

M.L. 2017 Project Abstract

For the Period Ending June 30, 2021

PROJECT TITLE: Rearing Native Mussels for Reintroduction and Expanding Water Quality Awareness

PROJECT MANAGER: Seth Stapleton

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2017, Chp. 96, Sec. 2, Subd. 04c as extended by M.L. 2020, First Special Session, Chp. 4, Sec. 2

APPROPRIATION AMOUNT: \$591,000

AMOUNT SPENT: \$590,760

AMOUNT REMAINING: \$240

Sound Bite of Project Outcomes and Results

The Minnesota Zoo increased capacity for rearing mussels to more than 10,000 individuals and researched methods to improve husbandry, enabling us to better support efforts to recover depleted populations. The Show Us Your Mussels challenge engaged >2,200 students, with student-created content reaching >150,000 citizens and encouraging action to benefit conservation.

Overall Project Outcome and Results

Native mussels are aquatic engineers, providing important ecosystem services such as water filtration and creating habitat for fish and other wildlife. However, many populations are depleted in Minnesota due to factors such as overharvest and pollution. With this project, the Minnesota Zoo sought to support state-wide recovery efforts led by the DNR and improve mussel conservation by 1) increasing our capacity to rear juvenile mussels for reintroduction; 2) advancing our understanding of mussel husbandry to improve the growth and survival of individuals in our care; and 3) raising public awareness about and encouraging action to benefit our aquatic resources.

We constructed a new mussel rearing and research facility on the Zoo's campus and installed associated systems for housing mussels. These improvements significantly increased our capacity for rearing mussels; we currently have space to accommodate >65,000 newly transformed mussels, surpassing our target of 10,000 individuals. This expansion significantly increases our conservation impact and positions us to better support ongoing recovery efforts that will restore ecosystem services.

We also conducted experiments to evaluate how substrate affects growth and survival of juvenile mussels. Our research documented that the presence of fine sand in rearing pans significantly increases growth rates for some species. As such, we have modified our husbandry methods to incorporate this finding, which will yield larger individuals more suitable for reintroduction and ultimately may accelerate reintroduction efforts.

To encourage local communities to take action on behalf of water quality, we established the Show US Your Mussel Challenge. This project engages middle and high school students in the creation of social media campaigns to expand communications efforts throughout local communities. To date, >2,200 students have participated in the challenge, sharing information about the importance of mussels, Minnesota's aquatic resources, and actions the public can take to protect Minnesota's waterways with >150,000 residents.

Project Results Use and Dissemination

Communicating the importance of native mussels and water quality was a key goal of this project. The Minnesota Zoo engaged with Minnesotans to share information about mussels, their conservation, and stewardship of aquatic resources via a variety of platforms, ranging from in-person and virtual presentations at schools, camps, and other forums to free school curriculum and teacher professional development in association with the Show Us Your Mussels challenge. We developed an interpretive area on Zoo campus, outfitted with signs, videos, and a water quality activity, shared research findings at scientific meetings and hosted Smart Salt workshops to reduce salt use.



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2017 LCCMR Work Plan Final Report

Date of Submission: August 16, 2021

Final Report

Date of Work Plan Approval: 06/07/2017

Project Completion Date: June 30, 2021

PROJECT TITLE: Rearing Native Mussels for Reintroduction and Expanding Water Quality Awareness

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Location: Minnesota Zoo

Total ENRTF Project Budget:	ENRTF Appropriation:	\$591,000
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Appropriation Language:

\$591,000 the first year is from the trust fund to the Minnesota Zoological Garden in cooperation with the Department of Natural Resources to accelerate the reintroduction of native mussels into Minnesota rivers and streams through expanded mussel rearing, research, and statewide educational activities promoting mussel conservation and water quality. This appropriation is available until June 30, 2020, by which time the project must be completed and final products delivered.

M.L. 2020 – Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2021]

I. PROJECT TITLE: Rearing native mussels for reintroduction and expanding water quality awareness

II. PROJECT STATEMENT:

Native mussels play a critical role in keeping our State's streams and rivers clean and in creating healthy fish habitat. Today, however, freshwater mussels are the most at-risk group of species in the United States. Pollution, dams, and historical harvest for the button industry are among the factors that have taken a heavy toll on mussels. Populations have been lost or numbers greatly diminished across many Minnesota water bodies, and about half of the mussel species found in the state are currently considered imperiled.

The Minnesota Zoo recently began a collaboration with the Minnesota DNR to help reverse this trend. Zoo staff are nurturing juvenile mussels originally produced at DNR facilities in the Zoo's main lake until they are large enough to be reintroduced into selected Minnesota waterways. However, current Zoo resources are insufficient to rear the thousands of mussels needed to effectively re-establish native mussel populations.

This ENRTF Project will allow the Zoo to use its unique site and expertise in aquatic systems and husbandry to expand the mussel-rearing program and optimize propagation techniques, accelerating reintroduction and increasing the likelihood of success. The ultimate goal of this project is to reestablish threatened and endangered mussel populations in Minnesota to provide clean waterways, improve ecosystem health, and expand fish habitat. The Zoo also will use its education expertise to build awareness in students across Minnesota about the relationship between native mussels and water quality, encouraging actions to support mussel conservation and improve water quality in state waterways.

The specific objectives of the project are:

- 1) To expand the Zoo's capacity to rear 10,000 reintroduction-ready individuals from seven threatened and endangered mussel species—mucket, elktoe, monkeyface, fluted shell, Higgins' eye, snuffbox, and winged mapleleaf;
- 2) To advance the science of mussel husbandry by evaluating key factors that promote mussel survivorship and growth, thereby informing propagation and expediting reintroduction efforts; and
- 3) To train 6,000 students as ambassadors for native mussels and clean waterways, ultimately reaching up to 60,000 people through student digital media campaigns including online videos, websites, and social media.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of June 1, 2018:

The Minnesota Zoo's mussel conservation program is expanding its capacity for rearing mussels and conducting research on their propagation with the support of ENRTF. Newly hired mussel staff have positioned the Zoo to grow current mussel propagation, research, and conservation efforts and to educate the general public about water quality issues that affect Minnesota's waterways and mussel reintroduction sites. We remain on-track to explore key issues in propagation with the construction of the new research pod. Community engagement through the first year of the Show us your Mussels digital media campaign has been well-received and has identified additional ways to communicate with our guests about the importance of water and mussels in their communities.

Amendment Request (6/1/2018):

To expand participation in Activity 3 ('Show us your mussels' digital media challenge for high school students), the Zoo needs to reach a larger and more diverse audience. This challenge was designed to appeal both to science teachers as well as language arts and media specialists. In our first year, we were successful in reaching the Zoo's usual audience of science teachers and wildlife enthusiasts, but we did not attract teachers from other

disciplines to participate in the challenge. To accomplish this, we need to increase our outreach to forums where the Minnesota Zoo hasn't traditionally participated, including conferences related to language arts, technology, media literacy, and other disciplines. We propose that Zoo staff both present and table at these conferences.

Because the Challenge is technology based, we have found that one of the most successful ways to reach teachers is through Facebook and other social media outlets. By boosting posts on these platforms, we are able to reach a broader audience. This is an effective way to expand participation among teachers and provides a vehicle to share educational resources about mussels and water quality with teachers across the state.

As part of our end-of-year evaluation for the Challenge, we received feedback from participating teachers suggesting that they would have benefitted from additional training on native freshwater mussels. This background would have allowed them to better facilitate the Challenge in their classrooms. As such, we propose hosting two professional development workshops each summer / fall for FY19 and FY20. The Zoo will host these events; ENRTF support would fund the printing of reference materials for teachers to bring back to their classrooms.

Amendment Approved by LCCMR 6/12/2018

Project Status as of December 1, 2018:

The Minnesota Zoo continues to increase its capacity for rearing mussels with the support of ENRTF. The expansion of holding space in two lakes on Zoo site ('A' or Mussel Lake and Main Lake) currently enables us to rear up to 7,000 mussels. We began construction on a new research pod this fall and are on-track to initiate studies during spring, 2019 that will allow us to evaluate those conditions that affect the survival and growth of mussels, thereby improving husbandry practices and accelerating their return to the wild. Additional systems that will be installed in the research facility and around the floating dock on Mussel Lake will increase capacity to our target of 10,000 individuals. Growth documented in Mussel Lake during 2018 exceeded what we have observed using other water sources in previous years, suggesting that this waterbody is an excellent site for husbandry activities. We concluded the first year of the Show us your Mussels digital media campaign with a series of field trips for the winning schools and are implementing the second year of the campaign. As part of this program, educators have developed curriculum associated with the campaign, and the Zoo hosted professional development workshops on mussel conservation and water resources. We continue to identify new ways to communicate key messages to the general public to improve the conservation of Minnesota's aquatic systems.

Project Status as of June 1, 2019:

With the completion of the mussel research pod and associated rearing systems this winter, the Minnesota Zoo has surpassed its capacity goal of 10,000 mussels. The research and propagation systems also will allow us to control variables that may affect mussel growth and survival to determine how best to improve mussel husbandry. We acquired and are currently housing more than 2,000 new mussels in these research systems, allowing us to evaluate how substrate type can impact growth and survival for black sandshell and mucket mussels; several hundred individuals also comprised a quarantine trial to establish an acquisition protocol to prevent the addition of deleterious species to water bodies at the Zoo. We completed a successful second year of the *Show Us Your Mussels Challenge*, with student-initiated projects about water quality and mussel conservation reaching some 40,000 individuals, and we hope to pilot the program with middle school students during the 2019 – 2020 academic year. Finally, we are sharing information about mussel conservation and Minnesota's water resources with the public through a variety of on- and off-site outlets and have scheduled numerous engagement opportunities for the summer.

Project Status as of December 1, 2019:

The Minnesota Zoo continues to modify rearing and research systems and husbandry practices to improve mussel health and production. Although our holding capacity will vary based on the size and growth rates of mussels that we house, we remain well-above our overall capacity targets. We are continuing research to quantify the impacts of sediment type on the growth and survival of mussels in our care. After some initial attrition, survival rates of the experimental cohort have remained very high, and preliminary results suggest that black sandshell mussels reared in finer-grained sediments may grow at higher rates than conspecifics reared in coarser sediments. We will complete more rigorous analyses to better evaluate this finding and will be completed in the months ahead. The *Show us Your Mussels* Challenge enters its third year with strong initial registration and has expanded to engage both middle and high school teachers and students. We have continued to work with Minnesota teachers to draft new curricula associated with mussel conservation and water quality. Finally, we are continuing to disseminate information about this work, the efforts of our partners, and the importance of healthy mussel populations and waterways to a variety of community groups.

Amendment Request as of (12/1/2019):

We are requesting funds be shifted from the Personnel budget line to Equipment and Supplies within Activity 1.

- The Personnel budget would be reduced by \$5,000 to a revised budget of \$270,238.
- Equipment and Supplies would increase by \$5,000 to a revised budget of \$48,000.

Creating and constructing systems for newly transformed mussels of some imperiled species was recently identified as an immediate research and conservation need. We are requesting this revision to accommodate this need. We hope that designing and building new systems will support the propagation of mussels at this sensitive life history stage and enable the Zoo to further contribute to mussel conservation within Minnesota and to the reintroduction goals of the DNR. The Zoo's mussel conservation specialist was hired later in FY 2017 than initially projected; this surplus would facilitate this request.

Amendment Approved by LCCMR 12/11/2019**Project Status as of June 1, 2020:**

To further benefit mussel conservation in the state, the Minnesota Zoo constructed a new rearing system at the ENRTF-funded research pod. Although initially intended for imperiled spectaclecase mussels, this system is currently hosting juvenile giant floater mussels, and individuals will be incorporated into a University of Minnesota-based study. We anticipate receiving >5,000 individuals from the DNR – representing several other species – to provide assistance to our partners during these uncertain and challenging times. In addition, we completed research trials evaluating the quality of substrates for rearing juvenile black sandshell and mucket mussels. Black sandshell mussels raised in silty sediments were significantly larger than individuals reared in other substrates. We are using this information to modify rearing protocols, allowing us to maximize growth and potentially facilitate earlier reintroductions. Finally, despite the challenges presented by the COVID-19 pandemic, we successfully implemented the third year of the Show Us Your Mussels Challenge and identified novel ways to engage both students and teachers remotely.

Amendment Request as of (6/1/2020):

We are requesting funds be shifted within the Other budget category within Activity 3.

- The Transportation (bussing) budget would be reduced by \$14,014 to a revised budget of \$8,486
- A new, Show Us Your Mussels Challenge student and chaperone ticketing category would be established with a budget of \$14,014

Given the COVID-19 pandemic, which has resulted in the closure of the Zoo and schools state-wide, as well as the upcoming end of the academic year, those schools that were the highest vote recipients for the Show Us Your Mussels challenge (Activity 3) are unable to complete the planned field trip to the Zoo. This field trip has

provided students the opportunity to learn more about the conservation of mussels and Minnesota's aquatic resources and witness first-hand the Zoo's specific conservation activities associated with mussel conservation. Students will be in new classes with new teachers next year.

As such, we are requesting a budget amendment to provide students from the winning schools the opportunity to visit the Zoo in the future. Specifically, 555 students registered in the Show Us Your Mussels challenge from the winning schools. We propose to provide a flexible, discounted group ticket to each winning student (\$10.25 / student) and an adult chaperone (\$15 / adult) to allow students to visit the Zoo to learn more about Minnesota's natural resources and the State's conservation efforts after we re-open. We propose to re-budget funds in the bussing / transportation line of the budget (which will go unspent due to the COVID-related closures) to cover these costs.

Amendment Approved by LCCMR 6/23/2020

Project extended to June 30, 2021 by LCCMR 6/18/20 as a result of M.L. 2020, First Special Session, Chp. 4, Sec. 2, legislative extension criteria being met.

Amendment Request as of (7/22/2020):

We are requesting funds be shifted from the Travel and Other budget categories within Activities 1 and 3 to the personnel category of Activity 1.

- The Travel budget category for Activities 1 and 3 would be reduced by a total of \$8,835.
- The Other budget category for Activity 3 also would be reduced by a total of \$5,552.
- The Personnel budget for Activity 1 would increase by \$14,387 to a revised budget total of \$284,625.

The Zoo's mussel conservation initiative is currently rearing >10,000 mussels on Zoo site. Although the program has received a tentative recommendation for a new ENRTF appropriation that would support our conservation efforts for the next 4 years, Legislature has not yet passed an associated ENRTF bill. However, the mussels in our care still require regular husbandry and maintenance to ensure their health and survival and ultimately support reintroduction efforts led by our partners.

Due to the Zoo's recent financial hardships and resultant layoffs associated with the COVID-19 pandemic, we do not have the capacity to implement the Show us your Mussels challenge this year without the support of a new ENRTF appropriation. [However, please note that the Zoo's Education staff have developed virtual and distance learning lessons (<https://mnzoo.org/education/schools-teachers/educator-corner/new-distance-learning/grades-6-8/>) and other downloadable lessons (<https://mnzoo.org/education/schools-teachers/educator-corner/free-downloadable-curriculum/>), focusing on the ecology and conservation of native mussels and Minnesota's aquatic resources, that may be used during the 2020 – 2021 academic year.] As such, we propose to reallocate funding remaining in the Show us your Mussels (Activity 3) budget to provide for the care of the mussels currently housed at the Zoo. This amendment request represents part of a broader strategy to allow critical husbandry work – the program's highest priority – to continue until an ENRTF bill may be considered.

Amendment Approved by LCCMR 8/24/2020

Project Status as of December 1, 2020:

Despite the ongoing challenges presented by the COVID-19 pandemic, we have continued to implement the Zoo's mussel rearing and research programs to improve the conservation of Minnesota's aquatic resources. Our research continues to suggest that silty sediments support the highest growth rates for black sandshell mussels until they reach a releasable size (~40 – 60 mm). In collaboration with the DNR, we released the ~1,100 black sandshell mussels incorporated in this study to the Cedar River in southern Minnesota (along with >2,000 black

sandshells reared by the DNR). We also reared juvenile giant floater mussels to support a University of Minnesota research project, with survival rates far exceeding expectations; of the ~7,000 individuals reared for this study, 6,000 were placed in the wild, and ~1,000 remain at the Zoo as a control group. The Zoo acquired 3,000 new mussels that the DNR was unable to maintain or reintroduce due to COVID-19 work and travel restrictions. The acquisition of these new mussels, including species listed as imperiled at the federal and state levels, has provided the opportunity to begin new research trials on novel improvements to rearing methodologies. We were unable to implement the *Show us Your Mussels* challenge in schools this fall, but we incorporated curriculum developed for that program in virtual camps offered by the Zoo. Zoo educators designed additional content and lessons to support distance learning; these materials are available as free resources via the Zoo's online Learning Corner.

Project Status as of June 1, 2021:

Although the COVID-19 pandemic still presents obstacles to the Zoo's conservation programs, we have continued to make progress on objectives associated with mussel propagation, research, and outreach. We made improvements to mussel rearing systems, continued ongoing research initiatives, and designed and implemented new studies to address questions about mussel husbandry and natural history. We also are poised to accept a new cohort of juvenile mussels early this summer. Endangered mussels received from the DNR last July have fared well through the spring, and measurements for the year suggest strong growth among all species. Outreach activities have remained relatively limited due to capacity and resource constraints.

Overall Project Outcomes and Results:

Native mussels are aquatic engineers, providing important ecosystem services such as water filtration and creating habitat for fish and other wildlife. However, many populations are depleted in Minnesota due to factors such as overharvest and pollution. With this project, the Minnesota Zoo sought to support state-wide recovery efforts led by the DNR and improve mussel conservation by 1) increasing our capacity to rear juvenile mussels for reintroduction; 2) advancing our understanding of mussel husbandry to improve the growth and survival of individuals in our care; and 3) raising public awareness about and encouraging action to benefit our aquatic resources.

We constructed a new mussel rearing and research facility on the Zoo's campus and installed associated systems for housing mussels. These improvements significantly increased our capacity for rearing mussels; we currently have space to accommodate >65,000 newly transformed mussels, surpassing our target of 10,000 individuals. This expansion significantly increases our conservation impact and positions us to better support ongoing recovery efforts that will restore ecosystem services.

We also conducted experiments to evaluate how substrate affects growth and survival of juvenile mussels. Our research documented that the presence of fine sand in rearing pans significantly increases growth rates for some species. As such, we have modified our husbandry methods to incorporate this finding, which will yield larger individuals more suitable for reintroduction and ultimately may accelerate reintroduction efforts.

To encourage local communities to take action on behalf of water quality, we established the Show US Your Mussel Challenge. This project engages middle and high school students in the creation of social media campaigns to expand communications efforts throughout local communities. To date, >2,200 students have participated in the challenge, sharing information about the importance of mussels, Minnesota's aquatic resources, and actions the public can take to protect Minnesota's waterways with >150,000 residents.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Expand capacity to rear mussels on-site at the Zoo for reintroduction

Description:

The Zoo will increase its capacity to rear mussels from 1,000 to 10,000 individuals, focusing on four state threatened and three federally endangered species. Using juveniles provided by the DNR, mussels will be reared in specialized systems at the Zoo for 2 – 3 years, until they are large enough to withstand most predation in the wild. Mussels then will be strategically released by the DNR, with Zoo staff participation, into pre-determined locations including the Cedar River, the Cannon River system, and the Mississippi River. To achieve the desired outcomes, the Minnesota Zoo must expand and improve its rearing systems and dedicate increased staffing to the project.

The existing mussel rearing project at the Zoo implements standard practices for operation of three systems: an upwelling bucket system, a pan system, and an exhibit system. The systems differ in how they recreate the natural environment and can be modified to suit different species based on their specific life history requirements.

We will construct replicates of the upwelling bucket and pan systems and may employ alternative systems (e.g., floating baskets), enabling the Zoo to increase capacity to house 10,000 mussels on-site. We also will modify existing systems to improve efficiency and better replicate the natural environment (i.e., increasing substrate variety with the exhibit system). Additional upwelling bucket systems will require regular maintenance (i.e., at least weekly during spring, summer and fall), including cleaning and scrubbing buckets and screens and rinsing mud from the mussels. Pan systems also require regular cleaning and maintenance of the pans, pump housings, and mussels. We will monitor water flow and water quality for all systems to ensure that conditions are suitable for captive reared mussels. During the growing season, we will also monitor survival on a weekly basis (during cleaning and maintenance operations) and record and remove any mortalities. We will measure a sample of all mussel species (including endangered species not covered under Activity 2) on a monthly basis to estimate growth. Maintenance and monitoring will require a significant increase in staff time, given the projected 10-fold increase in the Zoo's rearing capacity.

Support from the ENRTF will facilitate the expansion of mussel rearing capacity at the Zoo by providing the necessary funding for dedicated staff time and associated supplies and equipment.

Summary Budget Information for Activity 1:

ENRTF Budget:	\$ 336,475
Amount Spent:	\$ 336,341
Balance:	\$ 134

Outcome	Completion Date
1. Staffing is in place to expand rearing capacity to 10,000 juvenile mussels	10/15/2017
2. Systems are in place to expand rearing capacity to 10,000 juvenile mussels	11/30/2018
3. Up to 1,000 mussels per species are reared annually to sizes needed for reintroduction and provided to DNR for release	6/30/2020

Activity 1 Status as of June 1, 2018:

The Minnesota Zoo hired a full-time mussel conservation specialist (Ben Minerich) in August, 2017. We first worked to streamline the planning, transfer, and tracking processes that will guide future acquisitions of mussels from the Minnesota Department of Natural Resources and the U.S. Fish and Wildlife Service. We also reviewed mussel quarantine and transfer protocols and will implement protocol modifications associated with the new propagation and research facility to reduce the risk of disease transmission between sites.

We added a new floating dock (using the Zoo's Legacy appropriation) at the campus's 'A' Lake, which holds our current population of mussels, including 61 fatmucket, 225 plain pocketbook, two Wabash Pigtoe, one

pimpleback, and one threehorn wartyback (all housed in upwelling bucket systems) and 785 muckets (housed in new floating basket systems). We constructed a second floating rack in the Zoo's Main Lake, increasing mussel holding capacity by about 1,800 mussels, and a pan system is built and ready for installation to further increase holding at the Main Lake by 2,000 individuals. Our current holding capacity is ~6,000 mussels. We are on-track to reach our target capacity of 10,000 mussels, with the addition of buckets and baskets to the floating dock, along with the holding space available with the new research pod.

We completed full chemical tests for water bodies identified for holding additional mussels to confirm that heavy metals, acidity, and natural waste levels are conducive to rearing and growing mussel. In April, the Zoo acquired 616 mucket mussels (*Actinonaias ligamentina*; 1-3 mm long juveniles produced during winter, 2018) to evaluate new holding systems in the lake where the new facility will be constructed.

An aerator was installed in the Main Lake on Zoo site, which will increase dissolved oxygen levels that will support beneficial biological activity, thus improving conditions for rearing mussels. We anticipate expanding the population of mussels currently in our care to facilitate research for the 2019 summer growing season.

Activity 1 Status as of December 1, 2018:

The mucket mussels acquired during spring, 2018 and placed in 'A' Lake (hereafter Mussel Lake) in basket propagation systems were examined and measured multiple times during summer, 2018. These individuals were received as 1 – 3 mm juveniles, and their survival (92%) and growth rates (\bar{x} : 24 mm; maximum: 40 mm) far surpassed expectations and previous observations for mussels reared in the Zoo's Main Lake, where maximum growth did not exceed 11 mm.

For comparative purposes, another group of mussels from the same population of propagated, juvenile muckets was placed in Lake Pepin in basket systems during spring – summer, 2018. This comparison allowed us to evaluate the suitability of Mussel Lake alongside another water body commonly used for mussel rearing. Here, too, mussels reared in Mussel Lake exceeded the growth rates of the Lake Pepin group. Other species moved to Mussel Lake also have experienced improved growth rates (plain pocketbook mussels, \bar{x} : 30 mm growth in 2018 versus 8 mm during previous years; fatmucket mussels, \bar{x} : 22 mm in 2018 compared to 10 mm previously). These findings suggest that Mussel Lake is an excellent water source for future mussel rearing, and we have transitioned much of our core husbandry activities to this site. We hypothesize that three elements are contributing to increased growth rates observed in Mussel Lake: 1) improved food quality and availability, 2) increased depth of sand substrate in basket propagation systems, and 3) reduced stress due to decreased cleanings and system maintenance.

Additional basket systems have been constructed and will be deployed in the spring as incoming juvenile mussels arrive from the Minnesota DNR. Our current capacity for mussel propagation is roughly 7,000 individuals. The completion of the research facility (status update in Activity 2), combined with the installation of additional propagation systems in Mussel Lake, will allow us to achieve our goal of increasing the Zoo's rearing capacity to 10,000 mussels.

We outfitted Mussel Lake's floating dock with a dedicated air blower to keep the area ice-free during the winter and to stimulate water movement near mussel propagation systems. We installed a submersible pump in Mussel Lake to provide a water source for the research facility; the hose is ready for hookup once the facility can accommodate interior installations.

Given the high rates of juvenile mussel growth and survival on Zoo site, as well as the successes of the DNR in the reproduction of the federally endangered spectaclecase mussel, we are working with the USFWS to obtain the permits necessary to rear federally listed mussel species at the Zoo.

Activity 1 Status as of June 1, 2019:

With the construction of the mussel research and rearing pod this winter (see also Activity 2 status update), we surpassed our goal of developing the capacity to rear at least 10,000 juvenile mussels. Following the completion of the framing and ‘shell’ of the facility (i.e., the roof, walls and insulation), Zoo conservation staff were provided access to begin setup of the internal components (see Images 1, 2, 3, and 4 below). Initial work included the installation of filters to clean water of debris and an ultraviolet sterilizer to prevent infestation by parasites, insects, plants, or animals that may pass through, filters at very small life stages (Images 5 and 6). The first research system – built during the late fall – was then moved into the facility to identify locations for the electrical outlets. Water pipes were installed along the walls to deliver clean lake water to the systems, and two additional propagation and research systems were constructed in the building and tied into the water supply (image 7). A quarantine system was installed for new mussel acquisitions (image 8), and utility (e.g., electric, sanitary sewer) hook-ups were completed.

Each of the three research systems in the facility can hold up to 3,600 juveniles mussels; combined with the quarantine system (>500 mussels), our total capacity in the research and rearing facility alone is ~11,300 juvenile mussels. With our current setup, a total of ~1,000 mussels can be held in A Lake, 11,300 in the research facility, and 1,200 in the systems at the mussel conservation cabin on the Zoo’s Main Lake; following the reconnection of air lines, we will be able to house an additional 3,600 individuals in bucket - rack systems in Main Lake. We anticipate that our total holding capacity will be >17,000 mussels. Continued improvements to the dock at A Lake may further expand our capacity.

We successfully overwintered 99 plain pocketbook, 56 fat muckets, 303 muckets, 2 wabash pig toes, one three-horn wartyback, and one pimpleback mussel in basket and bucket systems in the Zoo’s A Lake. Survival was >94% for mussels maintained at Zoo previous winters and ~50% for the muckets acquired during spring, 2018. Despite troubleshooting with the DNR, we did not identify any specific causes of mortality with the 2018 acquisitions and determined that this loss may be consistent with the expected mortality during the first few years. In the weeks ahead, we will evaluate over-winter growth using photographs and associated length measurements and will attempt to determine when most mortality of muckets occurred among our overwintered individuals (i.e., during early or late winter).

In April, the DNR provided ~2,000 new black sandshell mussels to the Zoo. These individuals were distributed among pans in the research systems to evaluate the effects of substrate type on growth and survival (see also Activity 2 status update). Additionally, we received >550 new muckets from the DNR which were housed in the recently-constructed quarantine system until they were cleared of potentially harmful animals or plants (see Activity 2).

We are meeting with the DNR on June 6 to showcase the research pod and to determine future goals for animal transfers now that our holding capacity is set. We will establish a similar meeting with USFWS to assess how our space can complement their rearing program and to develop future research questions.



Images 1-4 (clockwise from top left): (1) Initial site preparations for the new propagation and research facility were completed during fall, 2018. The Zoo's A Lake, with the dock for mussel propagation, is shown in the background. (2) Excavation completed and forms installed for pouring the foundation. (3) Framing and roofing erected. (4) Walls, insulation, siding and all interior and exterior fixtures installed.



Images 5 (left) and 6 (right): (5) Drum filter installed on wooden shelf (circled in yellow); water reservoir (black tank; left of drum filter); and construction of initial propagation and research system. (6) Ultraviolet sterilizer unit (circled in red), which is located behind the water reservoir, cleans intake water from the Zoo's A lake.



Images 7 (left) and 8 (right): (7) New propagation and research systems were installed this winter and are now operational. (8) A quarantine system to evaluate condition of newly acquired mussels and identify and remove unwanted species was also installed. A pump delivers food regularly in this system to simulate the near constant supply of food that would exist in the wild.

Activity 1 Status as of December 1, 2019:

This summer, our focus was to establish baseline husbandry activities to project future labor needs, finish some aspects of our mussel rearing systems, and continue regular maintenance associated with our mussel rearing systems. We implemented routine preventative maintenance tasks, allowing us to reduce stress on mussels by handling them less frequently; we currently only need to disturb them for cleanings every two months (rather than weekly). Weekly cleanings of rearing systems and water pre-treatment systems in the research facility ensure that our systems remain free of nuisance algae, sponges, and aquatic insects. Additional, more intensive monthly cleanings of the drum filter screens remove buildup and keep the systems operational. A benefit of the new pan system design is that the mussels can be isolated and maintained with recirculating water while the incoming water system is shut down, cleaned, and flushed for additional, more intensive maintenance needs. Over the past 6 months, we have completed this task as needed to remove organic buildup in the pipes that decreases water delivery to the systems. Establishing this cleaning and maintenance baseline has allowed us to schedule these tasks for the coming year to ensure that they have minimal impact on the mussels and the rearing systems.

We installed an upgraded airline at the Mussel Lake dock that included the addition of 11 new airline drop downs to support additional basket and bucket systems as well as improved aeration to keep the area ice-free over-winter. We also established quarantine protocols for new acquisitions and implemented this plan with our first batch of mucket mussels received this spring. Results were promising, and conversations with the DNR about guidelines for supplemental feed for different species and sizes of mussels will help improve future processing of new mussels.

The Genoa National Fish Hatchery in Wisconsin has offered to process water samples from the Zoo's Main Lake and A Lake to document the presence and concentration of algae and bacteria. As such, we have been – and continue to – send samples there monthly to analyze the nutritional composition of on-site lake water. Initial results indicate peak particle sizes of 2-3 µm for bacteria and 5-20 µm for different algae species and organic matter (e.g., small pieces of leaves, woody debris, and soil). These findings are consistent with a healthy, nutritious river that is productive for mussel growth (based on communication with Megan Bradley, Mussel Biologist at the Genoa National Fish Hatchery). We are using temperature loggers to regularly document water

temperatures and improve our understanding of the relationships between food availability, mussel growth and temperature, thereby informing our husbandry practices.

Maximum, on-site holding capacity at the Zoo will vary based on the size and growth of individuals in our care. Simply put, larger animals need more space, and the high growth rates observed this summer suggest that we may need to revise our holding capacity, given the sizes of mussels generally provided by our partner organizations. Following additions and improvements to on-site propagation systems – and based on the sizes of mussels provisioned to us and projected growth rates – we now estimate that we can accommodate about 7,200 juvenile mussels in our research facility systems; ~500 mussels in the quarantine system; ~2,000 mussels in A Lake basket systems; ~800 mussels in A Lake bucket systems; and ~2,000 in the systems at the mussel conservation cabin on the Zoo's Main Lake. Our current maximum holding capacity is ~12,500 individuals, well above our initial goal of 10,000 individuals. Continued additions and improvements to the A Lake dock may further expand our holding capacity.

Since April, 2018, we have reared mucket mussels in basket systems in A Lake. During spring, 2019, we received the remaining mussels propagated from the same parent group, which was previously held by the DNR. This new cohort was used to test quarantine protocols (as noted above) and subsequently was placed in pan systems. Individuals were randomly distributed among the four substrate treatment types evaluated for the black sandshell mussels, thus providing an inter-specific comparison for response (growth and survival) to different substrates (see also Activity 2). Also, this group will allow us to evaluate growth and survival of muckets reared in both pan and basket systems.

Initial survival estimates for the newly acquired black sandshell mussels were concerning (56%). However, the mortalities were at the small end of the size range of newly acquired individuals, and none were longer than the minimum shell length of the group measured in July, by which point surviving mussels had grown to an average of 15.5 mm (maximum: 26 mm). As such, we assumed that nearly all mortalities occurred soon before or shortly after acquisition on May 13, 2019. The DNR also noted that this group of mussels was experiencing relatively high mortality prior to the Zoo's acquisition and that the observed mortality rate was likely a continuation of this attrition. Since the first measurement in July, 2019, virtually all (99.8%) black sandshell mussels have survived. Growth has averaged 20.5 mm (~350% increase in length), and the largest individuals are ~40 mm (seasonal growth >30 mm; Image 9). These black sandshell mussels have achieved a releasable size in one summer, ahead of our production goals. The Zoo will hold these mussels until next summer, at which point the DNR will release them into the Cedar River near Austin, MN. As such, we are on track for our production goal. We also may be able to revise future goals if we are able to release animals after 2 years rather than the planned 3 years.

This summer, we successfully reared 1,106 black sandshell, 81 plain pocketbook, 44 fat muckets, 478 muckets, 2 wabash pig toes, one three-horn wartyback, and one pimpleback mussel in pan, basket, and bucket systems using the Zoo's A Lake water.

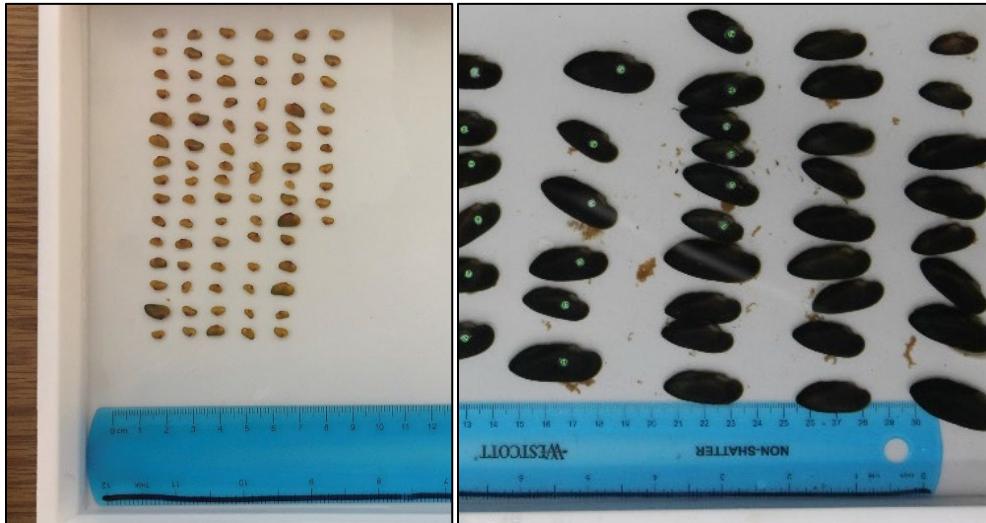


Image 9: Cropped and scaled images offer a side-by-side comparison of mussels from pan 3.2.2 on May 13, 2019 (left) and September 6, 2019 (right), illustrating the increase in size and color change of juvenile black sandshell mussels.

Activity 1 Status as of June 1, 2020:

The fish host for the spectaclecase mussel, a Minnesota threatened and federally endangered species, was only recently identified in 2018 by the DNR. This sensitive species is challenging to rear when very young; none of the DNR laboratory group survived through 2019. Through our collaboration with the DNR, we developed novel, juvenile mussel rearing tanks to address some of the species-specific propagation challenges (Image 13). However, due to the COVID-19 pandemic, the DNR currently is unable to conduct fieldwork associated with collection of fish hosts or female mussels with eggs to facilitate rearing spectaclecase mussels, place juvenile mussels that were reared in the laboratory overwinter into the wild, or fully staff their facility to accommodate the daily husbandry needs to care for mussels that would normally be growing in the wild before reintroduction. As such, the Zoo will be using a recently-built quarantine system and expanded floating dock systems to accept >5,000 mucket, Higgins eye, and black sandshell mussels in June to assist in continued care for these animals until they can be reintroduced.

Additionally, in partnership with the DNR, the Zoo is rearing giant floater mussels (~14,000 newly transformed individuals were acquisitioned this spring) to support a University of Minnesota study. We outlined a plan with the DNR and our partners at the USFWS (Genoa National Fish Hatchery) to expand our propagation space to accommodate newly transformed mussels. We adapted systems initially built for spectaclecase mussels to house the giant floaters (Images 11 and 12). Survival of giant floater mussels to releasable size is very low; we hope to produce a few hundred individuals for use in this study by the end of summer 2020 or 2021.

Our routine cleanings in early May identified higher than expected growth, and we documented only 8 overwinter mortalities. Our current, on-site mussel population is ~16,000 individuals, which we anticipate will grow to >21,000 next month based on projected acquisitions. The new mussel research and rearing facility and propagation systems on the floating dock have allowed the Zoo to support partner agencies in achieving their production goals and to help during the uncertain times of the COVID-19 pandemic. We have surpassed our goal of expanding holding capacity to at least 10,000 individuals, and we anticipate that the >1,000 black sandshell mussels in our care will be reintroduced to the Cedar River in late summer, 2020.



Image 13: New trough system installed in mussel research facility with novel rearing tank prototypes and basket systems being prepared to hold mussels from the DNR.



Image 14: Adaptations and new static systems to help raise mussels for a University of Minnesota research study.

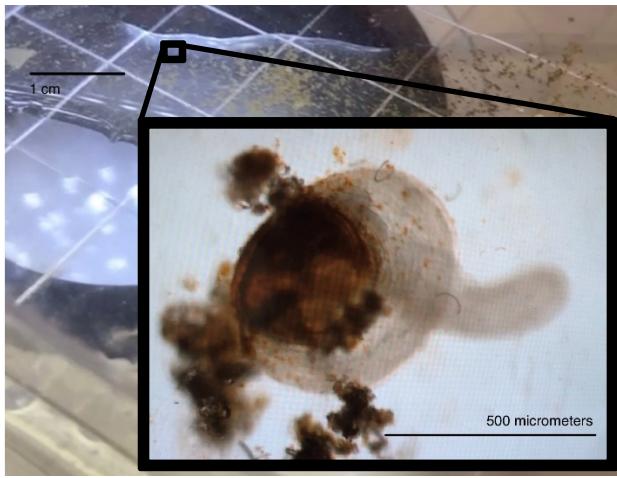


Image 15 (right): Actual size and inset of giant floater mussels under magnification. Visible is the golden shell from when the mussel fell from its host fish; clear shell that grew in the previous week; faint, vertical lines within the shell that distinguish the newly developed gills; and the well-developed foot that is sticky and essential for feeding at this stage.

Activity 1 Status as of December 1, 2020:

The Zoo's rearing systems have remained near capacity through fall, 2020. Improvements to quarantine protocols resulted in 100% survival and a clean inspection of newly acquired black sandshell, mucket, and Higgin's eye mussels (received in July from the DNR). Despite the cooler temperatures and reduced food availability during the fall, mussels in this group have already exhibited some growth over the last four months (Image 17). In addition to this mussel transfer, the DNR loaned the Zoo 10 fish baskets that were adapted for mussel rearing, further expanding our holding capacity.

Our husbandry system accommodating newly transformed individuals allowed us to rear giant floater mussels shortly after they fell off their host fish. The DNR coordinated the collection of host fish and adult female mussels with the University of Minnesota and produced the mussels that were transported to the Zoo in May. We anticipated low survival rates, but we successfully raised >7,000 of these microscopic mussels to an average size of 12 mm. A University of Minnesota graduate student studying how the morphology of these mussels differs with different environmental conditions marked and placed 6,000 individuals in streams and lakes throughout Minnesota (Images 18 and 19). Genetic samples were collected from a subset of each group, and ~1,000 giant floaters will remain at the Zoo in systems identical to those in which they were raised to provide a control for growth shape under laboratory conditions.

We are excited to have attained a benchmark of success that is at the heart of this program. In early October, about 1,100 black sandshells reared at the Zoo were released to the Cedar River in partnership with the MN DNR, marking our first cycle of head-starting and reintroducing mussels to the wild (Image 20). This contribution comprised about one-third of the total mussels in the reintroduction (the remainder were reared by the DNR near the release site). With this release, we are continuing to leverage our assets – specifically, a high quality water source, research facility, and husbandry expertise – to support the reintroduction goals of the DNR and improve their production capacity. We will coordinate with the DNR in early 2021 to plan rearing and research goals for the upcoming years.

We are currently housing ~5,500 mussels comprising 9 species; we are rearing >3,600 of these individuals to support the DNR's reintroduction efforts in 2021 and 2022.



Image 17: Mucket mussels received in July (left) and after four months at the Zoo (right). Pictures are cropped and scaled to show growth.



Image 18: Different body shapes resulting from Giant floater mussels growing in either stream or lake habitats.
Photo credit: Sean Keogh.



Image 19: UMN doctoral candidate Sean Keogh places black glue on Giant floater mussels at the Zoo. These mussels were placed in either streams or lakes to study how environmental conditions impact the way mussels grow.

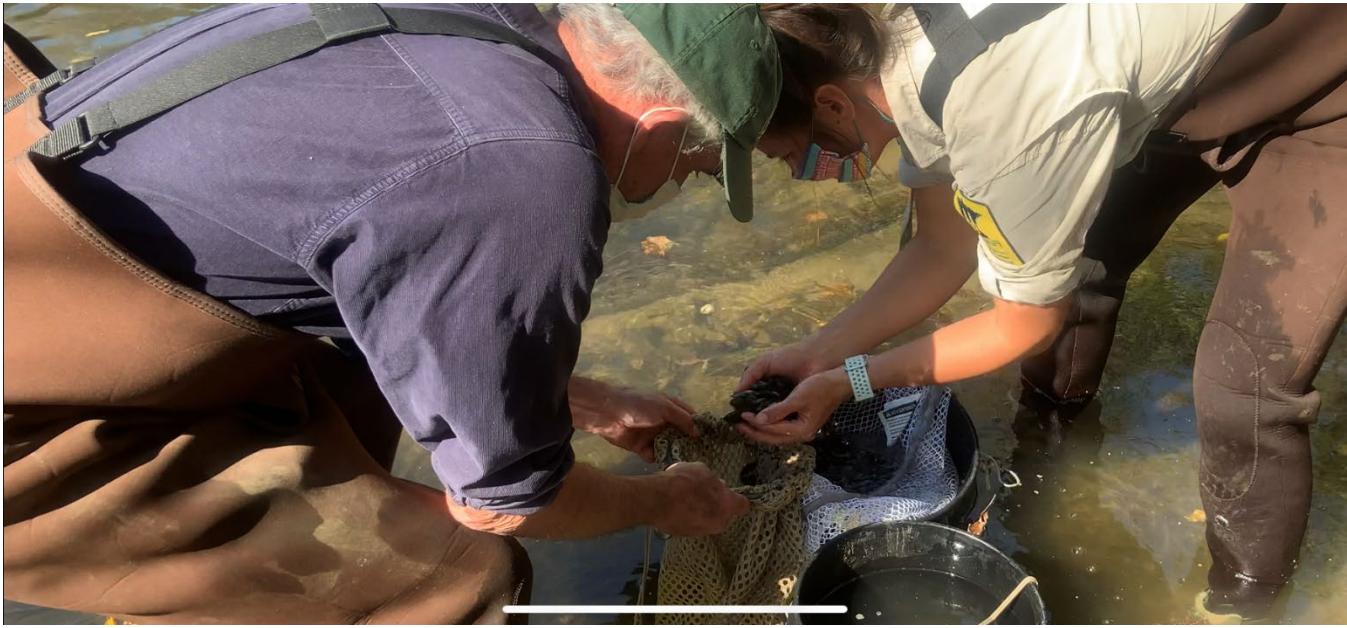


Image 20: Mike Davis (left) and Madeline Hayden (right) dividing black sandshell mussels into mesh bags so that the mussels may be distributed along a stretch of the Cedar River selected for reintroduction. The DNR recorded GPS locations of the upper and lower boundaries of the release sites to allow future assessment of survival and growth rates.

Activity 1 Status as of June 1, 2021:

We installed an electronic monitoring and control system in the mussel rearing and research facility, which allows us to continuously monitor and log water flow rate and temperature of incoming lake water. This important upgrade provides alerts if the system is operating outside of an acceptable range so that we can promptly correct potential issues. The control system also allowed us to automate critical steps in husbandry for the trough designed to rear newly transformed mussels. Specifically, a series of pumps are programmed to complete water changes and deliver micro-doses of highly concentrated prepared algae to the bucket systems maintained within the trough 4 times / day, providing essential food for the mussels. This automation replaces the manual labor that was required previously and frees staff to perform cleanings and focus on directly monitoring animal health via regular sample inspections under the microscope. As such, we are better able to monitor and track food uptake and respond to issues within the systems, and we have tripled the number of bucket systems used to raise newly transformed mussels, increasing our holding capacity of these microscopic individuals from ~14,000 to ~42,000. This expansion, in turn, has allowed the DNR to increase the number of mussels they rear. We anticipate receiving ~8,000 Snuffbox and ~5,000 Winged mapleleaf newly transformed mussels in June.

Juvenile Mucket and Higgin's eye mussels received in July, 2020, exhibited high survival rates and ongoing overwinter growth.

Final Report Summary:

With this project, the Minnesota Zoo established a goal of increasing our holding capacity for juvenile mussels from ~1,000 to 10,000 or more individuals, allowing us to better support conservation efforts by head-starting greater numbers of mussels and thus accelerating DNR-led reintroduction programs. This collaborative work seeks to recover depleted mussel populations and restore valuable ecosystem services like water filtration and sediment stabilization. With the support of the Environment and Natural Resources Trust Fund, we constructed additional holding systems and the infrastructure required to provide clean water and supplemental food to

head-started mussels. To further increase holding capacity, we also built holding systems for newly transformed mussels and currently are able to accommodate >65,000 newly transformed mussels. We further achieved our goal of releasing up to 1,000 individuals of a species per year in 2020 by providing >1,000 head-started black sandshell mussels to the DNR for reintroduction to the Cedar River.

Recently, we adapted surge bucket systems from designs for pulse-flow and static bucket systems; these new systems have allowed us to automate water changes with filtered water and food delivery to newly transformed mussels (Image 23). We are currently housing >25,000 mussels comprising 10 species; we expect that >8,000 of these individuals will survive to a size large enough to be integrated into the DNR's reintroduction efforts in 2022 and 2023.



Image 23: The surge bucket system allows for automated water changes in the buckets to provide new food and remove animal waste, creating optimal rearing conditions for newly transformed mussels.

ACTIVITY 2: Conduct research to optimize rearing protocols

Description:

Little is known about propagation techniques that best promote growth and survival in juvenile mussels. In collaboration with the Minnesota Department of Natural Resources, the Zoo has identified several key research questions that will inform mussel propagation and improve rearing protocols. Staff will systematically vary factors including over-wintering water temperatures, substrate (presence and type), and diet (e.g., food type and availability at different size classes) to understand their impacts on growth and survival. The Zoo will also examine the utility of alternative propagation systems (e.g., floating baskets). Since species' responses to treatments may vary, we will conduct research with up to four mussel species, as space and sample sizes permit. A subset of mussels (excluding the federally endangered species) will be used with these experiments.

Mussels will be individually marked or housed (to the extent possible) such that individual growth and survivorship can be monitored and compared across treatments. Marking individuals will provide for the most robust analyses and strongest inferences, and marking can facilitate tracking individual survival and growth after reintroduction. During the spring, summer, and fall, mortalities will be recorded weekly to monitor survivorship, and individuals will be measured monthly to estimate growth.

Funding from ENRTF will provide personnel and material support for implementing this research program. The construction of a new research and rearing pod will provide the controlled environment necessary for conducting this work and will contribute to the expansion of the Zoo's rearing capacity.

Summary Budget Information for Activity 2:

ENRTF Budget:	\$ 179,112
Amount Spent:	\$ 179,109
Balance:	\$ 3

Outcome	Completion Date
1. A new mussel pod is installed on Zoo site, increasing capacity to rear mussels in a controlled research environment.	11/30/2018
2. Experiments assessing factors that may impact mussel growth and survival are initiated.	5/15/2019
3. Mussel performance (growth and survival) is compared under different experimental treatments using statistical analysis.	5/1/2020
4. Rearing protocols are updated to reflect propagation protocols that optimize growth and survivorship of juvenile mussels.	6/30/2020

Activity 2 Status as of June 1, 2018:

We outlined needs for the mussel facility with Zoo operations and campus management staff. Following a competitive bidding process, we identified an architect to develop construction plans to build and outfit the pod with appropriate heating/cooling, water / sewer, electrical and data systems. Construction is expected to begin in July / August to install a concrete pad and tie in associated utilities; the facility will be completed during fall, 2018.

Mussel staff have completed plans for the installation of mussel systems and delivery and treatment of pond water to the facility. Incoming water will be filtered to 65 microns and treated with an ultraviolet filter to remove nuisance aquatic insects. Research systems are designed and being built to allow for variability in flow, substrate size and mix, temperature, and food size and density. Systems will have exact temperature control to replicate seasonal conditions, biofilters to maintain nitrogen waste at natural levels, modular hookups to change holding tanks, a supplemental food delivery system, and the ability to exchange substrate types. In particular, we hypothesize that substrate type may benefit the feeding strategy and orientation preference of juvenile mussels. We will adjust type, depth, and mix of substrates (while controlling all other variables) to determine if there is a significant difference in growth and survivorship.

We are also improving the air delivery system at the Main Lake systems that will allow for individual airflow control to each mussel holding system to analyze the effect of water flow on growth and development of mussels. Underwater video has been used to observe mussel behavior during different flow rates to inform how different species react to turbulence.

The Zoo's Legacy appropriation supported Zoo staff participation in the Freshwater Mollusk Health and Disease workshop. Presentations at this workshop offered insights for health considerations of mussels at reintroduction sites and in propagation facilities. Conversations enriched partnerships with other mussel propagation specialists that have helped guide research questions. Similarly, coursework on Risk Assessments and Reintroduction Plans informed additional checkpoints for quality assurance, and conversations with experts in the field have refined propagation design elements to offer more flexible housing that accommodate species-specific needs. Partners at the MN DNR, USFWS, USGS, Saint Anthony Falls Stream Lab, and various watershed

management organizations have offered their experiences to identify novel ways to track variables that mimic optimal natural environmental conditions throughout seasonal changes for different age groups of mussels.

Activity 2 Status as of December 1, 2018:

During summer, 2018, we hosted a series of planning meetings with the contracted architectural firm and mechanical engineers to establish specifications for the research facility and ensure that it would be outfitted with the necessary electrical, heating / cooling, and water / sanitary sewer systems. Construction plans were finalized during late summer, 2018, and we identified a construction contractor shortly thereafter through a competitive bidding process. The project was slightly behind schedule at this point (primarily due to staff turnover at the Zoo); unfortunately, the permitting process with State and local jurisdictions also took longer than anticipated. We discussed these delays with LCCMR staff during mid-November and presented a revised timeline, which identified the completion of the research facility by January 31, 2019 (with a target completion date of December 31, 2018), and installation of the rearing and research systems by February 28, 2019. This timeline allows one month for systems testing and adjustments, before the anticipated receipt of new mussels in April, 2019. We remain on-track to begin experiments to evaluate factors that may impact mussel growth and survival by mid-May, 2019.

The Zoo received the required construction permits in mid-November, and we broke ground on the project on November 19, 2018. To date, site preparation has been completed, and the footers and foundation of the facility have been poured. Construction progress is on-target with the revised timeline. In addition, the Zoo's Conservation staff have begun construction of the research and propagation systems for the new facility, which will be ready to install as soon as the internal water delivery and drainage systems are in place. The systems have been designed to provide flexibility and allow us to simultaneously test multiple parameters (e.g., flow rate, substrate, supplemental feed) that may impact mussel survival and growth.

The population of muckets at the Zoo (outlined in the Activity 1 Status Update) were uniquely identified with colored numeric tags that were glued to the shell to monitor individual growth rates. This approach is more statistically powerful than measuring a random sample of our population during each measurement session and generating a mean population growth rate. (Most propagation programs track growth using this latter approach.) For example, tagging will allow us to document whether different size-classes of individuals use resources or respond to the treatment variable(s) in different ways. Additionally, individually marking animals will facilitate tracking growth and survival post-release in native habitats.

Activity 2 Status as of June 1, 2019:

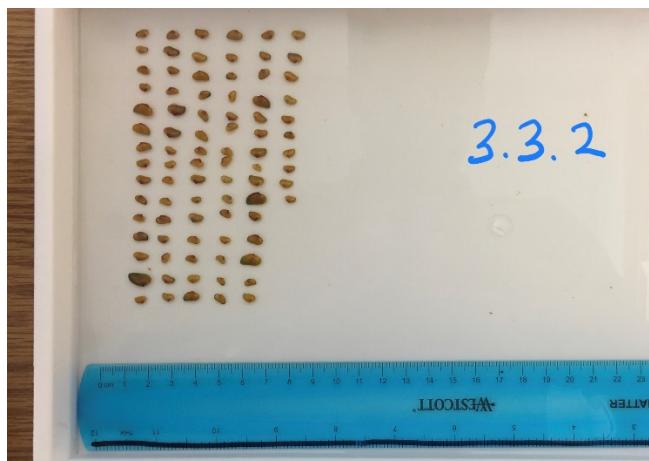
As outlined above, the research facility was constructed this winter, and we completed the installation of rearing and research systems in the new pod. We acquired ~2,000 black sandshell mussels and distributed these individuals among 24 replicate pans that were filled with one of four substrates: silt, sand, gravel, or a mixture of the three (Images 9 and 10). Each substrate 'treatment' includes 6 replicate pans. We measured initial lengths for each mussel and plotted the distribution of lengths by pan to facilitate estimation of growth (Image 11); at the end of the growing season (i.e., ~5 months), we will evaluate growth and survival to assess the optimal substrate for rearing juvenile black sandshells. We will compile preliminary results this fall, but the experiment will continue through the winter and into summer, 2020 to evaluate the impact of substrate on the growth and survival of larger animals.

Research systems allow for control of temperature, flow rate, feed amounts, turbidity, and percentage of lake water. Animals receive nutrition from lake water and supplemental feeds throughout the day. Lake water is heated to summer temperatures to match the conditions these mussels experienced during their first 8 months at the DNR facility and to extend the growing season. Conservation staff work daily to ensure the systems are running as intended, water quality and appropriate cleanliness are maintained, and fouling agents are excluded.

We also received >550 mucket mussels in April. These individuals were inspected and placed in the closed quarantine system, which does not allow water to flow back to A Lake. New batches of water are introduced daily, and the old water is discarded via the city sanitary sewer line. This is the first time we have relied on a non-lake-based food source (i.e., metered microalgae concentrate mixed with the recirculating lake water) to provide all nutrition to a group of mussels on site. Mussels were examined after 30 days (May 29th) and declared clean and healthy, tagged with individual numbers, measured, and placed in another research system (image 12). Modifications to the filtration assembly will be made based on the need to mediate waste (i.e., ammonia) accumulation in the system to benefit future quarantine groups.



Images 9 (left) and 10 (right): (9) Pans are set-up with silt, sand, pebble, and mixed substrate types. (10) The mixed substrate pans included relatively equal amounts of the three grades of substrate (shown prior to mixing).



Images 11 (left) and 12 (right): (11) Eight-month old black sandshell mussels are counted and measured prior to placing in one of 24 replicate pans, allowing us to examine the effect of substrate type on growth and survival. (12) Larger mucket mussels were individually tagged to facilitate tracking growth and survival in the different sediment treatments.

Activity 2 Status as of December 1, 2019:

We continued experiments to evaluate the impact of sediment type on black sandshell mussel growth and survival throughout the summer and into the fall. After black sandshell mussels reached an average size of ~25 mm (in early September, 2019), we placed unique bee tags on half of the individuals in each pan group to facilitate tracking individual metrics. Individually marking animals will increase our ability to evaluate the effects of different sediment treatments on mussel fitness. We hypothesized that the black sandshell mussels will survive at higher rates and show better growth rates in smaller grained sediments (silt and sand) than larger

grains (pebble and a mixture of silt, sand, and pebble). Preliminary data suggests that the mussels are growing in response to the substrate types as predicted, although we are not yet certain if these effects will be statistically significant (Table 1).

We are also documenting production of byssus as a proxy for mussel stress. We initially hypothesized that mussels with the poorest growth also will have lower byssus production because they are actively searching for new, more preferable substrate. Thus far, our data do not appear consistent with this hypothesis. Byssus is most commonly present on mussels reared in the mixed substrate (~73%), followed by pebble (68%), sand (65%), and silt (59%). We note, however, that these data are preliminary, and we recorded a high degree of variability within treatment groups. For example, byssus production ranged from 46% to 78% per pan for mussels reared in silt substrate. More rigorous analyses are needed to test this hypothesis. Additionally, as mussels grow, they gradually stop producing byssus, underscoring that individual size will need to be accommodated by future analyses. During the coming winter months, we will complete analyses to more comprehensively assess the relationships between substrate and survival, growth and byssus production for the mussels in our care.

Table 1: Average size of black sandshell mussels (mm) reared in four substrate types (silt, sand, pebble, and mixture) at the Minnesota Zoo, as documented during three measurement intervals approximately two months apart during 2019.

Substrate type	Average size in mm (Standard Deviation)		
	May, 2019	July, 2019	September, 2019
Silt	5.8 (1.1)	16.5 (3.2)	27.5 (4.2)
Sand	5.7 (1.1)	15.7 (3.5)	26.0 (4.5)
Pebble	5.8 (1.0)	15.4 (3.2)	25.2 (4.2)
Mixture	5.9 (1.2)	16.2 (3.3)	26.8 (4.7)

Activity 2 Status as of June 1, 2020:

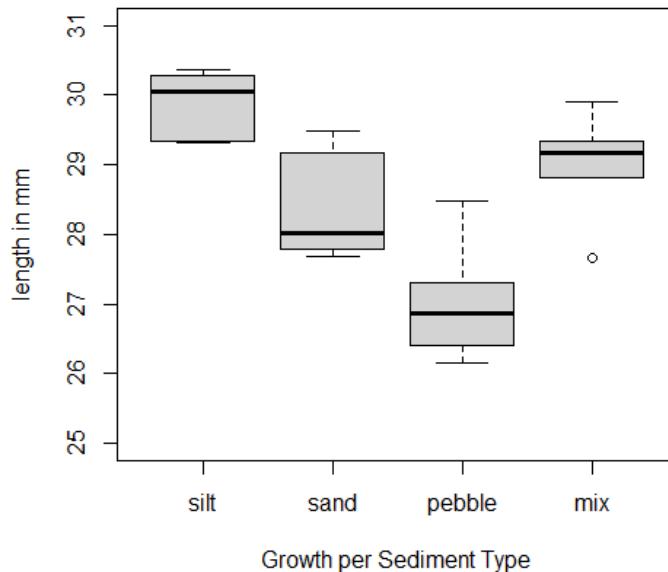
We collected final measurements for analysis of survival, growth and byssus production of black sandshell and mucket mussels in January, 2020. Analyses suggest better growth rates for individuals reared in silt sediment (Plot 1). Mucket mussels that were a year older and much bigger (i.e., individuals began this trial > 25 mm larger than the black sandshells) showed no difference in growth among different sediments used for rearing. We will continue to evaluate the performance of black sandshell mussels through summer, 2020 to evaluate if the improved growth rates in finer-grained sediments may be linked to the size and / or age-class of mussels. Going forward, we anticipate using silt for rearing black sandshell mussels to maximize growth, potentially allowing for earlier reintroductions.

Presence of byssus did not differ among the sediment types but did decrease over time. This was most pronounced for the black sandshell mussels (Plot 2), whereas mucket mussels were more consistent over time. However, both species exhibited reduced byssus production as mussels increased in size (Plot 3), which is consistent with the literature. Although previous studies suggest that mussels do not produce byssus once they are ~30 mm, we documented production of byssus in mussels up to 41 mm in size.

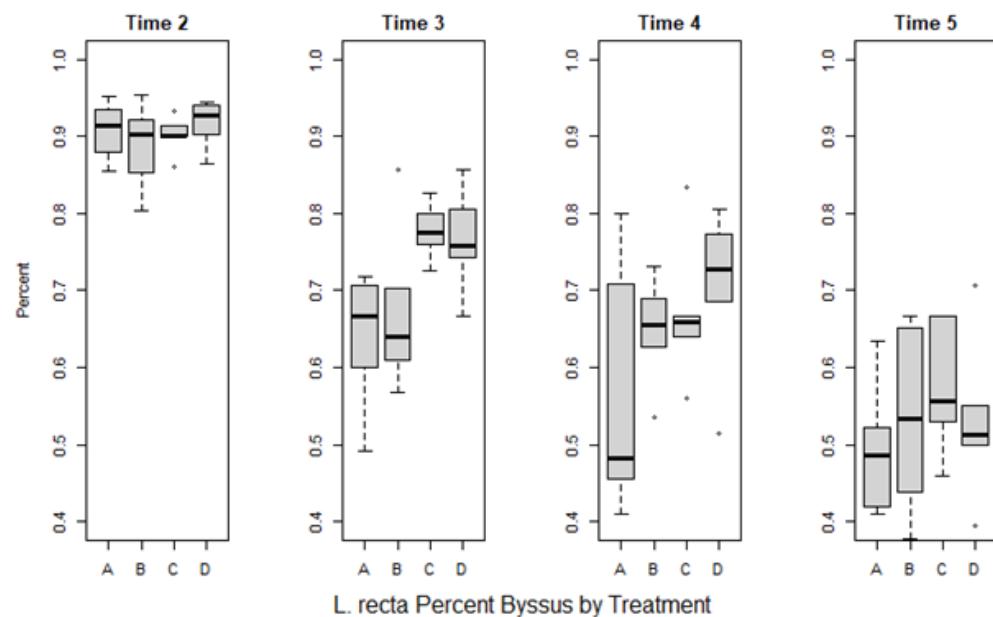
Recently, off-hours observations have suggested that our species of mussels tend to be very active at night. Early in the winter, we noticed that some individuals were unexpectedly on the substrate surface during night. We initiated daily logs to document mussel behavior (e.g., the number of individuals buried in the sediment; Image

16). Preliminary results suggest that mussels are ~5% more active at night. We will establish more rigorous protocols to quantify if this behavior is related to substrate type, temperature, season, or food availability.

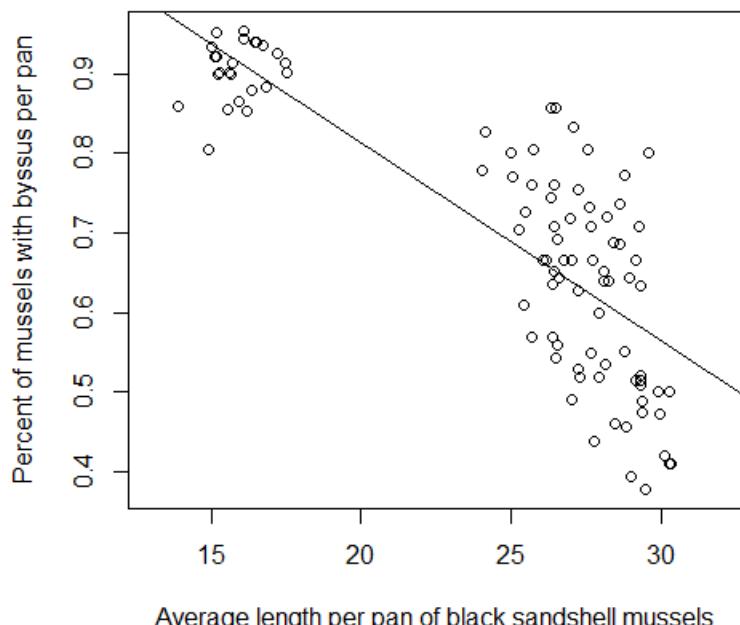
We have attained our goals of constructing our rearing facility, establishing replicate experimental systems, and completing experiments to examine best methods for rearing target mussel species, and we have used this information to revise our husbandry protocols.



Plot 1: Average length per pan of black sandshell mussels reared in different sediment treatments.



Plot 2: Percentage of black sandshell mussels (by pan) with byssus in different sediments at different time intervals. A: Silt; B: Sand; C: Pebble; D: Mixture.



Plot 3: Percent byssus plotted against average length of black sandshell mussels per pan, for sampling points 2 – 5. Fitted line shows diminishing byssus presence with increasing mussel size.



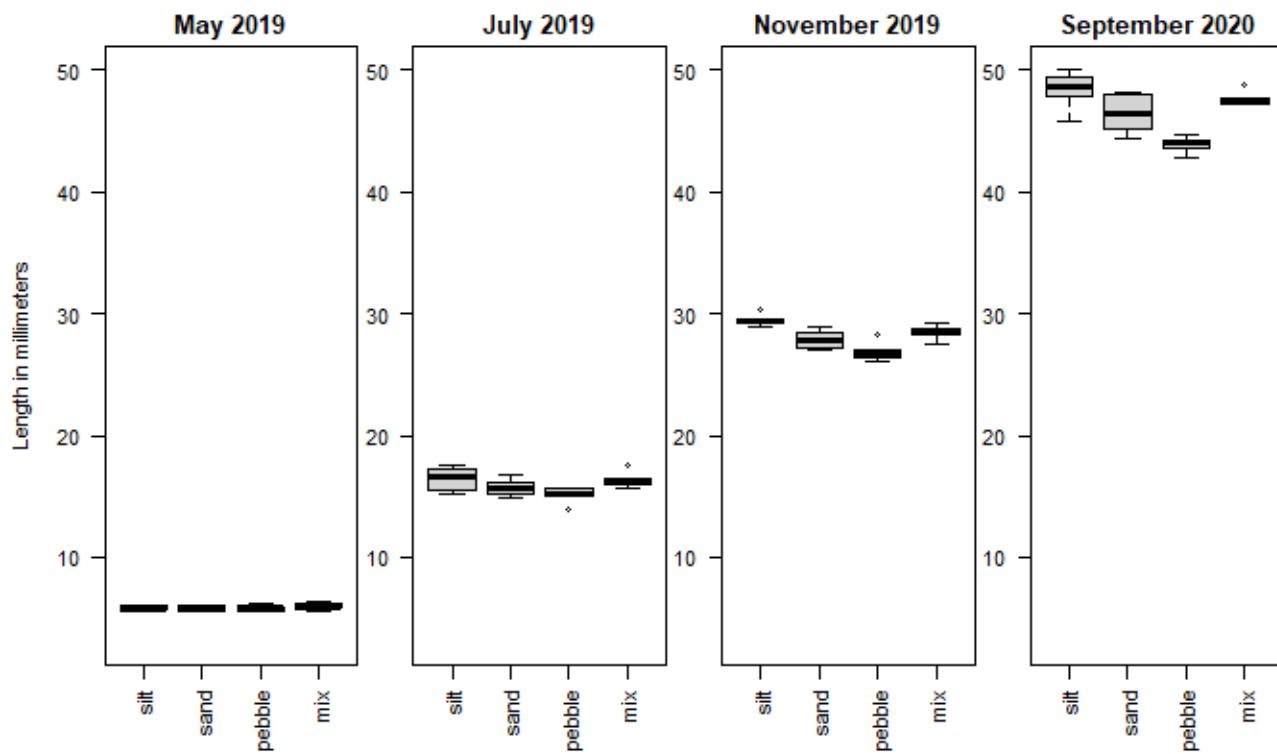
Image 16: Evening observation of mussels emerging from and moving around the substrate. We will analyze these data to determine if this behavior is related to substrate type, temperature, season, or food availability.

Activity 2 Status as of December 1, 2020:

Results from the second year of our study investigating the survival, growth and byssus production of black sandshell mussels in different sediment types indicate that individuals reared in silt continued to outgrow those reared in all other sediment types (Plot 4). We anticipate reanalyzing growth metrics in the coming months to

incorporate individual-based data, which is a more robust statistical approach. As possible, we also will evaluate recaptures of the black sandshell mussels released to the Cedar River during fall, 2020 to quantify reintroduction success and potential differences among initial rearing sediment types.

We placed the mucket and Higgin's eye mussels received from the DNR during summer, 2020 into a new experiment (Image 21). During spring, we observed that older, larger mussels reared at the Zoo were more active, resulting in cleaner sediments and holding systems, as the movement of mussels turned over the substrate. We hypothesized that this natural cleaning may benefit younger mussels reared at the Zoo that otherwise may be negatively impacted by overgrowth of bryozoans and accumulation of detritus. Additionally, our research (as well as that of other scientists) has demonstrated that sediment is important for the growth of juvenile mussels, perhaps because they derive essential nutrients from organic material on the sediment or the particles themselves. Adult mussel activity may create an environment that encourages the growth of beneficial algae and bacteria to supplement the dietary needs of juvenile mussels. As such, we established an experiment to evaluate if the presence of larger mussels improves the growth of juveniles. With the support of and assistance from the DNR, we placed mussels into pan and basket systems, with treatments including juvenile mussels placed with larger individuals and control groups comprised entirely of younger mussels. We will continue to collect data through 2021 and possibly into 2022, depending on when the juveniles attain a releasable size.



Plot 4: Boxplots summarizing average length per pan of black sandshell mussels reared in different sediment treatments from their acquisition in May, 2019 until their release to the Cedar River during fall, 2020.



Image 21: We established a new experiment to evaluate the impacts of housing different size classes of mussels in the same systems. We are rearing juvenile Higgin's eye and mucket mussels with adult plain pocketbook and mucket mussels.

Activity 2 Status as of June 1, 2021:

We completed additional analyses to investigate the effect of sediment type on growth and survivorship of juvenile Black sandshell mussels. This work supported previous findings that mussels reared in fine sand substrate exhibited higher growth rates than individuals reared in sand or pebble sediments. As such, we recommend using fine sand substrates for rearing Black sandshells at this life history stage.

We are not yet able to assess variation in survival and growth of juvenile mussels reared only with conspecifics of the same age relative to those reared with adult mussels. However, overhead photographs taken every two weeks illustrate a significant decrease in sediment on the surface of pans that house adults (Image 22). The highly active adults turn over the substrate and release settled organics back into the water column, resulting in much cleaner rearing tanks. This study will continue throughout the summer and yield additional data to help quantify the impacts of rearing mussels of different size classes together.

From this work, we developed a new study, specifically resulting from the inclusion of the adult Plain pocketbook mussels in research pans with juveniles. In late March, we observed that adult female mussels were displaying their mantle lures (used to attract potential fish hosts to carry their young larvae while they develop). This observation indicates that this group of adult Plain pocketbook mussels spawned during the fall of 2020, and adult female mussels developed their young in pouches nested in their gills over the winter. The lure of this species is well-documented, but there are conflicting reports regarding the timing (day or night), frequency, and longevity of display behavior. We installed infrared night vision trail cameras above pans to monitor the behavior of brooding females. Data collected from water temperature sensors and photos collected every 30 minutes will help us to better describe the activity of this species during brooding and identify seasonal variations in behavior and spawning. This information may help determine the best times to collect female mussels for spawning new broods and may suggest a preferred host fish whose feeding behavior matches the same rhythms as the mussel lure display (i.e., host fish species that could be stocked near reintroduction sites).



Image 22: Pans with adult mussels (left) are cleaned regularly by the active motion of adult mussels. Pans with only juveniles (right) tend to accumulate detritus. This study will evaluate the potential benefits conferred to smaller mussels by the removal of obstructions at the sediment surface.

Final Report Summary:

With the support of the Environment and Natural Resources Trust Fund, the Minnesota Zoo constructed a new mussel research and rearing building on Zoo campus. We outfitted this facility with mechanical and chemical water filtration systems and rearing systems to house mussels and facilitate replication for scientific experimentation. Our initial research project demonstrated that the inclusion of fine sand in rearing pans significantly improved the growth of juvenile Black sandshell mussels. As such, we have modified our rearing protocols to incorporate this finding, which we anticipate will produce larger individuals that are more suitable for reintroduction. We have since initiated a study evaluating the impacts of rearing larger mussels with smaller individuals, which we hypothesize may improve growth rates. We also are evaluating the lure flapping behavior of adult female Plain pocketbook mussels to improve our understanding of their breeding behavior and quantify their seasonal cycles. Continued research will inform and improve rearing methods and accelerate the reintroduction of native mussels to Minnesota's waterways, thereby improving water quality and creating habitat for fish and other wildlife.

ACTIVITY 3: “Show Us Your Mussels” Digital Media Challenge for High School Students

Description:

The Zoo will use its educational outreach expertise to recruit schools throughout Minnesota to participate in a project aimed at increasing Minnesotan’s knowledge of the importance of native mussels to water quality and encouraging actions to improve water quality in the state’s waterways.

The Zoo will develop materials and resources that align with Minnesota K-12 Academic Standards in Science (*interdependence among living systems* and *human interactions with living systems*) and in English Language Arts (*media literacy*). Resources will stress the critical role that native mussels play in maintaining aquatic ecosystem health and illustrate the positive actions and simple behavioral changes community members can make to benefit water quality. Materials will be provided to teachers from schools recruited from around the state to participate in the digital media challenge.

With guidance and support from the Zoo, students will further research local water quality issues and the role native mussels play in improving water quality. They will develop and deliver digital media campaigns — such as online videos, websites, and social media outreach — promoting mussel conservation, water quality awareness, and personal or community action. As a component of the challenge, students will be required to track and record the reach and impact of their campaigns, such as number of visits to a website over a period of time or number of views, ‘likes,’ or retweets of social media posts. Schools will be challenged to reach a large audience, and the five schools’ campaigns with the largest impact per year (based on the number of people reached) will be recognized with a free field trip to the Zoo. During this trip, students will visit our mussel rearing exhibit and

have an opportunity to directly interact with the animal care and conservation staff leading this project. One grand prize winner with the best campaign will earn the opportunity to participate in behind-the-scenes, hands-on mussel conservation work with project staff at the Zoo.

The Zoo will showcase winning projects on its website and will further promote mussel conservation through Zoo media-based educational outreach.

Summary Budget Information for Activity 3:

ENRTF Budget: \$ 75,413

Amount Spent: \$ 75,310

Balance: \$ 103

Outcome	Completion Date
1. 20 schools / 2,000 students recruited to create digital media projects.	12/31/2017
2. 20,000 community members reached by student digital media projects.	3/31/2018
3. 500 students visit the Zoo to view and participate in on-site mussel conservation work.	6/10/2018
4. 20 additional schools / 2,000 additional students recruited to create digital media projects.	12/31/2018
5. 20,000 additional community members reached by student digital media projects.	3/31/2019
6. 500 additional students visit the Zoo to view and participate in on-site mussel conservation work.	6/10/2019
7. 20 additional schools / 2,000 additional students recruited to create digital media projects (for a total of 60 schools / 6,000 students over the course of the project).	12/31/2019
8. 20,000 additional community members reached by student digital media projects (for a total of 60,000 community members reached over the course of the project).	3/31/2020
9. 500 additional students visit the Zoo to view and participate in on-site mussel conservation work (for a total of 1,500 students over the course of the project).	6/10/2020

Activity 3 Status as of June 1, 2018:

Contracting with Teachers and Development of Materials

In August, five high school teachers were selected through a competitive application process to help create materials in support of the *Show Us Your Mussels* digital media challenge and to provide feedback on the initial campaign design. These individuals represent rural and urban districts, private and public schools and teach subjects ranging from high school biology to language arts.

Teachers met with Zoo staff on September 30th, 2017, and provided input on the design of the challenge, received basic training on native freshwater mussels, and divided up tasks (i.e., developing supporting materials). The participants also agreed to pilot the project in their classrooms and provide feedback at the end of the year.

In late May, we held our last meeting with these partner teachers and received feedback on the digital media challenge, which will be integrated in the campaign during the coming year. Teachers have completed all requirements specified in the contract; funds are currently being distributed and will be reflected in the next project status update.

Updated Description of the *Show US Your Mussels* campaign

For the *Show Us Your Mussels* challenge, Minnesota high school students created original digital media campaigns informing the public about the impact of water quality on native mussels. The school groups that spread the message to the most people received complimentary field trips to the Minnesota Zoo including bussing, admission, parking and interactive sessions with the Zoo's mussel conservation staff.

Twelve schools registered for the Challenge before the deadline of December 15, 2017. Teachers received weekly emails with resources supporting the campaign, including webinars, PowerPoint slide presentations, and news articles focused on native freshwater mussels and water quality. In addition, participants were provided with materials developed specifically to support the Challenge such as Frequently Asked Questions, project description rubrics, and graphics. As part of the program, Zoo staff also made presentations to schools as requested.

At the end of January, participating teachers were sent a link for students to embed in their projects. Students then directed the public to go to the voting site and vote for their project; voting was open from Feb 1 – 28.

Results

During the open voting period, >63,000 votes were cast for student projects. Members of the public were only allowed to vote once per day for a particular project. The top five schools were:

Schools	Number Votes
Hmong College Prep Academy	29,099
Melrose High School	28,534
Minneapolis Washburn High School	2,331
Apollo High School	1,645
Orono High School	992

At the same time, students were tracking how many people they reached. This number was later self-reported through the Student Reflection Form. Not including voting, students reported reaching over 50,000 individuals with their projects during the course of the Challenge. The results from the top 5 schools were:

Schools	People Reached
Hmong College Prep Academy	20,000
Melrose High School	15,052
Minneapolis Washburn High School	3,105
Apollo High School	1,628
Orono High School	1,572

After completing the Challenge, nearly all students reported understanding why native mussels are important components of healthy aquatic ecosystems, as well as what the Zoo and our partners are doing to promote their conservation.

	Strongly Agree	Mostly Agree	Neither Agree or Disagree	Mostly Agree	Strongly Disagree
I understand why native mussels are important to the environment.	88%	11%	1%	0%	0%
I understand what I can do to save native mussels and improve water quality.	72%	28%	0%	0%	0%
I understand the difference between zebra mussels and native mussels.	85%	15%	0%	0%	0%
I understand what the Minnesota Zoo is doing to contribute to conservation and save species.	79%	21%	0%	0%	0%

Teachers also reported increasing their knowledge in these areas.

	Strongly Agree	Mostly Agree	Neither Agree or Disagree	Mostly Agree	Strongly Disagree
I understand why native mussels are important to the environment.	100%	0%	0%	0%	0%
I understand what I can do to save native mussels and improve water quality.	71%	29%	0%	0%	0%
I understand the difference between zebra mussels and native mussels.	100%	0%	0%	0%	0%
I understand what the Minnesota Zoo is doing to contribute to conservation and save species.	71%	29%	0%	0%	0%

Highlights of Student Projects

There were many projects that were unique and reached audiences with engaging and multifaceted approaches. For example, the Hmong Academy created a website in both English and Hmong. Because the Hmong have a culture of fishing, they tried to spread the word in their native language about how to take care of fishing boats and equipment to minimize the risk of spreading zebra mussels. In addition, this group created posters which they posted in local Asian grocery stores to spread the word within the community.

Apollo High School developed a website that included mussel memes, children books, videos and more. The integration of these platforms allowed them to reach a wide audience.

Field Trips

The top 5 schools received trips to the Minnesota Zoo, providing an opportunity for the students to get a first-hand look at the work that the Minnesota Zoo and our partners are conducting to help native freshwater mussels. Field trips were completed by the end of May, 2018. Transportation costs have been invoiced and will be reflected in the next project update.

Activity 3 Status as of December 1, 2018:

Updated Description of the *Show US Your Mussels Challenge*

We are excited to be engaging students and teachers for the second year of the *Show US Your Mussels Challenge*. In the Challenge, Minnesota High School students create original digital media campaigns informing the public about the impact of water quality on native mussels. The school groups that convey their messages to

the most people receive free field trips to the Minnesota Zoo including bussing, admission, parking, and interactive mussel conservation sessions.

Projects can be developed throughout the school year; however, the number of people reached is only tallied for the month of February to determine the Challenge winners. The Minnesota Zoo selects winning schools based on two criteria: 1) number of votes received and 2) how many people were reached. Students track how many people they reach and self-report this at the end of the Challenge by completing Project Reflection Form. Each project also embeds a link into their projects that directs the public to the Minnesota Zoo's website for voting.

Field Trips for 2017-2018 Winners

Last year's Challenge culminated with the five award-winning schools visiting the Zoo. ENRTF funds supported transportation costs via bussing, and the Minnesota Zoo provided admissions, parking, and staff time for interpretation. Each school received an interactive session at the Mussel Cabin focused on mussel conservation and celebrating the school's accomplishments.

School	Location of School	Date of Visit	Number of Students
Melrose High School	Melrose, MN	5/9/18	130
Hmong College Prep	St. Paul, MN	5/22/18	35
Washburn High School	Minneapolis, MN	5/1/18	80
Apollo High School	St. Cloud, MN	4/23/18	23
Orono High School	Orono, MN	4/27/18	35
TOTAL			303 students

Contracting with Teachers to Develop Curriculum

In June, 2018, five high school teachers were selected through a competitive application process to help create curriculum to support the *Show Us Your Mussel* Challenge. On Wednesday, June 27th the teachers met for an all-day planning and curriculum design session. This workshop included training with Zoo conservation staff about freshwater mussels and the Zoo's current conservation efforts. A detailed time line and deliverables were developed in order to ensure that materials were completed by the start of the 2018-2019 school year.

The curriculum is aligned to state and national standards. Lessons are designed to both support the Challenge and be utilized by teachers who are interested in teaching about mussels but unable to participate in the Challenge due to other barriers. Teachers can download all lessons for free by visiting mnzoo.org/digitalmussels/.

The *Show Us Your Mussels* curriculum includes the following:

Name of Lesson	Theme	Age Ranges	State Standards
1. Introduction to Native Freshwater Mussels	Ecology	4-12th grade	4.3.4.1.1 5.4.1.1.1 7.4.2.1.1 8.3.4.1.2 9.4.2.1.2
2. Aquatic Food Webs and Invasive Species	Food Webs Invasive Species	4-8th grade	5.4.2.1.1 5.4.2.1.2 7.4.2.1.1 7.4.2.1.2

3. Water Quality and Freshwater Mussels Lab	Water Quality	9-12th grade	9.1.1.1 9.1.1.2 9.1.3.4
4. Water Quality and Freshwater Mussels Lab (data given)	Water Quality	9-12th grade	9.1.1.1 9.1.1.2 9.1.3.4
5. Ecological Relationships	Symbiosis	9-12th grade	9.4.2.1
6. Who is this mussel pretending to be?	Mimicry	9-12th grade	9.4.3.3.5
7. What matters with missing mussels?	Nutrient Cycling	5-12th grade	5.4.1.1.1 7.4.2.1.1 9.4.2.1.2
8. Where are the zebra mussels?	Invasive Species	9-12th grade	9.4.2.1.1 9.4.2.1.2
9. Native and Zebra Mussels Population Dynamics	Invasive Species	9-12th grade	9.1.1.2.3 9.1.3.4.3 9.4.2.1.2 9.4.2.1.1 9.4.4.1.2 9.4.4.2.4 9.3.4.1.2
10. A River Runs Through It	Human Impact	4-12 th grade	4.3.4.1.1 8.3.4.1.2 9.4.2.1.2 9.4.4.1.2

Supporting materials were developed to help teachers implement projects in their classroom, including:

- Sample Project Description and Rubric
- FAQs for the Show Us Your Mussels Challenge
- How to use YouTube in the classroom (YouTube Story Board, Teacher Guide and Student Guide)
- ‘Tracking Website Visitors’ Teacher Guide
- Instagram Teacher Guide
- Checklist for students before they post their projects

Recruitment for 2018-2019 School Year

As of November 12, 2018, 10 schools had registered to compete in the Challenge, with a total of 660 students. We anticipate that additional schools will enroll before registration closes in mid-December. To date, outreach to teachers has included:

a. Promotional Materials

Postcards were sent to all high school science teachers in the state informing them of the Challenge and the professional development opportunity. In addition, we developed tabling materials for conferences and a 1-page fact sheet.

b. Social Media

Facebook posts were boosted to engage teachers who were already on social media. This increased our reach to >1,500 for informing our target audience about the professional development opportunity, and to >1,750 about the Challenge as a whole.

c. Professional Development for Teachers

On September 22, 2018, 10 teachers attended a half-day professional development workshop about native freshwater mussels and water quality at the Zoo. The goal of this free training program was to provide educators with the tools necessary to confidently teach this subject in their classrooms. The day included behind the scenes tours of the Zoo's mussel conservation initiative. Each participant received a printed copy of our mussel curriculum as well as tips on how to implement lessons in their classrooms. In addition, participants earned Continuing Education Units.

d. Outreach to Schools

We received feedback from teachers indicating that it was difficult to teach about mussels during the winter because students couldn't go outside and actually see them. As a result, we developed kits that include native freshwater mussel shells, marine clams and zebra mussels to help educators demonstrate the differences between these taxa to students. Teachers can check-out these teaching kits out for use with an introductory lesson. So far, we have piloted this approach with two schools.

Teachers also have inquired as to whether Zoo staff can visit their schools to kick-off the Challenge. To accommodate these requests, Zoo staff have presented mussel conservation content in two high schools, reaching more than 200 students. We also have received requests for mussel programming to be available when schools visit the Zoo. We piloted an interpretive program at the existing Mussel Cabin with a St. Paul school, reaching 131 students.

e. Tabling and Presentations at Conferences

This year we have highlighted the *Show US Your Mussels Challenge* in the following venues:

- Informational tables at:
 - Minnesota Field Trip Expo
 - Minnesota Educator Academy (MEA) Conference
 - MnSTA Conference on Science Education (MNCOSE)
- Presentations at:
 - Association of Zoos and Aquariums (AZA) National Conference
 - Carol Strecker, Director of Education, presented the *Show Us Your Mussels Challenge* results from the 2017-2018 school year at the poster session
 - Minnesota Independent School Forum STEM Conference
 - Liz Gilles, School Programs Supervisor, presented on Conservation in Water Systems and how to get involved in the Show Us Your Mussels Challenge
 - MnSTA Conference on Science Education (MNCOSE)
 - Kristi Berg, STEM Specialist, presented on Conservation in Water Systems and how to get involved in the Show Us Your Mussels Challenge
 - TIES 2018 Education Technology Conference (December 8-11)
 - Liz Gilles, School Programs Supervisor, will present a poster session on the Show US Your Mussels Challenge

Schedule of Activities for the Show Us Your Mussels Challenge during 2018-2019 School Year

Date	Activity
August 1, 2018	Registration opens for the Challenge
September 22, 2018	Free Professional Development for teachers on freshwater mussel conservation
December 14, 2018	Registration closes for the Challenge.
Late January, 2019	Teachers receive link to embed in student projects.
February 1-28, 2019	Site is open for voting.
March 8, 2019	Student reflection forms due to the Zoo via email.
March 12, 2019	Winners contacted by the Zoo.
April - June 2019	Field trips for winning schools.

Activity 3 Status as of June 1, 2019:

Summary of Participation in the Show Us Your Mussels Challenge

	2017-2018	2018-2019	2019-2020 ¹
Schools Registered	12	14	5
Students Registered	612	708	273
Public Reached by Projects	46,052	49,112	-
Winning Students visiting Zoo on Field Trips (not including ~150 student participants from the School for Environmental Studies, which is located on Minnesota Zoo site)	303	284	-

¹ Registration opened for the 2019-2020 Challenge on May 15, 2019 and will close on December 20, 2019. The numbers in red indicate registrants to date for next school year's Challenge.

Updated Description of the Show US Your Mussels Challenge

In the *Show Us Your Mussels Challenge*, Minnesota high school students created original digital media campaigns informing the public about the impact of water quality on native mussels. The school groups that shared the message with the most people received free field trips to the Minnesota Zoo including bussing, admission, parking and interactive sessions with our mussel education staff. Teachers were invited to attend a free professional development in September to learn about mussels, conservation and receive a free copy of 10 lesson plans specifically designed to support teaching about native freshwater mussels and water quality in the classroom.

Fourteen schools registered for the Challenge before the December 14, 2018 deadline. Teachers who registered received weekly emails with the resources developed to support the Challenge, including webinars, PowerPoints, news articles, and other content specifically about native freshwater mussels and water quality. In addition, participants were provided with materials developed specifically to support the Challenge such as Frequently Asked Questions, project description rubrics, and graphics.

As part of the program, Zoo staff also visited classrooms upon request to introduce the topic to students. In late January, participating teachers received a link for students to embed in their projects. Students then directed the public to visit the website and vote for their project. From February 1 – 28, the public was able to vote for participating projects.

School of Environmental Studies

The School of Environmental Studies is a high school for juniors and seniors located on the Zoo's campus. The entire senior class (~150 students) participated in their own version of the *Show Us Your Mussels Challenge* in the fall. This included receiving technical support (e.g., meetings with mussel conservation and education staff), visiting facilities, and hosting an online platform for voting. Their numbers are not presented below as they competed at a different type time of year and were not eligible for the final prize.

Results

This year, we changed the voting program in order to make voting more selective and to decrease the likelihood of impropriety. This meant that only one vote cast per IP address in a 24-hour period and that cell phones would not register for voting. The top five vote-receiving schools were:

Schools	Votes
Apollo High School	467
Cretin-Derham High School	357
Melrose High School	252
AFSA High School	180
Washburn High School	154

At the same time, students tracked how many people they reached. This number was later self-reported through the Student Reflection Form. Not including voting, students and teachers reported reaching ~40,000 individuals with their projects during the course of the Challenge. The top five schools in terms of reach were:

Schools	People Reached
Melrose High School	16,097
Washburn High School	7,480
Apollo High School	1,408
Cretin-Derham High School	1,045
AFSA High School	810

A combination of votes cast and the total public reached was used in order to determine the final winners. These schools were invited to the Zoo for a free field trip and mussel session:

- 1) Melrose High School

- Apollo High School
- 2) Cretin-Derham High School
 - Washburn High School
 - 3) AFSA High School
 - 4) Washington Technology Magnet

Field Trips

As winners of the Show Us Your Mussels Challenge, the following school participants received free trips to the Minnesota Zoo including admission, parking, bussing and a mussel program at the mussel biology lab led by Zoo educators.

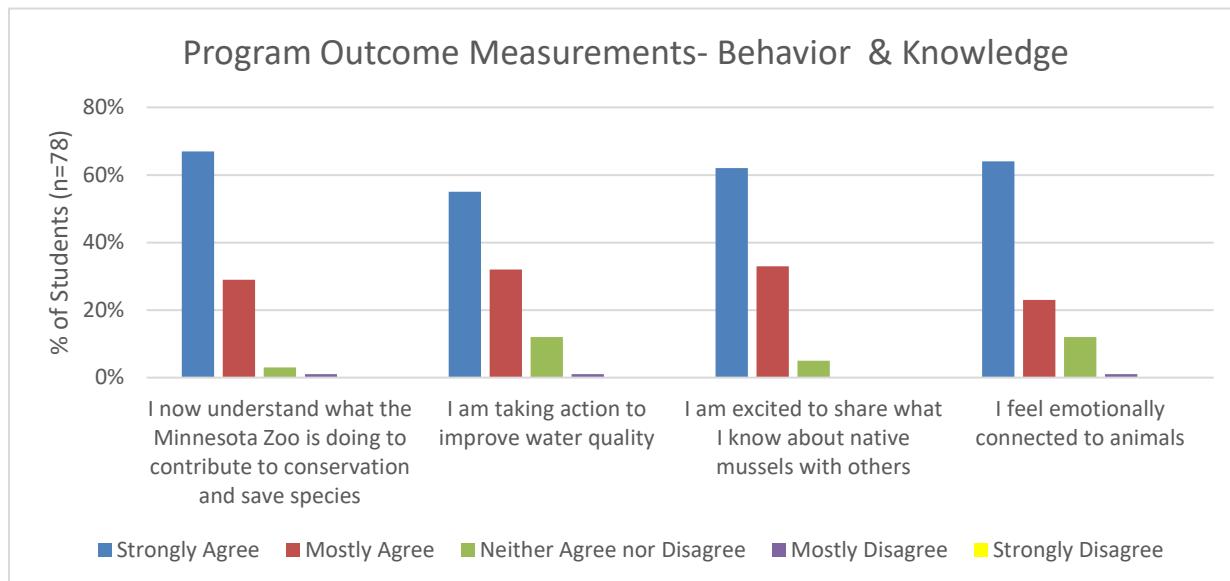
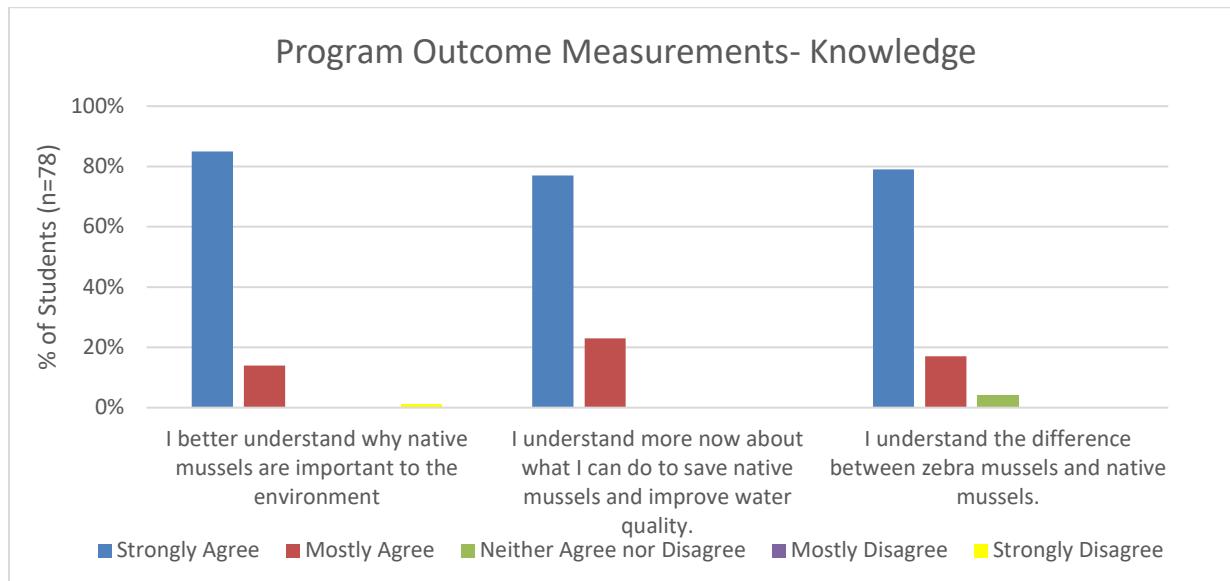
School	Date	Number of Students
Washburn High School, Minneapolis	4/23/19	68
Cretin-Derham Hall, St. Paul	4/29/19	8
AFSA High School, Vadnais Heights	5/2/19	24
Apollo High School, St. Cloud	5/6/19	78
Washington Technology Magnet, St. Paul	5/7/19	45
Melrose High School, Melrose	5/15/19	61

Highlights of Student Projects

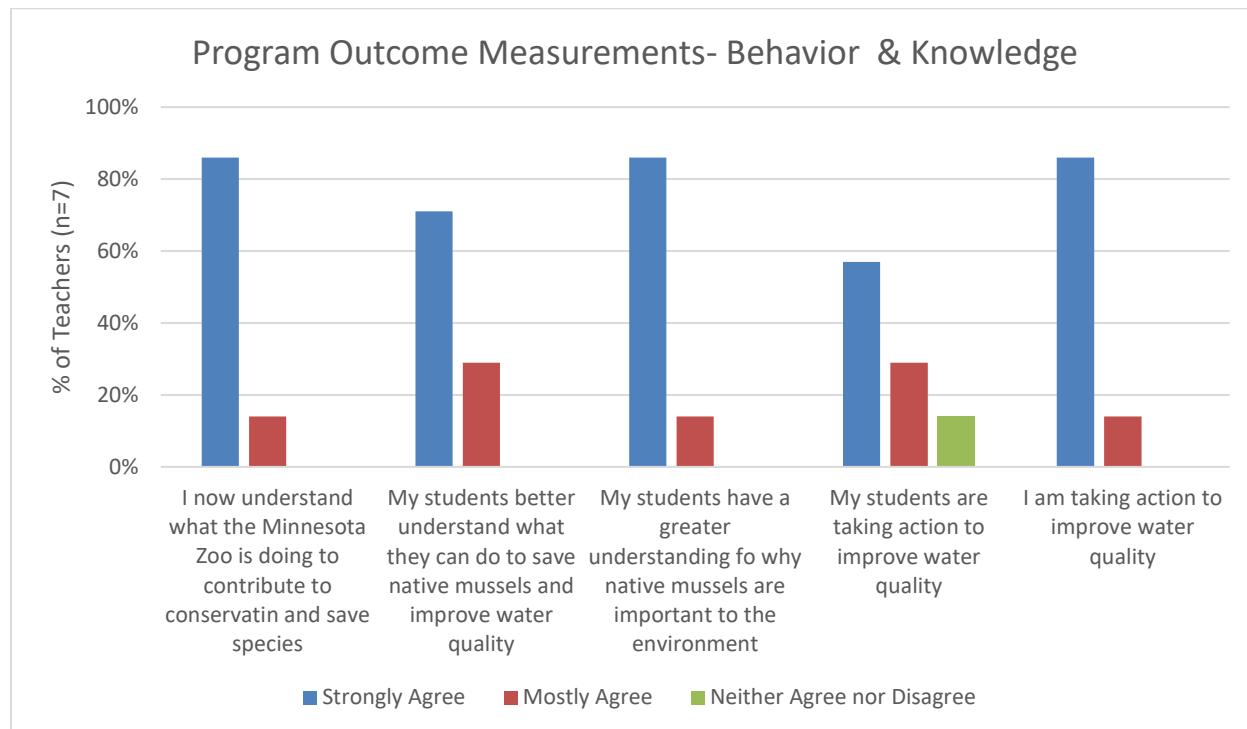
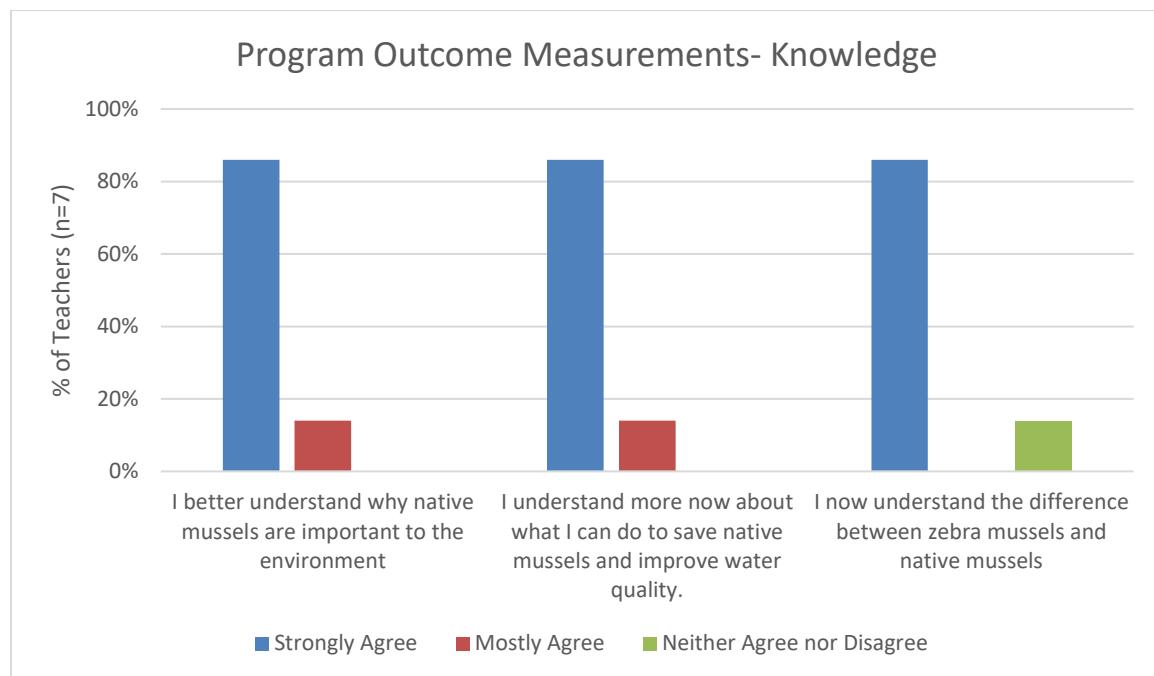
There were numerous projects that were unique and reached audiences using engaging and multi-faceted approaches. Many students created original artwork, songs, websites and videos; one group of students created a website completely in Hmong. Project examples are included in the attachment.

Evaluation Data

Overall, students self-reported increases in knowledge about mussels, water quality, and the role of the Minnesota Zoo in conservation. Students seemed excited to share what they know, and some students are taking actions to directly improve water quality. All of these measures demonstrate success at achieving program goals.



All of the teachers who responded to the post-survey indicated interest in participating in the Show Us Your Mussels challenge again, suggesting high satisfaction. Teachers also reported increases in knowledge about mussels, water quality, and the role of the Minnesota Zoo in conservation.



Recruiting for 2019-2020 School Year

In order to increase our reach, we explored including middle school teachers in the 2019-2020 Challenge. A survey was distributed to >200 middle school teachers and administrators. About 64% of

respondents reported being either very or extremely interested in participating in next year's Challenge. Based on this data, we would like to pilot the Challenge to middle school participants next year, with the aim of expanding it further in future years.

Activity 3 Status as of December 1, 2019:

Summary of Participation in the *Show Us Your Mussels Challenge*

	2017-2018	2018-2019	2019-2020¹
Schools Registered	12	14	12
Students Registered	612	708	725
Public Reached by Projects	46,052	49,112	-
Winning Students visiting Zoo on Field Trips (not including ~150 student participants from the School for Environmental Studies, which is located on Minnesota Zoo site)	303	284	-

¹ Registration opened for the 2019-2020 Challenge on May 15, 2019 and will close on December 20, 2019. The numbers in red indicate registrants to date for the current school year's Challenge.

Updated Description of the *Show Us Your Mussels Challenge*

For the third consecutive year, we are educating and engaging students and teachers in mussel conservation via the *Show US Your Mussels Challenge*. During the 2019 – 2020 academic year, we have increased the potential reach of the program by expanding the challenge to include middle schools as well as high schools. Participating Minnesota students design and develop original digital media campaigns to inform the general public about mussel conservation and the impact of water quality on native mussels. School groups that share their messages with the greatest number of people receive free field trips to the Minnesota Zoo to learn more about the state's native mussels and view the Zoo's conservation activities first-hand.

Although projects can be developed throughout the school year, the number of people reached will only be tallied during February and early March. The Zoo selects winning schools based on 1) how many votes a school has received and 2) how many people were reached. Students track how many people they reach and self-report their reach at the end of the Challenge. Each project also embeds a link into their projects that directs the public to the Minnesota Zoo's website for voting.

Contracting with Teachers to Develop Curriculum

In June, 2019, five high school teachers were selected through a competitive application process to help create curriculum to support the *Show Us Your Mussel Challenge*. Teachers met with the Zoo's Education staff for an initial planning and curriculum design session, which included training on native mussels and our current conservation efforts with the Zoo's Conservation staff. We developed a detailed timeline and associated deliverables to ensure that materials were completed by the start of the 2019-2020 school year.

The curriculum is aligned to current state and national standards in English Language Arts, Humanities and science. Lessons are designed both to support the Challenge and to be utilized by teachers who are interested in educating students about mussels but unable to participate in the Challenge due to other barriers. All lessons may be freely downloaded at mnzoo.org/digitalmussels. Ten additional lesson plans were developed this year and will be added to the website following formatting, bringing the total number of lessons to 20.

This year, the curriculum developed for the Challenge included:

Name of Lesson	Skills/Themes	Age Ranges	Type of Standards Addressed
11. Historical Impacts to Mussels	Using evidence to support a claim	6-12 th Grade	Social Studies and science
12. Minnesota's Most Endangered Animals	Analyzing and interpreting data	6-12 th grade	Science
13. Community Action	Persuasive writing	5-9 th Grade	English Language Arts and science
14. River Predictions and Inferences	Supporting conclusions with evidence	6-8 th Grade	English Language Arts and science
15. Limnology	Intro to study of freshwater ecosystems	9-12 th Grade	Life Science
16. Pet Waste Discussion	Interpreting graphs	9-12 th Grade	Life Science
17. Homeostasis	Lab/investigation	9-12 th Grade	Life Science
18. Climate Change	Using evidence to support a claim	9-12 th Grade	Life Science
19. Are there mussels near your school?	Lab/investigation	6-12 th Grade	Life Science
20. Mussel Investigation and Research (modified for universal abilities)	Student led research	6-12 th Grade	Life Science

Summary of Recruitment for 2019-2020 School Year

As of November 12, 12 schools were registered to compete in the Challenge, including 725 students. To date, our outreach to teachers has included the following:

Print Promotional Materials

Postcards were sent to all high school science teachers in the state informing them of the Challenge and of the professional development opportunity.

Email Newsletters

E-newsletters were sent to both high school and selected middle school teachers to inform them about the project.

Social Media

Posts to the Minnesota Zoo for Educators (MNZOOEdu) page 2-4 times per month, raising awareness about native freshwater mussels and how to becoming involved in the Challenge.

Professional Development for Teachers

On Saturday, September 21, 2019, 6 teachers attended a 4-hour professional development course at the Zoo about native freshwater mussels and water quality. The goal of this free training was to provide teachers the tools necessary to confidently teach about this subject in their classrooms. The day included behind the scenes tours of the Minnesota Zoo's native freshwater mussel program. Each participant received a printed copy of our mussel curriculum as well as tips on how to implement

lessons in their classrooms. Participants also received Continuing Education Units and could request reimbursement for travel to and from the Zoo.

Outreach to Schools

Feedback we received from teachers indicated that it was difficult to teach about mussels during the winter because students couldn't go outside to see them. In response, we developed kits that include shells of native freshwater mussels, marine clams and zebra mussels to help teach students about differences in their biology and life histories. Teachers can check-out these kits from the Zoo and return them after an introductory lesson. In addition, teachers have contacted us to inquire if Zoo staff are available to visit schools to help kick-off the Challenge.

Tabling at Conferences

This year we have highlighted the *Show US Your Mussels Challenge* in the following venues:

- Informational tables at:
 - Minnesota Field Trip Expo
 - Minnesota Educator Academy (MEA) Conference
 - MnSTA Conference on Science Education (MNCOSE)
 - Transcending Boundaries Conference
 - Impact Education Conference (formerly known as TIES)

Schedule of Activities for the Show Us Your Mussels Challenge during 2019-2020 School Year

Date	Activity
April, 2019	Registration opens for the Challenge
September 21, 2019	Free Professional Development for teachers on freshwater mussel conservation
December 20, 2019	Registration closes for the Challenge.
End of January, 2020	Teachers receive link to embed in student projects.
February 3 – March 2, 2020	Site is open for voting.
March 10, 2019	Student reflection forms due to the Zoo via email.
March 16, 2019	Winners contacted by the Zoo.
April - June 2019	Field trips for winning schools.

Activity 3 Status as of June 1, 2020:

Summary of Participation in the *Show Us Your Mussels Challenge*

	2017-2018	2018-2019	2019-2020	Total
Schools Registered	12	14	18	44
Students Registered	612	708	867	2,187
Public Reached by Projects	46,052	49,112	50,610	145,774

Winning Students visiting Zoo on Field Trips (not including ~150 student participants from the School for Environmental Studies, which is located on Minnesota Zoo site)	303	284	555 ¹	1,14
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¹ Due to the COVID-19 pandemic, students were provided with virtual tours of the mussel facility at the Zoo in lieu of field trips.

Updated Description of the *Show US Your Mussels Challenge*

In the *Show Us Your Mussels Challenge*, Minnesota middle and high school students create original digital media campaigns informing the public about the impact of water quality on native mussels. The school groups that get the message out to the most people receive a free field trip to the Minnesota Zoo including bussing, admission, parking, and interactive sessions with our mussel education staff. Each year, teachers are invited to attend a free professional development in September to learn about mussels and conservation of our aquatic resources; they receive a free copy of lesson plans specifically designed to support teaching about native freshwater mussels and water quality in the classroom.

Eighteen schools registered for the Challenge before the deadline of December 20, 2019. Teachers who registered received weekly emails with resources developed to support the Challenge. Resources included webinars, PowerPoints, news articles, and other content about native freshwater mussels and water quality. Also, participants were provided with materials developed specifically to support the Challenge such as Frequently Asked Questions, project description rubrics, and graphics. As part of the program, Zoo staff also visited classrooms and introduced the topic to schools that requested it. At the end of January, participating teachers received a link for students to embed in their projects. Students then directed the public to visit the voting site and vote for their projects.

School of Environmental Studies

The School of Environmental Studies is a high school for juniors and seniors located on the Zoo's campus. The entire senior class (~150 students) participated in their own version of the *Show Us Your Mussels Challenge* in the Fall, which included technical support such as special visits with mussel conservation and education staff, visiting facilities, and hosting an online platform for voting. Their numbers are not recorded below as they competed at a different time of year and were not eligible for the final award

Results

From February 3 – March 2, the public cast votes for participating projects. The ten schools receiving the most votes were:

Number of Votes Cast for Top Ten Schools	
School Name	Votes
1. Tracy High School	2,188
2. Melrose Area Public High School	2,057
3. Heritage E-STEM Middle School	2,007
4. St. Cloud Apollo High School	1,922
5. Minneapolis Washburn High School	1,897
6. DaVinci Academy	782
7. Brooklyn Middle STEAM School	721
8. Cretin-Derham Hall	410
9. Washington Technology Magnet	156
10. Humboldt High School	115
TOTAL	10,248

Students also tracked how many people they reached through their projects. This number was later self-reported through the Student Reflection Form. Teachers and students reported reaching almost 40,000 members of the community. Again, here is a breakdown of the top ten schools by voting:

Number of Community Members Reached by Top Ten Schools	
<i>School Name</i>	<i>Number reached</i>
1. Minneapolis Washburn High School	12,327
2. Tracy High School	12,292
3. Heritage E-STEM Middle School	7,772
4. Melrose Area Public High School	4,545
5. St. Cloud Apollo High School	1,105
6. Cretin-Derham Hall	228
7. Brooklyn Middle STEAM School	100
8. DaVinci Academy	46
9. Washington Technology Magnet	30
10. Humboldt High School	0*
TOTAL	38,445

*Did not provide evidence in reflection form

A combination of votes cast and the number of general public reached was used to determine the final winners. Normally, the top five schools would have been invited to the Zoo for a field trip, but due to COVID-19, we made alternative plans. The top ten schools by combined voting and reach were:

Top Ten Schools with Combined Voting and Community Reach			
<i>School Names</i>	<i>Votes</i>	<i>Reach</i>	<i>Combined</i>
1. Tracy High School	2,188	12,292	14,480
2. Minneapolis Washburn High School	1,897	12,237	14,134
3. Heritage E-STEM Middle School	2,007	7,772	9,779
4. Melrose Area Public High School	2,057	4,545	6,602
5. St. Cloud Apollo High School	1,922	1,105	3,027
6. Brooklyn Middle STEAM School	721	100	821
7. DaVinci Academy	782	46	828
8. Cretin-Derham Hall	410	228	638
9. Washington Technology Magnet	156	30	186
10. Humboldt	115	0*	115

*Did not provide evidence in reflection form

Highlights of Student Projects

Students used a variety of platforms to share their projects including websites, YouTube, Instagram, Tic Tac, Twitter, and Google Slides. Samples of projects that teachers were particularly proud of can be found at <http://mnzoo.org/show-us-mussels-challenge-2/>

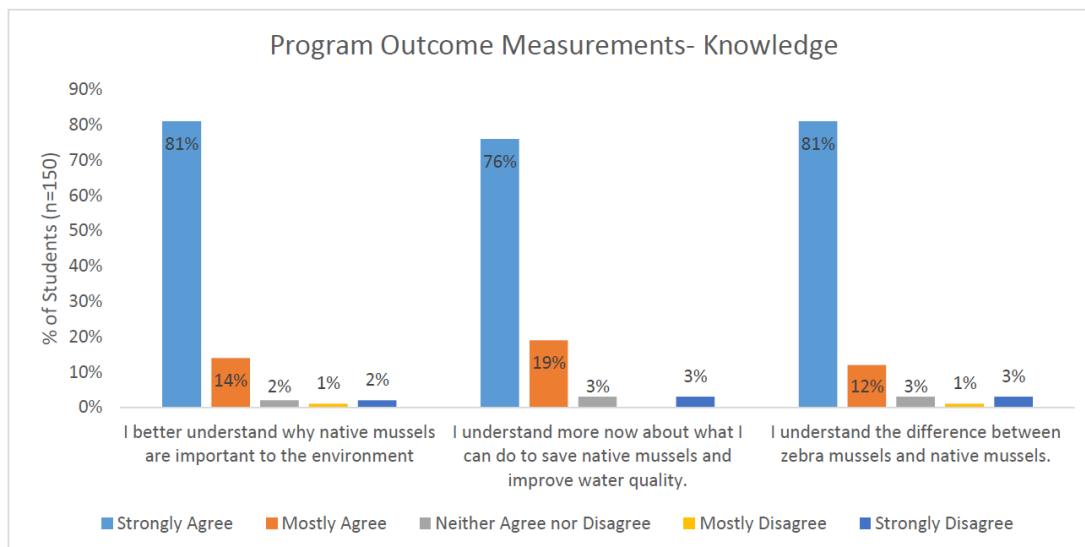
Field Trips for Winning Schools

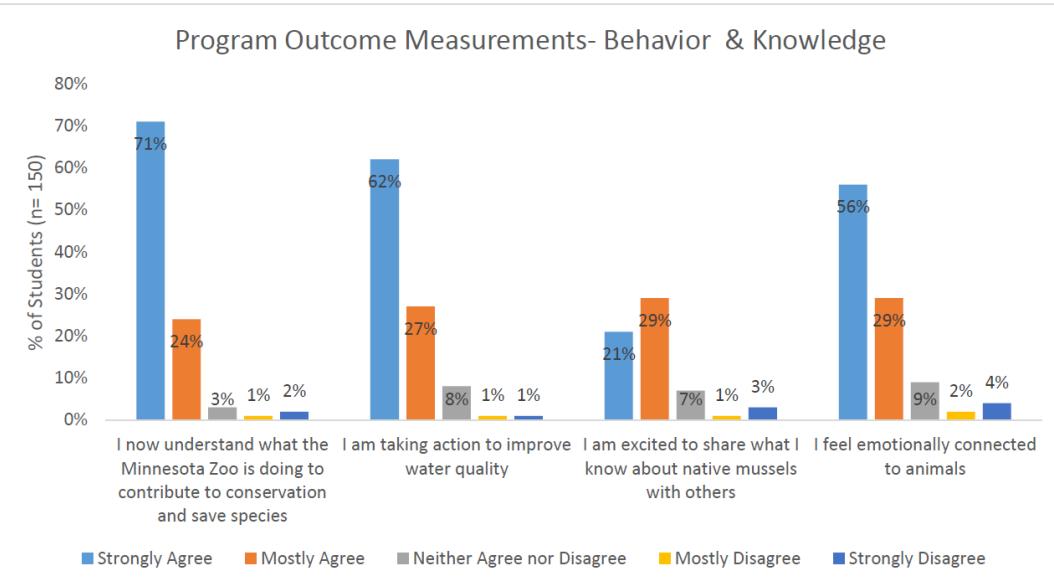
We originally planned to notify the top five schools on March 16, 2020, and invited them for a field trip to the Minnesota Zoo between April and June. However, on March 14, 2020, the Minnesota Zoo closed to the public due to the COVID-19 pandemic and remains closed..

As we recognized that an onsite field trip would not be possible this year, Zoo Education staff identified other ways to celebrate teacher and student success and continue learning related to mussels and water quality. First, we decided that we would provide virtual resources to all top ten schools this year. We created and distributed certificates to all participating schools to share with their students. We also created a Facebook video celebrating the schools' successes and posted it on the Minnesota Zoo's primary Facebook page, which has over 170,000 followers. We tagged the participating schools to ensure visibility to teachers and administrators alike. Next, Zoo Conservation staff created a virtual tour of our mussel facility. This link was provided to all teachers for use in their classrooms. Finally, we adapted several of the recently developed mussel lesson plans for use in distance learning and distributed them to teachers, providing resources to allow teachers to continue to teach about mussels remotely.

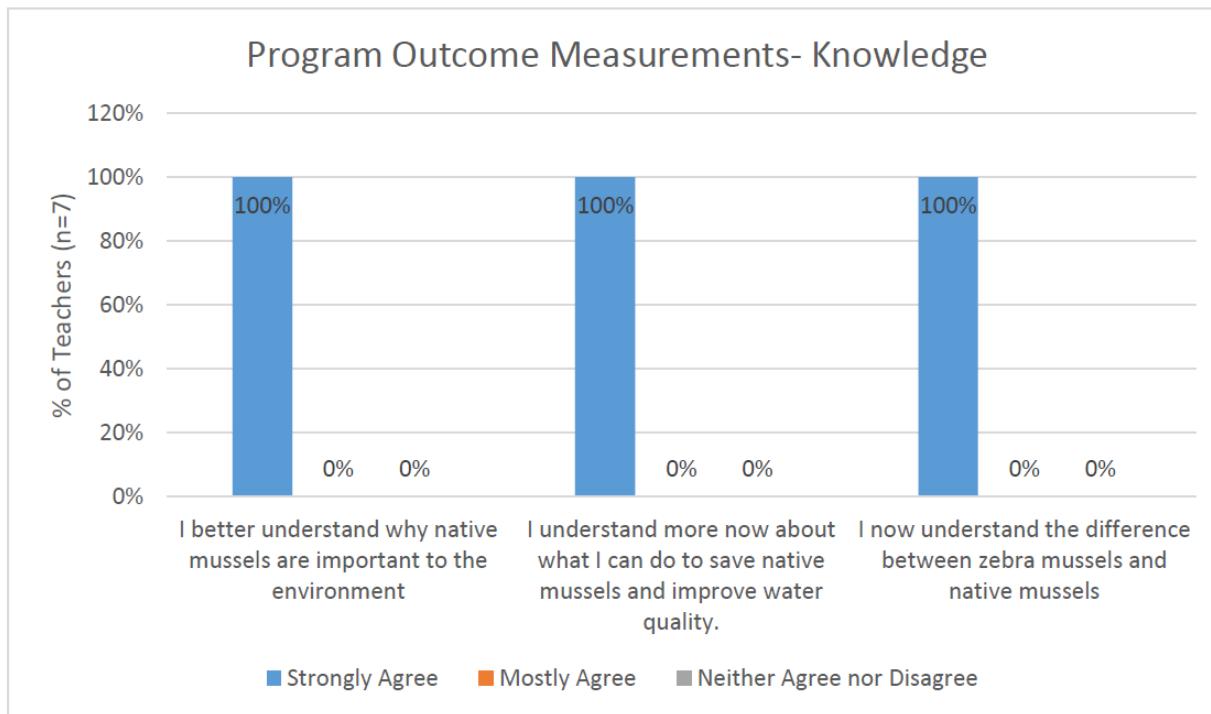
Evaluation Data

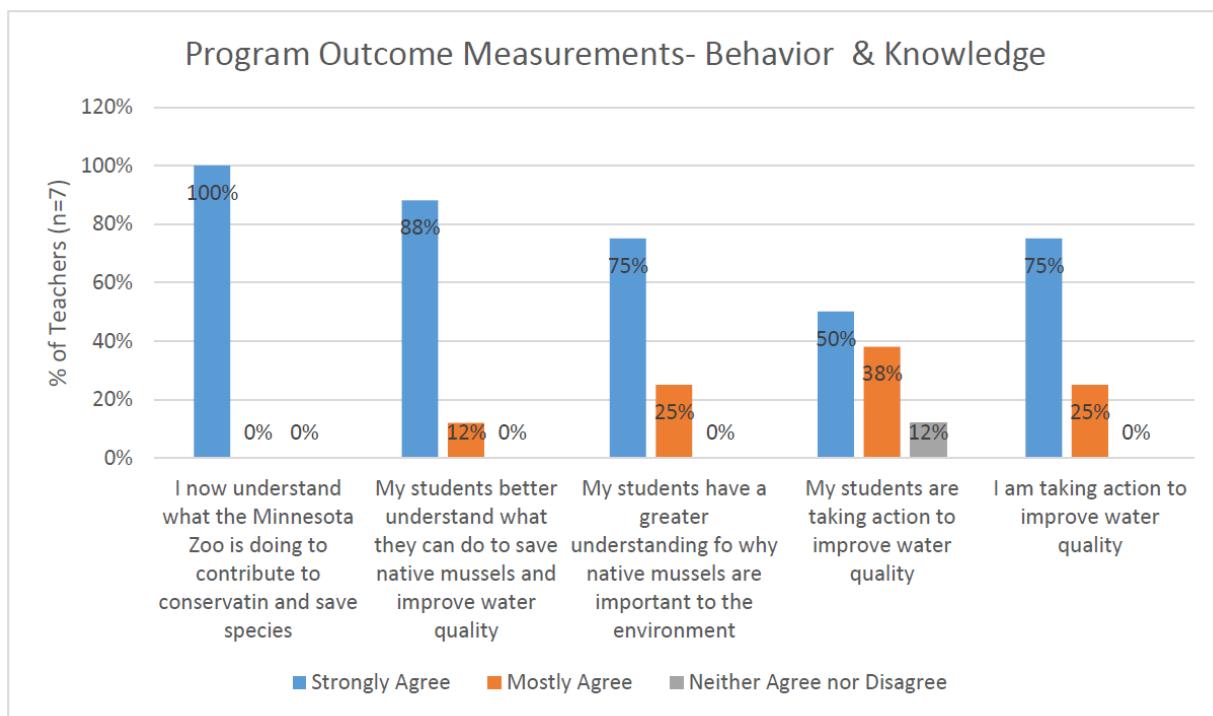
Overall, participating students report increases in knowledge about mussels, water quality, and the role of Minnesota Zoo in conservation. Students seem excited to share what they know, and some students are taking actions to directly improve water quality. All of these measures demonstrate success in achieving program goals.





All of the teachers who responded to the post-survey indicated an interest in participating in the challenge again, suggesting high satisfaction. Teachers also reported increases in knowledge about mussels, water quality, and the role of Minnesota Zoo in conservation.





Activity 3 Status as of December 1, 2020:

Because it was not possible for students participating in the *Show Us Your Mussels* challenge during the 2019 – 2020 academic year to complete field trips to the Zoo, we distributed flexible tickets to >500 students and their adult chaperones during summer, 2020. These tickets have allowed students to visit the Zoo to learn more about Minnesota’s natural resources and the State’s conservation efforts.

We were unable to continue the *Show Us Your Mussels* challenge during the 2020 – 2021 academic year due to the Zoo’s financial and capacity constraints resulting from the pandemic. However, materials developed over the course of this grant continue to benefit learners in Minnesota. Virtual and distance learning lessons developed by Zoo educators are freely available via the Zoo’s online Learning Corner. In addition, we are providing technical support and resources to facilitate bringing native mussels into the classrooms of two area schools, and we maintain relationships with teachers to share updates about our mussel conservation efforts.

Activity 3 Status as of June 1, 2021:

We did not have the capacity to support the implementation of the Show Us Your Mussels (SUYM) challenge during the 2020 – 2021 academic year and thus did not advertise about the program this year. However, one school contacted the Zoo and requested participation in the SUYM challenge. We supported their effort and hosted a virtual visit for the 21 students as an introduction to the challenge and facilitated voting for their projects on our webpage. In addition, the Zoo continued to provide distance learning lessons about mussels for teachers via our website and integrated messaging about mussel conservation into our virtual camps.

Final Report Summary:

Over the course of this project, >2,200 middle and high school students created digital projects to increase awareness about native freshwater mussels and their conservation, with student-created campaigns reaching >150,000 members of the public. We also created 20 lessons aligned to Minnesota state science standards as free resources for teachers, available through the Minnesota Zoo’s webpage. In evaluations of the Show Us Your Mussels challenge, teachers and students consistently reported high satisfaction in the program and increased

knowledge about mussels, water quality, and the role of Minnesota Zoo in conservation. Students seemed excited to share what they learned, and some reported taking actions to directly improve water quality after completing the challenge.

Despite the onset of the pandemic in 2020 and a subsequent reduction in capacity at the Zoo, outreach and mussel education continued. We adapted lessons for virtual or hybrid instruction and distributed these resources to all schools in the Minnesota Zoo's network. Students from schools winning the Show Us Your Mussels challenge during the 2019 – 2020 academic year were unable to visit the Zoo as originally planned but received a virtual behind-the-scenes tour of our mussel research facilities and tickets to visit the Zoo on their own. During the 2020-2021 school year, we supported schools that expressed an interest in learning about mussels by hosting a small-scale Show Us Your Mussels challenge, including voting on our site and doing a virtual site visit. Zoo education staff participated in a podcast with the Three Rivers Park district talking about the importance of native freshwater mussels as well as the Zoo's role in conservation and reintroduction efforts. The episode can be accessed [here](#).

V. DISSEMINATION:

Description:

Updates on project activities and progress will be shared with partners via annual reports. Information about the program will be disseminated to the general public by the Zoo's marketing and education departments as much as possible. These communications may include public presentations by staff and sharing information on the Minnesota Zoo's web page and social media outlets. In addition, Zoo staff and volunteers will be instructed in speaking with the public about mussels, the Zoo's rearing program, and the benefits of mussels for a healthy ecosystem and improved water quality. Results of the mussel propagation research will be submitted for publication in peer-reviewed scientific journals and presented at professional conferences.

Status as of: June 1, 2018

Information and results associated with the Zoo's mussel conservation appropriation are disseminated in cooperation with the MN DNR and project partners.

Zoo staff presented our work at 'Nerd Out' night (Moto-L, Minneapolis, MN) and set up a booth at Lake Monster Brewing during a public awareness event to connect with the public about mussel conservation efforts at the Zoo and across Minnesota

The mussel team competed for the title of Mr. Manuary, hosted by the MyTalk radio station, to raise awareness about water quality and mussel conservation.

We drafted an email blast that was sent to Zoo subscribers about how pet waste washed into our watershed increases harmful algae that harms mussels.

Staff presented for the Hmong College Prep Academy and at Trinity Lone Oak School to engage students about the Show Us Your Mussels campaign and to educate about how proper watershed management improves water resources for mussels and for humans.

To supplement a video that displays footage of mussel reproduction and Zoo conservation work, staff are developing a water quality laboratory interactive interpretation component to the Mussel Cabin. Mussel staff attended seminars hosted by the Metro Watershed Partners and the Twin Cities Water Monitoring and Data Assessment Group to develop ideas on how to engage Zoo guests on water quality, resource management and how our use of water affects mussels and our drinking supply.

Presentations for Zoo volunteers and summer educators have helped to enrich the knowledge base of Zoo representatives that interact most directly with Zoo guests. We are developing a mussel lure and host fish matching game for summer classes.

Status as of: December 1, 2018

We continue to disseminate information about this project and the conservation of native, freshwater mussels and aquatic systems through a variety of outlets:

- The Zoo hosted the National Public Radio's *Brains On!* podcast and distributed EarthEcho water quality test kits, empowering community members to monitor the health of their local water bodies.
- Zoo staff participated in *Imagine a Day Without Water*, during which we partnered with our freshwater turtle conservation staff and Dakota County Soil and Water District to inform guests about rain gardens and the ways in which our water resources impact the daily lives of humans, turtles, mussels, and other wildlife.
- The Zoo hosted two Smart Salt workshops for Zoo employees, municipal plow operators and winter maintenance practitioners, and private snow removal companies during November, 2018. These courses educate maintenance practitioners about the threats that chlorides can pose to our waterways and provide suggestions for minimizing the application of de-icing salts to levels that still keep winter surfaces safe for the public while reducing salt intrusion. Funding for these workshops was provided by the Minnesota Zoo's Legacy appropriation, the Minnesota Zoo Foundation, and other Zoo budgets.
- We drafted an email blast was sent to Zoo subscribers (including >40,000 member households) about the wise application of salts and tips for reducing salt use for de-icing applications at home. These messages were circulated to Zoo staff via email blasts as well.

Additional activities disseminating information about this project are highlighted in the Activity 3 status update.

Status as of: June 1, 2019

Although completing and outfitting the research facility was a primary focus for much of the winter, we were opportunistic and continued to disseminate information about mussel conservation and Minnesota's aquatic resources during the past 6 months. Activities included:

- Conservation staff participated in Minnesota Zoo Day at the Capital during the legislative session. We highlighted conservation of mussels and other aquatic resources, sharing information with members of the Minnesota legislature, their staff, and the general public.
- We disseminated information on Minnesota's water resources, mussel ecology and mussel conservation during public talks in Eagan and at the University of Minnesota.
- We are finalizing the development of an interpretive activity to engage guests in water quality monitoring. Zoo visitors will use a pre-set water quality probe to 'test' water from four different locations in Minnesota. Output will allow guests to visualize how water quality changes over the course of the year due to temperature and precipitation changes, road salt applications, and agricultural runoff. An interpretive station will be constructed in the coming months to host this activity as well as (1) a plastic, 3D model of a mussel illustrating how the specialized internal anatomy of mussels helps clean our waters and (2) new signs detailing the unique life histories of freshwater mussels.

- Content on mussel ecology and conservation – as well as tips for improving the health and conservation of our aquatic resources – will be shared with Zoo summer campers. We will be teaching 16 classes (3rd – 9th grade) and teacher workshops during camps over the next several months.
- Additional upcoming outreach events include:
 - Communicating the role that mussels play in water quality for a "Landscaping for Clean Water" class hosted by the Dakota County Water Conservation District;
 - Leading a session on water quality and mussel conservation for a Minnesota Master Naturalist course in early June; and
 - Teaching a university-level course at Metropolitan State University about the role of mussels in aquatic ecosystems.

Status as of: December 1, 2019

We continue to share information about the conservation of native freshwater mussels, water quality and aquatic systems via a diversity of outlets:

- LCCMR members and staff visited the Zoo in October to view first-hand the ENRTF-supported programs at the Zoo. The tour included a visit to the new mussel research and rearing facility and highlighted ways our work will benefit the conservation of mussels and other aquatic resources.
- We disseminated information about Minnesota's water resources, mussel ecology and mussel conservation during a lecture at Metropolitan State University and at public talks for the American Association of University Women in Minnetonka and the Minnesota Zoo Associates Board at the Royal Foundry in Minneapolis.
- Materials associated with the Mussel Lab interpretive center (as outlined in the last update) have been completed, including a water quality probe activity, 3D mussel model, and additional signs about mussel life history and conservation.
- We taught 16 classes for Minnesota Zoo campers (3rd – 9th graders) and at teacher workshops this summer. Talks focused on mussel ecology and conservation and included tips for improving the health and conservation of our aquatic resources.
- We communicated the role that mussels play in water quality during a "Landscaping for Clean Water" class hosted by the Dakota County Water Conservation District.
- We led a session on water quality and mussel conservation for a Minnesota Master Naturalist course in early June.

Status as of: June 1, 2020

We continued to disseminate information about mussel conservation and Minnesota's aquatic resources during the past 6 months, although our capacity to and methods for communication shifted significantly due to the COVID-19 pandemic.

- We shared information on mussel diversity, ecology and conservation at the Osher Lifelong Learning Institute (through the University of Minnesota).

- We hosted a unique, and first-of-its kind, adult-only overnight event at the Zoo themed for Valentine's Day. We presented content focusing on the ecology and science of mussel reproduction.
- We drafted a piece that was distributed to Zoo subscribers (including >40,000 member households) and posted an associated blog on the Zoo's website, detailing how mussels clean our water and identifying ways that people can help protect aquatic resources.
- Unfortunately, a spring "Landscaping for Clean Water" class hosted by the Dakota County Water Conservation District was canceled due to the COVID-19 pandemic. Zoo camps are also canceled; summer camps would have included a session focusing on invasive species and numerous other camps that would have addressed the conservation of our aquatic resources.

Status as of: December 1, 2020

The ongoing COVID-19 pandemic and associated financial and societal impacts restricted our ability to disseminate information about this project and native mussel conservation efforts in Minnesota over the past several months:

- We highlighted mussel conservation in Summer and School's Out camps through the Zoo's virtual detectives series.
- Zoo educators are collaborating with the Three Rivers Park District and will be featured in a podcast about mussel conservation in January, 2021.
- We shared information about the mussel conservation efforts of the Zoo and our partners, including the Minnesota DNR and the University of Minnesota, during presentations to all Zoo staff and the Zoo's Board.

Status as of June 1, 2021:

The COVID-19 pandemic and associated constraints continued to impact our ability to share information about native mussel conservation and healthy aquatic ecosystems:

- We shared information about the conservation of mussels and healthy aquatic ecosystems in virtual camps during the school year, reaching ~140 students. Additional content will be incorporated in this summer's virtual camps.
- Zoo Education staff were interviewed for a podcast (produced by the Three Rivers Parks District) about mussel conservation. The podcast is scheduled for release this summer.
- We presented research findings on the husbandry of black sandshell mussels at the virtual Freshwater Mollusk Conservation Society bi-annual symposium this spring.
- We highlighted our partnership with the Minnesota DNR's Center for Aquatic Mollusk Programs in our new, quarterly Conservation Connection newsletter, distributed to member households and donors during May, 2021.
- We continued to communicate about the conservation work of the Zoo, the Minnesota DNR, the University of Minnesota, and other partners during presentations to all Zoo staff and the Boards of Directors for the Minnesota Zoo and Minnesota Zoo Foundation.

Final Report Summary:

Communicating the importance of native mussels and water quality was a key objective of this project. The Minnesota Zoo engaged with Minnesotans to share information about mussels, their conservation and stewardship of our aquatic resources via a variety of platforms, ranging from in-person and virtual presentations for classes, camps, and other forums to free school curriculum and teacher professional development in association with the Show Us Your Mussels challenge. We developed an interpretive area on Zoo campus, outfitted with signs, videos and a water quality activity; shared research findings at professional scientific meetings; hosted Smart Salt workshops to reduce salt use on our roadways; shared information by tabling during *Imagine a Day Without Water*; guided a group of Zoo patrons on a canoe trip to discover mussels along the Cannon River; and much more, enabling us to broadly share the story of imperiled mussels and the collaborative work to restore depleted populations.

VI. PROJECT BUDGET SUMMARY:**A. ENRTF Budget Overview:**

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$352,109	Project Manager / Research Scientist Supervisor at 0.2 FTE for 3 years; Research Analyst Intermediate (or similar classification) at 1.0 FTE for 2.9 years and 0.2 for 1.0 years; Construction Project Manager for rearing pod at 0.1 FTE for 4 months; Education Project Coordinator at 0.1 FTE for 3 years (currently supported by soft money); Graphic Designer for education campaigns at 0.1 FTE for 2 months; Web Developer for exhibition of digital media campaigns at 0.1 FTE for 2.5 months; Education Project Evaluator at 0.1 FTE for 3 months
Professional/Technical/Service Contracts:	\$15,000	Teacher contracts for development of school materials (5 teachers x \$1,000 ea x 3 years). Selection through a competitive application process.
Equipment/Tools/Supplies:	\$63,671	Mussel rearing supplies and equipment: Estimated total of \$48,000 for materials associated with the source water supply, pan systems, upweller systems, floating rack, basket systems, lake aerator, and additional rearing systems. Lab supplies for in-house water quality testing: Estimated total of \$3,600 for supplies to fecal levels, nutrients, and water chemistry. Research to evaluate rearing methods: Estimated total of \$12,075 for materials to individually marking mussels, dissecting microscope, and other supplies.
Capital Expenditures over \$5,000:	\$130,000	Mussel research and rearing facility, including structure design and permits, site preparation, concrete slab and footings, building, insulation, water and sewer hook-ups, electrical hook-ups,

		and HVAC installation (contractors). Facility electric and design and installation of internal mussel rearing systems completed by MN Zoo staff.
Travel Expenses in MN:	\$592	Mileage and meals for travel to DNR facilities in Lake City and reintroduction sites around the state. Travel for teachers to attend professional development workshops at MN Zoo. Conference travel for Zoo staff to present and table at conferences specifically related to the digital media challenge.
Other:	\$29,388	Promotional post card printing and mailing to recruit teachers for student digital media campaign. Transportation for participating students to MN Zoo. Flexible tickets to students and chaperones from the winning schools participating in the 2019-2020 Show Us Your Mussels challenge. Zoo-based educational outreach materials. Online marketing for Show Us Your Mussels challenge. Promotional materials printed for conferences. Printing of materials for professional development workshops for teachers.
TOTAL ENRTF BUDGET:	\$590,760	

Explanation of Use of Classified Staff:

Most staff positions that will be supported by these ENRTF funds are classified. Current personnel have the necessary expertise to successfully implement Activities 1, 2, and 3, but without the support of the ENRTF funding, they would not have the support and ability to work on this project and instead would need to focus on other position responsibilities. Incorporating the existing expertise from across departments at the Zoo will be essential for the completion of this project.

Explanation of Capital Expenditures Greater Than \$5,000:

The mussel rearing program at the Minnesota Zoo requires indoor laboratory space to maintain a controlled environment suitable for experimentation with propagation techniques. In the research and rearing pod, we will manipulate the conditions under which a subset of mussels are reared to evaluate treatments that may increase juvenile mussel growth and improve survival. In addition, this building will house equipment and provide additional work space that will facilitate the expansion of the Zoo's mussel rearing program. We have budgeted \$130,000 for the construction of this facility. We project that the building's lifespan will extend beyond the 3-year duration of this grant, enabling us to continue partnering with the MN DNR to propagate mussels on site for eventual release into Minnesota waterways. We will seek funding from federal, state, and non-governmental sources to continue this program beyond 2020.

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 4.12

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0.3

B. Other Funds:

Source of Funds	\$ Amount Spent	Use of Other Funds
Non-state		
Minnesota Zoo Foundation	\$30,000	Supplies and equipment in support of the Zoo's current mussel rearing activities during FY18 – FY21. Estimate.
State		
Minnesota Zoo's General Operating Budget	\$88,614	Administrative costs, utilities, and other expenses associated with implementation of activities, estimated at 15% of total grant expenditures.
Minnesota Zoo's appropriation from State of Minnesota's Clean Water, Land and Legacy amendment	\$105,000	Additional costs associated with the construction of the mussel rearing and research facility (~\$75,000). Additional project expenses, including equipment, supplies, and staff time. Estimated at \$7,500 per year from FY18 – FY21.
TOTAL OTHER FUNDS:	\$223,614	

VII. PROJECT STRATEGY:

A. Project Partners:

The Minnesota Zoo was the sole recipient of ENRTF funds under this proposal. Zoo Conservation staff primarily planned and implemented Activities 1 and 2. Zoo education staff planned and implemented Activity 3. The Minnesota DNR, a primary partner for Activities 1 and 2, has received ENRTF funding supporting complementary mussel conservation efforts.

Partners receiving ENRTF funding

- Teachers (5), Contracts, \$15,000: Development of school materials in support of Activity 3.

Partners NOT receiving ENRTF funding

- Mike Davis, Bernard Sietman, Madeline Pletta, Zeb Secrist, and Lindsay Ohlman; Minnesota DNR. MN DNR was the primary partner for Activity 1, providing juvenile mussels for rearing at Zoo facilities and returning mussels to Minnesota waterways after they reach suitable size. The DNR was also a primary collaborator for Activity 2, which investigated propagation techniques that promote high growth rates and improved survival. The DNR provided expertise and served as a resource for the captive rearing of mussels.
- U.S. Fish and Wildlife Service, Tamara Smith: Resource for permitting under the US Endangered Species Act.
- U.S. Fish and Wildlife Service-Genoa National Fish Hatchery, Megan Bradley and Elizabeth Glidewell: Technical resource for rearing mussels in captivity and completing water quality testing to quantify food availability in Zoo's water bodies.
- Teachers at selected schools around the state: Assistance with implementation of Activity 3.

B. Project Impact and Long-term Strategy:

This proposal builds upon the DNR's ongoing research and propagation efforts and is part of a long-term, multi-partner effort to reintroduce threatened and endangered mussels across the upper Midwest. The ultimate goal of this work is to restore healthy mussel populations, thereby enhancing ecosystem health and improving water quality. Given the massive filtration rates performed by healthy mussel communities and mussels' abilities to remove harmful bacteria and contaminants from the water, restoring Minnesota's native mussel populations plays an important part in achieving the state's clean water goals. The combined efforts of the Zoo, the DNR, and other partners also advances the recovery of state and federally listed mussel species in Minnesota. In

addition, public awareness about mussel conservation and actions to improve water quality has increased by our activities.

Because successful restoration efforts require many years, the Zoo's mussel rearing activities will continue beyond the scope of this grant proposal. The Zoo has received additional support from the Environment and Natural Resources Trust Fund to implement mussel conservation activities (M.L. 2020, Expanding Restoration And Promoting Awareness Of Native Mussels) and will continue to explore non-ENRTF funding sources for this work.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
Minnesota's Clean Water, Land, and Legacy Amendment, Arts and Cultural Heritage Fund appropriation to the Minnesota Zoo. Purchase of equipment and supplies for initiating mussel rearing program. Outfitting the Zoo's existing cabin on the main lake for rearing a small number of mussels.	FY16	\$10,000
Donations managed by the Minnesota Zoo Foundation. Purchase of equipment and supplies for initiating mussel rearing program, outfitting the Zoo's existing cabin on the main lake for rearing a small number of mussels.	FY16	\$15,000

VIII. REPORTING REQUIREMENTS:

- The project is for 4 years, will begin on 07/01/17, and end on 06/30/21.
- Periodic project status update reports will be submitted December 1st and June 1st of each year.
- A final report and associated products will be submitted between June 30 and August 15, 2021.

IX. VISUAL COMPONENT or MAP(S):

Please see the attached graphic.

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

Environment and Natural Resources Trust Fund
M.L. 2017 Final Project Budget

Project Title: Rearing Native Mussels for Reintroduction and Expanding Water Quality Awareness

Legal Citation: M.L. 2017, Chp. 96, Sec. 2, Subd. 04c

Project Manager: Seth Stapleton

Organization: Minnesota Zoo

M.L. 2017 ENRTF Appropriation: \$591,000

Project Length and Completion Date: 4 Years, June 30, 2021

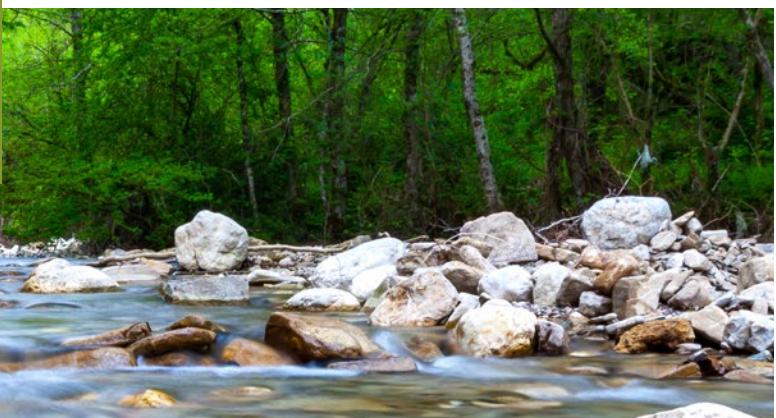
Date of Report: August 16, 2021



ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget 8/24/2020	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget 8/24/2020	Amount Spent	Activity 3 Balance	Revised TOTAL BUDGET	Revised TOTAL BALANCE
BUDGET ITEM	Expand capacity to rear mussels on-site at the Zoo for reintroduction			Conduct research to optimize rearing protocols			Show us your Mussels digital media challenge for high school students				
Personnel (Wages and Benefits) - Overall	\$284,625	\$284,625	\$0	\$37,037	\$37,037	\$0	\$30,550	\$30,447	\$103	\$352,212	\$103
Project Manager / Research Scientist Supervisor (1 person, 72% salary / 28% benefits), 0.2 FTE for 3 years - \$64,075											
Research Analyst Intermediate or similar classification (tbd; 1 person, 64% salary / 36% benefits), 1.0 FTE for 2.9 years and ~0.2 FTF for 1.0 year - \$252,587											
Construction Project Manager for rearing pod (1 person, 72% salary/28% benefits), 0.1 FTE for 4 months - \$5,000											
Education Project Coordinator (1 person, 72% salary/28% benefits; portion of position to be covered by ENRTF funds is currently supported by soft money), 0.1 FTE for 3 years - \$24,000											
Graphic Designer for education campaigns (1 person, 72% salary/28% benefits), 0.1 FTE for 2 months - \$1,100											
Web Developer for exhibition of digital media campaigns (1 person, 72% salary/28% benefits), 0.1 FTE for 2.5 months - \$2,750											
Education Project Evaluator (1 person, 72% salary/28% benefits), 0.1 FTE for 3 months - \$2,700											
Professional/Technical/Service Contracts											
Teacher contracts for school materials development (5 teachers x \$1000 ea x 3 years). Teachers selected through a competitive application process, with the objective of expanding annually to reach new communities. Curriculum development for this project is beyond the scope of their existing contracts and will occur during the duration of the project.							\$15,000	\$15,000	\$0	\$15,000	\$0
Equipment/Tools/Supplies											

Mussel rearing systems supplies & equipment, estimated costs. Source water supply (Estimated total: \$24,000 for drum filter; piping, valves, fittings, pump, pump housing, air blower, screening, adapters, storage tank, and other supplies). Pan systems (Estimated total: \$4,900 for bulkheads, pipes, fittings, filter socks, pumps, sumps, pans and other supplies). Upweller system, ~350 gallons (Estimated total \$5,200 for bulkheads, pipes, fittings, chiller, pumps, sumps, trough, and other supplies). Floating rack, 10 spots (Estimated total \$4,500 for floating dock, bulk metals, insulation, fittings, valves, tubing and other supplies). Basket systems (Estimated total \$900 for baskets, screening and other supplies). Aerator for lake (Estimated total \$3,500). Additional rearing and propagation systems and associated supplies	\$48,000	\$48,000	\$0							\$48,000	\$0
Lab supplies for in-house water quality testing of lake water used to rear mussels, estimated costs. Fecal testing supplies (millipore miliflex cassettes and millipore growth ampules): \$2,800. Kits and materials for testing nutrients and water chemistry: \$800	\$3,600	\$3,599	\$1							\$3,600	\$1
Research supplies/equipment, estimated costs. Tagging supplies for individually marking research mussels (PIT tags, PIT tag reader, shellfish tags): \$7,750. Dissecting microscope and associated supplies: \$2,400. Flat bottom boat for lake access: \$600. Scale and miscellaneous tools and supplies: \$1,225				\$12,075	\$12,072	\$3				\$12,075	\$3
Capital Expenditures Over \$5,000											
Mussel research and rearing facility, estimated costs ¹ . Structure design and permits (contractor): \$7,000. Site preparation (contractor): \$10,000. Installation of concrete slab with footings (contractor): \$30,000. Morton building (pole barn structure with metal siding), customized for mussel rearing (materials): \$20,000. Spray foam insulation (contractors): \$7,500. Water and sewer hook ups (contractors): \$27,500. Electrical hook-ups (contractor): \$18,000. Installation of HVAC systems (contractor): \$10,000. Facility electric: MN Zoo staff. Design and installation of internal mussel rearing systems: MN Zoo staff.				\$130,000	\$130,000	\$0				\$130,000	\$0
Travel expenses in Minnesota											
Mileage and meals associated with travel from MNZoo in Apple Valley to DNR mussel facility in Lake City (3 trips/year x 3 years) and 3 trips to reintroduction sites in the state. Reimbursement rates as allotted per the State of Minnesota travel regulations. <small>Budget field revised after actual expenditures incurred</small>	\$250	\$117	\$133							\$250	\$133
Travel for teachers to attend professional development workshops at MN Zoo (6/1/2018). Reimbursement for mileage @ 30 teachers / year x 2 years x ~150 mile roundtrip (average) x \$0.545 / mile. Budget field revised after actual expenditures incurred.							\$345	\$345	\$0	\$345	\$0

Conference travel for Zoo staff (6/1/2018). Zoo staff to present and table at conferences specifically related to the digital media challenge (language arts and technology focuses). Conferences to may include MN Council of Teachers of English (Fall and spring, ~\$580 x 1 yrs), TIES Education Technology Conference (~\$1,000 x 2 yrs), and Information and Technology Educators conference (~\$250 x 1 yrs). Budget field revised after actual expenditures incurred.							\$130	\$130	\$0	\$130	\$0
Other											
Promotional post card printing and mailing to recruit teachers to participate in "Show Us Your Mussels" Student Digital Media Campaign (approx. 6,000 post cards x \$0.18 / piece x 2 years) . <i>Budget field revised after actual expenditures incurred</i>							\$1,944	\$1,944	\$0	\$1,944	\$0
Transportation (bussing) costs for participating schools to attend "Show Us Your Mussels" student event at Minnesota Zoo -5 schools x 2 years. Budget field revised after actual expenditures incurred							\$6,491	\$6,491	\$0	\$6,491	\$0
Flexible tickets to the Zoo awarded to 555 students (\$10.25 / student) plus 1 chaperone / student (\$15 / adult) from the winning schools participating in the 2019 - 2020 Show Us Your Mussels challenge. Tickets allow students to visit the Zoo to learn more about the State's natural resources and conservation efforts, in lieu of the field trips which were canceled due to the COVID-19							\$14,014	\$14,014	\$0	\$14,014	\$0
Zoo-based mussel conservation educational outreach, including interpretive signage, displays, and artifacts (\$2,000/year x 3 years)							\$6,000	\$6,000	\$0	\$6,000	\$0
Online marketing for "Show us your Mussels" digital media challenge (6/1/2018). Advertising on Facebook and other platforms; posts 'boosted' to reach broader audience during campaign. Costs contingent on clicks, averaging \$5 - \$20 / boost. <i>Budget field revised after actual expenditures incurred</i>							\$106	\$106	\$0	\$106	\$0
Promotional materials printing for conferences (6/1/2018). Materials include postcard with campaign information and photos and FAQs document. ~1000 half pages \$0.40 x 2 1 years; ~750 full pages x \$0.80 x 1 years. Budget field revised after actual expenditures incurred							\$662	\$662	\$0	\$662	\$0
Printing of workshop materials for professional development workshop (6/1/2018): \$30 / teacher x 30 teachers x 2 years. Budget field revised after actual expenditures incurred							\$171	\$171	\$0	\$171	\$0
COLUMN TOTAL	\$336,475	\$336,341	\$134	\$179,112	\$179,109	\$3	\$75,413	\$75,310	\$103	\$591,000	\$240



mussel rearing and research

Native mussels are aquatic engineers, providing important ecosystem services such as water filtration and creating habitat for fish and other wildlife, but many populations are depleted across Minnesota. With the support of the ENRTF (M.L. 2017, Chp. 96, Sec. 2, Subd. 04c), the Minnesota Zoo has increased our rearing capacity to accommodate more than 10,000 juvenile mussels, positioning us to better support recovery efforts. We also are researching methods to improve husbandry and maximize growth and survival of mussels in our care.





MINNESOTA ZOO



ENVIRONMENT
AND NATURAL RESOURCES
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mussel education

The “Show Us Your Mussels” challenge has engaged students in creating digital media campaigns to promote the conservation of imperiled mussels and Minnesota’s aquatic resources. Student-created campaigns have reached more than 150,000 citizens, encouraging public action to benefit water conservation.

