Enhancing Water Quality by Restoring Freshwater Mussels in an Urban River: Assessing and Testing Prospects in Relation to Salinity

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

Project CWQIG 18-04

Partnership for the DELAWARE ESTUARY

May, 2021 | Report No. 21-02

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

Native species of freshwater mussels were found to tolerate and grow in size at all tidal study sites on the lower Christina River in New Castle County, Delaware. At least some mussels from a common, hatchery-propagated cohort were able to survive and grow at all sites, and the growth rates of the survivors exceeded the growth of mussels a non-tidal stream site (Winterthur) that served as a reference location. This positive result was in spite of variable water quality, relatively high suspended solid concentrations, and a few significant storm events that contributed to spikes in specific conductivity.

Nevertheless, the survival of deployed mussels at the tidal Christina study sites was lower than at the reference site due to several factors. Experimental errors in the form of cage losses due to severe storm events or vandalism were not considered in the survival analysis. After accounting for such losses, the lower survival at study sites compared to the reference appeared to mainly be associated with predation based on discrete shell breakage patterns witnessed on the recovered shells. The type of predator is unclear, but blue crabs, crayfish, raccoons and other animals exist within the study area.

Although not part of the original study design, a subset of mussels was also deployed into experimental "mussel enhancement habitats" at one of the study sites to preliminarily test whether stabilization of soft bottom habitats might increase habitat suitability for mussels. The experimental plots consisted of unbounded "V" and "W" shaped strings of small gabions filled with oyster shell, around which mussels were deployed, as compared to mussels deployed in an unprotected control plot. The preliminary design and budget precluded replication for statistical analyses, however, after nearly five months more than twice as many tagged mussels were re-surveyed in these gabion-stabilized plots compared to the untreated plot, and growth was comparable during the deployment period.

All surviving mussels from the initial cage and gabion study were relocated to small tributary streams that were deemed to be conducive for mussels based on the existence of small numbers of extant, wild mussels. A subsequent survey of the tributaries for these free-released, tagged mussels found that most had perished or disappeared, presumably due to predation.

Outcomes from this project confirm that the water quality and food conditions of the lower Christina, while not always ideal, are sufficient to support good growth and survival of native species of freshwater mussels. The early indications from the gabion study also suggest that habitat modification tactics may plausibly help to stabilize and enhance mussel habitat

suitability, which should be useful for designing living shorelines that could contain mussel beds as one feature in the subtidal terraces of the project area.

Results of this study also indicate, however, that predation pressure will be an important constraint on mussel restoration or mussel-based living shorelines in the tidal Christina River. Future studies should examine which species and sizes of mussels are most vulnerable to this predation, and then develop predator management practices to either protect sensitive sizes or species or to only release mussels that are of a species or size that has lower predation risk.

Both mussel bed restoration projects and mussel-based living shoreline projects are plausible tactics that merit greater investigation for tidal freshwater areas of Delaware, such as the lower Christina River. There is increasing interest in restoring and enhancing native mussel populations because of their increasingly recognized contribution to water clarity, particle removal, and nutrient pollutant recycling/sequestration. Results of this study suggest that food quality and quantity should not be a constraint on mussel carrying capacity in the particle-rich lower Christina River. Hence, projects aimed at enhancing mussel beds for ecosystem services are warranted, and we recommend that additional studies begin to develop and test different tactics for enhancing mussel habitat suitability and protecting mussels from predators, with a goal of boosting mussel densities and habitat carrying capacity per acre.

Introduction

Freshwater mussels, hereinafter mussels, are a group of bivalve mollusks (order Unionida) that are uniquely adapted to live in the benthos of freshwater ecosystems. Mussels burrow into the substrate (e.g. sand) and anchor themselves with a muscular foot. Similar to saltwater bivalves such as clams and oysters, mussels feed on seston (microparticulate matter) suspended in the water column using specialized body parts. However, mussels have a complex life history wherein they brood larvae and require an intermediate fish host for reproduction. Mussels are renowned for their species diversity, but also for their ecological roles and ecosystem services they provide.

Filter-feeding bivalves are often the functional dominant species in ecosystems due to their ability to filter quantities of microscopic particles from the water column (Dame 2012, Kreeger et al. 2018). This filtration behavior reduces turbidity and removes suspended particulate pollutants, such as nutrients (e.g., nitrogen, phosphorus). 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). Subject to environmental conditions, an adult mussel can filter up to ten or more gallons of water every day. Accordingly, 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).

Unfortunately, over 70% of the near 300 mussel species that exist across the United States are considered endangered, threatened, or of special concern. This means that mussels are one of the most imperiled animal taxonomic 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)*.

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	no data	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

To advance the goals of the FMRP, this study tested freshwater mussel stocking potential in the Christina River in New Castle County, Delaware. The River runs through a dense, historical urban landscape and is challenged by a myriad of water quality issues. Great progress has been made to improve the overall health and integrity of the Christina River, but continued development across the river's watershed is expected to continue to tax natural habitats and their vital ecological services. Mussels may be particularly vulnerable when positioned downstream of heavily urbanized watersheds (Gillis 2012). Additionally, the Christina River experiences fluctuations in salinity from tidal forces, periodically pushing brackish water in from the Delaware Bay. Non-point source pollution and road-salt runoff represent additional threats to natural resources such as bivalve molluscs.

The combined threats from runoff (e.g., road salt) and sea level rise and storm surge (e.g., seaward sourced salinity) threaten to periodically increase the conductivity or salinity of the water, effectively converting a freshwater system to an oligohaline system (0.5 – 5 ppt). Salinity pulses could affect mussel survivorship and condition because native unionid mussels are relatively salt-intolerant (Gillis 2011, Blakeslee et al. 2013, Patnode et al. 2015). As freshwater organisms, mussels can experience negative effects when exposed to salinities as low as 1-3 parts per thousand (ppt) and can exhibit 100% mortality at 3.5 ppt (Ercan & Tarkan 2014). It is thought that juvenile stages are particularly vulnerable to salinity inputs resulting in disturbances to reproduction (Gillis 2011). The results of this study will guide site selection where future expanded mussel restoration would likely be most successful. This is especially pertinent in heavily impacted watersheds across the state, which would also likely receive the greatest ecosystem service value enhancement from freshwater mussel reestablishment.

This project sought to delineate the bounds of potential mussel restoration within the tidal Christina River as a first step in determining where living shoreline or similar projects might be developed that would incorporate beds of mussels. Comparable to an oyster reef, the resilience and functional habitat value of a mussel bed depends on the density, health and extent of the mussel assemblage, which natural aggregates. Sites with mussel densities <1 per m^2 may provide some beneficial mussel-mediated services (e.g., pollutant filtration) but are below the "mussel bed" threshold needed to provide other benefits such as bottom stabilization or habitat enrichment. Ideally, candidate sites for living shorelines would have suitable water chemistry and food conditions to support mussel densities >10 per m^2, once the habitat suitability is amended to be more suitable for mussels. Emerging evidences suggests that mussel densities of >10 per m2 are sufficient to significantly alter benthic structure and function. When mussel size and density exceeds this threshold, their close proximity, physiological ecology and morphological complexity is sufficient to promote numerous positive biophysical feedbacks related to benthic stability, organic and nutrient enrichment, light availability, etc. Hence, at these densities the mussel bed can be considered as a habitat type that has its own structural properties, functional properties, and ecosystem services, compared to bottom areas that lack mussels.

The specific objectives of this study were to identify the spatial bounds of where freshwater mussels can simply persist and grow in the lower Christina River. The approach was to build upon previous surveys of extant mussels by deploying cohorts of mussels at various locations along the natural salinity gradient and then monitoring their performance and site-specific conditions (e.g. salinity, landscape complexity). Outcomes from this study are already being used to guide further investigations of candidate mussel restoration sites and suitable tactics in this important watershed of New Castle County, Delaware.

For more information on freshwater mussel ecology, life history, and Delaware River Basin species, refer to *Freshwater Mussels of the Delaware Estuary: Identification Guide & Volunteer Survey Handbook* (PDE 2014) and: delawareestuary.org/freshwater-mussels Additional educational material can be found at: mightymussel.com

Methods

Study Site

The Christina River drains a majority of north New Castle County, Delaware. Its course runs eastward to its confluence with the Delaware River, nearly 70 miles (113 km) from the Atlantic Ocean. This span of the Delaware River coincides with the estuary's turbidity maximum zone, as well as its average salt front location, where mean salinity is roughly 0.25 ppt (DRBC 2021). The lower Christina River is tidal and can be oligohaline through saltwater inputs from the mouth (though salinities are typically below 0.5 ppt), whereas the upper Christina River, which consists of two branching tributaries (north and south), is a non-tidal freshwater system.

The length of the Christina River spans a topographical and land use gradient. The upper River has steep topography, draining from the piedmont in the north, with mostly suburban land uses. Conversely, the lower River is a coastal plain system, with low grade topographies. The lower River is heavily urbanized (i.e. City of Wilmington). Two major interstate highways now converge above the Christina River. During the construction of these highways in the 1970's, the Christina River's path was rerouted to accommodate this infrastructure.

Field study activities took place in the tidally-influenced Christina River as well as a pond and tributary stream located at Winterthur Museum Garden & Library (hereinafter Winterthur). The Winterthur site served as a reference location which has documented good mussel growth and survival and which does not receive salt inputs via highway road salts nor brackish water from a connected waterbody. Specific site details are described for each activity below.

Mussel Deployment

To investigate growth and survival of freshwater mussels throughout the Christina River, juvenile mussels were deployed and monitored in cages as well as free released. Juvenile mussels were used because of their relatively faster growth and sensitivity to environmental stressors, compared to adult mussels. The mussel species used in this study was the Alewife Floater, *Utterbackiana implicata*. This species was chosen because it is commonly found throughout tidally-connected waters of the Delaware Estuary, exists in the tidal portion of the Christina River, and is readily propagated in hatcheries. Therefore, *U. implicata* serves as a reliable stock of mussels for research studies. The mussels used for this study were propagated in 2017, using broodstock from the tidal Delaware River, in collaboration with the Harrison Lake National Fish Hatchery. Prior to deployment mussels were cared for at Winterthur.

Christina River Cages

To compare mussel survivorship and growth along the Christina River, four test sites and one reference site were chosen. One site was a pool (Pool) within a marsh complex of the Christina River near the Dupont Environmental Education Center (DEEC). A second site (Mainstem) was geographically close to the Pool site but situated opposite of the tidal marsh and along the Christina River. The third Christina River site was near the Newport boat ramp (Newport). A fourth site, farthest upstream, was near the Churchmans Road boat ramp (Churchman). The reference site was a stream that drains the pond at Winterthur, where mussels are grown year-round (Fig. 1). To reliably contain and protect mussels for the duration of the study, researchers designed and fabricated benthic cages that discouraged predation and allowed water flow over the mussels (Fig. 2). The design and construction of the benthic cage was recorded as an FMRP method (#18) and this method is included as Appendix A for reference.

In June 2019, cages (*N*=3) were deployed at each site with at least one meter between each cage. The low profile of the cages was intended to minimize risk of dislodgement caused by floating trees or other detritus. For Churchman and Newport sites, kayaks were used to transport cages and access the sites for monitoring, while Pool and Mainstem sites were accessible by wading. Nearby sediment was added into deployed cages for weight and habitat.

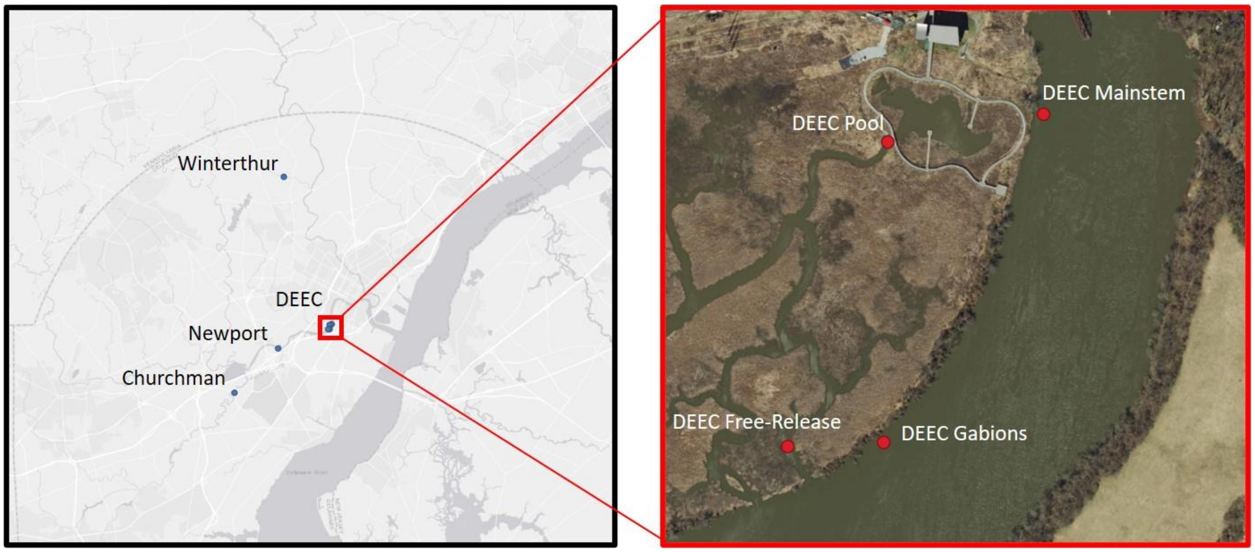

Figure 1. Study sites along Christina River and the Winterthur reference site. Cages were deployed at Winterthur, Newport, Churchman, DEEC Pool, and DEEC Mainstem sites. The gabion and free-release portion of this study were conducted near DEEC.

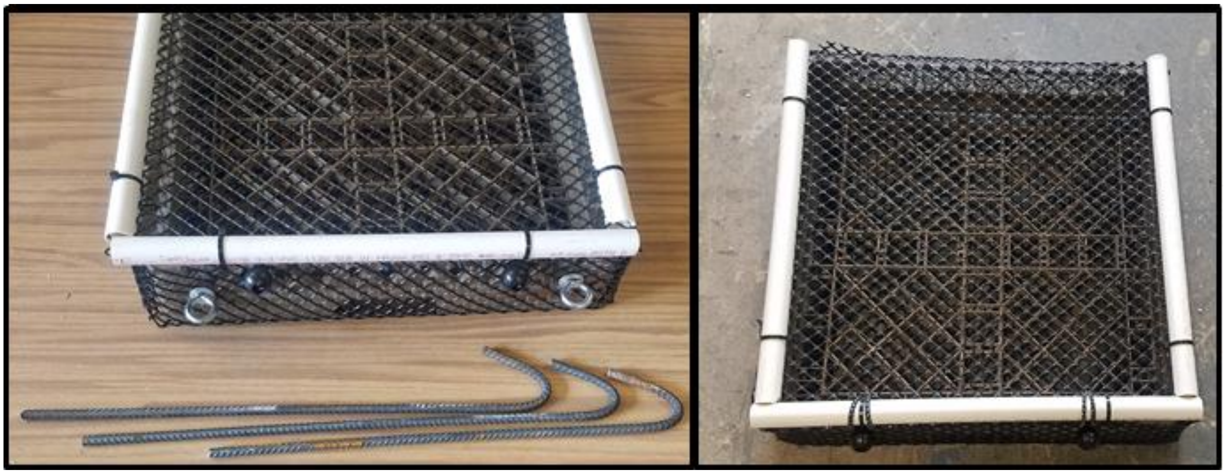

Figure 2. Newly designed benthic cages (shown above) were secured using rebar anchors through eyebolts on the cages. The plastic diamond mesh on the cages was intended to protect the mussels from predators and also to retain mussels within the cages.

A total of 225 mussels were tagged with alphanumeric plastic tags secured using cyanoacrylate, measured using digital calipers (Mitutoyo CD-6” CXR, ± 0.02 mm), and deployed in cages. Each cage received 15 mussels. At monitoring dates throughout the study, mussels were removed from cages, measured with digital calipers, assessed for any noteworthy shell erosion or deformities, and then deployed back into the cages.

Throughout the study, cages were monitored five times after initial deployment. Some cages were inaccessible on certain monitoring dates due to abnormal tide heights. The final monitoring, and subsequent cage removal, for Winterthur, Churchman, Newport, and Mainstem occurred on August 10th and 11th, 2020. The Pool site cages were removed on September 8th, 2020 due to access issues on the August date. Due to challenges posed by the Covid-19 pandemic and related safety concerns, some of 2020 field efforts were delayed or cancelled, leading to fewer monitoring efforts overall. Monitoring dates and associated time steps are summarized in Table 2.

Table 2. Monitoring dates for each time step by site. Days post-deployment are in parentheses.

Time Step	Field Site				
	Winterthur	Churchman	Newport	Pool	Mainstem
T-1	2019-07-22 (27)	2019-07-25 (30)	2019-07-25 (30)	2019-07-24 (29)	No Monitor
T-2	2019-08-22 (58)	2019-08-22 (58)	2019-08-22 (58)	2019-08-22 (58)	2019-08-22 (58)
T-3	2019-10-22 (119)	2019-10-22 (119)	No Monitor	2019-10-22 (119)	2019-10-30 (127)
T-4	2020-05-11 (321)	2020-05-11 (321)	2020-05-11 (321)	2020-05-11 (321)	2020-05-11 (321)
T-5	2020-08-10 (412)	2020-08-10 (412)	2020-08-10 (412)	2020-09-08 (441)	2020-08-11 (413)

Shell Measuring Comparison

A comparison of surveyor measurements was performed to determine a margin of error. While digital calipers are accurate to 0.02 mm, mussel shells are curved and orienting a shell to measure the longest anterior-posterior axis can vary, especially in a field setting. Slight differences could influence recorded shell lengths. The two main surveyors separately recorded 36 measurements of dead shells to compare results.

Shell Gabions

Although not part of the original scope for this study, an opportunity arose to preliminarily test the effectiveness of innovative living shoreline materials that might be useful in future projects for enhancing the benthic habitat suitability for mussels, such as in living shoreline projects. A common constraint on habitat suitability for mussels is insufficient benthic stability, whereby mussels can be dislodged from unstable soft bottom habitats during extreme hydrodynamic events.

At the Mainstem site, small gabions filled with oyster shell were used to modify benthic conditions, prior to being stocked with mussels. The (2x1x1)' gabions used were constructed by PDE staff from purchased galvanized steel wire mesh panels and galvanized steel hog rings. Half of the gabions were filled with loose oyster shell obtained from PDE's shell recycling program, and half were lined with coir fiber mats before being filled with the same oyster shell (Fig. 3). This factor was intended to test the retention of shells in lined vs unlined gabions.

On June 23rd, 2020, two experimental treatments were constructed by placing these gabion structures in either the shape of a "V" or a "W". A third, comparable plot was marked by PVC pipes but was otherwise untreated, serving as an experimental control (Fig. 4). The "V" structure was built from unlined gabions, and the "W" structure used lined gabions. Gabions were secured to each other using 1/8" diameter braided steel cable seals and anchored to the river bottom using 18" steel helical anchors shown in Figure 4.

On June 24th, 2020, 30 tagged mussels were deployed within each plot, referred to here as a subsite. Mussel locations within these subsites are represented Figure 5. Gabion mussels were tagged with both alphanumeric plastic tags, and Passive Integrated Transponder (PIT) tags. Plastic tags were secured using cyanoacrylate, and PIT tags were secured using a marine epoxy (Fig. 6). All mussels were measured using digital calipers before deployment. The use of PIT tags allowed for the surveying of released mussels using a PIT tag reader (Biomark HPR+) to determine mussel presence via tag detection. This technology aids in recapture for growth and mortality monitoring (Fig. 7). Gabion mussels were monitored on August 11th and November 13th 2020. On the November monitoring date, eight of the mussels were removed from the water, measured using digital calipers, and returned to their subsite.

Figure 3. Gabions filled with oyster shell (left) and additional coir liner (right).

Figure 4. Location of the gabion deployment in Wilmington, Delaware. The v-shaped structure is the most downstream, with the control area upstream.

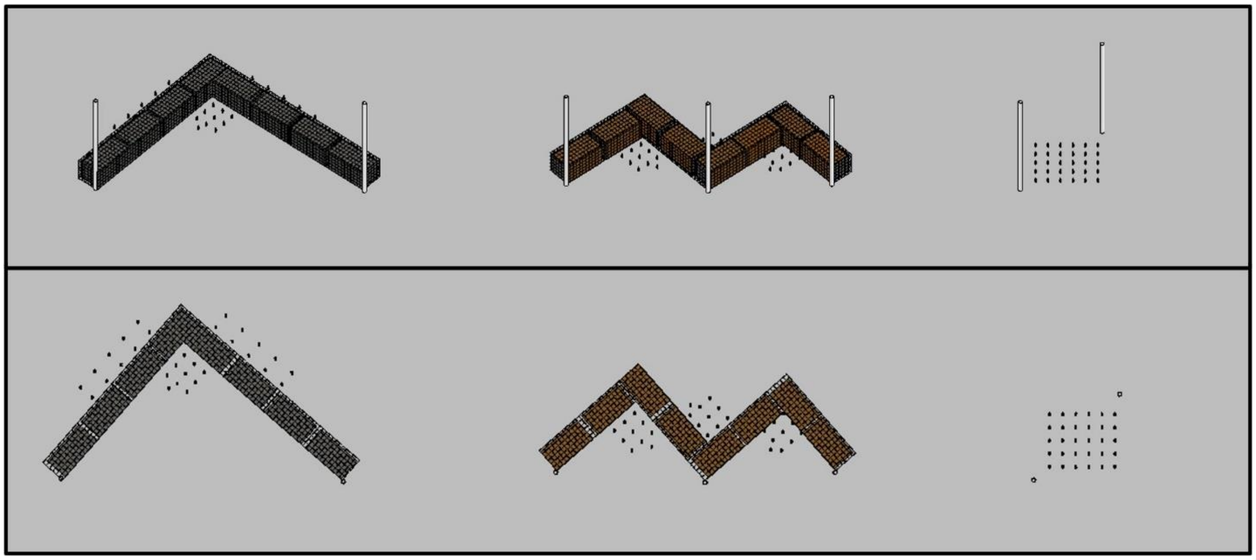

Figure 5. Design of gabion structures deployed in the Christina River in a three-dimensional view (top) and two-dimensional view (bottom). PVC pipes are represented by rods and mussels are represented by dots.

Figure 6. Alewife Floater mussels tagged with red alphanumeric plastic tags and PIT tags encased in white marine epoxy (left). Mussels were recovered in the field using PIT tags for detection (right).

Figure 7. PDE surveyor uses a PIT tag reader to scan the gabion structures for tagged mussels.

Free Release

Mussels that were removed from cages in August (*N*=91) were then free-released into two tributaries of the Christina River upstream from gabion deployment on August 11th, 2020. Tributary 1 (39.719699 N, -75.56267 W) received 30 mussels and Tributary 2 (39.719555 N, -75.563188 W) received 61 mussels. These small tributary creeks had previously been determined to support the long-term persistence of extant mussels, but pre-existing wild mussels were very low in abundance. Mussels that were removed in September (*N*=8) were instead released near the Pool site within the DEEC marsh complex. Mussels were released in tributaries by hand during low tide. Areas such as deeper runs and pools were targeted (Fig. 8).

Free released mussels were exposed to any flow events and disturbances. A free release survey was performed on December 10th, 2020 at a low tide for each tributary. Surveys lasted approximately 20 minutes each. Survey effort was influenced by field observations (i.e. additional detections warrant longer survey duration to continue detecting mussels). Any dead shells were collected for reference.

Figure 8. Mussels were deployed in the deepest observed areas of tributaries during a low tide.

Water Quality Assessment

Water quality monitoring consisted of *in situ* spot sampling, long-term water level and conductivity monitoring, and physical water collections (grab samples) for seston (microparticle) analysis. Dissolved water quality data were recorded using a Eureka Manta +35 unit throughout the study. The probe was calibrated prior to each field usage. The water quality parameters measured included dissolved oxygen (mg/L), water temperature (°C), pH, and specific conductance (µS/cm).

Long-term water level (HOBO U20-001-01) and conductivity (HOBO U24-001) loggers were deployed along the Christina River. One conductivity logger was deployed at the Churchman site (39.6850 N, -75.6322 W), in the upstream tidal Christina River (date range 2019-2020). Conductivity was converted to salinity using freshwater parameters in the HOBOware®—the analysis program developed for synthesizing data from HOBO loggers. Approximately 10.5 river km downstream, we deployed both a water level logger and a conductivity logger at the Peterson Urban Wildlife Refuge (39.7233 N, -75.5600 W) (date range 2018-2020). To avoid equipment freeze damage, loggers were extracted from January-March. This coincides with mussel dormancy. Loggers recorded every 15 minutes. Reference water levels were obtained by Real Time Kinematic (RTK) survey; these measurements were used to convert logger data into values relative to NAVD88. Spot sampling conductivity data were used to corroborate salinity values from conductivity loggers.

Drought can have consequences for estuarine salinity due to reduction in freshwater flow from upstream locations. Salinity pulses due to drought can negatively affect freshwater mussel populations when they occur during months when mussels are active. Drought information (Palmer's Drought Severity Index Z, or PSDI) from NOAA's National Center of Environmental Information (NOAA 2020) was used to investigate drivers in salinity variation. Regional drought information was obtained for southeastern Pennsylvania (SE PA), northern Delaware (N DE), as well as southern Delaware (S DE).

Water grab samples were collected at high tide at all caged mussel sites to measure seston quantity and quality, which comprises the mussels' diet. Seston composition was assessed following methods described by Kreeger et al. (1997). Per site and sampling period, 4-liter water samples were collected in triplicate using plastic cubitainers. 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 (Fig. 9).

Glass filters were previously combusted and weighed for 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 (VWR, ±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 percentage organic content of seston was calculated using the following formula:

$$Organic\ Content = \left(\frac{POM}{PM}\right) * 100$$

Figure 9. A technician collects a water seston sample using a filtration setup.

Results

Mussel Deployment

Caging

Cages retained both live mussels and any dead shells throughout the deployment period. Mussels that survived demonstrated healthy growth. Cage problems observed included loss due to a storm, incidental vandalism (a cast net damaged a cage), and periodic sedimentation. Sedimentation is preventable with maintenance, although the COVID-19 pandemic largely disrupted field work for 2020. Results on the measurement comparison, mussel growth, survivorship, and loss during the caging period are presented below.

Measurement Comparison

In a repeat assessment of 36 mussel shells, the measurements of different surveyors were within 1 mm 83% (33/36) of the time and were within 1.2 mm of each other 100% of the time.

Mussel Growth

Ninety-nine mussels (44% of deployed mussels) survived in cages for the study's duration. Seasonal patterns of shell length change were observed with steady growth from T-0 through T-3 (peak of summer through early fall), minimal growth from T-3 through T-4 (winter into spring), and resumed growth from T-4 through T-5 (late spring through summer) (Fig. 10). The initial mean shell length of mussels at sites ranged from 57.28 – 60.78 mm, and this was statistically similar across all sites (p=0.25, 1-way ANOVA).

Mean shell length of mussels was significantly different at T-2 ($p<0.05$. 1-way ANOVA), where Newport mussels were significantly larger than Winterthur and Newport mussels ($p<0.01$, $p<0.05$ respectively via post-hoc Tukey test). The mean shell length of mussels caged at all Christina River sites was greater than 82.0 mm at the study completion compared to 75.7 mm at Winterthur. A 1-way ANOVA determined mean shell length was significantly different by site upon study completion (i.e. T-5). Mussels were similar in size among all Christina River sites ($p>0.05$, post-hoc Tukey test), but larger than mussels at Winterthur ($p<0.05$ Pool comparison; $p<0.001$, Churchman, Mainstem, & Newport comparisons). Churchman mussels exhibited the greatest mean shell length increase (25.7 mm) from 59.7 to 85.4 mm.

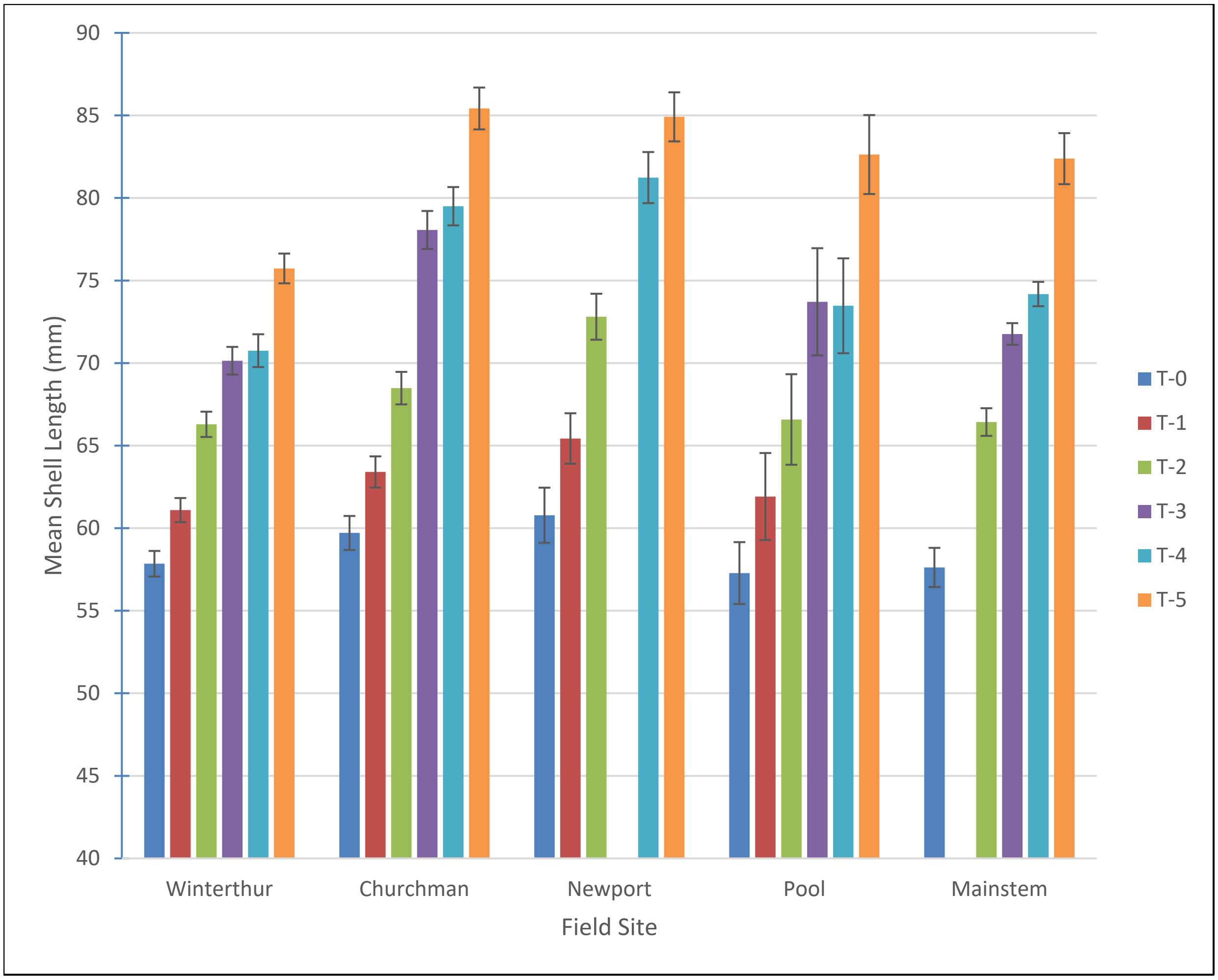

Figure 10. Mean shell length of caged mussels by time step for each site. Error bars represent standard error of the mean.

Missing Mussels

A total of 33 mussels were lost during this study, and 23 (70%) of these were from the Pool site (Table 3). At this site, an entire cage with mussels was lost during Tropical Storm Isaias. Another cage at Pool sustained damage to its mesh lid. An additional six mussels went missing at Mainstem. One mussel went missing at Churchman. Two mussels went missing at Newport.

Table 3. Number of missing mussels counted by field site and time step.

Time Step	Field Site				
	Winterthur	Churchman	Newport	Pool	Mainstem
T-1	0	0	0	2	No Monitor
T-2	0	1	1	0	4
T-3	0	0	No Monitor	0	1
T-4	0	0	1	5	0
T-5	0	0	1	16	1
Sum	0	1	3	23	6

Dead Mussels

Over the 441 days of the caging study, surveyors observed mortalities at all sites. These mortalities ranged from freshly dead (tissue still present) to dead shell. Some shells collected were characterized by a discrete pattern of shell damage along the ventral margin which is uncharacteristic of natural mussel mortality (Fig. 11). A total of 93 mussel mortalities were observed and are summarized by site and time step in Table 4.

All but 16 dead shells were measured to determine length at mortality. The 16 that were not measured were from the Mainstem site, where the shells were too heavily damaged to accurately measure.

Among the 77 dead mussel shells that were measured, 49 shells (63%) were found to be less than 1.2 mm from the mussel's previous live measurement, which represented an average change of 1.8% of the previous mussel size. Cages were often filled with sediment upon monitoring. Outside of physical removal, no mussel mortality pattern was observed with respect to time step or site.

Table 4. Number of dead mussels observed by field site and time step.

Time Step	Field Site				
	Winterthur	Churchman	Newport	Pool	Mainstem
T-1	4	9	7	4	No Monitor
T-2	2	0	0	2	2
T-3	0	1	No Monitor	0	17
T-4	0	8	15	3	2
T-5	1	0	11	5	0
Sum	7	18	33	14	21

Figure 11. Recovered mussel shells with damage along ventral margins.

Gabions

Mussels averaged just under 70 mm in shell length upon deployment (Table 5). Surveyors detected a subset of mussels among the V-gabion, W-gabion, and reference subsite during both monitoring events (Table 6). Survey #2 was more intensive and detected more mussels than survey #1. Detected mussels grew an average of 16.9 mm near the W-gabion, and 13.3 at the reference subsite. The sole mussel detected at the V-gabion grew 15.7 mm over the monitoring period. One dead mussel was found approximately 100 meters downstream from the experimental gabion area. Shell length for the dead mussel was 71.7 mm (2.5 mm increase). Visual inspection of mussels that persisted in the plots found healthy shells with minimal erosion or damage. (Fig. 12).

Anchored gabions filled with oyster shell appeared to work well with regard to their persistence. They retained oyster shell regardless whether they were lined with coir or not. Some leafy debris collected inside the gabion walls (Fig. 13), however the structures did not show any damage and no gabion was lost or dislodged. PVC pipes remained in place and some accretion of sand was noted within the vertices of the V and W structures.

Table 5. Initial mussel size at deployment to gabions and reference area. SEM = standard error of the mean; *N* = sample size (total deployed).

Subsite	Start Shell Length (mm)		
	Mean	SEM	*N*
V-Gabion	68.2	0.94	30
W-Gabion	68.6	0.84	30
Reference	67.0	0.78	30

Table 6. Survey detection results along with initial and final shell lengths of mussels measured at survey 2. SEM = standard error of the mean; *N* = sample size.

Subsite	Survey #1	Survey #2						
	Mussels Detected	Mussels Detected	Start Shell Length (mm)			Survey Shell Length (mm)		
			Mean	SEM	*N*	Mean	SEM	*N*
V-Gabion	5	15	61.8	-	1	77.5	-	1
W-Gabion	9	15	68.2	1.6	5	85.2	0.9	5
Reference	7	6	72.0	3.5	2	85.2	3.5	2

Figure 12. Subset of tagged mussels recovered from gabion area with no damage to shells, red tags, or PIT tags (in white epoxy).

Figure 13. V-gabion structure observed during a low tide.

Free Release

Surveyors carefully waded in and around the low water level to detect mussels visually and with clam rakes (Fig. 14). After 121 days, no mussels were detected in Tributary 1 and five mussels were detected in Tributary 2. Of the mussels found in Tributary 2, four were dead shell and one was alive. The live mussel was measured and grew 5.32 mm during the study.

Surveyors observed that the shells of dead mussel all exhibited a similar pattern of shell breakage that has not been seen in our other mussel studies, including at the Winterthur reference site (Fig.15). Damage included partial or complete breaks on either anterior or posterior ends of the shell as well as jagged edges along the ventral margin. One shell was found with light scratches and a hole punctured through the shell. Presumably, these mortalities were associated with predation of mussels.

Notably, during free release survey a wild Tidewater Mucket, *Atlanticoncha ochracea*) was found, which is one of the few living examples of this species that has been found by PDE staff to date. The surveyors also reported live crayfish (unknown species), and a claw of a Red Swamp Crayfish (*Procambarus clarkii*) roughly 3" in length. The claw had fresh tissue when a surveyor separated the claw. Photos of these observations are presented in Figures 16 & 17.

Figure 14. Water level of Tributary 2 at the mouth during the free release monitoring survey.

Figure 15. Recovered mussel shells from the free release observed to have similar breakage patterns (top) and puncture damage with surficial scratches (bottom).

Figure 16. A live crayfish (top) spotted in Tributary #2 alongside a large mussel, field identified as a Tidewater Mucket (bottom).

Figure 17. A claw of a Red Swamp Crayfish found in Tributary #2 (December 2020) of the Christina River where mussels were free released.

Water Quality Assessment

Spot Sampling and Long Term Monitoring

Water quality data taken via spot sampling at field sites are presented in Table 7. Water quality followed typical seasonal trends and dissolved oxygen was well above the necessary levels required for freshwater mussels in lentic and lotic environments. While Winterthur demonstrated consistent conductivity over the study, Christina River sites fluctuated in specific conductivity with very large spikes during September and October. These spikes coincide with the long-term conductivity dataset (Fig. 18).

Salinity was monitored at the Peterson Marsh from April 2018 to October 2020 and at the Churchmans from October 2019 to June 2020 (Fig. 18). Over the course of the monitoring period, salinities at the Peterson Marsh spiked above the oligohaline threshold (0.5 ppt) approximately four times. On three of these occasions, salinity did not exceed 1 ppt. In October 2019, however, salinities exceeded the oligohaline threshold for nearly 1 month, with peak values exceeding 3 ppt. This weeks-long event was also the only event to exceed the oligohaline threshold at Churchmans, nearly 8 km upstream, albeit the event appeared shorter in duration.

Drought indices during the long-term sampling period varied regionally through time (Fig. 19). Over time salinities in the Christina River appear most correlated with drought from SE PA (ρ=-0.49, p<0.01) and N DE (ρ=-0.50, p<0.01), which are the areas that drain into the Christina River basin (Table 8). Pluvials (wet periods) occurred in SE PA and N DE during the Christina's salinity spike (~October 2019), whereas drought conditions were recorded in S DE. Despite pluvial conditions in the Christina River's drainage basin, the long term (~1 month) salinity spike is likely a combination of drought in the Delaware Bay (south of the Christina River) and the passing of the Tropical Storm Melissa. TS Melissa approached Delaware nearly 200-km off of the coast in October 2019. As it approached, however, it began to stall causing significant storm surge in the Delaware Bay (Fig. 20). This storm surge likely pushed salty water farther up the estuary, causing a large, prolonged spike in salinity for the Christina River.

Table 7. Water quality data taken via spot sampling summarized in chronological order for sites with available data. * pond was sampled rather than the stream.

Site	Date	Temperature (C°)	Specific Conductivity (uS/cm)	Dissolved Oxygen (mg/L)	pH
Winterthur (Reference)	2019-07-30	27.8	253	7.7	6.7
	2019-09-17	23.8	284	8.5	7.5
	2019-10-29	15.4	225	9.4	6.4
	2020-05-11*	14.0	232	12.2	8.7
	2020-08-10	27.8	132	7.6	7.1
Churchman	2019-07-30	25.8	323	8.2	7.4
	2019-09-17	23.5	892	8.9	7.5
	2019-10-29	15.7	529	6.8	7.1
	2020-05-12	14.0	295	8.2	7.4
	2020-08-10	26.2	193	4.6	6.5
Newport	2019-07-30	28.2	339	8.2	7.5
	2019-09-17	23.9	1800	8.7	7.7
	2019-10-29	15.9	1480	8.4	7.1
	2020-05-12	13.0	356	9.8	7.5
	2020-08-10	25.4	240	5.2	6.7
Pool	2019-07-30	28.6	326	8.8	7.5
	2019-09-17	24.4	172	10.5	8.1
	2019-10-29	15.9	724	7.9	6.8
	2020-05-11	13.4	357	10.1	8.3
Mainstem	2019-07-30	28.0	339	6.9	7.5
	2019-09-17	24.3	3360	7.5	7.6
	2019-10-29	16.1	2210	8.0	7.2
	2020-05-11	13.1	359	9.9	7.9
	2020-08-11	26.8	225	5.9	7.1

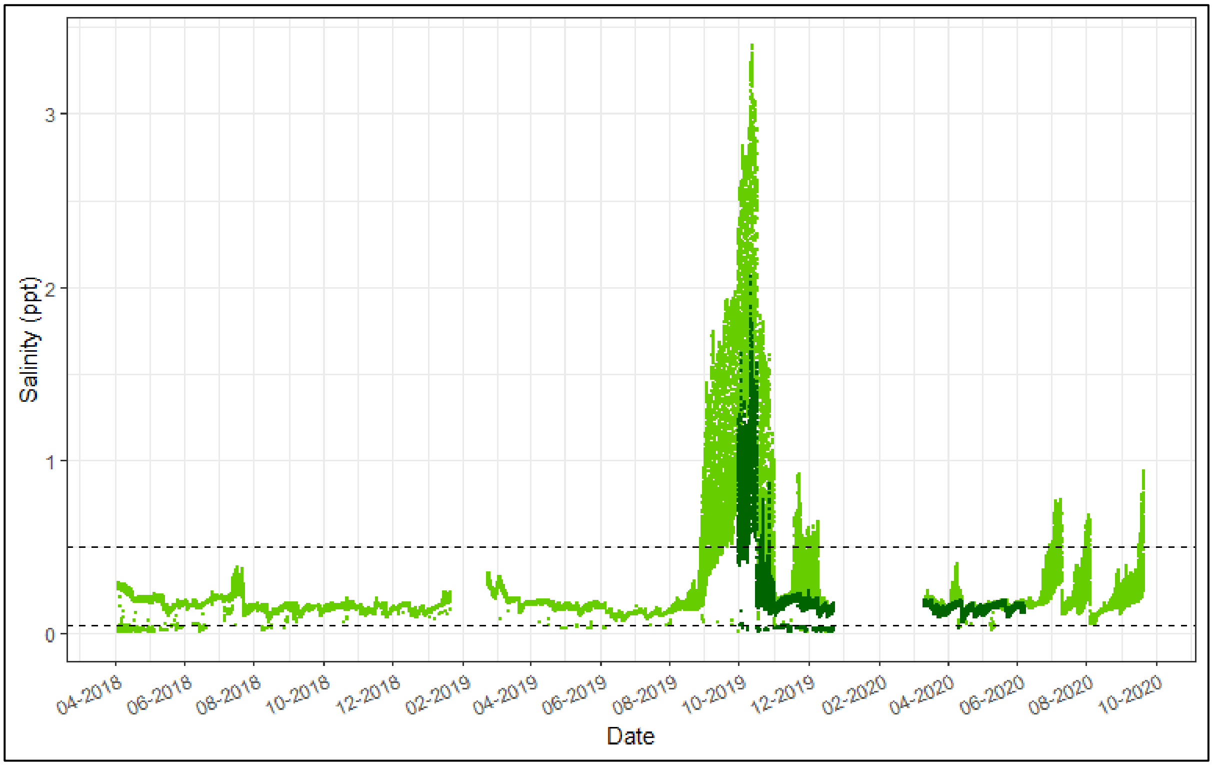

Figure 18. Salinity monitoring data. Light green is downstream (Peterson marsh), darker green is upstream (Churchman's boat ramp). Dashed lines are 0.5 ppt (oligohaline threshold, top) and 0.05 ppt (freshwater threshold, bottom).

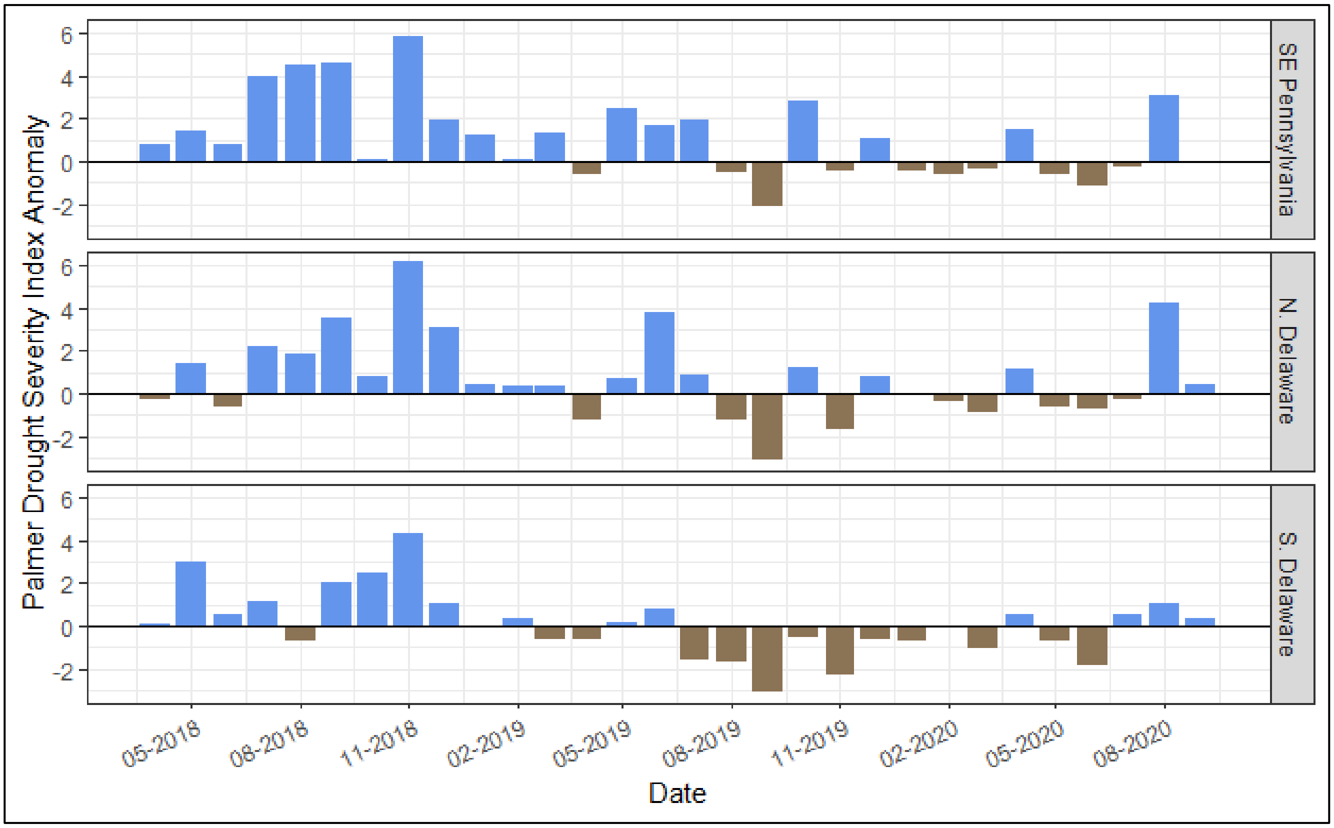

Figure 19. Drought indices. Positive PDSI anomalies are pluvials, negatives are droughts.

Table 8. Spearman's rank correlation test results, significant tests are in bold font (p<0.05).

Variable 1	Variable 2	ρ	*S, p*
SE Pennsylvania Drought	**Christina downstream salinity**	**-0.49**	**S = 5458, p-value = 0.008**
N DE Drought	**Christina downstream salinity**	**-0.50**	**S = 5489, p-value = 0.006**
S DE Drought	Christina downstream salinity	-0.32	S = 4840, p-value = 0.09
Reedy Point conductivity	SE Pennsylvania drought	-0.29	S = 4742, p-value = 0.12

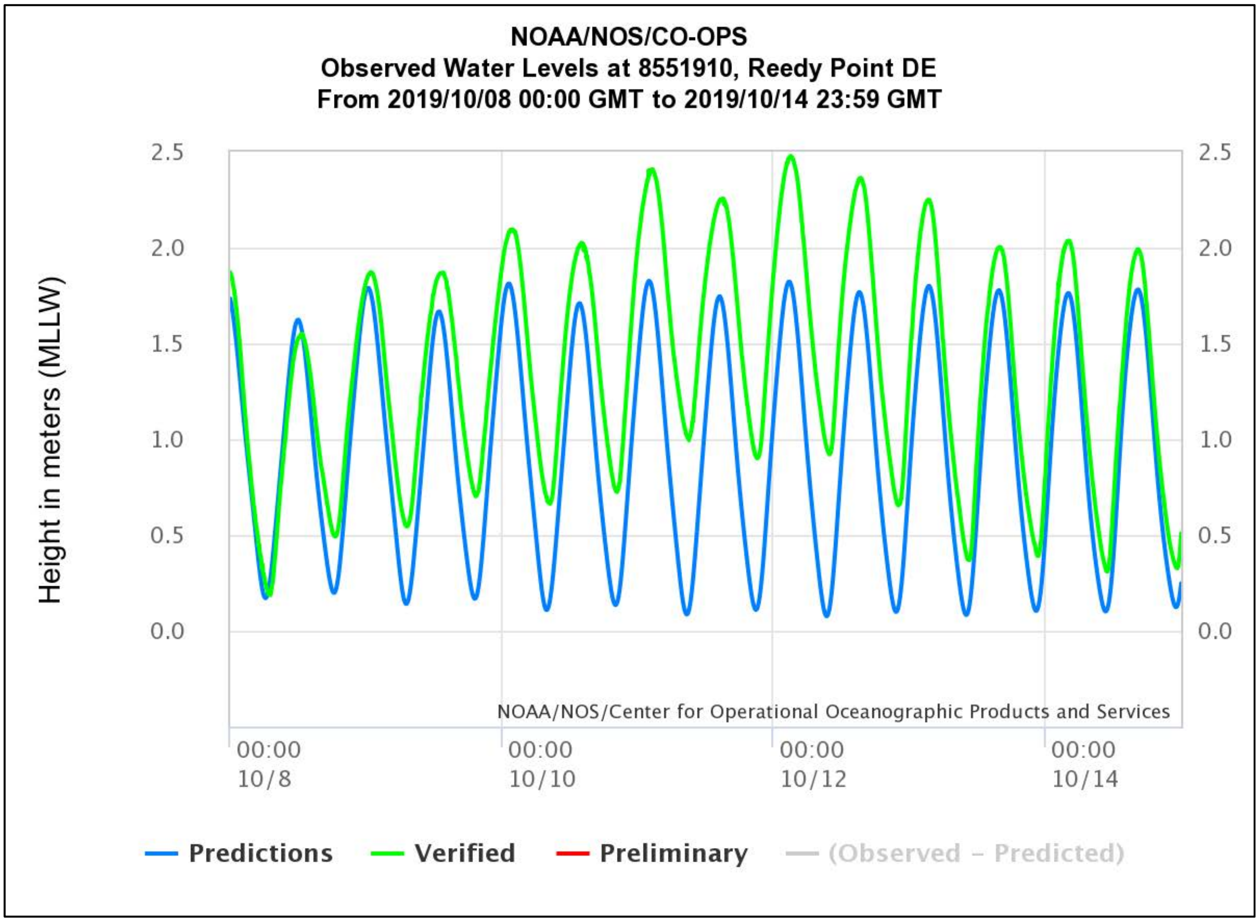

Figure 20. Water levels at Reedy Point Jetty, Delaware, from October 8 to 14, 2019. Tropical Storm Melissa, although >200 km off the coast of Delaware, perpetuated significant surge that affected the Delaware River. Reports of the storm's effects on water levels show that the surge at Reedy Point surpassed MHHW by about 0.74 m.

Seston Analyses

Seston data (i.e. PM, POM, and organic content) were gathered for two seasons during the study and are presented in Table 9. Mean PM varied by a factor of ten, from 3.88 mg/L at Winterthur to 37.8 mg/L at Newport. PM at all Christina River sites were at least four times greater than that observed at Winterthur. Accordingly, observed POM was greater in Christina River sites compared to Winterthur. However, the organic content was greatest in Winterthur compared to all sites. The water source at Winterthur is a 1.5 acre lake, compared to the tidally influenced lotic sites along the Christina River.

Within Christina River sites (i.e. Churchman, Newport, Pool, and Mainstem), mean PM varied significantly by month ($p<0.001$, 2- way ANOVA), however the interaction of site and month also was significant ($p<0.001$, 2-way ANOVA). Similarly, mean POM varied significantly by site, month, as well as the interaction of site and month ($p<0.001$ all tests, 2-way ANOVA).

While PM and POM had significant interactions, a 2-way ANOVA did not find a significant interaction of site and month for mean organic content of seston ($p=0.48$) but did find significance for each main effect i.e. site and month ($p<0.001$, each test). A post-hoc Tukey test determined that mean organic content for all sites was similar during July and September ($p=0.23$), significantly lower in October compared to July and September ($p<0.001$, each test). Comparing sites over the study period, mean organic content was greatest in Pool, followed by Churchman, Newport, and Mainstem. A post-hoc Tukey test determined that mean organic content was statistically greater in Pool over Newport ($p<0.05$) and Mainstem ($p<0.001$). Churchman also had significantly greater organic content over Mainstem ($p<0.01$).

Table 9. Particulate water quality data summarized in chronological order for each caging site.

Site	Date	Particulate Matter (mg/L)			Particulate Organic Matter (mg/L)			Organic Content (%)		
		Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
Winterthur (Reference)	2019-07-30	4.48	0.8	4	2.44	0.2	4	57	5.4	4
	2019-09-17	3.88	0.3	4	3.01	0.3	4	77	4.2	4
	2019-10-29	4.77	0.5	4	2.23	0.1	4	47	2.7	4
Churchman	2019-07-30	23.0	1.6	4	8.51	0.3	4	37	1.5	4
	2019-09-17	28.7	0.8	4	10.3	0.3	4	36	0.3	4
	2019-10-29	32.7	6.0	3	5.80	0.2	3	19	2.8	3
Newport	2019-07-30	12.8	1.8	4	4.74	0.5	4	38	1.5	4
	2019-09-17	27.5	0.8	4	8.60	0.2	4	31	0.9	4
	2019-10-29	37.8	2.2	4	7.28	0.2	4	19	1.3	4
Pool	2019-07-30	22.9	2.0	4	8.85	0.5	4	39	1.3	4
	2019-09-17	22.8	0.9	4	8.82	0.1	4	39	1.1	4
	2019-10-29	17.5	1.1	4	5.68	0.1	4	33	1.7	4
Mainstem	2019-07-30	15.5	0.7	4	4.98	0.3	4	32	0.5	4
	2019-09-17	21.5	1.7	4	5.53	0.3	4	26	1.6	4
	2019-10-29	36.2	1.3	4	6.33	0.2	4	18	0.8	4

Discussion

Native species of freshwater mussels were found to tolerate and grow in size at all tidal study sites on the lower Christina River in New Castle County, Delaware. At least some mussels from a common, hatchery-propagated cohort were able to survive and grow at all sites, and the growth rates of the survivors exceeded the growth of mussels a non-tidal stream site (Winterthur) that served as a reference location. This positive result was in spite of variable water quality, relatively high suspended solid concentrations, and a few significant storm events that contributed to spikes in specific conductivity.

Nevertheless, the survival of deployed mussels at the tidal Christina study sites was lower than at the reference site due to several factors. Experimental errors in the form of cage losses due to severe storm events or vandalism were not considered in the survival analysis. After accounting for such losses, the lower survival at study sites compared to the reference appeared to mainly be associated with predation based on discrete shell breakage patterns witnessed on the recovered shells. The type of predator is unclear, but blue crabs, crayfish, raccoons and other animals exist within the study area.

Although not part of the original study design, a subset of mussels was also deployed into experimental "mussel enhancement habitats" at one of the study sites to preliminarily test whether stabilization of soft bottom habitats might increase habitat suitability for mussels. The experimental plots consisted of unbounded "V" and "W" shaped strings of small gabions filled with oyster shell, around which mussels were deployed, as compared to mussels deployed in an unprotected control plot. The preliminary design and budget precluded replication for statistical analyses, however, after nearly five months more than twice as many tagged mussels were re-surveyed in these gabion-stabilized plots compared to the untreated plot, and growth was comparable during the deployment period.

All surviving mussels from the initial cage and gabion study were relocated to small tributary streams that were deemed to be conducive for mussels based on the existence of small numbers of extant, wild mussels. A subsequent survey of the tributaries for these free-released, tagged mussels found that most had perished or disappeared, presumably due to predation.

Outcomes from this project confirm that the water quality and food conditions of the lower Christina, while not always ideal, are sufficient to support good growth and survival of native species of freshwater mussels. The early indications from the gabion study also suggest that habitat modification tactics may plausibly help to stabilize and enhance mussel habitat suitability, which should be useful for designing living shorelines that could contain mussel beds as one feature in the subtidal terraces of the project area. However, our results also indicate that predation pressure will be an important constraint on mussel restoration or mussel-based living shorelines in the tidal Christina River. Future studies should examine which species and sizes of mussels are most vulnerable to this predation, and then develop predator management practices to either protect sensitive sizes or species or to only release mussels that are of a species or size that has lower predation risk. For projects aimed at enhancing mussel beds for ecosystem services (e.g. water quality improvement), additional work is also needed to further test different tactics for enhancing mussel habitat suitability, with a goal of boosting mussel densities and habitat carrying capacity. Based on our results, food quality and quantity should not be a constraint on mussel carrying capacity in the particle-rich lower Christina River.

Mussel Survivorship

Monitoring of caged mussels provided insights on whether the ambient water quality and food composition of the lower Christina was sufficient to sustain growing mussels. While some mussels simply went missing because of cage damage or cage loss (e.g., associated with storms), other dead mussels (empty shells) were found buried even deep within the cages. These buried shells were likely a result of cases where the entire cage became buried due to sedimentation, which would have led to decreased oxygen and food supply, thus smothering the mussels. Sedimentation, while possible in a natural setting, is preventable in future studies by modifying the cage designs, and for this study those data were considered artifacts of the study. Those mussels would have likely survived if cages were bigger and maintained more rigorously. However, some mortalities were not due to burial, evidenced by their shells (some dead shells were clean (i.e. not buried) and other shells were damaged along the ventral margin or partially smashed).

The majority of mussel mortalities observed in the study (63%) were mussels that were monitored and subsequently died before growing 1.2 mm. While 1.2 mm could be considered substantial growth for a small mussel, the measurement comparison conducted suggests that 1.2 mm could be within a margin of error (particularly for dead shell that may warrant additional shell manipulation to approach an accurate measurement). The 1.2 mm margin of error would likely reduce if the test were repeated with live mussels rather than empty shells requiring additional manipulation, and therefore introducing more variability. Accordingly. mussel researchers have reported slightly lower errors (< 1 mm, 95% of comparisons) in similar trials (Downing and Downing 1993). Still, the purpose of the test validates the data and provides context on mortalities in that most of the mussels that died did so shortly after deployment or redeployment following monitoring. Death resulting from stress caused by location change and handling should be considered as a possible factor, but there is no direct evidence to support this. Previous PDE studies involving similar caging protocols did not observe such high mortality numbers (Gray & Kreeger 2014, Cheng & Kreeger 2017).

Predation

Predation is a natural phenomenon and a consideration for any population ecology or restoration study. However, in more than 15 years of PDE-led studies to survey and restore native mussel populations we had not encountered a situation where predation was a significant factor in large scale mussel survival. Judging from the pronounced shell breakage patterns, deployed mussels experienced predation in all facets of this study, and to varying degrees at all tidal Christina study sites.

Initial observations of possible predation in this study were dead shells found in cages located at the Mainstem site. Shells were found to have jagged edges that would be inconsistent with mortality due to physiological causes. Instead, shell damage would suggest disturbance from another organism. Damage from outside forces such as wave action combined with debris or other natural shell breakage could be ruled out due to the shells having protection through the caging material. Furthermore, if abrasion was a cause, the fragile periostrocum of the shells would have shown significant wear, but was intact even on the broken shells.

The Blue Crab (*Callinectes sapidus*) is the most likely predator to have caused the observed shell breakage and associated mortality. *Callinectes sapidus* is a known predator of marine bivalves and has been documented to prefer thin shelled animals, such as *Mya arenaria*, but can also prey upon thicker shelled animals if available (Ebersole & Kennedy 1994). While *C. sapidus* primarily resides in mesohaline and polyhaline environments, crabs (especially males) seasonally travel into freshwater zones where their distribution overlaps with freshwater mussels (Mangum & Amende 1972, Ettinger & Blye 1981). Additionally, similar shell damage patterns have been reported for other freshwater mussels (Pla Ventura et al. 2018). This predator likely has substantial, but seasonally limited, impacts on benthic communities in freshwater zones of the estuary.

Near the end of this study, surveyors observed both a small live crayfish (unknown species) and a claw consistent with that of *P. clarkii*, the most commercially farmed species for human consumption across the globe (Huner 1993, 2002). *Procambarus clarkii* is considered to be a very concerning invasive species and has successfully invaded many freshwater systems beyond its native range of northern Mexico and southern United States (Geiger et al. 2005, Strayer 2010, Twardochleb et al. 2013, Souty-Grosset et al. 2016). As a generalist predator, *P. clarkii* preys upon a range of benthic organisms, including macroinvertebrates and snails, and it can switch prey items based on availability (Correia 2002, Alcorlo et al. 2004). While no established population may be recorded in Delaware and the claw could have been from discarded trash, the potential presence of this well-known invasive species warrants further investigation, particularly if it has the potential to impact local biota, i.e. mussel predation as suggested in this study.

Salinity and Drought

Although the study did not capture conductivity changes due to road salt (very mild winter), conductivity loggers recorded dramatic salinity spikes related to drought and storm surge. Over the course of this short monitoring period, salinity values in the lower Christina River peaked around 3.4 ppt and around 2 ppt in the upper River during a late summer/early autumn drought, which also coincided with tropical storm surge. This is approximately at the 100% mortality threshold described by Ercan and Tarkan (2014) for *Unio crassus*. Thus, freshwater mussel survival (>34%) for *U. implicata* in this study suggests that either: a) *in situ* mussel salinity tolerance in the Christina River is likely greater that what was found by Ercan and Tarkan (2014), or b) the duration of the high salinity event was not prolonged enough to cause mortality, since mussels can close up for short-term periods. Another factor could be differences in mussel age that relate to salinity sensitivity; i.e., mussels evaluated in this study were two years or more in age and do not represent the most vulnerable life stages of mussels (Wang et al. 2007, Wang et al. 2017).

Mussels experienced some mortalities due to cage loss as well as sedimentation during this time. However, the absence of significant mussel die-off outside of physical burial or removal during month-long salinity conditions in late summer/early autumn spike suggests that mussels in the Christina tolerated these conditions. Climate change, increased storm occurrence or intensity, drought, as well as rising sea levels, however, are likely to increase the frequency and severity of salinity spikes in the Christina River.

Road salt applications are most likely to affect mussels in late fall, when mussels have either not yet entered dormancy, or in late spring, when mussels have left dormancy. These occasions are also likely to occur more frequently with climate change, as erratic weather conditions are predicted to occur more often.

This study suggests that mussels likely tolerate salinity fluctuations and perhaps even tolerate events that are more than "mild" (e.g. 1 ppt for a few tidal cycles). However, the confounding mortalities could be partly explained by physiological stress related to this pulse. Survival of mussels was greater at Winterthur than Christina River sites. To our knowledge, this is the first instance on record where a salinity spike of this magnitude has been monitored with respect to the redeployment of mussels. The fact that extant mussels of two species (*U. implicata* and *A. ochracea*) also were found in near the Mainstem site (lowest in the system) supports our results that native mussels can tolerate periodic salinity excursions in the lower Christina River. Although mussels may be more resilient to salinity than expected, we caution that more study is needed to examine the effects of the duration of salinity spikes. In the long-term, increasing sea level and salinity will eventually elevate mean salinity and constrain mussel habitat at the seaward bounds.

Food Quality and Quantity

Seston data gathered through this study demonstrated that the concentration of both particulate matter and particulate organic matter is highly variable spatially and temporally in the lower Christina River. No consistent seasonal patterns were found. However, the organic content (a crude proxy for mussel food quality) followed the expected seasonal pattern whereby the mean organic content of seston was greatest in July, followed by declines in organic content through September and October. Additionally, the organic content generally followed an upstream to downstream gradient with greater organic content observed in the most upstream site (Churchman) followed by a slight decline in the next downstream site (Newport) and even less organic content in the furthest downstream site (Mainstem). The higher seston quality at upstream sites may result from greater phytoplankton concentrations fueled by nutrient runoff or less turbid conditions; however, chlorophyll concentrations were not assessed. Pool site organic content was nominally greater than any other Christina site for each sampling date. This may be explained in part due to its location within a productive wetland and its unique characteristic of retaining water during ebb tide (enabling stable productivity while other sites are continually flushed with each tide).

Generally, the seston available to mussels in the Christina River was found to be consistently plentiful when compared to the reference site of Winterthur. While the organic content of the seston was greater in Winterthur, seston was much more plentiful in the tidal Christina, presumably because of greater amounts of suspended sediments, detritus, and tidal-driven particle resupension of shallow bottom sediments. Like other bivalve molluscs, suspension-feeding freshwater mussels have complex sorting processes that are capable of compensating for diluted food quality within certain ranges. Although concentrations of suspended particulate matter in the Christina were higher than at Winterthur and typical streams in the region, they were always <50 mg/L, which is a typical threshold that would elicit decreased feeding efficiency (Safi et al. 2007), high rejection rates and energy balance constraints. Since extant populations of mussels are so scant in the tidal Christina River, other factors besides bottom-up food limitation are likely to blame.

Conclusions

Results from this study provide important new information to guide future directions of the Freshwater Mussel Recovery program that is being led by the Partnership for the Delaware Estuary. The goal of this program is to promote mussel conservation, restoration, and enhancement to both stem the decline of native mussel species and populations, as well as to promote shellfish-mediated ecosystem services such as their increasingly valued water quality benefits.

Different restoration strategies will be needed for tidal and non-tidal areas, and the habitats to be targeted vary with mussel species and existing environmental conditions. Regardless of species or area, a foundational question is whether mussel could survive there. Considering that historical data suggest the native mussels were formerly abundant in nearly every freshwater areas, why are they no longer present or abundant? Are the conditions that caused them to decline still present? What are their future prospects, considering large-scale anthropogenic alterations of the system and climate change? Results from this study do not address all of these questions for the tidal Christina River, but they do indicate that water quality and food conditions are not a constraint for mussels. Habitat degradation (e.g. over abundance of fine particles, instability) and possibly changes in the predator community are more likely explanations for limited mussel numbers in the tidal Christina River. While these constraints may be concerns for the restoration of natural mussel assemblages, they could be potentially addressed in designed enhancement projects aimed at ecosystem service uplift, such as living shorelines for water quality. Positive early indications from pilot mussel pen structures suggest that more study is warranted to develop and test structures that could increase mussel carrying capacity and protect mussels from predation, especially sensitive early life stages.

This study therefore expanded on previous studies in the region on the feasibility of freshwater mussel restoration (Cheng & Kreeger 2015, Cheng & Kreeger 2018), use of caging protocols to address certain site viability questions (Gray & Kreeger 2014), and more specifically on how to incorporate new technologies such as propagating mussels for water quality benefits (Cheng et al. 2020). While freshwater mussels have largely been extirpated from northern Delaware streams (Kreeger et al. 2014), the Christina River remains one of the last bastions of native populations in New Castle County. This research confirmed that large juveniles are able to persist under current conditions. Freshwater mussel populations are controlled by many interacting biotic and abiotic factors (Strayer 2008). This study furthered our understanding on some of the physical (e.g. conductivity) and biological (e.g. predators) factors that mussels face in the tidally influenced Christina River. Future studies may build on these results to contrast mussel fitness and predation sensitivity, between juveniles and adults, and among mussel species.

Literature Cited

Alcorlo, P., W. Geiger & M. Otero. 2004. Feeding Preferences and Food Selection of the Red Swamp Crayfish, *Procambarus clarkii*, in Habitats Differing in Food Item Diversity. *Crustaceana* 77(4): 435-453.

Atkinson, C. L., C. C. Vaughn, K. J. Forshay & J. T. Cooper. 2013. Aggregated filter-feeding consumers alter nutrient limitation: Consequences for ecosystem and community dynamics. *Ecology* 94(6): 1359-1369.

Atkinson, C. L, & C. C. Vaughn. 2015. Biogeochemical hotspots: temporal and spatial scaling of the impact of freshwater mussels on ecosystem function. *Fresh Biol*. 60: 563-574.

Blakeslee, C. J., H. S. Galbraith, L. S. Robertson & B. St. John White. 2013. The effects of salinity exposure on multiple life stages of a common freshwater mussel, *Elliptio complanata*. *Environmental Toxicology and Chemistry* 32(12): 2849-2854.

Cheng, K. M. & D. A. Kreeger. 2015. Current Status and Restoration of Freshwater Mussels in Northern Delaware. Partnership for the Delaware Estuary, Wilmington, DE. PDE Report No. 15-04.

Cheng, K. M. & D. A. Kreeger, 2017. Determination of Freshwater Mussels' Filtration Capacity and Pollutant Removal in Delaware Streams. Partnership for the Delaware Estuary, Wilmington, DE. PDE Report No. 17-04.

Cheng, K. M. & D. A. Kreeger, 2018. Juvenile Freshwater Mussel Stocking for Water Quality Enhancement in Southeast Pennsylvania Waters. Partnership for the Delaware Estuary, Wilmington, DE. PDE Report No. 18-06.

Cheng, K. M., D. A. Kreeger & M. J. Gentry. 2020. Juvenile Freshwater Mussel Rearing for Water Quality Improvement in Delaware. Partnership for the Delaware Estuary, Wilmington, DE. PDE Report No. 20-03.

Correia, A. M. 2002. Niche breadth and trophic diversity: feeding behaviour of the red swamp crayfish (*Procambarus clarkii*) towards environmental availability of aquatic macroinvertebrates in a rice field (Portugal). *Acta Oecologica* 23(6): 421-429.

Dame, R. F. 2012. Ecology of Marine Bivalves An Ecosystem Approach (2nd ed.). Boca Raton, FL: CRC Press.

Downing, W.L. & J. A. Downing. 1993. Molluscan shell growth and loss. *Nature* 362(6420): 506-506.

DRBC Delaware River Basin Commission, Salt Front, published January 2021, retrieved on January 12, 2021 from https://www.state.nj.us/drbc/hydrological/river/salt-front.html

Ebersole, E.L. & V.S. Kennedy. 1994. Size selection of Atlantic rangia clams, Rangia cuneata, by blue crabs, Callinectes sapidus. Estuaries 17, 668–673.

Ercan, E. & A. S. Tarkan. 2014. Effect of Salinity on the Growth and Survival of the Freshwater Mussel Unio crassus in an Environmentally Disturbed River. *Pakistan Journal of Zoology* 46(5).

Ettinger, W. & R. Blye. 1981. Occurrence of the Blue Crab *Callinectes sapidus* in the Tidal Freshwater Reaches of the Delaware and Schuylkill Rivers in 1976. *Journal of Crustacean Biology* 1(2): 177-182.

FMCS (Freshwater Mollusk Conservation Society). 2016. A national strategy for the conservation of native freshwater mollusks. *Freshw. Mollusk Biol. Conserv.* 19: 1-21.

Geist, J. 2010. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of conservation genetics and ecology. *Hydrobiologia* 644: 69-88.

Geiger, W., P. Alcorlo, A. Baltanás & C. Montes. 2005. Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions* 7: 49–73.

Gillis, P. L. 2011. Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters. *Environmental Pollution* 159(6): 1702-1708.

Gillis, P. L. 2012. Cumulative impacts of urban runoff and municipal wastewater effluents on wild freshwater mussels (*Lasmigona costata*). *Science of the Total Environment* 431: 348-356.

Gray, M. W. & D. A. Kreeger 2014. Monitoring fitness of caged mussels (*Elliptio complanata*) to assess and prioritize streams for restoration. Aquatic Conservation: *Marine and Freshwater Ecosystems* 24: 218-230.

Haag, W. R. & J. D. Williams. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia* 735(1): 45-60.

Hoellein, T. J., C. B. Zarnoch, D. A. Bruesewitz & J. DeMartini. 2017. Contributions of freshwater mussels (Unionidae) to nutrient cycling in an urban river: filtration, recycling, storage, and removal. *Biogeochem.* 135: 307-324.

Huner, J. V. 1993. International crayfish production. *International Association of Astacology Newsletter* 15(1): 4.

Huner, J. V. 2002. Procambarus. In D. Holdich (Ed.), Biology of freshwater crayfish (pp. 541–584). Oxford, UK: Blackwell Science Ltd.

Kreeger, D. A., C. E. Goulden, S. S. Kilham, S. G. Lynn, S. Datta & S. J. Interlandi. 1997. Seasonal changes in the biochemistry of lake seston. *Freshwater Biology* 38: 539 554.

Kreeger, D., P. Cole, M. Mills, L. Butler, A. Padeletti, R. Thomas & J. D'Agostino. 2013. Connecting people to aquatic biodiversity: freshwater mussel surveys in Pennsylvania's coastal zone. Partnership for the Delaware Estuary final report to the Pennsylvania Coastal Management Program. PDE Report No. 13-02. 65 p.

Kreeger, D.A., K. Cheng, P. Cole & A. Padeletti. 2014. Partnership for the Delaware Estuary. 2014. Reintroduction of Freshwater Mussels into the Red and White Clay Creeks, DE. PDE Report No.14-02

Kreeger, D. A., C. Gatenby & P. Bergstrom. 2018. Restoration potential of several native species of bivalve molluscs for water quality improvement in mid-Atlantic watersheds *J. Shellfish Res.* 37(5): 1121-1157.

Mangum, C., & L. Amende. 1972. Blood Osmotic Concentration of Blue Crabs (*Callinectes sapidus* Rathbun) Found in Fresh Water. *Chesapeake Science* 13(4): 318-320.

Neves, RJ. 1999. Conservation and commerce: management of freshwater mussel (Bivalvia: unionidea) resources in the United States. *Malacologia* 41(2): 461-474.

Nobles, T. & Y. Zhang. 2011. Biodiversity loss in freshwater mussels: importance, threats, and solutions. In: O. Grillo & G. Venora, editors. Biodiversity Loss in a Changing Planet. Rijeka:Intech. pp. 137-162.

NOAA National Centers for Environmental information, Climate at a Glance: Global Mapping, published December 2020, retrieved on January 12, 2021 from https://www.ncdc.noaa.gov/cag/

Parker M. & S. Bricker. 2020. Sustainable oyster aquaculture, water quality improvement, and ecosystem service value potential in Maryland Chesapeake Bay. *J. Shellfish Res*. 39(2): 269-281.

Partnership for the Delaware Estuary (PDE). 2012a. Technical Report for the Delaware Estuary & Basin. P. Cole and D. Kreeger (Eds.). PDE Report No. 12.01. 1-255 pp.

Partnership for the Delaware Estuary. 2012b. Freshwater Mussel Recovery Program in the Delaware Estuary. PDE Report No. 12-02b. 41 pp.

Patnode, K. A., Hittle, E., Anderson, R. M., Zimmerman, L., & Fulton, J. W. 2015. Effects of high salinity wastewater discharges on Unionid mussels in the Allegheny river, Pennsylvania. *Journal of Fish and Wildlife Management* 6(1): 55-70.

Pla Ventura, M., S. Quiñonero Salgado, J. Hernández Núñez, J. Velázquez Cano, P. Risueño Mata & J. López Soriano. 2018. Predation of the blue crab *Callinectes sapidus* Rathbun, 1896 on freshwater bivalves (Unionidae & Corbiculidae) in eastern Iberian Peninsula. *Folia Conch.* 47: 3-9.

Safi, K. A., J. E. Hewitt & S. G. Talman. 2007. The effect of high inorganic seston loads on prey selection by the suspension-feeding bivalve, Atrina zelandica. *Journal of Experimental Marine Biology and Ecology* 344(2): 136-148.

Souty-Grosset, C., P. M. Anastacio, L. Aquiloni, F. Banha, J. Choquer, C. Chucholl & E. Tricarico. 2016. The red swamp crayfish *Procambarus clarkii* in Europe: impacts on aquatic ecosystems and human well-being. *Limnologica* 58: 78-93.

Strayer, D., J. Downing, W. Haag, T. King, J. Layzer, T. Newton & S. Nichols. 2004. Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* 54(5): 429-439.

Strayer, D. L. 2008. Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance. Berkeley, California: University of California Press. 216 pp.

Strayer, D. L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater biology* 55: 152-174.

Twardochleb, L. A., J. D. Olden & E. R. Larson. 2013. A global meta- analysis of the ecological impacts of nonnative crayfish. *Freshwater Science* 32: 1367–1382

Vaughn, C. C. 2017. Ecosystem services provided by freshwater mussels. *Hydrobiologia* 810(1): 15-27.

Wang, N., C. G. Ingersoll, D. K. Hardesty, C. D. Ivey, J. L. Kunz, T. W. May. F. J. Dwyer, A. D. Roberts, T. Augspurger, C. M. Kane, R. J. Neves & M. C. Barnhart. 2007. Acute toxicity of copper, ammonia, and chlorine to glochidia and juvenile mussels. *Environmental Toxicology and Chemistry* 26: 2036-2047.

Wang, N., C. D. Ivey, R. A. Dorman, C. G. Ingersoll, J. Steevens, E. J. Hammer, C. R. Bauer & D. R. Mount. 2018. Acute toxicity of sodium chloride and potassium chloride to a unionid mussel (*Lampsilis siliquoidea*) in water exposures. *Environmental toxicology and chemistry* 37(12): 3041-3049.

Williams, J. D., M. L. Warren Jr, K. S. Cummings, J. L. Harris & R. J. Neves 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries* 18(9): 6-22.

Appendix A

PARTNERSHIP FOR THE DELAWARE ESTUARY

Freshwater Mussel Recovery Program Method No. 18

Benthic Cage

Prepared by: Kurt Cheng, Matthew Gentry, and Danielle Kreeger

Date Prepared: 2020-07-13

Version: 1.0

Version Date: 2020-07-13

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Benthic Cage

Partnership for the Delaware Estuary (PDE)

Freshwater Mussel Recovery Program Method No. 18
Version: 1.0

Description

This Freshwater Mussel Recovery Program Method (FMRPM) builds upon pre-existing designs for a benthic cage to contain bivalves. This FMRPM describes the general conduct of fabricating and deploying benthic cages for freshwater mussel studies.

Summary of Approach

The general approach is to create a low profile, robust cage to house freshwater mussels in riverine environments. The cage allows for good flow of water throughout, discourages predation, and is anchored in place to withstand regular tidally shifting flows.

Suggested Equipment and Materials

- 1 x Plastic Dishwasher Tray
- Plastic Diamond Mesh
- 3 x 1” Slotted PVC (18” length)
- Hog Rings
- Hog Ring Pliers
- 3 x Eyebolts with washers and nuts
- 2 x Ball Bungee (5”)
- 3 x 3/8” Rebar (24” length)
- 26 x Cable Tie (6”)
- Tin Snips/Wire Cutter
- 2’ Steel Pipe (3/4” + I.D.)
- 5’ Steel Pipe (3/4” + I.D.)

Methods

1. Cut Plastic Diamond Mesh

Refer to Figure 1 for the plastic mesh cutting specifications, based on an 18” square plastic dishwasher tray. If a different size dishwater tray is used, the plastic mesh specifications will be different, and must be measured based on the side length and height of the selected dishwasher tray. Plastic diamond mesh should be cut using tin snips, wire cutters, or some other heavy-duty cutting tool.

2. Install Mesh to Dishwasher Tray

Refer to the attached technical drawings for panel numbering of the plastic diamond mesh. This is required to understand the following fabrication steps.

2.1 Assembling Panels Together

- Place the dishwasher tray onto Panel A (Fig. 2). Use four evenly spaced cable ties to attach Panel A to the bottom of the dishwasher tray.

- Fold Panels C, D, E, and F up along the sides of the dishwasher tray. Use four evenly spaced cable ties on each side to secure the panels to the top of the dishwasher tray sides.

- Fold Panels E1 and D1 over Panel C, and Panels E2 and D2 over Panel F.

Use hog rings to attach the plastic diamond mesh panels to each other. Use as many hog rings as needed to secure panels (minimum 3).

At this point, the plastic dishwasher tray should have plastic diamond mesh attached on the bottom and all sides. Panel B (top) should be able to fold over the top of the cage.

3. Cage Lid

Panel B of the plastic diamond mesh will be the lid of the benthic cage. Slotted PVC is available from suppliers such as Ketcham Supply (https://ketchamsupply.com/) or can be created. These 20” slotted PVC sliders can be modified slightly for use in this benthic cage.

3.1 Attach PVC Sliders to Panel B

Cut PVC sliders to match the side lengths of Panel B. This can be accomplished with a hand saw or PVC pipe cutting tool. Carefully slide PVC onto the edges of Panel B. Use two cable ties on each PVC slider to secure them to the plastic diamond mesh

4. Attach Eyebolts for Anchoring

Eyebolts for anchoring are attached at three points on the benthic cage. Place a washer over the threaded end of an eyebolt and put that end through the outside of Panels D’, C, and the dishwasher tray side that they cover. Secure the eyebolt on the inside of the cage using another washer and a nut. Repeat this on the corner through Panels E’, C, and the dishwasher tray side that they cover. Install the third eyebolt through the middle of Panel F.

5. Attach Ball Bungees for Lid Closure

Place the bungee end of two ball bungees, evenly spaced, through Panel F and the dishwasher tray side that it covers. Tie an overhand knot inside the cage to prevent the bungee from slipping out. When closing the lid, thread the bungee loop up through Panel B and loop it around the corresponding ball component of the ball bungee. The finished product should resemble the graphic shown in Figure 3.

6. Anchoring and Deployment

Rebar anchors can be purchased or fabricated.

6.1 Create Anchors

Cut rebar to 24” if needed. Place rebar inside two steel pipes, ensuring the two pipes meet at the 6” mark of the rebar. Create a slight gap to allow the pipes to move. Stand on the short steel pipe and pull up and back on the longer pipe to form a “candy-cane” anchor. A fabricated cage complete with anchors is presented in Figure 4.

6.2 Deployment

Place one anchor through each eyebolt. In soft sediment, the anchors may be installed by hand. In some sediments, a hammer may be needed to install the anchors. Ensure anchors are as flush with bottom as possible to avoid catching passing debris.

Technical Diagrams and Photos

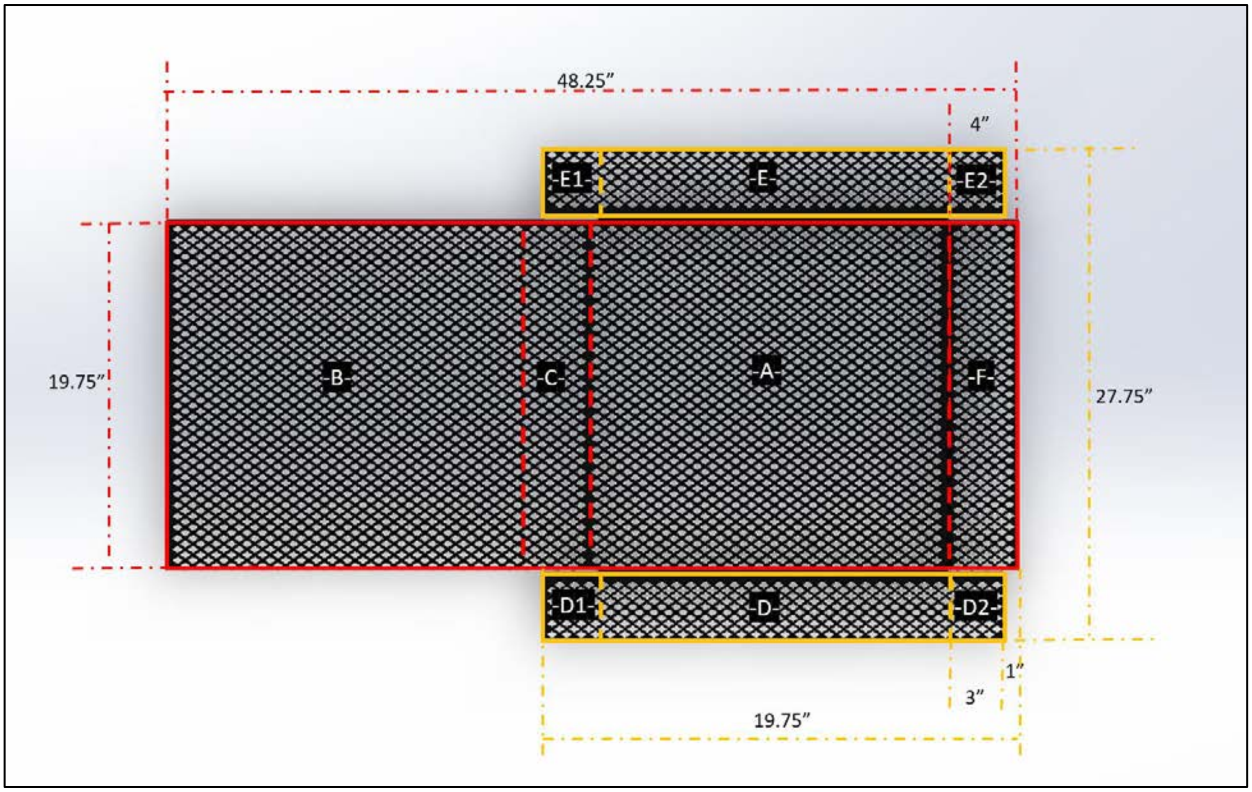

Figure 1. Panel diagram for mesh assembly.

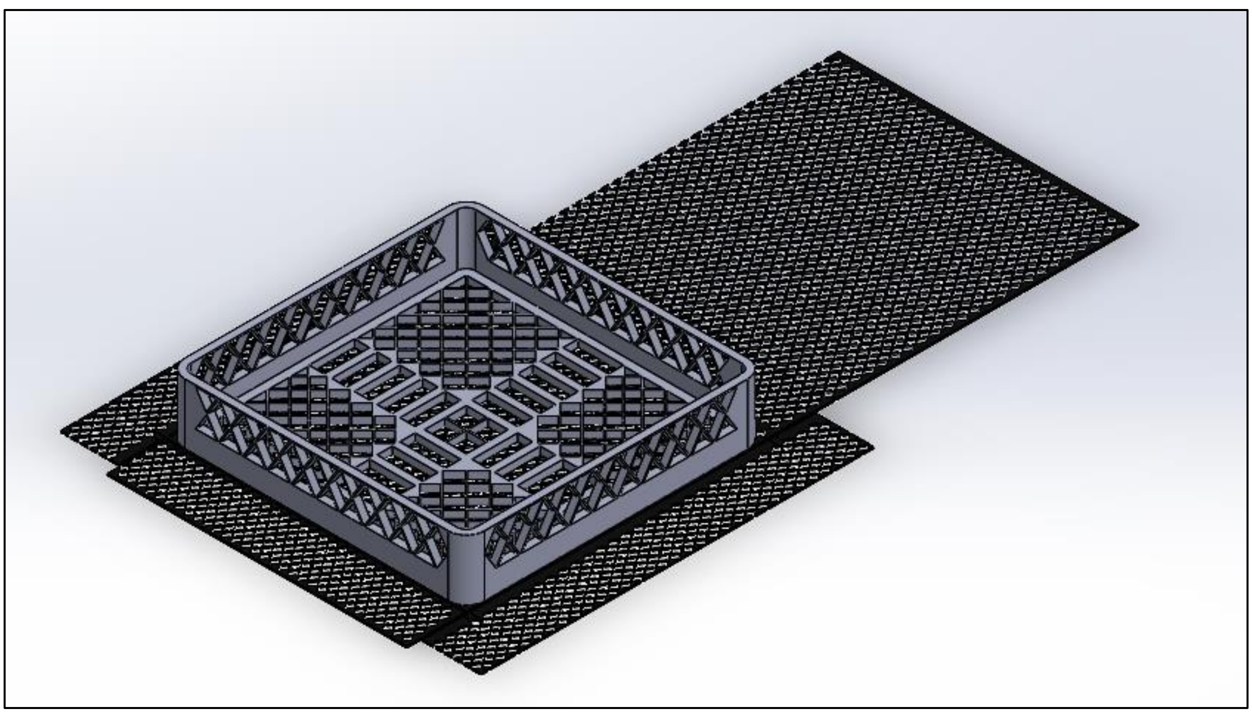

Figure 2. Tray positioned on top of mesh panels.

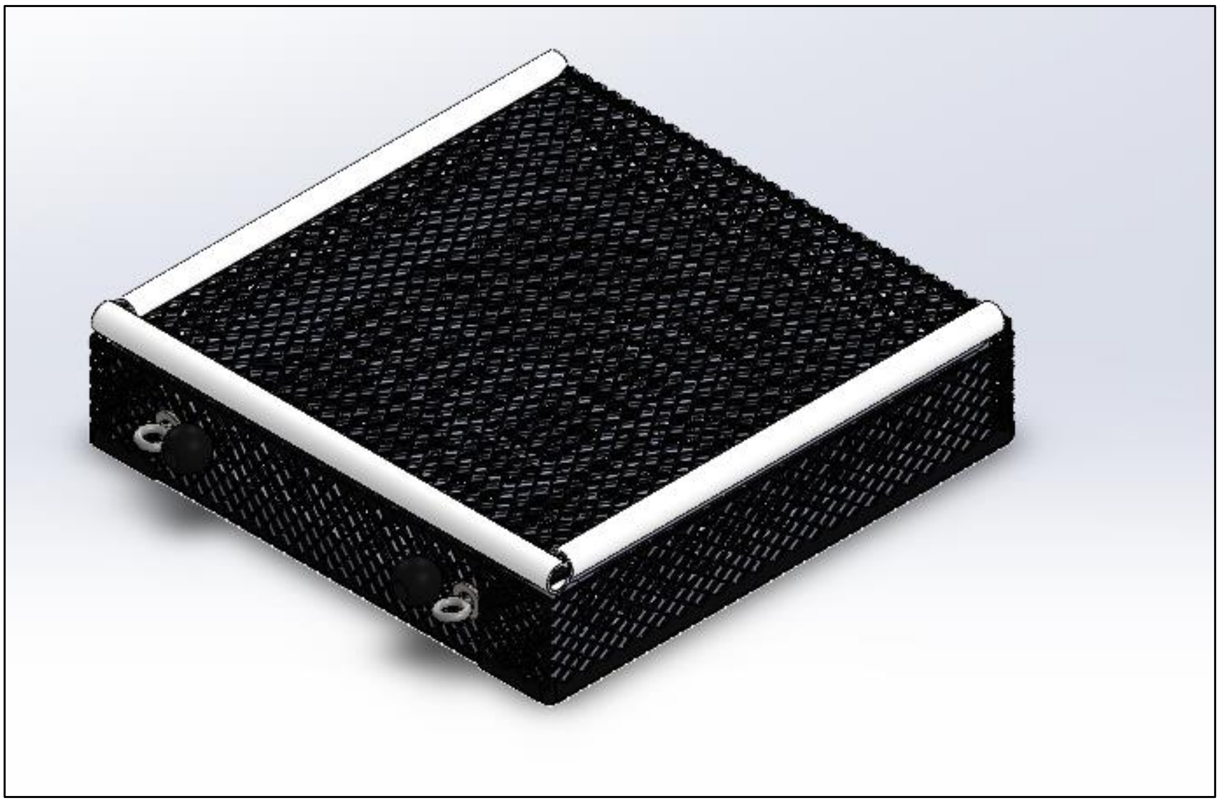

Figure 3. Completed cage with PVC-reinforced lid secured and eyebolts.

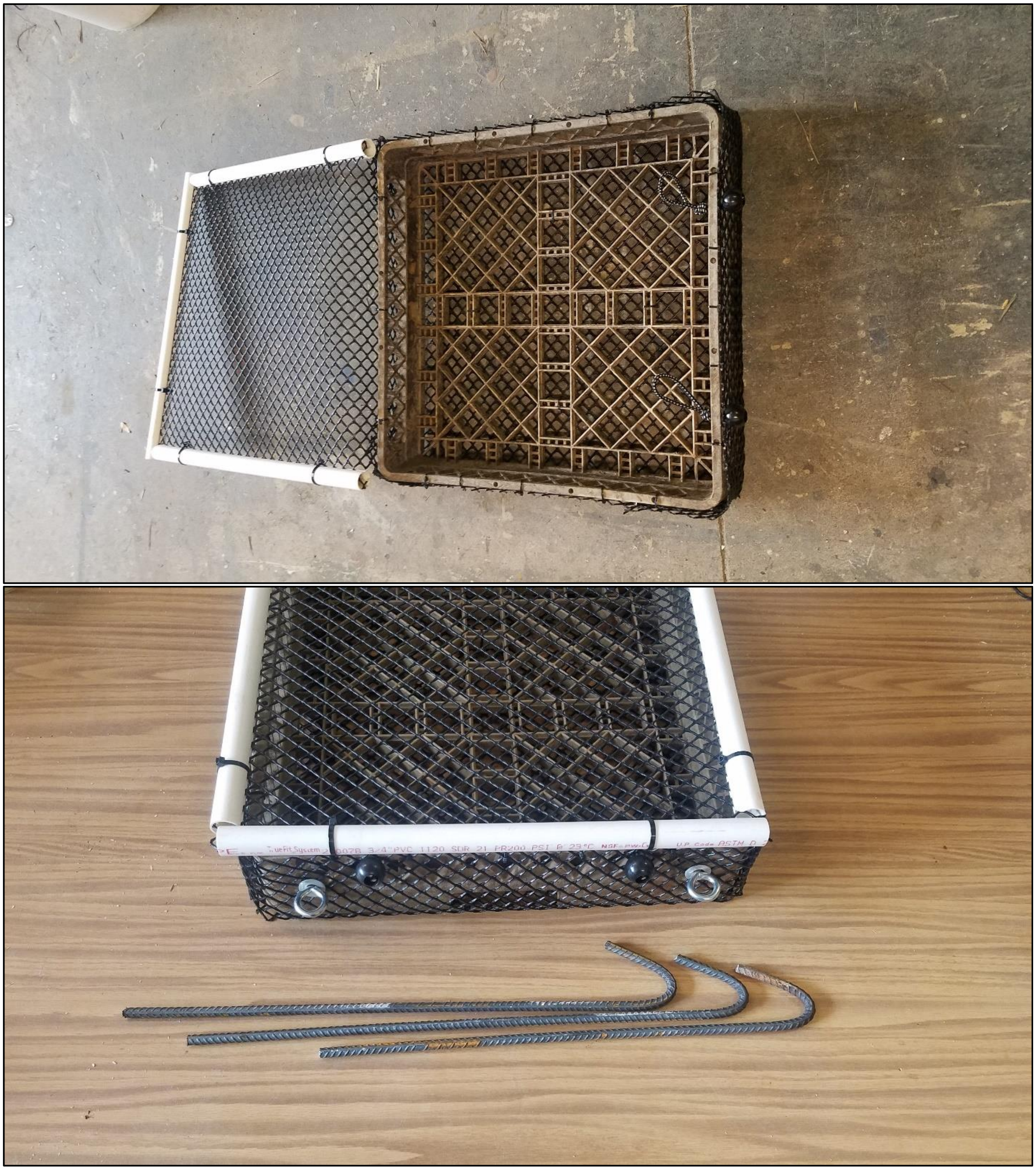

Figure 4. A completed cage open (top) and closed with rebar anchors (bottom).