AN INTRODUCTION AND OVERVIEW OF FRESHWATER MUSSEL PROPAGATION

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The propagation of freshwater mussels in the United States began in the early 1900's to augment the recruitment of Mississippi River populations being harvested for the pearl button industry. Research on life histories and culture methods for exploited species began at the Fairport Biological Station and provided a wealth of data on successes and failures through empirical aquaculture trials. Publications from these mussel pioneers gave evidence that success stories were few and far between. With no evidence of quantifiable results in the augmentation of wild populations, and the recognition that water pollution was likely a controlling variable for population viability, propagation efforts ceased by 1942. This investigative field lay fallow for nearly 50 years, until the Endangered Species Act of 1973 mandated efforts to protect and recover species federally listed as endangered and threatened. On June 14, 1976, there were 23 freshwater mussels placed on the list for federal protection, with subsequent recovery plans that identified propagation as one of the means for recovery. By 2002, 70 species of mussels were under federal protection, pushing the need for successful methods to propagate these species. Propagation research began at Virginia Tech in 1990, and subsequent efforts at various locations in the eastern United States have focused on endemic or localized populations in need of recovery. The first release of propagated juveniles of an endangered mussel occurred from our propagation facility into the Hiwassee River, Tennessee in September 1998. Since then, our propagation facility has released nearly 260,000 endangered juveniles of nine species into various streams and rivers in Tennessee and Virginia. A construction grant from the National Fish and Wildlife Foundation has allowed us to expand our culture operations, such that additional species and rivers can be included in this recovery work.

This first-of-its-kind workshop was convened by the Freshwater Mollusk Conservation Society with the goal to describe current methods of freshwater mussel propagation and provide mussel culture as a viable tool for species restoration and recovery. Specific objectives of the workshop are to 1) describe the reproductive biology and propagation of mussels at various locations in the United States, 2) exchange experiences and ideas on culture systems and methods, and 3) assist prospective culturists with information needs. With so much national and international interest in the conservation of biodiversity at all taxonomic levels, this workshop will hopefully stimulate governmental agencies and personnel to test the waters and begin pilot projects in the propagation of regionally important species, such that further extinctions from this highly endangered fauna will be prevented.

MUSSEL REPRODUCTIVE BIOLOGY

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The reproductive biology of Unionoid mussels is a wonder of nature. Male unionoids release sperm in aggregates (spermatozeugmata) that are carried by water currents to the female. Fertilization takes place in water passages within the gills of the female, and the fertilized eggs are brooded within these spaces. Embryonic development is completed within days to weeks. The mature glochidia may be brooded for several months (bradytictic) or may be released shortly after maturation (tachytictic). Many species produce glochidia in the late summer or fall and do not release them until the following spring or summer (winter-brooders). Many other species produce glochidia in the late spring or summer and release them in the late summer (summer-brooders).

Depending on species, glochidia may be "broadcast" from the female in the excurrent water stream. Larval threads may be deployed to aid suspension of the glochidia. Other species release glochidia within cohesive masses of eggs (conglutinates). Conglutinates act as baits to attract host fish. In many conglutinate-producing species (e.g. *Fusconaia*, *Pleurobema*, *Plethobasus*, and *Cyprogenia*) a large fraction of the eggs normally do not develop. These sterile (structural) eggs appear increase the durability and visibility of conglutinates. Improved host fish infection by conglutinates bearing sterile eggs presumably offsets the consequent reduction in the number of larvae that are produced. In some species, the ventral and posterior mantle is modified as a lure to attract carnivorous hosts. The glochidia may be freed when the host fish attacks and ruptures marsupial gills of the female.

Glochidia range in size from about 60 microns to nearly 400 microns. Although glochidia may survive for months during brooding, they seldom survive more than a few days after release unless they reach a compatible host. Encystment on the host occurs by overgrowth of host tissue. Metamorphosis occurs within days or weeks, depending on species and temperature. Typically, transformation can be completed only on one or a few species of immunologically compatible host fish. The degree of host specificity varies greatly among species and may also vary among populations within species. Literature reports of host relationships should not be extrapolated without great caution. Compatible hosts acquire immunity after one or more exposures to a mussel species, and this immunity may extend to other mussel species as well.

The peculiar reproductive biology of Unionoid mussels is probably a major reason for their decline in historical times. Reproduction is susceptible to disruption by any factor that reduces the abundance, distribution, or mobility of the host fish. The newly transformed juvenile is very small in size, and must fortuitously land in suitable habitat after leaving the host, forming another significant bottleneck. On the bright side, the parasitic habit of unionids is associated with very high fecundity, and both bottlenecks in the life cycle can be widened by human intervention.

HABITAT REQUIREMENTS.

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If stocking programs are to be successful, mussels must be stocked into suitable habitats. The difficulty of identifying suitable habitat varies depending on the purpose of the stocking program. For put-and-take stocking, in which mussels are moved out of harm's way into in a new site for only a short time (weeks to years) then returned to their site of origin, habitat requirements are modest. Animals must simply survive in good condition for a relatively short time, and neither reproduction nor sustainable population growth is required. Nevertheless, inadequate habitat selection probably is partly responsible for the mixed success of put-and-take programs. For population augmentation, in which animals are moved into a site that already supports the species, it again is relatively easy to identify suitable habitat, because the existence of living animals often indicates that habitat is at least suitable for short-term survival of the species. However, the ability of population augmentation to increase the viability of mussel populations seems doubtful. Finally, when stocking is used to establish new mussel populations, habitat identification is difficult. The habitat must support mussel survival, reproduction, and growth at sufficient levels to sustain the population over the long term. Traditional habitat descriptors have failed critical tests, and promising more functional approaches to mussel habitat are not yet well developed. Instead, mussel conservationists must rely on a combination of historical, mechanistic, empirical, and surrogate approaches (along with their intuition) to identify mussel habitat.

TECHNIQUES AND CONSIDERATIONS FOR CAPTIVE HOLDING OF ADULT FRESHWATER MUSSELS.

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Successful long-term captive holding of freshwater mussels may be necessary to establish: captive brood stock for propagation/conservation efforts, biological studies, salvage from a severe perturbation event, or establishment of arc populations for critically imperiled species. Over the last few years several researchers have developed a number of systems to hold freshwater mussels. Although many systems work well initially, long term holding success is more problematic. Because it is possible for some species of freshwater mussels to live well over a year in captivity with little or no food, evaluation of holding success requires a minimum of 18 months. To date, facility designs have focused on a variety of closed re-circulation and open flow-through systems. Initial evidence supports a preference for open flow-through systems. Because open systems more closely mimic the riverine conditions where mussels occur, flow-through systems may better support normal seasonal variation in mussel growth and maintenance. In addition to facility design, handling and transport of adult mussels and techniques for the evaluation of mussel health will be discussed.

FISH HOST DETERMINATION.

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A fundamental piece of information needed in mussel propagation and restoration is the identity of host fishes for a particular mussel species of concern. Host species can be inferred by: 1) examining natural infections on wild-caught fishes, or 2) conducting and monitoring laboratory-induced infection trials. Examining natural infections has the benefit of providing information about natural fish/glochidia associations but is unable to provide definitive answers about host suitability. Laboratory trials are able to provide definitive information about host suitability, as well as quantitative information about duration of parasitic stage, differences in transformation success among fish species, and general robustness of a host relationship. Generating ecologically comprehensive host information in which the host suitability of a taxonomically wide range of fishes that co-occur with the mussel species in question is evaluated is far more useful than more limited information on only a few species. Replication of host trials at two levels by conducting at least two trials using glochidia from different individual mussels and using multiple individuals of each fish species in each trial provides data that are more easily interpreted and generalized than unreplicated studies. Fish species that produce large numbers of juvenile mussels consistently among and within trials are considered “robust hosts” that are useful in propagation and are likely important hosts in the wild. Species that produce inconsistent results or low numbers of juveniles are considered “marginal hosts” that are not useful in propagation and are of questionable value as hosts in the wild.

PRODUCTION OF ENDANGERED JUVENILE MUSSELS (UNIONIDAE) AT THE FRESHWATER MOLLUSK CONSERVATION CENTER, VIRGINIA TECH.

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Biologists at the Freshwater Mollusk Conservation Center have developed methods to produce and culture endangered juvenile mussels for release into rivers of the upper Tennessee River drainage. Freshwater mussels have a unique life history, requiring the use of fish in the life cycle. Thus, the process of producing juvenile mussels begins by collecting suitable host fish from the river and holding them in captivity until gravid female mussels can be found. In the laboratory, the larvae (glochidia) in the gills of the female mussel are flushed out using a hypodermic needle filled with water. This non-lethal method allows us to return females to the river once her progeny have been removed. We have collected and transported female mussels of various species to our laboratory, removed their glochidia, and released them back to the site of capture. The following year we have then recaptured some of these female mussels finding them gravid. The larvae can number more than 200,000 per female. These larvae are then introduced into a bucket holding the host fish, and aeration is used to keep the water agitated to allow larvae to attach to the gills of the fish. After 1 hour of exposure, the fish are moved to large aquaria where the attached larvae begin the transformation process, which requires 2-3 weeks. Glochidia are transformed at cool temperatures between 19-22°, which increases survival of host fish and allows glochidia to transform unharmed to the juvenile stage. Once these young juveniles drop from their host fish, they are collected by siphoning the tank bottoms. Newly metamorphosed juveniles are held in small containers with cultured algae and sediments for 1-2 weeks before release to the wild, or cultured long-term in recirculating aquaculture streams. Between 1998 and 2001, nearly 260,000 juvenile mussels of 9 species were released into the Clinch, Powell and Hiwassee rivers in Tennessee.

CULTURE OF ENDANGERED JUVENILE MUSSELS (UNIONIDAE) IN RECIRCULATING AQUACULTURE SYSTEMS.

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Long-term (2-6 months) culture of juvenile mussels in recirculating aquaculture troughs is a feasible, cost-effective method to produce juveniles for population augmentation of endangered species, toxicity testing or other research needs. The process begins by placing newly metamorphosed juveniles in individual containers in the raceway of the recirculating aquaculture trough. The juveniles are cultured in dishes containing fine sediments. The culture unit is a 3 m long, 225 L plastic livestock feed trough. A 50:50 mixture of conditioned (dechlorinated) municipal water and well water is used in the culture system, with hardness ranging from 250 to 350 mg/L $CaCO_3$. A 50 L square, plastic container serves as a sump reservoir, and PVC piping is used for delivery and return lines. Water is pumped through the raceway using a centrifuge or magnetic drive pump, and gravity-fed back to the sump reservoir through a standpipe. The juveniles are fed small (5-10 μm) green algae, e.g., *Neochloris oleoabundans* or *Nannochloropsis oculata*, at a daily concentration of 20,000-30,000 cells/mL. For the best results, juveniles are cultured at temperatures ranging from 21-24°C. Sustained temperatures > 27°C seem to be detrimental to survival and growth of young juveniles in our recirculating aquaculture systems. Generally, survival of juvenile mussels is influenced by seasonal viability of newly metamorphosed juveniles, species differences, substrate composition, water quality, and predators. For example, the common rainbow mussel *Villosa iris* is much easier to culture than the endangered oyster mussel *Epioblasma capsaeformis* ($p<0.05$); additionally, the survival of transformed juveniles of both species is greater in the spring ($p<0.05$). Long-term (60-90 d) survival of endangered juveniles has ranged from 0-50%; however, techniques are now greatly improved and survival is expected to increase. These juveniles are typically between 700-1200 μm long at the time of their release into the wild.

PROPAGATION AND CULTURE OF FRESHWATER MUSSELS IN FISH HATCHERY RACEWAYS

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Since 1994, the Tennessee Cooperative Fishery Research Unit has been evaluating the use of fish hatchery raceways for production of mussels. Three methods of propagation were evaluated: introduction of adult mussels and known host; artificially infesting hosts with glochidia before introduction; and introducing various aged juvenile mussels propagated in the laboratory and cultured in an indoor recirculating system. Although mussels spawned in the raceway, and glochidial infestations on host fishes occurred, production was low. Juveniles of three species propagated by releasing glochidial-infested fish were cultured for three years; during their 4th growing season, they matured and spawned in the raceway. A third species, introduced as 1-day-old juveniles also spawned during the 4th growing season. Each method of propagation has advantages: artificially introduction of adult mussels and uninfested hosts is requires the least amount of time and effort; infesting hosts prior to release is more labor intensive but glochidial infestation rates can be controlled and maximized; juveniles propagated in the laboratory and kept in culture baskets in the raceways is the most labor intensive but it provides an opportunity for sampling juveniles throughout the growing season to determine growth and survival. Survival of juveniles cultured unrestrained in the raceway has been 3 to 5% during the first growing season; however, growth rates are high, and between year survival > 95%. In contrast, juveniles grown in culture baskets has been slower but survival has been much greater (up to 53%). The ability to propagate and culture some mussel species throughout their entire life cycle is encouraging; however, these techniques have not proven successful for all species tested.

DIETARY HABITS AND NUTRITIVE REQUIREMENTS OF FRESHWATER UNIONIDAE

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We compared the feeding habits and nutritive content of several unionid species in a detritus-dominated river and an algal-dominated lake using biochemical analyses, gut contents, and stable isotope ratios. There was little difference in diet or nutritive content between unionid species, regardless of body size or habitat preference. The main food source for all species of unionids was the <28 micron fine particulate organic matter (FPOM). This FPOM component was a mixture of items such as detritus, algae, zooplankton, bacteria and fungus. However, the delta13C values for algae and other food web components showed that all the unionids from both river and lake used bacterial carbons, not algal carbons, as their main diet source. This was in spite of the positive selection and concentration of diatoms and green algae from the water column into the gut and mantle cavity. Algae did provide key nutrients such as vitamins A, D, and phytosterols that were bioaccumulated in the tissues of all species. Biochemical data showed that all unionid species bioaccumulated the bacterially-derived vitamin, B12. The delta15Nratios for the multi-species unionid community in the Huron River indicated some differences in nitrogen enrichment between species, with P. grandis having the highest levels of enrichment. These nitrogen ratios indicate that most of the unionids were omnivorous in their feeding habits, and not pure herbivores.

COLLECTING, TRANSPORTING AND MAINTAINING SMALL, STREAM FISHES FOR USE WITH MUSSEL CULTURE.

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The ability to maintain healthy host fish under laboratory or hatchery conditions is an integral part of unionid propagation. Obviously, the specific nature of the mussel/fish host relationship severely limits the choice of fishes available for this purpose. Small stream fish can be collected and handled properly, so that mortality can be reduced to near zero. We will outline basic guidelines for safe capture, transport and maintenance of several different groups of small stream fishes. These techniques will reduce stress on the fish, increase the likelihood that they will adapt to aquaria, and therefore can be successfully used for mussel propagation.

We also suggest the potential of using captive propagated (vs. wild-collected fish) in mussel culture. Many mussel hosts are small, benthic species that respond well to artificial culture. In some instances, it is not practical to collect large numbers of a host fish, either because local populations are relatively small, or the species is protected by federal or state regulations. Many of these fish are simply difficult to collect or our ability to detect them is sporadic.

Some of the best hosts, darters of the genus *Percina*, can be difficult to maintain in good condition in aquaria. Wild collected specimens tend to be highly excitable and easily stressed. In addition, only a few species are easily collectable in numbers. Our observations are that F-1 propagated individuals do not exhibit these characteristics to the extreme, as they have adjusted to aquarium life. Theoretically, this would make them more suitable for use as hosts.

For any of these fish, wild collected or propagated, proper maintenance can determine the success or failure of a mussel propagation program. We hope to be able to share some of our accumulated knowledge and experience in this area to benefit mussel propagation.

PHYSIOLOGICAL CONDITION OF *IN VITRO*- AND *IN VIVO*-REARED JUVENILE MUSSELS

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Juvenile unionid mussels have been successfully reared in the laboratory using one of two techniques. Larvae can be placed on host fish to undergo metamorphosis, or they may be placed in a modified cell culture medium where development to the juvenile will occur. The current data available on juvenile freshwater mussels focuses on their use in toxicity testing, the identification of diets, and some aspects of their basic biology, but there is currently no information comparing the juveniles that result from these two different rearing techniques. The present study examined juveniles of *Utterbackia imbecillis* reared either in vitro or on host fish to determine if there were differences between the resulting animals. Morphologically the process of metamorphosis is similar for animals from both rearing conditions, but fish-reared larvae accumulated lipids and glycogen deposits at the base of the larval mantle cells while in vitro-reared larvae did not. Fish-reared juveniles also had better survival and growth rates in the weeks immediately following metamorphosis. In terms of the nutritional status of the postmetamorphic juveniles, fish-reared animals had higher levels of triglycerides, cholesterol, glycogen, and protein at one and two weeks following metamorphosis, as compared to their in vitro-reared counterparts. The response of these animals to thermal and hypoxic stress was also examined and fish-reared juveniles had higher survival rates at each combination of temperature and oxygen tension examined. When subjected to thermal stress alone, fish-reared juveniles responded in a manner similar to fed animals while in vitro-reared animals responded similar to starved individuals in terms of their protein content and RNA:DNA ratios. The results of this study indicate that fish-reared juveniles have higher energy stores, better survival and growth, and are more able to tolerate thermal and hypoxic stress than are in vitro-reared juveniles. If these observations prove applicable for other species of unionids, then perhaps the use of *in vitro*-reared juveniles for experimental and toxicological studies should be reassessed.

GENETIC ISSUES ASSOCIATED WITH THE CONSERVATION OF FRESHWATER MUSSELS

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The protection of biodiversity includes protection of variation within species, because genetic diversity provides the "raw material" for adaptation and natural selection. For a given species, this genetic diversity consists of two main factors: the total variation within the species (the result of accumulated mutations and extinctions of alleles) and the partitioning of that variation among populations. From a conservation standpoint, it is not enough to just protect variation within the target species. It is also critical to preserve any geographic structure that may exist. We have used allozyme electrophoresis to examine genetic structure in a number of common freshwater mussel species. In particular, we are interested in 1) quantifying variation within mussel populations; 2) quantifying variation among populations; 3) determining how among-population variation is partitioned at spatial scales ranging from local to regional; and 4) considering the conservation implications of protecting the genetic structure of target species.

Our work incorporates results from a number of species, including *Quadrula quadrula*, *Elliptio dilatata*, *Amblema plicata*, *Lampsilis siliquoidea*, and *Ptychobranchus fasciolaris*. We have found evidence that within-population variation is roughly correlated with river size. Conversely, among-population variation is inversely correlated with river size. Headwater species often show low within-population variation and high among-population variation, while the reverse seems to be true for large river species. Potential explanations for this pattern include: larger effective population sizes minimizing the effects of genetic drift in large river populations; greater movement among large river host fishes resulting in greater gene flow among mussel populations; greater habitat stability leading to less local adaptation in large rivers. Genetic variation is partitioned in a hierarchical fashion, with among-region and among-river variation exceeding that among populations and within populations. Thus, freshwater mussels show significant geographic structuring of genetic variation.

Genetic structure of target species must be considered when planning conservation strategies such as artificial propagation, augmentation and reintroduction, and translocation. Founder effects and genetic bottlenecks may occur if small numbers of individuals are used to establish captive breeding populations or reestablish wild populations. The source populations for brood stocks or reestablishment must be carefully considered. Care must be exercised when managing reproduction of brood stocks during propagation. We are in the process of building a simulation model that will allow us to develop guidelines for the maintenance of genetic diversity when creating captive populations, augmenting or reestablishing wild populations, or translocating populations. Such guidelines will allow managers to make informed decisions to protect biodiversity within threatened and endangered species.

USES OF MOLECULAR MARKERS IN THE CONSERVATION GENETICS OF UNIONIDS

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I. Conservation Genetics and the Species-Population-Individual Continuum
- A. Molecular genetic techniques for identifying genetic variation
  1. Allozyme electrophoresis
  2. DNA isolation (molecule and total genomic)
  3. Molecule-RFLP and PCR-RFLP
  4. DNA sequencing
  5. Fragment analysis
- B. Molecular Systematics
  1. Examples: *Lasmigona, Alasmidonta*
  2. Management role: species & subspecies designation, evolutionarily significant lineages (ESLs), Endangered Species Act (ESA)
- C. Phylogeography
  1. Example: *Lasmigona subviridis, Alasmidonta heterodon*
  2. Management role identifying ESLs and management units (MUs) for use in Recovery Plans
- D. Population Genetics
  1. Allozyme patterns
     a. Examples: *Quadrula, Elliptio*
     b. Large rivers vs. small streams
  2. Microsatellite DNA patterns
     a. Example: *Lampsilis abrupta*
     b. Cross-species amplification; example: *Lampsilis*
  3. Management role: delineation of fine-scale population structure; allow management of biodiversity at the finest level

II. Conservation strategies for small populations
  1. Genetic bottlenecks, founder effects
  2. Enlightened broodstock management
  3. Creation of guidelines for maintenance of genetic variation

POLICY REGARDING CONTROLLED PROPAGATION OF SPECIES LISTED UNDER THE ENDANGERED SPECIES ACT.

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This policy, published jointly by the Fish and Wildlife Service and the National Marine Fisheries Service, addresses the role of controlled propagation in the conservation and recovery of species listed as endangered or threatened under the Endangered Species Act of 1973 (as amended) (Act). The policy provides guidance and establishes consistency for use of controlled propagation as a component of a listed species recovery strategy. This policy will help to ensure smooth transitions between various phases of conservation efforts such as propagation, reintroduction and monitoring, and foster efficient use of available funds. The policy supports the controlled propagation of listed species when recommended in an approved recovery plan or when necessary to prevent extinction of a species. Appropriate uses of controlled propagation include supporting recovery related research, maintaining refugia populations, providing plants or animals for reintroduction or augmentation of existing populations, and conserving species or populations at risk of imminent extinction or extirpation. Species, as defined in section 3(15) of the Act, includes ‘‘any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature.’’ Though the Act emphasizes the restoration of listed species in their natural habitats, section 3(3) of the Act recognizes propagation as a tool available to us to achieve this end. The controlled propagation of animals and plants in certain situations is an essential tool for the conservation and recovery of listed species. In the past, we have used controlled propagation to reverse population declines and to successfully return listed species to suitable habitat in the wild.

To support the goal of restoring endangered and threatened animals and plants, we are obligated to develop sound policies based on the best available scientific
and commercial information. The Endangered Species Act specifically charges us with the responsibility for identification, protection, management, and recovery of species of plants and animals in danger of extinction. Fulfilling this responsibility requires the protection and conservation of not only individual organisms and populations, but also the genetic and ecological resources that listed species represent. Long-term viability depends on maintaining genetic adaptability within each species.

ISSUES REGARDING RESTORATION OF ENDANGERED FRESHWATER MUSSELS

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Issues to consider before implementing a mussel propagation/augmentation program.

- Is propagation essential to the species conservation and recovery?
- Who are your partners?
- What other actions besides propagation are needed for the species conservation and recovery?
- When are augmentations appropriate?
- What species will be released?
- What approvals are needed?
- Will the reintroduction be conducted under "experimental population" regulations?

MUSSEL PROPAGATION AND THE NATURE CONSERVANCY

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The Nature Conservancy (TNC) is increasing emphasis on conservation in freshwater ecosystems. Over 50% of the priority landscape sites from ecoregional planning efforts have significant freshwater ecosystem components. Freshwater mussels are a conservation target in most of these aquatic systems of eastern North America. Propagation is being evaluated as a tool in the recovery of mussels at several sites, including the Clinch Valley, VA-TN, Green River, KY, and Mackinaw River, IL. Decisions about the role of mussel augmentation at TNC conservation sites are developed with the advice of experts in mussel conservation and propagation. The Clinch Valley Program of TNC has developed relationships with partner agencies to help advance juvenile propagation as a conservation management tool. As a step towards playing a meaningful role in mussel propagation, TNC has begun to develop a mobile mussel culture facility in the Clinch Valley Program, sited along the Clinch River in Russell County, VA. The facility is designed to use natural river water for all mussel culture operations, but has the capacity to use well water for juvenile transformations on fish or culturing juveniles. The source river water comes from a reach of the Clinch River that supports a relatively healthy and diverse mussel assemblage, indicating suitability for propagation purposes. The use of natural river water provides the culture facility with the proper water chemistry and food supply for freshwater mussels, while requiring less intensive management from facility staff. Other design criteria of the facility include the ability to switch from a flow-through system under normal operation to a recirculating system in the event of a power outage or water quality problem, such as a chemical spill. This switch will be automatic with a power outage and will allow water circulation to continue for approximately 24 hours without any action from staff, a crucial feature of remotely located culture facilities. In addition, the availability of well water provides a backup source of culture water in the event of a sustained contaminant event. We hope to be a significant partner in the recovery of mussels in the upper Clinch Valley Program area by successfully producing and rearing juvenile mussels in a cost efficient manner with minimal staffing requirements. TNC's long-term goal is to evaluate the feasibility of operating a small culture facility, to determine the likelihood of success for a larger, permanent culture facility fed with natural river water in the Clinch River basin, and to provide a facility in which various culture and ecological parameters can be tested.

PATHOGEN AND DISEASE CONCERNS ASSOCIATED WITH MUSSELS, FISHES AND RELOCATION.

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A byproduct of the efforts at conservation of native freshwater mussels' is the concern for pathogens and diseases of the mussels, their fish hosts for glochidia development and resident fishes at safe refugia used for mussel propagation. Fish-rearing hatcheries were selected as safe refuges to hold mussels' for propagation after they are collected from imperiled rivers. With this, fisheries managers raised concerns about introduction of fish pathogens to hatcheries via the relocated bivalves; studies were initiated to address these questions. Once methods were developed to isolate bacteria from mussels, it was found that their bacterial flora was quite stable in terms of numbers of bacteria, but dynamic relative to changes in their aquatic environment. Mussels from open waters, i.e. Ohio River, were shown to harbor fish pathogenic bacteria. Although, after exhaustive efforts, *Flavobacterium columnare* was isolated from only a single *Amblema plicata*, nonetheless, pathogen harboring was demonstrated. Next, it was shown in the laboratory, with *A. plicata*, that mussels can readily serve to vector the fish pathogen *Aeromonas salmonicida* to Arctic char, which resulted in disease and death to the fish. However, if *A. plicata* are allowed to depurate for a period of less than 15 d, pathogen vectoring does not occur, owing to the dynamic nature of the flora. Pathogen and disease studies involving mussels are now focusing on the mussels', and not solely from the point of view of resident fish at hatcheries. The overall goal is to provide information so those persons working for mussel conservation can make informed decisions to prevent disease transmission and introduction. Work will begin soon to determine if the cause of natural mussel dieoffs are the result of an etiological agent(s). Bacterial flora databases will be developed for sites known to have experienced dieoffs, then in the event of the dieoff, the bacterial isolates can be compared with those for the expected normal flora to identify suspected causal agents. Another study will provide pathogen depuration and condition factor data to support the idea to reduce the length of quarantine that native mussels' must currently endure. The thought is that lesser time spent in quarantine yields a superior animal for relocation, thus, better equipped for survival, propagation and disease resistance. Another effort has been initiated to develop a non-lethal means for screening mussels for presence of pathogens. Current methods result in death to the animals, obviously an undesirable approach for imperiled species. If non-lethal sampling does not cause undue harm to mussels and the technique yields results similar to (lethal) whole tissue analyses, then mussels' to be relocated could be examined for pathogens prior to their relocation. This would provide a powerful tool for combating movement of pathogens; prevention of disease is always the most desirable option.

PROPAGATION OF *LAMPSILIS HIGGINSI* AT GENOA NATIONAL HATCHERY

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The Genoa National Fish Hatchery is participating in a multi-agency effort to recover Higgins-eye pearly mussel (*Lampsilis higginsi*) populations. The goals of this effort are to bolster existing populations, and to establish new populations of this Federally Endangered mussel. Projects carried out during 2000 and 2001 yielded varied results in survival and growth. Management schemes included hatchery propagation, free release of infested host fish, and cage release of infested host fish. Juvenile releases over the two seasons were 4,850 in 2000 and an estimated 178,650 in 2001. Survival of juveniles in hatchery propagation trials dropped from 48% through 70 d in 2000 to ~2.0% by 30 d across all treatments during 2001. The leading factors in reduced early survival of juveniles during 2001 appear to be predation by freshwater hydrozoans and flatworms. The majority of juvenile distribution carried out during 2001 was in the form of free release of infested host fish (1,698 fish), and cage release (1,645 fish). Results from cage releases show promise, with initial assessments of 4 cages yielding >250 juvenile *L. higginsi* ranging in size from 5 – 10 mm at 90 d post planting.

CAPTIVE REFUGIA & PROPAGATION WORK FOR FRESHWATER MUSSELS AT THE WARM SPRINGS NFH, WARM SPRINGS, GA

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Freshwater mussels are among the most endangered animals in the U.S. The Flint River Basin historically contained 29 species of mussels, but only 22 species are believed to exist today. Five of these species are considered either endangered or threatened under the Endangered Species Act. In June 2000, long stretches of Spring Creek, Miller County, GA (Southwest Georgia), which is an area of tremendous mussel diversity in the Flint River system, went dry. Thousands of dead mussels, fishes, turtles, crayfish, and snails littered the creek bed. Service biologists organized a major salvage effort to save as many of the mussel species as possible, including several hundred individuals of two federally endangered species; shiny-rayed pocketbook (*Lampsilis subangulata*) and oval pigtoe (*Pleurobema pyriforme*). Approximately 1,375 live mussels were salvaged from the few remaining pools and patches of mud. The mussels were transported to the Warm Springs National Fish Hatchery where temporary facilities were set up to hold them. Two weeks later, a permanent building was completed to hold and study the mussels throughout the year. The hatchery staff conducted water quality studies and tried to mimic their habitat to acquire information on how to maintain mussels in captivity, how to propagate certain species, and how to identify potential host species for mussel glochidia. During June 2001, after normal stream flows returned, hatchery and Ecological Services staff marked approximately 1,050 of the surviving mussels at Warm Springs for additional monitoring. The mussels were returned then to the original salvage sites within Spring Creek. A total of 1,123 of the salvaged mussels survived one year of captivity at the hatchery.

In 2001, while adult mussels were in captivity, propagation techniques were developed, glochidia were collected from several species, transformed juveniles were collected, and kept alive for six months. Juveniles from two surrogates: *Villosa lienosa* (little spectaclecase) and *Villosa vibex* (Southern rainbow) of endangered species and one federally listed species, *Lampsilis subangulata* (shiny-rayed pocketbook), were successfully stocked after six weeks in captivity in three different locations within Spring Creek. Total numbers of juveniles stocked: shiny-rayed - 5,532, Southern rainbow - 2,627 and little spectaclecase - 940. Another twenty thousand juvenile mussels were retained in the lab for additional work. Studies have continued throughout FY-02 in host identification, amount/rate of glochidia infection, adult and juvenile nutrition, transportation, captive refugia, water quality, marking, and monitoring, and other evaluation techniques.

MUSSEL CULTURE AT WHITE SULPHUR SPRINGS NATIONAL FISH HATCHERY, WV

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White Sulphur Springs National Fish Hatchery (WSSNFH) has been working to develop a mussel program since 1995. The program started when mussels were collected from the Ohio River under an emergency salvage order and brought to the hatchery for refuge. Some of the mussels collected in 1995 are still living. During this time period, mussels were held in both a pond and an earthen bottom, flow-through raceway. Survival was higher in the pond where water temperatures were warmer. The depth of the substrate in the containers appears to be an important factor affecting survival. Over winter mortality was very high when mussels were held in containers with < 5 cm of substrate. In subsequent years, mussels were placed in containers with 20 cm of substrate and over winter mortality was substantially reduced.

Juvenile rainbow mussels (*Villosa iris*) and wavy rayed lampmussels (*Lampsilis fasciola)* were successfully reared at WSSNFH by researchers from Virginia Tech. After approximately 90 days, mean survival of *V. iris* juveniles cultured with, and with out, fine silt was 49.8% (± 14.5) and 32.9% (± 11.7), respectively. Mean survival of *L. fasciola* was 6.3% (± 4.5) after approximately 90 days.

Researchers at Virginia Tech. are currently investigating habitat conditions of hatchery ponds at WSSNFH to determine the suitability of the ponds for holding adult mussels. A total of 210 adult mussels comprised of three non-endangered species (*Cyclonaias tuberculata*, *Actinonaias ligamentina*, and *Tritogonia verrucosa*) were tagged and stocked into the pond. Investigators will compare algae concentration in the pond to levels found in rivers, compare the condition of captive mussels to mussels collected from rivers, and determine if mussel fertilization takes place in the pond.

WSSNFH is constructing a new building in 2002 to conduct mussel culture activities. The new building, combined with the existing ponds and raceways, will facilitate all phases of propagation including algae culture, holding adult mussels, holding host fish, infesting host fish with glochidia, and enumerating and growing out juveniles. Hatchery ponds will be used to hold and rear juvenile and adult mussels and to supply algae rich water to mussels held inside the building.

WSSNFH can provide a location to conduct a variety of mussel research and propagation activities. The facility can be used to conduct applied mussel research on a production scale or to conduct smaller experimental research studies. Propagation techniques for mussels can be developed and enhanced at WSSNFH and the facility can serve as a refugia for endangered mussels.

PROPAGATION OF FRESHWATER MUSSELS IN A CLOSED RECIRCULATING SYSTEM

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We renovated a preexisting wet laboratory located at North Carolina State University for purposes of propagating Atlantic slope species of freshwater mussels. Our initial objective was to demonstrate the ability to produce juvenile mussels for augmentation and experimental purposes. Trials to propagate multiple species of mussels were conducted with a closed recirculating system designed to be cost effective and require minimal maintenance. In addition, we evaluated the use of automatic feeding systems, and techniques to hold host fish captive. Success of rearing juvenile mussels beyond 2 months was variable depending on the species and environmental conditions. We speculate that water quality and temperature, food quality and quantity, and condition of the substrate were the important factors that influenced growth and survivorship of our juvenile mussels. We provide an evaluation of our juvenile mussel culture system, and reveal some of the trials and tribulations of establishing a freshwater mussel propagation facility.

MUSSEL PROPAGATION IN MISSOURI

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Missouri's mussel propagation program is a multi-state, cooperative agreement between the Missouri Department of Conservation (MDC), U.S. Fish and Wildlife Service (Service), Southwest Missouri State University (SMSU), Kansas Department of Wildlife and Parks, Oklahoma's Langston University, and Arkansas Game and Fish. Funds for the program come primarily from a 75:25 cost-share agreement between the Service and MDC (U.S. Endangered Species Act, Section 6 monies). Fish host and mussel reproduction research are carried out at SMSU. MDC state hatcheries contribute space and staff time to help implement, on a larger scale, propagation techniques learned at the SMSU laboratory. Host fish are also contributed by Langston University. Propagation begins with fieldwork to find brooding females. Mussel biologists from MDC, USFWS, and SMSU typically complete this task. Gravid females are transported to SMSU and MDC hatcheries where transformation on host fish takes place. Juvenile mussels are transported as soon as possible after transformation to release sites, which have included rivers not only in Missouri but also Kansas and Arkansas. Mussels propagated and stocked to date include three federally endangered species (pink mucket, scaleshell, fat pocketbook), one federal candidate species (Neosho mucket), and the black sandshell, a species of conservation concern in Missouri.

The timing of events is critical to the success of our program. Factors to consider include the period of gravidity of the mussel species, the availability of the biologists for repeated field trips to find endangered broodstock, the availability of host fish, and hatchery space. Key factors for success include ongoing communication between partners, willingness to work together, and especially the flexibility of the hatchery staff to work around their sportfish production program to help endangered species.

ST. CROIX RIVER MUSSEL PROPAGATION FACILITY PROPOSAL

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A new mussel propagation facility is being proposed by the Science Museum of Minnesota, the Minnesota Department of Natural Resources, the U.S. Fish and Wildlife Service, and Watchable Wildlife. The facility will be constructed adjacent to the St. Croix River, a National Scenic Riverway, bordering Minnesota and Wisconsin. The St. Croix River supports a healthy mussel population of 40 endemic species, including the federally endangered winged mapleleaf (*Quadrula fragosa*), Higgins' eye pearly mussel *(Lampsilis higginsi*), and over 18 other Minnesota and Wisconsin endangered and threatened mussel species. The propagation facility would have immediate access to the river and be located on land owned by the Science Museum of Minnesota, which operates the St. Croix Watershed Research Station. Water would be withdrawn from the St. Croix River and piped into mussel holding tanks. The waste water would then be outletted back into the river. This mussel propagation facility thus meets multiple goals and strategies outlined in the *National Strategy for the Conservation of Native Freshwater Mussels*: The facility would develop and implement technology for propagation and reintroduction of mussels, specifically the winged mapleleaf. It would help to determine the specific fish hosts for winged mapleleaf and other species in need of management. It would help to determine the extent and mechanism of the immune response of host fish to glochidia. It would help to determine the period of spawning and gravidity as well as spawning and setting sites. It would help to determine the level of recruitment needed for species survival and long-term viability. And, it would enhance public awareness and support for mussel conservation through the Science Museum of Minnesota.

The national strategy includes a list of facility criteria for propagating mussels, that will be fully met by the proposed mussel propagation facility to be sited at the St. Croix Watershed Research Station. These criteria include the following:

1. Propagation, specifically of winged mapleleaf is essential and is justified in the winged mapleleaf recovery plan.
2. Water from the St. Croix River is known to be compatible with the winged mapleleaf, provides a known food source, and is free of zebra mussels.
3. The St. Croix Watershed Research Station currently operates a state-of-the-art wet lab.
4. The mussel propagation facility will involve the U.S. Fish and Wildlife Service, the Minnesota Department of Natural Resources, the Wisconsin Department of Natural Resources, Watchable Wildlife, the Science Museum of Minnesota, and other federal, state, tribal, or private organizations.
5. All federal and state permit requirements will be met.