

**Long-Term Conservation Efforts: Native Minnesota Freshwater Mussel  
Propagation, Survey, and Monitoring**

By

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## **Introduction**

Mussels are aquatic animals that inhabit both marine and freshwater benthos all over the world. They are members of the phylum Mollusca, and are closely related to other bivalves such as marine mussels and clams. Freshwater mussels play a key role in the rivers, lakes, and streams they inhabit because they link the water column and benthos by filtering particles from the water as their food and passing nutrients down into the benthic ecosystem (*Mussels of Minnesota*, 2018).

Freshwater mussels' life history and ecological role as key filter feeders makes them sensitive to environmental changes, and consequently they are often used as bioindicators of ecosystem health, water quality, and ecotoxicology (Cope et al., 2008; Vaughn, 2018). Mussels can be used as a live ecotoxicology indicator because they modify behavior (such as opening/closing their shells), filtration, and heart rate as a result of chemical stressors (Hartmann et al., 2016). They are long-lived and have limited mobility, so they cannot migrate to escape pollutants and thus provide a long historical record about pollution and water quality in the area. Mussels' shells incorporate and sequester materials ingested during that year's growth. Some contaminants, such as metals, are recorded in the annual lines added to the shells, just like tree rings. Mussel shell deposits provide a reference condition, such as which species were present in a prehistoric community, for example (Davis, 2021).

Because mussels are situated as the connection between pelagic and benthic environments, they directly affect nutrient cycling and food webs (Vaughn & Hoellein, 2018). Research has shown that mussel aggregates create biogeochemical hotspots of

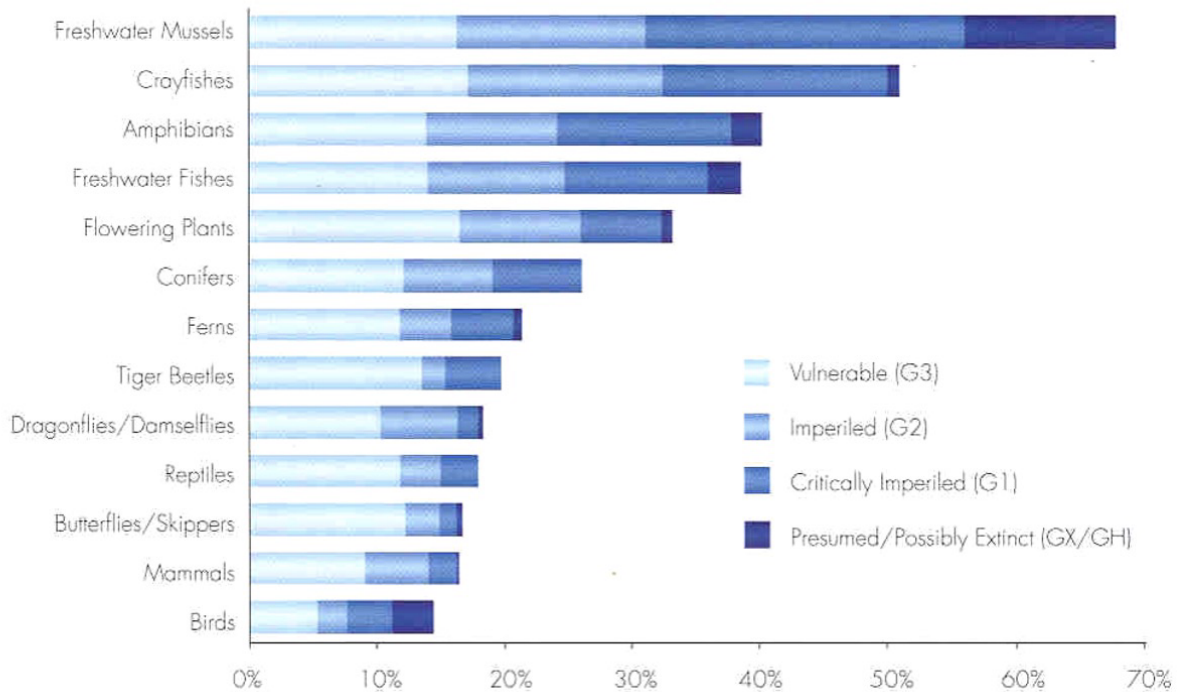
nutrient cycling and storage, and that these hotspots are especially impactful in streams that are more sensitive to mussel biomass and species composition (Atkinson & Vaughn, 2015). They provide many ecosystem services such as habitat structure, nutrient processing, including regeneration and storage, and contaminant removal (Atkinson & Vaughn, 2015). Mussel aggregates (i.e., beds) function similarly to coral reefs; fish are attracted to them because of the food resources. Their shells are substrate for algae growth, which alters the nitrogen-phosphorus ratio, reduces blue-green algae, and increases green algae (Davis, 2021). Mussels not only physically provide all these ecosystem services, but their species composition and biodiversity benefit their habitats as well. A spatial mixture of diverse species is desired because it contributes to the ecosystem's community structure, population dynamics, and most importantly, to this aspect of mussels, ecosystem patterns and processes (Winemiller et al., 2010). These ecosystem services and benefits are part of the reason why mussel conservation goals include restoring biodiversity (Spooner & Vaughn, 2008). More diverse systems are also less likely to be taken over by invasive species due to their resilience and resistance making them stronger than less diverse ecosystems (Strayer, 2007).

Anthropogenic use of mussels has been documented as far back as the early Neolithic period. Archaeological digs have found shell material at Eastern North American sites dating back to 8000 B.C., where they were used for food, pottery, jewelry, and tools. In the mid-1800s, Europeans in North America were hunting for natural pearls formed in mussel shells, and the search had spread west to the Mississippi by the end of the 1800s. Production of pearl buttons made of mussel shells

began in the 1890s and continued until approximately the 1940s. This multimillion-dollar industry harvested tons and tons of mussels, devastating the formerly abundant mussel beds throughout the Mississippi and its tributaries. Mussel propagation began in the early 1900s as a way to provide shells for the button factories but was unsuccessful due to pollution of the river systems. Mussels are still harvested today, primarily for use in the cultured pearl industry with oysters, but it has been restricted or shut down by many states due to their imperiled status (Minnesota DNR, *Importance of Mussels*, 2018).

Freshwater mussels have one of the highest rates of extinction and imperilment out of all animal groups on Earth (Haag & Williams, 2014; Johnson et al., 2013; Minnesota DNR, *Mussels of Minnesota*, 2018), as shown in **Figure 1** (Stein et al., 1997). Approximately 70% of mussels are imperiled, meaning they are vulnerable, threatened, or endangered (Johnson et al., 2013). At least thirty-five species have gone extinct within the past 100 years (Haag & Williams, 2014; Farmer, 2018). The decline of mussel populations to these extreme levels has been a long time coming, but the exact causes are not known. There are many possible reasons, primarily excessive habitat loss/deterioration, habitat fragmentation due to dam construction, anthropogenic use/commercial harvest, pollution, their fragile parasitic relationship with fish, extreme climatic events such as drought, and non-native mussel invasion (Bogan, 1993; Haag & Williams, 2014; Barnhart et al., 2015; DuBose et al., 2019). Presently, native mussels are severely threatened by invasion by the non-native Asiatic clam and zebra mussel (Williams et al., 1993) because of increased competition for food (Smith et al., 2012). This drastic decline in a group of organisms so critical to the health of freshwater ecosystems is why mussel restoration efforts are being studied and implemented all

over the world, through work such as that of the Minnesota Department of Natural Resources' Center for Aquatic Mollusk Programs.



**Figure 1.** Proportion of U.S. species at risk. The species groups that are proportionately the most imperiled - mussels, crayfishes, and amphibians - consist entirely or primarily of freshwater species (Stein and Flack, 1997).

## Internship Program

### **Background**

The Minnesota Department of Natural Resources' Center for Aquatic Mollusk Programs (CAMP) is a research facility and team based in Lake City, Minnesota, that began working in the late 1980s to research, rebuild, and restore populations of

threatened and endangered native mussel species. Much of this is done through lab propagation, presently of about ten threatened or endangered species, such as *Lampsilis higginsii*, or Higgins eye (Minnesota DNR, *Mussels of Minnesota*, 2018). Freshwater mussel propagation has the potential to play a critical role in the restoration of these native species to their former habitats and numbers, and long-term data are already showing its positive impacts on extirpated populations (Haag & Williams, 2014). Enabling juveniles to grow in captivity in a lab setting allows more of them to survive than naturally would in the wild because the juvenile stage is when a bottleneck mortality effect occurs (Sicuro, 2015). These juveniles are then released back into the wild to rebuild their former populations, with the goal of helping them eventually reach a point where they can be self-sustaining (Minnesota DNR, *Mussels of Minnesota*, 2018).

The Center's work is done with many different projects, grants, associations, and funding sources, a few being the National Park Service, Minnesota Department of Transportation, and U.S. Army Corps of Engineers. Collected data is managed in the DNR's Natural Heritage Information System (NHIS) and is used in many ways, such as selecting streams for reintroductions, developing educational materials for public audiences, measuring the success of watershed management projects, assigning legal conservation status to vulnerable mussel species, and can be utilized by other users upon request. CAMP and its summer internship have two main components: lab propagation and field work, which consists of surveys and monitoring. The primary lab staff handles all work related to mussel propagation, and field crews do the surveys and monitoring all over the region, including Minnesota, Wisconsin, and Iowa.

### ***Propagation***

The propagation aspect of the internship includes not only mussel propagation - both in and out of the lab - but also education/outreach, and any additional experiments. The purpose of propagating mussels in the lab is to eventually release them back into the watersheds from where they were originally collected and possibly even where they historically inhabited. The goal of these population boosters and reintroductions is to create restored mussel assemblage by adding both numbers and biodiversity to the existing wild mussel populations, in hopes that they will recover and be able to carry on in the future. CAMP serves as an education resource, providing lab tours to school groups and community outreach by staff members. The lab also conducts numerous experiments, as requested and funded by various groups. Some examples of studies include fish host trials, food delivery methods (constant pumping, scheduled pulse flow, manual feedings), sediment grain size, and countless others.

### ***Field Work***

Aspects of CAMP's mussel conservation efforts that are carried out in the field include tagging (**Figure S1**), water quality monitoring (**Figure S2**), site monitoring (**Figure S3**), strandings/rescues (**Figure S4**), and surveys (**Figure S5**). Mussels are tagged with either plastic or Passive Integrated Transponder (PIT) tags during routine monitoring of their grow-out containers (**Figure S1**). PIT tags are used in a variety of fields to track individual organisms by giving each a "barcode" for its lifetime that can be scanned by scientists for identification (Smyth & Nebel, 2013). PIT tags are placed on mussels when they are released from the grow-out containers into their wild habitats so

the CAMP team can monitor the area and collect accurate data on their post-release success. Water quality is routinely monitored at various sites relevant to CAMP's work (**Figure S2**). Water quality data is collected with multiprobes and ammonia and nitrate test kits. Sites are monitored regularly because another part of the Center's work is rescuing mussels stranded by low water levels, which becomes much more of a priority during droughts (**Figure S4**). For site monitoring (**Figure S3**) and surveys (**Figure S5**), two main methods of field data collection are utilized: quadrats (**Figure S3, right**) and timed searches. Quadrats are used to collect quantitative data on the spatial distribution density, and timed searches are used to obtain qualitative catch per unit effort data.

One of CAMP's longest-running monitoring projects is with the Army Corps of Engineers, done annually in the Mississippi River near Prairie du Chien, Wisconsin. A few interns were brought along for the monitoring trip in **Summer 2021** and we collected data with quadrats and timed searches via boat dives for two days. Quantitative surveys have been done regularly since 1985 at the Prairie du Chien East Channel Reference site, and less consistently at the Downstream and Turning Basin sites. CAMP provided data for this project since 2014, with the exception of 2020 due to COVID preventing most of the program and its field work from happening. The (field) methods and results sections of this report are focused on this project, due to it being the program's longest dataset (1985 to present day) with both field methods utilized in the data collection.



## Methods

### **Propagation Methods**

During field surveys, adult mussels of the target species are collected and brought back to the lab, where they are kept for the duration of propagation season and then returned to the sites where they were collected for release. The field crew also collects host fish as needed, their species depending on what the mussel species or project assignment requires. Ideal mussels collected are adult females that have already been fertilized, but all are closely monitored, at least twice daily, and checked for glochidia production. When the female mussels release glochidia, they (or the conglutinate packets, depending on the mussel species (**Figure 2**)) are collected and studied with microscopes for age and development analysis (**Figure 3**). Larvae that are ready to attach to their host fish's gills have distinguishable shells and should be seen opening and closing them. Once the larvae reach that developmental stage, they are used to inoculate, or infest, the host fish. Inoculation - specifically the amount of time, water, and number of larvae used - must be very precise to prevent over-inoculating because it is easy to do and could lead to death of the fish and the mussels growing on its gills. For the next few weeks after the host fish are inoculated (**Figure 4**), they are carefully observed and kept as healthy as possible while the larvae attached to their gills are metamorphosing into the juvenile phase. When the juveniles are sufficiently developed, they release from the fish's gills and settle to the bottom. During this process in the lab, they are subject to dangers such as tank drainages, so collection nets are used to safely remove them and prevent as few as possible from escaping and being

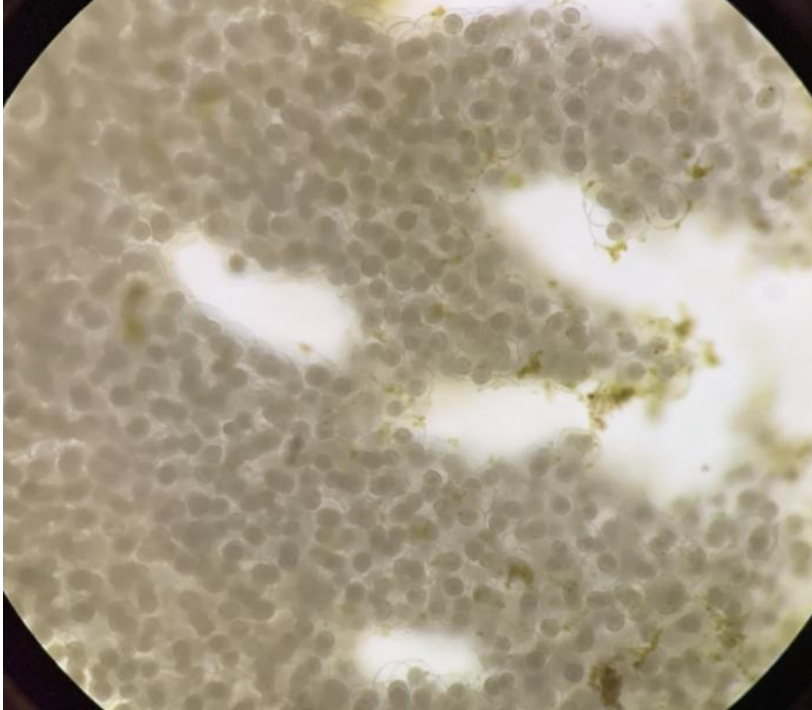
lost. Collection nets are rinsed into a tiered set of sieves, the sizes of which depend on the mussel species. The sieve stack is usually topped with a 500- $\mu\text{m}$  sieve to remove large debris such as fish feces and extra food pieces. A typical sieve stack used when the mussels begin to drop off the fish is 500- $\mu\text{m}$  on top to remove debris, followed by a 300- $\mu\text{m}$  sieve that most juveniles will rinse through, followed by a 150- $\mu\text{m}$  sieve on the bottom to catch the juveniles. The contents on the lowest (smallest mesh size) sieve are then rinsed into a petri dish with a scored counting grid, and its lid is labeled with the species and watershed from which the parent mussel was collected. River water is added to the dish until all mussels are fully covered. A microscope is used to better see the juveniles so they can be accurately counted. Some mussel species that have larger juveniles - such as *Cumberlandia monodonta* - use the same sieve sizes, but the contents on both the 300- $\mu\text{m}$  and 150- $\mu\text{m}$  meshes are kept and studied under a microscope. If the dish is crowded with too many mussels to accurately count, a volumetric count (details in **Supplemental Information**) can be done instead. After each day's dropped mussels have been counted and recorded, they are placed in new containers, such as buckets and AHAB tanks, for their next stage of growth. They are eventually placed in grow-out containers (**Figures 5 and 6**) in various bodies of water around the state to overwinter and continue growing. These containers are routinely checked over the next year(s) (**Figure 6C**) and the mussels are eventually released and monitored in the wild.

Some general lab methods that are especially relevant to the propagation side of things include river water collection and mussel equipment cleaning. Water is collected as needed from the Mississippi River for use in the lab whenever well water cannot be

used. It is pumped through a filter into a hauling tank on a trailer and brought to the lab where it goes through several filters of various sizes and cleaning mechanisms before entering a 1,000-gallon holding tank. There is a hose connected to this tank so the river water can be used as necessary around the lab facility. Every morning, the hose is run before it is used that day to drain any water that was sitting in it overnight. Cleaning of any equipment that is used in working with the mussels is done with either bleach or a vinegar solution, never soap, to prevent leaving a residue.



**Figure 2.** A *Cumberlandia monodonta* mussel in a bucket surrounded by conglutinates (white packets) she released.



**Figure 3.** Microscope view of *Cumberlandia monodonta* conglutinate. Mussel larvae appear as dark circles; more mature larvae have a visible flat side, which is where the shell hinges.



**Figure 4.** Microscope view of inoculated fish gills. Attached mussel larvae appear as small white dots.



**Figure 5.** Tote bin system used to hold juvenile mussels during the grow-out phase.





C.



D.

**Figure 6.** Basket system used to hold juvenile mussels during the grow-out phase, pictured in various stages of the process. **A.** Staff and interns tying five brand new baskets together before sinking them (Waterville, MN). **B.** Sunken basket groups after placement. Left: Waterville, MN; Right: Minnesota Zoo. **C.** An open basket during a monitoring check of the mussels inside. **D.** Retrieved basket groups. The DNR staff member pictured is Madeline Pletta, the designer of the basket system.

## Field Methods

The CAMP field crew annually monitors mussels at various sites (Reference, Turning Basin, and Downstream) in the Mississippi River near Prairie du Chien, Wisconsin (**Figure 7**). The monitoring trips conducted for the Prairie du Chien (PdC) project use both methods of field data collection: quadrats, for quantitative data, and timed searches, for qualitative data. 0.5-m<sup>2</sup> quadrats are randomly placed around the boat. The diver descends and excavates whatever is contained within the quadrat's frame, wherever it landed. To account for mussels burrowing into the substrate, everything within the top 15 centimeters is dug up and collected in the quadrat's net (**Figure 8**). The diver returns to the surface with the quadrat, its net is rinsed and emptied on the boat's table, the mussels are separated into species, all live mussels are measured with calipers, and the information is recorded on data sheets (either on paper or with tablets entering the information directly into the database). Timed searches are conducted by diver(s) searching by touch and retrieving any mussels at the site for a set amount of time. Mussels are placed in the diver's collection bag during the search. There is always one surface crew member on the boat, and that person is responsible for the timer and telling the divers - by pulling their rope, banging on the boat, or using an underwater communication device - when to come up after the search time has elapsed. After the search, the diver's collection bag is emptied onto the boat's table and the data is recorded in the same manner as with quadrats. All live mussels collected in both of these sampling methods are returned to the rivers near where they were removed.



**Figure 7.** Map of the Unionid sampling locations at the Upper Mississippi River (UMR) Pool 10 Prairie du Chien (East Channel) Higgins Eye Essential Habitat Area (Minnesota DNR, 2014, 2015, 2016, 2018, 2019).



**Figure 8.** Site monitoring conducted by digging quadrats to collect spatial distribution data.



## Results and Discussion

Consistently abundant mussel species such as *Amblema plicata* and *Truncilla truncata* are examples of the clear trends visible when examining the entire sampling period from 1985 to 2019 (**Figures 9 and 11**). There is a noticeable decline when considering the full time series, emphasizing the importance of long-term collections. The exact reason for this decline is not known, but there are many possible contributing factors, such as habitat loss/degradation/fragmentation, particularly as a result of dam construction, anthropogenic pollution, dramatic climatic events like droughts, and a precarious relationship with the various species of mussel host fish (Bogan, 1993; Haag & Williams, 2014; Barnhart et al., 2015; DuBose et al., 2019). Many of those potential reasons for decline are more applicable when considering mussel abundance over a far longer time scale than that of the data presented here, so it is more likely that this downward trend in native mussel abundance is at least partially attributed to the rise of zebra mussels as an invasive species, best seen when comparing **Figures 10 and 12**. Data from 2000-2002 show the largest boom in zebra mussel populations (**Figure 12**), which lines up precisely with the lowest mussel densities over the entire time series (**Figure 10**).

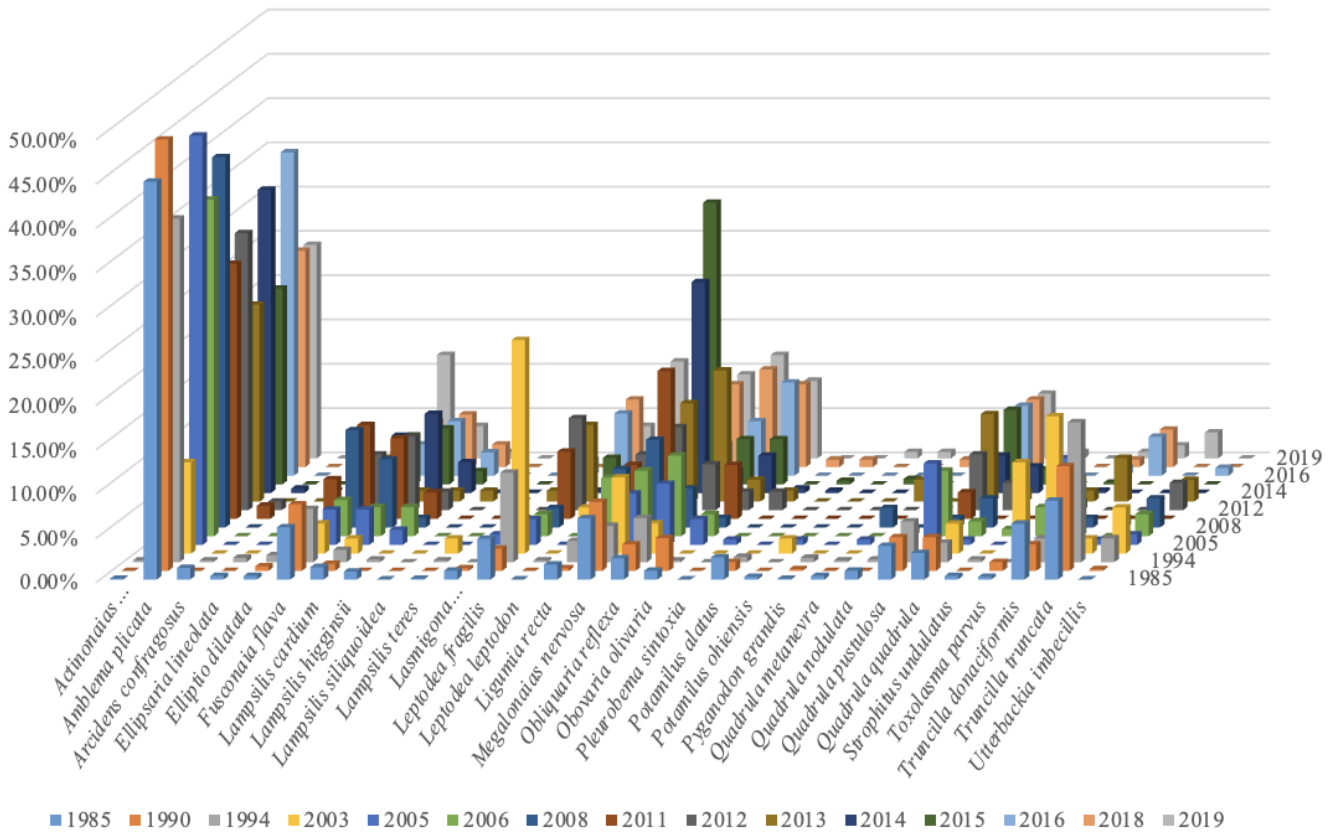
Patterns in the zebra mussel density data (**Figure 12**) show that massive die offs generally follow years with larger abundance. This trend is reflected in the composition of the substrate observed by the divers and recorded on the datasheets after each dive; after years with significant zebra mussel die offs, the substrate is comprised of much more zebra mussel shell than before the die-offs (Minnesota DNR, 2019). CAMP's goal

of improving native mussel species diversity and abundance stems from the expectation that a more diverse and flourishing community is more stable and less likely to be decimated by adverse conditions, such as colonization by invasive species or environmental perturbations (Strayer, 2007). The species richness data from all three sampling sites (Reference, Turning Basin and Downstream; **Table S1**) seems to support this conclusion because years with the highest number of species collected (from 2014 to 2016) also had some of the lowest number of zebra mussels in recent years (**Figure 12**). The relative abundance data (**Table S1**) also supports this conclusion because years with the highest catch per unit effort (CPUE; mussels/min), such as 2015 and 2016, are also the years with far fewer zebra mussels collected. It is known that zebra mussels are invasive and harmful to native mussel populations (Williams et al., 1993), so this data showing the inverse relationship between the abundance of native mussels and zebra mussels is reasonable.

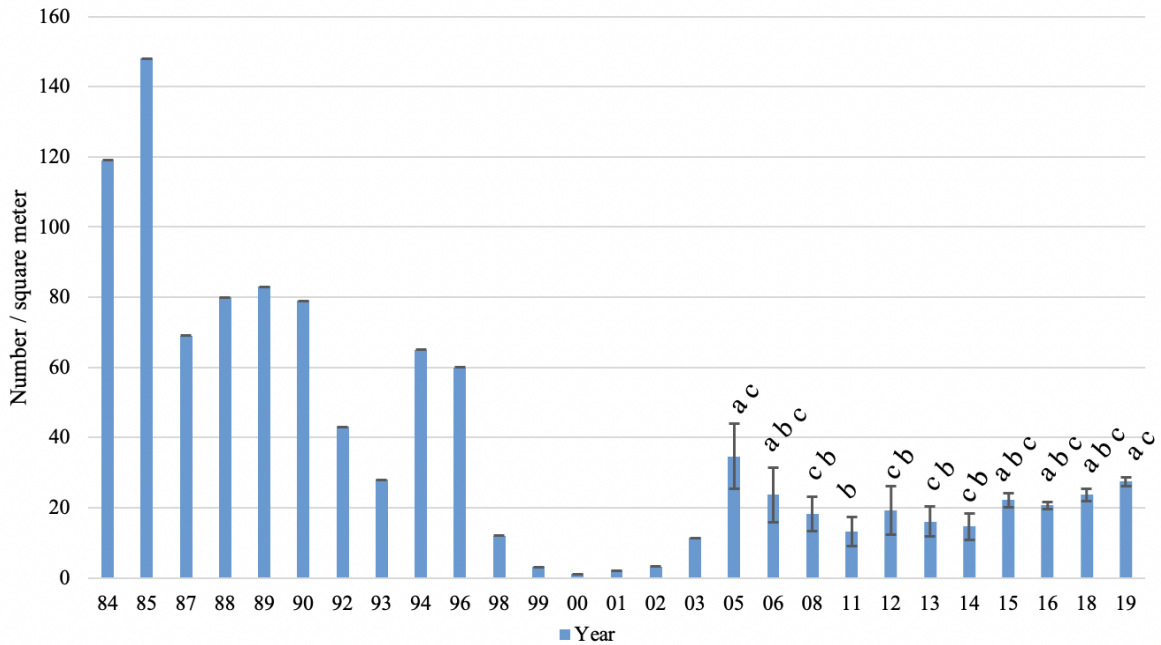
The year of 2019 may be an exception to the expectation that increased native species richness and abundance makes populations more resilient to stressors, such as invasive species (Strayer, 2007). The 2019 species richness data is counterintuitive to Strayer's expectation (Strayer, 2007) because 24 species were collected that year (**Table S1**), just as in 2016, but the zebra mussel count in 2019 is disproportionately higher than in 2016 (**Figure 12**). The 2021 data are still being processed by Minnesota DNR staff and were therefore not included in this report, so it remains to be seen if this 2019 anomaly is indeed an outlier or perhaps the beginning of a new dynamic where the increased native mussel abundance - possibly as a result of successful propagation efforts - continues year to year despite increased zebra mussel numbers, confirming

Strayer's hypothesis (Strayer, 2007). The 2019 CPUE is also by far the lowest out of the recent sampling years, potentially indicating the effects of such abundant zebra mussels that year. It can be inferred that the effect is due to zebra mussel numbers because other drastic, negative changes in environment, habitat, or water quality would also have some effect on the zebra mussel abundance. Quantitative native mussel density data (**Table S2**) is also inconsistent with Strayer's conclusion (Strayer, 2007) because 2019 had the highest native mussel density along with the most zebra mussels since 2011 (**Figure 12**). A high native mussel density coinciding with a high zebra mussel abundance could be related: if the native mussels are too crowded in the space, the benefits from their improved abundance may be outweighed by the drawbacks of overcrowding.

The National Strategy for the Conservation of Native Freshwater Mollusks has been the plan for mussel improvement projects since 1998, and was revised in 2016. There were several differences between the two versions, one of which is the issue of "technology to propagate and reintroduce mussels" ("A National Strategy for the Conservation of Native Freshwater Mollusks," 2016). The revised 2016 edition emphasizes mussel propagation efforts less than it did in 1998, implying that those efforts have been successful so far. This implication is supported by the longer term data set. It confirms a positive effect from CAMP's work all these years by showing that the species propagated there - including *Ligumia recta*, *Cumberlandia monodonta*, *Actinonaias ligamentina*, *Epioblasma triquetra*, and *Lampsilis higginsii* - do indeed seem to be making a comeback in their native habitats in the wild (**Figures 9 and 10**).

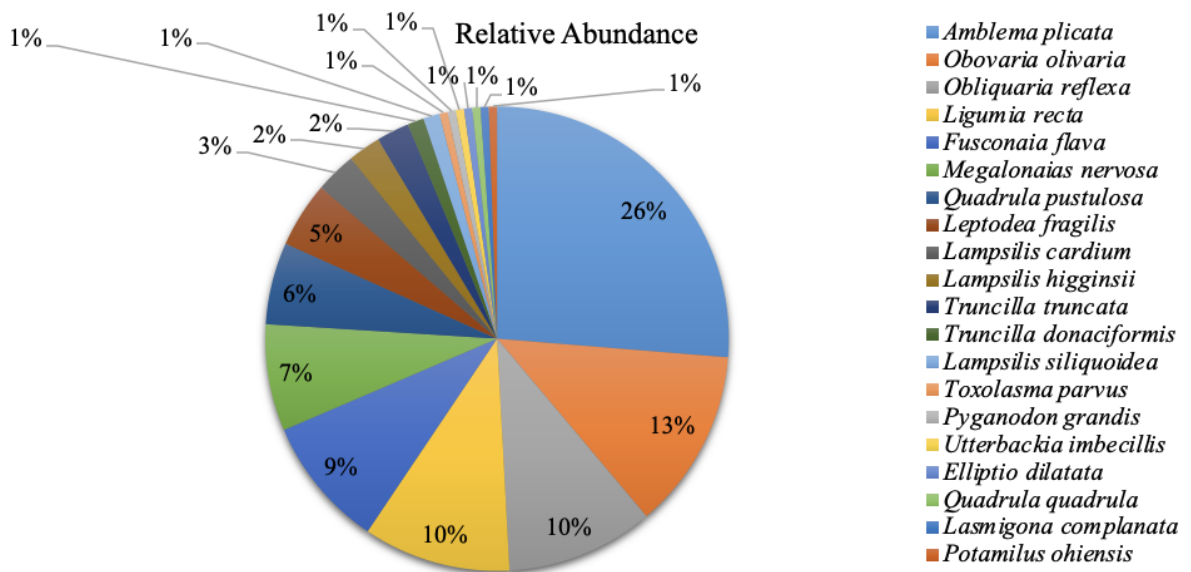


**Figure 9.** Relative abundance at the Prairie du Chien East Channel Reference Site for species with highest variability between 1985 and 2019 (Minnesota DNR, 2019).

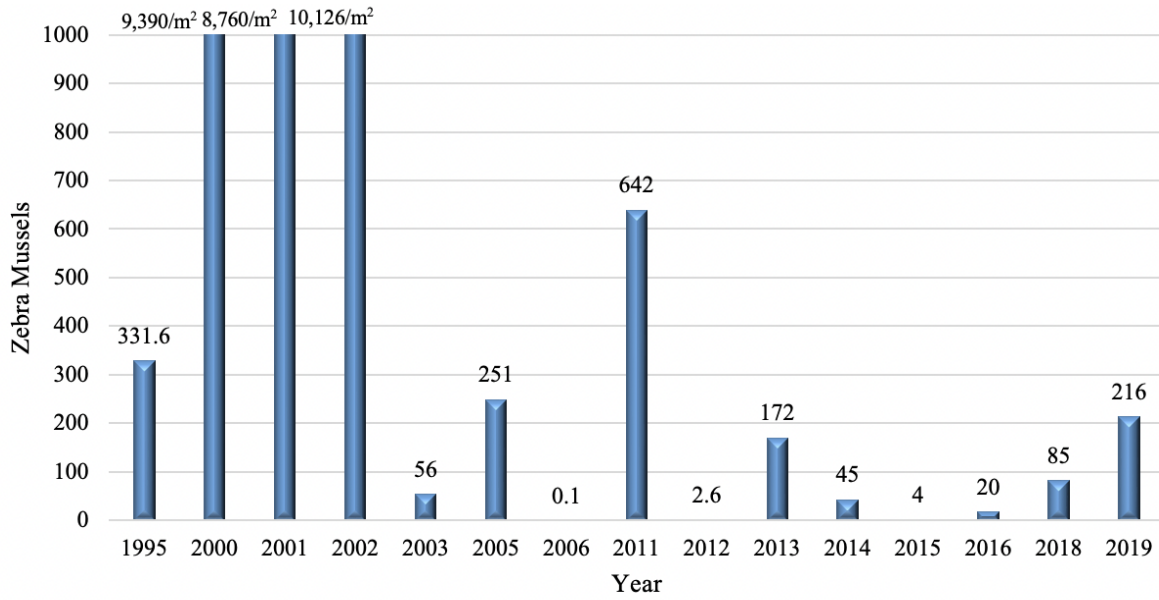


**Figure 10.** Mussel Densities at the Prairie du Chien East Channel Reference Site from 1985-2019 (Minnesota DNR, 2019).

\*Raw data were only available for 2005-2019. Mean densities were compared using one-way ANOVA. Mean densities without a shared letter were significantly different from each other (Tukey HSD test,  $p < 0.05$ ).



**Figure 11.** Relative abundance of all quadrat data at Prairie du Chien (Minnesota DNR, 2019)



**Figure 12.** Zebra mussel density at the Prairie du Chien East Channel Reference Site from 1995-2019 (Minnesota DNR, 2019).

## Conclusions

Long-term data sets such as CAMP’s are paramount in understanding the status and trends of native mussel populations. The relative abundance data shows that CAMP’s efforts to restore native Minnesota freshwater mussels seem to have been effective thus far, according to the two propagation species shown to be recovering in Figure 9. Propagation on the larger scale has been helpful to population restoration because there are more and more success stories, hence why it was less emphasized in the updated conservation plan than in the original from 1998 (“A National Strategy for the Conservation of Native Freshwater Mollusks,” 2016). Research conducted by CAMP and other facilities/organizations has studied other potential areas of improvement for mussel propagation and conservation such as host fish trials, testing of various

substrate types, feeding mechanisms, and countless other projects (Hart et al., 2018; Davis, 2021). Native mussel restoration efforts in the future will be influenced by studies such as these, with findings that have the potential to lead the work in many different directions for maximal positive impact. Discoveries such as improved methods for host fish husbandry, new techniques in larval propagation, additional host fish species, and others all may play a significant role in future mussel conservation work (Hart et al., 2018). Despite ongoing research to improve and continue the recovery of these species, propagation is only a reactionary solution with unknown self-sustainability in the long term. Many of these studies confirm the harmful effects of dam construction on native mussels, particularly because they interrupt the migrations of many key host fishes (Hart et al., 2018). It is for these reasons that habitat fragmentation caused by dams can only truly be remedied - and facilitate long-term mussel success that is self-sustaining without propagation assistance - through dam removal (Haag & Williams, 2014).

Before I learned of the Minnesota DNR program and interviewed for its CAMP internship, I did not know about mussels and the threats they are facing. There was once a guest lecturer, Dr. Peter Hazelton, in my Sustainable Aquaculture class who educated us about the situation with native freshwater mussels and thoroughly discussed the research and propagation efforts underway to combat their decline. It was a particularly relevant class because it was mere hours after my interview for the MN DNR internship position. My time as an intern taught me everything I know about mussel conservation, and I have since been applying for permanent jobs far more related to this field than I previously ever thought I would be interested in. It truly

changed the course of my career by significantly broadening the scope of things I thought I could do with my education and experience and affirming my desire for a career in conservation.



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## Supplemental Information

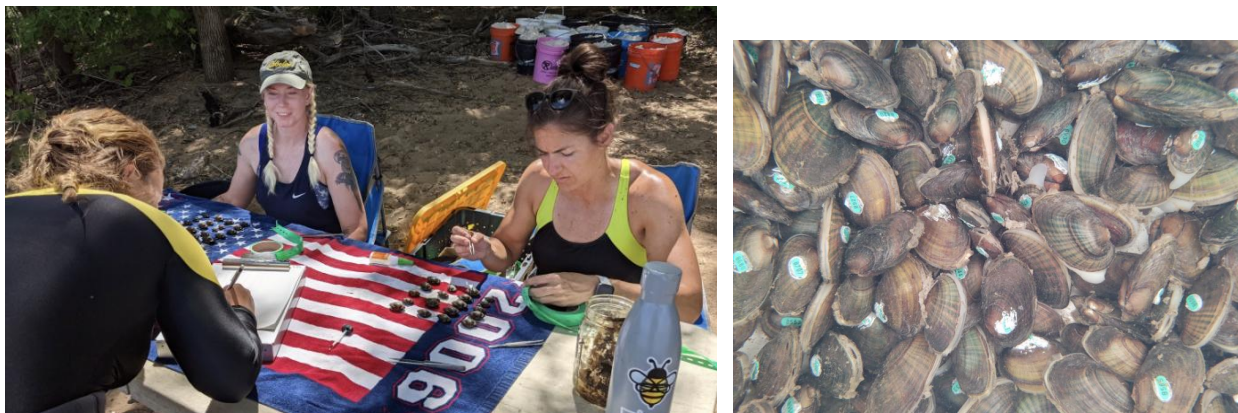
### **Propagation Methods Summary**

1. Adult mussels and host fish are collected and brought to the lab, where the mussels are then monitored for glochidia larvae release.
2. When the females release glochidia/conglutinates, they are collected and observed with microscopes to determine level of development.
3. Larvae that are mature enough to have a hinge line and visible shell opening/closing are promptly used to inoculate the host fish. Inoculated host fish are kept separately - divided by mussel species, watershed, and inoculation date.
4. After the larvae have been growing on the host fish for several weeks (specific time frame depends on the species, typically 2-3 weeks), collection nets are placed under the outflow of each tank. When juvenile mussels drop off the host fish, they are flushed out of the tanks into the collection nets.
5. Collection nets are rinsed out over a stack of sieves decreasing in size to eliminate debris and leave just the mussels at the bottom. The contents of the smallest (lowest) sieve are rinsed into a petri dish.
6. The number of juveniles in each petri dish is counted and recorded. The juveniles from each are placed in various containers (AHAB tanks, buckets, etc.) to continue growing.
7. Mussels are transferred to grow-out systems, like tote bins or baskets, that are placed in bodies of water where they overwinter and are contained until release.

## Volumetric Count Procedure

The petri dish is emptied and rinsed into a 500 mL beaker, and river water is added up to a designated volume. The best total volume depends on how concentrated the mussels were in the petri dish, because the mixture does not need to be too dilute. The mussels naturally settle to the bottom of the beaker, so they are stirred up with a baster until the mixture is as heterogeneous as possible. While the baster is still mixing, a micropipette is used to remove 0.5 mL and place the drop onto a large petri dish. This is repeated until the dish has 10 drops and repeated with a second large petri dish until both dishes have the same number of drops. Each dish is then examined under the microscope and the number of mussels per 0.5 mL drop is recorded. The numbers of mussels per drop are added to get a total number of mussels between the two dishes, which is then divided by 10 mL to get a count of mussels/mL. This is then scaled up according to the total volume of water in the beaker to get an estimated total number of mussels.

## Background Figures



**Figure S1.** Mussel tagging. Left: Placing tags on mussels from a tote bin and recording their sizes and tag numbers during a monitoring project. Right: Tagged mussels.



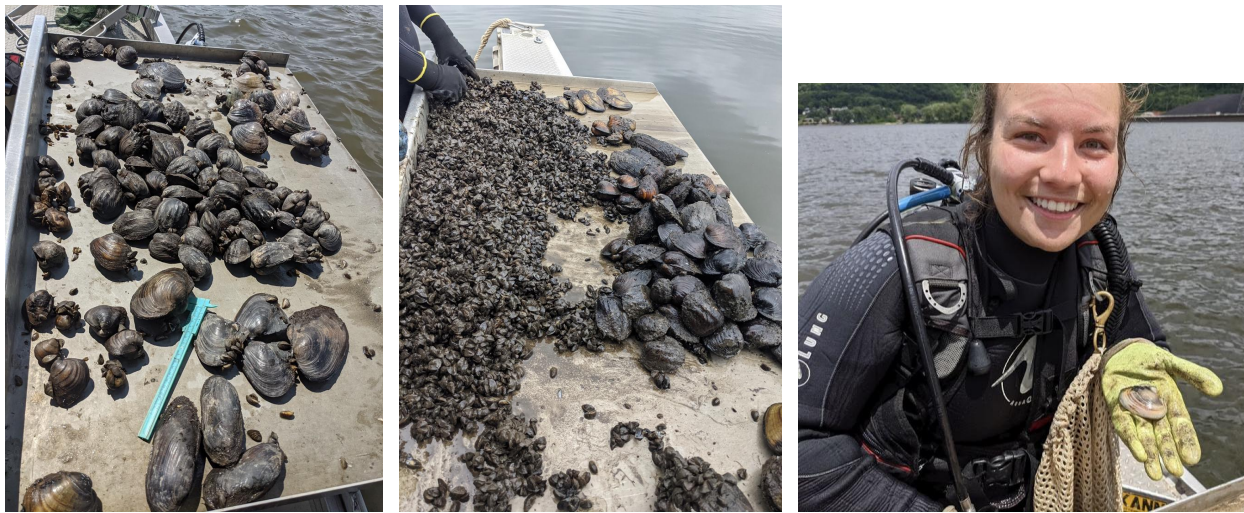
**Figure S2.** Water quality monitoring. Photos show collection of YSI data and water samples.



**Figure S3.** Site monitoring conducted by digging quadrats to collect spatial distribution data.



**Figure S4.** Strandings/Rescues. Intern throws a stranded mussel into deeper water.



**Figure S5.** Surveys. Photos show what mussels collected during various surveys, including both timed searches and quadrats. Recording data on mussel species, abundance, age, length, location, number of zebra mussels attached, etc.



## Supplemental Tables

**Table S1.** Species richness and relative abundance of unionids from qualitative and quantitative samples at the Reference Site, Downstream Site, and Turning Basin from 2014-2019 (Minnesota DNR, 2014, 2015, 2016, 2018, 2019).

	Year	Reference Site	Downstream Site	Turning Basin Site	Total
<b>Number of Mussels Collected</b>	2014	403	37	180	620
	2015	525	37	105	667
	2016	509	52	61	622
	2018	325	39	148	512
	2019	248	56	32	336
<b>Number of Species Collected</b>	2014	19	10	12	20
	2015	19	9	12	20
	2016	22	13	10	24
	2018	19	10	15	19
	2019	21	13	7	24
<b>(CPUE) Catch per Unit Effort (mussels / min)</b>	2014	5.5	1.23	6.0	4.4
	2015	8.8	1.2	3.5	5.6
	2016	8.48	1.73	2.03	4.9
	2018	5.42	1.30	2.03	4.27
	2019	1.85	0.83	0.87	1.35

**Table S2.** Quantitative Samples: Abundance and density of native mussels at Prairie du Chien East Channel Reference Site from 2014-2019 (Minnesota DNR, 2014, 2015, 2016, 2018, 2019).

	<b>Year</b>	<b>Reference Site</b>
<b>Number of Mussels Collected</b>	2014	73
	2015	111
	2016	104
	2018	118
	2019	137
<b>Number of Species Collected</b>	2014	16
	2015	14
	2016	15
	2018	16
	2019	17
<b>Mussel Density (mussels / m<sup>2</sup>)</b>	2014	14.6
	2015	22.2
	2016	20.6
	2018	23.6
	2019	27.4