ABUNDANCE, DIVERSITY AND HABITAT PREFERENCE OF FRESHWATER MUSSELS (BIVALVIA: UNIONOIDEA) IN SULPHUR FORK CREEK AND LOWER RED RIVER, TENNESSEE AND KENTUCKY

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A Thesis

Presented for the

Master of Science

Degree

Austin Peay State University

R. Adam Ray

May 1999

DEDICATION

For my fiancée, Kerry.

Her insurmountable love, forbearance and understanding have given me the fortitude to achieve all my academic goals.

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ABSTRACT

Populations of freshwater mussels in Tennessee have declined significantly in recent years. A freshwater mussel survey of the Sulphur Fork Creek/lower Red River watershed, approximately 74 km long, averaging between 29-35 km in width and part of the Cumberland River drainage, was performed. This freshwater mussel study is more extensive than previous studies in the watershed, both in terms of area investigated and overall effort. A total of 347 man-hours were devoted to research in the spring and summer and fall of 1998 to determine abundance, diversity and habitat of unionoid mussels in the watershed, based on living individuals and remnant shells. Mussels were hand-collected by snorkeling, canoeing, walking stream banks and utilizing a Needham bottom scraper at 23 sample sites. Living species encountered in the watershed were (greatest → least abundant): *Amblema plicata*, Threeridge; *Cyclonaias tuberculata*, Purple Wartyback; *Potamilus alatus*, Pink Heelsplitter; *Lampsilis ovata*, Pocketbook; *Ptychobranchus fasciolaris*, Kidneyshell; *Tritigonia verrucosa*, Pistolgrip; *Elliptio crassidens*, Elephantear; *Lampsilis fasciola*, Wavyrayed lampmussel; *Elliptio dilatata*, Spike; *Fusconaia flava*, Wabash Pigtoe; *Lasmigona costata*, Flutedshell; and

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Quadrula cylindrica, Rabbitsfoot. Species encountered only as remnant shells were: *Cumberlandia monodonta*, Spectaclecase; *Fusconaia ebena*, Ebonyshell; *Leptodea fragilis*, Fragile Papershell; *Obovaria subrotunda*, Round Hickorynut; *Toxolasma lividus*, Purple Lilliput; *Villosa iris*, Rainbow; *Villosa taeniata*, Painted Creekshell; *Villosa vanuxemensis*, Mountain Creekshell; and an unidentifiable *Villosa* sp. Lower Red River held the highest frequency of living species. No evidence of unionoid mussel recruitment was discovered, however, a variety of potential host fishes were observed during the survey. Judging by the numbers of remnant shells observed and results of the few investigations in this watershed, it appears that the mussel fauna has declined significantly in recent years, possibly due to excess silt deposition, chemical run-off, bacterial contamination and loss of habitat.

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CHAPTER I

INTRODUCTION

Stream Ecology Concepts

The world's streams and rivers are highly diversified, but only a minimal amount of the world's freshwaters are contained in these running waters (Allan, 1995). Streams and rivers come in all sizes, from temporary trickles that flow only after rains, to the miles-wide stretches of the great rivers such as the Mississippi and the Amazon (Brewer, 1994).

The classical term for flowing water is lotic. A basic arbitrary subdivision is between creeks or brooks, which are less than three meters wide, and rivers, which are three meters or more (Brewer, 1994). Lotic water pathways are affected greatly by topography, geology, riparian vegetation and climate (Allan, 1995; Brewer, 1994). Because of this phenomenon, streams of most sizes show two basic habitats, riffles or pools. Although there may be some distance separating pools from riffles, commonly the two habitats are close together (Allan, 1994; Brewer, 1994).

Animals of the riffles keep from being swept away by the current through three main mechanisms: they attach themselves to solid surfaces, swim strongly, or avoid the current by positioning themselves in the substrate (e.g. freshwater mussels) (Williams and Hynes, 1976).

Mobile substrates such as gravel, sand and sandy/mud have been subjected to a number of independent waves of colonization by bivalves, freshwater mussels (Purchon, 1968). The filter-feeding bivalves are in most cases only dependent on the substrate for an anchor, for protection against instability, and for concealment from predators (Purchon, 1968). They are characteristically dependent on the water for their supply of food and oxygen (Lock and Williams, 1981), maintaining contact with the overlying water by means of the incurrent and excurrent siphons.

Generally, bivalves occupying a superficial position in the substrate are comparatively small (Purchon, 1968). Thus, suspended sediment and substrate are important determinants to bivalve survival. Also, it is often difficult to anticipate freshwater mussel distribution in lotic systems, since these waters are variable in terms of velocity, depth and substrate make-up (Parmalee and Bogan, 1998). These factors can also affect mussel growth and survival (Purchon, 1968).

It is thought that freshwater unionoid mussels originated in lotic waters, "since the center of diversity appears to have been in the South, where natural lakes may have been relatively few in number (Parmalee and Bogan, 1998)." In addition, these mussels have been exploited for their insurmountable resources.

Importance of Freshwater Mussels

Freshwater mussels are an interesting group of animals that are not particularly well known by the public. Mussels can affect the human species both directly and indirectly. They can serve as a source of food for fish, raccoon, mink, otter, muskrat and several other mammals (Olszewski, 1978). In states such as Tennessee, mussels are harvested or collected for their shells that are sold to places like Japan on the multi-million dollar aquatic market (McGregor and Gordon, 1992; TWRA, 1996). Also, certain mussels are very sensitive to changes in our waterways, so it is possible to utilize them as a means of water quality biomonitoring (Anderson, 1978).

Exotic mussels, such as the zebra mussel (*Dreissena polymorpha*), are a primary cause of clogged water intakes in treatment plants and industrial pipelines. This mussel has invaded Tennessee's waterways only recently (McGregor and Gordon, 1992; Ahlstedt, 1993). The Asian clam (*Corbicula fluminea)*, also an exotic mussel, was introduced into North America from Asia sometime in the 1920's (Counts, 1986). The Asian clam has been introduced into virtually every major lotic system in the United States (Parmalee and Bogan, 1998).

Because they serve many ecological and economic functions, freshwater mussels represent an important and valuable part of the aquatic fauna of Tennessee. They were, and continue to be, an important part of the American economy. In the early 1900's, mussel shells were the primary source of pearl buttons. Various shapes were punched out, polished and used in the textile industry (Parmalee and Bogan, 1998; Parmalee, 1967; Murray and Leonard, 1962). As a result, many thousands of pounds of mussels were taken from American waterways each year, often with little regard to efficiency (Parmalee, 1967). However, after the development of plastics in the 1940's, less emphasis was placed on mussels as a source for button material, and native populations began to recover (Parmalee and Bogan, 1998; Neves et al., 1997).

The shells of native Tennessee mussels were ideal for the lucrative pearl industry. In recent years, Tennessee's mussels have gained popularity in the Japanese cultured pearl industry (Neves et al., 1997; McGregor and Gordon, 1992). A cultured pearl results when sections (seeds) from a freshwater mussel shell are collected, partitioned, rounded, polished and inserted into an oyster, where it is covered by multiple layers of nacre (Purchon, 1968).

Presently, the United States produces approximately 60 percent of the world's pearl seeds (Neves et al., 1997). This has increased the demand for these products, thus, helping stimulate the freshwater mussel industry in Tennessee. In 1989 and again in 1990, more pearl seeds were produced in Tennessee than any other state in the nation (McGregor and Gordon, 1992). The commercial musseling industry in Tennessee continues to expand (Parmalee and Bogan, 1998; Hubbs, 1993).

Classification and Anatomy of North American Freshwater Mussels

Historically, there have been approximately 300 species of freshwater mussels identified in North America. Scientific names used in this manuscript are based on *Common Scientific Names of Aquatic Invertebrates from the United States and Canada: Mollusks* (Turgeon et al., 1988).

Freshwater mussels found in the Southeastern United States belong to five families, three of which are monogeneric. Family Corbiculidae contains only *Corbicula fluminea*, Asian Clam. Family Dreissenidae is restricted to *Dreissena polymorpha*, Zebra Mussel. Family Margaritiferidae contains only *Cumberlandia monodonta*, Spectaclecase. Family Sphaeriidae, fingernail or peaclams, is represented by four genera: *Eupera, Musulium, Pisidium* and *Sphaerium* (Neves et al., 1997). Finally, the polygeneric family Unionidae consists of three subfamilies, Ambleminae, Anodontinae and Lampsilinae. The margaritiferids and unionids are classified in the superfamily Unionoidea and are the subject of this paper.

Freshwater mussels are extremely variable with respect to coloration, shape and size, both within and between species, however, basic structures (Fig. 1 a,b) remain the same in all species (Parmalee, 1967). Identification of species relies heavily on the color of the nacre, size and shape of the pseudocardinal and lateral teeth, the appearance of the periostracum, and the morphology of the outer shell (Parmalee, 1967; Parmalee and Bogan, 1998).

The nacre is the interior, iridescent thick layer of a mussel shell and can possess a number of different colors (e.g., white, pink, salmon, etc.). Pseudocardinal teeth are hinge teeth which lie near the anterior dorsal margin of the shell, and lateral teeth are raised, elongate, interconnected ridges which lie along the hinge line of the valve posterior to the umbo (Parmalee and Bogan, 1998). The periostracum is a thin, colorful epidermal covering on the outer portion of the shell. Furthermore, the outer shell can possess a variety of patterns, such as wrinkles, knobs, spines, etc. (Parmalee and Bogan, 1998; Parmalee, 1967; Cummings and Mayer, 1992).

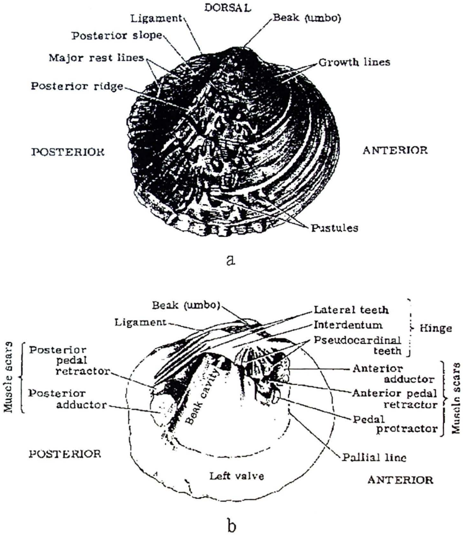

Figure 1. Basic morphology of a freshwater unionid mussel (*Cyclonaias tuberculata*). (a) Dorsal region of right valve, (b) Ventral region of left valve (Parmalee and Bogan, 1998).

The dark concentric lines on the periostracum indicate periods of rest, for example, winter (Parmalee and Bogan, 1998). By studying these lines, one can estimate the age of a freshwater mussel.

Mussels are long-lived, with many species living more than 10 years, and some reported to live more than 100 years (Kesler and Downing, 1997). The age of mussels is usually estimated by using the external growth ring method (Chamberlain, 1931; Crowley, 1957), but new evidence has suggested this method may in fact be incorrect. Past estimates of annual growth based on annuli may be too large. Kesler and Downing (1997) found that annual length changes determined by re-measurement were significantly lower than annual length changes predicted by length-at-age from internal annuli.

Freshwater Mussel Reproduction

Sexes are separate in most freshwater mussels (Shalie, 1970). Sperm is released into the water by the male and enters the female via the incurrent siphon. The eggs are fertilized internally within the water tubes of the female's gills. In the superfamily Unionoidea, fertilized eggs develop into an intermediate larval stage called a glochidium (Fig. 2 a,b). The female stores the glochidia in the water tubes of the gills, which function as a brood chamber or marsupium (Parmalee and Bogan, 1998), as well as a means for obtaining oxygen and capturing food particles (Purchon, 1968; Yeager and Saylor, 1995).

The female expells the glochidia into the water usually during the spring or summer, depending on the reproductive period of the species. This marks the beginning of the parasitic phase in their life cycle (Fig. 3). The glochidia, which have tiny teeth and byssal threads, attach to an appropriate host, normally the gills or fins of a fish, and form cysts. Some mussel species, for example, *Lampsilis ovata*, possess a modified portion of their mantle which acts as a lure (Fig. 4) for attracting potential host fishes (Parmalee and Bogan, 1998; Neves et al., 1997). Although some species are host-specific, others can use a wide variety of fishes as hosts. In fact, under laboratory conditions, glochidia of some mussel species have been found to undergo metamorphosis on amphibians and exotic fishes (Watters and O'Dee, 1998).

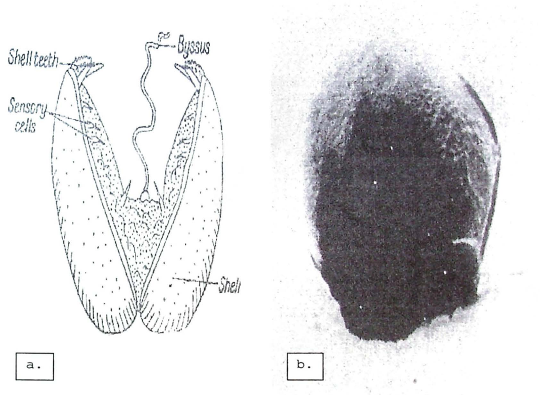

Figure 2. Schematic view of a freshwater mussel glochidium with developing shell, shell teeth, sensory cells and byssus, (b) *Ligumia recta* (Black Sandshell) glochidium at approximately 260 μm (Isom and Hudson, 1982).

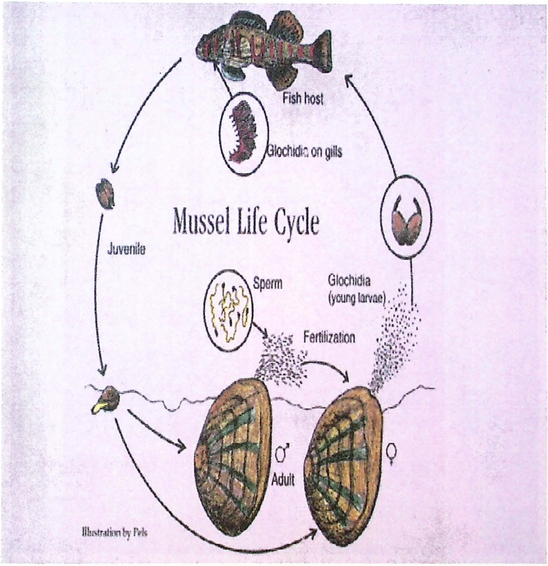

Figure 3. Parasitic life cycle of a freshwater unionoid mussel. Courtesy of Brian Watson, Virginia Tech University.

Figure 4. Minnow-shaped, modified mantle of *Lampsilis ovata,* used as a "lure" to attract potential host fishes.
from: http://www.uiuc.edu/cbd/main/collections/mollusk.html

After some time, one to six weeks or more, the parasitic larvae will transform into juveniles (Parmalee and Bogan, 1998; Parmalee, 1967; Murray and Leonard, 1962). At this point, they will disattach from the host fish or amphibian, drift to the river bottom and grow for an undetermined amount of time.

Conservation of declining mussel populations depends greatly on understanding the fish-host relationship (Yeager and Saylor, 1995). The same can be said when attempting to re-establish populations by transplanting species between streams. Therefore, a transplanted species, in the absence of a suitable fish host, will be unable to reproduce successfully.

Ecology of Freshwater Mussels

Freshwater mussels spend most of their juvenile and adult lives buried in mud, sandy mud and rock bottoms of streams, rivers, ponds and lakes (Parmalee and Bogan, 1998; Oesch, 1984; Murray and Leonard, 1962; Parmalee, 1967; Williams and Schuster, 1989). In the natural environment, mussels have their anterior portion buried in the substrate and their posterior region, with the openings of the incurrent and excurrent siphons, pointed upward into the water column.

Suspended materials in the water are drawn in through the incurrent siphon and filtered by the gills. Waste products are released, via the excurrent siphon, back into the water where they are carried off by the overlying water.

The chemical and biological conditions of the water, depth of the water and substrate type on the stream or river bottom usually determine the number and species of mussels present at a specific site. Also, vegetation, light, current, temperature, predatory animals and parasites found around and within the site can affect the abundance and species present (Parmalee and Bogan, 1998; Oesch, 1984; Murray and Leonard, 1962; Parmalee, 1967; Williams and Schuster, 1989). Furthermore, possibly the biggest factor affecting mussel richness today is the presence of fine sediment, specifically, silt and clay.

Following the introduction of impoundments, Tennessee waterways, for example, have exhibited increased rates of siltation. These silt loads primarily affect the recruitment of young mussels in mussel beds (Williams, 1975). Silt deposits on gills, which are the primary organs used in respiration and feeding in mussels, causing restriction of oxygen passage over gills and interference with food gathering (Purchon, 1968).

Tennessee's Mussel Fauna

Tennessee is home to approximately one-third of the nation's freshwater mussel species (Parmalee and Bogan, 1998; Neves et al., 1997). Historically, over 130 mussel species have been observed in Tennessee's waterways, which, nationally, ranks second only to Alabama in species diversity (Fig. 5) (Neves et al., 1997).

Presently, Tennessee's mussel fauna has been reduced to two-thirds its original species due, at least in part, to high rates of siltation, heavy metal concentrations, and other direct and indirect sources (coliforms, pesticides etc.) in streams and rivers. Metals, such as lead, cadmium and copper, affect mussel growth patterns and their ability to mature (Powell and Williams, 1975; Neves, et al., 1997).

Figure 5. Numbers of freshwater mussel species historically found in the southeastern United States (Neves et al., 1997).

Description of the Watershed

The Sulphur Fork Creek/lower Red River watershed (Fig. 6), as investigated in this study, occupies the majority of Robertson County, Tennessee. The lower Red River was studied only between Dot, Logan County, Kentucky and Port Royal, Montgomery County, Tennessee and includes neither the upper main-stem of the Red River, the South Fork of the Red River nor the lower Red River between Port Royal and Clarksville, Tennessee. The main-stem of Sulphur Fork Creek was investigated, as were several tributaries The segment of the watershed investigated in this study is approximately 74 kilometers long, averages about 29-35 kilometers in width and is part of the Cumberland River drainage.

The watershed lies within the Western Pennyroyal Karst subregion of the Interior Plateau Ecoregion (Griffith et al., 1998). Streams in the watershed carry a bed load of chert gravel and lesser amounts of sand, but limestone bedrock and outcrop controls the thalweg at frequent intervals.

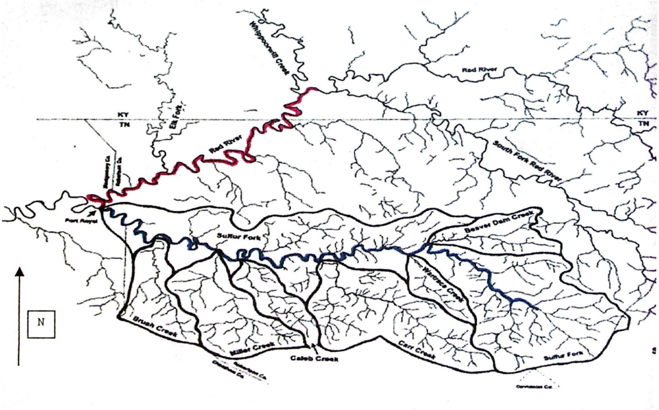

Figure 6. Map of Sulphur Fork Creek (blue)/lower Red River (red) watershed.

Sulphur Fork Creek and its tributaries lie mainly in the southern portion of Robertson County, Tennessee. There are five major, southern tributaries: Brush, Miller's, Caleb's, Carr and Spring creeks. The upper two-thirds of the Sulphur Fork Creek watershed, which has a limestone bed, is wadable (Finley et al., 1997). Sulphur Fork Creek typically remains at wadable levels year round, and its tributaries are constantly fed by springs, thus, making it an ideal stream for biomonitoring (Finley et al., 1997).

Studies by The Center for Field Biology at Austin Peay State University indicate a high rate of siltation in Sulphur Fork Creek watershed (Finley et al., 1997). In 1995, lead concentrations were around 39 µg/L in some areas of the western portion of the Red River (a fourth-order stream of the Cumberland River) in Montgomery County (Cole et al., 1995). However, in 1996, analyses of water samples from sites in the Red River/Sulphur Fork Creek watershed indicated lead concentrations (about 15 µg/L) well below EPA guidelines (Matthews et al., 1996).

Sulphur Fork Creek/Lower Red River Watershed Mussel Fauna: Previous Studies

Sulphur Fork Creek/lower Red River watershed is part of the Cumberland River drainage system. Gordon and Layzer (1989) stated that, historically, there have been 93 mussel species encountered in this drainage. They also noted that, today, eight of the species are federally endangered and nine others are presumed extinct. However, Starnes and Bogan (1988) and Parmalee and Bogan (1998) report that 87 species have been recorded in this drainage. This could be the result of misidentifications or miscommunication of anecdotal results.

Four freshwater mussel surveys have been performed, at two selective sites, on the Sulphur Fork Creek/lower Red River watershed. The first two surveys concentrated on examining mussel populations at Port Royal, Montgomery County, Tennessee, the confluence of Sulphur Fork Creek and Red River.

Wilson and Clark (1914) and Starnes and Bogan (1988) reported a total of eight and twenty-two mussel species, respectively, near Port Royal. Mussels observed in both studies were: *Actinonaias ligamentina*, Mucket; *Actinonaias* *pectorosa*, Pheasantshell; *Alasmidonta marginata*, Elktoe; *Alasmidonta viridis*, Slippershell Mussel; *Amblema plicata*, Threeridge; *Cyclonaias tuberculata*, Purple Wartyback; *Elliptio crassidens*, Elephantear; *Elliptio dilatata*, Spike; *Epioblasma florentina florentina*, Yellow Blossom; *Epioblasma florentina walkeri*, Tan Riffleshell; *Lampsilis fasciola*, Wavyrayed Lampmussel; *Lampsilis ovata*, Pocketbook; *Lampsilis teres*, Yellow Sandshell; *Lasmigona complanata*, White Heelsplitter; *Lasmigona costata*, Flutedshell; *Megalonaias nervosa*, Washboard; *Obovaria subrotunda*, Round Hickorynut; *Potamilus alatus*, Pink Heelsplitter; *Ptychobranchus fasciolaris*, Kidneyshell; *Strophitus undulatus*, Creeper; *Tritigonia verrucosa*, Pistolgrip; *Truncilla truncata*, Deertoe; and, *Villosa vanuxemensis*, Mountain Creekshell.

In 1990 and 1992, Aquatic Resource Center, under contract with Tennessee Wildlife Resources Agency (TWRA), examined the mussel populations in a portion of the Red River at Porter's Chapel Road in Robertson County, Tennessee (TWRA, 1993). In 1990, after only thirty-five minutes of observation, 137 individual mussels, representing 14 species, were collected.

In a 1992 study, quantitative sampling, using 36 randomly placed 0.25 m^2 quadrats, resulted in only fifty-two mussels, representing eight species, observed in a nine-man-hour effort. The most abundant species observed in the 1990 collection were, in order of most to least abundant, *Amblema plicata*, *Cyclonaias tuberculata*, *Elliptio crassidens* and *Elliptio dilatata*. The 1992 survey suggests that the original species richness had decreased. Also, mussels appeared to be old, suggesting that there may be no recruitment in these populations (TWRA, 1993).

Beyond these four studies, little information is known about the mussel fauna in this watershed. It is important that more research be carried out to provide new and additional information on the status of the mussel fauna in the watershed.

Significance of Study

Populations of freshwater mussels in Tennessee have declined significantly in recent years (Parmalee and Bogan, 1998; Neves et al., 1997). The Sulphur Fork Creek/lower Red River watershed possesses characteristics common to many of Tennessee's waterways, creating the potential for constructing a model applicable in other mussel studies in the state.

This freshwater mussel study is more extensive than previous studies in this watershed, both in terms of area investigated and overall effort. It will provide new information for the region. To maximize the value of this study, one or more follow-up studies should be carried out after a few years to measure the health of the mussel fauna and the watershed. It will also be imperative to study the direct rates of sedimentation in later studies. This could provide information on why the state of Tennessee, particularly the Cumberland River drainage, has seen such a decline in its mussel populations.

CHAPTER II

MATERIALS AND METHODS

Study Area and Sample Sites

A majority of the sample sites, investigated in the Sulphur Fork Creek/lower Red River watershed, were located in Robertson County, Tennessee. The lower Red River was studied only between Dot, Logan County, Kentucky and Port Royal, Montgomery County, Tennessee and included neither the upper main-stem of the Red River, the South Fork of the Red River nor the lower Red River between Port Royal and Clarksville, Tennessee.

Mussel collections were taken from 23 sample sites within the watershed. Sample sites were selected, in December 1997, based on good stream habitat and easy road access. Twenty one of these sites were located in Robertson County, Tennessee, one in Kentucky, and the furthest downstream site was located at the confluence of the Red River and Sulphur Fork Creek in Montgomery County, Tennessee.

One canoeing trip, from Glenn Raven Road to a location approximately 1 km upstream of Hills Mill dam near Walnut Grove, Robertson County, Tennessee, was taken in September 1998. This trip allowed investigation of mussel beds in areas of Sulphur Fork Creek, other than those accessible by roads near designated sample sites.

The distribution of sample sites within the watershed is as follows:

A.) Sulphur Fork Creek - 10 sites

B.) Lower Red River - 4 sites

C.) Confluence of Sulphur Fork Creek and lower Red River - 1 site

D.) Carr Creek - 2 site

E.) Miller's - 2 sites

F.) Brush Creek - 1 site

G.) Caleb's Creek - site

H.) Spring Creek - 1 site

I.) Confluence of Sulphur Fork and Spring Creeks - 1 site

Locations for each site were, from west to east (downstream → upstream):

1.) Confluence of Sulphur Fork Creek and lower Red River at Port Royal State Historical Park
2.) Sulphur Fork Creek at Walnut Grove, U.S. Highway 76
3.) Brush Creek at Edd Ross Road
4.) Sulphur Fork Creek at Glenn Raven Road
5.) Miller's Creek at Maxie Road, Turnersville
6.) Miller's Creek at Carr Road
7.) Caleb's Creek at Maxie Road
8.) Confluence of Sulphur Fork and Spring Creeks at Cedar Hill Road
9.) Spring Creek at Kenney Road
10.) Carr Creek at U.S. Highway 49
11.) Carr Creek at New Chapel Road
12.) Sulphur Fork Creek at Kenney's School Road
13.) Sulphur Fork Creek at Becky Lane, Springfield
14.) Sulphur Fork Creek at junction of U.S. Highways 49 and 76 East, Springfield
15.) Sulphur Fork Creek at Possum Trout Road
16.) Sulphur Fork Creek at Distillery Road

17.) Sulphur Fork Creek at New Mount Pleasant Road

18.) Sulphur Fork Creek at Hall Road off

19.) Webb Branch of Sulphur Fork Creek at U.S. Highway 257 east off of exit 104 on Interstate 65

20.) Red River at U.S. Highway 41

21.) Red River at Porter's Chapel Road

22.) Red River at Anderson Road

23.) Red River at U.S. Kentucky Highway 161 near Dot, Kentucky.

Mussel Collection

Mussel samples were collected throughout a twenty-month period (approximately 400 man-hours) using manual techniques such as snorkeling, hand collecting, and utilizing a Needham bottom scraper. Snorkeling and hand collecting were emphasized at all sites, while clam netting was implemented at sites with a depth of < 0.5 meters.

Snorkeling was accomplished using a 2.5 mm, nylon wetsuit, snorkel and goggles and a stainless steel, hand-held garden hook, for gouging the substrate. Snorkeling allowed the collector to scan the river bottom in search of living mussels and remnant shells.

A Needham bottom scraper (27x26x17 cm wire cage on a 1.6 m wooden handle) was primarily used to search for juvenile mussels in the substrate as evidence of recruitment.

Freshwater unionoid mussels, both live and dead (remnant shells), were collected from the stream substrate, exposed gravel bars and along stream banks. At most sites, sampling began approximately 150 m downstream of the sample site entrance, usually a bridge, and proceeded upstream for 300 meters. There was no timed sampling performed.

After live mussels were encountered, their microhabitat was categorized as sandy mud, mud, loose gravel or limestone bedrock. Mussels were then placed in a five-gallon bucket, filled with water, and transported to the laboratory. Remnant shells were collected in one-gallon plastic bags and were also transported to the laboratory.

Stream Analysis

Prior to mussel sampling, substrate type, water turbidity and depth and width of the stream were recorded to categorize the sample site(s) for that day. Substrate type was recorded as sand, mud, gravel or bedrock. Turbidity was determined using a HF Scientific© turbidimeter (nephlometer), Model DRT-15C, and recorded in nephlometric turbidity units (NTU). Depth was recorded in centimeters using a meter stick and width in meters using a measuring tape.

On each trip, other physicochemical parameters (temperature, dissolved oxygen, percent saturation of dissolved oxygen, specific conductance, pH and total dissolved solids), were measured using one of the following multiparameter water quality meters, HYDROLAB H20 (Model 11120) and YSI Model 600XLM. These apparati were calibrated for all measurements prior to recording data.

Periodically, water samples were taken at the following selected sample sites: 4; 6; 7; 11; 12; 16; and 21 (see ***Study Area and Sample Sites***, above). These samples were used for chemical analyses in the laboratory. An additional five water samples were taken at sample sites 4, 5, 7, 16, and 21 (see ***Study Area and Sample Sites***, above) to test for presence of bacterial contamination (fecal *Streptococcus* and fecal coliform). Samples were

Preservation of Voucher Specimens

Voucher specimens of remnant shells were preserved by scrubbing the inner and outer surfaces of the shell in an Alconox© detergent bath with a toothbrush and wire brush. Scrubbing with detergent removed most of the excess algae, sediment and discoloration from the shell. Shells tend to develop a dark color if exposed to anaerobic conditions.

After cleaning, the inner surface or nacre, was labeled using a black waterproof pen. The following information was included:

1.) Genus species

2.) Collector(s)

3.) Date

4.) Location (River, County and State).

Finally, the inner and outer surfaces of the shell were sprayed with Krylon© Crystal Clear Acrylic Coating 1303, to enhance and preserve the appearance of the nacre and periostracum.

All voucher specimens were photo-documented and placed in the Austin Peay State University Aquatic Invertebrate Collection.

Chemical and Bacterial Analysis

In the laboratory, nitrate-nitrite nitrogen, total phosphates, orthophosphates, sulfates, alkalinity, and hardness for water samples were determined following EPA standards and procedures of quality control and quality assurance (EPA, 1983). Water quality standards were determined by using the State of Tennessee Water Quality Standards manual (Tennessee Department Environment and Conservation, 1995).

Spectrophotometric methods were used to determine concentrations of nitrate-nitrite nitrogen (EPA method 353.3), total and orthophosphates (EPA method 365.2) and sulfates (EPA method 375.4).

Alkalinity, a measurement of the buffering capacity of the water, and hardness, primarily attributable to Mg and Ca ions, were calculated using the titrametric methods 310.1 and 130.2, respectively.

Total organic carbon (TOC) and dissolved organic carbon (DOC) were analyzed at Murray State University's Hancock Biological Station, Murray, Kentucky (EPA method 415.1).

Dr. Don Dailey and staff, Austin Peay State University, performed bacterial analyses on water samples, from designated sample sites, using the filtration method. Numbers of colonies of fecal coliform and fecal *Streptococcus* were counted to determine if contamination existed. The ratios of fecal coliform (fc) to fecal *Streptococcus* (fs) were determined to identify if contamination most likely resulted from human or other sources. A ratio of $^{fc}/_{fs} > 4$ indicates possible human fecal contamination (Csuros and Csuros, 1999)

Statistical Analysis

Frequency distributions and other descriptive statistics (mussel shell length, total numbers of mussels and diversity) were calculated using Microsoft Excel© software for Windows. JMP IN 3.0© for Windows software was used to perform a one-way ANOVA to test for significant differences between shell lengths and wet weights among species.

CHAPTER III

RESULTS

Mussel Analysis

A total of ninety living unionoid mussels, representing 12 species, was collected in Sulphur Fork Creek and lower Red River (Table 1).

Table 1. Total numbers of living, unionoid mussel species collected in Sulphur Fork Creek and lower Red River, Robertson County, Tennessee, May to October 1998.

	Sulphur Fork Creek	Lower Red River
Total living mussels	40	50
Total species	9	9
Species in common	6	6
Unique species	3	3

The following results are based on raw data in the Appendix (Table A1). Living species encountered in the watershed were (greatest→least abundant): *Amblema plicata*, Threeridge, (Plate 1); *Cyclonaias tuberculata*, Purple Wartyback, (Plate 2); *Potamilus alatus*, Pink Heelsplitter, (Plate 3); *Lampsilis ovata*, Pocketbook, (Plate 4); *Ptychobranchus fasciolaris*, Kidneyshell, (Plate 5); *Tritigonia verrucosa*, Pistolgrip, (Plate 6); *Elliptio crassidens*, Elephantear, (Plate 7); *Lampsilis fasciola*, Wavyrayed lampmussel, (Plate 8); *Elliptio dilatata*, Spike, (Plate 9); *Fusconaia flava*, Wabash Pigtoe, (Plate 10); *Lasmigona costata*, Flutedshell, (Plate 11); *Quadrula cylindrica*, Rabbitsfoot, (Plate 12).

Species encountered only as remnant shells were: *Cumberlandia monodonta*, Spectaclecase, (Plate 13); *Fusconaia ebena*, Ebonyshell, (Plate 14); *Leptodea fragilis*, Fragile Papershell, (Plate 15); *Obovaria subrotunda*, Round Hickorynut, (Plate 16); *Toxolasma lividus*, Purple Lilliput, (Plate 17); *Villosa iris*, Rainbow, (Plate 18); *Villosa taeniata*, Painted Creekshell, (Plate 19); *Villosa vanuxemensis*, Mountain Creekshell, (Plate 20); and an unidentifiable *Villosa* sp. (Plate 21).

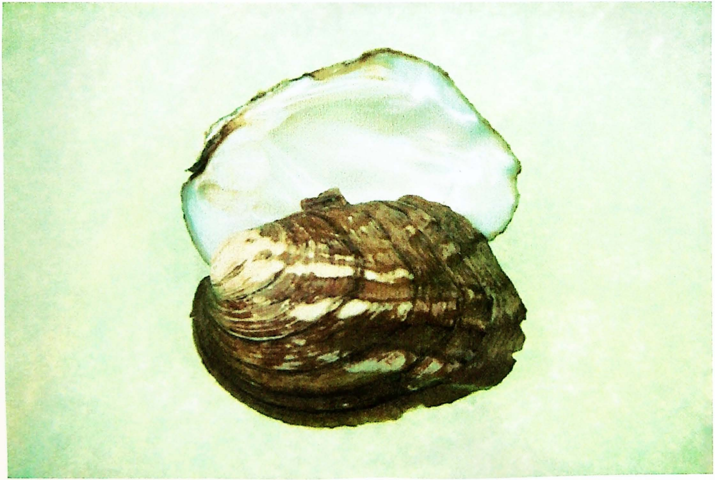

Plate 1. *Amblema plicata* (Say, 1817), Threeridge. Sulphur Fork Creek at Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, 9 September 1998. Length: 6.7 cm.

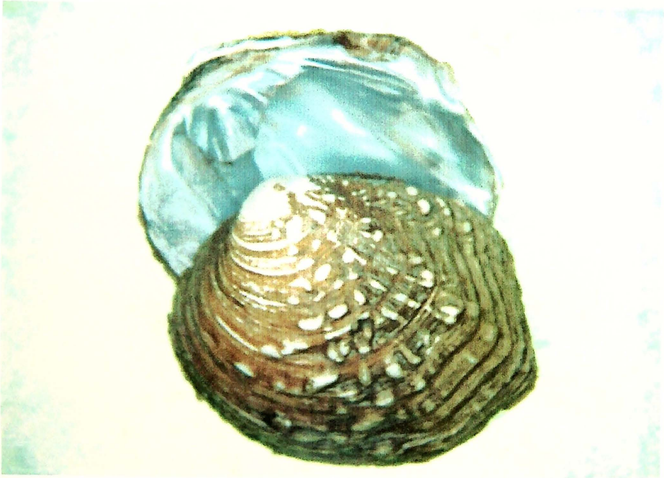

Plate 2. *Cyclonaias tuberculata* (Rafinesque, 1820), Purple Wartyback. Sulphur Fork Creek at Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, 9 September 1998. Length: 9.6 cm.

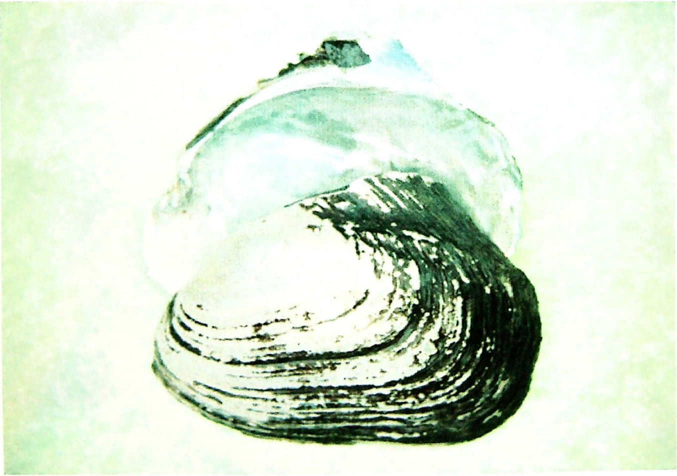

Plate 3. *Potamilus alatus* (Say, 1817), Pink Heelsplitter. Sulphur Fork Creek at Port Royal State Park, Robertson County, Tennessee, 10 July 1998. Length: 9.3 cm.

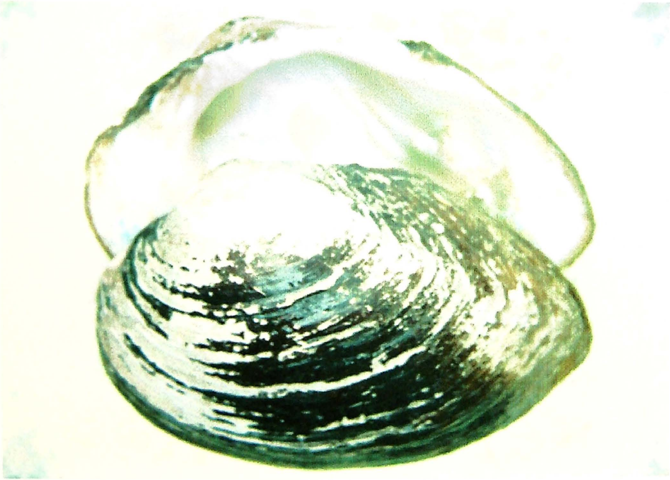

Plate 4. *Lampsilis ovata* (Say, 1817), Pocketbook. Sulphur Fork Creek at Glen Raven Road, Robertson County, Tennessee, 28 September 1998. Length: 12.4 cm.

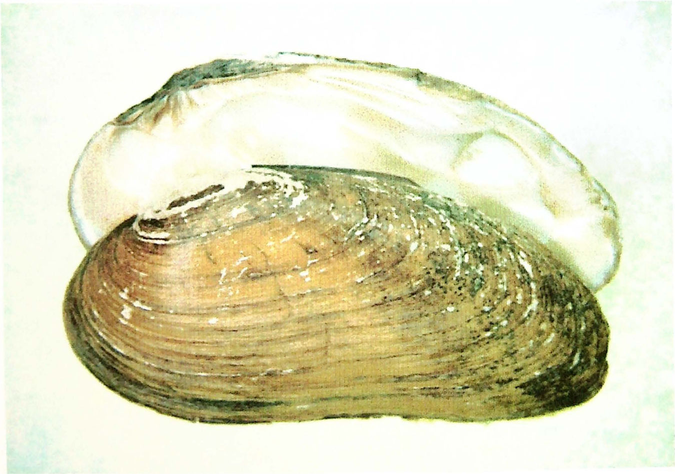

Plate 5. *Ptychobranchus fasciolaris* (Rafinesque, 1820), Kidneyshell. Sulphur Fork Creek between Glen Raven Road and Hills Mill, Robertson County, Tennessee, 28 September 1998. Length: 12.3 cm.

Plate 6. *Tritigonia verrucosa* (Rafinesque, 1820), Pistolgrip. Sulphur Fork Creek at Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, 9 September 1998. Length: 7.0 cm.

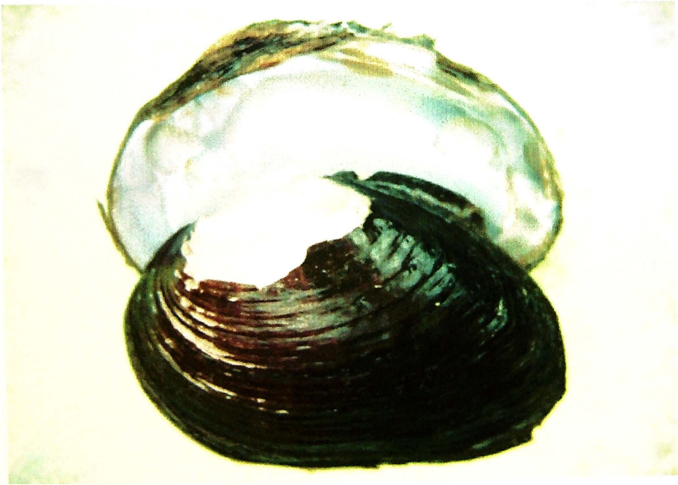

Plate 7. *Elliptio crassidens* (Lamarck, 1819), Elephantear. Red River at Porter's Chapel Road, Robertson County, Tennessee, 22 July 1998. Length: 11.2 cm.

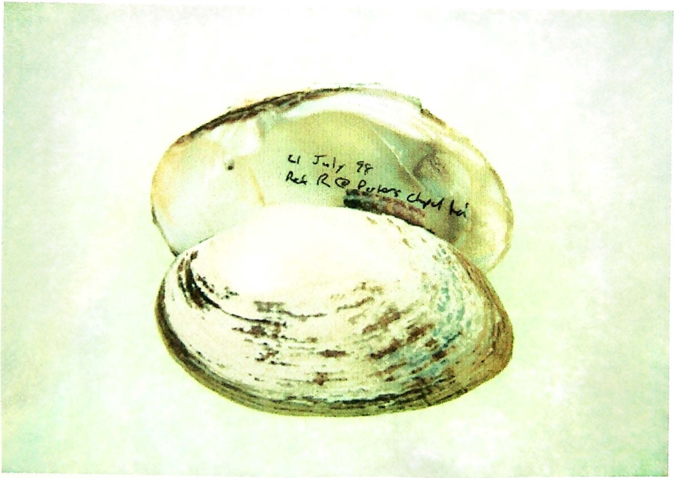

Plate 8. *Lampsilis fasciola* (Rafinesque, 1820), Wavyrayed Lampmussel. Red River at Porter's Chapel Road, Robertson County, Tennessee, 22 July 1998. Length: 7.4 cm.

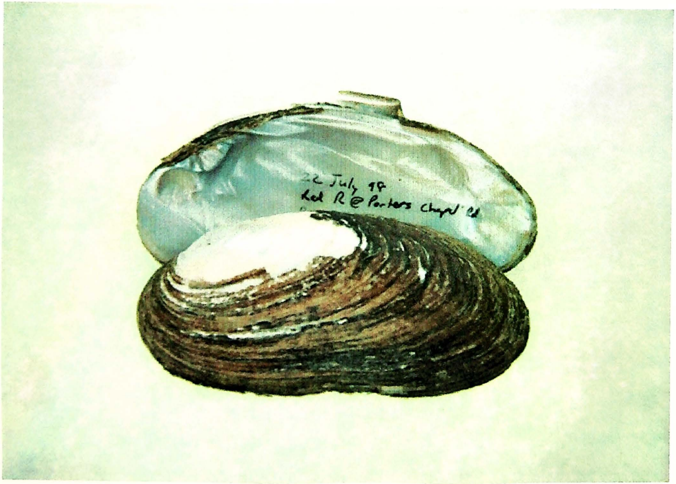

Plate 9. *Elliptio dilatata* (Rafinesque, 1820), Spike. Red River at Porter's Chapel Road, Robertson County, Tennessee, 22 July 1998. Length: 8.3 cm.

Plate 10. *Fusconaia flava* (Rafinesque, 1820), Wabash Pigtoe. Sulphur Fork Creek at Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, 9 September 1998. Length: 7.7 cm.

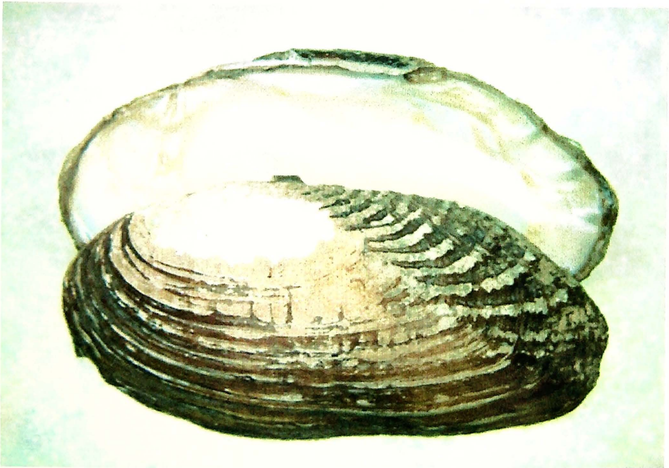

Plate 11. *Lasmigona costata* (Rafinesque, 1820), Flutedshell. Sulphur Fork Creek at Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, 9 September 1998. Length: 12.8 cm.

Plate 12. *Quadrula cylindrica* (Say, 1817), Rabbitsfoot. Red River at Porter's Chapel Road, Robertson County, Tennessee, 6 August 1998. Length: 10.2 cm.

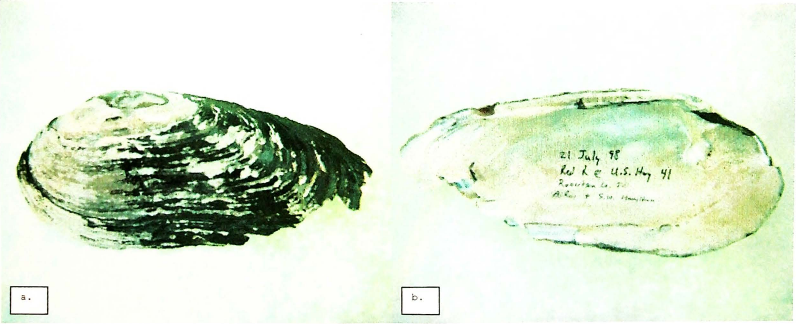

Plate 13. *Cumberlandia monodonta*, (a) dorsal view; (b) ventral view, (Say, 1829), Spectaclecase. Red River at U.S. Highway 41, Robertson County, Tennessee, 21 July 1998. Length: 11.6 cm.

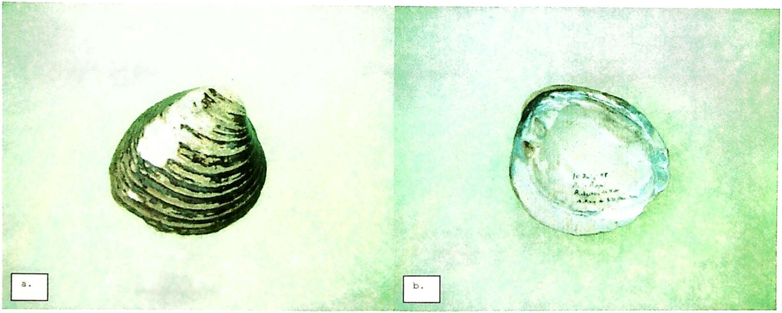

Plate 14. *Fusconaia ebena*, (a) dorsal view; (b) ventral view, (Lea, 1831), Ebonyshell. Sulphur Fork Creek at Port Royal State Park, Robertson County, Tennessee, 10 July 1998. Length: 5.2 cm.

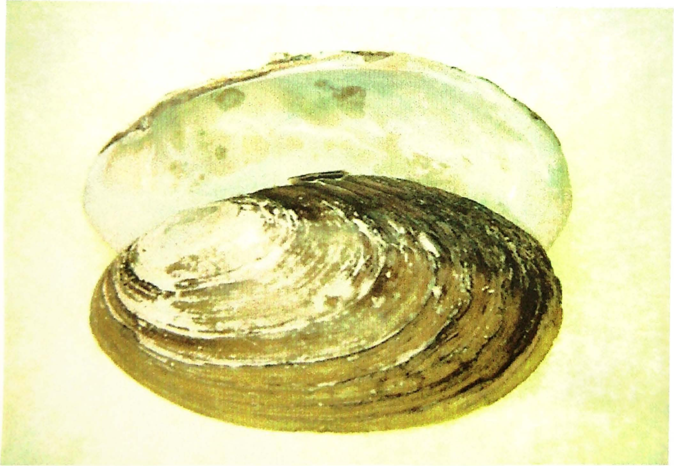

Plate 15. *Leptodea fragilis* (Rafinesque, 1820), Fragile Papershell. Red River at Porter's Chapel Road, Robertson County, Tennessee, 21 July 1998. Length: 9.0 cm.

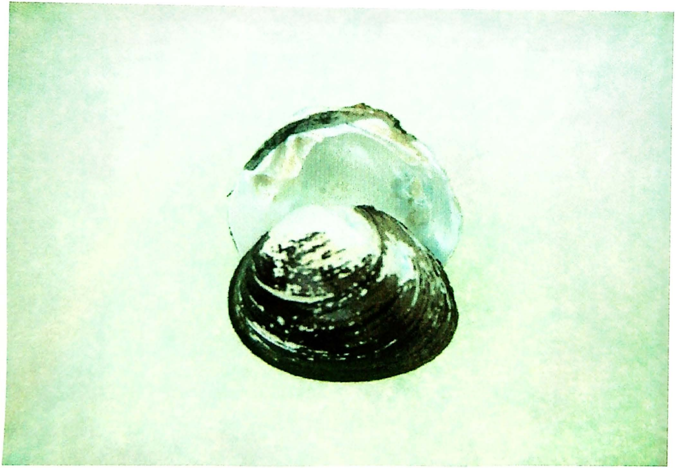

Plate 16. *Obovaria subrotunda* (Rafinesque, 1820), Round Hickorynut. Sulphur Fork Creek at Port Royal State Park, Robertson County, Tennessee, 10 July 1998. Length: 4.5 cm.

Plate 17. *Toxolasma lividus* (Rafinesque, 1831), Purple Lilliput. Sulphur Fork Creek at Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, 26 June 1998. Length: 6.4 cm.

Plate 18. *Villosa iris* (Lea, 1829), Rainbow. Sulphur Fork Creek at confluence with Spring Creek, Robertson County, Tennessee, 3 June 1998. Length: 4.0 cm.

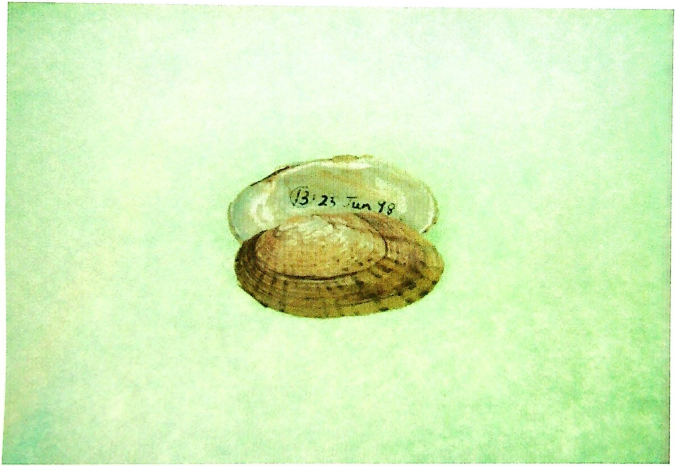

Plate 19. *Villosa taeniata* (Conrad, 1834), Painted Creekshell. Sulphur Fork Creek at Glen Raven Road, Robertson County, Tennessee, 25 June 1998. Length: 4.2 cm.

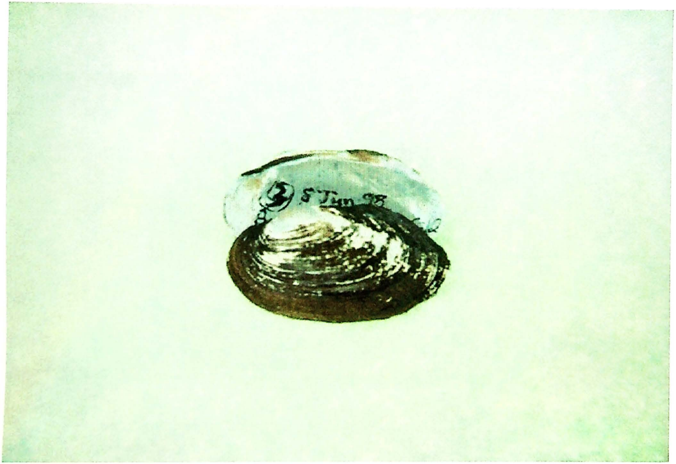

Plate 20. *Villosa vanuxemensis* (Lea, 1838), Mountain Creekshell. Sulphur Fork Creek at Becky Lane, Springfield, Robertson County, Tennessee, 8 June 1998. Length: 4.3 cm.

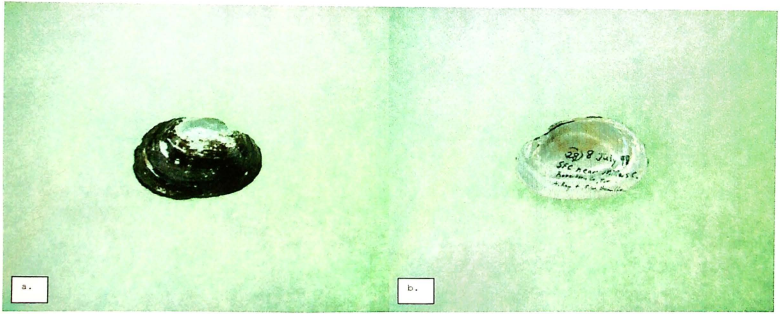

Plate 21. *Villosa* sp., (a) dorsal view; (b) ventral view, Sulphur Fork Creek, near confluence with Miller's Creek, Robertson County, Tennessee, 8 July 1998. Length: 4.3 cm.

Amblema plicata was the most abundant living species observed in both Sulphur Fork Creek (Fig. 7) and lower Red River (Fig. 8). Living mussels were collected at only three sites (Table 2). Nearly all living species encountered in Sulphur Fork Creek were located in a backwater region (Plate 22) located approximately 600 meters downstream of man-made impoundment (Plate 23). This backwater region exhibited no surface recharge and very minor surface discharge. The dissolved oxygen concentration was 1.43 mg/L (percent saturation, 16.4%). Mussels were buried in loose sediment with only their siphons exposed.

Two unusually large, living mussels, *Amblema plicata* (Plate 24) and *Potamilus alatus* (Plate 25), were observed in the main channel adjacent to the backwater region. Habitat preferences of other living mussels encountered in the watershed are located in Table 3.

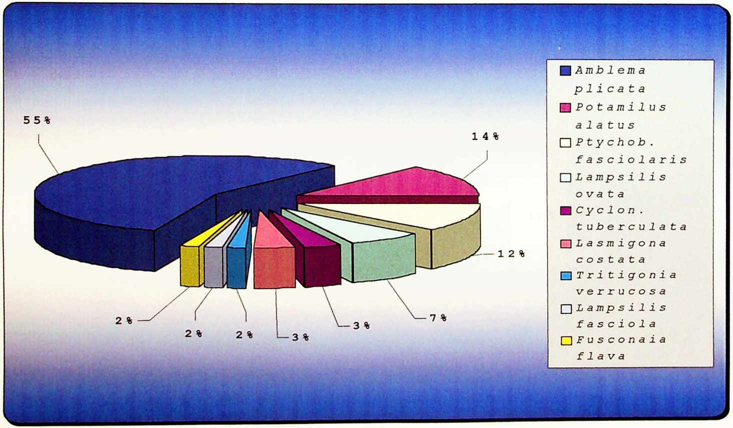

Figure 7. Relative abundance of living unionoid mussel species collected in Sulphur Fork Creek, May to October 1998.

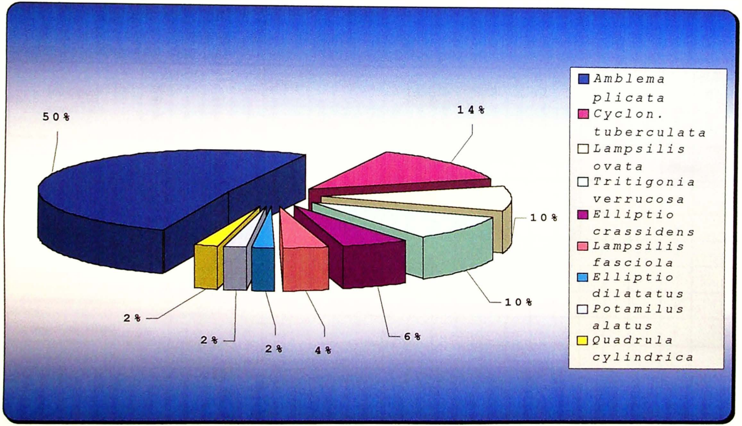

Figure 8. Relative abundance of living unionoid mussel species collected in lower Red River, May to October 1998.

Table 2. Site locations (refer to **Chapter II, *Study Area and Sample Sites***), in Sulphur Fork Creek and lower Red River indicating (•) where living unionoid mussels were collected from May to October 1998.

Mussel species	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	Site 15	Site 16	Site 17	Site 18	Site 19	Site 20	Site 21	Site 22	Site 23
Amblema plicata		•		•																	•		
Cyclonaias tuberculata		•																			•		
Elliptio crassidens																					•		
Elliptio dilatata																					•		
Fusconaia flava		•																					
Lampsilis fasciola		•																			•		
Lampsilis ovata		•		•																	•		
Lasmigona costata		•		•																			
Potamilus alatus		•		•																	•		
Ptychobranchus fasciolaris				•																			
Quadrula cylindrica																					•		
Tritigonia verrucosa		•																			•		

Plate 22. Backwater region where living unionoid mussels were initially observed in Sulphur Fork Creek, Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, approximately 100 m downstream of U.S. Highway 76 bridge.

Plate 23. Man-made impoundment at Hills Mill, Robertson County, Tennessee, approximately 500 m upstream of U.S. Highway 76 bridge, which restricts upstream movement of freshwater mussels and their fish hosts.

Plate 24. Comparison of old (length, 19.6 cm; wet weight, 1,302 g) versus young (length, 5 cm; wet weight, 49.5 g) living *Amblema plicata* collected in Sulphur Fork Creek, Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, 9 September 1998.

Plate 25. Comparison of old (length, 19.2 cm; wet weight, 847 g) versus young (length, 10 cm; wet weight, 144 g) living *Potamilus alatus* collected in Sulphur Fork Creek, Walnut Grove, U.S. Highway 76, Robertson County, Tennessee, 9 September 1998.

Table 3. Habitat preferences of living unionid mussels observed in the Sulphur Fork Creek/lower Red River watershed, May to October 1998.

Mussel species	Muddy substrate; Backwater region	On top of substrate; No flow	On top of substrate; moderate flow	Loose mud, sand; moderate→ high flow
Amblema plicata	•	•	•	•
Cyclonaias tuberculata	•		•	•
Elliptio crassidens		•		•
Elliptio dilatata		•		
Fusconaia flava			•	
Lampsilis fasciola		•	•	
Lampsilis ovata	•	•		•
Lasmigona costata	•			
Potamilus alatus	•	•	•	•
Ptychobranchus fasciolaris		•	•	•
Quadrula cylindrica				•
Tritigonia verrucosa	•	•	•	•

Inferential Statistical Analysis

Mussel shell lengths differed significantly between species ($p = 5.49$, $p < 0.001$, $R^2 = 40.99\%$), as did wet weights between species ($p = 2.08$, $0.03 < p < 0.04$, $R^2 = 20.82\%$).

Physicochemical Analysis

All physicochemical results were well under EPA guidelines. However, all fecal bacteria results exceeded EPA guidelines for recreational use. Raw data for physicochemical (Tables A2-A5) and fecal bacteria (Table A6) analyses, for selected sample sites (refer to **Chapter II, *Study Area and Sample Sites***), are located in the Appendix.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

From the three prior investigations in the Sulphur Fork Creek/lower Red River watershed, 22 unionoid mussel species have been documented (Wilson and Clark, 1914; Starnes and Bogan, 1988; TWRA, 1993). My investigation reveals only 12 living species and another nine species from shells only. Based on my results, there appears to be a significant decline in richness and abundance of freshwater unionoid mussels in this watershed. Unionoid mussel population declines have also been observed across the southeastern United States (Neves et al., 1997; Parmalee and Bogan, 1998).

Large quantities (>100) of remnant shells were observed at the lower Red River sites and in Sulphur Fork Creek at Port Royal State Historical Park, Robertson County, Tennessee. No living mussels were encountered in Sulphur Fork Creek at Port Royal. Only a decade ago Bogan and Starnes (1988) noted 22 living unionoid species in the Port Royal site. Excess silt deposition, chemical run-off, bacterial contamination and loss of habitat are possible factors affecting mussel survival in this watershed.

On a superficial level, siltation (sedimentation) seems to be detrimentally affecting the native mussel fauna. Heavy amounts of silt, probably resulting from run-off, were observed at every site. It is thought that increased rates of sedimentation have detrimental effects on mussel growth and development (Neves et al., 1997; Purchon, 1968; Parmalee, 1967; Parmalee and Bogan, 1998; Murray and Leonard, 1962; Schalie, 1938). However, the rate of sedimentation was not measured in this study.

In most areas, agricultural lands border the Sulphur Fork Creek/lower Red River watershed. After heavy rains, the watershed is susceptible to receiving high rates of agricultural chemicals, such as insecticides, fungicides, herbicides and fertilizers from run-off, dependent on the timing and intensity of the rain event. These chemicals could be affecting glochidial or juvenile mussel survival as well as the health and reproductive success of adults. These effects may be acute or chronic.

Bacterial contamination is another possible factor affecting the native mussel fauna. Nearly all fecal coliform (fc) and fecal *Streptococcus* (fs) counts exceeded EPA standards for recreational use (Table A6). On several occasions, ratios of these bacteria ($^{fc}/_{fs}$) exceeded four, indicating possible human fecal contamination (Table A6) (Csuros and Csuros, 1999). Although none were observed, these bacterial ratios suggest there could be failing residential septic systems or direct outlets of domestic wastewater to the stream in the watershed. In most cases, the $^{fc}/_{fs}$ ratio was less than four, indicating fecal contamination from other animal sources. High numbers of beef cattle utilize pasturelands, and livestock keeping practices allow access to the streams. In addition, even when animals do not have access to the streams, the riparian buffer is too narrow to prevent fecal matter from washing into the stream during rain events. These observations could explain the high non-human bacterial counts observed throughout the watershed.

The stream substrate was highly embedded at most sample sites (i.e., interstitial spaces filled with fine material), particularly in areas of low stream velocity. This results in loss of habitat for mussels, which are burrowing macroinvertebrates. In addition, embeddedness could inhibit settling and burrowing of juvenile unionoid mussels. Juvenile mussels of some species have been found up to two meters deep in the streambed or hyporheic zone (Parmalee and Bogan, 1998).

It is interesting to note that most living and remnant species encountered in Sulphur Fork Creek were limited to the lower one-fifth of the watershed downstream of Hills Mill dam. This dam has been a barrier to fish migration since the early 1900's. Prohibiting fish migration upstream interferes with the normal dispersal of unionoid mussels in the watershed.

One mussel species, *Ptychobranchus fasciolaris*, was only found upstream of Hills Mill. This species has the potential to move downstream of the impoundment, although this would require infected host fish to move through the slack water of the impoundment and over the dam. Species located downstream (e.g., *Cyclonaias tuberculata* and *Potamilus alatus*) will have no opportunity to advance upstream of the impoundment on their own.

The backwater region downstream of Hills Mill dam (Plate 22), an area of nearly stagnant water cut off from the main channel by a gravel bar, possibly resulted from a large flood event (>50 cm of rain over approximately three days during late May 1998). The mussels found in this pool may have been stranded in an area that had previously received high stream flow. Furthermore, this flood event seemed to "flush" the entire watershed prior to my survey of the mussel fauna. It is possible that living specimens and remnant shells were carried downstream from their prior locations. On several occasions, large and presumably old unionoid mussels were found lying on top of the stream substrate, yet, they showed evidence of being embedded in the substrate for very long periods of time. Specimens were observed with anterior discoloration and periostracal wear from burrowing, posterior algal growth, severe symmetrical truncation of the posterior ends of the shells from long-term abrasion while embedded and asymmetrical distortion of shell growth, presumably due to confined growth in the substrate. It would have been very interesting to observe the mussel fauna prior to this flood.

Living mussels encountered near the backwater region were large with many growth rings indicating they were very old, some as old as 20 years or more. This observation applies throughout the watershed; no living mussels observed were younger than seven years and most were 10 years or older. In a report to the Tennessee Wildlife Resources Agency (1993), it was noted that all living mussels observed in the Red River near Porter's Chapel Road were old (>8 years) and that no recruitment was evident.

In this study, mussel recruitment was not recorded in any region of the watershed. However, a variety of suitable host fish species were observed during the survey and have been documented by TWRA (Mack Finley, personal communication). Therefore, lack of recruitment may be due to several factors other than availability of suitable host fishes. These factors may include 1) relatively small adult mussel populations that limits potential for successful reproduction; 2) unhealthy and/or sterile surviving adults; 3) mortality of glochidia due to poor water quality; and 4) mortality of juveniles due to poor water quality and/or poor substrate characteristics.

Amblema plicata, the most abundant unionoid species observed living in the watershed, is a resilient and adaptable species which thrives in a variety of aquatic habitats, seemingly because its glochidia are capable of encysting and transforming on a variety of host fish species (Parmalee and Bogan, 1998). This species was encountered in large numbers compared to the other species and is listed as currently stable in Tennessee (Parmalee and Bogan, 1998).

Two other species observed living in the watershed are listed as either threatened, *Quadrula cylindrica*, or a species of special concern, *Cyclonaias tuberculata* (Parmalee and Bogan, 1998). Only one individual of *Quadrula cylindrica* was collected, while *Cyclonaias tuberculata* was the second most abundant unionoid species observed in this study. Of the nine remnant species collected, one, *Cumberlandia monodonta*, is listed as threatened and three others, *Obovaria subrotunda*, *Toxolasma lividus* and *Villosa vanuxemensis*, are listed as species of special concern (Parmalee and Bogan, 1998).

In the future, it will be imperative to attempt to identify factors affecting the native unionoid mussel fauna in the Sulphur Fork Creek/lower Red River watershed, such that these factors can be mitigated and native mussel populations can recover and be restored in the watershed.

Finally, it must be noted that unionoid species documented only as remnant shells in this study and species documented in previous studies, but not found in this study, may still be living in the watershed. Due to the burrowing nature of freshwater mussels, living specimens may have been overlooked.

CHAPTER V

RECOMMENDATIONS

The following are proposed recommendations for improving and restoring the health of the native freshwater unionoid mussel fauna in Sulphur Fork Creek and lower Red River:

1.) Re-investigate the unionoid mussel fauna in three to five years

2.) Restore the freshwater unionoid mussel populations to previous levels

   a.) Re-introduce extirpated species by translocating living specimens from other watersheds into the Sulphur Fork Creek/lower Red River watershed

   b.) Translocate healthy native mussels from below Hills Mill dam, Robertson County, Tennessee, (e.g., *Cyclonaias tuberculata* and *Potamilus alatus*) to suitable locations above the dam c.) Translocate healthy native mussels from above Hills Mill dam (e.g., *Ptychobranchus fasciolaris*) to suitable locations below the dam

3.) Enhance existing populations of mussels currently living in the watershed by translocating as above

4.) Measure the direct rates of sedimentation

5.) Continue to monitor bacterial levels

6.) Attempt to create proper habitat for living mussels at selected sample sites

7.) Carefully introduce native fish species known to be hosts for some mussel species

8.) Attempt to transform glochidia *in vitro*, using a liquid growth medium

9.) Attempt to rear native juvenile mussels in the laboratory to:

a.) test the effects of agrochemicals on juvenile mussels (recruits),

b.) cage recruits in the watershed for monitoring survival, and c.) enhance unionoid mussel recruitment in the watershed

10.) Protect and improve water quality in the watershed

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LIST OF REFERENCES

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