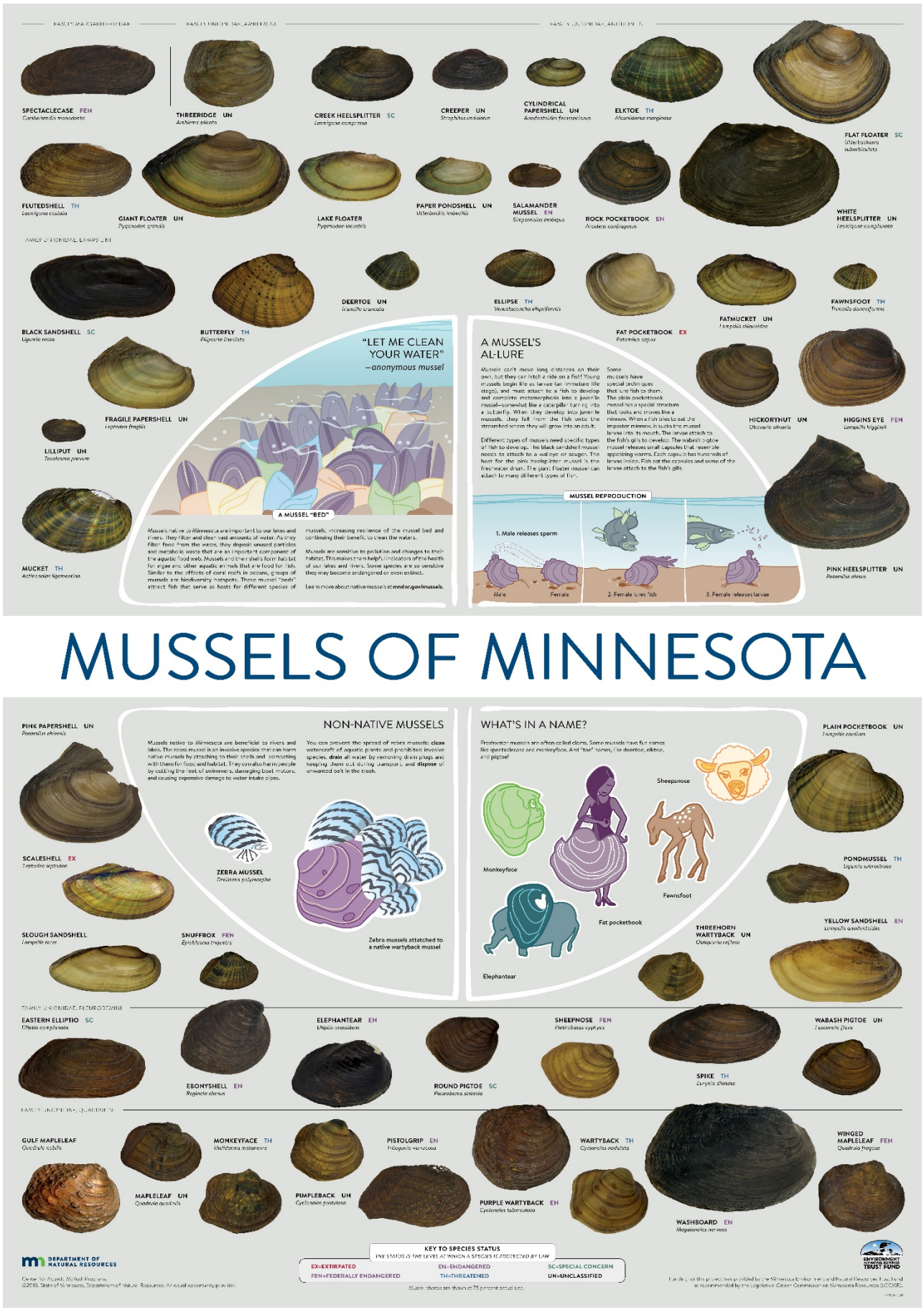


Figure 21. Recirculating trough for juvenile mussel growth. Trays are a mixture of fine sand and sediment.

Figure 22. Fall tote check of Blacksand Shell juveniles in East Side Lake. Black Sandshell juveniles collected in the sieve after rinsing the contents of a tote.



MUSSELS OF MINNESOTA

Figure 23. Draft version of Mussels of Minnesota Poster

Figure 24. Cannon River Mucket (Cohort 2018) that overwintered at CAMP.

Figure 25. Floating totes at Waterville Fish Hatchery, where several thousand of juveniles that overwintered at CAMP were placed in the summer of 2019 for continued growth.

Appendix A: Cannon River Inoculated Host Fishes or Newly Metamorphosis Juvenile Mussel Information

		Placement into Secondary Culture System						Retrieval from Secondary Culture	
Species	Cohort Year	Start Date	Location	No. of Fish	No. of Juveniles	Culture Type	No. of Sys	Date Removed	No. Live
Mucket	2016	Jun-16	Lake Byllesby	130		Metal Cage	5	Sep-17	138
Mucket	2018	Jun-18	Lake Byllesby	52		Tote	4		
Mucket	2019	May-19	Lake Byllesby	40		Tote	3		
			Total	222			12		138

Appendix A.2: Cannon River Secondary Culture System Information

		Placement in Secondary Culture System						Retrieval from Secondary Culture		
Species	Cohort Year	Date Moved	Sys Location	Size at Move (mm)	No. of Juveniles	Culture Type	No. of Sys	Date Removed	No. Live	Size (mm)
Mucket	2018	May-19	Lake Byllesby	8.7	1,930	Tote	3			

Appendix B: Cedar River Inoculated Host Fishes or Newly Metamorphosis Juvenile Mussel Information

		Placement into Secondary Culture System						Retrieval from Secondary Culture	
Species	Cohort Year	Start Date	Location	No. of Fish	No. of Juveniles	Culture Type	No. of Sys	Date Removed	No. Live
Black Sandshell	2017	Jul-17	East Side Lake	81		Tote	3	Jun-18	0
Black Sandshell	2017	Jul-17	East Side Lake	32		Metal Cage	1	Jun-18	0
Black Sandshell	2019	Jul-19	East Side Lake		21,800	Tote	4		
Mucket	2017	Jul-17	East Side Lake	45		Tote	3		
Mucket	2017	Jul-17	East Side Lake	15		Metal Cage	1		
Spike	2019	Jul-19	East Side Lake		19,800	Tote	3		
			Total	173	41,600		15		

Appendix B.2: Cedar River Secondary Culture System Information

		Placement in Secondary Culture System						Retrieval from Secondary Culture		
Species	Cohort Year	Date Moved	Sys Location	Size at Move (mm)	No. of Juveniles	Culture Type	No. of Sys	Date Removed	No.Live	Size (mm)
Black Sandshell	2016	Oct-16	East Side Lake	2 - 7	9,956	Metal Cage	3	Jul-19	99	56.3
Black Sandshell	2016	May-17	East Side Lake	7.6 (2.5 - 16.4)	1,841	Tote	2	Jul-19	1,044	56.3
Black Sandshell	2016	May-17	East Side Lake	7.6 (2.5 - 16.4)	1,160	Metal Cage	2	Jul-19	341	56.3
Black Sandshell	2016	May-17	MN Zoo	5.8	2,000	Pan System		May-19	1,106	15.9
Black Sandshell	2018	Jun-19	Waterville	6.76	3,682	Floating Tote	5	Sep-19	255	22.3
Black Sandshell	2018	Jul-19	East Side Lake	8.70	3,022	Tote	9			
Mucket	2017	Oct-17	East Side Lake	0.98 - 2.3	12,544	Tote	4	Nov-18	0	-
Mucket	2017	Jun-18	East Side Lake	8.20	932	Tote	4	Jul-19	417	36
Threeridge	2018	Jun-19	Waterville	5.05	9	Floating Tote	1	Sep-19	0	-
			Total		**35,146**		**30**		**3,262**	

Appendix C: St. Croix River Inoculated Host Fishes or Newly Metamorphosis Juvenile Mussel Information

		Placement into Secondary Culture System						Retrieval from Secondary Culture	
Species	Cohort Year	Start Date	Location	No. of Fish	No. of Juveniles	Culture Type	No. of Sys	Date Removed	No. Live
Higgin's Eye	2018	Aug-18	Hudson	78		Tote	3		
Mucket	2017	Jun-17	Hudson	75		Tote	3		
Mucket	2017	Jun-17	Hudson	50		Metal Cage	2		
Plain Pocketbook	2017	Jun-17	Lake Pepin		4,096	Floating Tote	1	Jun-18	0
Snuffbox	2017	Jun-17	Hudson	25		Tote	1	Sep-19	1
Snuffbox	2017	Jun-17	Hudson	35		Metal Cage	2	Sep-19	9
Snuffbox	2017	Jun-17	Lake Pepin		1,250	Floating Tote	1	Jun-18	0
Snuffbox	2018	Jun-18	Hudson	140		Tote	12		
Snuffbox	2018	Aug-18	Hudson		1,747	Tote	2		
Spectaclecase	2018	Aug-18	Hudson		12,777	Tote	2		
Winged Mapleleaf	2016	Nov-16	Lake Frontenac	24		Metal Cage	3	Jun-18	0
Winged Mapleleaf	2016	Nov-16	Hudson			Metal Cage	7	Aug-18	0
Winged Mapleleaf	2017	Sep-17	Hudson	107		Tote	10	Sep-19	1
Winged Mapleleaf	2017	Nov-17	Hudson		26,854	Tote	3	Sep-19	0
			Total	**534**	**46,724**		52		11

Appendix C.2: St. Croix River Secondary Culture System Information

		Placement in Secondary Culture System						Retrieval from Secondary Culture		
Species	Cohort Year	Date Moved	Sys Location	Size at Move (mm)	No. of Juveniles	Culture Type	No. of Sys	Date Removed	No. Live	Size (mm)
Higgin's Eye	2017	Oct-17	Hudson		7	Tote	1	Sep-19	0	-
Higgin's Eye	2018	Jun-19	Waterville	7.00	500	Floating Tote	1	Sep-19	131	20.11
Higgin's Eye	2018	Jul-19	Hudson	7.16	681	Tote	1			
Mucket	2017	Oct-17	Lake Frontenac	0.85 - 1.3	16,538	Tote	5	Jun-18	0	-
Mucket	2017	Oct-17	Hudson	0.9 - 1.5	10,483	Tote	4	Sep-19	0	-
Mucket	2016 - 2017	Oct-17	Hudson		1,403	Tote	1	Sep-19	661	39.71
Mucket	2018	Jun-19	Waterville	7.90	875	Floating Tote	2	Sep-19	133	19.07
Mucket	2018	Jul-19	Hudson	7.32	861	Tote	1			
Snuffbox	2017	Oct-17	Hudson	2.80	1,237	Tote	1	Sep-19	0	-
Snuffbox	2017	Jun-18	Hudson	5.90	44	Tote	1	Sep-19	39	18.87
Snuffbox	2018	Jun-19	Waterville	5.66	200	Floating Tote	1	Sep-19	6	9.89
Snuffbox	2018	Jul-19	Hudson	7.10	287	Tote	1			
Spectaclecase	2017	Oct-17	Lake Frontenac	1.1 - 1.5	1,604	Tote	1	Jun-18	0	-
Spectaclecase	2017	Oct-17	Hudson	1.4 - 1.6	2,463	Tote	1	Sep-19	0	-
Spectaclecase	2017	May-18	Hudson		234	Tote	1	Sep-19	120	29.07
Spectaclecase	2018	Jun-19	Waterville		51	Floating Tote	1	Sep-19	0	-
				Total	37,468		24		1,090	

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

Project Title: Restoring Native Mussels in Streams and Lakes
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04c
Project Manager: Mike Davis
Organization: MNDNR.
M.L. 2016 ENRTF Appropriation: $600,000
Project Length and Completion Date: 3 years - June 30, 2019
Date of Report: November 21, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Direct & Necessary	Spent	Balance	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL SPENT	TOTAL BALANCE
BUDGET ITEM	**MNDNR**			**Juvenile mussel production**			**Rearing juvenile mussels**			**Releasing and monitoring mussels**					
Personnel (Wages and Benefits)				$190,880	$190,880	$0	$272,355	$272,355	$0	$80,706	$80,706	$0	$543,941	$543,941	$0
Mussel Culture Biologist: $125,722(72% salary,28% benefits), 1 FTE for 2 years															
Aquarist/survey diver (Shelby Marr): $77,502 (72% salary, 28% benefits), .75 FTE for 2 years															
Malacologist (Bernard Sietman): $116,500; (72% salary, 28% benefits), .75 FTE for 2 years															
Lab, database and survey diver (Zeb Secrist): $79,869 (72% salary, 28% benefits), .75 FTE for 2 years															
Project Manager/malacologist (Mike Davis): $144,348; (72% salary, 28% benefits), .75 FTE for 2 years															
Printing														$0	$0
Remake and printing of mussel poster										$3,049	$3,049	$0	$3,049	$3,049	$0
Travel expenses in Minnesota														$0	$0
Travel is to collect female mussels for propagation and returning them and for travel to release sites for release and monitoring										$6,229	$6,229	$0	$6,229	$6,229	$0
Subtotal				$190,880	$190,880	$0	$272,355	$272,355	$0	$89,984	$89,984	$0	$553,219	$553,219	$0
MNDNR Direct and Necessary															
People support - $11,640															
Safety support - $2,744															
Financial support - $7,745															
Communication support - $1,236															
IT support - $22,352															
Planning support - $829															
Procurement support - $235															
Total Direct and Necessary cost	**$46,781**	**$46,781**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	**$0**	$46,781	$46,781	$0
COLUMN TOTAL	**$46,781**	**$46,781**	**$0**	**$190,880**	**$190,880**	**$0**	**$272,355**	**$272,355**	**$0**	**$89,984**	**$89,984**	**$0**	**$600,000**	**$600,000**	**$0**