

Department of Defense Legacy Resource Management Program

PROJECT NUMBER 09-450

Propagation of Species At Risk Atlantic Pigtoe on Military Installations

Eric D. Wolf Conservation Management Institute Virginia Tech

April 30, 2010



Propagation of Species At Risk Atlantic Pigtoe on Military Installations





Atlantic pigtoe (Fusconaia masoni)

Prepared by:

The Conservation Management Institute-Military Lands Division College of Natural Resources Virginia Polytechnic Institute and State University 1900 Kraft Dr. Suite 250 Blacksburg, VA 24061 540-231-7348

CONTENTS

<u>page</u>
ACKNOWLEDGEMENTS
1.0. BACKGROUND5
2.0. REPRODUCTION6
3.0. CURRENT STATUS8
4.0. PROPAGATION12
5.0. APPROACH14
6.0. PROCEDURES
6.1. Potential Host Fish Collection
6.2. Mussel Collection
7.0. IMPLICATIONS
8.0. CONCLUSIONS
9.0. SELECTED RESOURCES
APPENDIX A: Presentation of Project Results at 3 rd Annual Virginia Atlantic Slope Mussel Recovery Group Conference
APPENDIX B: Legacy Fact Sheet 2009 Project Number 09-450

AKNOWLEDGEMENTS:

Funding for this project was provided through the Department of Defense Legacy
Resource Management Program as a part of the ongoing effort to protect and enhance natural
resources on military lands while supporting the training mission. This project represents a
cooperative interagency collaboration incorporating resources and personnel of the DoD Legacy
Program, U.S. Fish and Wildlife Service White Sulphur Springs National Fish Hatchery,
Virginia Army National Guard Maneuver Training Center-Fort Pickett, and The Conservation
Management Institute at Virginia Tech. Military lands are a finite commodity and conservation
of their natural resources is essential to the maintenance of the high quality training environment
required for the success of the training mission.











1.0. BACKGROUND

Clean and healthy rivers and streams need freshwater mussels. Freshwater mussels (Order Unionoida) are an important component of many surface water systems. Mussels are bivalves like clams and oysters, and are filter feeders. They settle in to the substrate of rivers and streams and filter bacteria, algae, and other small particles out of the water by a siphoning action, drawing water in, passing it over its gills for oxygenation and filtration, and then expelling the filtered water out again (Figure 1). This means that the presence of freshwater mussels can actually work to improve water quality. But freshwater mussels have become the nation's most endangered group of animals.

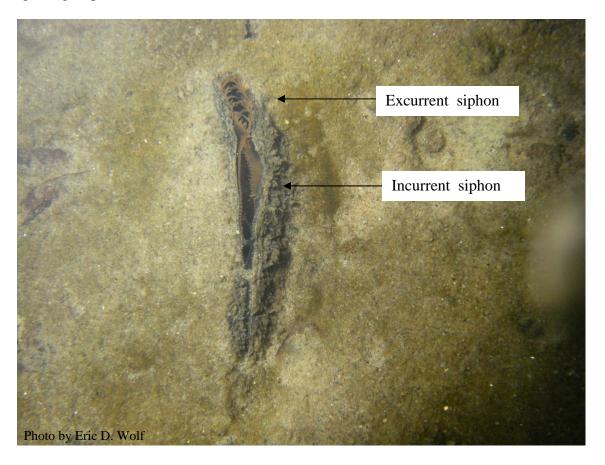


Figure 1. Live mussel embedded in substrate actively filter feeding by siphoning action.

Mussels serve as a vital trophic link in aquatic systems, harvesting smaller particles from the water stream, and completing nutrient and energy cycles by converting them into an important food source for a wide variety of fish, reptiles, birds and mammals. Their shells provide a foundation for the growth of algae, aquatic insects, and plants, and even when dead their shells can contribute to substrate diversity, providing foraging and egg-laying sites for fish like madtoms (*Noturus spp.*) and darters (Percidae family). Their primarily sedentary lifestyle and their feeding habits make mussels particularly vulnerable to environmental degradation and so they can be valuable indicator species for water quality monitoring, and can be used to indicate the presence (or absence) of environmental perturbations like excess sedimentation and organic waste or nutrient pollution.

2.0. REPRODUCTION

Freshwater mussel reproduction is seasonally and condition-dependant and involves a complex, multi-stage process. When conditions are right, male mussels release gametes (sperm) into the water column to hopefully be siphoned into the gills of by the female to fertilize the eggs held in her gills. At this stage, the females' gills become slightly inflated, and the female mussel is said to be gravid. Thousands of fertilized eggs can then obtain oxygen from the gills and brood there until they develop into glochidia, the mussel's larval stage.

Maturation is highly dependent on water temperature and can occur very rapidly as temperatures rise. Female mussels then release packets of larvae called conglutinates. These conglutinates simulate a fish food source such as an aquatic insect. When the host fish eats them, thousands of microscopic larvae become dislodged from the conglutinates and come in contact with the host fish. Larvae react to the slime on the surface of the fish's skin and clamp

down. Larval mussels (glochidia) can then survive and disperse by attaching themselves to the gills or fins of the host fish. Once the glochidia are attached, the fish's body reacts by covering them with epithelial cells until they are encased in a cyst. These larval mussels then live as parasites for several weeks, growing and feeding on the bodily fluids of the host fish, until the juvenile mussel matures and is large enough to drop off into the substrate to start the process again (Figure 2).

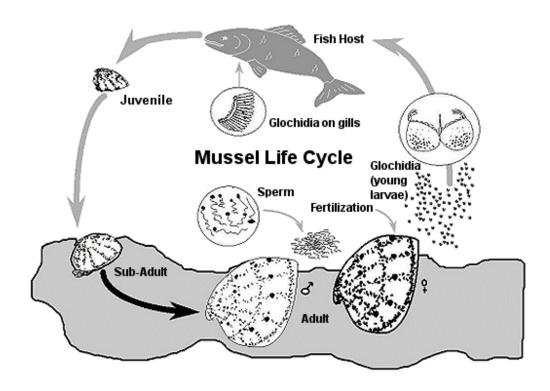


Figure 2. Typical life cycle of a freshwater mussel (U.S. Fish and Wildlife Service).

This is an indispensable step in the completion of their lifecycles, meaning that successful freshwater mussel reproduction is also dependent on the survival and movement of often species-specific host fish. The distribution and success of the host fish can also be impacted by degradation of water quality and stream alterations like impoundments and riparian disturbances. An individual mussel can produce over 200,000 tiny larvae, but without the presence of the

appropriate host fish, they will never mature into adults. The vital connection between specific mussels species and their particular host fish species has been well-documented in some species, but information on this all-important link for the Atlantic pigtoe (*Fusconaia masoni*) is limited, and suitable host fish species are unknown.

3.0. CURRENT STATUS

The Atlantic pigtoe is listed as a federal Species of Concern as well as a Priority Level 3 Species at Risk by the Army. Virginia Department of Game and Inland Fisheries list the species as State Threatened and a Wildlife Action Plan Tier II species indicating Very High Conservation Need. NatureServe (2008) classifies the Atlantic pigtoe as having G2 status as Globally Imperiled, and S2 State Status as Very Vulnerable to Extirpation in Virginia. The International Union for Conservation of Nature's (IUCN) Red List estimates that only 1,000-2,500 individuals remain, and in 2008, the U.S. Fish & Wildlife Service proposed that the Atlantic pigtoe be considered for Endangered Species Act protection.

The Atlantic pigtoe has historically been documented in Virginia, North Carolina, South Carolina, and Georgia, but populations in Georgia and both North and South Carolina have declined precipitously. Populations have similarly been declining throughout Virginia. In Virginia, the Atlantic pigtoe has been documented in the Chowan River Basin, the James River Basin, and the Roanoke River Basin but viability of all but two recorded populations was assessed as "poor" as of January 2008. Viability of the Atlantic pigtoe in the Nottoway River was ranked highest in Virginia, but populations in the lower Nottoway (below the fall line) have declined dramatically, making the upper reaches of the Nottoway River, including the portion of the river that flows through Fort Pickett Army National Guard Maneuver Training Center

(ARNG-MTC Fort Pickett) in southeastern Virginia (Figure 3) home of some of the few remaining stable populations in the state, and possibly the world.



Figure 3. Location of Fort Pickett Army National Guard Maneuver Training Center (ARNG-MTC Fort Pickett) in southeastern Virginia.

A qualitative biological survey conducted by the Conservation Management Institute (CMI) in 2006 in the Nottoway River on ARNG-MTC Fort Pickett documented an abundance of freshwater mussels thriving in the section of the river that crosses the southern portion of the installation. While far-outnumbered by the more common *Elliptio* genus, we found the Atlantic pigtoe to be the second most abundant genus of mussel encountered. We documented and marked Atlantic pigtoe mussels in sampling reaches along much of the length of the Nottoway on ARNG-MTC Fort Pickett. Of approximately 14.7 km of the Nottoway River that cross the installation, we identified Atlantic pigtoe in 10 separate reaches along 10.8 km (Figure 4). These sites are close enough in proximity, and have no apparent separation barriers between them, so mussel populations at these sites are likely to be genetically linked.

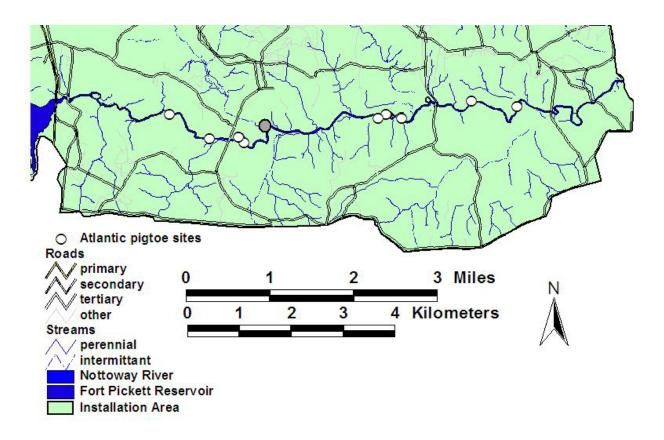


Figure 4. Distribution of sites where Atlantic pigtoe have been documented in the Nottoway River on ARNG-MTC Fort Pickett (gray marker shows the site of quantitative sampling).

In 2007 we conducted in-depth, quantitative surveys of reaches found to have the highest mussel diversity and greatest number of Atlantic pigtoe recorded to date. Highest density of Atlantic pigtoe encountered (site indicated by the gray circle in Figure 4) was nearly $1/10m^2$ (0.9/ m^2). We documented overall mussel density at the site as $30/10 m^2$ ($3/m^2$). These survey efforts have documented and marked a total of 22 Atlantic pigtoe in the Nottoway River on ARNG-MTC Fort Pickett (Table 1).

Table 1. Locations and identification tag numbers of Atlantic pigtoe mussels documented in the Nottoway River on ARNG-MTC Fort Pickett in 2006 and 2007 surveys (UTM Zone 18, NAD 83).

Date	Easting	Northing	Tag #	Length (mm)
June 2006	245140	4097716	A157	44.41
June 2006	245140	4097716	-drop	ped untagged-
June 2006	244712	4097681	A158	28.22
June 2006	244269	4097812	A159	40.54
June 2006	242917	4097492	A151	54.88
June 2006	242917	4097492	A152	57.82
June 2006	242621	4097557	A153	50.64
June 2006	242621	4097557	A154	46.3
June 2006	242621	4097557	A155	44.98
June 2006	242621	4097557	A156	35.3
June 2006	240310	4097343	A161	57.16
June 2006	240310	4097343	A162	54.88
June 2006	240310	4097343	A163	52.04
June 2006	239900	4097019	A160	58.??
June 2006	239786	4097123	A164	54.42
June 2006	239786	4097123	A165	51.38
June 2006	238468	4097565	A168	65.29
Oct 2007	240310	4097343	A366	68.9
Oct 2007	240310	4097343	A367	58.3
Oct 2007	240310	4097343	A368	42.1
Oct 2007	240310	4097343	A369	52.2
Oct 2007	240310	4097343	A370	53.8

Atlantic pigtoe require fast-flowing, well-oxygenated streams and are restricted to fairly pristine habitats. They are very sensitive to sedimentation and channel modification, and the glochidia appear to be extremely sensitive to pollution, so its presence in the Nottoway River on ARNG MTC-Fort Pickett is a good indication of positive environmental conditions.

4.0. PROPAGATION

Freshwater mussel propagation in the United States began in the early 1900's through the mid-20th century to provide shells used to supply the pearl button industry. In 1976 the rapidly declining status of freshwater mussel populations was acknowledged with their inclusion into the protections of The Endangered Species Act with the listing of 23 species, and propagation efforts emerged as an important means to provide for the mandated protection and recovery of threatened and endangered mussel species. By 2002, 70 species were listed as protected. For many species, population densities were so low that habitat improvement and protection alone could not support the natural reproduction required to sustain them, emphasizing the urgent need to develop and refine successful propagation methods. Since 1998, hundreds of thousands of propagated endangered mussel juveniles have been released into the wild, but the vital information required to successfully support scores of species remains undiscovered.

Propagation is a versatile and widely used tool that can be used not only to augment declining populations, but support stable populations as well. Propagation research relies in part on identifying the most successful techniques for holding adult and juvenile mussels in recirculating systems, refining water sources, food delivery systems and schedules, holding tank shape and size, food quality and stocking densities required to maintain freshwater mussels. These same techniques can be applied in the propagation of more common mussel species that can then be released as a means to improve water quality. Such procedures can also be used to preemptively protect mussel populations from negative impacts by providing refugia that can house, hold and maintain mussels threatened by construction projects or major pollution events until they can be reintroduced after the threat has passed.

Active propagation is included in most endangered mussel species recovery plans to enhance and expand current populations but it is perhaps an even more powerful a tool for use in supporting declining populations before they become threatened or endangered. Supporting populations through active propagation before species reach critical levels is not only more likely to be successful but it is often more cost effective.

The population of Atlantic pigtoe on ARNG-MTC Fort Pickett presents a rare and time-sensitive opportunity. Active propagation is the most proactive way to support declining populations and is the single best strategy to keep the species from federal listing, and prevent ensuing training restrictions. But current knowledge is incomplete pending further research to identify the host fish species upon which the mussels depend to complete their life cycle.

Host fish trials will allow propagation and growth of juveniles that can then be released back in to the river. In this manor the population on the installation could serve to support overall population levels in the State, as well as other suitable areas throughout its original range. Identification of host fish species is critical to this process, and will form the foundation upon which all subsequent recovery work can be based. The focus of these efforts will be to support a stable population over a broad geographical area. This project represents a uniquely cost-effective and time-sensitive opportunity to proactively avoid federal listing and subsequent encroachment of the training mission, while simultaneously building a working relationship with partners through which this same technique can be applied on many additional DoD lands with mussel species at risk.

5.0. APPROACH

The process of identification of suitable host fish species begins with targeted snorkeling surveys to collect gravid female mussels (those carrying developing young) from the population on ARNG-MTC Fort Pickett (Figure 5).



Figure 5. Snorkeling survey for freshwater mussels in the Nottoway River on ARNG-MTC Fort Pickett.

Potential host fish species are also collected from the area and held in environments mimicking existing stream conditions. Mussels are kept in specialized holding facilities and monitored for larval release, or larvae are extracted. Larvae are collected and introduced into containers holding the host fish while aeration is used to keep the water agitated, allowing the larvae to attach to the gills of the fish in the process referred to as infestation. Infested fish are then held under carefully controlled conditions in aquaria for a temperature-dependent period of

2 weeks to a month while larvae are encysted and allowed to develop until juveniles fall off their host fish (Figure 6). Juveniles can then be collected and the success of each potential host fish species can be assessed.



Figure 6. Representation of captive mussel propagation process

A "robust host" will consistently produce a large number of juvenile mussels while a "marginal host" will produce inconsistent or low numbers of juveniles. Juveniles can then be cultured in the laboratory for a period of 2-12 months. A portion of these newly metamorphosed juveniles can then be tagged for future identification and released to the original collection site along with the adult female mussels so as to assure no detrimental impacts to the original population. The remainder will be cultured and grown under suitable conditions for monitoring

and validation of survival and vigor, and ultimately will also be released. Recent advances in propagation and culture have allowed the grow-out of captive-reared juvenile to exceed 5-10 mm. Releasing these larger juvenile mussels has not only vastly increased their survival, but has also improved the potential for future monitoring of released juveniles, making it possible to better evaluate both short-term and long-term success.

Biologists at the White Sulphur Springs National Fish Hatchery's Aquatic Resource

Restoration Center (ARRC) in White Sulphur Springs, West Virginia have broad experience

propagating over 30 mussel species, including 16 federally listed species that have been used to
augment existing populations and re-establish historic populations. The ARRC facility hosts a
dedicated mussel building containing adult and juvenile holding systems and a fish building with
multiple sizes and types of recirculating tanks for both quarantining and holding various host fish
during the infestation period. In addition, the facility boasts a state of the art greenhouse and
clean lab for growing large quantities of algae that are used to create specialized feed mixes
designed to maintain the condition of adult freshwater mussels using techniques developed for
the commercial shellfish industry. Protocols to determine the most successful techniques for
holding adult and juvenile mussels are continually being refined and tested. Innovative
techniques for growing and delivering food to juvenile and adult mussels are constantly being
adapted to meet the needs of each species.

6.0. PROCEDURES

6.1. Potential Host Fish Collection

In order to prepare for the host fish identification trials, we collected potential host fish during three different trips to the Nottoway River. We gathered potential host fish species prior to mussel collection to keep from having to move gravid female mussels more times than

necessary in order to minimize the potential that larvae might be released prematurely as they are handled. Fish collection followed standard protocols using seine hauls and backpack electroshockers. During all three trips we collected and transported approximately 220 potential host fish back to White Sulphur Springs National Fish Hatchery (WSSNFH). Fish collected represented the *Cyprinidae*, *Anguillidae*, *Catostomidae*, *Ictaluridae*, *Centrarchidae*, and *Percidae* fish families. Since current information indicates that many other pigtoe mussel species tend to use fish from the family *Cyprinidae* as hosts, we collected 13 fish species of *Cyprinids*. Collected fish were transported in aerated coolers back to WSSNFH and held in state-of-the-art AHAB recirculating systems. All fish collected have now been released back into the Nottoway River at their original capture sites.

6.2. Mussel Collection

Very little is known about the life history of the Atlantic pigtoe. Some mussel species retain glochidia in the gills over winter and release them when conditions are right the following spring (long-term brooders) but the Atlantic pigtoe appears to be a short-term brooder that must be fertilized and release glochidia in the same season, making it all the more vulnerable to environmental variability. It has been presumed that reproduction takes place every year in late spring, making that the target window for collection of gravid mussels. However conditions in the Nottoway River on ARNG-MTC Fort Pickett in 2009 made mussel survey and collection impossible until late June due to high water levels, flow rate and turbidity (Figures 7 and 8).

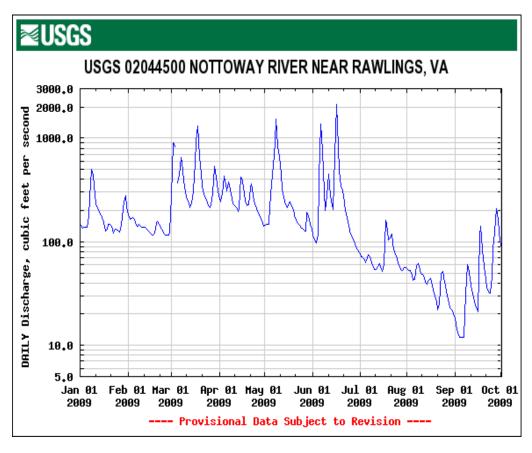


Figure 7. USGS discharge for the Nottoway River May-August 2009.



Figure 8. Example of high water level and extremely turbid conditions in the Nottoway River within ARNG-

MTC Fort Pickett during spring and early summer 2009 (shown here at discharge rate of 139 ft³/sec). Inset shows conditions during 2007 sampling with discharge rate of 4 ft³/sec.

After intensive sampling of the site previously identified as having the highest abundance of Atlantic pigtoe on ARNG-MTC Fort Pickett we were only able to collect 7 live individuals. Of those 7 mussels only one mussel had larvae in her gills; however, those larvae were comprised solely of egg material and were not usable for host fish or propagation purposes (Figure 9). None of the individuals collected in 2009 were already tagged at the time of capture and so represent different individuals than those captured and marked during previous sampling efforts.

Given the low numbers of gravid individuals found within ARNG-MTC Fort Pickett, we targeted another section of the Nottoway upstream from the installation with a site that has been

historically known to have large numbers of Atlantic pigtoe. We found another 7 specimens after 15 additional person hours but no individuals were gravid. In addition to many relic shells found at the site, we observed many shells that were more freshly dead as well, which may be another indication of the rapid decline of the Atlantic pigtoe at the sites we visited in the Nottoway River.

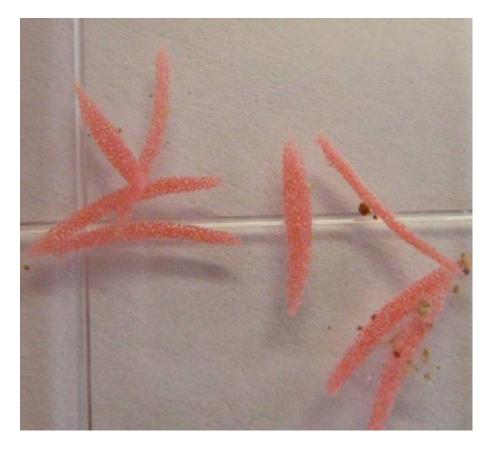


Figure 9. Atlantic pigtoe conglutinates collected from the Nottoway River on ARNG-MTC Fort Pickett in 2009 (non-viable).

Because no gravid females could be obtained from the Nottoway River, we attempted to collect gravid Atlantic pigtoe from another site of historic abundance in Craig Creek in Botetourt County, VA. During 2 collection trips, we found 33 individuals but again, none of them were gravid. And just as we observed in the Nottoway, we found numerous dead shells at the Craig Creek site (Figure 10). For all sites in the Nottoway and in Craig Creek combined, we

documented 47 live Atlantic pigtoe mussels (Table 2). All individuals were tagged and released to the original collection location (Figures 11, 12, and 13).

Table 2. Results of 2009 Atlantic pigtoe (*F. masoni*) sampling efforts at sites in The Nottoway River and Craig Creek, Virginia.

Date	Site	Water Temperature	Number live	Number gravid	Person-hours	Person-hours
		Temperature	F. masoni	F. masoni	Mussel collection	Fish collection
6/25/2009	Nottoway River Fort Pickett Hurricane Branch	79 F	1	0	10	6
7/1/2009	Nottoway River Fort Pickett Hurricane Branch	78 F	3	1, larvae all egg and not viable	15	9
7/15/2009	Nottoway River Falls, Route 49	76 F	7	0	15	5
7/23/2009	Craig Creek Oriskany, VA	72 F	21	0	8	
8/7/2009	Nottoway River Fort Pickett Hurricane Branch	76 F	3	0	4	
8/27/2009	Craig Creek Oriskany, VA	71 F	12	0	8	
Totals			47	1 NOT VIABLE	60	20



Figure 10. Dead shells of Atlantic pigtoe documented at Craig Creek sampling site in 2009.



Figure 11. Atlantic pigtoe collected from the Nottoway River, ARNG-MTC Fort Pickett.



Figure 12. Atlantic pigtoe collected from the Nottoway River outside of ARNG-MTC Fort Pickett in 2009.



Figure 13. Live Atlantic pigtoe collected from Craig Creek, VA in 2009.

7.0. IMPLICATIONS

The fact that we were unable to find any gravid Atlantic pigtoe has delayed gaining further insight into the identification of the all-important link to its host fish species until more individuals can be collected, but much has been gained during this portion of our work. The relatively lower numbers of Atlantic pigtoe encountered despite intensive and thorough sampling not only on ARNG-MTC Fort Pickett, but elsewhere in the Nottoway, as well as in Craig Creek, supports assumptions about overall declining population trends for the Atlantic pigtoe. Our experience has not only shown these areas to be sites of traditionally high abundance, but these sites have also been identified as home to some of the few remaining stable populations in the state, if not the world. The decline in the number of individuals encountered underscores the urgent need for active propagation efforts. The fact that virtually 100% of the 47 individuals we collected were not reproductive indicates that the reproductive cycle may not occur every year as previously believed. Whether this intermittent reproductive cycle is the result of environmental conditions or a facet of overall reproductive strategy is as yet unclear, but adding to the body of knowledge about Atlantic pigtoe life history is an important step as the lack of basic knowledge of a species' reproductive cycles is often cited as a fundamental impediment to recovery efforts, and is a feature common to many other species already federally protected under the Endangered Species Act.

The Atlantic pigtoe shares many other of the traits commonly associated with endangered/vulnerable species:

Limited geographic distribution: Limited geographic distribution makes a species more
vulnerable to extinction, and as supported by our recent survey efforts, occurrence of the
Atlantic pigtoe is in rapid decline across its historic range.

- Small population size An additional consequence of this overall decline is the
 increasingly small size of individual populations, a vulnerability commonly found in
 endangered species, and also observed for Atlantic pigtoe in our survey efforts.
- Specialization Specialization of important aspects of habitat requirement or life history is also common to many threatened and endangered species, and the highly specialized reproductive relationship between freshwater mussels and their dependence upon host fish is a prime example.
- Vulnerability to human impacts As human use of the land changes and grows, species that are sensitive to the common results of human activity like pollution, increased sedimentation, and destruction of riparian areas become increasingly at risk. Adult Atlantic pigtoe are very sensitive to increased sediment loads and the changes in flow structure and water temperature that can come with stream channel modification, and the glochidia appear to be extremely sensitive to many forms of pollution.
- Low motility While the use of host fish make range expansion possible for mussel species as a whole, adult mussels settle into the substrate and are primarily sedentary, making it nearly impossible to escape deleterious events. However, some movement is possible in response to local changes in water conditions. Mussels may move vertically by burrowing deeper into the substrate, or even horizontally (a few meters at most) in search of more favorable conditions (Figure 14), but clearly not enough to escape major perturbation events or the impacts of changing land use patterns.

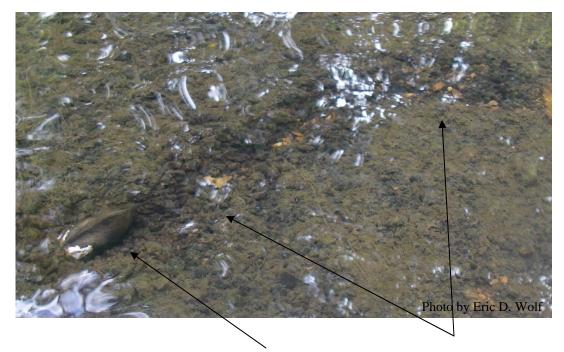


Figure 14. Documentation of a mussel moving horizontally (note track of disturbed substrate from upper right toward lower left) from shallower toward deeper water.

• Low reproductive rates - While little has been discovered so far about Atlantic pigtoe reproductive rates, only a tiny fraction of the tens of thousands of larvae produced by each mussel in the wild will live to reach reproductive status of their own.

While in many cases species that exhibit these traits can survive and thrive, all of these elements combine to make populations vulnerable to decline whether from sometimes subtle demographic shifts, or a triggering event like a natural disaster, unintended training or construction impacts, or even the coincidence of unfavorable environmental conditions such as high water events early in the season several years in a row or even ephemeral shifts in water temperature.

The implications for the Atlantic pigtoe are clear: swift action is required to prevent further decline and subsequent federally listing and protection under the Endangered Species Act.

8.0. CONCLUSIONS

Training demands on military installation are currently increasing at the same time that freshwater mussel populations are declining dramatically. Changes in training land infrastructure coupled with the expected increase in training loads will result in a variety of disturbances that alter soil structure, increase soil compaction, eliminate vegetative cover, and result in accelerated erosion, and degradation of surface water systems. Atlantic pigtoe (and many other mussel species) are very sensitive to the effects of these impacts.

The Atlantic pigtoe is in precipitous decline and is ripe for federal protection under the Endangered Species Act. Supporting active propagation of this Species at Risk now is the best way to prevent the species from federal listing and subsequent encroachment of the training mission. While conditions have delayed the identification of host fish species, it remains a vital step in this process, and will form the foundation upon which all subsequent recovery work can be based.

This project has strengthened interagency cooperation and formed a lasting partnerships, incorporating DoD, University, Virginia Wildlife Action Plan, and Fish and Wildlife Service support to fill in vital information gaps that are currently inhibiting efforts to support rapidly declining freshwater mussel populations. Building upon the information and relationships generated in this study we will make it possible to not only directly support populations of Atlantic pigtoe in Virginia, but throughout its range.

But the reach and benefit of this project are greater still. The Army Species at Risk List currently contains 10 freshwater mussel species found on 11 DoD properties in 8 states, and with many more mussel species on the decline across North America, the potential for negative impacts to the military mission from additional listings is growing rapidly. This strategy, and the

cooperative interagency relationships formed in this project, can not only be used for any number of mussel Species at Risk on DoD lands, but can be used to propagate more common species to improve water quality as well as protecting and/or restoring mussels populations threatened or damaged by accidents or training impacts.

The continuation of this effort has become even more important given the results of our work so far. We have learned important life history elements but more work is needed to identify the host fish species of the Atlantic pigtoe. Successful host fish trails will identify fish species and conditions best suited to support Atlantic pigtoe. We are now prepared to simultaneously establish the host fish relationship required to actively propagate and grow out older juveniles for direct release into the river, as well as conduct streamside infestations, where female mussels are collected from the river system, larvae extracted onsite. Host fish collected from the same area can be then be infested onsite and released, with a representative sample brought back to the lab to quantify success. We will also be able to develop criteria and methodologies for application on additional DoD facilities. This combination of strategies is extremely cost-effective and builds on a successful and expanding knowledge base. It is the best chance at keeping the Atlantic pigtoe from declining to the point that it requires federal protection, and once established, these techniques and the working partnerships formed can be applied over broad geographic areas and with a variety of mussel species.

Active propagation is the most proactive, cost-effective way to support declining populations of the Atlantic pigtoe and is one of the best strategies to keep the species from federal listing. We are gathering information vital to that effort and developing the propagation technology that will allow the release of large juveniles back in to the Nottoway River, within the confines of ARNG-MTC Fort Pickett as soon as conditions there allow. By rebuilding the

population on the installation, it can serve to support overall population levels in the State, as well as other suitable areas throughout its original range. Supporting active propagation of this Species at Risk is the best way to prevent the species from federal listing and subsequent encroachment of the training mission, while simultaneously building a working relationship with partners through which this same technique can be applied at many additional DoD lands with mussel species at risk.

9.0. SELECTED RESOURCES

Conservation Management Institute http://www.cmiweb.org/

White Sulphur Springs National Fish Hatchery http://www.fws.gov/northeast/wssnfh/index.html

Freshwater Mussel Conservation Society http://ellipse.inhs.uiuc.edu/fmcs/

North American Benthological Society http://www.benthos.org/index.cfm

The Virtual Aquarium at Virginia Tech http://www.cnr.vt.edu/efish/index.html

The Mussel/Fish Connection http://www.fws.gov/endangered/bulletin/96/mussel.html

The Status of Aquatic Mollusks in the Southeastern United States: A Downward Spiral of Diversity

http://www.fishwild.vt.edu/mussel/PDFfiles/Status%20of%20Aquatic%20Mollusks.pdf

Propagation of endangered freshwater mussels in North America http://www.fishwild.vt.edu/mussel/PDFfiles/Propagation_of_mussels.pdf

National Strategy for the conservation of native freshwater mussels http://www.fishwild.vt.edu/mussel/PDFfiles/National%20Strategy.pdf

Freshwater mussels: A neglected and declining aquatic resource http://www.fishwild.vt.edu/mussel/PDFfiles/Neglect_Declining_Resource.pdf

Conservation status of freshwater mussels of the United States and Canada http://www.fishwild.vt.edu/mussel/PDFfiles/Conservation_status.pdf

Muskrat predation on endangered freshwater mussels in Virginia http://www.fishwild.vt.edu/mussel/PDFfiles/muskrat.pdf

Models and model selection uncertainty in estimating growth rates of endangered freshwater mussel populations

http://www.fishwild.vt.edu/mussel/PDFfiles/model_selection_uncertainty.pdf

Factors affecting survival and growth of juvenile freshwater mussels cultured in recirculating aquaculture systems

http://www.fishwild.vt.edu/mussel/PDFfiles/Factors_affecting_survival.pdf

Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants

http://www.fishwild.vt.edu/mussel/PDFfiles/Differential_exposure.pdf

Conservation status of freshwater mussels of the United States and Canada http://www.fishwild.vt.edu/mussel/PDFfiles/Conservation_status.pdf

Native Freshwater Mussels

ftp://ftp-

 $\underline{fc.sc.egov.usda.gov/WHMI/WEB/pdf/TechnicalLeaflets/NativeFreshwater_\%20MusselsJan16.p}\\\underline{df}$

The Role of Military Lands in Maintaining Biodiversity http://www.dodbiodiversity.org/ch1/index 4.html

APPENDIX A:

Presentation of Project Results at 3rd Annual Virginia Atlantic Slope Mussel Recovery Group Converence

March 26, 2010 Forrest, VA

A.1. Meeting Agenda:

VA Atlantic Slope Mollusk Recover Group Meeting

Date: Friday, March 26, 2010

Time: 10AM - 4:00PM

Location: Forest Library, 15583 Forest Road, Forest, VA 24551

1) Mussel Propagation

- o Harrison Lake National Fish Hatchery (Watson)
 - > Propagation results for 2009
 - > Propagation plans for 2010
- o White Sulphur Springs National Fish Hatchery (Mair)
 - Propagation results for 2009
 - Propagation plans for 2010
- o Virginia Tech (Dan)
 - > Propagation results for 2009
 - > James spinymussel streamside infestations
 - > Propagation plans for 2010

2) Conservation Activities

- o James spinymussel streamside infestation at Cowpasture River (Kane)
- o EPA proposed ammonia criteria (Kane)
- o Dominion Power/Bremo Bluff Power Station thermal discharge (Watson)
- o Dominion Power/Lake Anna Nuclear Station (Watson)

3) Survey Updates; 2009 Surveys and Planned 2010 Surveys

- o North Fork Shenandoah watershed (Roble)
- o James River (Ostby)
- o Planned survey activities for 2010 (All)

4) Research Project Overview/Update

- o Lanceolate Elliptio genetics (Watson)
- o VA freshwater mussel web atlas (Watson)
- o Freshwater gastropod of VA web atlas, Atlantic slope (Watson)

5) Other Issues of Interest (All)

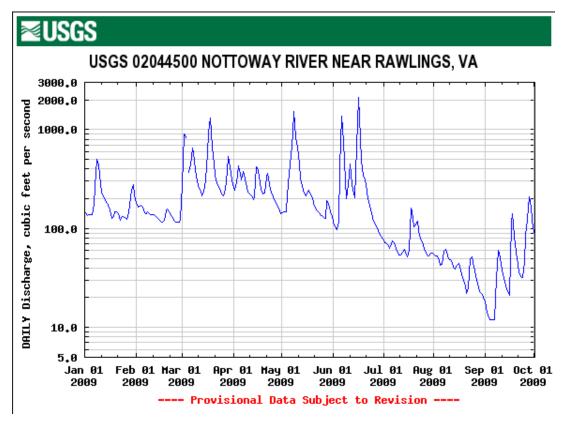
o FWS BO for Craig Creek ford maintenance (Ostby)

A.2. Slides from presentation of project results

Host fish tests for Atlantic pigtoe, *Fusconaia masoni*



Nottoway River Conditions During Atlantic pigtoe Collections





Collection of Atlantic pigtoe, *Fusconaia masoni,* from the Nottoway River, 2009

Date	Site	Water Temperature	Number live	Number gravid	Person-hours	Person-hours
		•	F. masoni	F. masoni	Mussel collection	Fish collection
6/25/2009	Nottoway River Fort Pickett	79 F	1	0	10	6
	Hurricane Branch					
7/1/2009	Nottoway River Fort Pickett Hurricane Branch	78 F	3	1, larvae all egg and not viable	15	9
7/15/2009	Nottoway River Falls, Route 49	76 F	7	0	15	5
7/23/2009	Craig Creek Oriskany, VA	72 F	21	0	8	
8/7/2009	Nottoway River Fort Pickett Hurricane Branch	76 F	3	0	4	
8/27/2009	Craig Creek Oriskany, VA	71 F	12	0	8	
Totals			47	1 NOT VIABLE	60	20

Atlantic pigtoe collection trips

Atlantic pigtoe collected from the Nottoway River, Fort Pickett



Atlantic pigtoe collected from the Nottoway River, Falls, Rte 49



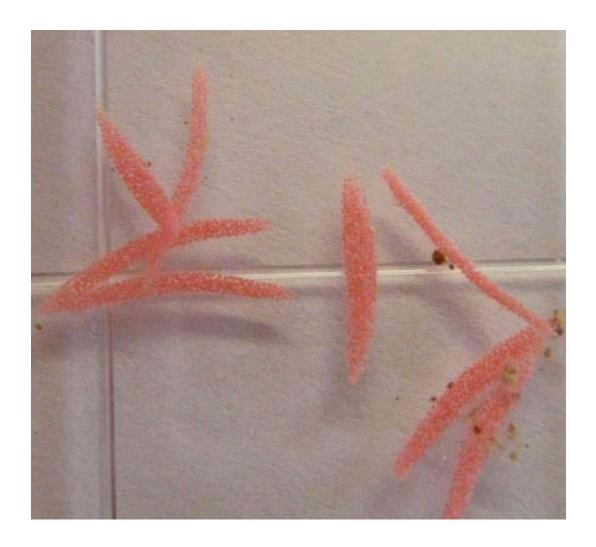
Shells of Atlantic pigtoe collected from Craig Creek, VA



Live Atlantic pigtoe collected from Craig Creek, VA



Atlantic pigtoe larvae



2010 Propagation Plan

- Grow notched rainbow
- —Toxicity testing
- PhD student juvenile settling study
- Continue JSM work
- -Streamside and outreach
- Concentrate on lab culture and cages
- Complete JSM propagation and augmentation plan
- Continue Atlantic pigtoe host fish tests
- Continue assisting with mussel surveys

APPENDIX B:

Legacy Fact Sheet 2009 Project Number 09-450



Propagation of Species At Risk Atlantic Pigtoe on Military Installations

Project # 09-450

Background:



Clean and healthy rivers and streams need freshwater mussels. Mussels actually work to improve water quality, and fill a host of important roles in aquatic ecosystems. But freshwater mussels have become the nation's most endangered group of animals.

The Department of Army has identified the Atlantic pigtoe mussel as a Species at Risk (SAR) with potential for detrimental impact on the military mission if federally listed as either threatened or endangered.

The Nottoway River on ARNG-MTC Fort Pickett, VA is home to one of only two known stable populations of the Atlantic Pigtoe mussel left in Virginia, and perhaps the world. Populations are in precipitous decline throughout the southeast, and expert consensus is that the species currently warrants federal listing.

Active propagation is the most proactive way to support declining populations and is the single best strategy to keep the species from federal listing, and prevent ensuing training restrictions. But current knowledge is incomplete pending further research into the species' unique reproductive cycle, including the identification of the host fish species upon which the mussels depend to complete their life cycle.

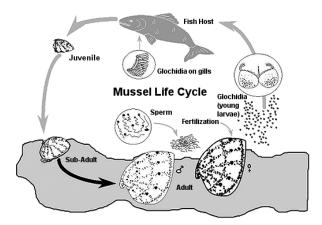
Objective:

Successful host fish trials will allow propagation and growth of juveniles that can then be released back in to the river. In this manor the population on the installation could serve to support overall population levels in the State, as well as other suitable areas throughout its original range. Identification of host fish species is critical to this process, and will form the foundation upon which all subsequent recovery work can be based.

This project represents a uniquely cost-effective and time-sensitive opportunity to support existing populations and proactively avoid federal listing and subsequent encroachment of the training mission, while simultaneously building a working relationship with partners through which this same technique can be applied many additional DoD lands with mussel species at risk.

Summary of Approach:

The remarkable life cycle of freshwater mussels depends on a parasitic stage where larvae survive by attaching themselves to specific host fish.



For propagation, mussels carrying developing young and potential host fish species are collected and kept in specialized holding facilities where larvae are introduced into aerated containers to allow them to attach to the gills of the fish. Infested fish are then held until juveniles fall off their host, and the success of each potential host fish species can be assessed.

A "robust host" will consistently produce a large number of juvenile mussels while a "marginal host" will produce inconsistent or low numbers of juveniles. Juveniles can then be cultured, tagged, and released to the original collection site along with the adult mussels so as to assure no detrimental impacts to the original population.



Biologists at the White Sulphur Springs National Fish Hatchery's Aquatic Resource Restoration Center in have broad experience propagating over 30 mussel species, including 16 federally listed species that have been used to augment existing populations and re-establish historic populations.

Benefit:

Training demands on military installation are currently increasing at the same time that freshwater mussel populations are declining dramatically, and Atlantic pigtoe (and many other mussel species) are very sensitive to the potential impacts. Supporting active propagation of this Species at Risk now is the best way to support the remaining population and keep the species from federal listing and subsequent encroachment of the training mission.

But the reach and benefit of this project are greater still. The Army SAR List currently contains 10 freshwater mussel species found on 11 DoD properties in 8 states, and with many more mussel species on the decline across North America, the potential for negative impacts to the military mission from additional listings is growing rapidly. This strategy, and the cooperative interagency relationships formed in this project, can not only be used for any number of mussel Species at Risk on DoD lands, but can be used to propagate more common species to improve water quality as well as protecting and/or restoring mussels populations threatened or damaged by accidents or training impacts.

Accomplishments:

Our intensive sampling in previously abundant areas clearly demonstrates the urgent need for active propagation efforts as we encountered historically low numbers of individuals and none with viable larvae.

The lack of basic knowledge of a species' reproductive cycle is often cited as a fundamental impediment to recovery efforts. Even though the lack of viable larvae has forced a delay in the identification of host fish species, we have filled in some of the critical gaps in the life history of the Atlantic pigtoe, gaining greater understanding of the seasonal and environmental conditions required for reproduction, and the discovery that the species may not be reproductive every year, much needed information for any recovery effort.

This project has also strengthened interagency cooperation and is solidifying a lasting partnership, between the DoD, Virginia Tech, the Virginia Wildlife Action Plan, and the U.S. Fish and Wildlife

Service to fill in vital information gaps that are currently inhibiting efforts to support rapidly declining freshwater mussel populations found on many DoD lands.

Building upon the information and relationships generated in this study we will make it possible to not only directly support populations of Atlantic pigtoe in Virginia, but throughout its range, as well as other mussel species in jeopardy.

Contact Information:

Name: Eric D. Wolf

Title: Project Manager

Org: Conservation Management Institute – VA Tech

Address: 1900 Kraft Drive Suite 250

Blacksburg, VA 24061

Phone: 540-231-9605

Fax: 540-231-7019

Email: ewolf6@vt.edu