Exploring the Use of Freshwater Mussels for Water Quality Improvement in Stormwater Management Ponds

Final report for Delaware Department of Natural Resources and Environmental Control's Clean Water Advisory Council

Project CWQIG 19-03

Partnership for the DELAWARE ESTUARY

February, 2022 | Report No. 22-01

A publication of the Partnership for the Delaware Estuary—A National Estuary Program

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The Partnership for the Delaware Estuary brings together people, businesses, and governments to restore and protect the Delaware River and Bay. We are the only organization that focuses on the entire environment affecting the River and Bay — beginning at Trenton, including the Greater Philadelphia metropolitan area, and ending in Cape May, New Jersey and Lewes, Delaware. We focus on science, encourage collaboration, and implement programs that help restore the natural vitality of the River and Bay, benefiting the plants, wildlife, people, and businesses that rely on a healthy estuary.

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Executive Summary

Two freshwater mussel species native to the region (i.e. *Utterbackiana implicata*, *Sagittunio nasutus*) demonstrated their ability to survive and grow in a diverse array of man-made environments throughout New Castle County, Delaware. Small ponds and basins currently used to manage stormwater runoff may provide a unique opportunity to couple research and restoration efforts and nutrient removal practices in the form of large scale mussel growing operations. Reference and experimental ponds were stocked with mussels and while there were differences in growth rates, mussels fared well in all ponds. While mussel growth structures were readily accessed by shore, relatively few instances of vandalism were observed over the course of the study. Most areas were not secluded and baskets were left alone. One pond was located near an industrial area behind an abandoned lot and baskets there were ultimately lost. Still, these observations were promising.

Interestingly, mussels deployed in some of the experimental ponds demonstrated superior growth rates over the entire study. Few researchers are monitoring mussel growth in nature with this level of rigor, particularly in areas where mussel do not naturally occur (i.e. stormwater pond). This study reveals the potential for greater growth in areas that may have previously been deemed too disturbed or impacted by runoff and pollution to support healthy mussel growth. Even more, this study suggests that mussels may survive and grow faster in some areas, and may even have better body condition than mussels living in what could be considered a less impacted area. These differences may be associated with microscopic food quantity and quality as well as other water quality metrics we may need to monitor in the future (e.g. calcium content).

Still, this study only compared a small cohort of mussels (<100 per pond) and their growth, condition, and survival over a few years to gather high resolution data. Now that data exist to support larger scale efforts, the next step will be to stock exponentially more mussels into these ponds to push forward larger growing techniques as well as determine the true efficacy of their water filtration as it relates to clean water goals.

This research has propelled freshwater mussel science in the region forward and has set up more potential projects to refine the practice of growing mussels for restoration while strategically placing them in areas where nutrient removal may be a goal. Identifying where mussels can provide the most valuable ecosystem services is critical to maximizing their usefulness and leveraging their natural abilities to meet restoration and environmental goals.

Introduction

Freshwater mussels (order Unionida), hereinafter mussels, are a diverse group of bivalve mollusks that are uniquely adapted to live in the benthos of freshwater ecosystems. Mussels live and burrow within a waterbody's bottom substrate (e.g. sand). Similar to saltwater bivalves (e.g. clams and oysters), mussels feed on microscopic matter, called seston, suspended in the water column using their gills and other specialized body parts. Filter-feeding bivalves are often the functional dominant species in ecosystems due to their ability to filter large quantities of microscopic particles out of the water column (Dame 2012, Kreeger et al. 2018). An average mussel filters up to ten gallons of water each day to meet its energetic demands. Where abundant, mussels have the ability to influence nutrient dynamics, maintain and improve water quality, as well as enhance habitat for other aquatic life (Atkinson et al. 2013, Kreeger et al. 2013, Hoellein et al. 2017, Vaughn, 2017). However, the actual water quality benefits depend not only on mussel population size but also on seston composition; i.e. the quantity and quality of suspended particles that comprise the mussels' diet (Atkinson & Vaughn 2015). Mussels are increasingly recognized for their ecological roles and ecosystem services they provide. Accordingly, the use of bivalve mollusks for water quality benefits has gained regional interest through an established Best Management Practice involving oysters in Chesapeake Bay (Parker and Bricker 2020).

Freshwater mussel life history is complex and requires females to brood larvae and interact with an intermediate fish host for reproduction. Unfortunately, more than 70% of the near 300 mussel species that exist across North America are considered endangered, threatened, or of special concern. Mussels are one of the most imperiled aquatic animal groups nationally (Williams et al. 1993, Strayer et al. 2004, Nobles & Zhang 2011, Kreeger et al. 2013). While efforts have been underway for decades across the world, more concerted efforts are being considered nationally (FMCS 2016) and globally to address critical conservation needs where few mussel species still exist (Geist 2010). In the Delaware River Basin, the historical range, abundance, and the species richness of mussel assemblages have undergone extensive reductions (PDE 2012a, 2012b) with few species considered secure in the basin (Table 1). While there are knowledge gaps in mussel biology and conservation (Haag & Williams 2014), mussels have generally been lost from regional waterways due to stressors such as streambed erosion, severe flooding, chemical spills, dam-mediated dispersal limitations, land use changes, and anthropogenic impacts (Neves 1999, Kreeger et al. 2013). Even after a stressor is removed, mussel populations often fail to repopulate due to their intricate life cycle and slow growth rate, among other unknown factors.

As the coordinator for the Delaware Estuary Program, the Partnership for the Delaware Estuary (PDE) is expected to establish measurable goals for sustaining and improving water and habitat conditions and to implement a Comprehensive Conservation and Management Plan (CCMP) to protect and restore natural resources. PDE has elevated healthy freshwater mussel populations as one of a limited subset of "driver" goals that facilitate ecosystem-based restoration in the Delaware River Basin. This goal is based on the observation that mussels are long-lived (species dependent, 30-100 years) and are sensitive to environmental and ecological disturbances such as water quality, water quantity, riparian cover, and fish passage. Hence, to achieve multiple goals for water and habitat conditions in any given water body, a simplified focus on achieving a healthy assemblage of native freshwater mussel species living in abundance will drive positive decision-making in support of broader CCMP actions and needs.

The Freshwater Mussel Recovery Program (FMRP) was launched in 2007 by PDE with the goal of conserving and restoring native freshwater mussels within the Delaware Estuary. This program complements PDE's comprehensive watershed-based shellfish restoration strategy, which also includes saltwater oysters (*Crassostrea virginica*) and saltwater ribbed mussels (*Geukensia demissa)*. For more information on freshwater mussel ecology, life history, and Delaware River Basin species, refer to the *Freshwater Mussels of the Delaware Estuary: Identification Guide & Volunteer Survey Handbook* (PDE 2014) as well as online at the following url: delawareestuary.org/freshwater-mussels

Table 1. Conservation status of freshwater mussel species in the Delaware River Basin.

Scientific Name	Common Name	State Conservation Status		
		DE	NJ	PA
Alasmidonta heterodon	Dwarf Wedgemussel	Possibly Extirpated	Endangered	Critically Imperiled
Alasmidonta undulata	Triangle Floater	Possibly Extirpated	Threatened	Vulnerable
Alasmidonta varicosa	Brook Floater	Extirpated	Endangered	Critically Imperiled
Atlanticoncha ochracea	Tidewater Mucket	Critically Imperiled	Threatened	Critically Imperiled
Elliptio complanata	Eastern Elliptio	Secure	Secure	Apparently Secure
Lampsilis cariosa	Yellow Lampmussel	Possibly Extirpated	Threatened	Apparently Secure
Lampsilis radiata	Eastern Lampmussel	Critically Imperiled	Threatened	Critically Imperiled
Lasmigona subviridis	Green Floater	no data	Endangered	Imperiled
Margaritifera margaritifera	Eastern Pearlshell	no data	Possibly Extirpated	Critically Imperiled
Pyganodon cataracta	Eastern Floater	Apparently Secure	Secure	Apparently Secure
Sagittunio nasutus	Eastern Pondmussel	Critically Imperiled	Threatened	Imperiled
Strophitus undulatus	Creeper	Critically Imperiled	Special Concern	Secure
Utterbackiana implicata	Alewife Floater	Critically Imperiled	Secure	Vulnerable

A growing field of research is hatchery propagation and subsequent grow-out of freshwater mussels. Hatchery propagation has been used as a tool to provide researchers with mussel stocks, thereby reducing pressures on wild populations, to advance our understanding and ability to conserve mussels in the future.

Many systems for growing juvenile freshwater mussels have been developed and improved to encourage growth and survival over the last 30 years. Across North America and Europe, methods range from intensive laboratory rearing with dosed algal diets to extensive pond grow-out where large numbers of mussels are housed in cages, baskets, or other equipment and feed on the natural seston (Gum et al. 2011). To better mimic natural pond conditions and the advantages they confer to juvenile mussels, laboratory systems often contain natural sediment or detritus (Eads and Levine 2012), and may also rely on flow-through of natural pond water (Patterson et al. 2018). Floating baskets described in Patterson et al. (2018) are commonly used for pond grow-out of freshwater mussels. Recent research supports the use of pond grow-out when possible, with growth rates of juvenile mussels an order of magnitude higher in pond baskets than in laboratory systems, even when those laboratory systems used pond water (Martell 2020). In this same study, survival did not differ significantly between pond and hatchery systems. With pond grow-out desirable both for its results and its relatively lower intensity, optimizing systems such as floating baskets and cages is vital. With large-scale mussel grow-out, optimization of these systems aims to minimize maintenance and maximize the stocking density of mussels.

As very small juveniles, mussels are often sensitive to a variety of environmental factors and their mortality can be considerable (Cheng et al. 2020). As mussels grow, they are less prone to mortality and may withstand more challenging environments, including areas influenced by road runoff (Cheng et al. 2021). This project specifically focused on the feasibility of growing mussels in stormwater ponds throughout New Castle County where they may provide water quality benefits. Stormwater ponds are necessary to contain and manage various types of land use and associated stormwater runoff. Researchers sought to determine whether mussels could flourish in man-made retention ponds and if so, what growth rates would be realized. Coupling freshwater mussel growth trials with stormwater ponds provided the opportunity to challenge and expand assumptions on optimal growth conditions for mussels, bring mussels into the public eye, and provide a net positive benefit from mussels filtering out nutrients from ponds.

Methods

Study Site Recon

To increase the probability of success and mitigate potential field challenges, PDE staff worked with New Castle County staff to identify a series of candidate stormwater ponds. Ponds were characterized as either "farm ponds" or "wet ponds". While the characterization of ponds indicated their origin (e.g. farm pond converted vs. engineered specifically for stormwater), each pond served the same purpose of collecting and managing stormwater. Therefore, PDE focused solely on optimizing mussel growth and field logistics at these ponds, regardless of their origin. A desktop analysis was performed to plot where all ponds existed to inform field reconnaissance. Up to six study sites were to be compared to one or more reference ponds where mussels were previously grown in. During field visits of candidate ponds, crews focused on key characteristics of ponds and surrounding areas such as:

- Overall Safety
- Parking Availability
- Shoreline Access
- Wadability
- Pond Depth
- Pond Volume
- Degree of Eutrophication

These criteria were used to rank ponds in relation to feasibility and the probability of mussel mortality vs. growth. In particular, ponds with larger volumes have the potential to buffer times of drought or dilute/mitigate other hazardous conditions. Additionally, while mussels may be able to assist and improve some water quality metrics when in abundance, mussels may not thrive under extremely eutrophic conditions. Mussels require baseline amounts of dissolved oxygen, a typical pH range, and an adequate food source. Finally, mussels contained within a pond would require enough water depth to avoid seasonal conditions including summer temperatures and possible low oxygen levels (especially at night) as well as winter freezing.

Mussel Deployment, Monitoring, and Assessment

Mussel Deployment

To safely hold and grow freshwater mussels in stormwater ponds, staff utilized existing methods of mussel containment consisting of floating baskets tethered to a small anchor. Within each basket, a small diameter mesh was placed at the bottom and approximately one inch worth of cleaned play sand was added to serve as burrowing substrate for mussels. Baskets had larger mesh covers to prevent large debris from filling the basket and to discourage predation (Fig. 1).

Baskets, in sets of three, were deployed into all study sites and received an initial stock of 15 juvenile Alewife Floater (*Utterbackiana implicata*) mussels. Mussels were measured for their shell length prior to deployment using digital calipers (±0.01 mm).

Upon realizing success during the first half of the study (2019-2020), a subsequent deployment of the Eastern Pondmussel (*Sagittunio nasutus*) occurred in 2021 and 15 additional mussels were added to existing baskets at all sites. The additional mussels were measured and monitored in the same manner as the original mussels. The newly added Eastern Pondmussel cohort was smaller in shell length and provided researchers with additional data on growth of a second species and a different size class.

Figure 1. Three floating baskets, complete with can-floats and mesh lids, loosely connected with a tether.

Mussel Monitoring

Field crews regularly monitored baskets to check on mussel mortality, growth, and overall condition of the baskets. If encountered, dead shells were counted and removed from baskets. All live mussels were measured for their shell length with digital calipers connected to a field laptop for immediate data entry and storage (Fig. 2). If needed, clean play sand was added to maintain substrate in all baskets. Any vandalism (e.g. damage) was noted and baskets were cleaned and maintained as needed. Any fouling agents were removed from baskets, floats were re-secured as necessary, and baskets were disentangled. Despite regional work and travel restrictions associated with COVID-19, mussel monitoring was generally unaffected and mussels were monitored with enough frequency to gather useful data. Laboratory analyses were shifted and postponed until work could be completed.

Figure 2. Mussels being monitored in the field with digital calipers connected to a field laptop.

Condition Index Assessment

An assessment of condition index was initiated during the second half of the study and was paired with a subsequent deployment of mussels. To investigate the condition index of mussels (relative assessment of overall health), subsamples were taken of mussels for sacrificial harvest and tissue assessment during the subsequent deployment and final monitoring event. Both *U. implicata* and *S. nasutus* were harvested from multiple study sites in 2021 to investigate how condition index was influenced by species and grow-out site. Mussels were weighed for their total wet weight and dissected for further drying. Tissues were freeze-dried in a freeze dryer for two days until a constant weight was obtained for dry tissue weight. Shells were dried in a drying oven for two days at 60 °C and weighed for dry shell weight. Condition Index (CI) was calculated from mussel dry tissue weight (DTW), total wet weight (TWW), and the dry shell weight (DSW) using the formula originally described by Crosby and Gale (1990):

$$CI = \frac{DTW * 1000}{TWW - DSW}$$

Figure 3. A subset of *U. implicata* mussels to be sacrificed for their condition index.

Water Quality Assessment

Water quality was monitored using two complementary methods, *in situ* spot sampling with a water quality sonde and water grab samples for further particulate analyses (quality and quantity of seston). All i*n situ* sampling was conducted using a Eureka Manta +35 water quality sonde. The water quality parameters measured included dissolved oxygen (mg/L), water temperature (°C), pH, and specific conductance (µS/cm). The water quality sonde was calibrated prior to each field usage.

Water grab samples were collected to characterize seston composition following methods described by Kreeger et al. (1997). At each study site, 4-liter water samples were collected in triplicate using plastic cubitainers. Cubitainers were rinsed prior to sampling using ambient pond water. Collectors submerged cubitainers beneath the water's surface and avoided kicking up sediment during sampling. In the lab, water was passed through a 53-µm sieve and subsequently filtered through glass fiber filters via vacuum filtration. Filters were previously combusted and weighed for their filter weight (FW). Sample filters were frozen until analyses could be performed. Frozen sample filters were held in a drying oven for 48 hours at 60 °C and weighed for dried sample weight (DSW). Dried filters were then combusted for 24 hours at 450 °C in a muffle furnace and weighed for ashed sample weight (ASW). PDE staff used an analytical balance for all gravimetric analyses (±0.01 mg).

The concentration of particulate matter (PM), expressed as mg/L, was calculated based on the volume of water filtered (V) using the formula:

$$[PM] = \frac{DSW - FW}{V}$$

Particulate Organic Matter (POM) was calculated using the formula:

$$[POM] = \frac{DSW - ASW}{V}$$

POM was expressed as mg/L. The organic content fraction of seston was calculated using the following formula:

$$Organic\ Content = \left(\frac{POM}{PM}\right)$$

Results

Study Site Recon

In August 2019, field staff visited a total of 14 ponds at 11 unique sites across New Castle County. Some sites were disqualified due to significant surficial macroalgae (too eutrophic) while others were discounted based on other concerns such as lack of public access (practical access only via private property), shallow depths coupled with very warm temperatures, or depths too deep to feasibly deploy mussels without major watercraft support. The study team ultimately decided upon four new stormwater ponds to test in relation to two existing ponds. Details on the stormwater and reference ponds assessed in this study are summarized in Table 2.

Table 2. Study site descriptions and locations in New Castle County.

Pond Location	Nickname	Latitude (N)	Longitude (W)	Type
Winterthur	WTR	39.80402	-75.59307	Reference
Bellevue Lake	BLV	39.78015	-75.48754	Reference
Tally Day Park	Tally	39.79362	-75.52278	Stormwater
Rockwood Park	Rock	39.77243	-75.51829	Stormwater
Papermill Park	Paper	39.73522	-75.72512	Stormwater
Airport Road	Air	39.65801	-75.59758	Stormwater

Mussel Growth, Mortality, and Condition Index

The growth trials for this project lasted a total of 736 days. During this time, mussels were assessed for their shell length as well as mortality over a series of time steps. A summary of time steps is presented in Table 3. Trials for *Utterbackiana implicata* growth trials encompassed all time steps while trials for *S. nasutus* spanned T-6 through T-7.

Growth was calculated as the difference in shell length from two distinct time steps, divided by the amount of time elapsed. Some baskets were lost due to strong storms (BLV) while others were affected by some vandalism (Air). Mortalities were counted if a dead shell was observed or if the mussel was missing from the basket (aside from basket loss or vandalism). All available data are reported below.

Table 3. Monitoring dates for each site and time step. Elapsed days since the start of the study are in parentheses; nd = no data.

Time Step	Field Site					
	WTR	BLV	Tally	Rock	Paper	Air
T-0	2019-09-05 (0)	2019-09-11 (0)	2019-09-04 (0)	2019-09-04 (0)	2019-09-04 (0)	2019-09-04 (0)
T-1	2019-10-17 (42)	2019-10-17 (36)	2019-10-17 (43)	2019-10-17 (43)	2019-10-17 (43)	2019-10-17 (43)
T-2	2020-04-14 (222)	2020-06-26 (289)	2020-04-15 (224)	2020-04-15 (224)	2020-04-15 (224)	2020-04-15 (224)
T-3	2020-09-16 (377)	nd	2020-09-16 (378)	2020-09-16 (378)	2020-09-16 (378)	2020-09-16 (378)
T-4	2020-12-10 (462)	nd	2020-12-10 (463)	2020-12-10 (463)	2020-12-10 (463)	2020-12-10 (463)
T-5	2021-03-12 (554)	nd	2021-03-05 (548)	2021-03-05 (548)	2021-03-05 (548)	2021-03-05 (548)
T-6	2021-06-07 (641)	nd	2021-06-07 (642)	2021-06-07 (642)	2021-06-07 (642)	2021-06-07 (642)
T-7	2021-09-09 (735)	nd	2021-09-09 (736)	2021-09-09 (736)	2021-09-09 (736)	2021-09-09 (736)

Mussel Growth

Growth trials for *U. implicata* were conducted through day 736. On day 642, subsets of mussels were sacrificed for condition index assessment, which adjusts the mean shell length by site. Only data through day 642 are discussed and represent nearly two full years of growth. Overall, mussels at all sites were able to demonstrate positive shell length change (growth), with mean change ranging from 10 – 34 mm (Table 4). Starting shell size ranged from 58 – 64 mm, which was statistically similar ($p>0.15$, 1-way ANOVA). Ending shell size ranged from 70 – 93 mm, which was found to be significantly different by site ($p<0.001$, 1-way ANOVA). Daily growth rate was 0.02 mm/day for all baskets at both Winterthur and Airport sites. Mussel growth rates were slightly greater at Papermill site (0.03 – 0.04 mm/day). Greatest growth rates were observed in Rockwood and Tally Day sites (0.05 – 0.06 mm/day). These rates are nearly three times greater than at the reference site of Winterthur as well as Airport and almost double the rate at Papermill site. Mussels at Rockwood and Tally Day sites similarly saw greatest change in mean shell length and surpassed 90 mm. A post-hoc Tukey test revealed that while nearly all site-to-site comparisons of mussel size were different ($p<0.001$), Winterthur and Airport mussels were similarly the smallest ($p>0.92$). The same test found mussels in Tally Day were significantly bigger than mussels in Rockwood ($p<0.02$).

The shorter growth trial for *S. nasutus* began on T-6 and lasted 94 days, ending on T-7 as a subset of mussels were then subsequently sacrificed for condition index. This growth trial spanned most of one growing season (June through September, 2021). Shell length data are summarized in Table 5. Mean shell lengths ranged from 55 – 63 mm at the onset of the trial and ranged from 61-73 mm upon termination of the trial. While both starting and ending cohorts of mussels varied significantly in size by site ($p<0.001$ each time period, 1-way ANOVA), mean shell length change was consistently greater for all sites (9-11 mm) over Winterthur (5-6 mm). Mussels at Winterthur demonstrated daily growth rates of 0.06 mm/day while mussels at all other sites grew nearly twice as fast ranging between 0.10 – 0.11 mm/day. A series of Tukey tests determined that mussels were similar in size between Winterthur and Tally Day during T-6 ($p>0.20$) but mussels in Tally Day simply outpaced mussels at Winterthur and grew significantly larger by T-7 ($p<0.001$).

Table 4. Summary of *U. implicata* shell length over the course of the growth trial with overall mean change and daily growth rate. SL = shell length; SEM = standard error of the mean; N = sample size.

Site-Basket	Day 0 (T-0)			Day 642 (T-6)			Mean SL Change (mm)	Daily Growth Rate (mm/day)
	Mean SL (mm)	SEM	N	Mean SL (mm)	SEM	N		
Paper-1	59	1.7	15	83	1.7	15	23	0.04
Paper-2	58	1.7	15	80	1.4	15	23	0.03
Paper-3	60	1.3	15	80	1.3	14	20	0.03
Rock-1	61	1.4	15	90	1.4	15	29	0.05
Rock-2	58	1.7	15	90	1.7	14	31	0.05
Rock-3	59	1.5	15	87	1.5	15	28	0.05
Tally-1	59	1.7	15	93	1.7	15	34	0.06
Tally-2	62	1.5	15	93	1.5	13	31	0.05
Tally-3	58	1.5	15	92	1.5	15	34	0.06
Air-1	58	1.1	15	71	1.1	15	13	0.02
Air-2	58	1.7	15	70	1.7	11	12	0.02
Air-3	60	0.7	15	73	0.7	15	13	0.02
WTR-1	60	1.4	15	72	1.4	15	12	0.02
WTR-2	61	1.3	15	72	1.3	15	11	0.02
WTR-3	64	1.4	15	74	1.4	13	10	0.02

Table 5. Summary of *S. nasutus* shell length over the course of the growth trial with overall mean change and daily growth rate. SL = shell length; SEM = standard error of the mean; N = sample size.

Site-Basket	Day 0 (T-6)			Day 94 (T-7)			Mean SL Change (mm)	Daily Growth Rate (mm/day)
	Mean SL (mm)	SEM	N	Mean SL (mm)	SEM	N		
Paper-1	60	1.5	15	72	1.8	15	10	0.10
Paper-2	63	1.6	15	73	2.0	15	11	0.11
Paper-3	55	1.9	15	65	2.5	15	9	0.10
Rock-1	55	0.7	15	65	1.6	15	10	0.11
Rock-2	55	1.7	15	64	2.1	15	9	0.10
Rock-3	55	1.6	15	64	2.1	15	9	0.10
Tally-1	57	1.8	15	66	2.3	15	9	0.10
Tally-2	60	1.5	15	70	2.1	15	10	0.11
Tally-3	60	1.1	15	70	1.7	15	10	0.11
WTR-1	56	1.2	15	61	1.8	15	5	0.06
WTR-2	59	1.4	15	64	2.2	15	6	0.06
WTR-3	55	0.8	15	61	1.3	15	6	0.06

Mussel Mortality

Mussel mortality for *U. implicata* was observed at all sites with no pattern or single large mortality event (Table 6). Natural mussel mortality was consistently low over the 736 project days. With 270 mussels initially deployed (45 per site), no site experienced more than 5 mortalities during a single time step. Many mortalities observed were isolated to a single mussel in a single basket. Some mussels were lost to the project from vandalism (theft or destruction). For sites where no vandalism occurred (i.e. WTR, Tally, Rock, Paper), survivorship was over 90% (169/180). With WTR as a reference site, both Tally and Rock experimental sites demonstrated identical mortalities.

A total of 225 mussels were deployed for the brief growth trial for *S. nasutus*. While all 45 mussels were lost due to vandalism at Airport site, no natural mortalities were observed for the 180 mussels deployed at all other sites.

Table 6. Number of dead mussels observed by field site and time step.

Time Step	Field Site						SUM
	WTR	BLV	Tally	Rock	Paper	Air	
T-0	0	0	0	0	0	0	0
T-1	0	0	1	0	0	0	1
T-2	0	3	0	0	0	0	3
T-3	0	nd	1	1	0	2	4
T-4	0	nd	0	0	0	0	0
T-5	0	nd	0	0	5	2	7
T-6	2	nd	0	0	0	0	2
T-7	0	nd	0	1	0	nd	1
SUM	2	3	2	2	5	4	18

Condition Index

Condition index samples were taken on June 7th, 2021 and September 9th, 2021. Summary statistics for condition indices calculated for mussels are presented in Tables 7 and 8 for *U. implicata* and *S. nasutus*. Mean condition index for either mussel species was consistently lowest at Winterthur during both time periods. Tally, Rock, and Paper all supported consistently greater condition indices. The condition index of *U. implicata* at Tally and Rock showed a sizeable change (decrease) of approximately 28% between June and September while mussels at Winterthur did not show a similar change. Rather, condition of *U. implicata* at Winterthur was fairly steady only dropping 3% in condition from June to September.

The mean condition index of *S. nasutus* was only measured for the standing stock at Winterthur in June and was relatively stable for those mussels at Winterthur in September decreasing approximately 6%. However, mussels transferred to Tally and Rock saw marked increases in their mean condition index by nearly 25% in September.

Mean condition index appeared to be greater for *S. nasutus* over *U. implicata*. However, *S. nasutus* mussels were slightly smaller and younger animals. Furthermore, condition index may not be comparable between these species of freshwater mussels given their difference in shell morphology and thickness (which influences the value of the index).

Table 7. Summary of Condition Index (CI) for *U. implicata* during two time periods. SEM = standard error of the mean; N = sample size.

Site	2021-06-07			2021-09-09		
	Mean CI	SEM	N	Mean CI	SEM	N
WTR	58	2.8	9	56	2.8	15
Tally	105	4.5	9	75	2.0	15
Rock	104	3.7	9	77	3.0	15
Paper	118	4.0	9	-	-	-
Air	73	4.7	9	-	-	-

Table 8. Summary of Condition Index (CI) for *S. nasutus* during two time periods. SEM = standard error of the mean; N = sample size.

Site	2021-06-07			2021-09-09		
	Mean CI	SEM	N	Mean CI	SEM	N
WTR	81	10.7	10	76	3.8	15
Tally	-	-	-	100	3.8	15
Rock	-	-	-	101	3.3	15

Water Quality Assessment

Spot Sampling

Summaries of spot sampled water quality data are presented in Tables 9 through 14 for each study site. Temperature generally followed typical seasonal patterns. Conductance was highly variable in stormwater ponds with readings often spanning orders of magnitude while specific conductance in WTR and BLV (reference ponds) was stable and predictable. Notable observations include a spike in specific conductance levels for all stormwater ponds in March 2021 and another smaller spike in June 2021. Both dissolved oxygen and pH levels were appropriate to support aquatic life and did not demonstrate erratic patterns nor concerning extremes.

Seston Analysis

Seston data (i.e. PM, POM, and organic content) varied significantly by site, season, and their interaction ($p<0.001$ all tests, 2-way ANOVA) and are summarized in Table 15. Mean PM for all sites ranged between 3.83 – 72.8 mg/L. Mean POM for all sites ranged between 1.71 – 16.1 mg/L. Mean organic content fraction ranged between 0.21 – 0.73. Mean PM, POM, and organic content fraction varied significantly by date for each site ($p<0.05$ all tests, 1-way ANOVA). Generally for all sites, concentration of particulate matter tended to be greater during the third and fifth time step (2020-04-15, 2021-06-07) and lower during the first two steps (2019-10-17, 2019-12-12). Annual variation was observed between the fourth and sixth time steps (2020-09-16, 2021-09-09) for all sites with available data with PM and POM being considerably more plentiful during 2021 compared to 2020. Concentration of particulate organic matter was greater in Tally and Rock compared to reference sites for almost all time steps. This pattern is not strong for other sites such as Air and Paper. The organic content of seston highlighted differences among dates and sites with no strong patterns.

Table 9. Water quality data recorded via *in situ* sampling for Winterthur.

Date	Temperature (C°)	Specific Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	pH
2019-10-17	13.6	225	9.4	7.2
2019-12-12	3.9	233	10.5	6.5
2020-02-05	7.6	218	10.7	6.9
2020-04-14	14.4	178	10.3	6.1
2020-09-16	19.5	243	9.8	8.2
2020-12-10	5.0	209	11.5	8.0
2021-03-12	9.7	248	10.7	7.5
2021-06-07	25.5	219	6.0	7.1
2021-09-09	22.1	155	7.3	7.8

Table 10. Water quality data recorded via *in situ* sampling for Bellevue Lake.

Date	Temperature (C°)	Specific Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	pH
2019-10-17	16.2	326	8.1	7.1
2019-12-12	6.0	386	11.0	7.1
2020-09-16	20.1	270	5.5	6.8

Table 11. Water quality data recorded via *in situ* sampling for Tally Day Park.

Date	Temperature (C°)	Specific Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	pH
2019-10-17	14.9	106	9.0	6.4
2019-12-12	4.4	85	10.5	6.9
2020-02-05	6.9	135	10.6	6.5
2020-04-15	13.5	110	7.7	6.4
2020-09-16	21.8	55	8.3	7.3
2020-12-10	4.5	63	8.3	8.8
2021-03-05	4.5	797	13.4	7.4
2021-06-07	29.1	834	8.2	7.7
2021-09-09	24.5	184	7.7	7.8

Table 12. Water quality data recorded via *in situ* sampling for Rockwood Park.

Date	Temperature (C°)	Specific Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	pH
2019-10-17	13.1	328	9.4	6.8
2019-12-12	3.9	213	12.0	7.1
2020-02-05	8.6	632	11.1	6.8
2020-04-15	12.9	333	8.7	6.7
2020-09-16	18.4	193	8.9	7.6
2020-12-10	4.6	333	12.0	8.4
2021-03-05	4.9	1352	13.4	7.5
2021-06-07	29.7	511	8.9	8.1
2021-09-09	23.9	219	7.6	7.7

Table 13. Water quality data recorded via *in situ* sampling for Papermill Park.

Date	Temperature (C°)	Specific Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	pH
2019-10-17	14.0	67	8.9	7.2
2019-12-12	4.7	85	10.4	7.2
2020-02-05	7.5	174	9.5	7.0
2020-04-15	13.3	92	8.5	7.0
2020-09-16	21.4	85	9.2	7.7
2020-12-10	4.9	152	10.3	8.3
2021-03-05	5.6	2391	11.9	7.9
2021-06-07	30.5	798	9.5	8.6
2021-09-09	23.1	105	4.8	7.5

Table 14. Water quality data recorded via *in situ* sampling for Airport Road.

Date	Temperature (C°)	Specific Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	pH
2019-10-17	15.1	181	9.3	7.3
2019-12-12	5.3	126	11.3	7.2
2020-02-05	7.2	355	11.3	7.1
2020-04-15	12.9	171	11.2	7.6
2020-09-16	21.2	54	6.8	7.5
2020-12-10	5.0	78	11.5	8.3
2021-03-05	5.4	4147	10.9	7.6
2021-06-07	29.6	1072	9.6	8.2
2021-09-09	24.6	278	8.2	7.6

Table 15. Particulate water quality data summarized for all study sites. SEM = Standard Error of the Mean; N = Sample Size; nd = no data.

Site	Date	Particulate Matter (mg/L)			Particulate Organic Matter (mg/L)			Organic Content Fraction		
		Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
WTR	2019-10-17	3.83	0.27	4	2.78	0.12	4	0.73	0.04	4
	2019-12-12	5.19	0.98	3	1.71	0.05	3	0.35	0.11	3
	2020-04-15	30.7	4.0	4	6.67	0.65	4	0.23	0.03	4
	2020-09-16	16.7	1.8	3	6.13	0.50	3	0.37	0.03	3
	2021-06-07	34.4	4.2	4	9.13	0.23	4	0.28	0.03	4
	2021-09-09	24.2	1.2	3	10.3	0.54	3	0.43	0.04	3
BLV	2019-10-17	6.60	0.11	4	3.49	0.08	4	0.53	0.02	4
	2019-12-12	14.0	2.4	3	5.96	0.16	3	0.45	0.07	3
	2020-09-16	17.7	2.3	4	6.46	0.36	4	0.38	0.04	4
Tally	2019-10-17	23.1	0.51	4	16.1	0.39	4	0.70	0.03	4
	2019-12-12	8.44	nd	1	4.70	nd	1	0.56	nd	1
	2020-04-15	22.2	1.2	4	6.69	0.08	4	0.30	0.02	4
	2020-09-16	8.67	0.51	4	6.80	0.33	4	0.79	0.02	4
	2021-06-07	26.5	1.7	4	11.8	0.32	4	0.45	0.02	4
	2021-09-09	20.7	1.9	4	10.6	0.18	4	0.52	0.04	4
Rock	2019-10-17	18.1	0.81	4	8.45	0.31	4	0.47	0.01	4
	2019-12-12	14.1	0.86	3	5.01	0.29	3	0.36	0.04	3
	2020-04-15	72.8	14	4	15.1	0.35	4	0.24	0.06	4
	2020-09-16	11.6	0.79	4	5.90	0.37	4	0.51	0.02	4
	2021-06-07	73.9	9.5	3	24.0	1.06	3	0.33	0.03	3
	2021-09-09	41.4	9.1	4	13.9	1.5	4	0.36	0.04	4
Air	2019-10-17	6.93	0.18	4	4.71	0.14	4	0.68	0.01	4
	2019-12-12	10.1	1.5	4	2.78	0.16	4	0.30	0.05	4
	2020-04-15	14.0	2.0	3	5.61	0.45	3	0.41	0.05	3
	2021-06-07	13.1	0.70	4	7.06	0.13	4	0.54	0.02	4
Paper	2019-10-17	8.34	0.37	4	4.31	0.06	4	0.52	0.02	4
	2019-12-12	13.0	0.50	2	4.64	0.16	2	0.36	0.002	2
	2020-04-15	30.0	2.1	3	6.29	0.61	3	0.21	0.02	3
	2020-09-16	6.20	1.7	3	2.91	0.34	3	0.51	0.08	3
	2021-06-07	24.6	2.4	2	8.28	0.04	2	0.34	0.03	2
	2021-09-09	13.1	1.2	2	4.79	0.54	2	0.37	0.01	2

Discussion

Site Selection

Environmental as well as social and pragmatic factors are critical to site selection. This study assessed multiple publicly accessible ponds for their capacity to support freshwater mussels and also served as a first foray into bringing floating aquaculture gear to open areas. Most sites were within public parks (Tally Day Park, Rockwood Park, Papermill Park) while one site was near industrial land use and behind a vacant parking lot (Airport Road site). Airport road site was the only site that experienced any vandalism in the form of debris thrown into baskets and upon completion of the study, likely theft of the baskets (evidenced by cut rope along the shore and crushed shells). All baskets at park sites were seemingly untouched over two years which is encouraging. Papermill Park baskets did become entangled with fishing gear (e.g. lures, hooks) which caused a nuisance in the field and likely signage or other outreach could help avoid this problem. Anecdotally, the baskets provided park goers a unique experience and this study provided a positive example of using public resources in a synergistic manner. The education and outreach potential may be great and could engage local school groups and other interested parties to explore many new avenues.

Mussel Growth and Survival

The growth and survival of young freshwater mussels is critical to the long term success of restoration efforts that continue to grow throughout the region and nation. Two species of freshwater mussels (*Utterbackiana implicata* and *Sagittunio nasutus*) were found to grow at different rates in different ponds. While variation in growing conditions and growth is expected, the experimental ponds supported significantly greater growth for both species than the reference pond. This is a clear indication that mussels have a greater growth potential than what may be considered “typical”. Mussels that experienced double or even triple the growth rate of reference pond mussels are at a considerable advantage as early life stages of mussels are at risk of predation and do not have the same energy reserves an older mussel may have. The larger a mussel grows, the better it will be able to withstand periods of low resources or other environmental disturbances. These data are important to consider when siting new growing operations and evaluating existing operations. Aside from mussel growth rate, it was encouraging to observe minimal natural mortality over two years during a critical life stage for all sites.

Condition Index

One of the insights gathered through this study was the difference in mean condition index calculated for different species living in different ponds. While condition index is a relative measure, it can help understand a bivalve's response to its environment beyond a binary live vs. dead or simple growth measurement approach. The mean condition index of *U. implicata* appeared to decrease from summer to fall in Tally and Rock while holding steady in Winterthur. This may suggest that the condition of mussels is deteriorating in fall but mean indices were still greater in Tally and Rock in fall than indices in Winterthur for either time step. Furthermore, the seasonal decrease is expected and suggests the bivalve is responding to environmental changes. In fact, it is plausible that *U. implicata* in Winterthur may not have enough resources to respond accordingly and therefore the condition index is closer to a low baseline.

Similarly for *S. nasutus*, mean condition indices were different for mussels held at Winterthur vs. Tally and Rock. With indices higher in Tally and Rock, *S. nasutus* seems to follow the same pattern at *U. implicata*. However, *S. nasutus* mean condition index in June was only analyzed on mussels held at Winterthur. One explanation for the marked increase in condition index into the fall for mussels transferred to Tally and Rock could be that mussels immediately transferred to those sites began increasing their condition index and then naturally decreased with fall (an unobserved "spike"). Alternatively, the mussels may have seem a gradual increase in condition through September. Overall, the results of mussel condition index suggests that Tally Day and Rockwood sites are adequate sites to grow mussels in and may support healthier mussel growth than current reference sites.

Water Quality and Seston Characteristics

The water quality of all ponds did not appear to be a limiting factor in freshwater mussel growth and survival. Conductivity pulses observed in test ponds were likely associated with runoff events and were not observed in reference sites. These observations highlight the critical difference between stormwater ponds and reference ponds where stormwater ponds regularly receive stormwater runoff that can introduce salts and other inputs that can influence the conditions of the water. Regardless, the conductivity pulses did not appear to affect freshwater mussels in a detectable way either in growth, survival, or condition index.

Seston was found to vary by year as well as by site. Seston differences by year indicate the dynamic nature of these systems and in particular, a late fall bloom may have been captured in September 2021 over September 2020. The concentration of particulate matter may have suggested some differences in overall productivity among ponds but there were no clear patterns. However, the particulate organic matter concentrations were either comparable or greater at Tally Day and Rockwood sites for every time step over reference sites (Winterthur and Bellevue). While the seston data is not uniform for any given site or time step, it suggests there is simply more abundant food conditions in some experimental ponds over the reference ponds. This holds true for the most important times (April, June, September) when spring blooms and possibly fall blooms should be fueling productivity in the ponds. Interestingly, Rockwood PM reached nearly 75 mg/L, which is above the 50 mg/L threshold that typically would prompt a decreased feeding efficiency (Safi et al. 2007). However, mussels at Rockwood were among the best growing mussels which could suggest that seston conditions were not consistently high or the type of seston was conducive to bivalve feeding behavior.

Conclusions

This study provided useful observations that confirmed the practicality of growing freshwater mussels in diverse environments, particularly those that may be dismissed as too impacted by urban land use. These types of discoveries and confirmation of efficacy helps move the Freshwater Mussel Recovery Program that is being led by the Partnership for the Delaware Estuary beyond standard practices and into new territories that diversifies restoration science and tactics. With goals such as promoting conservation, restoration, and enhancement of freshwater mussels, PDE seeks to promote shellfish-mediated ecosystem services where possible and appropriate. Expanding where these valued water quality benefits can be achieved is a win for all.

The observations of this study continued to build on previous studies in the region on the feasibility of freshwater mussel restoration (Cheng & Kreeger 2015, Cheng & Kreeger 2018), particularly how to incorporate new technologies such as propagating mussels for water quality benefits (Cheng et al. 2020) and where salinity concerns exist (Cheng et al. 2021). While there is a dearth of stable freshwater mussel populations in New Castle County (Kreeger et al. 2014), this type of restoration science provides tools and insights that future studies can leverage to continue pushing for cleaner water in the region.

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