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An Investigation of the Changes in Species Richness and Distribution of the Unionidae (Bivalvia: Mullusca) within the Pamunkey Watershed.

By William Robert Lees

For a Master's of Science in Biology at the University of Richmond in August of 2005
Thesis advisor: Dr. Roni J. Kingsley

Abstract

A survey of the Unionidae within the Pamunkey Watershed was conducted to investigate changes in distribution and species richness compared to a previous survey (Riddick, 1973) conducted over 30 years ago. Species richness decreased from 10 species in the previous survey to four species in the present survey. Notable increases of species richness were found in the upper South Anna and the lower North Anna regions, while sizable decreases were found in the lower South Anna and the upper and middle regions of the Pamunkey River. Regression analyses and t-tests comparing mussel survey data with habitat parameters were conducted to investigate the habitat characteristics most linked to unionid populations. The results indicated that greater composition of larger substrates and lower levels of silt deposition were significantly correlated to mussel presence. Crop land was the only quantified land use to correlate significantly (negatively) with changes in species richness and regional abundance parameters.

An Investigation of the Changes in Species Richness and Distribution of the Unionidae (Bivalvia: Mollusca) Within the Pamunkey Watershed

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AN INVESTIGATION OF THE CHANGES IN SPECIES RICHNESS AND DISTRIBUTION OF THE UNIONIDAE (BIVALVIA: MOLLUSCA) WITHIN THE PAMUNKEY WATERSHED

By

WILLIAM ROBERT LEES

B.S., Virginia Commonwealth University, 1987

M. Ed., Virginia Commonwealth University, 1988

A Thesis

Submitted to the Graduate Faculty

Of the University of Richmond

In Candidacy

For the degree of MASTER OF SCIENCE

In

Biology

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INTRODUCTION

Unionids and Freshwater Ecosystems

Freshwater mussels are currently considered the most imperiled fauna within North America (Master et. al. 2000). Many species of freshwater mussels (unionids) have become extinct or are presumed extinct (over 10% of the total number of species) and many more are considered imperiled (Master et. al, 2000). Among the approximately 300 species of Unionidae that remain, 69% are considered in trouble (Master et. al, 2000). Sixty nine (23%) are currently federally listed as endangered (Neves, 1999). The state of Virginia contains 19 species that are federally listed (Neves, 1999) and many more that are identified as species of concern. Although unionids are declining rapidly in diversity, a sufficient body of research demonstrating changes in species richness and connections to habitat characteristics and land uses is lacking, especially among smaller drainages such as the Pamunkey River. In addition to determining the status of species richness, surveys of Unoinidae may also serve as an indicator to overall health among freshwater ecosystems.

Widespread reported declines in mussel presence and diversity indicate that these freshwater ecosystems are deteriorating. Excessive sedimentation and nutrients entering streams in addition to water flow changes from impoundments are often mentioned as factors that change habitats creating stresses for native species (Bogan, 1993). Sensitivity to deteriorating water quality conditions, disturbance, and changes to substrate composition may enable freshwater mussels to function as effective indicators to changes in habitat conditions.

Functionally, freshwater mussels are valuable members of freshwater ecosystems. While filter feeding, freshwater mussels increase water clarity while providing an important link within the food web of stream habitats. In a study by Welker and Waltz (1998), an exponential reduction in the presence of plankton was recorded from a lake to river interface which was attributed to an increase in predation rate through filter feeding by freshwater mussels. The significant reduction in plankton levels demonstrated the potential for freshwater mussels to play an important ecological role in cycling nutrients within freshwater stream ecosystems. By reducing nutrient levels within the water column, freshwater mussels may also serve to reduce the effects of eutrophication which are associated with reductions in water quality. By maintaining higher dissolved oxygen levels and greater water clarity, freshwater mussels can contribute to improved conditions for underwater plants, fish, macroinvertebrates and other freshwater organisms.

Sedimentation, Nutrient Loading and Habitat

The U.S. Environmental Protection Agency (1990) identified excess sedimentation as the biggest problem impairing over 40% of the river miles within the United States. Sedimentation through erosion, along with nutrient run-off and impoundments, are among the most frequent causes suggested for reductions of unionid species present in freshwater ecosystems. Although mussel declines have occurred rapidly, changes in their habitats are based primarily on descriptive or anecdotal information (Bogan, 1993). However, a few studies have demonstrated convincing links between substrate types and mussel populations. In a study by Neves and Widlak (1987), the composition and number of mussels found were directly related to the distribution of

bed sediments. Several studies, such as those by Houp (1993) and Stein (1972), have demonstrated how coal mining, logging, and road construction can completely change mussel assemblages through the elimination of species adapted to stable stream environments and the proliferation of those species that can tolerate heavy sedimentation.

Instances of heavy sedimentation caused by storms or flooding in unstable stream environments can kill mussels by burying them underneath sediment accumulation. In laboratory experiments, Marking and Bills (1980) found that large percentages of some species died when buried under 10 cm of silt, while others that were more adept at moving vertically up through sediments had much lower mortality rates. Studies have also been conducted in the lab to identify the effects of sedimentation on unionid physiology. As reported by Aldridge et al. (1987) excess sediment interferes with filtering mechanisms used by freshwater mussels for feeding and respiration. Studies on marine bivalves have shown that excess sediment decreases the feeding efficiency and reduces growth rates in juveniles (Bricelj and Malouf, 1984). An excessive sediment load can change the heterogeneous substrate causing it to become filled in and compacted essentially creating an embedded hardpan layer, making it difficult for the mussels to become established (Gordon et al. 1992).

Although lab studies have provided some compelling evidence demonstrating the effects of sedimentation on unionid physiology, relating these effects to land use changes and declines in species richness is a more complicated relationship to demonstrate.

According to Box and Mossa (1999), the focus of research should be on identifying the composition of bed material, the movement of the suspended bed material load, and the changes of substrate that occur in connection with land use. In their article, Box and

Mossa (1999) describe appropriate procedures for analyzing and sampling sediments and identifying the nature of sediment sources. They state that more rigorous procedures are needed in order to assess relationships between substrate types, sediments, and the presence of unionid mussels.

Quantifying and identifying sediment loads and channel morphology are methodologically challenging. Determining exactly what to measure and using appropriate sampling schemes are two of the biggest stumbling blocks. Box and Mossa (1999) outline problems with some of the methodologies used for examining sediment and channel morphology. They cite that one of the reasons substrate size is not consistently associated with mussel species distributions is that sampling is inadequate in quantity (less than 50) and categories of particle size (usually just six) are too limited. In cases of adequate sampling, they indicate substrate composition by size can be a strong indicator of habitat suitability, but in some species water velocity or host fish populations may be more important predictors of mussel populations.

Studies have focused on different aspects of habitat conditions and had different purposes in their investigations of freshwater mussel populations. Neves and Widlak (1987) found that in the Holston River in Virginia, the greatest species densities were associated with stable mixed sand, pebble, and gravel substrates. Other studies like the one by Winterringer et al. (2003), using multiple regression analyses, found that channel condition, slope, stream morphology, fish barriers, velocity, and bank stability were the most effective indicators of mussel bed presence. Parameters not significantly associated with mussel bed presence in the the Winterringer et. al study included riparian zone composition, water appearance, nutrient enrichment, pool presence, canopy, particle size

and embeddedness along with water quality factors including pH, dissolved oxygen, fecal coliforms, and suspended and dissolved solids.

Variable results from habitat studies indicate the likelihood that parameters important in indicating the quality of mussel habitat are often unique to watersheds and depend on the species present at the time of the study, as well as specific local factors. Therefore, generalizing results from one watershed to another is difficult, especially without having data on past surveys and historical ranges of the species present.

Although some habitat parameters may be widely generalized, many are probably specific to watersheds that have unique geologic histories that influence stream morphologies over time. Some watersheds with greater topographical relief are more likely to have sediment enter streams unless some types of barriers are in place to prevent excessive run-off. Soils also have varying degrees of permeability and water absorbing capabilities. Water tends to run off soil with high clay content and erode sandy soils. Thus, geologic factors along with land use practices are both instrumental in shaping changing stream morphologies and sediment loads within the system.

Stream stability is important for mussel habitat because of the potential for mussels to be washed away or lethally covered by sediment if flooding and erosion substantially alter substrate conditions. Unionids are fairly long lived species capable of surviving more than 15 years. Since mussels have limited mobility, they are dependent on being able to control their position within a limited area of substrate both horizontally and vertically. Changing substrate conditions can greatly compromise their growth, health, and survival. Stream reaches that are not stable can accumulate sediment covering areas of heterogeneous stable substrate or degrade by having the substrate

scoured down as the channel widens through the erosion of its banks. Stream reaches that are stable have slower rates of erosion and maintain substrate conditions that do not change rapidly over time. Some of the characteristics generally thought to enhance the stability of streams include density of riparian coverage, the presence of a flood plain, a mixed loamy soil with a moderate amount of sand and clay, gradual elevation change adjacent to the channel, the presence of sinuosity in the stream channel, and moderate flow rates with less fluctuation.

To measure stream stability in a quantitatively rigorous way requires timeconsuming sampling techniques repeated in regular intervals over extended periods of
time. No doubt these studies would provide valuable insight into how sediment loads and
suspended sediments affect stream habitats in small microhabitat spatial scales. To
investigate areas that are candidates for more rigorous quantitative analysis, it may also
be possible to find sampling methodologies used with larger spatial scales that can target
areas of concern. In previous studies, a variety of methods have been used to identify
levels of suspended sediments and sediment deposition including many water sampling
devices, filters, sediment traps, and various sampling regimes. Most often these measures
have been applied to site-specific habitats and have not been used with data collected at
larger spatial scales. Stream stability data on a larger spatial scale along with GIS land
cover and riparian data may provide a means to investigate relationships between land
uses and evidence of erosion occurring within freshwater habitats.

Land Use

A variety of land use changes can significantly impact unionid species diversity and distribution. The presence of dams in recent years has likely impacted the diversity and distribution of freshwater mussels by drastically changing flow conditions and limiting the upstream distribution of host fish that unionids need to complete their larval development. When dams in streams are created there is a distinct change in depth, flow rates, and substrate composition both upstream and downstream of the impoundment. Over time, conditions around a dam or impoundment may stabilize, however the upstream migration of mussel species are effectively cut off. Haag and Warren (1998) found that mussel species are often restricted to sites that have stable host-fish densities and in a study by Watters (1996), dams were found to effectively restrict the upstream distribution of mussels. Because fish populations are also affected by human alterations of habitat in the form of sedimentation, nutrification, and toxins, mussel populations are especially vulnerable due to their dependence on specific hosts for larval development and distribution, and their own sensitivity to sediment loads and changing flow patterns.

Other land uses have had more general effects on nearby freshwater ecosystems. Acid mine drainage can strip a stream of its native flora and fauna. When pH values drop below 3, the organisms are typically limited to iron oxidizing bacteria specialized for thriving in acidic environments and algal populations (USGS 1999). Streams adjacent to agricultural crop land or pastures are impacted through increased nutrient loads that decrease water quality. Some areas along streams lack riparian buffers and others are accessible to cows creating opportunities for sedimentation along with nutrient and fecal coliform contamination. Other detrimental conditions to stream habitats occur when

areas undergo substantial human development. An increase of impervious surfaces through road construction increases run-off into streams creating a major source of sedimentation and potential toxins. The contents in the run-off are deposited on the stream bottom or continue downstream as suspended sediment increasing turbidity and eventually settling out and adding to the accumulating sediment in downstream locations.

Incorporating Data from GIS

In order to investigate land use impacts on stream ecology, it is becoming more common for biologists and ecologists to utilize land use data within Geographical Information Systems (GIS) to examine changes in species diversity at a larger spatial scale. Several studies in the past several years have looked at changes in populations of fish, macroinvertebrates, and mussel populations in connection with patterns of land use and geological features. In a study by Richards and Host (1994), land use/land cover data were used with a variety of physical habitat parameters to identify relationships between land use and habitat parameters and macroinvertebrate species assemblages. Using principal component and correlation analysis, relationships were found among the parameters tested. Agriculture and urban land use were found correlated to habitat conditions and substrate conditions and levels of woody debris were correlated to macroinvertebrate species richness and composition. The authors suggest more subtle influences might be detected through higher resolution land cover analysis than with the data available to them (16 ha). In a study by Joy and Death (2004) a variety of land use, geomorphological, spatial, and climatic data were used to create a predictive model by spatially mapping fish and decapod populations. Large spatial scale data were used

within an artificial neural network to construct a predictive model to identify the presence of specific species within the region. Among the many parameters that were predictive of freshwater communities were vegetation type, land use proportions, and geological structure. In a highly agricultural area of Iowa, mean watershed slope and alluvial deposit features characterized by GIS data bases were found to be the most highly correlated landscape scale features associated with mean density and species richness of mussel assemblages (Arbuckle and Downing, 2002). The alluvial deposits were identified as being instrumental in moderating groundwater flux to streams so that stream and substrate stability could be maintained. By incorporating land use data the researchers in these three studies were able to identify more specifically the factors of land use and geology that are most likely impacting freshwater habitats.

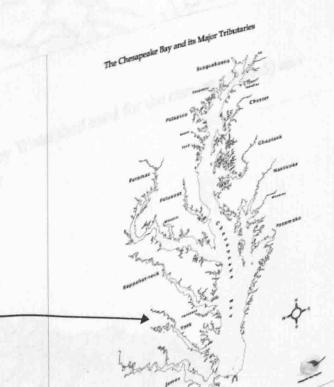
Regional Land Use Issues

The Pamunkey Drainage area used in this study is part of the Atlantic Slope
Region. The bio-geographical region described by Johnson (1970) includes the area east
of the Appalachian Mountains with streams that flow into the Atlantic Ocean from
Georgia to Canada. This region of the United States is characterized by land use
predominantly of forest and agriculture in more rural areas and highly developed land
uses in the urban areas. Many agricultural and human developmental land uses are
associated with the degradation of freshwater ecosystems. The Chesapeake Bay
Watershed encompasses a large portion of the Northern Atlantic Slope region. The
Chesapeake Bay has been the recipient of deteriorating water quality entering the bay
from all its tributaries. Declining populations of bay grasses, blue crabs, and fish

populations have been used by the Chesapeake Bay Foundation as indicators of the reduction in water quality within the bay. Sediments and nutrients entering the bay from its tributaries are thought to be among the most important factors causing the water quality to decline. Nutrients and sediment coming from river tributaries are most likely responsible for increasing "dead zones" in which dissolved oxygen levels have become so low that fish and other aquatic organisms can no longer survive. Habitats within the tributaries upstream are also likely impacted by sediment and nutrients entering streams through erosion and groundwater. The Pamunkey Watershed, investigated in this study, through erosion and groundwater which is one of the major tributaries flowing into the Chesapeake Bay. (Figure 1)

Figure 1. The major tributaries of the Chesapeake Bay

The Pamunkey and Matoponi Rivers converging and becoming the York River that is a major tributary of the Chesapeake Bay



The Study Area

Within the Paumkey River drainage there are 2 major tributaries: the North Anna River and the South Anna River which along with the Little River join together to form the Pamunkey River. The Pamunkey River then converges with the Mataponi River to form the York River which drains into the Chesapeake Bay (Fig. 1). For purposes of comparison the Pamunkey Watershed (Fig. 2) was divided into subhydrological units identified by the United States Geological Survey (USGS). (Fig. 3)

Figure 2. The Pamunkey Watershed among the watersheds of Virginia

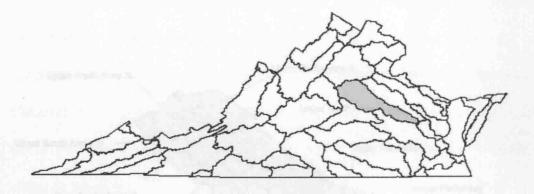
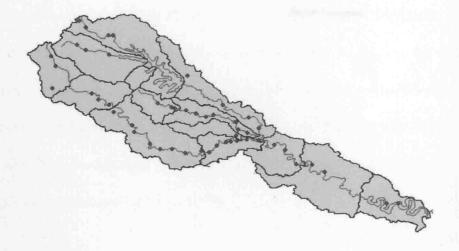
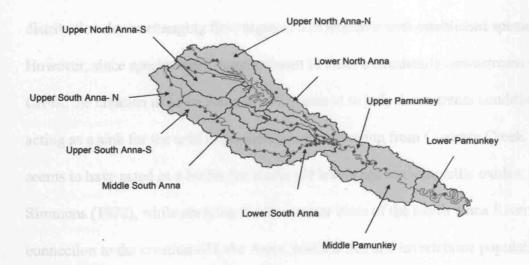


Figure 3. Sites within the Pamunkey Watershed used for the current (2004-5) and previous (1972-3) unionidae survey



Sites were located in 12 subdrainages (Figure 4) dividing the North Anna River into 3 regions, the Little River into 2 regions, the South Anna into 4 regions, and the Pamunkey into 3 regions. The 12 sub-hydrological regions identified in a USGS spatial data layer provided a regional unit of spatial scale with which to compare temporal changes of species richness and distribution between surveys and a means to compare spatial differences in the present survey.

Figure 4. Regions of the Pamunkey Watershed with survey sites



Land Use Issues Within the Pamunkey Watershed

The Pamunkey Watershed contains a variety of land use issues that have shaped community structure within its tributaries. One of the tributaries of the North Anna River, Contrary Creek, has become known for its devastating effects on downstream habitats due to acid mine drainage from old mine sites dating back to the 19th century. Episodes of flooding were associated with reported fish kills apparently as a result of the influx of contaminants into the river system. The North Anna Watershed was also altered through the creation of Lake Anna in 1972, built in part to function as a cooling site for a nuclear power plant created by VEPCO (now Dominion Virginia Power). Impoundments usually are associated with negative impacts on freshwater fauna diversity and distribution due to changing flow regimes that interfere with established species. However, since species were largely absent in areas immediately downstream of Contrary Creek, the creation of Lake Anna in 1972 seemed to help downstream conditions by acting as a sink for the acid mine contamination coming from Contrary Creek. The lake seems to have acted as a buffer for acidic pH levels and toxic metallic oxides. Reed and Simmons (1972), while studying the freshwater biota of the North Anna River in connection to the creation of Lake Anna, noticed fish and invertebrate populations in lower stretches of the North Anna River, but no unionid species, which led them to conclude that freshwater mussels may be more sensitive indicators of water quality. It was hypothesized that water quality conditions over time created by the dilution effect of Lake Anna would create downstream conditions once again favorable for freshwater mussel populations (Riddick, 1973).

In the Pamunkey Watershed the most rapid development has occurred around the town of Ashland, with some of the northern sprawl located within the Upper Pamnkey portion of the watershed. The development has possibly created moderate increases in run-off into this region through an increase in the area of impervious surfaces. The lower South Anna and upper and middle Pamunkey regions also include a large amount of agricultural crop land use, creating the possibility of even greater erosion through increases in sedimentation and nutrification. Other areas within the watershed have encountered less development and other than forested land contain primarily rural and agricultural land use for pasture or hay, according to the USGS 1991 GIS data layer for

Land cover / land use. (Figure 5)

Figure 5. Landuse/landcover data by region of the Pamunkey study area.										
	% Barren	% Developed	% Forested	% Pasture/Hay	% Cropland	% Rec/Grass	% Water	% Wetlands	% Rip (100 feet)	
Upper North Anna-north	1.462	1.134	61.952	24.456	2.019	0	8.452	0.524	2.3754	
Upper North Anna- south	2.663	0.955	71.595	20.776	0.888	0	1.79	1.332	2.2962	
Lower North Anna	4.408	1.408	73.082	10.786	6.78	0	0.985	2.55	5.0346	
Upper Little River	0.155	0.234	73.197	18.913	2.579	0	0.741	4.182	4.5696	
Lower Little River	1.051	0.676	69.446	16.114	7.656	0	0.602	4.45	5.4419	
Upper South Anna- north	3.501	1.451	61.736	26.425	1.813	0	1.002	4.071	2.0148	
Upper South Anna- south	2.881	1.919	75.220	15.483	0.977	0	0.814	2.706	3.6811	
Middle South Anna	2.068	0.956	67.332	19.941	3.787	0	1.02	4.898	4.1434	
Lower South Anna	0.613	1,732	60.587	23.591	9.151	0.409	1.951	1,966	4.4463	
Upper Pamunkey	0.665	4.550	52,508	13.713	16.965	0	1.602	9.995	4.758	
Middle Pamunkey	2.520	1.818	55.160	14.344	16.414	0.017	1.666	8.062	5.2723	
Lower Pamunkey	1.594	1,258	52.827	5.466	10.246	0	11.136	17.474	3.8061	

The upper reaches of the South Anna have also shown evidence of being affected by acid mine drainage (Riddick, 1973). The two northwestern most survey sites of the South Anna River had no unionid species present in the 1973 survey in addition to fish kills reported in 1968 in connection with increased acid mine leakage in the headwaters of the South Anna Watershed (Riddick, 1973). More recently habitat conditions have apparently improved, judging from water quality measurements and the return of several species of freshwater mussels.

Along the South Anna River and in upstream locations of the Little River many old mill and hydrologic dams are present. These dams represent blockages to upstream migration of mussels through the impediment of host fish which are necessary for successful development of mussel larvae. Over time these barriers may limit the sustainability of upstream populations for some species through the elimination of upstream migration.

Previous surveys

In 1970 Richard Johnson published *The systematics and zoogeography of the Unionidae (Mollusca:Bivalia) of the Atlantic slope region*, a highly influential bulletin addressing the systematics and zoogeography of Unionoidae of the Atlantic Slope Region. Many river basins were surveyed and compared with respect to geography and the species present. The Pamunkey Basin was reported to contain seven species of unionoids including *Elliptio complanata*, *Elliptio lanceolata*, *Lasmigona subviridis*, *Alasmidonta undulata*, *Strophitus undulatus*, *Lampsilis cariosa*, and *Lampsilis ochracea* (Johnson, 1970). In a survey conducted by Riddick (1973), *S. undulatus* and *L. ochracea* were not found, although they had been reported in earlier surveys in the Pamunkey

Region (Johnson, 1970). New records of species discovered in the 1973 survey included: Alasmidonta heterodon, Pygonodon cataracta, Elliptio angustus (now considered E. producta or E. fisheriana), Ligumia nasuta, and Lampsilis radiata radiata. The study conducted by Riddick (1973) confirmed at least 10 species present in the Pamunkey Drainage with the two additional species reported in other surveys that are also known to occur in other nearby river systems. (Refer to Table 1 to compare the species present by site number and sub-drainage for the survey conducted in 1972-3 to the current survey conducted in 2004-5.)

Since 1973 unoinoids have likely been impacted by stream altering conditions and land use practices. Among the 10 to 12 species historically present within the Pamunkey Basin, one is federally listed as an endangered species, A heterodon and other species, A. undulata, E. lanceolata, L. radiata radiata, L. cariosa, and L. subvirdis, are identified by state agencies as species of concern in the Pamunkey Watershed. In 1991 the Division for Natural Heritage of the Virginia Department of Conservation and Recreation followed up on the survey conducted by Riddick and revisited sites within her survey that included rare species. A. heterodon and A. undulata were absent from both sites identified in Riddick's survey and L. subviridis was absent from all three previous locations, while L. cariosa and L. radiata were present in only one of the four sites identified previously for each of these species. Other scattered surveys by various agencies have also been conducted within the drainage with relatively consistent results indicating decreases of the rare more habitat sensitive species over time (Table 2). It is likely that changes in habitat conditions have occurred at least partly from land use consequences and these

changes have contributed to the reduction in species richness occurring within the watershed.

Project Overview

By repeating the Riddick survey that took place from 1972-1973, a temporal comparison was made of the change in species richness and distribution within the Pamunkey Watershed. From the current survey, habitat parameters were analyzed to investigate relationships to the presence and abundance of mussels at individual sites. Additionally, spatial comparisons of the Pamunkey Drainage were investigated through a regional analysis of habitat data collected, the creation of a GIS quantifying regional land use and riparian extent, and regional survey results for abundance and species richness.

It was hypothesized prior to the survey that there would be a reduction of the number of species found in the current (2004-2005) survey of the Pamunkey Watershed compared to the survey conducted by Riddick in 1972-1973. Changes in species richness and distributions within the drainage were expected to correspond to historical land use issues including acid mine drainage, the formation of Lake Anna, and the long term effects of erosion due to agricultural land uses. Sites downstream of Lake Anna were expected to have freshwater mussels present where historically they were not observed as Riddick and her advisors predicted. Regions with large proportions of agricultural land use and development were expected to be associated with losses of species richness and lower survey abundances. Regions of the watershed with higher percentages of forested riparian coverage were hypothesized to be associated higher levels of species richness and abundance. (see Figure 5)

Substrate and water quality parameters associated with erosion and stream instability were expected to differentiate areas according to the presence, abundance, and change of species richness of freshwater mussels. Areas with higher turbidity were expected to be associated with losses of species richness and relatively low abundances of mussels. Some of the substrate parameters measured were expected to be associated with increased erosion and less favorable mussel habitats. The percentage of the substrate composed of silt and sand and average silt depth within areas of run were hypothesized to be negatively related to the presence and richness of unionid species. Average substrate size and the percentage of the substrate composed of pebble, gravel, or cobble substrate were anticipated to indicate favorable mussel habitat.

Qualitative stream stability indicators were expected to differentiate regions that were more heavily impacted by erosion than others. Degree of channelization, bank angles, bank vegetative cover, soil characteristics, and algal extent were included in an Index of Stream Stability. The index was hypothesized to rank regions from low to high in regard to levels of erosion, which would correspond to the favorability of habitat conditions and the presence of mussels. Regions with low evidence of erosion were expected to be associated with a greater likelihood of mussel presence, species richness, and positive changes in species richness from the Riddick (1972-3) survey.

Methods and Materials

Mussel Species Surveys

The Pamunkey River drainage including North Anna, South Anna, and Little River tributaries as well as sites on the Pamunkey River itself was surveyed for

freshwater mussel diversity. The sites visited were identical to those surveyed by Riddick (1973). Searches included a minimum of 90 minute search effort per person (SEP), at each of the 50 sites through an accumulation of search time by visits both in the summer/fall of 2004 and the spring of 2005. Many sites that had favorable habitat and higher mussel abundances received additional visits and considerably more than the 90 minute minimum. Stream sediment searches for live individuals using view scopes, snorkeling, and sifting through substrate were conducted along with searches along banks for shell piles created by mussel predators. The view tube was frequently the most efficient method for searching the stream beds for live mussels and offered an efficiency advantage over the previous survey. Although the search time in the survey by Riddick was not quantified, the combination of the multiple search visits, a minimum search time of 90 minutes per site, and the efficiency advantage of a view tube were used in the current study to ensure that any decreases in diversity found would have a greater likelihood of coming from changes in habitat conditions rather than insufficient sampling.

The abundance of mussels found at each study site was estimated through determining the quantity of mussels found per unit of search time in minutes and hours per person (MPSEh and MPSEm). The MPSEm parameter used in spatial comparisons was calculated by using the spring 2005 site visits which were more successful and conducted under more favorable weather conditions than site visits in the summer/fall of 2004. For statistical purposes the MPSEm were log transformed to create a more normal distribution. From the log transformed site values of MPSEm, regional averages were also calculated for quantitative comparison. Additionally sites were also assessed values of Presence or Absence based on whether live mussels were found at a particular site.

Species richness values were also noted for sites, regions, river systems, and the entire watershed.

Habitat Data Collection

Water Quality

Baseline water quality was measured by visiting all 50 sites in a 2 day window of time when the weather was essentially constant with no recent precipitation. A Hydrolab mini-sonde unit was used to measure temperature, pH, dissolved oxygen levels (ppt), and turbidity (NTU) at each site. Measurements were repeated three times within the channel at each site and averaged to determine water quality values. Measurements were taken in an upstream to downstream sequence of sites during the two day time period covering all three tributaries and the Pamunkey itself.

Substrate Conditions

Substrate conditions were measured at each site by using a methodology similar to Wolman Pebble Counts including transects of riffle (if present) and run stream segments. Riffle sites were characterized by rippling water over relatively shallow bottoms and run segments were portions of the stream that had no ripples in the water where the water was deeper. To conduct the survey, a tape transect was stretched over the stream. The width was divided by 30 to identify sampling locations for the 30 samples procedurally used in Wolman Pebble Counts. The sediment samples from equidistant points across the stream were sampled by picking up substrate and measuring the diameter of the widest dimensions of the particle picked up at the designated location across the stream. The protocol used for sampling included facing away and lowering the

index finger to the substrate on to a substrate particle which was picked up, brought to the surface and measured using a metric ruler. The technique was repeated for each condition present (riffle and run, or two run transects if no riffle was present). For sites 48 to 50, the technique was modified due to stream width, depth, and access conditions. At these sites the sampling points were estimated without use of the measuring tape and often required the use of a fabric net to scoop substrate from the deepest points. At sites 49 and 50 where the river is greater than 800m wide samples were only taken across the first 75 meters perpendicular to the bank. The substrate was almost entirely sand so it is unlikely that other significantly different substrate sizes would have been encountered further across the channel.

Average substrate sizes were calculated for both riffle (if present) and run transects. Each particle recorded was categorized in 1 of 24 size categories ranging from silt (<0.06 mm) to bedrock (> 4096 mm boulders). Substrate composition was identified by combining size categories into silt (.062mm or less), sand (.063-2.0 mm), pebbles (2.1 – 12 mm), gravel (12.1 – 64 mm), cobble (64.1 – 256 mm), boulder (256.1 – 4096 mm), and bedrock (larger than 4096mm). Percentages of substrate types were calculated individually and in functional combinations of sand-silt, pebble-gravel-cobble-, and boulder-bedrock combinations. Average substrate size was calculated by using each particle sampled excluding those that were larger than 256mm (boulders and bedrock), which were considered not suitable as habitat substrate for freshwater mussels. Each size averaged into the calculation was a median among 19 possible particle diameter ranges including substrates from silt (.06 mm in diameter) to large cobble (256mm in diameter).

Deposition of silt was measured by longitudinal transects of 5 meters at right bank, right channel, center, left channel, and left bank for all sites. Measurements were taken at points along longitudinal transects at 1, 3, and 5 meters within a section of stream starting 5 meters into a section of run downstream of a riffle or the shallowest run section within the survey area. Silt accumulation was measured by inserting a plastic metric ruler slowly into the sediment until the ruler stopped penetrating or bent from substrate resistance. Silt depth was recorded at each of the designated grid points (1, 3, and 5 meters). Average silt depth was determined among the 15 measurements taken at each site.

Regression analyses of the survey results for mussel abundance (MPSEm) and changes in species richness were performed for all the water quality and substrate parameters.

Stream Stability Index

Field equipment and qualitative observations were used to collect data on stream channel features, substrate features, and riparian buffer features at each study site.

Stream width was measured using a measuring tape and top of bank and bank full heights were estimated using a surveying measurement stick. The degree of channelization was estimated through calculating the ratio of bank height (water mark of how high along the bank water rises under normal conditions) to full bank height (how high water must rise to reach the flood plane). Bank exposure, bank angle, algal growth, and soil type were characterized through visual estimating conditions at each site and assigning ratings of stream stability ranked from 1 to 3 (see Appendix I. Stream Data Form for descriptions of categories). An overall stream stability index was created by combining the factors and

averaging qualitative estimates described above to characterize stream stability at each of the survey locations. Stream stability indexes were also averaged to characterize regional stream stability. Regional stream stability measures were ranked for use in Spearman correlations, a nonparametric correlation used with non-interval data. The regions of stream stability were ranked sequentially from the region with the lowest average stability rating to the region with the highest stability rating. Identical scores were given the same rating. Rankings of stream stability were then compared to rankings of regions in regard to positive changes in species richness, starting with regions with the lowest negative change in species richness and preceding to the region with the highest positive change in richness. The same procedure was followed pairing stream stability with regional mussel abundance (MPSEm).

General observations regarding substrate condition were also noted and identified as being predominantly bare/loose, stable, embedded, or covered, as descriptions of the general condition of the stream bottom at each survey site. The riparian buffer, within 30 m on each side of the stream at each site was visually estimated in regard to coverage (effective vegetation of tree, shrub, and herbaceous cover), canopy coverage (shading of riparian area), and stream shading and recorded as low, present, or high for each study site. Levels of observed abundances of fish, woody debris, and the exotic Asian clam, *Corbicula fluminea*, were also noted among the site field data along with the presence or absence of cows in the area.

Landscape Data Collection

Land use and riparian extent were quantified regionally through the synthesis and manipulation of GIS data. The 1991 USGS data on land cover/land use was downloaded from the USGS website and incorporated into a GIS with USGS layers containing spatial data on Virginia watersheds and regional sub-hydrological units. The 1991 land use/land cover data layer was reclassified by combining the different forest types into one forest cover layer. Agricultural land cover remained characterized as pasture/hay land cover or crop land cover. High and low developed land use was combined to create a developed land category. The other land use categories were left classified as barren land cover, recreational, water, and wetlands. The sub-hydrological units were used to divide the land use data layer into separate regional units. The number of pixels representing each land use classification within each region was obtained from the data tables of the newly created map layers. The total number of pixels, each representing 30 by 30 meter squares, was calculated and used to determine the percent composition of each classification of land cover represented.

The extent of a forested riparian area was estimated by using a geographic data layer from a joint effort of the Chesapeake Bay Program and Pennsylvania State University that characterized the extent of a forested riparian area within the Chesapeake Bay Watershed (USDA: Bulletin prepared by the National Consortium for Rural Geospatial Innovations, 2001). The extent of forested riparian area within 30 meters to the right or left of stream channels within the sub-drainages of the Pamunkey Watershed was characterized as present or not present. The riparian area was considered present only if it extended at least 30 meters. Widths less than 30 meters were characterized as

not present. The sub-hydrological unit layer was again used to divide the riparian data layer into regions then quantified by making a calculation to determine the percent of the potential riparian that met the 30 meter extent minimum.

Results

Temporal Comparison

The current survey of mussel populations within the Pamunkey Watershed indicates a reduction in species diversity from 10 species in the survey conducted by Riddick in 1972-73 to 4 species present in the current survey (2004-05) (refer to Table 1). Absent from the current survey were Alasmidonta heterodon, a federally listed endangered species, as well as Elliptio lanceolata, Alasmodonta undulata, Ligumia nasuta, Lampsilis radiata radiata and Lasmigona subviridis. The absence of E. lanceolata in the present survey is a little surprising given its prevalence in the previous survey, appearing at 9 sites, and the fairly consistent appearance in some of the subsequent surveys (1990's), although many of the most recent site surveys have indicated a rapidly dwindling presence or absence at sites in which evidence of E. lanceolata was previously found (Table 2). All the sites containing A. heterodon, A. undulata, L. cariosa, L. radiata radiate, L. subviridis, and E. lanceolata in Riddick's survey (1972-1973) were revisited by staff from the Virginia Department of Conservation and Recreation in 1991. Other site specific surveys in the area have also taken place in the area. The chronology of site survey results indicates a steady decline in species richness during the past 30 years (see Table 2).

Evidence of the presence of E. complanata, by far the most common unionid species within the Pamunkey Watershed, was found at 30 of the 50 sites with live samples present in 22; shells from recently dead organisms were found at 8 additional sites. In the 1972-3 survey, evidence of E. complanata was found at 27 of the 50 sites including live specimens present at 18 sites and shells of recently dead organisms at 9 additional sites (Table 1). The species E. producta, formerly known as E. augusta was found at five sites (four with live specimens) in the current survey compared to four (three with live specimens) found in the 1972-3 survey. P. cataracta, only found as recently dead shells in the 1972-3 survey, was found live at three of the sites in the current survey and as a shell from a recently dead individual at another site (Table 1). In the current survey Lampsilis cariosa was found as one live specimen and two shells from recently dead mussels at site 23 in the upper South Anna and at site 47 (one shell from a recently dead organism in the middle Pamunkey region). In the previous survey L. cariosa was found at four sites including the lower South Anna and upper and middle Pamunkey regions (Table 1).

In regard to distribution within the watershed, there are significant regional differences in species richness and presence in the current survey compared to the previous survey. The upper portion of the South Anna River that was devoid of species in the survey by Riddick, now supports the highest level of species richness in the current survey (four species). The lower South Anna Region and upper and middle Pamunkey regions, which had the highest levels of species richness historically, eight, four, and six species respectively, were found to be much lower in the 2004-5 survey. The lower South Anna and upper Pamunkey region was represented by only one species and the

middle Pamunkey region was represented by just two species (shells only) (figure 8). Below Lake Anna, the North Anna River sites have established unionid populations that were absent from this region in the 1972-3 survey (figures 6, 7, and 8). A minor change occurred in the upper region of the Little River with the presence of *E. complanata* represented by one site (14), while in the previous survey none were observed.

Figure 6. Species richness by site in the 1972-3 survey

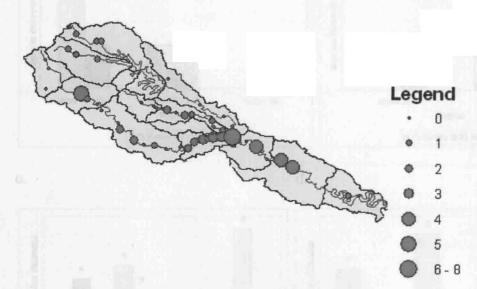


Figure 7. Species richness by site in the 2004-5 survey

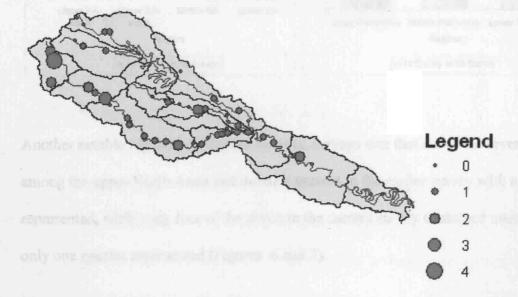
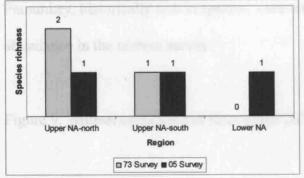
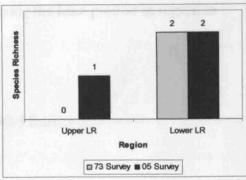


Figure 8. Comparison of species richness by region for the surveys conducted in 1972-3 and 2004-5. a) North Anna River b) Little River River c) South Anna River d)

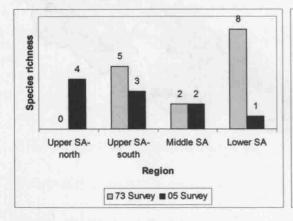
Pamunkey River

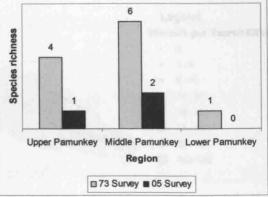
a.





c. d.



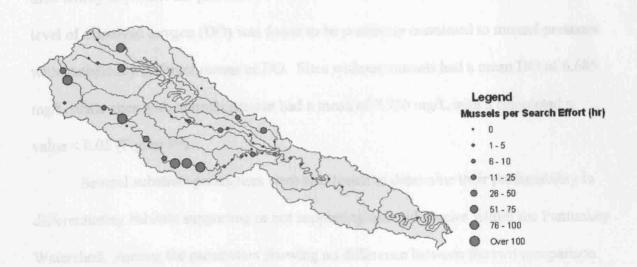


Another notable regional difference between surveys was that six of the seven sites among the upper North Anna had mussels present in the earlier survey with two species represented, while only four of the seven in the current survey contained mussels with only one species represented (Figures 6 and 7).

Overview of Live Mussel Abundance within the 2004-5 Survey

Figure 9 depicts abundances of mussels found per search hour at each site through graduating the size of each site symbol. The highest MPSEh averages occurred in the upper northern and middle regions of the South Anna River. The lower South Anna and Pamunkey, historically rich in species, were among the least prolific in regard to abundance in the current survey.

Figure 9. Mussel abundance at Sites in the 2004-5 Survey



Site Level Comparisons of the 2004-5 Survey

Presence / Absence Comparisons

Water quality, substrate, and stream stability parameters were tested to investigate relationships to the presence or absence of mussels among survey sites. Using presence/absence values, sites were compared by performing t-tests, grouping those sites that had live mussels (22) and those sites that did not have live mussels (20). Eight sites

used to investigate temporal changes were eliminated from the spatial analysis because they were either in tributaries to the main river channel that historically had no evidence of mussel populations or were at upstream or downstream extents of the Pamunkey Watershed that historically had no evidence of supporting unionid species. To avoid skewing results comparing survey results to habitat parameters, these eight sites were also excluded from other site level and regional analyses so that all spatial analyses were based on 42 sites among the main tributaries within the watershed covering known range of freshwater mussel habitat.

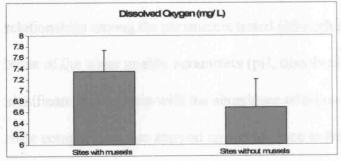
Among the water quality parameters pH and turbidity showed no significance in their ability to predict the presence or absence of mussels at specific sites. However, level of dissolved oxygen (DO) was found to be positively correlated to mussel presence with statistically different means of DO. Sites without mussels had a mean DO of 6.685 mg/L while sites with mussels present had a mean of 7.350 mg/L with a calculated p value < 0.05 (Figure 10).

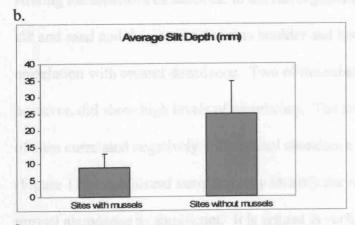
Several substrate parameters were also tested to determine their predictability in differentiating habitats supporting or not supporting unionid species within the Pamunkey Watershed. Among the parameters showing no difference between the two comparison groups included: average substrate size in transects of both riffle and run segments, the percent composition in riffle samples of sand/silt, pebble/gravel/cobble, and boulder/bedrock, and the percent of silt/sand and percent boulder/bedrock substrate in samples of run segments. A comparison of average silt depth among sites with mussels and sites without mussels resulted in significantly different means (p value < 0.01). Sites without mussels had an average silt depth of 24.90 mm, while sites with mussels had an

average silt depth of 8.86 mm (Figure 10). The mean percentage of the substrate composed of pebble, gravel, or cobble sized substrate was significantly higher at sites with mussels, mean=39.77%, compared to sites without mussels, mean=23.00% with a p value < 0.01 (Figure 10).

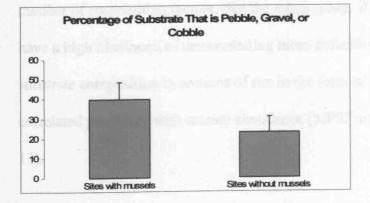
Figure 10. Bar graphs with SEM showing habitat parameters differentiating sites with and without mussels within the survey area: a. levels of dissolved oxygen, b. average silt depth, c. percentage of substrate size composition that is pebble, gravel, and cobble







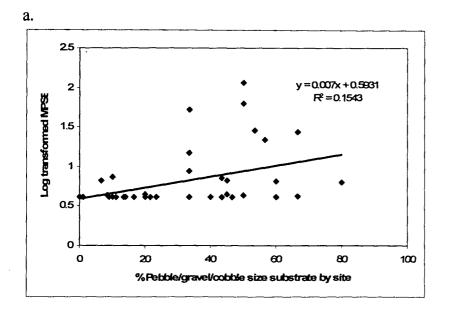
c.

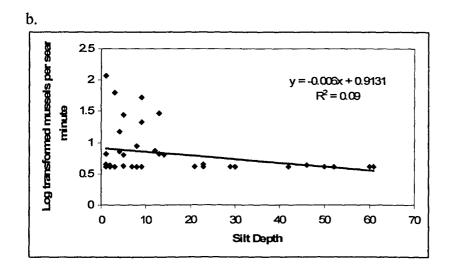


Comparisons using Mussel Abundance (MPSEm)

Habitat measures among sites were also investigated using a measure of site abundance as the predictor variable. The abundance measure at each site, mussels per search effort minute (MPSEm), was determined by dividing the number of mussels found by the search time in minutes from the spring of 04/05 and log transformed to create a more normal distribution. Regression analysis of correlations between the MPSEm predictor variable and other parameters resulted in some significantly correlated relationships among the parameters tested although several others showed no correlation. None of the water quality parameters (pH, dissolved oxygen, turbidity) showed a significant relationship with the abundance of mussels (MPSEm). Substrate measures of riffle composition also showed no significance to the abundance of mussels found. Among the measures of substrate in the run segments, the percent of substrate that was silt and sand and the percent that was boulder and bedrock also did not show a significant correlation with mussel abundance. Two of the substrate measures in run segments, however, did show high levels of correlation. The average depth of silt in run segments of sites correlated negatively with mussel abundance with a p = 0.05 and an $r^2 = .09$ (Figure 11). Additional sampling may identify the relationship of silt depth at sites and mussel abundance as significant. It is argued in such a variable habitat with a large number of contributing factors, that the relationship, if sampled more intensively, would have a high likelihood of demonstrating more definitive significance. The percent of the substrate composition in sections of run in the form of pebble, gravel, and cobble correlated positively with mussel abundance (MPSEm) with a p < .01 and $r^2 = .15$ (Figure 11).

Figure 11. Substrate parameters at sites indicating relationships to mussel abundance (MPSEm) through regression analyses: a.percentage of substrate composition including pebble, gravel, and cobble, b. average silt depth





Regional Comparisons Within the Pamunkey Watershed

Comparisons of Regionally Averaged Habitat Parameters and Regional Averages of Mussel Abundance (MPSEm)

Larger spatial scales were investigated by creating regional averages of the site parameters measured. The values for mussels per search effort minute (MPSEm) for each site were averaged to compile regional averages that were used as an indicator of mussel abundance at a larger spatial scale. Water quality and substrate parameters calculated at each site were averaged in the same way to create regional averages corresponding to the sub-drainages found within the Pamunkey study area. At a larger spatial scale, the strength of correlations and the level of significance among several parameters increased in comparison to site level comparisons. Dissolved oxygen (DO), which was not significantly correlated with mussel abundance at the site level, when compared regionally resulted in an $r^2 = .33$ and a p value = 0.05. Additional samples are required to determine if the correlation between DO and regional MPSEm meets the threshold of significance. Turbidity and pH parameters remained unrelated to mussel abundance among sub-drainages.

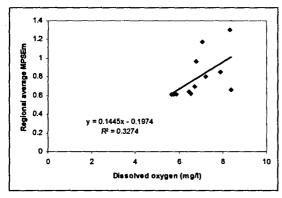
Several of the substrate parameters showed consistently higher levels of correlation with mussel abundance on a regional scale. Substrate size, not significantly correlated at the site level of analysis, was positively correlated to mussel abundance at the regional level, r^2 =.56 and p< 0.01, while the percent silt and sand in transects of run segments, not significantly correlated at the site level, was negatively correlated with abundance values at the regional spatial scale, r^2 =.46 and p value < 0.015 (Figure 12).

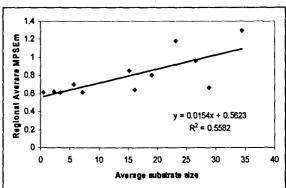
Silt depth, did not correlate significantly with mussel abundance, r^2 = .27 and p <0.1. The percentage of the substrate in run segments composed of pebble, gravel, and cobble substrate, r^2 =.71 and p< 0.01, was highly correlated to mussel abundance values on a regional spatial scale (Figure 12). The percent of the substrate that was boulder and bedrock remained uncorrelated to mussel abundance regionally.

Figure 12. Regional relationships between water quality and substrate parameters with regional mussel abundance (regional average of log transformed MPSEm site values) a. Dissolved oxygen b. Average substrate size, c. % substrate composed of pebble, gravel, and cobble d. % substrate composed of silt and sand

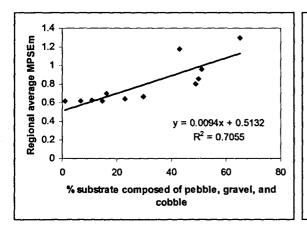
b.

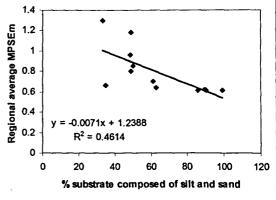
a.





c. d.





Regional abundances (average log transformed MPSEm) were also correlated with regional land use proportions to see if any relationships might be suggested between land use practices and mussel abundance. Of the land use types characterized (Figure 14), the percentage of developed land, land used for pasture/hay, wetlands, water, recreational areas, and the percentage of forest land cover were found to have no significant relationship to the regional mussel abundance found in the current survey. The percent crop land cover (r^2 =.35, and p<0.05) did show a negative correlation with regional mussel abundance found in the current survey. (Figure 13)

Figure 13. Regional mussel abundance (MPSEm) correlated to crop land use

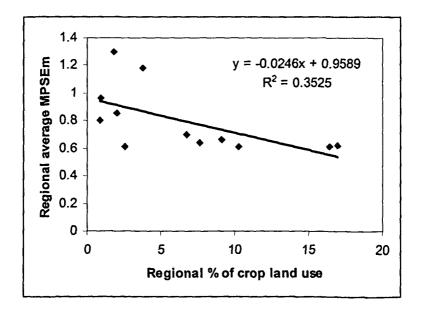
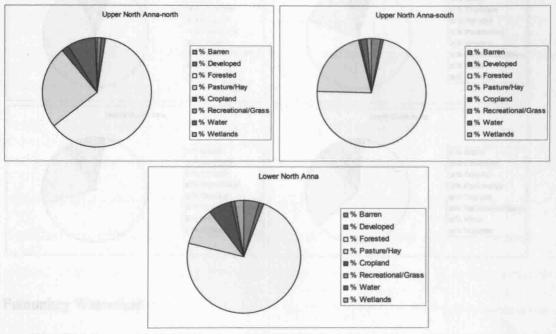
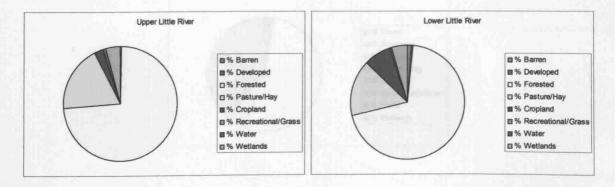


Figure 14. Regional quantifications of percent land use by category

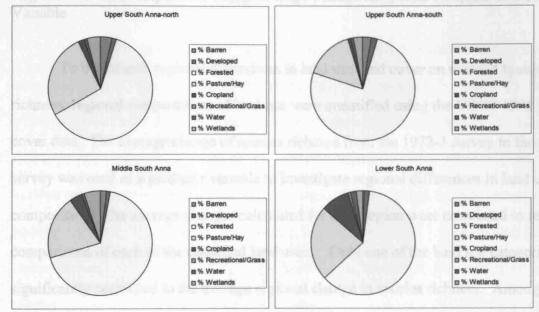
North Anna Watershed



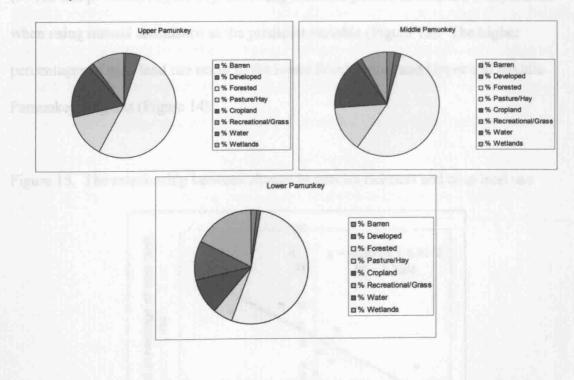
Little River Watershed



South Anna Watershed



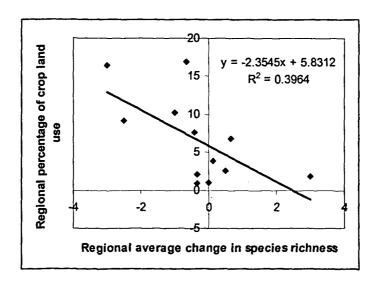
Pamunkey Watershed



Regional Habitat Comparisons Using Average Change in Species Richness as a Predictor Variable

To investigate regional differences in land use/land cover on unionid species richness, regional compositions of land use were quantified using the USGS 1992 land cover data. The average change of species richness from the 1972-3 survey to the present survey was used as a predictor variable to investigate regional differences in land use composition. The average changes calculated for each region were correlated to regional comparisons of each of the classified land uses. Only one of the land use categories was significantly correlated to the average regional change in species richness. Among regions, crop land use was negatively correlated to positive change in species richness $(r^2=.40 \text{ and p} < 0.05)$ (figure 15), following the same pattern as land use comparisons when using mussel abundance as the predictor variable (Figure 13). The higher percentages of crop land use occur in the lower South Anna, and Upper and Middle Pamunkey Regions (Figure 14).

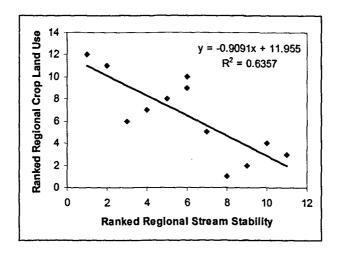
Figure 15. The relationship between change in species richness and crop land use



Comparisons Using Estimates of Stream Stability

Stream stability was estimated at each site through a protocol of measurements and observations. The characteristics observed included bank height ratio, bank angle, soil type, extent of bank cover vegetation, and algal extent. (See Appendix 1) At each site, each characteristic was rated as 1, 2 or 3 with regard to estimated stream stability, or in other words the absence of site erosion. Ratings of 1 indicated the least stream stability and the highest level of site erosion, whereas a rating of 3 indicated the most stream stability and the least site erosion. Scores from the five different observational characters were averaged to give an overall site stream stability score. These scores were averaged regionally to provide a regional indication of stream stability. Because the observational data collected was not quantitative, the final scores were ranked regionally and used in Spearman correlations with ranked regional proportions of land use/land cover data. The most statistically significant relationship was found when comparing stream stability and crop land use. An increase in crop land use corresponded to a decrease in stream stability, $r^2 = .65$ and p < .05(Figure 16).

Figure 16. Ranked regional crop land use and regional ranked stream stability



Discussion

In this study, the Pamunkey Watershed was compared temporally and spatially for unionid species richness, abundance, and presence. Additionally, habitat data were collected to establish more clear relationships between habitat parameters and the presence, abundance, and species richness of unionids in the Pamunkey Drainage. Land use characteristics were also quantified in an attempt to identify land use issues that may be impacting mussel habitats. Although several strong correlations were found in this study, the sampling was based on a temporal comparison to the survey conducted by Riddick (1972-3). Sampling strategies focused on some of the most correlated parameters identified in this study should be conducted to more definitively define potential correlations between land use practices, habitat characteristics and actual mussel populations.

Some of the analyses conducted suggest the presence and abundance of unionids within the study area appears to be related to some of the water quality and substrate parameters measured. Silt depth and predominance of silt and sand substrates are also closely related to regions with relatively high percentages of crop land use. Mussel survey results which were correlated to some of the substrate parameters and to regional crop land use do suggest that there may be negative effects due to erosion occurring in regions of the Pamunkey Watershed. Areas with the largest declines in species and those with the lowest survey abundances were associated with high percentages of silt and sand, larger silt depth measurements, and lower percentages of substrate that were composed of pebble, gravel, or cobble. The same regions were also composed of relatively larger proportions of crop land use. Overall the reduction of species seemed to

signal a change of conditions that have disproportionately affected the more rare species known to inhabit the watershed. For example, *A. heterodon* and many of the other previously surveyed species of concern that are now absent are typically found in stable substrate mixtures of pebbles, gravel, and sand. Five of these species (*A. heterodon, A. undulata, L. subviridis, L. radiata radiata, and E. lanceolata*) were absent in the present survey, whereas the species that remain are generally regarded as being more tolerant of silt accumulation and less sensitive to turbid water conditions. The overall decreases in the number of species present between the 1972-3 survey (10 species) and the current survey (4 species) suggest changes in habitat conditions for unionid species.

Regional Changes

Upper North Anna

There were many interesting changes in unionid assemblages within the Pamunkey Watershed. Sites among the upper North Anna regions were less species rich and more inconsistent with regard to mussel presence. One of the sites (5) was worth noting due to the complete absence of any evidence of mussels and the presence of metallic tailings not unlike that expected in old mine areas. The most upstream sites (1 and 2) containing no mussels were on small narrow streams and adjoined by vast agricultural lands with almost no forested riparian buffer.

Sites in the lower North Anna Region had no evidence of mussels in the previous survey, but as predicted by Riddick (1973), have returned to this region, supporting the idea that Lake Anna has helped downstream habitats by neutralizing the harmful acid mine drainage coming from Contrary Creek. Substrate west of Route 1 appears to be a

more suitable unionid habitat compared to the section of river at site 10 (intersection with Route 1), and to the east which seems to be increasingly composed of shifting sand and silt within in a highly channelized stretch of the river. Previously, Riddick (1973) identified this site (10) as the fall line composed of a more rocky substrate.

Slow flow rates are common among sites in the upper region of the Little River. Two of the sites (12 and 15) had old mill dams creating pond-like conditions including lily pads and high levels of algae throughout much of the region. In the lower portion of the Little River, flow rates were faster and the substrate changed from predominately silt to abundant bedrock and boulders in sections of riffle and rocky substrate in sections of run. Many places seemed suitable habitat for mussel species, yet little evidence of unionid presence was found. Interestingly during the spring 2005 survey visits at sites 17 and 18, there was evidence of a large mussel kill. At site 17 there was a mussel bed with a far greater number of recently dead mussels found within it than the relative few live mussels present. In the previous 2004 visit, fewer shells were found in the same area containing live mussels. Downstream at site 18, over 50 recently dead mussel shells and no live organisms were found in substrate on the bottom of the stream in a somewhat concentrated area. The recently dead mussels were positioned in the sand as they would be if alive. The shells of the recently dead organisms at both sites felt soft to the touch as though decalcifying.

Historically devoid of species, the upper South Anna had the highest level of species richness. The most upstream site (22) had several pieces of metallic rock, possibly evidence of historic mine activity, however, live specimens of two species were found there. The next site (23) downstream had by far the highest level of species

richness containing all 4 of the species found within the entire watershed. The abundance of live mussels here was also unusually high. Live examples of *L. cariosa* (1), previously found only in locations much farther downstream, were present at this site along with hundreds of *E. complanata* and a few live *E. producta*. In addition shells of *P. cataracta* and shells of recently dead individuals of the 3 live species found here were also present. The gap in distribution between historic ranges of *L. cariosa*, previously found much farther downstream, and this upstream site is very perplexing. The distribution from downstream locations seems unlikely due to old mill dams, which may mean they have been present historically, but have gone unobserved during the previous surveys.

The middle region of the South Anna contains at least two old mill dams that have been present so long that stream conditions have probably long ago stabilized. The sites within the region consistently contained live mussels at almost all of the sites. The stretch of river containing sites 32 to 34 had particularly dense beds of mussels, almost exclusively *E. complanata*. The survey results from the earlier survey were fairly similar except for the presence of *E. lanceolata*, which was missing from the current survey. The absence is consistent with site surveys over time which indicated reductions in its presence among surveys over time (Table 2).

Although the substrate often appears to be a suitable habitat for mussel populations, in the lower South Anna region sites were not only less species rich, but surprisingly sparse in abundance even for *E. complanata*. Consistent with these findings were other recent site specific surveys that also resulted in very low numbers of live individuals. Perhaps heavy layers of algae, covering the shallower areas of substrate, at some of the sites is partially to blame. Other possible limiting factors include a shallow

hydraulic dam present at one of the sites (38), and a large tall dam upstream of site 42 that effectively blocks any upstream migration of host fish from this location. A sewage treatment station may also be contributing to declining conditions.

Survey results for the upper and middle Pamunkey regions indicated decreases in species richness. Highly diverse historically, these regions appear to be the least stable with habitat degrading faster than other regions. Riddick (1973) characterized the stretch as having steep cliff banks and predominately a sandy substrate with areas of pebble and gravel substrate adjacent to outcroppings and channel islands. These sections of pebble and gravel were sited as being the most prolific for finding mussel species. Currently the stream still has steep clay banks and is highly channelized, but now it seems to have widened when compared to stream widths reported by Riddick in 1973. Additional signs of erosion include numerous trees that have eroded into the stream creating barriers capturing deep layers of silt along the banks. There also appear to be fewer outcroppings with pebble and gravel, translating into less suitable habitat area for mussels. The water also appears highly turbid carrying abundant suspended solids that are probably also contributing to the decline in habitat conditions. The sewage treatment plant just upstream in the Lower South Anna might also be playing a part in the reduction of water quality.

The lower Pamunkey only had a single shell as evidence of unionid habitat in the earlier survey, making the lack of any species found in the present survey not surprising. This tidewater region of the watershed encounters shifts in salinity and other conditions that may effectively limit downstream distributions of mussels. A landowner at one of

the sites reported that she had remembered seeing mussels as a child, but had not noticed seeing any in recent years.

Site Analyses

In a highly variable system such as freshwater stream habitats a variety of factors combine to create circumstances that are either favorable or unfavorable for species. In order to identify what appear to be the most important factors determining habitat conditions, it becomes necessary to compare survey data to a variety of habitat parameters. One method used in this study was to compare the presence or absence of mussels at sites to water quality and habitat parameters and analyze average means statistically through t-tests to investigate relationships between mussel presence and habitat conditions. According to this analysis, levels of dissolved oxygen in this study were found to be reasonable predictors of mussel presence. Other water quality parameters, pH and turbidity, revealed no statistically significant pattern suggesting that within this watershed, levels of pH and baseline turbidity may not be as important in determining habitat suitability. Turbidity, which was hypothesized to correlate with declines in mussel presence and abundance did not show significant correlations. Perhaps, monitoring the change in turbidity after heavy rain events may identify stronger relationships between mussel presence and regional levels of erosion.

Comparison of mussel presence and habitat parameters also revealed some substrate conditions as significantly correlated to mussel presence. The significant correlation between mussel presence and the measure of substrate in run segments composed of substrate sizes characterized as pebbles, gravel, and cobble, may indicate

areas with less impact from erosion. Although the percent sand and silt and silt depth within sections of run was not found to be significantly correlated to mussel presence, there was a trend that suggested more sand and silt may be associated with a lower likelihood of mussel presence. The negative correlation to silt depth also suggests that erosion may be an important factor contributing to less favorable mussel habitat. The lack of significance of the riffle composition parameters may come from the tendency of mussel populations in this drainage to occur in sections of run or areas of transition leading into or out of riffle segments.

Using Mussel Abundance in Comparison to Habitat Characteristics

Another predictor variable used to compare habitat conditions was the abundance of mussels found at sites measured as mussels per search effort minute (MPSEm) in the spring 2005 site visit. Regression analysis of correlations resulted in similar findings to the presence/absence t-tests with a few notable differences. The correlation between the substrate parameters percent pebble-gravel-cobble and silt depth according to mussel abundance were again significant. Dissolved oxygen was not significantly related to mussel abundance as it may have been with regard to the mussel presence analysis.

Possibly this indicates that once a suitable level of dissolved oxygen is present, more dissolved oxygen does not necessarily mean more abundance. All other substrate parameters as in the presence/absence analysis were not significantly correlated to mussel abundance. The relative compositions of substrate and amount of silt seem to have some impact on not only the presence of unionids, but their abundance in site habitats.

Regional Analyses

One issue that is often encountered when researching particular groups of species and their habitats is that of spatial scale. In this study, data from the site level were averaged to create regional measures of mussel abundance and habitat parameters to see if using larger spatial comparisons resulted in stronger or weaker correlative relationships. A significant limitation in this analysis was using averaged site measures as measures of regional conditions. The number of sampling sites varied between regions due to the arrangement of the historical site locations. Because the study was the replication of a historical study, the sites were predetermined. Future investigations of the Pamunkey Watershed using a regional approach would benefit from the addition of sites in regions of the watershed that were underrepresented. Although some of the regions were based on a limited number of sites, the results do suggest some interesting patterns not dissimilar from the findings analyzed among the individual sites. In most cases in this study the correlations strengthened when compared on a regional scale.

Land Use analysis

In the Pamunkey Watershed land use is predominantly forested along with areas characterized as rural and agricultural with some developed suburban sprawl north of Ashland. Forest land cover is generally still fairly high (between 52 and 76 percent of each regions land cover). The other prominent land uses include agricultural land used for pasture and hay (up to 26% regionally), agricultural land used for crops (up to 17%

regionally), with water and wetlands comprising modest proportions (regionally 10% or less) and developed and barren land occupying less than 5% of the land in a given region.

Investigations of changes in species richness resulted in only agricultural crop land showing significant correlations. The proportion of land for crops was negatively correlated to positive change in species richness. Interestingly, agricultural land use designated as pasture/hay did not seem to be negatively related to positive changes in species richness, although not statistically significant the trend of the regional proportion of land used for pasture/hay was fairly positive in regard to trends in species richness. At least in this drainage, the potential effects of the two major types of agricultural land use seem to vary drastically. Forested land use although statistically insignificant also tended to be positively related to positive changes in species richness. Lack of significance may be due to the relatively similar amounts of forested land among regions. Contrary to what was hypothesized, developed land was not at all significantly related to changes in species richness, possibly because there is so little of it within the Pamunkey Watershed.

Surprisingly, the relationship between regionally quantified riparian areas within the drainage and mussel presence and abundance were unrelated. Not only was there not a statistically positive relationship between the regional percentages of potential riparian buffer and regional changes in species richness, but the trend tended to be negative with the areas having the lowest percentage of riparian buffer showing the most positive changes in species richness. The quantification of the riparian zone, however, may be largely to blame. The GIS layer created from land cover / land use and forestation was created such that a forested riparian area was counted present only if it spanned 30 meters on the right or left side of the stream. The percentage of stream in each region satisfying

this criterion was relatively small (less than 6% in all regions). It may be that regions with consistent but narrower riparian areas were misrepresented by the resolution used in quantification of forested riparian. Riparian areas of 15 meters or less may be enough to constitute effective riparian for the Pamunkey Watershed which has relatively low topographical relief. Currently higher resolution of the extent of forested riparian areas is not available. Additionally, more work needs to be done in defining what constitutes an effective riparian area. Vegetation compositions and their ability to stabilize stream channels probably depends heavily on topographical relief and soil and compositions.

Stream Stability Observations

The five factors selected for the stream stability index generated for this study were chosen to reflect what seemed to be the most important signals of erosion in the Pamunkey Watershed. The characterizations are somewhat subjective and limited in the level of categorization. These weaknesses aside, the stability index was investigated to see if on a larger spatial scale relatively quick and easy observations are useful in identifying regional extents of erosion that are impacting stream habitats. Cropland had the strongest correlation with the stream stability index rankings showing areas with the highest percentage of cropland tending to have the lowest stream stability averages. The negative correlation between crop land and species richness was similar to the land use findings when using mussel abundance as the predictor variable. The results suggest there may be potential for utilizing observations of site erosion to identify general relationships between land uses and their consequences. However, the index values of stream stability were not statistically correlated with unionid presence or abundance,

indicating a lack of usefulness in predicting mussel populations. A refined or improved stream stability index would likely prove more effective than the one used in this study.

Other Factors not Addressed Directly in the Present Study

Other factors not addressed in the study are likely making significant impacts to the populations of freshwater mussels. Host fish are requisite to the successful development of mussels. Species of mussels have specific fish that can serve as hosts although not all unionids have had host species identified. Appendix II is a list of the mussel species found in the Pamunkey Watershed and their known hosts. A fish study by King (1986) included a table of fish species found among sampling that was conducted in the North Anna and South Anna River. Species identified in his study are indicated by an asterisk in Appendix II. It is interesting to note that of the known host species it seems that the North Anna would be a more successful reproductive habitat for unionids, although historically it contains far fewer species. Increasing water quality along with several potential host fish species may indicate the possibility for the reintroduction of species into the lower North Anna. Other factors not a part of this study that may influence unionid populations include flow rates and measures of nutrients. Levels of nitrogen and phosphorus would be particularly interesting to find out in the lower South Anna and upper and middle Pamunkey regions to determine if excess nutrification could be playing a role in the reduction of mussel species in these areas.

Although several water quality and substrate parameters appeared highly suggestive in relation to mussel populations, future research is still needed to establish more rigorous quantitative connections between substrate stability and erosion. By using

time-series habitat data collection and quantifications of land use and land use change over time, more convincing arguments can be made that link land use practices, habitat conditions, and the status of freshwater organisms. These types of studies would prove useful both as tools for resource managers to lessen developmental impacts to streams, but as evidence to those in county, state, and federal positions making decisions regarding how our natural resources are to be used most wisely.

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Table 1. Species Presense in the Surveys Taking Place in '872-3 and 2004-5

and 1. Species rieselise il the Suiveys Laking Fiace iii 812-5 and 2004-5	He on keys 18	aking Flace iii	07 BUR C-7/A	ę.							
Species	Upper North Anna Sites 1 to 4	Upper North Mid North Anna Sites Anna Sties 1 to 4 5 to 7	Lower Uppe North Anna River Sites 8 to 11 12 to	Upper Little Lower Little River Sites River 12 to 14 Sites 15 to 21	Upper South Anna 1 Sites 22 to 24	Upper South Upper South Middle South / Anna 1 Sites Anna 2 Sites Sites 29 to 36 22 to 24 25 to 28	Upper South Middle South Anna Anna 2 Sites Sites 29 to 36 25 to 28	Lower South Anna Sites 37 to 43	Upper Pamunkey Sites 44 to 4	Upper Middle Pamunkey Pamunkey Sites 44 to 46 Sites 47 to 48	Lower Pamunkey Sites 49 to 50
Eliptio complanata 73 Eliptio complanata 05	3, 4,	5, 6, 7* 6, 7	9, 10	15, 17, 18, 19°, 21° 14 17, 18, 21°	*, 21* 22, 23	25*, 26*, 25, 26*, 27,	30, 31°, 33, 36 37, 38, 39, 40, 42, 43 29, 30°, 31, 32, 33, 34, 36 38, 38°, 40, 42°, 43	37, 38, 39, 40, 42, 43 38, 39*, 40, 42*, 43	44*, 45	45 47, 48	* 0
Elliptio producta 73 Elliptio producta 05				15, 17		25* 23 25, 27	м	26			
Pygonodon cataracta 73 Pygonodon cataracta 05					22, 24	25* 27		43*		*7*	
Lampsilis cariosa 73 Lampsilis cariosa 05					``	23		42,43	•	45 47*	
Species Not Found in 05 Survey	٠.										
Elliptio lanceolata 73	-					25*	30*, 31*, 36*	37*, 38, 40, 42, 43			
Alasmodonta heterodon 73						25•		43*			
Alasmodonta undulata 73						25*,		43*			
Lasmigona subviridis 73								40*, 43*		48 •	
Lampsilis radiata 73								43*	45.	47*, 48*	
Ligumia nasuta 73								,	45*	48 •	

* denotes that only shells from recently dead mussels were found

Table 2. Chronological Survey Data for Rare Species of Unionidae in the Pamunkey System

North Anna		1972-3 Survey (Riddick)	1991 Survey (DNH)	Other Surveys 1	Other Surveys 2	s 2 Other Surveys 3	3 2004-5 Survey (Lees)
E. lanceolata	Site 3	Present*	Absent	Absent 97 DNH			Absent
South Anna							
E. lanceolata	Site 25 Site 30 Site 31 Site 32	Present* Present* Present* Absent	Present 2 Absent Present 2 Present 2 Present 9				Absent Absent Absent Absent Absent
	Site 36 Site 36 Site 40 Site 42 Site 42	Present Present Present Present Present Present	Present 2 Absent Absent Absent Absent Absent	Absent 02 survey Present*1 98 VDOT Present 2 94 VDOT Present 5 91 VDOT Absent 04 survey	Absent Absent	96 (DNH) Absent 02: 02 survey 04 survey	Absent 02 survey Absent Absent Absent Absent Absent Absent
L. cariosa	Site 23 Site 42 Site 43	Absent Present Present	Absent Absent				Present 1 Absent Absent
L. radiata	Site 43	Present*	Absent				Absent
L. subviridis	Site 40 Site 43	Present* Present*	Absent Absent				Absent Absent
A. undulata		Present* Present*	Absent Absent				Absent Absent Absent
A. neterodon	Site 43	Present*	Absent				Absent
Pamunkey L. cariosa	Site 45 Site 47	Present Present*	Present 1 Absent				Absent Present*
L. radiata	Site 45 Site 47 Site 48	Present* Present* Present*	Absent Present 1 Absent				Absent Absent Absent
L. subviridis Site 48 Present* * indicates that only recently dead shell(s) were found	Site 48 ently dead s	Present* shell(s) were found	Absent				Absent

the Virginia Department of Conservation and Recreation. DNH- indicates surveys by the Department of Natural Heritage, VDOT - indicates surveys conducted for the Virginia Department of Transportation

^{*} indicates that only recently dead shell(s) were found
^survey entailed 23.5 hours per person search effort
Results from surveys other than the Riddick (1972-3) study and Lees (2004-5) study were obtained from field notes and records from the Department of Natural Heritage of
Results from surveys other than the Riddick (1972-3) study and Lees (2004-5) study were obtained from field notes and records from the Riddick (1972-3) study and Lees (2004-5) study were obtained from field notes and records from the Department of Natural Heritage of

Appendix 1. Site #	DATA	A SHEET	
Water Quality pH-	DO-	Turbidity-	
Substrate Types: Pebble count- on	Pebble Count Sheet		
Sediment Layer Right Bank 1-	2-	3-	
Rt. Channel 1-	2-	3-	
Ct. Channel 1-	2-	3-	
Lt. Channel 1-	2-	3-	
Left Bank 1-	2-	3-	
	STREAM STA	ABILITY INDEX	
plain/channel Bank Angle- Rt. 2. Bank Angle: (1) Soil- %Clay- 3. Soil type: fix Bank Cover- 4. Exposed bank annual vegeta Eutrophication-	Lft Lft less than 45 degrees (3 %Silt %Sand sed loamy soil(3), clay	Avg 3), 45-90 degrees (d compacted imperentation annual vegetation 5% bare or annual	
Riparian Coverage-	Lft Bank %	Rt. Bank %	L. Width- R. Width-
Stream width-	Ripple Depth-	Run Depth	Pool Depth-
Sinuosity-			

Other Species and observations:

Mussel Data:

Land Use-

Appendix II. Known Host Fish of Mussels Historically Present in the Pamunkey Watershed

Unkown Hosts

A. heterdon

*Tesselated Darter E. olmstedi

Johnny Darter E. nigrum E. lanceolata Mottled Sculpin C. bairdi E. producta L. nasuta

A. undulate L. subviridis-direct dev

Blacknose Dace R. artratulus
Common Shiner L. cornutus
*Largemouth Bass M. salmoides
Longnose Dace R. catarctae
*Pumpkinseed L. gibbosus
Slimy Sculpin C. cognatus
*White Sucker C. commersoni

.....

E. complanata

Banded Killifish
Green Sunfish

*Largemouth Bass
White Crappie

^Yellow Perch

F. diaphanous
L. cyanellus
M. salmoides
P. annularis
P. flavescens

L. cariosa

^Yellow Perch P. flavescens ^White Perch M. americana

L. radiata

*Black Crappie P. nigromaculates
*Largemouth Bass M. salmoides
*Pumpkinseed L. gibbosus
Rockbass A. rupestris
*Smallmouth Bass M. dolomieu
^Yellow Perch P. flavenscens

P. cataracta

Common Carp

*Bluegill

*Pumpkinseed

Threespine stickleback

*White Sucker

^Yellow Perch

C. carpio

L. macrochirus

L. gibbosus

G. aculeatus

C. commersoni

^{*} included among the fish reported in a thesis by King (1986) found in the North Anna and South Anna Rivers

[^] found only in the North Anna River in the King (1986) survey

Appendix III. Sites Within the Pamunkey Study

Sites on the North Anna Drainage

- 1 Pamunkey Creek, State Route 615, Orange County
- 2 Pamunkey Creek, State Route 631, Orange County
- 3 Pamunkey Creek, State Route 630, Orange County
- 4 North Fork-Anna River, State Route 669 Orange County
- 5 Madison Run, State Route 643 Orange County
- 6 North Anna River, State Route 639, Orange/Louisa County
- North Anna River, State Route 651, Orange/Louisa County
- 8 Northeast Creek, State Route 622, Spotsylvania County
- 9 North Anna River, State Route 601, Caroline/Hanover County
- 10 North Anna River, U. S. Route 1, Caroline/Hanover County
- North Anna River, State Route 30, Caroline/Hanover County

Sites on the Little River Drainage

- 12 Little River, State Route 609, Louisa County
- 13 Little River, State Route 701, Louisa County
- 14 Little River, State Route 654, Louisa County
- 15 Little River, State Route 680, Hanover County
- 16 Little River, State Route 715, Hanover County
- 17 Little River, State Route 738, Hanover County
- 18 Little River, State Route 601, Hanover County
- 19 Little River, State Route 685, Hanover County
- 20 Little River, State Route 688, Hanover County
- 21 Little River, U. S. Route 1, Hanover County

Sites on the South Anna Drainage

- 22 South Anna River, State Route 660, Louisa County
- 23 South Anna River, U. S. Route 15, Louisa County
- 24 Camp Creek, U. S. Route 15, Louisa County
- 25 South Anna River, State Route 649, Louisa County
- South Anna River, State Route 604, Louisa County
- 27 South Anna River, State Route 646, Louisa County
- Fork Creek, State Route 605, Louisa County
- 29 South Anna River, State Route 522, Louisa County
- 30 South Anna River, State Route 601, Louisa County
- 31 South Anna River, State Route 610, Louisa County
- 32 South Anna River, State Route 635, Louisa County
- 33 South Anna River, State Route 617, Hanover County
- 34 South Anna River, State Route 677, Hanover County
- 35 Taylors Creek, State Route 611, Hanover County
- 36 South Anna River, State Route 33, Hanover County

South Anna River, State Route 657, Hanover County South Anna River, State Route 54, Hanover County South Anna River, State Route 686, Hanover County South Anna River, State Route 677, Hanover County New Found River, State Route 677, Hanover County South Anna River, U. S. Route 1, Hanover County

Sites on the Pamunkey River

43

44 Pamunkey River, U. S. Route 301, Caroline/Hanover County

South Anna River, State Route 738, Hanover County

- 45 Pamunkey River, State Route 614, King William/Hanover County
- 46 Pamunkey River, State Route 615, King William/Hanover County
- 47 Pamunkey River, State Route 602, King William/Hanover County
- 48 Pamunkey River, U. S. Route 360, King William/Hanover County
- 49 Pamunkey River, State Route 630 (private property), King William County
- Pamunkey River, State Route 634 (private property), King William County