Assessing the potential for restoring freshwater mussels to urban streams.

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Abstract

We conducted field trials to assess the potential for stocking native unionid mussels in urban streams of the Chesapeake watershed. Juvenile mussels (*Utterbackiana implicata*) were placed in enclosures at 5 urban streams (2 restored, 3 non-restored) and 2 nearby rural streams. Mussel growth and survivorship were assessed in relation to stream conditions (hydrology, water quality and food resources). Among the non-restored urban streams we found that the main impediment to assessing mussel performance was the frequent occurrence of high discharge events, which resulted in burial and downstream loss of enclosures. At the restored

urban streams and the rural streams, washout effects were less severe. Apart from washout effects, mortality, as indicated by the presence of dead mussels, was low. We observed positive growth rates at all sites, though in-stream growth rates were lower in comparison to individuals maintained in the hatchery. Water quality conditions were generally suitable for mussels with respect to temperature, pH and dissolved oxygen. Occasional spikes in conductivity or chloride were observed, though these were not linked with mortality of mussels. Food resource conditions, as indicated by the quantity and quality of suspended and benthic particulate matter, were higher among rural sites and lower in non-restored urban streams. Overall, we found suitable conditions for stocking native mussels in rural and restored urban streams, whereas in non-restored urban streams, bed and bank instability during high discharge events resulted in high attrition of mussels.



Introduction

Urban streams are typically degraded systems. Impairments include poor water quality (e.g., low dissolved oxygen following sewage overflows, high salinity associated with road runoff) and poor biological condition, as typified by low-diversity, pollution-tolerant assemblages (Winter and Duthie 1998; Paul and Meyer 2001; Meyer et al. 2005; Violin et al. 2011; Kaushal and Belt 2012; Reisinger et al. 2016). A major stressor in these systems is water itself because runoff from impervious surfaces results in large, rapid fluctuations in stream flow. High flows cause bed and bank erosion, which displace and bury macroinvertebrate communities, and lead to an incised channel. With greater bank height and steepness, the incised stream becomes disconnected from its floodplain, further exacerbating flow velocity and erosion within the channel. A common remedy for restoring streams is to re-engineer channel morphology from a U-shaped cross section to a V-shaped cross-section by reducing bank steepness and allowing for greater lateral expansion during rising stage. Engineering-based approaches to stream restoration have generated controversy within the scientific community, and within residential communities where the projects are carried out. For the latter, extensive earthworks required to repair incised channels become a focus of opposition, particularly when this results in the removal of mature riparian trees. Scientific debate regarding the benefits of stream channel restoration centers on the uncertainty of successful outcomes (Seavy et al. 2009; Bernhardt and Palmer 2011; Doyle and Shields 2012; Kenney et al. 2012). Key questions surrounding the practice of stream restoration include: What is the likelihood of attaining long-term stability of bed and bank materials in the absence of remediating urban stromwater runoff? What is the likelihood of attaining measureable improvements in stream functioning through engineering of channel form?

Key goals of restoring stream functioning are the enhancement of nutrient and sediment retentionl, and supporting diverse biological communities. Assurance of upgrading ecosystem services is of particular concern in cases where nutrient or sediment reduction credits are granted for stream restoration projects. As is typical for environmental remediation practices, implementation is rarely accompanied by monitoring. In cases where assessments were carried out, results have been mixed as some projects have shown improvements in nutrient and sediment retention, while others have not (Bukaveckas 2007; Kail et al. 2007; Kasahara and Hill 2007; Filoso and Palmer 2011; Sudduth et al. 2011). Improvements in biological condition following restoration are based on an "if you build it, they will come" approach, whereby modification of habitat conditions (e.g., construction of pools and riffles, addition of woody debris) is expected to facilitate colonization and more diverse communities. There are potential benefits to augmenting this practice via active biological restoration by rearing or transplanting of species. The introduction of native unionid mussels to restored streams offers potential benefits at both the local (stream reach) and regional (catchment) scale (Kreeger et al. 2019; Strayer et al. 2019). Direct benefits are those associated with establishing mussel populations in areas where they were likely historically present. The use of hatcherypropagated individuals and the potential for establishing self-sustaining local and downstream populations aids in the conservation of species that are declining throughout their range (Strayer and Dudgeon 2010). Comprehensive data on their occurrence is lacking, but sitespecific studies indicate that mussel abundance is at historic lows (Williams et al. 1993). Their widespread decline is attributed to various factors including habitat alteration (damming, channelization, dredging), channel erosion and sediment burial, current or legacy water quality conditions and loss of fish host species for reproduction (Nedeau et al. 2003; Haag and Williams 2014; Haag et al. 2019). Despite their complex life histories, recent advances in propagation methods allow for the rearing of hatchery-raised mussels in sufficient numbers to undertake ecologically relevant stocking efforts (Jones et al. 2003; Patterson et al. 2018). As with any stocking effort, careful consideration must be given as to which species are appropriate for given locations, which depends in part on restoration goals.

Indirect benefits of stocking are those arising from the ability of mussels to alter the structural and functional properties of stream ecosystems. Mussels are akin to oysters, corals and other "ecosystem engineers" in that their production of hard substrates benefits a range of other organisms (Gutiérrez et al. 2003; Kreeger et al. 2019). Mussels produce shells that break down slowly; their accumulation over time provides habitat for spawning fish and a variety of macroinvertebrates that colonize the interstitial spaces. In addition, mussels have the potential to enhance ecosystem tropho-dynamics and biogeochemical cycling through their filtering activities (Atkinson et al. 2013; Atkinson and Vaughn 2015; Hoellein et al. 2017; Vaughn 2018). Large, dense beds of mussels are capable of altering nutrient cycling through their ability to filter suspended particulate matter (Hoellein et al. 2017; Nickerson et al. 2019). Particulates and their nutritionally important constituents (N, P, lipids, etc.) that would otherwise pass downstream are captured and retained within the stream. A small portion of this material resides in mussel biomass and shells, potentially over times scales up to decades, whereas the bulk of this material is released in dissolved or particulate form. Broader benefits include stimulation of benthic algal production by excreted ammonia, utilization of biodeposits by bacteria and detritivores, and attenuation of downstream nitrogen fluxes via denitrification.

In this paper, we assess the potential for stocking mussels into restored and un-restored urban streams and consider the success of these efforts in relation to stream condition (flow, water quality and food resources). We do not seek a generic answer to the question of whether mussel stocking efforts should or should not encompass urban streams, but rather describe an approach for assessing the suitability of individual sites and provide benchmark data on mussel performance in relation to habitat characteristics.

Methods

Study Sites

Stocking sites were first and second order streams located in northern (Reston) and central (Richmond) Virginia. The Richmond sites (Broad Rock, Gillies and Reedy Creeks) are un-

restored urban streams that are monitored bi-monthly for water quality (since 2016). The Reston sites (The Glade and Snakeden) are restored urban streams in which the channels were modified to enhance floodplain connectivity and stabilized by the use of hardscaping (placement of boulders and cobble) to reduce bed and bank erosion. The streams were restored in 2008 and monitored since that time to assess bed and bank stability, riparian vegetation and benthic macroinvertebrates. In addition to the 5 urban sites, two rural streams (Kimages and Herring Creeks) were selected to provide comparative data on habitat quality and mussel growth and survivorship in a non-urban setting. The rural sites are Coastal Plain streams located at the VCU Rice Rivers Center (Kimages Creek) and the USF&WS Harrison Lake National Fish Hatchery (Harrison Creek). We also assessed water quality, food conditions and mussel growth for individuals maintained in a hatchery pond at the Harrison Lake facility.

Mussel Stocking

Alewife Floater (Utterbackiana implicata) mussels were chosen for stocking because they are native to the region and have been used in prior stocking efforts (Kreeger et al., 2019). The mussels were derived from brood stock collected in the nearby Rappahannock River and raised at the Harrison Lake National Fish Hatchery. Mussel stocking trials were conducted in 2019 at the 3 Richmond sites and two rural sites, and in 2020-21 at the Reston sites and one of the rural streams (Kimages). Growth rates of mussels at the hatchery ponds were monitored through both intervals. The initial (2019) stocking used 2 year old mussels (length 40-55 mm); the subsequent stocking (2020-21) used individuals from the same reproductive cohort (length 55-70 mm at stocking). Mussels were placed in enclosures constructed from plastic crates (approx. 25 x 25 x 25 cm) with plastic mesh (1 cm) added along all sides to prevent loss of mussels. Local substrate (from the stream bed) was added to each enclosure to a depth of 3-5 cm and the enclosure was partially imbedded in the stream bottom to a comparable depth. A variety of measures were used to secure the enclosures including metal poles (rebar) driven into the stream bed and the use of "duckbill" anchors. At the hatchery pond, mussels were deployed in three floating baskets (Patterson et al. 2018). All mussels were given a unique ID number using either a Zing laser engraver (Richmond sites) or a glue-on shell tag (Reston sites).

The three Richmond sites and two rural sites were stocked in May 2019 and monitored though December 2019. Approximately 20 mussels were placed in each of 6 enclosures at each of the 5 sites (total = ~600). The 6 enclosures were deployed over a 20-50 m reach in each stream. Two cohorts of mussels were stocked at the Reston sites (November 2020 and April 2021) and monitored through November 2021. Five mussels were placed in each of six cages on each stocking date at both sites (total ~120). At Snakeden, the enclosures were installed at a single location; at The Glade, the enclosures were deployed at two locations located ~1 km apart. We did not find differences in mussel growth or survivorship between the two locations, and therefore report results for the pooled dataset. In addition to the hatchery-raised mussels, 50 *Elliptio complanata* mussels collected from Bull Run Creek were transplanted to the Reston sites.

At all sites, mussels were monitored for survivorship and growth at ~50-day intervals. Mussels in enclosures were removed and measured individually. Length and mass were recorded for mussels in the Richmond streams; length only was measured for the Reston mussels. Free-ranging (transplanted) mussels were located using their PIT tags. A subset of these (~30% on each census date) were removed from the stream bed to check condition and measure shell length. Survivorship was determined from the number of alive mussels on each census date. Separate tallies were recorded for dead mussels (recovery of empty shells) vs. lost mussels (arising from loss of cages or missing mussels within recovered cages). Growth rates were derived for each monitoring interval based on repeat measurements of tagged individuals. In the later stages, some of the laser tags became unreadable. We recorded length and weight for these individuals, and derived the average growth rate for the cohort based on the change in mean length and mass among all individuals.

Habitat Conditions

Habitat suitability was assessed based on discharge, water quality and food conditions. At the Richmond sites and one of the rural streams (Kimages), discharge and water quality data were collected twice per month throughout the mussel stocking trials. At the Reston sites and Herring Creek, discharge and water quality were measured on each mussel census date. Stream discharge was measured by obtaining cross-sectional data on water level and flow velocity. Water quality (temperature, pH, dissolved oxygen, turbidity and conductivity) was measured using a YSI Pro DSS sonde calibrated according to manufacturer's protocols. At the Reston sites, continuous discharge and water quality data were collected during the period starting with the second deployment of mussels (April – December 2021; USGS stations 0164579522 and 0164578734). The stations are located ~75 m (Snakeden) and ~510 m (The Glade) downstream of the mussel enclosures.

We assessed the quantity and quality of food resources by measuring the mass, organic matter content, N and P content and chlorophyll-a content of suspended and sedimented (benthic) particulate matter. Samples of suspended particulate matter were collected from the thalweg of the stream (in proximity to the mussel enclosures) concurrent with the water quality monitoring (i.e., at half- to one- month intervals). Fine benthic material was collected by handmixing bottom deposits into an enclosed, measured volume of overlying water and collecting a sample of the suspended material (Mulholland et al. 2001). Samples of fine benthic matter were collected on a minimum of three dates at each site (excluding pond). Triplicate samples were collected in proximity to the mussel enclosures and analyzed as for suspended material.

Analytical Methods

Water samples were filtered upon return to the laboratory through pre-weighed and combusted GF/A glass fiber filters (0.5-µm nominal pore size). Filters were dried at 60° C for 48 h and re—weighed to determine the dry mass of suspended materials and fine sediments. A sub-sample of the filter was run on a Perkin-Elmer CHN Analyzer to determine the carbon (C)

and nitrogen (N) content. Filters for CHLa analyses were extracted for 18 h in buffered acetone and analyzed on a Turner Design TD-700 Fluorometer. Sample analysis followed protocols developed for the VCU Environmental Analysis Lab, a state-accredited water quality testing facility (VA ID #450147).

Results

<u>Hydrology</u>

Baseflow discharge was similar among two of the urban sites (Broad Rock, Reedy) and one of the rural sites (Kimages) with median values ~30 L s⁻¹ (Figure 1). Higher discharge was observed at Gillies and Herring (medians = 103 and 135 L s⁻¹, respectively). Long-term (2016-2021) data show that high discharge events occur regularly at these sites with peak measured values in the range of 1500-3500 L s⁻¹. Spot measurements are useful for characterizing baseflow conditions, and occasionally capture events, but do not represent the full range of discharge at a given site. Continuous water level data were recorded for one of the Richmond sites (Reedy) during 2015-2018. Although the monitoring period did not overlap with the mussel trials, these data are included to illustrate the range of hydrologic variability. Water level ranged from ~15 cm at baseflow to almost 3 m during events. Estimated discharge derived from a stage rating curve varied by 4 orders of magnitude with peak estimated flows exceeding 10,000 L s⁻¹ (Figure 2). Continuous monitoring at the Reston sites yielded median discharge values of 14 (The Glade) and 19 (Snakeden) L s⁻¹ for the period of mussel deployment. Highest daily mean values were 242 and 428 L s⁻¹ (respectively), though these do not reflect the highest recorded stage measurements as the stage-discharge relationship for these sites is still under development. Median water levels were 41 (Snakeden) and 76 (The Glade) cm, with peak values reaching 213 and 175 cm (respectively) during passage of Hurricane Ida on September 1st (Figure 2). Overall, these data show that the sites exhibit the typical "flashy" hydrographs of urban streams susceptible to stormwater runoff from impervious surfaces.

Water Quality

Monitoring data indicate that water quality conditions were generally suitable for mussel stocking trials (Figure 1). Among the Richmond urban streams and Kimages Creek, peak (90%-tile) water temperatures were 26° C. At the Reston sites, daily maximum water temperatures were 24° C. All of the streams are relatively dilute, as indicated by low specific conductance (means across sites = 150 to 250 μ S cm⁻¹). Spikes in conductivity were observed in winter with peak values at the Richmond sites of 500-1500 μ S cm⁻¹ (N = 2-3 observations per site during 2016-2021). At the Reston sites, daily maximum conductivity values exceeding 500 μ S cm⁻¹ were observed on three dates during the period of mussel deployment. Despite dilute conditions, the streams were typically circumneutral with median pH ranging from 6.5 to 7.5. Occasional low values (pH<6) were recorded at Broad Rock (3 dates) and Gillies (4 dates). Average dissolved oxygen ranged from 8.2 to 10.2 mg L⁻¹ across sites and was typically near atmospheric equilibrium. Minimum dissolved oxygen did not fall below 5 mg L⁻¹, except for one

date at Kimages Creek (4.6 mg L⁻¹). Turbidity was generally low (medians = 2-6 NTU) at the Richmond urban streams and higher at Kimages Creek (median = 14.0 NTU). High turbidity occurred during discharge events when peak values reached 30-100 NTU (Richmond streams and Kimages) and daily maxima up to 350 FTU were recorded at the Reston sites. We do not provide statistical summaries of water quality data collected at one of the rural sites (Herring) and the hatchery ponds owing to the relatively small sample size (N = 6 and 11, respectively). The limited data suggest that water quality conditions were generally similar to the other sites with respect to temperature, pH and dissolved oxygen, though we note that conductivity was low in the hatchery ponds (~50 μ S cm⁻¹) in comparison to the stream sites.

Mussel Growth & Survivorship

Juvenile mussels exhibited high attrition rates among enclosures installed at the urban, unrestored (Richmond) streams (Figure 3). Highest mortality was observed at Reedy Creek (65%) where the overall attrition rate (including missing individuals) was 74% by the first census date (48 days). High mortality was also observed at Gillies Creek (44%), though 42% of individuals survived through 160 days. At both sites, mortality was largely associated with enclosures that had been displaced downstream or buried by shifting bed materials. Washout was also an issue at Broad Rock where 90% of individuals were lost prior to the first census date. A second stocking at this site resulted in comparable attrition (77%), though over a longer time span (100 days). Higher survivorship was observed among mussels over-wintering in the restored urban stream (The Glade) and at the rural sites (Kimages, Herring). At The Glade and Kimages, greater than 80% of mussels were recovered over a time span exceeding 100 days. Somewhat greater attrition was observed at the other rural site (Herring) where ~50% of mussels were lost through the first 100 days, but the remainder persisted through 200 days. Mortality was less than 10% at these sites. Among the spring cohort of mussels stocked at the urban restored streams, ~60% were recovered at 100 days (mortality = 18%). A high discharge event associated with passage of Hurricane Ida (early September) resulted in the loss of most remaining mussels. The translocation of adult mussels (not held in enclosures) was more successful with 80% (The Glade) and 60% (Snakeden) recovered over a span of nearly one year. Mortality rates of translocated adults were 4% (The Glade) and 10% (Snakeden). Overall, washout effects were found to be the main cause of attrition due to burial and loss of enclosures downstream.

Growth rates were derived using two metrics (mass and shell length) and by two computational approaches (individual- and cohort- based). We found good agreement between growth rates derived by the two computational approaches ($R^2 = 0.96$, p < 0.001) and between the lengthand mass- based estimates ($R^2 = 0.87$, p < 0.001; Figure 4). Hereafter, we focus on the lengthbased data derived from repeated measurements of tracked individuals, as these were available for all sites, and allowed for assessment of variation in growth rates among individuals at a given site. Despite the high attrition rates, we obtained 28 measurements of growth rates across sites and dates, each based on a minimum of 10 individuals. In total, over 900 measurements were used with an average of 33 individuals per determination. The average growth rate across all sites was 0.040 ± 0.009 mm d⁻¹, which equates to an average increase in length of 4.1 ± 0.9% for the typical census interval of 50 days. Average growth rates (across census dates) were more than 2-fold higher in the rural streams and hatchery pond (>0.050 mm d⁻¹) relative to the urban streams (<0.025 mm d⁻¹). Across all urban sites (Richmond and Reston) growth rates averaged 0.013 ± 0.006 mm d⁻¹ (no data for Reedy Creek due to high attrition). Among the stream sites, highest growth rates were observed at Herring Creek (0.120 \pm 0.053 mm d⁻¹) and Kimages (0.034 \pm 0.014 mm d⁻¹), with intermediate values recorded at the hatchery pond (0.050 \pm 0.011 mm d⁻¹). Growth rates at the hatchery pond were similar between the 2019 (0.047 \pm 0.011 mm d⁻¹) and 2020-21 (0.053 \pm 0.022 mm d⁻¹) census periods. The hatchery mussels were tracked continuously for over a year (May 2019 to September 2021), which allowed us to derive an annualized estimate of growth (15.7 mm y^{-1}), corresponding to a 33% increase in length. Growth rates were seasonally variable, but highest during May-July. As the translocated *Elliptio complanata* were tracked for almost one year (November 2020 to October 2021), we derived annualized estimates of growth. The average growth rate at both sites was 0.012 mm d⁻¹, which represents an annualized increase in shell length of 4.6% (The Glade) and 5.1% (Snakeden).

Food Conditions

Analysis of the quantity and composition of suspended and benthic particulate matter revealed differences among urban, urban restored and rural streams (Figure 5). Concentrations of suspended particulate matter were generally similar across all sites (mean = $4.5 \pm 1.7 \text{ mg L}^{-1}$), with the exception of Kimages Creek (mean = $16.2 \pm 2.5 \text{ mg L}^{-1}$). The C fraction of suspended particulate matter was lower among the urban and restored urban streams (0.23 to 0.39 mg L⁻¹) and higher among the rural streams and in the hatchery pond (0.66 to 1.15 mg L⁻¹). The chlorophyll-a content of suspended particulate matter was highest in the hatchery pond (6.7 \pm 1.0 µg L⁻¹), but otherwise similar among the stream sites (0.8 to 3.0 µg L⁻¹). Benthic particulate matter was lowest in the urban streams (20-46 g m⁻²), intermediate in the restored urban streams (34-41 g m⁻²), and highest among rural streams (234-522 g m⁻²). The C fraction of benthic particulate matter was similar across sites (5.8 \pm 0.9%) such that benthic C followed a similar pattern with lowest values among the urban streams (1.5-1.8 g m⁻²), intermediate values in restored urban streams (7.9-10.5 g m⁻²), and highest values in rural streams (11.6-14.2 g m⁻²). Overall, these findings suggest that food resource conditions were most favorable in the rural streams and hatchery pond, and least favorable in the urban streams.

Discussion

We deployed hatchery-raised juvenile mussels in enclosures as a means of assessing the suitability of urban streams as potential stocking sites. The central challenge to this approach was maintaining enclosures within the streams during high discharge vents. Urban streams are

known for their "flashy" hydrology and instability of bed and bank materials (Walsh et al., 2016). Though we lack site-specific continuous discharge data, long-term (Reedy Creek) and recent (The Glade and Snakeden) continuous stage data show rapid and large changes in stream water level during events. High discharge and shifting bed materials resulted in displacement, loss and burial of enclosures, particularly at the unrestored urban sites. Attrition rates at these sites were appreciably higher (75 ± 9 % within 50 days) relative to rural and restored urban streams (23 ± 7 % over 50 days). Prior studies using the Asian Clam (Corbicula) also reported high rates of attrition in urban streams (Nobles and Zhang, 2015), whereas enclosures in non-urban systems yielded higher survivorship (e.g., 60-90%; Haag et al., 2019), comparable to those observed at our rural sites and the restored urban streams. Our restored urban sites are headwater streams that have been extensively modified to prevent erosion by re-grading the formerly incised channel and through the use of local stone to armor banks. Unlike the unrestored urban sites, sediment transport and burial of enclosures was not an issue at these sites, though the predominance of cobbles in the stream bed limited the locations where enclosures could be installed. High attrition rates encountered in this study reflect in part the use of enclosures, which were needed to recover individuals for assessment of growth and survivorship. Mussels stocked directly into streams are better able to avoid washout, as indicated by the high recovery rates for the translocated adults in the restored urban streams. Mortality rates were low among translocated mussels, suggesting that water quality and food conditions were suitable to support resident populations. Overall, our findings document the challenges to establishing mussel populations in urban streams susceptible to large variations in discharge, but suggest that restoring streams through channel stabilization may allow for successful stocking.

Growth rates of Alewife Floater mussels used in this study ranged from 0.001 to 0.23 mm d⁻¹, comparable to those reported for other mussels (e.g., *Lampsilis cardium* = 0.001 - 0.020 mm d⁻¹; Ohlman and Pegg, 2020). Highest growth rates were observed at one of the rural sites (Herring Creek), which were comparable to those reported for this species in hatchery conditions (0.22 mm d⁻¹; Kreeger et al., 2019). Growth rates vary in response to a number of factors including species, age, water temperature and food resources. We found that growth rates were higher in rural streams compared to urban streams. Differences in growth rates generally tracked differences in food quality and quantity between urban and rural sites. High concentrations of suspended particulate matter are potentially indicative of greater food resources, though the response of mussels to higher food concentrations is complex and varies by species (Yeager et al. 1994; Tuttle-Raycraft and Ackerman 2019). In addition, concentration alone provides little information regarding food quality as a fraction of particulate matter may be of low quality (e.g., silt and clay). Studies have shown that the clearance rate of mussels decreases with high levels of suspended particulate matter (Tuttle-Raycraft and Ackerman, 2019). Our data show that the quantity and organic matter content of suspended and benthic particulate matter was higher among rural streams in comparison to the unrestored urban sites. At the restored urban sites, the quantity and organic matter content of benthic materials was higher in comparison to

the non-restored urban sites. In the restored streams, greater lateral connectivity with reductions in bank height may favor the transport of terrestrial plant material into the stream channel, though our sample size is too small to draw broad conclusions about the accumulation of fine benthic materials in these systems.

Conclusions

Prior studies have documented the effects of urbanization on stream hydrology and geomorphology. Typically, these are associated with high runoff from impervious surfaces during storm events, which lead to bed and bank erosion. These conditions are often associated with impairment of stream function (e.g., reduced sediment and nutrient retention; low biodiversity). Results from this study further highlight the challenges to restoring urban streams as exemplified by the high rate of washout and burial of introduced mussels. Restoration efforts within the catchment are needed to reduce urban runoff, which may then allow for successful re-introduction of mussels and associated improvements in stream ecosystem services. Data from this study show that while introduction of mussels to urban streams had limited success, growth and survivorship in rural and restored urban streams indicate greater potential for success. The stocking of freshwater mussels into these streams may be a useful approach to mitigating nutrient and sediment transport to Chesapeake Bay. Further studies are needed to better understand water quality and food conditions that are conducive to successful establishment of mussels, how stream restoration efforts may be geared to accommodating mussel stocking, and how stocking mussels may improve local and downstream water quality.

Recommendations for Future Work

The findings of this study suggest that the restored urban streams located in Reston, VA provide a suitable habitat for mussel restoration. This finding is based on the short-term growth and survivorship of juvenile mussels stocked within enclosures, and the longer-term (1 year) growth and survivorship of translocated adult mussels. In contrast to the un-restored (Richmond) sites, the restoration efforts at The Glade and Snakeden have stabilized bed and bank materials, preventing washout and burial of mussels. A mussel stocking demonstration project at this site would entail two components: (1) provisioning, stocking and monitoring of mussels, and (2) assessment of their impacts on stream functioning.

The goal of the stocking effort would be to establish a multi-species, mixed-age community of mussels. Data collection in support of this objective should include monitoring of mussel growth and survivorship, as well as assessment of mussel health and reproductive status. For The Glade, projected stocking rates are for 300 mussels for each of 4 species per year. In Snakeden, due to its larger stream size, over 750 mussels may be stocked per year. Total projected stocking rates are 6,000 and 7,500 individuals for The Glade and Snakeden (respectively) over a 5-year period. Mussels released should be greater than 20 mm in length, tagged and measured prior to release. Mussel broodstock, including some or all of the following

species should be collected locally (e.g., from the Potomac River and tributaries): Eastern Pondmussel (Ligumia nasuta), Eastern Elliptio (Elliptio complanata), Eastern Floater (Pyganodon cataracta), Eastern Lampmussel (Lampsilis radiata), Creeper (Strophitus undulatus), and Triangle Floater (Alasmidonta undulata). Monitoring may include collection of seasonal growth data, annual gravidity checks to determine reproduction, and qualitative assessment of survival.

The second objective is to assess whether the stocking of mussels enhances stream ecosystem services, particularly with respect to attenuating downstream transport of nitrogen. Data collection in support of this objective should include an assessment of stream nitrogen uptake and retention via denitrification. It is anticipated that the establishment of mussel beds will create biological hotspots, resulting in greater autotrophic and heterotrophic assimilative capacity for dissolved inorganic N (DIN). Filtering activities of mussels produce biodeposits, which provide an organic matter source for bacteria, thereby enhancing denitrification. These hotspots may also serve to improve habitat structure (i.e., the presence of living and dead mussel shells) and provide a broader uplift to stream communities including fish and macroinvertebrates. By these mechanisms, the establishment of mussel beds can enhance stream capacity to support biodiverse communities, and create biogeochemical hotspots for N removal and sequestration.

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Figure 1. Time series of stage measurements for urban streams located in Richmond, VA (Reedy Creek) and Reston, VA (The Glade and Snakeden).

Figure 2. Discharge and water quality conditions in three urban streams (Broad Rock, Gillies and Reedy), one rural stream (Kimages) and two restored urban streams (The Glade, Snakeden). Blue bars denote conditions during mussel stocking trials; black bars are long-term data.



120% 120% Broad Rock Urban 100% 100% Hatchery 80% 80% Recovered (%) Recovered (%) Kimages 60% 60% Gillies Herring 40% 40% Reedy 20% 20% Rural 0% 0% 50 100 150 200 50 100 150 0 0 200 120% 120% 100% 100% Glade (over winter) Glade 80% Recovered (%) 80% Recovered (%) 60% 60% Snakeden Glade (summer) 40% 40% **Urban Restored** Urban 20% 20% **Translocated Adults** Restored Snakeden 0% 0% 100 200 300 0 400 0 50 100 150 200 Days Days

Figure 3. Attrition rates of juvenile *Utterbackiana implicata* mussels stocked at rural, urban and restored urban streams, and adult *Elliptio complanata* mussels translocated to two restored urban streams.

Figure 4. Relationships between individual-based and cohort-based estimates of growth rate (upper panel) and between mass- and shell length- based estimates of growth rate (lower panel) for juvenile *Utterbackiana implicata* mussels stocked at rural and urban streams.



Figure 5. Growth rates (shell length) of juvenile *Utterbackiana implicata* mussels stocked at urban, rural, and urban restored streams in comparison to individuals maintained under hatchery (pond) conditions (note difference in y-axis scales).



Figure 6. Quantity and C content of suspended and benthic particulate matter in urban (Gillies, Broad Rock, Reedy), urban, restored (Snakeden, The Glade) and rural (Herring, Kimages) streams. Also shown for comparison are SPM values for a hatchery pond and BPM values for three other rural streams (Courthouse, Powell, Crump).

