# PROGRAM AND ABSTRACTS

# FRESHWATER MOLLUSK CONSERVATION SOCIETY 2006 WORKSHOP

## COLUMBUS ZOO & AQUARIUM, POWELL, OHIO 5-7 MARCH 2006







*Edited by:* G. Thomas Watters The Ohio State University



Cover mussel art from: Poupart, 1707. Rémarques sur les coquillages à deux coquilles, & premièrement sur les moules. Histoire de l'Academie Royale des Sciences à Paris, Mémoires [for 1706]: 51-61, 5 figs.

Workshop logo by: Dustin Collins and Chris Lutmerding

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	Sunday	Monday	Tuesday
8:30 AM	Continental	Continental	Continental
	Breakfast	Breakfast	Breakfast
9:00 AM	Welcome	Welcome	Welcome
9:15 AM	Overview	Health	Legal Matters
	Jones	Gustafson	Koch
9:45 AM	Host ID	Acarology	Jones
	Hove	Mitchell	Navarro
10:15 AM	Propagation I	Serum Methods	Discussion
	Barnhart	McGregor	
10:45 AM	Break	Break	Break
11:00 AM	Nutrition I	Rearing Fish	
11.00.004	Orcutt	Muller	
11:30 AM	Nutrition II Nichols	Rearing Snails Johnson	
12:00 PM	NICHOIS	JOHNSON	
12.00 PIVI			
	LUNCH	LUNCH	
	LONOIT	LONOTI	
1:30 PM	Assessing Success	Facility Design	
	Layzer	Brittsan	
2:00 PM	Disease & Parasites	Reintroductions	
	Wolf	Kuehnl	
2:30 PM	Genetics	Case Study I	
	King	Neves	
3:00 PM	Break	Break	
3:15 PM	Testing Water	Case Study II	
	Gibula	Brady	
3:45 PM	Toxicology	Case Study III	
	Farris	Oetker	
4:15 PM	Propagation II	Case Study IV	
	Sweet	Johnson	
4:45 PM	Riffleshell Redux		
	Watters		

Program - Propagation and Captive Care of Freshwater Molluscs

### Mixers: PLEASE WEAR YOUR NAME BADGES

Sunday - 7 PM till ? Hors d'oeuvres and open bar (beer-wine) mixer at the Mussel Facility

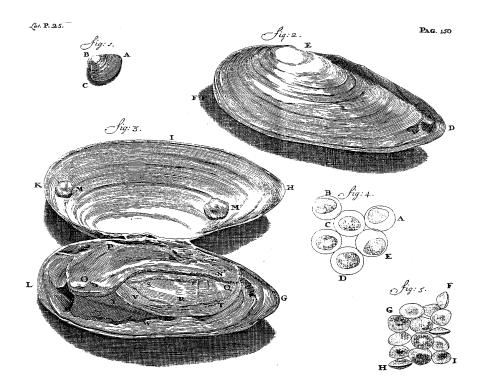
Monday - 7 PM till ? Buffet dinner and open bar (beer-wine) behind the scenes at the Columbus Zoo and Aquarium at the Living Reef and Manatee exhibits

Sunday		
8:30 - 9:00	Continental Breakfast	
9:00 – 9:15	<b>Welcome.</b> Robert Anderson, President of the Freshwater Mollusk Conservation Society.	
9:15 – 9:45	Propagation and Culture of Freshwater Mussels in the United States. Jess Jones.	
9:45 – 10:15	Identifying Unionid Glochidia Hosts. Mark Hove.	
10:15 – 10:45	More Fun than Pet Rocks. Methods for Propagating and Culturing Native Freshwater Mussels. Chris Barnhart.	
10:45 – 11:00	Break	
11:00 – 11:30	Algae Culture: From Agar Slants to Photo-Bioreactors. David Orcutt.	
11:30 – 12:00	A Review of Unionid Feeding and Nutrition. Jerrie Nichols.	
12:00 – 1:30	Lunch (provided)	
1:30 – 2:00	Assessing the Success of a Mussel-stocking Program. James Layzer.	
2:00 – 2:30	Mussel Maladies: Diseases and Parasites. Tiffany Wolf.	
2:30 - 3:00	Genetic Considerations in Captive Populations. Tim King.	
3:00 – 3:15	Break	
3:15 – 3:45	A Guide to Basic Water Quality for the Care of Captive, Freshwater Mussels. Jeff Gibula.	
3:45 – 4:15	Challenges to Water Quality Guidance for Protection and Propagation of Freshwater Mussels. Jerry Farris.	
4:15 – 4:30	Mussel Propagation Equipment made from Ordinary Household Hardware. Doug Sweet.	
4:30- 4:45	Northern Riffleshell Redux. Tom Watters.	
7:00 –?	Mixer, Open Bar, and Hors d'oeurves at Mussel Facility	

Monday		
8:30 - 9:00	Continental Breakfast	
9:00 – 9:15	Welcome	
9:15 – 9:45	Recommendations for the Non-lethal Health Assessment of Freshwater Mussel Populations. Lori Gustafson.	
9:45 – 10:15	Mites and Mussels. Rodger Mitchell.	
10:15 – 10:45	Rearing Freshwater Mussels without Their Hosts. Monte McGregor.	
10:45 – 11:00	Break	
11:00 – 11:30	Rearing Darters for Host Work. Bob Muller.	
11:30 – 12:00	Artificial Propagation and Culture of North American Freshwater Snails. Paul Johnson.	
12:00 – 1:30	Lunch (provided)	
1:30 – 2:00	Designing a Fresh Water Mussel Facility. Mike Brittsan.	
2:00 – 2:30	Criteria for Selecting Reintroduction Sites. Kody Kuehnl.	
2:30 – 3:00	Case Study I. A Case Study of Propagation and Juvenile Mussel Releases in Virginia and Tennessee. Dick Neves.	
3:00 – 3:15	Break	
3:15 – 3:45	Case Study II. The Use of MusselCulture Cages in the Recovery of Endangered Higgins Eye Pearlymussel. Tony Brady.	
3:45 – 4:15	Case Study III. Propagation of Winged Mapleleaf in Cages in the St. Croix River. Susan Oetker.	
4:15 – 4:45	Case Study IV. Propagation of Freshwater Snails. Paul Johnson.	
7:00 –?	Mixer, Open Bar, and Buffet at Shores, Columbus Zoo & Aquarium	

#### Tuesday

- 8:30 9:00 Continental Breakfast
- 9:00 9:15 Welcome
- 9:15 9:45 Legal Considerations. US Fish & Wildlife Service Discussion Regarding Mussel Propagation Activity at Propagation Facilities. Leroy Koch & Jess Jones.
- 10:00 10:15 Legal Considerations. Legal Ramifications of Propagation and Reintroduction – A State's Perspective. John Navarro.
- 10:15 11:00 Break



*First illustration of glochidia.* van Leeuwenhoek, Antonii. 1697. *Continuatio Arcanorum Naturæ Detectorum.* Henricum a-Kroonevelt. 192 pp.

#### More Fun than Pet Rocks: Methods for Propagating and Culturing Native Freshwater Mussels

BARNHART, CHRIS. Biology Department, Missouri State University, 901 S. National Ave. Springfield, MO 65897. chrisbarnhart@missouristate.edu

The propagation and culture of native mussels presents several challenges because of the parasitic stage, the near-microscopic size of glochidia and early juveniles, and suspension feeding. Over the past several years, three systems were developed at Missouri State University to facilitate host evaluation, captive propagation, handling, and grow-out of unionids. First, for host evaluation, a commercial multi-unit research aquarium system ("AHAB") was modified with recovery filters to enable daily recovery of transformed juveniles from individual host fish without the labor-intensive step of siphoning aquaria. Second, a recirculating propagation system ("RPS") was developed for large-scale captive transformation using hundreds of host fish. The RPS incorporates paired 400-gallon conical-bottom tanks with a shared sump for recovery of juveniles and for biological filtration. These systems have been used to transform over two million juvenile mussels since 2002 and are in use at Missouri State, Missouri Dept. of Conservation Lost Valley Hatchery, and the Virginia Aquatic Conservation Center. Third, a compact recirculating system ("Mucket Bucket") was developed in 2004 for captive grow-out of juveniles. The bucket systems are economical, occupy minimal space, and should facilitate research as well as serving production purposes. A small submersible pump moves water from a lower to an upper compartment, and the water returns to the lower compartment through cylindrical screen-capped chambers containing the juveniles. Feeding is accomplished by a pump and manifold system that delivers algal suspensions to each system at controlled rates. Thousands of early juveniles, in up to seven separate groups, can be held in each system. Unionids of 10 species have been held in these systems, some for over 18 months, most with good survival and growth. The design facilitates handling, containment, and examination of the juveniles. The bucket rearing systems may be particularly useful for conducting studies of water quality and feeding regimes that require replication to account for container effects.

### The Use of Mussel Culture Cages in the Recovery of Endangered Higgin's Eye Pearlymussel

BRADY, TONY R. AND ROGER GORDON. Genoa National Fish Hatchery, S5689 St. Rd. 35, Genoa, WI 54632. tony\_brady@fws.gov

The Higgin's eye pearly mussels *Lampsilis higginsii* has been listed since 1976 under the Federal Endangered Species Act of 1973. With the invasion of the exotic zebra mussel *Dreissena polymorpha* in the late 1990's increasing the risk of extinction, biologists from the upper Mississippi River basin began to take action to prevent their

loss. As part of the Mussel Coordination Team (MCT), a multi-agency task force, Genoa National Fish Hatchery was charged with exploring and developing methods for producing sub-adults Higgins eye for recovery efforts. Modifying the cage culture techniques used by Howard in the early 1900's to fit today's recreational uses of the Mississippi River, aluminum framed cages 914-mm x 609-mm x 457-mm (3' x 2' x 18") covered with 12.7-mm mesh (1/2") hardware cloth were built to fit inside a collection basin (base). Inoculated fish are placed inside the cages and then cages are submerged in the Mississippi River. Transformed juveniles are then cultured in the substrate filled base and harvested approximately 120 days after cage placement. Higgins eye sub-adult mussels harvested from cages are equally distributed back into the cages for up to two additional years of culture. To date over 11,000 Higgins eye mussels have been harvested from cages in the Mississippi River. Progeny from the 2002 and 2003 cohort of Higgins eye cultured in cages have now reached maturity with females producing viable glochidia.

### Designing a Fresh Water Mussel Facility

BRITTSAN, MIKE. Columbus Zoo and Aquarium, Powell, OH 43065. Mike.Brittsan@columbuszoo.org

Successful design of any aquatic facility needs to take into account the organisms (knowing the biology and ecology), husbandry factors, and probably most importantly water composition and quality. This paper will discuss types of systems (Open, Semiclosed, Closed), and is designed to help those holding adult mussels. Factors to consider are food source, flow requirements, substrate depth/type, and filtration (particularly in semi-closed and closed systems) including water quality. Food source may include both natural i.e. source water, and cultured food sources. Flow requirements may depend upon the species, whereas pump capacity, type of pump, and redundancy can be critical. Substrate depth should create natural conditions especially for adult mussels. Substrate composition needs to consider natural origin of the species, as many commercially available substrates are generally silica and may not provide adequate buffering capacity. Filtration and disinfection will be discussed. Mechanical (particulate), biological (bacterial and algal based), and chemical adsorption (foam fractionation, carbon) will be highlighted. Disinfection through heat and ozone are briefly touched on. Materials of construction including concrete, fiberglass and plastics are considered.

#### Challenges to Water Quality Guidance for Protection and Propagation of Freshwater Mussels (Unionidae)

FARRIS, JERRY L.<sup>1</sup> AND W. GREGORY COPE<sup>2</sup>. <sup>1</sup>College of Sciences and Mathematics, Arkansas State University, State University, AR 72467. <sup>2</sup>Department of

Environmental & Molecular Toxicology, North Carolina State University, Raleigh, NC 27695-7633.

The widespread reduction in density and diversity of freshwater mussels in aquatic ecosystems suggests that subtle changes in water quality characteristics can have pervasive effects. Mollusk populations adhere to the laws of thermodynamics and their relative condition reflects adjustments to altered resource availability or simply disturbance. Critical interactions specific to freshwater mussels make it extremely difficult to identify limiting stressors exerting the greatest effect upon oxygen consumption, filtration, burrowing, detrital processing, or nitrogenous excretion. Recent attempts to link bioavailability of contaminants and exposure to adverse effects have highlighted the need for better toxicological data from the variety of routes (surface water, pore water, sediments, and food). Establishing water quality criteria that includes freshwater mussel responses requires an understanding of conventional test approaches, scientific considerations for standardized techniques specific to life stages, as well the context and ramifications of the demand for test organisms.

## A Guide to Basic Water Quality for the Care of Captive, Freshwater Mussels

GIBULA, JEFFREY M. Newport Aquarium, One Aquarium Way, Newport, KY 41075. jgibula@newportaquarium.com

A basic explanation of general water quality parameters and insight on the life support component selection for freshwater mussels. The detrimental effects of organic waste, toxic forms of nitrogen, undesirable pH levels and low dissolved oxygen concentrations on such life forms identifies the essential need for life support components that are capable of meeting specific, preset, life-sustaining parameters. The goals of mechanical, biological and chemical filtration; dissolved organic and particulate removal; and sterilization can be achieved through a combination of varied applications. Although component selection is at the discretion of the life support designer, professionals agree that ideal water quality parameters should not be compromised.

#### Recommendations for the Non-lethal Health Assessment of Freshwater Mussel Populations

GUSTAFSON, LORI. USDA APHIS Veterinary Services, Eastport, ME 04631. Lori.L.Gustafson@aphis.usda.gov

Non-lethal techniques for the assessment of freshwater mussel health are few and far between. Consequently, monitoring efforts all too often depend upon late-stage indicators such as mortality or delays in growth. I'll review non-lethal techniques in the making that should help better position practitioners of mussel health towards

preventive medicine and early response. I'll outline techniques for the safe harvest of hemolymph and foot biopsy sections. I'll outline available reference ranges for hemolymph chemistry and stable isotope parameters, highlighting those parameters that seem the most promising for health evaluation. Lastly, I'll recommend steps towards the design of a population health assessment program aiming to simultaneously improve knowledge, and monitor the status, of mussel health.

## Identifying Unionid Glochidia Hosts

HOVE, MARK C.<sup>1, 2</sup> AND DANIEL J. HORNBACH<sup>2</sup>. <sup>1</sup>University of Minnesota, St. Paul, MN 55108. <sup>1,2</sup>Macalester College, St. Paul, MN 55105. mark.hove@umn.edu

Unionid conservation efforts are frequently enhanced with knowledge of glochidia host requirements. Thorough analysis of these requirements has been conducted for a minority of species. Currently, hosts are usually identified using a combination of studies: (1) laboratory studies determining species that facilitate glochidia metamorphosis (suitable host species), and (2) observing species naturally infested with glochidia (naturally infested species). Very useful information comes from studies that identify juvenile mussels excysted from naturally infested animals (host species). However, identification of these small mussels frequently requires developing molecular or visualization (*e.g.*, describing qualitative and quantitative valve characters using scanning electron microscopy) tools. Other tools used in deducing unionid glochidia-host relationships include immunological techniques, correlation analysis of fish and mussel species distributions, and by inference. Methods for conducting these studies will be discussed. Knowledge of host species can help culturists select hosts best suited for propagating mussels, and improve the likelihood of long-term survival of re-introduced mussel populations.

### Artificial Propagation and Culture of North American Freshwater Gastropods

JOHNSON, PAUL. Alabama Aquatic Biodiversity Center, Marion, AL 36756. leptoxis@hotmail.com

With approximately 60 species extinct and 50% of remaining species highly imperiled (G1 & G2), the conservation status of North American freshwater gastropods rivals that of freshwater mussels. Species losses have primarily been restricted to several families of the Caenogastropoda that contain gill breathing, separately sexed species, generally restricted to discrete river drainages. Following recent developments for freshwater mussels, artificial propagation methods have been successfully developed for approximately 20 species of the Pleuroceridae in addition to several species of Hydrobiidae and Viviparidae. Although different species can have vastly different life histories, most have responded well to captive propagation attempts. Unlike mussels,

most female snails attach eggs to firm substrates and juveniles hatch after a period of direct development. Ovipostion for many species examined appears to be cued by temperature, current velocity, or some combination. Fecundity, period of ovipostion, and placement of the eggs is vastly different among closely related species. Culture of hatched juveniles has been very successful although mortality increases with culture duration. This presentation will focus on the methodology of propagation and culture of juvenile snails, including the design of holding systems, hatching strategies, and juvenile culture techniques. Restoration methods alternative to direct propagation will also be discussed.

#### Propagation and Culture of Juvenile Mussels (Unionidae) in the United States: How Federal and State Hatchery Programs are Turning the Corner Toward Success.

JONES, JESS W. U.S. Fish and Wildlife Service, Department of Fisheries and Wildlife Sciences, Virginia Tech, Blacksburg, VA 24061. vtaquaculture@hotmail.com

The U.S. Bureau of Fisheries established the Fairport Biological Station at Fairport, lowa in 1914 to conduct mussel culture research in response to declining commercial shell harvests of the late 19th century. Located on the banks of the Mississippi River, the lab conducted research for nearly two decades to develop techniques to grow-out juvenile mussels to supplement waning river populations. These early studies established the basic tenets of mussel culture, but were unable to turn the corner toward development of methods capable of reliably culturing juvenile mussels to older and larger sizes. In response to stemming the continental wide freshwater mollusk extinction crisis of the late 20th century, biologists around the country recently have developed various methods to produce and culture juvenile mussels to help recover imperiled populations. Currently, 15 federal and state facilities propagate mussels in the Southeast and Midwest. These facilities have conducted critical life history studies on freshwater mussels and, during the past several years, have released over 1 million juveniles of more than a dozen endangered species into rivers throughout the eastern United States. Importantly, advances in culture technology have now turned the critical corner toward growing juveniles to larger sizes to facilitate survival in natal rivers. Survival of laboratory-reared juveniles 1-3 years of age after release already has been documented. For example, juvenile Neosho mucket were reintroduced in 2000 into historical habitat in the Fall and Verdigris rivers, Kansas. Biologists recovered 28 juveniles of this species at release sites in 2002. The endangered Higgin's-eye pearlymussel and endangered oyster mussel have been propagated, outplanted, and recovered at release sites in the upper Mississippi River, Wisconsin, and Clinch River, Tennessee, respectively. Therefore, propagation of mussels now offers state and federal hatcheries an opportunity to expand their mission and assume an important role in conservation of biological diversity in the United States.

#### U.S. Fish and Wildlife Service Discussion Regarding Mussel Propagation Activity at Propagation Facilities

KOCH, LEROY<sup>1</sup> AND JESS W. JONES<sup>2</sup>. <sup>1</sup>U.S. Fish and Wildlife Service, 3761 Georgetown Road, Frankfort, KY 40601. <sup>2</sup>U.S. Fish and Wildlife Service, Department of Fisheries and Wildlife Sciences, Virginia Tech, Blacksburg, VA 24061. Leroy\_Koch@fws.gov

The Service will provide some information for discussion, of mussel propagation activities at selected facilities, especially regarding the Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act.; and, how the Service has been interpreting the implementation of this policy. Another topic for discussion involves permitting to conduct mussel propagation activities for federally listed species. Discussion on these topics is intended to help provide guidance to those involved in propagation activities; and, provide an opportunity to identify any concerns and/or problems associated with these topics. An example of a propagation plan will be presented and discussed so participants can learn about the major components involved in preparing such plans.

### **Criteria for Selecting Unionid Reintroduction Sites**

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The vast majority of North America's freshwater mussels (Bivalvia: Unionidae) have been decimated by various anthropogenic influences leaving them in a state vulnerable to extinction. The majority of these factors can be directly linked to loss, degradation, or changes in essential habitat needed to fulfill essential life-history attributes. In response, multiple state and federal institutions have developed recovery and/or reintroduction plans, all of which incorporate the use of relocation of reproducing adults or captive propagation of juvenile individuals for release into current or historic ranges in order to facilitate recovery of the species. However, very few of these plans identify even general criteria used to select reintroduction sites, and none address potential requirements for early life history stages, even though juveniles and adults have been found to respond differently under the same environmental conditions (e.g. toxicity studies). As a result, the likelihood of success of many of these reintroduction efforts may be hindered. While quantifying suitable habitat sites for unionids is complex and much of the information required to accurately assess existing sites is lacking on a species to species basis (e.g. basic life history, reproductive biology, ecology, and habitat requirements at different life stages), several general criteria should be considered when any reintroduction is attempted. Here we review the literature pertaining to selecting sites for the purposes of reintroduction, relocation, and augmentation in an effort to better understand the criteria needed to facilitate successful integration of a new population of unionids.

### Assessing the Success of a Mussel-Stocking Program

LAYZER, JAMES B. U.S. Geological Survey, Tennessee Cooperative Fishery Research Unit, Tennessee Tech University, Box 5114, Cookeville, TN 38505. jim\_layzer@tntech.edu

Most mussels are stocked to reestablish populations within the historic range of a species or to augment existing populations. A true reintroduction does not require the ability to recognize an individual beyond the species level; however, because some extirpated species seemingly have the innate ability for spontaneous regeneration, most reintroductions should be treated as augmentations. Evaluating the success of a population augmentation program requires comprehensive prestocking planning that includes: 1.) defining the goal and specific objectives of stocking, 2.) establishing criteria for determining if objectives are met, 3.) selecting locations suitable for stocking and monitoring, 4.) choosing a method to recognize stocked individuals, and 5.) establishing a statistically rigorous sampling design. Results of most of these planning steps will be unique to each program. The primary focus of this paper is to review methods that have been used to identify stocked individuals, suggest alternative methods, and discuss the advantages and disadvantages of each method.

### Rearing Freshwater Mussels without the Host: Artificial Culture of Glochidia *in vitro* using Modern Cell Culture Methods

MCGREGOR, MONTE A.<sup>1</sup>, ROBERT.G. HUDSON<sup>2</sup>, JEFF JACK<sup>3</sup>, CHRISTOPHER OWEN<sup>3</sup> AND RONALD DIMMOCK<sup>4</sup>. <sup>1</sup>Kentucky Department of Fish and Wildlife Resources, Center for Mollusk Conservation, Frankfort, KY 40601. <sup>2</sup>Department of Biology, Presbyterian College, Clinton, SC 29325. <sup>3</sup>Department of Biology, University of Louisville, Louisville, KY 40292. <sup>4</sup>Department of Biology, Wake Forest University, Winston-Salem, NC 27109. Monte.McGregor@ky.gov

Freshwater mussel propagation has been limited by the availability and/or difficultly of handling host fishes. Even if glochidia and hosts are available in the best laboratory conditions, transformation rates to the juvenile stage are mostly unpredictable. Pioneer biologists from the early 1900's reported these same problems and came up with innovative solutions to bypass the host stage. Lefevre and Curtis (1910) tried placing glochidia in drops of fish or mudpuppy blood on a microscope slide sealed with vaseline, but had no success. Ellis and Ellis (1926) reported the growth and transformation of glochidia in a physiological nutrient solution. In their notes, glochidia were extracted

from the gills of infested hosts a few days post infestation for placement into the mystery solution. However, their methods were never published. Over fifty years later, lsom and Hudson (1982) reported findings of a nutrient medium that could be used to bypass the fish host in rearing juvenile mussels. The medium consisted of physiological salts, amino acids, glucose, vitamins, antibiotics, and non-specific fish blood plasma. Keller and Zam (1990) later simplified the technique using commercially available tissue culture products and reported good transformation success. In recent years, several studies have been completed with modifications of either lsom and Hudson's or Keller and Zam's techniques. Nineteen North American species have been transformed in artificial media: 11 species in media with fish plasma and 8 in combinations of rabbit or horse serum, fish plasma, and serum replacements. In an effort to promote the artificial culture of mussels for recovery purposes, we provide a summary of old and new *in vitro* methods. We examine the equipment, materials and supplies, costs, pro and cons, and other issues with the techniques that apply to mussel conservation.

### **Rearing Darters for Host Work**

MULLER, ROBERT. North American Native Fishes Association, Royal Oak, MI 48067. michiganfish@wideopenwest.com

Darter brood stock, used in captive production of host fish, can be wild caught just prior to spawning season; or maintained throughout the year and conditioned to spawn in season. Timing for acquiring wild brood stock is critical. Photoperiod and temperature manipulations, simulating natural conditions, are used to stimulate breeding and can advantageously accelerate spawning in brood stock maintained year round. Three common darter-spawning strategies: cave, plant and gravel, and aquarium set-ups facilitating these strategies as well as egg harvesting are described. Exceptions to these spawning strategies will also be given. Hatching times and feeding of fry are covered in detail. Aquarium setups for the spawning and rearing of native minnows are also discussed. This presentation is the culmination of eight years experience breeding native North American fishes, including 16 darter and 14 minnow species, some of which were maintained for several generations.

#### Legal Ramifications of Propagation and Reintroduction – A State's Perspective

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The mission of the Ohio Department of Natural Resources, Division of Wildlife is to conserve and improve fish and wildlife resources and their habitats while promoting

their use and appreciation by people. Consequently, the general public is an important component of our mission. The Division of Wildlife's Strategic Plan for 2001-2010 switched from an emphasis on single species management (ex. whitetail deer) to habitat management (ex. forest) which has benefited non-game species. Recently, more focus has been placed on the management of species that could potentially become endangered (a.k.a. Species of Greatest Conservation Need) through the State Wildlife Grant Program. But inevitably, certain species will become imperiled and need the protection of the Endangered Species Act (USFWS). Endangered species recovery can only be accomplished after the habitat and water quality are restored. Once this is accomplished, endangered species can either recover on their own or with outside help. This is where the dilemma begins because resource agencies need to be sensitive to private landowner rights while protecting wildlife resources and their habitat. Introduction of endangered species to locations where they already exist (augmentation) is an accepted practice because no additional restrictions to private land use will be enforced. Reintroduction of endangered species to a new location could potentially restrict private land use which complicates the issue. Consequently, each state has a different philosophy when it comes to endangered species reintroductions.

#### A Case Study of Propagation and Juvenile Mussel Releases in Virginia and Tennessee

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The Freshwater Mollusk Conservation Center (FMCC) at Virginia Tech has been conducting propagation of endangered mussel species since 1997. The facility has continued to expand its propagation and release efforts in recent years, such that a total of 1.28 million juveniles have been produced, and 495,000 of those have been released mostly into various tributaries in the Upper Tennessee River (UTR) system. From our first modest release of 72 juvenile tan riffleshells in the Hiwassee River, TN in 1997, we now average approximately 50,000-100,000 endangered juveniles per year. A total of 39 species have been produced and cultured, to include 25 federally listed species. In this paper, we report the efforts to augment or re-establish federally endangered species at three sites in the UTR. The 3 sites represent different streams and a varied set of environmental parameters which likely affected the success or failure of our releases. Indian Creek, tributary to the Clinch River in Tazewell County, VA has received juveniles of the tan riffleshell and purple bean for several years, and the jury is still out on whether these releases have survived to augment limited natural recruitment. Our releases of oystermussel juveniles at Horton Ford, Clinch River, TN have been successful in augmenting and re-establishing this species at that site. The

release of several endangered species at McDowell and Bales Fords, Powell River, TN has not resulted in the recovery of older juveniles and seems to have failed. One possible factor in survival of released juveniles may be their age and physiological condition at release. Early augmentation efforts consisted of releasing young juvenile mussels (1-2 wks), while recovered juveniles at Horton Ford came from the release of older, more robust juveniles 8 wks of age. The suite of environmental factors and anthropogenic impacts to these rivers and locations also play a significant role in each of these case studies. Chronic and episodic perturbations in Indian Creek and the Powell River are presumably responsible for contributing to water and substrate conditions unsuitable for survival of released juveniles.

### A Review Of Unionid Feeding And Nutrition

NICHOLS, JERRIE<sup>1</sup>, CATHERINE GATENBY<sup>2</sup> AND JULIE DEVERS<sup>2</sup>. <sup>1</sup>U.S. Geological Survey, 1451 Green Rd., Ann Arbor, MI 48105. <sup>2</sup>U.S. Fish and Wildlife Service, White Sulphur Springs National Fish Hatchery, 400 E. Main St., White Sulphur Springs, WV 24986. s\_jerrine\_nichols@usgs.gov

We will review available information on unionid feeding behavior and nutritional needs in both wild and captive individuals. A key component of captive care of any animal is providing a diet that will be eaten and that meets the nutritional needs of all life stages. Filter-feeding organisms have an additional dietary component regarding how food supplies are delivered. Unfortunately, diet development has proven to be a major bottleneck in the aquaculture of adult and juvenile unionids. Developing a suitable diet has been difficult for a number of reasons, including limited information on diets in wild individuals and how these diets change by season, reproductive state, or species. Recent studies on wild populations have yielded some information on adult unionid diets, but the data are still incomplete. Nor are there any data on juvenile diets in the wild. The use of algal species high in long-chain fatty acids has supported growth and survival of juveniles of many species under captive conditions, but adult mussels usually do not survive more than a year on such feed. Keeping adult mussels in captivity for longer than a year is still rare, even if they are kept in ponds and allowed to feed on a "natural assemblage" of foods. Raceway culture or use of natural river water has proven more successful in keeping adults. Ongoing studies on adult mussels are showing that adult survival rates can be improved by increasing the food ration, increasing lipid levels in the feed, and improving the food delivery system. Long-term survival rates of adult and juvenile mussels in captivity are increasing, but much work remains to be done.

## Propagation of Winged Mapleleaf (*Quadrula fragosa*) in Cages in the St. Croix River.

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Following the determination of host fish for the winged mapleleaf (Quadrula fragosa) in 2003, a propagation and augmentation plan was developed by a team of biologists working on this rare species. In the fall of 2004, two gravid females were collected from the St. Croix River and used to infest 100 channel catfish, which were held at Genoa National Fish Hatchery at St. Croix River temperatures. Because research indicates winged mapleleaf glochidia overwinter on host fish and drop off in the spring, infested fish must be held at winter river temperatures so that resulting juveniles are collected when river temperatures are warm enough for the juveniles to survive and grow. To determine if fish may be held in the river over the winter until juvenile transformation, 100 uninfested catfish were placed in cages in successful cage sites in the St. Croix and Mississippi Rivers. In May 2005, immediately prior to the expected dropoff period, the infested catfish were placed at two sites in six cages similar to those used for Higgin's eve (Lampsilis higginsii) propagation but modified such that catfish will not disturb juveniles that drop to the bottom of the cage. In October, these cages were examined for juvenile survival. The lower, more riverine site yielded no winged mapleleaf juveniles, but the first cage examined at the upper site yielded 11 juveniles. The remaining cages at the upper site were not disturbed. Should cage transformation and rearing be successful for this species, the resulting juveniles will become part of a long term propagation and reintroduction effort in the Upper Mississippi River basin as well as throughout the former range of the species, including Arkansas, Tennessee, and Missouri.

## Algae Culture: From Agar Slants to Photo-Bioreactors

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Successful algae culture requires a basic knowledge of microbiological techniques and specific knowledge regarding algal growth requirements for nutrients, light, temperature, and pH. Algae are not created equal, in that different species may have specific nutrient and growth requirements to achieve optimum production. Methods of

culturing algae depend on the amount of algae needed and the level of purity required. Where large amounts of algae are needed multiple levels/scales of production may be necessary starting with agar slant stock cultures, followed by culture in test tubes, flasks, carboys, vats, plastic bags, Kalwall tubes and large bioreactors. Care must be taken at all levels of production to insure algal purity but as the scale of production increases it becomes more difficult to maintain a sterile/uni-algal state. A common method of algae culture is the "batch culture" technique where algae are grown in a finite amount of nutrients and harvested after a certain density is reached. This method can be quite labor intensive depending on the amount of algae required. Other methods include continuous/semi-continuous culture where the algae are continuously/ periodically "fed" fresh nutrients at a particular rate and harvested at the same rate. Continuous/semi-continuous techniques have an advantage over batch methods in that a constant supply of algae are available, nutritional quality can be controlled and manipulated, while maintaining a steady state growth phase. Recent developments in commercially available photo-bioreactors that operate in continuous/semi-continuous mode, provide an efficient and highly productive means for growing algae. Advantages of such systems, over previously mentioned methods, include: increased light, nutrient and water use efficiency, fewer culture crashes, easier to maintain uni-algal cultures, and require less space and personnel to maintain. Success in growing algae, no matter what the scale, requires skilled interested individuals that have a primary focus only on algae production.

## Mussel Propagation Equipment Made from Ordinary Household Hardware

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Laboratory and hatchery production of freshwater mussels can be accomplished with inexpensive equipment constructed from ordinary household hardware. Host fish transformation chambers (for small host fish) can be constructed from two-liter soft drink containers, needlepoint square material, vinyl or Nitex screen, CPVC or PVC ball valves, cable ties and vinyl tubing. Sieves for capturing transformed juveniles are constructed of Nitex screen and PVC pipe and couplers cut down to size. Juvenile rearing containers are made from plastic shoeboxes fitted with Nitex screen covers, and small bulkhead fittings as overflows. Finally, brood-stock over-wintering containers can be constructed from various stainless steel or plastic containers fitted with hardware cloth wire covers painted with epoxy paint.

## Mussel Maladies: Diseases and Parasites

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Although their marine counterparts experience a wide spectrum of commercially significant parasites and diseases, few highly lethal maladies of freshwater mussels have been identified. While documentation exists of infestation by many symbionts, most have yet to be implicated as having a parasitic relationship with their host. Organisms known to cause lesions in freshwater mussels include trematodes, protozoans, copepods, mites and annelids. Often, mussels are intermediate hosts for the developmental stages of these parasites, such as the trematodes, serving as a source of energy and protection until the parasite emerges ready to infest its definitive host— usually fish or birds. Other parasites, including some trematodes, parasitize the mussel for their entire life cycle. Protozoan parasites are often found in association with the mantle, but some are known to be part of the mussel diet. Ophryoglena hemophaga is a ciliate protozoan that has been demonstrated to feed on the hemolymph within the digestive gland, and has been associated with loss of body condition. Evidence indicates that high intensity infections with this organism may be lethal. Mites of the family Unionicolidae may be parasitic or commensal, depending on the species of mite and its life cycle. Heavy mite infestation may cause shredding of the gills or even death. Parasites have been found in virtually all organs, including the digestive gland, gonad, kidney, mantle, foot, and gills, with varying effects on the mussel host. Complete castration, reduced body condition, and epithelial erosion are some of the reported results of parasitism in freshwater mussels. While not generally lethal, trematode infestation and its consequences can be deleterious to captive propagation efforts. A recent study of the tolerance of Quadrula pustulosa to treatment with the anthelmintic praziguantel has identified a dose of the drug that appears to be safe for freshwater mussels.



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