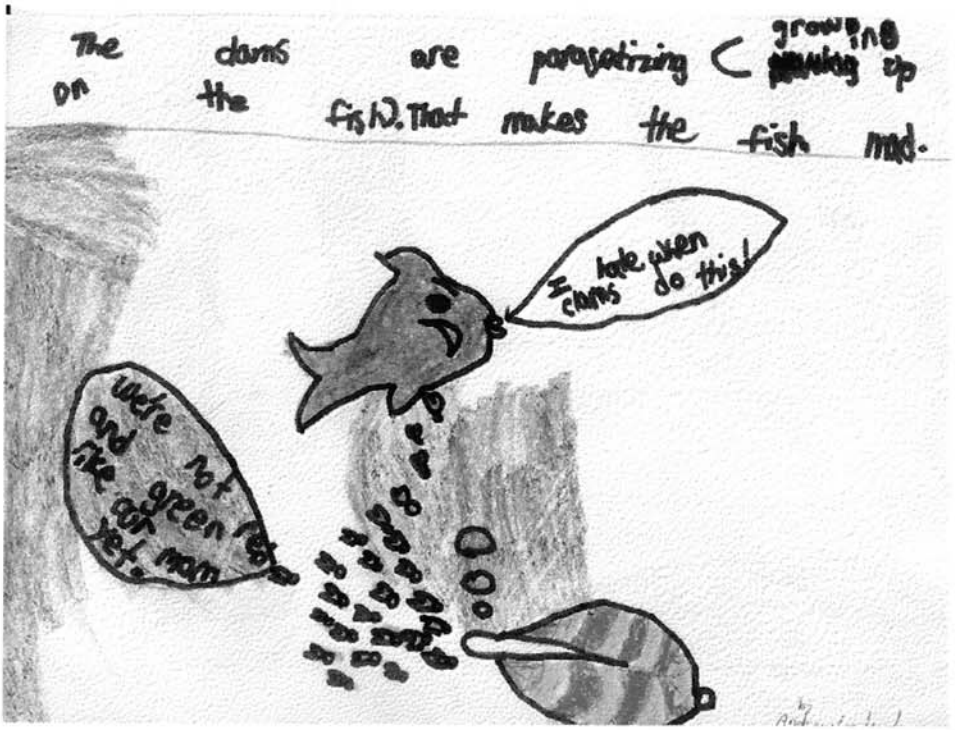
TRIANNUAL UNIONID REPORT

Report No. 14

March 1998

A forum for the informal exchange of information on the status of North American unionid research, management, and conservation



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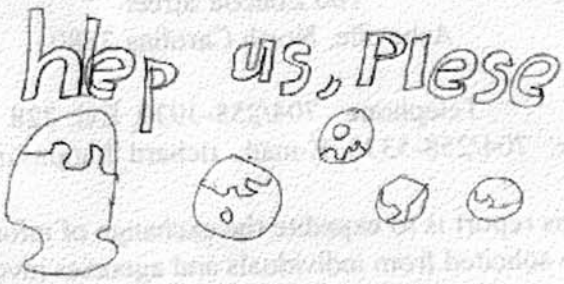
NOTE: The intent of this report is to expedite the exchange of information in an informal format. Report submissions were solicited from individuals and agencies involved in unionid conservation, copied as received, and assembled into this report. The submissions were **not** edited and were **not** peer reviewed.

FRONT COVER MUSSEL ART: THANKS TO ANDREW LYDEARD, TUSCALOOSA, ALABAMA.

INSIDE COVER MUSSEL ART: THANKS TO BECKY AND CALEB, 2ND GRADE, CANE CREEK ELEMENTARY SCHOOL, PUTNAM COUNTY, TENNESSEE.



TRIANNUAL UNIONID REPORT NO. 14
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TABLE OF CONTENTS

	Page
National Native Mussel Conservation Committee. -- Announcement of the 3rd National Freshwater mussel Symposium to be held March 17-19, 1999, Chattanooga, Tennessee	1
Watson, C. N., Jr. -- Lillard Mill mussel survey, 1997	2
Anderson, B. -- Possible impact of river otter (*Lutra canadensis*) on muskrat (*Ondatra zibethicus*) and mussel populations in selected Tennessee streams	3
Havlik, M. E. -- One year follow-up of a unionid mollusk translocation from an area with a moderate infestation of *Dreissena polymorpha* (Pallas, 1771), Mississippi River mile 725.8, T.H. 43 Bridge, Winona, MN/WI	4
Matcalfe-Smith, J. L., G. L. Mackie, S. K. Staton, and E. L. West. -- Current status of rare species of freshwater mussels in Southern Ontario	5
Hubbs, D. -- Commercial mussel regulations: Tennessee	6
Ahlstedt, S. -- Mussel poster produced depicting 48 native mussels of the upper Clinch River, Tennessee and Virginia	6
Watson, B. T., and R. J. Neves. -- Fish host identification for two federally endangered unionids in the upper Tennessee River drainage	7
Hove, M.C., and J. E. Kurth. -- Darters, sculpins, and sticklebacks serve as suitable hosts for *Venustaconcha ellipsiformis* glochidia	8
Watters, T. G. -- New publication: 1998. The trouble with zebras. *Timeline, Ohio Historical Society 15(2):32-41*	9
Watters, T. G., and S. H. O'Dee. -- New publication: 1998. Metamorphosis of freshwater mussel glochidia (Bilalvia: Unionidae) on anphibians and exotic fishes. *American Midland Naturalist* 139:49-57	9
Watters, T. G., and S. H. O'Dee. -- Potential host for the washboard, *Megalonaias nervosa* (Rafinesque, 1820)	9
Watters, T. G., and S. H. O'Dee. -- Continuing saga of *Anodonta woodiana*	10
Drees, L. -- Mussel speakers wanted	10
Dunn, H. L. -- Recent unionid studies by Ecological Specialists, Inc.	11

The following are abstracts of papers and posters given at the Conservation, Captive Care, and Propagation Freshwater Mussel Symposium held March 6-8, 1998, in Columbus, Ohio. The symposium was hosted by the Columbus Zoo; UMBC (An Honors University in Maryland); Ohio Biological Survey; and Ohio Department of Natural Resources, Division of Wildlife. Richard Tankersley and Doug Warmolts were Co-chairmen.

Araujo, R., S. Jimenez, and M. A. Ramos. -- Reproduction in captivity and glochidial development of *Margaritifera auricularia* (Spengler, 1793) in Spain	15
Badra, P., and M. M. White. -- Conservation of a unionid (*Lampsilis siliquoidae*)	16

Barnhart, M. C., and A. D. Roberts. -- The effect of temperature, CO_2 and pH on the transformation success of glochidia of fish hosts and *in vitro* 16
Berg. J. B., and S. I. Guttman. -- Genetic structure of unionid populations: Implications for captive propagation and reintroduction 16
Blalock, H. N., J. D. Williams, and D. N. Shelton. -- Historical and current distribution of the freshwater mussels (Unionidae and Margaritiferidae of the Escambia and Yellow River drainages of southern Alabama and Western Florida 17
Blodgett, K. D., J. A. Stoeckel, S. D. Whitney, and R. E. Sparks. -- A dynamic population model as a tool for evaluating mussel management strategies 17
Chen, L. Y., A. G. Heath, and R. J. Neves. -- Physiological effects of transportation in water and in air on freshwater mussels (Unionidae) 18
Christian, A. D., and D. J. Berg. -- Community and population characteristics of unionid assemblages in the headwaters of the Big Darby Creek basin, OH 18
Cordeiro, J. R. -- Distribution and habitat of Colorado freshwater mussels (Mollusca: Bivalvia: Unionidae) 19
Cummings, K. S., A. E. Bogan, S. A. Bruenderman, T. J. Frest, R. G. Howells, T. Muir, D. G. Smith, G. T. Watters, and J. D. Williams. -- North American freshwater mussels: Distribution, biology, and conservation 19
Dimock, R. V., Jr. -- Oxygen consumption by juvenile *Pyganodon cataracta* in declining Po_2 (Bivalvia: Unionidae) 20
Dimock, R. V., Jr., R. A. Tankersley, and M. Whitton. -- Effect of commercial algal preparation on growth and survival of juvenile *Pyganodon cataracta* and *Utterbackia imbecillis* (Bivalvia: Unionidae) 20
Ferris, J. L., C. D. Milam, and J. L. Harris. -- Relocation and subsequent evaluation of condition for adult mussel populations relocated to hatchery refugia 21
Gatenby, C. M., M. A. Patterson, B. C. Parker, and R. J. Neves. -- Filtration rates over 24 h for adult rainbow mussels (*Villosa iris*, Lea 1829) held in culture 21
Gatenby, C. M., M. A. Patterson, B. C. Parker, P. Morrison, and R. J. Neves. -- A protocol for the salvage and quarantine of mussels from zebra-infested waters ... 22
Hallac, D., and J. E. Marsden. -- Differences in tolerance to and recovery from zebra mussel (*Dreissena polymorpha*) fouling by *Elliptio complanta* and *Lampsilis radiata* 22
Havlik, M. E. -- External aging of unionids revisited: Height versus age of 4800 *Megalonaias nervosa* (Rafinesque 1820), Mississippi River reaches (Pools) 9-19, Lansing, IA-Fort Madison, IA, 1 July-14 September 1997 23
Hoggarth, M. A., D. L. Rice, and T. L. Grove. -- The correlation of mussels with fish in the upper Blanchard River in Hardin and Handcock Counties, Ohio, with special regard to the rayed beam (*Villosa fabalis*) 23
Hove, M. C., K. R. Hillegass, J. E. Kurth, V. E. Pepi, C. J. Lee, P. A. Mahoney, A. R. Kapuscinski, and M. Bomier. -- Considerations for conducting host suitability studies 24
Howells, R. G. -- Reproductive seasonality of freshwater mussels (family Unionidae) in Texas 24
Hubbs, D. -- Practical propagation of freshwater mussels for shell harvest 24

Keller, A. E., N. J. Kernagham, and L. Straub. -- Seasonal changes in respiration, cholinesterase activity and glycogen stores in three species of unionid mussels ... 25
Kenyon, R., K. Welke, P. Thiel, T. Naimo, and E. Monroe. -- Survival and growth of unionids after relocation into an artificial pond ... 25
Khym, J. R., J. B. Layzer, and J. M. Redding. -- Efficacy of cortisol administration to induce glochidial metamorphosis ... 26
King, T. L., M. S. Eackles, R. F. Villella, and B. Gjetvag. -- Discontinuity in the genetic population structure of freshwater mussels: Conservation implications ... 26
King, T. L., R. F. Villella, M. E. Smith, and P. I. Washington. -- Conservation of freshwater mussel species: A tissue repository for genetic and systematics ... 27
Kloeck, R. -- 1997 unionid surveys in the Fox River and Springbrook Creek, Illinois ... 27
Lellis, W. A., and C. S. Johnson. -- Experiences with captive maintenance of Atlantic Slope unionids in small research systems ... 27
McClane, M. B. -- Mississippi River precision brailing: Utilizing-GPS, GIS and the Internet ... 28
Milam, C. D., J. L. Ferris, M. L. Barfield, J. Van Hassel, and L. A. Locum. -- Reintroduction of native freshwater mussels into a recovering stream using both *in viro* and *in vitro* propagation techniques ... 28
Monroe, E. M., and T. J. Naimo. -- Effects of relocation on physiological condition of *Amblema plicata plicata* ... 29
Myers-Kinzie, M., and A. Spacie. -- Non-destructive glochidial harvest and artificial transformation of *Lampsilis siliquoidea*: A suitable species for glochidial and juvenile research ... 29
Nedeau, E. J., M. Kaufman, and R. W. Merritt. -- Digging deeper to solve the missing mussel mystery ... 30
Neves, R. J., F. X. O'Beirn, B. B. Beaty, and M. B. Steg. -- The survival and growth of freshwater mussels in a recirculating aquaculture system ... 30
Nichols, S. J., and D. Garling. -- Food web dynamics of Unionidae in a canopied river and a non-canopied lake ... 30
Nichols, S. J., G. Black, and J. Allen. -- Feasibility of using microhabitat selection to provide *in situ* protection of unionid populations from the impact of zebra mussels ... 31
O'Brien, C. A. -- Reproductive biology for four mussel species of the Gulf Coastal Plain ... 31
O'Dee, S. H., and G. T. Watters. -- New and confirmed host identification for ten freshwater mollusks ... 32
Parker, B. C., M. A. Patterson, and R. J. Neves. -- Feeding interactions between native freshwater mussels (Bivalvia: Unionidae) and zebra mussels (*Dreissena polymorpha)* in the Ohio River ... 32
Patterson, M. A., C. M. Gatenby, B. C. Parker, and R. J. Neves. -- Ingestion and assimilation of ^{14}C labeled algae by the freshwater mussel, *Villosa iris* (Lea, 1829) ... 33
Patterson, M. A., B. C. Parker, and R. J. Neves. -- Glycogen levels of unionids during starvation and controlled feeding in quarantine ... 33

Quinn, R. D., and J. B. Layzer. -- Extreme variation in survival rates of mussels during a 30-day quarantine period 34
Riusech, F. A., and M. C. Barnhart. -- Host suitability differences among *Venustaconcha ellipsiformis* (Bivalvia: Unionidae) from different river drainages 34
Ruessler, D. S., and A. E. Keller. -- Survival of juvenile unionid mussels cultured under several food and water regimes 34
Schwartz, M. L., and R. V. Dimock, Jr. -- Ultrastructural analysis of changes in the marsupial demibranch during brooding of glochidia in *Pyganodon cataracta* and *Utterbackia imbecillis* (Bivalvia: Unionidae) 35
Schwegman, J. E. -- Lure behavior in *Toxolasma texasensis* 35
Seal, U. S. -- Conservation of threatened mussel species as a multiple stakeholder process 36
Shadoan, M. K., and R. V. Dimock, Jr. -- Differential sensitivity of hooked and hookless glochidia to chemical and mechanical stimuli 36
Smith, D. G. -- Investigations of the byssal gland in juvenile unionids 36
Starkey, R. W., A. G. Eversole, T. E. Schwedler, D. E. Brune, G. Schwartz, and J. A. Collier. -- Growth and survival of juvenile and adult freshwater mussels in the partitioned aquaculture system 37
Starliper, C. E., R. Villella, P. Morrison, and J. Mathias. -- Preliminary studies on the potential bacterial pathogen contagion between freshwater mussels and salmonid fish 37
Tankersley, R. A. -- Fluorescence techniques for evaluating the lipid content of larval and juvenile mussels 38
Tankersley, R. A., and S. Butz. -- Design, construction, and evaluation of a laboratory-scale recirculating aquaculture system for the captive care of freshwater mussels 38
Tankersley, R. A., M. G. Wieber, K. Kachurak, and S. Butz. -- Use of condition indices, protein biomarkers, and RNA:DNA ratios for detecting nutritional stress in freshwater unionid mussels 39
Villella, R. F., W. J. Bartles, and D. A. Weller. -- Freshwater mussel (*Elliptio complanta*) movement and condition with relation to temperature, flow, and substrate type ... 39
Waller, D., M. Bartsch, S. Jennings, H. L. Dunn, W. G. Cope, and R. Rada. -- Conservation of unionid mussels in the St. Croix River: Development *in situ* refugia 40
Watters, G. T., and S. H. O'Dee. -- Glochidial release as a function of water temperature 40
Welke, K., R. Kenyon, T. Naimo, E. Monroe, and P. Thiel. -- Logistic considerations in the relocation of unionids into artificial refugia 41
Westbrook, O. J., and J. B. Layzer. -- Survival and growth of hatchery-reared juvenile *Lampsilis cardium* 41
Wicklow, B. J., and P. M. Beisheim. -- Life history studies of the squawfoot mussel *Strophitus undulatus* in the Piscataquog River watershed, New Hampshire 42

Tennessee Aquarium

Southeast Aquatic Research Institute

National Native Mussel Conservation Committee

Announces the
3rd National Freshwater Mussel Symposium
"Musseling in on Biodiversity"
hosted by
The Southeast Aquatic Research Institute
and
The Tennessee Aquarium
March 17th - 19th, 1999
at the
Clarion Hotel
407 Chestnut Street
Chattanooga, Tennessee

The National Native Mussel Conservation Committee (NNMCC) announces the 3rd National Freshwater Mussel Symposium will be held on March 17-19, in Chattanooga Tennessee. The meeting will be similar in format to the Conservation and Management of Freshwater Mussel meetings held in St. Louis, Missouri in 1992 and 1995. The theme of this symposium will be freshwater mussel biodiversity. Like the earlier meetings, this symposium will focus on status surveys, life histories, habitat requirements, biology, techniques, etc. in both oral and poster presentation formats. In addition to the freshwater mussel presentations, there will be a special session dealing with the conservation of freshwater gastropods. As with the previous meetings, a proceedings will be published. A call for papers will follow in the fall of 1998. Scientists, researchers, conservation agency personnel, federal and state biologists with an interest in freshwater mollusks are encouraged to participate. Current meeting information will soon be available on the Southeast Aquatic Research Institute web site at http://www.sari.org, or call Paul Johnson at (423) 785-4074.

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Lillard Mill Mussel Survey, 1997

In August 1997 Aquatic Resources Center performed a mussel survey at Lillard Mill on the Duck River for TWRA. The purpose of this survey was to determine the density, distribution and population demography of the unionid fauna with emphasis on the endangered species *Lemiox rimosus*, *Epioblasma capsaeformis* and *Quadrula intermedia*. Lillard Mill is of particular interest because it harbors one of the last sizable populations of *L. rimosus*. Previous surveys were made in 1993, 1994 and 1995.

Sampling occurred along 24 transects, 12.5 m apart. Ten quadrats were randomly selected on each transect. All mussels in a transect were enumerated and identified. *Lemiox rimosus*, *E. capsaeformis* and *Q. intermedia* were measured, sexed and aged.

A total of 23 species and 965 individuals was found. Mean density was 16.1/m^2. These figures compared favorably with those from previous surveys (21 - 25 species, 17.7 - 15.1/m^2). The five most abundant species in 1997 were *Cyclonais tuberculata* - 253 (26.2%), *Elliptio dilatata* - 131 (13.5%), *Lemiox rimosus* - 93 (9.6%), *Truncilla truncata* 88 (9.1%) and *Quadrula pustulosa* - 76 (7.8%). *Cyclonais tuberculata* ranked first in all previous surveys. *Elliptio dilatata* ranked seventh in 1993 and has steadily increased in rank. *Lemiox rimosus* has fluctuated in rank (1993 - 5th, 1994 - 3rd, 1995 - 6th). *Truncilla truncata* declined, having consistently ranked second in all previous surveys. *Quadrula pustulosa* also declined in rank, having been fourth in previous surveys.

Lemiox. rimosus ranged in age from 2 - 15 years. There were 59 males, 23 females and 11 juveniles. At least one individual was found on every transect. *Epioblasma capsaeformis* ranged in age from 3 - 10+ years. There were 7 males, 9 females and 2 juveniles. *Epioblasma* numbers have been increasing (1993 - 0, 1994 - 5, 1996 - 11) but remain low. Only two *Quadrula intermedia* were found. One was 10+ years old, the other was too eroded to age. No attempt was made to sex them. Previously, one *Q. intermedia* was found in 1995, and none in 1993 and 1994.

Few muskrat middens were found in 1997. This contrasted with 1995, when several large middens were found at the site. Sixteen *L. rimosus* and four *E. capsaeformis* were found in middens in 1997. In 1995, 638 *L. rimosus* and 8 *E. capsaeformis* were recovered from middens.

The mussel population at Lillard Mill appears to be healthy. Many species, including the endangered *L. rimosus* and *E. capsaeformis* are reproducing, as evidenced by the presence of juveniles and gravid females. Runoff from the access road and parking area is a potential threat. A large silt plume was observed following a heavy rain during sampling. We recommend that steps be taken to minimize runoff at Lillard Mill.

Possible impact of river otter (Lutra canadensis) on muskrat (Ondatra zibethicus) and mussel populations in selected Tennessee streams

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The river otter (Lutra canadensis), once endemic statewide in Tennessee, was largely extirpated over much of the state in the early 1900's (Toweill and Tabor, 1982). Muskrats (Ondatra zibethicus) remained indigenous to most, if not all streams in the state and were known to feed on resident mussels where those were available. Muskrats were considered a normal part of the ecosystem and were not considered to have an unusually detrimental impact on mussel populations. However, evidence of significant changes in muskrat densities resulting from otter restoration may indicate a need to examine the otter/muskrat/mussel interrelationship more closely.

Reintroduction of the river otter in Tennessee began in 1984 with the release of six otters into the Obed Wild and Scenic River. At the time of otter release, the Obed sustained an abundant muskrat population. Muskrat "middens," composed mainly of mussel shells, were common on the river. By 1986, Dean Bryson, a local trapper who trapped along the Obed, reported a decline in muskrat numbers there. Subsequent observations revealed a steady and ultimately, enormous decline in the occurrence of muskrat sign, primarily middens, on the Obed. Similar observations have been made on other streams, including the Big South Fork of the Cumberland River and the Hiwassee River, after otter reintroduction (Steve Bakaletz, US Park Service, Big S. Fork NRRA; Jim Herrig, US Forest Service, Cherokee National Forest).

While virtually all research encompassing food habits of river otter suggests that small mammals constitute only a small percentage of otter diet (Toweill and Tabor, 1982), no research assessing the impact of otter presence on muskrat densities can be found. Some otter food analysis was conducted using scats from otter released on the Obed (Griess and Anderson, 1987), and the Great Smokey Mountains National Park (Griess, 1987; Miller, 1992). No muskrat remains were found in any scat collected. However, these samples were small and were undertaken as an incidental addition to the primary research and were not designed to assess impact of otters on muskrats.

Analysis specifically aimed at assessing otter impact on the muskrat populations in the above streams is now impossible since prerelease muskrat population levels cannot be determined. However, research to determine such impact would be useful given the field observations noted above. Otter extirpation may have resulted in an abnormal rise in muskrat populations, possibly adversely affecting resident mussels. Conversely, reintroduction of river otter may serve as a significant natural control of muskrat populations, thereby enhancing survival of mussels.

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ONE YEAR FOLLOW-UP OF A UNIONID MOLLUSK TRANSLOCATION FROM AN AREA WITH A MODERATE INFESTATION OF Dreissena polymorpha (Pallas, 1771), MISSISSIPPI RIVER MILE 725.8, T.H. 43 BRIDGE, WINONA, MN/WI.

A mussel translocation from this site, September 1996, yielded 6199 unionids (23 species, mean density 1.88/m^2) in a primarily sand substrata. No federally listed unionids were found but 2% represented seven Minnesota and Wisconsin endangered, threatened and special status species. Most of 80 Obovaria olivaria (Rafinesque 1820) were juveniles. Ellipsaria lineolata (Rafinesque 1820) (1), Pleurobema sintoxia (Rafinesque 1820) (4), Ligumia recta (Lamarck 1819) (12), and Utterbackia imbecillis (Say 1829) (11), were also represented by juveniles. Other special status species were Arcidens confragosus (Say 1829) (2), and Megalonaias nervosa (Rafinesque 1820) (2). On 18 September 1997 quantitative and random sampling at the translocation site showed an overall survival of 93.77% of the translocated unionids. Spring 1997 high water apparently caused changes in the river since 5-7.5 cm of sand had accumulated over much of the sand, gravel, and mud substrata we found at the translocation site in 1996. Most of the living unionids were nearly buried in packed sand. The mean density of hash-marked common unionids from 32-0.25 quadrats was 4.25/m^2 and included 11 of the 23 translocated species. The density of unmarked unionids was 17.25/m^2. Besides marked unionids in quadrats, and 38 (of 112) numbered and 185 hash-marked unionids were found during random searches for a 1997 total of 241 live and 16 dead marked unionids (N = 257) from the translocation site. Twelve unmarked special status unionids were among 600 unmarked unionids identified during follow-up; included were Arcidens confragosus (1), Pleurobema sintoxia (5), and Obovaria olivaria (6). Of the 38 numbered unionids, two were dead for a survival of 94.74% of the special status species. Both at this translocation site, and at a similar translocation site near La Crosse, WI, the species that seems to be most affected by translocation is Ligumia recta. We have the impression that this species might do better if it were just placed on the substrata, rather than being planted, since there was no other obvious cause of the mortality rate seen for that species (2 of 4 numbered specimens were dead at this site). Hove (1997) reported seeing L. recta lying on the substrate. Many special status unionids, particularly young Obovaria olivaria (Rafinesque 1820), clearly showed growth, both after the 1996 translocation (evidenced by a false rest or interruption ring) and again in 1997 with another 5 mm of growth after the usual winter rest ring. There is good recruitment and growth of a number of species at the translocation site in spite of obvious impacts from spring high water. Only small numbers of Dreissena polymorpha were found. This exotic species does not appear to have seriously impacted this translocation site, possibly because of the predominantly sand substrata and the number of juvenile unionids involved in this project.

Current Status of Rare Species of Freshwater Mussels in Southern Ontario

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Nearly half of the 40 species of freshwater mussels native to the Canadian waters of the lower Great Lakes drainage basin are presently ranked as very rare (S2), extremely rare (S1) or known from historical records only (SH) by the Natural Heritage Information Centre in Peterborough, Ontario. In this study, we determined the current conservation status of 21 species believed to be at risk in Ontario. Most of these species have severely declined in the Great Lakes themselves due to the impact of the zebra mussel (*Dreissena polymorpha*). The last refugia for many of Canada's native mussel species are the Grand, Thames and Sydenham Rivers in southwestern Ontario. Thirty-seven sites on these rivers that historically supported the target species were intensively surveyed during the summer of 1997 to determine the true status of these species. Current data on species distributions from this and other recent (post-1990) surveys on these rivers were combined and compared with the historical data to determine if there have been changes over time. The major findings of this study are as follows:

We successfully located more living species on all three rivers than other recent surveyors, due to our greater sampling effort. However, we still observed species losses: 27%, 41% and 24% of the species known from the Grand, Thames and Sydenham Rivers, respectively, according to historical records dating back to the late 1800s, were not found alive in 1997. Although 30 of the 36 species previously known from the study area were found alive, 13 of these species now occur in fewer rivers than they did in the past, i.e., their ranges have been reduced. The Sydenham River still supports the richest and most productive mussel community of any small river in Canada, with 25 living species, an average diversity of 13 species/site, and an average abundance of over 150 individual mussels/site based on a sampling effort of 4.5 person-hours. In 1992, Arthur H. Clarke urged "...that the Sydenham River be made an ecological preserve and that its fauna be protected by legislation." To this, we would add that time is of the essence.

The conservation status of 21 species of freshwater mussels was assessed by comparing the current (post-1990) distribution of each species with its historical distribution. On the basis of these comparisons, changes to the official conservation status ranks (Ontario's SRANKS) of 11 species were proposed. Three species currently ranked SH were found alive and could therefore be downlisted to S1 (*Epioblasma torulosa rangiana, Obliquaria reflexa* and *Toxolasma parvus*). As the persistence of *Simpsonaias ambigua* is highly likely due to the presence of fresh shells at many sites, this species was also tentatively downlisted to S1. Five species appear to have declined significantly (*Fusconaia flava, Ligumia nasuta, Pleurobema coccineum, Truncilla donaciformis* and *Villosa iris*) and are therefore recommended for uplisting. Two other species (*Cyclonaias tuberculata* and *Truncilla truncata*) were more common than expected, and could be downlisted. We recommended that 11 species with current or proposed ranks of S1 be given first consideration for national status designation by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), as measures will have to be taken soon to prevent their extirpation. These species are: *E. t. rangiana, O. reflexa, S. ambigua, T. parvus, Lampsilis fasciola, Obovaria subrotunda, Ptychobranchus fasciolaris, Utterbackia imbecillis, Villosa fabalis, T. donaciformis* and *P. coccineum*. The authors have been commissioned by COSEWIC and COSSARO (Committee on the Status of Species at Risk in Ontario) to prepare status reports on three of these species (*Epioblasma torulosa rangiana, Lampsilis fasciola* and *Villosa fabalis*). During this study, a conservation status score system was devised for identifying and prioritizing areas of prime mussel habitat that should be protected. This system may be a useful tool for agencies that are responsible for managing the water and habitat quality of Ontario's rivers. This research was partly funded by the Endangered Species Recovery Fund.

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TWRA fish division personnel in conjunction with the mussel advisory board have conducted regular public meetings for the past four years. These meetings have been a forum for the exchange of information between TWRA and the mussel industry. Since March of 1996, much of the discussion has centered around the unification of mussel shell size limits for the major shell producing states of Alabama, Arkansas, Louisiana, Kentucky, Tennessee and Texas. Because of the illegal trafficking of undersized shells from other states into Tennessee, parity of shell size limits was defined as the number one priority at a meeting held in Nashville in March 1996. This meeting was hosted by TWRA, with 13 states and four shell companies attending. Tennessee is the only state that has yet to pass a proposal that conforms to the established minimum shell size limits. At the June 1997 mussel advisory board meeting, TWRA informed the mussel industry of its intention to propose an increase in shell size limits. Much of the in suing comments dealt with the economic impact this would have on the shell harvester. Finally, a proposal was put forth by a shell diver to increase the size limits one-sixteenth inch per year to reach the proposed minimum size limits. Since the June meeting, TWRA has met Twice in Bowling Green, KY with Kentucky Department of Fish & Wildlife and the shell industry. A joint proposal has been developed between the two states to reach the new minimum size limits and already passed by Kentucky. The size increase schedule is as follows: Washboard Mussel (currently 3 3/4") - 3 13/16" for 1998, 3 14/16" 1999, 3 15/16" 2000, and 4" in 2001and thereafter, Lake Shells (Threeridge, mapleleaf, pigtoe etc. currently 2 5/8") - 2 11/16" for 1998, and 2 3/4" in 2000 and thereafter, ebony and monkeyface shells will remain at 2 3/8"; threehorns will become legal at 1 3/4".

This proposal was presented to the Tennessee Wildlife Resources Commission (TWRC) during its January meeting at Reelfoot Lake. After more than one hour of public comment, the TWRC deferred voting on the proposal until its next meeting to be held at the Legislative Plaza in Nashville, February 25th and 26th. The vote was deferred at the request of 74th district State Representative Mr. John Tidwell. Representative Tidwell requested the deferral so that he could further study the need for this proposal. TWRA's Fish Division met with Representative Tidwell, explained in detail the necessity of this regulation. After this meeting, Mr. Tidwell stated that "he thought the size limits should remain the same....but it would be good if the proposal passed."

The proposal was presented for the second time at the February TWRC meeting in Nashville, and subsequently deferred for one year.

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In collaboration with Dr. Paul Parmalee, McClung Museum, University of Tennessee, we are in the final stages of a color poster on the mussels of the upper Clinch River, Tennessee and Virginia. The poster will feature 48 native mussels (7 showing sexual dimorphism) and the Asian clam. Both common and scientific names and federal status are included along with a paragraph concerning mussel conservation. The poster was funded by the U. S. Fish and Wildlife Service (Regions 4 and 5), The Nature Conservancy (Virginia and Tennessee Chapters), Virginia Department of Game and Inland Fisheries, Virginia Department of Conservation and Recreation, Tennessee Wildlife Resources Agency, and Tennessee Valley Authority. Posters will be distributed throughout the upper Clinch River to schools, businesses, 4-H clubs, city community buildings, and local, state, and federal agencies.

Fish Host Identification for Two Federally Endangered Unionids in the Upper Tennessee River Drainage

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Fish hosts were identified for the federally endangered dromedary pearlymussel (*Dromus dromas*) and birdwing pearlymussel (*Lemiox rimosus*) during 1997. A total of 31 fish species were tested as hosts for these mussels. The only host fish identified for the dromedary pearlymussel was the fantail darter. Thirty-four juveniles were collected from four infested fantail darters over a period of 18-25 days (average water temperature was 23.0°C). Examination of the gills of eight infested fantail darters that died, before transformation could occur, revealed the presence of approximately 265 encysted glochidia. Four additional percids also were identified as potential host fish; tangerine darter, gilt darter, banded darter, and logperch. In addition, the snubnose darter was identified as the second percid to serve as a host for the birdwing pearlymussel, with the greenside darter implicated as a host. Twelve juveniles were collected from two infested snubnose darters over a period of 20-32 days (average water temperature was 23.0°C). Five encysted glochidia were present on these two snubnose darters after they were sacrificed at 38 days post-infestation, indicating that the transformation period may be longer. The following table summarizes the results for all the fish species tested.

FISH SPECIES	*Dromus dromas*	*Lemiox rimosus*
Campostoma anomalum	NSH	X
Catostomus commersoni	NSH	X
Cottus carolinae	NSH	X
Cyprinella galactura	NSH(?)	X
Cyprinella spiloptera	NSH(?)	X
Erimystax dissimilis	NSH(?)	X
Erimystax insignis	NSH(?)	X
Etheostoma blennioides	NSH	POTENTIAL
Etheostoma camurum	NSH(?)	X
Etheostoma flabellare	HOST	NSH
Etheostoma rufilineatum	NSH	NSH
Etheostoma simoterum	NSH	HOST
Etheostoma stigmaeum	NSH(?)	X
Etheostoma vulneratum	NSH	NSH(?)
Etheostoma zonale	POTENTIAL	X
Hybopsis amblops	NSH(?)	X
Hypentelium nigricans	NSH(?)	X
Luxilus chrysocephalus	NSH(?)	X
Luxilus coccogenis	NSH(?)	X
Nocomis micropogon	NSH	X
Notropis leuciodus	NSH(?)	X
Notropis rubellus	NSH(?)	X
Notropis sp.	NSH(?)	X
Notropis telescopus	NSH(?)	X
Noturus insignis	NSH	X
Percina aurantiaca	POTENTIAL	X
Pecina caprodes	POTENTIAL	X
Percina evides	POTENTIAL	NSH
Phenacobius uranops	NSH(?)	X
Pimephales notatus	NSH	X
Rhinichthys atratulus	NSH(?)	X

NSH = Non-Suitable Host; NSH(?) = Non-Suitable Host, although fish died before transformation;
HOST = Successful host; POTENTIAL = Fish died before transformation but glochidia still on gills;
X = Fish species not infested for that particular mussel species

Darters, sculpins, and sticklebacks serve as suitable hosts for *Venustaconcha ellipsiformis* glochidia

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Laboratory transformation studies have shown slimy sculpins and Johnny darters will facilitate metamorphosis of *V. ellipsiformis* glochidia (Hove and Anderson 1997).

Metamorphosis of *V. ellipsiformis* glochidia was observed on six of eight fish species tested (Tables 1 and 2) during this fall and winter. Between 40-80 juveniles were collected from aquaria holding brook sticklebacks, Iowa darters, fantail darters, and mottled sculpin. Only three juveniles were collected from logperch and three from blackside darters.

Table 1. Trials where *V. ellipsiformis* transformation observed.

Species	Number tested	Juvenile collection period (d)
b. stickleback	8	18-37
Iowa darter	8	18-30
fantail darter	8	18-35
blackside darter	4	30-34
logperch II	3	25-32
mottled sculpin I	4	19-36
mottled sculpin II	4	19-28

Average water temperature was 21 ± 2 °C

Table 2. *V. ellipsiformis* transformation not observed.

Species	Number tested	Encystment period (d)
river darter	1	21-23
logperch I	8	18
logperch III	8	8-11
bluntnose minnow	8	5

Gravid females were collected from the Zumbro River, Minnesota in early November 1997. Mantle waving by gravid females in the laboratory observed in previous years (Hove and Anderson 1997) was not observed in four females held this year.

Funding and support for this study was provided by the Minnesota Department of Natural Resources - Natural Heritage & Nongame Research Program, Legislative Commission on Minnesota Resources, University of Minnesota - Undergraduate Research Opportunities Program, and Bell Museum of Natural History.

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Hove, M. C., and T. W. Anderson. 1997. Mantle-waving behavior and suitable fish hosts of the ellipse. Triannual Unionid Report. Report No. 11, p. 3.

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New publications

Watters, G.T. 1998. The trouble with zebras. *Timeline, Ohio Historical Society* 15(2): 32-41.

A popular article on zebra mussels. Neat photography by Gary Meszaros.

Watters, G.T. & S.H. O'Dee. 1998. Metamorphosis of freshwater mussel glochidia (Bivalvia: Unionidae) on amphibians and exotic fishes. *American Midland Naturalist* 139: 49-57.

Abstract. - This study determined that inexpensive and easily maintained amphibians and exotic fishes could act as hosts for two species of native North American unionid mussels, and bypass the need to identify native hosts when the object is to culture mussels. Two mussel species, *Lampsilis cardium* and *Utterbackia imbecillis*, were used to parasitize 42 exotic fishes and seven potential nonpiscine hosts. Nonpiscine hosts included amphibians and decapod crustaceans. *Lampsilis cardium* successfully metamorphosed on six species of exotic fishes, as well as on larval tiger salamanders. *Utterbackia imbecillis* successfully metamorphosed on 30 species of exotic fishes and all four amphibian species tested. No glochidia metamorphosed on crustaceans. Successful metamorphosis on amphibians indicates that mussel zoogeography may be more complicated than previously thought. Using surrogate hosts may be a valuable alternative to natural hosts in laboratory culture of mussels.

Potential hosts for the Washboard, *Megalonaias nervosa* (Rafinesque, 1820)

Laboratory transformations occurred on the following species:

Largemouth Bass	Longear Sunfish	Bluegill
Longnose Gar	Yellow Perch	

And to a much lesser extent on:

Logperch	Slenderhead Darter	Central Stoneroller

The following fishes did not act as hosts:

Banded Darter	Variegate Darter	Bluntnose Minnow
Suckermouth Minnow	Striped Shiner	Rosyface Shiner
Sand Shiner	Spotfin Shiner	

The continuing saga of *Anodonta woodiana*

In my article on this species in *Veliger* 40: 152-156, I mentioned a report by Ng et al. (1993) that stated that *Pseudodon vomdembuschianus* (Lea, 1840) had been introduced to Singapore on exotic fishes. Dr. Chow Sow-Yan of Singapore kindly supplied me with specimens from the Lower Seletar Reservoir. As he suspected, these actually are *Anodonta woodiana*. They were introduced on carp and/or tilapia from hatcheries in Taiwan.

Ms. Stephanie Clark of New South Wales, Australia, recently sent specimens of a unionid from Laguna de Bay, Luzon Island, Philippines, collected in 1992. Laguna de Bay is the largest freshwater lake in the Philippines. The Philippines have no native unionids, but they now have *Anodonta woodiana*. The specimens were found at or adjacent to the Bureau of Fisheries at Tanay, Rizal Province.

Mussel Speakers Wanted

The National Native Mussel Strategy Outreach Committee is developing a volunteer "Native Mussel Speakers Bureau". Volunteers with a desire to share their expertise on all aspects of native mussels including the national mussel conservation effort are sought. The speakers list would be made available to state and federal agencies, conservation and sport organizations, schools, and museums. Unfortunately, funding is not available at this time to pay for travel expenses. The joy of saving mussels must be our compensation. If you are interested in participating on the National Native Mussel Conservation Volunteer Speakers Bureau please send your name and topic of interest to Linda Drees, 315 Houston St. St. E. Manhattan, KS. 66502, 785-539-3474X20 Fax 785-539-8567. Linda_Drees@fws.gov

Name_____

Address _____

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Phone_____

E-Mail_____

FAX_____

Topic_____

Geographic Scope You Can Lecture Within_____

Recent unionid studies by Ecological Specialists, Inc.

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St. Croix River I-94 Unionid Relocations,

ESI relocated over 8,500 unionids of 30 species from I-94 construction areas and over 14,000 unionids of 28 species from bridge demolition areas of the St. Croix River near Hudson, Wisconsin in 1994 and 1995, respectively. Federal endangered species (*Lampsilis higginsi*) and Wisconsin threatened (*Quadrula metanevra* and *Tritogonia verrucosa*) and endangered (*Cumberlandia monodonta*, *Cyclonaias tuberculata*, *Ellipsaria lineolata*, and *Elliptio c. crassidens*) species were marked by etching shells with a unique number and placed in grids for future monitoring. The remaining animals were marked and placed in an existing unionid bed upstream of the bridge. Animals relocated in 1994 were monitored in October 1994, August 1995, and June 1996 and animals relocated in 1995 were monitored in October 1995, June 1996, and June 1997. Recovery was over 70% and mortality was less than 10% after one year in both relocations.

St. Croix River Stillwater Bridge Unionid Relocation,

ESI relocated over 18,100 unionids of 25 species from the proposed bridge area; including nine individuals of Federal or Wisconsin protected species. Animals were relocated to areas with similar habitat and unionid communities. Most animals were distributed in a 6,000m2 area. Experimental grids were established to monitor effects of relocation on three common species, as well as protected species. Over 88% of common species and 60% of protected species were recovered, observed mortality was <3%, and most animals had grown one year following relocation (1997). Relocated and resident animals also will be monitored in 1998 to assess effects of relocation on recovery, mortality, and growth, and assess effects of increased density on the resident unionid community.

St. Croix River Unionid Refugia Relocation,

Large midwestern rivers once supported diverse unionid communities. However, many unionid species are now on the brink of extinction due to pollution, habitat modification, and zebra mussel infestation. The recent invasion of the zebra mussel is currently the most serious threat to unionid communities in many of our large midwestern rivers. Relocating unionids into hatcheries or smaller rivers, which may not be invaded by zebra mussels, is considered a possible means of saving endangered species. In 1996, 100 *Lampsilis higginsi* (Federally endangered) and 100 each of two common species (*Elliptio dilatata* and *Quadrula pustulosa*) were relocated from the lower St. Croix River to an area approximately 50 miles upstream. Flow, depth, substrate, and unionid communities vary between the areas. Unionids in both collection and relocation areas will be monitored for at least two years to determine if large river species can survive in a smaller river environment and whether an "in river" refugia is a viable alternative to maintaining unionids in artificial systems. High survival, growth, and evidence of reproduction were observed one year after relocation.

Wolf River Highway 29 Bridge Unionid Relocation,

Wisconsin Department of Transportation plans on building a bypass for Highway 29 around the city of Shawano. The bypass will cross the Wolf River in a section known to contain *Epioblasma triquetra*, endangered in Wisconsin. In July 1995, over 27,000 unionids were collected from construction impact areas and relocated to an area upstream. Methods used for relocation and monitoring were similar to those used in the St. Croix River. No mortality of *E. triquetra* and minimal mortality of other species was observed one month following the relocation. Recovery after one year was near 80% and mortality was less than 5%. Monitoring will continue through 1997.

Relocated Unionid Monitoring, Ohio River Mile 418.9,

In 1987 over 5000 unionids of 22 species were relocated from an area of potential impact on the Ohio River near Cincinnati, Ohio. Unionids were tagged, measured, weighed, identified and relocated to a recorded location. The relocated community was monitored for four years. Only 35% of the relocated animals were alive and within the relocation area in 1990, however growth was apparent in most species. Movement was related to substrate type and discharge. After 10 years (1997), only 6% of the animals appear to be alive and within the relocation area. However, movement in this area is considerable, due to the rocky nature of the substrate; 81% of the marked animals were found away from their initial location, some as far as 75ft. Mortality of marked animals was evident, particularly in 1997. Some mortality is probably natural, as many of the relocated animals were probably 25 to 30 years old in 1997, and some mortality is probably due to zebra mussel infestation.

Campbell's Island, Illinois, Unionid Relocation,

Illinois Department of Transportation plans on replacing the existing bridge which spans the side channel of the Mississippi River at mile 490.3 near Rock Island, Illinois. One of the richest unionid communities in the upper Mississippi River occurs in this river reach and previous surveys near the Campbell's Island bridge indicated 33 species occurred in the area, including Illinois and Federal threatened and endangered species. Unionids were relocated from construction impact areas to a unionid bed on the channel side of Campbell's Island in September 1997. Far fewer unionids were present in the area than original expected, possibly due to zebra mussel effects. Fifteen species were found and relocated, including *Lampsilis higginsi* (Federally endangered) and *Ellipsaria lineolata* (Illinois threatened). Endangered and threatened species were marked and placed in grids to facilitate monitoring of survival. Two grids with three common species were established to assess survival and growth of relocated animals. Relocated animals were inspected one month after the relocation and all animals were live. Monitoring will be conducted in 1998.

Ohio River Unionid Monitoring, Ohio River Miles 204.3 and 218.0,

Ohio Municipal Electric Generation Agency contracted ESI to conduct a systematic dive survey of two unionid beds, and monitor these beds before and during construction and following operation of the hydropower facility. Pre-construction monitoring was conducted in 1993 and 1994, construction began in 1995, and post-construction monitoring was conducted in 1995, 1996, and 1997. Over 5,000 unionids were qualitatively collected each year to estimate species composition and relative abundance within the beds. Quantitative samples were randomly collected in each bed to determine mean unionid density and juvenile to adult ratios for comparison among years. Unionid density did not differ among years, and *C. stegaria* was collected during 1994 and 1997 qualitative sampling. Data from qualitative and quantitative sampling will be compared with future monitoring data to evaluate possible hydropower impacts on unionid communities.

Unionid Monitoring Plan Development and Long-Term Monitoring, Elk River, West Virginia,

Wolfpen Knob Development Company proposes constructing and operating a mining complex in Braxton County, West Virginia adjacent to the Laurel Fork of Tate Creek. Tate Creek flows into the Elk River, which supports a diverse unionid community, including three Federally endangered species; *Lampsilis abrupta*, *Epioblasma t. rangiana*, and *Pleurobema clava*. U.S. Fish and Wildlife Service and West Virginia Department of Natural Resources are concerned the project may adversely impact unionids and required Wolfpen to develop a monitoring plan to assess mining effects on unionids. ESI surveyed the Elk River for unionids upstream and downstream of the mine site, selected suitable monitoring sites, and developed a monitoring plan. The plan was presented to and approved by resource agencies in 1996. Monitoring, using the scope of work outlined in the plan, was initiated in 1997. Density, species richness, and age structure were estimated for unionid communities at four sites; two upstream and two downstream of the mine. Pre-construction data will be used to estimate natural and sampling variability.

Ohio River Unionid Impact Assessment and Monitoring, Ohio River Miles 252.6 to 257.5,

ESI was contracted by Southern Ohio Coal Company to evaluate unionid communities upstream and downstream of Leading Creek; an Ohio River tributary receiving discharge from Meigs Mine. Unionids were surveyed using quantitative and qualitative diving techniques at three locations; one site upstream and two sites downstream of Leading Creek. Unionids were sampled a few weeks following discharge, and one, two, and four years following discharge. No unionid mortality was detected due to mine water release in the sampled areas.

Unionid and Fish Habitat Survey in the Mullet River, Wisconsin,

The State Historical Society of Wisconsin (SHSW) proposes reconstructing a water-powered sawmill at Wade House State Park, located in Greenbush, Wisconsin. Two unionid species, *Venustaconcha ellipsiformis* and *Alasmidonta viridis*, which are threatened in Wisconsin occur in the project area. Unionid and fish communities, and their habitat, were characterized in the Mullet River from downstream of Glenbeulah dam to Mullet Marsh to facilitate project design, impact evaluation, and mitigation development. Sampling was conducted between 15 and 31 July 1997. *Alasmidonta viridis* and *V. ellipsiformis*, and their reported fish host (*Etheostoma nigrum*) were collected throughout three isolated reaches of suitable habitat. The project is within the longest reach of suitable habitat. ESI worked with engineers at Woodward/Clyde and SHSW to develop a construction and operation plan that would serve the project purpose, yet minimally affect the stream and its biota. The impoundment needed to drive the sawmill turbines will be constructed to the side of the river to minimize in stream disturbance, fish passage will be provided around the dam structure, and a re-regulation pool downstream of the dam should dissipate the effects of flow pulses.

Straight River Unionid Mollusk Survey, Minnesota,

Northern Natural Gas Co. proposes replacing a pipeline that crosses the Straight River (Cannon River Drainage) in Steele County, Minnesota. The unionid fauna of the Cannon River Drainage includes the Minnesota threatened *Pleurobema coccineum* and *Venustaconcha ellipsiformis*, and Minnesota special concern species *Elliptio dilatata*, *Lasmigona compressa*, *Lasmigona costata*, and *Ligumia recta*. Because unionids could be affected by pipeline replacement, the unionid community was sampled near the proposed pipeline crossing and downstream, to determine if protected unionids were present. The results of this survey indicated that a unionid community, similar to the historic fauna of the river, exists within the stream reach that may be impacted by in-stream disturbance from pipeline construction. This community includes the Minnesota threatened *V. ellipsiformis* and *P. coccineum*. If drilling under the river is successful, no impacts on the unionid community are expected. If in-stream disturbance cannot be avoided, mitigative measures may be needed to reduce construction impacts.

Minnesota and Pomme de Terre Rivers Unionid Mollusk Survey, Minnesota,

The proposed Alliance Pipeline Project crosses the Minnesota River (LeSure and Nicollet Counties) and the Pomme de Terre River (Stevens County), Minnesota. Both rivers have diverse unionid faunas that include state protected species, and the Federally endangered *Lampsilis higginsi* historically occurred in the Minnesota River. Because unionids may be affected by pipeline replacement, unionid communities near the proposed pipeline crossings and downstream were sampled to determine if protected unionids occur in these areas. A depauperate unionid community exists within the river reach surveyed on the Minnesota River, which is likely due to the unstable sand that predominates the substrate. Only three live unionids were collected, and no live or freshly dead individuals of any protected species were found, therefore, pipeline construction should not affect protected unionids. The study area on the Pomme de Terre River, however, supports a well established unionid community that harbors up to ten species. One freshly dead shell of the Minnesota threatened *Pleurobema coccineum* was found at Site 1, indicating that the population within this river reach is probably small. Since *P. coccineum* was not observed in the vicinity of the proposed pipeline crossing, the Alliance Pipeline Project is not expected to affect this species. However, unionids were common at the proposed pipeline crossing and sites downstream, and may be affected by in-stream disturbance from pipeline construction.

Ohio River Pomeroy/Mason Bridge Unionid Assessment, Ohio River Miles 248.2 to 254.4.

The Ohio Department of Transportation proposes replacing a Ohio River bridge at Ohio River Mile (ORM) 251.3 near Mason, West Virginia and Pomeroy, Ohio. Two alternative corridors for the replacement of structure are being evaluated. ESI was contracted to document all recent unionid surveys between ORM 248.2 to 254.4 (5km above and below the bridge) and conduct a brail survey of three potential alternatives to assess project affects on unionids. Three different sites (13% of the study area) had been previously surveyed for unionids, and relatively diverse unionid beds occurred at two of the three sites. An established unionid community does not appear to exist at any of the alternatives. No unionids were collected by brail or along the riverbank within Corridor A, only two individuals of one species (*Amblema p. plicata*) were collected within Corridor B, and no unionids were collected from the corridor at ORM 249.6. Although a brail is an inefficient method of characterizing unionid communities, if a significant bed existed in these areas at least a few individuals should have been collected on most transects, and more than one species should have been observed. Since unionid communities do not appear to occur in the proposed corridors, neither alternative should affect protected unionids in this river reach.

Unionid Survey near Mississippi River Mile 467.4,

Harza Environmental Services is assisting a client with environmental permitting for a water intake in Pool 16 of the upper Mississippi River. The range of two Federally endangered species (*Lampsilis higginsi* and *Potamilus capax*), as well as, several species considered threatened or endangered in Illinois and Iowa includes this area. The study's objectives were to 1) characterize the community (i.e., species composition, age structure), 2) determine if protected species occur in the community, and 3) estimate the project's impacts on the community (particularly protected species). Sampling was completed on 10 and 11 September 1997. Unionids were present within proposed construction areas, however, diversity was low and *Amblema p. plicata* comprised over 50% of the community. *Ellipsaria lineolata* was the only species collected live that is protected in Illinois and Iowa. A diverse unionid community appears to occur in the downstream portion of the study area, which is heavily colonized by zebra mussels. The downstream area may be affected during construction by sedimentation and during operation by alteration in local hydrology.

Unionid Survey at a Proposed Water Intake on the Mississippi River, Near Alton, Illinois,

Illinois-American Water Co. proposes installing a water intake system upstream of the current facility on the Mississippi River (approximate River Mile 204.3), Madison County, Illinois. Historically at least 28 unionid species occurred in this reach of the Mississippi River, including the Federally endangered *Potamilus capax*, and Illinois protected *Ellipsaria lineolata*, *Elliptio crassidens*, *Elliptio dilatata*, and *Simpsonaias ambigua*. This study characterized the unionid community near the proposed construction location and downstream to determine if protected unionids occur in the area. This area currently does not appear to support a unionid community, as only the shells of eight species were collected. *Leptodea fragilis* was the only species represented by freshly dead shells; the remaining species were collected as weathered or subfossil shells. Given that habitat conditions within the study area are unsuitable for unionid colonization, and no unionids were found, construction and operation of the water intake and treatment discharge should not impact unionids.

Abstracts of Papers and Posters

presented at

Conservation, Captive Care, and Propagation Freshwater Mussel Symposium

March 6-8, 1998

Columbus, Ohio

hosted by

Columbus Zoo
UMBC (An Honors University in Maryland)
Ohio Biological Survey
Ohio Department of Natural Resources Division of Wildlife

Co-Chairman
Richard Tankersley, University of Maryland Baltimore County
and
Dour Warmolts, Columbus Zoo

Reproduction in Captivity and Glochidial Development of *Margaritifera auricularia* (Spengler, 1793) in Spain [GT]

Rafael Araujo, Silvia Jimenez and Maria Angeles Ramos

Museo Nacional de Ciencias Naturales. C. S. I. C., José Gutiérrez Abascal 2, 28006 Madrid Spain

Margaritifera auricularia was described by Spengler in 1793. Since then it has been found in several localities in Western Europe and North Africa, many of them as fossils, but no living specimens have been recorded in any place since 1917. In a recent survey of Spanish Rivers to inventory the European Habitats Directive (Directive 92/43/CEE) freshwater species, we found a living population of this giant pearl mussel in a very ancient channel of the Ebro River. Timing and length of the glochidial release period at the natural habitat has been determined by collection of drift samples. We introduced sturgeons into the aquaria with gravid mussels, realizing that all mussels began to spawn. Immediately after releasing, the glochidia attached to the fishes gills. We excised the gill filaments of the sturgeons 1, 2, 3, 4, 5 hours and 2, 3, 5, 13, 34 and 60 days after the beginning of glochidia release. Histological and scanning samples of these gill filaments are being studied in order to investigate the encystment process and to find out the period that the glochidia need to complete their metamorphosis. However, up to now, no juvenile specimens have been found in the aquarium. We are also evaluating, by artificial infestation, the possibility that other fish species (native species) may be parasitized by the glochidia. This kind of results, including the ones from molecular studies currently in course, are the basis for subsequent application of species recovery plans and reintroduction policies.

Conservation Genetics of a Unionid (*Lampsilis siliquoidea*) [Poster]

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Protein electrophoresis is being used to measure genetic variation and population differentiation in a Unionid mussel (*Lampsilis siliquoidea*) and three species of its fish hosts. Most Unionidae have a larval stage which are obligate parasites of fish. Adult mussels are sessile, thus any migration between populations must occur while larvae are attached to fish hosts. Barriers to dispersal and gene flow between fish populations are expected to act as barriers to Unionid dispersal and gene flow. The hypothesis that population differentiation in the Unionid mussel reflects the population differentiation of its fish hosts is being tested. Mussels and host fish were collected from five sites within the Raisin River drainage in southeast Michigan. Thus far, the products of 16 loci have been assayed for two mussel populations. Four of these are polymorphic (P = 0.25, H = 0.21). A description of the population genetics of the Unionidae and an improved understanding of the role of fish hosts in influencing genetic variation and population differentiation will enhance conservation and management of this declining group.

The Effects of Temperature, CO_2 and pH on the Transformation Success of Glochidia on Fish Hosts and *In Vitro* [GT]

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Low temperature is known to suppress immune function in ectothermic vertebrates, including fish. Therefore, we hypothesized that low temperature might facilitate successful encystment and transformation of glochidia on fish hosts. The glochidia of the flat floater, *Anodonta suborbiculata*, are normally present on fish hosts from January through March, when water temperature is low. The percent transformation success of attached glochidia in laboratory infections on fish hosts (golden shiners) was statistically significantly higher at 10°C (67%) and 15°C (62%) than at 21°C (42%). In contrast, transformation success of glochidia *in vitro* was significantly lower at 10°C (39%) than at 15°C (89%) or 20°C (93%). Thus, improved survival at low temperature on fish hosts does not appear to involve direct effects of temperature on the glochidia. These results are consistent with the hypothesis that immune suppression of fish hosts by low temperature enhances transformation success of this winter-breeding unionid species. We also tested the effects of CO_2 and pH on transformation of flat floater glochidia *in vitro*. Transformation success was highest at the lowest pH tested (7.6) and the highest level of CO_2 tested (5%). This result is consistent with previous studies of other species, but is puzzling, because the physiological pH of fish body fluids is higher (ca. 8.0) and the level of CO_2 is much lower. Although most workers have incubated glochidia in 5% CO_2, we found that transformation was reasonably successful (68%) without elevated CO_2, provided that pH was kept low.

Genetic Structure of Unionid Populations: Implications for Captive Propagation and Reintroduction [JR]

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Captive care, propagation, and reintroduction programs are often used by natural resource managers charged with protection of endangered species. Experiences with a variety of taxa show that such programs must consider the population genetic structure of target species to ensure long–term survival. We used allozyme electrophoresis to quantify genetic structure of several unionid species and compare relative amounts of within–population (w–p) and among population (a–p) genetic variation. W–p variation was lower in populations of *Elliptio dilatata* (average of

1.6 alleles/locus, 36% polymorphic loci, 12% heterozygosity) than in populations of *Quadrula quadrula* (2.1, 61%, 24%). *Amblema plicata* showed intermediate levels of w–p variation (2.0, 58%, 11.1%). Allele frequency differences were relatively large among populations of *E. dilatata* separated by short distances. Populations of *Q. quadrula* showed no variation in allele frequencies over distances > 1000 km. At least two models of genetic structure are found in unionid populations. Model I species such as *Q. quadrula* exhibit high levels of w–p genetic variation, while a–p variation is much lower. Model II species such as *E. dilatata* are characterized by low w–p variation and high a–p variation. Conservation of diversity in a Model I species requires that captive populations consist of large numbers of individuals from a given population, while conservation of a Model II species requires maintenance of separate stocks from a number of populations. Design of captive propagation, reintroduction, and translocation programs must account for genetic structure of target species.

Historical and Current Distribution of the Freshwater Mussels (Unionidae and Margaritiferidae) of the Escambia and Yellow River Drainages of Southern Alabama and Western Florida [Poster]

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The Escambia and Yellow river systems were surveyed from 1990 to 1996 to determine the presence/absence of thirteen rare mussel species. Historical distributions were determined from over 700 museum records from 64 sites in the Escambia River drainage, and from 71 museum records from 11 sites in the Yellow River Drainage. These records date from the late 1800's to 1989. In the Escambia River Drainage, 36 (56%) of the historical localities were resurveyed, as well as 59 new sites. Twenty-eight of the 30 species historically known from the basin were found in the current survey. In the Yellow River Drainage, seven (64%) of the historical sites were resurveyed, as well as 71 new sites. Eleven of the 15 species historically known from the drainage were found in the current survey, and two species previously unreported, *Utterbackia imbecillis* and *U. peggyae*, were also collected. The rare mussels known from the basins, *Anodontoides radiatus, Elliptio arctata, Fusconaia escambia, F. rotulata, F. succissa, Lampsilis australis, L. ornata, Margaritifera marrianae, Pleurobema strodeanum, Strophitus subvexus*, and *Villosa choctawensis* were found only at a few sites. *Medionidus acutissimus* and *Ptychobranchus jonesi*, historically known from both basins, were not found in the current survey. Future efforts will concentrate on resurveying the remaining historical sites.

A Dynamic Population Model as a Tool for Evaluating Mussel Management Strategies [Poster]

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Today, management of commercially important mussel species is hampered by an inability to quantitatively assess cumulative impacts of natural and harvest mortality on populations of this economically and ecologically significant resource. Sound science-based management will benefit from a tool that combines data on current populations with knowledge of population dynamics to predict future conditions under various management scenarios. We developed a single-species dynamic population model for native mussels using Stella II software running on a personal computer. The model was developed using data for the threeridge mussel (*Amblema plicata*) from Reach 15 of the Upper Mississippi River at Rock Island, IL, but it can be parameterized for other locations and species when pertinent data exist. Input parameters include initial density (number per m^2), age-frequency distribution, growth rate, age at maturity, and age-specific mortality rates. In our model, recruitment is density dependent and incorporates

fecundity, sex ratio, density-dependent fertilization rate, and glochidial attachment, transformation, and survival rates. The model outputs standing stocks (numbers per m^2) of different age classes over time. Our model allows harvest which can be regulated by minimum size (currently a common management practice), minimum density, maximum harvest rate (number per m^2), assorted closure strategies (e.g., harvest once every 1, 5, 10, or 20 years), or various combinations of these. Model outputs include annual and cumulative harvest (numbers and pounds per m^2). The model uses size-dependant price estimates to calculate dollar values for standing stocks and both annual and cumulative harvest.

Physiological Effects of Transportation in Water and in Air on Freshwater Mussels (Unionidae) [CC]

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To determine the best method for transporting freshwater mussels, fifty specimens of each of five species: *Elliptio complanata*, *Villosa iris*, and *Fusconaia ebena*, *Quadrula quadrula*, and *Amblema plicata* were collected in the field and subsamples of each species were frozen in liquid nitrogen. The rest of the samples were divided into two groups: one group was placed in a water-filled, aerated tank; the other group was placed in coolers with wet burlap. At 6 hr intervals up to 24 hr, a subsample of each group was removed and frozen in liquid nitrogen. All samples were then stored at -81 °C until analyzed for physiological stress. The concentration of glucose and glycogen in the posterior adductor muscle, gill, and mantle were chosen for estimating stress caused by transportation. In *V. iris*, the glucose levels of those from both transportation modes increased in the mantle tissues for the first 12 hr, but those transported in water recovered gradually after 12 hr. For the remaining four mussel species, transportation in water did not result in elevations of glucose levels, but transportation in air did cause an increase of glucose in some tissues. For all five species, there were no significant changes of glycogen content during either transportation modes. Overall, the transportation in water was less stressful than that in air. However, for the tolerant species, transportation in air for a short-term period might be an economic and feasible way to transport mussels.

Community and Population Characteristics of Unionid Assemblages in the Headwaters of the Big Darby Creek Basin, OH [Poster]

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The Darby Creek ecosystem has been designated a "Last Great Place" by The Nature Conservancy based on its high diversity of unionids. Investigation of community and population characteristics in this system will lead to greater understanding of unionid ecology and to more effective conservation. Unionids were collected using two sets of parallel transects at sites on Big Darby (BDC) and Little Darby (LDC) creeks. Transects were orientated parallel to stream flow with 2 m separating each member of a pair. The BDC site contained 12 species, 357 individuals, and had an H′ of 1.9. The dominant taxa for this site were *Strophitus undulatus*, *Lampsilis siliquoidea*, *Ptychobranchus fasciolaris* and *Elliptio dilatata*. The LDC site contained 10 species, 271individuals, and had an H′ of 1.5. The dominant taxa for this site were *E. dilatata*, *P. fasciolaris* and *Fusconaia flava*. Two state-endangered species, *Pleurobema clava* and *Quadrula cylindrica*, represented by two individuals each, were identified from this site. A Community Similarity Index between the two sites of 0.571 indicated that these two communities were moderately similar. Based on external annuli counts, *E. dilatata* from BDC and LDC sites ranged

from 2-10 and 2-11 years in age, respectively and *P. fasciolaris* from BDC and LDC ranged from 2-19 and 4-16 years in age, respectively. Analysis of frequency histograms of age cohorts indicated no difference in structure of BDC and LDC populations of *E. dilatata* or *P. fasciolaris*. This information will be useful in understanding dynamics of unionid populations from headwater streams.

Distribution and Habitat of Colorado Freshwater Mussels (Mollusca: Bivalvia: Unionidae) [Poster]

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North America has the greatest diversity of freshwater mussels in the world, yet members of the superfamily Unionacea are one of the most highly threatened groups of organisms in the United States. Colorado represents the westernmost range of some plains species. Past diversity in Colorado included upwards of seven different species. This study hypothesizes the diversity of unionid bivalves in Colorado lakes and streams is in rapid decline both at the species and the population level. The cylindrical papershell, *Anodontoides ferussacianus* (Lea), was once the most common species in the state, but it is currently found only in a slow-moving foothills stream with sand/cobble substrate and a small foothills lake with high turnover and mud/cobble substrate. A single dead shell of *Uniomerus tetralasmus* (Say), the pondhorn, found in a southeastern plains lake with mud/clay bottom is the only recent evidence of the this species in Colorado. *Anodonta grandis grandis* Say, the giant floater, has maintained steady populations in Colorado plains reservoirs with hard, alkaline water, but no longer occurs in streams. Management of these species requires knowledge of the location of population sources, the status of those populations, the habitat conditions under which they exist, and a reintroduction program to prevent further decline. Potentially stable population sources include Boulder Reservoir, Cherry Creek Reservoir, Pueblo Reservoir, and Colorado Fuel & Iron Reservoirs 1-3 for *A. g. grandis* and Valmont Reservoir for *A. ferussacianus*. It is likely already too late for *U. tetralasmus* as all former populations seem to have disappeared.

North American Freshwater Mussels: Distribution, Biology and Conservation [Poster]

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One of the objectives of the National Strategy on the Conservation of Freshwater Mussels (Biggins *et al.* (1995) was to prepare an atlas of North American freshwater mussels. An organizational meeting was held 20-21 October 1997 in Arlington, Virginia attended by the above (minus B. Howells and T. Frest) to discuss the preparation of a book entitled "North American Freshwater Mussels: Distribution, Biology and Conservation." It will be a reference volume largely based on the Atlas of North American Freshwater Fishes by Lee *et al.* 1980 (North Carolina State Museum of Natural History). The goals of the meeting were to: 1) compile initial lists of regional coordinators, compilers and species to be included, 2) develop a draft format for the maps and an associated database of the data sources, 3) develop a budget, 4) target potential funding sources, and 5) develop a timetable for completion of the project. Authorship of the

entire volume would be the regional coordinators with the species compilers credited on the pages of individual accounts as was done in Lee *et al.* (1980). Each species account will include: a color photograph, common and scientific name (including author and date), original description citation, type locality, systematics, key characters, general distribution, habitat, biology, and conservation status. Appendices will likely include: state references, synonyms, and fish host(s). The layout will be similar to the fish atlas but will include a two page format for some species. Although a fully geo-referenced database and computer generated maps would be desirable, it is unlikely that such a project could be completed in a reasonable time frame. However, one of the goals is to compile the data in a GIS usable format for the future. Pre-formatted software (PC and Mac compatible) will be distributed to all compilers to document the source of the distributional data used. Exact locality data are desired (citing museum collection numbers) but previously published maps and other sources could be used if documented (*i.e.* all Missouri data from R.D. Oesch, 1995. Missouri Naiades: a guide to the mussels of Missouri).

Oxygen Consumption by Juvenile *Pyganodon cataracta* in Declining P_{O_2} (Bivalvia: Unionidae) [MP]

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The physiology of very young unionids is largely unstudied. Historically, the near impossibility of acquiring sufficient numbers of young mussels from the field precluded experimental investigation. Success with *in vitro* and *in vivo* transformation of glochidia larvae in the laboratory has been tempered by generally low survivorship and relatively poor growth of the resulting juveniles. As a result, except for work on nutrition, most physiological studies have been limited to toxicological analyses of 1-2 week old juveniles of very few species. My laboratory has characterized the tolerance of 1-week old *Utterbackia imbecillis* and *Pyganodon cataracta* to thermal, acidic and hypoxic stress and the effects of temperature and P_{O_2} on heart rate of 2-week old juveniles. Recently we were able to culture 31 *P. cataracta* for 24 weeks. Of these, 13 attained a mean length of 6.2 mm (range = 3.3-8.3) and mean wet wgt (including shells) of 27.3 mg, large enough to monitor V_{O_2} of individual animals using microrespirometry (1.0 ml chambers, Strathkelvin oxygen sensors). Although the pattern of response to declining P_{O_2} varied among individuals, especially in respect to whether animals remained open and siphoning for the duration of an experiment, consistent data were acquired for 9 animals. Mean V_{O_2} near full air saturation was about 0.086 ml O_2 gm^{-1} hr^{-1}. Juveniles exhibited moderate oxyregulation from full saturation to P_{O_2} ~30 mm Hg, a pattern that is consistent with that of adults. These results constitute the first data on respiratory physiology of such young freshwater mussels.

Effects of Commercial Algal Preparations on Growth and Survival of Juvenile *Pyganodon cataracta* and *Utterbackia imbecillis* (Bivalvia: Unionidae) [Poster]

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Although commercially available algal diets, including spray-dried powders and concentrated microalgal pastes, have been used extensively by marine aquaculturalists to rear larval and juvenile shellfish, their application for culturing unionid mussels in captivity has been limited. We examined the growth and survivorship of newly transformed juvenile *Utterbackia imbecillis* fed spray-dried *Schizochytrium* and *Chlorella*, or a multialgae paste composed of *Thalassiosira pseudonana*, *Skeletonema* sp., *Chaetoceros calcitrans* and *Isochrysis galbana*. Ten days following transformation, juvenile mussels were assigned to one of five diets: *Schizochytrium* alone, *Chlorella* alone, multialgal paste alone, a 1:1:1 mixture of all three diets, and a starved (no algae) control. Although initial growth rates of mussels were similar among treatments, after 3-wks mussels fed *Schizochytrium* alone had significantly higher growth rates (shell length, 54.4 μm wk^{-1}) and survival (77.8%) than all other treatments. Longer-term experiments were

conducted to compare growth rates of juvenile *Pyganodon cataracta* (2-wks post-transformation) reared on spray-dried *Schizochytrium* alone and in combination with fresh cultures of *Chlorella vulgaris*. Juvenile mussels were assigned to 3 diets, *Schizochytrum*, live *Chlorella*, and a 1:1 mix of both diets, plus silt. Only juveniles fed the combination of *Chlorella* and *Schizochytrum* survived to 10-wks (17%). At the end of the experiment (22-wks), survivorship of juvenile *Pyganodon cataracta* on the combination diet was nearly 16%. Mussels reached a mean shell length of 5.3 mm, representing about a 12 fold increase, and had a mean growth rate of about 271 $\mu m\ wk^{-1}$.

Relocation and Subsequent Evaluation of Condition for Adult Mussel Populations Relocated to Hatchery Refugia [CC]

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Evidence of zebra mussel (*Dressiena polymorpha*) impacts on native mussels is growing as the invasion extends to Arkansas' waterways. The Arkansas River experienced the greatest initial invasion, while zebra mussels are now distributed along navigable sections of the White River. The objectives of this study were to transfer viable, native mussel populations, absent of any attached *D. polymorpha* to a refugium in order to determine the functionality and effectiveness of spring-fed ponds and raceways to shelter at-risk populations. Another objective was to monitor the physiological adaptations of transferred populations into "closed" systems located at the Mammoth Springs National Fish Hatchery for a specific period of time. Twenty-seven species from the White River were collected in the fall of 1996 for transfer to the hatchery. After initial size and weight were measured, mussels were tagged, cleaned and inspected for infestation before being transferred to holding chambers. Mussels were held for a two-week quarantine period before establishing permanent residence in the ponds. Mussels collected in the fall of 1997 from the Ouachita River included twenty-nine species, and similar techniques for transfer and isolation were conducted. To date, survival and mortality have been monitored for the White River species following one year after transfer, and <30% mortality has occurred in these populations. Physiological indices have been established for these mussels using *Amblema plicata* as a surrogate species. Cellulolytic enzyme activity and glycogen content have been monitored as biomarkers of relative condition to help evaluate the current holding facilities as effective habitats for refuge of threatened mussel populations.

Filtration Rates Over 24 h for Adult Rainbow Mussels (*Villosa iris*, Lea 1829) Held in Culture [MP]

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The success of propagation of unionids for stock enhancement and endangered species management depends on understanding the feeding ecology of these aquatic organisms. Most bivalves feed by filtering suspended material from the water. Unfortunately, little data exist for filtering rates of unionids. Thus, our specific research objective was to determine the amount of algae cleared from suspension by adult rainbow mussels over 24 h. From the clearance of suspensions, we estimated the filtering rate of this species under our culture conditions. Filtration rates were monitored and compared using repeated analysis of variance with a sequential profile to examine successive differences. We tested for the effect of shell length, time, and interaction between time and length on filtration rates. Not surprising, filtration rates are different for different sized animals. In addtion, filtration rates change significantly over 24 h. Mussels, 37-52 mm in length, were fed 1 x 10^6 c/ mL. Little to no algae settled in our culture chambers. For all mussels, filtration rates varied between 14 and 280 mL/h in the first hour, and after 24 h, the filtration rates varied between 9 and 34 mL/h. Mussels appeared to feed (filter) furiously in the first 2 h, then filter at lower rates for several hours, begin feeding and then lowered their filtration

rate again. From this study, we estimated that 1, 45 mm sized rainbow mussel cleared over 900,000 cells of algae in 24 h.

A Protocol for the Salvage and Quarantine of Mussels from Zebra-Infested Waters [CC]

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In the summer of 1995, a quarantine facility was assembled on Middle Island, of the USFWS Ohio River Islands National Wildlife Refuge, that could accomodate the salvage of several thousand unionids from the zebra mussel-infested Ohio River. The facility was supplied by well water and equipped with 14, 500 L tanks, aerated by a 0.5 hp regenerative blower, and plumbed so each could be drained easily. Because no biofilters were installed, water was changed every 2 d. Protocols for the salvage and quarantine of unionids were developed by state, federal, and university biologists. Three thousand unionids of 6 common species were collected in 1995, and several hundred unionids in 1996 and in 1997. Unionids were scrubbed on site to remove zebra mussels, and transported in ice-cooled containers to the quarantine facility. Unionids were quarantined for a minimum of 30 d, reinspected for zebra mussels, and transported to pond refugia if uninfested. Unionids were fed 10 L of a dense algal suspension 3 times/week in 1995, starved in 1996, and then were fed 1 x 10^5 cells/mL twice/day in 1997. Survival in quarantine was over 95% in all three years; however, survival after one year in pond refugia was different for fed (94%) versus starved unionids (70%). Suggestions for salvage and quarantine of unionids include: keep unionids cool (<28°C) during handling, transport in ice-cooled containers or in aerated water supplied with food, check for zebra mussels every 7 d and minimize the quarantine period to less than 30 d, and feed unionids at 1x10^5 cells/mL twice daily. If possible, collect unionids during cool months, when metabolism is low and energy reserves are high prior to reproductive activity.

Differences in Tolerance to and Recovery from Zebra Mussel (*Dreissena polymorpha*) Fouling by *Elliptio complanata* and *Lampsilis radiata* [MP]

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Since their discovery in 1993, zebra mussels (*Dreissena polymorpha*) in Lake Champlain have colonized the shells of many native unionids. Periodically cleaning zebra mussels from unionids may be an effective conservation technique if unionids can recover from the stress induced by zebra mussels. Conservation efforts will need to target those species that are most vulnerable to fouling and subsequent energetic losses. Because glycogen is the primary energy store in many bivalves, a decrease in glycogen content indicates loss of fitness. As dreissenid-unionid mass ratios increase in *Elliptio complanata* from Button Bay Vermont, there is no evidence that glycogen levels decrease even with mass ratios as high as 1.25. In contrast, dreissenid-unionid mass ratios as low as 0.25 in *Lampsilis radiata* are correlated with a significant decline in glycogen content. Results are consistent with a higher mortality of *L. radiata* than *E. complanata* in wild populations. In a second experiment, 32 mussels from each species were collected from the mouth of Lewis Creek, cleaned of zebra mussels (mean mass ratio: 1.45 ± 0.57) and returned to the creek. After 10 weeks, cleaned mussels and an equivalent number of heavily fouled mussels (mean mass ratio: 1.76 ±0.71) of each species were collected. Twenty *E. complanata* and *L. radiata* were also collected from the Lamoille River where zebra mussels are absent. Mean glycogen levels in heavily fouled populations of both species were lower than control levels while cleaned mussels did not differ from controls. Results suggest that heavily fouled *E. complanata* and *L. radiata* can recover glycogen levels if cleaned of zebra mussels.

External Aging of Unionids Revisited: Height versus Age of 4800 *Megalonaias nervosa* (Rafinesque 1820), Mississippi River Reaches (Pools) 9-19, Lansing, IA-Fort Madison, IA, 1 July-14 September 1997 [Poster]

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The Shell Exporters of America (SEA) sponsored a commercial unionid mollusk survey on the Upper Mississippi River Mile 663.0-386.0, Reaches 9-19, with an emphasis on *M. nervosa*, using modified 0.25 m^2 Surber samplers I designed. Over 20,400 unionids were processed (692 quadrats, 252 random dives). Over 4800 living *M. nervosa* were scrubbed, measured for height and length, and aged. Mean density/Reach ranged from 2.7-7.6/m^2. Mean height/age class/Reach, and mean age/ height class/Reach show age does not equal size. Growth rates increase further downstream (south). Two 80 mm high specimens, 23 and 6 years of age, were from Lynxville, WI, and Dallas City, IL respectively. Commercial *M. nervosa* (101.6 mm ht) were 9-38 years of age. In most Reaches 2.4-10.8% (mean 7.8%) of a commercial bed is of legal height. The most legal unionids were found in the Sylvan Slough Sanctuary (18.5%) indicating little illegal harvest. The 6 year age class (N = 130) had the largest height range (65 mm), while the height of 118-21 year olds varied 37 mm. The mean difference of all height classes was 41 mm/class. Number/size class for *M. nervosa* showed bell curves, but every Reach showed a marked shift to the left with number/age class peaks usually between 10-13 years; this is likely the species' response to the mid-1980's die-off. 3.18% of *M. nervosa* were less than 50 mm (2-7 years) indicating that the Mississippi River may be near a low point in the breeding cycle for *M. nervosa*.

The Correlation of Mussels With Fish in the Upper Blanchard River in Hardin and Hancock Counties, Ohio, With Special Regard to the Rayed Bean (*Villosa fabalis*) [Poster]

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The rayed bean, *Villosa fabalis*, is a state endangered species in Ohio. It is among the smallest unionids to occur in the Midwest and has always been considered a rare species wherever it is found. The species was being considered for listing as endangered by the U.S. Fish and Wildlife Service before that agency eliminated its Category 2 Candidate list. However, *V. fabalis* is one of the most abundant species of mussels in the upper Blanchard River above Findlay in Hardin and Hancock counties in northwestern Ohio. It is the third most common species in that reach of stream behind the spike, *Elliptio dilatata*, and the fatmucket, *Lampsilis radiata luteola*. The current paper reports on collections of fish and mussels from 11 stations in the upper Blanchard River. The mussel community of this reach is represented by 16 extant species while the extant fish community totals 36 species. Records for a total of 17 species of mussels and 45 species of fish have been recorded for this reach of the Blanchard River. It was observed that the mussel community changed dramatically from station to station in this river mostly as a consequence of habitat availability. It was found that the fish community changed as well. This paper will report on the correlation of fish species with mussel species within this reach of stream. The objective is to suggest some potential natural hosts for these mussels, and to provide a protocol for the management of *V. fabalis* in the upper Blanchard River.

Considerations for Conducting Host Suitability Studies [GT]

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A well-designed protocol is important for identification of glochidial host(s) and propagation of juvenile mussels. We conduct our studies in flow-through and static aquaria. Fish are infested with glochidia by pipetting glochidia onto gills or placing larger fishes in a bath with several thousand glochidia under vigorous aeration. Aquaria are siphoned and siphonate checked for presence of glochidia and juveniles three times a week. We have increased the survivorship of hosts and maximized juvenile mussel production by: (1) scrutinizing host fish welfare just before and during juvenile mussel excystment, (2) conducting studies in flow-through aquaria, and (3) keeping tanks free of excess food. We have improved juvenile mussel recovery by: (1) holding small host fishes in suspended nets, (2) recording presence of pre-metamorphosed juveniles, (3) washing siphonate, (4) cleaning juvenile collection sieve with a cloth, and (5) increasing the number of hosts tested.These techniques were important in identification of glochidial hosts for a variety of local mussels. Metamorphosis of *V. ellipsiformis* glochidia was observed on *Percina maculata*, *Etheostoma exile*, *E. flabellare*, and *Cottus bairdi*. Four of seven Ictalurids were found to be suitable hosts for *Cyclonaias tuberculata* glochidia. Of twelve fish species tested only the *Ameiurus natalis* facilitated *T. verrucosa* glochidia metamorphosis. *Epioblasma triquetra* glochidia were exposed to four fish species and *Percina maculata* and *P. caprodes* served as hosts.

Reproductive Seasonality of Freshwater Mussels (Family Unionidae) in Texas [Poster]

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Most historical reports of spawning and brooding periods for freshwater mussels (Unionidae) have focused on work conducted in central and northern regions of the United States and Canada. Comparatively little has been documented in southern states, particularly in drainages west of the Mississippi. From 1992 through 1997, Texas Parks and Wildlife Department conducted statewide surveys of unionid abundance and distribution in Texas. Collectively, from specimens obtained during these surveys, over 5,000 specimens of 40 species were examined for the presence or absence of eggs and glochidia in female marsupia. Taxa studied included both widely-ranging species like *Amblema plicata*, *Megalonaias nervosa*, and *Pyganodon grandis* as well as locally-endemic forms like *Quadrula petrina*, *Quadrula aurea*, and *Lampsilis bracteata*. Some observations include species not covered in previous literature. Results of these examinations are presented.

Practical Propagation of Freshwater Mussels for Shell Harvest [JR]

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Commercial harvest of freshwater mussel shell has grown into a multimillion dollar business in Tennessee. Shell harvest fluctuates from 1,500 to 4,000 tons annually according to market demand. Approximately 50% of the shells exported from the United States are harvested in Tennessee. This harvest feeds an industry that employs 2,000 to 3,000 people and produces revenues exceeding $60 million a year in Tennessee. Greater than 90% of Tennessee's mussel harvest is taken from Kentucky Reservoir on the Tennessee River. Intensive harvesting of freshwater mussels has routinely depleted areas of their legal sized commercial shell stocks. In most areas of the reservoir, mussel stocks are harvested almost immediately after attaining legal size. Natural reproduction requires expelled glochidia to attach themselves to a proper host,

normally a fish. Most studies have indicated that infestation rates of wild fish are very low; however, through artificial propagation, heavy infestations can be obtained on nearly 100% of exposed fish. Because of the continued demand for mussel shells and the tenuous position of the resource, TWRA began experimenting with techniques for propagating commercial mussels during 1994. Objectives include identifying practical methods for augmenting commercially valuable mussel populations within selected Tennessee waters. Primary species employed in propagation procedures are the washboard *(Megalonaias nervosa)* and the threeridge *(Amblema plicata plicata)*. Over 5,000 fish have been infested and released back into the reservoir since 1994. Juvenile *A. p. plicata* were recovered from a site where infested fish were held in a cage.

Seasonal Changes in Respiration, Cholinesterase Activity and Glycogen Stores in Three Species of Unionid Mussels [MP]

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Glycogen stores, respiration rate and excretion were assayed once a month for twelve months in adult *Utterbackia imbecillis*, *Elliptio icterina* and *Lampsilis teres* mussels collected from the Suwannee River, FL. These species were selected because they have different habitat preferences, were locally abundant and had different reproductive cycles. Glycogen was found at the highest concentrations in the mantle and was lowest in the gill. *E. icterina* mussels contained the largest stores of glycogen overall and the highest adductor muscle cholinesterase activity of the three species. *U. imbecillis*, the fastest growing, thin-shelled member of the group had the highest respiration rate, followed by *L. teres*.

Survival and Growth of Unionids After Relocation Into an Artificial Pond [CC]

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We evaluated the suitability of hatchery ponds as temporary refugia for unionids as a result of the zebra mussel invasion in the Upper Mississippi River (UMR). We compared the growth and survival of unionids between a hatchery pond and an *in situ* riverine control; among four holding options (treatments) in each location; and among species. Five species of unionids (*Amblema plicata*, *Fusconaia flava*, *Leptodea fragilis*, *Obliquaria reflexa*, and *Quadrula quadrula*) were obtained from the UMR, cleaned of zebra mussels, quarantined, and allocated into one of four replicates of each treatment (n=96 per treatment). Treatments included mesh bags, corrals, buried trays, and suspended trays. Survival, after 2.5 years of relocation, averaged 36% in the hatchery pond and 80% in the riverine control. In the riverine control, survival was substantially reduced in the corrals, but similar among the three other treatments. In the hatchery pond, survival was highest in the suspended trays and lowest in the mesh bags. Survival also varied among species, with highest survival in *Quadrula* and *Amblema* and lowest survival in *Leptodea*. Little or no new shell material was produced by mussels in the hatchery pond, whereas some growth was evident in the riverine control. These findings suggest handling and transportation stress did not adversely affect the short-term survival of unionids, because survival exceeded 80% during the first 2.5 years in the riverine control. In contrast, the poor survival in the hatchery pond suggests that these mussels were not receiving adequate nutritional resources.

Efficacy of Cortisol Administration to Induce Glochidial Metamorphosis [GT]

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Host specificity of glochidia is believed to be immunologically based. Cortisol is a corticosteriod produced by teleost fish during stress. Prolonged elevations of cortisol can have an immunosuppressive effect on fish. Results from recent research have demonstrated the ability to induced glochidial metamorphosis on certain cortisol-treated nonhost fish. Our objective was to test the efficacy of administering cortisol to induce glochidial metamorphosis using a readily obtainable fish, largemouth bass *Micropterus salmoides*. Cortisol-injected night crawlers *Lubricus terrestris* were fed to largemouth bass prior to infesting the fish with glochidia of *Potamilus alatus* or *Medionidus conradicus*. Although this treatment successfully elevated plasma cortisol levels (≥21µg/100ml), glochidial metamorphosis did not occur on these nonhost fish in two trials. Further, cortisol treatment did not have a significant effect ($P>0.05$) on how long glochidia remained attached to the gills. In a third trial, cortisol was orally administered to largemouth bass, a reported host of *Ligumia recta*. Metamorphosis of *L. recta* glochidia occurred on two of four cortisol-treated largemouth bass, and on two of six untreated fish. Juvenile metamorphosis was highly variable within and among treatments. Plasma cortisol levels of largemouth bass were not correlated ($P>0.05$) with juvenile mussel production. These results suggest that cortisol treatment is not an effective means to induce glochidia metamorphosis on largemouth bass.

Discontinuity in the Genetic Population Structure of Freshwater Mussels: Conservation Implications [Poster]

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Molecular genetics has recently achieved an important place in contemporary conservation biology. It has proven to be a robust tool for identifying reproductive isolation among populations, permitting the delineation of management units and allowing assessment of conservation priorities from an evolutionary perspective. BRD recently funded nucleotide sequence analysis of the first internal transcribed spacer region (ITS-1) between 5.8S and 18S ribosomal DNA genes in *Lasmigona subviridis* representing the entire range of the species. Results indicate considerable genetic differentiation (including the absence of gene flow) between *L. subviridis* inhabiting the Susquehanna-Potomac Rivers and more southerly distributed populations. Similar levels of genetic differentiation have been observed in mitochondrial DNA in all freshwater mussels studied to date including the cytochrome oxidase I subunit in *Alasmidonta heterodon* and the 16S rRNA region in *Megalonais nervosa*, *Amblema neislerii*, and *A. plicata*. It appears that freshwater mussels are subdivided into more or less distinct genetic units. Knowledge of this structure is of major importance both for management and conservation of genetic resources. If it becomes necessary to culture the species targeted by conservation programs, sufficient numbers of viable individuals should be used as broodstock to eliminate inbreeding depression and the drift of alleles out of the population. Moreover, it is imperative that geographic populations of species targeted by such programs be segregated in holding facilities until such time genetic structure among populations is determined. This precaution is necessary to prevent the occurrence of outbreeding depression, hybridization, and disease transmission.

Conservation of Freshwater Mussel Species: A Tissue Repository for Genetics and Systematics [Poster]

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The continuous decline of native North American freshwater mussels underscores the need for genetics research to determine population structure for conservation and management. Knowledge of mussel taxonomy is such that identification of species, subspecies, or distinct populations can often be problematic. Recognizing a need for phylogenetics research, the Aquatic Ecology Laboratory of the Leetown Science Center established a tissue repository and associated database to coordinate tissue samples collected for genetics and systematics research. The repository provides a centralized location to obtain properly catalogued and preserved adductor muscle, mantle, foot, gill and digestive gland tissue samples. All samples are preserved for both protein and DNA analyses. Data generated for the repository are maintained in the PARADOX for Windows relational database package. Collection information for each specimen includes date, site name, site description, and habitat characteristics. Reports of database content are generated and provided to interested researchers. Currently the database contains 388 individuals representing 53 species inhabiting the Atlantic Slope and interior basin drainages. Researchers utilizing the repository are required to accommodate a standard numbering scheme to allow comparisons of the same individuals among diverse studies and methodologies. Potentially, the repository would conserve precious resources by reducing the number of animals sacrificed and provide comprehensive data to multiple researchers. A single collection of mussels can provide ecophenotypic, protein, DNA, and immunological information for species and population structure delineation. This poster describes the development of the repository and presents data collection protocols, preservation methods, database structure, and a report of the database contents.

1997 Unionid Surveys in the Fox River and Springbrook Creek, Illinois

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Illinois unionid mussels are declining rapidly in almost all waterways including Lake Michigan. Exotic zebra mussels are quickly colonizing many northeastern Illinois waterways and decimating native mussels. This three-year project will document and map two important native mussel populations, one of which may soon come under attack from zebra mussels. Focus areas will include the lower Fox River in Kane County and Springbrook creek in DuPage County, which has the highest biodiversity of any remaining county stream. The project includes an outreach program about the Fox River for Kane County schoolchildren, as well as well as investigating the potential for cleaning zebra mussels from unionids in-situ as a means of zebra mussel control.

Experiences With Captive Maintenance of Atlantic Slope Unionids in Small Research Systems [CC]

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Seven species of Atlantic slope unionids have been maintained in captivity for up to 3 years for use in reproductive and behavioral research. Mussels are housed in one of four culture systems that use either rectangular glass (120-240 l), rectangular fiberglass (540 l), or circular fiberglass (350 l) tanks. Tanks are supplied with well water heated or chilled within the range of 0.5-23.0°C and illuminated with timer-controlled overhead lights to simulate natural seasonal fluctuations.

Riverine species are provided mixtures of sand and gravel for substrate whereas pond species are provided with silt. Water current is established and regulated using airstones at the end of rectangular tanks and at the center of circular tanks. Mussels are batch-fed twice daily with one or more foods that may include cultured phytoplankton (*Nanochloropsis sp.*), detritus siphoned from concrete fish ponds, commercial preserved algal paste (*Chlorella vulgaris*), or bacteria collected from biofiltration units of recirculating fish culture systems. One-year survival varied with species, culture system, experimental treatment, and incidence of fatal mishaps, with an average 97% *Elliptio complanata*, 91% *Alasmidonta marginata*, 83% *Alasmidonta undulata*, 83% *Pyganodon cataracta*, 78% *Lasmigona subviridis*, 72% *Strophitus undulatus*, and 71% *Alasmidonta varicosa*. Three-year survival of *Elliptio complanata* has ranged from 20-80% with most mortality occurring in the third year. *Elliptio complanata* have spawned and produced viable glochidia within captivity. All other species have produced live glochidia, but fertilization may have occurred prior to capture. Development and testing of culture methods in ongoing with emphasis on water quality, diet and feeding, substrate, and metabolic and behavioral indices of unionid health.

Mississippi River Precision Brailing: Utilizing-GPS, GIS and the Internet [Poster]

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The U.S. Army Corps of Engineers requested a brail survey be performed within specific areas above and below Mississippi River Locks and Dams 20, 22, 24 and 25. Numerous areas were located in the middle of the river with few landmarks readily available. Corps' ArcView GIS base maps depicting specific areas to be surveyed were downloaded via the Internet. Navigational waypoints were selected from these base maps using GPS software. Navigation to brail transect start and end points was performed using a GPS datalogger receiving real-time data transmitted from U.S. Coast Guard radiobeacon transmitters. An on-board weatherized notebook computer displaying Corps base maps and brail transects, assured that specified areas were efficiently surveyed. In-field labor was greatly reduced by having the ability to precisely navigate to selected sites, as well as easily record sample locality data. Following field activities, positioning data were uploaded to a desktop computer and differentially corrected using GPS base files downloaded from available Internet sites. All files were converted to ArcView GIS format. Report maps and tables were generated using ArcView and electronic files were provided to the Corps for use in conjunction with their base maps during additional planning phases.

Reintroduction of Native Freshwater Mussels into a Recovering Stream Using Both *In Vivo* and *In Vitro* Propagation Techniques [JR]

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The release of partially-treated mine water from a coal outfall in southeastern Ohio in 1992 gave rise to a restoration project involving native freshwater mussels. The discharge comprised 100% of the instream flow in Leading Creek and as a result of elevated metal concentrations, conductivity and suspended solids and low pH, most of the indigenous biota including fish, benthos and molluscs, were depleted. Adult gravid mussels were shipped from the Ohio River basin to the Ecotoxicology Research Facility at Arkansas State University for the propagation of eight species. The species were chosen for their historical presence and abundance during the propagation efforts with the goal of reintroduction into the recovering creek. Mussels selected for transformation included *Lampsilis ventricosa* (Rafinesque, 1820), *Quadrula quadrula* (Rafinesque, 1820), *Lampsilis siliquoidea* (Barnes, 1823), *Leptodea fragilis* (Rafinesque, 1820),

Fusconaia flava (Rafinesque, 1820), *Pyganadon grandis* (Say, 1829), *Strophitus undulatus* (Say, 1817) and *Lasmigona complanata* (Barnes, 1823). Propagation techniques were established to transform glochidia to juvenile in two different media and using specific fish hosts. To date, four species have been successfully transformed and reintroduced into the recovering creek. In-situ chambers, containing transformed juveniles, have been placed throughout the creek at locations previously known to have viable mussel populations. These are being monitored for long-term survival in attempts to validate the success of these propagation techniques on future restoration projects and as part of this particular watershed enhancement plan.

Effects of Relocation on Physiological Condition of *Amblema plicata plicata* [MP]

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We evaluated the use of artificial ponds as temporary refugia for unionids threatened by zebra mussels. We removed 5 species of unionids from the Upper Mississippi River (UMR) and placed them into an experimental location in a hatchery pond and a control location in the UMR. In the experimental and control locations, we placed 24 unionids into one of four treatments (mesh bags, corrals, suspended trays, and benthic trays). To assess physiological condition, we annually measured glycogen concentrations in foot and mantle tissue and the tissue condition index (TCI; tissue dry mass/shell dry mass) on one *A. plicata* from each treatment and on an additional 10 *A. plicata* from an undisturbed location in the UMR (reference). Three years after relocation, mean glycogen concentrations (dry weight) in mantle tissue were similar in the reference (250 mg/g) and the control (262 mg/g), but were substantially lower in the hatchery pond (168 mg/g). In contrast, glycogen concentrations in foot tissue from the three locations did not differ. TCI was similar in mussels in the reference and the control (means of 4.2 and 4.1, respectively), but averaged only 2.8 in the hatchery pond. Furthermore, both glycogen and TCI varied among treatments in the experimental and control locations. These data suggest that the physiological condition of mussels was compromised when relocated into the hatchery pond. Thus, conservation efforts should focus on identifying the biological variables that are most critical not only to survival, but to maintenance of unionids in a healthy physiological condition.

Non-Destructive Glochidial Harvest and Artificial Transformation of *Lampsilis siliquoidea*: A Suitable Species for Glochidial and Juvenile Research [GT]

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Glochidia of the freshwater mussel *Lampsilis siliquoidea* (fatmucket) were transformed to juveniles using artificial culture techniques. This species is common in the midwest, and has not previously reported to have been transformed by artificial culture. Gravid females were collected locally from a small stream and maintained in flow-through tanks in well water until initiation of cultures. Females remained gravid in captivity for up to 6 months after collection. Non-destructive glochidial harvest was accomplished by flushing the marsupia with sterile filtered EPA reconstituted hard water. Glochidia were inoculated into sterile petri dishes with media consisting of Eagle's minimal essential medium, rabbit serum, antibiotics and serum replacements. Glochidia were incubated in a CO_2 atmosphere at room temperature. At the end of the transformation period, juveniles were removed from the media and rinsed in EPA reconstituted hard water. Viable juveniles were obtained from incubation periods of 11 days to 22 days, with peak transformation rates of up to 81% being from 14 to 18 days. Juveniles maintained in water for up to 19 days without feeding showed survival rates of at least 72%. These results indicate that *Lampsilis siliquoidea* is a suitable species for studies involving glochidia and juvenile mussels.

Digging Deeper to Solve the Missing Mussel Mystery [Poster]

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Abstract: Hundreds of shells of 7 common mussel species were found in riffles downstream of an industrial effluent in southwestern Michigan. No live individuals were found. Our initial hypothesis was that a toxic spill from the effluent extirpated the entire mussel community. However, no mussels - live or dead - were found upstream after many hours of searching at multiple sites. Among other things, the effluent increases the total stream discharge by as much as 45%; this input causes a significant improvement in habitat quality, improving substrate from 100% sand and silt upstream to large proportions of gravel and cobble downstream. Thus, our initial hypothesis might be supported if we assumed that upstream habitat was simply inappropriate for mussels. A catastrophic rainfall in June 1997 caused sand and silt to be scoured from an upstream site, deepening the channel by 1-2 feet, and exposing a gravel/cobble substrate littered with mussel shells. This suggests that some unknown factor further upstream caused the mussel community to be extirpated. Although we cannot determine what caused the mussels' demise, it is ironic that it took a point-source pollutant to improve habitat quality enough to reveal what a history of non-point source pollution had hidden.

The Survival and Growth of Freshwater Mussels in a Recirculating Aquaculture System [JR]

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An indoor recirculating aquaculture system was constructed to provide suitable conditions to culture juvenile freshwater mussels. In the first of three growth trials, *Villosa iris* juveniles were cultured for 22 wk, and grew from an initial mean length of 0.4 mm to a mean length of 2.7 mm. Overall, survival was 26.8%. In the second trial, growth and survival were compared between juveniles of *V. iris* held in sediment and without sediment. The initial mean length of both groups was 2.7 mm, and this study ran for 17 wk. The juvenile mussels in sediment grew to a mean length of 5.7 mm and had 85% survival, whereas those juveniles held without sediment grew to a mean length of 4.5 mm with 74% survival. A third trial monitored the growth and survival of two cohorts of juvenile *Lampsilis fasciola*. Both cohorts increased in length, from 1.1 mm and 1.4 mm to 3.3 mm and 4.1 mm, respectively. Survival for cohorts 1 and 2 was 78.7% and 64.5%, respectively. Additional experiments testing water hardness and organic content of sediments were completed to refine the most suitable conditions for juvenile mussel propagation. Results of all trials demonstrate that juvenile mussels can be reared successfully within recirculating systems. One of the factors deemed important in successful culture is continuous feeding of an appropriate food source. Regular cleaning of the system and water replacement also was important. Finally, the culture of juvenile mussels in sediments appears to be an important factor in ensuring good growth and survival. This phenomenon could be related to pedal feeding behavior, proper orientation of the mussels for filtering efficiency, or stability from physical disturbance.

Food Web Dynamics of Unionidae in a Canopied River and a Non-Canopied Lake [MP]

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Recently, we studied the food web dynamics of selected unionids colonizing a heavily- canopied river and a non-canopied lake. Using a combination of stable isotope analyses, gut content, and biochemical analyses of community components and unionid tissue, we examined the utilization

of food web constituents by unionids, and suggest key dietary components which appear to be critical for enhanced growth under captive management. Our unionids were omnivorous, feeding on algae, bacteria, detritus, and zooplankton, but the relative importance of each dietary component varied between species and sampling site. Proximate analysis showed both unionids and their food resources were high in protein (60-72%), low in water soluble vitamins, and contained more vitamin D than A. Lipid levels were low (7-12%), with cholesterol the dominant compound. Lake unionids contained less nutrients, and grew slower than river populations. All unionids contained high levels of the bacterially-derived vitamin B_{12}. Gut examination revealed no residential bacteria capable of digesting cellulose or chitin. Free-living cellulose and chitin degraders were found in the river unionids, but appear to be transients obtained from the environment. When feeding ceased during winter or the animals were kept in the laboratory for long periods of time, such bacteria disappeared. Lake animals rarely contained even transient cellulolytic or chitinolytic bacteria. Residential starch-degrading bacteria (cellobiose degraders) were found in all unionid species examined. Applications to captive management: Growth rates improved for our multi-specie population in the laboratory when cholesterol, vitamin D and bacterially-enriched detritus were added to the diet.

Feasibility of Using Microhabitat Selection to Provide *In situ* Protection of Unionid Populations from the Impact of Zebra Mussels [PO]

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Protecting unionid populations as zebra mussels spread into inland waterways has relied mainly on relocating at-risk animals into aquaculture facilities. While such relocations are the only viable management technique for some populations, facility availability is limited, leaving many unionids facing extirpation. We recently field-tested the use of microhabitat selection to provide *in-situ* protection for unionids from zebra mussel biofouling and food competition. Study sites include: small inland lakes, a small regulated river with both impoundments and free-running stretches, and shallow, soft-sediment estuaries. All sites contained 18-21 species of Unionidae and had been colonized by zebra mussels for at least 4 years. We found no suitable microhabitats in small lakes. Food resource depletion has been extensive enough to impair zebra mussel reproduction and body condition. The unionids are extirpated. Zebra mussel distribution in the regulated river is concentrated in upstream impoundments and just below low-head dams. The most effective technique at providing refugia has been to move unionids into the free-flowing river sections (downstream ~ 4km) or closer to the spillway of the low-head dam. Unionids in the free-flowing river do not become biofouled, but face future food limitations due to increasing zebra mussel densities upstream. Unionids near the spillway biofoul, but have first access to food supplies. Removal of encrusted zebra mussels by SCUBA divers once yearly improves unionid survival and has permitted unionid reproduction to continue. Estuaries and wetlands provide excellent *in-situ* protection for unionids; biofouling by zebra mussels rarely occurs, and food supplies, due to allochthonous input, remain substantial. Natural refugia for unionids can be found on site in many types of aquatic habitats. Such sites may be limited in size, and perhaps duration, depending on future zebra mussel population dynamics. However, they provide an additional management strategy for enhancing survival of unionid populations.

Reproductive Biology for Four Mussel Species of the Gulf Coastal Plain [Poster]

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A study on the reproductive biology of *Amblema neislerii*, *Elliptoideus sloatianus*, *Medionidus penicillatus*, and *Pleurobema pyriforme* was conducted from May 1995 to May 1997. These mussel species are currently proposed for federal endangered species status. Periods of gravidity and host fish were determined for each mussel species and glochidia morphology was evaluated

using a scanning electron microscope. *Amblema neislerii* are tachytictic breeders and mature glochidia were found in May. *Elliptoideus sloatianus* are tachytictic breeders and mature glochidia were found from late February to early April. *Medionidus penicillatus* are bradytictic breeders and mature glochidia were found in November and February to April. *Pleurobema pyriforme* are tachytictic breeders and mature glochidia were found from March to July. The following fish species served as hosts for *A. neislerii*: *Notropis texanus*, *Lepomis macrochirus*, *L. microlophus*, *Micropterus salmoides*, and *Percina nigrofasciata*. The following fish species served as hosts for *E. sloatianus*: *Gambusia holbrooki*, *Poecilia reticulata*, and *P. nigrofasciata*. The following fish species served as hosts for *M. penicillatus*: *G. holbrooki*, *P. reticulata*, *Etheostoma edwini*, and *P. nigrofasciata*. The following fish species served as hosts for *P. pyriforme*: *Pteronotropis hypselopterus*, *G. holbrooki*, and *P. reticulata*. Glochidia from *E. sloatianus*, a monotypic species, were morphologically similar to *Epioblasma* spp. glochidia. Glochidia from *A. neislerii*, *M. penicillatus*, and *P. pyriforme* were morphologically similar to the glochidia of their respected genera.

New or Confirmed Host Identifications for Ten Freshwater Mollusks [Poster]

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New or confirmed (*) host identifications were performed on 10 freshwater mussels. Fish species were infested with glochidia and monitored during laboratory experiments. Three methods were used: 1) pipetting of glochidia onto gill membranes; 2) feeding conglutinants to fish; 3) placing fish in buckets of water containing glochidia in suspension, agitated with airstones for 1 hour. Aquaria contents were sampled every other day and examined for untransformed glochidia and transformed juveniles under polarized light. Untransformed glochidia suggested unsuitable hosts while transformed juveniles indicated potential suitable hosts. Johnny darter, largemouth bass, bluegill, and white shiner were hosts for *Elliptio fischeriana*. Bluegill and shield darter were hosts for *Fusconaia masoni*. Silver shiner and creek chub were hosts for *Fusconaia flava*. Striped shiner, streamline chub, smallmouth bass (*), largemouth bass (*), green sunfish, bluebreast darter, greenside darter, rainbow darter, and yellow perch were hosts for *Villosa iris*. Bluntnose minnow, striped shiner, smallmouth bass (*), largemouth bass (*), bluegill (*) and longear sunfish were hosts for *Lampsilis radiata luteola*. Green sunfish and western banded killifish were hosts for *Lampsilis cardium*. Banded darter, bluebreast darter, brown trout and banded sculpin were hosts for the federally endangered *Epioblasma torulosa rangiana*. Longnose gar, largemouth bass, yellow perch, longear sunfish, bluegill, slenderhead darter and logperch were hosts for *Megalonaias nervosa*. Largemouth bass and bluegill were hosts for *Anodontoides ferussacianus*. Blackside darter, striped shiner and logperch were hosts for the federally endangered *Pleurobema clava*.

Feeding Interactions Between Native Freshwater Mussels (Bivalvia: Unionidae) and Zebra Mussels (*Dreissena polymorpha*) in the Ohio River [MP]

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The effects of zebra mussel infestation on the feeding of native unionids in the Ohio River were evaluated through gut content analysis. In 1996, mean algal cell numbers in guts of heavily infested and lightly infested *Amblema plicata* (5.7×10^5 vs. 9.1×10^5, respectively) were not significantly different ($p=0.17$). Heavily infested *A. plicata*, however, had significantly lower ($p<0.01$) mean ash-free dry weight (AFDW) in the guts (1.4 mg) than lightly infested specimens (4.6 mg). Mean algal cell number and AFDW in gut samples from heavily infested *Quadrula pustulosa* (1.8×10^4 and 0.6 mg AFDW, respectively) also were significantly lower ($p<0.05$) than that of lightly infested specimens (3.9×10^5 and 1.8 mg AFDW, respectively). In 1997,

significant reductions ($p<0.05$) in total algal cells and AFDW in gut samples again occurred for heavily vs. lightly infested *A. plicata* and *Q. pustulosa*. Also, the guts of all zebra mussels (18-33 mm in length) attached to individual unionids from Ohio River Mile 397 contained 50% more ($p<0.05$) cells (2.3×10^5 vs. $9\ 4 \times 10^4$, respectively) and AFDW (4.55 mg vs. 0.92 mg, respectively) than individual infested unionids. Algal taxa in the river at the sediment-water interface at each sample site were essentially the same as those in guts of unionids and zebras, confirming significant diet overlap. Thus, algal and detrital food resources in the guts of unionids are greatly reduced by the presence and feeding of zebra mussels. Reductions in unionid ingestion is likely a primary mechanism for reduced glycogen levels previously reported for heavily-infested and lightly infested unionids.

Ingestion and Assimilation of ^{14}C Labeled Algae by the Freshwater Mussel, *Villosa iris* (Lea, 1829) [MP]

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While some information exists on particle selection, filtration rates, and gut contents of unionids, no published information currently exists on the efficiency of unionid assimilation. In this study, assimilation efficiencies and carbon budgets were established for the rainbow mussel, *Villosa iris*, using radiolabelled cultures of *Neochloris oleoabundans*. Individual mussels were fed approximately 0.78 mg dry weight of ^{14}C labeled *N. oleoabundans* . At algal cell concentrations of 3.4 mg dry weight l^{-1} (1×10^5 cells ml^{-1}), individual mussels readily ingested ca. 35% (0.27 ± 0.21 mg) of the available carbon in two hours. Of the total carbon ingested, 35% was defecated (0.09 ± 0.07 mg), 8% excreted as waste (0.02 ± 0.03 mg), 14% respired (0.04 ± 0.03 mg) , and 43% incorporated into tissues (0.12 ± 0.09 mg). Consequently, mussels assimilated (incorporation + respiration) 57% of the ingested carbon. Thus, at relatively high cell concentrations, *N. oleoabundans* has high nutritional value as a food for maintaining fitness in captive freshwater mussels.

Glycogen Levels of Unionids During Starvation and Controlled Feeding in Quarantine [CC]

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During 1996, the effects of starvation during quarantine on the glycogen levels of *Amblema plicata* (Say, 1817) and *Quadrula pustulosa* (I. Lea, 1831) were evaluated. Significant declines in unionid glycogen stores were found after only 7 days of starvation and after 30 days, glycogen levels in *A. plicata* and *Q. pustulosa* (1.22 mg/g and 1.90 mg/g) were less than 70% of wild caught specimens (8.08 mg/g and 6.20 mg/g). Due to significant reductions in unionid glycogen stores, the quarantine period was repeated during 1997 with the addition of an algal feeding regime. Mussels were fed mixtures of *Neochloris oleoabundans* and *Scenedesmus* spp. twice a day at cell concentrations of 1×10^5 cells/ml (3 mg dry weight / l). With the addition of food, unionid glycogen levels after 7, 14, and 30 days of quarantine were not significantly different ($p>0.3$) from levels in the Ohio River. Thus, batch feeding of unionids may allow for the maintenance of glycogen stores during a 30 day quarantine period and subsequently improve the chances of survival after relocation. The large-scale relocation of unionids, however, would require the production of large amounts of algae. Therefore, methods to remove zebra mussels from the shells of unionids and shorten the required quarantine may be a more effective technique for reducing the effects of quarantine on unionid energy stores.

Extreme Variation in Survival Rates of Mussels During a 30-Day Quarantine Period [CC]

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To investigate factors that may affect survival of mussels during quarantine, we conducted five experiments, using the freshwater mussel *Fusconaia ebena*, between May and December of 1996. During most experiments, *F. ebena* were held for 30 days at densities of 50, 100, 150, 200, 250, and 300/760 L. Survival rates of these unionids varied among seasons. For instance, an inverse relationship between survival and density occurred in October and ranged from 32% in the highest density tank to 74% in the lowest density tank; however, survival in November was $\geq$ 99% for all densities tested. In 1997, we conducted three experiments using *F. ebena* held at densities of 50, 100, and 200/760 L. Two replicates of each density were used in these experiments conducted during late-spring, early-summer, and late-summer. The results of these experiments indicated that season had little effect on survival rates of unionids quarantined at low densities during 1997. For instance, survival was $\geq$ 88% for all replicates during each experiment. Conversely, *F. ebena* quarantined at high densities exhibited extreme variation between replicates; survival ranged from 41% to 91% during September for mussels quarantined at a density of 200/760 L.

Host Suitability Differences Among *Venustaconcha ellipsiformis* (Bivalvia: Unionidae) from Different River Drainage's [Poster]

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The glochidia larvae of unionid mussels are obligate parasites of fish. Little is known of the host-specificity of unionids. In particular, it is not known whether host requirements differ among closely related taxa. This information may be vital for introduction or reintroduction of unionids to natural habitats. Host fish suitability was compared among ellipse mussel (*Venustaconcha ellipsiformis*) from the Meramec River and Spring River (Arkansas River drainage) and Pleas' mussel (*Venustaconcha pleasii*) from the James River (White River drainage) in Missouri. Some authors consider these taxa to be subspecies. Test hosts were rainbow darters (*Etheostoma caeruleum*) from the James River. Fishes were infected by pipetting glochidia directly onto the gills. After infection, fishes were kept individually in 2-liter containers, which were drained through a filter every 2 days to recover detached glochidia and juveniles. Transformation success of Pleas' mussel on the sympatric rainbow darters was high and most attached glochidia transformed into juveniles. In contrast, transformation success of the ellipse mussel glochidia to juveniles on these fish was low. Ellipse juveniles were recovered from only 8 of 27 infected fish, and only 17 of 1560 attached glochidia transformed. These results suggest that closely related unionid taxa may exhibit different host specificity.

Survival of Juvenile Unionid Mussels Cultured Under Several Food and Water Regimes [JR]

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Juvenile mussel culture is a crucial link in the reestablishment and reintroduction of mussels. To date, the ability to raise juvenile unionid mussels in a laboratory setting has been met with only limited success. Several researchers have attempted to develop systems that will support juvenile mussels for six months to a year and maintain high survival rates (~50% or greater). We used a simple method that combines daily feeding, silt substrate, and flowing well water to culture five species of mussels, *Utterbackia imbecillis*, *Villosa villosa*, *Lampsilis teres*, *Lampsilis straminea claibornensis*, and *Epioblasma triquetra*. Several other test regimes were also considered, but

none were as successful as the well water, algae, and silt combination. Survival varied by species and among various food, substrate, and water combinations. Species survival vs habitat preferences and culture conditions will be discussed, as well as the potential of this method to produce large numbers of juvenile unionids for reintroduction or experimental purposes.

Ultrastructural Analysis of Changes in the Marsupial Demibranch During Brooding of Glochidia in *Pyganodon cataracta* and *Utterbackia imbecillis* (Bivalvia: Unionidae) [Poster]

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The life history of unionid mussels includes sequestration and brooding of glochidia larvae within the demibranchs of the gills. This arrangement has been suggested to facilitate nutrient transfer between parental mussels and developing glochidia. Transmission electron microscopy was used to examine the morphological relationships between the brood chamber of Pyganodon cataracta and Utterbackia imbecillis and their glochidia to determine whether the arrangement and composition of these tissues might support a nutrient transfer hypothesis. In early brooding, glochidia are surrounded by a vitelline membrane that contacts numerous cilia and microvilli of the epithelial cells lining the marsupium. This inter-lamellar tissue has large deposits of glycogen. The septum between the primary and secondary water tubes contains numerous mitochondria, and the surface facing the lumen of the secondary water tube has scattered microvilli. Later in brooding, the glochidia have lost the vitelline membrane and are not in close contact with the tissues of the marsupium. Glycogen in the inter-lamellar tissues becomes greatly reduced, and the cilia and microvilli lining the interior of the brood chamber are less numerous. In contrast to the brooding demibranch, the primary water tubes of the non-brooding inner demibranch are lined with a simple squamous epithelium with some microvilli. These observations may indicate nutrient transfer between a parental mussel and glochidia via glycogen from the inter-lamellar tissues and/or active transport by the septum of the secondary water tube.

Lure Behavior in *Toxolasma texasensis* [Poster]

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On June 19 and 20, 1997 three female Texas Liliput mussels (*Toxolasma texasensis*) were observed displaying worm-like lures in Bear Creek Ditch, a seasonally dry drainage ditch in southern Pope County, IL. The centimeter long worm-like lures were created by extending the caruncles, which exist along the lower margin of the female's mantle, into orange-brown structures the diameter and color of small earthworms. These lures were then waved wildly, apparently to attract potential fish hosts for its glochidia. To expose the lures the animals had taken sharp turns to the left while inverting their shells so that the umbo was deepest in the substrate and the ventral and posterior margins of the shell were exposed. After waving their lures for at least 30 minutes, they stopped and began making wave-like pulses along the edge of the mantle. These behaviors were observed between 2 and 4 PM central daylight time in the subdued light beneath a highway bridge. Efforts were made to stimulate visible glochidia releases by striking at a lure. Rapid closure of the valves at this and other times during lure display may have been associated with glochidial releases but this was not confirmed. The substrate was fine gravel to mud and the mussels were in only 1 to 2 cm of water at the edge of a deeper pool. The shallow water made it ideal for videotaping and photographing the behavior. No host fish were observed to be attracted to the lure but the stream did support *Lepomis cyanellus*, *Lepomis humilis*, *Gambusia affinis*, *Fundulus olivaceou*, *Semotilus atromaculatus*, *Cyprinella lutrensis*, *Pimephales notatus*, *Fundulus olivaceous*. Other unionids present were *Pyganodon grandis*, *Ligumia subrostrata* and *Uniomerus tetralasmus*. This poster will consist of both photographs and video of the lure display and associated movements.

Conservation of Threatened Mussel Species as a Multiple Stakeholder Process [PO]

U.S. Seal, Philip Miller, Onnie Byers, and Jenna Borovansky
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Protection and recovery of the great diversity of threatened mussel species in the United States present a tangle of problems stemming from the multiple use demands made upon their freshwater habitats, their diversity, the lack of knowledge of critical features of their reproduction and life history, the paucity of demographic information, and the uncertainties of their vulnerability to diverse threats. These problems are engaging the participation of people from diverse scientific disciplines who need to cross disciplinary boundaries to integrate their findings into testable management scenarios. The uses of these freshwater systems for power generation, for boating, fishing, drinking water, discharge of effluents from sewage plants, and agricultural run-off involve stakeholders with concerns about the impact of management proposals. CBSG Workshop processes, based upon biological and sociological science, provide an objective environment, expert knowledge, and a neutral facilitation process that supports sharing of available information across institutions and stakeholder groups, reaching agreement on the issues and available information, and then making practical management recommendations for the taxon and habitat system. The process has been successful in integrating previously unpublished information, using simulation modeling with VORTEX as one tool, for the decision making process. A successful workshop for the winged mapleleaf mussel (*Quadrula fragosa*), conducted in January 1998, is our first application of the multiple stakeholder PHVA process to a mussel species. The process, results, and an assessment of the participants' evaluation of the process will be presented.

Differential Sensitivity of Hooked and Hookless Glochidia to Chemical and Mechanical Stimuli [Poster]

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While the response of selected species of glochidia to pharmacological agents and inorganic salts has been fairly well studied, little is known of the chemo- and mechano-sensory stimuli that might relate to the attachment of larvae to a suitable host fish. Specificity in the site of attachment, with hookless glochidia generally encysting on gills and hooked forms attaching to fins or opercula, may also involve differential sensitivity to specific stimuli. To test this hypothesis, individual glochidia of *Utterbackia imbecillis* (hooked) and *Megalonaias nervosa* (hookless) were exposed to mucus of 3 species of fish, to fractionated mucus of bluegill, and to 3 putatitive mucous components. Larvae also were exposed to fish-conditioned water, to 2 fish pheromones and to 11 amino acids. The sensory hairs of mantle cells of gaping glochidia were mechanically stroked with a glass micropipet, both in artificial pond water and in 3 amino acids. The number of valve adductions min^{-1}, the time to on-set of tonic valve closure (or the number strokes leading to closure), and the duration of closure were monitored. All fish mucus induced tonic closure, with the duration being longer for *U. imbecillis*. Larvae only responded to the <3 kD fraction of bluegill mucus. Among all non-amino acids, only sialic acid induced rhythmic adduction, with *M. nervosa* being most responsive. *Megalonaias* was also more responsive to all amino acids, but significantly less so to mechanical stimulation. Both species exhibited increased sensitivity to physical stroking in the presence of each of 4 amino acids.

Investigations of the Byssal Gland in Juvenile Unionids [Poster]

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All bivalve mollusk families inhabiting North American fresh waters, including the exotic Corbiculidae and Dreissenidae, produce a byssal thread or threads in some or all species during at least part of their life cycles. Yet there is almost nothing known about the morphology of the

gland apparatus or the nature of the byssal thread. Concerning unionids, the production of a byssal thread during the post-metamorphic juvenile stage has been known for some time. It is assumed that as the animal grows, the byssal thread is cast off and the byssal gland becomes non-functional and eventually is resorbed by the animal's tissues. However, the biology of byssus formation in unionids, and unionoids in general, is virtually unknown and based on the most fragmentary evidence, not all unionids produce a byssal thread. A small collection of young (10 to 20 mm shell length) unionid specimens with or without a byssal thread has been investigated by dissection, histological methods for light microscopy, and with SEM to determine some aspects of the anatomy of the byssal gland apparatus and the byssal thread. Three species were examined, *Alasmidonta undulata, Elliptio complanata* and *Lampsilis radiata*. Preliminary findings from dissections indicate that in *E. complanata* and *L. radiata*, a gland is present in the visceral mass of the foot just posterior to the cerebral-pleural ganglia and extending from the gland is a "channel" coursing ventrally to the foot margin. A distinct groove is present along the ventral margin of the foot anterior to the opening of the "channel".

Growth and Survival of Juvenile and Adult Freshwater Mussels in the Partitioned Aquaculture System [CC]

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The Partitioned Aquaculture System (PAS), primarily designed for intensive catfish culture, utilizes separate channels for outdoor mass algal culture. This unique design incorporates mass algal production and flow regimes to remove nutrients. Two filter feeders, the Nile tilapia *Oreochromis niloticus* and the freshwater mussel *Elliptio complanata* were used to decrease algal cell retention times for maximization of oxygen production and nutrient removal. A total of 5,322 adult *E. complanata* were collected and stocked in nested baskets in the PAS starting June 1996. Despite high summer water temperatures (34°C), survival was estimated at 96.6% as of November 28, 1997. Mussel growth was demonstrated using juvenile *Utterbakia imbecillis* approximately 400 µm shell length (SL). Juveniles placed in a floating upwelling unit in the PAS on July 24, 1997 averaged 18.9 mm SL (14-25 mm SL) after 91 days. Juvenile survival over this period was 7.0% but research to decrease juvenile mortality is currently underway. The low adult mortality and rapid juvenile growth from this preliminary research suggests the PAS is a suitable freshwater mussel rearing facility.

Preliminary Studies on the Potential for Bacterial Pathogen Contagion Between Freshwater Mussels and Salmonid Fish [Poster]

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Over 70% of the approximately 300 species and subspecies of freshwater mussels native to the United States are categorized as endangered, threatened or of special concern. There are a number of factors contributing to the decline and include those that affect mussels and there fish hosts. In recent years a new threat to native populations in many large river systems has been the introduction of the zebra mussel*Dreissena polymorpha*. Because of the combined threats to native populations, effort was initiated in 1995 by the U.S. Fish and Wildlife Service and other federal, state and private partners to collect and hold native animals from large rivers in areas of zebra mussel infestation. These individuals would be maintained and propagated in some federal facilities, including fish hatcheries that rear salmonid fishes such as rainbow *Oncorhynchus*

mykiss and brook *Salvelinus fontinali* trout. With this relocation, questions arose regarding the potential for transmission of pathogenic microorganisms from mussels to fish, or fish to mussels. Our studies were initiated to evaluate and study the bacterial flora of common, native freshwater mussels collected from the Ohio River. Techniques of bacterial sampling are presented and tissues were used to inoculate a battery of general and specific media for isolation of salmonid fish pathogenic bacteria.

Fluorescence Techniques for Evaluating the Lipid Content of Larval and Juvenile Mussels [MP]

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Early growth and viability of juvenile unionid mussels is thought to depend upon the accumulation of neutral-lipid energy reserves. To test this hypothesis, the lipid-specific fluorophore nile red (NR) was used to document the concentration and distribution of lipid stores in glochidia and newly transformed juvenile mussels. When viewed under epifluorescence illumination (450-490 nm excitation), lipid droplets stained with NR fluoresce a bright yellow/orange, making them easy to detect and visualize through the thin, semi-transparent shells of larval and juvenile mussels. Digital image analysis was used to quantify the relative concentration of neutral lipid reserves and to determine the optimal concentration and time-course for staining. To examine the spatial distribution of lipid deposits, confocal microscopy was used to optically section and three-dimensionally reconstruct intact larvae and juveniles. In mature larvae, lipid droplets (2-8 µm diameter) were dispersed throughout the mantle, with the highest concentrations occurring in the lateral pit cells. Following transformation, lipid levels increased rapidly in developing juveniles and were concentrated in the foot, gills and ventral margin of the mantle. In subsequent experiments, NR was used to 1) document the accumulation of lipid deposits in glochidia during metamorphosis, 2) compare the lipid content of juveniles transformed using traditional and "lipid fortified" culture media, and 3) determine the effect of starvation on the lipid reserves of juveniles. Results of these studies indicate NR is a sensitive, inexpensive probe for assessing the lipid content of individual mussels and may serve as a valuable, non-destructive tool for evaluating the physiological condition of glochidia and the nutritional status of juveniles.

Design, Construction and Evaluation of a Laboratory-Scale Recirculating Aquaculture System for the Captive Care of Freshwater Mussels [CC]

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We constructed a closed, recirculating freshwater aquaculture system suitable for the long-term maintenance and quarantine of adult unionid mussels in captivity. The system was designed 1) to accommodate mussels with different habitat requirements, 2) to enable strict control of environmental conditions and prevent the accumulation of debris and metabolic wastes, 3) to include several tanks that could serve as independent experimental units (replicates) in laboratory studies, and 4) to incorporate integrated filtration and feeding systems to help minimize the time required for daily maintenance and care. The system consists of two duplicate units, each witheighteen 38 L rectangular glass tanks. Conditions within each tank, including substrate, temperature, water level and flow rate, can be adjusted independently. Water draining from the tanks empties into a 170 L insulated holding tank and is filtered by a 120 L min^{-1} modular system equipped with mechanical and chemical filters and an ultraviolet sterilizer. Nitrogenous wastes are removed using a trickle-style biological filter, and temperature within each unit is controlled ($\pm$ 0.5 °C) using a flow-through chiller unit. Water quality parameters, including pH, temperature, and oxidation-reduction potential (ORP), are monitored continuously and logged to a remote computer. An automatic feeder is used to dose the system with concentrated suspensions of live and spray-dried algae at programmed intervals. The system is capable of holding up to 1000 mussels and has been used to maintain populations of *Elliptio complanata* for >5 months

with 100% survival. A smaller-scale intermittent-flow system employing many of the same design features and components is currently being evaluated for culturing juvenile mussels.

Use of Condition Indices, Protein Biomarkers, and RNA:DNA Ratios for Detecting Nutritional Stress in Freshwater Unionid Mussels [MP]

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With the growing concern for the protection of North American unionid mussels and the increased acceptance of captive rearing and relocation programs as methods for preventing the extirpation of threatened and endangered species, there is an immediate need to develop sensitive, non-destructive methods for assessing the physiological condition and health of existing populations. Although numerous methods have been developed for evaluating the nutritive condition of bivalve molluscs, especially commercially important marine species, their application to unionid mussel conservation efforts has been limited. Using *Elliptio complanata* as a model, we determined the impact of starvation on the physiological condition of adult mussels and investigated the sensitivity of several common morphological and biochemical condition indices for detecting nutritional stress in captive mussels. To assess the effects of starvation on physiological condition, we compared the nucleic acid, glycogen, lipid, protein, carbohydrate and organic content of the mantle tissue of mussels maintained on one of three feeding regimes: (1) starved, (2) fed (*i.e.*, fed mixed algal cultures daily), and (3) partially fed (*i.e.* fed mixed algal cultures on alternate days). The biochemical composition of the mantle was also compared to more traditional condition indices based upon soft-tissue weight, shell weight, and shell cavity volume. In general, biochemical indices were better predictors of physiological condition and feeding regime than gross morphological indices based upon tissue mass and shell size. Although glycogen, lipid, protein and carbohydrate levels declined rapidly in starved mussels, RNA:DNA ratios in the mantle tissue were the most sensitive indicators of nutritive stress. Throughout the experiment, protein and lipid levels and RNA:DNA ratios of starved mussels were significantly lower than in fed and partially fed mussels, but glycogen levels among the three treatments were highly variable and did not display a consistent pattern. These results suggest that glycogen may not be reliable indicator of nutritional stress in unionid mussels.

Freshwater Mussel (*Elliptio complanata*) Movement and Condition with Relation to Temperature, Flow, and Substrate Type [Poster]

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The extent of movement patterns of fresh water mussels is believed to be based on variables such as water quality, flow rates, substrate type, temperature, and the availability of food. In this study, movement and condition of individual *Elliptio complanata* is being monitored in controlled environments of 2 x 8 foot rectangular tanks. Substrates of sand, gravel, and silt were introduced individually among 8 tanks at approximately 12" depths. On site reservoirs provided water and a natural food base. Water is pumped to the tanks on a flow through basis to mimic natural stream conditions. Forty five animals were randomly distributed to each of the eight tanks. All the mussels received two Hallprint Shellfish tags,(Hallprint Pty. LTD.) one adhered directly to the left valve of the mussel, and the other attached to a 35 cm section of (Courtland intermediate sink Rocket tapered) fly line. The adhesive medium used was (Krazy Glue brand) cyanoacrylate. Each tank was divided into nine equal quadrant units, and flows taken in the center of each unit, for the assessment of horizontal movement. The above mentioned fly line is used in the determination of vertical positioning within substrates with little disturbance to individual mussels. Temperature and dissolved oxygen are monitored daily for animal health and their relationship to possible movement patterns. This poster illustrates the design and structure of rectangular tank facilities

with the intent of natural stream assimilation, and it's effects on fresh water mussel movement and condition. Preliminary comparisons of four substrates, flow rates, and temperatures will be estimated among tanks.

Conservation of Unionid Mussels in the St. Croix River: Development *In Situ* Refugia [Poster]

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Relocation of native mussels to refugia is one mechanism for protecting unionid populations at risk from colonization by the zebra mussel *Dreissena polymorpha*. In 1996, we established a system-specific *in situ* mussel refugia in the St. Croix National Riverway. Our specific objective was to evaluate survival, growth and reproduction of mussels between the refuge and a source-site control. Three species of unionids (*Quadrula pustulosa*, *Elliptio dilatata*, and *Lampsilis higginsi*) were collected from the lower St. Croix River and relocated to three 5 x 5 m study grids, two in the refuge and one at the source site. The refuge site is located upstream of a navigation control point, which was established to restrict passage of boats that have been in zebra mussel infested waters. The source site is located below the control point in an area with a rich and diverse unionid fauna. In June 1997, we evaluated mussel survival, growth, and reproduction and substrate characteristics at the refuge and source sites. We found that overall survival and recovery of mussels at the refuge and control site were very high. Total recovery of all species at the three sites was 82-86%; total survival of recaptured animals was 96-99%. However, survival of mussels at the source site was lower in areas with shifting sand substrate. *Lampsilis higginsi* mussels showed evidence of reproduction in both areas as 28-57% of females were gravid. Monitoring will continue for at least two years to determine the long-term success of relocated mussels in the refugia.

Glochidial Release as a Function of Water Temperature [CC]

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The timing of glochidial release in five species of freshwater mussels were compared to water temperature. The mussels were *Amblema plicata*, *Elliptio dilatata*, *Lampsilis radiata luteola*, *Leptodea fragilis*, and *Pyganodon grandis*. Mussels were maintained in outdoor enclosures at ~40° North latitude. Each species released glochidia at different temperatures, such that some were winter-releasers and others were summer-releasers. These did not necessarily correspond to the categories of "bradytictic" and "tachytictic" brooders, as widely used in the literature. Species considered "bradytictic" were actually winter-releasers, apparently overwintering their glochidia on the host, rather than brooding them in the marsupia. The applicability of the terms "bradytictic" and "tachytictic" to freshwater mussels must be reevaluated. Circumstantial evidence was found that *Lampsilis radiata luteola* and *Elliptio dilatata* spawn twice per year, resulting in a summer and a winter brood. *Amblema plicata* released at 23°C (July); *Elliptio dilatata* at 4 and 18°C (November and August); *Lampsilis radiata luteola* nearly year-round, but primarily from May through October, with a peak release at 19°C; *Leptodea fragilis* at 11°C (September through November); and *Pyganodon grandis* at 4°C (October through February).

Logistic Considerations in the Relocation of Unionids into Artificial Refugia [CC]

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In 1995, we began an experimental unionid relocation project to identify conservation strategies for unionid populations imperiled by zebra mussels in the Upper Mississippi River. Before beginning the relocation process, it was necessary to develop methods for collecting, screening, quarantining, handling, transferring, and maintaining unionids subjected to relocation into artificial refugia. Our criteria for this methodology were that it would (1) produce a large supply of disease-free and zebra mussel-free unionids, (2) minimize handling and transportation stress of unionids, (3) ensure that unionids could be recovered, individually identified, and returned to the relocation environment over the long term (> 5 years), (4) be easily implemented and cost effective, and (5) provide a suitable habitat, including necessary physical and nutritional resources for the maintenance of physiological condition. Through initial planning and critical evaluation of annual recovery efforts, the methods we developed satisfied the first four relocation requirements. However, our data suggests that the fifth criterion, the maintenance of physiological condition in unionids, was not met at the end of three years. An evaluation of water and sediment quality data suggests that inadequate nutritional resources likely limited the physiological condition of unionids. Thus, a greater understanding of the nutritional requirements of unionids may be required to ensure their well-being and survival after relocation into artificial environments.

Survival and Growth of Hatchery-Reared Juvenile *Lampsilis cardium* [JR]

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Recovery of threatened and endangered freshwater mussel species may require large scale propagation of juvenile mussels for reintroduction into areas where populations are low or have been extirpated. The small size (<0.3 mm) of juvenile mussels at excystment makes them vulnerable to the environment. Therefore, it is important not only to produce large numbers of juvenile mussels, but also to rear them to a size that will ensure high survival following stocking. In April 1996, 64 fish of three known host species were infested with glochidia of *L. cardium*; 5 were held in laboratory aquaria and the remaining fish were released into a raceway at the Minor E. Clark Fish Hatchery. Juvenile mussels produced in the laboratory were transferred to petri dishes filled with substrate and suspended in a wire cage in the raceway. Survival was assessed at 60, 90, and 120 days by removing one or two petri dishes and examining the contents under a dissecting microscope (10X). Survival declined from 17% at 60 days to 3.5% at 120 days. However, mean length increased from 1.88 mm to 18.2 mm during the same period. We quantitatively sampled the raceway in October 1996 and estimated the population of juveniles to be 874 ± 387 with a mean length of 21.5 mm. One year later, we recovered 760 ± 300 juveniles with a mean length of 54.5 mm.

Life history Studies of the Squawfoot Mussel *Strophitus undulatus* in the Piscataquog River Watershed, New Hampshire

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Strophitus undulatus, considered a species at risk, occurs scattered in low numbers in the Piscataquog River Watershed. A long term brooder, *S. undulatus* releases white, vermiform conglutinates with attached glochidia. Stream drift studies show that conglutinate release begins in mid-April and continues through mid-June. We observed laboratory fish actively attacking freshly released conglutinates, mouthing the conglutinate repeatedly before rejecting it as food; glochidia may attach to epithelia of the mouth and gills at this time. Eventually the conglutinate degrades, freeing glochidia that are available to attach to fins and soft outer tissues of benthic species. This two-tiered strategy would increase the probability of host infestation by initially targeting fish feeding in the water column, subsequently targeting benthic feeders/nesters. Using laboratory infestation experiments we identified 4 host fish species: longnose dace (Rhinichthys cataractae), fallfish (*Semotilus corporalis*). golden shiner (*Notemigonus crysoleucas*), slimy sculpin (*Cottus cognatus*). In addition the larvae (but not adults) of the northern two-lined salamander (*Eurycea bislineata*) served as a suitable host species for *S. undulatus*; this salamander inhabits the same streams as *S. undulatus*. Laboratory experiments show that stream drift captured glochidia successfully attach to host fish, encyst, metamorphose then are released as juveniles. We documented morphology and behavior of both glochidia and juveniles using video microscopy and SEM.

Program Code:

[CC] Captive Care and Maintenance of Adult Mussels
[GT] Glochidia Transformation Techniques/Methods
[JR] Juvenile Rearing, Propagation and Reintroduction
[MP] Mussel Physiology and Nutrition
[PO] Public Outreach and Conservation Programs
[Poster] Poster Session and Reception