

**Ohio River Restoration**

**2014 Planned Activities**

**West Virginia Division of Natural Resources**

**March 20, 2014**

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## **Introduction**

The West Virginia Division of Natural Resources (WVDNR) is a trustee in the Ohio River Aquatic Restoration NRDAR Project 0237, and an active participant in the restoration effort.

## **Belleville Complex (BC)**

Three upweller holding tanks are currently being utilized to overwinter mussel broodstock at the WVDNR Belleville Complex. These tanks receive Ohio River water after passing through an additional tank which allows sediment to settle out. These tanks are available for holding mussel broodstock throughout the summer in order to monitor for glochidia development and/or conduct fish host inoculations. This facility is used for winter holding of broodstock for use the following spring by cooperators. The BC has also been used to overwinter juveniles.

## **Propagation Efforts – Palestine Fish Hatchery**

In previous years we have conducted propagation and grow-out of juvenile mussels in cages at Stonewall Jackson Lake and the French Creek Embayment of the Ohio River with some success. This year's propagation efforts will be concentrated within laboratory facilities of other cooperators where juveniles can be collected immediately after transformation and release from the host fish. These juveniles will then be distributed to various cooperators for grow-out in various systems. We propose to attempt grow-out of juveniles in fine mesh cages placed in elevated outdoor raceways at Palestine Hatchery. Water supplied to these raceways is gravity fed from a pond filled by pumping water from the Little Kanawha River. The Little Kanawha River is a highly diverse mussel stream and thus should provide adequate water quality and food resources. In 2014 we propose to attempt grow-out of several species which may include elephant ear (*Elliptio crassidens*), black sandshell (*Ligumia recta*), Ohio pigtoe (*Pleurobema cordatum*), sheepsnose (*Plethobasus cyphus*), pink mucket (*Lampsilis abrupta*), butterfly (*Ellipsaria lineolata*) and *Truncilla* spp.

## **Host Fish Collections**

In 2014 we propose to attempt collection of wild skipjack herring for host fish studies and/or propagation of elephant ear by Ohio State University (OSU)/Columbus Zoo (CZ).

## **Broodstock Collection**

Effort will again be directed toward the collection of gravid broodstock for propagation work to be conducted by project cooperators in 2014. Targeted species include round hickorynut (*Obovaria subrotunda*) as well as the species listed above targeted for grow-out. The pink mucket propagation activities including acquisition of broodstock will be conducted by Tennessee Tech University (TTU).

## **Monitoring**

In 2010, 200 endangered fanshells (*Cyprogenia stegaria*) were stocked at Muskingum Island and their survival will continue to be assessed in 2014. In 2013 we had proposed to conduct the 5 year monitoring at the head of Blennerhassett Island and the Degussa monitoring sites which were established in 2008. Only limited transects were able to be completed due to poor river conditions. This year we will attempt to conduct additional surveys at these locations. Active restoration with adults began at Blennerhassett Island in 2013 and the Degussa site is not being actively restored. The results of these surveys will be a good indicator of natural recovery in the river. We will also attempt to re-survey the site at Buckley Island that was surveyed in 2000.

## **Adult Translocations and Juvenile Stocking**

Coordination will be conducted on all construction projects being conducted within the Belleville Pool. Any project requiring mussel salvage and relocation will be requested to relocate

mussels to the head of Blennerhassett Island. This was a new area targeted for active restoration in 2013. Juveniles reaching taggable size will be stocked in potentially three locations, Muskingum Island (federally endangered species), Neal Island, and/or Blennerhassett Island.

**Summary of Expenses**

Cost estimates are provided in Table 2.

Table 2. Estimated expenses for 2014 proposed Ohio River mussel restoration activities by WV Division of Natural Resources.

Broodstock Collection Costs	\$7,010.87
Juvenile Grow-out Palestine Hatchery and Stocking	\$10,509.83
Broodstock and Juvenile Holding Belleville Complex	\$3,684.60
Fish Collection and Delivery	\$1,927.95
Monitoring	\$2,090.66
<b>Grand Total</b>	<b>\$25,223.91</b>

## Proposal to the Ohio River Restoration Work Group

### Submitted by:

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31 March 2014

### Host Identifications for Freshwater Mussels

The Facility has a history of determining potential hosts for mussels using state-of-the-art equipment. We have identified numerous hosts, including hosts for three federally endangered species. The Facility currently has two full-time staff dedicated to mussel propagation and host identification and all necessary equipment is in place. The Facility now has an agreement with the Newport (KY) Aquarium in which their personnel will supply Ohio River fish, including some rarely used species, to the Facility for host work. This is in addition to our fish supplies from ODNR and our own collections.

We have independently contracted a dive team to collect mussels in April-May of this year for host identification work and brood stock. Of the 26 species listed as impacted by the spill, we anticipate receiving eight of these species in this effort. We will take delivery of host fishes from commercial hatcheries in preparation for the study. Additional host fishes are being supplied by the Thomas Moore College Field Station and OSU personnel. Host identifications take place throughout most of the year, with the remainder of the time devoted to caring for the fish, mussels, and maintenance of the facility.

Our 2014 plan calls for continued host identifications for *Elliptio crassidens*. The following species may be included as well as specimens become available: *Ellipsaria lineolata*, *Truncilla donaciformis*, *Truncilla truncata*, *Pleurobema cordatum*, and *Plethobasus cyphus*. *In vitro* transformations studies will begin on these species as well.

### Mussel Propagation

The Advisory Committee has identified 10 species of mussels for work in 2014. We advocate propagating mussels by several methods:

1. Releasing newly transformed juveniles to the river. This method delivers the largest number of juveniles but probably has the greatest mortality rate. It is also more difficult to gage success as the juveniles are too small to tag.

2. Growing juveniles in the laboratory using a variety of grow-out chambers, such as those in use by Missouri State University and the Frankfort, KY, facility.
3. Growing juveniles in cages within Ohio from captive hosts. The Facility already has a program with The Wilds to use their existing ponds for this purpose. No fish will be released or placed into the Ohio River.
4. Transformation *in vitro* followed by juvenile grow out in Methods 3 & 4.

Our 2014 plan calls for propagation of the following species: *Ellipsaria lineolata*, *Lampsilis abrupta*, *Truncilla donaciformis*, *Truncilla truncata*, *Pleurobema cordatum*, and *Plethobasus cyphus*.

**Total requested: \$75,000.**

### **Matching**

The major expenses for this mitigation project have already been paid by other parties. The main Facility has been on line for several years. We do not request any addition funds for the existing Facility beyond defraying maintenance costs and a single dedicated personnel position. Currently over \$500,000 has been spent in personnel and equipment to bring the Facility to its present state. This represents a significant commitment of the Ohio Department of Natural Resources Division of Wildlife, the US Fish and Wildlife Service, the Ohio State University, the Columbus Zoo and Aquarium, and many other parties to this propagation effort. The Ohio Department of Natural Resources Division of Wildlife also will supply funding for the *in vitro* CO2 incubator and necessary supplies.

**Budget. Columbus Zoo and Aquarium Freshwater Mussel Facility**  
**Ohio River Restoration 2014**  
**Submitted 31 March 2014**

**Host work**

	hr/week	cost/week	weeks	totals
Elliptio crassidens	15	\$558.37	8	\$4,466.96
Pleurobema cordatum	15	\$558.37	8	\$4,466.96
Plethobasus cyphus	15	\$558.37	8	\$4,466.96
Truncilla sp.	15	\$558.37	8	\$4,466.96
Ellipsaria lineolata	15	\$558.37	8	\$4,466.96
<b>totals</b>				<b>\$22,334.80</b>

**Propagate**

	hr/week	cost/week	weeks	totals
<b>Infest</b>				
Ellipsaria lineolata	7.5	\$279.16	5	\$1,395.80
Lampsilis abrupta	7.5	\$279.16	5	\$1,395.80
Truncilla donaciformis	7.5	\$279.16	5	\$1,395.80
Plethobasus cyphus	7.5	\$279.16	5	\$1,395.80
Pleurobema cordatum	7.5	\$279.16	5	\$1,395.80
Truncilla truncata	7.5	\$279.16	5	\$1,395.80
<b>totals</b>				<b>\$8,374.80</b>

	hr/week	cost/week	weeks	totals
<b>Monitor</b>				
Ellipsaria lineolata	7	\$260.57	16	\$4,169.12
Lampsilis abrupta	7	\$260.57	16	\$4,169.12
Truncilla donaciformis	7	\$260.57	16	\$4,169.12
Plethobasus cyphus	7	\$260.57	16	\$4,169.12
Pleurobema cordatum	7	\$260.57	16	\$4,169.12
Truncilla truncata	7	\$260.57	16	\$4,169.12
<b>totals</b>				<b>\$25,014.72</b>

	hr/week	cost/week	weeks	totals
<b>In vitro</b>				
Ellipsaria lineolata	7.5	\$279.16	10	\$2,771.60
Lampsilis abrupta	7.5	\$279.16	10	\$2,771.60
Truncilla donaciformis	7.5	\$279.16	10	\$2,771.60
Plethobasus cyphus	7.5	\$279.16	10	\$2,771.60
Pleurobema cordatum	7.5	\$279.16	10	\$2,771.60
Truncilla truncata	7.5	\$279.16	10	\$2,771.60
<b>totals</b>				<b>\$16,749.60</b>

Facility costs \$2,525.08

**Total \$75,000.00**

## Budget for 2014 Ohio River Restoration: Juvenile Propagation and Culture at White Sulphur Springs National Fish Hatchery

The table below represents an estimate of costs based on grow out of 3 mussel species to be shipped to us by Tennessee Tech and or Genoa NFH, and the Propagation/grow out of 1 species at WSSNFH. We are scheduled to grow out the following species: Pink Mucket, Butterfly, and Black Sandshell. We will propagate and grow out Round Hickorynut. Effort and personnel hours for juvenile culture are based on producing enough mussels to release and average of 500 tagged juveniles at 15-30+ mm in length. WSSNFH will continue to grow-out the remaining 6,856 juveniles from 2012-2013. We will grow mussels out in floating cages and in the laboratory Upwelling systems.

<b>Task</b>	<b>Effort (hours)</b>	<b>Rate/hour</b>	<b>Number of personnel required</b>	<b>Total (\$)</b>
Mussel broodstock collection	16	20	1	320
Fish collection	30	60(total)	3	1,800
Operational costs (Utilities and supplies)				12,000
Juvenile mussel collection and tagging	125	20	2	5,000
Juvenile culture assuming 1-2 year grow-out	1040	15.15	1.0	15,756
Juvenile cage culture	60	30	2	3,600
Development of work plans, data entry, writing annual reports	40	45	1	1,800

Note\* the 1040 hours is based on half of an employee's yearly hours dedicated to the above listed production. The other half of time is dedicated host fish, algae production, coldwater fish production and many other hatchery duties.

**\$40,276**

**Table 1. WSSNFH 2014 Target Species for Ohio River Restoration**

Target High Priority Mussel Species <sup>1</sup>	Laboratory Grow-out	Cage Infestation/Grow-out	Host Fish Work	Total current #
Plain pocketbook <i>Lampsilis cardium</i>	Yes	Yes	No	5421
Pimpleback <i>Quadrula pustulosa</i>	Yes	Yes	No	34
Round hickorynut <i>Obovaria subrotunda</i>	Yes	Yes	No	214
Fat mucket <i>Lampsilis siliquoidea</i>	Yes	Yes	No	956
Black sandshell <i>Ligumia recta</i>	Yes	Yes	No	22
Mucket <i>Actinonaias ligamentina</i>	Yes	Yes	No	194
Pink heelsplitter <i>Potamilus alatus</i>	Yes	Yes	No	517
<b>PROPAGATION 2014</b>				
Round hickorynut <i>Obovaria subrotunda</i>	Yes	Yes	No	
<b>GROW OUT FROM 2014 PROPAGATION @ TN Tech/Genoa NFH</b>				
Pink mucket <i>Lampsilis abrupta</i>	Yes	Yes	No	
Butterfly <i>Ellipsaria lineolata</i>	Yes	Yes	No	
Black sandshell <i>Ligumia recta</i>	Yes	Yes	No	



**Proposed Ohio River Aquatic Restoration Work FY 2014**  
**USFWS-Ohio River Islands NWR**  
**NRDAR Project 0237**

Collect broodstock mussels and snails, and transport as needed for propagation facilities (*O. subrotunda*, *E. crassidens*, *E. lineolata*, *L. recta*, *Truncilla spp.*, *P. cyphus*, and *Lithasia spp.*). 8 (2-person) dive team days = \$ 4800

Conduct quantitative and qualitative monitoring at passive restoration sites (DeGussa and Blennerhassett Island), possibly Buckley Island reference site, and fanshell re-introduction sites, 6 (3-person) dive team days = \$ 5280

Transport, hold and observe gravid mussels in refuge lab and Belleville to check for larval maturity 6 days = \$ 1200

Coordination with Technical Committee, Trustees, write up annual work plans and accomplishment reports. 6 days = \$ 2100

**TOTAL for 2014**

**\$ 13,380**

**Production of Juvenile butterfly, deertoe, fawnsfoot and black sandshell mussels**

**for**

**Ohio River Restoration Project 0237**

Genoa National Fish Hatchery (NFH) has been actively involved in mussel propagation since 2000. Currently, Genoa NFH is producing four federally endangered species, one federal candidate and several more of interest on local state levels. Genoa NFH proposes to propagate two target species which use freshwater drum (*Aplodinotus grunniens*) as a primary host for the Ohio River Restoration Project 0237 in 2014. Four mussel species targeted for restoration under the project use the freshwater drum as primary host; the butterfly (*Ellipsaria lineolata*), pink heelsplitter (*Potamilus alatus*), deertoe (*Truncilla truncata*) and fawnsfoot (*Truncilla donaciformes*). For this year the butterfly, deertoe and fawnsfoot will be the focus of restoration efforts. In addition to this effort the black sandshell (*Ligumia recta*) will also be propagated using walleye (*Sander vitreus*) produced at Genoa NFH. Biologists from West Virginia/Ohio will collect gravid mussels of the target species during their brooding period and ship them to Genoa NFH. Genoa NFH biologists will extract glochidia, inoculate host fish, and monitor infested fish for juvenile mussel transformation. All propagation will be done at Genoa NFH in disease free conditions; no mussels will be either produced or cultured off-station or in wild stream water. At the end of each infestation newly metamorphosed juvenile mussels will be shipped to restoration partners for further culture before release.

**Budget**

<b>Host Fish Collection</b>	<b>\$3,500</b>
<b>Host Fish Maintenance</b>	<b>\$2,000</b>
<b>Salary</b>	<b>\$8,000</b>
<b>Utilities/Shipping</b>	<b>\$1,000</b>
<b>Total</b>	<b>\$14,500</b>

# Proposal for 2014 Ohio River Restoration: Propagation and Culture of Juvenile Mussels at Tennessee Technological University

**Submitted by:**  
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**Tennessee Technological University**  
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For 2014 we propose to propagate and culture four species of freshwater mussels (*Lampsilis abrupta*, *Ligumia recta*, *Cyprogenia stegaria*, and *Obovaria subrotunda*) for restoration efforts in the Ohio River. The goal of the Tennessee Cooperative Fishery Research Unit will be to produce a total of 5,000 juvenile *Lampsilis abrupta* of 3 year classes. Each year class will be cultured for 16 to 18 months. This long-term culture should provide juveniles of a size sufficient for tagging, 20-30 mm long. Culturing activities will occur at Tennessee Wildlife Resources Agency's Normandy Fish Hatchery, Cumberland River Aquatic Center, and also cage culture at Dale Hollow National Fish Hatchery. Broodstock will be a combination of a captive population, currently held at the Normandy Fish Hatchery, and additional individuals collected from the Tennessee River. In addition to growing out juveniles to a taggable size, the Tennessee Cooperative Fishery Research Unit will also transfer 1-7 day old juveniles to other cooperators for grow-out in their respective mussel culture facilities. Depending on availability, up to 10,000 newly metamorphosed juvenile *L. abrupta* will be distributed each year among the other cooperators. These juveniles will be delivered to cooperators using overnight shipping with next day delivery service provided by shipping companies. Juvenile pink muckets (~125) of a taggable size produced in previous years will be transferred to WVDNR for stocking in the summer of 2014. In addition to providing newly metamorphosed and taggable juvenile pink muckets, the Tennessee Cooperative Fishery Research Unit will provide 3 gravid female *L. abrupta* to Ohio State University to be used as broodstock in captive propagation activities.

In addition to the pink mucket, the Tennessee Cooperative Fishery Research Unit will attempt to produce a total of 5,000 taggable juvenile *Ligumia recta* and 2,000 taggable juveniles of *Obovaria subrotunda* and *Cyprogenia stegaria* of 3 year classes. Each year class will be cultured for 16 to 18 months to allow juveniles to grow to a size sufficient for tagging. In addition to growing out juveniles to a taggable size, Tennessee Cooperative Fishery Research Unit will also transfer 1-7 day old juvenile *L. recta*, *C. stegaria*, and *O. subrotunda* each year to other cooperators for grow-out in their respective mussel culture facilities. Depending on availability, up to 5,000 newly metamorphosed juvenile *L. recta* and 1,000 newly metamorphosed juvenile *O. subrotunda* and *C.*

*stegaria* will be distributed each year among cooperators' facilities. Gravid female *L. recta* and *O. subrotunda* used for broodstock would be provided to the Tennessee Cooperatives Fishery Research Unit by other cooperators. Individuals from source populations in or near the Ohio River would be collected by other cooperators and transferred to the Tennessee Cooperative Fishery Research Unit. Broodstock for *Cyprogenia stegaria* would be collected from the Green River, KY by Tennessee Cooperative Fishery Research Unit personnel. All broodstock individuals would be held at the Normandy Fish Hatchery until the completion of propagation activities at which time they would be returned to the original providers.

**Table 1. Proposed species and culture methods of propagation activities at TCFRU and number of newly metamorphosed juveniles to be produced for cooperators facilities for grow out in 2014.**

Species	Laboratory culture	Cage culture	Number to be shipped	Receiving Facility*
<i>Lampsilis abrupta</i>	Yes	Yes	10,000	WSS, OSU
<i>Ligumia recta</i>	Yes	Yes	5,000	WSS, WVDNR
<i>Obovaria subrotunda</i>	Yes	No	1,000	WSS
<i>Cyprogenia stegaria</i>	Yes	No	1,000	MSU

\*WSS: White sulfur Springs National Fish Hatchery, OSU: Ohio State University, WVDNR: West Virginia Department of Natural Resources, MS: Missouri State University

**Budget for proposed propagation and culture of mussels at Tennessee Technological University for Ohio River Restoration.**

Task	Estimated Cost
Propagate, collect and grow out juveniles	\$33,887
Collect adult mussels for propagation	\$2,136
Captive care of fish and mussel broodstock	\$5,875
Acquire host fish (for mussel propagation)	\$6,093
Annual report, work plan etc.	\$2,437
<b>Total Cost</b>	<b>\$50,428</b>

**Propagation and captive culture of *Cyprogenia stegaria*, and  
captive culture of *Ellipsaria lineolata* and *Truncilla truncata*.**

Ohio River Restoration NRDA  
Technical Committee

2014 Proposal

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## Summary:

Missouri State University (MSU) will collaborate with the Ohio River Restoration NRDA Technical Committee (ORR) to propagate and restore native freshwater mussels. Specifically, MSU will grow out two species propagated by Genoa National Hatchery (*Truncilla truncata* and *Ellipsaria lineolata*), and will propagate and grow out a third species, *Cyprogenia stegaria*. In each of two production years, MSU will culture up to 5,000 individuals of each species in laboratory systems. In the second and third years, 1-year old juveniles from lab culture will be transferred to upwellers fed on lake water at the Kansas City Zoo for grow-out to at least 2 cm. Mussels will be tagged by laser engraving and supplied to ORR for release. An average annual budget of \$26,404 per year over 3 years is proposed.

## Qualifications:

Missouri State University (MSU) has 20 years of experience in the propagation and captive culture of native mussels. We have metamorphosed 35 species of Unionidae and Margaritiferidae on fish hosts, more than 1/10 of the North American fauna. Of these species, we have cultured 21 species to more than one year of age, 11 species to more than 3 years, and at least 8 species to sexual maturity (Table 1). We developed many of the methods and equipment currently in use, including the use of modified AHAB systems for host work, "mucket bucket" downweller systems for culture of postmetamorphic juveniles, floating upweller systems (flupsys) and raceway upwellers for pond culture, and Bernoulli flow cages (mussel silos) for in-stream caging. We have carried out studies of mussel host specificity, immunity, toxicology, culture and feeding ecology (CV, attached). We have collaborated with a wide variety of agencies to investigate mussel biology and to supply mussels for research and restoration (Table 2 and CV). We have propagated mussels for population restoration projects in Kansas, Missouri, Arkansas, Oklahoma, Tennessee, and California.

## Approach:

- 1) The target species in the first two years will be *Truncilla truncata* and *Ellipsaria lineolata*, which will be propagated on drum by Genoa National Hatchery, and *Cyprogenia stegaria*, which will be propagated by MSU. MSU will grow out up to 5,000 individuals of each species for two years. We have worked with *Ellipsaria* previously and cultured hundreds to sexual maturity for EPA-sponsored work on environmental estrogens (Figure 1). We are currently working on the spawning biology of *Truncilla truncata*. *Truncilla* species spawn in the early spring, which is unusual among lampsiline taxa. Therefore searches for brooding females should be made in the spring months, not the fall. We have previously propagated *Cyprogenia* for host work (Eckert 2003, Serb and Barnhart 2008) but have not previously attempted grow-out of this species.

- 2) In each project year, juveniles of *Ellipsaria* and *Truncilla* will be provided by Genoa. If needed, we are readily able to propagate these species at MSU using drum obtained from Langston University.
- 3) Brooding female *Cyprogenia* will be collected by ORR collaborators from source populations between November 1 and June 1 and transported to MSU. Females will be acclimated to reconstituted water, and then held in isolation at MSU at 10C in recirculating aquarium systems designed for this purpose. The females will not be fed during the holding period. Before removal of glochidia the females will be weighed, measured, and permanently marked by engraving so that they can be identified if recollected after return to the source population. Glochidia larvae will be obtained from female *Cyprogenia* by withdrawing conglutinates from the distal margin of the demibranchs. Glochidia will be dislodged by spraying through a screen. Glochidia will be counted, and the proportion of viable glochidia and unfertilized eggs will be determined for calculation of fertilization success. Host fish will be logperch (*Percina caprodes*) which is the most widespread and abundant host. Fish will be inoculated and juvenile mussels will be recovered quantitatively using standard methods. Metamorphosis success will be determined as the proportion of attached glochidia that are recovered as metamorphosed juveniles.
- 4) We will seek to obtain from Genoa or to produce by propagation on fish hosts 50,000 or more juveniles of each species per year, of which we expect about 10% may survive two years in culture. This estimate is based on experience with these and other species. Culture for the first 6 months will take place in laboratory downweller systems (mucket buckets) using artificial food (Barnhart 2006). After mussels reach 1 mm they are moved to larger (300 liter) laboratory upweller systems. Food is delivered hourly by computer-driven peristaltic pumps and food levels are monitored using a Beckman-Coulter MS-4.
- 5) When the mussels reach 3 mm or larger in size, will be moved to larger upweller systems at the Kansas City Zoo for at least one summer of growth on natural food (Figure 1). Growth rates, survival, and maturity will be monitored approximately monthly during the growing season. Survival generally approaches 100%/month in these systems.
- 6) When the mussels reach at least 2 cm shell length, they will be individually laser-engraved and will be available for release (Figure 2). All decisions and actions regarding release and subsequent monitoring will be the responsibility of ORR.
- 7) The project will contribute to the training of graduate and undergraduate university students in the biology and conservation of freshwater mussels. MSU has a 20-year history of training students in the biology and conservation of freshwater mollusks and has produced leaders in the field. Through our partnership with the Kansas City Zoo, we also promote

public recognition of the importance of freshwater conservation. We will provide ORR and others with high quality photo and video resources to help document this work for the public.

- 8) The proposal is based on a funding level of \$25,254 for the first year (lab culture only). Cost of the second year (\$30,650) includes culture at KC Zoo of first year juveniles and lab culture of second year production, while the third year (\$23,308) assumes only pond upweller culture of mussels from the second propagation year (see budget).

**Literature Cited:**

Barnhart, M.C. 2006. A compact system for rearing juvenile freshwater bivalves. *Aquaculture* 254:227-233. doi:10.1016/j.aquaculture.2005.08.028.

Eckert, Nathan 2003. Reproductive biology and host requirements among isolated populations of *Cyprogenia aberti* (Conrad 1850). Master of Science, Missouri State University.

Serb, J. M. and M. C. Barnhart. 2008. Congruence and conflict between molecular and reproductive characters when assessing biological diversity in the western fanshell *Cyprogenia aberti* (Bivalvia: Unionidae). *Annals of the Missouri Botanical Garden* 95(2): 248-261.



Table 1. List of mussel species which MSU has propagated (metamorphosis on fish hosts), species which were captive-cultured for more than 1 year, and species have cultured to 3 or more years of age. Asterisk indicates culture to sexual maturity.

Species		Metamorphosis	>1 year	>3 years	
1	<i>Ellipsaria lineolata</i>	Butterfly	X	X	X*
2	<i>Lampsilis abrupta</i>	Pink mucket	X	X	X*
3	<i>Lampsilis rafinesqueana</i>	Neosho mucket	X	X	X*
4	<i>Lampsilis reeveiana</i>	Broken-rays	X	X	X*
5	<i>Lampsilis siliquoidea</i>	Fatmucket	X	X	X*
6	<i>Leptodea leptodon</i>	Scaleshell	X	X	X*
7	<i>Ligumia recta</i>	Black sandshell	X	X	X*
8	<i>Megaloniaias nervosa</i>	Washboard	X	X	X*
9	<i>Potamilus alatus</i>	Pink heelsplitter	X	X	X*
10	<i>Quadrula cylindrica</i>	Rabbitsfoot	X	X	X*
11	<i>Quadrula fragosa</i>	Winged mapleleaf	X	X	X
12	<i>Amblema plicata</i>	Threeridge	X	X	
13	<i>Anodontoides ferrusacianus</i>	Cylindrical papershell	X	X	
14	<i>Epioblasma triquetra</i>	Snuffbox	X	X	
15	<i>Lampsilis cardium</i>	Plain pocketbook	X	X	
16	<i>Margaritifera falcata</i>	Western pearlshell	X	X	
17	<i>Plectomerus dombeyanus</i>	Bankclimber	X	X	
18	<i>Potamilus capax</i>	Fat pocketbook	X	X	
19	<i>Ptychobranchus occidentalis</i>	Ouachita kidneyshell	X	X	
20	<i>Utterbackia imbecillis</i>	Paper pondshell	X	X	
21	<i>Villosa iris</i>	Rainbow mussel	X	X	
22	<i>Actinonaias ligamentina</i>	Mucket	X		
23	<i>Anodonta suborbiculata</i>	Flat floater	X		
24	<i>Anodonta californiensis</i>	California floater	X		
25	<i>Arkansia wheeleri</i>	Ouachita rock pocketbook	X		
26	<i>Cyprogenia aberti</i>	Western fanshell	X		
27	<i>Fusconaia ebena</i>	Ebonysell	X		
28	<i>Fusconaia flava</i>	Wabash pigtoe	X		
29	<i>Fusconaia ozarkensis</i>	Ozark pigtoe	X		
30	<i>Obliquaria reflexa</i>	Threehorn wartyback	X		
31	<i>Quadrula pustulosa</i>	Pimpleback	X		
32	<i>Simpsonaias ambigua</i>	Salamander mussel	X		
33	<i>Truncilla truncata</i>	Deertoe	X		
34	<i>Venustaconcha ellipsiformis</i>	Ellipse	X		
35	<i>Venustaconcha pleasii</i>	Pleas' mussel	X		

Table 2. Agencies with whom we have worked and/or supplied cultured freshwater mussels for research and restoration.

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- 1 Arkansas Game and Fish Commission
  - 2 Arkansas State Highway Commission
  - 3 Clemson University
  - 4 Environment Canada
  - 5 Kansas Department of Wildlife and Parks
  - 6 King County, Washington
  - 7 Marrone BioInnovations, Inc.
  - 8 McMaster University
  - 9 Missouri Department of Conservation
  - 10 National Park Service, Buffalo River
  - 11 North Carolina State University
  - 12 Oklahoma Department of Wildlife Conservation
  - 13 Osage Tribe of Indians of Oklahoma
  - 14 Peoria Tribe of Indians of Oklahoma
  - 15 Presidio Trust, San Francisco CA
  - 16 U.S. Army Corps of Engineers, Memphis District
  - 17 U.S. Environmental Protection Agency
  - 18 U.S. Fish and Wildlife Service Region 2
  - 19 U.S. Fish and Wildlife Service Region 3
  - 20 U.S. Fish and Wildlife Service Region 4
  - 21 U.S. Fish and Wildlife Service Region 6
  - 22 U.S. Geological Survey, Columbia Environmental Research Center
  - 23 U.S. Geological Survey, Upper Midwest Environmental Sciences Center
  - 24 University of Oklahoma
  - 25 University of Georgia
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Figure 1. A. Raceway upweller at Kansas City Zoo. B. Detail of raceway upweller showing 2-year pink mucket, about 500 mussels per bucket. C. 2-year butterfly, *Ellipsaria lineolata*, D. 1.5 year scaleshell, *Leptodea leptodon*, E. 3-year rabbitsfoot, *Quadrula cylindrica*. F. 0.5 year black sandshell, *Ligumia recta*

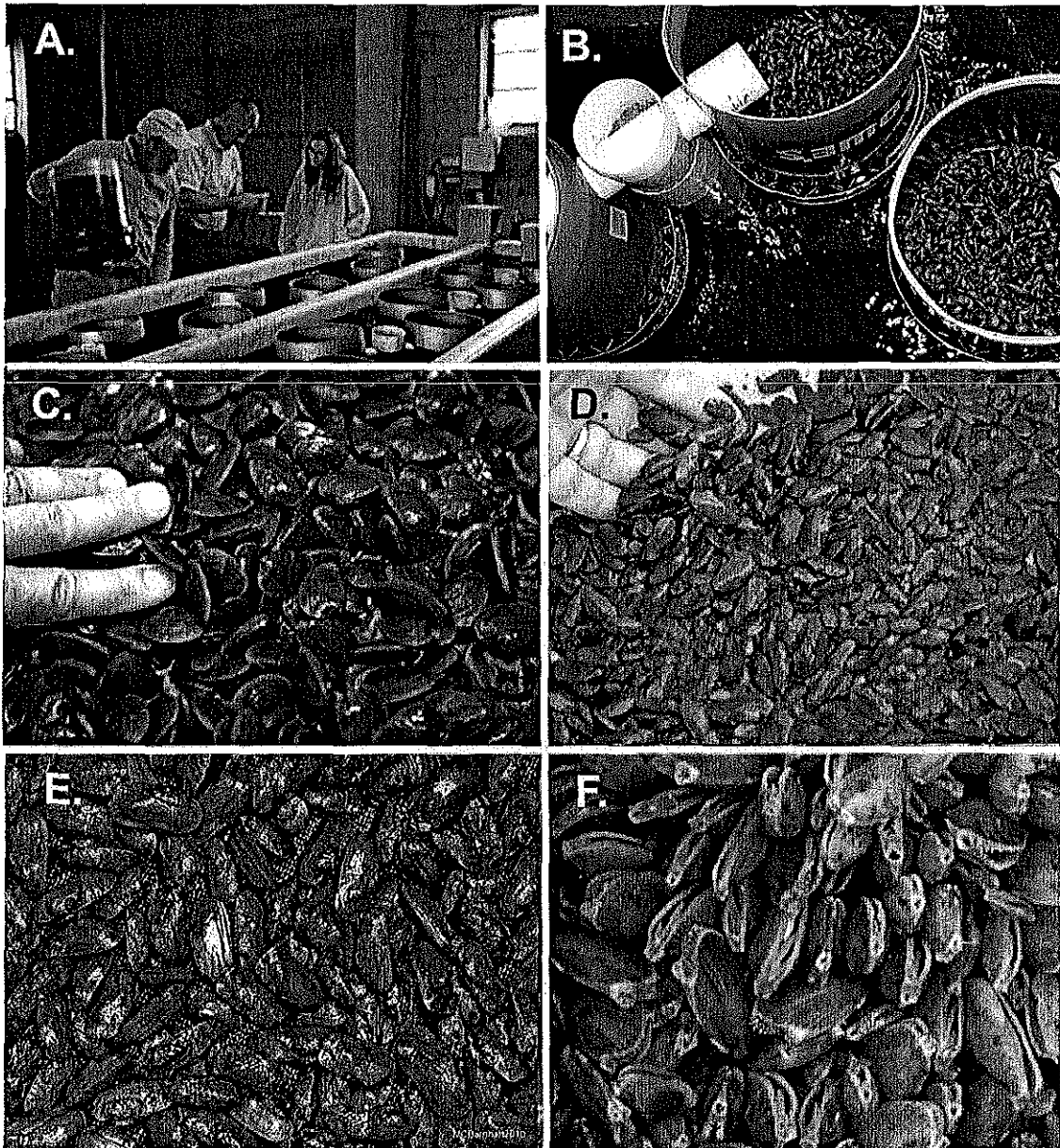


Figure 2. Laser-engraving propagated pink mucket for identification



Tasks and Expenses	Year 1 - 2014	Year 2 - 2015	Year 3 - 2016
Salaries for propagation and grow out	\$17,460	\$21,152	\$14,768
Supplies and materials	\$3,500	\$3,500	\$3,500
Travel (host fish collection, mussel delivery, work at the KC Zoo)	\$1,000	\$2,000	\$2,000
Facilities and Administration	\$3,294	\$3,998	\$3,040
<b>Total Project Cost</b>	<b>\$25,254</b>	<b>\$30,650</b>	<b>\$23,308</b>

Culture and propagation of the fanshell, *Cyprogenia stegaria*, elephantear, *Elliptio crassidens*, and sheepnose, *Plethobasus cyphus*, from the Ohio River drainage

Prepared by

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for

Ohio River Restoration Project 0237

**Project:** Culture and propagation of the fanshell, *Cyprogenia stegaria*, elephantear, *Elliptio crassidens*, and sheepnose, *Plethobasus cyphus*, from the Ohio River drainage

**Location:** Kentucky Dept. of Fish and Wildlife Resources Center for Mollusk Conservation, Frankfort, KY

**Contact:** Dr. Monte McGregor, Kentucky Dept. of Fish and Wildlife Resources, 3761 Georgetown Road, Frankfort, KY 40601, 502-573-0330x221, monte.mcgregor@ky.gov

**Species:** fanshell, *Cyprogenia stegaria*, elephantear, *Elliptio crassidens*, and sheepnose, *Plethobasus cyphus*

**Locations:** Ohio River and tributaries

**Problem and Need:**

Freshwater mussels are the most at-risk group of animals in North America. Of the 297 native mussel species in the United States, 71.7% are considered endangered, threatened, or of special concern, including 21 mussels that are endangered and presumed extinct (Williams et al., 1993). Seventy species (23.6%) are considered to have stable populations, although information is lacking for many of these species. Mussel and host fish populations are projected to decline if habitats are not restored and individual species numbers increased. Modern day threats to mussels include habitat destruction from a variety of factors, including sedimentation from agricultural land, logging and mining operations, construction projects, stream channelization and dredging, toxic spills (oil, gas, industrial acids, pesticides, fertilizers) and resulting fish kills, and invasion from exotic species (Parmalee and Bogan 1998). With the low numbers of mussels and continuing population declines, protecting each stage in the life cycle of the mussel becomes critical. Critical life history stages include the production of gametes (currently inhibited by low density levels), host fish attachment and development, and juvenile survival. Suitable hosts must be present in adequate numbers to increase the chance of "catching" a larvae. Sufficient habitat must also be present for grow-out of juveniles to the adult stage. Good water quality and habitat is critical to all stages of development, especially so for the larval and juvenile stage. Culture of juveniles to a stockable size improves the chances for increasing population size, as larger juveniles have much higher survival. Larger juveniles may reach a reproductive age in 2-3 years, thus creating more opportunities for reproductive success and future recruitment.

The Ohio River is a large tributary to the Mississippi River located in the eastern US. The River supports a diverse fauna with around ~80 species of freshwater mussels found in the mainstem portion bordering Kentucky. Kentucky has 104 species of mussels and currently has 27 federally listed freshwater mussels; 46 species are on the states' list of species of greatest conservation need.

KDFWR's Center for Mollusk Conservation has developed over the past few months into a multiphase facility, capable of holding adults in semi-natural conditions, culture of juvenile mussels, and growout of juveniles to a larger, stockable size. We have successfully reared several species of *Lampsilis* (*L. abrupta*, *L. fasciola*, *L. siliquioidea*, *L. hydiana*, *L. teres*, and *L. cardium*) and have shown significant growth of each of these

species. The project will promote a cooperative effort among interstate organizations and state fish and wildlife agencies.

We propose to work with the staff at the Ohio River Islands National Wildlife Refuge and the state of West Virginia to culture and propagate the fanshell, *Cyprogenia stegaria*, elephantear, *Elliptio crassidens*, and sheepsnose, *Plethobasus cyphus*, from the Ohio River drainage. As part of this proposal, we will take brood stock from the Ohio River and/or its tributaries for use in the culture of juveniles for grow out at the Center for Mollusk Conservation in Frankfort, KY. We propose to attempt to grow to a stockable size (at least 15-20 mm) a minimum of 1,000 juveniles of each of the three species for release at selected sites in the Ohio River and its tributaries. Additional individuals (up to 1,000) of the fanshell adults will be provided from the Licking River population for translocation to at least 3 sites in West Virginia and Ohio. This translocation is conditional upon approval by the lead office for the fanshell. The site (s) will be selected by Ohio River Islands staff in coordination with the Center for Mollusk Conservation and other federal and state agencies as needed. We will provide advice on where and how to release the mussels, and how to monitor the success of the project.

**Schedule/Cost**

Spring 2014: Select Sites and Collect Brood Stock (recommended early spring, May to June). A minimum of 3 up to 20 females are needed. These females will be handled by CMC staff or methods provided to the collector to reduce stress during handling.

Spring 2014: Fish Collection (at least 1-2 weeks prior to brood stock collection). Fish will be collected by CMC staff. We anticipate needing 100-150 fish for each species except the elephantear, which will only be attempted using *in vitro* culture methods due to difficulty of handling and holding the host (skipjack heering).

Staff Needs

Broodstock Collection, Infestation, Invitro Culture and Juvenile Production

Year 1 (2014)	
Two Staff: fish collection/transport	\$1,500
Fish Purchase	\$500
Equipment (fish transport tank, salt treatment, fuel)	\$1,500
Fish Infestation (1 day, 3 staff) x 6 events	\$3,000
Fish Husbandry (use of tanks, feed, staff-up to 21 days)	\$3,000
Equipment (invitro culture: antibiotics, materials, methods, pipette tips, sterile filters, dishes, chemicals)	\$1,500
Culture Serum (carp serum and rabbit serum)	\$3,000
<i>In vitro</i> Husbandry (use of incubators, and staff-up to 21 days)-2 events	\$4,500
Project Oversight (CMC director and Senior Biologists)	\$2,000
Facility Maintenance, Equipment, and Use Fees (10%)	\$2,500
Subtotal 1	\$23,000

Grow Out (2014-2015)

Culture and Propagation and Growout (mussel culture tanks, algae food, husbandry, tank maintenance, captive care for 6-18 months) **		
Captive Care and Maintenance (4 hours/day x 5 months-May to Sept)	\$6,000	
Captive Care and Maintenance (Minor Clark Hatchery-5 hours/ week Winter/7 months)	\$1,500	
Facility Maintenance, Equipment, and Use Fees (10%)	\$750	
	Subtotal 2	\$8,250
<u>First Year Total:</u>		<u>\$31,250</u>

Year 2 (2015-2016)

Summer growing season (5 months - May to Sept)		
Captive Care and Maintenance (Summer-4 hours/day x 6 months)	\$6,000	
Captive Care and Maintenance (Minor Clark Hatchery-5 hours/ week Winter/6-7 months) ***	\$1,500	
PIT tags (500)	\$3,000	
	Project Oversight (CMC director and Senior Biologists)	\$2,000
	Facility Maintenance, Equipment, and Use Fees (10%)	\$1,250
	Subtotal 3	\$13,750

\*\* We anticipate using seasonal Fish and Wildlife Technicians to monitor and feed juvenile mussels during the year. These positions are entry level positions that last up to 9 months at \$11.33/hour.

\*\*\* This cost will permit the last group of each of the three species to be held a second winter so a second batch could be released in 2016 from the original 2014 batch.



2014 Work Plan Chart

Potential Mussel and Snail species  (time gravid females available)	Facility/Cooperator							Comments
	CZ/OSU	Genoa	TN Tech	WSSNFH	WVDNR	ORINWR	KY DFWR Mo. State U.	
<b>Lampsilis abrupta</b> , pink mucket (LT, March-May)	PF, G		PF, G, B	G	G			F2 broodstock from TN Tech; some go to CZ/OSU
<b>Cyprogenia stegaria</b> , fanshell (LT, Sept - June)			PF, G				PF, PI, G	PF, G broodstock from KY, through Monte McGregor
<b>Plethobasus cyphus</b> , sheepnose (ST, June-July)	PF or PI, G (5)				B, G	B	PI, PF, G	Muskingum and Ohio Rivers source (5)
<b>Obovaria subrotunda</b> , round hickorynut (LT, now-April)			PF, G (25)	PF, G (5)	B	B		7 in holding now
<b>Elliptio crassidens</b> , elephantear (ST, late May-June) **	H, PF (10), PI?				B, G	B	PI (10)	skipjack herring, goldeye, mooneye targeted
<b>Ellipsaria lineolata</b> , butterfly (LT, now-May)		PF (3 +)		G	B, G	B		G 3 in holding now
<b>Ligumia recta</b> , black sandshell (LT, now-April)		PF (2)	PF cage (3)	G	B, G	B		5 in holding now
<b>Pleurobema cordatum</b> , Ohio pigtoe (ST, early-late June)	H, PI, B							
<b>Truncilla</b> spp., deertoed and fawnfoot (LT, May-June?)	B, PF, PI	PF (10)			B, G	B		G L. Kanawha., Muskingum River
<b>Lithasia</b> spp. Snails	P, G				B	B		Blennerhassett and Neal Islands
** WVDNR will try to collect fish for CZ/OSU								
Code to enter:								
PF = propagate w/ fish								
PI = propagate in vitro								
G = grow out (Lab, or Cage)								
H = host fish work								
B = broodstock collection								
# = number of females requested								
<b>Bold = federally endangered species</b>								