**FY 2016 Annual Report: Year 1 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 [*Plethosasus 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:**
    Patricia Morrison, Refuge Biologist, Ohio River Islands NWR, 304-375-2923 x 124, patricia_morrison@fws.gov

    Barbara Douglas, Senior Endangered Species Biologist, West Virginia Field Office, 304-636-6586 x 19, barbara_douglas@fws.gov

    **Cooperators:** Ohio River Islands NWR, 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:** As of the close of 2016, many of the cooperators had not yet received their funding. For some reason, the schedule for transfer of the funds from Washington to the field stations was delayed. Funds did not get to the Service cooperators until July 2016, and the transfer to partners via grants and cooperative agreements took many months. Nevertheless, there was substantial work accomplished in the short field season.

    A new pilot population of 99 fanshells (*Cyprogenia stegaria*) was established in the Ohio River, with adults provided by KY CMC from the source population in the Licking River, KY (Table 1). WSSNFH propagated juveniles on mottled sculpin, but lost those fish due to the late June flood and its aftermath. The KY CMC successfully produced 5000 juvenile fanshell in April 2016 using logperch as host fish, and they are growing out at the facility.

    Cooperators at WSSNFH inoculated golden shiners with sheepnose (*Plethobasus cyphyus*) larvae in late June of 2016, but the facility had a record flood which devastated operations for months. The fish subsequently died. The KY CMC successfully propagated sheepnose in vitro in July 2016, producing over 3000 juveniles. Unfortunately, the culture facility experienced a spike in ammonia concentration, and the juveniles died.

    In 2016, a total of 20,487 tagged clubshell (*Pleurobema clava*) were added to the five new sites; three sites in the mainstem Ohio River, one in the Little Kanawha and one in Middle Island Creek (Table 1). All sites have sufficient numbers now to constitute a founder population! This number includes the first-ever stocking of captively propagated juvenile clubshells. These 104 juveniles were propagated by WSSNFH, grown out by the KY CMC, and tagged and stocked by the refuge and WVDNR team. This actually represents a typical example of how much of this project work is done – a variety of agencies and facilities working together to accomplish the objective.

    This project has had a significant benefit accrue to another endangered mussel – the northern riffleshell (*Epioblasma torulosa rangiana*). This species is a close relative of the purple cat's paw and is being used as an indicator of likely survival of future purple cat's paw stockings. In concert with the work with the clubshell, we had to opportunity to translocate thousands of northern riffleshell into four of our restoration areas: three sites in the mainstem Ohio River, and one site in the Kanawha River (Table 1).

    Propagation of purple cat's paw (*Epioblasma obliquata obliquata*) hit a milestone in 2016, as the KY CMC successfully transformed 3000 juveniles using in vitro techniques. Previous efforts yielded numbers in the tens.

    Attempts were made at captive propagation of orange-foot pimpleback (*Plethobasus cooperianu*) and spectaclecase (*Cumberlandia monodonta*) in 2016, but no juveniles survived. Both species are aggregated in wild water to improve reproductive success (Table 2).

Table 1. Number of endangered mussels stocked at six sites during 2016.

| Species | Site Locations | | | | | | Total Individuals |
|---|---|---|---|---|---|---|---|
| | Ohio River at Greenup | Ohio River Buckley Island | Ohio River Muskingum Island | Little Kanawha River at Annamoriah | Middle Island Creek at Falls Mills | Kanawha River below Falls | |
| **Fanshell** | 99 | | | | | | **99** |
| **Northern riffleshell** | 1468 | 1830 | 2076 | Not an historic species here | Not an historic species here | 1829 | **7203** |
| **Clubshell** | 4396 | 4101 | 3999 | 1995 | 5996 | Not an historic species here | **20,487** |

Table 2. Aggregation and propagation activities of endangered mussels in 2016.

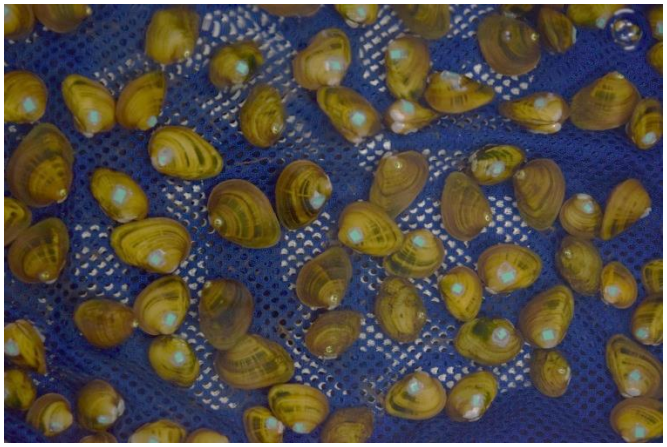
| Species | Aggregation or Propagation Site | Number of Individuals | Comments |
|---|---|---|---|
| Spectaclecase | Aggregated in Green River, KY | 30 | Larvae collected and placed in vitro in 2016, but no transformation to juvenile occurred |
| Orange-foot pimpleback | Aggregated in two sites:<br>1. Lower Tennessee River, KY<br>2. Gallatin Hatchery, TN; propagated by KY CMC | 1. 30<br>2. 6 | Larvae transformed to juvenile in vitro in 2016, but died later due to ammonia problem |
| Purple cat's paw | Aggregated in Killbuck Creek, OH; propagated by KY CMC | 12 | Over 3000 juveniles produced in vitro by KY CMC |
| Sheepnose | Aggregated in the Ohio River behind the Refuge headquarters (19), and Thomas More College (5). | 24 | Propagation attempted by WSSNFH on fish and KY CMC in vitro, but juveniles did not survive |
| Fanshell | Adults collected from the Licking River, KY and propagated by KY CMC | N/A | 5000 juveniles produced in vitro by KY CMC |

**13. High resolution photos of Target Species and Work:**


Tagged clubshells ready for the Little Kanawha River site. Photo by Patricia Morrison, USFWS

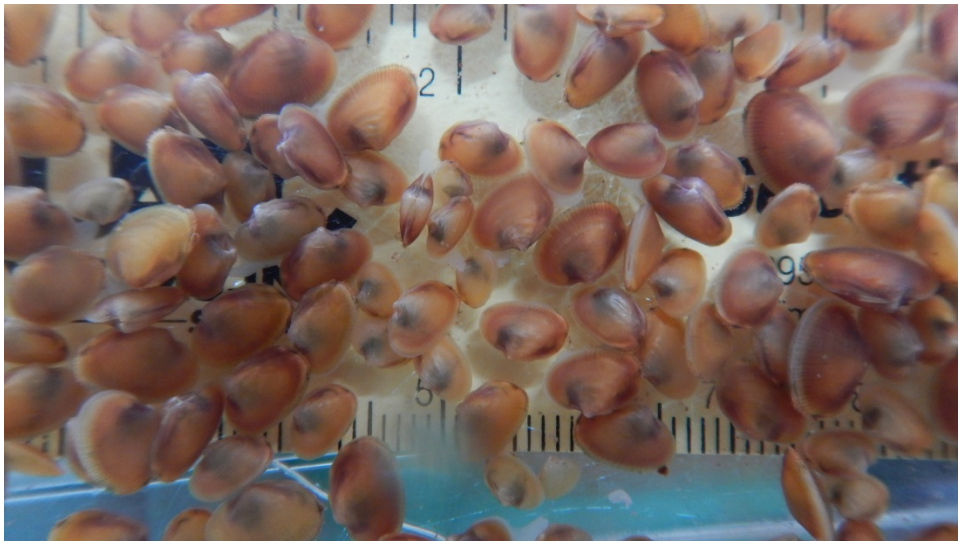

Tagged northern riffleshell tucks in to the riverbed on the refuge. Photo by Janet Clayton, WVDNR.

Matt Bristol tagging juvenile clubshell and northern riffleshell.
Photos by Patricia Morrison, USFWS



Both species tagged and ready to be stocked into the Ohio River.

Refuge divers Collette Johnson (left) and Patty Morrison (right) preparing to stock mussels.  Photo by Matt Bristol, USFWS



Four-month old purple cat's paws propagated in vitro in 2016.  Photo by Monte McGregor, KDFWR.

Purple cat's paw at 12-months old.  Photo by Monte McGregor, KDFWR..

Janet Clayton, WVDNR, taking a peek inside a female sheepnose to check for gravidity.  Photo by Matt Bristol, USFWS

Female sheepnose released her mature larvae in captivity.

A close up look at the sheepnose larvae packets, tinged with pink.

Both photos by Patricia Morrison, USFWS.

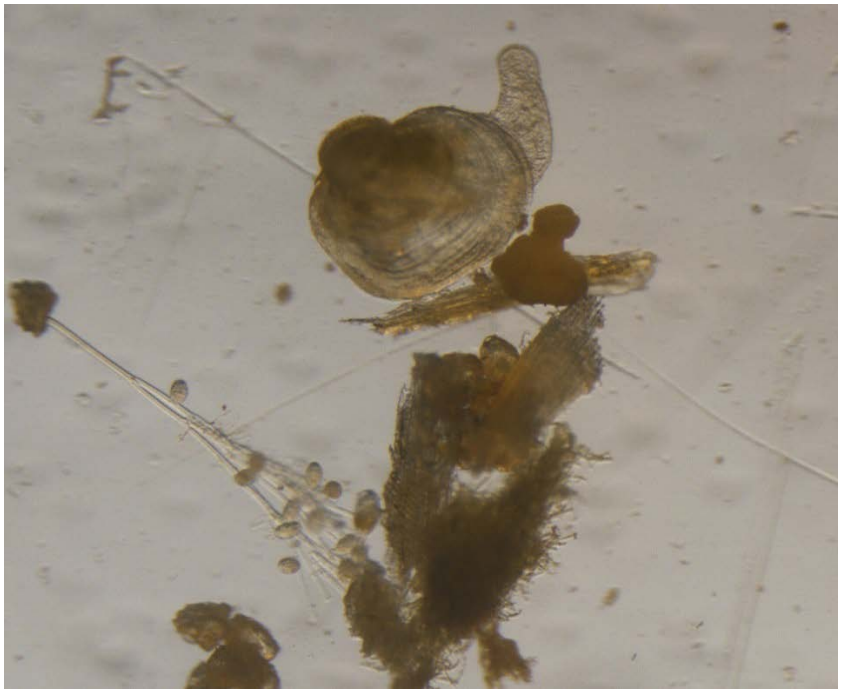
Purple cat's paw females being sorted from the cages in Killbuck Creek, OH.  Photo by Marty Heuhner, Enviroscience, Inc.

Biologist Angela Boyer, Columbus Field Office, sorting through substrate from cages to retrieve female purple cat's paw. Photo by Marty Heuhner, Enviroscience, Inc.


Genetics investigations in the lab of Dr. David Berg, Miami University (OH). Tissue sample from orange-foot pimpleback. Photo by David Berg.


The instrument is a Bio-Rad T100 Thermal Cycler, which is used for conducting polymerase chain reactions (PCR) for amplifying DNA. Photo by David Berg.

A collection of orange-foot pimpleback adults from the lower Tennessee River in KY. Photo by Monte McGregor, KDFWR.



A propagated juvenile orange-foot pimpleback, just a few days old, note the foot and young shell.  Photo by Monte McGregor, KDFWR.

Tagging fanshells prior to stocking at a new Ohio River site.

One of the 99 comprising the new pilot population in the Ohio River.

Both photos by Matt Bristol, USFWS.

One of the rarest mussels in the Ohio River basin, this ring pink was recently discovered in the Green River, KY. Cooperators will follow up with eDNA sampling to locate potential remaining populations. Photo by Chad Lewis, Lewis Environmental Consulting.