Mussel reestablishment as a best management practice: scaling their nutrient effects from the individual to the stream.

Annual Research Report for RPG Project: Mussel Performance in an Urban Stream Environment

Report 1, February 20, 2024.

Project lead, Sally Entrekin (email: sallye@vt.edu) and collaborators with their affiliations:

Sally Entrekin, PhD, is an aquatic entomologist and ecosystem ecologist in the department of Entomology at Virginia Tech. She is the lead PI responsible for management and coordination of all aspects of the project. She is working with the USGS on understanding how macroinvertebrates and organic matter dynamics change and interact with restoration efforts in the context of mussels and without mussels. She will direct the Postdoctoral Fellow, co-advise a PhD student and mentor undergraduates.

Donya Mohamed, B.S. is pursuing her degree in Entomology. She has been fully funded through college and departmental fellowships but her project is addressing mussel sensitivity to secondary salinization. She has also worked as a research technician on this project.

Jess Jones, PhD, is a restoration biologist with the US Fish and Wildlife Service stationed at Virginia Tech. He is responsible for collecting and/or cultivating mussels, introducing them into the two streams in baskets and free and his crew is monitoring the survivorship, movement, and fitness of the mussels throughout the project duration. He is working with a full-time technician, a Postdoctoral Fellow, and plans to co-advise a PhD student, and work with fisheries on a mussel-fish action plan that supports mussel sustainability.

Athan Anderson, B.S. is working as a technician on this project by supporting the efforts of Jess Jones. In particular, Athan worked on mussel stocking, GPS coordinates for the stream reaches and mussel monitoring.

Chester Zarnoch, PhD, studies the structure and functioning of bivalves in estuarine and freshwater habitats, Baruch College, New York. He is responsible for measuring mussel feeding and filtration rates in-situ following mussel re-introductions in baskets and in the benthos. He is working closely with Entrekin, Jones, and Bruesewitz to collate data on how much mussels filter and the nutritive characteristics of their egesta and excreta. These data will be combined with the USGS real time data as a way to upscale dissolved and particulate nutrient dynamics to inform how many mussels are needed to mitigate or augment nutrient retention and flux.

Denise Bruesewitz is a biogeochemist and ecosystem ecologist in Environmental Studies at Colby College, Maine. She is measuring the role of mussels in baskets and in the benthos on nitrogen removal as a long-term storage via assimilation vs permanent removal via facilitating denitrification in mussel beds with higher organic matter, ammonium and nitrate. Dr. Bruesewitz is working in collaboration with Dr. Zarnoch to use in-situ chambers and collecting sediment cores for denitrification. She is also working with Zarnoch and Entrekin on whole-stream nutrient uptake, metabolism and denitrification.

Paul Angermeier, PhD, is a fishery biologist and Assistant Unit Leader with the USGS Cooperative Fish and Wildlife Research Unit at Virginia Tech. He is collaborating with the USGS fish-sampling crew and Jones to develop a fisheries management plan that informs mussel translocations based on existing fish assemblages and outlines management actions to ensure long-term maintenance of mussel populations. The plan will synthesize data on fish assemblage composition, population densities and age

structure, dispersal abilities, and habitat suitability in the study streams – with an emphasis on species that are hosts for reintroduced mussels.

Brendan Foster is a hydrologist at the USGS VA/WV Water Science Center, MS. His group is collecting water samples for nutrients and sediment using discrete and continuous monitoring approaches. USGS is monitoring water downstream of the two restored streams, collecting benthic macroinvertebrates, and monitoring fish. USGS has also expand their monitoring to incorporate transect sampling using hand held nutrient sensors as a way to accommodate additional sampling closer to proposed mussel beds (see Figure 1)

Kathy Morrow, PhD is an educator at the local high school in Reston, VA. As an expert in microbial ecology and genomics, Kathy is working with high school students to characterize the mussel microbiome.

Executive Summary

A total of 2,816 adult mussels of the Eastern Elliptio (*Elliptio complanata*) were collected in the spring and early summer of 2023 by hand via snorkeling in the mainstem of the Potomac River and several of its tributaries (Table 1). Mussels were added to Snakeden and Glade Run in July 2023 and as of December 21st, 98% of the mussels have survived. In September 2023, we completed mussel chamber experiments in-situ to measure their contribution to nutrient cycling and metabolism. We also measured their filtering rates, biodeposition and excretion using side-channel experimental chambers that sourced stream water. In addition, the USGS has continued to maintain gaging stations for basic water quality parameters in addition to adding fine spatial scale measurements of nitrogen and conductivity in fall and winter. The research group is on-target (and slightly ahead) of our proposed timeline in Figure 2. Our next steps will be spring benthic sampling for macroinvertebrates and fish, servicing of the deployed sondes for water quality and preparation for the summer field season. More mussels will be added this summer, in addition to more in-situ mussel sampling to quantify their contribution to nutrient cycling and biodeposition. We will also add lab experiments to identify how NaCl influences mussel functions and interacts with the benthic macroinvertebrates.

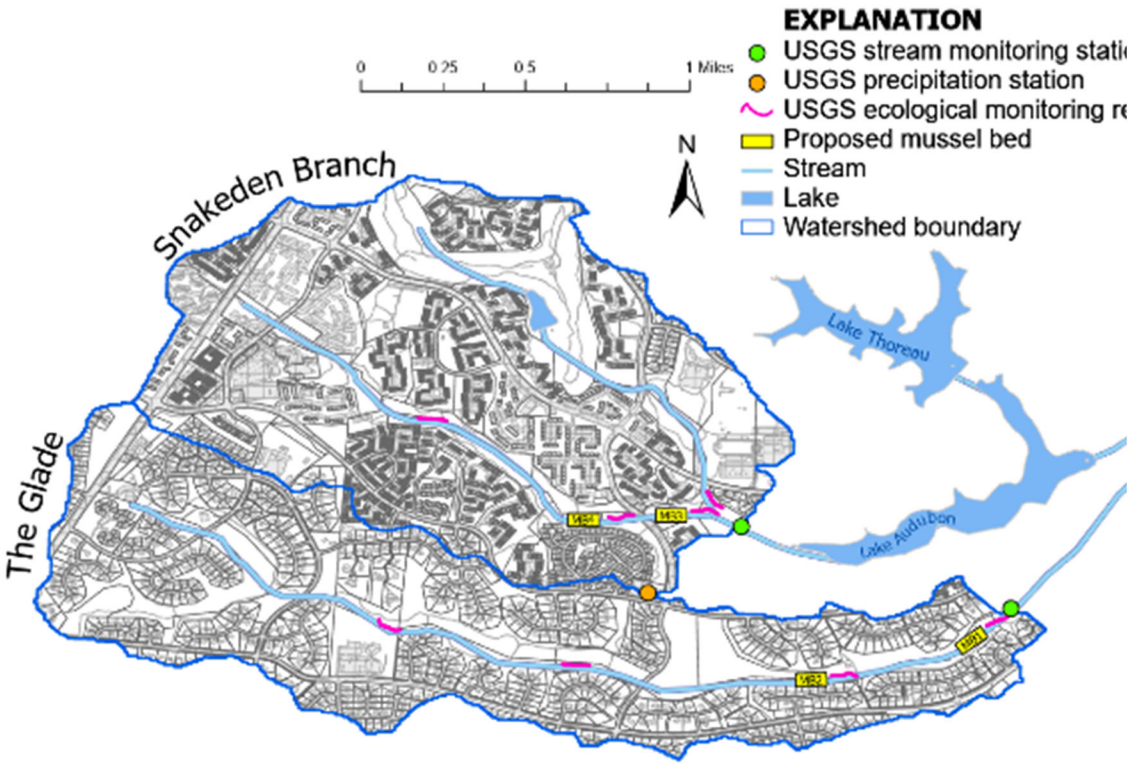

Figure 1. The sub-basin of Snakeden and Glade Run. Gages are indicated by dots and sample reaches are pink. Mussels were added to the reaches closest to the USGS gaging stations.

Scope of Work and Schedule: We are providing a process for reintroduction/tracking (*obj 1*) and monitoring (*obj 2*) of mussels and associated water quality effects as a way to mitigate nutrients and stabilize substrates in restored urban headwaters. Project tasks organized by lead institutions in Figure 2 show the approach, general timeline, and minimum collaborative products. Mussels and monitoring began in mid-July 2023 (year 1), experiments were conducted in the summer of year 1 and will continue in the summer and autumn of years 2-4 (*obj 3*). We will have an in-person team meeting in year 2 so goals towards mussel establishment and nutrient and FPOM effects can be assessed, mussels stocking assessed and possible additional mussel species added and experimented in years 3 or 4 informed by convergence meeting a possible fish management plan (*objs 4-6*). Modeling and scaling will occur as a collaborative exercise led by VT by the Postdoctoral Fellow starting at the end of year 2. The official Postdoctoral Fellow ad can be found here (Figure 2): https://careers.pageuppeople.com/968/cw/en-us/job/527778/postdoctoral-research-associate

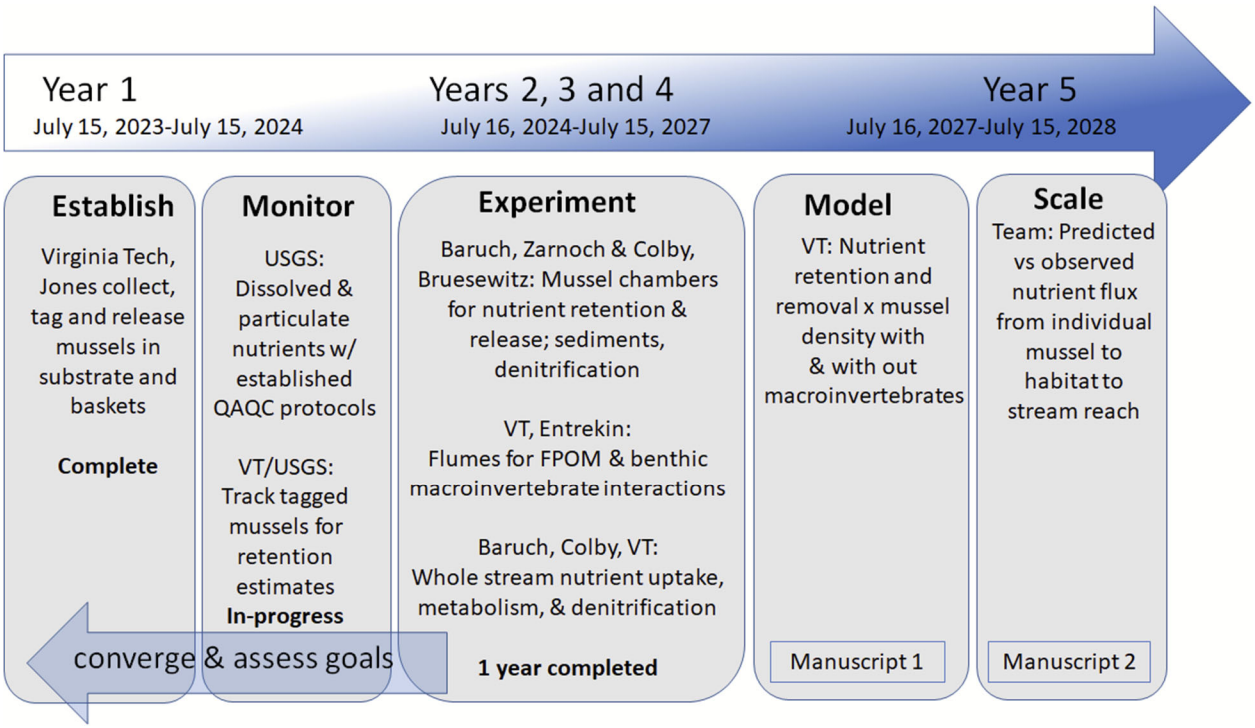

Figure 2. Major project tasks organized by lead institutions. This organizational timeline shows when mussels were added to Glade and Snakden and mussel monitoring began in mid-July 2023 (year 1), experiments were conducted in the summer of year 1 and will continue in the summer and autumn of years 2-4 (*obj 3*). We will have an in-person team meeting in the fall of year 2 so goals towards mussel establishment and nutrient and FPOM effects can be assessed, mussels stocking assessed and possible additional mussel species added and experimented in years 3 or 4 informed by convergence meeting a possible fish management plan (*objs 4-6*). The project began in Fall 2022, sensors for water dissolved oxygen, temperature, specific conductance and algal biomass were deployed in January 2023.

Summary of the progress since January 2023:

- January 18th, 2023: YSI water quality sensors that measure dissolved oxygen (DO), water temperature, specific conductance, and algae pigments every 2 hours were deployed at Snakeden and Glade Run in the upstream control reach where there are no mussels.
    - See map for sensor locations:
    - https://www.google.com/maps/d/u/1/viewer?mid=1784_OVukUiDd0VwIx2b9eG5a7luyBk9A&ll=38.93206720391859%2C-77.34770085196618&z=14
- March 8th: Downloaded and checked all sensors, redeployed.
- April 7th: Sonde was downloaded and benthic macroinvertebrates were sampled in pools using a quantitative Hess sampler.
- April 20th: Angermeier and Jones (Entrekin as the straw PI) submitted a proposal (Restoring freshwater mussels and host-fishes to urban headwaters of Chesapeake Bay) to the NFWF WILD PTA program-not funded but can be used for future proposals.
- June 27th: Benthic macroinvertebrates were sampled in pools using a Hess sampler: 5 in both streams in Snakeden, Broad, and Glade Run. A conductivity sensor was deployed in Broad Run.
- mid-July (Before mussels): Brendan Foster led USGS effort to capture dissolved oxygen, specific conductance and nitrate concentrations across the control and mussel added reaches using sondes in Glade Run and in Snakeden Run. These data are currently under USGS internal review: specific conductivity, dissolved oxygen, temperature, specific conductivity, nitrate, and turbidity.

- July 26th and 27th: ~1500 adult *Elliptio complanta* added to each Snakeden and Glad Run Creeks
    - News coverage: https://www.wusa9.com/article/tech/science/environment/virginia-tech-researchers-mussels-streams-project/65-581097d3-e8ed-490a-855b-acbf0639f2d1
- July 25-28th: Trays of substrate and mussels added to each reach in preparation for the nutrient and gas exchange experiments (Jess, Athan, Donya, Sally, Denise, Paul)
    - Map of pools with mussels added: https://www.google.com/maps/d/u/0/edit?mid=1U3_3gxlAIHBZHu5rM3NJZGNjXRIgBH4&ll=38.92692772375668%2C-77.33739808055475&z=16
- August 14-17th: Experimental trays with mussels and substrate were used for approximating mussel respiration and excretion. Flow-thru chambers with mussels were used to estimate filtering and assimilation (Chester, Tim, Denise, Sally). Water samples were also taken by VT and analyzed at the VT Soil Lab for DOC, nutrients, and trace elements. Benthic macroinvertebrate samples were taken in pools in the Control and Treatment sites in Snakeden and Glade Run.
- September 14th: Mussels relocated on banks or downstream were placed back in the stream (Snakeden Creek).
- October 7th: Dr. Kathy Morrow conducted preliminary sampling on mussel shells for microbe analysis.
- mid-October (after mussels): Brendan Foster led USGS effort to capture using sondes that were run across transects in Glade Run and in Snakeden Run. These data are currently under USGS internal review: specific conductivity, dissolved oxygen, temperature, specific conductivity, nitrate, and turbidity.
- November 16-17th: Benthic macroinvertebrates samples were taken in pools in the Control and Treatment sites in Snakeden and Glade Run. Sondes were downloaded.
- December 19th: Mussel shells collected and both streams walked. Pools were mapped. This trip followed a big storm that resulted in a lot of bedload movement. Retrieved 2 dead mussels in Glade Run and 10 in Snakeden.
- **Media Coverage of the Project** – Four Washington D.C. TV stations covered the project to restore mussels in Glade and Snakeden Runs this summer and the story aired multiple times in late July. NBC 4 Washington - Researchers restore mussels to clean up Potomac River watershed
    - Virginia Tech filmmaker Ray Meese produced "Restoring Filter Feeders in the Potomac River": https://news.vt.edu/videos/k/2023/08/1_b9qmxi01.html to showcase the project and the role that mussels play in water quality in streams in Northern Virginia and Washington, D.C.

Progress by objective:

Objective 1. Mussel collection, tagging and release into the streams was successful (lead by Jess Jones). All observations suggest that mussels have established in Glade Run and Snakeden but are more mobile in Snakeden Run. The following information details the mussel reintroduction process.

Mussel Collection – A total of 2,816 adult mussels of the Eastern Elliptio (*Elliptio complanata*) were collected in the spring and early summer of 2023 by hand via snorkeling in the mainstem of the Potomac River and several of its tributaries (Table 1). All mussels were collected under permits obtained by Jess Jones from West Virginia Department of Natural Resources, Maryland Department of Natural Resources, and Virginia Department of Wildlife Resources. Collectors included Jess Jones, Athan Anderson, Kayla Howard, and Richard Neves from the Freshwater Mollusk Conservation Center (FMCC), Department of Fish and Wildlife Conservation at Virginia Tech, Donya Mohamed and Sydney Haney from the Department of Entomology at Virginia Tech, Andrew Phipps, U.S. Fish and Wildlife Service, and Zachary Taylor from

the Maryland Department of Natural Resources. All mussels were transported to the FMCC where they were cared for and tagged by staff. Captive care included separating the mussels by site into holding systems connected to a pond which continuously supplied water with fine particulate organic matter (FPOM) and phytoplankton to the mussels for feeding and maintaining physiological condition.

Mussel Tagging – Once at FMCC, all mussels were tagged using Hallprint tags and fast-acting superglue. Each Hallprint tag has a unique letter and number identifier to facilitate future monitoring of the mussels in each stream. We let the glue dry for approximately ten minutes, and while drying, the length and weight of each mussel were measured in millimeters and grams, respectively, and recorded in an Excel spreadsheet. All tagged mussels were then returned to their holding systems at FMCC. Mussels were tagged in July over a 2-3 week period and many people participated in the tagging, including Athan Anderson, Rachel Joe Sussman, Dawn Mercer, and Jess Jones from FMCC, and Donya Mohamed, Elizabeth Sicking, Holly Stillwell, Sydney Haney, Marlaina Marvin, and Ty Christian from Sally Entrekin's group in the Department of Entomology at Virginia Tech.

Mussel Stocking – All mussels were gathered from their holding containers at FMCC on the morning of July 26 and transported to Reston, Virginia the same day by Donya Mohamed, Athan Anderson and Jess Jones. Mussels were transported in large coolers under constant aeration. Once mussels arrived at each stream they were placed in baskets for acclimation and counting and stocking to each habitat unit (Figure 3). Both Snakeden and Glade Runs received mussels in approximate equal proportions from each collection site, i.e., ~25% from the Potomac River, Cacapon River, Cedar Run, and Broad Run, respectively. On July 26, a total of 1,224 mussels were stocked into 11 pool habitat units in Glade Run at an overall density of 1.5 mussels m^{-2}, a density estimate that includes the riffle habitat units (Table 2). On July 27, a total of 1,578 mussels were stocked into 8 pool habitat units in Snakeden Run at an overall density of 1.9 mussels m^{-2}, a density estimate that also includes the riffle habitat units (Table 3). Thus, a total of 2,802 mussels were stocked in both streams. This total does not include the 14 mussels that died at FMCC before stocking (Figure 3, Table 1).

Mussel Survival to Date

A total of 2,802 mussels were stocked in Snakeden and Glade Runs, with 49 mussels collected dead in Snakeden over a five-month period from August to December 2023, for a survival rate of 98.2% overall for the project so far. However, all dead mussels were collected in Snakeden Run where 1,578 mussels were

stocked, resulting in 96.9% survival of mussels in this stream. An additional 14 mussels died at FMCC before relocation to each stream.

Figure 3. Mussel introduction day where the press interviewed Donya Mohamad (left) and Athan Anderson (right) as they prepared to place the mussels (in red baskets) upstream.

Figure 4. Biodeposition experiments where the mussels were removed from the stream and allowed to feed and egest in the individual chambers shown above. This design allowed us to estimate how much fine particulate matter the mussels were able to assimilate.

Objective 2. Mussel and water monitoring are led by all group members.

Mussel observations. During stocking none of the mussels were initially put into the riffle habitat units. All mussels were only initially stocked into pools. However, after several high-stream discharge events during the summer, mussels moved into the riffles, especially in Snakeden Run. Thus, an overall mussel density that includes the riffle habitat units is reported here for both streams (Tables 4 and 5).

YSI Sonde and grab sample data have been conducted prior to mussel addition and periodically since. We have two sondes deployed at the most downstream end of the control reaches in Glade Run and Snakeden Run Creeks. The sondes are equipped with specific conductivity, turbidity, chlorophyll a, temperature and dissolved oxygen sensors. Grab samples were taken for water column trace elements and nutrients in August and again in September. These discrete samples were taken in the control and treatment reaches of Snakeden Run and Glade Run. Analyses were conducted by the VT soils lab in SPES.

USGS transect data were taken with a portable sonde equipped with dissolved oxygen, specific conductivity, temperature, nitrate and turbidity sensors. These transects were conducted in the control and treatment reaches of Glade Run and Snakeden Run Creeks. Data were collected in July prior to the mussel addition and in mid-July following stocking by about 2 months. Data are currently shared with the group but not widely available until the USGS internal review is complete.

USGS Stream Gage Data are monitored continuously and the gage data can be found at the following locations:
Snakeden Run: https://waterdata.usgs.gov/monitoring-location/0164578734/#parameterCode=00065&period=P365D&showMedian=false
Glade Run: https://waterdata.usgs.gov/monitoring-location/0164579522/#parameterCode=00065&period=P365D&showMedian=false

Objective 3. Experiments to measure mussel influence on carbon, nitrogen and phosphorus dynamics.

Mussel impacts on stream nitrogen cycling

Mussel and sediment treatments were created in July by filling $0.051 m^{-2}$ benthic chamber bases with sediment from the control (upstream and downstream) and mussel reaches in each stream. Six mussels were added to the bases in the mussel reach treatment (density = 118 m^{-2}). The chamber bases were incubated in the streams for ~3 weeks to re-establish the microbial communities and redox gradients. Benthic chamber incubations were performed in August. Plexiglass chambers (~16 L) were attached to the bases and a magnetic impeller was used to stir the water to prevent stratification within the chamber. Incubations were performed for ~ 3h with samples taken at the start of the incubation and 3 times over the course of the incubation. Sample collection included those for gas (O_2 and N_2) and nutrients (NO_3^-, NO_2^-, NH_4^+, and PO_4^-) analysis.
At the end of the incubations, mussels were removed and stored for analysis of tissue and shell dry mass. Sediment samples were collected from each chamber base to determine ash free dry mass and carbon:nitrogen ratio.
Sample analysis is underway at Baruch College, City University of New York.

Mussel feeding behaviors.

In August, mussel feeding behaviors were measured at each stream using the biodeposition method (Figure 4).

- Feeding chambers were set up along the back of each stream and stream water was continuously pumped into the chambers.
- Mussel biodeposits were collected in timed trials with feces and pseudofeces collected separately.
- Biodeposits were filtered on previously ashed and preweighed glass fiber filters.
- Biodeposit analysis is underway and being led by Dr. Zarnoch at Baruch College, City University of New York.

Figure 4. on the right shows the flow thru experimental chambers for feeding behavior and left shows filtered feces from the stream with Glade on the left and Snakeden on the right indicating differences in the trophic basis of production.

Additional project progress that supports and extends beyond objectives and have been conducted without funding through this grant:

- **Mussel microbiome:** Dr. Kathy Morrow microbiome experiment as a capstone project for the high school students in her class. Progress to date: Sample Collection 10/7/23 1-5PM; Rained in the morning. Link to maps: https://www.google.com/maps/d/u/0/edit?mid=1JCU9siVY-Y97FHzueM2b7tu_7zkCuFs&ll=38.930446447160726%2C-77.34307878645609&z=19
- At each location collect:
    - 5 sediment samples in duplicate = 10 2 mL tubes * 3 locations * 2 streams = 60 tubes
    - 5 biofilm samples = 5 sterile sponges in whirl-pak * 3 * 2 = 30 sponges
        - 5 biofilm samples by scraping rocks at Glade only = 5 * 3 = 15 - Same rocks as sponge biofilm
- **Fish habitat assessment and outreach: proposal written and submitted by Paul Angermeier and Jess Jones to the National Fish and Wildlife Foundation – Chesapeake Bay WILD Grants- Planning and Technical Assistance program. Submitted on April 20th. Not funded.**
    - **Title:** Restoring freshwater mussels and host-fishes to urban headwaters of Chesapeake Bay
    - **A. Goals and Objectives:**
        - *a. What are the overall goals and objectives for the project?*
        - Goals: 1) Develop background knowledge of required host-fishes to ensure long-term success of ongoing reintroduction of freshwater mussels into two Chesapeake Bay streams; 2) Engage with local K-12 schools to enhance environmental education regarding the importance of restoring freshwater mussels in Chesapeake Bay streams.
    - Objectives: 1) Conduct literature review to identify known host-fishes for six reintroduced mussel species; 2) Assess habitat suitability in two restored streams for known host-fishes identified above; 3) For host-fishes not present in the restored streams, identify nearby streams as sources for translocation and develop stocking plans to maintain host-fish populations in the restored streams; 4) Collaborate with key teachers in K-12 schools in Reston, VA who are involved in environmental education related to Chesapeake Bay; 5) Co-create or enhance environmental education programs to increase awareness of the importance of restoring freshwater mussels in Chesapeake Bay streams.

- **Does secondary salinization in urban streams alter the role of mussels in nutrient cycling: Donya Mohamed, Master's thesis and she is a student in Entomology who has proposed additional experiments.** Anthropogenic impacts are the main cause of freshwater mussel decline. Agricultural and commercial activities that can result in increased nutrient loadings and increased salinization in freshwaters are altering mussel populations. The use of salt as de-icing agents on roads in the United States are causing sodium and chloride concentrations to increase in urban streams. The effects of salinization can often be detected by an increase in chloride, which is more commonly studied than sodium in urban streams. There is a growing concern over the effects of increasing chloride levels on aquatic organisms, particularly unionid mussels, who are sensitive to ionic changes in water quality. Unionid mussels are generally limited in their mobility, have complex life-histories, and maintain the lowest internal salt concentration of any freshwater organism, therefore, even the smallest increase in the external medium can result in their stress. Mussels contribute to nutrient cycling in aquatic ecosystems by the retention of carbon (C), nitrogen (N), and phosphorus (P) in tissues, increases of soluble C, N, and P to the sediment via excretion and biodeposition, and enhancing denitrification through the enhancing of microbial activity on sediments. Unionid mussels' water filtration, retention, nutrient excretion,

and biodeposition can have strong effects on the nutrient cycle and there is a growing concern for how rising NaCl is affecting their functions. During a chronic 28-day laboratory study, *Elliptio complanata* will be exposed to an urban gradient of sodium chloride (reference, 250, 500, and 1000 mg/L) to quantify the rates of filtration (measured as clearance rates), retention, and biodeposition of C, N, and P. On days 1, 7, 14, and 28, mussels will be randomly selected for experimental assessments. Quantifying how mussels' filtering behavior, retention, excretion rate, and biodeposition of C, N, and P change with elevated NaCl can be used to support mussel restorations. If mussels can mitigate excess nitrogen in urban streams that are being salinized, the findings could potentially inform the implementation of mussel-mediated denitrification as a mitigation tool for excess nitrogen in urban streams.

- Dr. Tim Hoellein, Professor, Biology Department at Loyola University in Chicago, has participated in the biogeochemical sampling and all of the meetings since the mussels were introduced.

Summary of Progress to Date

The first round of mussel additions have been completed with success. After 8 months, we have documented a remarkable survival rate of 98%. The USGS has continued water quality monitoring that will allow us to calculate the Before After Control Impact that we aimed to achieve. In addition, we deployed dissolved oxygen, temperature, conductivity and turbidity sensors at the end of the control reaches for additional analysis. The USGS also conducted water quality analysis across shorter spatial intervals using a handheld sonde as a way to detect mussel-specific changes to nitrate concentrations in the water column. In addition to the comprehensive water sampling, we have also sampled benthic macroinvertebrates in two ways: qualitative and quantitative measurements that will allow us to identify how the stream condition is changing using the Virginia State Condition Index and quantitative measurements in the mussel beds to understand how the mussels and benthic macroinvertebrates could be interacting. Finally, the first year of mussel-driven changes in stream biogeochemistry have been conducted and are currently being analyzed. The experimental chambers where mussels were confined to an air tight system will provide measurements of nitrogen cycling and dissolved oxygen changes. The feeding experiments that were conducted near-channel will allow us to quantify the quality and amount of fine particulate matter that the mussels are filtering, excreting and rejecting. In addition to these studies, Dr. Morrow, a local high school teacher, is working on how mussel microbiomes support nutrient cycling and a Virginia Tech graduate student, Donya Mohamed, is measuring changes in mussel function from the addition of NaCl, a common urban contaminant.

Acknowledgments: We thank Kevin Eliason with West Virginia Division of Natural Resources, Matt Ashton with Maryland Department of Natural Resources, and Brian Watson with Virginia Department of Wildlife Resources for coordinating the mussel collection permits in their respective agencies, without which this work would not be possible. We also thank Marlaina Marvin for the support in project management and event coordination in year 1. Ben Rhoades is the Reston Association Watershed Manager and he has been invaluable in supporting our project by coordinating with the homeowners, storing gear, and checking on the mussels after storms.

Table 1. Collection and location information for *Elliptio complanata* translocated to Glade and Snakeden Runs in Reston, Fairfax County, Virginia in the spring and summer of 2023. Mortality before translocation occurred while the mussels were held at FMCC and mortality after translocation occurred in Snakeden Run and were observed dead on July 27. *Unknown were individuals that escaped their holding baskets at FMCC before they were tagged and the collection location could not be determined. "-" Site 1 measurements are from individuals representing all five sites in Broad Run.

Stream	Number Collected	Date Collected	Mean Length (mm)	Minimum Length (mm)	Maximum Length (mm)	Latitude and Longitude	Mortality Before Translocation	Mortality After Translocation
Potomac River - Bles Park	582	May 24	86	39	123	39.0196780, -772447780	6	1
Potomac River - River Bend Park	10	May 24	86	39	123	39.0717800, -77.4463560	0	0
Cacapon - Site 1	200	June 21	67	48	90	39.6174810, -78.2824920	0	4
Cacapon - Site 2	405	June 21	67	48	90	39.6151920, -78.2788630	1	0
Broad Run - Site 1	190	June 27	89	72	122	38.7240580, -77.5285050	6	4
Broad Run - Site 2	200	June 27	-	-	-	38.7240240, -77.5258390	0	0
Broad Run - Site 3	200	June 27	-	-	-	38.7236680, -77.5231720	0	0
Broad Run - Site 4	200	June 27	-	-	-	38.7229000, -77.5214100	0	0
Broad Run - Site 5	200	June 27	-	-	-	38.6921800, -77.4989030	0	0
Cedar Run	603	June 28	77	45	109	38.6169270, -77.5555160	1	1
*Unknown	26						0	0
Total:	**2816**							

Table 2. Summary of July 26, 2023 mussel stocking in Glade Run, Reston, Fairfax County, Virginia. Species stocked was Eastern Elliptio (*Elliptio complanata*). Mean mussel density for the total stream reach area, including riffle habitat units, is 1.5 mussels per square meter (m^{-2}). NA = data not recorded.

Stream	Habitat Unit #	Habitat Type	Length (m)	Mean Width (m)	Area (m^2)	Mussels Stocked	Mussel Density (m^{-2})	GPS Coordinates	Notes
Glade	1	Pool	14.3	4.1	58.4	165	2.8	38.9238, -77.33168	First pool downstream of bench
Glade	2	Riffle	5.8	2.4	32.6	0	0.0	N/A	
Glade	3	Pool	6.7	3.2	26.6	40	1.5	38.92387, -77.33152	
Glade	4	Riffle	6.4	1.9	23.0	0	0.0	N/A	
Glade	5	Pool	10.1	2.8	30.6	65	2.1	38.92397, -77.33136	
Glade	6	Riffle	18.9	1.7	21.3	0	0.0	N/A	
Glade	7	Pool	15.9	4.2	54.6	245	4.5	38.92415, -77.33123	Dead tree in middle
Glade	8	Riffle	10.7	2.6	27.9	0	0.0	N/A	
Glade	9	Pool	12.2	4.2	51.2	175	3.4	38.92435, -77.33096	2 trays stocked with mussels
Glade	10	Riffle	20.4	2.4	37.8	0	0.0	N/A	
Glade	11	Pool	11.3	3.2	39.3	70	1.8	38.92465, -77.33091	
Glade	12	Riffle	11.3	2.4	27.4	0	0.0	N/A	
Glade	13	Pool	13.1	3.0	39.7	49	1.2	38.92479, -77.3308	4 trays stocked with mussels
Glade	14	Riffle	2.4	2.4	43.9	0	0.0	N/A	
Glade	15	Pool	14.6	3.9	56.8	133	2.3	38.92495, -77.33071	Immediately upstream of bridge
Glade	16	Riffle	23.8	2.4	33.3	0	0.0	N/A	
Glade	17	Pool	13.4	2.9	39.0	73	1.9	38.92516, -77.33024	6 trays stocked with mussels
Glade	18	Riffle	11.0	2.3	24.8	0	0.0	N/A	
Glade	19	Pool	12.5	3.4	41.9	95	2.3	38.92531, -77.32998	
Glade	20	Riffle	7.8	2.4	42.3	0	0.0	N/A	
Glade	21	Pool	14.0	4.3	60.0	114	1.9	38.92539, -77.32986	
Totals or Means:			**257**	**3.0**	**812**	**1,224**	**1.5**		

Table 3. Summary of July 27, 2023 mussel stocking in Snakeden Run, Reston, Fairfax County, Virginia. Species stocked was Eastern Elliptio (*Elliptio complanata*). Mean mussel density for the total stream reach area, including riffle habitat units, is 1.9 mussels per square meter (m^{-2}). NA = data not recorded.

Stream	Habitat Unit #	Habitat Type	Length (m)	Mean Width (m)	Area (m^2)	Mussels Stocked	Mussel Density (m^{-2})	GPS Coordinates	Notes
Snakeden	1	Pool	17.7	4.1	72.0	400	5.6	38.93031, -77.34245	3 trays stocked with mussels
Snakeden	2	Riffle	20.4	3.5	71.5	0	0.0	N/A	
Snakeden	3	Pool	15.0	4.1	60.9	100	1.6	38.93034, -77.34264	
Snakeden	4	Riffle	10.7	3.5	37.9	0	0.0	N/A	
Snakeden	5	Pool	19.2	4.8	92.9	400	4.3	38.93026, -77.34282	
Snakeden	6	Riffle	6.4	3.8	24.4	0	0.0	N/A	
Snakeden	7	Pool	11.9	3.8	45.0	0	0.0	38.93021, -77.34299	
Snakeden	8	Pool	19.5	5.1	99.1	400	4.0	38.93019, -77.34321	4 trays stocked with mussels
Snakeden	9	Riffle	24.1	2.9	69.8	0	0.0	N/A	
Snakeden	10	Pool	13.4	4.7	62.4	0	0.0	38.93028, -77.34337	
Snakeden	11	Pool	19.5	5.2	100.8	278	2.8	38.93026, -77.34367	
Snakeden	12	Riffle	5.8	5.7	27.0	0	0.0	N/A	
Snakeden	13	Pool	14.6	4.8	70.6	0	0.0	38.93024, -77.34385	
Totals or Means:			**198**	**4.3**	**834**	**1,578**	**1.9**		

Table 4: Record of dead mussels observed at FMCC and after translocation to Snakeden Run in Reston, Fairfax County, Virginia in July of 2023.

Date Found Dead	Mussel ID	Died Before or After Translocation
7/10/2023	B130	Before
7/26/2023	B171	Before
7/26/2023	B185	Before
7/10/2023	B190	Before
7/12/2023	B425	Before
7/12/2023	B465	Before
7/13/2023	B650	Before
7/13/2023	B768	Before
7/10/2023	B940	Before
7/10/2023	C048	Before
7/18/2023	C262	Before
7/26/2023	C359	Before
7/20/2023	C990	Before
7/26/2023	D709	Before
8/15/2023	B335	After
8/15/2023	B748	After
8/15/2023	B875	After
8/15/2023	C186	After
8/15/2023	C411	After
8/15/2023	C637	After
8/15/2023	C676	After
8/15/2023	D103	After
8/15/2023	D169	After
8/15/2023	D198	After

9/26/2023	B891	After
9/26/2023	C217	After
9/26/2023	C825	After
9/26/2023	C903	After
9/26/2023	D045	After
9/26/2023	D619	After
12/22/2023	B280	After
12/22/2023	B446	After
12/22/2023	B499	After
12/22/2023	B569	After
12/22/2023	B597	After
12/22/2023	B841	After
12/22/2023	C137	After
12/22/2023	C616	After
12/22/2023	C633	After
12/22/2023	D189	After
12/22/2023	D266	After
12/22/2023	D267	After
12/22/2023	D300	After
12/22/2023	D788	After
9/14/2023	B498	After
9/14/2023	D059	After
9/14/2023	D257	After
9/14/2023	D710	After
9/14/2023	D073	After
9/28/2023	B374	After
9/28/2023	C369	After
10/4/2023	C623	After
10/4/2023	D777	After

10/4/2023	C366	After
10/4/2023	B446	After
10/4/2023	B228	After
12/18/2023	B811	After
12/18/2023	C124	After
12/18/2023	C854	After
12/18/2023	B751	After
12/18/2023	D210	After
12/18/2023	D142	After
12/18/2023	C811	After

Table 5: Record of dead mussels observed at FMCC and after translocation to Glade Run in Reston, Fairfax County, Virginia in 2023.

Date Found Dead	Mussel ID	Died Before or After Translocation
12/22/2023	B841	After
12/22/2023	Lost tag	After