Final Restoration Completion Report

Ohio River Fish and Mussel Kill Natural Resource Damage Assessment and Restoration

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Final Report, Ohio River Restoration Project

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In 1999 a major fish and mollusk kill occurred within the Belleville Pool of the Ohio River near Parkersburg, WV and just downstream of Marietta, OH. Aquatic resources were impacted in more than a 20 mile reach. The kill resulted from a release of hazardous substances (hexavalent chromium and chemical used to precipitate it out of solution) from a ferro-alloy manufacturing facility, Eramet Marietta, Inc. The following report outlines the investigation and subsequent damage settlement (USFWS *et al.* 2007) and provides a record of restoration activities that were conducted.

INVESTIGATION AND RESOURCE DAMAGE ASSESSMENT

On June 7, 1999, a major fish kill was reported on the Ohio River between River Mile (RM) 176.5 and 185.0. On June 8, 1999, a fish kill investigation was conducted by the West Virginia Division of Natural Resources (WVDNR) between RM 175.0 and 187.0. Based upon the numbers of dead fish found, it was estimated that approximately 4,000 fish were killed, 90% of which were freshwater drum (*Aplodinotus grunniens*). US Fish and Wildlife Service (USFWS) personnel continued to observe dead or distressed fish between RM 176.0 and 181.5 through June 18, 1999. A second major fish kill was reported on October 4, 1999. The WVDNR investigated and found over 5,000 additional dead fish from RM 176 to 187. Again, about 96% of the fish killed were freshwater drum.

On June 8, 1999, USFWS divers inspected mussel beds at Muskingum Island (upstream of the Eramet facility), Vienna Island (approximately 600 feet downstream from the Eramet facility), and Site 11 (approximately three miles downstream from the Eramet facility). Native mussels and invasive zebra mussels (*Dreissena polymorpha*) were alive and siphoning normally at all three sites. However, on September 2, 1999, USFWS divers, conducting a routine survey, discovered that a major kill of freshwater mussels had and was occurring at Site 11 (RM 179 to 180). Live zebra mussels, which in years leading up to the kill had been abundant, were absent. Many dead or distressed native mussels were observed. See Figure 1 for a map of the impact/study area.

In order to pinpoint the source of impact, on September 8 and 9, 1999, USFWS divers collected mussel samples and videotaped the substrate at selected locations between RM 175 and 181. Native and zebra mussels at Muskingum Island, upstream of the suspect facility, were alive and siphoning normally. However, downstream of the facility, zebra mussels were absent or dead for more than a four-mile segment of the river below the facility's discharge. Native mussels were also dead or distressed at Vienna Island and Site 11. On October 18 and 19, 1999, biologists from the USFWS and WVDNR inspected the bottom of the Ohio River between RM 175.3
(upstream of the facility) and RM 179.9 (Site 11) using a drop camera. Native and zebra mussels were alive and siphoning normally from RM 175.3 to 176.9 (upstream of the facility's discharge). No live mussels were observed near the Ohio shoreline between RM 176.9 and 179.9 (downstream from the facility).

In order to enumerate the loss of native mussels, a quantitative survey of Site 11 was conducted by Ecological Specialists, Inc. (ESI) in May 2000 (ESI 2000). One hundred percent mortality was found in both native and zebra mussels. In addition, ESI reported that gastropod (snail) mortality was 99.8% and that there were no living benthic invertebrates within 260 feet (80m) of the Ohio shoreline. A second quantitative survey in October 2000 (ESI 2002), found similar

results in the segment of the Ohio River from the facility's discharge to the head of Neal Island, which is part of the Ohio River Islands National Wildlife Refuge (ORINWR). In all, ESI assessed mussel beds at five locations within the Belleville Pool downstream of the facility (ESI 2002). These included Site 11 (Old Lock 18), Neal Island, Blennerhassett Island, Dunfee Run area, and Newberry Island. Results of these surveys estimated that over 990,000 native mussels and over 12,000,000 snails were lost from discrete sampling locations over a 20 mile stretch of the Ohio River. The actual kill was likely much larger extending downstream into the Racine Pool and may have extended for more than 30 miles.

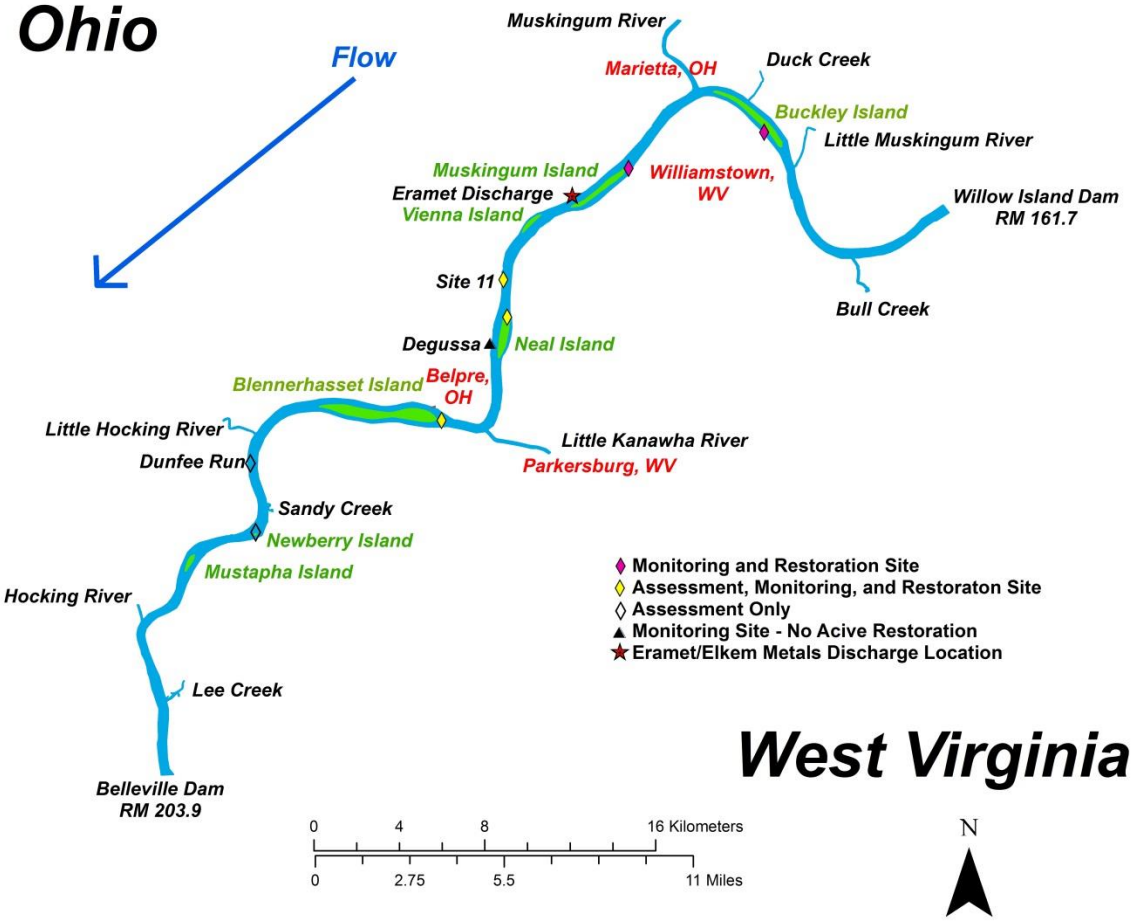

Figure 1. Map showing the Belleville Pool of the Ohio River (Wood and Pleasants counties, WV; Washington, Athens, and Meigs counties, OH) showing the assessment, monitoring, and restoration sites associated with the Eramet/Elkem Metals Natural Resources Damage Assessment.

TOXIC DISCHARGE SOURCE

All studies confirmed that the source of the impact came from the discharge pipe at the Eramet Marietta, Inc. facility. Eramet Marietta, Inc. owned and operated the ferro-alloy production facility located on the right descending bank (OH shoreline) of the Ohio River approximately four miles southwest of Marietta, OH. The facility was a 1,350 acre site that was originally part of a large Union Carbide Corporation complex that was constructed between the late 1940's and the mid

1950's. In 1981, Elkem Metals Company, L.P, purchased the ferro-alloy division from Union Carbide. In 1988, a coal burning power plant, which was part of the ferro-alloy division, was sold by Elkem to American Municipal Power-Ohio, Inc. Eramet purchased the remainder of the ferro-alloy operations from Elkem on or around June 30, 1999.

The facility discharged wastewater to the Ohio River through an outfall located at RM 176.9. This discharge was permitted through the National Pollutant Discharge Elimination System (NPDES) by the Ohio Environmental Protection Agency (OEPA). Releases of hazardous substances in excess of the limitations set forth in the facility's wastewater discharge permit were documented and allegedly resulted in a series of fish, mussel, and snail kills within a 20 mile segment of the river, which forms the boundary between the states of Ohio and West Virginia.

Unusually low flows were experienced in the Ohio River in 1999, resulting in accumulation and concentration of hazardous substances. The available data suggest that both zebra and native mussels were directly exposed by filtering particulates from contaminated water near the bottom of the Ohio River. Fish species, primarily freshwater drum which are known to feed on mussels, were then exposed by consumption of the contaminated mussels.

SUMMARY OF FINDINGS

Injuries to natural resources were documented within a 20 mile reach of the Ohio River resulting from the hazardous substances released from the ferro-alloy manufacturing facility are summarized as follows.

- Injury to surface water occurred as evidenced by sediment concentrations of chromium sufficient to cause injury to biological resources.

- Injury to biological resources occurred as evidenced by death of:
    - Fish (estimated over 8,600 killed), including freshwater drum, sauger (*Sander canadense*), hybrid striped bass (*Morone saxatilis x M. schrysops*), channel catfish (*Ictalurus punctatus*), bluegill (*Lepomis macrochirus*), walleye (*Sander vitreus*), gizzard shad (*Dorosoma cepeianum*), and suckers.
    - Freshwater mussels (estimated over 990,000 killed at 5 bed locations (ESI, 2002)) including, but not limited to, the natives *Actinonaias ligamentina*, *Amblema plicata*, *Elliptio crassidens*, *Eurynia* (=*Elliptio*) *dilalata*, *Ellipsaria lineolata*, *Fusconaia flava*, *Lampsilis cardium*, *L. siliquoidea*, *Lasmigona complanata*, *Leptodea fragilis*, *Ligumia recta*, *Megalonaias nervosa*, *Obliquaria reflexa*, *Obovaria subrotunda*, *Plethobasus cyphyus*, *Pleurobema cordatum*, *P. sintox*ia, *Potamilus alatus*, *P. ohiensis*, *Pyganodon grandis*, *Theliderma* (=*Quadrula*) *metanevra*, *Cyclonaias* (=*Quadrula*) *pustulosa*, *Q. quadrula*, *Truncilla donaciformis*, *T. truncata*, *Utterbackia imbecillis*, and non-native zebra mussels.
    - Gastropods (snails -- estimated over 12,000,000 killed at 5 bed locations (ESI, 2002)) including, but not limited to, one species of *Pleurocera*, one species of *Campeloma*, and one species of *Lithasia*.
    - Other benthic invertebrates (100 percent mortality at Site 11 within 80 meters of the Ohio shore; not quantified elsewhere).

Further it should be noted that Site 11 had been inhabited in the recent years leading up to the kill by two federally-listed endangered species, the Pink Mucket (*Lampsilis abrupta*) and the

Fanshell (*Cyprogenia stegaria*). Pink Mucket juveniles were documented at Site 11 as recently as 1991, and Fanshell juveniles were documented at Site 11 as recently as 1994. Although none of these species were found dead during assessment surveys, it is reasonable to conclude some were in the area and, therefore, affected by the release.

SUMMARY OF THE SETTLEMENT

Elkem Metals Company, LP" its two partners (Ferro Invest III, Inc., and Ferro Invest II, LLC), and Eramet Marietta, Inc. agreed to pay $3.25 million to settle allegations that the companies injured natural resources (under the Natural Resource Damage Assessment [NRDA] provisions of the Comprehensive Environmental Response, Compensation, and Liability Act [CERCLA]) and violated the Clean Water Act (CWA) as a result of wastewater discharges from their ferro-alloy manufacturing facility in Marietta, OH. As a result of the settlement, the companies paid a combined total of $2,040,000 towards restoration of injured natural resources in the Ohio River. They also paid a total of $460,000 as reimbursement to the Department of Interior (DOI) and the states of West Virginia and Ohio for natural resource assessment costs under CERCLA. In addition, Elkem paid a penalty of $245,000 and Eramet paid a penalty of $525,000 to the US Environmental Protection Agency (USEPA) for alleged CWA violations.

A Consent Decree among the parties was entered by the US District Court for the Southern District of Ohio on April 19, 2006. The portion of the Consent Decree dealing with settlement of natural resource damage claims stipulated that such funds were to be used jointly by the natural resource trustees to restore, replace, or acquire the equivalent of the injured natural resources. The settlement enabled the natural resource trustees to work together to restore freshwater aquatic life that experienced substantial impact and loss within the affected reach of the Ohio River.

Under CERCLA, federal agencies who administer natural resources, all state governors, and federally-recognized Indian tribes are designated as natural resource trustees for those natural resources for which they have statutory authorities and responsibilities. These designated natural resource trustees have the responsibility to restore, rehabilitate, replace, or acquire the equivalent of natural resources injured as a result of a hazardous substance release.

For the purposes of this incident, the Region 3 Regional Director of the USFWS was designated as DOI's authorized official, to act as the natural resource trustee on behalf of the DOI Secretary. The governor of the state of West Virginia designated the WVDNR and the WVDEP to act as the CERCLA trustees on behalf of the state of West Virginia for all cases that affect natural resources for which West Virginia is a trustee. The governor of the state of Ohio designated the Ohio Environmental Protection Agency (OEPA) to act as the CERCLA trustee on behalf of the state of Ohio for all incidents that affect natural resources for which Ohio is a trustee.

It was further stipulated that restoration efforts were to be conducted within the Belleville Pool of the Ohio River, a 42 mile reach which extends from RM 161.8 to RM 203.9 bordering Pleasants and Wood counties, WV and Washington, Athens, and Meigs counties, OH. The area directly affected by the discharges was RM 176.9 to RM 203.9, with decreasing mortality as one moves downstream from the discharge point. However, other reaches of the Belleville Pool were indirectly affected by the resource losses, as there were fewer mussels, snails, and fish to support the larger ecosystem services and functions. The Belleville Pool, a large river warm water ecosystem, is impounded for navigation by the Belleville Dam. Water averages 24 feet (7.3 meters) deep and 1,327 feet (404.5 meters) wide with an average bottom slope of 0.5 feet

per mile (0.3m/km). Water temperatures in the mainstem typically range from 32 to 85 ^{o}F (0 to 29 ^{o}C), with occasional icing over. The usual low flow period is August through October.

SITE DESCRIPTION

There are seven islands in the Belleville Pool, three of which are a part of the ORINWR (Buckley Island, Muskingum Island, and Neal Island). Only Neal Island was within the kill-zone. The islands provide habitat diversity in the form of extensive shallow water features (less than 15 feet or 4.6 meters). River substrates are primarily mixtures of sand, gravel, and cobble, with occasional boulders, woody debris, submerged and emergent aquatic beds, and nearshore clay and silty sand benches. Major tributaries (upstream to down) include the Little Muskingum River, Duck Creek, Muskingum River, Little Kanawha River, Little Hocking River, Hocking River, and Lee Creek.

Over 100 species of fish are known to inhabit the West Virginia portion of the Ohio River, and many if not all of these species likely occur in the Belleville Pool. From 1980 to 2000, 35 species of native mussels and the zebra mussel have been collected either alive or freshly dead in the Belleville Pool.

The floodplain through this reach of the Ohio River includes urbanized areas such as the cities of Marietta OH, Williamstown WV, Belpre OH, and Parkersburg WV; agricultural areas and rural settlements; industrial facilities such as Eramet, Chevron, Marietta Industrial Enterprises, DeGussa, DuPont, GE Plastics, Huntsman Chemical, Kraton Chemical; and some tracts of wetlands and undeveloped bottomland hardwood forests. Federally listed species in this reach include the bald eagle (*Haliaeelus leucocephalus*), Indiana bat (*Myotis soldalis*); Pink Mucket, Fanshell, and Sheepnose mussels.

The aquatic resources in the Belleville Pool experienced further insult during a winter flood event in January 2005. A towboat pushing barges upstream out of the Belleville Locks lost control, and the barges broke away and wedged into the gates of the dam. As the water levels fell, the gates could not be closed to hold normal pool level. The pool dropped for a period of almost 30 days, and as the pool dropped, aquatic habitat was exposed to drying and freezing conditions (Figure 2). By the time the gates were cleared and could be closed, the river had dropped 18 feet in the lower reaches of the pool, and had started to expose mussel beds at the upper reach near Buckley Island (RM 169). Thousands of mussels, snails, and other invertebrates died, as well as some fish in the tributaries.

Figure 2. The few remaining mussels located at the head of Blennerhassett were further insulted when the pool level dropped during freezing conditions due to runaway barges blocking three gates open on the Belleville Dam, January and February 2005. Clayton Photo

RESTORATION PLAN

In 2007 a Technical Committee was convened to assist the Trustee Council with advising, evaluating, developing, and implementing the technical aspects of the restoration effort. Initial participants on the Technical Committee included:

Dave DeVault, USFWS, Region 3 Contaminants Program
Patricia Morrison, USFWS, Biologist, Ohio River Islands NWR
Dr. G. Thomas Watters, Columbus Zoo and OSU Center for Mussel Conservation (OSUMCC)
John Navarro, ODNR Ohio Division of Wildlife Program Administrator
Scott Morrison, WVDNR, District VI Fisheries Biologist
Janet Clayton, WVDNR, Mussel Program Biologist
Barbara Douglas, USFWS, Elkins, Endangered Species Biologist
Dr. Catherine Gatenby, USFWS, White Sulphur Springs National Fish Hatchery (WSSNFH) Manager
Tony Brady, USFWS, Genoa NFH (GNFH) Biologist

Due to the extended timeframe of the project and the extensive work conducted, additional technical committee members and partners were added later. These included:

Nathan Eckert, USFWS, GNFH Biologist – Replaced Tony Brady
Jeremy Tiemann, Illinois Natural History Survey
Rachael Mair, USFWS, WSSNFH Mussel Propagation Specialist
Keith McGilvray, USFWS, WSSNFH Project Leader -- Replaced Catherine Gatenby)
Deborah Millsap, USFWS, Ohio Field Office – Replaced Dave DeVault
Tyler Hern, USFWS, WSSNFH, Biologist (Replaced Rachael Mair)
Pennsylvania Fish and Boat Commission
The Wilds (a private conservation organization in central Ohio)
USEPA Wheeling scientific dive team
Monte McGregor, Kentucky Center for Mollusk Conservation (KCMC)
Tennessee Tech University Cooperative Fisheries Research Unit (TTU)
Dr. Chris Barnhart, Missouri State University
Dale Hollow and Wolf Creek National Fish Hatcheries in TN
USFWS Ecological Service Field Offices in WV, PA, OH, and KY.

Restoration Goals

The restoration plan was designed to restore mussels, snails, and fish populations to the geographic area in which the natural resource injury occurred. Injuries to fishery resources were localized within a relatively small area. Although significant numbers of fish were killed, the trustees believed the injured fish species (primarily freshwater drum) had already recolonized the impacted area to pre-release conditions. Therefore, the trustees proposed to restore historic native Ohio River fish species that had not fully recovered from past ecosystem problems, such as shovelnose sturgeon (*Scaphirhynchus platorynchus*), lake sturgeon (*Acipenser fulvescens*), blue catfish (*Ictalurus furcatus*), blue sucker (*Cycleptus elongates*), speckled chub (*Macrhybobsis aeslivalis*), and big river perch species including channel darter (*Percina copelandi*), river darter, (*Percina shumardi*), crystal darter (*Ammocrypta asprella*), walleye, and longhead darter (*Percina macrocephala*).

Mussel and snail restoration was to first target mussel beds at Site 11 and Neal Island between RM 176.9 and 181. These two mussel beds experienced greater than 99% mortality due to the toxic release. If additional money became available through matching grants or other means, additional historic mussel bed locations between RM 176.9 and the Belleville Dam were also to be actively restored or augmented. Pre-kill mussel community density at Site 11 and Neal Island mussel beds exceeded five mussels per square meter ($5/m^2$). The proposed action was not expected to fully restore the two mussel beds to pre-kill densities, but in the short-term would aid in recreating a minimally viable and ecologically sustainable mussel community. Long-term restoration to pre-kill densities would rely on natural recolonization from other Ohio River and tributary mussel resources, successful reproduction by restored species, and most importantly continued improvements to the physical and chemical habitat at these two mussel beds as mussels become reestablished.

The presence of mussels helps stabilize the substrate and increase the likelihood of colonization by other invertebrates including other mussels. By anchoring into the substrate, mussels help promote aeration of the substrate. As filter feeders, they convert planktonic food to a form available to other benthic invertebrates and fish. Mussels also filter large quantities of suspended materials (silt, sediment, etc.) from the water column and help purify the water. Their shells are colonized by other macroinvertebrates and snails that provide food for fish. Over time, as the physical, chemical, and biological habitat of the mussel beds improves, natural recolonization by mussels and other aquatic species is more likely to occur.

The targeted restored density was set at one mussel/m^2 or 20 percent of the pre-kill density. This targeted density was based on the consensus of malacologists at the time of a minimum ecologically significant mussel community in the mid-Ohio River. If additional money were to become available through matching grants or other funds, then the target restoration density would be increased through additional stocking and relocation to approach the pre-kill mussel density of $5/m^2$. The targeted restoration density for aquatic snails was $80/m^2$, which is the mean number of live snails collected in quantitative surveys upstream of the Eramet facility at Muskingum Island prior to 2000.

Individuals of 27 species of native mussels were found dead in 2000 at sampling sites within the Belleville Pool below RM 176.9, although 35 species are known from the pool since 1990. The proposed plan was to actively restore a subset of species known to occur within the Belleville Pool, based on the following criteria: the rarity of the species in the Ohio River (as evidenced by state listings); the importance of individual populations within the Ohio River; habitat preference as big river fauna; the species zoogeography and overall abundance; the species abundance and distribution elsewhere in the mid-Ohio River Basin; availability of and ease of working with broodstock mussels; scientific knowledge of fish hosts; ease or difficulty of holding fish hosts in captivity; and distance to the next known population source.

Methods

Fish Restoration Strategies

Fish recovery efforts were to focus on: (I) propagation and release of a suite of big river fish species that historically inhabited the Ohio River; and (2) release of host fish species used for mussel restoration. Big river fish species that were planned to be restored included the shovelnose sturgeon, lake sturgeon, blue catfish, blue sucker, speckled chub, channel darter, river darter, crystal darter, and longhead darter. The shovelnose sturgeon was selected, for example, because this species historically inhabited the entire reach of the Ohio River within

West Virginia. Over the last 100 years, populations have declined in all historically inhabited water bodies due to degraded water quality and habitat, as well as commercial overexploitation. This resulted in the extirpation of this species from West Virginia by the mid-1900s. With improved habitat conditions and closure of commercial and recreational fishing for this species, interest had been expressed in restoring shovelnose sturgeon, as well as other historic big river species in the Ohio River. Candidate host fish species for mussel restoration included: hybrid striped bass, largemouth bass (*Micropterus salmoides*), bluegill, channel catfish, flathead catfish (*Pylodictis olivaris*), blue catfish, sauger, walleye, white crappie (*Pomoxis annularis*), and logperch (*Percina caprodes*). Fish species used as hosts for mussel restoration were to be raised in hatchery settings and used in mussel propagation either through captive propagation, infest and release, or caged propagation. Shovelnose sturgeon and several species of host fish for mussels would be reared at existing state and federal facilities. To meet this new demand, some renovations to existing state and federal facilities were required. To create a viable shovelnose sturgeon population, the trustees believed 1,000 to 5,000 fingerling shovelnose sturgeon needed to be stocked into the Belleville Pool each year, for ten years. It was planned to rear at least two species of mussel host fish annually at state and federal facilities.

Mussel Restoration Strategies

Active restoration of mussels was conducted by implementation of three different strategies:

- adult mussel translocation;
- infestation of host fish with glochidia (mussel larvae) and subsequent release of the fish directly to the river, or placement of the fish in cages in the river;
- propagation and release of tagged juvenile mussels. All animal translocations and releases followed "Aquatic Nuisance Species and Disease Control Procedures" established by the WVDNR.

The goal of active restoration was to recreate a viable and ecologically stable mussel community. Follow-up surveys at Neal Island and Site 11 in 2002 and 2006 showed that native mussels were recolonizing these two sites naturally. Although only a few young individuals of three species were collected, this indicated the habitat was suitable for mussel recolonization.

For the affected area represented by Site 11 and Neal Island alone, the combined mussel bed surface area was 195,000 m^2; restoration of one mussel per m^2 would require a long-term survival of 195,000 mature (age 5) mussels restored via adult translocation and propagation of juveniles at a hatchery (Southwick and Loftus 2003). Although survival of mussel juveniles varies by species, some general estimates could be used to help establish the number of juvenile mussels that needed to be reared in order to generate the needed number of adults. For example, if the cumulative mussel survival rate is only 1 percent from newly transformed juvenile to age 5 adult, then hatchery facilities must raise 19.5 million juveniles; with a 2 percent survival, the number would be 9.75 million. This illustrates why different strategies were to be used for different species of mussels, and why the restoration project overall was planned for at least ten years.

Translocation of Adult Mussels

This low-risk strategy of directly translocating adult mussels into impacted reaches was only employed in limited instances. Unfortunately because of environmental insults over the last two centuries, most mussel species are not found in adequate numbers within their endemic range to provide transplant candidates in numbers needed for restoration. Additionally, the

translocation of any species represents no net gain to a given water system, but merely reallocates an already depleted resource. However, it was planned to take advantage of mussels from those areas where large scale relocations were already taking place to avoid impacts (e.g., bridge crossings and river dredging); these were opportunities to acquire animals for translocation that needed to be moved anyway. The objective of adult translocations was to stabilize the substrate in the affected areas and make the habitat more suitable for other invertebrates and mussels to co-habit. Species likely to be available in large numbers were Three-ridge, Mapleleaf, Washboard, Mucket, and Spike. Biologists used the appropriate scientific collection and importation permits as needed to collect and transport individuals. Depending on the source site, individual mussels were quarantined at the ORINWR facility or WVDNR's Belleville Complex for an appropriate time period prior to their tagging and release to restoration areas.

Infest and Release of Host Fishes

Most native freshwater mussels require a fish host in order to complete their reproductive cycle. The female mussel siphons sperm out of the water column to fertilize her eggs. The fertilized eggs develop into larval mussels, called glochidia, and then develop into juvenile mussels only after spending some period of time attached as parasites to a fish host. The female mussel expels the glochidia onto a fish, where they attach to the skin, fins, or gills. Some species of mussels require one particular species of fish, whereas other mussel species can use a variety of fish species as hosts. Many species of mussels have adapted techniques to attract fish in order to maximize the likelihood of successful attachment of glochidia to the fish. Once the glochidia are attached, they become encysted and ride around on the fish for a number of weeks or months, getting nourishment from the fish tissue and growing in size. Host fish do not suffer any ill effects from this process. Over time, the glochidia grow, transform into juvenile mussels, and drop off the fish. If the habitat is suitable, the juvenile mussels burrow in and grow into adults.

Host fish were obtained from a hatchery and infested with glochidia. Once the fish were infested, they were released directly to the river in hopes that they would drop off in suitable habitat within the Belleville Pool. It is difficult to measure the success of this technique in terms of actual juveniles produced and settling on the river bottom, but it was hoped to obtain estimates based on parallel cage propagation.

Cage propagation involved placing glochidia infested host fish in cages in the river. The idea was that glochidia metamorphose into juveniles on the fish, and the juvenile mussels drop off and land in the bottom of the cage. The fish are subsequently released to the river and the mussels kept in the cages long enough to grow into a size suitable for direct stocking to the river. This method would allow measurement of the actual number of juveniles produced and stocked.

Hatchery Propagation of Mussels

Under this strategy, mussel broodstock (i.e., gravid females) were brought into captivity in a laboratory or hatchery setting. Glochidia were removed from the female and fish were infested. Fish were then held in tanks in the laboratory or cages within lakes or ponds until the juvenile mussels dropped off. The juveniles were then held in captivity until they grew to sufficient size to tag and then stocked. Using this method it was possible to measure the actual number of juveniles produced and stocked.

Snail Restoration Strategies

Follow up surveys of Site 11 in 2002 and 2006 showed that snails had not begun to recolonize the area. Active restoration of snail populations at Site 11, Neal Island, and possibly other locations within the Belleville Pool were planned to consist of: (I) direct translocation; (2) captive propagation; and (3) egg traps.

Snail Translocation

Similar to mussel translocation, this technique involved moving snails from locations in the main stem or tributaries of the Ohio River where they were plentiful to the restoration areas.

Snail Propagation

Captive propagation involved rearing snails in captivity and releasing young snails to the river. Adult broodstock were collected from the main stem or tributaries of the Ohio River, and transferred to a laboratory or hatchery setting. The snails reproduced in captivity and the young snails were tagged and released to restoration areas. The techniques used for raising snails are less challenging than those for mussels, but it was still believed that it would require substantial effort over a long time period to achieve pre-kill densities of 80 snails per m^2. At least three species were injured by the discharges: *Pleurocera canaliculata*, *Campeloma decisum*, and *Lithasia verrusoca*. Researchers at OSUMCC Research Facility had been experimenting with raising snails, and had good success raising them in aquaria. Since their reproduction in the river is less complicated than that of native mussels, it was believed there would also be substantial on-site recruitment of stocked snails.

Egg Traps

This new technique was evaluated in 2007. Hard substrates (e.g., clay tiles, Plexiglas, and PVC pipe) were placed on the river bed for snails to lay their eggs upon. After two to three weeks, the traps were pulled and checked for the presence of snail eggs. Once colonized by eggs, the traps were moved directly to the restoration sites where the eggs would hopefully hatch and young snails would recolonize the sites. Two such efforts were conducted in 2007 with eggs from lower Belleville Pool (Old Lock 20, RM 202.5) being moved to Site 11.

Mollusk Monitoring

The goal of the monitoring program was to ascertain the status of mussel, and snail communities at the restored beds over a ten-year period. The final monitoring efforts under this restoration program would take place no sooner than five years after the last stocking event. The monitoring sites are all now part of the WVDNR long-term monitoring project. Quantitative monitoring was conducted at approximately 5-year intervals by establishing an 80m by 80m study area and then surveyed using the three random start systematic sampling methodology (Strayer and Smith 2003). In many cases these sites were then further assessed using semi-quantitative transects in an effort to capture more species diversity. Transects were broken into 10m by 1m segments and surveyed perpendicular to flow across the 80m by 80m area. Federally listed species were individually tagged and a subset was placed within a cell and monitored periodically via mark/recapture.

RESULTS

Fish Restoration

Blue catfish broodstock were obtained from Ohio, Kentucky, and Arkansas. A total of 252,996 fingerlings were stocked into the Belleville Pool at 4 locations over 11 years, 2004 to 2015 (Table 1). In 2009, 1236 of those blue catfish were infested with Washboard glochidia and released into the Bellville Pool near Belpre, OH. In 2008, 23 shovelnose sturgeon (10 females and 23 males) were obtained from the Wabash River, IN. After completion of spawning, these individuals were stocked into the Little Kanawha River, a direct tributary to the Belleville Pool of the Ohio River.

Table 1. Number and location of fingerling blue catfish stocked into the Belleville Pool of the Ohio River, Wood County, West Virginia from 2004 through 2016.

Year	Location	Number Stocked
2004	Lock 19	6,085
2006	Williamstown	871
2006	Lock 19	3,514
2006	Williamstown	3,521
2006	Belleville	2,000
2007	Lock 19	5,659
2008	Lock 19	7,957
2008	Williamstown	9,913
2009	Parkersburg	3,300
2009	Williamstown	7,569
2009	Lock 19	7,750
2009	Belleville	1,270
2010	Belleville	10,503
2010	Williamstown	8,222
2011	Belleville	10,802
2011	Lock 19	3,995
2011	Williamstown	3,995
2011	Williamstown	3,995
2011	Williamstown	10,921
2012	Williamstown	15,075
2012	Lock 19	14,950
2012	Belleville	14,028
2013	Williamstown	21,625
2013	Belleville	4,997
2013	Lock 19	4,872
2014	Lock 19	8,687
2014	Lock 19	8,687
2014	Williamstown	5,787
2015	Williamstown	8,060

Table 1. Continued

Year	Location	Number Stocked
2015	Lock 19	8,622
2015	Belleville	5,099
2016	Un-reported	20,665
Total		**252,996**

Host Fish Production

From 2008 to 2016 numerous fish were provided by the WVDNR for propagation efforts. These included reared or purchased largemouth bass, green sunfish, bluegills, channel catfish, blue catfish, and fathead minnows. Additional wild caught host fish were also provided. These included sauger, walleye, creek chubs, stonerollers and mooneye (*Hiodon tergisus*).

The WVDNR did not charge this project for fish supplied or expenses associated with the Belleville Complex but were provided funds in order to contract a fish hatchery engineering firm to prepare specifications for a replacement water supply pump for the Palestine Hatchery. The ODNR provided fish to the OSU facility, and WSSNFH utilized fish from the WVDNR, private hatcheries, and wild caught fish. GNFH obtained freshwater drum a small impoundment at Langston University, Langston OK. Other cooperators made arrangements for their own fish needs and data were not provided.

Mollusk Restoration

Summary

A total of 54,772 mussels of 34 species were either stocked as juveniles (30,259) or translocated as adults (24,513) into the Belleville Pool of the Ohio River from 2007 to 2017. Translocations were either from areas outside the Belleville pool or within the pool from areas outside to the kill-zone. See Table 2 for summary of propagation and recovery of the species known to possibly occur within Belleville Pool. Only five species originally targeted for restoration met their target number to restore. Two of these, Mucket and Spike were met due to adult translocation. The other three met their targets through hatchery propagation of juveniles (Plain Pocketbook, Fat Mucket , and Black Sandshell). Thirteen species were successfully propagated at some level with an additional three species translocated as juveniles obtained as volunteers from propagation cages. By 2016 and 2017 16 species showed some level of natural recruitment at Site 11 (Figure 2) and Neal Island respectively. Since 2008 many restoration activities have been ongoing within the Belleville Pool of the Ohio River. Although all stocking was not funded by the NRDA settlement, they did benefit the overall recovery of the mollusk population within the Pool and their numbers are included in project totals. Besides Site 11 and Neal Island, restoration activities were conducted at Blennerhassett Island, and federally endangered species restoration at Muskingum Island and Buckley Island.

Figure 2. Naturally recruited mollusks observed during monitoring of Site 11, Ohio River, Belleville Pool, Wood County, West Virginia 2012.

Table 2. Species noted as potentially still extant within the Belleville Pool of the Ohio River, Wood County, West Virginia, or those species that were slated for restoration. Green cells are those species in which restoration efforts met or exceeded target levels. Grey cells are those species in which natural recruitment was observed within the kill-zone during post-kill monitoring events.

Species	**Cumulative**		**Total**	**Target # to Restore per EA (2007)**	**Broodstock or Translocated Adult Source Populations Outside of the Belleville Pool**
	adults	juveniles			
Actinonaias ligamentina – Mucket	3,013	373	3,386	2,000	New River, Allegheny River (PA)
Amblema plicata – Threeridge	268	19	287	204,000	
Cyclonaias (=*Quadrula*) *pustulosa* – Pimpleback	153	194	347	15,620	
Cyclonaias tuberculata - Purple Wartyback	7	0	7	0	
Cyprogenia stegaria – Fanshell **FE**	400	0	400	5,000	Licking River (KY)
Ellipsaria lineolata – Butterfly	34	0	34	5,000	
Elliptio crassidens – Elephantear	117	0	117	5,000	Ohio River Greenup Pool
Eurynia (=*Elliptio*) *dilatata* – Spike	3,108	0	3,108	2,000	Allegheny River (PA)
Epioblasma rangiana - Northern Riffleshell **FE**	5,472	215	5,687	0	Allegheny River (PA)
Epioblasma triquetra – snuffbox **FE**	0	0	0	0	
Fusconaia flava – Wabash Pigtoe	348	0	348	0	Allegheny River (PA)
Lampsilis abrupta – Pink Mucket **FE**	21	1,601	1,622	5,000	Tennessee River (TN)
Lampsilis cardium – Plain Pocketbook	86	11,254	11,340	5,000	Elk River, South Fork Hughes River, Middle Island Creek, Allegheny River (PA)
Lampsilis ovata - Pocketbook	41	0	41	0	Elk River, Allegheny River
Lampsilis siliquoidea – Fatmucket	226	7,970	8,196	5,000	South Fork Hughes River, Fishing Creek, Allegheny River (PA)
Lasmigona complanata – White Heelsplitter	37	0	37	0	
Lasmigona compressa - *creek heelsplitter*	0	0	0	0	
Lasmigona costata - Flutedshell	80	0	80	0	Allegheny River (PA)
Leptodea fragilis – Fragile Papershell	19	0	19	0	Allegheny River (PA)
Ligumia recta – black sandshell	77	5,748	5,825	5,000	Elk River, Upper Kanawha River, Allegheny River (PA)
Megalonaias nervosa – Washboard	53	3	56	16,500	
Obliquaria reflexa – Threehorn Wartyback	354	0	354	0	
Obovaria subrotunda – Round Hickorynut	0	43	43	2,000	South Fork Hughes River, Middle Island Creek, McElroy Creek, Upper Kanawha River, Elk River
Plethobasus cyphyus – Sheepnose **FE**	4	0	4	5,000	Ohio River Greenup Pool, Upper Kanawha River
Pleurobema clava - Clubshell **FE**	9,432	104	9,536	0	Allegheny River (PA)
Pleurobema cordatum – Ohio Pigtoe	32	0	32	5,000	Ohio River Greenup Pool

Table 2. Continued

Species	Cumulative		Total	Target # to Restore per EA (2007)	Broodstock or Translocated Adult Source Populations Outside of the Belleville Pool
Pleurobema sintoxia -- Round Pigtoe	0	0	0	0	
Potamilus alatus – Pink Heelsplitter	916	1,611	2,527	7,700	Allegheny River, PA
Potamilus ohiensis – Ohio Heelsplitter	3	0	3	0	
Pyganodon grandis – Giant Floater	1	0	1	0	Allegheny River (PA)
Quadrula quadrula – Mapleleaf	35	98	133	53,480	Middle Fork Lee Creek, Ohio River Willow Island Pool, Allegheny River (PA)
Theliderma (=Quadrula) metanevra – *Monkeyface*	39	0	39	16,150	Ohio River Greenup Pool
Toxolasma parvus - Lilliput	0	9	9	0	Ohio River Willow Island Pool
Truncilla donaciformis – Fawnfoot	7	0	7	5,000	Little Kanawha River, Allegheny River (PA)
Truncilla truncata - Deertoe	16	0	16	5,000	Little Kanawha River
Utterbackia imbecillis – Paper Pondshell	0	69	69	0	Ohio River Willow Island Pool
Utterbackiana (=*Anodonta*) *suborbiculata* – Flat Floater	0	2	2	0	Ohio River Willow Island Pool
Lithasia armigera - Armored Rock Snail	0	946	946	0	Mississippi River and Ohio River, IN
Lithasia verrucosa --					
Pleurocera caniculata --					
TOTAL	**24,513**	**30,259**	**54,772**	**374,450**	
FE = federally endangered					

As would be expected, short-term brooders (mussels which breed over a very short time frame) were much more difficult to propagate and the river did not always cooperate when the mussels were expected to be gravid. While diving was the preferred method for collection of broodstock, some years collections had to be made via brailing due to poor river conditions. A few years, the river was in such poor condition that some mussel species did not appear to spawn. Only four of the propagated juvenile species were short-term brooders. Over the years of the project, much was learned in developing the methodology for collecting and handling gravid short-term brooders. The first few years, learning the time of year and temperature needs for gravid species was critical. Then years following, this time period was targeted for collection of gravid broodstock. A few individuals would be collected and their gravid condition assessed. If only eggs or young embryos were found then the mussels were returned and depending on what stage of development was found, additional mussels would be collected the next week or later if so determined. Once viable glochidia were observed, additional collections would be conducted to collect adequate numbers of broodstock to send to hatchery cooperators. The best methodology was to allow the mussels to expel their larvae rather than physically extract them. This tended to allow for collection of more mature larvae. Too much poking and prodding would cause the mussels to abort and expel larvae, in many cases before they were fully mature. Much of the holding to assess gravid conditions was conducted either at the ORINWR lab and later at the WVDNR Belleville Complex. Additionally, one of the common problems experienced was the difficulty in holding host fish in stressful summer conditions for the amount of time needed for the summer spawning mussels to transform. For example, in 2016 Elephantear (*Elliptio crassidens*) broodstock was collected for WSSNFH. Seven days after infesting wild collected Mooneye (*Hiodon tergisus*), the fish died.

No monitoring sites, including the upstream reference sites at Muskingum and Buckley Islands met the target goal of 80 snails/m^2. Follow-up surveys of Site 11 during 2002 and 2006 showed that no snails had yet recolonized the area, but 2007 surveys revealed that they are finally starting to come back. Although snails continued to make a slow recovery post-kill, they showed a decline since 2012 monitoring efforts. This may be due to the impact from zebra mussel populations or the numerous high flow events. Zebra mussels continue to settle and grow episodically in the Belleville Pool, and their colonization of the smaller mollusks (i.e., the snails) hampers their mobility, energy reserves, and survival. Site 11 snail density was 4 species at 5.5/m^2 in 2012 and dropped to 3 species at 1.9/m^2 in 2017. Muskingum Island reference site had 3 species at 5.3/m^2 in 2012 and 2 species at 3.3/m^2 in 2017. Neal Island restoration site had 2 species at 19.2/m^2 in 2010. While 3 species were observed in 2016, density dropped to 6.6/m^2. The next quantitative monitoring for the primary restoration site at Neal Island is scheduled for 2021. Additional monitoring for the Site 11 restoration site and reference site at Muskingum Island is scheduled for 2022. The most recent monitoring was conducted at the restoration site at the head of Blennerhassett Island in 2018 and data were included below.

Translocation of Adult Mussels

Development of an Aquatic Nuisance Species and Disease Control Plan was developed. There were concerns over the introduction of aquatic nuisance species, fish related diseases (notably VHS, which was documented in the Great Lakes Watershed), and non-endemic genotypes of native species. The WVDNR guidelines and procedures were applicable to the restoration being implemented. A draft document was circulated in April 2007. Because of these concerns, all mussels translocated from the Allegheny River in PA had to undergo a disease quarantine procedure based on USGS published parameters. The timeframe was further reduced following additional studies at Ohio State University.

Translocation of adults began in 2007 in conjunction with ongoing mussel relocation for the East Brady and Foxburg bridges in the Allegheny River, PA. The majority of the work was done at no cost to the Restoration Fund, as the mussel collecting and relocation was required under another program. Approximately 5000 common mussels were relocated from the primary impact area of the two new bridges, and were subsequently stocked at Site 11 after going through 15-day quarantine at the WVDNR Belleville property. The mussels were tagged and hand-placed in the substrate at an approximate density of $4/m^2$. Other mussels relocated to Site 11 came from the refuge property, and other mussels which were rescued from the Belleville Pool drawdown in 2005 and held at the OSUMCC. Survival was low for the rescued mussels and those that survived were moved back to the Pool in 2006. During 2008, an additional 1000 mussels were moved from the Willow Island Dam tailwaters (upper Belleville Pool) due to hydropower development.

Other translocations occurred throughout the project and included Muckets from the Pugh Bridge replacement project on the New River to the head of Blennerhassett. This translocation was only conducted following a genetic study to determine individuals were of similar genetic stock. Additional mussels were moved from the Allegheny River prior to commercial sand and gravel dredging operations. Mussels were translocated from the North Fork Hughes River prior to bank stabilization and channelization efforts. Elephantear were translocated from the Ohio River, Greenup Pool. Fanshells were translocated from the Licking River in KY to augment populations at Muskingum Island and in the lower Muskingum River in OH.

Identification of Fish Hosts

For many of the mussel species targeted for restoration, their fish host(s) was not yet known; for other species, a suite of potential hosts had been identified but the most effective or practical host for large scale propagation was not known. Over the life of the project, cooperators conducted host fish studies on 15 mussel species. Data are provided in Table 3. Sometimes the best host fish with respect to produced transformers may not be the best host for production which needed to be determined.

Table 3. Fish host identification studies were undertaken as part of the Ohio River NRDA restoration project 2007 to 2014.

Species	Lab	Year	Fish Attempted	Host Confirmation
Amblema plicata	OSUMCC	2007	yellow perch, bluegill	Affirmative
Amblema plicata	OSUMCC	2010	largemouth bass	Affirmative
Amblema plicata	OSUMCC	2010	green sunfish, channel catfish, largemouth bass, pumkinseed	Negative
Cyclonaias pustulosa	OSUMCC	2007	channel catfish, blue catfish	Affirmative
Cyclonaias pustulosa	WSSNFH	2011	channel catfish *, crappie	Affirmative
Ellipsaria lineolata	WSSNFH	2009	bluegill, largemouth bass	Affirmative
Ellipsaria lineolata	WSSNFH	2009	green sunfish, fathead minnow	Negative
Ellipsaria lineolata	OSUMCC	2014	sauger, bluegill, freshwater drum	Negative
Elliptio crassidens	OSUMCC	2013	madtom catfish, sunfish, blacknose dace, stoneroller, striped shiner, largemouth bass, fathead minnow	Negative
Lampsilis cardium	OSUMCC	2009	largemouth bass*, green sunfish*	Affirmative
Lampsilis cardium	OSUMCC	2009	bluntnose minnow, bluegill, longear sunfish	Negative
Lampsilis cardium	OSUMCC	2010	largemouth bass	Affirmative
Lampsilis siliquoidea	OSUMCC	2011	largemouth bass	Affirmative
Ligumia recta	OSUMCC	2008	largemouth bass	Affirmative
Ligumia recta	OSUMCC	2008	rainbow darter, spotted darter, tadpole madtom, bluegill, shovelnose sturgeon, mottled sculpin, striped shiner, redbelly dace, channel catfish	Negative
Ligumia recta	OSUMCC	2009	largemouth bass*	Affirmative
Ligumia recta	OCUMCC	2009	rainbow darter, madtoms, bluegill, shovelnose sturgeon, spotted darter, mottled sculpin, striped shiner, red belly dace, channel catfish, rosefin shiner, yellow bullhead, black bullhead	Negative
Ligumia recta	OSUMCC	2010	largemouth bass	Affirmative
Megalonaias nervosa	GNFH	2008	blue catfish	Affirmative
Megalonaias nervosa	OSUMCC	2008	bluegill, mottled sculpin, redbelly dace, spotted darter, rainbow darter, bluntnose minnow, rosefin shiner, striped shiner, goldfish, spotted sunfish	Negative
Megalonaias nervosa	OSUMCC	2008	green sunfish, yellow and black bullhead catfish, studfish, shovelnose sturgeon, striped shiner, largemouth bass	Affirmative
Megalonaias nervosa	WSSNFH	2009	white crappie*, blue catfish*, channel/blue catfish hybrid*, channel catfish, bluegill	Affirmative
Megalonaias nervosa	OSUMCC	2009	black bullhead	Affirmative

Table 3. Continued

Species	Lab	Year	Fish Attempted	Host Confirmation
Megalonaias nervosa	OSUMCC	2009	green sunfish, spotted sunfish, shovelnose sturgeon, bluntnose minnow, bluegill, rosefin shiner, largemouth bass, red belly dace, spotted darter, mottled sculpin, rainbow darter, studfish, longear sunfish, madtom, drum	Negative
Megalonaias nervosa	WSSNFH	2011	green sunfish/bluegill, channel catfish, crappie	Affirmative
Megalonaias nervosa	OSUMCC	2011	common shiner, creek chub, northern hogsucker, longear sunfish, blacknose dace, fathead minnow, white sucker, channel catfish, largemouth bass, black bullhead catfish	Negative
Obovaria subrotunda	WSSNFH	2011	fantail, banded darter, greenside darter, banded sculpin, bluegill, largemouth bass, streamlined chub	Affirmative
Obovaria subrotunda	WSSNFH	2011	blacknose dace, stoneroller, longhead darter, logperch	Negative
Obovaria subrotunda	WSSNFH	2012	fantail darter, rainbow darter	Affirmative
Plethobasus cyphyus	GNFH	2007	stonerollers, creek chubs, fathead minnows	Affirmative
Plethobasus cyphyus	WSSNFH	2009	bluegill, green sunfish, largemouth bass, stoneroller, blacknose dace	Negative
Plethobasus cyphyus	KYDFWR	2011	stonerollers	
Plethobasus cyphyus	WSSNFH	2011	Stonerollers, blacknose dace, bluehead chub, fathead minnows	All fish died
Plethobasus cyphyus	OSUMCC	2011	rainbow darter, blacknose dace, yellow bullhead, emerald shiner, longear sunfish, creek chub, central stoneroller	Negative
Plethobasus cyphyus	WSSNFH	2012	central stoneroller, blacknose dace	Affirmative
Plethobasus cyphyus	WSSNFH	2012	creek chub, fathead minnow, bluegill, channel cat	All fish died
Plethobasus cyphyus	KYDFWR	2012	central stoneroller	
Pleurobema cordatum	WSSNFH	2008	common shiner, spot tail shiner, stoneroller, blacknose dace, rockbass	Affirmative
Pleurobema cordatum	WSSNFH	2008	sculpin	Negative
Pleurobema cordatum	WSSNFH	2009	fathead minnow, green sunfish, blacknose dace*, stoneroller	Affirmative
Pleurobema cordatum	WSSNFH	2009	bluegill	Negative
Pleurobema cordatum	WSSNFH	2011	blacknose dace	Affirmative
Pleurobema cordatum	WSSNFH	2011	fantail darter, stoneroller, bluehead chub, crappie, fathead minnows, bluegill	Negative

Table 3. Continued

Species	Lab	Year	Fish Attempted	Host Confirmation
Pleurobema cordatum	OSUMCC	2010	creek chub, hogsucker rosefin shiner, studfish, bluntnose minnow, largemouth bass, stoneroller, guppies, redeared sunfish, black bullhead catfish, rosefin shiner, bluegill, fathead minnow, yellow bullhead catfish, common shiner, greenside darter, longhead darter	Negative
Pleurobema cordatum	OSUMCC	2011	creek chub	Affirmative
Pleurobema cordatum	OSUMCC	2011	bluegill, green sunfish, largemouth bass, pumpkinseed	Negative
Pleurobema cordatum	OSUMCC	2014	bluntnose minnow, guppies, scarlet fish shiner, creek chub, blacknose dace, white sucker spotfin shiner	Affirmative
Pleurobema cordatum	OSUMCC	2014	striped shiner, red dace, stoneroller, madtom, largemouth bass, emerald shiner, yellow perch, long-eared sunfish	Negative
Pleurobema sintoxia	OSUMCC	2007	largemouth bass	Affirmative
Quadrula quadrula	WSSNFH	2009	channel catfish	Affirmative
Theliderma cylindrica	OSUMCC	2010	striped shiner	Affirmative
Theliderma cylindrica	OSUMCC	2010	creek chub, common shiner, redside dace, hogsucker, rainbow darter	Negative
Theliderma metanevra	OSUMCC	2007	bluntnose minnow, fathead minnow	Affirmative
Theliderma metanevra	WSSNFH	2011	bluegill	Negative

Mussel Broodstock collection and holding

Mussel broodstock was collected from a variety of locations. An attempt was made to collect these individuals from a genetically close location. The priority was to collect broodstock from the Belleville Pool however often times the river conditions were not suitable or species was not at densities making collections inefficient. Many broodstock mussels were collected from the Little Kanawha River Watershed, a direct tributary to the Belleville Pool, and Middle Island Creek, a direct tributary to the Willow Island Pool, the next pool upstream of Belleville. Some broodstock had to be collected from much further abroad. Pink Mucket broodstock originated from the Tennessee River downstream of Pickwick Dam, TN. Although unsuccessful, Fanshell broodstock originated from the Licking River, KY and also was the source for translocated individuals. The Allegheny River, PA was the source of many individuals of many species for translocation and broodstock. Of significance was the number of individuals of Riffleshell and Clubshell that were translocated to avoid impacts from bridge construction and demolition. Location information for broodstock and translocated individuals by species is provided in Table 2.

Early attempts at broodstock collecting involved the sequester of individuals into a “corral” located within the backchannel of Buckley Island behind the refuge headquarters in 11 feet (3.4m) of water. This was undertaken to make it easier to collect certain species, now aggregated, that typically were found in low numbers. The first corral consisted of a cinderblock square. This was eventually replaced by two aluminum frames built by GNFH. The interagency dive team installed them and set a fixed line from shore in order to be able to easily find the animals when needed. These were eventually dislodged by high flow events, one lost and other replaced. When it was again dislodged and finally removed. The corral area then relied on rebar corners and string to delineate the area. The area around the corral was also used to congregate large numbers of mussels, in particular short-term brooders to facilitate quicker collection in the spring. For example:

- over 70 Washboards were collected from the near shore area of Muskingum Island in the Ohio River in September 2008 and placed around the corral. In mid-November, 30 were removed and placed into two four-foot diameter circular tanks with flow-through Ohio River water located at the WVDNR Belleville Complex (Figure 3). At that time, some Washboards were also provided to OSUMCC for their propagation efforts. Continued use of these individuals overtime showed reduced reproductive potential. As Washboards are not a significant component of the mussel diversity in the backchannel, it was thought that fertilization may have been reduced. Whereas the first year, spawning would have taken place at Muskingum Island where Washboards are common.
- In 2009 over 200 Mapleleaf were collected over a large area of the Belleville, Hannibal, and Willow Island pools of the Ohio River and its tributaries. Methods included boat and hand brailing, diving, bucketing, and snorkeling. They were placed into the corral area for host fish inoculation planned for 2010.

Attempts were also made by WSSNFH to hold individuals in captivity in hopes that they would spawn naturally; this was not shown to be a viable option. Long-term brooders collected in the fall of the year were typically held at the WVDNR Belleville Complex, WSSNFH or OSUMCC. As previously noted short-term brooder’s gravid condition was assessed by holding adult mussels at the ORINWR lab or the Belleville Complex. Once determined that mature larvae were being produced they were dispersed to cooperating propagation facilities.

Notes on gravid condition and timing:

- Washboard: mucousy conglutinates were released from December through February at temperatures of approximately 5 to 6^{o}C (Cody Kuehnl, personal communication, OSU doctoral student). Those mussels held at the Belleville Complex were also observed releasing a glochidia filled mesh (Figure 4).

Figure 3. Three upweller holding tanks designed by ORINWR personnel were located at the WVDNR Belleville Complex. They were used for over-wintering broodstock and eventually modified in attempts to grow-out juvenile mussels.

Glochidia Harvest

Long-term brooders are typically very fecund and hold their larvae over an extended period of time. Larvae were easily extracted by prying the mussel open, inserting a plug to hold it open, and then using a water filled syringe to flush out the glochidia. The glochidia were then used to infest the host fish. Short-term brooders are typically less fecund and hold mature larvae for a short period making them more difficult to collect with mature larvae. Short-term brooding mussel species release mature glochidia based on time of year and water temperature. Over the course of this project we were able to refine our ability to predict the general time frame for this release for the few select species we attempted to propagate. This aided our timely collection of gravid broodstock. Even being able to predict the approximate timing of mature larvae did not guarantee collection. For example, in 2011 high river flows made early summer collection very difficult. We had to time collections between high flow events. In 2012, spring came extremely early causing water temperatures to rise quickly. Temperatures at the Site 11 restoration area rose over 10^{o}C from 5 Mar to 25 Mar 2012 before stabilizing and then continuing to follow the 2011 trend but at a higher level.

Figure 4. *Megalonaias nervosa* glochidia and associated mucous matrix being siphoned from upweller tanks at the WVDNR Belleville Complex.

After numerous attempts to collect mature larvae, we found that for short-term brooders such as Threeridge, Ohio Pigtoe, Wartyback, Monkeyface, and Mapleleaf; the easiest method for collection of glochidia was to allow them to abort. A small number of animals were collected and held in aerated containers typically in Ohio River water at the ORINWR lab and later the Belleville Complex. Usually overnight the females aborted a portion of their brood. The brood was then checked for stage of maturity. This may have been from egg stage to fully mature glochidia. A glochidium is determined to be mature if its valves are ajar and snapping occurs. A mature glochidium also typically snaps voluntarily or as a result of salt shocking. If the glochidia were not mature then the mussels were returned to the river, at which time the next collection was conducted the following week and the process repeated. Once a number of mature larvae were observed, a larger number of animals were collected, held, and allowed to abort their brood. Glochidia release and subsequent infestations may occur over a series of days. The WVDNR's method was then to collect the aborted larvae, transport to PFH, where a glochidia soup was made in an aerated bucket to which the host fish were introduced. Inoculation time depended on the infestation rate. A fish was checked periodically to determine if infestation was occurring. During potential reproductive periods of short-term brooders, facilities needed to be ready to respond at a moment's notice when glochidia were mature as the window was usually very short. In a few cases, aborted larvae were transported to cooperating propagation facilities but in most cases a new batch of potential broodstock was collected and shipped or transported.

In 2010 during one inoculation event, transportation temperature change became an issue. The weather was hot and it took a minimum of 45 minutes to drive from ORINWR lab to PFH. The continued viability of the glochidia was in question. Potential for zebra mussel contamination was the primary reason for not transporting the adult broodstock to PFH. Likewise collection of broodstock during those same hot periods required containers of ice to be placed in coolers to maintain stable temperatures. Reducing the temperature of transportation water following collection of broodstock was thought to reduce stress and inhibit premature release of glochidia.

This appeared to reduce glochidia abortions. The duration of time between inoculation and caging of host fish was shortened to reduce stress on the host fish and to capture the early release of juvenile mussels. We found hosts for Threeridge began releasing juvenile mussels within one week.

One of the limiting factors for captive propagation of Pink Heelsplitter, Fragile Papershell, Fawnsfoot, Deertoe and Butterfly *was* the lack of any reliable supply of captive-cultured freshwater drum. Drum is the sole host for these species. GNFH had access to cultured freshwater drum and thus Ohio River broodstock of Butterfly and Pink Heelsplitter were shipped to them and they in turn shipped back newly transformed juveniles to various cooperators for grow-out. This was initiated in 2012. GNFH also transformed and shipped Black Sandshell.

Cage Culture

As the WVDNR did not have a mussel hatchery facility, their method of propagation was to rely on cage culture. During spring 2007, WVDNR biologists Janet Clayton and Scott Morrison traveled to GNFH to observe and participate in various aspects of captive propagation of native mussels, including inoculation of fish with mussel glochidia, captive care of fish, putting fish in cages in river environments, and checking cages used for growing out sub-adult mussels from previous years' propagation. In March 2008, with the assistance of GNFH, 11 cage support racks were installed under the floating docks at Stonewall Jackson Resort State Park marina, Lewis County, West Virginia. The first fish were inoculated on 15 April and placed into cages at the marina on 29 April.

Limited propagation success was observed at Stonewall Jackson marina. Issues included prop-wash from nearby boat traffic causing some of the mussels to be expelled from their cages, one cage was found completely devoid of sand and mussels. The effects of prop-wash were also seen in the distribution of juvenile Fat Muckets; while Fat Mucket infested fish were placed into four cages, they were harvested from eight cages. It was later reported by the WVDNR District Fish Biologist that the lake stratifies during the summer with the lower levels being nearly devoid of oxygen. Further impacts to cage culturing appeared to have resulted from discharges from docked houseboats as evidenced by black, septic looking material located in several of the harvested cages in 2010. An attempt was then made to relocate all racks and associated cages to the upstream area of the dock. The other significant issue with propagation success appeared to be a result of poor fish health following the stress of fish capture and inoculation. In 2010 this stress was attempted to be reduced by caging the fish as soon as possible following inoculation rather than holding them for a time. Stress was not the only reason for the change to caging earlier; as it was noted in our 2009 efforts that Threeridge does not remain on the fish for more than one or two weeks. ORINWR personnel held a couple bass in an aquarium and within a week viable juveniles had been released. Those fish slated for caging were still probably in the hatchery at the time. The 2008 caging efforts resulted in the harvest and subsequent stocking of the first propagated juveniles (Fat Mucket and Plain Pocketbook) for this project in October 2009 at Site 11. The WVDNR continued with caging efforts (Figure 5) until 2014 at which time it was decided resources would be best used to benefit the project elsewhere.

In contrast to the WVDNR efforts, OSUMCC had great success in cage culture in the embayment behind their facility. In 2009 the facility produced Black Sandshells, Plain Pocketbooks and Washboards. 2009 methods involved grow-out of the juveniles in cages, "Barnhart buckets," a PVC/mesh compartment placed in an established snail tank with flow through river water, and a recirculating self-contained plastic cylinder with flow through river water with cages demonstrating the only success. WSSNFH had good success with cage culture in several ponds in Greenbrier County. Cage culturing was also the preferred method for

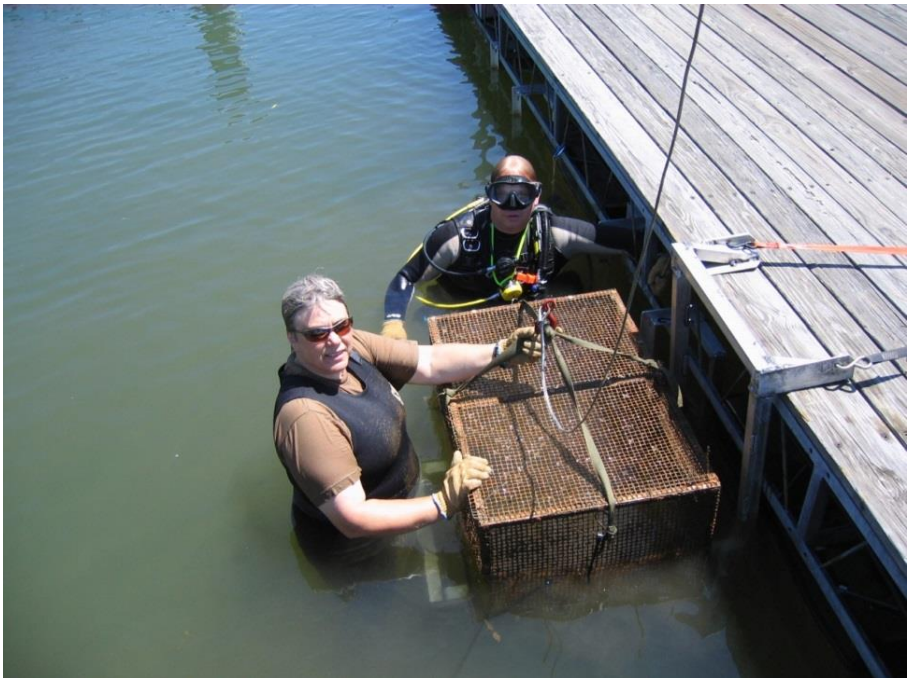

Figure 5. WVDNR biologists, Janet Clayton and Mike Everhart, retrieving cages from under the dock at SJSP to harvest mussels, 24 Jun 2009.

Black Sandshell and Pink Muckets provided by TTU. It appeared that cage cultured juvenile mussels had faster growth rates than lab reared juveniles. Only three species, all long-term brooders, reached their target numbers to restore through propagation efforts, and largely due to cage culturing success.

In June 2009, eight cages were placed in the Ohio River within the backchannel of Buckley Island. The cage legs with attached bottoms were embedded in the substrate, and the cages subsequently attached to them. They were placed near the island shore in approximately 8 ft (2.5 m) of water. Blue catfish inoculated with Wartyback were placed into one of the cages and the other seven cages received largemouth bass, which had been inoculated with Threeridge. Current breaks were attached to the upstream end of each cage. Rebar (4 ft lengths with 2 ft exposed) was placed in front of the cages, slanting toward the cages, to deflect potential debris moving downstream. In October 2009, two of the cages where checked for juvenile mussels. No propagated mussels were found although numerous *Corbicula* were observed. The cages were removed from their bases and stored. The bases were left in place on the bottom of the river. The remaining cages were left in place over-winter. In June 2010, WVDNR and ORINWR personnel attempted to locate the remaining six cages. Only one was recovered and contained no native juvenile mussels. The other five had apparently been washed out by high flows.

Captive Propagation – Hatchery Production

Several cooperating hatcheries had mixed success with propagating mussels for restoration of the Ohio River. The main producer was WSSNFH. Facilities like GNFH, Missouri State University, and KCMC worked with a limited number of fewer species. While success was slow, much was learned in the propagation of short-term brooders. Table 4 provides a list of the 24 species in which propagation efforts were attempted. Of the 24 species attempted success was only achieved with 14 species.

In-vitro Propagation

KCMC joined the project in 2014 providing the expertise for propagation using *in-vitro* technology. They were able to transform juveniles of Fanshell, Sheepnose, Round Hickorynut, and Elephantear but no juveniles survived to stocking. However, as part of another project, they were successful in the *in-vitro* propagation of the critically endangered Catspaw (*Epioblasma obliquata*) that was reintroduced into the backchannel of Buckley Island in 2017.

Table 4. Results of freshwater mussel propagation attempts for the Ohio River NRDA 2008 to 2017. Attempts were by fish host inoculations unless otherwise noted. i =*in-vitro*, f=fish

Species	Broodstock collected	Infestations	Juveniles transformed	Juveniles Stocked
Actinonaias ligamentina	2007, 2008, 2012, 2013	2007, 2008, 2012, 2013	2007, 2008, 2012,2013	2010, 2011, 2014, 2015
Amblema plicata	2007, 2008, 2009, 2010, 2011, 2012	2007, 2008, 2009, 2010, 2011, 2012	2007, 2008, 2009, 2010, 2011, 2012	2011, 2013
Cyclonaias pustulosa	2008, 2009, 2010, 2011, 2012, 2013	2009, 2010, 2011, 2012	2011, 2012	2013, 2014
Cyprogenia stegaria		2014, 2016	2014: 2016 (i&f)	0
Ellipsaria lineolata	2009, 2011, 2010, 2012, 2013, 2014, 2015	2009, 2011, 2012, 2013, 2014, 2015	2009, 2012, 2013, 2015	0
Elliptio crassidens	2012, 2013, 2015, 2016	2013, 2016-fish died at 7 days	2016i	0
Epioblasma obliquata			2016i	2017
Epioblasma rangiana				2015, 2016
Lampsilis abrupta		2011, 2012, 2013, 2014, 2015, 2016	2011, 2012, 2013, 2014, 2015(i&f), 2016(i&f)	2013, 2014, 2015, 2016, 2017
Lampsilis cardium	2008, 2009, 2010, 2011, 2012, 2013	2008, 2009, 2010, 2011, 2012, 2013	2008, 2009, 2010, 2011, 2012, 2013	2009, 2010, 2011, 2012, 2013, 2014, 2015
Lampsilis ovata	2013	2013		0
Lampsilis siliquoidea	2008, 2009, 2010, 2011, 2012, 2013	2008, 2009, 2010, 2011, 2012, 2013	2008, 2009, 2010, 2011, 2012, 2013	2009, 2010, 2011, 2012, 2013, 2014, 2015
Lasmigona costata		2013		0
Ligumia recta	2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016	2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016	2008, 2009, 2010, 2012, 2013, 2014, 2015, 2016	2010, 2011, 2014, 2015, 2016, 2017
Megalonaias nervosa	2007, 2008, 2009, 2010, 2011, 2012, 2013, 2016	2008, 2009, 2010, 2011, 2012, 2013, 2016	2008, 2009, 2011, 2012, 2013, 2016	2010, 2011
Obovaria subrotunda	2011, 2012, 2013, 2014, 2015, 2016	2011, 2012, 2013, 2014, 2015, 2016	2011, 2012, 2013, 2014, 2015: 2016(i&f)	2013
Plethobasus cyphyus	2008, 2009, 2010, 2011, 2012, 2013, 2014, 2016	2011, 2012, 2013, 2016	2011, 2012: 2014, 2016 i	0
Pleurobema clava				2016
Pleurobema cordatum	2008, 2009, 2010, 2011, 2012, 2013, 2014	2008, 2009, 2010, 2011, 2012, 2013, 2014	2008, 2009, 2011, 2012, 2014	0
Potamilus alatus	2008, 2011, 2012, 2013	2011, 2012, 2013	2011, 2012, 2013	2012, 2013, 2014, 2015
Quadrula quadrula	2008, 2009, 2010, 2011, 2012, 2013, 2015	2009, 2010, 2011	2009, 2011	2011, 2012
Theliderma metanevra	2008, 2009, 2010, 2011, 2012, 2013	2009, 2010, 2011, 2012, 2013		0
Truncilla donaciformis	2014	2014		0
Truncilla truncata	2010, 2014, 2015	2014, 2015		0

Snail Propagation

Only one cooperator worked on propagation of snails for recovery, OSUMCC. They built a new snail culture tank system and USFWS divers provided them with 110 adult Varicose Rocksnails

(*Lithasia verrucosa*) in early November 2008 to begin testing the new system. Survival was good to 2009, and then a system failure caused a loss of most. Upon fixing the problem, a second group of snails of two species were received from Illinois Natural History researchers working in the lower Ohio River, Olmstead Pool: approximately 100 Varicose Rocksnails and 200 Armored Rocksnails (*Lithasia armigera*). These snails began actively feeding on algae in the snail culture system and were not expected to breed until summer.

The original snail propagation system consisted of a 400 gallon tank with a self-contained flow-through, high velocity water system including a chiller unit and bio-ball tower for water purification. Within the tank were numerous 6" diameter sections of PCV pipes. The pipes significantly increased the available surface area and were able to be removed to count snails and egg masses. For algal growth, four grow lights were mounted on a spinning "propeller." The propeller served to evenly distribute the light and cool the high intensity bulbs.

Unfortunately by 2010 90% mortality in the Varicose Rocksnails and 83% mortality in the Armored Rocksnails (no eggs or juveniles produced) was experienced. Given the facilities success with related species, this was unexpected. It was possible that the smooth plastic bottom of the tank did not give the snails any purchase when attempting to right themselves if they fell from the PVC columns. Waste also had accumulated in the bottom. Snails showed a marked preference for the high velocity water of the water inlet, ignoring the majority of the remaining tank. OSUMCC modified the holding tank which included a more natural sand/cobble bottom, and increasing water velocity through multiple port manifolds. As of 2011 only 2 Varicose Rocksnails and 4 Armored Rocksnails had survived in captivity. No egg sacs or juveniles.

In October 2013, OSUMCC received a new batch of Armored Rocksnails from the INHS that were collected from a site on the Mississippi River and thought possibly to be an introduced population. The snails were placed into 20-gallon aquaria with natural sunlight. They began laying eggs in February 2014, and continued through May. Some eggs were artificially warmed to about 24-25 ^{o}C whereas others continued in ambient temperatures. Regardless of treatment, eggs hatched after 3-4 weeks once water reached about 15 ^{o}C suggesting hatching occurred when a threshold temperature was reached rather than after a set duration. Most pleurocerids deposit eggs during the summer but OSUMCC's previous work did not observe summer egg laying in this species. Egg laying during the winter in near freezing temperatures was unexpected and may represent a novel adaptation. A total of 765 (2015 cohort) and 181 (2016 cohort) Armored Rocksnails were tagged with gold and copper glitter respectively. Of the 2015 cohort, approximately half were stocked at Site 11 and half were stocked at Muskingum Island. When they were stocked by hand at depth, divers observed them immediately crawling up onto rocks on the riverbed. The 2016 cohort was stocked at Neal Island.

<u>Assisting Future Mussel Restoration Efforts</u>

One of the most important tools used in the initial development of mussel restoration costs for this project was what is known as "AFS Special Publication 30" – Investigation and Monetary Values of Fish and Mussel Kills, published by the American Fisheries Society in 2003 (Southwick and Loftus 2003). This document provided the scientifically accepted guidelines for conducting fish and mussel kill assessments, and estimating replacement costs for those resources lost. However, the data that went into the 2003 version were outdated. This 10-year Ohio River Restoration project had added much more realistic information on the true costs of species-specific mussel replacement, from collecting and holding broodstock, to securing fish hosts, conducting the propagation and growing-out of juveniles to a taggable and stockable

size. The project funding for 2016 included $2500 contributed to the synthesis and writing of an updated version. The final document was published as Southwick and Loftus (2017).

Mollusk Monitoring

Below are the results of the site specific monitoring conducted throughout the project. Site 11 and Neal Island were the original sites slated for restoration activities. Only these two sites exceeded the target goal of 20 mussel species at a mussel density of 1/m^2 by the end of the project. Blennehassett Island, once slated for monitoring recovery via natural recruitment only, underwent active restoration starting in 2013. The Degussa site was monitored for recovery via natural recruitment. Muskingum Island, the original control site, and later Buckley Island were monitored as upstream controls and were the sites primarily used for federally endangered species restoration activities. Snail target levels were not reach at any site, including the control sites and was most likely due to continued impacts from zebra mussel colonization of the pool.

Site 11

The results of the freshwater mussel monitoring conducted at Site 11 (Old Lock 18) are provided in Table 5. Data provided for 2000 are from the initial kill assessment in which there was nearly 100% mortality exhibited (ESI 2000). Only 3 live individuals were observed within qualitative surveys and were located near the river's channel, the toxins hugged the Ohio shoreline. Data provided for 2005 and 2007 were collected as part of pre-active restoration efforts and depict natural recruitment. By 2007 mussel densities had recovered to 7 species at 0.5/m^2. The year 2007 represented the first NRDA project monitoring event. Every third of the first 108 quads were excavated in May and an additional 99 quads were excavated in June for a total area of 51.75m^2 surveyed. Subsequent surveys typically planned for around 120 quad (30m^2) excavations. Additional monitoring surveys were conducted at 5-year intervals (2012 and 2017). This site is now a part of the WVDNR monitoring network and is currently scheduled to be resurveyed in 2022.

Mussel restoration at this site began with the translocation of 4931 individuals representing16 species in 2007. While good records were kept on the number of mussels of each species stocked each year, some data are lacking on numbers stocked at each site. Thus the data provided in Table 6 may not fully represent the number of mussels stocked into Site 11. The mussel community restoration targets for Site 11 were met in 2012 with 22 species at a density of 3.4/m^2. However, in 2017 species richness dropped below target levels to 16. Although significantly less, mussel density continued to remain above target levels with 1.6/m^2. The reduction in species diversity and density observed in 2017 was experienced pool wide and may have been a result of poor reproduction over the high flow, high turbidity years.

Snail density was calculated based on the excavated quads only. Snail monitoring data is provided in Table 7. No snail recovery was observed at Site 11 until 2007. A total of 1089 snails of 3 species were observed in 2007 at a density of 32.3/m^2. It is not known how much of this recovery was the result of the egg traps placed at this site in 2007. Hard substrates (e.g., clay tiles, Plexiglas, and PVC pipe) were placed on the river bed for snails to lay their eggs upon at Old Lock 20, RM 202.5. After two to three weeks, the traps were pulled and checked for the presence of snail eggs (Figure 6). Once colonized by eggs, the traps were moved directly to the Site 11 where the eggs hopefully hatched and young snails recolonized. Two trap translocations were conducted in 2007. Multiple size classes of Silty Hornsnail (*Pleurocera canaliculata*) and a new species, Globe Siltsnail (*Birgella subglobosa*), along with Pointed Campeloma (*Campeloma decisum*) were found at Site 11 in 2007 after placement of the traps. No Rocksnails (*Lithasia spp*.) were encountered. Approximately 382 Armored Rocknails (*Lithasia armigera*) were

stocked at this site in 2015. Subsequent monitoring events again found snails in decline with 5.6/m^2 and 1.9/m^2 found in 2012 and 2017 respectively. Varicose Rocksnails were observed in 2012 and 2017 however only 1 individual of Armored Rocksnail was observed in 2012.

Habitat monitoring was conducted from 2007 to 2017 by conducting random Wolman pebble counts systematically throughout the survey area. Data are provided in Table 8. Figure 7 depicts location of several of the activities carried out at this site over the life of the project.

Figure 6. PVC pipe was colonized with snail eggs at Old Lock 20, RM 202.5, Belleville Pool, Wood County, West Virginia. This and others were taken to Site 11 (Old Lock18) to allow hatching and hopeful recolonization of the site

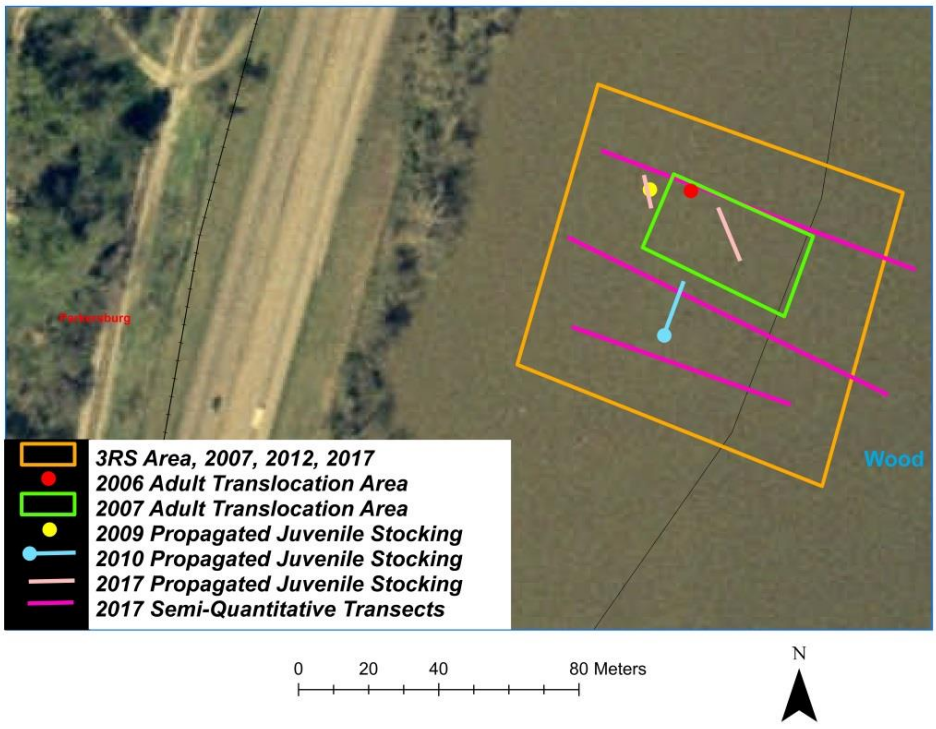

Figure 7. Overall view of restoration and survey efforts conducted at Site 11 (Old Lock 18) on the Ohio River, Belleville Pool, Wood County, West Virginia, 2007 to 2017.

Table 5. Semi-quantitative and quantitative freshwater bivalve data collected from Site 11 (Old Lock 18) on the Ohio River, Belleville Pool, Wood County, West Virginia, 2000 to 2017. The year 2000 was the initial kill assessment. Active restoration began post 2007 assessment. FD=Fresh Dead, WD=Weathered Dead

Species	Quan 2000	SQuan 2000	Quan 2002	Quan 2007	Quan 2012	SQuan 2012	Quan 2017	SQuan 2017
Actinonaias ligamentina		2FD			7	22	5	FD
Amblema plicata	41^{FD}	1, 388^{FD}	21^{FD}	5	1	8	3	3
Cyclonaias pustulosa	4^{FD}	1, 37^{FD}	12^{FD}		7	4	6	4
Cyprogenia stegaria					1		WD	WD
Ellipsaria lineolata		2^{FD}	1^{FD}	2	2	4	2	2
Elliptio crassidens		SF						
Eurynaia dilatata					17	40	7	1
Fusconaia flava		1^{FD}			WD			
Lampsilis cardium		2^{FD}				4	1	6
Lampsilis ovata					1			1
Lampsilis siliquoidea		3^{FD}				3	2	WD
Lasmigona complanata		7^{FD}			1	5		1
Lasmigona costata			1^{FD}			2		WD
Leptodea fragilis	1^{FD}	1^{FD}	2	8	12	5	4	1
Ligumia recta		1^{FD}			3	9	3	10
Megalonaias nervosa	3^{FD}	2, 53^{FD}	4^{FD}		10	15	3	15
Obliquaria reflexa	10^{FD}	9^{FD}	1, 3^{FD}	5	15	9	13	12
Obovaria subrotunda		1^{FD}						
Pleurobema clava	SF						SF	
Pleurobema cordatum	1^{FD}	9^{FD}			WD	6	WD	2
Pleurobema sintoxia							WD	
Potamilus alatus		18^{FD}		3, 1^{FD}	13	69	2	6
Potamilus ohiensis		1^{FD}						
Quadrula quadrula	29^{FD}	156^{FD}	10^{FD}		WD	4	2	5
Theliderma metanevra	2^{FD}	46^{FD}	2^{FD}	1	WD	22	WD	1
Tritogonia verrucosa								
Truncilla donaciformis	13^{FD}	2^{FD}		1	1		WD	
Truncilla truncata	4^{FD}	1^{FD}			1			
Truncilla sp.				1				
Utterbackia imbecillis					3	2		
Number Live (Dead) Species	**0 (10)**	**3(20)**	**2, 7^{FD}**	**7**	**16 (4)**	**18**	**13 (6)**	**15 (4)**
Number Live Individuals (Dead)	**(108)**	**4(744)**	**3**	**26**	**89**	**213**	**53**	**70**
Density (mussels/m^2)	**0 (5.8)**		**0.4**	**0.50**	**3.36**		**1.58**	
Area Surveyed (m^2)	**75**	**~750**	**7.5**	**51.75**	**26.5**	**~600**	**33.5**	**~300**

Table 6. Freshwater mussels stocked at Site 11 (Old Lock 18) long-term monitoring/ restoration site on the Ohio River, Belleville Pool, Wood County, West Virginia 2007 through 2017. The years 2007 to 2009 consisted mostly of translocated adults. The other years were primarily propagated juveniles.

Species	2007	2007	2008	2009	2010	2012	2014	2017
Actinonaias ligamentina (A&J)	1725		10		135			
Amblema plicata (A)	34		177	56				
Cyclonaias pustulosa (A)	15		120	18				
Ellipsaria lineolata (A)	1		33					
Eurynaia dilatata (A)	2799		1					
Fusconaia flava (A)	1		2	2				
Lampsilis cardium (A&J)	65	46	18	1	2008	388	37	
Lampsilis siliquoidea (A&J)	4	47	8	33	112	30		
Lasmigona complanata (A)			23	12				
Lasmigona costata (A)	44		6					
Leptodea fragilis (A)	18		1					
Ligumia recta (A&J)	45		12	10	1			+832
Megalonaias nervosa (A&J)	44		4	5	3			
Obliquaria reflexa (A)			283	69				
Pleurobema cordatum (A)	14		4	3				
Potamilus alatus (A&J)	14		260	406		5	1	
Potamilus ohiensis (A)				1				
Quadrula quadrula (A)	6		11					
Theliderma metanevra (A)	9			4				
Tritogonia verrucosa (A)			1					
Number Species	**16**	**2**	**18**	**13**	**5**	**3**	**2**	**1**
Number Stocked	**4838**	**93**	**974**	**620**	**2259**	**423**	**38**	**+832**

Table 7. Quantitative freshwater gastropod data collected from Site 11 (Old Lock 18) Long-term monitoring/Restoration site on the Ohio River, Belleville Pool, Wood County, West Virginia, 2002 to 2017. The year 2000 was the initial kill investigation.

Species	2000	2002	2007	2012	2017
Burgella subglobosus			4	D	9, 387[D]
Campeloma decisum	93	10[D]	21	D	12[D]
Lithasia armigera				1	
Lithasia verrucosa				18	6, 29[D]
Pleurocera canaliculata	4, 1230[D]	350[D]	1063	130	49, 764[D]
Lithasia spp.	1[D]				
Pleurocera spp.	1[D]				
Unidentified			1		
Number Live (Dead) Species	**1**	**0**	**3**	**3 (2)**	**3 (1)**
Number Live Individuals	**4**	**0**	**1,089**	**149**	**64**
Density (snails/m^2)	**<.01**	**48.0[D]**	**32.27**	**5.6**	**1.91**
Area surveyed (m^2)	**75**	**7.5**	**33.75**	**26.5**	**33.5**

Table 8. Percent composition of Wolman pebble counts conducted at Site 11 (Old Lock18) on the Ohio River, Belleville Pool, Wood County, West Virginia, 2007 to 2017.

Substrate Type	**2007**	**2009**	**2012**	**2017**
Silt and Clay			3	
Sand	21	30	17	23
Gravel	76	68	72	70
Cobble	4	1	7	6
Boulder			1	1
Wood			1	
Number Pebbles Characterized	**324**	**164**	**164**	**117**

Neal Island

Monitoring results conducted at Neal Island are provided in Table 9. Data provided for 2000 are from the initial kill assessment (ESI 2002) in which there was nearly 100% mortality exhibited. Mussels were recovering, at least 5 species, as early as 2005 but was set back by the loss of pool kill event. The monitoring survey conducted in 2010 was done so prior to active restoration efforts and depicts natural recruitment. By 2010 mussel densities had recovered to 10 species at 0.69/m^2. The mussel community restoration density target for Neal Island was met in 2012 with 1.83/m^2. A total of 20 species have been observed since 2010 which meets the diversity target however no single year reported the collection of 20 species. The 2014 data resulted from a survey conducted by ORINWR personnel for the US Army Corp of Engineers (USCOE) who was proposing a bank stabilization project on the island. Subsequently a mussel relocation was authorized. However, one Fanshell (*Cyprogenia stegaria*) was found which put the project on hold. The 2014 data includes those individuals observed during the initial survey as well as those salvaged. The Fanshell observed during the USCOE 2014 salvage effort brings the total species count post-kill at Neal Island to 21. This site is now a part of the WVDNR monitoring network and is currently scheduled to be resurveyed in 2021.

Mussel restoration at this site began with stocking of 2,313 individuals representing 14 species in 2011. While good records were kept on the total number of mussels by species stocked each year, some data are lacking on numbers stocked at each site. Thus the restoration data provided in Table 10 may not fully represent the total number of mussels stocked at Neal Island.

Based on the collection of tagged mussels, 11% of the mussels collected during the 2016 qualitative searches were stocked either as adults or juveniles. Mussels stocked as juveniles in years past showed good growth rates and appeared to be healthy (Figure 8). See Table 11 for percent recapture by species.

Figure 8. Previously stocked mussels collected during 2016 monitoring at Neal Island, Ohio River, Belleville Pool, Wood County, West Virginia. Mussels showed substantial growth since stocked. (Left to right: *Lampsilis siliquoidea, Potamilus alatus, Lampsilis cardium*) WVDNR Photos

Zebra mussel densities were estimated during the 2016 monitoring by weighing the total zebra biomass within 22 quadrat samples. Three 100 gram subsamples were taken from all the zebra mussels collected, and the number of zebra mussels/gram was calculated. This allowed for the estimated density of zebra mussels to be calculated. Density was estimated at 212/m^2 and biomass was estimated at 68.7g/m^2.

Snail recovery was occurring by 2005 as evidenced by the 0.9 snails/m^2 that were observed during the loss of pool kill assessment. The number of snails were counted during transect surveys however they were not identified to species. Snail densities were also estimated during quantitative monitoring events and data are provided in Table 12. In 2010 two species of live snails were also collected and density was estimated at 18.6/m^2. Typically within the Belleville Pool the most common species was the Silty Hornsnail (*Pleurocera caniculata*). While it was indeed the most common snail collected at Neal Island post-kill, 43% of the snails collected in 2010 were Varicose Rocksnails. Following similar trends as other monitoring sites within the pool, snail densities dropped during the 2016 monitoring to 6.6/m^2. While Varicose Rocksnails were still present, their numbers only made up 25% of the snail population. One additional snail species was represented by dead shell only, Globe Siltsnail. A total of 181 Armored Rocksnails were stocked at this site in 2016 (Figure 9).

Habitat monitoring was conducted 2016 by conducting 120 random Wolman pebble counts systematically throughout the survey area. The data showed the site consisted of 51% gravel, 48% sand, and 2& boulder. Figure 10 depicts location of several of the activities carried out at this site over the life of the project.

Figure 9. Propagated juvenile Armored Rocknail (*Lithasia armigera*) tagged and ready for stocking into the Ohio River, Belleville Pool, Wood County West Virginia.

Table 9. Freshwater mussels collected during various survey events at the Neal Island long-term monitoring/restoration site, Ohio River, Wood County, West Virginia. The year 2000 was surveys conducted for the initial kill assessment. The year 2002 was a zebra mussel monitoring event. The year 2005 were dead mussels assessed as part of the loss of pool kill event. The year 2014 are results of the survey/salvage efforts conducted for the USCOE proposed bank stabilization project. The years 2010 and 2016 were long-term monitoring events. (FD=Fresh Dead, WD=Weathered Dead: Quan=Quantitative, SQuan=SemiQuantitative, Salv=Salvage).

Species	**Quan 2000**	**SQuan 2000**	**Quan 2002**	**SQuan 2005**	**Quan 2010**	**SQuan/ Salv 2014**	**Quan 2016**	**SQuan 2016**
Actinonaias ligamentina		FD						2
Amblema plicata	FD	29	2	23 FD	5	49	8	20
Cyclonaias pustulosa	FD	3	3	11 FD	5	60	14	53
Cyprogenia stegaria						1		
*Elliptio dilitata**							3	9
Ellipsaria lineolata		FD				10	1	10
*Fusconaia flava**	WD	FD						3
*Lampsilis cardium**		FD	WD	1 FD		48	6	34
Lampsilis fasciola								1
*Lampsilis siliquoidea**						16		17
Lasmigona complanata		FD	1		1	6		2
Leptodea fragilis				1 FD	2	7	WD	
Ligumia recta			1	2 FD	2	63	7	36
Megalonaias nervosa		1						
Obliquaria reflexa		FD	WD		2	35	2	27
Obovaria subrotunda								1
Pleurobema cordatum						7		2
*Potamilus alatus**	WD	FD		5 FD	3	143	8	10
Potamilus ohiensis					1			
Ptychobranchus fasciolaris						1		
Quadrula quadrula		1	WD		1	6	1	8
Theliderma metanevra	FD	FD	WD			7	4	8
Truncilla donaciformis					1		WD	
Total Number Live	**0**	**34**	**7**	**0**	**23**	**463**	**54**	**243**
Total Live (Dead) Species	**(7)**	**4(8)**	**4**	**(6)**	**10**	**15**	**10**	**17**
Surveyed Area (m^2)	**15.0**	**~600**	**6.0**	**~664**	**33.25**	**~3200**	**29.5**	**~340**
Density Live (mussels/m^2)	**0.0**	**~0.01**	**1.17**	**0.0**	**0.69**	**~0.14**	**1.83**	**~0.71**

* Species stocked as part of NRDA restoration efforts

Table 10. Freshwater mussels stocked at Neal Island long-term monitoring site, Ohio River, Wood County, West Virginia. The year 2011 consisted of translocated adults. J=juveniles, A=adults

Species	2011	2011	2012	2013	2016
Actinonaias ligamentina (A)	43				
Amblema plicata (J)		1			
Cyclonaias pustulosa (J)				163	
Eurynia dilatata (A)	308				
Fusconaia flava (A)	340				
Lampsilis abrupta (J)				11	644
Lampsilis cardium (J)		217		5584	
Lampsilis siliquoidea (A&J)	117	999		6139	
Lasmigona costata (A)	30				
Ligumia recta (A&J)	5				2,221
Obovaria subrotunda (J)				43	
Potamilus alatus (A&J)	142			1329	
Pyganodon grandis (A)	1				
Quadrula quadrula (A&J)	1	64	34		
Toxolasma parvum (J)		9			
Truncilla donaciformis (A)	2				
Utterbackia imbecillis (J)		34		9	
Utterbackiana suborbiculata (J)				2	
Total Number Live Stocked	**989**	**1,324**	**34**	**13,280**	**2,865**
Total Species	**10**	**6**	**1**	**8**	**2**

Table 11. Percent of species represented by stocked mussels, either as adults, juveniles, or both. Individuals were collected during semi-quantitative transect surveys at Neal Island long-term monitoring/restoration site, Ohio River, Belleville Pool, Wood County, West Virginia, July 2016.

Species	Percent of Stocked
Elliptio dilitata	100
Fusconaia flava	100
Lampsilis cardium	17.6
Lampsilis siliquoidea	41.1
Potamilus alatus	27.3

Table 12. Gastropods collected during various survey events at the Neal Island long-term monitoring/restoration site, Ohio River, Wood County, West Virginia. (D=Dead). The year 2000 was survey conducted for the initial kill assessment. The year 2002 was a zebra mussel monitoring event. The year 2005 were dead gastropods collected as part of the loss of pool kill event. The years 2010 and 2016 were long-term monitoring events.

Species	2000 Quan	2002 Quan	2005 SQuan	2010 Quan	2016 Quan
Pleurocera canaliculata	45	88		351	146
Lithasia verrucosa				269	48
Burgella subglubosus					D
Campeloma decisum	D				
Unidentified (most likely *P. canaliculata*)			355[D]		
Snail Density (snails/m^2)	**3.0**	**14.7**	**~0.9[D]**	**18.6**	**6.6**
Surveyed Area (m^2)	**15.0**	**6.0**	**~644**	**33.25**	**29.5**

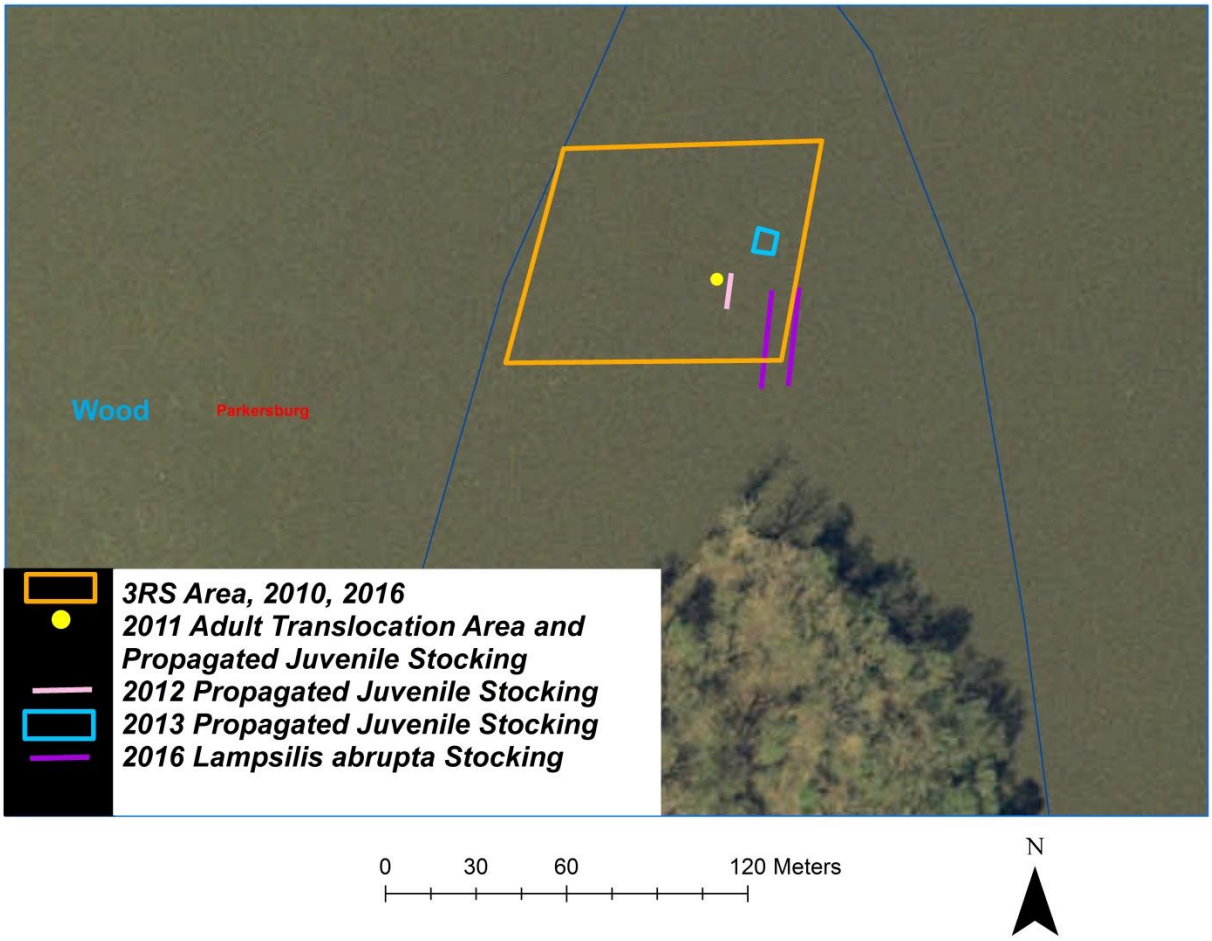

Figure 10. Overall view of restoration and survey efforts conducted at Neal Island on the Ohio River, Belleville Pool, Wood County, West Virginia, 2010 to 2016.

Blennerhassett Island

Blennerhassett Island was impacted by the 1999 kill event with 95.5% mortality observed as of October 2000 (ESI 2002). It was further impacted in 2005 by the loss of pool event with the mussel bed at the head of Blennerhassett Island being one of the mussel beds that was exposed (Figure 11). Significant water level drop occurred over a period of 10 days in freezing conditions. Mussels literally baked in the sun during the day and froze overnight.

In September 2008 baseline monitoring was conducted at Blennerhassett Island. Originally this site was not planned to receive active restoration but allowed to recover naturally. It was believed that data from this site would make a good comparison to active restoration sites, similar to Muskingum Island though this site was within the kill-zone. This site was surveyed in 2008 using the 3RS methodology. Two separate areas were surveyed since after the first 53 quads only one Pink Heelsplitter was observed. Another site was selected and additional 108 quads only resulted in one additional Pink Heelsplitter. Observations indicated possible unstable substrate, due to the lack of mussels, possibly resulting in minimal natural recruitment. It should be noted that the navigational channel passes close by this site. Obviously natural recovery was not occurring at this site similar to other sites pre-restoration and was of concern. Thus in 2013 it was decided to begin active restoration of this site when nearly 1000 adult Muckets from the New River were translocated in hopes that they would aid in stabilizing the substrate and thus improve conditions for natural recovery. A total of 7417 individuals of 9 species were stocked through 2016 (Table 13).

Figure 11. Exposed Blennerhassett Island mussel bed being assessed as a result of the 2005 loss of pool kill event. This site was the most downstream, and thus the most exposed, of the islands that were a part of the Ohio River NRDA restoration project. Ohio River, Belleville Pool, Wood County, West Virginia, January 2005.

Not having settled on a location for long-term monitoring, in 2013 a semi-quantitative survey of a 1 x 100m transect line and four 1 x 50m lines found natural recruitment of 11 live species and one species represented by fresh dead shell, indicating slow but gradual recovery. Of note was the large number of Varicose Rocksnails observed (Figure 12). The result of the above survey was used to establish the long-term monitoring site surveyed in June 2018. In addition to the 3RS survey, three 100m semi-quantitative transects were also surveyed through the 3RS area. The survey data are provided in Tables 14 and 15. Mussel density in 2018 was estimated at 0.16 mussels/m^2. While this appears low, semi-quantitative density was estimated at 0.66 mussels/m^2 which does not include excavations. This is an increase from the 2013 semi-quantitative density estimate of 0.19 mussels/m^2. Mussel diversity also increased from 11 species in 2013 to 13 live species in 2018. Three new species observed were Muckets (stocked October 2013), Wartyback and Ohio Pigtoe. One species, Paper pondshell, observed in 2013 was not observed in 2018. Snail density of the two live species was estimated at 1.48 snails/m^2 in 2018. Varicose Rocksnails again dominated the population, not typical of the Belleville Pool. This site is now part of the WVDNR monitoring network and is currently scheduled to be resurveyed in 2023.

Figure 12. Varicose Rocksnails, *Lithasia verrucosa*, observed at Blennerhassett Island, Ohio River, Belleville Pool, West Virginia, 2013.

Habitat was only formally characterized in 2018 when 121 pebbles were characterized using random Wolman pebble counts collected systematically through the 3RS area. This indicated that the habitat consisted of 86% gravel, 8% sand, 4% cobble and 2% silt/clay. Figure 13 depicts location of several of the activities carried out at this site over the life of the project.

Table 13. Freshwater mussels stocked at Blennerhassett Island long-term monitoring site, Ohio River, Wood County, West Virginia.

Species	2013	2014	2015	2016
Actinonaias ligamentina (A&J)	949	417	107	
*Lampsilis cardium** (J)		2101	439	
Lampsilis ovata (A)		6		
*Lampsilis siliquoidea** (J)		248	132	
Ligumia recta (J)		343	135	2,220
*Potamilus alatus** (J)		251	25	
Quadrula pustulosa (J)		30		
Truncilla donaciformis (A)		5		
Truncilla truncata (A)		9		
Total Number Individuals Stocked	**949**	**3410**	**838**	**2,220**
Total Number Species	**1**	**9**	**5**	**1**

Table 14. Semi-quantitative (SQuan) and quantitative (Quan) freshwater mussel data collected from the head of Blennerhassett Island on the Ohio River, Belleville Pool, Wood County, West Virginia, 2000 to 2018. The 2005 count were all fresh dead as data was collected from the de-watered area but would have been an indicator of natural recovery had they not died. WD = weathered dead, FD = fresh dead

Species	Quan 2000	SQuan 2000	SQuan 2005	Quan 2008	SQuan 2013	Quan 2018	SQuan 2018
Actinonaias ligamentina						1	133
Amblema plicata	FD	1	26 FD		1		1
Cyclonaias pustulosa		FD	14 FD		WD	WD	4
Ellipsaria lineolata	WD	FD			2		3
Elliptio crassidens					WD		
Eurynia dilatata		FD					
Fusconaia flava			1 FD				
Lampsilis cardium		FD			1		2
Lampsilis siliquoidea		FD			1	1	4
Lasmigona complanata		FD			1	1	1
Leptodea fragilis	1	FD	4 FD		FD		
Ligumia recta			2 FD		8	2	19
Megalonaias nervosa		1			10		6
Obliquaria reflexa	FD	FD			2		
Obovaria subrotunda						WD	
Pleurobema cordatum							2
Potamilus alatus		FD	3 FD	2	28	WD	11, 2 FD
Quadrula quadrula	WD	FD			2	WD	2
Theliderma metanevra		1	1 FD		2	WD	WD
Truncilla donaciformis	FD						
Number Live (Dead) Species	**1(5)**	**3(10)**	**7**	**1**	**11 (3)**	**4 (5)**	**14 (1)**
Number Live Individuals	**1**	**3**	**51**	**2**	**58**	**5**	**197**
Live Density (mussels/m^2)	**0.07**	**~0.01**	**~0.15**	**0.05**	**~0.19**	**0.16**	**~0.66**
Surveyed Area	**15**	**~600**	**~350**	**40.25**	**~300**	**31.75**	**~300**

Table 15. Gastropods observed at the head of Blennerhassett Island on the Ohio River, Belleville Pool, Wood County, West Virginia 2000 to 2018. The year 2000 was the survey conducted for the initial kill estimate at this site. The year 2005 was the kill estimate for the loss of pool event.

Species	2000	2005	2013	2018
Campeloma decisum				2D
Pleurocera canaliculata	74			18
Lithasia verrucosa			Many!	29
Unidentified		108 D		
Snail Density (snails/m^2)	**4.93**	**~1.53**		**1.48**
Surveyed Area (m^2)	**15.0**	**~350**		**31.75**

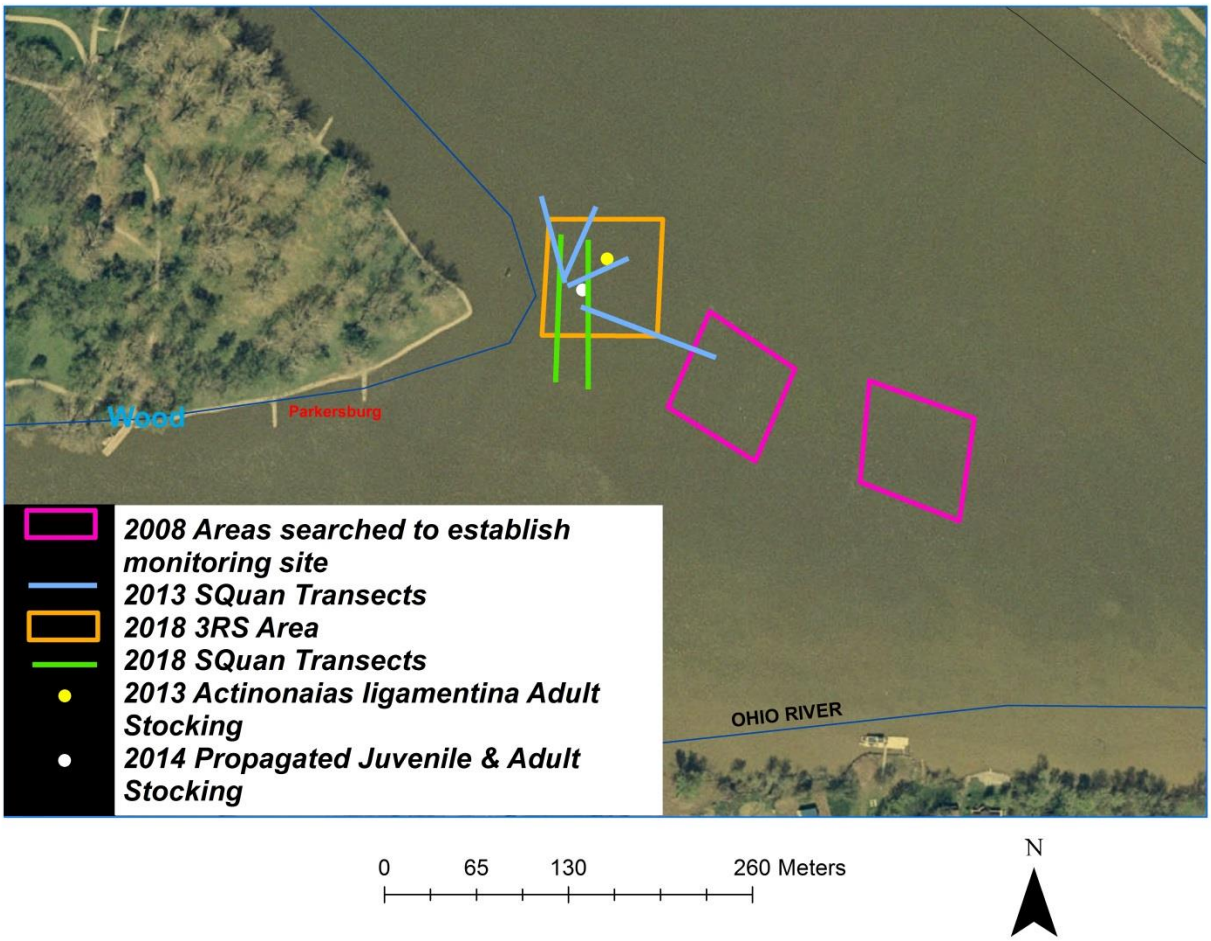

Figure 13. Overall view of restoration and survey efforts conducted at Blennerhassett Island on the Ohio River, Belleville Pool, Wood County, West Virginia, 2008 to 2018.

Degussa Site

In September 2008 there was a release of carbon black feedstock (heavy oil) from a burst pipe at the Degussa plant (RM 182, RDB) which required an assessment of mollusk mortality. Since oil recovery actions were ongoing at the plant, the WVDNR chose a sampling site along the right descending bank approximately 400 meters upstream of the Degussa plant for a surrogate assessment site. This site was later chosen to serve as an assessment of natural recovery in the area previously affected by the Eramet kill. The 2008 assessment was conducted in September using the same 3RS methodology used for the Ohio River NRDA monitoring. A total of 219 quads were excavated from an area beginning at the Ohio shoreline and extending 101m toward the channel with a length of 80 m. Five species of mussels at an approximate density of 0.514/m^2 were observed. In addition, two species of aquatic snails were observed with an approximate density of 21.92/m^2. This is slightly less than the density of mussels documented at Site 11 in 2007, but the habitat immediately above Degussa appeared less suitable.

A semi-quantitative survey was attempted at the Degussa monitoring site in 2013 but due to poor river conditions only one 1 x 70m transect line was completed. Five naturally recruited live native mussel species and two additional species represented by fresh dead individuals were observed. Full monitoring was completed in 2019 (Figure 14). All data for this site is provided in Table 16. Given its location, along the right descending bank adjacent to Neal Island, it is assumed that near 100% mortality probably occurred at this site in 1999. It was most likely unaffected by the loss of pool event in 2005 except in the near shore area since the bank dropped off fairly quickly. A total of 9 species have been observed alive at this site since 2008 with the greatest mussel density of 0.65/m^2 observed in 2019. Snail density was estimated at 5.53 snails/m^2 in 2019, which is an increase from 4.07 snails/m^2 estimated in 2008. In total, 152 snails representing four species were observed in 2019 (Table 17).

Figure 11. Natural recruitment of Washboard (Megalonaias nervosa) observed at the Degussa monitoring site on the Ohio River, Belleville Pool, Wood County, West Virginia, 2019. Clayton Photo

Percent substrate composition was characterized by conducting random Wolman pebble counts systematic at each 5m mark and between each 5m mark along the guidelines used to conduct the 3RS survey in 2019. Data are provided in Table 18.

Table 16. Qualitative (Qual), semi-quantitative (SQuan) and quantitative (Quan) freshwater mussel data collected from the Degussa monitoring site on the Ohio River, Belleville Pool, Wood County, West Virginia. No active restoration was conducted at this site. WD = weathered dead, FD = fresh dead

Species	Quan 2008	SQuan 2013	Quan 2019	Qual 2019
Amblema plicata	3	WD	1, 14^{WD}	1, 6^{WD}
Cyclonaias pustulosa		FD	5^{WD}	2^{WD}
Ellipsaria lineolata	1			
Lasmigona complanata				1^{WD}
Leptodea fragilis	5	FD	3, 1^{WD}	
Ligumia recta		1	1, 1^{WD}	5
Megalonaias nervosa		1	4, 2^{WD}	6
Obliquaria reflexa	7	3	5, 4^{WD}	2
Pleurobema cordatum		WD		1
Potamilus alatus	12	2	4, 6^{WD}	1, 5^{WD}
Quadrula quadrula		WD	19^{WD}	9^{WD}
Theliderma metanevra			3^{WD}	
Utterbackia imbecillis		1		
Number Live (Dead) Species	**5**	**5(5)**	**6 (3)**	**6 (3)**
Number Live (Dead) Individuals	**28**	**8**	**18 (55)**	**16 (23)**
Surveyed Area (m^2)	**55.0**	**~70**	**27.5**	
Effort (Minutes)				**210**
Density (Mussels/m^2)	**0.51**	**~0.11**	**0.65**	

Table 17. Quantitative gastropod data collected from the long-term monitoring site at Degussa, Belleville Pool, Ohio River, Wood County, West Virginia, in 2008 and 2019. No active restoration was undertaken at this site.

Species	2008	2019
Birgella subglobosus		12
Campeloma decisum	12	4
Lithasia verrucosa		1
Pleurocera canaliculata	212	135
Number Live (Dead) Species	**2**	**4**
Number Live Individuals	**224**	**152**
Density (snails/m^2)	**4.07**	**5.53**
Surveyed Area (m^2)	**55.0**	**27.5**

Table 18. Percent substrate composition from random Wolman pebble counts conducted systematically at the long-term monitoring site at Degussa, Belleville Pool, Ohio River, Wood County, West Virginia.

Substrate Type	2019
Silt and Clay	16
Sand	13
Gravel	64
Cobble	5
Boulder	1
Wood	1
Number Pebbles Characterized	**170**

Muskingum Island, Upstream Control

Muskingum Island is part of the ORINWR, and as such has been extensively surveyed for mussels over the past 25 years. Its diverse mussel population of over 23 species includes the federally endangered Pink Mucket, Fanshell, and Sheepnose. This area (outside of the monitoring area) was used extensively for the collection of many broodstock used throughout the life of the NRDA project. This site served as the upstream control for the NRDA restoration efforts as it was just upstream of the Eramet facility and not impacted by the 1999 kill event.

This site was used primarily for the restoration of federally listed species. The initial stocking consisted of adult Fanshells translocated from the Licking River, KY in 2010. They were placed into a 5 by 10m cell at a rate of 4 mussels/m^2 where their status could be assessed frequently. Survival has been great with typical recovery rates were around 50% each assessment. Additionally Riffleshells (*Epioblasma rangiana*) and Clubshell (*Pleurobema clava*) translocated from the Allegheny River, Hunter Station Bridge salvage project, was initiated in 2014. While similar cells were established for monitoring as with the Fanshell, most of the individuals were hand-placed throughout the full monitoring site. The year 2013 marked the first stocking of propagated juvenile Pink Muckets for the project. They similarly were stocked into a cell for ease of monitoring. Only limited non-federally listed species were stocked at this site. The one exception was the translocation of the 97 Elephantear (*Elliptio crassidens*) from the Greenup Pool. As Elephantears were rare within the Belleville Pool, even before the 1999 kill event, they were placed alongside one of the above smaller monitoring cells. See Table 19 for mussel restoration efforts undertaken at Muskingum Island.

Table 19. Freshwater mussel stocking at Muskingum Island long-term monitoring site on the Ohio River, Belleville Pool, Wood County, West Virginia, 2010 to 2017. A=adults, J=juveniles

Species	**2010**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
Amblema plicata (J)			18				
Cyclonaias pustulosa (J)			1				
Cyclonaias tuberculata (A)			7				
Cyprogenia stegaria (A)	203						
Elliptio crassidens (A)		92					
Epioblasma rangiana (A&J)				100	998	1,994	
Lampsilis abrupta (J)			389	250	300		7
Pleurobema clava (A&J)				100	520	3,999	
Utterbackia imbecillis (J)			26				
Total Stocked	**203**	**92**	**441**	**450**	**1,818**	**5,993**	**7**

Although this site has been monitored for many years it was not until 2007 that it was surveyed using the 3RS methodology. Prior to 2007 limited quantitative surveys were conducted as part of the ORINWR zebra mussel monitoring program. A survey was conducted in 2000 along both the left and right banks near the head of the island as a control site for comparison to surveys conducted within the kill-zone (ESI 2002). Monitoring at Muskingum Island for the NRDA project was initiated in 2007. The final assessment was conducted in 2017. Data are provided in Tables 20 and 21 and includes the native mussel data collected during the 2002 zebra mussel monitoring. This site is now a part of WVDNR's long-term monitoring network and is scheduled to be resurveyed in 2022.

Table 20. Semi-quantitative (SQuan) and quantitative (Quan) freshwater mussel data collected from the long-term monitoring site at Muskingum Island, Belleville Pool, Ohio River, Wood County, West Virginia, 2000 to 2017. This site is the upstream control site for the Ohio River NRDA. The year 2000 data was collected along both right and left banks at the head while the actual long-term monitoring site is only along the right bank. FD=Fresh Dead, WD=Weathered Dead

Species	**Quan 2000**	**SQuan 2000**	**Quan 2002**	**Quan 2007**	**Quan 2009**	**Quan 2012**	**SQuan 2012**	**Quan 2017**	**SQuan 2017**
Actinonaias ligamentina	1FD		1	2		4	13	1	4
Amblema plicata	21, 10^{FD}	351,356^{FD}	14, 9^{FD}	19	11	27	267	47, 1^{FD}	129, 1^{FD},
Cyclonaias pustulosa	10,1^{FD}	5,12^{FD}	6	16	16	17	137	30	114, 2^{FD}
Cyprogenia stegaria							3	2	
Ellipsaria lineolata		1	2	3	4	6	43	6	28, 1^{FD}
Elliptio crassidens						WD		1	
Epioblasma rangiana									2
Fusconaia flava		1		1			1		WD
Lampsilis abrupta								3	
Lampsilis cardium		4,6^{FD}		1	1	3	9	1	7
Lampsilis siliqouidea				1	1		3		
Lasmigona complanata	1	6,7^{FD}		1			4		1
Lasmigona costata		1,1^{FD}					1		1
Leptodea fragilis	2	1^{FD}	3, 1^{FD}	4		WD	1	1^{FD}	4
Ligumia recta				2	1	1	26	8	17
Megalonaias nervosa		7	1				12	4	9
Obliquaria reflexa	4, 2^{FD}	17,12^{FD}	8, 1^{FD}	6	8	6	45	13, 3^{FD}	42
Obovaria subrotunda						WD		WD	WD
Pleurobema clava								WD	2
Pleurobema cordatum	2	10,2^{FD}	2	8	6	11	68	15	82
Pleurobema sintoxia								WD	
Plethobasus cyphyus		2,1^{FD}	2				5	1	4
Potamilus alatus	2^{FD}	2,6^{FD}	1	5		4	26	1, 1^{FD}	8, 2^{FD}
Potamilus ohiensis		1^{FD}							
Quadrula quadrula	1,1^{FD}	13,27^{FD}	1	3	1	5	8	2	15
Theliderma metanevra	1,1^{FD}	24,5^{FD}	5, 1^{FD}	7	6	15	98	9	67, 1^{FD}
Truncilla donaciformis	1^{FD}					1	2	2	
Truncilla truncata	1		1	1	1	1			
Number Live (Dead) Species	**9(3)**		**13**	**16**	**11**	**13 (3)**	**20**	**17 (4)**	**18 (2)**
Number Live Individuals	**43**	**444**	**47**	**80**	**56**	**101**	**773**	**146**	**536**
Density (mussels/m^2)	**2.9**	**~7.4**	**9.0**	**3.44**	**2.87**	**3.74**		**2.88**	**~1.79**
Survey Area (m^2)	**15.0**	**~600**	**5.2**	**23.25**	**19.5**	**27**	**~600**	**50.75**	**~300**

Table 21. Quantitative freshwater gastropod data collected from Muskingum Island on the Ohio River, Belleville Pool, Wood County, West Virginia, 22 to 24 Aug 2017. This site is the upstream control site for the Ohio River NRDA. The year 2000 data was collected along both right and left banks at the head while the actual long-term monitoring site is only along the right bank. FD=Fresh Dead, D= Dead

Species	**2000 Quan**	**2002 Quan**	**2017 Quan**
Burgella subglobosus			9[D]
Campeloma decisum			2[D]
Lithasia verrucosa			32, 3[D]
Pleurocera canaliculata	199,291[FD]	147,14[D]	135, 1[FD], 635[D]
HYDRBIIDAE	1[FD]		
Cipangopaludina chinensis malleatus (Chinese Mystery Snail)			1, 1[D]
Number Live (Dead) Species	**1(2)**	**1**	**3(2)**
Number Live Individuals	**199**	**147**	**168**
Density (mussels/m^2)	**13.27**	**28.0**	**3.29**
Surveyed Area (m^2)	**15.0**	**5.25**	**50.75**

A total of 25 freshwater mussel species have been observed at the Muskingum Island monitoring site since 2005. Two additional species were represented by dead shell material only, Round Hickorynut and Round Pigtoe. Excluding the 2002 quantitative data due to the limited number of samples collected, densities ranged from 2.87/m^2 in 2009 to 3.74/m^2 in 2012. Prior to 2000, densities were much higher but nearly 25% pool-wide mortality occurred as a result of high zebra mussel densities (ESI 2002). Mortality at Muskingum Island due to zebra mussels was estimated at 30.6% of freshwater mussels and 59.5% for snails. Zebra mussels also appeared to crash between 1999 and 2001. Results of the annual USFWS zebra mussel monitoring from 1995 to 2003 is provided in Table 22. Although snail densities appeared to rebound in 2002, continued decline was observed in 2017. However, Varicose Rocksnails increased (Figure 15). One possibility is that the habitat preference of the Varicose Rocksnails may make them less susceptible than the Silty Hornsnail to impacts from zebra mussels.

Table 22. Results of the USFWS annual zebra mussel monitoring conducted at Muskingum Island, Belleville Pool, Wood County, West Virginia, 1995 through 2003.

Year	**# Live Mussel Species**	**Live Mussel Density**	**Live Zebra Mussel Density**	**Avg. # Zebras/Live Mussel**	**% Live Mussels Infested**
1995	12	10.9	5.3	0.1	10.3
1996	9	8.9	0.3	0.03	3.2
1997	13	7.3	224.7	6.5	80.3
1998	18	17.8	5,709.8	61	89.9
1999	12	16.5	10,429.7	141	98.8
2000	13	9.1	2,311.4	140	89.5
2001	9	4.4	16.8	0.015	15.0
2002	12	6.2	27.4	0.42	25.4
2003	11	4.6	405.4	16.5	80.5

In Aug 2017 this long-term monitoring site was assessed. The survey area during the previous survey events was 80 by 80m. In 2017 the 80 by 80m area was moved 50m upstream to capture the Clubshell and Fanshell restoration cells. Later it was decided to capture all the original survey area thereby sampling an area approximately 80 by 130m. The 3RS survey was complimented with three semi-quantitative transects through the 3RS area.

Figure 12. Varicose Rocksnail (Lithasia verrucosa) observed during long-term monitoring at Muskingum Island, Ohio River, Belleville Pool, Wood County. West Virginia. Stihler Photo

A total of 23 live species were observed at an estimated density of 2.88 mussels/m^2 in 2017. This is down from the estimated density of 3.74 mussels/m^2 observed in 2012. A similar trend was also noted at the other sites surveyed during the same time period within Belleville Pool. This trend may have been a result of reduced reproduction due to higher water years. Propagated Pink Muckets stocked at Muskingum Island showed good growth and survival. Ten individuals (appeared to be mostly propagated juveniles) were found during 2017 monitoring events (Figure 16)

Figure 13. Propagated Lampsilis abrupta observed during 2017 monitoring events at Muskingum Island long-term monitoring site, Belleville Pool, Ohio River, Wood County, West Virginia.

In 2017 the 3RS survey effort was aborted temporarily due to severe storm threat and unfortunately guidelines were pulled prior to conducting the Wolman pebble counts. Random Wolman pebble counts were ultimately characterized at each 5m mark along the three semi-quantitative lines which significantly reduced the number of counts from the 2012 survey and most likely resulted in the change in habitat noted (23). Figure 17 depicts location of several of the activities carried out at this site over the life of the project.

Table 23. Percent composition of random Wolman pebble counts conducted systematically on the Ohio River at the Muskingum Island long-term monitoring site, Belleville Pool, Wood County, West Virginia 2009 to 2017.

Substrate Type	2009	2012	2017
Silt and Clay			3
Sand	30	26	35
Gravel	68	74	62
Cobble	1	1	
Bolder			
Wood			
Number Pebbles Characterized	**164**	**164**	**66**

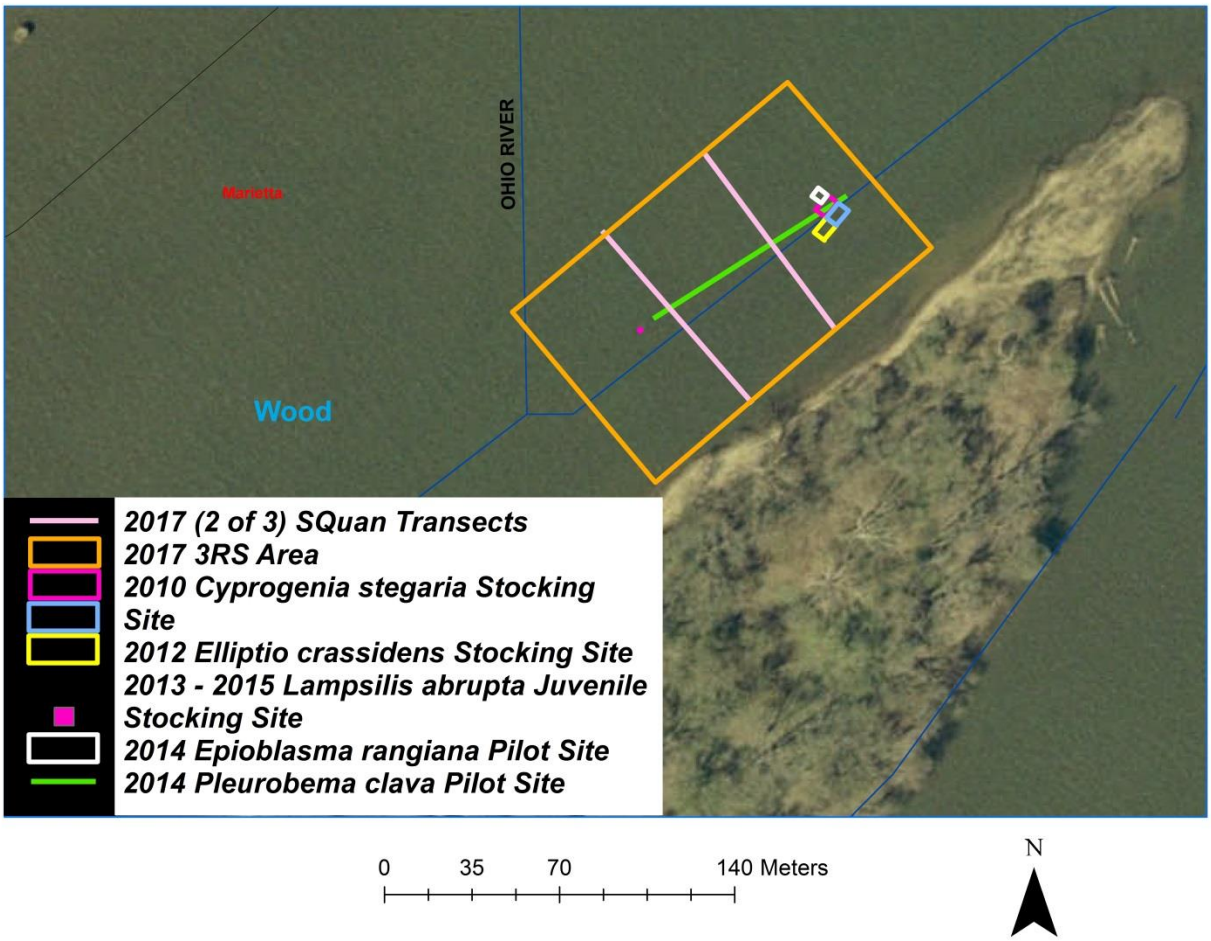

Figure 17. Overall view of restoration and survey efforts conducted at Muskingum Island on the Ohio River, Belleville Pool, Wood County, West Virginia, 2010 to 2017.

Buckley Island

Buckley Island is part of the ORINWR but has been less extensively studied than Muskingum Island. A corral was established within the backchannel in 2008 to hold broodstock for the NRDA restoration efforts. In 2014 Riffleshell and Clubshell restoration efforts were established with pilot studies initiated under a Cooperative Recovery Initiative (CRI) project. The mussels were obtained from salvage efforts for the Hunter Station Bridge, PA. Additional individuals of each species were stocked in 2015 and 2016. In 2016, the largest of all restoration efforts at this site was a translocation conducted by Enviroscience, Inc. Enviroscience translocated over 5,500 mussels but unfortunately they chose to place them further downstream in less optimal habitat.

In 2016 21 F2 adult Pink Muckets were also stocked in the backchannel of Buckley Island. Most significant however, was the introduction of Catspaw (*Epioblasma obliquata*) in 2017, also part of the CRI project. A total of 49 *in-vitro* produced juveniles of this critically endangered species (Figure 18) were stocked near the head of Buckley Island in the backchannel. They were hand-planted into a 2 by 1m cell next to a large bolder in clean swept cobble/gravel/sand. These juveniles ranged from 14.3 to 20.2 mm and were propagated by KCMC. Table 24 provides the total of all mussels stocked within the backchannel of Buckley Island.

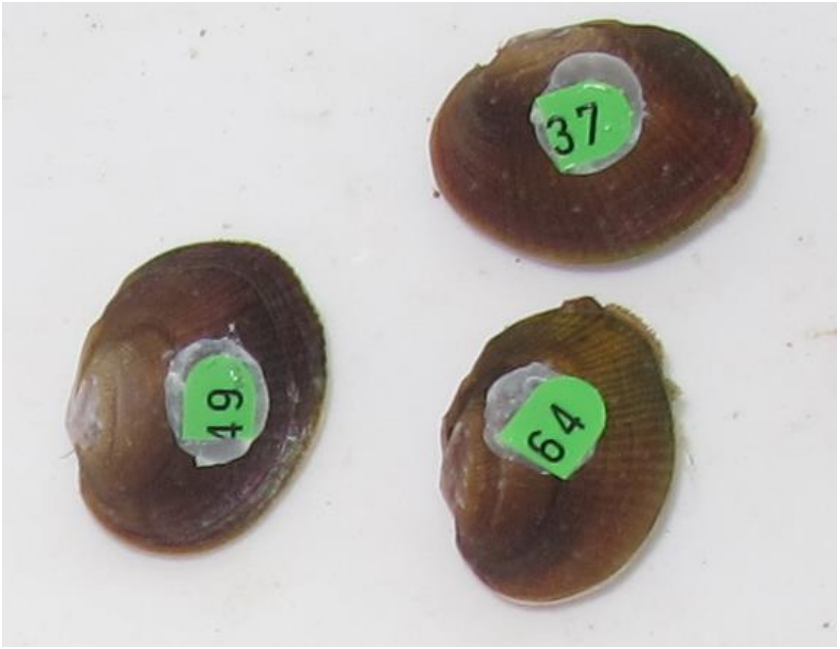

Figure 18. Critically imperiled Epioblasma obliquata ready for stocking within the backchannel of the Ohio River at Buckley Island, Belleville Pool, Wood County, West Virginia, September 2017. Clayton Photo.

Table 24. Freshwater mussel stocking within the backchannel of Buckley Island on the Ohio River, Belleville Pool, Wood County, West Virginia, 2010 to 2017. A=adults, J=juveniles

Species	**2010**	**2013**	**2014**	**2015**	**2016**	**2017**
Elliptio crassidens (A)		25				
Epioblasma obliquata (J)						49
Epioblasma rangiana (A&J)			100	665	1,830	
Lampsilis abrupta (A)					21	
Pleurobema clava (A&J)			100	716	4,101	
Quadrula quadrula (A)	200+					
Total Stocked	**200+**	**25**	**200**	**1,381**	**5,952**	**49**

Monitoring was a requirement of the Hunter Station Bridge Biological Opinion. As a result, a long-term 3RS monitoring site was established in the backchannel of Buckley Island in August

2017. Due to the widespread placement of the above activities it was difficult to capture them all in a single survey event. Typical monitoring areas on the Ohio River established by the WVDNR and ORINWR have been 80 by 80m. The mussel bed in this area is associated with a narrow lens area along the right descending side of the backchannel. As such it was decided to conduct a 60 by 100m monitoring area which was centered over the area previously determined to have the better mussel population and which included the area in which the pilot Riffleshell and Clubshell cells were established. The 2016 stocking area was too far downstream, in poorer habitat, and much deeper water. Typical survey methodology also included 3 semi-quantitative transects placed across the 3RS (Figure 19) area to ensure adequate samples to determine species richness of the area. At this site, three 50m transects were set as planned and then an additional three were placed downstream to cover the 2016 general stocking area. Data are provided in Tables 25 and 26. In 2017 a total of 21 species were observed including the federally endangered Sheepnose as well as the translocated Riffleshell and Clubshell. One additional species (Flutedshell, *L. costata*) was represented by dead shell only. Mussel density was 2.43/m^2 and snail density was estimated at 14.83/m^2. While mussel density was comparable to that observed at Muskingum Island (2.88/m^2), snail density was significantly higher than the 3.29/m^2 observed at Muskingum Island.

Random Wolman pebble counts collected sysematically at every 5m along the 3RS guidelines indicated the substrate consisted largely of gravel and sand (Table 27). Figure 20 depicts all stocking and monitoring areas conducted within the backchannel of Buckley Island.

Figure 19. WVDNR Biologist, Craig Stihler-retired, sorting a substrate sample collected from the 3RS long-term monitoring site at Buckley Island on the Ohio River, Belleville Pool, Wood County, West Virginia, August 2017.

Table 25. Semi-quantitative and quantitative freshwater bivalve data collected from Buckley Island long-term monitoring site on the Ohio River, Belleville Pool, Wood County, West Virginia, August 2017.

Species	Quan	SQuan 3RS Area	SQuan *E. rangiana* & *P.clava* 2016 Stocking Area
Actinonaias ligamentina	1	7, 1[WD]	1, 1[WD]
Amblema plicata	13, 67[WD]	29, 202[WD]	21, 145[WD]
Cyclonaias pustulosa	32, 3[WD]	92, 7[WD]	43, 12[WD]
Ellipsaria lineolata	1, 1[WD]	8, 3[WD]	7, 2[WD]
Epioblasma rangiana	1, 1[FD]	2, 3[WD]	6, 2[WD]
Fusconaia flava		1, 2[WD]	1
Lampsilis cardium	1, 1[WD]	9	3, 3[WD]
Lampsilis siliquoidea		3[WD]	1
Lasmigona complanata			1, 1[WD]
Lasmigona costata	1[WD]		
Leptodea fragilis		1	
Ligumia recta	3	3, 1[WD]	6, 1[WD]
Megalonaias nervosa	1	4, 1[WD]	4, 1[WD]
Obliquaria reflexa	7, 3[WD]	16, 3[WD]	11, 3[WD]
Obovaria subrotunda		1	1[FD]
Pleurobema clava		6, 1[SF]	14
Pleurobema cordatum	6	39, 2[WD]	12, 1[WD]
Plethobasus cyphyus			1
Potamilus alatus	1, 6[WD]	8, 3[WD]	5, 1[FD,] 14[WD]
Quadrula quadrula	1, 1[WD]	1, 6[WD]	8[WD]
Theliderma metanevra	5	10	9, 1[WD]
Tritogonia verrucosa		1	1, 1[WD]
Number Live (Dead) Species	**13 (1)**	**18 (1)**	**18, (2)**
Number Live Individuals	**73**	**238**	**147**
Effort (Minutes)		**~566**	**~530**
Surveyed Area (m^2)	**30.0**	**~150**	**~150**
Density (mussels/m^2)	**2.43**	**~1.59**	**~0.98**

Table 26. Quantitative freshwater gastropod data collected from the long-term monitoring site within the backchannel of Buckley Island on the Ohio River, Belleville Pool, Wood County, West Virginia, August 2017.

Species	Number
Burgella subglobosus	1[WD]
Pleurocera canaliculata	445, 1[FD], 467[WD]
Number Live (Dead) Species	**1 (1)**
Number Live Individuals	**445**
Density (mussels/m^2)	**14.83**
Surveyed Area (m^2)	**30.0**

Table 27. Percent composition of random Wolman pebble counts collected systematically throughout the long-term monitoring site within the Buckley Island backchannel on the Ohio River, Belleville Pool, Wood County, West Virginia, August 2017.

Substrate Type	2017
Silt/Clay	1
Sand	11
Gravel	84
Cobble	4
Number Pebbles Characterized	**112**

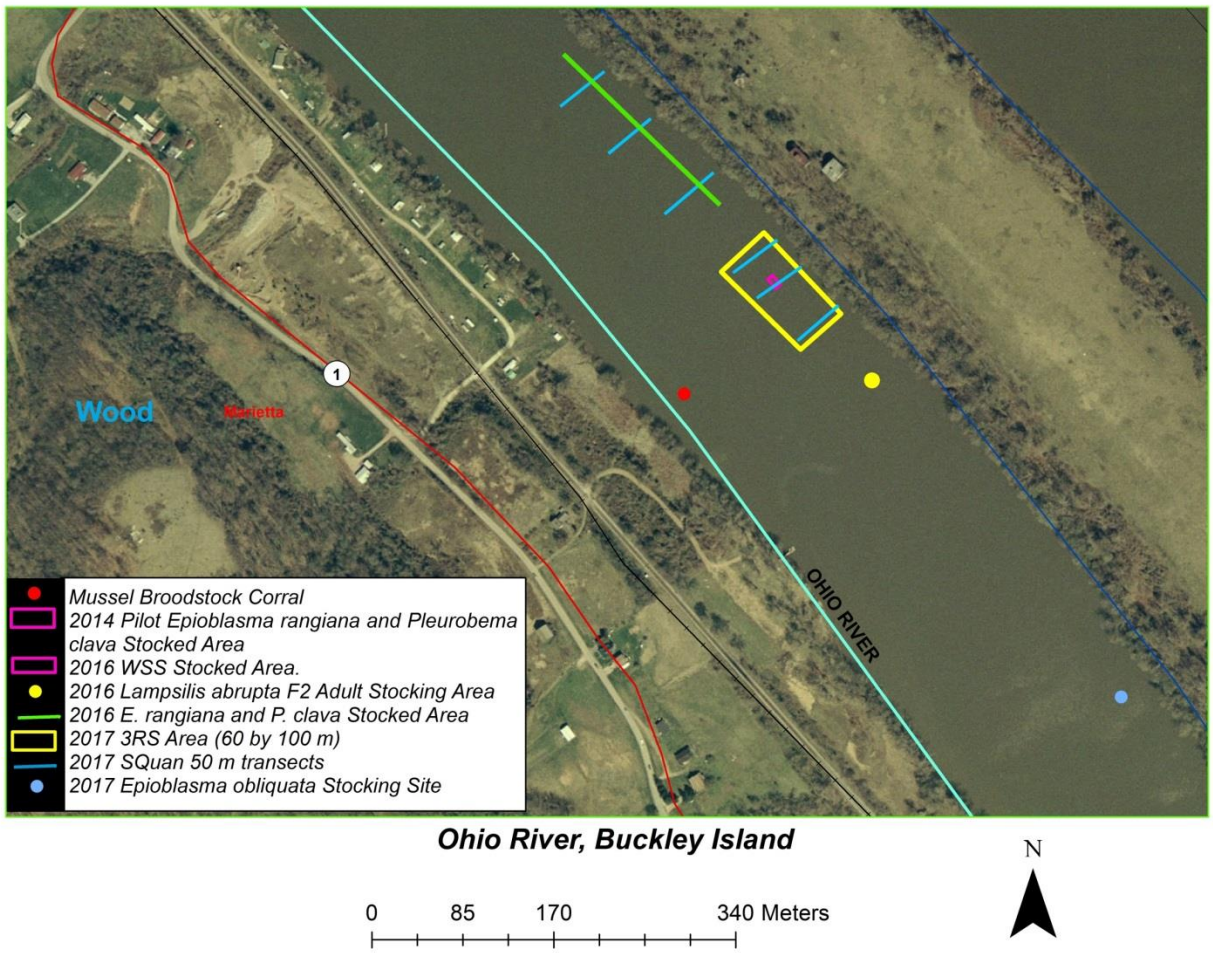

Figure 20. Overall view of restoration and survey efforts conducted at Buckley Island on the Ohio River, Belleville Pool, Wood County, West Virginia, 2007 to 2017.

Assisting Future Mussel Restoration Efforts

One of the most important tools used in the initial development of mussel restoration costs for this project was what is known as “AFS Special Publication 30” – Investigation and Monetary Values of Fish and Mussel Kills, published by the American Fisheries Society (AFS) in 2003. This document provided the scientifically accepted guidelines for conducting fish and mussel kill assessments, and estimating replacement costs for those resources. However, the data that

went into the 2003 version was outdated. This 10-year Ohio River Restoration project added much more realistic information on the true costs of species-specific mussel replacement, from collecting and holding broodstock, to securing fish hosts, doing the propagation and growing out the juveniles to a taggable and stockable size. The project funding for 2016 included $2500 to contribute to the synthesis and writing of updated mussel replacement guidelines and funding was provided to AFS for this important work.

Cumulative Accomplishments

Since 2007 we have met our active restoration target for five mussel species: Spike (*Eurynaia dilatata*), with over 3,100 restored to the area; Mucket (*Actinonaias ligamentina*), with over 3,300; Pocketbook (*Lampsilis cardium*), with over 11,000; Fatmucket (*Lampsilis siliquoidea*), with over 8,000; and Black Sandshell (*Ligumia recta*) with over 5000 stocked. There were also 13 species for which restoration targets were not established, yet positive results were obtained. A total of 54,772 adults and tagged juveniles were stocked within the restoration area, including five federally endangered species (Pink Mucket [*Lampsilis abrupta*], Fanshell [*Cyprogenia stegaria*], Sheepnose [*Plethobasus cyphyus*], Clubshell [*Pleurobema clava*], and Northern Riffleshell [*Epioblasma rangiana*]). However, after ten years of restoration activities, the effort fell well short of the 374,000 mussels which were presumed to be needed in the area to reach community goals. But results, based on the 2012 to 2017 community monitoring, showed target densities and diversities were met, at the two initial restoration sites. For future NRDA projects, we can advise with confidence that some level of freshwater mussel natural recruitment likely will occur to help advance the recovery if enough available broodstock and host fish remain within the recovery area. In summary, this restoration project has been a success, generating significant scientific advancement in propagation, culture, transport, tagging, stocking, and monitoring techniques.

Snails are another story completely. While the lifecycle of a mussel is much more complicated, their dispersal mechanism appears to have been more affective. No monitoring sites, including the upstream reference sites at Muskingum and Buckley Islands have met the target goal of 80 snails per m^2. Although snails made a slight recovery post kill, they have shown a decline since the 2012 monitoring efforts. This appears to be due to the impact from zebra mussel populations. The snail crash was observed at Muskingum Island along with the 30% mortality in native mussels in 2000 caused by the explosion of zebra mussels in 1999 and 2000. Zebra mussels continue to settle and grow episodically in the Belleville Pool, and their colonization of the smaller mollusks (i.e., the snails) hampers their mobility, energy reserves, and survival of the snails. Site 11 snail density was 4 species at 5.5 per m^2 in 2012 and dropped to 3 species at 1.9 per m^2 in 2017. Muskingum Island reference site had 3 species at 5.3 per m^2 in 2012 and 2 species at 3.3 per m^2 in 2017. Neal Island restoration site had 2 species at 19.2 per m^2 in 2010. While 3 species were observed in 2016, density dropped to 6.6 per m^2. Figure 21 shows the colonization of snails by zebra mussels. Divers commonly observe zebra balls crawling along the stream bottom as the snails are completed encased with zebra mussels. Data suggests that Varicose Rocksnails may be better able to withstand the impacts from zebra mussels than Silty Hornsnails.

Several presentations were provided to the scientific community providing updates on the status of this project. At least one other non-peer reviewed article was published. Copies of these abstracts and articles are provided in Appendix A.

Figure 21. Zebra balls, zebra mussel encased snails, observed in the Ohio River, Belleville Pool, Wood County, West Virginia in 2014. Note the Varicose Rocksnail, *Lithasia verrucosa*, that was found devoid of zebra mussel attachment.

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APPENDIX A

FMCS 2018 Workshop - Workshop Freshwater Mollusk Health and Disease Assessment
La Crosse, Wisconsin

1999 OHIO RIVER MOLLUSK KILL ASSESSMENT: THE GASTROPOD STORY. Janet L. Clayton[1] and Patricia A. Morrison[2]. [1]West Virginia Division of Natural Resources, PO Box 67, Elkins, WV 26241, janet.l.clayton@wv.gov; [2]USFWS Ohio River Islands National Wildlife Refuge (Retired), pearlymussel@gmail.com.

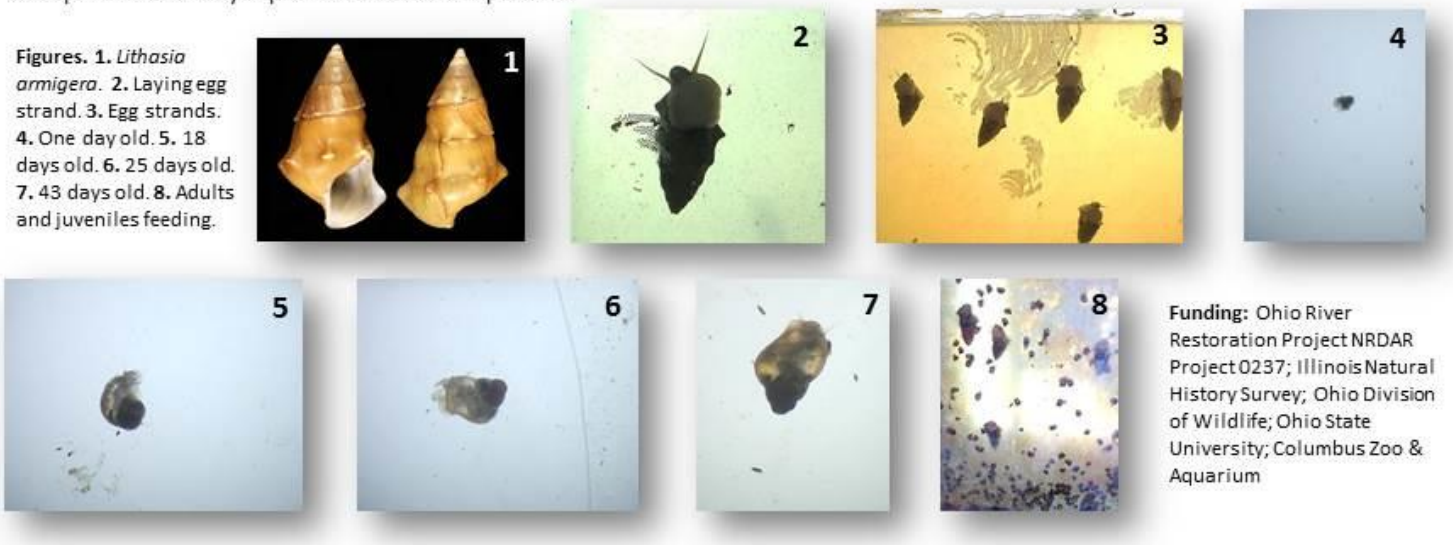
An extensive mollusk kill occurred on the Ohio River in 1999 resulting from a toxic release of hexavalent chromium and the treatment thereof with a molluscicide compound. The kill originated in the Belleville Pool below Marietta, OH. The kill assessment was conducted by Ecological Specialists and consisted of semi-quantitative transects and excavated quadrats at five known mussel beds below the alleged discharge. Impacts were observed nearly 30 miles downstream. While the primary objective was to assess the unionid kill, the quadrat excavations provided an opportunity to also assess the impact to the snails. The dominant snail observed was *Pleurocera canaliculata* with three additional species observed. It was believed that 100% mortality of unionids occurred at the most upstream assessed bed, and total mortality at the five beds was estimated at 870,000 individuals; approximately 11 million gastropods died at those sites alone. Losses in between and beyond those sites are immeasurable. Both unionid and gastropod recovery has been monitored over the past 18 years. Snails were much slower to rebound and whereas the population was primarily made up of *P. canaliculata* prior to the kill it is now composed of *P. canaliculata* and *Lithasia verrucosa*. An explosion of *Birgella subglobosus* occurred in 2007 but has only barely persisted since. Episodic settlement and growth of zebra mussels continue to threaten the full recovery of both the unionids and gastropods.

EGG LAYING IN THE PLEUROCERID *LITHASIA ARMIGERA* (SAY, 1821)

A WINTER'S TALE

Trisha Gibson[1], Jacqualyn Halmbacher[1], Jeremy Tiemann[2] and G. Thomas Watters[1]
[1]Ohio State University and Columbus Zoo and Aquarium, Columbus, OH 43212; [2]Illinois Natural History Survey, Prairie Research Institute at the University of Illinois, Champaign. IL 61820

The pleurocerid snail *Lithasia armigera* (Say, 1821) is a globally vulnerable freshwater snail in the upper Cumberland, Ohio, and Tennessee river basins. This species was one of several impacted by a chemical spill on the Ohio River mainstem near Marietta, OH. Mitigation efforts to restore the species include propagating the snail in captivity for release into the wild. Specimens of *L. armigera* were collected in October, 2013, from a newly discovered population in the Mississippi River near St. Louis, Missouri. These were maintained at the Columbus Zoo & Aquarium Freshwater Mussel Research Facility near Shawnee Hills, Ohio, in 75 L glass aquaria with flow through river water and natural sunlight. Numerous egg masses were deposited on the aquarium glass beginning on February 4, 2013, at a water temperature of 5.7 C. Eggs masses consisted of several back and forth passes of gelatinous material in which were embedded several hundreds of eggs in 2-3 parallel rows. Eggs were laid continuously well into May. Some eggs were artificially warmed to ca. 24-25 C whereas others continued in ambient temperatures. Regardless of treatment, eggs hatched after 3-4 weeks once water reached ca. 15 C suggesting hatching occurred when a threshold temperature was reached rather than after a set duration. Most pleurocerids deposit eggs during the summer but our previous work did not observe summer egg laying in this species. Egg laying during the winter in near freezing temperatures was unexpected and may represent a novel adaptation.



Figures. 1. *Lithasia armigera*. **2.** Laying egg strand. **3.** Egg strands. **4.** One day old. **5.** 18 days old. **6.** 25 days old. **7.** 43 days old. **8.** Adults and juveniles feeding.

Funding: Ohio River Restoration Project NRDAR Project 0237; Illinois Natural History Survey; Ohio Division of Wildlife; Ohio State University; Columbus Zoo & Aquarium

FMCS 2013 Symposium: Lake Guntersville State Park - Guntersville, AL. Species Recovery and Restoration- from Concept to Implementation.

RESTORING THE FRESHWATER MOLLUSK COMMUNITY FOLLOWING A TOXIC EVENT: PART I – ASSESSING THE DAMAGES AND DEVELOPING A RESTORATION PLAN.

Patricia Morrison[1], Janet Clayton[2].[1]US Fish and Wildlife Service, Ohio River Islands NWR, Williamstown, WV; [2]West Virginia Division of Natural Resources, Elkins, WV.

In 1999, the middle Ohio River experienced a significant aquatic mortality event due to an alleged discharge of toxic materials from a metal plating plant. Over 1 million mussels and over 12 million snails perished. A concurrent fish kill occurred, affecting over 8600 fish, mostly (> 96%) freshwater drum. Fish and mollusk kill assessments were done in 1999 and 2000. The pre-existing mussel community was comprised of 35 species, with three species of snails. An interjurisdictional team of state, federal, and academic investigators pieced together the likely pathway of injury and brought a claim for Natural Resources Damages under CERCLA. The case eventually settled for $3.2 million, of which $2.04 million was set aside for natural resources restoration. A restoration trust fund was established, managed by an interagency Trustee Council. On-the-ground restoration is accomplished through a Technical Committee that advises the Council, partners with cooperators, and implements annual work plans. Cooperators use a combination of techniques to restore the aquatic fauna including translocation of adult mussels, propagation and stocking of tagged juveniles, setting and relocation of snail egg traps, and release of fish directly infested with mussel glochidea. The stated goal is to restore ecologically viable populations of mussel, snails, and fish within the affected area of the Ohio River within ten years of initiation of activities. For mussels, that goal is > 1 mussel per square meter, and a community represented by > 20 species.

FMCS 2013 Symposium: Lake Guntersville State Park - Guntersville, AL. Species Recovery and Restoration- from Concept to Implementation.

RESTORING THE FRESHWATER MOLLUSK COMMUNITY FOLLOWING A TOXIC EVENT: PART 2 – RESTORATION ACTIVITIES AND SUCCESSES. Janet L. Clayton[1] and Patricia A. Morrison[2]. [1]West Virginia Division of Natural Resources, Elkins, WV, 26241; [2]U. S. Fish and Wildlife Service, Ohio River Islands NWR, Williamstown, WV, 26187.

In 1999, the Ohio River in West Virginia experienced a significant aquatic mortality event in which over 1 million mussels and over 12 million snails perished. The pre-existing mollusk community was comprised of 35 unionid and three snail species. Our goal is to restore ecologically viable populations of mussels, snails, and fish within the affected area of the Ohio River within ten years of initiation of activities. For mussels, that goal is > 1 mussel per square meter, and a community represented by > 20 species. Restoration activities have been undertaken by numerous cooperators using a combination of techniques. Our initial efforts targeted translocations of adults to establish a bed into which propagated juveniles would be stocked. Animals used for translocations were salvaged from areas within the Belleville Pool of the Ohio River, WV and the Allegheny River, PA. Propagation on this scale required the collection and holding of adult broodstock consisting of a few individuals for long term brooders and 50 or more for short term brooders. The collection and holding of a large number of host fish of different species can also be challenging. Propagation activities have included laboratory reared and cage cultured juveniles which have required four months to two years to reach taggable size. To date over 12,000 mussels of 21 species (adults and tagged juveniles) have been stocked into two restoration sites. Following 5 years of restoration activities, the 2012 assessment of the primary restoration site reported 21 unionid species, 14 of which are showing natural recruitment. This bed was completely killed in 1999 and, in 2012, the population density was estimated at 3.4 mussels per square meter. While limited snail restoration activities have been undertaken, two of the three snail species lost have shown some recovery and three additional species have appeared. The collection of the first naturally recruited federally endangered species, *Cyprogenia stegaria*, provides encouragement for moving forward with propagation and release of endangered species within the restoration areas.

2014 Gibson, T., Halmbacher, J. and G.T. Watters. Laboratory-determined hosts for the Ohio Pigtoe, *Pleurobema cordatum* (Rafinesque, 1820). *Ellipsaria* 16(4): 33.

Laboratory transformations are reported for the Ohio Pigtoe, *Pleurobema cordatum* (Rafinesque, 1820), an Ohio Endangered species. The mussels were collected from the Muskingum River below Devola Lock & Dam #2 in Washington County, Ohio. Potential hosts were derived from a variety of sources, but none were from the Muskingum River. Female Ohio Pigtoes released numerous, ladder-shaped, white conglutinates, some of which were fed to the test fishes to initiate infections. In addition to the fishes listed in the table, the following fishes were tested but did not transform any glochidia: bluntnose minnow, striped shiner (two trials), emerald shiner, red dace, stoneroller (four trials), madtom, yellow perch, largemouth bass (two trials), and long-eared sunfish.

Fish species	Days to transformation	# Juveniles	% Transformed	Temp C	Date Infected
scarlet fin shiner	9	27	57.50%	21.5	7/15/2014
scarlet fin shiner	13	32	38.10%	21.5	7/15/2014
blacknose dace	8	44	31.80%	21.5	7/15/2014
blacknose dace	N/A			21.5	7/15/2014
blacknose dace	N/A			21.5	7/15/2014
white sucker	9	6	8.60%	21.5	7/15/2014
guppy	15	3	8.33%	21.7	7/15/2014
creek chub	13	4	7.27%	21.5	7/15/2014
bluntnose minnow	8	10	7.14%	21.5	7/15/2014
creek chub	8	12	20.00%	21.5	7/15/2014
creek chub	13	3	3.23%	21.5	7/15/2014
creek chub	8	1	1.30%	21.5	7/15/2014
creek chub	8	1	1.00%	21.5	7/15/2014
creek chub	13	1	0.70%	21.5	7/15/2014
creek chub	N/A			21.5	7/15/2014
spotfin shiner	9	2	0.30%	21.5	7/17/2014