Annual Report: FY 2013 Cooperative Recovery Initiative Project

Project Name: The Return of Big River Endangered Freshwater Mussels

Project Manager/Primary Contact: Patricia Morrison, Ohio River Islands NWR, Region 5
Phone Number: 304-375-2923 x 124
Email: patricia_morrison@fws.gov

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 was indeed a problematic year for water levels and flows.

Project Measurable Objectives (Year 1, FY 2013), (with progress in BLUE):

Species and Tasks (FY 2013)
Establish baseline mussel community and habitat conditions at two to four restoration sites on and near refuge (to be determined by the restoration team)
COMPLETED – three restoration sites established (2 on the Ohio River in the Belleville and Greenup Pools and 1 on the Little Kanawha River at Annamoriah). We were unable to survey a fourth site (Manchester Islands) due to persistent high water in the KY portion of the Ohio River this year, but will attempt in 2014.
Clubshell (*Pleurobema clava*)
Translocate trial populations of adults (> 50 each) to 2 – 4 sites and collect genetic samples from Middle Island Cr.
Collect broodstock, and propagate juveniles
ALL TASKS COMPLETED. Two-hundred (200) clubshells were collected from the Allegheny River at Hunter Station, quarantined, and relocated to three sites (100 to Muskingum Island, 50 to Greenup Pool, and 44 to the Little Kanawha River above Annamoriah). Six individuals died in quarantine. Genetic samples were collected from Middle Island Creek and Hackers Creek. Juveniles were propagated from Allegheny River broodstock females.
Purple cat's paw (*Epioblasma obliquata obliquata*)
Stock cultured juveniles of northern rifffleshell as a congener surrogate species in silos at 2 – 4 sites
Propagate juvenile purple cat's paw and culture to taggable size (*ahead of schedule*)
ALL TASKS COMPLETED, and then some. The first ever successful collection of gravid females and propagation of juvenile purple cat's paw was achieved, using mottled sculpin as a host fish. Replicate sets of three silos each were set at three Ohio River sites (Muskingum Island, Greenup Pool, and Buckley Island) to evaluate potential suitability of sites for *Epioblasma* re-introduction.
Orange-foot pimpleback (*Plethobasus cooperianus*)
Aggregate 20 to 100 adults into river refugia to monitor reproductive condition
60% COMPLETED. Persistent high water prevented in river work until this October, but so far 12 individuals

have been aggregated into the lower Tennessee River. None are gravid.
Spectaclecase (*Cumberlandia monodonta*)
Collect genetic samples from additional streams
Collect gravid females
Refine propagation techniques
66% COMPLETION. Persistent high water prevented in-river work until September. Genetic samples have been taken from 37 individuals. None of the females were gravid at that time, so propagation testing was not possible. These mussels have been caged in the Green River for examination in Spring 2014.

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).

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			
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 1) Performance:

○ Less than 70% conservation objectives achieved
○ 70 – 79% conservation objectives achieved
√ **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 only Year 1 of a 4+ year project)**
○ 70 – 79% conservation objectives achieved
○ 80 – 89% conservation objectives achieved
○ 90% or more conservation objectives achieved

Detailed Status Report Follows

Potential restoration sites: The refuge and WVDNR established **three restoration sites so far** (two on the mainstem Ohio River in the Belleville and Greenup Pools and one on the Little Kanawha River at Annamoriah)(see Figure 1). A fourth site is yet to be established at the Manchester Islands in KY (persistent high water during 2013 prevented baseline surveys).

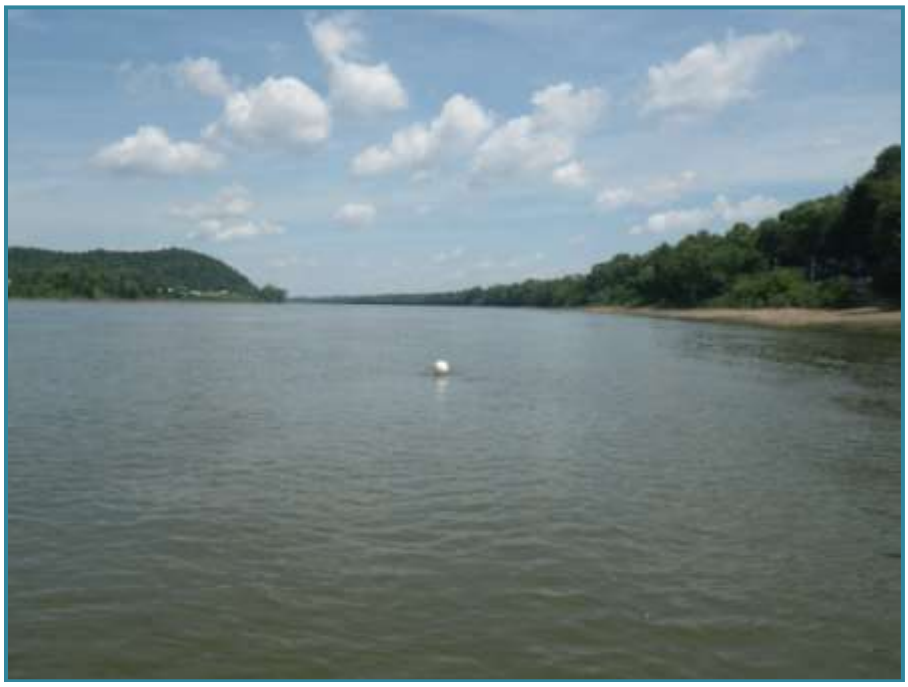

Figure 1. Endangered mussels return home to the Ohio River, as divers stock clubshells in the Greenup Pool site.

Clubshell: Sampling kits were prepared by the Northeast Fishery Center (NEFC) and distributed for tissue collection from both wild and hatchery-maintained mussels. Microsatellite primers have been ordered and are being optimized for analysis of the P. clava samples. WVDNR collected tissue samples from six (6) clubshells in Meathouse Fork of Middle Island Creek, and nine (9) from Hacker's Creek, and submitted the samples to the NEFC for genetics analysis, and comparison to previous samples from other streams in the basin (see Figures 2 and 3). Divers and snorkelers from ORINWR, WVDNR, WV Field Office, and WSSNFH collected two-hundred (200) clubshells from the Allegheny River at Hunter Station on June 26, 2013, just as the rain began to fall. The mussels were brought to the WVDNR Belleville complex for a planned 3-day quarantine period that ended up lasting over 30 days due to persistent high water at the restoration sites (Figure 4). Six individuals died during this period. All mussels were tagged with PIT tags and plastic Hallprint shellfish tags (Figure 5). On July 31st, 100 clubshells were stocked at Muskingum Island in the Belleville Pool of the Ohio River; on August 2nd, 50 were stocked in the Greenup Pool (above Lesage Island); and on August 5th, the remaining 44 were stocked in the Little Kanawha River above Annamoriah (see Figure 6). Gravid females were also collected by WSSNFH and were used to produce 265 juveniles, although they did not survive. Mature larvae collected during the quarantine period were also sent to KY Center for Mollusk Conservation, where they attempted culture on rosefin shiners, striped shiners, and spotfin shiners, but none of these transformed to juveniles. 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 available for release in 2014 for this CRI project (Figure 7).

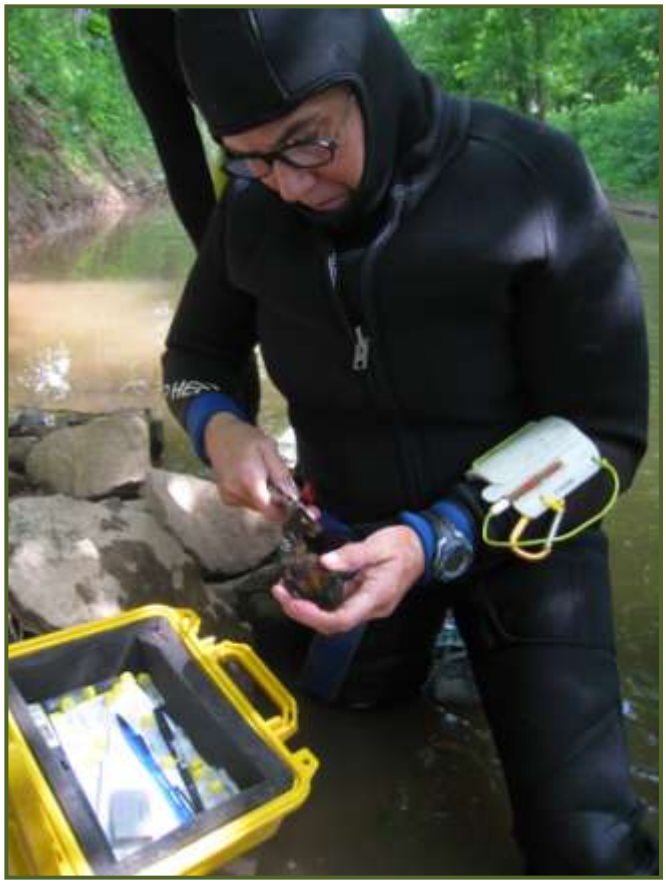

Figure 2. WVDNR biologist Janet Clayton opens clubshell on Hacker's Creek in WV.

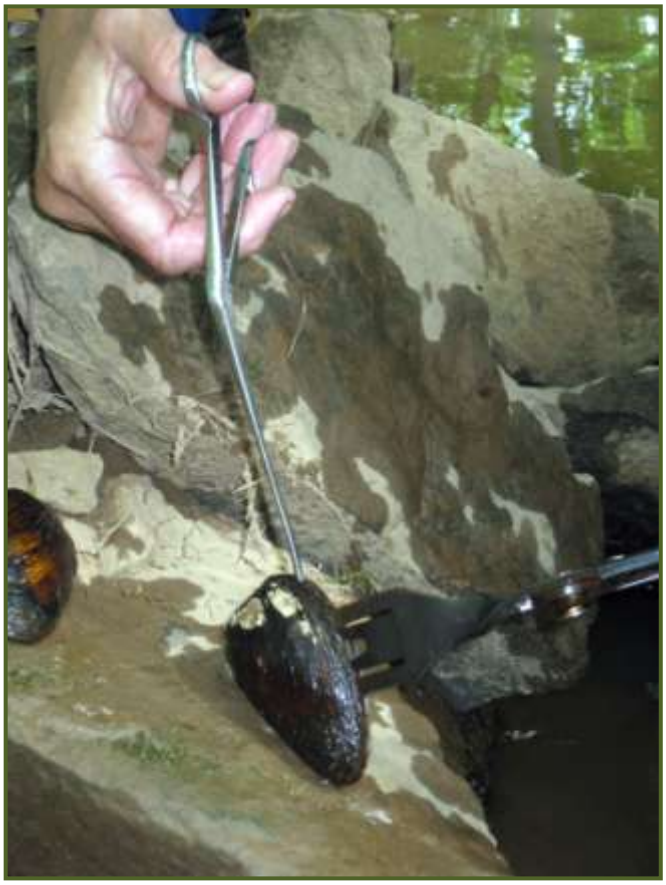

Figure 3. Extracting a tissue snip for genetics analysis.

Figure 4. Clubshells from the Allegheny River were quarantined to prevent transfer of disease and non-desirable biological organisms.

Figure 5. Clubshells get PIT tags and shellfish tags prior to stocking.

Figure 6. Tagged adult clubshells in the Little Kanawha R.

Figure 7. Juvenile clubshells raised by WSSNFH

<u>Purple cat's paw:</u> The first ever successful collection of gravid females and propagation of juvenile purple cat's paw was achieved this year as part of this project!! Purple cat's paw is one of the rarest freshwater mussels on the planet, and this marks a major milestone towards potential recovery. A total of six (6) gravid females were collected by cooperators from the Ohio Field Office, volunteers, and private consultants. The various propagation facilities involved used different techniques and species of fish as potential hosts. All females were retuned alive to Ohio. The Columbus Zoo/OSU facility used a variety of darters for host fish (11 rainbow dater, 2 fantail darter, 2 banded darter, 1 greenside darter, and 2 spotted darter). None of those were successful at transforming larvae to juvenile mussels. KY Center for Mollusk Conservation used fish host protocols and attempted *in vitro* transformation. Approximately 30-40% of the larvae went into media and the remaining 60-70% was placed on 26 logperch (~460 per fish). No juveniles were ultimately raised from either technique this year. Two gravid females were taken to WSSNFH on April 1. Mussels began to display readily and were infested the following day (see Figure 8). Because every known *Epioblasma* uses sculpin as a host fish, WSSNFH chose to inoculate mottled sculpin*, Cottus bairdi*, with catspaw larvae. Over 1300 juveniles dropped off of the sculpin; however movement was observed in just over 900 juveniles. Juveniles were cultured at WSSNFH and currently 13 remain alive, measuring 2-4 mm in length (Figure 9).

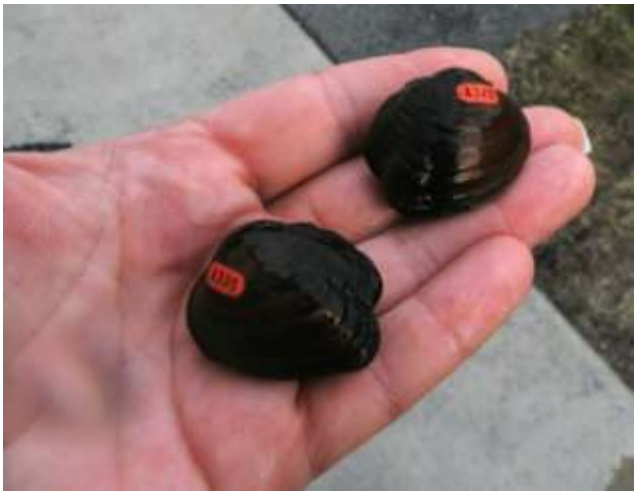

Figure 8. Adult purple cat's paw females

Figure 9. Juvenile purple cat's paw from WSSNFH

Replicate sets of three silos each were set at three Ohio River sites (Muskingum Island, Greenup Pool, and Buckley Island) to evaluate potential suitability of sites for *Epioblasma* re-introduction (see Figures 10 and 11). WSSNFH provided 300 juveniles of the northern riffleshells for this trial assessment. A fourth set will be established at the Manchester Islands in KY (persistent high water prevented this task in 2013).

Figure 10. Refuge volunteers built 24 silos as juvenile mussel culture chambers.

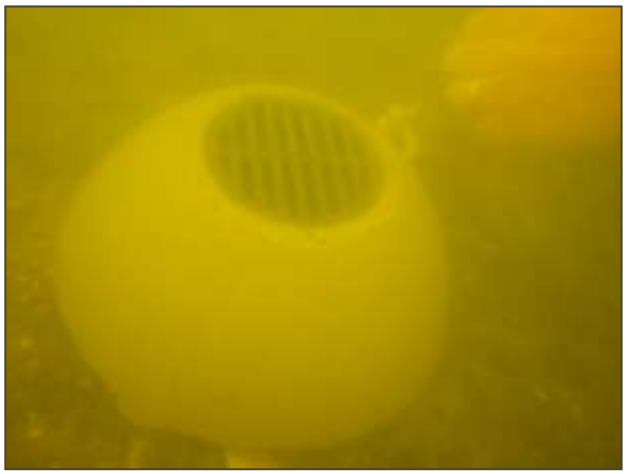

Figure 11. A silo in place on the bottom of the Ohio R.

Orange-foot pimpleback: Persistent high water in the Ohio and Tennessee Rivers throughout this year has delayed the search for this species. As of the week of October 28th, TN researchers had located three (3) individuals from the Tennessee River. The search will continue as flow and river conditions allow, and into next year. The lower Ohio River is now workable and private contractors spent eight days and collected nine (9) individuals thus far (Figure 12). All 12 have now been aggregated into a holding site in the lower Tennessee River.

Figure 12. Orange-foot pimpleback collected from the Ohio River in KY

Spectaclecase: Persistent high water in the Ohio River Basin this year also impacted the work with this species. Finally, in September, contractors were able to find and place 37 individuals in two cages and position them in the Green River so that they can be retrieved in the spring of 2014 (Figure 13). None of the individuals examined were gravid, but hopefully they will be in spring. Students of Dr. David Berg from Miami University (OH) were able to swab all 37 individuals from the Green River in KY for genetic analysis. The plan is to have a comprehensive analysis of genetic variation for multiple populations from each of six rivers (Clinch, Ouachita, Osage, Meramec,

Gasconade, and St. Croix) plus the Green River population. This encompasses most of the known significant populations of this species in the world.

Figure 13. A nice variety of ages of spectaclecase mussels collected in the Green River in September 2013.