FY 2017 Annual Report: Year 2 of 2016 Cooperative Recovery Initiative Project

1. **Project Title: Restoring Endangered Freshwater Mussels on a Landscape Scale**

2. **Is your project completed?:** No

3. **Interim or Final?:** Interim

4. **Project Summary:** The long-term goal of this multi-species recovery project is to prevent the extinction of eight critically endangered mussels. This partnership has been working together for 10-20 years, building capability and refining techniques, and recently successfully completed a CRI project funded in 2013. Four of the mussel species targeted in the current project are a continuation of the progress made in the first project, furthering the recovery of those species within their historic range. The additional four species are in dire need of assistance and require cooperation across multiple state and regional boundaries in order to effect a positive change.

   fanshell [*Cyprogenia stegaria*]
   sheepnose [*Plethobasus cyphyus*]
   ring pink [*Obovaria retusa*]
   white wartyback [*Plethobasus cicatricosus*]
   clubshell [*Pleurobema clava*],
   orange-foot pimpleback [*Plethobasus cooperianus*],
   purple cat's paw pearlymussel [*Epioblasma obliquata obliquata*], and
   spectaclecase [*Cumberlandia monodonta*]

5. **Lead Region:** 5

6. **Other Participating Regions:** 3 and 4

7. **National Wildlife Refuge(s) Involved:** Ohio River Islands NWR, Region 5, (PA, WV, and KY)

8. **Any Private Lands Involved?:** No

9. **All FWS Programs Involved:** Refuges, Fish and Aquatic Conservation, and Ecological Services (in 3 Regions)

10. **Project Contacts:**
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    Cooperators: Ohio River Islands NWR (ORINWR), White Sulphur Springs National Fish Hatchery (WSSNFH), Northeast Fishery Center, West Virginia Field Office, West Virginia Division of Natural Resources (WVDNR), Pennsylvania Fish and Boat Commission, Pennsylvania Field Office, Ohio Field Office, Columbus Zoo/OSU Mussel Conservation Facility, Kentucky Field Office, Kentucky Department of Fish and Wildlife Resources' (KDFWR) Center for Mollusk Conservation (KY CMC), Tennessee Fish and Wildlife Resources Agency (TWRA),

Alabama Dept. of Conservation and Natural Resources (ALDCNR), Lewis Environmental Consulting, Minnesota Field Office, Eastern Kentucky University, and Miami University of Ohio.

11. Project Data Table: see attached spreadsheet.

12. Explanations and Justifications:

Considering this project has numerous target species, the following explanation has been broken out by activities per species.

Cyprogenia stegaria (FANSHELL)

Adult fanshell were collected by cooperators in Kentucky and translocated to cooperators in West Virginia from ORINWR and the WVDNR. On 25 Sep 2017, 101 adult *C. stegaria* were received from the Licking River in Kentucky. The previous *C. stegaria* restoration cell (containing 99 individuals) was expanded downstream another 5m by 5m and mussels were hand-placed into the cell at a density of $4/m^2$ (Table 1). On 29 Jun 2017, the *C. stegaria* pilot cells were assessed. The *C. stegaria* have shown good survival and extremely limited horizontal movement over the seven years of monitoring. Only seven of the 201 translocated mussels are known to have died and only 11 have not been observed since translocation in 2010. Although they have shown limited horizontal movement, they have shown a great deal of vertical movement. In the last seven years, 31 have only been observed once, though each year previously undetected mussels are observed, including two in 2017. Ten individuals have been observed during all five survey efforts. *C. stegaria* recapture has remained around 50% during each survey effort, a very high rate for recapturing mussels. It should be noted the survey effort entails significant sweeping of the substrate, but no excavations.

Cooperators in Kentucky still have several dozen juveniles of this species in culture from last year's efforts (Table 2). They will remain in culture until they are large enough to be stocked out.

Plethobasus cyphyus (SHEEPNOSE)

During long-term monitoring at Kanawha Falls on the Kanawha River from 26 to 27 Jun 2017, six gravid *P. cyphyus* were collected for White Sulphur Springs National Fish Hatchery to use as broodstock. On 20 Jul 2017, the area was revisited in search of additional broodstock with the assistance of WSSNFH staff and four additional gravid *P. cyphyus* were collected. Sheepnose were successfully transformed using Golden Shiners as a host at WSSNFH. Juveniles were placed in recirculating culture systems and growth and survival was monitored for about 8 weeks when, due to an ostracod contamination, the juveniles were lost. Cooperators in Kentucky successfully produced *P. cyphyus* using in-vitro methods and approximately 40 individuals from the 2017 cohort are currently in culture.

Additionally, cooperators collected 11 *P. cyphyus* from the Tennessee River. Gravid females were transported to the KDFWR Center for Mollusk Conservation. Personnel were also able to retrieve 20 sheepnose from a stockpile grid in the Tennessee River. The gravid females were transported to be used for propagation efforts in Kentucky. Overall, a total of 27 sheepnose have been placed within the grid so they can be readily accessed for future propagation efforts (Table 2). There are plans to expend more search effort in 2018 collecting *P. cyphyus*.

Staff and students at the Thomas More College Biology Field Station are maintaining a system of aquaculture tanks with Ohio River water running through them, in order to hold adult and juvenile mussels and to propagate and culture of federally endangered species (i.e. sheepnose). These individuals are

monitored and cared for daily, including feeding, cleaning and periodic growth measurements. In 2017, about 4000 larvae were extracted from two female sheepnose mussels. They were taken to the Center for Mollusk Conservation in Frankfort, Kentucky for further development via in-vitro methods. Also, cooperators from Eastern Kentucky University visited Thomas More College Biology Field Station to take water and tissue samples for eDNA analysis.

Obovaria retusa (RING PINK)

Visual searches for this species continued in 2017, but were unsuccessful. More effort will be expended in 2018 to locate individuals of this species, including more visual searching and collecting eDNA samples. The last known live individual died in 2016. If more can be found, this would be an excellent candidate for in-vitro culture methods. In-vitro methods used by cooperators in Kentucky are largely to thank for the success this project has had with the Purple cat's paw pearly mussel (see below).

Plethobasus cicatricosus (WHITE WARTYBACK)

During 2017, weather and some logistical problems hampered our search for the White Wartyback Mussel in the Wilson Dam tailwater reach of Tennessee River. As such, only 42 hours of bottom time were expended during the effort. No White Wartybacks were found among the 36 species encountered. All species encountered were represented by live individuals with the exception of one found only as weathered dead shell and three found only as relics. Three federally-protected species were encountered: sheepnose (1 individual), rough pigtoe (9 individuals), and pink mucket (11 individuals). Most of these were relocated to corrals, where any White Wartybacks that are found in future searches will be placed. The corrals are also located in the Wilson Dam tailwater reach and will allow easy recovery of the mussels when needed for propagation or research. Funds remain for another 35 days of searching for White Wartybacks, during which our goal is 6 hours of bottom time per day (210 hours of bottom time total). The mussel diversity and numbers of federally protected species encountered during the first year of the search exceeded expectations, so we have reason for optimism going into our second dive season.

Pleurobema clava (CLUBSHELL)

Mussel restoration of *P. clava* has been ongoing on the Little Kanawha River at Annamoriah, Middle Island Creek, and the mainstem Ohio River (in 3 sites) since establishment of the pilot populations in 2013. As a follow up to this, a long-term monitoring site was established at Annamoriah in 2014. On 8 Jun 2017, a 5m by 10m monitoring cell was assessed, as well as the 2013 pilot population and the overall restoration area. A PIT-tag reader was scanned over both monitoring cells and snorkelers found tagged mussels. A total of 153 tags were detected. Only three of the original *P. clava* stocked in 2013 have not been seen since they were stocked and mortality remains low. This is an excellent result. The temperature logger installed in 2013 and set to record every two hours was retrieved on 8 Jun 2017 and replaced with a new logger. On 6 Jul 2017, an additional 111 *P. clava* from Hunter Station Bridge, Pennsylvania were placed at the Annamoriah restoration site on the Little Kanawha River (Table 1). Prior to stocking, these mussels were sent to WSSNFH for use as broodstock. WSSNFH staff inoculated dusky darters and successfully transformed juveniles. Juvenile growth and survival was good for approximately 8 weeks when the juveniles perished for currently unknown reasons.

Plethobasus cooperianus (ORANGEFOOTED PIMPLEBACK)

Attempts to collect gravid *P. cooperianus* were unsuccessful in 2017, but several individuals are being held in a cage in the lower Tennessee River (Table 2). Additional *P. cooperianus* individuals were added to the cage in 2017 after cooperators successfully relocated 31 individuals in a stockpile grid. Adults will be checked in early August for gravidity. From the 31 new mussels collected, 27 larvae were recovered,

as the females mostly contained eggs. Overall, a total of 49 *P. cooperianus* have been placed within the grid from 2013 – 2017.

Epioblasma obliquata obliquata (PURPLE CAT'S PAW PEARLY MUSSEL)
The first stocking of this critically endangered species occurred in 2017 (Table 1). This achievement marks a significant milestone for not only this species, but for the mussel recovery community around the world. This species, which was once thought to be extinct, is now present at nine locations in five watersheds from which it was previously extirpated. We have released 400 (50 per site) cultured juveniles of *E. o. obliquata* into 2 sites in the Licking River (Kentucky), 2 sites in the Green River (Kentucky), 2 sites in the Duck River (Tennessee), and 1 site in the Ohio River (West Virginia), and 1 site in the Walhonding River (Ohio). The releases were from 1.5 year old juveniles cultured in 2017. Additional individuals are being held at the Center for Mollusk Conservation for release in 2018. Approximately 50 adults are being held in captivity by cooperators in Kentucky, and several thousand larvae were obtained in 2018 for culture (Table 2). Thanks to this project, and the extraordinary efforts of cooperators working across states and regions, this species has been moved a little further back from the risk of extinction.

Cumberlandia monodonta (SPECTACLECASE)
Larvae of this species were obtained from cooperators late in the year and sent to WSSNFH where they were inoculated on American eels for host identification. Although no juveniles were transformed, we have not ruled American eels out as a potential host because the poor quality and small number of larvae obtained for this portion of the study made the results inconclusive.

Cooperators in Kentucky successfully transformed the spectaclecase using in-vitro culture methods. Larvae were obtained from the Cumberland River Aquatic Center (where the spectaclecase broodstock from the Green River, Kentucky were being held). They tested multiple mixtures over the past 3 years, with no success. However, a combination of enhanced vitamins and serum from the smallmouth buffalo after 4 weeks of culture produced juveniles of the species. The juveniles are currently in culture and will be grown until they are large enough for stocking (Table 2).

The WVDNR and the ORINWR staff collected water samples for eDNA analysis from three sites (two samples each) within the Marmet Pool of the Kanawha River. This is the only known location of the spectaclecase in West Virginia. Sites were selected based on habitat preference of this species (large, undercut boulders) using side-scan sonar data collected by the U.S. Army Corp of Engineers. Samples were filtered using 0.45 micron filters, fixed in 95% ethanol, and shipped to Dr. David Hayes at Eastern Kentucky University for analysis. In addition, qualitative mussel surveys were conducted at four locations. One individual of C. *monodonta* was observed during these efforts.

Cooperators at Eastern Kentucky University have obtained, in addition to the four eDNA samples from the Kanawha River, West Virginia, three samples from the Green River, Kentucky. Additional samples will be obtained in spring of 2018. In previous eDNA studies of mussels, we have found it difficult to identify some species using short DNA sequences generated from Illumina MiSeq sequencing. To increase the amount of information for species identification, we have been developing protocols based on a previous study that utilize long-polymerase chain reaction (PCR) to amplify whole mitochondrial genomes from water samples. To fully utilize the longer DNA sequences, we have opted to utilize the Oxford Nanopore MinION sequencer. The advantage of using the MinION sequencer is the ease of sample prep, portability, and rapid time to result. We have successfully generated mitochondrial genome sequences in our trial runs using the MinION USB DNA sequencer on DNA obtained from tissue samples. We will be attempting sequencing runs on water samples from the Green and Kanawha Rivers in summer 2018. We are currently in the process of optimizing our extraction protocols for obtaining high-

molecular weight DNA from water samples. We have developed a novel eDNA collection method using a handheld pump that is capable of filtering up to 40L of water in a single sample. Our hope is that the larger volume of water sampled will lead to an increase in detection probability of rare species in large river systems. Our initial laboratory trials indicate that the total amount of DNA recovered is similar to methods using lab-based filtration with 0.45-1.0um filters. Ongoing work on this aspect of the project includes obtaining additional samples from the Kanawha River and the Green River, comparing the filtering and extraction methods on eDNA detection from large river water samples, and developing rapid sequencing protocols using the MinION sequencer.

We spent two days within the stretch of the Green River that was previously pooled by Dam 6 searching for spectaclecase. Any area that was visually assessed as being good habitat was searched, including areas of boulders and cobble with good flow. Eighteen sites were sampled throughout this portion of the river. Although no live or dead individuals of this species were collected, many sites were documented as being good habitat now that the water level is lower, and six sites were evaluated as being good for potential future stocking efforts (Table 2).

Cooperators in Tennessee observed on 18 March 2017 that *C. monodonta* started to release conglutinates (packets of larvae). These conglutinates were collected on March 19th through 20th. Most of the glochidia were sent to Kentucky cooperators for in-vitro culture. They were successfully transformed to juvenile stage. Tennessee cooperators also tried to culture larvae using in-vitro methods, but were unsuccessful.

Table 1. Number of endangered mussels stocked during 2017.

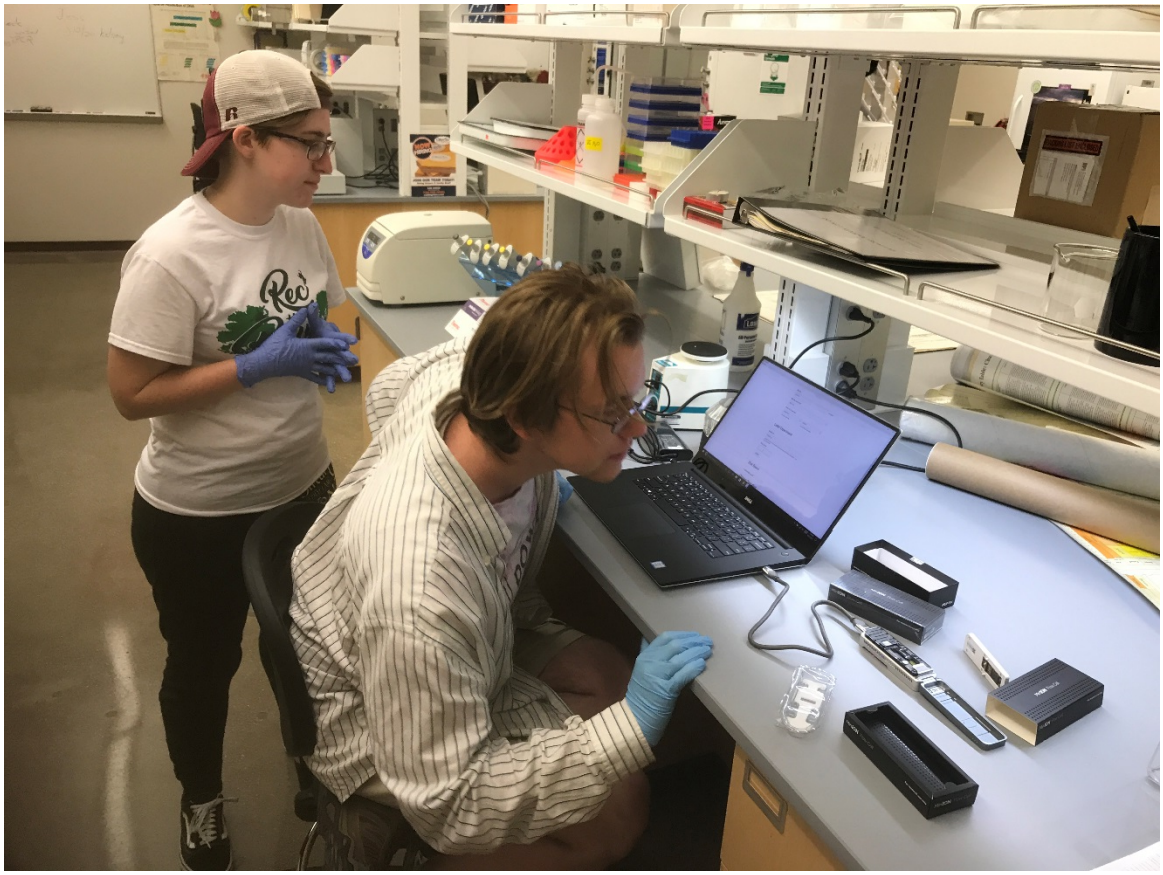
Species	Number stocked in 2017
Fanshell	101
Clubshell	111
Purple cat's paw pearlymussel	400

Table 2. Aggregation and propagation activities of endangered mussels in 2017.

Species	Aggregation or Propagation Site	Number of Individuals	Comments
Spectaclecase	Aggregated in the Green River in cages: Propagation efforts occurred at KY CMC and WSSNFH	30	18 sites were assessed in the Green River, no individuals found
Orange-foot pimpleback	Lower Tennessee River, KY	17 new 49 project total	No juveniles surviving from 2016 trials. No larvae were available in 2017
Purple cat's paw	Aggregated in Killbuck Creek, OH; propagated by KY CMC	10 – Killbuck 50 – CMC/Minor Clark	First ever stocking occurred this year. A large number of juveniles should be available in 2018 for stocking
Sheepnose	Aggregated in the Ohio River behind the Refuge headquarters (19), and Thomas More College (5).	11 new 27 project total	Propagation attempts were successful at WSSNFH on fish and KY CMC in vitro, some individuals still surviving in culture
Fanshell	Adults collected from the Licking River, KY and propagated by KY CMC	N/A	Juveniles from 2016 propagation efforts produced in vitro by KY CMC still in culture

13. High resolution photos of Target Species and Work:

WVDNR Biologist - Janet Clayton and USFWS Refuge Biologist- Patricia Morrison with the single *Cumberlandia monodonta* observed in Marmet Pool, Kanawha River, Kanawha County, West Virginia, 8 Sep 2017. Photo credit: Stockton photo (WVDNR)



EKU Honors students Tanya Cifranik and Henry Richburg using the Oxford Nanopore MinION sequencer to generate reference sequences from tissue samples. (Photo credit: David Hayes - EKU)

eDNA sampling kit designed to use for this project. Kit consists of a battery operated hand pump, tubing, filters, and collection tubes for storage of filters. (Photo credit: David Hayes - EKU)

USWFS and WVDNR cooperators tagging juvenile Purple cat's paw pearlymussels. Photo credit: USFWS Ryan Hagerty

USWFS and WVDNR cooperators tagging juvenile Purple cat's paw pearlymussels. Photo credit: USFWS Ryan Hagerty

Juvenile Purple cat’s paw pearlymussels ready to be stocked. Photo credit: USFWS Ryan Hagerty

Juvenile Purple cat’s paw Pearlymussels. Photo credit: USFWS Ryan Hagerty


Stocking juvenile Purple cat’s paw pearlymussels. Photo credit: USFWS Ryan Hagerty

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