Johnson Benz 1997

ARTIFICIAL PROPAGATION OF UNIONID MUSSELS OF THE COOSA RIVER DRAINAGE

A proposal submitted to the
United States Fish and Wildlife Service
Region 4 Office
160 Zillicoa Street
Asheville, NC 28801

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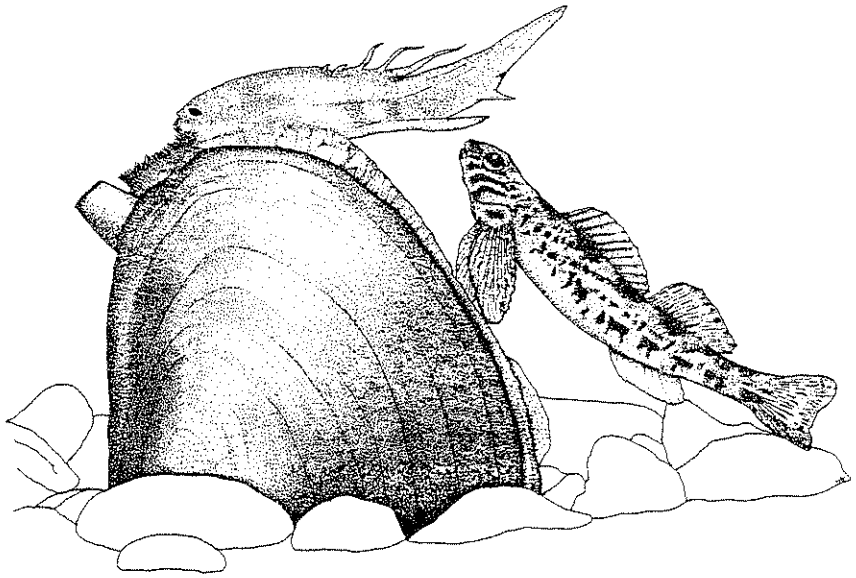

by

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PROJECT OVERVIEW

In this proposal, we request funds to begin an artificial propagation program aimed at augmenting existing populations of threatened and endangered (T&E) unionids to be reintroduced into headwaters of the Coosa River system. This program will involve 3 main steps and will target species in the Conasauga, Etowah, and Oostanaula river drainages in southeastern Tennessee and north-central Georgia. Initially we will propagate commonly found, unlisted species representing each unionid subfamily found in the upper Coosa drainage to establish culturing techniques. Secondly, we will determine hitherto unknown host identities for T&E species found within the Coosa system. After these steps have been completed, the propagation of T&E species will begin. Additional information on reproductive strategies, juvenile diet, and glochidial descriptions of T&E unionids will be secondary products of this research. The host determinations and surrogate species propagation efforts are the focus of this initial proposal, however the T&E species produced via these efforts will be stocked out into the Conasauga River.

We view this proposed propagation work critical to insuring the viability of natural unionid populations in the Coosa River system, where many mussel populations are perilously close to extirpation.

INTRODUCTION

The status of riverine unionids is one of the most critical conservation problems in the southeastern United States (Williams *et al.* 1992; Neves *et al.* 1997). Currently, 71% of the southeastern freshwater mussel fauna is federally listed or has candidate species status (Williams *et al.* 1992). The southeastern U.S. is the global epicenter of freshwater molluscan diversity (Burch 1973). Tennessee and Alabama historically contain the most diverse unionid fauna with 132 and 175 species respectively (Neves *et al* 1997). Unionid diversity in Georgia is also high, with 98 species making it the fourth most diverse state in the U.S. (Neves *et al.* 1997). Unionids have become imperiled mostly through population fragmentation resulting from habitat degradation (Williams *et al.* 1992; Neves *et al.* 1997). The complicated life-history strategies of unionids, as well as the modification of river channels for inland shipping, siltation and sedimentation from improper land use, and non-point source pollution are the factors most responsible for unionid declines (Williams *et al.* 1992; Neves *et al.* 1997). Unlike free-living organisms, unionids are particularly vulnerable to extirpation because they have an obligatory parasitic larval stage that requires a fish host, and host specificity is generally high (Watters 1994). Therefore, impacts on unionid populations can be produced by either directly influencing the mussels themselves or by influencing the host species on which they rely.

In the southeastern U.S., at least 21 unionid species have relatively recently gone extinct (Neves *et al.* 1997), and 213 taxa (71% of extant species) are considered endangered, threatened, or of special concern. Much of the endangered fauna is perilously close to extinction, and it is

not uncommon for fragmented populations to remain in small to medium size drainages. Experts agree (e.g., see Williams *et al.* 1992; Neves *et al.* 1997) that without drastic efforts to save this unique fauna, many more species will go extinct within the next few years.

Currently, the U.S. Geological Survey (USGS), the USFWS, and state and private conservation agencies (e.g., The Nature Conservancy), are targeting various southeastern rivers for restoration efforts, and some of these efforts have already begun. Because the headwaters of the Conasauga River remain in relatively good condition, they have been selected by the Nature Conservancy and the USFWS to receive restoration attention. Part of the Conasauga restoration plan includes intentions to reestablish unionid populations in appropriate river stretches (R. Biggins, USFWS, pers. comm.). Although no major restoration efforts have begun on the Etowah River, Burkhead *et al.* (1997) have crafted a detailed prescription to begin this work.

Poor water quality is the primary factor responsible for the loss of fish and unionids in the Conasauga (B. Freeman, University of Georgia, pers. comm.) and Etowah drainages (Burkhead *et al.* 1997). Although efforts are currently underway or have been planned to improve water quality in these drainages, many unionid populations found in these waters are at critically low levels (Burkhead *et al.* 1997; J. Williams, USGS, pers. comm.; D. Sheldon, AMRC, pers. comm.).

In light of all of the above, artificial propagation efforts now seem crucially needed to maintain viable unionid populations while restoration efforts improve living conditions in the Conasauga and Etowah river drainages. Currently, however, no propagation program is focusing specifically on the unionid fauna of the Coosa River system. With the importance of this system as a center of North American aquatic biodiversity in mind, we propose to begin an artificial

propagation program aimed at raising significant numbers of threatened and endangered unionids in an effort to shield these species from possible extinction.

OBJECTIVES AND OVERVIEW OF METHODS

Aquaculture techniques have recently been developed to hold and actively propagate unionids (e.g., see Gatenby and Neves 1996; Dunn and Layzer 1997), and using these techniques researchers have successfully propagated and raised juvenile unionids that have survived several years and have exhibited good growth (e.g., see Gatenby and Neves 1996; Westbrook and Layzer 1997). We propose to establish a propagation program for T&E unionids native to the Coosa River system with initial focus placed on species found within the Conasauga and Etowah river drainages. This program will aim to raise significant numbers of T&E unionids to augment and reintroduce these Mobile basin species. The proposed propagation program will consist of 3 major steps as follows:

Step 1 - Establishing propagation methods and facilities: this step will involve the collection and propagation of common, unlisted unionid species (with known hosts) found within the Coosa River system to establish propagation and rearing methods and facilities. Reintroductions of these propagated mussels would take place only in the Conasauga River drainage.

Step 2 - Host determinations for T&E species: this step will involve life history investigations of T&E unionids to identify hitherto unknown fish hosts.

Step 3 - Propagation of T&E species: this step will involve the propagation of T&E unionids for eventual transfer into the wild. Step 3 will officially proceed only after successful propagation results have been established with the non-listed species and after the hosts of the T&E species to be propagated have been determined (Steps 1 and 2 above). Of course, when viable T&E juveniles are produced via the life history work (Step 2) we will immediately attempt to rear those individuals. Juvenile T&E mussels successfully reared from the life history work will be released into the Conasauga River. Permission to release juvenile mussels will be obtained from the USFWS, TWRA, and GDNR, prior to any release.

METHODS

Step 1 ~ Establishing propagation methods and facilities

Selection of surrogate species for methods establishment

We will restrict our initial propagation efforts to common (i.e., unlisted) species from each of the 3 major unionid subfamilies (i.e., Ambleminae, Lampsilinae, and Anodontinae) so that we can establish a successful propagation process capable of handling the phylogenetic scope of the T&E species represented in the Coosa River system. Potential propagation candidates for this work are listed below (see Table 1). Final selection of these species will be made after consultation with USFWS biologists. Most of these species currently reside in the Conasauga or Etowah river drainages.

Currently, 4 species listed in Table 1 are being propagated at facilities in Cooksville, Tennessee and Blacksburg, Virginia. We will propagate several of these same species and assess our success relative to that of others so that we can identify the most productive propagation methods. All of the fish species required for these initial propagation efforts are now currently held on site at the Tennessee Aquarium (TA) or at the Aquarium's aquaculture facility (the Cohutta Fisheries Science Center) in Cohutta, Georgia (see Appendix 1).

Propagation protocol

Glochidia infection protocols will generally follow the techniques of Zale and Neves (1982). Fish will be transported from the Cohutta Fisheries Science Center (CFSC) to the quarantine room at the Tennessee Aquarium during late winter-early spring and acclimated to a holding temperature of 20° C. Fishes will be examined for glochidia infections after they have been acclimated at the aquarium, and held for a minium of 2 weeks in an attempt purge them of any encysted glochidia. Holding ponds at the CFSC have been colonized by and contain reproducing populations of *Pyganodon cataracta* and *Utterbackia imbecillis.* Therefore, care will be taken to develop protocols which will insure that glochidia and juvenile mussels collected from propagation chambers were not the result of natural infections. Anodontids are tachytactic (i.e., spring and fall brooders) and produce glochida in the fall which may overwinter attached to their fish hosts (Watters 1994). Anodontids have very large glochidia (400 μm) with prominent teeth and they are easily recognizable from glochidia representing other unionid subfamilies (McMahon 1991). Once fishes have been cleared of glochidia (as determined by visual

inspection), the artificial propagation of target species will begin.

Unionids will be collected from various localities in late winter - early spring and females will be examined for evidence of brooding with an otoscope. Brooding females will be transported to the laboratory, and demibranch development will be monitored. When gravid female unionids are subjected to warmer laboratory conditions, many species will release glochidia spontaneously (see Haag and Warren 1997). The glochidia of some individuals, however, may need to be removed by flushing gravid demibranchs with a hypodermic syringe filled with sterile water. When flushing demibranchs, only a small number of larvae will be initially harvested and the viability of these will be tested by exposing them to sodium chloride (i.e., uniodized table salt). Functionally viable glochida snap when exposed to salt (Hagg and Warren 1997), and if a high percentage of the harvested glochidia are judged nonviable, further harvesting of glochida will cease and the remaining embryos will be given more time to complete their development. If mature glochidia are present, appropriate fish will be exposed to them, by placing the fish in a 4 L plastic beaker containing 1.5 L water and the infective glochida. Fish will remain in the beaker for approximately 40 minutes to facilitate infection (R. Neves, USFWS, pers. comm.). However, a direct method of infection carried out by pipetting a solution of glochidia directly onto the gills of an anaesthetized (tricane methanesulonate) fish will be attempted if the bath exposure technique fails (Haag and Warren 1997). After exposure, infected fish will be placed in 19 L aquaria with continuous aeration and slow trickle filtration for the duration of the glochidia incubation period. Fish will be examined 1-3 days post-exposure to determine if infections were successful.

Infected fish will be placed in holding tanks similar to those used by Trdan and Hoeh

(1982). These aquaria will have false bottoms made of mesh that will allow the small transformed juveniles to drop through, preventing possible predation by fishes. Fish will not be fed after the first week to reduce bottom debris that may confound juvenile mussel collection. After 10 days, the bottom of each aquarium will be examined for transformed and untransformed juvenile mussels by gently siphoning the bottom contents through a 100 μm sieve. Sieve contents will be back washed into a gridded petri dish and examined under a stereomicroscope. The stereoscope will be fitted with cross-polarization filters, to facilitate the search for glochidia using polarized light. Juvenile mussels will be identified by the presence of a well-defined foot and mantle. Viable juveniles are usually quite active (Hove and Neves 1994). Untransformed glochidia will be discarded after their number is recorded. This procedure will be repeated every 2 days over a 2-week period so that all glochidia will have opportunity to transform. Juvenile harvesting will cease after a 6-day interval (i.e., 3 harvesting attempts) that yields no additional juveniles.

Juvenile mussels will be placed in 150 x 20 mm glass petri dishes filled with a mixture of heterogenous sediments consisting of fine aquarium gravel and large sand. Dish lids will be placed over the contents during transport to grow-out facilities at the TA's quarantine room or the CFSC. Juvenile mussels will be fed varied diets based on information provided by Gatenby and Neves (1996) and (S. Nichols, USGS Great Lakes Research Center, pers. comm.). Nichols (loc. cit.) stated that unionids have difficulty completing glycolysis without certain lipids, and demonstrated outstanding growth of both adult and juvenile mussels when a lipid supplement was added to their diets. Depending on our transformation success rate, we may design several

experiments to evaluate juvenile growth in the laboratory under different feeding regimes. Algal cultures would be maintained using techniques patterned after Gatenby and Neves (1996) or the marine algal mix (Tankersley pers. comm.) to produce food stock for laboratory reared juveniles.

Additional grow-out treatments will also occur in the hatchery raceways at the CFSC. Water from ambient temperature stock ponds will be diverted into the hatchery raceway where juvenile mussels will be kept in the above mentioned petri dishes. These stock ponds maintain high phytoplankton densities that will supply the food for these mussels. If possible, we will maintain juveniles in 2 separate channels; the mussels in one channel receiving the lipid supplement, and the mussels in the other channel maintained as a control (i.e., no lipid supplement used).

Maintenance of adults in holding tanks and stock ponds

As part of our study we will investigate various procedures to maintain and grow captive adult mussels, and as with the juvenile rearing explained above, protocols related to adult rearing will be developed both for the TA quarantine facility and the CFSC. Initially, adult unionids, taken from zebra mussel infested waters will be quarantined at 15° C for 30 days to observe them for zebra mussel contamination. Supply water at the CFSC is nearly isothermal and usually remains 14°-16° C year-round, and it can be utilized to control holding water temperature. Spring-fed raceways at the CFSC will be modified to hold mussels during quarantine, and food will be supplied during this period. The establishment of zebra mussels into systems at CSFC is unlikely due to the naturally high CO_2 content of CSFC waters. Furthermore, discharge from the CSFC is sporadic and leaves the receiving channel dry for much of the year. Mussel species

taken from different systems will be held separately in different raceways.

Investigators attempting mussel translocation experiments, have periodically had problems with mortality during quarantine or shortly thereafter (J. Layzer, USFWS, pers. comm.). We will attempt to develop techniques to minimize mortality and tissue degrowth (*sensu* Russell-Hunter 1985) during quarantine. Once unionids complete quarantine and are determined to be uncontaminated with zebra mussels, they will be transferred to different raceways or placed in pocket nets and put in stock ponds for permanent holding. Some post-quarantine mussels may also be moved to the quarantine room at the TA to assess survivorship differences between adults held at the CFSC and the TA. Survivorship, weight gain, and size increase data will be compiled to determine the best handling protocols for T&E unionids. Adult unionids held at the TA will be fed the algal culture mix recommended by Gatenby and Neves (1996), as well as the lipid supplements recommended by (S. Nichols, USGS Great Lakes Research Center, pers. comm.).

Step 2 ~ Host determinations for T&E species

Host fish determinations will be carried out on several unionid species native to the Coosa River system that have not yet been identified. Host information for all federally listed unionids and species currently proposed for federal listing in the Conasauga and Etowah river drainages are presented in Table 2.

We plan to initiate fish host investigations following the basic methods of Zale and Neves (1982) and Haag and Warren (1997). Brooding mussels will be collected from the field and

transported to the TA holding facilities. Glochidia maturity will be periodically assessed using the salt method described above. Once mature glochidia are identified, host fish infections will be attempted using the aforementioned direct infection technique by pipetting a glochidia suspension onto the gills (Haag and Warren 1997). Direct counts of encysted glochidia on experimental hosts will be carried out 1-3 days after inoculation. Following the incubation period, the viability and subsequent survival of transformed juveniles from different unionid-host species combinations will be evaluated. Unionid glochidia often exhibit strong survivorship differences among successful host species (W. Haag, USFS, pers. comm.), and we will document these differences so that we can utilize hosts which will optimize propagation efforts. Viable juvenile T&E species collected during the life history work will be transferred into the rearing facilities at the CFSC and rearing will be attempted (see Step 3). Once mature glochidia have been obtained from a listed species, several individuals will be properly fixed and retained for taxonomic description using scanning electron microscopy. Stripped female unionids will be returned to the locality from which they were originally collected. Prior to attempting fish host identification for T&E unionids, the Atlanta USFWS office and the appropriate state office(s) will be notified so that proper federal and state permits to take mussels will be in place.

Step 3 ~ Propagation of T&E species (not budgeted as part of this proposal)

After propagation protocols have been established using common, unlisted species (see Step 1 above) and after the hosts of T&E species have been determined (see Step 2 above), we will begin to propagate select T&E species of unionids using our most successful protocols. Input regarding the prioritization of species for propagation will come from the USFWS field

offices in Asheville, NC and Jackson, MS, as well as from appropriate state natural resources offices in Tennessee and Georgia. Throughout the project we will request assistance from the USFWS, the Tennessee Wildlife Resources Agency (TWRA), and Georgia's Department of Natural Resources to help coordinate work on fish host identifications, to avoid duplication of effort. In particular, efforts of the following researchers should be coordinated: Mark Hove (University of Minnesota, Department of Fisheries and Wildlife, St. Paul, MN), Tom Watters (Ohio Biological Survey, The Ohio State University, Columbus, OH), Chris Barnhardt (Southwest Missouri State University, Department of Biology, Springfield, MO), Jim Layzer (USFWS Cooperative Research Unit, Tennessee Technological University, Cookville, TN), Richard Neves (Virginia Polytechnic Institute, Department of Fisheries and Wildlife, Blacksburg, VA), and Wendell Haag (USFS Research Center, Oxford, MS).

PROJECT TIME LINE

Results from this first proposal regarding the surrogate species propagation and T&E species host identification will take 24 months to achieve. Ideally, propagation of unlisted species and host identification for T&E species would begin in late-winter 1998. Results from the adult holding treatments should be obtained by the end of the first study year. However, growth studies of propagated mussels would take a minimum of 1 year to complete. This length of time will be needed to reliably assess the growth of the small juveniles (300 - 1000 μm).

The work on T&E life histories may be more challenging. Locating gravid individuals of

several of the proposed species, particularly *Epioblasma* spp. and *Pleurobema* spp., may be difficult because of their limited distribution. Furthermore, *Pleurobema* spp. have a very narrow window (1-2 weeks) in the early spring when they are gravid (W. Haag, USFS pers. comm.) and requires collection efforts will need to be concentrated during these critical periods. In all, we believe that with careful planning, all phases of this initial project should be complete after a 2-year period.

DISSEMINATION OF RESULTS

Preliminary fish host information, as well as preliminary data and results stemming from the proposed project will be freely shared with other investigators via routine means and via the Triannual Unionid Report, the Unio-listserver, the SARI website (www.sari.org), and TWRA's annual Rare and Endangered Mollusk Committee meeting. A progress report (first-year results) and a final project report (to be submitted at the end of the second year) will be submitted to the USFWS, and the dissemination of significant project results will be pursued via appropriate scientific meetings and publications.

Table 1. List of prospective unionids for initial propagation efforts. Species marked with asterisks are currently being cultured at Virginia Polytechnic Institute and/or Tennessee Technological University.

Subfamily: species	Known host(s)
Lampsilinae:	
Lampsilis excavatus	*Micropterus salmoides*
Villosa nebulosa *	*Micropterus salmoides*
	Micropterus punctulatus
	Ambloplites rupestris
Villosa vanuxemensis	*Cottus carolinae*
	Cottus bairdi
	Cottus cognatus
Leptodea fragilis	*Aplodinotus grunniens*
*Lampsilis radiata**	*Micropterus salmoides*
	Micropterus punctulatus
	Ambloplites rupestris
Ambleminae:	
Fusconaia ebena	*Micropterus salmoides*
	Pomoxis nigromaculatus
	Pomoxis annularis
Amblema plicata *	*Pomoxis nigromaculatus*
	Lepomis macrochirus
	Pomoxis annularis
Megalonaias nervosa	*Lepomis cyanellus*
	Ictalurus punctatus
	Aplodinotus grunniens
Quadrula quadrula	*Pylodictis olivaris*
Anodontinae:	
Pyganodon cataracta	*Lepomis macrochirus*
	Pomoxis nigromaculatus
	Micropterus salmoides
Utterbackia imbecillis	*Lepomis cyanellus*
	Lepomis macrochirus
	Micropterus salmoides
Lasmigona complanata	*Cyprinus carpio*
	Lepomis cyanellus
	Micropterus salmoides

Table 2. T&E, special concern unionids and their fish hosts which are found in the Conasauga and Etowah river drainages.

Scientific name	Common name	Host(s)
Epioblasma metastriata	Upland combshell	**Unknown**
Epioblasma othcaloogensis	Southern acornshell	**Unknown**
Lampsilis altilis	Fine-lined pocketbook	*Fundulus olivaceus*
		Micropterus coosae
		Micropterus punctulatus
		Micropterus salmoides
Lampsilis perovalis	Orange-nacre mucket	*Micropterus coosae*
		Micropterus punctulatus
		Micropterus salmoides
Lasmigonia holstonia	Tennessee heelspliter	**Unknown**
Medionidus acutissimus	Alabama moccasinshell	*Fundulus olivaceus*
		Etheostoma douglasi
		Etheostoma whipplei
		Percina nigrofasciata
		Percina caprodes
Medionidus parvulus	Coosa moccasinshell	**Unknown**
Pleurobema decisum	Southern clubshell	**Unknown**
Pleurobema chattanoogaense	Painted clubshell	**Unknown** (located by M. Hughes 1996)
Pleurovema furvum	Dark pigtoe	*Cyprinella* spp.
Pleurobema georgianum	Southern pigtoe	**Unknown**
Pleurobema perovatum	Ovate clubshell	**Unknown**
Ptychobranchus greeni	Triangular kidneyshell	*Etheostoma bellator*
		Etheostoma douglasi
		Etheostoma whipplei
		Percina nigrofasciata
		Percina caprodes

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BUDGET

Category	Request Year 1	Request Year 2	Total
Salaries and wages:			
Project Leader, Paul Johnson (two months) (does not include benefits)	2,334.00	2,334.00	4,668.00
Graduate student (one year research assistantship)	8,200.00		8,200.00
Equipment and supplies:			
Propagation and lifehistory:			
Polarizing lenses	150.00		150.00
SEM supplies and microscope beam time	250.00	250.00	500.00
Petri dishes (Pyrex 150 x 20)	320.00		320.00
Marking tags	100.00		100.00
19 L aquaria (15 aquaria @ $ 7.50 each)	112.50		112.50
415 L fiberglass aquarium (2 @ 135.00)	270.00		270.00
Materials & tools for pocket net construction	400.00		400.00
Lipid food supplement	250.00	250.00	500.00
Misc. propagation and life history supplies	750.00	750.00	1,500.00
Algal culture and commercial mix:			
95 L Algal culture tank	192.00		192.00
Algal culture chemicals and supplies	750.00	750.00	1,500.00
Prepared marine algal mix	325.00	325.00	750.00
Cohutta Fisheries Science Center costs:			
Rental of 2 larval fish raceways	1,000.00	1,000.00	2,000.00
Rental of 2 holding raceways	750.00	750.00	1,500.00
Travel costs:			
Mileage for trips to CFSC: (1 round trip = 42 miles @ 0.26 / mile = 10.92 trip x 156 trips)	851.76	851.76	1,703.52
Mileage for unionid collection (1500 miles at $ 0.26 / mile)	195.00	195.00	390.00
Total direct costs	17,300.26	7,455.76	24,756.02
Indirect costs (5% of direct costs)			1,867.20
Grand total (requested to fund a 2-year study)			26,623.22

does not include non-federal contributions totaling $ 17,266.60 (see Non-federal contributions p. 23)

BUDGET JUSTIFICATION

Salaries and wages

The Project Leader, Paul Johnson, will devote approximately ⅓ of his total time to this project. He will supervise all aspects of the project, and will design specific experimental protocols, collect and analyze data, and write reports. Additionally, he will train a graduate student to assist in performing the host determination work and aid with animal maintenance at the TA and CFSC. This work will represent the thesis research of a master's student (Environmental Sciences graduate program) at the University of Tennessee at Chattanooga.

Propagation and life history

A polarizing filter will be fitted to the objective lens of SARI's Zeiss Stemi SV 11 stereomicroscope and a similar filter will be placed below the microscope stage. This filter configuration will facilitate locating glochida and transformed juveniles in aquaria water samples by using polarized light. Petri dishes, pocket nets, 415 L fiberglass aquaria, numbered marking tags, and lipid supplements will be used in rearing juvenile and adult mussels at the TA and CFSC. Costs will be incurred at the CFSC for the use of raceways. SEM costs will be incurred for supplies and beam time at Dupont's Fiber Research Laboratory (Chattanooga, TN). SEM photographs and descriptions of previously undescribed glochidia will be added to the existing USFWS glochidia data base.

Algal culture supplies

Algal culture facilities will be set up at SARI. Currently the TA has only a small algal culture facility, and the amount of algae required for this project will exceed its daily production capacity. The algal culture chemicals will be used to prepare stock nutrient media, which will be autoclaved at the TA before use at SARI.

capacity. The algal culture chemicals will be used to prepare stock nutrient media, which will be autoclaved at the TA before use at SARI.

Travel costs

Field trips will be necessary to collect mussels used in this study, and tri-weekly trips to the CFSC will be required for maintenance of juvenile and adult unionds being held there.

NON-FEDERAL CONTRIBUTIONS

Below is a list of non-federal contributions to be committed by SARI and the Tennessee Aquarium which will help support the proposed project.

Item	Value		
	Year 1	Year 2	Total
Salary:			
Paul Johnson (two months; does not include benefits)	2,334.00	2,334.00	$ 4,668.00
Benefits:			
Tennessee Aquarium cost corresponds to 10 % above salary	233.40	233.40	$ 466.80
Facilities:			
Unlimited use of SARI's laboratory and equipment to fulfill project objectives: estimated use of 20 days at $100.00 per day	1,000.00	1,000.00	$ 2,000.00
Use of limited quarantine room space and water-quality laboratory equipment and minor supplies at the Tennessee Aquarium to fulfill project objectives: estimated use 24 months at $ 500.00 per month.	6,000.00	6,000.00	$12,000.00
Sub-total of non-federal contributions	9,567.40	9,567.40	$ 19,134.80
Indirect costs charged to project (from Budget section)			$ 1,867.20
Grand total of Non-federal contributions (calculated as above Sub-total minus above Indirect costs)			$ 17,266.60

Appendix 1. List of fishes currently maintained at the Tennessee Aquarium aquaculture facility, Cohutta Fisheries Science Center, Cohutta, Georgia.

SCIENTIFIC NAME	COMMON NAME
Acipenseridae	
Acipenser fulvescens	Lake sturgeon
Scaphirhynchus platorynchus	Shovelnose sturgeon
Polydontidae	
Polydon spathula	Paddlefish
Lepisosteidae	
Atractrosteus spatula	Alligator gar
Lepisosteus ocelatus	Longnose gar
Lepisosteus osseus	Spotted gar
Salmonidae	
Oncorhynchus mykiss	Rainbow trout
Salmo trutta	Brown trout
Salvelinus fontinalis	Brook trout
Esocidae	
Esox lucius	Northern pike
Esox niger	Chain pickerel
Cyprinidae	
Campostoma anomalum	Stoneroller
Luxilus chrysocephalus	Striped shiner
Luxilus coccogenis	Warpaint shiner
Notropis maculatus	Taillight shiner
Notropis signipinnis	Flagfin shiner
Castostomidae	
Carpiodes carpio	River carpsucker
Carpiodes cyprinus	Quillback
Catostomus commersoni	White sucker
Ctenopharyngodon idella	Grass carp
Cyprinus carpio	Common carp
Hypophthalmichthys nobilis	Bighead carp
Ictiobus bubalus	Small mouth buffalo
Ictiobus cyprinellus	Big mouth buffalo
Ictiobus niger	Black buffalo
Minytrema melanops	Spotted sucker
Moxostoma carinatum	River redhorse
Moxostoma duquesnei	Black redhorse
Moxostoma erythrurum	Golden redhorse
Moxostoma poecilurum	Blacktail redhorse

Appendix 1. Continued.

SCIENTIFIC NAME	COMMON NAME
Ictaluridae	
Ictalurus furcatus	Blue catfish
Ictalurus punctatus	Channel catfish
Pylodictis olivaris	Flathead catfish
Percichthyidae	
Morone chrysops	White bass
Morone mississippiensis	Yellow bass
Centrarchidae	
Ambloplites rupestris	Rock bass
Lepomis auritus	Redbreast sunfish
Lepomis cyanellus	Green sunfish
Lepomis gulosus	Warmouth
Lepomis machrochirus	Bluegill
Lepomis megalotis	Longear sunfish
Lepomis microlophus	Red sunfish
Micropterus dolomieu	Smallmouth bass
Micropterus punctulatus	Spotted bass
Micropterus salmoides	Largemouth bass
Pomoxis annularis	White crappie
Pomoxis nigromaculatus	Black crappie
Percidae	
Perca flavescens	Yellow perch
Stizostedion canadense	Sauger
Stizostedion vitreum	Walleye
Sciaenidae	
Aplodinotus grunniens	Freshwater drum

Appendix 2. List of native fishes held in the quarantine holding rooms at the Tennessee Aquarium.

FAMILY	SCIENTIFIC NAME	COMMON NAME
Acipenseridae	*Acipenser fulvescens*	lake sturgeon
	Scaphirhynchus platorynchus	shovelnose sturgeon
Polyodontidae	*Polyodon spathula*	paddlefish
Lepisosteidae	*Lepisosteus oculatus*	spotted gar
	Lepisosteus osseus	longnose gar
	Lepisosteus platostomus	shortnose gar
	Lepisosteus spatula	alligator gar
Amiidae	*Amia calva*	bowfin
Cyprinidae	*Campostoma anomalum*	central stoneroller
	Clinostomus funduloides	rosyside dace
	Cyprinella camura	bluntface shiner
	Cyprinella galactura	whitetail shiner
	Cyprinella monacha	spotfin chub
	Cyprinella spiloptera	spotfin shiner
	Cyprinella venusta	blacktail shiner
	Erimystax insignis	blotched chub
	Gila pandora	Rio Grande chub
	Gila robusta	roundtail chub
	Hemitremia flammea	flame chub
	Hypentelium etowanum	Alabama hog sucker
	Luxilus chrysocephalus	striped shiner
	Luxilus coccogenis	warpaint shiner
	Lythrurus ardens	rosefin shiner
	Lythrurus umbratilis	redfin shiner
	Nocomis micropogon	river chub
	Notemigonus crysoleucas	golden shiner
	Notropis boops	bigeye shiner
	Notropis hypselopterus	sailfin shiner
	Notropis leuciodus	Tennessee shiner
	Notropis maculatus	taillight shiner
	Notropis photogenis	silver shiner
	Notropis rubricroceus	saffron shiner
	Notropis signipinnis	flagfin shiner
	Notropis sp. A	sawfin shiner
	Notropis spectrunculus	mirror shiner
	Pimephales promelas	fathead minnow
	Rhinichthys atratulus	blacknose dace

Appendix 2. Continued

FAMILY	SCIENTIFIC NAME	COMMON NAME
Cyprinidae (continued)	*Rhinichthys cataractae*	longnose dace
	Rhinichthys osculus	speckled dace
	Semotilus atromaculatus	creek chub
Catostomidae	*Carpiodes carpio*	river carpsucker
	Carpiodes cyprinus	quillback
	Catostomus clarki	desert sucker
	Catostomus commersoni	white sucker
	Catostomus discobolus	bluehead sucker
	Catostomus insignis	Sonora sucker
	Erimyzon oblongus	creek chubsucker
	Erimyzon sucetta	lake chubsucker
	Erimyzon tenius	sharpfin chubsucker
	Hypentelium nigricans	northern hog sucker
	Ictiobus bubalus	smallmouth buffalo
	Ictiobus cyprinellus	bigmouth buffalo
	Ictiobus niger	black buffalo
	Minytrema melanops	spotted sucker
	Moxostoma carinatum	river redhorse
	Moxostoma duquesnei	black redhorse
	Moxostoma erythrurum	golden redhorse
	Moxostoma macrolepidotum	shorthead redhorse
	Moxostoma poecilurum	blacktail redhorse
Ictaluridae	*Ictalurus furcatus*	blue catfish
	Ictalurus punctatus	channel catfish
	Pylodictis olivaris	flathead catfish
Esocidae	*Esox lucius*	northern pike
	Esox lucius x *E. masquinongy*	tiger muskie
	Esox masquinongy	muskellunge
	Esox niger	chain pickerel
Umbridae	*Umbra limi*	central mudminnow
Salmonidae	*Oncorhynchus aguabonita*	golden trout
	Oncorhynchus mykiss	rainbow trout
	Orcorhynchus apache	Apache trout
	Salmo salar	Atlantic salmon
	Salvelinus fontinalis	brook trout
	Salvelinus namaycush	lake trout
Gadidae	*Lota lota*	burbot
Poeciliidae	*Gambusia affinis*	western mosquitofish
	Heterandria formosa	least killifish
	Poecilia latipinna	sailfin molly

Appendix 2. Continued

FAMILY	SCIENTIFIC NAME	COMMON NAME
Cyprinodontidae	*Cyprinodon variegatus*	sheepshead minnow
	Fundulus catenotus	northern studfish
	Fundulus chrysotus	golden topminnow
	Fundulus dispar	starhead topminnow
	Fundulus escambiae	russetfin topminnow
	Fundulus grandis	gulf killifish
	Fundulus julisia	Barrens topminnow
	Fundulus notatus	blackstripe topminnow
	Fundulus similis	longnose killifish
	Leptolucania ommata	pygmy killifish
	Lucania goodei	bluefin killifish
Cottidae	*Cottus carolinae*	banded sculpin
Percichthyidae	*Morone chrysops*	white bass
	Morone chrysops x *M. saxatilis*	hybrid striped bass
	Morone mississippiensis	yellow bass
	Morone saxatilis	striped bass
Centrarchidae	*Ambloplites rupestris*	rock bass
	Centrarchus macropterus	flier
	Elassoma okefenokee	okefenokee pygmy sunfish
	Elassoma zonatum	banded pygmy sunfish
	Enneacanthus gloriosus	bluespotted sunfish
	Enneacanthus obesus	banded sunfish
	Lepomis auritus	redbreast sunfish
	Lepomis cyanellus	green sunfish
	Lepomis gibbosus	pumpkinseed
	Lepomis gulosus	warmouth
	Lepomis humilis	orangespotted sunfish
	Lepomis macrochirus	bluegill
	Lepomis megalotis	longear sunfish
	Lepomis microlophus	redear sunfish
	Lepomis punctatus	spotted sunfish
	Micropterus coosae	redeye bass
	Micropterus dolomieu	smallmouth bass
	Micropterus punctulatus	spotted bass
	Micropterus salmoides	largemouth bass
	Pomoxis annularis	white crappie
	Pomoxis nigromaculatus	black crappie
Percidae	*Etheostoma blennioides*	greenside darter
	Etheostoma caeruleum	rainbow darter
	Etheostoma camurum	bluebreast darter

Appendix 2. Continued

FAMILY	SCIENTIFIC NAME	COMMON NAME
Percidae (continued)	*Etheostoma chlorobranchium*	greenfin darter
	Etheostoma crossopterum	fringed darter
	Etheostoma edwini	brown darter
	Etheostoma flabellare	fantail darter
	Etheostoma fusiforme	swamp darter
	Etheostoma jessiae	blueside darter
	Etheostoma kennicotti	stripetail darter
	Etheostoma lynceum	brighteye darter
	Etheostoma nigrum	Johnny darter
	Etheostoma parvipinne	goldstripe darter
	Etheostoma pyrrhogaster	firebelly darter
	Etheostoma ruflineatum	redline darter
	Etheostoma sagitta	arrow darter
	Etheostoma simoterum	Tennessee snubnose darter
	Etheostoma spectabile	orangethroat darter
	Etheostoma swaini	gulf darter
	Etheostoma swannanoa	Swannanoa darter
	Etheostoma vulneratum	wounded darter
	Perca flavescens	yellow perch
	Percina aurantiaca	tangerine darter
	Percina caprodes	logperch
	Percina copelandi	channel darter
	Percina evides	gilt darter
	Percina maculata	blackside darter
	Percina nigrofasciata	blackbanded darter
	Percina sciera	dusky darter
	Percina vigil	saddleback darter
	Stizostedion canadense	sauger
	Stizostedion vitreum	walleye
Sciaenidae	*Aplodinotus grunniens*	freshwater drum