[2012]

Freshwater Mussel Recovery Program in the Delaware Estuary

Final report for
DuPont Clear into the Future

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

March, 2012
PDE Report No. 12-02

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

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Acknowledgements:

This work was made possible through funding from DuPont Clear into the Future.

Established in 1996, the Partnership for the Delaware Estuary is a non-profit organization based in Wilmington, Delaware. The Partnership manages the Delaware Estuary Program, one of 28 estuaries recognized by the U.S. Congress for its national significance under the Clean Water Act. PDE is the only tri-state, multi-agency National Estuary Program in the country. In collaboration with a broad spectrum of governmental agencies, non-profit corporations, businesses, and citizens, the Partnership works to implement the Delaware Estuary's Comprehensive Conservation Management Plan to restore and protect the natural and economic resources of the Delaware Estuary and its tributaries.

Project Background

In 2010, DuPont generously provided the Partnership for the Delaware Estuary (PDE) with funding in the amount of just over $20,000, which was used to contiue the Freshwater Mussel Recovery Program (FMRP) for the Delaware Estuary.

Thanks to the funding provided by DuPont, PDE has made significant progress over the past two years toward our eventual goal of restocking native freshwater mussels such as *Elliptio complanata*, as further described in the remainder of this report.

Figure 1: Pictures of freshwater mussels from Pennsylvanian's streams.

Executive Summary

Freshwater mussels provide many benefits to the Delaware Estuary. These mussels create habitat for other species and help stabilize the bottom of creeks while filtering copious amounts of water. This filtering creates a cleaner downstream environment, meaning that freshwater mussels also benefit the bay ecosystems. These animals are also the most imperiled of all animals and plants in the world.

The FMRP was developed to restore native species, distributions, and population abundance of freshwater mussels across the Delaware Estuary watershed and adjacent areas. The FMRP consists of a multi-pronged effort to 1) perform surveys to fill vital data gaps and guide restoration needs, 2) stream suitability tests to rank restoration readiness for mussels, 3) mussel reintroduction using electronically tagged relocated adults, 4) mussel propagation and reseeding, and 5) outreach and education programs. The relative balance of these FMRP activities varies year to year and among different areas depending on funding and capacity. This DuPont grant focused on 1, 3, 4 and 5.

1) Surveys- Stream surveys were conducted not only on the main Stem Delaware but in 14 streams and creeks throughout Southeastern PA. These creeks include Crum, Cobb, Darby, Chester, Brandywine (East and West branch), Broad Run, Valley Creek, Doe Run, Schuylkill, Mill, Perkiomen, Pickering, Skipjack and Swamp. Fifty-four mussels were found in these various streams.

3) Reintroduction- The biggest success of this project was the completion of our first ever reintroduction of adult mussels to local creeks. One hundred *Pyganodon cataracta* and one hundred *Elliptio complanata* were collected from the Delaware River in the area around Tacony-Palmyra Bridge, and each mussel was tagged with a uniquely numbered plastic tag as well as with a PIT (radio frequency identification) tag. These mussels were then relocated to 6 stream reaches, three in Chester Creek and three in Ridley Creek. This is probably the first time that Chester Creek has had any mussels in decades, and the first time that either stream has had *P. cataracta* in perhaps 100 years. The new PIT (passive induction transponder) tag reader system has worked well and will greatly improve PDE reintroduction and mark-recapture studies with freshwater mussels.

4) Propagation- The FMRP has accomplished many important discoveries and outcomes. This project has allowed PDE to set-up an aquaculture laboratory on the campus of our project partner, Cheyney University of Pennsylvania. This alliance with Cheyney has provided many of their

students with the opportunity to gain hand-on, laboratory experience. At the laboratory, we developed the first hatchery propagation methods of their kind for the eastern elliptio (*Elliptio complanata*) mussel. These methods simulate natural reproduction but allow us to grow the juvenile mussels without predators, thereby producing more mussels than would, on average, survive in the wild. The development of these methods has led to the successful transformation of larvae mussels (that attach themselves to fish for approximately 2 weeks then fall off) to juvenile mussels in the laboratory.

<u>5) Outreach and Education-</u> During the week of July 11th teachers and local naturalists were brought to the Brandywine Creek as part of our Delaware Estuary Watershed Teacher Workshop, where they were taught about freshwater mussels and their beneficial roles in our local waterways. The teachers waded through the creek discovering mussel beds and shells. PDE staff presented photographs and data on some of our field and laboratory work.

The project has been featured in at least two issues of *Estuary News*, PDE's newsletter with a circulation of over 22,000 in the Delaware Estuary region and beyond. In addition, a project description and photo were included in PDE's 2011 annual report, which is printed and distributed to over 500 people throughout the year. Results from this project have been presented at the biennial Delaware Estuary Science and Environmental Summit in January 2011 and will also be presented at the January 2013 conference. This project has also been presented at Restore America's Estuaries in 2010, the Atlantic Estuarine Research Federation 2011, and the 2012 National Shellfish Association.

Why Freshwater Mussels?

Freshwater mussels are the most imperiled of all the flora and fauna across North America. In the Delaware River Basin approximately 12 to 14 species of freshwater mussels (Unionidae) are considered native; however only a few of these species can still be readily found. Within the three states of the estaury, each designates the status of species differently (Table 1). Not only have many species been lost, the remaining species are patchy in location and greatly reduced in abundance. The eastern elliptio (*Elliptio comoplanata*), the only species that all three states consider "common" is very patchy and increasingly difficult to find. There are also questions to whether the current population is stable and reproductive from a regional standpoint. Despite surveying more than 60 stream reaches in Southeastern Pennsylvania, only two populations of this "common" mussel were found and we have observed little to no juvenile mussels anywhere. This is despite the fact that this and many other species were once abundant across the area. For example in Pennsylvania, Ortmann (1919) recorded approximately 8 species of mussels in Darby, Chester, Ridley, and Brandywine Creeks, and the lower Schuylkill River. This mixed species assemblage would have filled different ecological niches and promoted many benefits to stream health. The loss of mussel species, range and population abundance signifies substantial declines in ecological interity because bivalves are regareded as some of the wourlds best bio-indicators.

Table 1: List of species and State listings

Scientific Name	Common Name	State Conservation Status		
		DE	**NJ**	**PA**
ALASMIDONTA HETERODON	DWARF WEDGEMUSSEL	Endangered	Endangered	Critically Imperiled
ALASMIDONTA UNDULATA	TRIANGLE FLOATER	Extirpated ?	Threatened	Vulnerable
ALASMIDONTA VARICOSA	BROOK FLOATER	Endangered	Endangered	Imperiled
ANODONTA IMPLICATA	ALEWIFE FLOATER	Extremely Rare	no data	Extirpated ?
ELLIPTIO COMPLANATA	EASTERN ELLIPTIO	common	common	Secure
LAMPSILIS CARIOSA	YELLOW LAMPMUSSEL	Endangered	Threatened	Vulnerable
LAMPSILIS RADIATA	EASTERN LAMPMUSSEL	Endangered	Threatened	Imperiled
LASMIGONA SUBVIRIDIS	GREEN FLOATER	no data	Endangered	Imperiled
LEPTODEA OCHRACEA	TIDEWATER MUCKET	Endangered	Threatened	Extirpated ?
LIGUMIA NASUTA	EASTERN PONDMUSSEL	Endangered	Threatened	Critically Imperiled
MARGARITIFERA MARGARITIFERA	EASTERN PEARLSHELL	no data	no data	Imperiled
PYGANODON CATARACTA	EASTERN FLOATER	no data	no data	Vulnerable
STROPHITUS UNDULATUS	SQUAWFOOT	Extremely Rare	Species of Concern	Apparently Secure

Healthy and abundance freshwater mussel beds impart many ecological services to streams and other aquaitc habitats. They stabilize bottom habitats and enrich sediments for other fauna. Most important perhapse they collectively filter a tremendous amount of water. For instance, one relic population of 500,000 eastern elliptio in the lower Brandywine Creek was estimated to filter greater than1 billion liters and remove 26 metric tons of dry total suspended solids each summer season (Kreeger, 2007). This population is old and may not be reproducing, representing a fraction of the system's carrying capacity for mussels. By rebuilding the natural mussel assemblage of mixed species in streams that today have few or no mussels we waim to increase water quality and achieve diverse habitat restoration goals across the Delaware Estuary. The FMRP is a core part of a holistic shellfish restoration strategy that extends from headwater streams to Delawre Bay (see below).

Regionally Important

The decline of bivalve biodiversity and abundance and the ecosystem services they provide, such as biofiltration, signifies a drop in environmental integrity at both local and watershed scales. Freshwater mussels and marine bivalves (such as oysters, scallops and clams) are often the dominant functional component in aquatic food webs from the headwaters to the coast, improving water quality, enriching sediments, and adding habitat complexity (Table 2). Estimated summertime water processing rates in the Delaware Estuary by eastern oysters (~10 billion L/h), a freshwater mussel species (~10 billion L/h), and an estuarine mussel (~60 billion L/h) suggests that populations of all three species furnish important service across the entire freshwater to estuarine gradient (Burgstrom, 2011). Unfortunately, all three bivalves are severely threatened by changing conditions (PDE, 2010)

Table 2: Some natural capital values of key types of bivalve shellfish in the Delaware Estuary, and their relative importance (number of check marks).

Importance of Shellfish to the Delaware Estuary Watershed			Oysters *Crassostrea virginica*	Marsh Mussels *Geukensia demissa*	FW Mussels *Elliptio complanata*
Natural Capital Value	Commercial	***Dockside Product + Secondary Value***	✓✓✓		
	Ecological	***Structural Habitat*** biological hot spots, bottom-binding	✓✓	✓✓	✓✓
		Prey	✓	✓	✓✓
		Biofiltration top-down grazing, TSS removal, light)	✓✓	✓✓✓	✓✓
		Biogeochemistry enrichment/turnover, benthic production	✓	✓	✓
		Shoreline Protection - nearshore reefs	✓		
		Shoreline Stabilization - living edges	✓	✓✓	
	Cultural-Historical	***Waterman Lifestyle, Ecotourism***	✓✓		
		Native American - jewelry, dietary staple	✓		✓✓
	Bioindicator	***Watershed Indicators*** hallmark resource status/trends	✓✓	✓	✓
		Site-specific Bioassessment NS&T, caged sentinels	✓	✓	✓✓
	Conservation	**Biodiversity** fw mussels most critically impaired biota			✓✓✓

Bivalves live throughout non-tidal and tidal waters, occupy diverse niches, and can dominate the structural and functional ecology. They are also world renowned as top-tier bioindicators, meaning that their health and abundance reflects that of the aquatic system. For example, the International Mussel Watch has proven for more than 50-years that filter-feeding bivalve molluscs are some of our best tools for bioassessment of both specific contaminants as well as overall ecological conditions. This is because large filter feeders must process large amounts of water and particles across soft tissues, leading to relativly greater contaminant exposture. They also do not swim around and so their fitness is diagnostic of local conditions. Arguably, freshwater mussels are even better indictors than marine bivalves because they have long life spans and have reproductive strategies that are easily disrupted by habitat alterations (e.g. dams that interfeer with cruical fish needed by mussel larvae).

For these reasons, we regard bivalves as the best taxonomic group to target for integrating an ecosystem-based approach to conserving, managing, and restoring aquatic resources. The Partnership for the Delaware Estuary is currently promoting a new watershed-based restoration initiative, titled “Healthy Bivalves = Healthy Watersheds”. This initiative seeks to rebuild native bivalve species and abundance across the watershed and estuary, not only because these animals will signify a return to better conditions, but also because they also are part of the solution due to the ecosystem services that they furnish. Bivalves can facilitate the overall recovery of degraded aquatic ecosystems.

Integrating conservation and restoration efforts of multiple bivalve species could potentially provide synergistic opportunities to improve the health of coastal watersheds, because of the connectivity between tidal and non-tidal areas. For example, revitalization of native freshwater mussels in rivers may improve water quality downstream, thereby benefiting estuarine species. In turn, reef-building oysters can furnish estuarine habitat for diadromous fish that, when upstream, serve as hosts for juvenile freshwater mussels.

Figure 2: Delaware Estuary map

Our tactics are tailored to the unique features of the Delaware Estuary and focus on the benefits of mussels to water quality and functional conditions. Therefore, we don't simply target rare species , the FMRP also seeks to expand the range and rebuild abundance of even common species to help other measurable benefits to water quality.

Restoration efforts are also strategic and mindful of ecological trajectories of watershed and climate change, focusing on outcomes that will be sustainable. This project is the first of its kind to address freshwater mussel restoration within a holistic watershed context, and represents a unique opportunity to bring together environmental managers, government agencies, communities, and top bivalve experts in the Delaware Estuary Region to achieve significant habitat restoration.

A Complicated Life

Although freshwater mussels provide many water quality benefits and natural capital benefits (Table 2), their life history cycle is quite complicated and this is the main reason why they are the most imperiled of all animals and plants in North America. The first important trait that seperates freshwater mussels (unionids) from marine mussels is their long lifespan and slow growth rate to reach maturity. Freshwater mussels can live to be 80- to 100-years old and most species do not begin reproducing until 8- to 10-years old. In contrast, marine mussels (and oysters and clams) can reach maturity in 1-year under good growing conditions, and they rarely live more than 5 to 10 years. Their slow growth and long lifespan means that freshwater mussels cannot quickly recover from any disturbance that claims lives. In the Delaware Estuary watershed, our rich history of human development has led to many impairments over the 300- to 400-years since European settlement. During this time, mussels likely became progressively extirpated (i.e., extinct) from local streams due to specific incidents such as spills that cause acute mortality in a single event, as well as overall declines in water and habitat conditions. The long lifespan means that recovery rates are slow following disturbances.

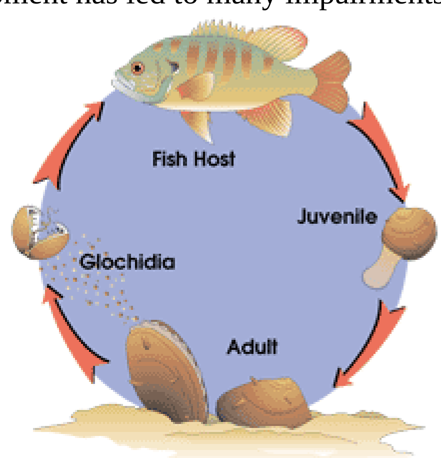

Figure 3: Life Cycle of freshwater mussels (North Carolina Wildlife Resource Commission)

The second important life history trait that makes freshwater mussels distinct from their marine counterparts is the need for a fish host to complete their life cycle. All freshwater mussels produce fewer larvae than marine species, and these larvae are brooded by their mothers; whereas, marine species have abundant planktonic larvae. First, a male mussel needs to be close in proximity to a female for fertilzation to occur. Second, often a specific species of fish needs to be in close proximity to the female mussel having developed larve. In some instances the fish is attracted to the open shell of a mussel, sensing the fish the mussel then propels baby mussels, called glochidia, into the fish's mouth where they attach to the gills or sometimes the fins.

This reproductive strategy is thought to have evolved as a tactic for dispersal, including upstream, thereby enabling a population to maintain themselves upstream and disperse. The glochidia are attached to the gills for approximately 2-3 weeks until they release and are called juvenile mussels (Figure 3).

This reproductive strategy means that for freshwater mussels to complete their life cycle, they need a healthy population of suitable fish hosts in addition to decent water and habitat quality. Many of the imperiled mussel species are very specific about which species and size of fish host they can use. Therefore, anything that affects the health or the movements of these fish hosts (e.g. dams, water quality, habitat degradation) will directly affect the mussel species that rely on them as hosts. Of particular concern are mussel species that rely on diadromous fish such as American eels or shad. In addition, are native local speices (eastern pearlshell) relies on brook trout, which is in decline due to warming conditions.

Project Overview

The FMRP consists of five linked activities;

1) Perform surveys to fill vital data gaps and guide restoration needs,
2) Stream suitability tests to rank restoration readiness for mussels,
3) Reintroduction using electronically tagged relocated adults,
4) Propagation and reseeding, and
5) Outreach and education programs

The relative emphasis on these five activities areas is tailored to specific watershed areas and priorities of funders, but generally flows from Task 1 to 5 in phases. For example, to set mussel restoration goals and identify needs, it is critical to begin with baseline information about the status of the mussel assemblage within an area. But existing survey data are often limited because freshwater mussels are not routinely assessed as part of stream impairment studies (they are left out of "macro invertebrate" assessments). Once candidate restoration streams are identified, they should be examined for their modern ability to sustain mussels. Some streams having no mussels are shown to support them nicely, but the mussels have no natural means of dispersing to the streams. However, conditions in some other streams have been found to be poor still once restoration streams are chosen and mussel species targeted for recovery, there are two direct methods to reintroduce and boost mussels in stream. One approach is to identify suitable local broodstock populations and to relocate gravid adults (usually tagged to facilitate monitoring), relying on fish in the receiving stream for natural reproduction. Our second approach is to take broodstock adults into a hatchery along with fish hosts and to propagate new juvenile mussels, with subsequent out-planting of seed to restoration streams once they attain suitable size. Both tactics have advantages and disadvantages, and we currently favor some of each to boost success through the diversification of tactics. As methods become more established and outcomes more predictable, we expect hatchery propagation to become more prevalent because it provides faster and more qualitative outcomes.

Finally outreach and education can occur at any stage, depending on local interests and needs. For this study we focused on tasks 1, 3, 4 and 5.

Task 2.Surveys

The goals of the mussel survey for this project were to identify new broodstock sources to support restoration since existing study populations in the Brandywine and Ridley creeks are limited in size. Specifically we aimed to increase our spatial coverage of streams investigated in Southeast Pennsylvania and to explore the tidal Delaware River which had been the focus of new attention based on the Delaware Estuary Benthic Inventory (DEBI) discovery.

Figure 4. Shoreline sampling.

PDE trained staff from the Academy of Natural Sciences of Drexel University's Patrick Center for Environmental Research (ANS PCER) on mussel survey methods for this study. The majority of the 2010 small stream surveys were conducted by the same two person team. Bank walks and wading methods were implemented with the aid of polarized sunglasses, viewing buckets, and other tools (Figure 6). Total search time was recorded from each site to determine level of effort (# mussels per unit time). While predetermined sites were chosen by PDE staff, the locations of some sites were moved for better accessibility, and the new locations recorded with a handheld GPS. Following training, ANS carried out a survey of freshwater mussels in the lower Schuylkill River watershed and other tributaries in southeastern PA that the project had not previously sampled. Selected sites on the tidal freshwater portion of the Delaware River, in both Pennsylvania and New Jersey, were also surveyed. Thirty-seven sites in sixteen water bodies were surveyed (Table 3) for the presence of freshwater mussels by ANS staff, lead by Dr. Kreeger from PDE (Figure 5). In summary, very few stream reaches were found to contain any mussels at all, but a major diverse bed of mussels was discovered in the tidal Delaware River (Figures 7-9).

All of the stream sites surveyed (Figure 10) were located in areas with varied types of anthropogenic impacts e.g., urbanization, decreased forest or riparian cover, dam presence, agriculture. Darby and Cobbs Creeks are excellent examples of typical urban streams. While Darby Creek has a representative amount of riparian cover, this densely populated area houses a golf course, shopping malls, and an industrial park in close proximity to the creek. Cobbs Creek offers similar urban pressures, including two large cemetery plots and very densely populated creek borders, with little riparian cover. Neither Darby nor Cobbs Creeks were dammed near our study sites, but current and relic dams exist downstream and potentially have interfered with fish host passage. No mussels were found in either Darby or Cobbs creek.

For the most part Crum Creek has a dense riparian buffer, with dams that isolate the sites south of the Springton Reservoir from the sites north of the reservoir, and from each other (Figure 13). The three Crum Creek sites above the Springton Reservoir were located in suburban areas with some agriculture and shopping centers. The two Crum Creek sites below the Springton Reservoir were located in more densely populated urban areas with numerous shopping malls. No mussels were found in Crum Creek drainage basin.

The three southern Chester Creek sites were located in rural areas, while the northern most site (Chester 4) was located in a developed suburban area just downstream of the West Chester Reservoir (Figure 14), adjoining large open spaces dedicated for sports, recreation, and shopping. Damming of Chester Creek is apparent on either side of the northern site and downstream of the southern site. No native mussels were found at any of the four Chester sites, supporting earlier negative survey data in this watershed.

The Brandywine Creek sites were located over a large rural area extending throughout the East and West branches, and included three tributaries, Broad Run, Valley Creek, and Doe Run (Figure 21). These are areas of the system that the Partnership and ANS had not previously investigated. Mussels were found at a few locations which is not surprising since previous PDE surveys have found considerable populations of mussels below Chad's Ford. There was some agriculture along the East Branch of the Brandywine (one such field is just along the stream); however, large dense patches of trees provided an extensive riparian zone. While the Brandywine is not heavily dammed, one dam does exist on the East Branch, just downstream of the site where mussels were found. This dam may serve to isolate freshwater mussel populations by preventing host fish from migrating upstream. The mussel bed located at BW1 was only a few steps from the creek bank, at a designated fishing/picnic area. The Broad Run site, BW3, was in a sparsely populated, heavily forested, suburban area with some agriculture in the vicinity. The Valley Creek site, BW4, was located at a public park with a designated fishing area. While the waterway has some riparian cover and was, for the most part forested, the immediate surrounding area is farmland (Paradise Farm Camp). About 1 mile upstream, the land use changes to a more suburban/industrial area where most of the trees have been cleared, in addition to an industrial park and quarry. The Brandywine West Branch site, BW2, was located in a densely vegetated rural area. Within less than a mile radius housing communities and farm lands exist. The site on the Brandywine West Branch Tributary, Doe Run, benefits from a large riparian zone and low population density, with adjoining fields being farmed. All mussels found in the Brandywine in 2010 were *E. complanata*, the only species known to still exist in the watershed.

Pickering Creek is in a heavily vegetated, moderately populated, suburban, agricultural area. There are two known dams on this water body, one at Pickering Creek Reservoir where the creek meets the Schuylkill River, and another one near Pick 2 (Figure 16). No mussels were found in Pickering creek watershed (Table 3).

Perkiomen Creek and its tributaries (Skippack and Swamp Creeks) are in heavily vegetated suburban areas. The segments of this waterway which were sampled are in a heavily dammed area (Figure 17). One mussel was found in Perkiomen Creek it was of the common species, *E. complanata*.

While the Schuylkill River goes through various stages of urbanization, all sites sampled were in urban areas with industrial development along the river banks (Figure 18). The Schuylkill River is also heavily dammed above and below the sampled stream reaches, and intermittently between sites. While there was no evidence of mussels in the Schuylkill (where water visibility and depth prevented a more extensive survey), it is suggested to resurvey using a mask and snorkel.

In summary the stream surveys showed that live specimens of only *Elliptio complanata* were recorded from one (new) site on Brandywine Creek (19 mussels) and one site on Perkiomen Creek (1 mussel) (Table 3). Additionally, relic mussel shells were found at several sites, but it is unclear if those were recent or historic shells (shell can wash from banks and be hundreds of years old). Unfortunately, no mussels were found at 34 of 36 stream segments visited, confirming PDE surveys suggesting that very few mussels remain in non-tidal streams and rivers of southeastern PA. In fact, over the last ten years, PDE/ANSP surveys have found only the one mussel species in greater than 70 stream reaches in the area that are above the head of tide (i.e., above dams in most cases.) Taken together, our survey data and surveys from state heritage programs in the same area suggest that most of the dozen or more native mussel species are extirpated from streams in the lower basin (Figure9).

Variability in water quality and available habitat may have influenced freshwater mussel populations throughout the study area. The majority of stream reaches searched were slightly to moderately affected by urban development and siltation. The two sites where live mussels were found had significant riparian cover. While the riparian zone of the Brandywine includes trees, shrubs, and grasses, the Perkiomen had a dense riparian zone (mostly trees) right up to edge of the creek. Land use surrounding both sites is mostly residential, with a point source of pollution within 400m from the water (the Brandywine has a farm, and the Perkiomen has a shopping center). While both water bodies have a similar depth (~50cm to ~100cm), the Brandywine is much narrower (~10m vs. ~50m) and the substrate is more constant (sand/silt/gravel vs. sand/silt/cobble/gravel/boulder). The single Perkiomen site mussel was not found in the main channel, but was in a backwater area, with sediments composed of sand/silt/gravel. These environmental factors may shed light on the reasons why live mussels were only found in two sites. It also may account for the difference in number of individuals found.

Importantly, however, a significant biodiversity discovery occurred as part of the PDE-ANSP exploratory surveys in the tidal freshwater (undammed) portion of the Delaware River and lower Schuylkill River. Seven species of native unionid mussels were found, including *Ligumia nasuta, Leptodea ochracea, Anodonta implicata, Strophitus undulatus, Pyganodon cataracta, Lampsilis cariosa, and Elliptio complanata. Ligumia nasuta* is a PA state listed species (critically imperiled), and *Lampsilis cariosa* is listed as vulnerable. *Leptodea ochracea* was believed extirpated from the entire basin, not having been seen for perhaps 50 or more years (subject to further literature review.) Due to the sensitive nature of location data for these sensitive species, PDE and ANSP are prohibited from sharing locations in this public-friendly report, but appropriate state agencies have been notified. Future surveys should include more sites throughout Pennsylvania as well as in Delaware and New Jersey portions of the Delaware Estuary and River Basin.

In 2011, new surveys of rare Delaware River mussel species were accomplished in June in concert with the Academy of Natural Sciences. In addition to finding the same rare species discovered in 2010 (two of which were previously believed extirpated from PA), at least two additional new species of rare freshwater mussels may have been discovered this year between Trenton and Philadelphia (to be formally identified this coming winter). If confirmed, this would bring the total number of species found in the urban corridor to nine, which is surprising considering that most streams have none left. Another success was the discovery of natural juvenile mussels in the discovered beds, indicating that at least one species is still naturally reproducing.

New beds were found closer to Philadelphia as well, including at Petty Island and adjacent shorelines. These discoveries generated national news coverage by more than a dozen outlets, between November 2010 and spring 2011. These discoveries offer promise that critical broodstock can be used to restore the bulk of the native mussel assemblage to streams of the basin if the vestigial populations in the urban river can be protected and managed carefully. Using other resources quantitative population surveys are planned for summer 2012 to establish the range and size of these new beds. Plausibly, the beds could be sufficient in size to help maintain water quality in the urban river corridor.

Figure 5. ANS partners measuring mussels found in the main stem Delaware River during surveying.

Figure 6. Needham scraper used to search for mussels in deeper waters.

Table 3: Survey site locations conducted 2010.

Water Body Name	Site Name	Lat	Long	Effort (# Individuals found per hour)
Crum Creek	Crum 1	39.93643	-75.37041	0
Crum Creek	Crum 2 (above reservoir)	39.97705	-75.43172	0
Crum Creek	Crum 3	39.91092	-75.35918	0
Crum Creek	Crum 4	39.97137	-75.43322	0
Crum Creek	Crum 5	39.98946	-75.43607	0
Darby Creek	Darby 1	39.96159	-75.33314	0
Darby Creek	Darby 2	39.94319	-75.31996	0
Darby Creek	Darby 3	39.93436	-75.27771	0
Cobbs Creek	Cobbs 1	39.96465	-75.24941	0
Cobbs Creek	Cobbs 2	39.93248	-75.23789	0
Cobbs Creek	Cobbs 3	39.90835	-75.25016	0
Chester Creek	Chester 1	39.89424	-75.45057	0
Chester Creek	Chester 2	39.93213	-75.49463	0
Chester Creek	Chester 3	39.95247	-75.54836	0
Chester Creek	Chester 4	39.98435	-75.56245	0
Brandywine Creek (East Branch)	BW 1	40.09465	-75.77688	19
Brandywine Creek (East Branch)	BW 5	39.97608	-75.68296	0
Brandywine Creek (West Branch)	BW 2	39.99370	-75.82698	0
Broad Run (East Branch Trib)	BW 3	39.98455	-75.65620	0
Valley Creek (East Branch Trib)	BW 4	39.99019	-75.66636	0
Doe Run (West Branch Trib)	Doe 1	39.92473	-75.78452	0
Schuylkill River	SK1 (Backwater)	39.96870	-75.18583	0
Schuylkill River	SK2 (Canal)	40.03142	-75.23408	0
Schuylkill River	SK4	40.07342	-75.28228	0
Mill Creek	SK3 (Schuylkill trib 1)	40.04520	-75.25624	0
Mills Creek	SK5 (Schuylkill trib 2)	40.07236	-75.32204	0
Pickering Creek	Pick 1	40.09415	-75.61072	0
Pickering Creek	Pick 2	40.10077	-75.54325	0
Delaware River	PA DR 052A			7
Delaware River	NJ-DR-53			19
Delaware River	PA-DR-054			8
Perkiomen Creek	PK1	40.13246	-75.44470	1
Perkiomen Creek	PK2	40.22704	-75.45257	0
Perkiomen Creek	PK3	40.29067	-75.45622	0
Skippack Creek	PKT1	40.18069	-75.42143	0
Skippack Creek	PKT2	40.22108	-75.38470	0
Swamp Creek	PKT3	40.27258	-75.51486	0

Figure 7. Underwater picture of mussels in Delaware River

Figure 8. Underwater picture of mussel bed in Delaware River

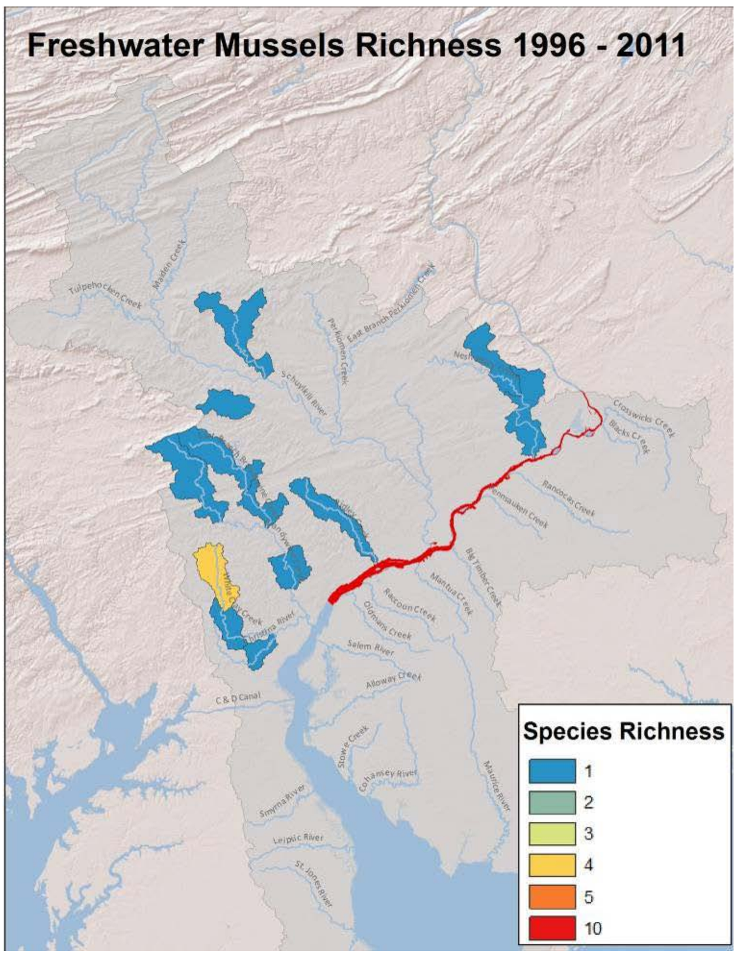

Figure 9. Using PA heritage data and PDE data mussel occurrence by watershed. Only one segment has more than one species found in it in the last 25 years.

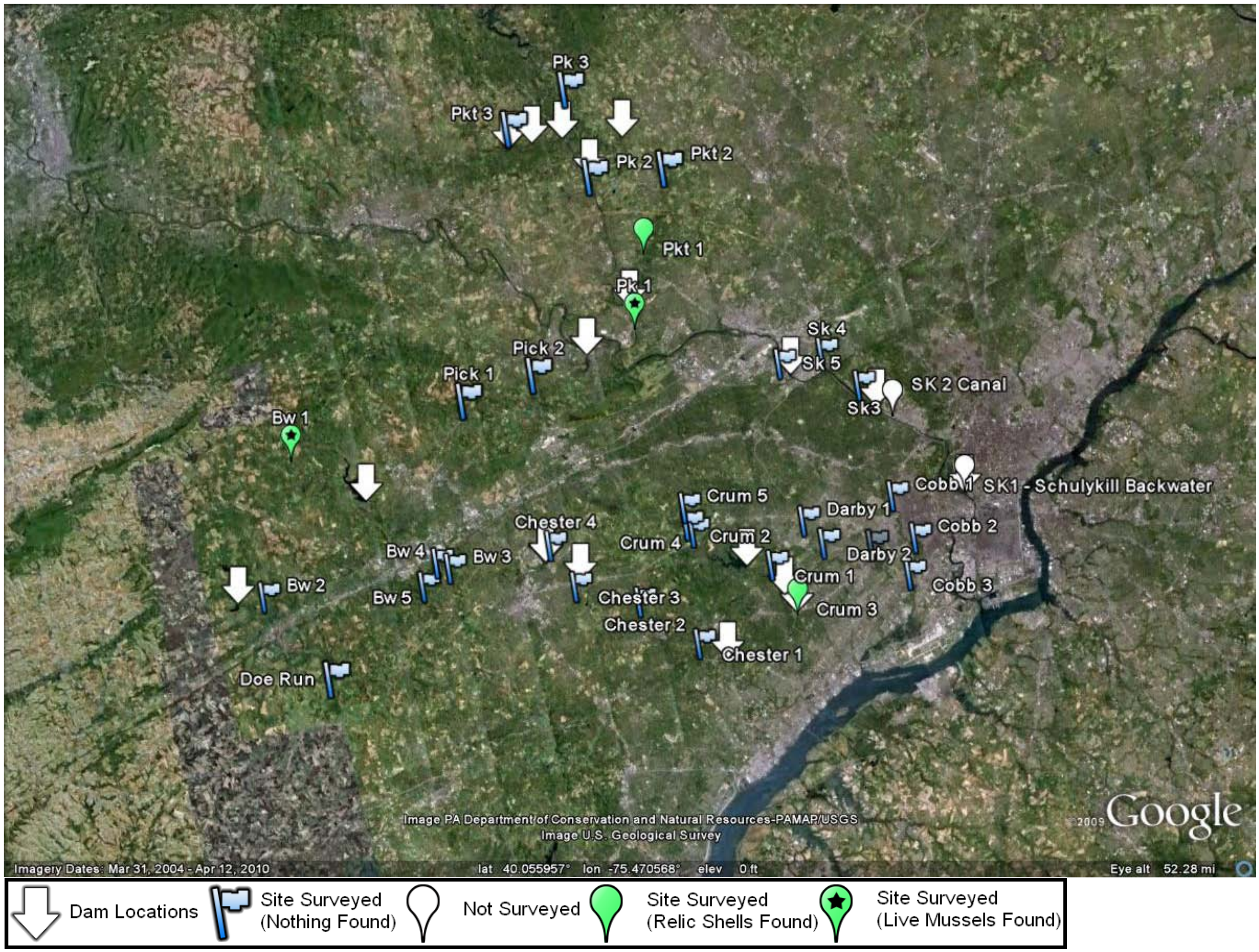

Figure 10. Map of all sites surveyed by ANS for 2010

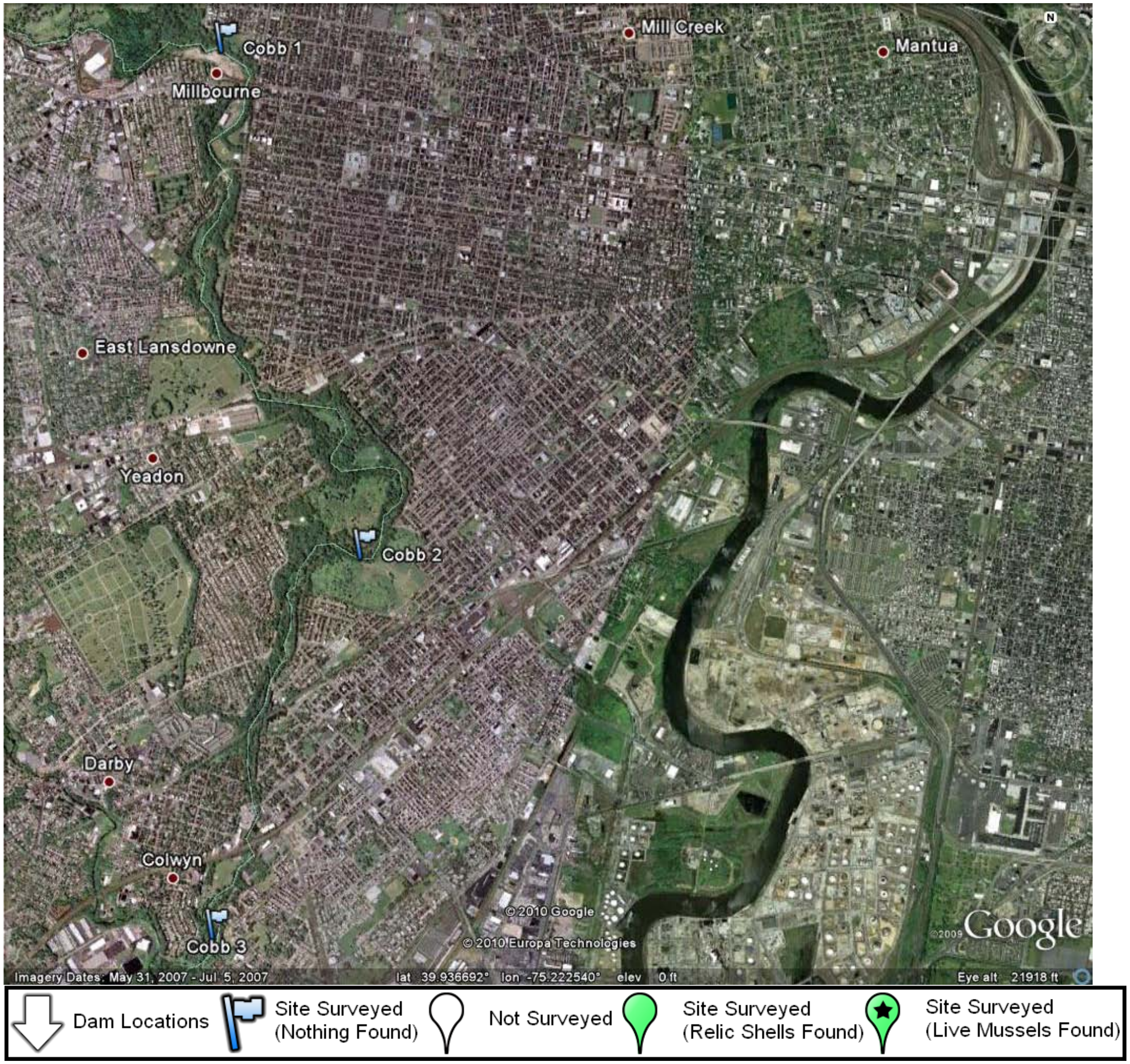

Figure 11. Map of Darby Creek sites during 2010 survey

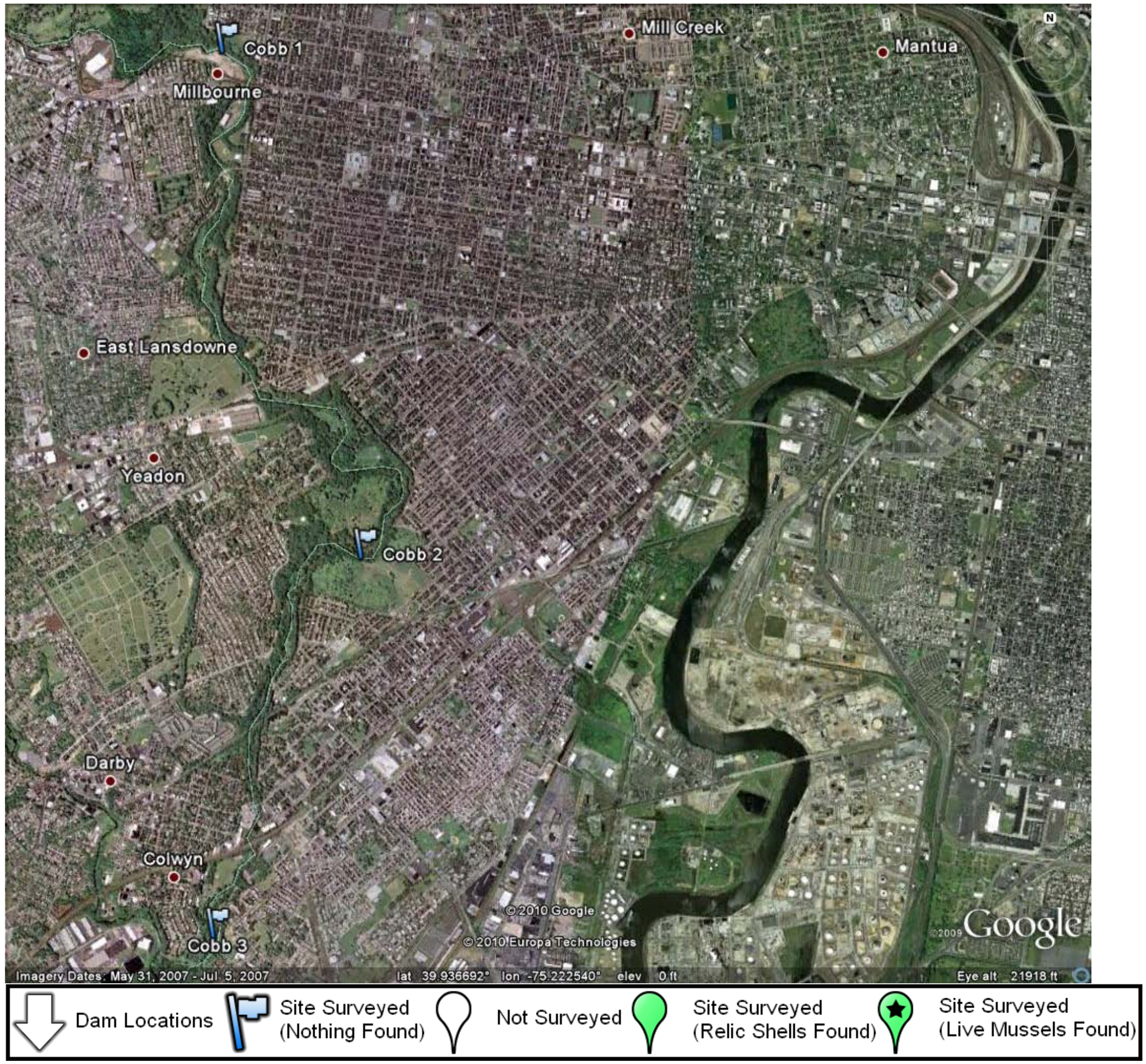

Figure 12. Map of Cobbs Creek sites during 2010 survey

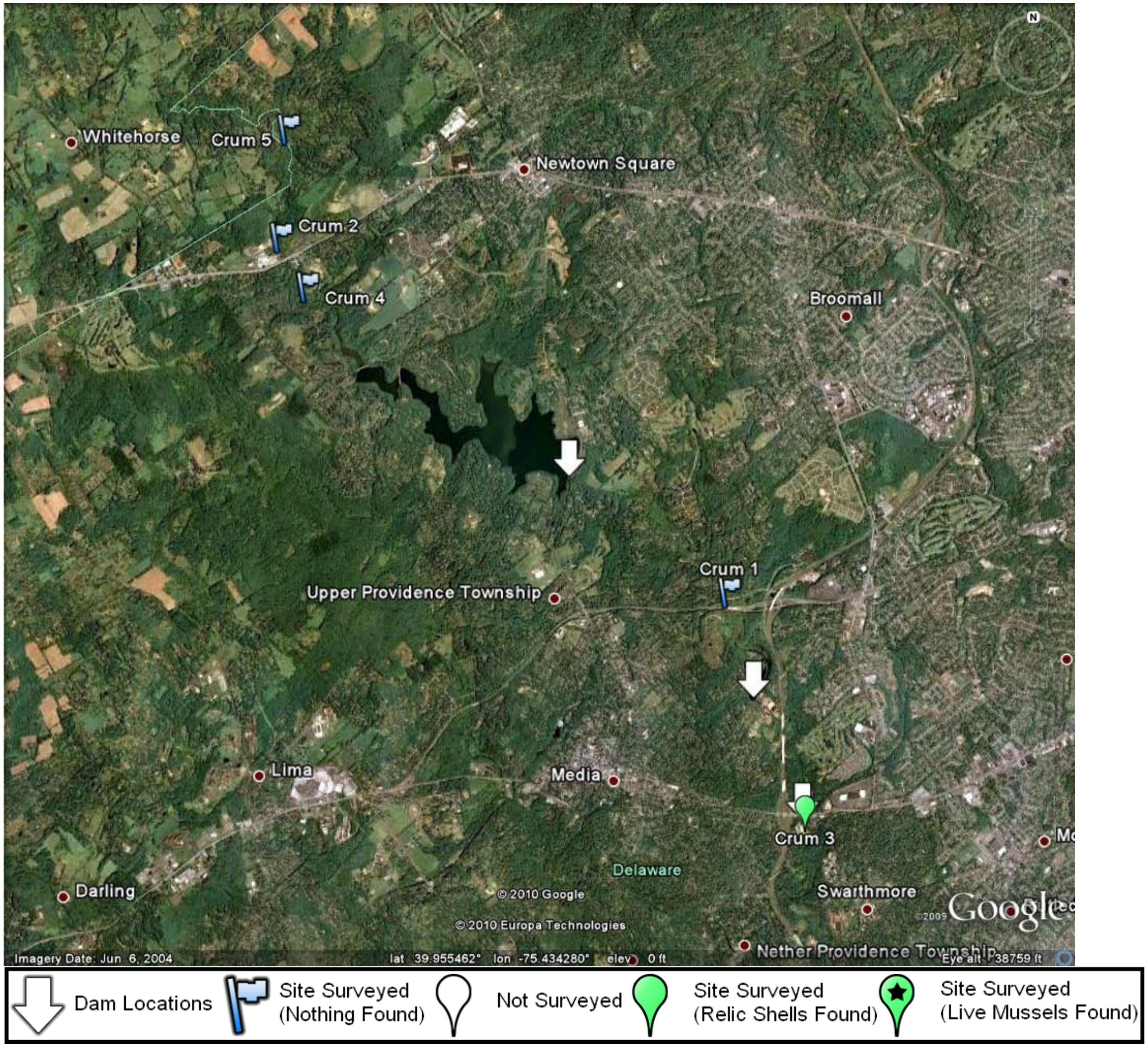

Figure 13. Map of Crum Creek sites during 2010 survey

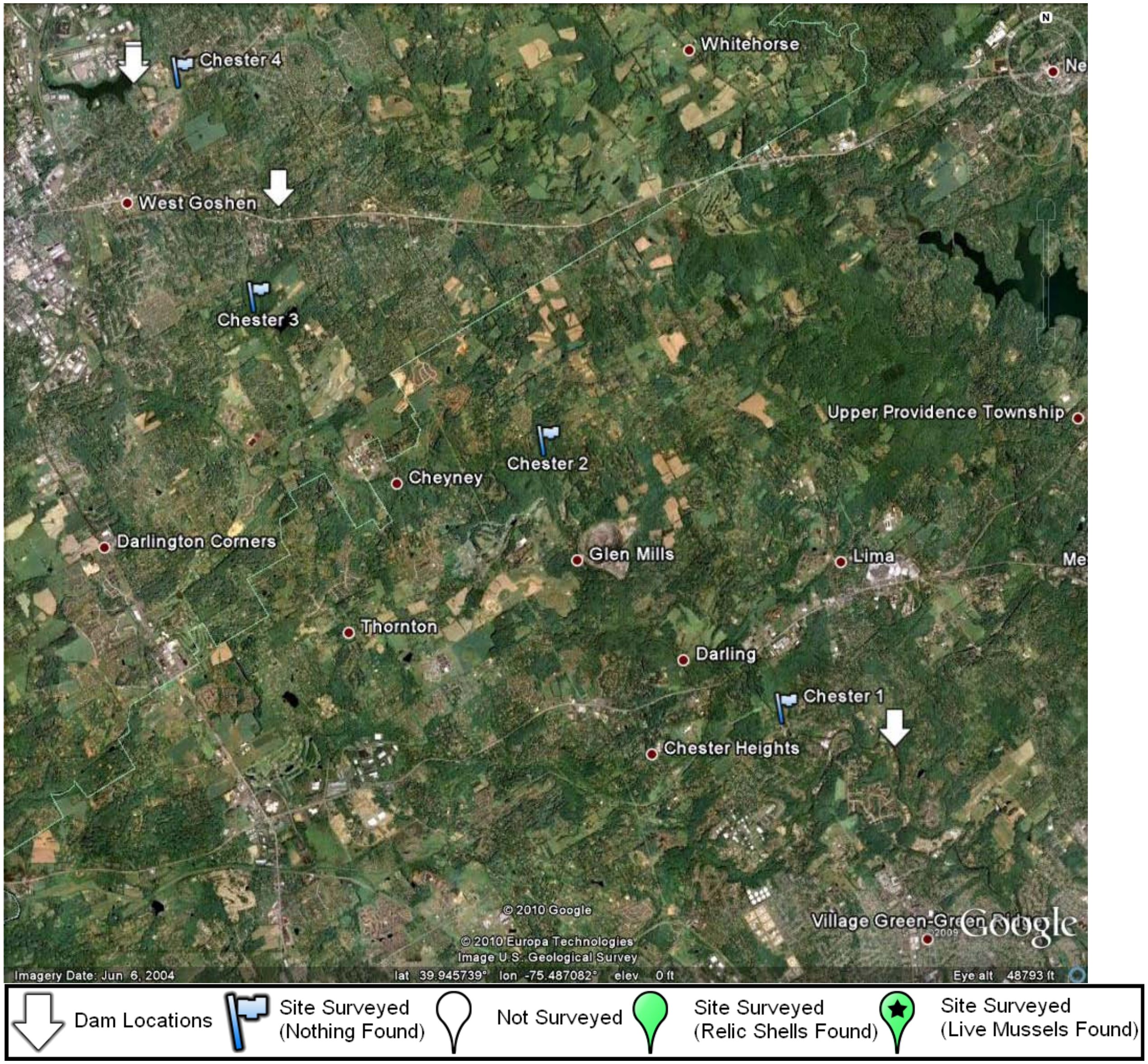

Figure 14. Map of Chester Creek sites during 2010 survey

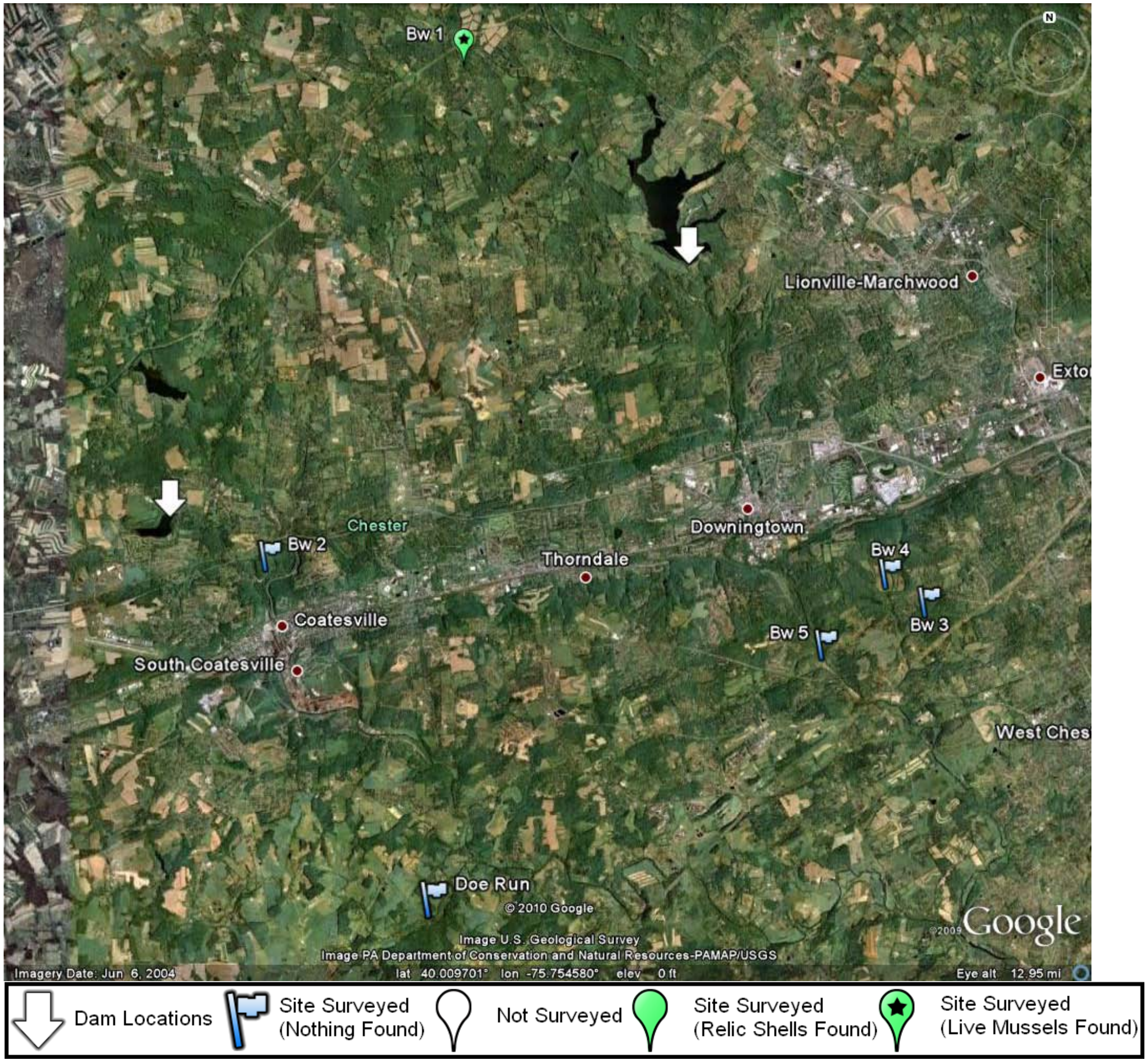

Figure 15. Map of Brandywine Creek sites during 2010 survey

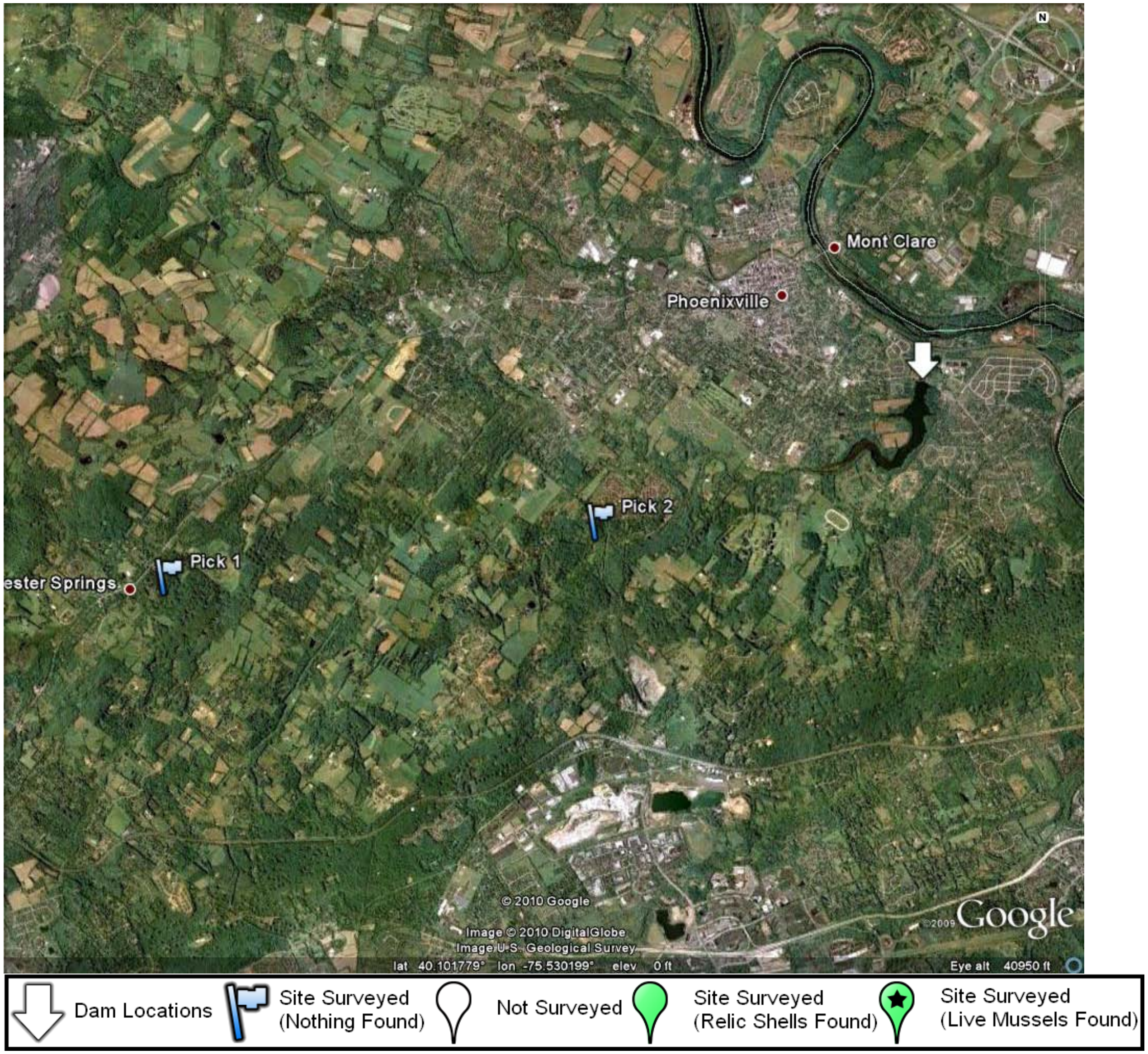

Figure 16. Map of Pickering Creek sites during 2010 survey

Figure 17. Map of Perkiomen Creek sites during 2010 survey

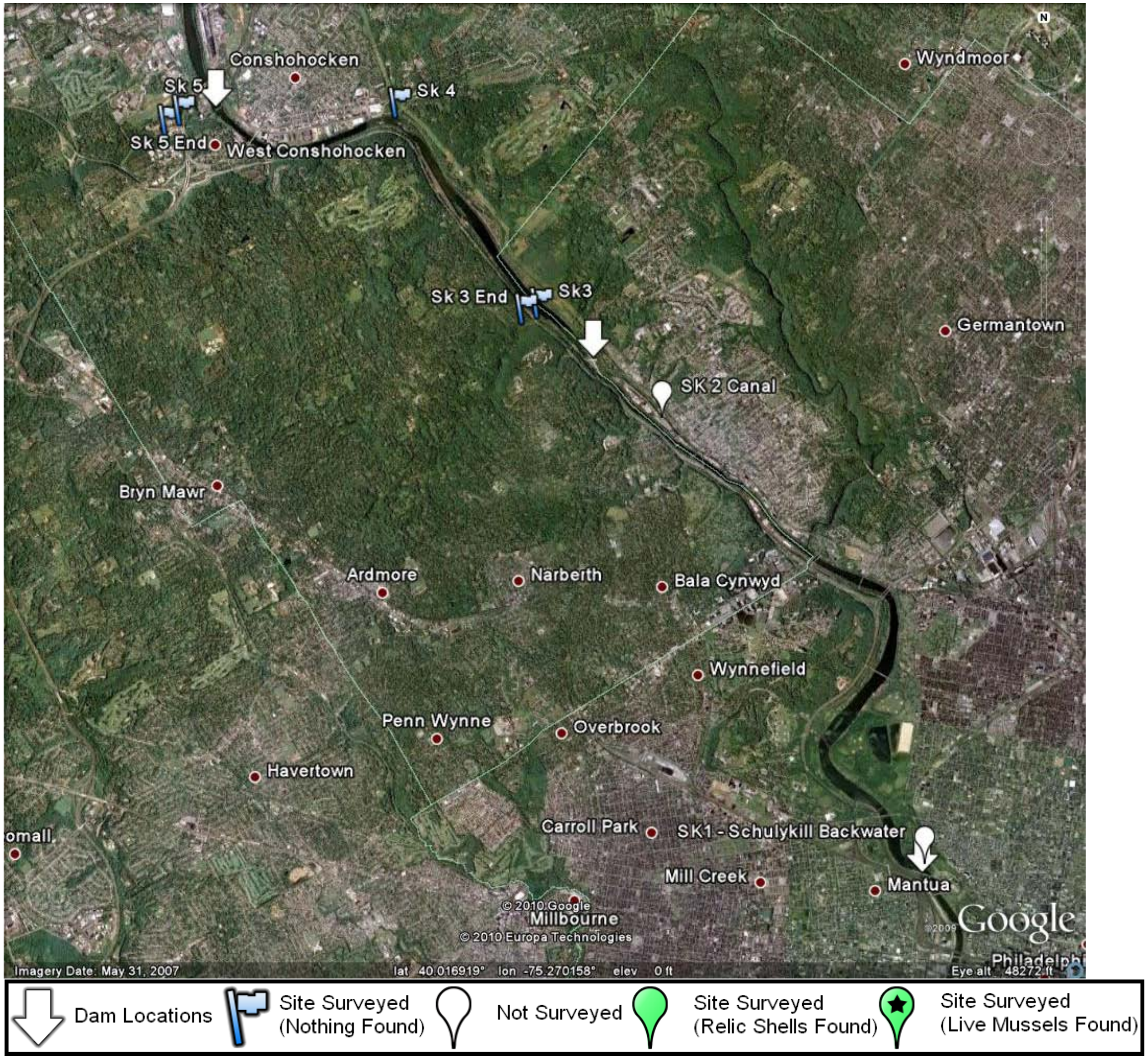

Figure 18. Map of Schuylkill sites during 2010 survey

Table 4. Survey data

Scientific Name	Date	Basin	State	Waypoint	Quantitative Method	Number of Searchers	Person Hours	Individual Live
Ligumia nasuta	6/9/11	Delaware	NJ	NJ-DR-055	Beach Walk	5	200.00	1
Ligumia nasuta	6/9/11	Delaware	NJ	NJ-DR-053	Beach Walk	5		201
Elliptio complanata	6/9/11	Delaware	NJ	NJ-DR-053	Beach Walk	5	100.00	13
Leptodea ochracea	6/9/11	Delaware	NJ	NJ-DR-053	Snorkeling	5		12
Lampsilis cariosa	6/9/11	Delaware	NJ	NJ-DR-053	Snorkeling	5		24
Ligumia nasuta	6/9/11	Delaware	NJ	NJ-DR-053	Snorkeling	5		7
Elliptio complanata	6/9/11	Delaware	NJ	NJ-DR-053	Snorkeling	5		242
Pyganodon cataracta	6/9/11	Delaware	NJ	NJ-DR-053	Snorkeling	5		22
Elliptio complanata	6/9/11	Delaware	PA	PA-DR-052	Snorkeling	5	105.00	106
Pyganodon cataracta	6/9/11	Delaware	PA	PA-DR-052	Snorkeling	5		26
Elliptio complanata	6/10/11	Delaware	NJ	NJ-DR-056A	Snorkeling, 1st	5	100.00	5
Pyganodon cataracta	6/10/11	Delaware	NJ	NJ-DR-056A	Snorkeling, 2nd	5		41
Elliptio complanata	6/10/11	Delaware	NJ	NJ-DR-056A	Snorkeling, 2nd	5		55
Pyganodon cataracta	6/10/11	Delaware	PA	PA-DR-057	Snorkeling	5		24
Elliptio complanata	6/10/11	Delaware	PA	PA-DR-057	Snorkeling	5		3
*Ligumia nasuta**	6/10/11	Delaware	NJ	NJ-DR-058	Beach Walk	5		2
Ligumia nasuta	6/10/11	Delaware	NJ	NJ-DR-058	Snorkeling	5		23
Pyganodon	6/10/11	Delaware	NJ	NJ-DR-	Snorkeling	5		31

cataracta				058			
Elliptio complanata	6/10/11	Delaware	NJ	NJ-DR-58	Snorkeling	5	50
Elliptio complanata	6/16/11	Delaware	PA	PA-DR-062	Snorkeling	4	98
Pyganodon cataracta	6/16/11	Delaware	PA	PA-DR-062	Snorkeling	4	49
Ligumia nasuta	6/16/11	Delaware	PA	PA-DR-062	Snorkeling	4	5

Task 3.Reintroduction

There are numberous case studies where freshwater mussel have been relocated. This happens mainly as an emergency measure wehreby endangered or threatened populations of mussels are moved out of harm's way such as when disturbance to their habibat might occur due to construction projects. These relocations can be short term, moving mussels hundreds of feet upriver to avoid unnecissary deaths, or can be long term, when moving populations miles from their orignial habitat due to a permanent change to the landscape. Relocation can alsobe used to reintroduce populations to areas where they once inhabitated, but fewer examles exist of this restoration tactic.

With help from the Academy of Natural Sciences, the Partnership undertook our first ever reintroduction in July 2011. Approximatly 100 eastern elliptios (*Elliptio companata*) and 100 eastern floaters (*Pyganodon cataracta*) were collected from Pennsylvania waters of the Delaware River in the urban corridor in the vicinity of the Tacony-Palmyra bridge. Mussels were then transported to shore (Figure 19) where they were cleaned and labeled with a plastic individually numbered ID tag as well as a electronic Passive Integrated Transponder or PIT tag (Biomark HPT8, 8.4mm 124.2kHz) (Figure 21). Each tag has a uniqe number that is read when swiping the reader over the mussel (Figure 22). Each individual mussel's ID, electronic number and size were recorded. Mussels were then divided into six groups of equal numbers of each species. The six groups of mussels were then relocated to suitable habitats in Ridley and Chester Creeks. Three Areas of Interest (AOI) were previously scouted for reintroduction for each of the sites (Figure 24). These sites were choosen based on being ideal mussel habitat found in the reach; i.e.; above a riffle, moderate water flow, sitly sediment near bank.

Figure 19. Mussels being brought to shore to be tagged for reintroduction.

An additional 30 eastern elliptios were collected from the local Ridley creek study population to use for a control group. The population in Ridley is smaller than that found in the Delaware River; therefore a smaller number of mussels were collected to be used in this experiment as controls to test whether source population affects the adjustment of the speices to a new location. These mussels were also cleaned, sized and tagged with both the plastic and PIT tags. These thirty mussels were then also divided among the six locations. A new Biomark tag reader system was purchased to monitor locations of the mussels over time, allowing us to more efficiently track the fate of the relocated.

The survivorship and locations of mussels are bieng monitored over time to compare the success of streams, locations and species to help guide future restoration efforts. The first post-reintroduction monitoring was undertaken after two months in Chester Creek, and a second monitoring survey was undertaken in both Chestser and Ridley Creeks after three months (September 2011, following Hurricane Irene and Tropical Storm Lee). In Chester creek we found

Figure 20. Mussel with blue plastic tag of ID number and white glob made of marine epoxy holding radio PIT tag.

mussels in each of the three AOI. Unfortunatly this area experienced severe flooding from both Hurricane Irene and Tropical Storm Lee at the end of August through the middle of September. Partnership staff were not able to assess how the mussels survived until the flooding had subsided on September 14th.. Some portion of the animals were smothered due to being buried too rapidly, and some were possibly washed downstream making them difficult to find. New channels were cut, banks were heavily eroded, and large trees were felled on top of one mussel bed. Despite record floods, we were surprized to find live, tagged mussels remained at all six sites post hurricane (Table 2). A higher percentage of both species were recovered in Ridley creek(Figure 26) post hurricane than in Chester creek. A higher percentage (from the Delaware River) of Ridley elliptio were recovered in both streams than any other of the two groups. The hightest recovery create was of elliptio in Ridley creek (Figure 27).

These surveys afer two and three months will be repeated in spring 2012 to deduce if mussels survived their first winter. Additional effort will also be spent downriver from the deployment locatins to determine how many washed downstream by the 2011 floods. Non-detection with the PIT tag reader does not mean they died since the unit must pass within twelve inches of a mussel to record a hit, and some deep pools can not be monitored with the device. Although preliminary, our data suggests that this first reintroduction was successful. This experiment marks the first time that any freshwater mussels have lived in Chester Creek in several decades, and it’s the first time that eastern floaters have been in either creek in perhaps 100 years. We believe that reintroduction will be an integral part of the ovarall Freshwater Mussel Restoration Program, and that more mussels and other species will be reintroduced into more streams in the region, contingent on resources.

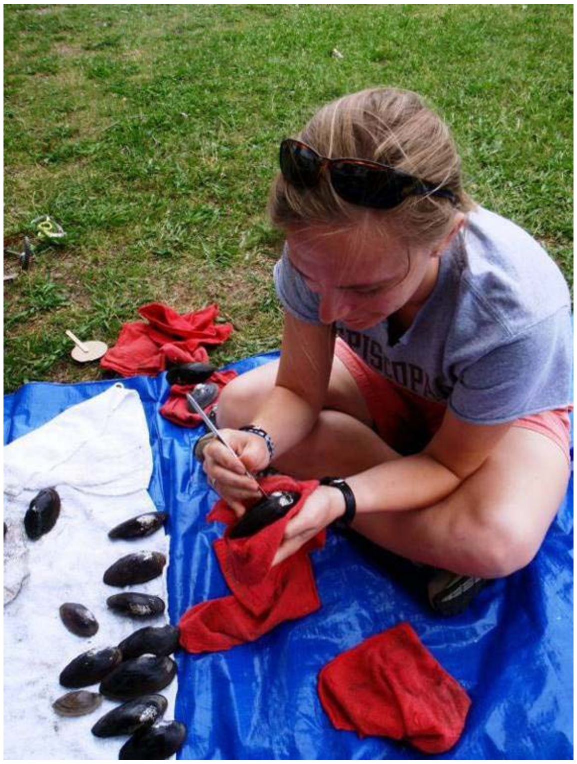

Figure 21 & 22. Cleaning mussels to prepare for tagging (left)., and PIT tag reading with hand held reader, yellow circle (right).

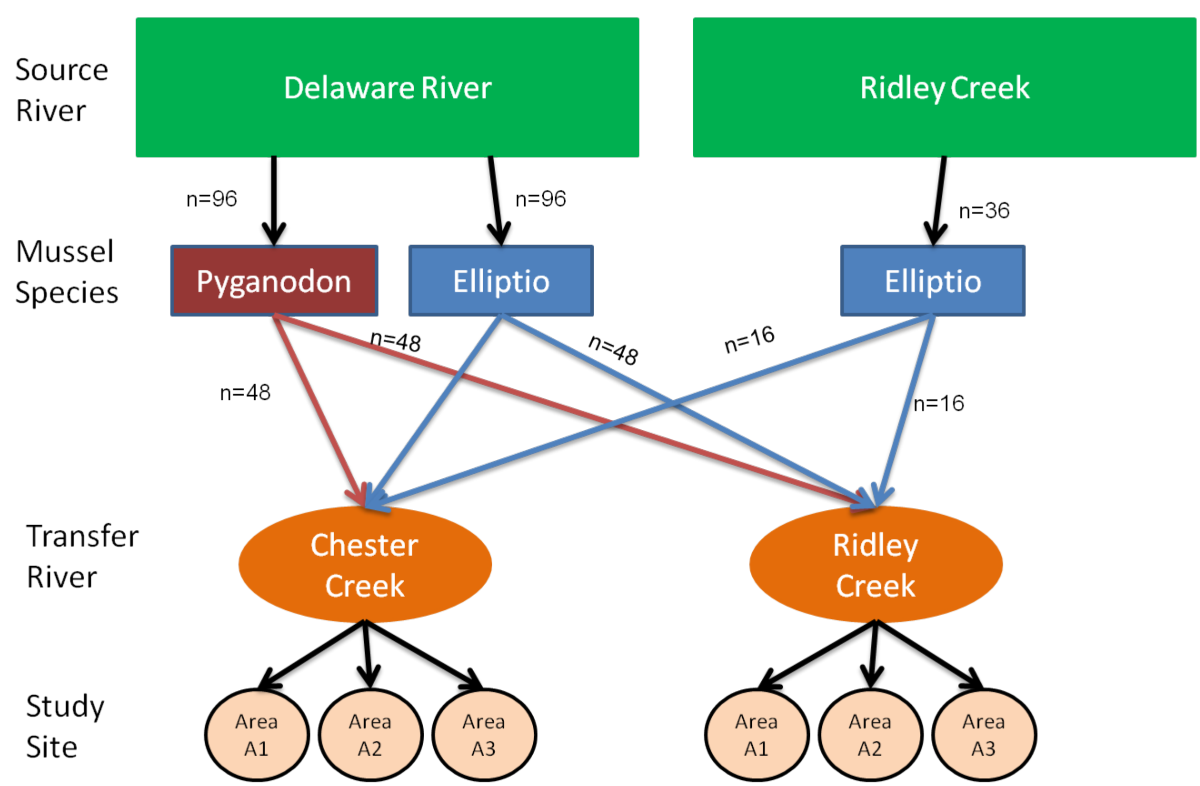

Figure23. Experimental design of the 2011 reintroduction trial, denoting source locations (green boxes), mussel species (red/blue boxes) and recipient streams (orange circles).

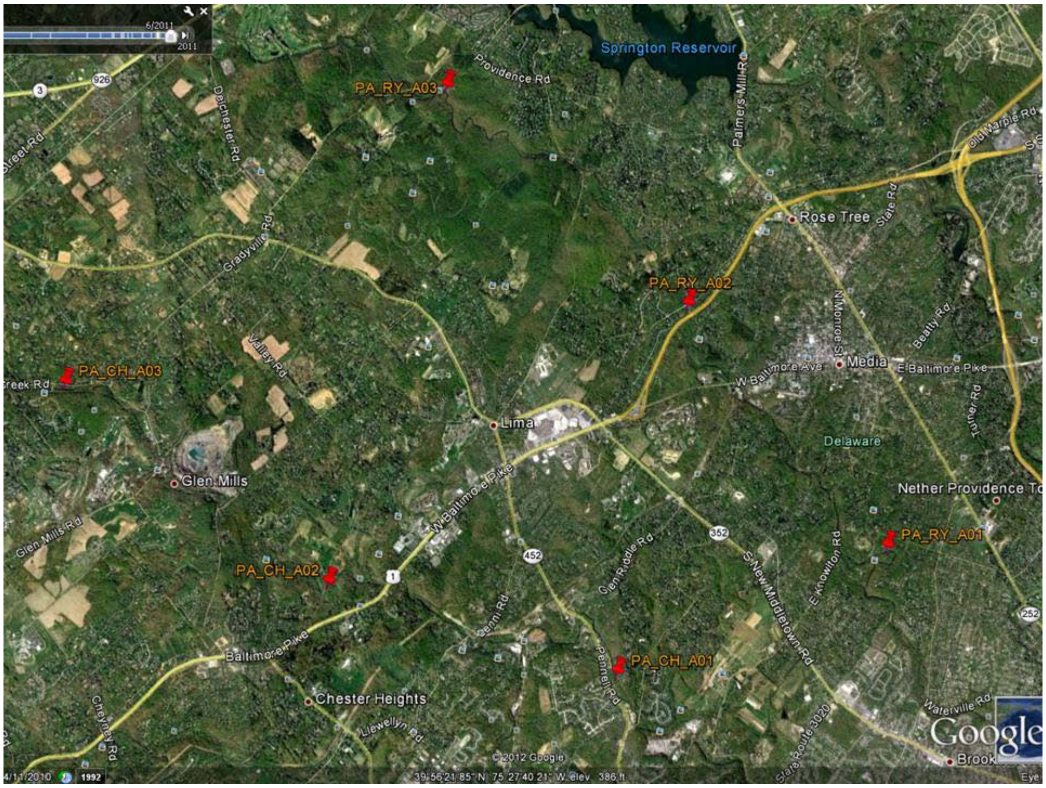

Figure 24. Map of recipient areas in Chester and Ridley Creeks for relocation eastern ellitptio (*E. companata*) and eastern floaters (*Pyganodon cataracta*).

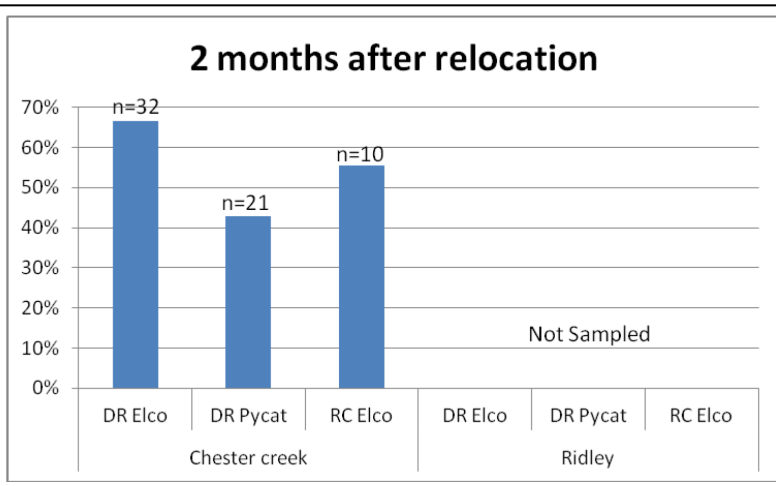

Figure 25. Percentage of relocated mussels (3 treatment groups) that were detected in recipient streams two months after being reintroduced.

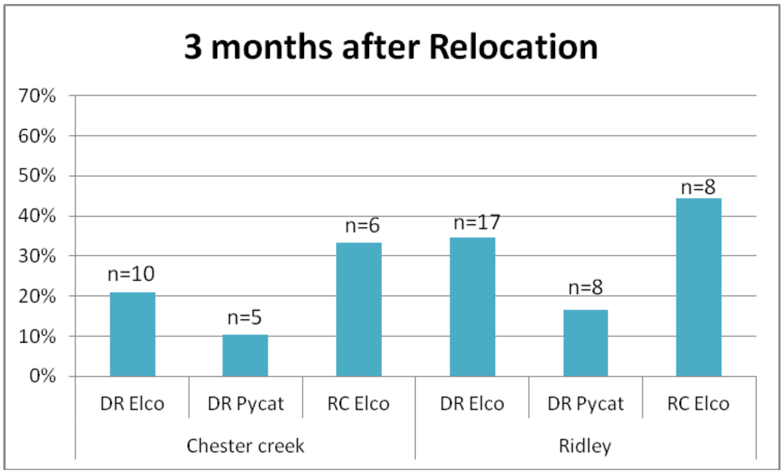

Figure 26. Percentage of relocated mussels (3 treatment groups) that were detected in recipient streams three months after being reintroduced.

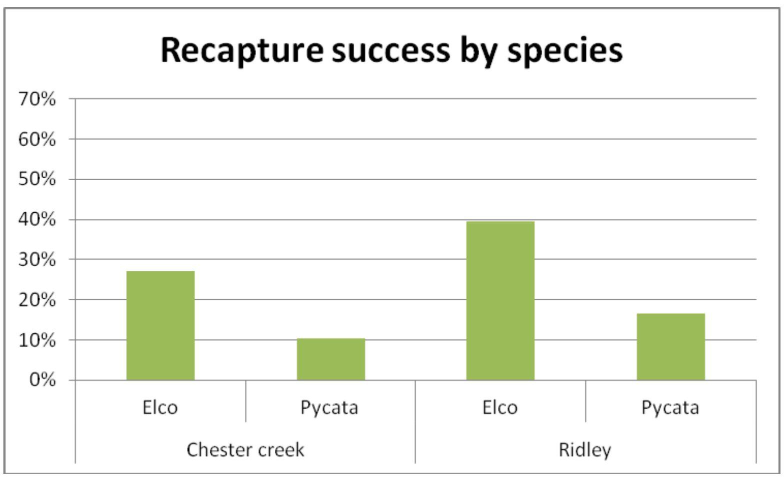

Figure 27. Percentage of relocated mussels detected three months following reintroduction, contrasted by species and stream.

Table 5. Monitoring data for detections of relocated mussels for reintroduction trial in 2011.

				Mussel Abundance (# detected)			**Percent Still in Their** Beds	
				Deployment		Post-Hurr.		Post-Hurr.
Original Stream	Mussel Group	Mussel Species	Mussel Source	June '11 0 months	Aug '11 2 months	Sept. '11 3 months	Aug '11 2 months	Sept. '11 3 months
Ridley	DR-Pycat	*Pyganodon cataracta*	Delaware River	16	na	0	na	0.0%
Ridley	DR-Pycat	*Pyganodon cataracta*	Delaware River	16	na	1	na	6.3%
Ridley	DR-Pycat	*Pyganodon cataracta*	Delaware River	16	na	7	na	43.8%
Ridley	DR-Elco	*Elliptio complanata*	Delaware River	15	na	5	na	33.3%
Ridley	DR-Elco	*Elliptio complanata*	Delaware River	16	na	0	na	0.0%
Ridley	DR-Elco	*Elliptio complanata*	Delaware River	17	na	12	na	70.6%
Ridley	RC-Elco	*Elliptio complanata*	Ridley Creek	6	na	3	na	50.0%
Ridley	RC-Elco	*Elliptio complanata*	Ridley Creek	5	na	0	na	0.0%
Ridley	RC-Elco	*Elliptio complanata*	Ridley Creek	6	na	5	na	83.3%
Chester	DR-Pycat	*Pyganodon cataracta*	Delaware River	16	13	3	81.3%	18.8%
Chester	DR-Pycat	*Pyganodon cataracta*	Delaware River	17	7	1	41.2%	5.9%
Chester	DR-Pycat	*Pyganodon cataracta*	Delaware River	16	1	1	6.3%	6.3%
					21	5	**42.9%**	**10.3%**
Chester	DR-Elco	*Elliptio complanata*	Delaware River	16	11	2	68.8%	12.5%
Chester	DR-Elco	*Elliptio complanata*	Delaware River	16	11	7	68.8%	43.8%
Chester	DR-Elco	*Elliptio complanata*	Delaware River	16	10	1	62.5%	6.3%
Chester	RC-Elco	*Elliptio complanata*	Ridley Creek	6	6	2	100.0%	33.3%
Chester	RC-Elco	*Elliptio complanata*	Ridley Creek	6	2	4	33.3%	66.7%
Chester	RC-Elco	*Elliptio complanata*	Ridley Creek	6	2	0	33.3%	0.0%

Task 4. Propagation

Propagation of offspring from female, gravid (i.e., full of eggs) freshwater mussel broodstock; raising the offspring in the laboratory; and then using the juvenile mussels to "seed" streams that were earlier prioritized for restoration. Work began in early spring 2008 with first attempts at laboratory propagation of juvenile mussels. The freshwater mussel's complicated life cycle can cause impediments in the laboratory when trying to reproduce these mussels (see previous "A Complicated Life" section). Cheyney University of Pennsylvania partnered with PDE and supplied their Aquaculture Research Education Center (AREC) as a space to begin rearing juvenile mussels. Cheyney University of Pennsylvania is the oldest of the Historically Black Colleges and Universities in America, and continues its commitment to academic excellence by offering diverse learning opportunities for students of diverse backgrounds.

Figure 28: Fish being infested in 20L bucket with approximately 1L of water.

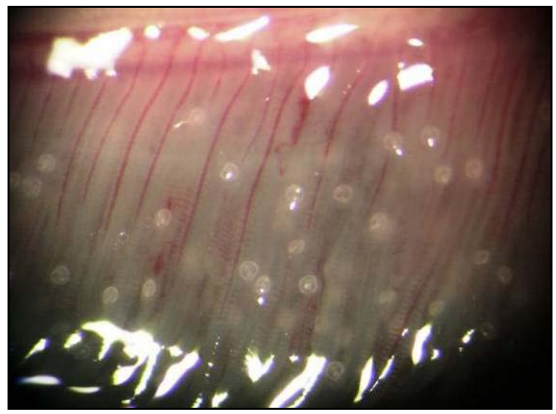

Figure 29: Gill of fish with attached glochidia (white dots).

In spring of 2008, Angela Padeletti, PDE's Science Specialist and Heidi Tucker-Wood, Cheyney's Fisheries Biologist visited the White Sulphur Springs National Fish Hatchery in White Sulphur Springs, West Virginia to be trained in aquaculture techniques specific to freshwater mussels. This hatchery is run by the United States Fish and Wildlife Service a partner of the FMRP. While at the hatchery, Ms. Padeletti and Ms. Tucker-Woods learned about the latest methodologies and supplies needed to propagate freshwater mussels in the hatchery.

The reproductive cycle for some species of freshwater mussels can happen over a very short period of time, and this is the case for the eastern elliptio, *Elliptio complanata*. Elliptios are called "short-term brooders" because they have a short period of time between gametogenesis (i.e. the development and maturation of sex cells through meiosis) and larval brooding, leading to dispersal of glochidia larvae onto fish hosts in late spring to early summer. The timing of these processes is especially sensitive to water temperature, and we regularly monitor ambient water temperatures during each spring propagation period. Typically, mussels begin releasing larvae onto fish in the late spring when the water hits 18°C and peak spawning and larval release occur at 22°C.

At the time that we received notice of the DuPont CiF award (5/26/10), we were already at the midpoint of the spring reproductive season for the targeted species of mussel. Although we had a limited propagation effort already underway at that time, it was too late in the season to significanty boost ur efforts. This delayed start was inconsequential howerever.

Figure 30: Juvenile mussel with foot

Due to factors that we do not understand, in spring 2010 we observed a near total shut-down of natural reproduction in both of our long-term study populations of mussels (Brandywine and Ridley Creeks). These are the only two remaining populations of native mussels in southeastern PA streams (south of the Schuylkill drainage). This was the first time that we observed such an event in the three years of intensive monitoring of reproduction patterns for the target species (and less intensive monitoring back to 1999) and we suspect that the unusual winter (record snowfall) could have altered or shut down reproduction.

Typically, larval development occurs within the inflated gills of adult mussels (Elliptio complanata) beginning in March or April when stream temperatures rise above 10^{o}C, and fully developed larvae are being brooded within the adults when stream temperatures hit 18^{o}C. During at least a one month period, more than half of adults sampled typically have inflated gills that are engorged with thousands of developing larvae and this is readily observed by gently prying apart the shell valves, confirmed with microscopic analysis of gill fluids that are flushed from the animals with a syringe. In the same way as the two years previous, in 2010 we began to monitor larval development in this way in March and continued doing so into July. Several hundred animals were examined over the March-July period from each of the two study streams, and only a few individuals were oberved to have larvae over this period, and laboratory attempts to transform these limited larvae on fish hosts (metamorphsis into juveniles) failed, suggesting that they were not viable.

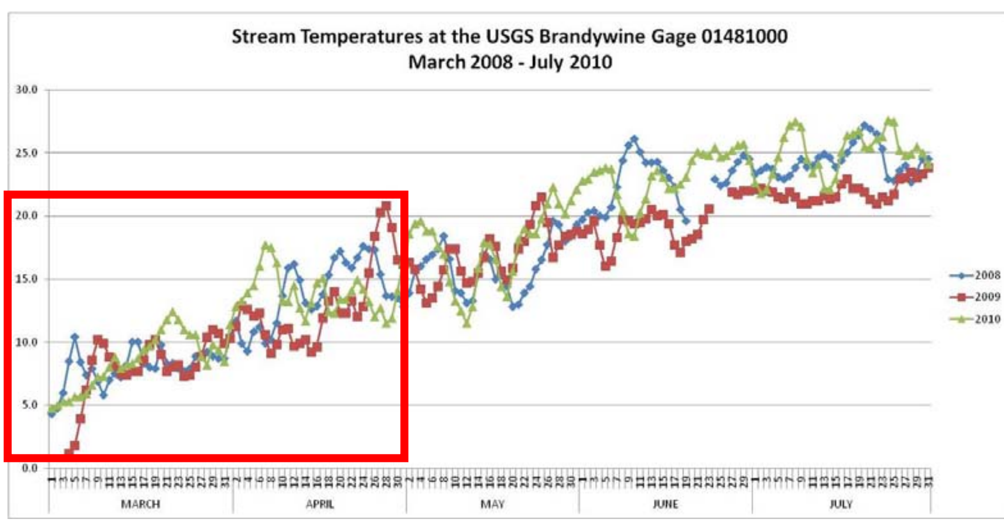

Figure 31. USGS temperature readings for spring 2008, 2009, 2010 at Brandywine gage. Red square around important spring time (March through April) in egg development.

Although it is unclear why natural reproduction failed in spring 2010 in southeast PA, we surmise that extreme climate fluctuations were responsible. Record snowfall during winter was followed by rapid spring warm-up (see March to early April in Figure 31). It is plausible that adult mussels had used up more of their overwintering energy reserves due to the harsh winter of 2010. Energy stores are typically accumulated in the form of glycogen in the fall as fuel for winter maintenance and ensuing gametogenesis. Alternatively, the sudden rise in early spring temperatures may not have been sufficiently gradual for successful gametogensis, which typically occurs gradually over months. Finally, mussels in these two streams might have been stressed by other factors associated with the unusual climate, including abnormal water quality or food quality associated wth runoff. Interannual variability in mussel reproductive patterns is poorly understood; however, literature information indicates that the general growth and productivity of freshwater mussels can be strongly linked to annual climate patterns in temperate regions. Typically, there is a cessation of somatic tissue growth in freshwater mussels during reproduction, and so it is likely that reproductive investment would be affected by climate anomalies in the same way as growth rates are. A survey of the literature failed to find references to reproductive shut-downs, and so it remains unclear how common the phenomenon is.

In 2011, water temperatures starting reaching the critical temperature of 18ºC in late April, yet would not remain at this threshold for very long. The first two weeks of May the temperatures within the creeks were slowly rising, a trend that is favorable to mussels becoming ripe. Broodstock from both Brandywine Creek and Ridley Creek were collected and brought to the Cheyney University aquaculture facility. Throughout the first three weeks of May multiple cohorts of mussels were brought into the laboratory and brought up to temperature (18ºC) and held there till glochidia were released by the mussels. Surprisingly Brandywine mussels did not produce glochidia whereas, mussel from Ridley Creek did. This finding may be linked to same facet of the health of the population from that source stream because in the past we have also found suboptimal health of mussels in the Brandywine source population, compared to Ridley Creek.

Initial work resulted in the infesting of over 100 lake trout with glochidia and also a number of eels. Compared to previous years this represented substantially improved infestation rates and we were on track to produce record numbers of juvenile mussels. Although the technical elements of the propagation effort went well, the air conditioning for the mussel culture laboratory was lost during the heat wave of May 30-June 1, 2011 and the water chillers were not able to maintain proper rearing temperatures. Water temperatures during this time exceeded 22ºC, causing all lake trout and juvenile mussels to perish. In addition, the adult mussels which we had held in colder water for spawning later in the spring, released all of their glochidia before we could make arrangements to acquire more fish for infestation. Due to the timing of this unfortunate event, no other gravid adults could be found in their natural habitats to allow more fish to be infested with mussel larvae for the 2011 propagation cycle. In preparation for 2012 hatchery propagation, lessons learned from 2011 were used to avoid a repeat of this unfortunate climate control problem (two hatcheries will be used for redundancy).

Despite this set back, the research project was able to continue on a limited basis. Approximately 500 transformed juveniles were obtained from Julie L. Devers (U.S. Fish and Wildlife Service, Maryland Fishery Resources Office, Annapolis, MD) on June 27, 2011.

After one week of acclimation, 384 of the remaining 395 mussels were divided equally into 8 sieve units (48 mussels/sieve) in eight Barnhart buckets (one sieve/bucket) (Figure 32). Each sieve was randomly assigned to one of four treatments; control, standard algae diet plus calcium, enhanced diet, enhanced diet and calcium. Each treatment had two replicates. All treatments were fed the same high density algal culture obtained frozen from Dr. Catherine Gatenby (U.S. Fish and Wildlife Service, White Sulfur Springs Hatchery, White Sulfur Springs, WV) for the five weeks that the experiment was conducted.

Calcium levels were determined in all units by use of Atomic Absorption Spectroscopy (AOAC, 2006). Levels were determined biweekly on Monday and Thursday and adjusted as necessary through the addition of API Eco-Calcium.

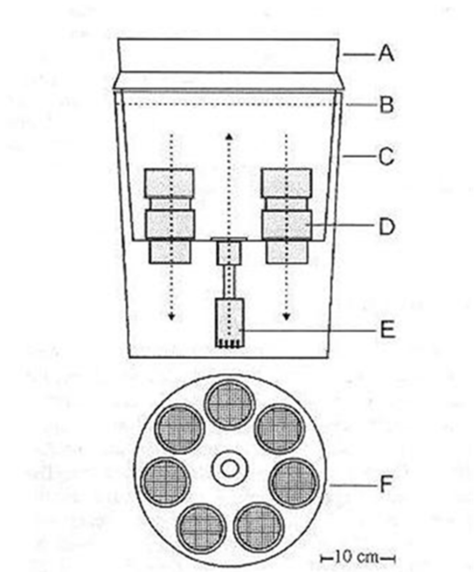

Figure 32. Barnhart Bucket system for juvenile rearing.

Survival data at the termination of the study was analyzed using the Chi-Square statistical procedure to determine if the mean values for any of the treatments are significantly different from one another using a significance level of 0.05 as the limit (Steel and Torrie 1980) (Table 7). Actual water quality readings from each treatment are shown in Table 8.

Table 6. Feeding and dietary treatments delivered to juvenile *Elliptio complanata.*

Treatment Number	Description
1 (Control)	Standard algal diet and standard water conditions (Ca approximately 30 ppm)
2	Standard algal diet and enhanced Ca aqueous level (>250 ppm by addition of API Eco-Calcium)
3	Enhanced algal diet (Kent Marine Microvert (Invertebrate Food for fine filter feeders) about .5 ml per 5 gallon bucket per day and standard water conditions
4	Enhanced algal diet (Kent Marine Microvert Invertebrate Food for Fine Filter Feeders at a rate of 0.5 ml per 5 gallon bucket per day) and enhanced aqueous Ca level

Table 7. Mean survival rates of juvenile *Elliptio complanata* reared under four different water quality regimes					
	Number of juveniles				5-Week Survival (%)
Treatment	Initial	Week 1	Week 3	Week 5	
Control	48	31	15	4	8.3^{A}
Added Ca	48	38	30	10	20.8^{B}
Added Microvert	48	39	31	7	14.6^{AB}
Added Ca and Microvert	48	43	40	18	37.5^{C}

The addition of both calcium and an algal enhancer improved the 5-week survival of the juvenile mussels in this study (Table 7), but the survival rate of the juveniles was still poor overall with the additional food and calcium treatment only providing a survival rate of approximately 38%. Though this is a marked improvement, it does raise the question of what "optimal" survival rates are in the laboratory. Ecologically, species that produce large numbers of relatively small and undeveloped progeny are doing so because the survival rates are expected to be low less than 5% whereas, species that invest more per offspring such as by brooding larvae tend to have better survival (greater than 25%). Additional seasons of culture with additional refinements to the feeding parameters for this species will need to be conducted to determine whether the juvenile survival rate can be increased further.

The treatment where the culture conditions were enhanced with calcium alone did provide improved survival, but not as much of an improvement as was seen when the calcium was paired with the feed enhancer. This would indicate that our original concerns from previous years were founded and that there is a requirement for higher levels of aqueous calcium than is provided in the standard water conditions of the facility. This also indicates that this should be a concern when the culture of this species (and probably all freshwater mussel species) is undertaken at other locations.

The addition of the Kent Microvert by itself also provided a numerical boost in survival but it was not significantly different from that of the mussels in the control group. These data when taken into consideration with the mussels given both the Microvert and higher levels of aqueous calcium would indicate that there is room for improvement in the algal diet being fed as part of our standard protocol. There is not currently a true characterization of the species, their relative ratios to one another, or the nutritional profile of the algae which comprise the standard diet of *Elliptio complanata*. The absence of this information makes it difficult to determine what advantage the Microvert was providing relative to the basal diet and what further improvements need to be incorporated into future feeding regimes.

These results provide important culture guidance for this species in future seasons. It is now part of the laboratory protocol that calcium levels are to be maintained above 250 ppm and Microvert will be added to supplement the algal diet for the mussels. Additional work is needed to determine the best natural diet for these mussels, and required ration levels for various nutritional components of the diet. The actual levels of required aqueous calcium need to be determined and correlated with both survival and growth. Tests also need to be conducted to assess the impact of temperature on the survival and growth rates of these juvenile mussels.

Table 8. Four treatment groups with water quality readings during the juvenile mussel rearing experiment.

Control Group					
	Initial	Week 1	Week 3	Week 5	
Mussel Count	48	31	15	4	
Temp Average °C	22.1	22	22.5	22.4	
Avg. Calcium Levels	58.7	53.5	46.8	31.4	
Avg. pH	7.62	7.45	7.32	7.21	

Added Ca Group					
	Initial	Week 1	Week 3	Week 5	
Mussel Count	48	38	30	10	
Temp Average °C	22.1	22	22.5	22.4	
Avg. Calcium Levels	418.1	320.7	339.2	406.6	5ml of Ca added to the buckets on Monday and Thursdays
Avg. pH	7.65	7.53	7.56	7.41	

Added Microvert Group					
	Initial	Week 1	Week 3	Week 5	
Mussel Count	48	39	31	7	
Temp Average °C	22.1	22	22.5	22.4	
Calcium Levels	54.5	50.1	42.1	40.2	
Avg. pH	7.63	7.57	7.42	7.33	2ml of Microvert added daily

Added Ca & Microvert Group					
	Initial	Week 1	Week 3	Week 5	
Mussel Count	48	43	40	18	
Temp Average °C	22.1	22	22.5	22.4	
Calcium Levels	405.7	341.5	351.2	386.7	5ml of Ca added to the buckets on Monday and Thursdays
Avg. pH	7.59	7.48	7.31	7.48	2ml of Microvert added daily

In future years, our goal will be to stock at least 10,000 seed of the eastern elliptio species into each suitable restoration stream from which they have become extirpated. There are approximately 1,000 gill filaments on a single gill arch of a lake trout and a total of 8 arches on each fish. If we consider that approximately two glochidia will attach to each filament when the fish is infested, under optimal conditions more than 8,000 juvenile mussels could be collected and reared to adulthood for each fish infested using our current methodology. Since we had more than 100 fish infested in 2011, we were on track to potentially produce 800,000 seed. Even if only 5% had survived to a size suitable for out-planting this would have been four times (40,000) needed.

By re-introducing propagated seed mussels along with relocation reproductive adults, we expect to eventually boost population biomass in areas where they have been lost or impaired. For each batch of 10,000 seed that we can produce, we project that they will filter 0.8 million liters of water in their first year in-stream and 2 billion liters of water over their first 30-years, after accounting for expected mortality and not including water filtered by any progeny (Figure 33).

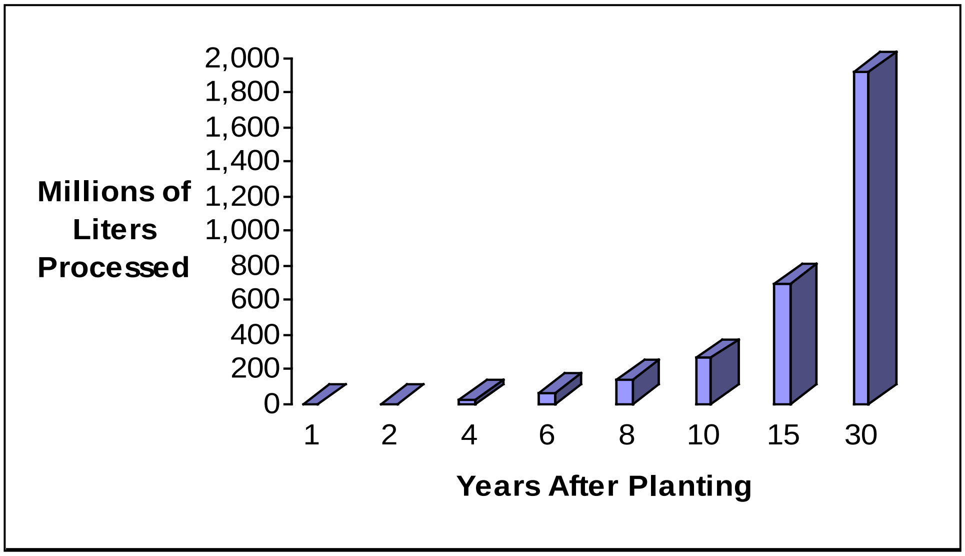

Figure 33. Projected potential water processing by 10,000 planted seed over first 30 years, not including progeny.

FMRP 5. Outreach and Education

During the week of July 11th teachers and local naturalists were brought to the Brandywine Creek as part of our Delaware Estuary Watershed Teacher Workshop, where they were taught about freshwater mussels and their beneficial roles in our local waterways. The teachers waded through the creek discovering mussel beds and shells. PDE staff presented photographs and data on some of our field and laboratory work.

The project has been featured in at least two issues of *Estuary News*, PDE's newsletter with a circulation of over 22,000 in the Delaware Estuary region and beyond. In addition, a project description and photo were included in PDE's 2011 annual report, which is printed and distributed to over 500 people throughout the year. Results from this project have been presented at the biennial Delaware Estuary Science and Environmental Summit in January 2011 and will also be presented at the January 2013 conference. This project has also been presented at Restore America's Estuaries in 2010, the Atlantic Estuarine Research Federation 2011, and the 2012 National Shellfish Association.

Figure 34. Teachers from the Watershed Workshop view mussel shells shown by Angela Padeletti, Science Coordinator, before looking for live mussels in the Brandywine.

Future Program Development

The Partnership for the Delaware Estuary is working with numerous collaborators to restore the species assemblage, range and abundance of native freshwater mussels in our region (Figure 35). Surveys help us determine what populations are left which are prioritized as critical habitat areas in need of protection. These areas are also critical broodstock sources from which we can restore from.

A second tactic is stream suitability tests. These tests determine if streams are once again healthy enough to reintroduce adults or juveniles and sustain populations, helping us prioritize limited funds.

Through hatchery propagation we hope to introduce juvenile mussels back into streams. Reintroduction of gravid adults into stream reaches provides another strategy to assist restoration, instantly providing not only filtering capacity but also seeding the way for future generations.

Education and outreach to communities about freshwater mussels and the need for restoration is critical to build awareness, support and potentially also to fill science rolls in monitoring projects and surveying new areas. One such project is currently underway. The Partnership is working with local watershed groups to train their volunteers to perform mussel surveys. These data will be collected and uploaded to a website. The volunteers will be trained by Partnership staff, and a Cheyney University student will be trained as well to help with connecting scientists to volunteers. We believe that by training volunteers, more survey data can be collected to augment scientific efforts being conducted with limited resources.

The focus of the FMRP is through direct actions to rebuild populations, but there are many other activities that can help restore native mussel populations. Dam removal or other fish passage projects, can restore fish host availability enabling freshwater mussels to complete their life cycle and disperse to new (old) areas. Mussel population health appears also closely linked to the health and depth of riparian forests, and so any activity that improves riparian cover along streams should benefit mussels. The maintenance of water quality, food conditions, and base flow will also benefit the FMRP. Finally storm water controls will aid mussels by reducing bed transport and scouring.

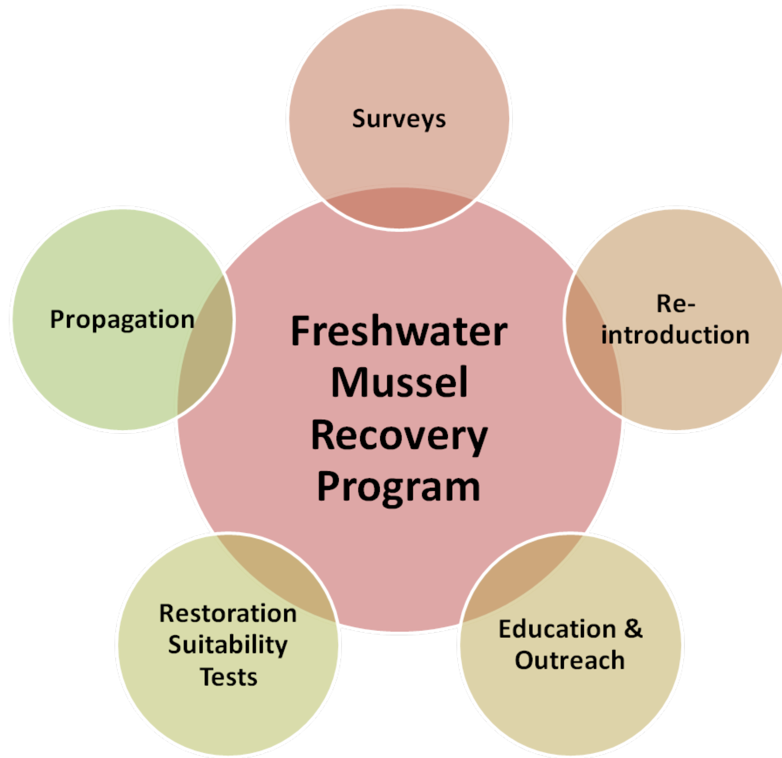

Figure 35. the Partnership for the Delaware Estuary's Freshwater Mussel Recovery Program five tiered strategy.

The vision of the FMRP, part of the Partnership's watershed-wide bivalve restoration strategy, is to refill vacant ecological niches once occupied by a diverse and abundant mussel assemblage. In doing so, the FMRP should eventually help to sustain clean water by enhanced filtering and processing by mussel beds, potentially helping meet TMDL needs and overall goals to the Comprehensive Conservation Management Plan.

The FMRPs future goals will be to expand production of eastern elliptio and to subsequently rear sufficient quantities in the hatchery for at least six-months until they reach a size (at least 1 cm shell length) and hardiness that is conducive to out planting. Seed viability and performance will be monitored thereafter; contingent on additional funding that is being sought. Also contingent on funding will be the assessment of actual in-stream water and habitat quality benefits. Eventually, the FMRP intends to be expanded to include many of the other dozen native mussel species that are in need of restoration,

Partners

The FMRP is coordinated by the Partnership, with the lead Principal Investigator being Dr. Danielle Kreeger, Science Director. Dr. Kreeger has more than 25 years of expertise as a shellfish and freshwater mussel ecologist. Dr. Kreeger oversees technical aspects of this project, field efforts, and coordinates activities of other PI's. Laboratory samples, such as mussel fitness analyses, were performed in Dr. Kreeger's laboratory at Drexel University. Ms. Angela Padeletti, PDE's Science Specialist, serves as project manager for the FMRP. Ms. Padeletti handles the day-to-day aspects of the project as well as helping coordinate the multiple partners.

The Academy of Natural Sciences of Drexel University (ANS) has collaborated via a sub-award from PDE, primarily to provide field project support for fish host collections, under the supervision of Mr. Roger Thomas (Section Leader, Staff Scientist IV). Dr. Richard Horwitz (Senior Scientist, Patrick Center Fisheries Section) has furnished many necessary fish hosts, technical advice and electro-shocking gear as appropriate. ANS has also been a core partner completing most mussel surveys along the Delaware and streams in recent years.

Figure 36: Freshwater Mussel partners.

Cheyney University of Pennsylvania collaborates with PDE by providing expertise and aquaculture facilities. The lead PI for Cheyney University is Dr. Stephen Hughes, Director of the AREC. Dr. Hughes contributes expertise in fish physiology, culture, and nutrition. He provides the hatchery infrastructure and has contributed space for use as a base of operations for field work. Dr. Hughes has overseen development of fish culture techniques and recirculation aquaria systems for holding mussels and fish. Dr. Hughes also recruits and advises several Cheyney undergraduate students who benefit from the unique learning experiences that this project provides. Cheyney University also provides the expertise of Heidi Tucker-Wood who is a fisheries biologist, who oversees the day-to-day running of the hatchery.

The United States Fish and Wildlife Service (USFWS) provide technology transfer and technical support by staff from the White Sulfur Springs National Fish Hatchery, WV. Dr. Catherine Gatenby, Project Leader, is the PI for USFWS. In particular, she provides expertise regarding captive care needs for mussels and fish hosts and propagation techniques. Beginning in 2012, we anticipate also partnering with USFWS on hatchery propagation to diversity our hatchery program.

William Lellis of the United States Geological Survey has helped by advising the project. He and his team work on the ecology and biology of declining freshwater mussels, mostly in the upper Delaware River Basin. Dr. Lellis has worked at the Northern Appalachian Research Branch in Wellsboro, PA, but recently moved to Washington, DC.

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Figure 8. Projected potential water processing