

**ASSESSMENT OF A FRESHWATER MUSSEL (MOLLUSCA:  
BIVALVIA) COMMUNITY IN THE LICKING RIVER, AT  
BUTLER, PENDLETON COUNTY, KENTUCKY**

**FINAL REPORT**

**By**

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## EXECUTIVE SUMMARY

This study was undertaken to assess the current condition of the freshwater mussel (also called unionid) (*Bivalvia: Unionidae*) community in the Licking River at the State Route 177 bridge crossing in Butler, Pendleton County, Kentucky. A recent study indicated a drastic decline in the reproduction occurring in this historically diverse assemblage, evident through findings of few glochidia in stream drift and the absence of glochidial encystment on any of the fishes collected. An intensive community analysis provided information on the current size demography of the resident populations, and monitored the presence of species historically known from this site. It also provided evidence of juvenile recruitment and reproduction, through presence/absence of juveniles at the site and glochidia (mussel larvae) in drift net samples or encysted upon fishes. Results from this survey would be useful for future monitoring of this community, and would provide important information that can be used in comparison with other mussel assemblages for which various have been quantified. Land use practices that had been or are currently being employed upstream of the proposed study site were considered to better understand the point and nonpoint source pollution factors that might be influencing community changes. It was hypothesized that upstream land use practices were contributing to a previously documented decline of reproduction at this locality. Qualitative and quantitative searches yielded 27 extant unionid species within the area, and resulted in the discovery of juveniles of eight species, including two juveniles of the state and federally endangered fanshell, *Cyprogenia stegaria*. Quantitative sampling, consisting of substrate excavation of randomly selected 0.25 m<sup>2</sup> plots yielded 130 individual unionids, representing 17 species, at a total density of approximately 4.3 unionids / m<sup>2</sup>. Calculations showed the most abundant species, the spike, *Elliptio dilatata*, to have had the highest importance value in the quantitatively sampled areas. Analysis of drift net samples yielded 159 unionid glochidia and 434 juvenile *Corbicula fluminea*, the exotic Asian clam, out of approximately 730 m<sup>3</sup> of stream drift. Examination of 545 fishes collected from the site yielded 241 glochidia encysted on either gills or fins of 47 individual fish hosts, for an average of 5.13 glochidia per infected fish. Eight species of fish were represented by hosts bearing encysted glochidia. These eight species comprised 71.74% of all fishes collected. The emerald shiner, *Notropis atherinoides*, was the most abundant fish from the three collections, bore the most glochidia, and had the highest prevalence of encystment. Twenty-seven emerald shiners were encysted with 195 unionid larvae; one individual was found bearing 105 glochidia encysted upon its gills. The dominant human influence in the lower Licking River drainage is agriculture, which has led to a decrease of riparian buffer along a large proportion of the banks within the watershed, and has also allowed livestock access to the streams. The decrease of a riparian buffer and cattle access allows for more direct introduction of chemicals (i.e., pesticides and fertilizers), as well as decreases bank stability, which, in turn, leads to excessive sediment introduction. Such anthropogenic factors and large numbers of *C. fluminea* have likely impacted the resident mussel fauna. Although this mussel community remains diverse, the lack of juvenile representation for all but eight species

indicated a breakdown in a very important stage in the life history for the rest of the community. Recent reproduction was evident through the presence of glochidia encysted on host fishes and being broadcast into the water column; however, accurate recruitment patterns for resident populations could not be obtained without identification of the glochidia. The only definitive sign of a particular species having recently recruited new members was through the presence of juveniles in the quantitative and/or qualitative samples. These analyses provided baseline population data for each species which can be used in future monitoring of this biologically and historically significant site. There is a great need for future monitoring of such communities, so dynamics of the community can be more definitively recorded, and fluctuations in population structures can be analyzed. Compilations of long-term monitoring efforts on such diverse mussel communities should allow more accurate speculation, in comparison to watersheds with similar land uses, on the effects of such anthropogenic factors as point and nonpoint source pollutants on unionids. Better understanding of the impacts of these factors to natural communities should lead to better management strategies to ameliorate these impacts, conserving existing communities.

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## CHAPTER I

### INTRODUCTION

Throughout the past century, much attention was directed toward the conservation and management of our native freshwater mussels (Bivalvia: Unionidae). Of the 297 freshwater mussels (also called unionids, bivalves, and/or clams) reported from the United States and Canada (Turgeon et al. 1988), 213 taxa were considered threatened, endangered, or of special concern by state and/or federal agencies (Williams et al. 1992). Of these, 19 species were known to be extinct (Bogan 1993). Only 70 were considered stable in their current status (Williams et al. 1993). Stream modification, pollution, siltation, introduction of nonindigenous species, loss of obligate fish hosts, and resulting habitat and resource destruction were all factors proposed as causes for the current endangerment of the fauna (Bogan 1993). Researchers warned of an extinction crisis unless prompt conservation efforts were implemented (Williams et al. 1992).

Historically, Kentucky's 103 reported unionid taxa was exceeded only by Alabama's 170 (Cicerello et al. 1991) and Tennessee's 155 taxa (Starnes and Bogan 1988, Parmalee and Bogan 1998). Freshwater environments in Kentucky also supported a very diverse fish fauna, with more than 230 native taxa reported, and again with only the faunas of Tennessee and Alabama known to have had more (Burr and Warren 1986). Thirty-six of Kentucky's unionids were listed as endangered, threatened, or of special concern by the United States Fish and Wildlife Service [USFWS] (1990) and/or the Kentucky State Nature Preserves Commission [KSNPC], and only 48 taxa were considered stable in their current distribution (KSNPC 1996). As a result of

anthropogenic factors altering natural habitats of unionids, 19 taxa were considered extinct or were probably extirpated from the state. Most of the extinct or extirpated animals in Kentucky were unionids and fish (KSNPC 1996). This indicated drastic declines in the integrity of aquatic ecosystems. Impoundments and channelization used to improve navigation or flood control have affected the flow regimes and physical integrity of virtually every large river ecosystem in the state (United States Army Corps of Engineers [USACE] 1993 *in* KSNPC 1996). Virtually all small riverine environments were also physically or chemically disturbed (KSNPC 1996). These trends showed that Kentucky's unionid fauna was probably the most endangered group of organisms in the state (Cicerello et al. 1991).

The Licking River, a major tributary to the Ohio River, historically supported 53 of Kentucky's known unionid taxa (Cicerello et al. 1991), evident from 314 collections at 172 collecting sites within the drainage (Laudermilk 1993). Of these, 25 taxa (47%) were afforded some conservation status, and were considered sporadic and rare, or were known from less than three drainage records. Recent collections (post-1979) indicated that 11 taxa (21%) may have already been extirpated from the drainage, and species richness appeared to have decreased at all investigated river stretches. Laudermilk (1993) concluded that 42 unionid taxa were still extant in the Licking River basin.

The mussel assemblage in the Licking River at the State Route (SR) 177 bridge crossing in Butler, Pendleton County, Kentucky had been thoroughly surveyed. More than 25 collections have been made since 1944, and they documented the presence of 36 taxa (Table 1) (Laudermilk 1993, McMurray 1997). Four of these taxa (Cyrogenia

Table 1. Unionid species previously documented from the Licking River at the State Route 177 crossing in Butler, Pendleton County, Kentucky. (LE = Federally Endangered Species; LT = Federally Threatened; E = State Endangered; T = State Threatened).

<u>Actinonaias l. ligamentina</u>	<u>Obliquaria reflexa</u>
<u>Alasmidonta marginata</u> (T)	<u>Obovaria subrotunda</u>
<u>Amblema p. plicata</u>	<u>Pleurobema clava</u> (LE, E)*
<u>Cyclonaias tuberculata</u>	<u>Pleurobema plenum</u> (LE, E)*
<u>Cyprogenia stegaria</u> (LE, E)	<u>Pleurobema pyrimidatum</u> (E)*
<u>Ellipsaria lineolata</u>	<u>Potamilus alatus</u>
<u>Elliptio dilatata</u>	<u>Potamilus ohioensis</u> *
<u>Epioblasma t. torulosa</u> (LE, E)*	<u>Ptychobranchus fasciolaris</u>
<u>Fusconaia flava</u>	<u>Pyganodon g. grandis</u>
<u>Lampsilis cardium</u>	<u>Quadrula c. cylindrica</u> (LT, T)*
<u>Lampsilis fasciola</u>	<u>Quadrula metanevra</u>
<u>Lampsilis siliquoidea</u>	<u>Quadrula nodulata</u>
<u>Lampsilis teres</u>	<u>Quadrula pustulosa</u>
<u>Lasmigona c. complanata</u>	<u>Quadrula quadrula</u>
<u>Lasmigona costata</u>	<u>Strophitus undulatus</u>
<u>Leptodea fragilis</u>	<u>Tritogonia verrucosa</u>
<u>Ligumia recta</u>	<u>Truncilla donaciformis</u>
<u>Megalonaias nervosa</u>	<u>Truncilla truncata</u>

\*Possibly extirpated from Licking River drainage.

stegaria, Pleurobema clava, P. plenum, and P. pyrimidatum) were federally endangered, while two others (Quadrula c. cylindrica and Alasmidonta marginata) had threatened status with the state (KSNPC 1996). Of these rare taxa, all except C. stegaria and A. marginata were probably extirpated from the Licking River drainage. In addition, Potamilus ohioensis was probably also extirpated. The only historic occurrence of E. t. torulosa within the drainage was reported from this site (Laudermilk 1993), and Bogan (1993) reported this species as extinct.

McMurray (1997) suggested that reproduction in the mussel community at Butler had drastically declined. Only 14 glochidia (mussel larvae) were retrieved from one drift net sample (26 August 1995), out of five samples, taken from July to October 1995. In addition, none of the 307 fishes collected during this period were encysted with glochidia. It indicated a breakdown in a very important part of the life history of unionids living at this site. Reproduction in nearly all unionids involves the release of larvae known as glochidia from the female. Subsequently, they are obligate parasites encysted on the scales, fins, or gill filaments of a host fish, where they metamorphose into a juvenile before dropping into the substrate (Howard and Anson 1922, Neves et al. 1985, McMahan 1991). In a healthy reproductive community like this was assumed to have been, it would be expected that some fish would have been encysted with glochidia. McMurray (1997) compared reproductive characteristics of the mussels at Butler with another mussel community in the Licking River at Moores Ferry, near Farmers, Kentucky, a community in which Kane (1990) and Smathers (1990) concluded there was no evidence of juvenile recruitment. Smathers (1990) showed that the community at

Moore's Ferry was made up of large individuals. No recent recruitment was evident, because no juvenile unionids (<20 mm shell length) were found. Her findings resulted from an extensive community analysis, which consisted of complete substrate excavation that quantified ecological characteristics and provided relative size distributions for all species. While studying the feeding habits of muskrats (*Ondatra zibethicus*) in the vicinity of Moore's Ferry, Kane (1990) also found no evidence of recent recruitment. He found no juvenile unionids in the middens of muskrats, which are predators of freshwater mussels.

Although these previous studies explored different facets of the ecology of the mussel community at Moore's Ferry, they had similar conclusions. The cause for the lack of reproduction and recruitment in these studies at Moore's Ferry was attributed directly or indirectly to temperature fluctuations produced by hypolimnetic discharge from the dam forming Cave Run Reservoir, which was located approximately 35 km upstream. The effects of the dam were believed to have been ameliorated at Butler, because the location was approximately 194 km downstream of the dam (McMurray 1997).

In addition to the dam, nonpoint source pollution has been identified as a problem potentially affecting unionid communities, as well as the ecology of the Licking River in general. The land use activities in the immediate study area revolved around agriculture, but there was some urbanization in the surrounding area due to the small city of Butler, Kentucky, and the proximity of the site to Falmouth, Kentucky, which was located upstream at the confluence of the Licking River and the South Fork Licking River. As a result of poor land use practices, portions of the river have carried a heavy silt load

(Harker et al. 1979). Much of the lower half of the mainstream below Cave Run Lake, as well as the North and South Forks of the Licking River, have been subjected to excessive siltation from poor agricultural practices as well as sewage pollution (Burr and Warren 1986). Agricultural and silvicultural practices have greatly modified the slopes of the Eden Shale Belt Subsection through soil erosion (Bryant 1981). Such modification has led to an increase in sediment introduction and deposition in many areas of the river. Houp and Smathers (1995) cited habitat alterations from sedimentation as possible reasons for the decline of mussel species in the Rockcastle River at Billows, Kentucky. Vannote and Minshall (1982) suggested an influx of sediment was responsible for a shift in species dominance in the Salmon River Canyon, Idaho. Varying depths of sand and silt overlays caused death in three Mississippi River species in a laboratory study on the effects of silt and sand sedimentation on freshwater mussels (Marking and Bills 1979). Erosional sand and silt have been responsible for the decimation of many mussel populations (Stansbury 1970). Generally, excessive sediment and silt loadings are probably the greatest factors degrading the quality of aquatic ecosystems.

Regardless of cause, without reproduction or juvenile recruitment, the destiny of native mussel populations is certain doom. If most species in a mussel community are "functionally extinct," (i.e., senescent adults which have ceased reproducing), then they contribute very little to the future success of the community (Bogan 1993). Recolonization of reproductive individuals would have to be achieved from adjacent communities or through artificial introductions, if a community is going to persist at the locale.

Since little was known about the mussel community demographics at the study site, it was difficult to determine the influences that nonpoint sources such as land use practices have had on the assemblage. The demography of the populations of this community, prior to anthropogenic disturbances, would have been important data with which to compare current findings. Most previous studies were general inventories for which such data were not collected. Early mussel research focused on taxonomy, zoogeography, and life history of unionids. Only in more recent years have quantitative techniques been employed to assess unionid community attributes.

Species richness, relative abundance, density, growth, recruitment, and mortality should always be the basis for an evaluation of the condition of a mussel bed (Miller and Payne 1988). Collection of unionids in defined unit areas has been the most commonly used method for quantifying community characteristics (Gale 1975, cited in Brice and Lewis 1979). Quantitative information will be important for future modeling and monitoring of mussel communities. Quantitative data will lead to a better understanding of community dynamics. Monitoring of selected beds is probably the best investment for the limited funds provided for unionid conservation (Payne et al. 1997).

Surveys to map the boundaries of a particular unionid bed must identify where a contiguous and dense assemblage of mussels becomes less contiguous and less dense, which is often difficult to determine (Payne et al. 1997). Determining upstream, downstream, far-shore, and near-shore limits of a mussel bed is done by a systematic area survey (Isom and Gooch 1986). A combination of less rigorous qualitative sampling and more rigorous quantitative sampling has almost always been appropriate in the mapping

of a defined unionid community (Isom and Gooch 1986, Payne et al. 1997). Quantitative studies are expensive and time consuming, but total substrate excavation has been deemed the most accurate means with which to quantify community parameters (Miller and Payne 1988, Richardson 1989, Hornbach and Deneka 1996). Traditional methods of qualitative or semi-quantitative collecting often did not detect juvenile mussels (Neves and Widlak 1987, Miller and Payne 1988, Amyot and Downing 1991).

Patterns in population recruitment were observed through quantitative sampling, for example Amblema p. plicata in the Mississippi River and Fusconaia ebena in the Ohio River, and large annual variations in recruitment should be expected in dominant species such as these (Miller and Payne 1988). Such quantitative studies have provided us with knowledge of demographics, recruitment patterns, and densities of populations sampled (Smathers 1990, Miller and Payne 1993, Cicerello and Rudd 1996, Strayer et al. 1997). These data can be compared to the population parameters of the same species in different communities in order to determine what is needed to maintain a viable "healthy" population.

The objective of the present study was to determine whether recent recruitment was evident in the mussel assemblage in the Licking River at Butler, Kentucky. Specifically, collections were made to document the presence, or absence of unionid glochidia in stream drift or encysted on fish hosts, and to detect any juvenile unionids within the sampled areas. To better understand the factors that were affecting the mussel community, land use data was gathered in the watershed above the study site to substantiate the hypothesis that the practices in the watershed were producing nonpoint



source disturbances that were directly or indirectly inhibiting the normal life history events that would have sustained healthy, viable populations.

## CHAPTER II

### STUDY SITE DESCRIPTION

#### Locality Information

The mussel community for this study was located in the vicinity of the State Road (SR) 177 bridge crossing over the Licking River at Butler, Pendleton County, Kentucky. This site was approximately 13 km north-northeast of Falmouth, Kentucky (McMurray 1997). The actual dimensions of this bed were delineated during initial visits to the locality.

#### Physical Environment

The Licking River watershed drains 9,601 km<sup>2</sup> (3,707 mi<sup>2</sup>), or approximately 10% of the state on its northwesterly route of 496 km (310 mi) from its headwaters in southeastern Magoffin County, Kentucky to its confluence with the Ohio River near Covington, Kentucky (Harker et al. 1979, Burr and Warren 1986, Kornman 1989, Laudermilk 1993). All or part of 21 counties are encompassed by this drainage (Harker et al. 1979, Laudermilk 1993). Cave Run Lake, a warm oligotrophic lake (Clinger 1974), was constructed in 1974 on the mainstream Licking River, near Morehead, Kentucky, and impounds 61 km (38 mi) of the mainstream and the lower reaches of several of its tributaries (Burr and Warren 1986).

Streams of this basin have generally been characterized as upland, with moderate to high gradient, well-developed riffles and shoals, rocky substrates, and poor to moderate development of floodplains (Burr and Warren 1986). The major tributaries of the Licking River with their respective drainage areas are as follows: Fox Creek (303 km<sup>2</sup>); Grassy

Creek (311 km<sup>2</sup>); North Fork Creek (259 km<sup>2</sup>); North Fork Licking River (798 km<sup>2</sup>); Slate Creek (596 km<sup>2</sup>); South Fork Licking River (2,401 km<sup>2</sup>); and Triplett Creek (484 km<sup>2</sup>) (Harker et al. 1979).

The portion of the Licking River that flows past Butler, Pendleton County, Kentucky is found within the Eden Shale Belt Subsection of the Blue Grass Section of the Interior Low Plateaus Physiographic Province. The Blue Grass Section is a structural dome which has been reduced in elevation by erosion to about 274-305 m at the center and somewhat less at the periphery (Burr and Warren 1986). The Eden Shale Belt Subsection of the Blue Grass Section has been characterized topographically by variable, steep-sided hills and sinuous ridges separated by angular, closely-spaced, narrow valleys (Burr and Warren 1986, Harker et al. 1979). This lower downstream portion of the Licking River basin drains limestones, sandstones, and shales of the Blue Grass (Smathers 1990).

The study area was a shoal with two channels. The two channels were a result of a gravel bar below a bridge piling that extended approximately 150 m downstream, ending in a water willow (Justicea americana) island. The right downstream channel had much higher flow, more depth, and was comprised of a higher proportion of boulders, than the left downstream channel. Upstream from the bridge was a pool with a backwater area extending up the left upstream side of the channel into a large bar of water willows. The active channel upstream of the bridge was more narrow than downstream. Substrate upstream was generally composed of sand and gravel, with some cobble and boulders, but in considerable less proportions than downstream of the bridge pilings. Natural debris

such as large logs, masses of intermingled tree branches, and leaf litter was common in this stretch of river, along with tires and other domestic garbage.

Substrate at the immediate study site consisted mainly of cobble and boulders, with large amounts of intermixed sand and gravel, especially in the riffle areas. Sand was abundant and intermixed with the cobble and large boulders along the stream margins, forming sand and cobble beaches. A sandy depositional run extended downstream of the island of water willows below the bridge.

**CHAPTER III**  
**MATERIALS AND METHODS**  
**FIELD METHODS**

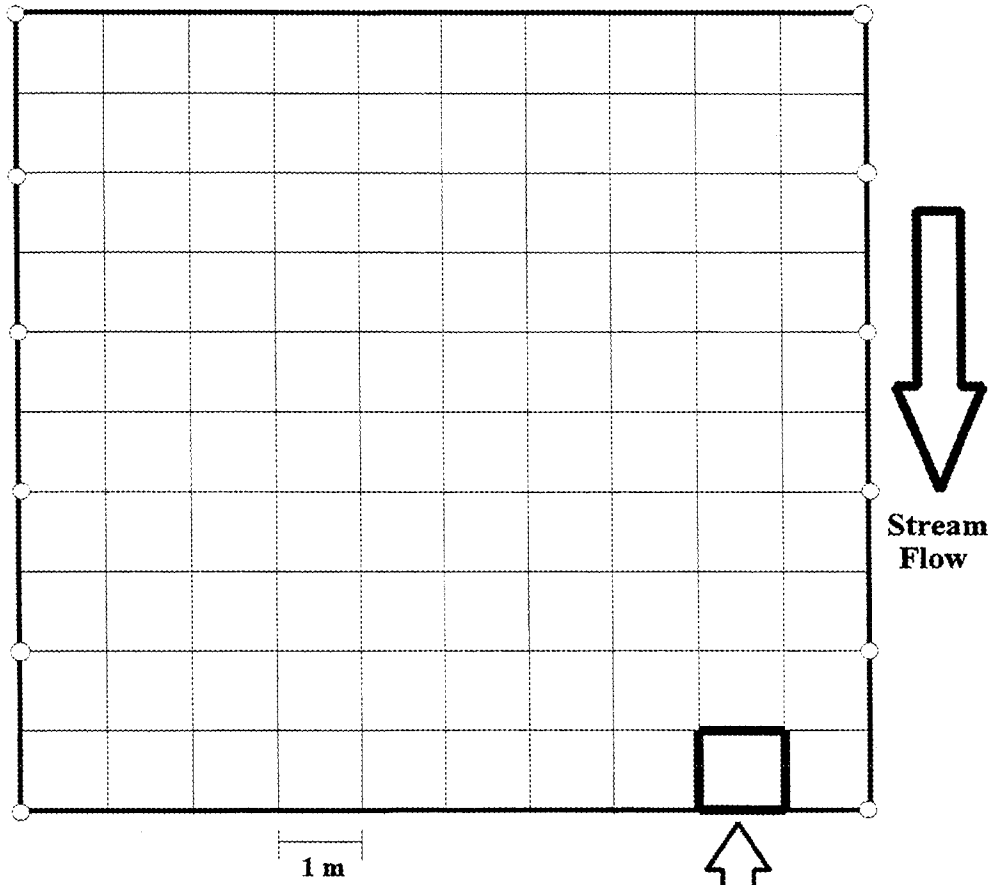
**Unionid Sampling**

Reconnaissance searches were carried out above and below the SR 177 bridge, with use of water scopes, snorkels, or by grubbing through the substrate, to provide an idea of the dimensions of this mussel bed, and to detect the presence of all extant species. Following these initial searches, two 100 m<sup>2</sup> grids were established to identify locations for quantitative sampling. A "chain ladder," consisting of ten successive 1 m<sup>2</sup> plots, was used to span the transects established within the grid, insuring that the defined area to be randomly sampled was precisely located along each transect. The transects were situated perpendicular to river flow (Figure 1)(Richardson 1989, Cicerello and Rudd 1996).

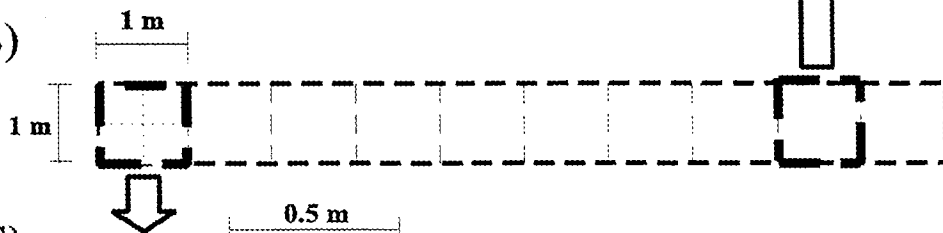
The iron stakes at each corner and along the lateral margins of the grid kept the chain ladder in place while sampling. Once the chain ladder was in place, numbered quadrats were selected within the transect (see Figure 1). Quadrat numbers were selected from a random numbers table. At least 15 m<sup>2</sup> of all 400 -- 0.25 m<sup>2</sup> quadrats within the grid were sampled (R. Cicerello, KSNPC, pers. comm.). After the selection of a particular sampling quadrat, a 0.25 m<sup>2</sup> PVC quadrat frame was placed on the surface of the substrate for sampling (Miller and Payne 1988, Miller and Payne 1992, Miller and Payne 1993, Hornbach and Deneka 1996, Vaughn et al. 1997, Strayer et al. 1997, Miller et al. 1997). The quadrat was first searched for larger, more visible unionids, and then

Figure 1. Area markers that were used to quantify various aspects of the unionid community at Butler, KY. *A)* the 100-m<sup>2</sup> sampling grid, *B)* illustration of the “chain ladder” with its 1 m<sup>2</sup> sections, and *C)* the single square meter plot within the grid network from which quadrats were located.

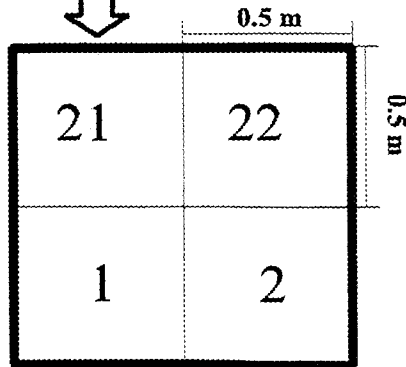
A)



B)



C)



total substratum (quantitative) sampling was carried out by removing all materials within the quadrat to a depth of 10-15 cm, or until a clay lens was encountered. The materials were then rinsed through a sieve with a 5 mm wire mesh (Cicerello and Rudd 1996). The trapped materials were searched for all unionids and Asian clams (Brice and Lewis 1979, Way and Shelton 1997).

A recording station was established on the closest dry spot (i.e., gravel bar), and all unionids collected were kept submerged in order to minimize aerial exposure and handling time, which has been shown to be detrimental to mussels (Waller et al. 1995). After various data were taken from each mussel, they were carefully returned to their original location, along with excavated materials. Each collected mussel was identified and measured. Shell length, width, and breadth were measured with a caliper to the nearest millimeter (Smathers 1990). Caliper measurements were taken twice for accuracy. Figure 2 is an example of a data sheet that was used in the field to record all mussel data. One data sheet was completed for each quadrat sampled. During the study, a single undetermined specimen had to be sacrificed and returned to laboratory for identification. Otherwise, no live mussels were sacrificed.

### **Glochidia Sampling**

To collect glochidia from the water column, drift nets were set up for one hour sampling periods, on 29 July, and 27 August, 1997. The samples were washed from collecting cups at the end of the drift nets into quart jars of 70% ethyl alcohol immediately following the one hour sampling period. All drift net samples were labeled with waterproof ink on 100% cotton bond paper. They were labeled according to the



Figure 2. A sample field data sheet used to record the physical parameters of each unionid encountered within a quadrat.



date, time of day, length of time the drift sample was collected (1 hour), average velocity of the current during sampling, and height of the water column on the vertical margins of the drift net frame. The velocity of the current was measured with an electronic current meter at two locations directly in front of the drift nets, and an average velocity of water entering each net was calculated. By measuring the width and the depth of the current, and by calculating the average velocity of the current, the approximate volume of water passing through the drift net during the collection period was calculated. Drift net samples, once preserved, were transported to the Branson Museum of Zoology for examination and curation.

### **Fish Sampling**

Fishes were collected with a minnow seine on 28 July, 9 September, and 4 October, 1997. They were collected from all possible habitats within the sampling area. Fishes were preserved following the procedures from Etnier and Starnes (1993) in a 10% buffered formalin solution. They were put into one gallon plastic collecting jars, in a loose fashion to ensure the quality of the specimens for later identification and museum curation. A label identifying location, date, and collectors was put into each collection jar.

### **Identification of Point and Nonpoint Sources**

A survey of the identifiable point and nonpoint sources of pollution was conducted for the Licking River and its major tributaries upstream from the study site. Observations were recorded while floating the mainstream of the Licking River and South Fork Licking River from the vicinity of Falmouth, down to Butler, Kentucky.

While floating, notes were taken about possible point and nonpoint sources of pollution. An intensive survey site data sheet, which was a modified site data sheet from Kentucky Division of Water (KDOW 1993), that included the accounts of surrounding environmental conditions (i.e., riparian cover, substrate types), was also completed at various points along the river.

## **DATA ANALYSIS/LABORATORY PROCEDURES**

### **Analysis of Mussel Data**

Collected data allowed for an overall community assessment, as well as assessment of individual populations. The mussels were identified, and taxonomic nomenclature was consistent with Turgeon et al. (1988), except where name changes had occurred since 1988. The length, width, and breadth measurements were used to determine the size distribution of each species, and also provided valuable evidence for recruitment. These data were used to calculate relative abundance, relative frequency, and relative volume of each unionid species within each sample. The resulting values were summed to produce an importance value for each species. The importance value was used to determine ecological dominance within the community. Additionally, size distributions, based on shell length, for the most common species encountered in the quantitative survey were graphed.

### **Analysis of Drift Net Samples for Glochidia**

A technique developed by Johnson (1995) to examine Dreissena spp. veligers by using cross-polarized light microscopy was used to analyze drift net samples for the

presence of glochidia and Corbicula fluminea juveniles. A piece of polarizing filter paper was attached under the stage of a binocular dissecting microscope. A polarizing camera lens was affixed to the nosepiece of the microscope. Light was polarized as it passed through the filter paper, and directed through a watch-glass containing the sample. The polarized light then passed through the polarizing camera lens, which was rotated until the light waves were cross-polarized. Under this light, the birefringent glochidial shell reoriented the light waves, causing the shell to glow, exhibiting a characteristic commonly known as the "Maltese Cross" (Watters 1996).

The first drift net sample (29 July 1997, 2:30P) was entirely searched to detect the presence of mussel larvae. Due to time constraints, it was decided that subsampling would be the best time investment to detect mussel larvae from the remainder of the samples. These samples (29 July 1997, 10:50A; 27 August 1997, 10:30A) were quartered with a plankton splitter, and one quarter of the original sample was searched. Any glochidia and Corbicula fluminea juveniles found using this technique were counted and retained, and the number of glochidia and C. fluminea juveniles per unit volume of stream drift was then calculated. For the subsamples, the number of glochidia and C. fluminea actually counted was extrapolated (4x) for the entire sample.

#### **Analysis and Identification of Fishes**

After the fishes were fixed in the buffered formalin solution 3-4 days, they were thoroughly rinsed with water and transferred to 70% ethyl alcohol for permanent preservation (Etnier and Starnes 1993). All fishes were identified, following the nomenclature of Page and Burr (1991), except where known name changes had occurred,

and all possible host fish were noted. The fishes collected were compared with the fish host list from Watters (1994) and other pertinent literature to determine the presence/absence of known fish hosts for unionids. Following identification, all fishes were examined under a dissecting microscope (10 -40X) to detect any glochidia. The fins and scales of every fish were examined for the presence of glochidial encystment. Gill filaments of each fish were also examined for glochidia by removing the opercular flaps (Bruenderman and Neves 1993, McMurray 1997). All collected specimens were deposited in the Branley A. Branson Museum of Zoology at Eastern Kentucky University.

#### **Land Usage Analysis**

In addition to surveying the river for identifiable nonpoint sources of pollution, literature concerning the land uses in the surrounding areas [i.e., Licking River Basin Status Report (KDOW 1998)] were researched to obtain the most up-to-date land usage information available. This information helped provide a better understanding of the most dominant land use practices occurring in the drainage immediately upstream of the Butler mussel community.

## CHAPTER IV

### RESULTS AND DISCUSSION

#### **Mussel Community**

Previous studies have found 36 unionid taxa from the Butler mussel bed. Qualitative and quantitative methods in this study found 27 species of unionids currently living in the study area (Table 2). The introduced Asian clam, Corbicula fluminea was also found in great abundance. None of the six possibly extirpated species (Laudermilk 1993) were encountered alive. Of these taxa, only two badly eroded, subfossil valves of the clubshell, Pleurobema clava, were encountered. Also, no relics of any of these species were found through qualitative searches upstream of Butler to the confluence of the South Fork Licking River. It is likely that these species have, indeed, been extirpated from the watershed. As for the remaining three species previously known to reside here, two (Lampsilis teres and Ligumia recta) were represented only by single weather-dried valves, and the other, Ellipsaria lineolata, was represented only much further upstream by a single living juvenile. Prior to the current survey (1995), however, two living specimens of Ligumia recta were found in the vicinity through quantitative surveys (J. Layzer, Tennessee Cooperative Fisheries Unit, unpubl. data).

These latter two species (Ellipsaria lineolata and Ligumia recta) were noted, since this lower stretch of the Licking River is free-flowing with no manmade dams or artificial impoundments hindering either drift of glochidia or migrations of potential fish hosts for these species. Laudermilk (1993) reported 42 taxa known from the study area

Table 2. Unionid species from qualitative and quantitative collections in the Licking River at Butler, KY, in 1997 and 1998. (WD = Weather-dried shells only; SF = Sub-fossil shells only).

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<u>Actinonaias l. ligamentina</u>	<u>Megalonaias nervosa</u>
<u>Alasmidonta marginata</u>	<u>Obliquaria reflexa</u>
<u>Amblema p. plicata</u>	<u>Obovaria subrotunda</u>
<u>Cyclonaias tuberculata</u>	<u>Pleurobema clava (SF)</u>
<u>Cyprogenia stegaria</u>	<u>Potamilus alatus</u>
<u>Elliptio dilatata</u>	<u>Ptychobranhus fasciolaris</u>
<u>Fusconaia flava</u>	<u>Pyganodon g. grandis</u>
<u>Lampsilis cardium</u>	<u>Quadrula metanevra</u>
<u>Lampsilis fasciola</u>	<u>Quadrula nodulata</u>
<u>Lampsilis siliquoidea</u>	<u>Quadrula pustulosa</u>
<u>Lampsilis teres (WD)</u>	<u>Quadrula quadrula</u>
<u>Lasmigona c. complanata</u>	<u>Strophitus undulatus</u>
<u>Lasmigona costata</u>	<u>Tritogonia verrucosa</u>
<u>Leptodea fragilis</u>	<u>Truncilla donaciformis</u>
<u>Ligumia recta (WD)</u>	<u>Truncilla truncata</u>

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downstream to the confluence with the Ohio River, and 38 taxa from this site upstream to the confluence of the South Fork Licking River at Falmouth, Kentucky. All of these species recorded by Laudermilk (1993) have potential to inhabit the area, since they have been documented from upstream or downstream locations, even if they've never been identified from the study site. For example, McMurray (1997) reported the first record of Alasmidonta marginata at the SR 177 bridge at Butler, but it had been previously recorded only from the adjacent upstream and downstream sites (Laudermilk 1993), so it is possible that this species may have inhabited this area before, but was just not detected. For this reason, long-term monitoring should be implemented in species rich assemblages such as this, to detect all species that inhabit the area over a span of time, as well as to detect population fluctuations within the community.

Qualitative and quantitative surveys have resulted in the discovery of juveniles of eight species of unionids, including two juveniles of the state and federally endangered fanshell, Cyprogenia stegaria (Table 3). The only other species with multiple juvenile specimens collected were the spike, Elliptio dilatata, and the purple wartyback, Cyclonaias tuberculata, with four and three juveniles, respectively. A single juvenile was collected for each of the other species. Although substrate excavation has been deemed the most efficient method by which to detect juvenile presence, a need remains for the supplemental qualitative survey. During qualitative searches at this site, juveniles of three additional species were encountered that were not represented by juveniles in the quantitatively sampled areas.

Table 3. Species with number of juvenile representatives from the Licking River at Butler, Kentucky, in 1997 and 1998.

Species	Common Name
<u>Actinonaias l. ligamentina</u> (1)	Mucket
<u>Cyclonaias tuberculata</u> (3)	Purple wartyback
<u>Cyprogenia stegaria</u> (2)	Fanshell
<u>Elliptio dilata</u> (4)	Spike
<u>Leptodea fragilis</u> (1)	Fragile papershell
<u>Obliquaria reflexa</u> (1)	Threehorn wartyback
<u>Quadrula metanevra</u> (1)	Monkeyface
<u>Quadrula pustulosa</u> (1)	Pimpleback

There was a total bivalve density of 23.27 individuals / m<sup>2</sup>; however, the exotic Asian clam, Corbicula fluminea, accounted for most of this number, comprising over 81% of the collection. Quantitative sampling yielded 130 individual unionids, representing 17 species, and a total unionid density of approximately 4.33 unionids / m<sup>2</sup>. This is in comparison to 568 individual Asian clams in the same sampling quadrats, with a density of 18.93 / m<sup>2</sup>. Smathers (1990) also found Asian clams dominated the bivalve fauna in the Licking River at Moores Ferry; however, the total bivalve density of 300.66 / m<sup>2</sup> was much greater than the density of bivalves in the current study. Unionid density in the survey at Moores Ferry was almost two times greater, with 7.98 unionids / m<sup>2</sup>. Previous quantitative collections at Butler also revealed higher unionid densities than were found in the current study, with 27.36, 19.84, and 16.80 unionids / m<sup>2</sup>, respectively, in 1994, '95, and '96 (J. Layzer, Tennessee Cooperative Fisheries Unit, unpubl. data). This shows a continuous decrease in unionid density over these three surveys, which continues through the current survey. Although the previous quantitative assessments at Butler were not conducted in the same location within the channel as the current survey (e.g., further downstream), there were few differences between the species lists of the two surveys. The reason for the decline in unionid density at Butler is unknown. It is possible that the higher unionid densities reported in 1994, '95, and '96 were due to the sampling taking place in a different spot in the river.

Miller and Payne (1988) defined a mussel bed as a "contiguous area of stable substratum where densities were at least 10 individuals per m<sup>2</sup>;" the density found in this survey were was much less than this prediction, as was the density found by Smathers

(1990), at Moores Ferry. Based on the findings of Layzer (unpubl. data), a mussel "bed" once existed in the vicinity of the SR 177 bridge. By the definition of Miller and Payne (1988), however, the area surveyed in the current study would not be considered a mussel "bed."

Species collected in the quantitative surveys and their importance values are shown in Table 4. Calculations showed the spike, E. dilatata, to have the highest importance value (1.317). This species also had the highest abundance (64) of all species found in the quantitatively sampled areas. The next most abundant species within the grids was the purple wartyback, Cyclonaias tuberculata (15), which also had the second-highest importance value (0.388). These two species were also the most abundant species encountered in qualitative surveys. Miller et al. (1992) predicted a population could remain reproductively viable with only two to three individuals per 100 m<sup>2</sup>. According to this prediction, every species encountered in the quantitative survey would be considered reproductively viable, since even those species with the lowest densities were calculated to have at least three individuals per 100 m<sup>2</sup>.

The length-frequency distributions (histograms) of the most abundant species collected from the quantitative survey are illustrated in the Appendix to depict relative size/age class distribution. The spike, E. dilatata had a distribution ranging from young to old, with most of the individuals falling into the mid-range. As illustrated, the other species were generally composed of older individuals; however, all species presented in the Appendix had juvenile representatives. It is interesting to note that the most common species encountered in the quantitative surveys were the only species to have juvenile

Table 4. Importance values for unionids collected during quantitative surveys in the Licking River at Butler, Kentucky, in 1997 and 1998.

Species	Common Name	Abundance	Density	Importance Value*
<u>Actinonaias ligamentina</u>	Mucket	2	0.07	0.077
<u>Amblema p. plicata</u>	Threeridge	2	0.07	0.082
<u>Cyclonaias tuberculata</u>	Purple wartyback	15	0.27	0.388
<u>Cyprogenia stegaria</u>	Fanshell	8	0.50	0.160
<u>Elliptio dilatata</u>	Spike	64	2.13	1.317
<u>Lampsilis cardium</u>	Pocketbook	5	0.17	0.179
<u>Lampsilis complanata</u>	Elephantear	1	0.03	0.039
<u>Lasmigona costata</u>	Flutedshell	3	0.10	0.077
<u>Leptodea fragilis</u>	Fragile papershell	2	0.07	0.040
<u>Megalonaias nervosa</u>	Washboard	3	0.10	0.119
<u>Obliquaria reflexa</u>	Threehorn wartyback	9	0.30	0.178
<u>Ptychobranthus fasciolaris</u>	Kidneyshell	1	0.03	0.024
<u>Quadrula metanevra</u>	Monkeyface	1	0.03	0.031
<u>Quadrula nodulata</u>	Wartyback	1	0.03	0.023
<u>Quadrula pustulosa</u>	Pimpleback	9	0.30	0.194
<u>Truncilla donaciformis</u>	Fawnsfoot	3	0.10	0.054
<u>Truncilla truncata</u>	Deersfoot	1	0.03	0.018
Total Unionid Abundance and Density		130	4.33	
<u>Corbicula fluminea</u>	Asian clam	568	18.93	

\* Importance Value = Relative Abundance + Relative Frequency + Relative Volume

representatives. The other juvenile specimens were encountered during the qualitative searches, and these species generally had very low abundance in the quantitatively sampled areas. The length-frequency distributions of the other species in quantitatively sampled areas were not presented due to their relatively low abundances, since an adequate histogram could not be produced for such low numbers.

### **Glochidia in Stream Drift**

Cross-polarized microscopic analysis of drift net samples yielded 159 unionid glochidia and 434 juvenile Asian clams out of approximately 730 m<sup>3</sup> of stream drift (Table 5). All glochidia were collected from two samples on 29 July 1997; no glochidia and 60 juvenile Asian clams were collected on 27 August 1997. Most of the unionid glochidia (140) and juvenile Asian clams (272) were collected in the morning sample (10:50 AM) on 29 July 1997; the afternoon sample on that same day (2:30 PM) yielded only 19 glochidia and 102 juvenile Asian clams. These times of day corresponded to the documented period when glochidial densities should have been their highest (10:00 AM - 5:00 PM) (Kitchell 1985). The August sample was also in the late morning (10:30 AM), and yielded no glochidia. Previous drift net collections yielded only 14 unionid glochidia from one sample (26 August 1995) out of five analyzed from July through October 1995 (McMurray 1997). This date corresponded to the second collecting period from the current study that yielded no glochidia. It is probable that the very small sampling net in respect to the river's width would miss glochidia being expelled into the water column. The average number of glochidia collected per unit volume of stream drift was 0.22 / m<sup>3</sup>,

Table 5. Results of drift net samples taken in the Licking River at Butler, Kentucky, in 1997.

Taxa	29 July		27 August	Totals
	10:50A*	2:30P	10:30A*	
Unionidae spp.	140	19	0	159
<u>Corbicula fluminea</u>	272	102	60	434
Water Volume Sampled (m <sup>3</sup> )	463.45	142.42	122.71	728.48
Average # Unionidae glochidia / m <sup>3</sup>	0.30	0.13	0	0.22
Average # <u>C. fluminea</u> juveniles / m <sup>3</sup>	0.59	0.72	0.49	0.60

\* Representative samples were quartered for subsampling, and actual numbers were multiplied (4x) to calculate listed totals.

and the average number of Asian clams was 0.60 / m<sup>3</sup>, from the three collections combined.

### **Encystment of Host Fish**

A total of 545 fishes were identified from the three collections. Eight families were represented by 34 species (Table 6). Through comparisons with previous literature from Watters (1994), 178 individuals (32.7%), representing 16 species had been documented as suitable hosts for unionid species present (termed resident species) at this locality (Table 6). Eight of the other species, represented by 202 individuals (37.1%) had been documented as suitable fish hosts for other unionid species not known (termed non-resident species) from this site, and the remaining 165 individuals (30.3%), representing 10 species had never been documented as host fish. Numerically, the fish assemblage was dominated by minnows (Family Cyprinidae: 70%). Darters (Family Percidae: 21%) also comprised a fairly large proportion of the fish fauna.

Specimens from eight species of fish had encysted glochidia (Table 7). Cyprinids had the greatest number of confirmed hosts, with 39 of 47 individuals (83%) with glochidia. The emerald shiner, Notropis atherinoides, was the most abundant fish from the three collections, and had the highest number of encysted individuals (27). Previously, this species was only known to be a host for non-resident unionids (Watters1994). Approximately 21% of individuals from all collections of this species were found to have glochidia. This percentage of infestation was exceeded only by the infestation percentage of Micropterus dolomieu and Etheostoma n. nigrum, each with half (50%) of its individuals encysted. These two species were represented only by six



Table 6. Fish species (number of individuals) collected from the Licking River at Butler, KY, from three collections, July – October, 1997, and documented fish host suitability.

Lepisosteidae	Centrarchidae
<u>Lepisosteus osseus</u> (2) <sup>b</sup>	<u>Ambloplites rupestris</u> (1) <sup>b</sup>
Clupeidae	<u>Lepomis macrochirus</u> (18) <sup>b</sup>
<u>Alosa chrysochloris</u> (1) <sup>b</sup>	<u>Lepomis megalotis</u> (3) <sup>b</sup>
Cyprinidae	<u>Micropterus dolomieu</u> (6) <sup>b</sup>
<u>Campostoma anomalum</u> (18) <sup>b</sup>	<u>Micropterus punctulatus</u> (1) <sup>a</sup>
<u>Cyprinella spiloptera</u> (77) <sup>bc</sup>	<u>Micropterus salmoides</u> (1) <sup>bc</sup>
<u>Cyprinella whipplei</u> (100) <sup>c</sup>	Percidae
<u>Semotilus atromaculatus</u> (4) <sup>b</sup>	<u>Ammocrypta pellucida</u> (3)
<u>Ericymba buccata</u> (2)	<u>Etheostoma blennioides</u> (7) <sup>ac</sup>
<u>Notropis atherinoides</u> (127) <sup>ac</sup>	<u>Etheostoma caeruleum</u> (8) <sup>b</sup>
<u>Notropis rubellus</u> (20) <sup>a</sup>	<u>Etheostoma camurum</u> (1)
<u>Notropis volucellus</u> (5)	<u>Etheostoma flabellare</u> (5) <sup>a</sup>
<u>Pimephales notatus</u> (27) <sup>bc</sup>	<u>Etheostoma n. nigrum</u> (2) <sup>bc</sup>
Catastomidae	<u>Etheostoma tippecanoe</u> (45) <sup>c</sup>
<u>Carpionodes carpio</u> (2) <sup>b</sup>	<u>Etheostoma zonale</u> (36) <sup>a</sup>
<u>Moxostoma anisurum</u> (1)	<u>Percina copelandi</u> (1) <sup>a</sup>
Ictaluridae	<u>Percina oxvrhyncha</u> (5) <sup>a</sup>
<u>Ictalurus punctatus</u> (7) <sup>b</sup>	Sciaenidae
<u>Noturus eleutherus</u> (1)	<u>Aplodinotus grunniens</u> (1) <sup>b</sup>
<u>Noturus flavus</u> (5)	
<u>Noturus stigmosus</u> (2)	

<sup>a</sup> Documented hosts for nonresident unionid species (Watters 1994)

<sup>b</sup> Documented hosts for resident unionid species at this site (Watters 1994)

<sup>c</sup> Documented hosts from present study.

Table 7. Individual fish hosts and prevalence of glochidial encystment from three collections in the Licking River at Butler, KY, July – October, 1997.

Species	Number Examined	Number Encysted	Percent Encystment
<b>Cyprinidae</b>			
Emerald shiner ( <u>Notropis atherinoides</u> )	127	27	21
Steelcolor shiner ( <u>Cyprinella whipplei</u> )	100	10	10
Spotfin shiner ( <u>C. spiloptera</u> )	77	1	1
Bluntnose minnow ( <u>Pimephales notatus</u> )	27	1	4
<b>Percidae</b>			
Tippecanoe darter ( <u>Etheostoma tippecanoe</u> )	45	3	7
Greenside darter ( <u>E. blennioides</u> )	7	1	14
Johnny darter ( <u>E. n. nigrum</u> )	2	1	50
<b>Centrarchidae</b>			
Smallmouth bass ( <u>Micropterus dolomieu</u> )	6	3	50
Total	391	47	12

and two individuals, respectively, from all collections. The steelcolor shiner, Cyprinella whipplei, was the second most abundant fish collected (100), and it also had the second highest number of encysted individuals (10). This species was never before documented as a host for any unionid species. The remaining host cyprinids (C. spiloptera and Pimephales notatus) each had a single encysted glochidia, and were previously documented as hosts for resident unionids (Watters 1994). The only additional fish that had multiple individuals encysted was Etheostoma tippecanoe, a species that has never been documented as a host for any unionid species.

The eight species of fish with encysted glochidia comprised nearly 72% of all fishes collected, indicating high host specificity (Table 8). The July and September collections had the highest rate of encystment, each having nearly 10% of the individuals with glochidia. In July, the four species with encysted glochidia comprised nearly 83% of the collection. In September, the six species with encysted glochidia comprised nearly 71% of the collection. The October collection had the lowest incidence of encystment, approaching 7%, with the only two species with encysted individuals comprising about 65% of the collection.

Because the two most abundant species (N. atherinoides and C. whipplei) had high percentages of individuals infested with glochidia on multiple sampling dates, host specificity was again evident. However, some of the other 25 species of fishes that were also relatively abundant were not infested, such as Campostoma anomalum, Lepomis macrochirus, and Etheostoma zonale. Campostoma anomalum and L. macrochirus have been documented as hosts for resident unionid species, whereas E. zonale is not a known

Table 8. Species confirmed as fish hosts from the present study, with percent composition (%) of encysted individuals reported from number sampled (n) by sampling date, from the Licking River in 1997.

Confirmed host species	28 July		9 Sept.		4 Oct.		Total	
	(n)	(%)	(n)	(%)	(n)	(%)	(n)	(%)
<u>Cyprinella spiloptera</u>	20	5	35	--	22	--	77	1.3
<u>C. whipplei</u>	53	15.1	17	11.8	30	--	100	10
<u>Notropis atherinoides</u>	8	12.5	69	23.2	50	20	127	21.3
<u>Pimephales notatus</u>	0	--	12	8.3	15	--	27	3.7
<u>Micropterus dolomieu</u>	1	--	2	--	3	100	6	50
<u>Etheostoma blennioides</u>	0	--	3	33.3	4	--	7	14.3
<u>E. n. nigrum</u>	0	--	1	100	1	--	2	50
<u>E. tippecanoe</u>	31	9.7	13	--	1	--	45	6.7
Total	113	11.5	152	13.8	126	10.3	391	12
% all encysted individuals	9.5		9.8		6.7		8.6	
% confirmed host species	82.5		70.7		65.3		71.7	

host for any unionid species (Watters 1994). Similar findings were reported by Neves and Widlak (1988), where Hypentelium nigricans and E. zonale were very common unencysted fish, and on various sampling dates, many of the other abundant fish species were frequently encysted. Since some of the most common fish have never been shown to be hosts, it is sufficient to assume that host specificity is not merely determined on percent composition of individuals, rather it must be determined by some other factor, such as fish immunity or the incompatibility of the fish to harbor glochidia.

The findings from this study were analyzed and compared with those of Neves and Widlak (1988), in which the encystment occurrence of amblemine glochidia was highest in July; however, they noted that encystments declined in August, and none were seen after late August. Also, their rates of encystment were 29% in July, and dropped to 8% in August. This could have suggested that the rate of amblemine encystment for the fishes in the Licking River were lower than those in the Holston River study area; however, the July collection in the present study was late in the month (28 July), so many of the encysted glochidia could have fallen off of their respective fish hosts by the time of collection. With no fish host data from June, though, this was mere speculation. Also, lampsiline glochidia had lower rates of encystment than amblemine glochidia in July and August, but were the only glochidia detected in October and through the winter (Neves and Widlak 1988), which may have suggested that the glochidia found in October in the current study were lampsiline. In the Holston River study, amblemine glochidia were found only on cyprinids, while lampsiline glochidia were found on percids, centrarchids, and cottids, indicative of high host specificity (Neves and Widlak 1988). In a similar

study in Big Moccasin Creek, a tributary in the Houston River basin, Zale and Neves (1982) also found lampsiline glochidia infesting members of these three families, and none encysted on any of the cyprinids.

Examination of the fishes yielded 241 glochidia encysted on either gills or fins of the 47 individual fish, for an average of 5.13 glochidia per infected fish. Encysted representatives of *N. atherinoides* harbored the largest number of glochidia, bearing 195 unionid larvae, although the average number of glochidia per infested specimen (7.22) was skewed by one individual, collected on 28 July, which had 105 glochidia encysted in its gills. In previous population studies, an average of less than 10 glochidia per infested fish was typical (Coker et al. 1921, Trdan 1981).

### **Land Use Influences**

Anthropogenic factors, such as increased siltation and industrial and domestic pollution have contributed to rapid declines in the aquatic diversity of North America. We must better understand the effects of such factors on our biota, if the causative practices are allowed to persist. As a result of poor land use practices, portions of the Licking River have carried a heavy silt load (Harker et al. 1979). In a study of Illinois streams, Smith (1971) cited excessive siltation as the principal cause for the extirpation of two native species and the decimation of 14 others. Much of the lower half of the mainstream below Cave Run Lake as well as the North and South Forks of the Licking River are subject to increased siltation from poor agricultural practices and sewage pollution (Burr and Warren 1986). Agricultural and silvicultural practices also modified the slopes of the Eden Shale Belt Subsection, which encompasses much of the lower

Licking River, by soil erosion (Bryant 1981). Land use in the study area is primarily agricultural, but there is some urbanization (the towns of Butler and Falmouth).

The clearing of the land associated with agricultural and urban development leads to increased siltation, because there is a lack of vegetation to lessen the erosion caused by surface runoff (Smith 1971). Sediment has not only been shown to lead to mussel dieoff, effects include impairment of light penetration disrupting phototaxic responses and clogging of the mussels' filtration mechanisms (Fuller 1974, McMahon 1991), which they use for feeding and reproduction. Bauer (1994) reported that juvenile survival and establishment of Margaritifera margaritifera were negatively associated with phosphate loadings, and adult survival was negatively correlated with nitrate loadings. Rivers passing through open agricultural lands, such as much of the lower Licking River watershed, have been shown to have significantly higher annual mean concentrations of ammonium than rivers with forested margins (Morris and Corkum 1996).

Although the mainstream of the Licking River from its confluence with the South Fork down to Butler, Kentucky, had a riparian buffer zone present along much of its interface to the agricultural lands it drains, overnutrification was evident, especially in the South Fork Licking River at Falmouth. This was evident through massive algae mats existing the entire distance from the US 27 bridge in Falmouth down to the South Fork's confluence with the Licking River.

The entire stretch of the South Fork bordering Falmouth was heavily impacted by urbanization, and bank removal through erosion was the most evident source. Only one live mussel, the fat mucket (Lampsilis siliquoidea) was found alive in the vicinity of the

US 27 bridge in Falmouth, with many weathered and fresh dead shells in the vicinity. A low-water dam existed just upstream of the bridge crossing, which inhibits fish migrations most of the year, and may act as a sink for nutrients entering the system; however, high water events probably flush the sink and may provide downstream connectivity to fishes during parts of the year.

Heavy recreation use was noted at the confluence of the South Fork and the mainstream Licking River. A boat access ramp descended one bank and was being used by five vehicles as we passed. Such activities often provide a direct connection for sediment loads, especially when the roads/ramps are composed only of dirt. Much of the lane being used was cobble, though, which may work to lessen the amount of soil erosion from vehicle use.

Strayer (1980) found the factor determining the present state of a mussel fauna is the degree to which urban pollution has affected the stream stretch. From collections in the Clinton River in Michigan, he found that all stretches subjected to urban pollution had lost their mussel faunas, whereas most of the stretches free of urban pollution retained dense and diverse assemblages, in spite of other disturbances. Although the major influence in the Licking River basin is from agricultural usage, there is some urbanization along the river, due the cities of Butler and Falmouth. Additionally, further downstream, the lower Licking River borders highly urbanized areas (i.e., Newport and Covington, Kentucky) within the urban sprawl of Cincinnati, Ohio. The continued growth of this area in northern Kentucky will inevitably influence the lands draining to the study area, in the future.



With much of the lower Licking River watershed draining agricultural lands, nonpoint source pollution factors remain constant. The use of pesticides and fertilizers on croplands continues to compromise the chemical integrity of this freshwater system, and the presence of large numbers of livestock will compromise both the chemical and the physical integrity of the watershed.

Until more containing means of pesticide control and nutrient enrichment are contrived, toxic and/or harmful chemicals will be in constant connectivity with the system. However, the presence of a riparian buffer on all surface streams within the system should be important in maintaining the physical integrity of the watershed, as well as providing an adequate buffer for the uptake and capture of much of the foreign nutrient loads being transported from the surrounding crop and grazing lands. Limiting access for livestock to roam on stream banks and in the water will greatly enhance the watershed management effort by cutting down on streambank erosion and increasing bank stability. This will lead to a decrease of sediments and silt introduced into the system, and should provide an overall increase in the physical integrity of the managed areas.

The lower Licking River represents one of the few remaining free-flowing riverine systems in Kentucky (Laudermilk 1993). Recent flooding of the Licking River spurred a popular call for a flood control dam on the Licking River on this lower section. A priority to keep this lower stretch free-flowing has been acknowledged, so dams would not be future factors to lead to a decrease in the diversity of the remaining mussel populations.

In response to the proposed hypothesis, land use practices were not arresting the reproduction and juvenile recruitment of all species present; however, it was assumed that

resulting factors of these practices are at least indirectly affecting these life history events for many species within this diverse assemblage. Because extensive sampling techniques resulted in finding juveniles of only eight of the 27 resident species, recent recruitment among these other 19 species was assumed to be very low or not to have occurred in the area. Land use practices and their resulting influences on the watershed were hypothesized as the most likely cause inhibiting juvenile recruitment for these species. It is possible that juveniles of these species occur outside of the study area. It is recommended that the watershed be actively managed in an effort to sustain the unionid populations present, in hopes of establishing healthy, reproductive populations of all resident unionids. Mussel populations generally have had a low but prolonged rate of recruitment to maintain their densities (Neves and Widlak, 1988), so the sampling in this study may merely represent a snapshot of the actual rate of recruitment occurring in these populations. The possibility remains that the resident populations are reproductively viable, since they generally consisted of more than two individuals per 100 m<sup>2</sup>. The only means by which to determine the reproductive viability of the species in the study area is by further long-term monitoring of the area, using similar, extensive measures by which to detect the presence of juveniles.

It was evident through these analyses that at least eight species in this community had successfully recruited new members, recently, and, therefore, were considered to have had viable populations. However, the lack of juveniles for the other 19 species, as well as low numbers of many of these species indicates a lack of recruitment. This reflects a lack of viability for these populations. The possibility of these species

maintaining their presence at this site is doubtful, unless recolonization of viable members of these species is achieved from adjacent locations.

## CHAPTER V

### SUMMARY AND CONCLUSIONS

This study was undertaken to assess the current condition of the freshwater mussel (Bivalvia: Unionidae) community in the Licking River at the State Route 177 bridge crossing in Butler, Pendleton County, Kentucky. A recent study indicated a drastic decline in the reproduction occurring in this historically diverse assemblage, evident through findings of few glochidia in stream drift and the absence of glochidial encystment on any of the fishes collected. An intensive community analysis provides information on the current size demography of the resident populations, and monitors the presence of species historically known from this site. It also provides evidence of juvenile recruitment and reproduction, through presence/absence of juveniles at the site and glochidia in drift net samples or encysted upon fishes. Results from this survey will be useful for future monitoring of this community, and will provide important information that can be used in comparison with other mussel assemblages for which aspects have been quantified. Land use practices that have been or are currently being employed upstream of the proposed study site were considered to better understand the point and nonpoint source pollution factors that may be influencing community changes. It was hypothesized that upstream land use practices were contributing to a previously documented decline of reproduction at this locality.

Qualitative and quantitative searches have yielded 27 extant unionid species within the area, and resulted in the discovery of juveniles of eight species, including two juveniles of the state and federally endangered fanshell, Cyprogenia stegaria.

Quantitative sampling, consisting of substrate excavation of randomly selected 0.25 m<sup>2</sup> plots yielded 130 individual unionids, representing 17 species, at a total density of approximately 4.3 unionids / m<sup>2</sup>. Calculations showed the most abundant species, the spike, Elliptio dilatata, to have the highest importance value in the quantitatively sampled areas. Analysis of drift net samples yielded 159 unionid glochidia and 434 juvenile Corbicula fluminea, the exotic Asian clam, out of approximately 730 m<sup>3</sup> of stream drift. Examination of 545 fishes collected from the site yielded 241 glochidia encysted on either gills or fins of 47 individual fish hosts, for an average of 5.13 glochidia per infected fish. Eight species of fish were represented by hosts bearing encysted glochidia. These eight species comprised 71.74% of all fishes collected. The emerald shiner, Notropis atherinoides, was the most abundant fish from the three collections, bore the most glochidia, and had the highest prevalence of encystment. Twenty-seven emerald shiners were encysted with 195 unionid larvae; one individual was found bearing 105 glochidia encysted upon its gills.

The dominant human influence in the lower Licking River drainage is agriculture, which has led to a decrease of riparian buffer along a large proportion of the banks within the watershed, and has also allowed livestock access to the streams. The decrease of a riparian buffer and cattle access allows for more direct introduction of chemicals (i.e., pesticides and fertilizers), as well as decreases bank stability, which, in turn, leads to excessive sediment introduction. Such anthropogenic factors and large numbers of C. fluminea have likely impacted the resident mussel fauna. Although this mussel community remains diverse, the lack of juvenile representation for all but eight species

indicates a breakdown in a very important stage in the life history for the rest of the community. Recent reproduction was evident through the presence of glochidia encysted on host fishes and being broadcast into the water column; however, accurate recruitment patterns for resident populations could not be obtained without identification of the glochidia. The only definitive sign of a particular species having recently recruited new members was through the presence of juveniles in the quantitative and/or qualitative samples.

It was evident through these analyses that, recently, at least eight species in this community had successfully recruited new members, and, therefore, were considered to have had viable populations. However, the lack of juveniles for the other 19 species, as well as low numbers of many of these species, indicates a lack of recruitment. This reflects a lack of viability for these populations. The possibility of these species to maintain their presence at this site is doubtful, unless recolonization of viable members of these species is achieved from adjacent locations.

These analyses provided baseline population data for each species which can be used in future monitoring of this biologically and historically significant site. There is a great need for future monitoring of such communities, so dynamics of the community can be more definitively recorded, and fluctuations in population structures can be analyzed. Compilations of long-term monitoring efforts should allow more accurate speculation on the effects of such anthropogenic factors as point and nonpoint source pollutants. Better understanding of the impacts of these factors to natural communities should lead to better management strategies to ameliorate these impacts, conserving existing communities.

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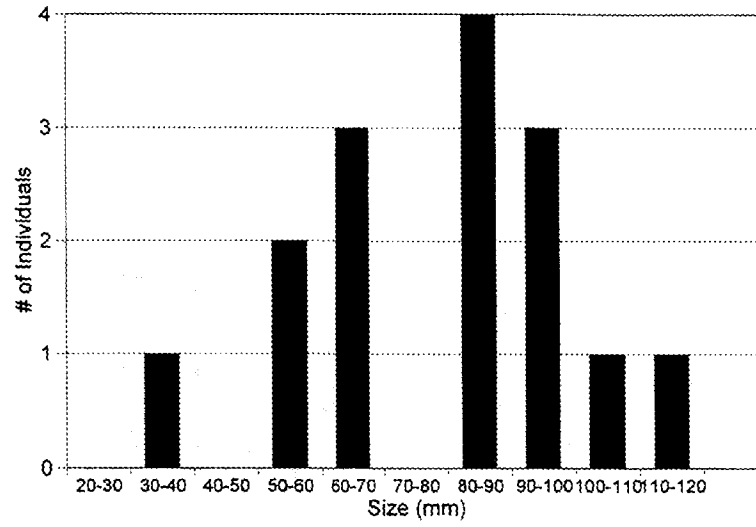
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## APPENDIX

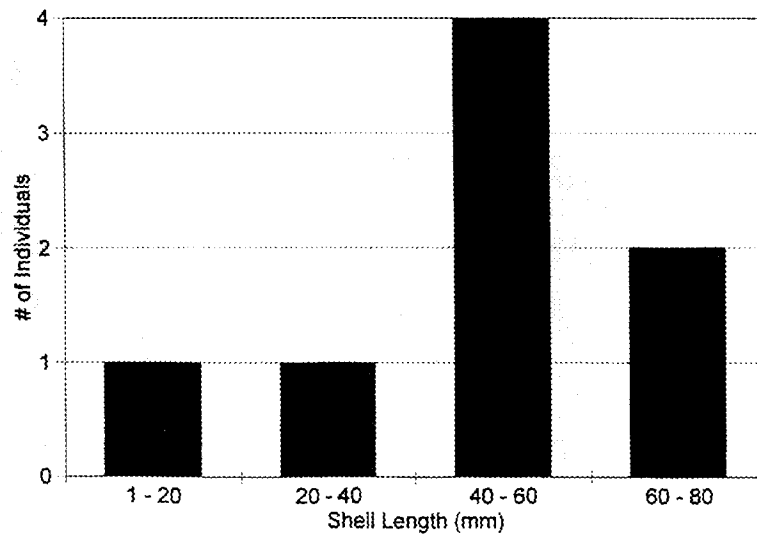
Length/size distribution of unionid populations found within quantitatively surveyed areas within the Licking River at the State Road 177 bridge in Butler, Pendleton County, Kentucky.

**Cyclonaias tuberculata**

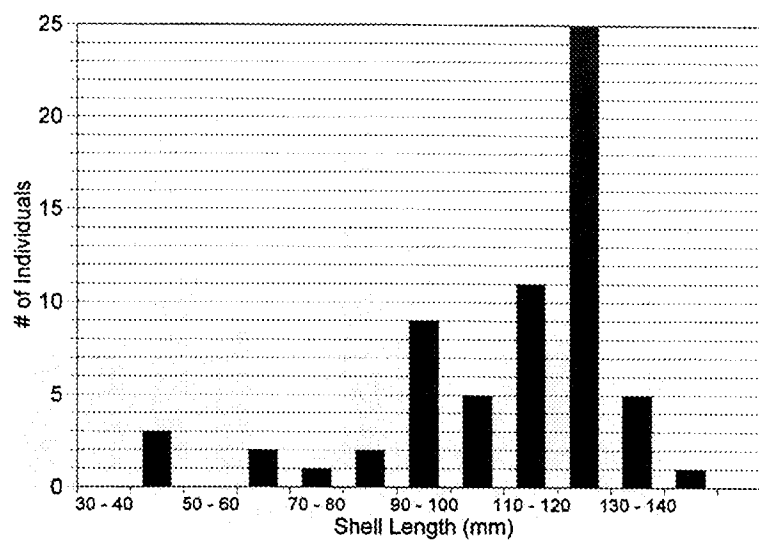
Purple wartyback

**Cyprogenia stegaria**

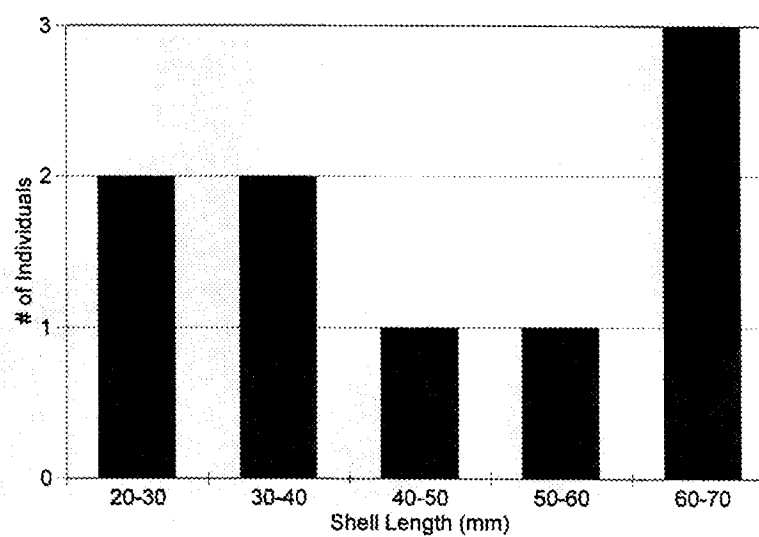
Fanshell



**Elliptio dilatata**  
Spike



**Obliquaria reflexa**  
Threehorn wartyback



Quadrula pustulosa  
Pimpleback

