Annual Report: FY 2014 Cooperative Recovery Initiative Project

Project Name: The Return of Big River Endangered Freshwater Mussels

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Cooperators: Ohio River Islands NWR, White Sulphur Springs NFH, Northeast Fishery Center, West Virginia Field Office, WV Division of Natural Resources (WVDNR), PA Fish and Boat Commission, PA Field Office, OH Field Office, Columbus Zoo/OSU Mussel Conservation Facility, KY Field Office, KY Dept. of Fish and Wildlife Resources' Center for Mollusk Conservation (CMC), TN Fish and Wildlife Resources Agency (TWRA), Lewis Environmental Services, MN Field Office, Miami University of Ohio.

Project Location: Ohio River Valley Ecosystem (PA, WV, KY, OH)

Project Goal: Establish two to four new populations of endangered freshwater mussel species on or near the Ohio River Islands NWR.

Expected Conservation Outcome of the Project: New populations of at least 100 individuals each of two to four endangered mussel species at two to four sites by the end of FY15. Four species were chosen (clubshell, purple cat's paw, orange-foot pimpleback, and spectaclecase) which will allow the team to maximize the chances for success over a three-year period. There is inherent uncertainty and risk in working with only one species of mussel in any given year – many factors may combine to undermine success, such as unworkable water conditions when it is time to collect adult mussels, loss of fish hosts in captivity, etc. The species chosen also represent different levels of imperilment, requiring different sets of recovery actions and activities. As it turned out, 2013 and 2014 were both problematic years for water levels and flows.

Project Measurable Objectives (Year 2, FY 2014 only), (with 2014 progress in BLUE):

Species and Tasks (FY 2014)
Clubshell (*Pleurobema clava*)
Monitor adult survival
Collect broodstock, propagate juveniles and culture to taggable (stockable) size
Stock cultured juveniles
95% OF 2014 EFFORT COMPLETED, PLUS 2 MORE PILOT POPULATIONS ESTABLISHED. Two-hundred (200) more clubshells were collected from the Allegheny River at Hunter Station, quarantined, and relocated to two more sites (100 to Buckley Island, 100 to Middle Island Creek), taking the total number of new populations to five. Adult survival from the three 2013 pilot populations establishment is excellent, no dead shells were found. PIT tag readers were used in deep water for the first time ever, with good success. Juveniles were propagated from Allegheny River broodstock females; they were transferred to KY Center for Mollusk Conservation for continued grow-out. At present, they are not large enough to stock, so that task will be done in 2015. That task represents a very small percentage of actual effort (1/2 day).
Purple cat's paw (*Epioblasma obliquata obliquata*)
Monitor survival of test juveniles
Propagate juvenile cat's paw and culture to taggable size and collect genetics samples
85% OF 2014 TASKS COMPLETED, PLUS ADULTS OF CONGENERS STOCKED AT 3 SITES. The second ever successful collection of gravid females and propagation of juvenile purple cat's paw was achieved this year at the KY Center for Mollusk Conservation, with female mussels provided by the Ohio Field Office and private cooperators. The fish hosts, mottled sculpin, were provided by White Sulphur Springs NFH. There are now 30

juvenile purple cat's paw mussels growing at the KY Center for Mollusk Conservation, representing two distinct year classes. None are large enough yet to stock, so they will remain in grow out for another year. Test silos of northern riffleshell (surrogates) were checked from 3 sites in the Ohio River, and survival was assessed. Good growth occurred. An additional 300 adult northern riffleshell from PA were stocked at these three sites.
Orange-foot pimpleback (*Plethobasus cooperianus*)
Propagate juveniles and culture to taggable size
0% COMPLETED. Although 12 adult mussels were aggregated last year in the Ohio and Tennessee Rivers, none of the mussels contained mature larvae when assessed this year. An additional 13 adult mussels were collected in the Tennessee River, which should improve the chances of getting gravid females for 2015.
Spectaclecase (*Cumberlandia monodonta*)
Collect genetic samples and refine propagation techniques
Propagate juveniles and culture to taggable size
50% COMPLETION. Genetic samples were taken in 2013, and the DNA extracted and analyzed. Cooperators have completed the analysis for this population and six other river systems within the specie's range. The Green River caged females were checked on two occasions, larvae extracted, but no juveniles transformed. The best timeframe for gravidity has been determined, and cooperators are hoping for success in 2015.

Project Measureable Objectives (Long Term): Establish 2 to 4 new populations of endangered species on or near the refuge. Species specific objectives are:

Clubshell – establish trial populations (minimum 50 adults each) at 2 to 4 sites on or near the refuge, from the Allegheny River source population, and augment with 100 – 1000 propagated juveniles (minimum two year classes). Collect tissue or buccal swabs from individuals in source populations for genetics analysis.

Spectaclecase – Obtain 5 to 15 gravid females, develop propagation techniques (i.e., identify workable fish host or in vitro techniques) needed to stock 100 – 1000 juveniles at a minimum of two sites on or near the refuge (one to two year classes). Collect tissue or buccal swabs from individuals in source populations for genetics analysis.

Orange-foot pimpleback – Collect and aggregate 20 to 100 adults into in-river refugia to identify gravidity periods, identify workable fish hosts and in vitro techniques for propagating juveniles and stocking 100 – 1000 juveniles (one to two year classes) at a minimum of two sites on or near the refuge.

Purple cat's paw – Propagate juveniles of the related northern riffleshell (congener) using known fish hosts, set out 2 to 4 silos (in-situ containment) of juveniles as trial populations on the refuge, propagate juveniles of purple cat's paw using fish hosts or in vitro techniques, and stock 100 – 1000 (one year class) at one or two sites on or near the refuge after successful survival of test juveniles (> 40% survival after one year).

Those items highlighted below in blue are mostly completed:

SPECIES AND PLANNED TASKS	TIMELINE (BY FISCAL YEAR)			
	FY 13	FY 14	FY 15	FY 16 ++
Establish baseline mussel community and habitat conditions at two to four restoration sites on and near refuge (to be determined by the restoration team)	X	Total of 5		
Clubshell (*Pleurobema clava*)				
Translocate trial populations of adults (> 50 each) to 2 – 4 sites and collect genetic samples from Middle Island Cr.	X			
Monitor adult survival		X		

Collect broodstock, propagate juveniles and culture to taggable (stockable) size	X	X		
Stock cultured juveniles *		X	X	
Monitor the survival and growth of juveniles and FY13 adults **				X
Purple cat's paw (*Epioblasma obliquata obliquata*)				
Stock cultured juveniles of northern riffleshell as a congener surrogate species in silos at 2 – 4 sites	X			
Monitor survival of test juveniles		X		
Propagate juvenile cat's paw and culture to taggable size and collect genetics samples		X	X	
Stock cultured juveniles			X	
Monitor survival and growth of juveniles				X
Orange-foot pimpleback (*Plethobasus cooperianus*)				
Aggregate 20 to 100 adults into river refugia to monitor reproductive condition	X			
Propagate juveniles and culture to taggable size		X	X	
Stock cultured juveniles			X	
Monitor survival and growth of juveniles				X
Spectaclecase (*Cumberlandia monodonta*)				
Collect gravid females from the wild	X			
Collect genetic samples and refine propagation techniques	X	X		
Propagate juveniles and culture to taggable size		X	X	
Stock cultured juveniles			X	
Monitor survival and growth of juveniles				X

* Stocking of juvenile mussels rarely occurs the same year as propagation. Culture of juvenile mussels to taggable (and therefore, trackable) size requires 6 months to 2 years, depending upon the species growth rate and culture facility.

++ Monitoring the long term "success" of stocked juveniles extends many years into the future, as it takes years for them to reach sexual maturity and begin to reproduce on their own.

Assessment of Short Term (Year 2) Performance:

○ Less than 70% conservation objectives achieved
√ **70 – 79% conservation objectives achieved (significant success with 3 of the 4 species)**
○ 80 – 89% conservation objectives achieved
○ 90% or more conservation objectives achieved

Assessment of Long Term Performance this year:

√ **Less than 70% conservation objectives achieved (it's Year 2 of a 4+ year project)**
○ 70 – 79% conservation objectives achieved
○ 80 – 89% conservation objectives achieved
○ 90% or more conservation objectives achieved

Detailed Status Report for 2014

Potential restoration sites: The refuge and WVDNR have established **five restoration sites so far** (three on the mainstem Ohio River in the Belleville and Greenup Pools, one on the Little Kanawha River at Annamoriah, and one on Middle Island Creek at Falls Mills. This exceeds the original project goal of two to four sites. A potential sixth site is yet to be established at the Manchester Islands in KY (persistent high water during 2013 and 2014 prevented baseline surveys) but it will be attempted in 2015.

Clubshell: On 26 Aug 2014, divers from the WVDNR and USFWS collected another 200 clubshells from the Allegheny River at Hunter Station, PA. These individuals underwent quarantine at the ORINWR Middle Island Facility (Figure 1). All clubshells received a PIT-tag and two Hallprint number tags. No mortality was observed during quarantine. On 3 Sep 2014, 100 clubshells were stocked in Middle Island Creek near Falls Mills, Pleasants County, WV and 100 clubshells were stocked in the Ohio River within the back channel of Buckley Island. The three pilot population of clubshells stocked in 2013 were checked for detectability and survival. No dead shells were found at any of the sites. Thirty-nine of the 194 clubshell were observed with an additional 35 PIT tags recorded from other individuals that were not located visually. This was the first attempt at using the PIT tag system in a non-wadable stream. Once the boat was positioned near the study area, a diver was deployed with the antenna which was attached by a 50 foot cable to the reader on the surface (Figure 2). Surface to diver communication was used to relay readings to the diver who then attempted to locate the buried mussel. In 2012, WSSNFH successfully propagated and cultured *P. clava* using common shiner, river chub, and mountain redbelly dace as fish hosts, and approximately 100 juveniles remain in culture and are growing at the KY Center for Mollusk Conservation (Figure 3). These juveniles should be of sufficient size to tag and release in 2015.

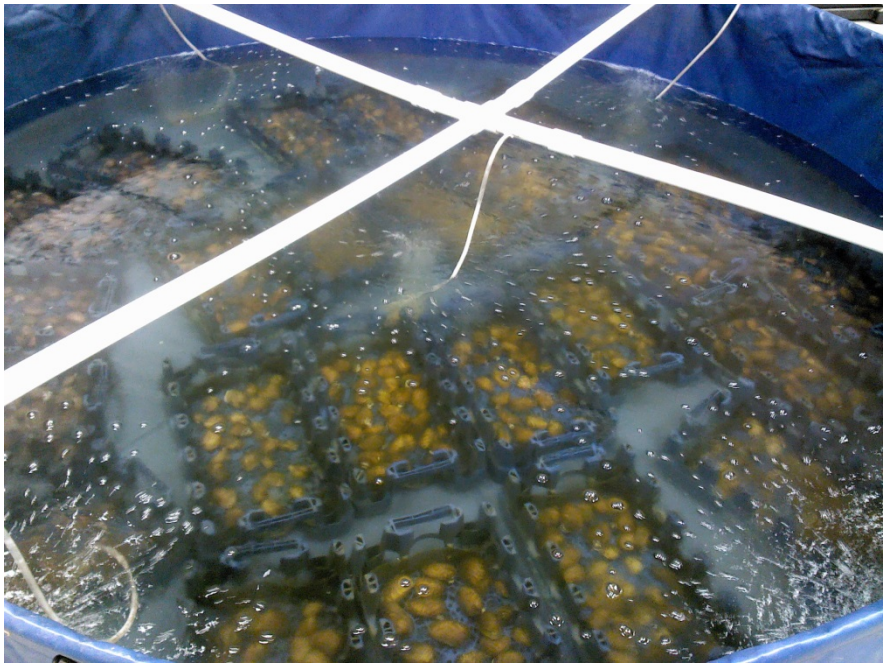


Figure 1. Adult mussels from the Allegheny River in PA, in aerated quarantine tanks on the refuge.

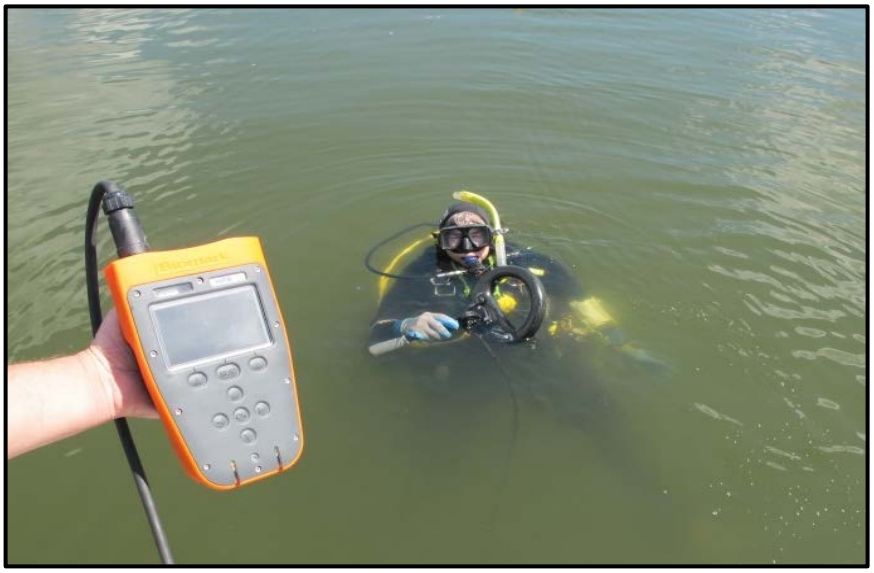


Figure 2. WVDNR diver Janet Clayton using PIT tag reader in deep water.



Figure 3. Juvenile clubshells propagated by WSSNFH, now growing at KY Center for Mollusk Conservation.

<u>Purple cat's paw:</u> Against all odds, the team was successful in propagating a second year class of purple cat's paw mussels in 2014. Purple cat's paw is one of the rarest freshwater mussels on the planet, and this marks a major milestone towards potential recovery. This species is known from only one stream in Ohio – Killbuck Creek. A total of six (3) gravid females were collected in early spring by cooperators from the Ohio Field Office, volunteers, and private consultants. The females and their larvae went to the Columbus Zoo/OSU facility and KY Center for Mollusk Conservation (CMC) (Figure 4). Building on the success in 2013 of WSSNFH personnel in using mottled sculpin successfully as a host, KY received 150 mottled sculpin from WSSNFH and were successful in

propagating more juveniles. At this date, there are 13 juveniles from 2013 and 17 juveniles from 2014 growing out in KY (Figure 5).

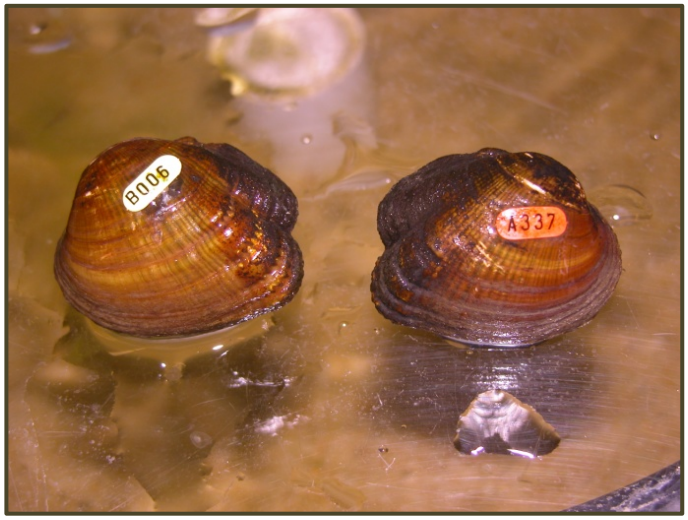


Figure 4. Gravid female purple cat's paw mussels at the KY Center for Mollusk Conservation in 2014.



Figure 5. Second year purple cat's paw juveniles growing in KY Center for Mollusk Conservation; notice the purple nacre visible through the young shell.

Replicate sets of three silos each were set in 2013 and checked in 2014 to evaluate potential suitability of Ohio River sites for *Epioblasma* re-introduction. WSSNFH provided 300 juveniles of the northern riffleshell for this trial assessment. Some of the silos experienced occlusion due to high sediment loads, but overall, survival of juvenile northern riffleshell was good. The live individuals grew an average of 60% in one year. An additional 300 adult northern riffleshell were subsequently stocked at three Ohio River sites and 100 in the upper Kanawha River. A fourth set of test congeners will be established at the Manchester Islands in KY (high water prevented this task in 2013 and 2014).

Orange-foot pimpleback: Persistent high water in the Ohio and Tennessee Rivers throughout 2013 and 2014 hampered the search for this species. In late fall 2103, cooperators in KY and TN finally located and aggregated 12 individuals into a holding area in the lower Tennessee River in KY. During 2014, the TWRA dive team spent a total

of 77 hours underwater at two sites, and collected an additional 13 individual mussels, a CPUE of 0.17 mussels per hour (Figure 6). The rarity of this species, challenges of finding them in big river ecosystems, and river conditions in the lower basin over the past two years confounds attempts at propagation. Of the total 25 specimens, none have yet produced any larvae to work with.



Figure 6. Some of the 13 orange-foot pimpleback collected by TWRA from the Tennessee River in KY.

Spectaclecase: In September of 2013, contractors were finally able to find and place 37 individuals in two cages and position them in Pool 4 of the Green River in KY (Figure 7). None of the individuals examined were gravid at that time, but the hope was that they would be in spring 2014. Tissue was collected in 2013 for genetics analysis, and cooperators at Miami University of Ohio completed DNA extraction, mitochondrial DNA sequencing, and microsatellite genotyping (16 loci) of 416 total individuals from all rivers. This concludes all of the laboratory work required. Dr. David Berg and his colleagues completed population genetic analysis for all of these populations, and constructed ecological niche models using bioclimatic and landscape data. They are currently modeling changes in genetic variation that might occur with future climate change, and are working on developing a population viability analysis (PVA) for this species that will include examining the effects of genetic stochasticity on extinction risk. There is a manuscript which is currently undergoing in-house review before it is submitted to a peer-reviewed journal. On the propagation task, mussels held in the Green River cages were checked in late March (3-26-14), but none were gravid. KYDFWR personnel brought 6 individuals back to the CMC where they released unfertilized eggs in Mid-April in chilled tanks (matching the Green River temperatures). They checked the cages again on May 30, four were dead, but they found six gravid females in cages, which were returned to CMC for culture/propagation research. The staff tested 400,000 larvae on the following fish hosts: shorthead redhorse, golden redhorse, striped shiners, spotfin shiners, rosefin shiner, and big eye chubs. No juveniles transformed on any of the tested fish. The staff also tested ~ 2,000,000 larvae using in vitro culture media. The larvae grew in the media from 50-60 microns to ~ 180 microns, but no transformation occurred. However, lessons learned in 2014 on the timing of gravidity will hopefully increase the chances of successful propagation in 2015.



Figure 7. A nice variety of ages of spectaclecase mussels collected in the Green River in September 2013, these are being held in mesh cages in the river to facilitate propagation efforts in early spring.