COPE

Triannual Unionid Report

Report No. 7

July 1995

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

Compiled by

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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. The submissions were not edited. They were copied as received and assembled into the report.

NOTE: If you are receiving duplicate mailings, have a change of address, no longer wish to receive this report, or know someone who would like to be added to our mailing list, please contact Sherrie Jager at the above address or phone 704/665-1195, Ext. 221

Submission for July Triannual Unionid Report

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Workshop on Protocal for Relocation of Unionid Mussels

Relocating mussels continues to be an uncertain and controversial procedure that would benefit from the development of standardized peer reviewed guidelines. The process of developing these guidelines includes receiving comments from the 'experts'. The upcoming meeting on the Conservation and Management of Mussels; Initiatives for the Future in St. Louis, Missouri is an excellent opportunity to take advantage of the collected house of experts to discuss, review, and develop guidelines for relocation procedures and practices.

A Workshop on Relocation Protocals will be conducted on Monday October, 16, 1995 tentatively scheduled from 5pm to 6pm, after the presentations of that day. Please bring your experiences, expertise, any existing procedures, guidelines, protocals, or methodologies for and relocating mussels that you have been involved with or are aware of, as well as opinions and sage advise.

The information gained from this pool of expertise will greatly enhance the development of relocation procedures. Although we would all like to see mussels not be relocated, the future of relocating mussels is here to stay.

For further information or questions please contact;

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A program has been initiated to further develop artificial medium for glochidia culture. The initial objective is to generate methods to produce glochidia from the same species year-round through controlled reproduction, hopefully on a planned schedule. The second phase is to develop a more universally applicable media, starting with the techniques developed by Anne Keller, then testing other commercial media, solid media, and metamorphosis inducers. The third objective is to better define the relationship between the glochidia and host fish, particularly the nutrition gained upon initial attachment and during the subsequent encystment period.

At present we are working with a group of 480 *Elliptio complanata* taken from Pine Creek, Ansonia, Tioga County, PA. Elliptio's were chosen because they are a common short-term breeder, thus we felt they would be easier to control than a long-term breeder. Mussels were collected in late December 1994 when temperature (0.5°C) and photoperiod were at a seasonal low. They were housed in groups of 60 within eight 4 ft diameter round fiberglass tanks containing 25 cm of sand/gravel substrate. Each tank has individual temperature and photoperiod control and mussels are fed daily algae obtained from a concrete pond. Four environmental treatments are being applied to 2 replicate tanks of mussels. In the first treatment, photoperiod and temperature follow natural conditions for the river from which the mussels were collected. The second and third treatments have seasonal delays of 6 and 12 weeks inserted on January 1. The fourth treatment follows natural conditions except the winter low temperature was 10°C opposed to 0.5°C. Observations on reproductive activity are recorded daily, and all mussels are counted and aggregational behavior recorded weekly.

Females from the first and fourth treatment began releasing conglutinates at approximately 15°C. Initial conglutinates contained undeveloped glochidia, but subsequent releases were fully developed with glochidia still packaged within an intact conglutinate. Later glochidia were released after the conglutinate had deteriorated into a mucus-like strand. Mussels on the 6-week winter delay have just begun releasing premature glochidia, essentially on the predicted schedule.

No evidence of male sperm release has been detected since the mussels were captured. However, aggregation increased as temperature and photoperiod increased, and a certain behavior in which mussels align siphon-to-siphon (aperture-to-aperture?) was particularly evident in the weeks prior to initial glochidia release. On many occasions, a mussel would travel throughout much of the tank, encountering numerous other mussels before stopping and aligning with the final mussel on its' journey. This has led to the suspicion that the mussels are selectively mating and passing sperm directly between pairs. This same activity has now been observed in both *Strophitus undulatus* and *Alasmidonta undulata* several weeks after completion of glochidia release.

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Shinyrayed Pocketbook (*Lampsilis subangulata*) Confirmed As Superconglutinate Producer

Cooleewahee Creek is a high quality tributary to the Flint River in Baker County, Georgia (Apalachicola River system). During a 23 May 1995 foray to Cooleewahee Creek to collect gravid mussels for my (CO) master's research project at the University of Florida, the NBS crew (including Andre Daniels) made a significant discovery. After having worked the site for a few hours, we found four superconglutinate strands snagged on woody debris in the low, clear water. A superconglutinate consists of a double row of conglutinates approximately 4-5 cm long that is expelled through the excurrent siphon and tethered to the female mussel by a transparent mucous strand up to 2.5 m long. The superconglutinate resembles a small fish in shape, size, color, and acts like a fishing lure in the way it gyrates at the end of the strand. The fish-like appearance and motions of this prey mimic are thought to be an extraordinary method of attracting a potential piscivorous host fish. Once detached from the female, the strand becomes snagged around rocks or branches in the stream and the prey mimic is suspended in the water column where it continues to resemble a minnow. When a piscivorous fish attempts to consume the prey mimic, glochidia are released into the mouth of the deceived predator.

Heretofore, the only mussels associated with superconglutinates were two allopatric species, the federally threatened orange-nacre mucket (*Lampsilis perovalis*), a Mobile Basin endemic, and the candidate southern sandshell (*Lampsilis australis*), restricted to the Choctawhatchee, Yellow, and Escambia river systems. We predicted that the superconglutinates found in Cooleewahee Creek were produced by the federally proposed endangered shinyrayed pocketbook (*Lampsilis subangulata*). A subsequent NBS trip on 1 June 1995 verified our assumptions: specimens of shinyrayed pocketbook were found in the process of producing superconglutinates. Using an underwater video camera, a female shinyrayed pocketbook was recorded releasing a 44 cm long superconglutinate strand over a 4-hour period at 23.5 °C water.

Several gravid shinyrayed pocketbooks were collected on 23 May 1995 for observation at the NBS laboratory facility. On 22 June 1995 three of the five specimens collected released superconglutinates in a 22 °C current tank within a 24-hour period. Video footage documented one of the shinyrayed pocketbooks releasing a 4.5 cm superconglutinate tethered by a 71 cm strand. Twenty-four hours later, the two other females released superconglutinates 4.5 and 4.2 cm long tethered by strands 37 and 33 cm long, respectively.

Host fishes for the shinyrayed pocketbook are unknown. As part of my master's project, I used glochidia collected from the shinyrayed pocketbook to infest six species of fish from four families in an effort to identify appropriate host fishes. Centrarchids are assumed to be host fish for this species; while filming the release of a superconglutinate in Cooleewahee

Creek, several sunfish attempted to strike at the superconglutinate. A poster with video footage of superconglutinates will be presented at the October 1995 Upper Mississippi River Conservation Committee Symposium in St. Louis, Missouri.

Wendell Haag (U.S. Forest Service, Oxford, Mississippi), RSB, and Paul Hartfield (U.S. Fish and Wildlife Service, Jackson, Mississippi) have authored a manuscript on the description of this phenomenal discovery which has been accepted by Freshwater Biology. Randy Hoeh (Dalhousie University, Halifax, Nova Scotia) is investigating the molecular genetic relationships of the three species known to produce superconglutinates along with another suspected superconglutinate producer, the federally threatened finelined pocketbook (*Lampsilis altilis*), also a Mobile Basin endemic. The evolutionary significance of superconglutinates is intriguing with the placement of an entire years reproductive effort in a single package that so cannily resembles a fish. In addition, the development of a superconglutinate as a highly specialized mechanism used to attract potential host fishes is an indicator that freshwater mussels and fishes have undergone co-evolution. Equally significant, but highly disturbing, is the fact that known superconglutinate producers are imperilled species with shrinking ranges. High levels of turbidity during the spawning season have made this ingenious fishing lure's success in attracting the proper fish host increasingly problematic. These factors have no doubt contributed to the need for these species Federal protection.

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One focus of our research is on the conservation biology and systematics of unionids using molecular genetic techniques. Presently, one of us (CL) is completing two projects done in collaboration with Dr. Margaret Mulvey and others (Savannah River Ecology Lab, Drawer E, Aiken, SC 29802). The first project is on the systematics of North American unionacean genera as inferred from 16S rRNA DNA sequences, and the second is the conservation genetics of two unionid genera (*Megalonaias* and *Amblema*). Both manuscripts will be submitted this summer.

Recently, we have begun a study examining genetic differentiation within and among the genera *Potamilus* and *Lastena*, with an emphasis on the federally threatened species, *Potamilus inflatus*. In addition, KR has begun a study to determine the host of *Potamilus inflatus*.

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Anodonta woodiana (Lea, 1834) in Costa Rica

In the Triannual Unionid Report No. 6 of February, 1995, G. Thomas Watters reported that Anodonta woodiana had become established in the Dominican Republic. On March 24, 1994, I collected A. woodiana from Laguna de Arenal (Lake Arenal) at San Luis, Costa Rica. I spoke to a biologist who had studied the fish of Lake Arenal and he said that there were no mussels in the lake before the tilapia were introduced. It seems likely that A. woodiana will follow other tilapia introductions.

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Chemosensory Abilities of Female Freshwater Mussels and Glochidia (Unionidae)

Behavioral responses in gravid *Lampsilis fasciola* and *Villosa iris* indicate their ability to distinguish host fish (*Micropterus dolomieu*) from non-host fish (*Cyprinus carpio*), and their mucus. Behavioral observations of adult mussels included degree of mantle presentation, pulse rate, glochidial ejection, shell spread, and inhalant aperture length. Measurements associated with these observations were used to create a composite behavioral index. Whereas *L. fasciola* was more active with exposure to host fish and mucus, behavioral responses decreased with exposure to non-host fish and mucus. Also, activity levels were higher with exposure to host fish than to their mucus. Similar behavioral responses were noted with *V. iris*. *Lampsilis fasciola* was found to be more active during the day, whereas *V. iris* was more active at night.

Glochidia of *V. iris* were tested for valve closure time and percentage of total glochidia closed after one minute exposure to components of host fish and non-host fish mucus and blood. Fibrinogen was found to induce the strongest responses in glochidia.

6/30/95

Status Survey on three endemic fresh-water mussels found in the Altamaha River System

This report summarizes the portion of the survey completed at the end October, 1993. The status of the following three mussels is being examined; Alasmidonta arcula (Lea, 1838), the Altamaha arcmussel; Elliptio spinosa (Lea, 1836), the Georgia spinymussel; and Elliptio sheparidana (Lea, 1834), the Altamaha lance.

A. The first field season began on June 30, 1993 and ended on October 23, 1993.

1. Surveyed for the target species at 131 different stations. One site was repeated in the Ohoopee River.

Altamaha River----93 stations
Ocmulgee River----19 stations
Oconee River-------5 stations
Ohoopee River------4 stations (One repeated)
Little Ocmulgee River System---10 stations

2. Examined 180 sites, many stations were subdivided by different habitats.

3. Conducted timed searches at 150 different sites.

4. Found 15 species of mussels, 1 species of clam and 8 species of gastropods.

Summary of Observations for the Target Species and other Endemic Mussels

Species	--Observed Alive- Number	Percent	---Shell Only--- Number	Percent	--Total Counted- Number	Percent	----Stations---- Number	Percent
Elliptio hopetonensis (Altamaha slabshell)	4,985	49.2%	1,419	56.2%	6,404	50.6%	109	82.5%
Elliptio dariensis (Georgia elephantear)	548	5.4%	143	5.7%	691	5.5%	76	57.5%
Elliptio spinosa (Altamaha spinymussel)	41	0.4%	53	2.1%	94	0.7%	27	20.6%
Elliptio shepardiana (Altamaha lance)	1,309	12.9%	104	7.4%	1,413	11.2%	95	72.5%
Alasmidonta arcula (Altamaha arcmussel)	117	1.2%	9	0.4%	126	1.0%	43	32.8%
Pyganodon gibbosa (inflated floater)	86	0.8%	31	1.2%	117	0.9%	29	22.1%
Lampsilis dolabraeformis (Altamaha pocketbook)	853	8.4%	453	18.0%	1,306	10.3%	83	62.9%
Totals of Species Listed	7,939	78.4%	2,212	87.7%	10,151	80.3%		
Totals for all Observations made in 1993	10,125		2,523		12,648		132	

B. The second field season (1994) was interrupted by tropical storm "Alberto". "Alberto" elevated all the streams and swamps in the Altamaha River system to flood levels and they remained high for the rest of the field season. I attempted to make some observations on the Altamaha River in September, but they were left incomplete because of high water (7.7 feet at the U.S. 301 bridge).

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New publications:

Watters, G. T. 1994. North American Freshwater Mussels. Part 2. **American Conchologist** 22(3): 11-13.

This paper was published in the bulletin of the Conchologists of America as part two of a popular introduction to unionids.

Watters, G. T. 1994. Function and form of unionoidean shell shape and sculpture. **American Malacological Bulletin** 11: 1-20.

The functions of Recent and fossil unionoidean sculpture are proposed in light of similar theories on the function of marine bivalve sculpture. Functional models are given for shell morphologies for both soft and hard substrata. Most soft substratum taxa have shells of reduced thickness and dentition, are laterally compressed, and generally are sculptureless. These characteristics minimize the specific gravity of soft substratum unionoideans. Sculptured taxa generally are found in hard substrata in large rivers. Shell sculpture is derived from an ancestral divaricate pattern, and has been modified into the spectrum of unionoidean sculpture found in Recent and fossil species. Shell sculpture in this group is modified for anchoring and anti-scouring functions. Burrowing sculpture, found in many marine bivalves, may not occur in unionoideans, but was exapted from ancestral burrowing sculpture for other roles. Big river taxa have evolved mechanisms for remaining buried, while headwater species have emphasized the ability to rebury if dislodged. It is proposed that unsculptured big river taxa evolved in headwater situations and reinvaded large rivers with alternate methods to facilitate anchoring and reduce scour. These methods form the morphological facies known as the "Big River Effect."

Addenda and errata available for *Annotated Bibliography*

Free on request to the author, updated periodically.

New hosts for *Anodontoides ferussacianus* (Lea, 1834)

Gravid *Anodontoides ferussacianus* were collected in the Little Scioto River in southern Ohio in September, 1994. Largemouth bass and bluegill were obtained from the Hebron State Fish

Hatchery. Both fish species had been raised in a mussel-free enclosure. Specimens of mussels and fishes were held in flow-through 10 gallon tanks at 15°C. Fish were anaesthetized with MS222 prior to infestation. Glochidia were removed by pasteur pipette and placed on the gills of both fish species. Metamorphosed juveniles appeared between 21-25 days post infestation from both largemouth bass and bluegill. The glochidia-filled mucus strands released by *Anodontoides ferussacianus* reported by Hove et al, 1995 (Triannual Unionid Report 6) also were seen in these specimens.

Distribution and Population Structure of Mussel Beds in the Flambeau River, Wisconsin

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The overall purpose of this study was to provide a quantitative assessment of areas of high unionid density and define the physical boundaries of the beds within a portion of the Flambeau River, Wisconsin. Parameters such as total area and location of the mussel beds, species richness, distribution, diversity, individual and overall densities, recruitment, species associations, and substrate type were determined for four mussel beds along a 13 km reach of river. A systematic sampling design was used by a diver in SCUBA which determined the boundary and as a result the total area of the bed by sampling at one meter intervals through the bed until three quadrats in a row had less than three unionids per 0.25 m^2, signifying the edge of the unionid bed. For each unionid bed, species association analyses were conducted using 2 x 2 contingency tables with no significant associations found. Chi-square tests of species distribution revealed the species were distributed evenly throughout all the beds regardless of substrate type or depth. However, the distribution of unionid densities (without respect to species) within the beds and their relation to substrate type was statistically significant for three of the four unionid beds. Among the beds the lowest densities occurred in a sand gravel substrate probably because of its shifting properties subjecting unionids to an unstable substrate. Overall, the highest densities occurred in a sand, gravel, or combination of sand gravel substrate with a boulder combination of 33-50%.

This study establishes baseline data on the community composition within these well defined mussel beds which will be used by myself as part of a follow up study I will be conducting this summer. Last Fall (1994) the Flambeau River was subjected to a 200-300 year flood event and I plan on returning to these mussel beds to see how the shape, size, and community composition has changed. This is of particular concern because two state endangered species, *Cyclonaias tuberculata* and *Plethobasus cyphyus* and a species of special concern, *Pleurobema coccineum* were all found within the mussel beds.

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Cooperative conservation plan for the freshwater mussel fauna in the Ohio River as a result of the invasion of zebra mussels (*Dreissena polymorpha*).

The globally significant freshwater mussel diversity of the Ohio River and its tributaries is currently critically threatened by the invasion of the exotic zebra mussel. Zebra mussels were first found in the lower Ohio River in 1991. By 1994, zebra mussel densities were immense in the lower 1/2 of the River (every native unionid observed in the lower river was infested with zebra mussels) and low densities were observed in the upper river. The immediate threat posed by the zebra mussel led to efforts by concerned natural resource management agencies, researchers, and organizations to cooperatively develop an action plan to preserve and manage the diverse unionid fauna of the Ohio River.

Approximately 40 surveys were sent out to those who may be experiencing, or may soon experience, the zebra mussel invasion in the Ohio River. The very high survey return rate of 87% showed strong interest in this issue. We proceeded to coordinate a meeting to develop a strategic action plan. There was a lot of interest in this issue and keeping the number of attendees at a manageable level was a task in itself. We developed a database which includes everyone who has expressed interest in participating in this effort (approximately 60 contacts); they will receive minutes from meetings, schedules of activities, and will participate in finalizing the plan and other potential activities as the plan is implemented. (contact Cindy Chaffee at the above address if you want to be added to the data base).

On April 4 & 5, 1995, a meeting was held in Columbus, Ohio to discuss freshwater mussel management in the Ohio River as a result of the zebra mussel threat. The meeting involved multiple states bordering the Ohio River, FWS (ES) field offices in each bordering state (involving 3 FWS regions), Ohio River Islands National Wildlife Refuge (ORINWR), National Biological Service (NBS) researchers active in this issue, U.S. Geolocial Survey (USGS), Ohio River Valley Water Sanitation Commission (ORSANCO), and Sea Grant. Our meeting was a success. In approximately 10 hours we were able to develop the 1st phase of a plan that included: 1) monitoring activities, 2) specific rescue protocols, 3) addressing tributary issues, 4) listing research requirements, and 5) outreach for education and support for our project. Each topic was assigned a contact person who will continue to coordinate the components of their assigned topic and will report back to the group at the next meeting.

Activities are already underway: monitoring protocols and standardized zebra mussel monitoring forms have been developed (contact Patricia Morrison 304-422-0752, monitoring coordinator), monitoring surveys have begun, rescue and transportation protocols for protected species have been developed, a salvage permit has been established to rescue Federally protected species, research is ongoing, as well as outreach efforts.

This cooperative effort takes an ecosystem approach to develop and implement a conservation plan in an effort to prevent a wave of extirpations. The results and implications of this management strategy will have wide application to other watersheds throughout the country as we battle the zebra mussel invasion and try to secure a future for native mussels.

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QUALIFIED MUSSEL SURVEYORS SOUGHT

Two federally-listed endangered mussel species, the clubshell mussel (*Pleurobema clava*) and northern riffleshell mussel (*Epioblasma torulosa rangiana*) are known to occur in northwestern Pennsylvania. Because several projects are and will continue to be proposed in streams occupied by these species, the U.S. Fish and Wildlife Service, pursuant to Section 7 of the Federal Endangered Species Act, has been requesting that project proponents/permit applicants conduct mussel surveys in these streams to determine whether or not endangered mussels may be affected by project activities. Project applicants often seek our input regarding qualified surveyors, however, at this time, we know of relatively few people qualified to do such surveys and willing to do so as a contractor. Note that the U.S. Fish and Wildlife Service does not endorse any individuals or companies, rather, we provide a list of highly qualified surveyors to project applicants/proponents to assist them in making contact with appropriate professional services. If you are qualified (see below) and interested in doing mussel survey contract work in Pennsylvania, please call me at the above number.

Qualifications

Due to the high degree of skill required in locating and correctly identifying freshwater mussels, the following are considered minimum requirements for contractors interested in surveying for federally-listed mussel species:

* Extensive field experience conducting surveys for (searching for and finding) native freshwater Unionid mussels, using a variety of techniques, including snorkeling, raking, viewing with glass bottom bucket, and searches of muskrat middens. Experience in conducting diving surveys using air support (e.g., SCUBA) is especially sought;

* Skill in identifying various ages of native freshwater mussels to species in the field, based on external shell morphology.

* Experience in identifying potentially suitable habitat for federally-listed endangered mussels found in Pennsylvania, based on substrate characteristics, flow, etc.

* Persons performing surveys for freshwater mussels are required to possess a valid Pennsylvania Fish and Boat Commission Scientific Collector's Permit. This permit is required to collect spent shells of endangered mussels, and also to remove live endangered mussels from the substrate, identify them, and return them in a life position to the substrate.

CLAM BAKE

Where: North Fork Holston River - eastern Tennessee, southwest Virginia
When: August 1 - 15, 1995

The U. S. Geological Survey, Upper Tennessee River Study Unit will be evaluating mussel transplants made into the North Fork Holston River back in the mid-1970s. We will be looking for adult survival and reproduction of 16 mussel species at five sites that were translocated from the Clinch. Since a number of individuals have expressed an interest in participating in this project, I have decided to also survey the whole river. The North Fork is a beautifull stream that is still in the process of recovery because of pollution problems near Saltville, Virginia. Individuals will need to bring mask, snorkel, wet suit or shortie, knee pads, and dive gloves (because of glass) for those that want to help looking for live mussels in the water. A water scope would be helpfull if you have access to one. I will also need help searching the streambanks for fresh dead and relict specimens in muskrat middens. Water should be very warm so swim suit or waders are fine and wading shoes (no bare feet). If you have mesh collecting bags bring them along. Bring your fishing pole if you feel up to it in the evenings. Smallmouth fishing is great. Plan to have some fun because biology is fun!

Motel Reservations can be made at: Days Inn - Downtown Kingsport, 805 Lynn Garden Drive, Kingsport, TN 37660, Phone (615) 246-7126, located on State Route 36 North. Gov't rate $30-33

The Holiday Inn (which will change to Quality Inn) is located across the street, 700 Lynn Garden Drive, Kingsport, TN 37660, Phone (615) 247-3133, Gov't rate $38.

We will leave at 8:00 AM on August 1 (Tuesday) from the parking lot of the Days Inn to start work. Those that can make it on Monday (July 31), we will leave from Days Inn at 1:00 PM. Plan to bring your own lunch and drinks each day. Supermarket is across the street. We will eventually move upriver to Abington, Virginia, (Empire Motor Lodge, 703 - 628-7131). If you need more information please call me at (615) 632-4167.

Steve Ahlstedt

Lampsilis higginsi
(Lea, 1857)

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1 May 1995

Triannual Unionid Newsletter readers:

All of us have been involved in discussions about unionid translocations, either as project mitigation, or to offset effects of Dreissena polymorpha (Pallas, 1771). A letter sent 15 years ago is presented (below) to stimulate discussion in this forum. Won't you please write your "horror story" so that all may benefit?

This will be a hot topic in St. Louis, October 1995, but there may not be time to hear all of the undocumented tales, or to be made aware of experiences with limited distribution. Thank you.

==========

23 January 1981

Dr. D. H. Stansbery
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Dear Dave:

Just a note to document the following events. On Tuesday, 18 November 1980, around 1:00 p.m., I collected (at the water's edge) a live Lampsilis ventricosa (Barnes, 1823) in Hunter's Channel, SW of Prairie du Chien, WI, Mississippi River Mile 632.3 (MEH:1980:89). Air temperature was 30 to 35° F. The same day at 2:00 p.m. I collected a live Elliptio dilatata (Rafinesque, 1820) from the water's edge of the East Channel below Prairie du Chien, WI, Mississippi R.M. 633.4 on the N end of Indian Isle (Bergman Island) (MEH:1980:90). Both specimens were probably disturbed by barge fleeting activities.

Each live specimen was put into a dry plastic bag along with dead shells collected at each site. These 2 specimens sat on the floor of my house (temperature probably around 64° F) for 1 1/2 weeks as I completely forgot about the two live specimens out of water. While preparing shells for shipment to OSUMZ I discovered the Lampsilis ventricosa closed tightly, but alive, on the evening of 28 November 1980, 10 days after collection. I put the specimen in my one gallon aquarium (tap water), and by the next morning the specimen had dug into the sand substrate and was siphoning.

On 29 November 1980, 11 days after collection, I found the Elliptio dilatata still alive, and also put it in the aquarium. This specimen didn't respond as quickly, but after 3 or 4 days it too had dug into the sand substrate and was siphoning. As of this date, over two months after collection, both specimens are still alive in my aquarium and apparently doing well. Amazing!

Marian E Havlik

Sincerely, Marian E. Havlik

United States Department of the Interior

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Aquatic Ecology Laboratory
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March 13, 1995

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Dear Colleague:

This letter is to update you on what has been happening in the field of freshwater mussels at the Leetown Science Center (LSC) since last summer.

Questionnaire Results. I would first like to thank everyone for taking the time to respond to the questionnaire on forming a freshwater molluscan group. We received 98 questionnaires with 85% seeing a need to formalize in some manner. Seventy-seven percent wanted to pursue the subgroup possibility with the North American Benthological Society (NABS) prior to creating a separate society. A letter has been sent to the president of NABS formalizing our request to the Executive Committee to permit the formation of a freshwater mollusk subgroup or section under NABS. The request was sent in early December and we are awaiting a response.

Genetic Repository of Freshwater Mussel Tissue. The LSC Genetics Tissue Repository (GTR) catalogued its first mussel (an *Elliptio complanata*) on July 1, 1995. Since that initial entry, 253 mussels representing 44 species, have been incorporated into the GTR. Five tissues (foot, adductor muscle, gill including glochidia, mantle, and digestive gland) were taken from live or fresh dead mussels . Each tissue was divided into two equal portions; one submerged in a homogenizing/preserving buffer and frozen at -40 C for protein analysis and the other in 95% ethanol, refrigerated for 24 hours, and shelved for DNA analysis. Shells were initially placed in buffered formalin and then transferred to 70% isopropanol for storage. A repository for preserved shells has not been identified.

If you have field sampling capabilities, please examine the accompanying database report (Enclosure 1) to determine if you can contribute a new species or can add a sample from a new watershed for an existing species. The GTR data collection sheet (Enclosure 2) should be used when contributing to the repository; the requested information is vital to the GTR.

LSC Genetics Research. The need for genetics and systematics information to augment freshwater mussel conservation efforts was highlighted at the Freshwater Mussel Workshop hosted by the LSC on May 11-12, 1994. This forum was also used by the Fish and Wildlife Service (FWS) to identify and prioritize listing and recovery activities for freshwater mussels in FY95. FWS identified an acute need for information on the population structures of the dwarf wedge mussel *(Alasmidonta heterodon)* and the green floater (*Lasmigona subviridis*). Subsequently, the FWS asked LSC's Aquatic Ecology Laboratory for assistance in assessing the genetic structure of geographic populations throughout the range of these rare species. A proposal has been submitted to NBS to fund research to determine population structure of these two species.

Research has begun on the development of genetic-based techniques for identification of cryptic species. Emphasis has been placed on techniques which allow minimally invasive sampling (i.e., a snip of foot tissue). Mussels of the *Elliptio* and *Lampsilis* genera are being examined for species specific banding patterns using: 1) general proteins analyzed by isoelectric focusing, 2) mitochondrial DNA haplotypes generated by restriction enzyme digests (RFLPs) of polymerase chain reaction (PCR) amplified 12SRNA, 16SRNA, and cytochrome oxidase I regions, and 3) phenotypes generated from RFLP analysis of the PCR amplified internal transcribed spacer region (ITS1) between 18S and 5.8S ribosomal DNA genes. Preliminary results suggest the variation identified in ITS1 and in general proteins will allow delineation of closely related species.

Holding Riverine Mussels in Ponds. With the zebra mussel rapidly expanding its range up the Ohio River, the NBS is funding a new cooperative project with the Leetown Science Center, Dick Neves at the Virginia Cooperative Research Unit, the Ohio River Islands National Wildlife Refuge, and the West Virginia DNR to collect and transfer representative numbers of mussel species at greatest risk from the refuge to .25-acre ponds at Leetown. This project will evaluate survival of large river animals in culture ponds, develop a non-invasive method to monitor physical condition, and determine whether gravidity and spawning are feasible in pond environments. We expect to have mussels in the ponds this summer.

Evaluating Sampling Designs. We will be evaluating a multi-stage sampling design for estimating mussel population density in the Cacapon River watershed in West Virginia. The design is an extension of double sampling for stratification. The stages of sampling are streams within a watershed, stream reaches within a stream, and line transects or plots within a stream reach. A distinctive feature of the design is the use of a rapid assesssment at the stream reach level to stratify reaches into density categories. For higher density strata, all or most reaches are sampled intensively; however, few of the lower density reaches are sampled further. As a result, the time spent sampling where mussels are at low density or absent is minimized. An added advantage is that sampling method used within a reach (as long as it is unbiased) can depend on strata, thereby increasing the flexibility and efficiency of the design. Average estimates within each strata and stream will be combined using appropriate weighting to arrive at an estimate of density within a watershed.

To facilitate future communications, please provide me with your EMAIL address as soon as possible. If you have any questions about any of the above mentioned research, or about sending mussel tissue for the repository, contact either Rita Villella at FTS (700)925-5322 or Comm. (304)725-8461, extension 322, or Tim King at FTS (700)925-5381 or 5278, or Comm. (304)725-8461, extension 381 or 278.

Sincerely,

Rita Villella

Rita Villella

3/10/95

FRESHWATER MUSSEL RESPOSITORY DATA BASE REPORT

Species Code	Common Name	Scientific Name	Collection County	Collection State	Total Number
20101	mucket	Actinonaias ligamentina	Kanawha	WV	1
20204	elktoe	Alasmidonta marginata	Pocahontas	WV	1
20209	triangle floater	Alasmidonta undulata	Knox	ME	3
20209	triangle floater	Alasmidonta undulata	Penobscot	ME	1
20210	brook floater	Alasmidonta varicosa	Knox	ME	1
20303	threeridge	Amblema plicata plicata	Kanawha	WV	1
20303	threeridge	Amblema plicata plicata	Lewis	WV	3
20303	threeridge	Amblema plicata plicata	Vanderburgh	IN	15
21301	butterfly	Ellipsaria lineolata	Vanderburgh	IN	4
21408	eastern elliptio	Elliptio complanata	Culpepper	VA	1
21408	eastern elliptio	Elliptio complanata	Dinwiddle	VA	2
21408	eastern elliptio	Elliptio complanata	King William	VA	2
21408	eastern elliptio	Elliptio complanata	Mercer	NJ	3
21408	eastern elliptio	Elliptio complanata	Montgomery	MD	6
21408	eastern elliptio	Elliptio complanata	Penobscot	ME	2
21408	eastern elliptio	Elliptio complanata	Prince William	VA	2
21408	eastern elliptio	Elliptio complanata	Spotsylvania	VA	5
21408	eastern elliptio	Elliptio complanata	Washington	MD	5
21410	elephantear	Elliptio crassidens	Kanawha	WV	1
21412	spike	Elliptio dilatata	Cherokee	KS	5
21412	spike	Elliptio dilatata	Kanawha	WV	1
21412	spike	Elliptio dilatata	Pocahontas	WV	1
21414	northern lance	Elliptio fisheriana	Fauquier	VA	1
21418	variable spike	Elliptio icterina	Dinwiddle	VA	2
21418	variable spike	Elliptio icterina	Nottoway	VA	2
21421	yellow lance	Elliptio lanceolata	Fauquier	VA	1
21425	Atlantic spike	Elliptio producta	Culpeper	VA	1
21425	Atlantic spike	Elliptio producta	Prince William	VA	1
21425	Atlantic spike	Elliptio producta	Washington	MD	1
21708	Wabash pigtoe	Fusconaia flava	Cherokee	KS	5
21708	Wabash pigtoe	Fusconaia flava	Vanderburgh	IN	2
21712	longsolid	Fusconaia subrotunda	Kanawha	WV	1
22105	plain pocketbook	Lampsilis cardium	Kanawha	WV	1
22106	yellow lampmussel	Lampsilis cariosa	Hampshire	WV	4
22106	yellow lampmussel	Lampsilis cariosa	Knox	ME	5
22106	yellow lampmussel	Lampsilis cariosa	Mercer	NJ	5
22106	yellow lampmussel	Lampsilis cariosa	Schoharie	NY	1
22106	yellow lampmussel	Lampsilis cariosa	Washington	MD	2
22108	wavyrayed lampmussel	Lampsilis fasciola	Kanawha	WV	1
22114	pocketbook	Lampsilis ovata	Cherokee	KS	5
22119	Neosho mucket	Lampsilis rafinesqueana	Cherokee	KS	4
22124	fatmucket	Lampsilis siliquoidea	Harrison	WV	1
22124	fatmucket	Lampsilis siliquoidea	Lewis	WV	3
22202	white heelsplitter	Lasmigona complanata complanata	Cherokee	KS	3
22204	flutedshell	Lasmigona costata	Cherokee	KS	2
22204	flutedshell	Lasmigona costata	Harrison	WV	1
22204	flutedshell	Lasmigona costata	Lewis	WV	2
22204	flutedshell	Lasmigona costata	Wood	WV	1
22207	green floater	Lasmigona subviridis	Huntington	NJ	4
22207	green floater	Lasmigona subviridis	Pocahontas	WV	4
22207	green floater	Lasmigona subviridis	Pocahontus	WV	3
22207	green floater	Lasmigona subviridis	Wake	NC	7

Species Code	Common Name	Scientific Name	Collection County	Collection State	Total Number
22403	tidewater mucket	Leptodea ochracca	Knox	ME	3
22602	black sandshell	Ligumia recta	Kanawha	WV	1
22602	black sandshell	Ligumia recta	Vanderburgh	IN	4
22802	washboard	Megalonaias nervosa	Vanderburgh	IN	2
22901	threehorn wartyback	Obliquaria reflexa	Vanderburgh	IN	11
23005	round hickorynut	Obovaria subrotunda	Kanawha	WV	1
23005	round hickorynut	Obovaria subrotunda	Lewis	WV	2
23407	James spinymussel	Pleurobema collina	Monroe	WV	1
23408	Ohio pigtoe	Pleurobema cordatum	Vanderburgh	IN	7
23427	round pigtoe	Pleurobema sintoxia	Cherokee	KS	3
23601	pink heelsplitter	Potamilus alatus	Vanderburgh	IN	4
23701	kidneyshell	Ptychobranchus fasciolaris	Kanawha	WV	1
23704	Ouachita kidneyshell	Ptychobranchus occidentalis	Cherokee	KS	4
23801	eastern floater	Pyganodon cataracta	Culpeper	VA	1
23801	eastern floater	Pyganodon cataracta	Knox	ME	1
23801	eastern floater	Pyganodon cataracta	Penobscot	ME	1
23801	eastern floater	Pyganodon cataracta	Prince William	VA	1
23801	eastern floater	Pyganodon cataracta	Washington	MD	1
23910	monkeyface	Quadrula metanevra	Vanderburgh	IN	5
23911	wartyback	Quadrula nodulata	Vanderburgh	IN	10
23914	pimpleback	Quadrula pustulosa pustulosa	Cherokee	KS	5
23914	pimpleback	Quadrula pustulosa pustulosa	Vanderburgh	IN	5
23915	mapleleaf	Quadrula quadrula	Cherokee	KS	4
23915	mapleleaf	Quadrula quadrula	Vanderburgh	IN	9
24203	squawfoot	Strophitus undulatus	Hampshire	WV	5
24203	squawfoot	Strophitus undulatus	Lewis	WV	1
24203	squawfoot	Strophitus undulatus	Monroe	WV	1
24203	squawfoot	Strophitus undulatus	Prince William	VA	1
24203	squawfoot	Strophitus undulatus	Spotsylvania	VA	1
24203	squawfoot	Strophitus undulatus	Tucker	WV	4
24401	pistolgrip	Tritogonia verrucosa	Cherokee	KS	3
24401	pistolgrip	Tritogonia verrucosa	Vanderburgh	IN	4
24908	rainbow	Villosa iris	Lewis	WV	2
24908	rainbow	Villosa iris	Scott	VA	1
24921	unspecified	Villosa	Scott	VA	1

FRESHWATER MUSSEL COLLECTION DATA SHEET

FORM NUMBER_____
(TO BE ASSIGNED AT LAB e.g. YY###)

Species Name: _____ _____ Collection Date: _____
(COMMON) (GENUS, SPECIES, SUBSPECIES)

Number Collected: ____ Collector: _____ Collectors Sample ID: _____
(First , Middle Initial and Last Name) (Optional)

Collection Site Description: (i.e., directions and landmarks) _____

_____

_____

Habitat type: Riffle ☐ Substrate type: Silt ☐ Sand ☐ Gravel (25-75 mm) ☐ Small Cobble (76-150 mm) ☐
(Predominant) Pool ☐ (Predominant) Large cobble (151-300 mm) ☐ Boulder (>300 mm) ☐

Specific Stream/Reach Name: _____ River: _____

Major River Drainage: _____ Final Destination: _____
(Atlantic Ocean, Gulf of Mexico, Great Lakes etc,)

Collection City: _____ County: _____ State: _____

Comments: _____

_____

******* COMPLETE UPPER PORTION OF FORM AND RETURN IT AND SPECIMEN(S) TO TIM KING, LEETOWN SCIENCE CENTER, *******
AQUATIC ECOLOGY LABORATORY, 1700 LEETOWN ROAD, KEARNEYSVILLE, WV 25430

THIS SECTION TO BE COMPLETED IN THE LABORATORY

STORAGE METHOD (F=Frozen*, E=Ethanol, B=Both) _____

Species Code: _____

Sample Number_____ Date Received_____ Date Processed_____ Foot____ Muscle____ Digestive Gland____ Gill____ Glochidia____ Mantle____

Sample Number_____ Date Received_____ Date Processed_____ Foot____ Muscle____ Digestive Gland____ Gill____ Glochidia____ Mantle____

Sample Number_____ Date Received_____ Date Processed_____ Foot____ Muscle____ Digestive Gland____ Gill____ Glochidia____ Mantle____

Sample Number_____ Date Received_____ Date Processed_____ Foot____ Muscle____ Digestive Gland____ Gill____ Glochidia____ Mantle____

COMMENTS: (Processed by) _____ (Data Entered by) _____

_____

_____

*Frozen specimens stored in Selander (1972) homogenizing buffer unless otherwise noted.

LABORATORY CONTINUATION SHEET FOR FORM NUMBER________

Species Code:______________________________

			STORAGE METHOD (F=Freezer*, E=Ethanol, B=Both)					
Sample Number________	Date Received__________	Date Processed__________	Foot____	Muscle____	Digestive Gland____	Gill____	Glochidia____	Mantle____
Sample Number________	Date Received__________	Date Processed__________	Foot____	Muscle____	Digestive Gland____	Gill____	Glochidia____	Mantle____
Sample Number________	Date Received__________	Date Processed__________	Foot____	Muscle____	Digestive Gland____	Gill____	Glochidia____	Mantle____
Sample Number________	Date Received__________	Date Processed__________	Foot____	Muscle____	Digestive Gland____	Gill____	Glochidia____	Mantle____
Sample Number________	Date Received__________	Date Processed__________	Foot____	Muscle____	Digestive Gland____	Gill____	Glochidia____	Mantle____
Sample Number________	Date Received__________	Date Processed__________	Foot____	Muscle____	Digestive Gland____	Gill____	Glochidia____	Mantle____
Sample Number________	Date Received__________	Date Processed__________	Foot____	Muscle____	Digestive Gland____	Gill____	Glochidia____	Mantle____
Sample Number________	Date Received__________	Date Processed__________	Foot____	Muscle____	Digestive Gland____	Gill____	Glochidia____	Mantle____

COMMENTS:(Processed by)______________________(Data Entered by)______________________________

____

____

*Frozen specimens stored in Selander (1972) homogenizing buffer unless otherwise noted.

Author(s): Jonathan W. Burress and Richard Neves
Address(s): National Biological Service
Virginia Cooperative Fish and Wildlife Research Unit
Department of Fisheries and Wildlife Sciences
Virginia Tech
Blacksburg, VA 24061-0321 (703) 231-5927

USE OF PONDS AS A REFUGIA FOR ADULT FRESHWATER MUSSELS

Freshwater mussels of 15 species were collected during 1992-1994. These adults were placed in ponds at 4 study sites throughout Virginia. This report represents survival data collected for holding periods of 2-29 months at these various locations.

Reynolds Homestead (Critz): Mussels collected from the Tennessee, Cumberland ,New and Hazel rivers are held in suspended cages (1m x 1m x 0.5m) in a 0.25 acre pond. Mussels are held within 100 mm plastic sleeves hung horizontally from the cage tops.

After 29 months, survival in the pond was 71% overall. There were significant differences in percent survival among the species held. *Elliptio* spp. exhibited a high survival ($\geq$ 77%), whereas *Pleurobema cordatum* and *Lampsilis ovata* was much lower at 54% and 14%, respectively. We suspect that low alkalinity (17 mg/L) of the pond may be a limiting factor for some species. Dissolution of the umbonal region of valves of *L. ovata* was evident after several months, and likely contributed to the mortality of this species.

Marion Fish Hatchery: In 1993, large raceways were made available for this project. The raceways were approximately 75 ft. x 30 ft. x 3 ft. (water depth). These raceways were concurrently being used for trout production. Mussels collected from the Tennessee River were held within cages placed on the bottom of the raceway. Mussels were held within plastic sleeves and placed unrestricted on the cage bottoms.

After 14 months, survival in the raceways was 30% and 5% overall for the sleeve and unrestricted holding methods, respectively. We suspect that the deposition of particulates derived from unconsumed trout pellets and fish wastes contributed to the low survival at this location.

Prices Fork Pond: A lined irrigation pond (36 m x 36 m) located at the Prices Fork Research Station, Blacksburg, VA., was obtained during 1993. Mussels collected from the Tennessee and Cumberland rivers were held within suspended cages in the pond. Mussels were held as described above.

After 7 months, survival was good, with most species exhibiting a range of 70% to 95% survival. During the summer months the water temperature reached 31° C. Following this temperature rise was an almost complete die-off of all adult mussels. *Lasmigona complanata* and *Megalonaias nervosa* were the only species to demonstrate an initial resistance to this temperature increase. After 9 months, the percent survival for these two species are 44% and 14%, respectively.

Hoge's Pond: During the fall of 1994 a 3 acre pond was obtained in Blacksburg, VA. Mussels from Kentucky Lake were held unrestricted within suspended cages in the pond.

Survival after 2 months, was 100% for the four species of mussels held. Survival was checked during early January, 1995 with ice cover of 1-2 inches, which was broken to obtain these results.

Braven Beaty and Dick Neves
Virginia Coop Unit, Virginia Tech, Blacksburg, VA 24061
(703) 231-5927

Attempts to culture juvenile mussels at the Clinch River Steam Plant, Carbo, VA

Trials were conducted again this year to test the feasibility of rearing juvenile mussels in an artificial stream channel at the Clinch River Power Plant in Carbo, VA. Transformed juveniles of *Villosa iris* were held in small (4 inch diameter round or 3 inch square) plastic containers in the troughs in either small (<120 µm) or large (120-600 µm) size sediment. Two sediment depths were used for each sediment size, 5 mm and 20 mm.

The first batch of juveniles placed in the artificial stream were delivered on June 22, 1994. The entire group consisted of 11 containers with 100 juveniles in each one. They remained in the troughs until October 12, a total of 112 days. These animals had a low survival rate, ranging from 0% to 17% live animals in October. Only three of the containers had more than 1 live animal at the end of the experiment (17, 6, and 2). The mean surface area of these animals was only 1.87 mm^2, significantly less than the 8.9 mm^2 for juveniles held about 20 days longer in 1993.

The second batch of juveniles was placed in the artificial stream on September 2 and 9. These animals were left in the artificial stream until December 11, a total of 92 days. Survival of this batch of animals was even worse, with no more than 3 live animals found in any container of 100. They also did not grow very much during this time. None of the containers showed growth of more than 50% of the initial size. The valves left by dead animals were readily found and allowed the accounting of approximately half the animals (range of 33 - 173 valves or 16 -86 animals worth). The presence of so many shells indicates that predation was not the cause of death for most of the juveniles.

These results tell us that juvenile mussels did not grow appreciably after the first part of September in the channel fed by natural Clinch River water. Therefore, to successfully rear juvenile mussels it is imperative to get the transformed juveniles by early summer. We are now planning to investigate what parameters are important for the growth and survival of juvenile mussels by conducting experiments under more controlled conditions, in parallel with the rearing in the artificial stream channel.

One other item of interest was noted during this experiment. While a substantial number of asian clams were found in the experimental containers in the 1993 season, none were found in the containers held in the fall of 1994. This could be due to the later date at which the experiment was begun or to some other unknown event or condition which prevented asian clams from successfully settling in containers this year.

M. Christopher Barnhart, Andrew D. Roberts and Ashley P. Farnsworth

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FISH HOSTS OF FOUR UNIONIDS FROM MISSOURI AND KANSAS

We made field observations of reproductive status and performed laboratory tests to determine potential fish hosts of *Ptychobranchus occidentalis* (Ouachita kidneyshell), *Lampsilis reeviana brevicula* (broken rays), *L. rafinesqueana* (Neosho mucket), and *Anodonta suborbiculata* (flat floater). Kidneyshells in the North Fork of the White River (Douglas Co. MO) released mimetic glochidial packets resembling larval fish between March 6 and April 8. Transformation of glochidia to juveniles occurred in 26-31 days at 21°C on darters (*Etheostoma blennoides, E. juliae, E. caeruleum*). Broken rays in White River tributaries displayed the mantle lure between April 10 and August 8. Transformation occurred in 22-34 days at 21°C on smallmouth bass (*Micropterus dolomieui*), green sunfish, (*Lepomis cyanellus*), and banded sculpin (*Cottus carolinae*). Neosho muckets in the Elk River (McDonald Co. MO) displayed the mantle lure in July and August (previous reports of display in September and October). Transformation occurred in 27 days at 21°C on *M. dolomieui* and *M. salmoides*. Flat floaters (Marais des Cygnes drainage, Linn Co. KS) released glochidia between December 19 and February 25. Transformation occurred in 51-63 days at 10°C on golden shiners (*Notemigonus crysoleucus*), warmouth (*Lepomis gulosus*), white crappie (*Pomoxis annularis*), and *M. salmoides*; transformation was generally unsuccessful at 21°C.

(abstract for report at 1995 UMRCC symposium: Conservation and Management of Freshwater Mussels)

"EDUCATION IS OUR BUSINESS" The Alabama Cooperative Extension Service

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DEPARTMENT OF FISHERIES & ALLIED AQUACULTURES

Memorandum

TO: Alabama/Mississippi Zebra Mussel Network

FROM: Richard (Rick) K. Wallace, Extension Marine Specialist
Auburn University Marine Extension & Research Center
E-Mail: rwallace@acenet.auburn.edu

DATE: June 27, 1995

SUBJECT: **ZEBRA MUSSEL REPORTS**

We are somewhat amazed (concerned?) that we haven't heard of any new zebra mussel sightings in Alabama or Mississippi. Have any zebra mussels been found outside of the Tennessee River System in Alabama or Mississippi this summer? If you know of any official (or even unofficial) sightings, please let us know so that we can alert the rest of the network. Given the experience in Louisiana, it seems unlikely that zebra mussels have not spread further into Mississippi and Alabama.

A revised Alabama/Mississippi Zebra Mussel Network roster is enclosed for your information. If you have any changes, please let us know.

RKW/kjb

NOTE: REVISED LIST IS NOT ENCLOSED. FOR REVISED LIST PLEASE CONTACT THE ABOVE LISTED ADDRESS.

AN ABSTRACT OF A THESIS

IMMUNOSUPPRESSION OF NONHOST FISH SPECIES AND ITS EFFECT ON GLOCHIDIAL METAMORPHOSIS

Sheila G. Kirk

Master of Science in Biology

Intraperitoneal implants of cortisol suspended in liquid cocoa butter were administered to nonhost fish species. Fish were then infested with glochidia of freshwater mussels to determine if inducement of transformation on nonhost species was possible after immune system manipulation.

Glochidia from *Venustaconcha sima* transformed on orangethroat darters (*Etheostoma spectabile*) after injection of cortisol at concentrations of 0.005, 0.010, 0.020, and 0.040 mg cortisol per gram of fish weight. Juvenile mussels were collected from orangethroat darters from experiments conducted between late March and July; however, no juveniles were collected from experimental fish during August through February. Creek chubs (*Semotilus atromaculatus*) similarly treated failed to produce juveniles of *V. sima* or *Villosa taeniata*. Banded sculpins (*Cottus carolinae*) transformed glochidia of *V. taeniata* after injections of cortisol at concentrations of 0.005, 0.010, and 0.020 mg/g. Juveniles were collected from experiments that began in November and June. No juveniles were collected from sham injected fish during any trial.

Results of some experiments suggest that cortisol-induced immunosuppression can facilitate metamorphosis of glochidia of freshwater mussels on nonhost fish species. However, a seasonal as well as a species-specific effect possibly exists. Orangethroat darters transformed glochidia during the spring and early summer only. Banded sculpins transformed fewer juveniles than orangethroat darters during the winter and early summer. Cortisol concentrations tested had no effect on creek chubs.

Tennessee Cooperative Fishery Research Unit
Tennessee Technological University
PO Box 5114
Cookeville, Tennessee 38505

REGULATED RIVERS: RESEARCH & MANAGEMENT, VOL. 10, 000–000 (1995)

MICROHABITAT USE BY FRESHWATER MUSSELS AND RECOMMENDATIONS FOR DETERMINING THEIR INSTREAM FLOW NEEDS

JAMES B. LAYZER AND LESA M. MADISON

National Biological Service, Tennessee Technological University, PO Box 5114, Cookeville, TN 38505, USA

ABSTRACT

A conventional application of the instream flow incremental methodology (IFIM) assumes that target organisms have specific microhabitat preferences and the ability to move to areas of suitable hydraulic conditions in response to changes in stream discharge. We investigated the use of the IFIM for determining the instream flow needs of a diverse mussel assemblage in Horse Lick Creek, a fourth-order stream in the upper Cumberland River drainage in Kentucky. We determined habitat availability by measuring water depth, velocity and substrate at 60 cm intervals along 23 transects during low, medium and high flows. The distribution of mussels within the study site was highly contagious. Although habitat suitability curves developed from data collected on 2004 mussels indicated a clear preference for particular hydraulic conditions, the limited mobility of mussels in the coarse substrate of Horse Lick Creek implies that these curves are flow-conditional—that is, mussels appear to prefer different hydraulic conditions at different stream discharges. Consequently, these curves are of limited value for determining conservation flows for mussels. Nonetheless, water depth and velocity were important factors limiting the distribution of mussels during base flow periods. Similarly, substrate characteristics were of limited value in defining mussel distributions; unfractured bedrock excluded mussels from portions of the study site, but mussels did not utilize all areas with preferred substrate. Because the larvae (glochidia) of mussels in Horse Lick Creek are obligate parasites on fish, data were also collected on habitat preferences of the host fishes. These data were incorporated in the physical habitat simulation system (PHABSIM) to determine the relationship between the availability of host fish habitat and stream discharge during periods of glochidia release and juvenile settlement.

Unlike simple hydraulic variables, complex hydraulic characteristics such as shear stress were significantly correlated with mussel abundance for flows ranging from 0·03 to 2·18 $m^3 s^{-1}$. This range encompasses most flows during the period of juvenile settlement. We suggest that the high shear stress in some portions of the study site is a major factor limiting mussel recruitment. The lack of a significant correlation between mussel abundance and shear stress at high flow (9·35 $m^3 s^{-1}$) resulted from a variable relationship between shear stress and discharge among transects due to channel morphology. The higher shear stresses at most transects over mussel beds during a discharge of 9·35 $m^3 s^{-1}$ suggests that spates occurring during or shortly after juvenile settlement may result in a loss of juveniles.

The unique life history and limited mobility of mussels necessitates a more complicated procedure than generally used for fish and other macroinvertebrates for determining conservation flows. Specifically, we recommend an approach that incorporates concepts of hydraulic stream ecology with the more common practice of modelling only simple hydraulic variables in habitat simulations. Estimating the complex hydraulic key characteristics can be performed with minimal effort through the selection of appropriate subroutines with PHABSIM. This approach may also be suitable for simulating habitat of other sessile organisms.

KEY WORDS mussels habitat IFIM hydraulic stream ecology instream flows mussel hosts

PAPER IN PRESS – Reprints will not be available until about September 1.

North Carolina Wildlife Resources Commission

512 N. Salisbury Street, Raleigh, North Carolina 27604-1188, 919-733-3391
Charles R. Fullwood, Executive Director

19 June 1995

TO: John Alderman, Piedmont Project Leader

FROM: Mark Hartman, Nongame Biologist

RE: Shocco Creek dwarf wedgemussel population update

On 13 April 1995, the known extent of the Shocco Creek dwarf wedgemussel population was extended to an area upstream of the Warren County SR 1133 bridge. In 50 minutes of surveying, 10 live specimens were found ranging in size from 33 mm to 54 mm.

The occupied stream habitat in this stretch of Shocco Creek is part of a palustrine wetland system dominated by scrub/shrub and emergent plant species. The main creek channel is 1-3 m wide and from 0.5 to 1.5 m deep. Within the creek, small patches of relatively firm sand and silt substrates are present; however, most areas are dominated by abundant woody debris, submerged and emergent aquatic vegetation, and flocculent organic ooze. Dwarf wedgemussels are found in the sandy substrate and suspended in the organic floc.

Historically, this portion of Shocco Creek was associated with forested wetlands; however, within the last 15-20 years saturated soils have killed most of the canopy trees up to approximately 200 m from the main creek channel. This was possibly due to beaver damming of tributary streams feeding into Shocco Creek from the bottomland areas. According to local citizens, the section of Shocco Creek upstream from the SR 1133 bridge has never been completely inundated for extended periods, as would occur in a beaver pond.

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1995 MRRC meeting abstracts available

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Below is a list of presentations and posters about freshwater bivalves presented at the 27th annual meeting of the Mississippi River Research Consortium (MRRC) in LaCrosse, WI. If you would like a copy of any of the abstracts contact me via e-mail at mh@finsandfur.fw.umn.edu, or at 200 Hodson Hall, 1980 Folwell Ave., St. Paul, MN 55108.

The next meeting will take place during 25-26 April, 1996 at LaCrosse, WI. All are invited to attend!

Titles and authors of presentations and posters

Three years of fingernail clam (Sphaeriidae) and mayfly (Ephemeroptera) sampling in the upper Mississippi River system. Jennifer S. Sauer

Does water velocity and depth affect fingernail clam distributions in Lake Onalaska? Randy Burkhardt

Genetic adaptation to pollution and response to a bottleneck effect by fingernail clams (Musculium transversum). Brian L. Sloss, Michael A. Romano, Richard V. Anderson

Mussel distribution patterns in habitats of Pool 19, Mississippi River. Richard V. Anderson, Jack Grubaugh, Jennifer L. Owens, Dave Day

Are unionid translocations a viable mitigation technique? The Wolf River, WI, experience, Part III: August 1994. Marian E. Havlik and Michael G. Havlik

Temporal patterns in the density, size-distribution, and settlement of zebra mussel veligers in the Illinois River. James A. Stoeckel, Lori Camlin, K. Douglas Blodgett, and Richard E. Sparks

Differences in shell morphology between a riverine and lacustrine population of zebra mussels. W. Gregory Cope, Ronald R. Hayden, and Michelle R. Bartsch

Effects of zebra mussel colonization of native unionids in the Illinois River. Scott D. Whitney, K. Douglas Blodgett, Richard E. Sparks

Changes in zebra mussel densities in the upper Mississippi River: 1991-1994. David C. Beckett, B. Will Green, and Andrew C. Miller

An experiment with zebra mussels, freshwater drum and juvenile bluegills. William Richardson & Lynn Bartsch

Growth of juvenile bluegill sunfish in the presence of zebra mussels: a mesocosm experiment. William B. Richardson and Lynn A. Bartsch

Potential for zebra mussel transport by divers. K. Douglas Blodgett, Lori S. Camlin, Richard E. Sparks, and Clifford E. Kraft

Do migrating ducks affect the population dynamics of fingernail clams? Randy Burkhardt

Population dynamics of zebra mussels in the upper Mississippi River three years after invasion. W. Gregory Cope, Michelle R. Bartsch, and Ronald R. Hayden

Effect of zebra mussel density on macroinvertebrate community structure on hard substrata in the upper Mississippi River. Trisha A. Ellings, M.D. Delong, and J.H. Thorp

Colonization rate of zebra mussels on hard substrata in the upper Mississippi River. Sharon R. Hersom, Michael D. Delong, and James H. Thorp

Habitat characteristics and mussel assemblages associated with the federally endangered mussel Lampsilis higginsi in the lower Saint Croix River, MN and WI. Daniel J. Hornbach, Tony Deneka, Patrick Baker

Bivalve survey of the Sandy River drainage, Minnesota. Mark Hove, Chris Freiburger, and Robin Engelking

Suitable fish hosts of six freshwater mussels. Mark C. Hove, Robin A. Engelking. Elaine R. Evers, Margaret E. Peteler, and Eric M. Peterson

Population dynamics of zebra mussels in the lower Illinois River, 1993-94. Scott D. Whitney, K. Douglas Blodgett, and Richard E. Sparks

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Early life history research on the squawfoot, Strophitus undulatus

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Strophitus undulatus is found in streams and rivers throughout central, southern, and eastern United States, and portions of central and eastern Canada (Williams et al. 1992). Strophitus undulatus is listed as threatened in Iowa (Cummings and Mayer 1992).

Little is known about the fish host requirements for this species whose glochidia are facultative parasites (Lefevre and Curtis 1911, Howard in Baker 1928). Laboratory tests show that largemouth bass and creek chubs are suitable hosts for the glochidia (Howard in Baker 1928).

Two gravid S. undulatus were collected from a tributary to the Mississippi River in northern Minnesota during August 1994. When inflated with glochidia, the gills of gravid females are brown. Most glochidia were encased in conglutinates. Observed conglutinates were tube-shaped, 5-10 mm long, 1-2 mm wide, and rounded at both ends. A conglutinate was comprised of a clear gelatinous sheath with brown glochidia inside. Glochidia were actively snapping, as described in Ellis and Keim (1918), even without exposure to NaCl.

Eleven species of fish collected from lakes and streams not known to contain S. undulatus were exposed to S. undulatus glochidia in the laboratory during October-November 1994. Spotfin shiners, fathead minnows, yellow bullheads, black bullheads, bluegill, largemouth bass, and walleye facilitated glochidia metamorphosis to the juvenile stage (Table 1). Roughly ten times as many juveniles were collected from walleye than from any other species tested.

Table 1. Suitable and unsuitable fish hosts for Strophitus undulatus glochidia.

Transformation observed	No transformation observed

Species tested	Number	Days to metamorphosis	Species tested	Number	Period of attachment
spotfin shiner	11	10-13	longnose gar	1	8-11 d
fathead minnow	5	14	bowfin	2	17-19 d
yellow bullhead	2	14-22	white sucker	6	1-4 d
black bullhead	3	18-20	banded killifish	6	15-18 d
bluegill	3	10-19			
largemouth bass	1	13-15			
walleye	4	10-29			

Average water temperature was 19 1 2!C.

The eleven spotfin shiners were tested in two groups to determine if separating the fish from juveniles would increase the number of juveniles recovered. Eleven juveniles were recovered from five unrestricted shiners, while fifty-five juveniles were collected from six shiners that were prevented access to the bottom of the aquarium by a plastic screen. It is suspected that the unrestricted shiners consumed the juvenile S. undulatus, however, the G.I. tracts of these shiners were not analyzed.

Baker, F. C. 1928. The fresh water mollusca of Wisconsin. Part II. Pelecypoda. Bulletin of the Wisconsin Geologic and Natural History Survey, 70: 1-495.

Cummings, K. S., and C. A. Mayer. 1992. Field guide to freshwater mussels of the Midwest. Illinois Natural History Survey Manual 5. 194 pp.

Ellis, M. M., and M. Keim. 1918. Notes on the glochidia of Strophitus edentulus pavonius (Lea) from Colorado. The Nautilus 32(1): 17-18.

Lefevre, G., and W. C. Curtis. 1911. Metamorphosis without parasitism in the Unionid>. Science 33: 863-865.

Williams, J. D., M. L. Warren, Jr., K. S. Cummings, J. L. Harris, and R. J. Neves. 1992. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18(9): 6-22.

NATIONAL BIOLOGICAL SERVICE
U.S. DEPARTMENT OF THE INTERIOR

NBS INFORMATION BULLETIN

No. 11 1995

FRESHWATER MUSSEL RELOCATION PROJECTS EVALUATED

The North American freshwater unionacean mussel fauna, once represented by about 297 taxa, has declined to about 276 taxa since the early 1900's because of overharvest, commercial navigation, pollution, and habitat degradation. Presently, 58 mussel species (21% of remaining species) are listed as federally threatened or endangered. Because of the drastic decline in mussel fauna and with the authority of the Endangered Species Act of 1973, resource agencies have attempted to mitigate the effects of human activities on unionacean mussels.

Relocation has been used as a conservation and management technique by state and federal agencies to recolonize areas where mussel populations have been eliminated by prior pollution events, to remove mussels from construction zones, and to reestablish populations of endangered species. More recently, relocation has been used to protect unionid populations from colonization by the zebra mussel (*Dreissena polymorpha*).

Although relocations have been conducted for more than 20 years, their effectiveness for the conservation and management of unionacean populations has not been assessed. Moreover, little guidance is available on methods for relocation projects or for monitoring the subsequent longterm status of relocated mussels. Little is known about the habitat requirements of mussels or the biological responses of mussels to removal from the substrate, handling, transporting, and relocating to a new site. Our objectives were to summarize the literature on mussel relocation, evaluate the relative success of mussel relocation projects, and identify research needs.

SUMMARY AND RELATIVE SUCCESS OF MUSSEL RELOCATION PROJECTS

We found 31 papers on mussel relocation—only three appeared in the peer-reviewed literature. The rest were either in the published gray literature or in unpublished reports, which were not widely available. We found that nearly 90,000 mussels have been relocated in 34 discrete projects.

The main reasons for mussel relocation were protection from construction projects, management efforts such as reintroductions, and research. Most (47%) relocations were conducted because of construction projects that were forced to comply with the Endangered Species Act of 1973 (Fig. 1*a*). Construction projects included those associated with bridge construction, bridge demolition, and dredging and channel maintenance. The rest of mussel relocations were attributed to management efforts (32%) such as reintroductions and to research (21%).

The longterm survival of relocated mussels was not routinely monitored. Only 76% of all relocation projects reported followup monitoring. Most (43%) projects were monitored for 1 year or less, and only 12% were monitored for 5 or more consecutive years (Fig. 1*b*).

The mortality of relocated mussels varied widely among projects and species, and was difficult to assess. To ensure equitable assessment of mortality among projects, we evaluated mortality on the basis of the percentage of mussels recovered in relation to the total number of mussels relocated. Mortality was unreported in 32% of projects and was greater than 70% in 24% of projects (Fig. 1*c*). Mean mortality of relocated mussels was 47%, based on an average recovery rate of 45%. Mortality was greater than 90% in some projects, and the greatest percentage often occurred within the first year after relocation.

About 50% of the mussel relocations occurred in the southern and southeastern United States, regions that are known to contain the highest diversity of mussel species. The timing of relocation projects coincided with the warmest season of a geographic region. Most (40%) relocation projects were conducted from July through September (Fig. 1*d*), presumably a period when reproductive stress is relatively low for most species and metabolic rate is sufficient for reburrowing in the substrate.

Critical Factors Influencing Mussel Survival

On the basis of our evaluation, the physical characteristics of mussel habitat at both source and destination sites and the methods of relocation are especially critical to the survival of mussels that are relocated. Existing criteria for selecting a suitable relocation site have been largely qualitative and observational. The presence of live mussels or the apparent similarity of habitat have often been used as criteria for site selection, but do not ensure that a site is suitable for relocation. For example, decreased survival of relocated mussels has been attributed to changes in habitat at the destination site, primarily due to substrate instability.

Standard protocols for conducting mussel relocations do not presently exist. Moreover, there is little guidance in the literature regarding relocation related variables such as methods for handling, transporting, and tagging mussels; the appropriate time of year to relocate mussels; minimum and maximum allowable water temperatures; maximum allowable period of aerial exposure; and methods for replacing mussels in the substrate. We found that the methods described for most of the relocation projects that we reviewed were generally insufficient in detail to repeat the project.

Monitoring Relocation Success

The greatest obstacles to evaluating the relative success of the mussel relocation projects that we reviewed were the lack of longterm, quantitative monitoring and the lack of universal reporting of mortality and recovery data. A majority (67%) of relocation projects were not monitored or were monitored for 1 year or less. An estimated 22,000 mussels (25% of those relocated) perished in 34 relocation projects; however, this number is an underestimate of actual mortality because 24% of projects were not monitored and only 68% of the projects that were monitored reported mortality. The relatively low recovery rate (45%) of relocated mussels in the projects evaluated does not necessarily correspond to mussel mortality, but may be partly attributed to sampling design, selection of an inadequate relocation site, or other factors. Alternatively, the lack of recovery may be due to mussel mortality and the movement of empty valves downstream with water currents.

Recommendations and Identification of Future Research Needs

Our review of the literature on mussel relocation revealed that the methods of relocation–when reported–varied widely among projects, the survival of the relocated mussels was generally poor (~50%), and the factors influencing survival of relocated mussels were poorly understood. For mussel relocation to be a successful conservation and management technique, more consideration must be given to habitat characterization, at both source and destination sites. Optimally, the water and sediment conditions should be monitored at both source and proposed destination sites over at least an annual cycle, not just once during the year, because flow regime and other key variables may change seasonally. Moreover, this type of information could be used to develop a complete set of site selection criteria.

In addition, future mussel relocation projects should be monitored for at least 2 years, but 5 years would allow documentation of recruitment–the true indicator of a successful relocation. Mortality, recovery, and sublethal indicators of relative condition should be measured for each species to assess variation in the sensitivity to relocation. Research is needed to develop criteria for selecting a suitable relocation site and to establish appropriate methods and guidelines for conducting relocation projects.

Finally, our literature search demonstrated the need for better access to methods and results of relocation projects. Most results from relocation projects were available only as intraagency reports that are not widely available. Studies evaluating mussel relocation, as well as those evaluating mussel communities, should be designed to yield quantitative and statistically valid results, and should be published in the peer-reviewed literature so that others may benefit from this information.

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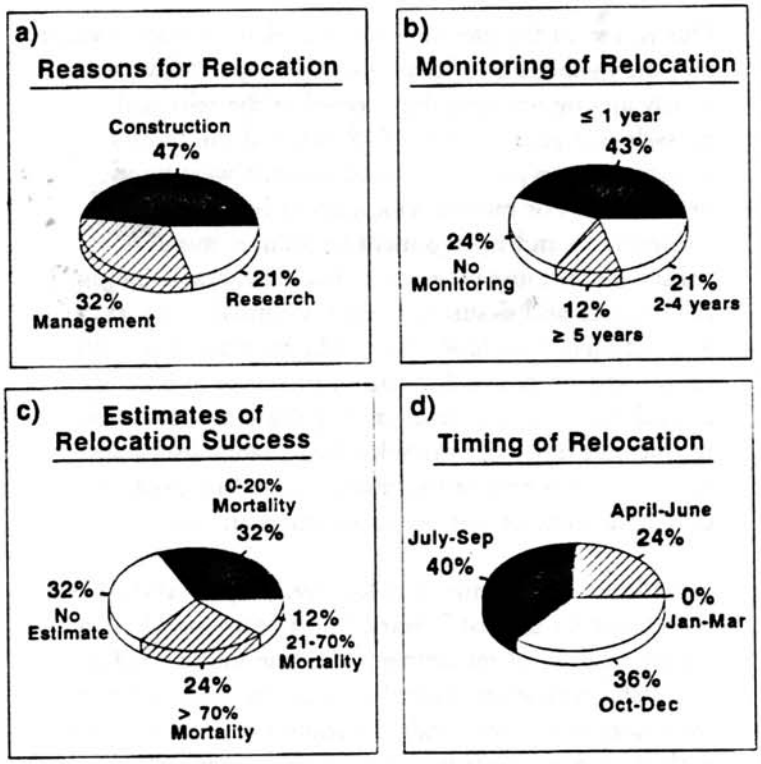

Figure. Pie charts showing *a*) the primary reasons for mussel relocation, *b*) the frequency of monitoring mussel relocation projects, *c*) the estimates of success for mussel relocation projects, and *d*) the timing of mussel relocations.

NATIONAL BIOLOGICAL SERVICE
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NBS INFORMATION BULLETIN

NO. 17 1995

A DIET FOR REARING JUVENILE FRESHWATER MUSSELS

Nearly 60 species of freshwater mussels (Unionidae) are listed as endangered in the United States, and many other species are declining because of habitat degradation and the invading zebra mussel (*Dreissena polymorpha*). We studied ways to develop a diet for rearing juvenile mussels, with the goal of long-term propagation of rare species. The published literature on mariculture of bivalves listed algae as the principal food, and polyunsaturated fatty acids (PUFA) as essential to larval and juvenile life stages. Similarly, Hudson and Isom reported that growth was enhanced and that survival increased for juvenile freshwater mussels with the addition of silt to aquaria. Our objectives were to determine the role of fine sediment in culturing juvenile mussels and develop a suitable diet for rearing juvenile mussels.

METHODS

The influence of river sediment and different foods on recently metamorphosed rainbow mussels (*Villosa iris*) and giant floaters (*Pyganodon grandis*) was investigated by culturing juvenile mussels on various combinations of algae, a live-cultured bacterium, a commercial suspension of several species of bacteria (Aqua-Bacta Aid, ABA), yeast, kaolin, and sediment, including autoclave-sterilized sediment. Two trials focused on determining the value of a substratum to juvenile mussels; a third trial tested various food sources to establish a suitable diet for rearing juvenile mussels (Table).

FEEDING TRIALS ONE AND TWO

After 45 days postmetamorphosis in fine sediment, juvenile *V. iris* fed algae exhibited a twofold increase in shell length (532 µm), and 63.5% mean survival. Juvenile *P. grandis* exhibited similar growth after 45 days postmetamorphosis with greater than a 2.2-fold increase in average shell length (806 µm) and 52.0% survival. In comparison, all juvenile mussels fed algae without sediment and algae plus bacteria (ABA), without sediment, exhibited no increase in shell length after 45 days postmetamorphosis.

Shell lengths of *P. grandis* juveniles fed algae in the kaolin substratum, in sterilized sediment, in sterilized sediment plus ABA, and in bacteria-colonized sediment were not significantly different. Shell lengths of *V. iris* juveniles fed algae with kaolin, or fed algae with colonized sediment also were similar after 60 days. Ingestion of bacteria was seemingly inconsequential to juvenile digestion and nutrition during this period. Juvenile mussels were observed to pedal-feed for about 120 ± 30 days; hence, sediment served as a physical substratum for pedal-feeding juveniles to collect food particles. Survival varied among mussels fed only algae without sediment. After 45 days, *V. iris* had 5.0% survival, whereas *P. grandis* exhibited 43.3% survival. Analysis of covariance indicated that growth rates of *P. grandis* and *V. iris* after 120 days were significantly different, with

P. grandis exhibiting a growth rate about twice that of *V. iris*.

After 272 days postmetamorphosis, *V. iris* fed *Chlorella*, *Ankistrodesmus*, and *Chlamydomonas* (CAC) with sediment achieved a mean shell length of 2,968 ± 405 µm (11-fold increase in length) and about 5% survival. After 195 days postmetamorphosis, *P. grandis* achieved a mean shell length of 4,877 ± 1,099 µm (13-fold increase in length) and about 12% survival. At 9 months postmetamorphosis, juvenile *P. grandis* were seen positioned with anterior end in the sediment and posterior end visible at the sediment-water interface with apertures visible.

Feeding Trial Three

Subsequent diet tests indicated that greater growth was correlated with algae high in oils containing PUFA. After 60 days, shell lengths of juveniles were similar among treatments, indicating that fine sediment provides some nutritional value. However, analysis of covariance indicated that *V. iris* juveniles in sediment, fed a tri-algal diet consisting of *Neochloris oleoabundans*, *Phaeodactylum tricornutum*, and *Bracteacoccus grandis* (NPB), showed the best growth over time (Figure). Shell lengths of juveniles fed NPB were significantly greater than those of juveniles fed other tested diets($P < 0.02$). Individuals fed NPB achieved a mean shell length of 1,747 ± 301 µm, and had 30.0% survival after 140 days postmetamorphosis. The oil-rich NPE and NNiC enhanced growth over the commonly used oil-poor green tri-algal mix of CAC, whereas commercial yeast diets did not support growth.

Conclusions

Results of this study indicated that juvenile freshwater mussels pedal-feed for about 4 months postmetamorphosis. A fine substratum associated with organic and inorganic materials is required presumably for pedal-feeding juveniles to collect food particles. Juveniles may begin filter-feeding in conjunction with pedal-feeding before 140 days postmetamorphosis as gill development occurs. By 9 months postmetamorphosis, they are filter-feeders. A substratum may also provide grit or a grinding surface for the crystalline style, thus enhancing digestion of algae. Algae are a suitable food source for rearing juvenile mussels, especially species high in polyunsaturated fatty acids. A tri-algal mixture of *Neochloris oleoabundans*, *Bracteacoccus grandis*, and *Phaeodactylum tricornutum* enhanced growth better than all other diets tested. Bacteria did not seem to contribute appreciably to juvenile growth and survival. Diet studies should be conducted for at least 60 days—and preferably 100 days—as juvenile mussels probably have fatty acid reserves that allow them to survive without food for several weeks postmetamorphosis.

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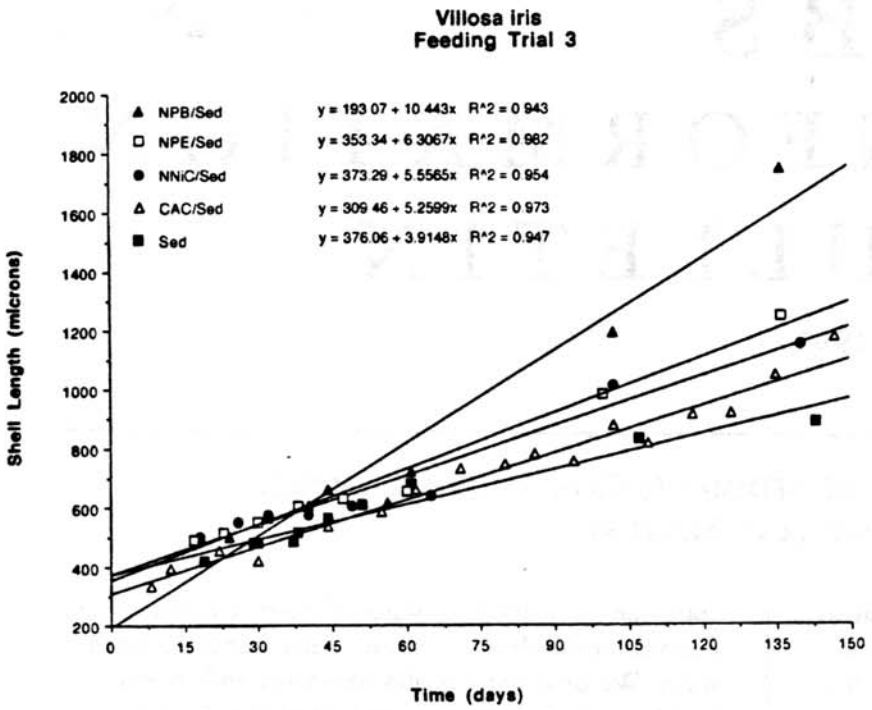

Figure. Comparison of growth equations of *Villosa iris* fed various diets for 140 days postmetamorphosis.

Table. Summary of experimental treatments to determine a suitable diet for rearing juvenile freshwater mussels. CAC = *Chlorella vulgaris*, *Ankistrodesmus falcatus*, and *Chlamydomonas reinhardtii*. NOC = *Neochloris oleoabundans*, *Oocystis marsonii*, and *Cyclotella menenghiana*. NNiC = *Neochloris oleoabundans*, *Nitszchia acicularis*, and *Cyclotella menenghiana*. NPB = *Neochloris oleoabundans*, *Phaeodactylum tricornutum*, and *Bracteacoccus grandis*. NPE = *Neochloris oleoabundans*, *Phaeodactylum tricornutum*, and *Enterobacter aerogenes*. Sed = fine sediment. StSed = autoclave-sterilized fine sediment.

Trial	Mussel	Host fish	Treatment (replicates)	Number of juveniles
1	*Villosa iris* (rainbow mussel)	*Ambloplites rupestris* (rock bass)	CAC (3)	200, 206, 205
			CAC/ABA (3)	200, 200, 200
		Micropterus salmoides (largemouth bass)	CAC/Sed (3)	200, 200, 215
2	*Pyganodon grandis* (giant floater)	*Ambloplites rupestris* (rock bass)	CAC only (3)	150, 153, 200
			CAC/Kaolin(3)	100, 100, 100
		Lepomis macrochirus (bluegill)	CAC/Sed (3)	201, 256, 258
			CAC/StSed(3)	204, 205, 205
		Micropterus salmoides (largemouth bass)	CAC/StSed/ABA (3)	200, 206, 202
		Carassuis auratus (goldfish)		
3	*Villosa iris* (rainbow mussel)	*Amploplites rupestris* (rock bass)	Unfed (1)	45
			Kaolin only (3)	50, 50, 50
		Micropterus salmoides (largemouth bass)	Sed only (2)	71, 70
			Yeast/Sed (3)	100, 100, 100
			CAC/Kaolin(2)	92, 100
			NOC/Sed (3)	100, 100, 100
			NNiC/Sed (3)	111, 100, 103
			NPB/Sed (3)	100, 101, 108
			NPE/Sed (3)	100, 103, 100

NATIONAL BIOLOGICAL SERVICE
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NBS INFORMATION BULLETIN

No. 23 1995

PREDICTION OF TOXICITY OF SEDIMENTS CONTAINING COMPLEX CONTAMINANT MIXTURES

Depositional-zone sediments in urban and industrial areas are often heavily contaminated with complex mixtures of chemicals from historic discharges of toxic pollutants. We developed a model that estimates aquatic toxicity from information on sediment chemistry to evaluate the potential toxicity of sediments that may contain combinations of many contaminants.

MODEL DEVELOPMENT

We needed a method for evaluating the combined effects of sediment mixtures comprising numerous contaminants of varying toxicity. Historically, concentrations of individual metals in mixtures were expressed in relation to incipient lethal concentrations–the concentrations of individual constituents beyond which organisms cannot survive for an indefinite period. These ratios, or toxic units, were then summed over all constituents in the mixture to estimate the total toxicity of that mixture. The toxic units approach remains in use for metals.

We expanded the toxic units method to incorporate information on organic contaminants, bioavailability (i.e., the proportion of a contaminant in a sediment that is available for uptake by organisms), and chronic toxicity. For sediments, this bioavailable fraction is the concentration in the interstitial waters of the sediment–the pore-water concentration. We define a toxic unit as the ratio of the estimated concentration of a contaminant in the pore water of a test sediment to an estimate of the chronic toxicity of that contaminant in water. We used water quality standards and criteria, mostly the U.S. Environmental Protection Agency Ambient Water Quality Criteria (AWQC), for chronic toxicity. These criteria are founded on information from a wide range of taxa and, where appropriate, incorporate bioaccumulation potential (i.e., the potential for concentrations to increase in upper trophic level organisms). The equation for toxic units is

$$\textit{Toxic unit} = \frac{C_{wp}}{C_{wqs}}$$

C_{wp} = *Estimated pore-water concentration*
C_{wqs} = *Water quality standard*

We estimate toxic units attributable to selenium according to a proposed sediment criteria. The toxic units for all contaminants in a sediment are then summed to produce a total toxicity estimate for that sediment.

For organic contaminants, we used information on the differential solubility of compounds in water and in organic carbon and the amount of organic carbon present

in the sediment to estimate the pore-water concentration. We assumed that the concentration of organic contaminants in water is controlled by organic carbon, and that the contaminant concentrations are in equilibrium with sediment organic carbon and pore water. Analogous to the approach used for organic contaminants, we used the concentration of sulfide released along with metals when the sediments were leached with a weak acid; that is, the acid-volatile sulfides (AVS) and simultaneously extracted metals (SEM), as estimates of the bioavailable fraction of each metal present in the sediments. In using the AVS approach, we assumed that equilibrium concentrations of metals in pore waters are controlled by the presence of sulfides, which combine with the metals to produce relatively insoluble sulfide salts, much as organic carbon controls pore-water concentrations of organic chemicals. For mercury, soluble and highly toxic in its methylated form, we assumed that all methyl mercury was soluble and hence bioavailable; for the remainder, bioavailable mercury was estimated using the AVS-SEM approach.

MODEL TESTING AND EVALUATION

To evaluate the ability of the model to predict toxicity from chemical information we examined data from 12 sites located in three Great Lakes harbors and tributaries–Indiana Harbor, Indiana; Saginaw River, Michigan; and Buffalo River, New York–containing different mixtures of contaminants. Sediments were collected with a Ponar grab and split into two portions–one for laboratory toxicity tests, the other for chemical analysis. Laboratory toxicity tests were performed with fish, zooplankters, benthic macroinvertebrates, phytoplankters, macrophytes, and microbes. The list of contaminants measured included polychlorinated biphenyls, polycyclic aromatic hydrocarbons, organochlorine pesticides, simultaneously extracted metals, total metals, and others. Organic carbon and acid volatile sulfide content of the sediments were also measured.

We evaluated the predictions of the toxic units model by comparing it with sediment toxicity determined in the laboratory. To estimate toxicity for each sediment sample as determined in the laboratory, the data from each of the series of toxicity tests, including multiple endpoints measured within some tests, were scaled similarly to the toxic units scaling performed on the chemistry measures–by normalizing the measured response associated with an endpoint to the control sediment response (i.e., the same test, run simultaneously with sediment from a clean site) for that endpoint:

$$\textit{Control-adjusted laboratory toxicity response} = 1 - \frac{\textit{Endpoint value for test sediment}}{\textit{Endpoint value for control sediment}}$$

These estimates of hazard–the control-adjusted laboratory toxicity responses for each endpoint–were averaged (i.e., arithmetic mean) over all measured endpoints for a site to estimate the mean hazard for each site on the basis of laboratory-determined toxicity.

TOXIC UNIT ESTIMATION FOR CONTAMINANT MIXTURES IS A VALUABLE TOOL

The relation between laboratory toxicity and toxic units was nearly linear, but included a quadratic term that was marginally significant (Figure). This model accounted for more than 93% of the variability present in mean laboratory toxicity. Our findings support the hypothesis that toxicity, as measured by short-term toxicity tests, is mediated by pore-water contaminant concentrations and that toxicity may be additive, as has been suggested by others. The short-term laboratory toxicity tests by themselves reflect toxicity of the sediments in the immediate vicinity from which they are collected, but not necessarily the ecosystem risk represented by the transfer of the contaminants they contain through the food chain. However, short-term laboratory toxicity tests, as documented in the strength of the relation between toxic units and toxic response, do seem to characterize the overall extent to which sediments are contaminated.

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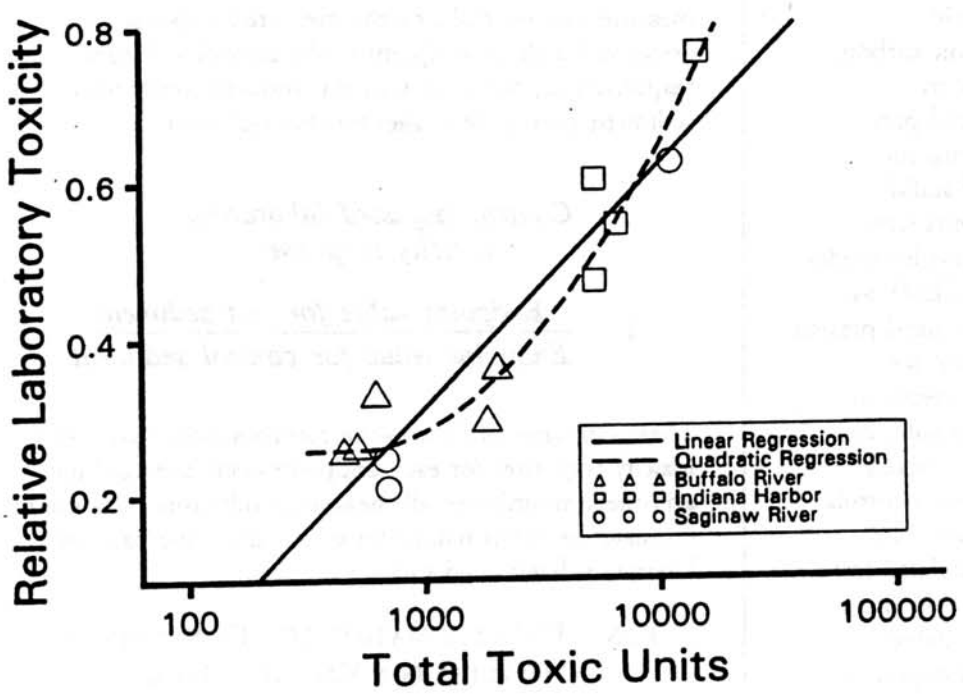

Figure. Relative toxicity of sediment versus total toxic units estimated from bioavailable fractions of all contaminants (for the line $R^2 = 0.88$; for the curve $R^2 = 0.94$; for the line and the curve, $n = 12$, $P = 0.0001$).