

POPULATION DEMOGRAPHICS OF SIX FRESHWATER MUSSEL SPECIES  
(BIVALVIA:UNIONIDAE) IN THE UPPER CLINCH RIVER,  
VIRGINIA AND TENNESSEE

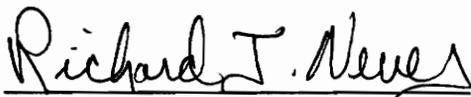
by

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(ABSTRACT)

Growth rates, age-frequency distributions, and mortality rates of six mussel species were examined at four sites in the Clinch River, Virginia and Tennessee, to identify potentially impacted sites in the upper river. The bioaccumulation of copper in mussel shells also was examined as a possible contributing factor to the declining mussel fauna.

Higher growth rates observed at Hackneys site, river kilometer (RK) 433.7, may have been due to the discharge of domestic sewage from the town of Cleveland into the Clinch River. However, a sewage treatment facility was constructed in 1986 which removed the source of enrichment. Growth rates of female *Lampsilis fasciola* were significantly less than growth rates of male *L. fasciola* after 3 years of age, probably due to the onset of sexual maturity.

Age-class distributions revealed an absence of recruited juveniles at the Slant site (RK 359.3) after 1977 to 1979 for four mussel species examined. Substantial erosion of stream banks in tributaries of the Clinch River and

deposition of sediment in the Clinch River were observed at Slant, indicating a potential cause of decline. Mean annual mortality rates of adult mussels were extraordinarily high for all species at all sites, which confirms the overall decline of freshwater mussels in the upper Clinch River.

There were no statistically significant differences in accumulation of copper in shells of *L. fasciola* among sites or sexes. Although Appalachian Power Company's Clinch River Plant (CRP) had a history of high copper levels in the effluent discharge, it is apparently not the cause of reduced recruitment at the Slant site, located 72 km downstream. As indicated by this research, water quality or habitat conditions of the upper Clinch River continue to be insufficient to sustain freshwater mussels. The CRP has improved the effluent discharge to contain less than 12  $\mu\text{g}$  Cu/L, which should improve conditions directly downstream; however, erosion of stream banks, sewage treatment facilities, and agricultural and urban runoff, continue to contribute to the demise of a rich freshwater mussel fauna.

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## Chapter One

### INTRODUCTION

The Clinch River, a tributary of the Tennessee River, holds one of the most diverse mussel faunas remaining in the world today. There are 45 species found there, of which 21 are endemic to the Cumberland region and 11 are federally listed as endangered species (Jacobson 1990). Due to this unique endemism and diverse mussel fauna, surveys of the mussels in the Clinch River and its' tributaries date back for 135 years and extensive research since the late 1960's (Ortmann 1918, Ortmann 1920, Stansbery 1973, Bates and Dennis 1978, Neves and Zale 1982, Zale and Neves 1982a, Zale and Neves 1982b, Ahlstedt 1986, Neves and Widlak 1987, Bruenderman 1989, Dennis 1989, Neves and Odom 1989, Goudreau et al. 1993). As early as 1858 researchers were concerned with the changing mussel fauna in the Clinch River; however, concerns have intensified and rates of decline increased in recent years.

Freshwater mussels are relatively sedentary, long-lived organisms with complex life cycles, leaving them particularly vulnerable to anthropogenic disturbances (Neves 1991). River reaches of the upper Clinch River, above Norris Reservoir, have remained unaltered by impoundment and relatively undeveloped. One major power plant, coal mines, small towns, and crop and

dairy farms are found along the river, each potentially contributing to mussel decline.

An examination of mussel population demographics such as growth rates, recruitment, and mortality rates provide insight to the response of mussels to human perturbations. Enhanced growth rates may indicate enrichment from town sewage runoff or cattle inputs. Decreased growth rates and high adult mortality may indicate impairment from point and non-point source discharges from the power plant, coal mines, and road or agricultural runoff. Missing cohorts may indicate impairment at specific points in time due to reduced recruitment of juveniles or high mortality.

Annual formation of growth bands in shells of freshwater mussels in temperate climates has been extensively studied and accepted by most researchers as an accurate estimation of age (Chamberlain 1931, Negus 1966, Lutz and Rhoads 1977, Neves and Moyer 1988). The thin-section aging technique (Clark 1980) provides a means of aging such that growth rates, recruitment of juveniles, and mortality rates of adults may be compared among sites, species, and males and females within species (Neves and Moyer 1988). In addition, it is believed that the presence of growth bands may provide an historical record of environmental perturbations, limited only by the lifespan and location of the mussels (Imlay 1982).

Appalachian Power Company's Clinch River Plant (CRP) is an electric power generating plant located in Carbo, Virginia on the upper Clinch River. Between 1967 and 1970, two catastrophic spills occurred at the CRP which peaked awareness of environmental impact on the Clinch River. The first spill in June 1967 was caused by the collapse of a dike wall surrounding a fly ash settling pond. A 490,000 m<sup>3</sup> slug entered the Clinch River during low flow conditions and traveled 145 km downstream, killing over 200,000 fish (Crossman et al. 1973). The Virginia State Water Control Board reported that the spill removed all bottom-dwelling "fish-food" organisms for 5 to 6 km below the site; all mollusks for 18 km downstream; and reduced the number of "fish-food" organisms for 124 km downstream. Three years later in June 1970, a second spill occurred at the CRP which released an undetermined amount of sulfuric acid to the river. The acidic slug traveled 22 km downstream, killing ~5,300 fish in its path (Crossman et al. 1973).

In addition to the two spills from the CRP, the power plant discharge had a history of high copper and zinc levels. Exposure to copper has been shown to accumulate in the soft tissues and significantly retard growth of the Asian clam (Belanger et al. 1990) and may be a contributing factor to native mussel declines. Therefore, in 1987, the CRP began to address the problem by implementing a program to reduce heavy metals in the effluent. In 1988 and 1989 levels of zinc and copper were reduced by 75% and 30%, respectively

(Clements et al. 1992). In 1993, an additional treatment facility was constructed to reduce copper and zinc in the effluent to below background river levels (personal communication with D. S. Cherry).

### Study sites

The upper Clinch River, ~300 kilometers, flows in a southwesterly direction from Tazewell, Virginia to Norris Reservoir, Tennessee. It increases from a third order stream at it's headwaters, to a sixth order stream at the Tennessee border.

Four study sites were chosen for this research based on river location and availability of muskrat foraging midden shells (Figure 1.1). Site 1, Pounding Mill, is the most headwater site of this study, located in Tazewell County, Virginia at Clinch River Kilometer (CRK) 527. Four mussel species were commonly found in muskrat middens, with *Villosa iris* dominating the species composition. Site 2, Hackneys, is located 2.4 km above the CRP (CRK 433.7), with 14 species comprising the mussel assemblage (Ahlstedt 1986). Site 3, Slant, is the first study site below the power plant, located ~72 km downstream (CRK 359.3) with 23 species found at this site between 1978 and 1983 (Ahlstedt 1986). Site 4, Kyle's Ford, is a very prolific mussel bed located south of the Virginia/Tennessee border (CRK 304). As many as 33 species are found at Kyle's Ford with 31 mussels/m<sup>2</sup> (Ahlstedt 1986).

## Study Objectives

The objectives of this study were to use growth rates, recruitment, and mortality rates as indicators of the ecological integrity (structural/functional) of four mussel assemblages in the upper Clinch River and to determine if historically high copper levels have affected these sites. This thesis is partitioned into four subsequent chapters: 1) Chapter Two -- Growth rates, 2) Chapter Three -- Age-frequency distributions, 3) Chapter Four -- Bioaccumulation of copper in mussel shells, and 4) Chapter Five -- Summary and Conclusions. Chapter 2 compares growth rates of six mussel species among study sites to identify impaired or enriched mussel assemblages in the upper Clinch River. Chapter 3 examines age-class distributions of 16 populations of mussels at the four study sites to identify periods of low and high recruitment of juveniles and reaches of high adult mortality. Chapter 4 addresses the correlation between accumulation of copper in mussel shells and reduced recruitment below the CRP. Chapter 5 summarizes the results of these studies and discusses the overall implications of the declining mussel fauna in the Clinch River. Each data chapter is complete with an abstract, introduction, materials and methods, results, discussion and literature cited. Tables and figures are included at the end of each chapter.

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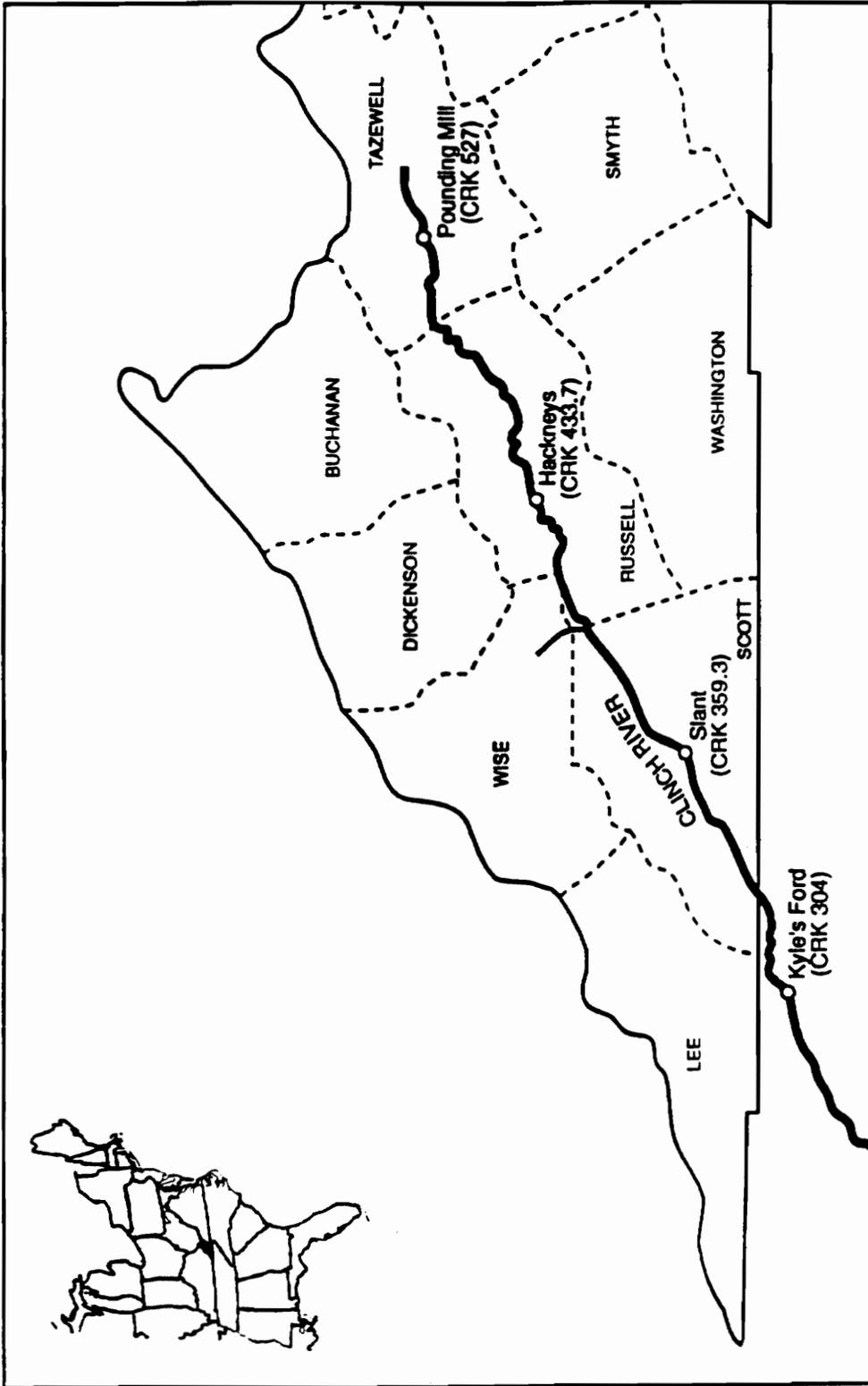


Figure 1.1: Four study site locations in the Clinch River.

## Chapter Two

### GROWTH RATES

#### Abstract:

Growth rates of 6 freshwater mussel species were determined to identify enriched or impaired mussel assemblages at 4 sites in the upper Clinch River. Growth rates between males and females of a sexually dimorphic species, *Lampsilis fasciola*, also were evaluated. The thin-section aging technique was used to determine total shell lengths-at-age and back-measured shell lengths-at-age. Shell lengths-at-age were fitted to the von Bertalanffy growth model and compared among sites and sexes. Five of the mussel species had higher growth rates at the Hackneys site when compared to other sites. The source of enrichment was presumed to be the town Cleveland, Virginia located approximately 5 km upstream of the site. Prior to June 1986, Cleveland had no sewage treatment facility, which resulted in the release of untreated sewage directly in the Clinch River. Since that time, a sewage treatment plant has been established which is likely contributing a new source of impact to mussels downstream. Secondly, male *L. fasciola* exhibited higher growth

rates than female *L. fasciola* at all sites. *L. fasciola* is known to reach sexually maturity in the Clinch River by age 4; therefore, females may begin to put more energy into reproduction and less into shell growth which results in an asymptotic growth pattern. Growth rates of freshwater mussels in the Clinch River indicated no impairment at the 4 sites examined.

#### Introduction:

A correlation between shell morphology and growth rates of freshwater mussels and their habitat has been documented by many researchers (Ortmann 1920, Ball 1922, Eagar 1948, Bailey and Green 1988). Shell size, measured as anterior to posterior length, is positively correlated to stream size and negatively correlated to river velocity, such that total shell length increases from river headwaters toward the river mouth, with largest forms found in lakes (Tevesz and Carter 1980). Ortmann (1920) documented this phenomenon in the Clinch River and found that species of more primitive genera; *Fusconaia*, *Amblema*, *Quadrula*, *Lexingtonia* and *Pleurobema*, exhibited a correlation between obesity (shell thickness/shell length) and location in the river. However, he concluded that "comparatively few [genera exhibit this phenomenon] while others positively do not show it". Bailey and Green (1988) found a positive correlation between growth rate of *Lampsilis radiata* and the

degree of exposed habitat characteristic of turbulent waters and sandy sediments.

Numerous factors such as 1) road and agricultural runoff, 2) industrial discharges, 3) habitat destruction or loss, and 4) siltation have potentially contributed to diminishing density and diversity of freshwater mussels for nearly a century (Ortmann 1918, Bates 1962, Negus 1966, Neves and Zale 1982, Aldridge et al. 1987). Small amounts of organic pollution may enrich mussel communities resulting in enhanced growth and high densities, but too much organic pollution can cause eutrophication increasing vegetative growth and siltation and posing a threat to mussel assemblages (Fuller 1974). Wastewater treatment plants, developed to reduce eutrophication, can release chlorine and ammonia into receiving systems which have detrimental effects on mussel assemblages (Goudreau et al. 1993). It was thus hypothesized that low level anthropogenic insults may stimulate or inhibit growth of freshwater mussels which would provide a means of identifying potentially impacted sites.

The objectives of this phase of the study were: 1) to explore a correlation between growth rates of six freshwater mussel species and longitudinal river position, 2) to identify potentially impacted mussel communities based on their enhanced or impaired growth rates, and 3) to compare growth rates of males and females of sexually dimorphic *Lampsilis fasciola*.

## Materials and Methods:

Shells collected in muskrat middens provided by R. J. Neves, J. L. Farris, G. W. Church and recently collected midden shells were used for growth rate determinations. Middens were collected from Pounding Mill at Clinch River Kilometer (CRK) 527 during the summers of 1992 and 1993, from Hackneys (CRK 433.7) in fall 1988 and summers 1992 and 1993, from Slant (CRK 359.3) during 1986, and from Kyle's Ford (CRK 304) in fall 1991 and summers 1992 and 1993.

Three to six freshwater mussel species; *Medionidus conradicus* (Cumberland moccasinshell), *Villosa iris* (rainbow), *Lampsilis fasciola* (wavy-rayed lampmussel), *Fusconaia cor* (shiny pigtoe), *Actinonaias pectorosa* (pheasantshell), and *Elliptio dilatata* (spike), were removed from middens at these sites, sectioned and aged to compute growth rates (Moyer 1984). *L. fasciola* is a sexually dimorphic species; therefore, males and females were separated based on shell morphology (Tevesz and Carter 1980). A size range (1 - 2 mm increments) of left valves for each species at each site was measured to the nearest 0.01 mm; the right valves were retained for metal concentration analysis (Chapter Four).

Left valves were sectioned using a Buehler Isomet low speed diamond saw. Imperfections were removed from the surface of the sectioned valve by grinding with 400  $\mu\text{m}$  and 600  $\mu\text{m}$  grit sizes. Valves were bonded to

petrographic micro-slides with Ward's Bioplastic epoxy and cut again to make the thin section. The thickness of each section was adjusted to approximately 280  $\mu\text{m}$  using a thin-section grinding wheel. The thin sections were then polished on a Buehler polishing wheel to remove all imperfections. Each slide was viewed under a stereoscope and aged by counting internal annuli (Lutz and Rhoads 1980, Neves and Moyer 1988). Annuli were marked with a pencil on the thin sections at the external surface of the shell to determine shell length at each age (back-measured length-at-age) on right valves (Moyer 1984). Back-measurements were made by placing the cut left valve on the thin section and marking annuli on the valve. The cut left valve was overlaid on the corresponding right valve, and annuli were marked and measured to the nearest 0.01 mm.

Total shell lengths-at-age and back-measured shell lengths-at-age were fitted to the von Bertalanffy asymptotic growth equation (1):

$$L_t = L_{\infty}(1 - e^{-k(t-t_0)})$$

- $L_t$  = length at time  $t$
- $L_{\infty}$  = theoretical maximum length
- $k$  = growth constant
- $t_0$  = theoretical time when  $L = 0$

which is the best model for estimates of freshwater mussel growth (Moyer 1984). Predicted shell lengths-at-age from the growth model and observed shell lengths-at-age were compared by regression analysis as a measure of goodness of fit. Although the von Bertalanffy model is widely accepted as a

model for freshwater mussel growth, the best statistical comparison method for this model is debated. The model parameters,  $L_{\infty}$ ,  $k$ , and  $t$ , are highly intercorrelated making interpretations of comparisons between growth rates difficult (Gallucci and Quinn 1979). There have been suggestions of utilizing a fourth parameter,  $w = L_{\infty} * k$ , to improve the robustness of the statistical analysis (Gallucci and Quinn 1979, Bruenderman 1989); however, one parameter does not fully describe the mussel growth. Therefore, growth equations of species, among sites and between sexually dimorphic males and females, are best compared by using dummy variables in the von Bertalanffy equation as follows (2):

$$L_t = (L_{\infty} + B_0 * Z) (1 - e^{(-k + B_1 * Z) (t - (t_0 + B_2 * Z))})$$

for 2 site comparisons and (3):

$$L_t = (L_{\infty} + B_0 * Z_1 + C_0 * Z_2) (1 - e^{(-k + B_1 * Z_1 + C_1 * Z_2) (t - (t_0 + B_2 * Z_1 + C_2 * Z_2))})$$

for 3 or 4 site comparisons utilizing a Statistical Analysis System (SAS) nonlinear procedure (Appendix A).  $Z$  is a dummy variable with a value of 0 or 1, and the parameters  $B$  and  $C$  represent the difference in model parameters:  $L_{\infty}$ ,  $k$  and  $t$  for each site (Chism et al. 1992). Pairwise comparisons (f-test) between the Sums of Square Error (SSE) of equation 1 and the SSE of equation 2 or 3 were made to detect differences in growth rates. Differences were detected among two sites with 95% confidence ( $\alpha = 0.05$ ), among three sites

with 99% confidence ( $\alpha=0.01$ ), and among four sites with 99.2% confidence ( $\alpha=0.008$ ).

United States Environmental Protection Agency (USEPA) STOrage and RETrieval (STORET) database was obtained for a sampling station 3.2 km upstream of Hackneys site. Parameters such as fecal coliforms, nitrate, and ortho-phosphorus were evaluated to determine possible relation to growth rates. The parameters were measured between 2 and 9 times a year from 1976 to 1979 and between 5 and 10 times a year from 1992 to 1993.

#### Results:

##### *Medionidus conradicus*

*M. conradicus* occurred at three of the four study sites: Pounding Mill, Hackneys, and Kyle's Ford. Specimens ranged from 5 to 24 years old and 27.90 to 50.88 mm in total shell length at Pounding Mill, 3 to 18 years and 25.89 to 62.29 mm at Hackneys, and 2 to 17 years and 22.42 to 55.15 mm at Kyle's Ford (Table 2.1). Sample sizes of shells thin-sectioned and back-measured for growth rate determinations are listed in Table 2.1. There was a high correlation between predicted and observed shell lengths per age ( $R^2 \geq 0.98$ ) at each site (Appendix B).

Von Bertalanffy growth equations for *M. conradicus* of ages 1 to 24 were:

$L_t = 52.84(1 - e^{0.22(t-0.24)})$	$R^2 = 0.99$	<i>Pounding Mill</i>	N = 29
$L_t = 60.90(1 - e^{0.19(t+0.12)})$	$R^2 = 0.99$	<i>Hackneys</i>	N = 52
$L_t = 57.05(1 - e^{0.16(t+0.84)})$	$R^2 = 0.98$	<i>Kyle's Ford</i>	N = 46

Growth rate of *M. conradicus* at Hackneys was significantly greater than at Pounding Mill and Kyle's Ford ( $\alpha = 0.01$ ,  $p < 0.001$ ); however, the growth rates at Pounding Mill and Kyle's Ford were not significantly different from each other ( $\alpha = 0.01$ ,  $p = 0.02$ ) (Figure 2.1).

There was no evidence of a correlation between growth rates or shell lengths and longitudinal river position for *M. conradicus*. Growth rates at the two extreme sites, Pounding Mill and Kyle's Ford located ~ 224 km apart, were not significantly different from each other (Figure 2.1), and predicted maximum shell lengths ( $L_\infty$ ) differed marginally among sites, regardless of river location (Table 2.2).

### *Villosa iris*

*V. iris* occurred at three of the four study sites: Pounding Mill, Hackneys, and Kyle's Ford. Specimens ranged from 4 to 20 years old and 17.91 to 77.22 mm in total shell length at Pounding Mill, 4 to 25 years and 27.35 to 75.15 mm at Hackneys, and 5 to 23 years and 32.74 to 70.57 mm at Kyle's Ford (Table 2.1). Sample sizes of shells thin-sectioned and back-measured for growth rate determinations are listed in Table 2.1. There was a high correlation between predicted and observed shell lengths per age ( $R^2 \geq 0.95$ ) at each site (Appendix B).

Von Bertalanffy growth equations for *V. iris* of ages 1 to 25 were:

$$\begin{array}{llll} L_t = 78.82(1-e^{0.09(t+0.23)}) & R^2 = 0.97 & \text{Pounding Mill} & N = 40 \\ L_t = 76.88(1-e^{0.14(t-0.67)}) & R^2 = 0.99 & \text{Hackneys} & N = 49 \\ L_t = 67.89(1-e^{0.13(t+0.80)}) & R^2 = 0.95 & \text{Kyle's Ford} & N = 33 \end{array}$$

Growth rate of *V. iris* at Hackneys was significantly greater than at Pounding Mill ( $\alpha=0.01$ ,  $p<0.001$ ) and significantly greater than at Kyle's Ford beyond 6 years of age ( $\alpha=0.01$ ,  $p<0.001$ ). Growth rates at Pounding Mill and Kyle's Ford were not significantly different from each other throughout the lifespan ( $\alpha=0.01$ ,  $p=0.10$ ); however, the growth rate at Kyle's Ford was significantly greater than at Pounding Mill prior to 17 years of age ( $\alpha=0.01$ ,  $p<0.001$ ) (Figure 2.2).

There was no evidence of a correlation between growth rates or shell lengths and longitudinal river position for *V. iris*. Growth rates at the two extreme sites, Pounding Mill and Kyle's Ford located ~224 km apart, were not significantly different from each other (Figure 2.2). In addition, predicted maximum shell lengths ( $L_\infty$ ) differed by ~11 mm among sites, with the smallest  $L_\infty$  at Kyle's Ford (Table 2.2).

### *Fusconaia cor*

*F. cor* occurred at Hackneys and Slant sites. Specimens ranged from 4 to 25 years old and 26.08 to 79.05 mm in total shell length at Hackneys, and 14 to 30 years old and 53.07 to 75.81 mm at Slant (Table 2.1). Sample sizes of shells thin-sectioned and back-measured for growth rate determinations are

listed in Table 2.1. There was a high correlation between predicted and observed shell lengths per age ( $R^2 \geq 0.99$ ) at both sites (Appendix B).

Von Bertalanffy growth equations for *F. cor* of ages 1 to 30 were:

$$L_t = 72.26(1 - e^{-0.13(t-0.20)}) \quad R^2 = 0.99 \quad \text{Hackneys} \quad N = 43$$
$$L_t = 75.03(1 - e^{-0.12(t+0.38)}) \quad R^2 = 0.99 \quad \text{Slant} \quad N = 18$$

Growth rates of *F. cor* at Hackneys and Slant were not significantly different from each other ( $\alpha = 0.05$ ,  $p = 0.95$ ) (Figure 2.3).

There was no evidence of a correlation between growth rates or shell lengths and longitudinal river position for *F. cor*. Growth rates were not significantly different at two sites ~74 km apart (Figure 2.3), and predicted maximum shell lengths ( $L_\infty$ ) differed by ~3 mm at the two sites (Table 2.2).

#### *Actinonaias pectorosa*

*A. pectorosa* occurred at three of the four study sites: Hackneys, Slant, and Kyle's Ford. Specimens ranged from 4 to 13 years old and 40.61 to 96.53 mm in total shell length at Hackneys, 5 to 18 years and 57.55 to 122.79 mm at Slant, and 4 to 21 years and 37.64 to 95.04 mm at Kyle's Ford (Table 2.1). Sample sizes of shells thin-sectioned and back-measured for growth rate determinations are listed in Table 2.1. There was a high correlation between predicted and observed shell lengths per age ( $R^2 \geq 0.99$ ) at each site (Appendix B).

Von Bertalanffy growth equations for *A. pectorosa* of age 1 to 21 were:

$L_t = 106.97(1 - e^{-0.16(t-0.25)})$	$R^2 = 0.99$	<i>Hackneys</i>	N = 53
$L_t = 146.10(1 - e^{-0.08(t+0.86)})$	$R^2 = 0.99$	<i>Slant</i>	N = 27
$L_t = 97.56(1 - e^{-0.18(t-0.28)})$	$R^2 = 0.99$	<i>Kyle's Ford</i>	N = 38

Overall growth rates of *A. pectorosa* at Hackneys and Slant were not significantly different ( $\alpha=0.01$   $p=0.05$ ); however, the growth rates diverge beyond age 10. Growth rates at Hackneys and Kyle's Ford were not significantly different from each other ( $\alpha=0.01$ ,  $p=0.04$ ); however, growth rate at Hackneys indicated a trend of faster growth. Growth rate at Kyle's Ford was significantly less than growth rate at Slant ( $\alpha=0.01$ ,  $p<0.001$ ) (Figure 2.4).

There was no evidence of a correlation between growth rates or shell lengths and longitudinal river position for *A. pectorosa*. Growth rates at the two extreme sites, Hackneys and Kyle's Ford, were not significantly different from each other, and the growth rate at Kyle's Ford was significantly less than at Slant, upstream (Figure 2.4). In addition, the predicted maximum shell lengths ( $L_\infty$ ) did not increase in a downstream direction, from Hackneys to Kyle's Ford (Table 2.2). The smaller  $L_\infty$  at Hackneys (~107 mm) and Kyle's Ford (~98 mm) when compared to that at Slant (~146 mm) may be due to muskrat predation bias against older, larger cohorts at the Hackneys and Kyle's Ford sites.

*Elliptio dilatata*

*E. dilatata* occurred at three of the four study sites: Hackneys, Slant, and Kyle's Ford. Specimens ranged from 3 to 23 years old and 32.18 to 87.36 mm in total shell length at Hackneys, 8 to 61 years and 47.87 to 103.32 mm at Slant, and 4 to 21 years and 35.56 to 75.43 mm at Kyle's Ford (Table 2.1). Two extreme outliers, ages 48 and 61 at Slant, were omitted from growth rate comparisons such that comparisons were made among sites for ages 1 to 40 years. Sample sizes of shells thin-sectioned and back-measured for growth rate determinations are listed in Table 2.1. There was a high correlation between predicted and observed shell lengths per age ( $R^2 \geq 0.99$ ) at each site (Appendix B).

Von Bertalanffy growth equations for *E. dilatata* of ages 1 to 40 were:

$$\begin{array}{llll} L_t = 80.41(1-e^{-0.19(t+0.22)}) & R^2 = 0.99 & \text{Hackneys} & N = 55 \\ L_t = 97.84(1-e^{-0.10(t+0.00)}) & R^2 = 0.99 & \text{Slant} & N = 45 \\ L_t = 72.81(1-e^{-0.18(t-0.01)}) & R^2 = 0.99 & \text{Kyle's Ford} & N = 33 \end{array}$$

Growth rate of *E. dilatata* at Hackneys was significantly different from growth rate at Slant ( $\alpha = 0.01$ ,  $p < 0.001$ ), and significantly greater than growth rate at Kyle's Ford ( $\alpha = 0.01$ ,  $p < 0.001$ ). Growth rate at Slant was significantly greater than growth rate at Kyle's Ford beyond 10 years of age ( $\alpha = 0.01$ ,  $p < 0.001$ ) (Figure 2.5).

There was no evidence of a correlation between growth rates or shell lengths and longitudinal river position for *E. dilatata*. Growth rates at Hackneys

was significantly greater than at Kyle's Ford, located ~120 km downstream (Figure 2.5), and the predicted maximum shell length ( $L_{\infty}$ ) was smallest at the downstream site (Table 2.2). The smaller  $L_{\infty}$  at Hackneys (~80 mm) and Kyle's Ford (~73 mm), when compared to Slant (~98 mm), may be due to muskrat predation bias against the older, larger cohorts at the Hackneys and Kyle's Ford sites.

*Lampsilis fasciola*

*L. fasciola* occurred at all study sites: Pounding Mill, Hackneys, Slant, and Kyle's Ford. Female specimens ranged from 6 to 12 years and 26.04 to 58.16 mm in total shell length at Pounding Mill, 3 to 24 years and 39.31 to 89.78 mm at Hackneys, 13 to 20 years and 51.29 to 80.31 mm at Slant, and 4 to 19 years and 40.50 to 68.38 mm at Kyle's Ford (Table 2.1). Sample sizes of shells thin-sectioned and back-measured for growth rate determinations are listed in Table 2.1. There was a high correlation between predicted and observed shell lengths per age ( $R^2 \geq 0.98$ ) at each site (Appendix B). Male specimens ranged from 6 to 15 years old and 48.61 to 75.32 mm in total shell length at Pounding Mill, 3 to 21 years and 32.94 to 85.39 mm at Hackneys, 10 to 32 years and 54.80 to 85.71 mm at Slant, and 4 to 19 years and 38.10 to 77.82 mm at Kyle's Ford (Table 2.1). One extreme outlier, age 32 at Slant, was omitted from growth rate comparisons, such that comparisons were made among sites for ages 1 to 21 years. Sample sizes of shells thin-sectioned and

back-measured for growth rate determinations are listed in Table 2.1. There was a high correlation between predicted and observed shell lengths per age ( $R^2 \geq 0.99$ ) at each site (Appendix B).

Von Bertalanffy growth rate equations for *L. fasciola* females of ages 1 to 24 were:

$L_t = 60.82(1 - e^{-0.21(t+0.12)})$	$R^2 = 0.99$	<i>Pounding Mill</i>	N = 12
$L_t = 77.72(1 - e^{-0.16(t+1.24)})$	$R^2 = 0.98$	<i>Hackneys</i>	N = 45
$L_t = 73.20(1 - e^{-0.17(t+0.00)})$	$R^2 = 0.99$	<i>Slant</i>	N = 19
$L_t = 69.87(1 - e^{-0.18(t+0.77)})$	$R^2 = 0.99$	<i>Kyle's Ford</i>	N = 15

Growth rate of *L. fasciola* females at Pounding Mill was significantly less than growth rates at Hackneys and Kyle's Ford ( $\alpha = 0.008$ ,  $p < 0.001$ ). The growth rate at Pounding Mill was not significantly different than growth rate at Slant over the lifespan ( $\alpha = 0.008$ ,  $p = 0.04$ ); however, beyond age 8, the growth rate at Pounding Mill decreased from 4.8 mm/yr to 0.6 mm/yr while growth rate at Slant decreased from 4.6 mm/yr to 1.2 mm/yr. Growth rate at Hackneys was significantly greater than growth rates at Pounding Mill ( $\alpha = 0.008$ ,  $p < 0.001$ ), Slant ( $\alpha = 0.008$ ,  $p = 0.001$ ), and Kyle's Ford ( $\alpha = 0.008$ ,  $p = 0.004$ ). Growth rates at Slant and Kyle's Ford were not significantly different from each other ( $\alpha = 0.008$ ,  $p = 0.45$ ) (Figure 2.6).

There was no indication of a correlation between growth rates and longitudinal river position for female *L. fasciola*. Although the growth rate at the most upstream site, Pounding Mill, was less than the growth rates at all

downstream sites, the growth rate at Hackneys was significantly greater than growth rates downstream (Figure 2.6). In addition, there was no indication of a correlation between shell length and longitudinal river position. The predicted maximum shell length ( $L_{\infty}$ ) at the most upstream site, Pounding Mill (~ 61 mm), was not substantially smaller than the most downstream site, Kyle's Ford (~ 70 mm), and  $L_{\infty}$  values were larger at the intermediate sites, Hackneys (~ 78 mm) and Slant (~ 73 mm) (Table 2.2).

Von Bertalanffy growth rate equations for *L. fasciola* males of ages 1 to 21 were:

$L_t = 78.02(1 - e^{-0.20(t-0.11)})$	$R^2 = 0.99$	<i>Pounding Mill</i>	N = 7
$L_t = 80.87(1 - e^{-0.19(t+0.24)})$	$R^2 = 0.99$	<i>Hackneys</i>	N = 52
$L_t = 87.44(1 - e^{-0.13(t+0.92)})$	$R^2 = 0.99$	<i>Slant</i>	N = 26
$L_t = 73.89(1 - e^{-0.18(t+0.53)})$	$R^2 = 0.99$	<i>Kyle's Ford</i>	N = 20

Growth rate of *L. fasciola* males at Pounding Mill was significantly less than at Hackneys ( $\alpha = 0.008$ ,  $p < 0.001$ ), significantly different from Slant ( $\alpha = 0.008$ ,  $p < 0.001$ ), and significantly greater than at Kyle's Ford ( $\alpha = 0.008$ ,  $p < 0.001$ ). Growth rate of males at Hackneys was significantly greater than at Slant prior to age 15 ( $\alpha = 0.008$ ,  $p < 0.001$ ), and significantly greater than at Kyle's Ford ( $\alpha = 0.008$ ,  $p < 0.001$ ). Growth rate of males at Slant was significantly greater than at Kyle's Ford beyond ~ 8 years of age ( $\alpha = 0.008$ ,  $p < 0.001$ ) (Figure 2.7).

There was no evidence of a correlation between growth rates or shell lengths and longitudinal river position for male *L. fasciola*. Growth rate at

Pounding Mill was significantly greater than at Kyle's Ford, located ~ 224 km downstream (Figure 2.7) and the predicted maximum shell length ( $L_{\infty}$ ) was smallest at Kyle's Ford (Table 2.2).

Sexual dimorphism in the shell of *L. fasciola* from the Clinch River was readily apparent (Figure 2.8). Von Bertalanffy growth rate equations for males and females at Pounding Mill of ages 1 to 21 were:

$$\begin{aligned} L_t &= 60.82(1-e^{-0.21(t+0.12)}) \quad R^2 = 0.99 \quad \textit{female} \quad N = 12 \\ L_t &= 78.02(1-e^{-0.20(t-0.11)}) \quad R^2 = 0.99 \quad \textit{male} \quad N = 7 \end{aligned}$$

Growth rate of males at Pounding Mill was significantly greater than that of females throughout their lifespans ( $\alpha=0.05$ ,  $p<0.001$ ) (Figure 2.9).

Von Bertalanffy growth equations for males and females at Hackneys of ages 1 to 21 were:

$$\begin{aligned} L_t &= 77.72(1-e^{-0.16(t+1.24)}) \quad R^2 = 0.99 \quad \textit{female} \quad N = 45 \\ L_t &= 80.87(1-e^{-0.19(t+0.24)}) \quad R^2 = 0.99 \quad \textit{male} \quad N = 52 \end{aligned}$$

Growth rate of males at Hackneys was significantly greater than that of females beyond 3 years of age ( $\alpha=0.05$ ,  $p<0.001$ ) (Figure 2.10).

Von Bertalanffy growth rate equations for males and females at Slant of ages 1 to 21 were:

$$\begin{aligned} L_t &= 73.20(1-e^{-0.17(t+0.00)}) \quad R^2 = 0.99 \quad \textit{female} \quad N = 19 \\ L_t &= 87.44(1-e^{-0.13(t+0.92)}) \quad R^2 = 0.99 \quad \textit{male} \quad N = 26 \end{aligned}$$

Growth rate of males at Slant was significantly greater than that of females throughout their lifespans ( $\alpha=0.05$ ,  $p<0.001$ ), with divergence of growth increasing with age (Figure 2.11).

Von Bertalanffy growth rate equations for males and females at Kyle's Ford of ages 1 to 21 were:

$$\begin{array}{llll} L_t = 69.87(1-e^{-0.18(t+0.77)}) & R^2 = 0.98 & \textit{female} & N = 15 \\ L_t = 73.89(1-e^{-0.18(t+0.53)}) & R^2 = 0.99 & \textit{male} & N = 20 \end{array}$$

Growth rates of males and females at Kyle's Ford were not significantly different from each other ( $\alpha=0.05$ ,  $p=0.11$ ); however, males indicated a trend of slightly faster growth than females beyond age 3 (Figure 2.12).

#### Discussion:

##### Muskrat predation

Size selectivity through muskrat predation was evident for large mussel species, *A. pectorosa* ( $L_\infty = 117$  mm) and *E. dilatata* ( $L_\infty = 84$  mm), shown by smaller predicted maximum shell lengths at Hackneys and Kyle's Ford than at the Slant site (Table 2.2). Muskrats select for mid-sized mussel species, 50 to 70 mm, and against larger mussel species, greater than 70 mm (Neves and Odom 1989); therefore, it is highly probable that muskrats select for mid-sized age classes, if available, within large shelled species.

The mussel community at Slant was largely skewed toward older age classes (Chapter Three) for all species present, such that very few mussels less than 70 mm were present. Therefore, in the absence of optimal size classes, muskrats selected larger prey which tended to increase  $L_{\infty}$  for these large-shelled species, when compared to other sites. The small mussel species showed very little  $L_{\infty}$  differences among sites. See Chapter Three for more detail of muskrat predation bias.

#### Longitudinal position in river

No correlation was found between growth rates and longitudinal river position for the six mussel species. Based on previous research of shell shape relating to river position (Ortmann 1920, Ball 1922), and growth rates relating to lake habitats (McCuaig and Green 1983, Bailey and Green 1988), it was anticipated that growth rates of species would increase in the downstream direction. However, growth rates were variable suggesting that freshwater mussels in lotic environments are influenced by many micro-physical and micro-chemical parameters (Strayer and Ralley 1993), such that it is difficult to determine a baseline growth model over a large scale. Mitchell and Collins (1984) found a great deal of variability in mussel growth among 10 lentic sites sampled. They support freshwater mussel growth data as water quality indicators; however, many sampling sites need to be investigated to develop a reliable baseline growth model (Mitchell and Collins 1984). Without a

baseline growth model, only relative comparisons between sites can be made to make inferences about water quality or environmental suitability.

#### Enrichment at Hackneys

There was evidence of organic enrichment for five of the seven groups of mussels examined at Hackneys. *M. conradicus*, *V. iris*, *E. dilatata*, *L. fasciola* (females) and *L. fasciola* (males) showed enhanced growth when compared to the other sites. Cleveland (population of approximately 400) is located 4.8 km upstream of Hackneys. In June 1986 a 50,000 gallon per day sewage treatment plant was built to service 137 homes and 10 businesses (USEPA 1989). Prior to 1986, Cleveland had no wastewater treatment facility which, as indicated by high fecal coliform levels, resulted in the release of untreated sewage directly into the Clinch River.

The STORET database for 1976 to 1979 revealed an average level of 2371 fecal coliforms/100 ml of Clinch River water directly below the town. During the summers of 1976 and 1977, a maximum of 6000 fecal coliforms/100 ml was detected on several occasions. Presently, the level of fecal coliforms averages 223/100 ml of Clinch River water for 1992 and 1993, with a maximum value of 1700/100 ml in 1992 (Table 2.3). Data are not available from 1979 to 1992 at this sampling station. Mean levels of nitrate have decreased slightly since the sewage treatment plant was built and average levels of phosphorus have decreased by 80% in recent years (Table 2.3).

The mussels used for growth analyses lived approximately 5 km downstream of a source of untreated sewage. Organic pollution, such as town sewage, causes an increase in eutrophication directly below the source such that an increase in macrophyte biomass occurs (Fuller 1974). Downstream of the initial impact zone, benthic organisms benefit from the breakdown of increased biomass showing increased population densities or growth rates (Fuller 1974, Hawkes and Davies 1974, Aston and Milner 1980).

Comparisons between growth rates of mussels greater than two years old from the 1988 collection and less than seven years old from the 1993 collection may support the occurrence of organic enrichment. Unfortunately, the sample of young individuals, < 7 years, in the 1993 collection was not sufficient to conduct this study. Therefore, the occurrence of enrichment from small town sewage such as Cleveland, Virginia resulting in enhanced growth of freshwater mussels was not confirmed.

#### Sexual dimorphism of *Lampsilis fasciola*

Male and female *L. fasciola* were separated based on shell shape. Females have a greater degree of shell inflation and a rounded posterior margin, while males have a more compressed shell and elongate posterior margin. Many species of mussels, particularly those in the subfamily Lampsilinae, show sexual dimorphism by differences in shell shape and obesity (Tevesz and Carter 1980). Grier (1920) described the occurrence of sexual dimorphism in twelve

sexual dimorphism by differences in shell shape and obesity (Tevesz and Carter 1980). Grier (1920) described the occurrence of sexual dimorphism in twelve species of freshwater mussels from the upper Ohio River drainage. In some species he observed males with a greater degree of shell inflation than females; however, both species of *Lampsilis* were observed with females having a greater degree of shell inflation (Grier 1920).

Growth rates of male *L. fasciola* were greater than female *L. fasciola* beyond 3 years of age at Hackneys, Slant and Kyle's Ford. Zale and Neves (1982), noted that *L. fasciola* reached sexual maturity "in the fourth summer of life", which supports the divergence of growth between males and females beyond age 3 in the current study. Most mussel assemblages exist in energy limiting environments, such that a trade off between reproduction and shell growth occurs (Tevesz and Carter 1980). At sexual maturity, females seemingly begin to put more energy into reproduction and less into shell growth.

## LITERATURE CITED

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Table 2.1: Mussels used for aging and range of age and shell lengths obtained for each species at 4 study sites in the upper Clinch River.

Sites	Species	Age (yrs)	Shell lengths (mm)	Number of mussels thin-sectioned	Number of back-measurements
Pounding Mill	<i>M. conradicus</i>	5-24	27.90-50.88	29	96
	<i>V. iris</i>	4-20	17.91-77.22	40	120
	<i>L. fasciola</i> (♀)	6-12	26.04-58.16	12	83
	<i>L. fasciola</i> (♂)	6-15	48.61-75.32	7	64
Hackneys	<i>M. conradicus</i>	3-18	25.89-62.29	52	95
	<i>V. iris</i>	4-25	27.35-75.15	49	95
	<i>L. fasciola</i> (♀)	3-24	39.31-89.78	45	76
	<i>L. fasciola</i> (♂)	3-21	32.94-85.39	52	106
	<i>F. cor</i>	4-25	26.08-79.05	43	174
	<i>A. pectorosa</i>	4-13	40.61-96.53	53	104
	<i>E. dilatata</i>	3-23	32.18-87.36	55	120

Table 2.1 (con't): Mussels used for aging and range of age and shell lengths obtained for each species at 4 study sites in the upper Clinch River.

Sites	Species	Age (yrs)	Shell lengths (mm)	Number of mussels thin-sectioned	Number of back-measurements
Slant	<i>L. fasciola</i> (♀)	13-20	51.29-80.31	19	75
	<i>L. fasciola</i> (♂)	10-32	54.80-85.71	26	114
	<i>F. cor</i>	14-30	53.07-75.81	18	139
	<i>A. pectorosa</i>	5-18	57.55-122.79	27	106
	<i>E. dilatata</i>	8-61	47.87-103.32	45	153
	<i>M. conradicus</i>	2-17	22.42-55.15	46	76
	<i>V. iris</i>	5-23	32.74-70.57	33	77
Kyle's Ford	<i>L. fasciola</i> (♀)	4-19	40.50-68.38	15	94
	<i>L. fasciola</i> (♂)	4-19	38.10-77.82	20	102
	<i>A. pectorosa</i>	4-21	37.64-95.04	38	118
	<i>E. dilatata</i>	4-21	35.56-75.43	33	113

Table 2.2: Von Bertalanffy growth parameters,  $L_{\infty}$ ,  $k$ , and  $t_0$ , of freshwater mussels at 4 sites in the upper Clinch River. Pounding Mill is the most upstream study site and Kyle's Ford is the most downstream site, river kilometers are included below.

Species		Pounding Mill (RK 527)	Hackneys (RK 433.7)	Slant (RK 359.3)	Kyle's Ford (RK 304)
<i>M. conradicus</i>	$L_{\infty} =$	52.84	60.90		57.05
	$k =$	0.22	0.19	-----	0.16
	$t_0 =$	0.24	-0.12		-0.84
<i>V. iris</i>	$L_{\infty} =$	78.82	76.88		67.89
	$k =$	0.09	0.14	-----	0.13
	$t_0 =$	-0.23	0.67		-0.80
<i>F. cor</i>	$L_{\infty} =$		72.26	75.03	
	$k =$	-----	0.13	0.12	-----
	$t_0 =$		0.20	-0.38	
<i>A. pectorosa</i>	$L_{\infty} =$		106.97	146.10	97.56
	$k =$	-----	0.16	0.08	0.18
	$t_0 =$		0.25	-0.86	0.26
<i>E. dilatata</i>	$L_{\infty} =$		80.41	97.84	72.81
	$k =$	-----	0.19	0.10	0.18
	$t_0 =$		-0.22	-0.00	0.01
<i>L. fasciola</i> (♀)	$L_{\infty} =$	60.82	77.72	73.20	69.87
	$k =$	0.21	0.16	0.17	0.18
	$t_0 =$	-0.12	-1.24	-0.00	-0.77
<i>L. fasciola</i> (♂)	$L_{\infty} =$	78.02	80.87	87.44	73.89
	$k =$	0.20	0.19	0.13	0.18
	$t_0 =$	0.11	-0.24	-0.92	-0.53

**Table 2.3: Water quality parameters at Hackneys, from the STORET database, before and after the establishment of a sewage treatment facility at Cleveland, Virginia.**

<b>Parameters</b>	<b>Before plant 1976 to 1979</b>	<b>After plant 1992 to 1993</b>
<b>Mean fecal coliforms/100ml (minimum-maximum)</b>	<b>2371 (n = 21) (100-6000)</b>	<b>211 (n = 13) (10-1700)</b>
<b>Mean nitrate (mg/L) (minimum-maximum)</b>	<b>0.658 (n = 18) (0.50-1.199)</b>	<b>0.565 (n = 13) (0.070-1.340)</b>
<b>Mean ortho-phosphorus (mg/L) (minimum-maximum)</b>	<b>0.051 (n = 30) (0.010-0.600)</b>	<b>0.015 (n = 15) (0.010-0.030)</b>

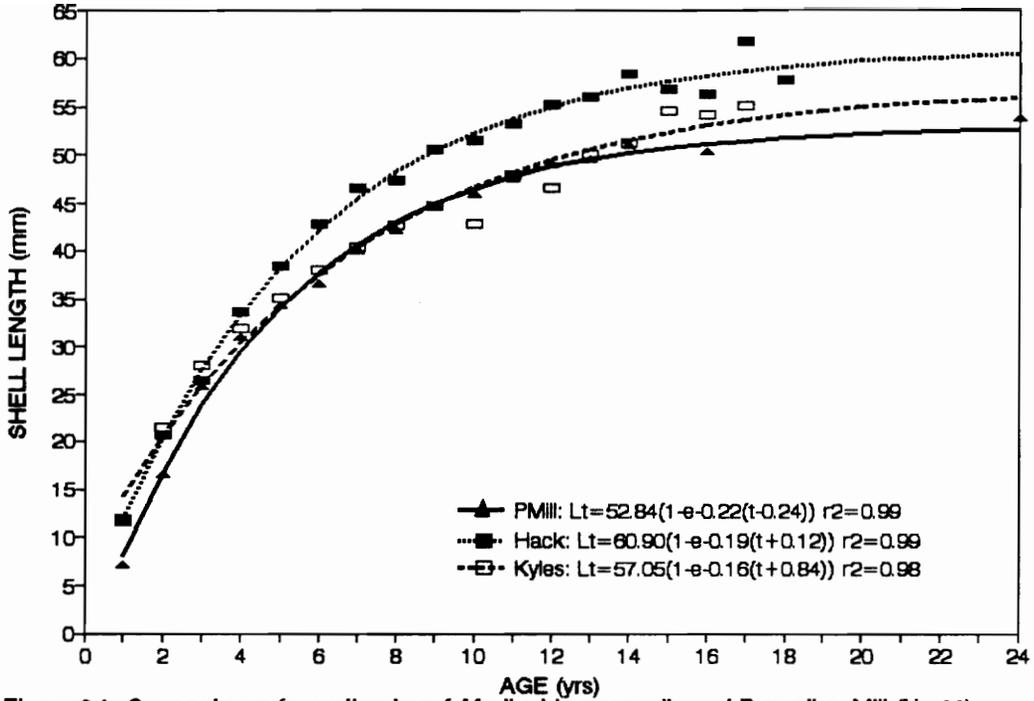


Figure 2.1: Comparison of growth rates of *Medionidus conradicus* at Pounding Mill (N=29), Hackneys (N=52), and Kyle's Ford (N=46) in the Clinch River. Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

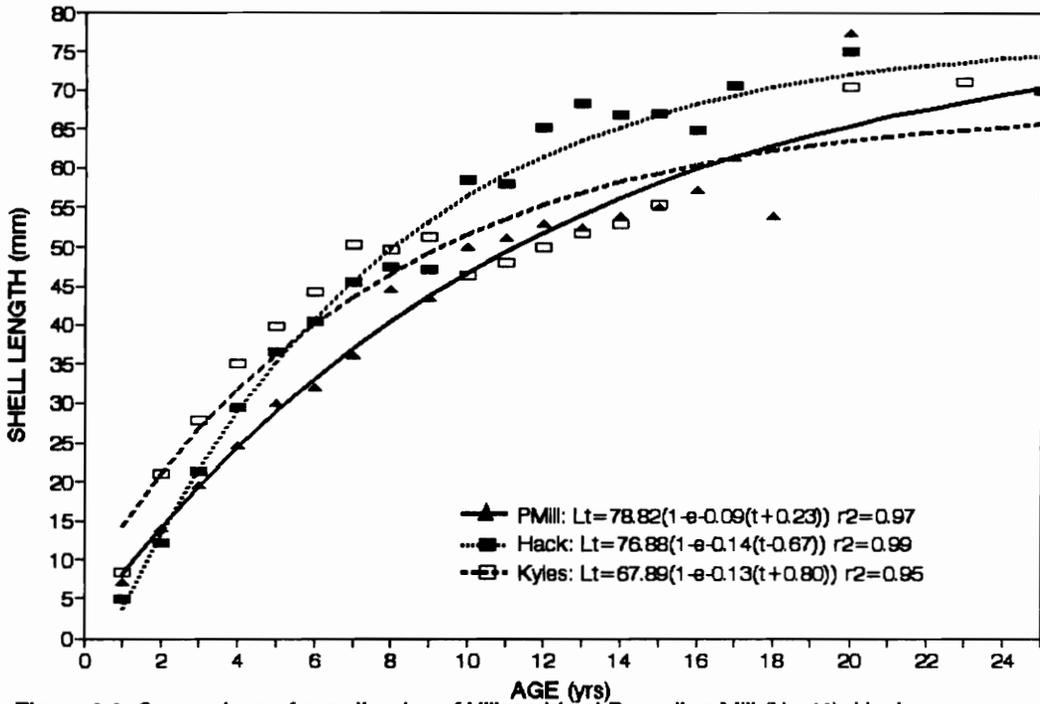


Figure 2.2: Comparison of growth rates of *Villosa iris* at Pounding Mill (N=40), Hackneys (N=49), and Kyle's Ford (N=33) in the Clinch River. Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

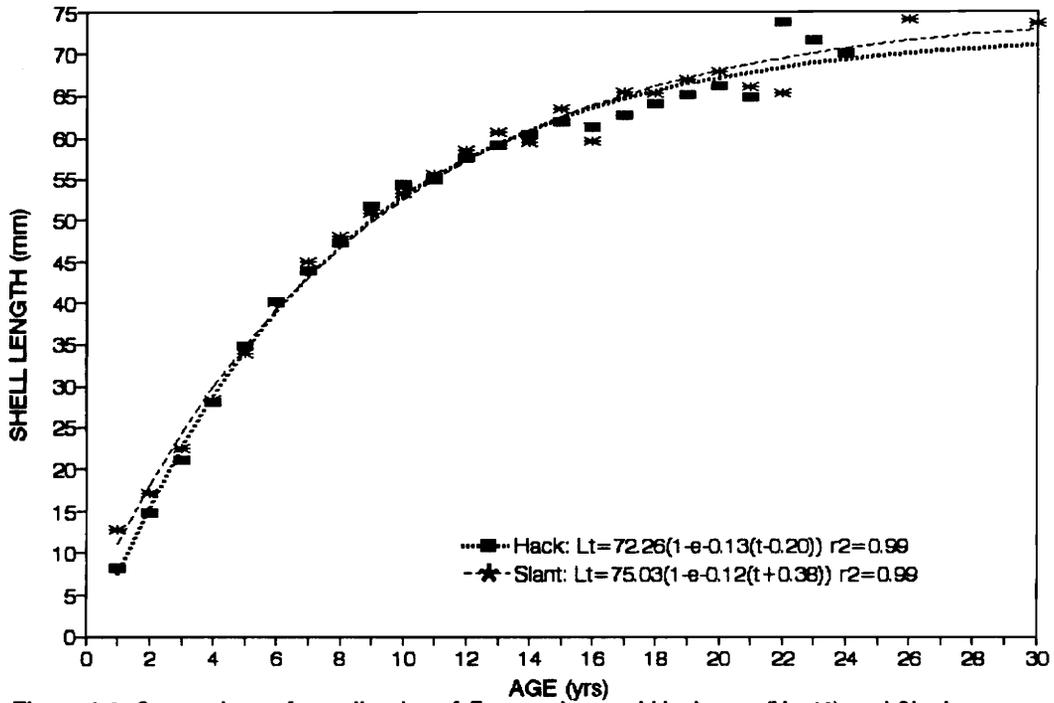


Figure 2.3: Comparison of growth rates of *Fusconaia cor* at Hackneys (N=43) and Slant (N=18) in the Clinch River. Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

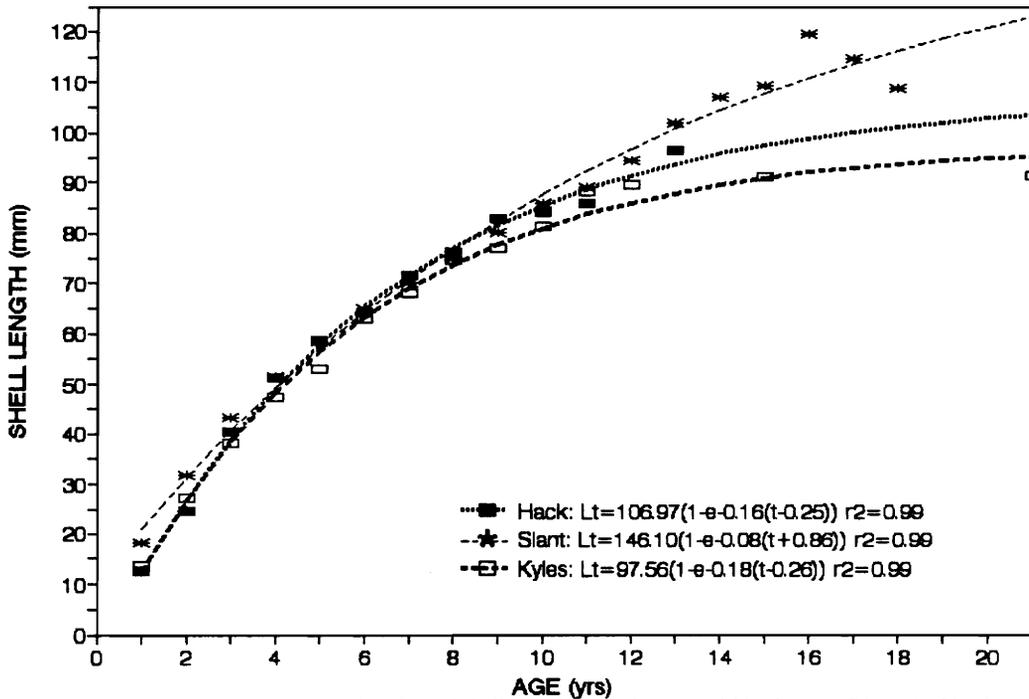


Figure 2.4: Comparison of growth rates of *Actinonaias pectorosa* at Hackneys (N=53), Slant (N=27), and Kyle's Ford (N=38) in the Clinch River. Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

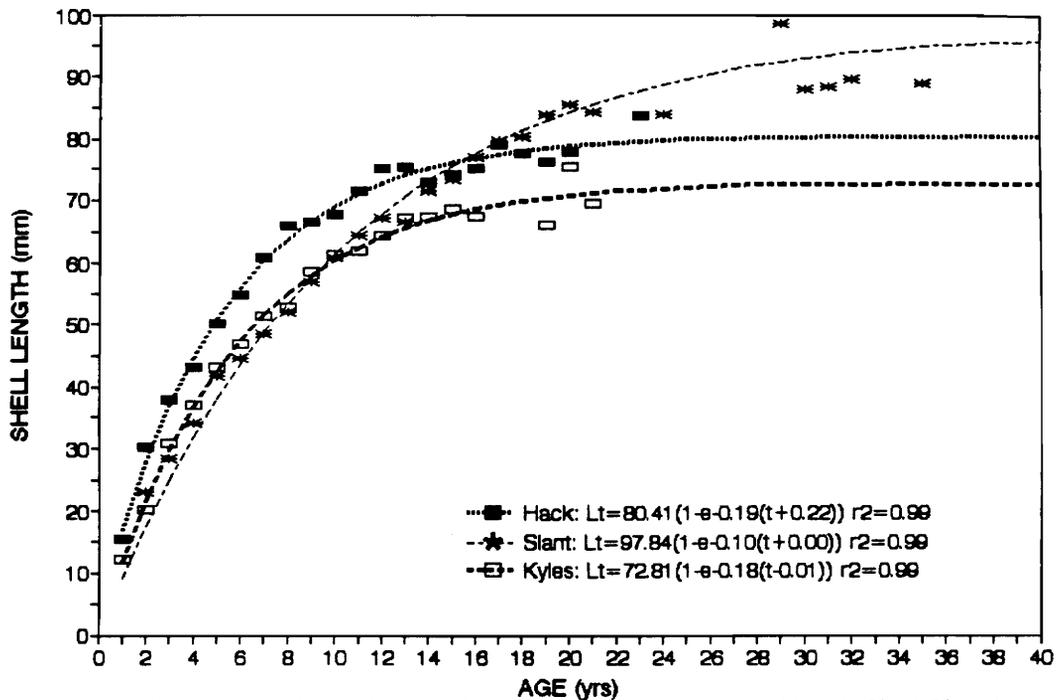


Figure 2.5: Comparison of growth rates of *Elliptio dilatata* at Hackneys (N=55), Slant (N=45), and Kyle's Ford (N=33) in the Clinch River. Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

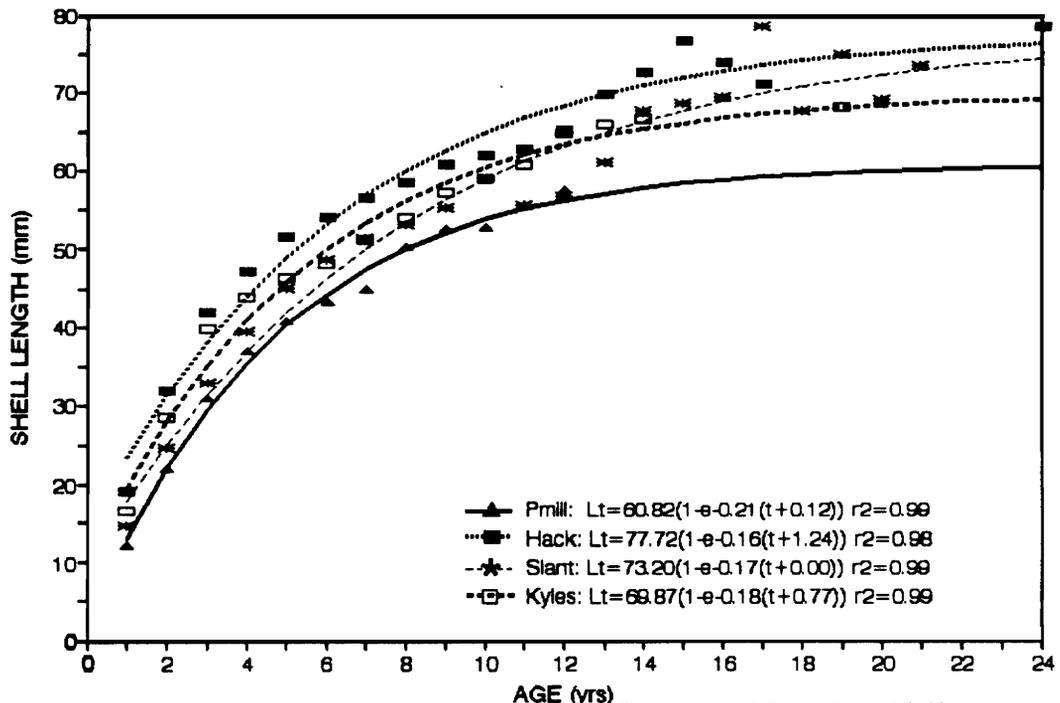


Figure 2.6: Comparison of growth rates of female *Lamproliss fasciola* at Pounding Mill (N=12), Hackneys (N=45), Slant (N=19), and Kyle's Ford (N=15) in the Clinch River. Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

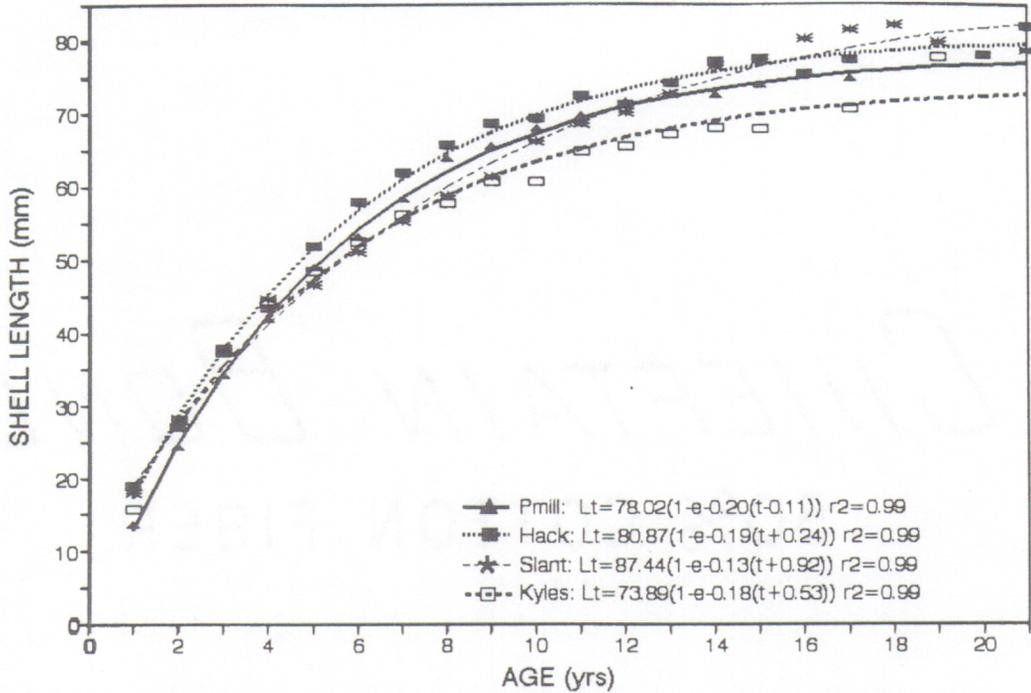


Figure 2.7: Comparison of growth rates of male *Lampsilis fasciola* at Pounding Mill (N=7), Hackneys (N=52), Slant (N=26), and Kyle's Ford (N=20) in the Clinch River. Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.



Figure 2.8: Female of *Lampsilis fasciola* with a rounded posterior margin and inflated shell (left valve). Male with an elongate posterior margin and compressed shell (right valve).

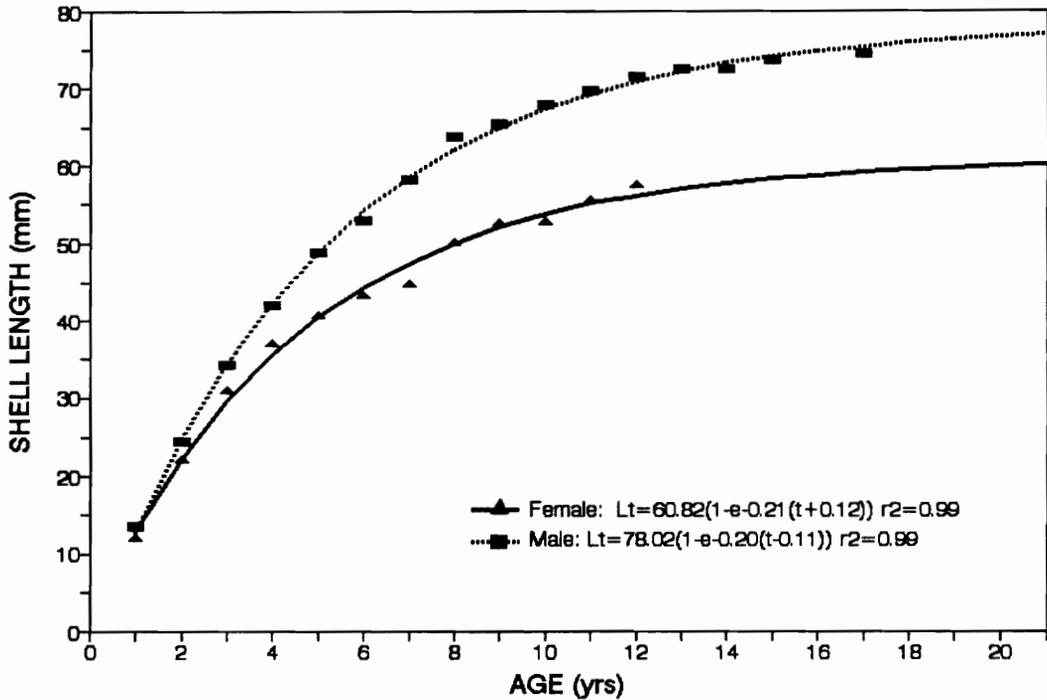


Figure 2.9: Comparison of growth rates of male (N=7) and female (N=12) *Lampsilis fasciola* at Pounding Mill in the Clinch River (KM 527). Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

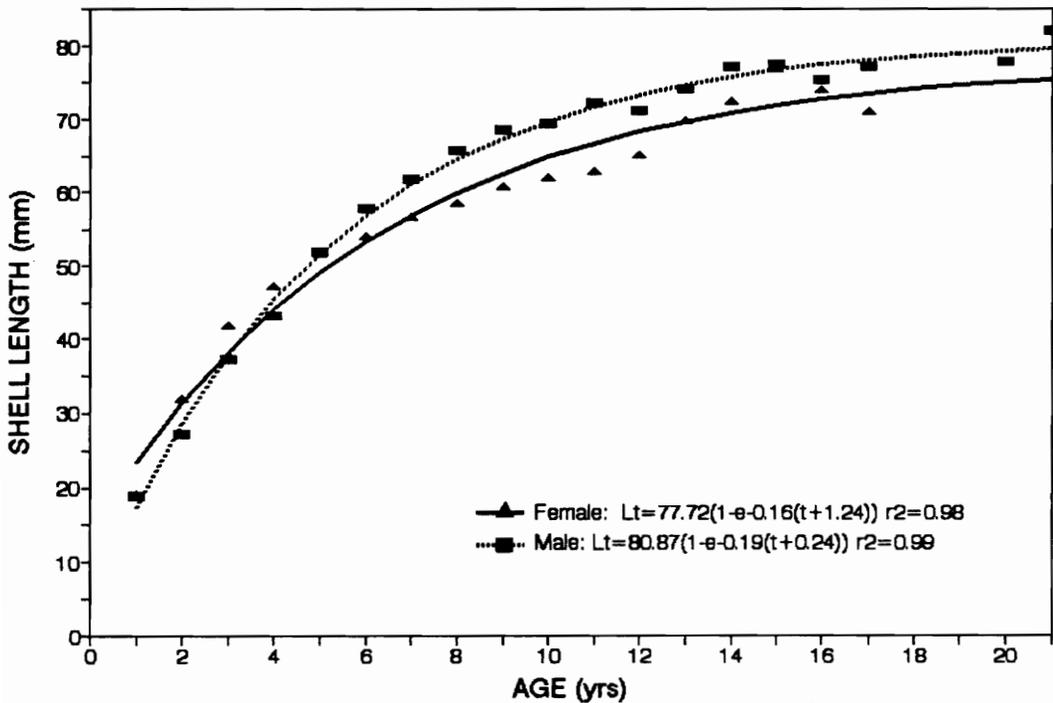


Figure 2.10: Comparison of growth rates of male (N=52) and female (N=45) *Lampsilis fasciola* at Hackneys in the Clinch River (KM 433.7). Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

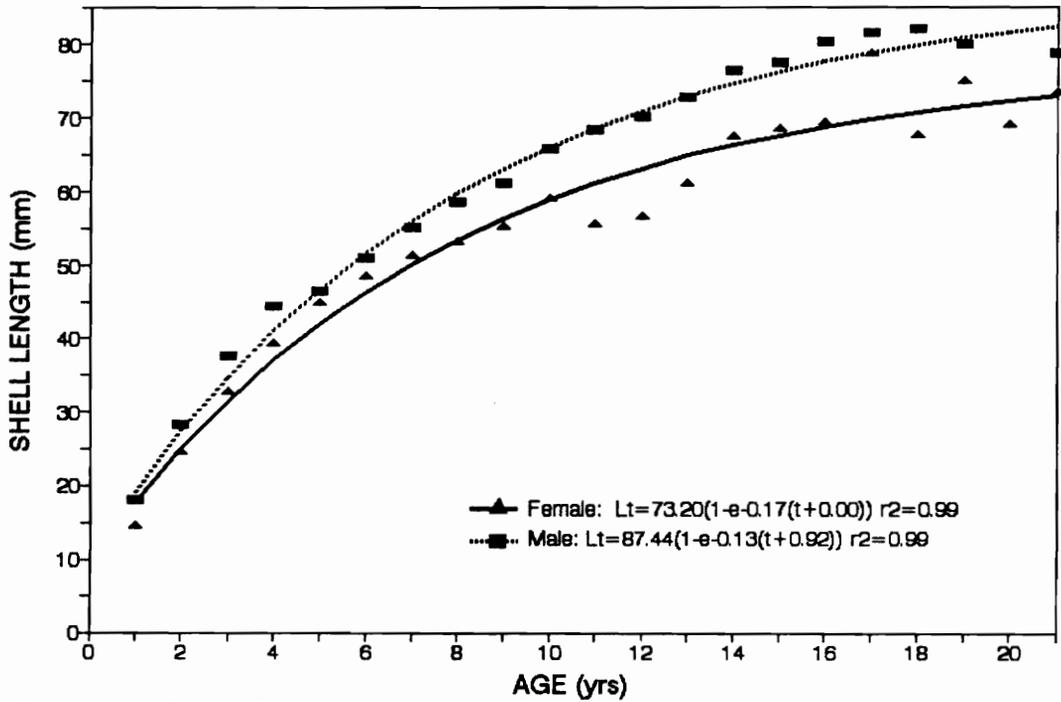


Figure 2.11: Comparison of growth rates of male (N=26) and female (N=19) *Lampsilis fasciola* at Slant in the Clinch River (KM 359.3). Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

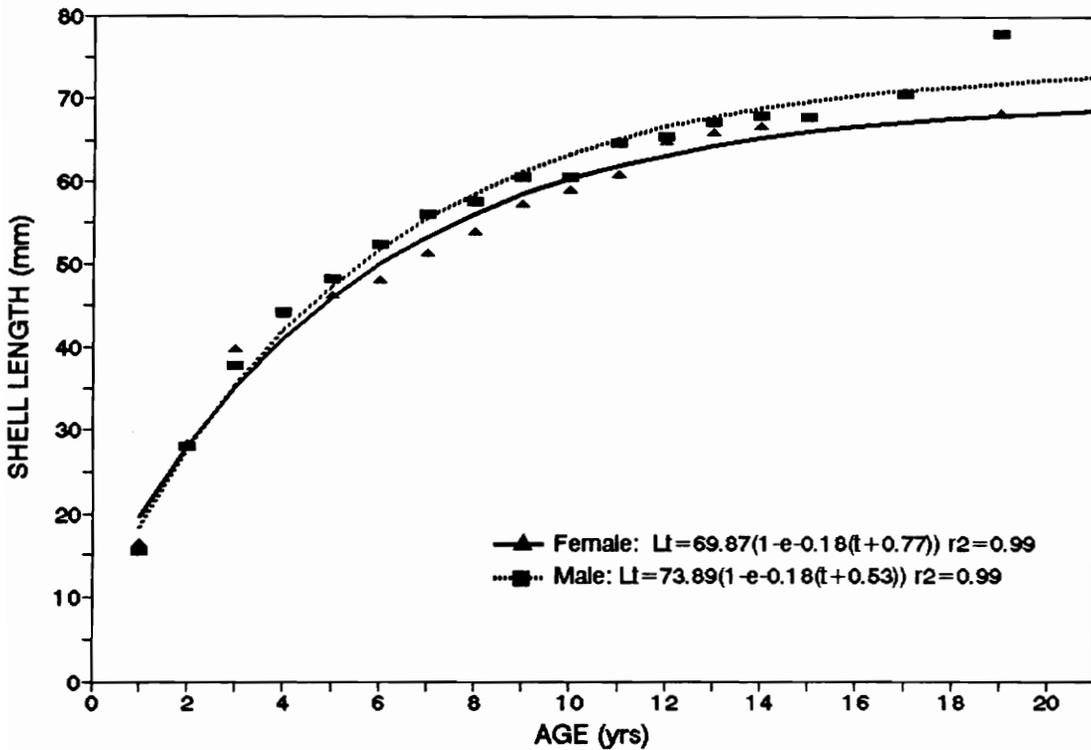


Figure 2.12: Comparison of growth rates of male (N=20) and female (N=15) *Lampsilis fasciola* at Kyles Ford in the Clinch River (KM 304). Symbols indicate mean observed shell lengths and lines indicate predicted shell lengths.

## Chapter Three

### AGE-FREQUENCY DISTRIBUTIONS

#### Abstract:

Age-frequency distributions of 6 freshwater mussel species were constructed such that periods of recruitment and mortality rate of adults were determined at 4 sites in the upper Clinch River. Muskrat midden samples were used for all analyses; therefore, the degree of muskrat predation bias for small and large mussels was evaluated. Periods of recruitment were determined by comparing age-frequency distributions among sites, and annual mortality was determined by the Robson and Chapman (1961) mortality rate estimator. Age-class frequencies at Pounding Mill and Kyle's Ford indicated reduced recruitment for most species between 1982 to 1984 and comparatively improved recruitment in recent years. A "die-off" of freshwater mussels was documented in 1983 in the Powell River, a tributary of the Clinch River, suggesting that a similar situation may have occurred in the Clinch River resulting in an absence of cohorts at that time. Age-class frequencies at Slant revealed absence of recruitment beginning in the late 1970's for all four species examined. The abrupt decline in recruitment

was possibly due to siltation by erosion of stream banks in the Cove Creek watershed, upstream of the Slant site. Mortality rates of all species examined throughout the river were high, 36 to 52%; however, the cause of fatality in adult mussels is unknown. Muskrat predation bias was shown for small and large mussel shells. Age-classes less than 5 years showed lower abundance than other age classes in muskrat middens and significantly lower frequency when compared to the instream population of *Villosa iris*. It was concluded that mussels less than 5 years old are apparently less available to predators by occupying different habitat than the adults or by burrowing deeper into the sediments. Prey selectivity of larger, older mussels by muskrats was observed likely due to the inability of predators to manipulate shells greater than ~ 70 mm in length.

#### Introduction:

Successful recruitment of freshwater mussels depends upon: 1) the density of reproductively active adults, 2) availability and immunity of fish hosts, and 3) sensitivity of juveniles to perturbations. First, female adult mussels siphon sperm from the water column, enabling internal fertilization to

occur (Fuller 1974). Once the density of adult mussels declines, the probability of fertilization is decreased and a drop in recruitment may follow.

Secondly, fish host specificity is narrow for most mussel species, with as few as one species in a particular stream (Zale and Neves 1982). In addition, survival of glochidia is decreased when parasitized on older (> 2 years) fish hosts that have developed immune responses (Bauer 1988). Therefore, unless the appropriate fish hosts are present, the reproductive cycle of the mussels is interrupted and densities decline.

Lastly, juveniles likely excyst in riffles where fish hosts reside and are carried to depositional areas behind rocks, boulders, or along stream banks. Mortality is high during this life stage, presumably from the inability to find suitable substratum (Neves and Widlak 1987). Juvenile mussels are more sensitive to certain toxicants than are adults. For example, lethal concentrations ( $LC_{50}$ ) of copper in the water column to juvenile and adult *Villosa nebulosa* (= *V. iris*) indicated that juveniles were significantly more sensitive ( $LC_{50} = 83 \mu\text{g/L}$ ) than adults ( $LC_{50} > 1000 \mu\text{g/L}$ ) (Jacobson 1990); therefore, suitable habitat may be more narrowly defined for juvenile mussels than adults.

Age-frequency distributions provide a means to determine periods of recruitment of mussels which indicates the suitability of habitat for settlement and appropriate densities of reproductive mussels and fish hosts. Age-

frequency distributions also provide a means to determine mortality of adult mussels. Mortality of adults is due to natural causes or to a number of anthropogenic effects such as impoundment, farming, eutrophication and predation (Ortmann 1918, Bates 1962, Negus 1966, Neves and Zale 1982, Aldridge et al. 1987). Mortality and survival estimates assume constant recruitment of juveniles among age classes (Robson and Chapman 1961); however, recruitment of juvenile mussels is irregular (Negus 1966, Miller and Payne 1988), causing an irregular distribution of age classes. In order to determine mortality for mussels, some age classes must be grouped to remove the variability of recruitment (Robson and Chapman 1961).

The objectives of this phase of the study were: 1) to determine the first age class of each mussel species depredated by muskrats, 2) to examine periods of recruitment of juveniles at each of the 4 study sites, and 3) to determine mortality rates of adults at each study site.

#### **Materials and Methods:**

Shells collected in muskrat middens provided by R. J. Neves, J. L. Farris, G. W. Church and recently collected midden shells were used for age determinations of mussel assemblages. Middens used for age-frequency determinations were collected from Pounding Mill at Clinch River Kilometer (CRK) 527 during the summers of 1992 and 1993, from Hackneys (CRK 433.7)

in fall 1988 and summers of 1992 and 1993, from Slant (CRK 359.3) during 1986, and from Kyle's Ford (CRK 304) in fall 1991 and summers 1992 and 1993.

Three to six freshwater mussel species; *Medionidus conradicus* (Cumberland moccasinshell), *Villosa iris* (rainbow), *Lampsilis fasciola* (wavy-rayed lampmussel), *Fusconaia cor* (shiny pigtoe), *Actinonaias pectorosa* (pheasantshell), and *Elliptio dilatata* (spike) were removed from middens at these sites and measured anterior to posterior length to the nearest 0.01 mm. An age-length key was computed for each species per site based on shells aged by thin-sectioning (Moyer 1984).

Age-class distributions were constructed for visual comparisons of recruitment, and mean annual mortality was determined with 95% confidence intervals. Mortality was determined using the Robson and Chapman (1961) estimator:

$$\text{mortality} = 1 - T / \sum N_x + T - 1$$

$$\text{variance} = (T / \sum N_x + T - 1) ((T / \sum N_x + T - 1) - (T - 1 / \sum N_x + T - 2))$$

where  $N_x$  is the abundance of each successive age class (x) and  $T = N_1 + 2N_2 + 3N_3 + \dots + xN_x$ . This estimator assumes constant cohort recruitment and survival rate, and equal vulnerability to muskrat predation (Hove and Neves 1994). Therefore, age classes were grouped by two to five year cohorts to compensate for irregular recruitment, and cohorts less than 5 years of age were

eliminated from examination to avoid the influence of muskrat predation bias (Robson and Chapman 1961). Statistically significant differences were determined based on no overlap of confidence intervals between sites.

Age distribution of a 1993 instream sample was determined for *Villosa iris* at Pounding Mill and compared to the age distribution of 1993 muskrat midden samples. On September 1, 1993 a 4 X 4 m grid, divided into four 1 X 4 m lanes, was placed in a sand and gravel substratum and held in place with tent stakes. The quadrat was snorkeled and substratum removed to a depth of ~ 15 cm. Mussels collected were identified, enumerated, measured anterior to posterior shell length, returned to the quadrat, and later aged using an age-length correlation key determined previously. The same instream snorkeling methods were used at the Slant site on October 21, 1993.

Vulnerability of age classes to muskrat predation was determined for *V. iris* at Pounding Mill by comparing the instream population to specimens from the muskrat midden sample. Frequencies of age classes from 1993 muskrat midden collections were compared to frequencies of age classes from 4 pooled lanes in the 1993 instream sample by a Chi-square test for age-classes 3 through 13 (SAS 1990). Frequencies between age classes among all other species at each site were compared from muskrat midden collections, and predicted shell lengths at ages examined (Appendix B). The assumed youngest preyed upon age class was compared among mussel species.

## Results:

### Muskrat predation

Comparisons between age classes of *V. iris* in muskrat middens and instream snorkel samples revealed some predation selectivity for particular age classes of mussels. Few 3 year old individuals were found by either sampling method (Figure 3.1). The 4 year old age class was found in significantly higher abundance in the instream sample than in the muskrat midden sample ( $0.010 < P < 0.025$ ). There were no significant differences in the abundances of 5, 6, 7, and 9 year age classes among instream and muskrat midden samples ( $P > 0.100$ ). Age classes 8, 10, 11, 12, and 13 all showed significantly greater abundances in the muskrat midden samples than in the instream samples ( $0.050 < P < 0.100$ ) (Table 3.1).

The youngest age classes fully preyed upon by muskrats from midden collections at Pounding Mill, Hackneys, and Kyle's Ford appeared to be between 4 and 6 years for all species where shells were available. The abundance of age classes for *F. cor* at Hackneys was too low and skewed toward older individuals to determine the youngest preyed upon age class. The age distribution at Slant was also skewed toward older individuals for all species examined, so youngest preyed upon age classes could not be determined. The smallest depredated size class varied from ~30 mm for

*Medionidus conradicus* and *Villosa iris* to ~50 mm for *Actinonaias pectorosa* (Appendix B).

Age distribution of the larger-shelled species, *E. dilatata* and *A. pectorosa*, indicated muskrat selectivity against the older, larger individuals. Frequency of age classes greater than 12 years for *E. dilatata*, shell lengths  $\geq 68$  mm, was significantly greater at Slant (76.9%) than at Hackneys (17.6%) ( $X^2 = 18.6$ ,  $P > 0.005$ ) (Table 3.2). The frequency of age classes greater than 7 years for *A. pectorosa*, shell lengths  $\geq 71$  mm, was also significantly greater at Slant (95.4%) than at Hackneys (26.3%) ( $X^2 = 19.6$ ,  $P > 0.005$ ) (Table 3.2).

#### Recruitment

Periods of recruitment were determined for three mussel species, *Medionidus conradicus*, *Villosa iris*, and *Lampsilis fasciola* at Pounding Mill using shells aged by thin-sectioning and age-length correlation keys from the 1992 sampling year (Table 3.3). The age distributions of two of three mussel species examined at Pounding Mill were skewed toward younger age classes. Approximately 81% of *M. conradicus* cohorts were recruited from 1984 to 1988 (Figure 3.2), and approximately 72% *V. iris* cohorts were recruited from 1983 to 1987 (Figure 3.3). *L. fasciola* showed an absence of recruitment since 1986, and prior to 1986 recruitment was low (Figure 3.4).

Periods of recruitment were determined for six mussel species, *Medionidus conradicus*, *Villosa iris*, *Lampsilis fasciola*, *Elliptio dilatata*,

*Actinonaias pectorosa*, and *Fusconaia cor*, at Hackneys using shells aged by thin-sectioning and age-length correlation keys from the 1988 sampling year (Table 3.3). The age distribution, based on muskrat middens of most mussel species examined, showed substantial recruitment of juveniles for 13 to 18 years prior to the 1988 collection date. With the exception of a few years, recruitment was good for *M. conradicus* after 1969 (Figure 3.5), for *V. iris* (Figure 3.6) and *L. fasciola* (Figure 3.7) after 1970, and for *E. dilatata* after 1974 (Figure 3.8). The age distribution for *A. pectorosa* showed few individuals recruited prior to 1980 (Figure 3.9). *F. cor* had a few years of high recruitment, 1964-66 and 1978-79, but overall abundance was low (Figure 3.10).

Periods of recruitment were determined for four mussel species, *Lampsilis fasciola*, *Actinonaias pectorosa*, *Elliptio dilatata*, and *Fusconaia cor*, at Slant using shells aged by thin-sectioning and age-length correlation keys from the 1986 sampling year (Table 3.3). The age distributions of all species examined at Slant were largely skewed toward older age classes. Age distributions of *L. fasciola* (Figure 3.11) and *A. pectorosa* (Figure 3.12) show an absence of year classes since 1979. *E. dilatata* had high recruitment prior to 1978, but since 1979 had essentially no recruitment of juveniles (Figure 3.13). *F. cor* showed an absence of year classes since 1977 (Figure 3.14). Five individual mussels were found in 4 X 4 m quadrat during instream

snorkeling. All mussels were the same species, *Actinonaias ligamentina*, and were greater than 95 mm in length.

Periods of recruitment were determined for five mussel species, *Medionidus conradicus*, *Villosa iris*, *Elliptio dilatata*, *Lampsilis fasciola*, and *Actinonaias pectorosa*, at Kyle's Ford using shells aged by thin-sectioning and age-length correlation keys from the 1991 sampling year (Table 3.3). The age distributions of all mussel species examined at Kyle's Ford were skewed toward younger age classes. *M. conradicus* had ~78% (Figure 3.15) and *L. fasciola* ~84% (Figure 3.16) of their cohorts recruited from 1982 to 1987. *V. iris* had ~88% (Figure 3.17) and *E. dilatata* had ~75% (Figure 3.18) of their cohorts recruited from 1982 to 1986. *A. pectorosa* had ~79% of the cohorts recruited from 1984 to 1986 (Figure 3.19).

### Mortality

Mean annual adult mortality was determined by thin-sectioning and using age-length correlation keys for all sampling years at Pounding Mill, Hackneys, and Kyle's Ford (Table 3.3). Mortality was not calculated for *F. cor* at Hackneys or for any species at Slant.

Mortality was determined for *M. conradicus* adults from 5 to 20 years of age grouped by 1 to 4 year age increments at Pounding Mill, Hackneys, and Kyle's Ford (Table 3.4a). Mean annual mortality at Pounding Mill, 52.2% ( $\pm 7.2$ ), was significantly greater than at Hackneys which had 36.6% ( $\pm 5.5$ );

however, neither was significantly different from Kyle's Ford, 37.4% ( $\pm 8.0$ ) at the 95% confidence level (Table 3.5).

Mortality was determined for *V. iris* adults from 6 to 20 years of age grouped by 1 to 4 year age increments at Pounding Mill, Hackneys, and Kyle's Ford (Table 3.4b). There were no significant differences in mean annual mortality between Pounding Mill, 47.7% ( $\pm 5.9$ ), Hackneys 46.0% ( $\pm 10.9$ ), and Kyle's Ford 51.2% ( $\pm 15.4$ ) (Table 3.5).

Mortality was determined for *A. pectorosa* adults from 4 to 15 years of age at Hackneys and Kyle's Ford grouped by 1 to 3 year increments at each site (Table 3.4c). There were no significant differences in mean annual mortality at Hackneys, 48.9% ( $\pm 6.0$ ), and Kyle's Ford, 43.4% ( $\pm 4.4$ ) (Table 3.5).

Mortality was determined for *E. dilatata* adults from 5 to 20 years of age at Hackneys and Kyle's Ford grouped by 1 to 3 year increments at each site (Table 3.4d). No significant differences of mean annual mortality were shown at Hackneys, 36.4% ( $\pm 3.4$ ), and Kyle's Ford, 49.9% ( $\pm 11.6$ ) at the 95% confidence level (Table 3.5).

Mortality was determined for *L. fasciola* adults from 5 to 20 years of age at Pounding Mill, Hackneys and Kyle's Ford grouped by 1 to 4 year increments at each site (Table 3.4e). There were no significant differences in mean annual

mortality at Pounding Mill, 43.9% ( $\pm 15.7$ ), Hackneys, 40.5% ( $\pm 4.9$ ), and Kyle's Ford, 38.7% ( $\pm 8.2$ ) (Table 3.5).

#### Discussion:

##### Muskrat predation

The lower limit of prey selectivity by muskrats was found to be more closely related to mussel age than shell size for particular species. It has been previously thought that predators of freshwater mussels are size selective probably due to their handling capacity or visual ability (Neves and Odom 1989, Berrow 1991). However, comparison of predation among different sized mussel species provided contradictory results.

*M. conradicus*, *V. iris*, *F. cor*, and *L. fasciola* are smaller mussel species ( $L_{\infty} \leq 80$  mm) than *A. pectorosa* and *E. dilatata* ( $L_{\infty} > 80$  mm) (Chapter Two). The youngest depredated age class ranged between 4 to 6 years for all mussel species examined, but the smallest preyed upon shell size varied by  $\sim 20$  mm, depending on mussel species. In addition, the theory of selective predation against smaller bivalves due to shell size is refuted by the presence of Asian clams (*Corbicula fluminea*) predominating muskrat middens in the Clinch River. The Asian clam rarely exceeds a maximum shell length of 20 mm in the Clinch River (Cherry et al. 1993), but, due to the active nature and abundance of

Asian clams, muskrats have little difficulty foraging on them (Neves and Odom 1989).

Muskrats will select food, plants, crayfish, and mussels, which are readily available in their foraging range at specific times during the year (Butler 1940, Schwartz and Schwartz 1981). Juvenile mussels seemingly occupy habitats different from adults, and after several years may seek out the adult habitats (Neves and Widlak 1989). Therefore, it is possible that muskrats are not foraging in juvenile mussel habitats. The age at which young adults begin to migrate to adult habitat is unknown, but may be related to onset of sexual maturity. Most mussel species reach sexual maturity by 3 to 5 years of age (van der Schalie and van der Schalie 1963, Stein 1969, Zale and Neves 1982), at which time they must be aggregated for reproductive success. In addition, once juveniles move to the main riffles, they are often found more deeply burrowed in the sediment than the adults (personal observation).

Finally, muskrat middens are most commonly found during the summer and early fall (personal observation), when stream flow is low and mussels are spawning. During the spawning season, mussels are close to the surface and easily removed from the substratum. Adult mussels, usually greater than 4 years of age, found actively spawning are more readily available, thus, particularly vulnerable to muskrat predation. The 1 to 3 year old juveniles are in depositional habitats where muskrats may not forage and, as shown by the

instream snorkel sample, the 4 year old juveniles are burrowed deep in the sediment. Due to reduced encounters, these age classes ( $\leq 4$  years old) are often absent from muskrat middens.

In contrast to reduced predation by muskrats on young mussels due to limited encounters, reduced predation of older mussels by muskrats is likely due to the inability of muskrats to manipulate larger mussel shells. The larger mussel species, *A. pectorosa* and *E. dilatata*, show an absence of older, larger individuals in muskrat middens when medium size classes are available. In fact, large, live mussels are often found near muskrat midden piles apparently discarded by the predator (personnel communication with J. L. Farris). At Hackneys, muskrats selected the younger, medium-sized individuals, but at Slant, mussels less than  $\sim 70$  mm were not readily available so the muskrats preyed on larger size classes (Chapter Two).

The shell length of *E. dilatata* at 13 years of age is 71 - 74 mm at Hackneys and Slant (Appendix B); however, the frequency of individuals greater than 12 years was significantly greater at Slant than at Hackneys (Table 3.2). The shell length of *A. pectorosa* at 8 years is approximately 77 mm at Hackneys and Slant (Appendix B), and the frequency of individuals greater than 7 years was significantly greater at Slant than at Hackneys (Table 3.2). Therefore, muskrats apparently avoided mussel shells greater than  $\sim 70$  mm for both species at Hackneys.

## Recruitment

Age-class frequencies of mussel species at Pounding Mill and Kyle's Ford were largely skewed toward younger age classes. Both sites showed absence of year classes for most species prior to 1982 to 1984 and comparatively high frequencies of year classes in recent years. Documented die-offs of freshwater mussels were reported from rivers and lakes of the eastern United States between 1977 and 1986. In 1983 a die-off was reported in the Powell River, a tributary of the Clinch River; however, the cause of the die-off was undetermined (Neves 1987). Approximately 5% of the mussel assemblage in the Powell River was said to be affected by the die-off (Ahlstedt and Jenkinson 1987). Although the Clinch River was not identified as one of the rivers exhibiting a mussel die-off, the source which created the die-off in the Powell River may also have reduced the recruitment or removed adults in the Clinch River as shown by the absence of cohorts between 1982 and 1984.

At the Slant site, there is an absence of cohorts since 1977 to 1979 for all four species examined. Slant is located less than 5 km downstream of The Nature Conservancy mussel preserve at Pendleton Island. Pendleton Island is a very diverse, dense mussel assemblage in the Clinch River (Dennis 1989); however, just 4.8 km downstream few adults remain and recruitment of juveniles has ceased.

Coal mining activities were investigated as possible impact sources, but relatively little mining activity occurs in this stretch of the river. Active coal mines were evaluated under the Surface Mine Control Regulation Act (SMCRA) in October 1979 and more rigorous regulations implemented. Therefore, water quality should have improved during the late 1970's to early 1980's. In addition, pollutant discharge permits were investigated to identify new sewage treatment facilities or other industries; however, no significant input was identified along this stretch. It was thus concluded that an isolated, yet abrupt, decline in recruitment was caused by increased siltation from eroding stream banks in the Cove Creek watershed.

Cove Creek is a tributary of the Clinch River less than 1.5 km above the Slant site. Much of the Cove Creek watershed occurs in the Jefferson National Forest, and greater than 3000 acres have been logged over the past 100 years. Continuous Inventory of Stand Conditions (CISC) documents provided by Jefferson National Forest Clinch River Ranger District showed that much of the logging activities in this area included clearcut stands, several of which line the banks of Cove Creek. Therefore, it is possible that clearcutting activities may have caused bank erosion and increased siltation of Cove Creek which flowed downstream to the Clinch River. More importantly, however, several hundred meters above the mouth of Cove Creek, land was clearcut to the edge of the stream as farming pastures, and serious erosion of stream banks was observed

(Figure 3.20). The sediment in Cove Creek was fine silt, several inches deep, which may be carried downstream into the Clinch River where a thick silty layer of sediment in the stream and deposits of sediment along the flood plain were observed at the Slant site (Figure 3.21). Negative effects of increased siltation from dam construction, barge traffic, dredging, and recreational activities is well documented (Fuller 1974, Rosenberg and Henschen 1986, Aldridge et al. 1987). Therefore, siltation from clearcutting or poor farming activities in the Cove Creek watershed appear to be possible causes of decline in recruitment of mussels at the Slant site. Instream sampling confirmed the continual decline of the mussel assemblage at this site. Only one species was found during the 1993 instream sampling, and all were large individuals. Ahlstedt (1986) found 23 species and a total of 365 individuals at this site. Unfortunately, he did not measure shell length, so a decline in recruitment was not noted at that time.

### Mortality

The percent mortality of all evaluated species was very high at each site. A few populations showed greater than 50% mortality of adults, while all others had at least 36%. Mortality was not determined for *F. cor*, a federally endangered species, due to the low abundance of each age class. Mortality was not determined for any species at Slant for lack of young adults less than 8 years.

Mortality rates were determined from muskrats midden samples at each site based on total abundance of age classes. It was shown that selective predation exists against larger mussel shells (>72 mm); therefore, the avoidance of larger, older individuals of large species may create falsely high mortality rates. *A. pectorosa* reaches 70 mm by ~7 years of age and *E. dilatata* reaches 70 mm by ~11 years of age at Hackneys (Appendix B). Hence, the high mortalities for these species may be an artifact of size selectivity in muskrat midden samples.

*M. conradicus* and *V. iris*, however, rarely exceed a shell length of 70 mm in the Clinch River. The comparison between muskrat middens and instream snorkel samples for *V. iris* indicated some selective predation for the older (8 to 13 years), larger individuals which may show increased abundance of older age classes. Therefore, mortality of these two species should be considered an accurate representation or slight underestimation of instream adult mortality.

It has been well documented that the mussel fauna in the Clinch River is declining (Ortmann 1918, Bates and Dennis 1978, Neves and Zale 1982, Ahlstedt 1986, Bruenderman 1989, Dennis 1989, Neves and Odom 1989) and, according to the results of the current study, adult mortality is a large factor contributing to the decline throughout the river. Each of these studies has speculated about the poor water quality and habitat degradation in the Clinch

**River. Future studies need to focus on the sources impacting the mussel fauna and attempt to offer solutions to arrest freshwater mussel declines.**

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Table 3.1: Chi-squares (p-values) and standard errors of age classes of *Villosa iris* from muskrat midden samples (N = 79) compared to instream sampling (N = 94).

Age	N	$\chi^2$	Standard error
3	3	0.30 (P > 0.100)	0.29
4*	13	7.23 (0.010 < P < 0.025)	0.11
5	31	2.57 (P > 0.100)	0.09
6	34	0.61 (P > 0.100)	0.08
7	31	0.01 (P > 0.100)	0.09
8*	21	3.27 (0.050 < P < 0.100)	0.10
9	20	1.93 (P > 0.100)	0.10
10*	6	4.28 (0.025 < P < 0.050)	0.14
11*	4	6.63 (0.050 < P < 0.100)	0.11
12*	4	6.63 (0.050 < P < 0.100)	0.11
13*	6	4.28 (0.050 < P < 0.100)	0.14

\*denotes significant differences between sampling techniques

Table 3.2: Frequencies of age classes with shell length greater than ~71 mm (> 7 years) for *Actinonaias pectorosa* and greater than ~68 mm (> 12 years) for *Elliptio dilatata* at 2 study sites. Small size classes were available at Hackneys, but not at Slant. Chi-squares with p-values and standard errors for each species are provided.

Species	Hackneys	Slant	$\chi^2$	Standard error
<i>A. pectorosa</i>	26.3%	95.4%	19.6 (P > 0.005)	0.074
<i>E. dilatata</i>	17.6%	76.9%	18.6 (P > 0.005)	0.080

Table 3.3: Sample sizes of mussels aged for recruitment (columns 3&4) and mortality (columns 3&5) determinations.

Sites	Species	Number of mussels aged by thin section	Number of mussels aged by age/length key from 1 sampling year	Total number of mussels aged by age/length keys from all sampling years
Pounding Mill	<i>M. conradicus</i>	29	89 (1992)	93
	<i>V. iris</i>	40	125 (1992)	204
	<i>L. fasciola</i>	19	0 (1992)	0
Hackneys	<i>M. conradicus</i>	52	79 (1988)	80
	<i>V. iris</i>	49	0 (1988)	9
	<i>L. fasciola</i>	97	92 (1988)	158
	<i>E. dilatata</i>	55	268 (1988)	474
	<i>A. pectorosa</i>	53	84 (1988)	147
Slant	<i>F. cor</i>	43	30 (1988)	62
	<i>L. fasciola</i>	45	15 (1986)	15
	<i>E. dilatata</i>	45	129 (1986)	129
	<i>A. pectorosa</i>	27	23 (1986)	23
	<i>F. cor</i>	18	19 (1986)	19

Table 3.3 (con't): Sample sizes of mussels aged for recruitment (columns 3&4) and mortality (columns 3&5) determinations.

Sites	Species	Number of mussels aged by thin section	Number of mussels aged by age/length key from 1 sampling year	Total number of mussels aged by age/length keys from all sampling years
	<i>M. conradicus</i>	46	22 (1991)	46
	<i>V. iris</i>	33	0 (1991)	45
Kyle's Ford	<i>L. fasciola</i>	35	29 (1991)	47
	<i>E. dilatata</i>	33	8 (1991)	36
	<i>A. pectorosa</i>	38	192 (1991)	588

**Table 3.4a: Abundances of grouped age classes for mean annual mortality rate determinations of *Medionidus conradicus* at 3 study sites. Mortality was determined for ages 5 to 20 years at each site.**

Pounding Mill		Hackneys		Kyle's Ford	
Age	Abundance	Age	Abundance	Age	Abundance
2-4	16	2-4	18	2-4	13
5-6	41	5	31	5	19
7-8	40	6-7	28	6	13
9-11	11	8-10	21	7-8	8
12-13	7	11-13	16	9-10	6
14-15	1	14-16	10	11-13	4
16-17	1	17-20	7	14-15	3
18-20	0	---	---	16-17	2
---	---	---	---	18-20	0
<b>Total</b>	<b>117</b>		<b>131</b>		<b>68</b>

**Table 3.4b: Abundances of grouped age classes for mean annual mortality rate determinations of *Villosa iris* at 3 study sites. Mortality was determined for ages 6 to 20 years at each site.**

Pounding Mill		Hackneys		Kyle's Ford	
Age	Abundance	Age	Abundance	Age	Abundance
3-5	27	3-5	8	3-5	9
6-7	58	6-8	14	6	13
8-9	41	9-11	13	7-8	5
10-12	16	12-15	8	9-10	2
13-15	14	16-18	4	11-13	1
16-18	8	19-20	1	14-16	1
19-20	1	---	---	17-20	1
<b>Total</b>	<b>165</b>		<b>48</b>		<b>32</b>

Table 3.4c: Abundances of grouped age classes for mean annual mortality rate determinations of *Actinonaias pectorosa* at 3 study sites. Mortality was determined for ages 4 to 15 years at Hackneys and Kyle's Ford. Mortality was not determined at Slant due to the low abundances of young cohorts.

Hackneys		Slant		Kyle's Ford	
Age	Abundance	Age	Abundance	Age	Abundance
2-3	1	3-4	1	2-3	5
4-5	51	5-6	3	4-5	76
6	49	7-8	9	6	60
7	21	9-10	8	7	46
8-9	12	11-12	20	8-9	30
10-11	2	13-14	5	10-12	10
12-13	1	15-15 <sup>+</sup>	4	13-15	2
14-15	0	---	---	---	---
<b>Total</b>	<b>137</b>		<b>50</b>		<b>229</b>

**Table 3.4d: Abundances of grouped age classes for mean annual mortality rate determinations of *Elliptio dilatata* at 3 study sites. Mortality was determined for ages 5 to 20 years at Hackneys and Kyle's Ford. Mortality was not determined at Slant due to the low abundances of young cohorts.**

Hackneys		Slant		Kyle's Ford	
Age	Abundance	Age	Abundance	Age	Abundance
2-4	29	3-4	0	2-4	2
5-7	94	5-6	0	5-7	17
8	68	7-8	5	8-10	14
9-10	46	9-10	17	11-13	2
11-12	30	11-12	18	14-16	2
13-14	28	13-14	21	17-19	2
15-17	12	15-16	23	20	1
18-19	9	17-18	22	---	---
20	4	19-20	27	---	---
<b>Total</b>	<b>320</b>		<b>133</b>		<b>40</b>

Table 3.4e: Abundances of grouped age classes for mean annual mortality rate of *Lampsilis fasciola* at 4 study sites. Mortality was determined for ages 5 to 20 years at Pounding Mill, Hackneys and Kyle's Ford. Mortality was not determined at Slant due to the low abundances of young cohorts.

Pounding Mill		Hackneys		Slant		Kyle's Ford	
Age	Abund	Age	Abund	Age	Abund	Age	Abund
2-4	0	2-4	21	3-4	1	2-4	9
5-7	7	5-7	48	5-6	3	5-6	21
8-10	5	8-10	47	7-8	9	7	14
11-13	4	11-12	35	9-10	8	8	8
14-16	2	13-15	20	11-12	20	9-10	4
17-19	1	16-17	13	13-14	5	11-13	3
20	0	18-20	3	15-16	4	14-17	2
---	---	---	---	17-18	6	18-19	2
---	---	---	---	19-20	6	20	1
<b>Total</b>	<b>19</b>		<b>187</b>		<b>61</b>		<b>64</b>

Table 3.5: Percent mean annual mortality with a 95% confidence limit for 5 mussel species at 3 study sites in the upper Clinch River.

Species	Sites		
	Pounding Mill	Hackneys	Kyle's Ford
<i>M. conradicus</i>	52.2 ± 7.2%	36.6 ± 5.5%	37.4 ± 8.0%
<i>V. iris</i>	47.7 ± 5.9%	46.0 ± 10.9%	51.2 ± 15.4%
<i>A. pectorosa</i>	NA	48.9 ± 6.0%	43.4 ± 4.4%
<i>E. dilatata</i>	NA	36.4 ± 3.4%	49.9 ± 11.6%
<i>L. fasciola</i>	43.9 ± 15.7%	40.5 ± 4.9%	38.7 ± 8.2%

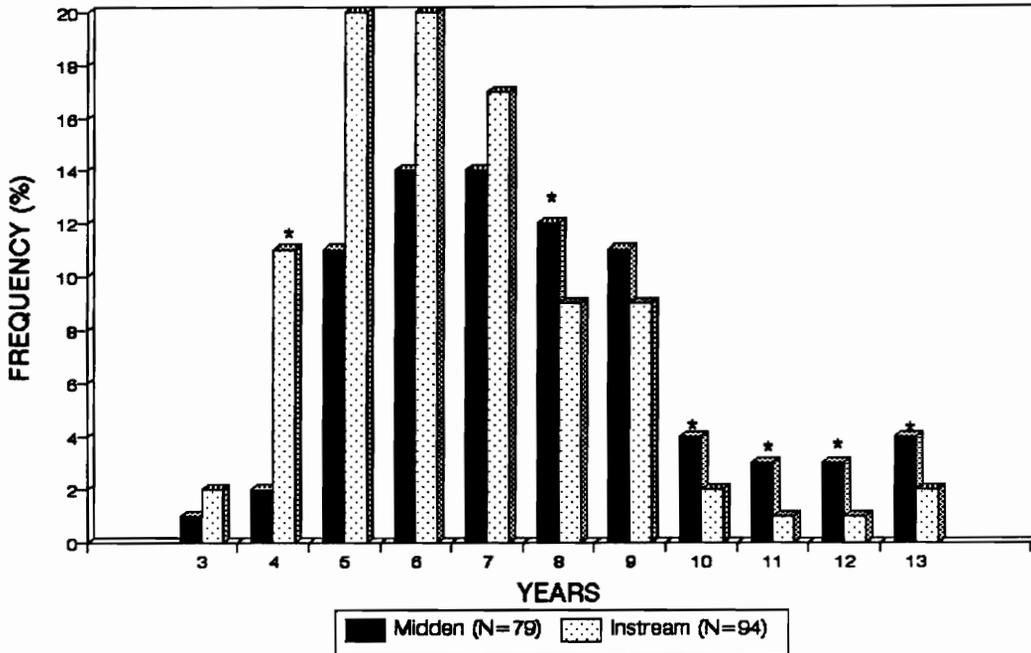


Figure 3.1: Comparison of age distribution between 1993 muskrat midsen samples and 1993 instream samples of *V. iris* at Pounding Mill (CRK 433.7).

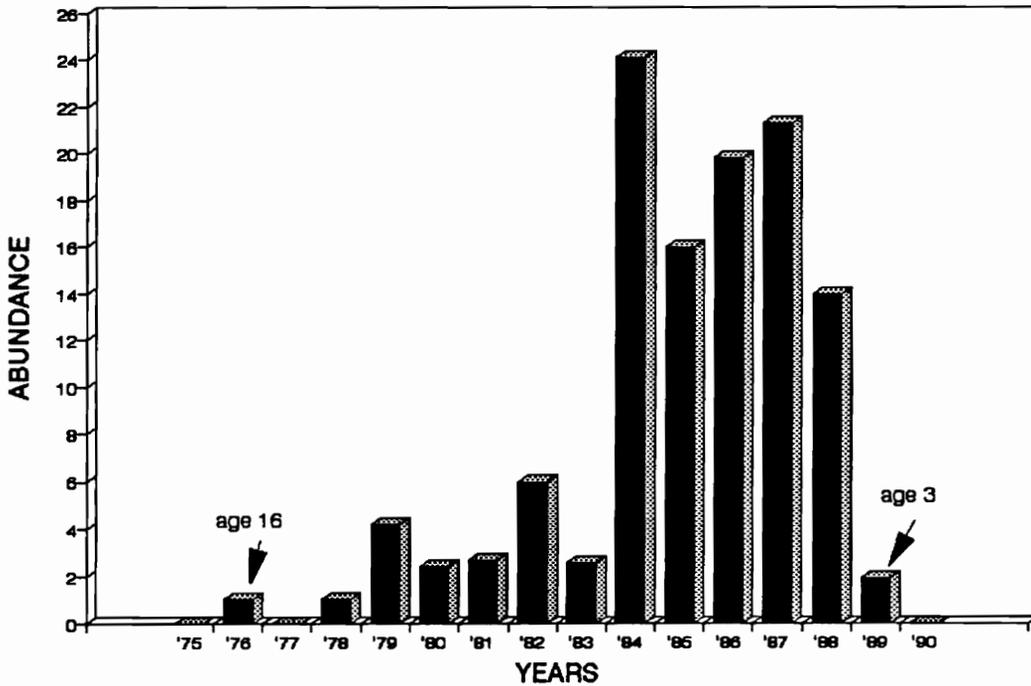


Figure 3.2: Year classes of *M. conradicus* from a 1992 collection at Pounding Mill (N=117).

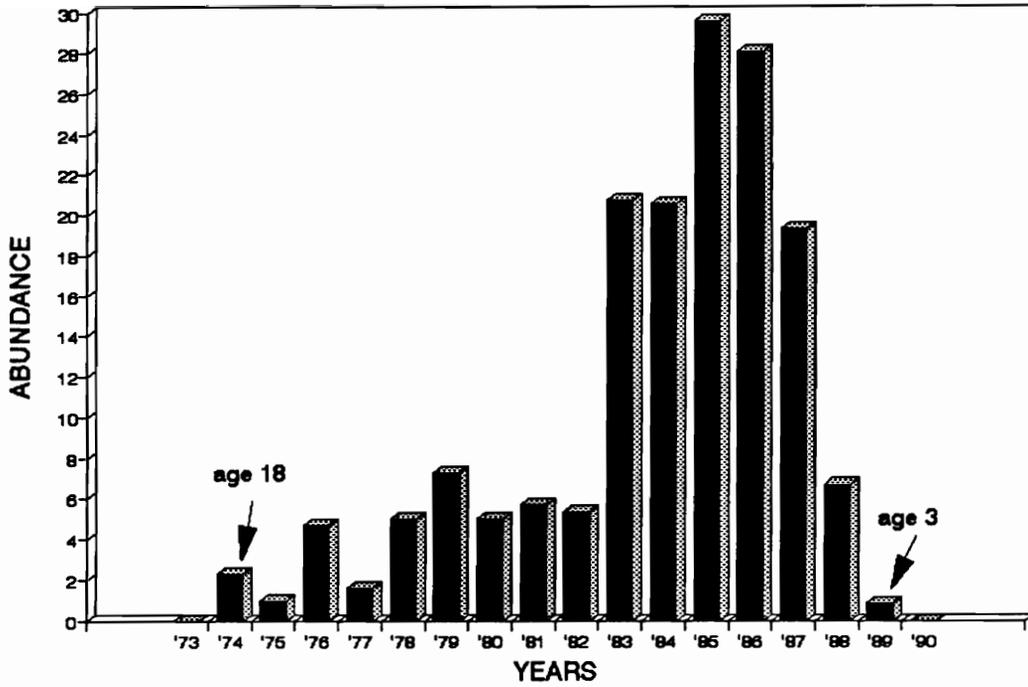


Figure 3.3: Year classes of *V. iris* from a 1992 collection at Pounding Mill (N=164).

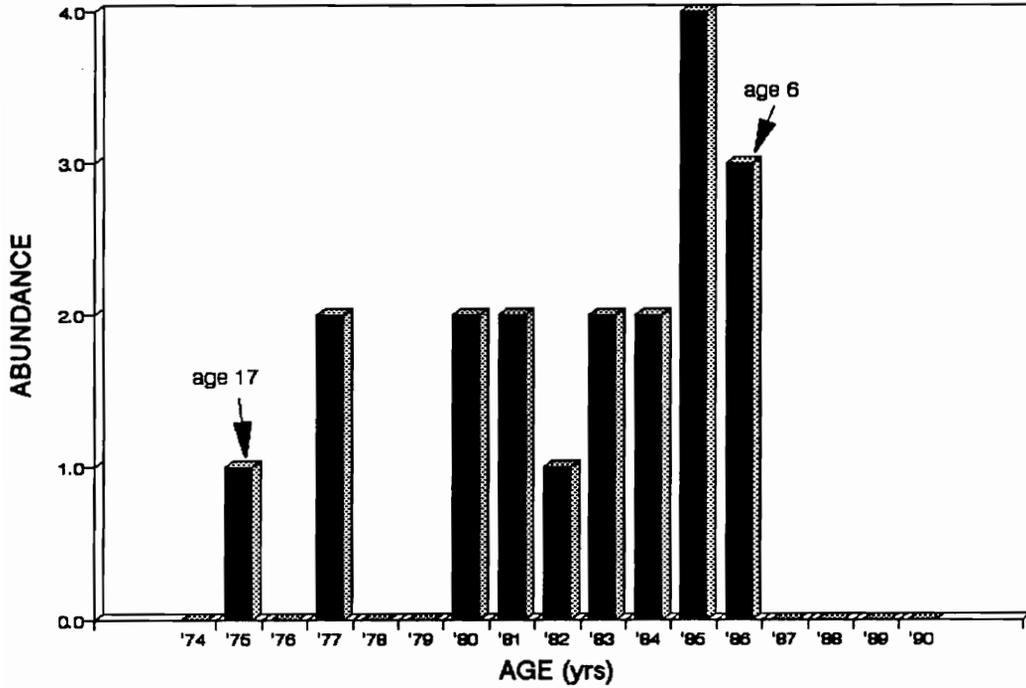


Figure 3.4: Year classes of *L. fasciola* from a 1992 collection at Pounding Mill (N=19).

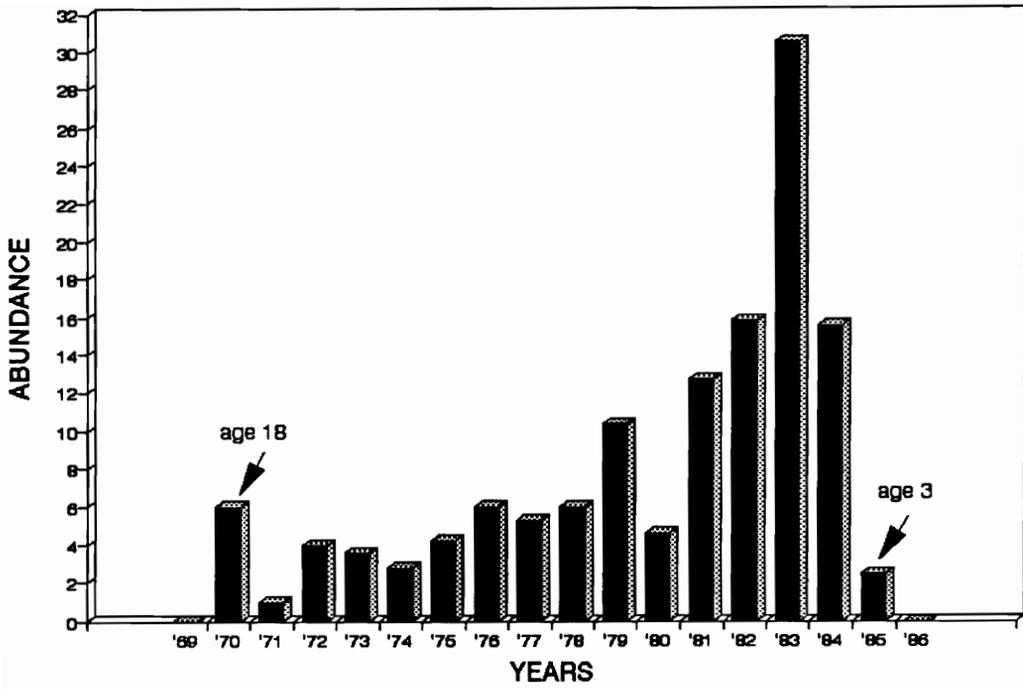


Figure 3.5: Year classes of *M. conradicus* from a 1988 collection at Hackneys (N=131).

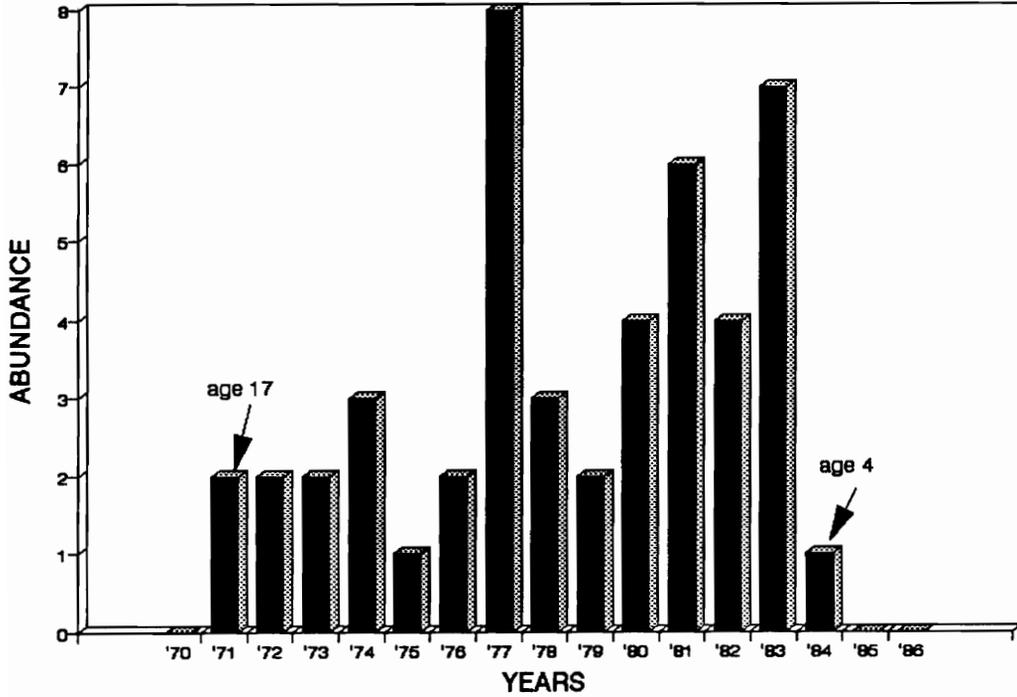


Figure 3.6: Year classes of *V. iris* from a 1988 collection at Hackneys (N=47).

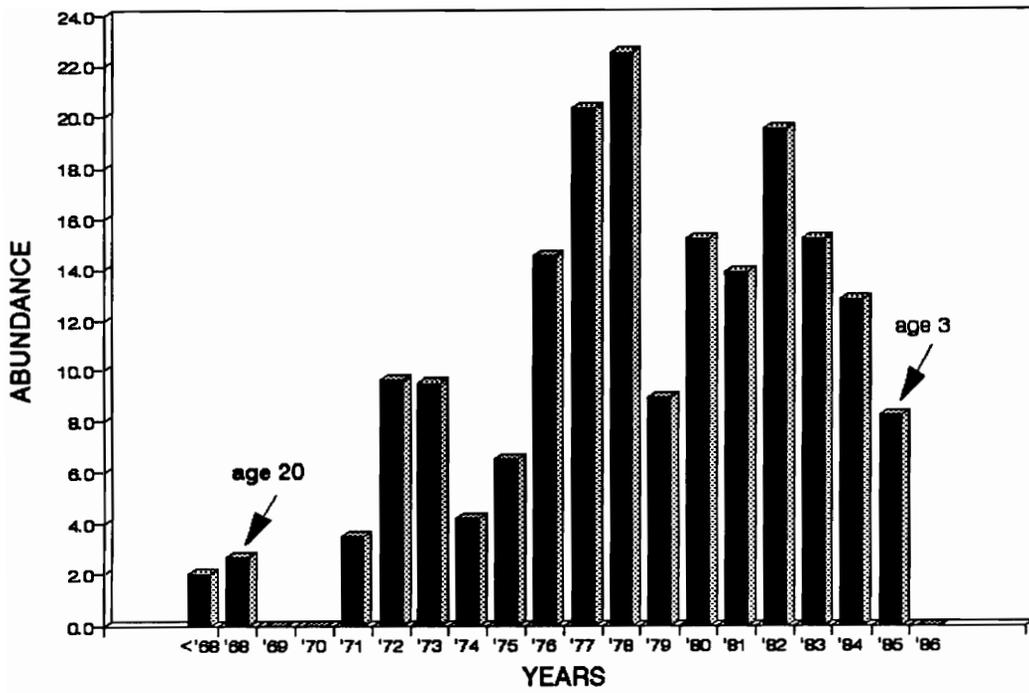


Figure 3.7: Year classes of *L. fasciola* from a 1988 collection at Hackneys (N=189).

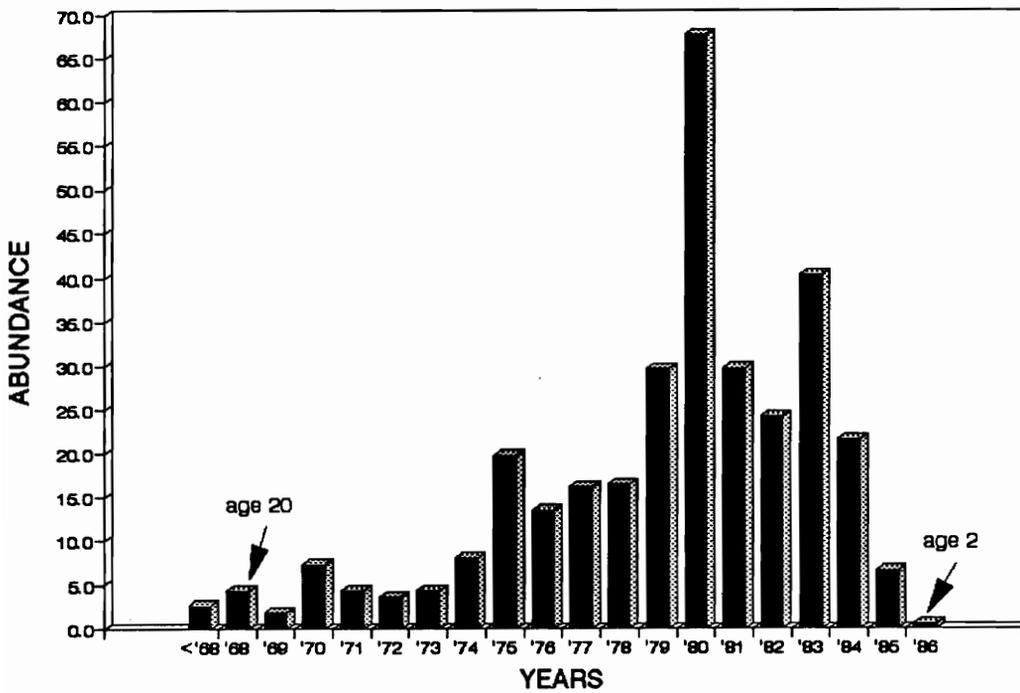


Figure 3.8: Year classes of *E. dilatata* from a 1988 collection at Hackneys (N=323).

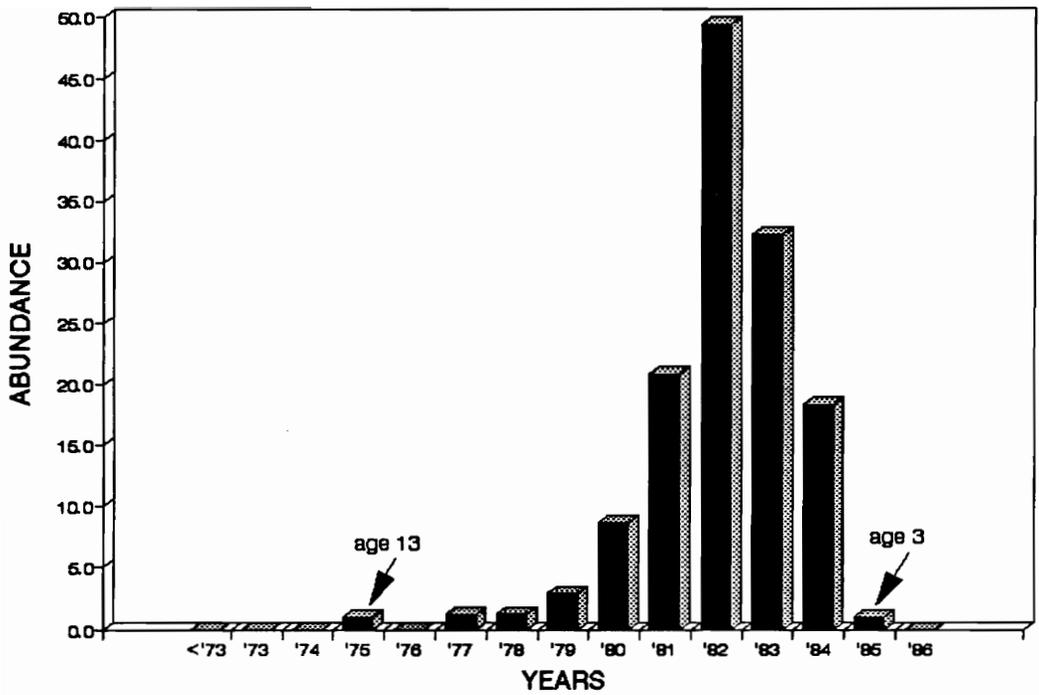


Figure 3.9: Year classes of *A. pectorosa* from a 1988 collection at Hackneys (N=137).

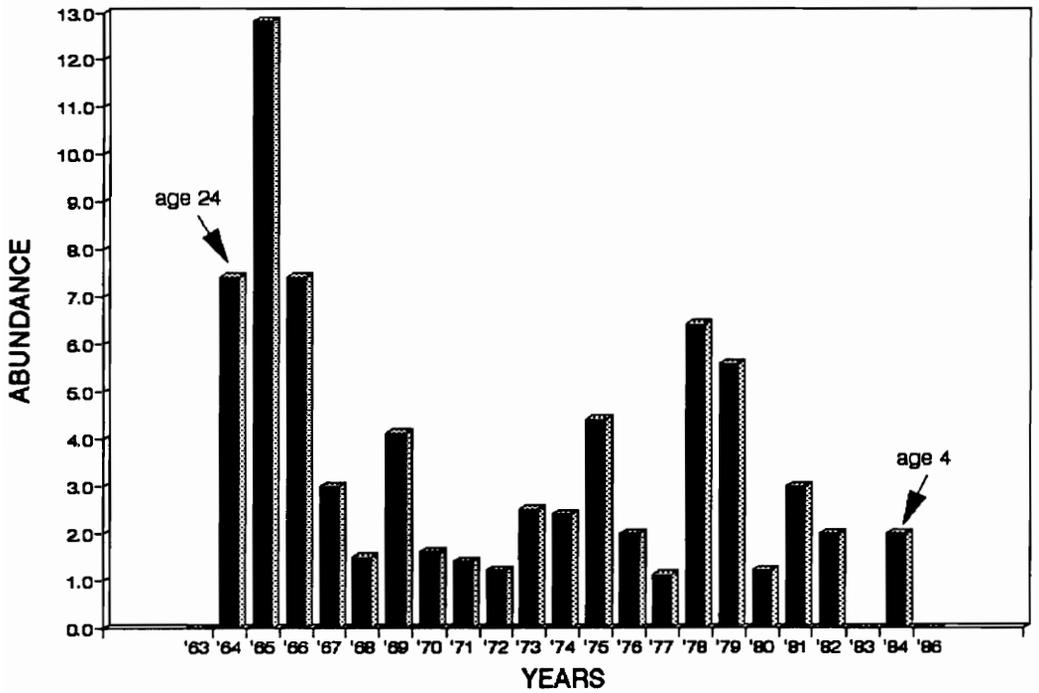


Figure 3.10: Year classes of *F. cor* from a 1988 collection at Hackneys (N=73).

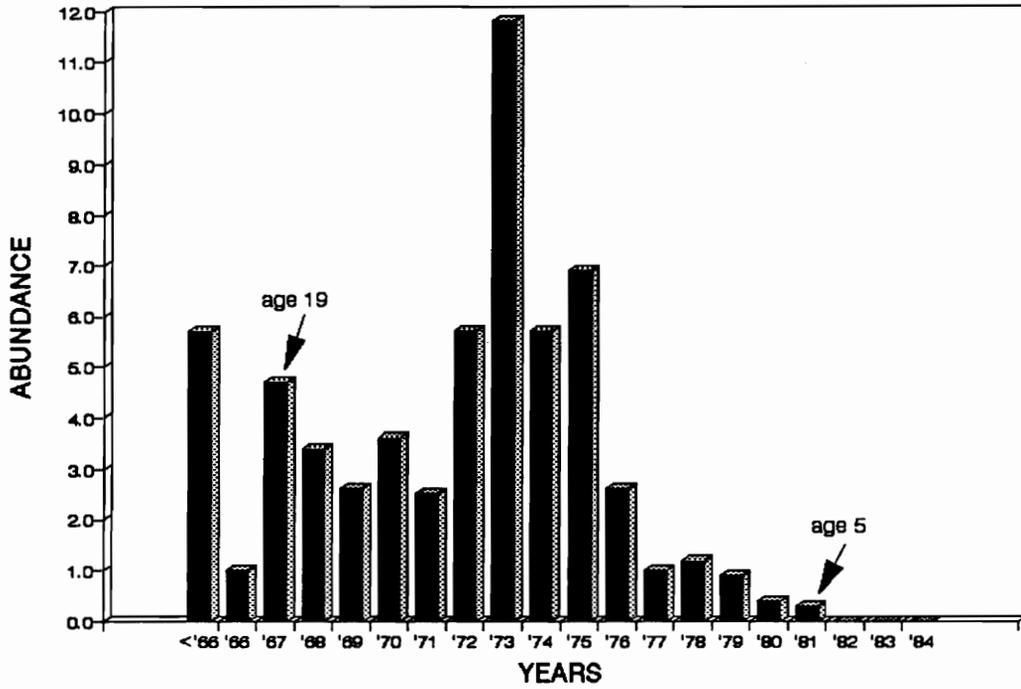


Figure 3.11: Year classes of *L. fasciola* from a 1986 collection at Slant (N=60).

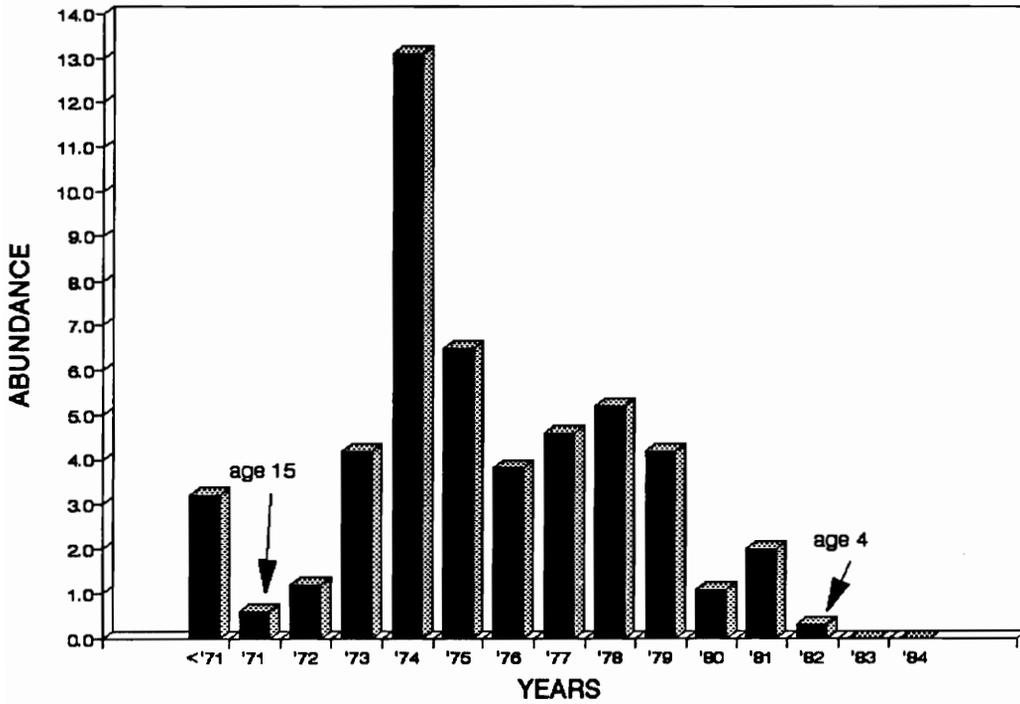


Figure 3.12: Year classes of *A. pectorosa* from a 1986 collection at Slant (N=50).

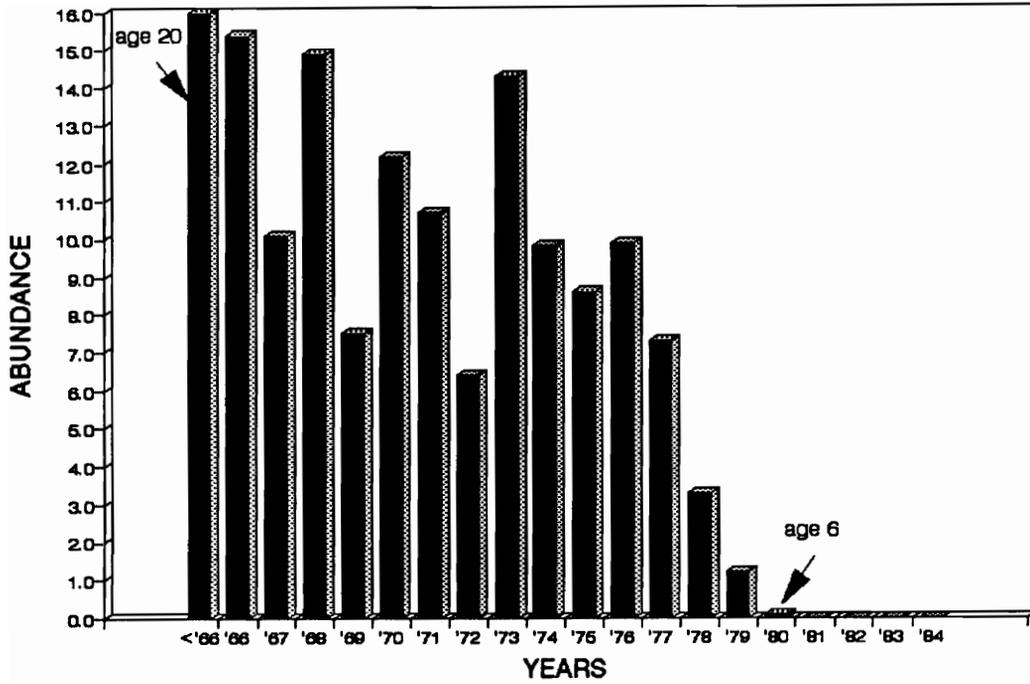


Figure 3.13: Year classes of *E. dilatata* from a 1986 collection at Slant (N=140).

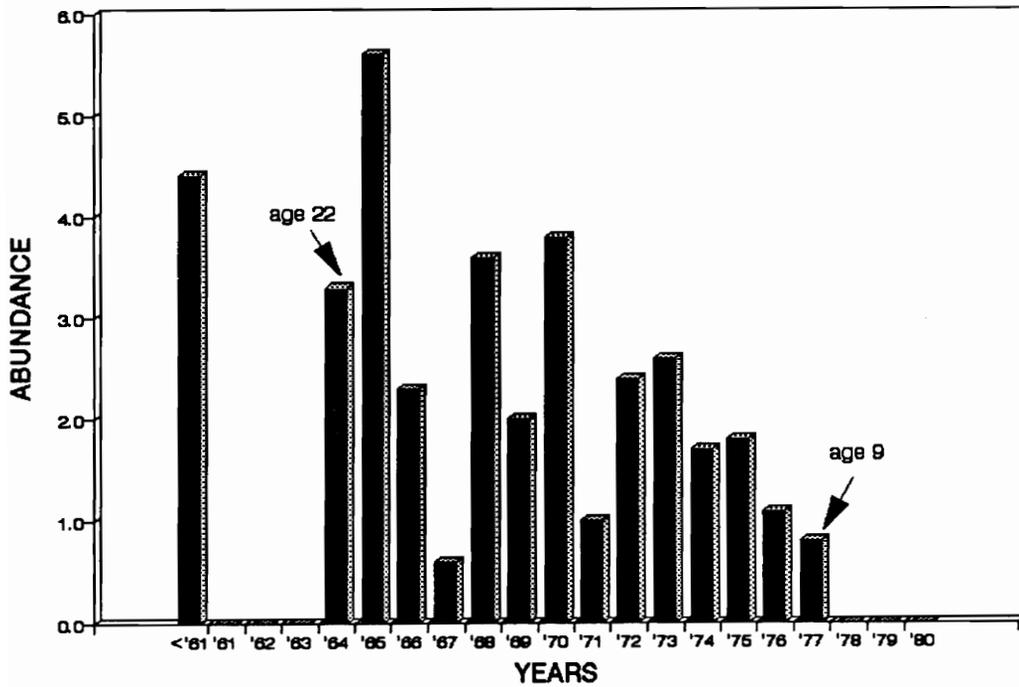


Figure 3.14: Year classes of *F. cor* from a 1986 collection at Slant (N=37).

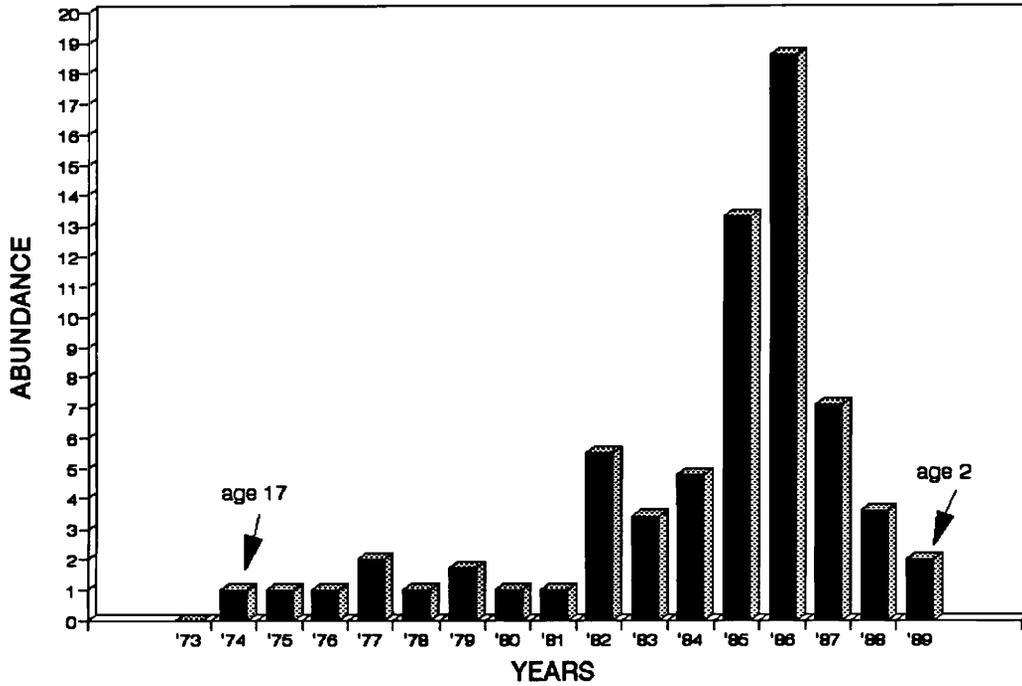


Figure 3.15: Year classes of *M. conradicus* from a 1992 collection at Kyle's Ford (N=68).

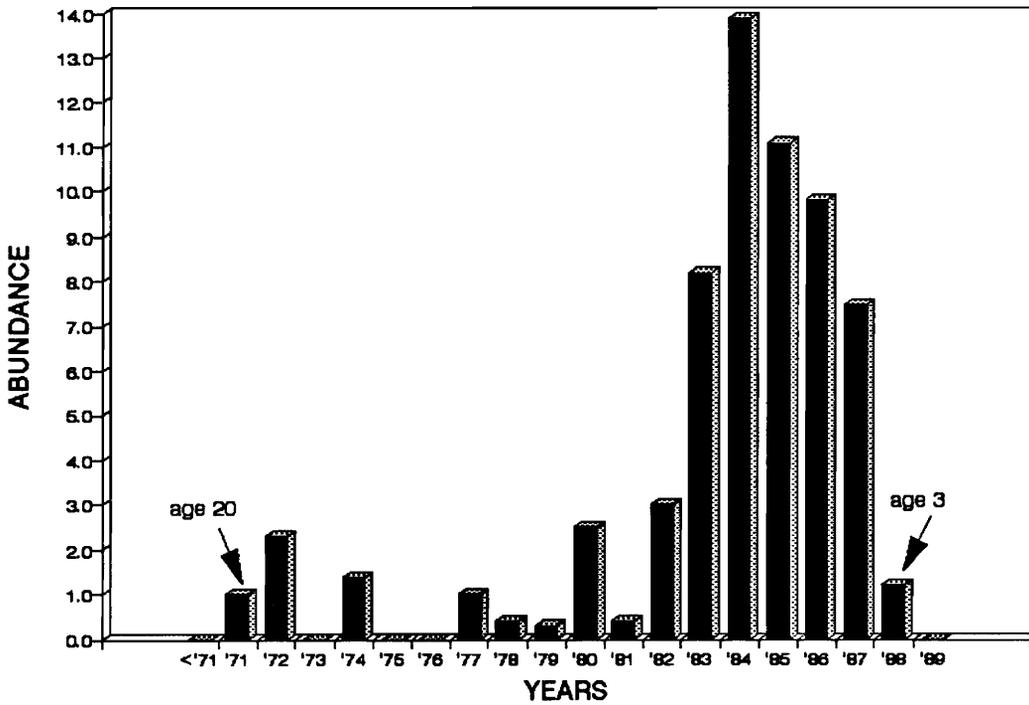


Figure 3.16: Year classes of *L. fasciola* from a 1991 collection at Kyle's Ford (N=64).

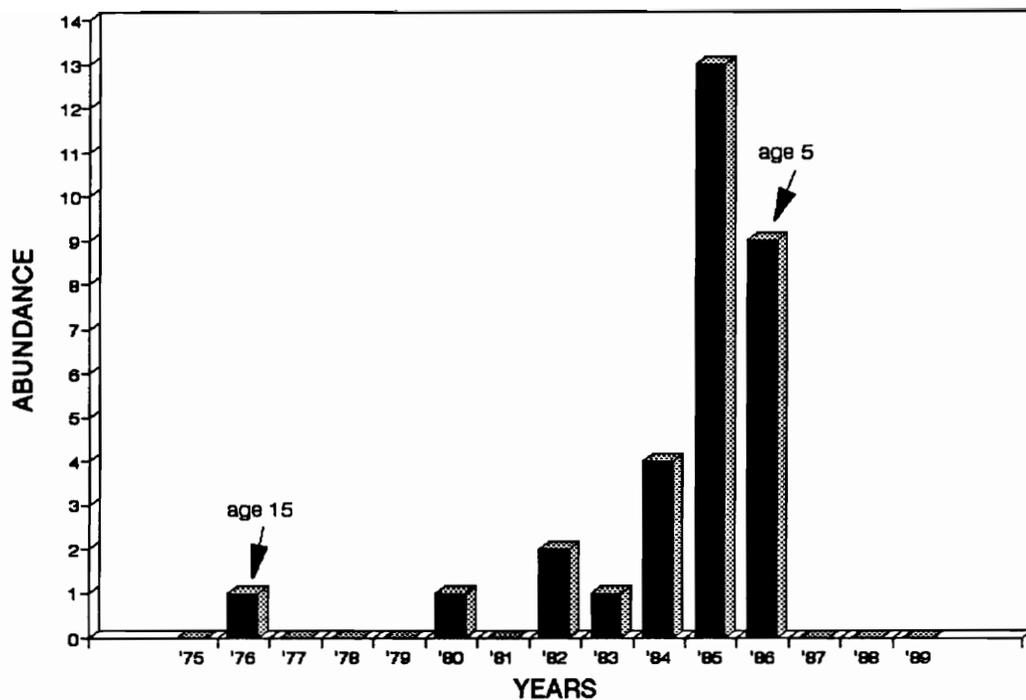


Figure 3.17: Year classes of *V. iris* from a 1991 collection at Kyle's Ford (N=31).

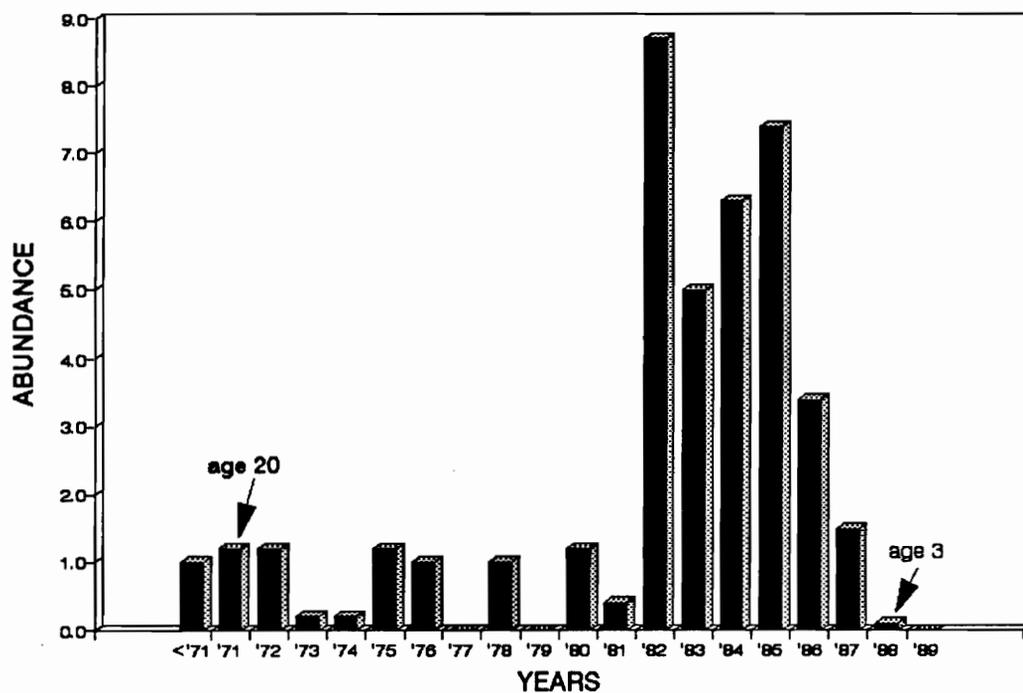


Figure 3.18: Year classes of *E. dilatata* from a 1991 collection at Kyle's Ford (N=41).

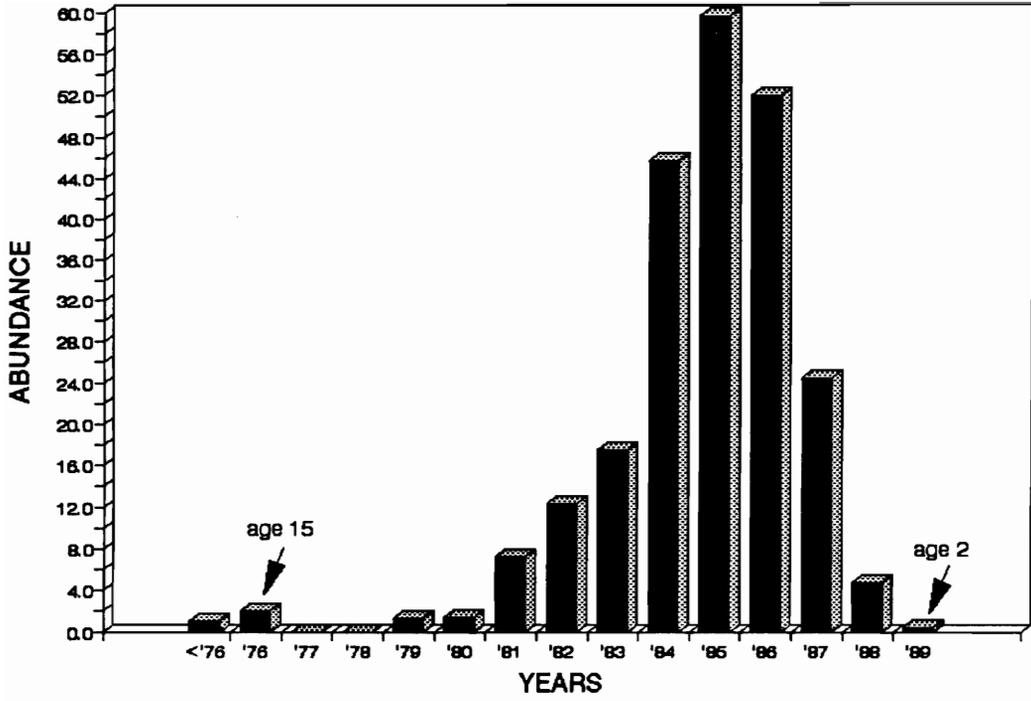


Figure 3.19: Year classes of *A. pectorosa* from a 1991 collection at Kyle's Ford (N=230).



Figure 3.20: Erosion of stream banks in Cove Creek approximately 100 meters upstream of the confluence with the Clinch River (March 1994).



Figure 3.21: Sedimentation in the flood plain at the Slant site (CRK 359.3). Possibly a cause of decreased recruitment at this site (March 1994).

## Chapter Four

### BIOACCUMULATION OF COPPER IN MUSSEL SHELLS

#### Abstract:

The bioaccumulation of copper in mussel shells was determined at 4 study site locations in the upper Clinch River. Ten valves of *Lampsilis fasciola*, 4 to 6 of each sex, were examined at each site. Approximately 300 to 350 mg of ashed shell was digested in 2 mL of concentrated trace metal grade HNO<sub>3</sub> and 2 mL H<sub>2</sub>O<sub>2</sub>. Shell digests were analyzed using atomic absorption graphite furnace technique with background correction. Comparisons were made with nonparametric ranked General Linear Model Statistical Analysis System. Mean concentrations of copper in mussel shells were 141.9  $\mu\text{g}/\text{kg}$ , 129.4  $\mu\text{g}/\text{kg}$ , 113.6  $\mu\text{g}/\text{kg}$ , and 115.1  $\mu\text{g}/\text{kg}$  at Pounding Mill, Hackneys, Slant, and Kyle's Ford, respectively. Mean concentrations of copper in shells of males were 108.1  $\mu\text{g}/\text{kg}$ , and 143.6  $\mu\text{g}/\text{kg}$  for females. No statistically significant differences were found among sites or between sexes ( $p > 0.10$ ). At low copper levels, CaCO<sub>3</sub> in the shell interferes with the recovery of copper by atomic absorption technique, such that the mean accumulated copper in mussel

shells riverwide may be as high as 800  $\mu\text{g}/\text{kg}$ . Copper levels obtained from the USEPA STORET database throughout the river were less than 10  $\mu\text{g}$  Cu/L in most cases from the water column, and averaged 19.9 mg Cu/kg dry weight sediment. As indicated by water column, sediments, and mussel shells, the copper levels are relatively uniform throughout the upper Clinch River. Therefore, a common occurrence throughout the watershed, such as coal mining practices or pesticide use, may be releasing low levels of copper to the water, which is being concentrated in the sediments and taken up to some degree by the mussels.

#### Introduction:

Bivalve mollusks may accumulate and concentrate trace metals, such as copper, in soft tissues and shells. Mussels are considered ideal biological indicators, and thus, have been used extensively to monitor metal contamination of marine and freshwater systems (Manly and George 1977, Imlay 1982, Bourgoin 1991). Suitable biological indicators, such as freshwater mussels, are organisms that are sedentary throughout their lives; long-lived; accumulate toxicants without being killed; large enough for analysis; abundant where found; and easily sampled (Phillips 1980).

Freshwater mussels move within the mussel bed due to changes in flow, temperature or food availability, although they may not travel long distances out of their original habitat (Imlay 1982). They are long-lived organisms; thin-shelled species live for 4 to 10 years and thicker-shelled species live for 20 to 40 years and some as long as 100 years (Imlay 1982). It has been hypothesized that trace metals may accumulate in annual growth increments such that the time at which a disturbance occurred may be determined (Imlay 1982).

Metals will accumulate in varying degrees in the soft tissues and shells, depending on the metal of interest (Anderson 1977, Phillips 1980, Imlay 1982, Tevesz et al. 1989). For example, copper accumulates in shell but becomes concentrated in soft tissues of bivalves, whereas lead accumulates equally in both areas of the organism (Anderson 1977). Manganese was found to be 2 to 25 times more concentrated in soft tissues than in shells (Tevesz et al. 1989). Many freshwater mussels are listed as threatened or endangered species (Neves et al. 1980); therefore, the use of fresh dead mussel shells for trace metal analysis rather than soft tissue is desirable. In the Clinch River, muskrat predation provides an adequate supply of fresh dead mussel shells for such analyses.

Appalachian Power Company's Clinch River Plant (CRP) located in Carbo, Virginia, had a history of high copper levels in the effluent discharge. From

1977 to 1984, copper was leached from pipes averaging levels of 857  $\mu\text{g Cu/L}$  effluent (Van Hassel and Gaulke 1986). Insect and bivalve impairment below the CRP was shown to result from exposure to high copper levels (Farris et al. 1988, Clements et al. 1992). Clean up efforts between 1986 and 1989 lowered copper levels in the effluent to 260.4  $\mu\text{g/L}$ , which resulted in 52.2  $\mu\text{g Cu/L}$  river water directly below the discharge (Clements et al. 1992). However, diversity and abundance of insect communities were reduced when exposed to 12  $\mu\text{g Cu/L}$  (Clements et al. 1988). The Asian clam showed reduced cellulolytic activity at 16  $\mu\text{g Cu/L}$  (Farris et al. 1988) in the Clinch River (water hardness  $\approx$  180 mg  $\text{CaCO}_3/\text{L}$ ) and reduced growth at 8.4  $\mu\text{g Cu/L}$  (Belanger et al. 1990) in the New River (water hardness  $\approx$  60 mg  $\text{CaCO}_3/\text{L}$ ). In response to these findings, CRP constructed an extensive waste treatment facility in May 1993, to reduce the levels of copper in the effluent to less than 12  $\mu\text{g/L}$  (personal communication with D. S. Cherry).

The objectives of this phase of the study were: 1) to examine the levels of bioaccumulation of copper in mussel shells above and below the Clinch River Power Plant and 2) to compare the accumulation of copper between the mussel shells of male and female *Lampsilis fasciola* (wavy-rayed lampmussel).

## Materials and Methods:

Right valves of *L. fasciola* collected in muskrat middens and retained from growth rate and age class determinations were used for accumulation of copper analyses. Ten valves, 4 to 6 of each gender, were examined from four study locations, Pounding Mill at Clinch River Kilometer (CRK 527), Hackneys (CRK 433.7), Slant (CRK 359.3), and Kyle's Ford (CRK 304). Individuals of known age (Chapter Two) were selected to minimize time of exposure as a variable, such that each was recruited between 1972 and 1983.

Valves were weighed to the nearest 0.01 g and placed overnight in a drying oven at 105 °C. They were removed and reweighed to confirm less than 1% wet weight loss. The periostracum was volatilized from the surface of the shells in a muffle furnace, at 500 °C for 45 to 60 min. The valves were removed from the muffle furnace, and any remaining organic residues were chipped away by hand (Standard Methods 1992).

Fifty-five 30 mL glass test tubes and polyethylene screw caps were soaked in 50% HNO<sub>3</sub> for approximately 30 min. and rinsed with distilled, deionized water (DDW). A small sample, approximately 300 to 350 mg, of the umbonal region of the shells was weighed and placed in each test tube. The samples were digested in 2 ml of concentrated trace metal grade HNO<sub>3</sub> and 2 ml of 30% H<sub>2</sub>O<sub>2</sub> in a Fisher Scientific dry bath incubator and brought up to 20 ml volume with DDW (USEPA 1991).

The fifty-five samples prepared included 10 shell samples from each of four sites, 3 blanks, and 12 spikes. The blanks were prepared without shell to determine the amount of copper contamination in the glassware. The spikes were prepared utilizing combined shell material from female *L. fasciola* at Kyle's Ford to determine the percent recovery of copper. Three spikes contained no dosed copper, and the remaining 9 spikes contained three samples each dosed with 4  $\mu\text{g/L}$ , 6  $\mu\text{g/L}$ , and 10  $\mu\text{g/L}$ , respectively.

Shell digests were analyzed using atomic absorption graphite furnace technique which was programmed as follows: preheating time of 20 s at 90 °C; drying time of 20 s at 130 °C; cooling time of 10 s at 20 °C; charring time of 25 s at 1200 °C; atomization time of 5 s at 2300 °C; cleaning time of 3 s at 2650 °C; and cooling time of 4 s at 20 °C; wavelength 324.7; integration time 5 s; background correction on; and sample size of 20  $\mu\text{L}$ . Comparisons of levels of copper in bivalve shells among sites and between males and females were made utilizing nonparametric ranked General Linear Model (GLM) Statistical Analysis System (SAS) with 95% confidence (SAS 1990).

#### Results:

Forty valves of the wavy-rayed lampmussel (21 males and 19 females) were examined for the accumulation of copper. Mussels represented were recruited from 1975 to 1983 at Pounding Mill, 1975 to 1978 at Hackneys,

1973 to 1975 at Slant, and 1972 to 1983 at Kyle's Ford. Levels of accumulation ranged from 36.4 to 587.5  $\mu\text{g}$  Cu/kg shell (Table 4.1); however, there were no significant differences in mean copper levels at the four study sites ( $p > 0.10$ ) (Table 4.1). In addition, there were no statistically significant differences between the accumulation of copper by male and female wavy-rayed lampmussels ( $p > 0.10$ ) (Table 4.2).

#### Discussion:

Comparisons of accumulation of copper in mussel shells among sites indicated that mussels at each site did not accumulate copper to varying degrees. This objective was undertaken to determine if historically high copper levels released from the CRP in Carbo, Virginia (Van Hassel and Gaulke 1986, Farris et al. 1988, Clements et al. 1992), influenced the decline in recruitment of juvenile mussels to the Slant site, located 72 km downstream (Chapter Three).

Mussel valves at Slant had the lowest mean amount of copper in the shells (113  $\mu\text{g}/\text{kg}$ ) when compared to the other three sites, although not significantly less. Mussels at Pounding Mill had the highest mean level of copper accumulated (141  $\mu\text{g}/\text{kg}$ ) and Hackneys (129  $\mu\text{g}/\text{kg}$ ) and Kyle's Ford (115  $\mu\text{g}/\text{kg}$ ) were intermediate. Therefore, although the CRP may have been responsible for the elimination of all unionid mussels along the left bank below

the plant (Ahlstedt 1986, Stansbery et al. 1986), copper in the plant discharge is seemingly not the cause of reduced recruitment at the Slant site.

It should be emphasized at this time that the levels of copper determined in bivalve shells are not absolute values, but are likely gross underestimations of actual copper levels. High salts in shell, such as  $\text{CaCO}_3$ , interfere with the recovery of copper by the atomic absorption technique (Standard Methods 1992). Spiked shell samples indicated a semi-linear increase in recovery of copper from sample levels of 4 to 10  $\mu\text{g/L}$  (Table 4.3) which translates to approximately 250 to 600  $\mu\text{g Cu/kg shell}$ . Less than 200  $\mu\text{g Cu/kg shell}$  was recovered from all but 4 samples analyzed. As demonstrated by spiked samples, less than 15% of the total copper was recovered at these low levels. Therefore, mean copper levels in shells at the four sites may not be 125  $\mu\text{g Cu/kg}$  as indicated, but rather  $> 800 \mu\text{g Cu/kg}$ . An 80% recovery of copper from mussel shells is approached when samples contain more than 600  $\mu\text{g/kg}$  (Table 4.3). Without more extensive sampling of spiked shells, percent recovery of less than 600  $\mu\text{g Cu/kg shell}$  is dubious and would be considered below detection limits. Most of the research conducted with metal accumulation in bivalve shells detected high levels of metals ( $\mu\text{g/g}$ ) from heavily contaminated sites (Anderson 1977, Manly and George 1977, Imlay 1982, Bourgoin 1991) such that  $\text{CaCO}_3$  did not interfere with recovery of the metals.

The United States Environmental Protection Agency (USEPA) STORET database measured copper in the water column at less than 10  $\mu\text{g/L}$  for 85 of 89 samples throughout the upper Clinch River since 1972. In May 1982, 430  $\mu\text{g Cu/L}$  was detected, 50  $\mu\text{g Cu/L}$  in September 1989, and 20  $\mu\text{g Cu/L}$  was detected on two other occasions. Mean copper in the sediment measured at 2 sites, 168 km apart, was  $19.9 \pm 10.3 \text{ mg/kg}$  from 1977 to 1991 (N=21). Assuming 15% recovery of copper in mussel shells, approximately 800  $\mu\text{g/kg}$  was accumulated in mussel shells at 4 sites in the river. In each case, copper levels are relatively uniform throughout the upper Clinch River system. There are a number of potential sources of copper in the watershed from coal mining practices to pesticide usage.

Previous studies showed adult *Anodonta grandis* to have no acute toxicity response to 96-hour exposures of  $> 1000 \mu\text{g Cu/L}$  (Jacobson 1990). However, juvenile mussels exhibit valve closure to copper levels of 17 to 24  $\mu\text{g Cu/L}$  in the water column (Jacobson et al. 1993) and mortality when exposed to water column levels of 44 to 83  $\mu\text{g Cu/L}$  (Jacobson et al. 1993) and 171  $\mu\text{g Cu/L}$  (Keller and Zam 1991). Acute toxicity varied based on mussel species tested and water hardness (Keller and Zam 1991). Acute and chronic toxicity responses to sediment exposure as mentioned above is not known, although juvenile mussels burrow and feed in the interstitial spaces of the sediment where copper exposure is maximized (Yeager et al. 1994). Therefore, an

average of 20 mg Cu/kg of sediment throughout the river system may have an adverse effect on juvenile mussels.

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**Table 4.1: Relative levels of copper accumulated in *Lampsilis fasciola* shells at 4 study sites in the upper Clinch River.**

Site	Sample	Gender	Copper ( $\mu\text{g}/\text{kg}$ )
Pounding Mill	1P	male	161.29
	2P	male	62.50
	3P	male	62.50
	4P	male	80.00
	5P	female	125.00
	6P	female	41.18
	7P	female	587.50
	8P	female	90.32
	9P	female	102.86
	10P	female	106.25
Mean copper $\pm$ std. err. ( $\mu\text{g}/\text{kg}$ )			141.9 $\pm$ 50.7
Hackneys	1H	female	115.15
	2H	female	68.57
	3H	female	68.75
	4H	female	78.79
	5H	male	72.73
	6H	male	150.00
	7H	male	58.82
	8H	male	112.50
	9H	male	118.75
	10H	male	450.00
Mean copper $\pm$ std. err. ( $\mu\text{g}/\text{kg}$ )			129.4 $\pm$ 36.8

**Table 4.1 (con't):** Relative levels of copper accumulated in *Lampsilis fasciola* shells at 4 study sites in the upper Clinch River.

Site	Sample	Gender	Copper ( $\mu\text{g}/\text{kg}$ )
Slant	1S	female	112.50
	2S	female	133.33
	3S	female	70.59
	4S	female	125.00
	5S	female	312.50
	6S	male	66.67
	7S	male	54.55
	8S	male	66.67
	9S	male	81.25
	10S	male	112.50
Mean copper $\pm$ std. err. ( $\mu\text{g}/\text{kg}$ )			113.6 $\pm$ 23.8
Kyle's Ford	1K	female	109.09
	2K	female	80.00
	3K	female	76.47
	4K	female	325.00
	5K	male	56.25
	6K	male	84.85
	7K	male	36.36
	8K	male	143.75
	9K	male	121.21
	10K	male	117.65
Mean copper $\pm$ std. err. ( $\mu\text{g}/\text{kg}$ )			115.1 $\pm$ 25.4

**Table 4.2: Relative mean concentrations of copper in shells of male and female *Lampsilis fasciola* in the upper Clinch River. No statistically significant differences were found ( $p > 0.10$ ).**

Gender	Sample size (N)	Mean copper ( $\mu\text{g}/\text{kg}$ )	Std. error
male	21	108.1	18.7
female	19	143.6	30.0

**Table 4.3: Mean recovery of copper from three spiked shell concentrations utilizing the atomic absorption furnace technique.**

Shell sample	Sample size (N)	Conc. recovered ( $\mu\text{g Cu}/\text{kg}$ )	Std. error ( $\mu\text{g Cu}/\text{kg}$ )	Exp. conc. ( $\mu\text{g Cu}/\text{kg}$ )	% Recov.
Spk 4	3	33.7	50.2	240.0	14.1
Spk 6	3	213.7	62.8	367.6	58.1
Spk 10	3	461.8	175.0	594.2	77.7

## Chapter Five

### SUMMARY AND CONCLUSIONS

The primary objectives of this study were to identify potentially enhanced or impaired mussel assemblages over a 223 km reach in the upper Clinch River and to attempt to identify possible sources of impairment. Six freshwater mussel species, *Medionidus conradicus* (Cumberland moccasinshell), *Villosa iris* (rainbow), *Lampsilis fasciola* (wavy-rayed lampmussel), *Elliptio dilatata* (spike), *Actinonaias pectorosa* (pheasantshell), and *Fusconaia cor* (shiny pigtoe) were evaluated. Population demographics including growth rates, periods of recruitment, and mortality rates of adults at four study site locations were used to identify enhanced or impaired assemblages. In addition, the bioaccumulation of copper in bivalve shells was examined specifically as an indication of exposure to metals, causing impairment. Secondary objectives of this study were to: 1) compare the growth rates of a sexually dimorphic species, *L. fasciola*, 2) examine the correlation between growth rates of 6 mussel species and longitudinal position in the river, and 3) evaluate muskrat predation bias on mussels of the Clinch River.

Before the primary objectives were addressed, secondary objectives were examined to clarify preliminary questions of growth and sampling design. Growth rates of male *L. fasciola* were greater than female *L. fasciola* at all 4

study sites. *L. fasciola* is known to reach sexual maturity in the Clinch River by age 4 (Neves and Zale 1982); therefore, at sexual maturity females apparently begin to put more energy into reproduction and less energy into shell elongation such that growth rate decreases.

Secondly, before growth rates can be used as a biological indicator of the ecological integrity of freshwater mussel assemblages, the correlation between growth and river position must be established. Growth rates were compared within species found in the headwaters and those found 223 km downstream. No correlation was found between growth rates and longitudinal river position for any of the 6 mussel species evaluated, so relative comparisons between growth rates of mussels at the study sites were made. Sites with mussel species exhibiting enhanced growth were deemed enriched and those with diminished growth were deemed impaired.

Thirdly, validation of the use of muskrat midden samples was necessary to determine representation of instream populations. Muskrats selected against mussels less than 5 years of age for all species examined, due to the presumed inaccessibility of younger cohorts. Young mussels are in different habitats than adults for a period of time and burrow more deeply than adults, reducing predator/prey encounters; therefore, cohorts less than 5 years of age are often absent from muskrat midden samples. Muskrats selected against older cohorts of the large mussel species, *A. pectorosa* and *E. dilatata*. It is known that

muskrats avoid mussels greater than 70 mm in length (Neves and Odom 1989). *A. pectorosa* reaches 70 mm by ~7 years of age, and *E. dilatata* reaches 70 mm by ~13 years of age; therefore, these age classes are often missing from muskrat midden samples. The sampling bias was considered when examining recruitment of juveniles and mortality rates of adults.

Growth rates of five mussel species at the Hackneys site (CRK 433.7) indicated enriched conditions. A small town, Cleveland, located approximately 5 km upstream of the Hackneys site is one possible source of enrichment. Prior to June 1986, no sewage treatment facility for Cleveland resulted in the release of untreated sewage directly into the Clinch River. Since that time, a 50,000 gallon per day Sewage Treatment Plant (STP) was built presenting a different source of impact for mussel assemblages downstream (Goudreau et al. 1993).

Absence of juvenile recruitment for 4 mussel species at the Slant site (CRK 359.3) indicated impaired conditions. Impairment is believed to be caused by increased siltation from erosion of stream banks in the Cove Creek watershed. Cove Creek is a tributary of the Clinch River, entering less than 1.5 kilometers upstream of the Slant site. A high degree of bank erosion and fine, silty sediment was observed in Cove Creek, and large deposits of sediment were observed in the flood plain downstream at the Slant site. Erosion of the stream banks in Cove Creek may have increased siltation downstream in the Clinch River and smothered the filter-feeding organisms at Slant. Snorkeling

the streambed at Slant revealed a loss of all *L. fasciola*, *F. cor*, *A. pectorosa*, and *E. dilatata* between 1986 and 1993. Five individuals of *Actinonaias ligamentina* were found, all greater than 95 mm in length indicating a highly skewed age class structure. Without improvement of water quality, there will be little opportunity for recruitment of juvenile mussels to the Slant site.

Adult mortality of all species at study sites was high, indicating a riverwide declining mussel fauna throughout the upper Clinch River. Examination of United States Environmental Protection Agency (USEPA) STORET water quality parameters revealed no specific problematic source. In addition, there were no significant differences in bioaccumulation of copper in *L. fasciola* shells among any of the four study sites. A mean of 125  $\mu\text{g}$  Cu/kg shell was found in the upper Clinch River. It was determined that, with  $\text{CaCO}_3$  interfering with the recovery of copper from bivalve shells, 15% recovery should be expected. Therefore, as much as 800  $\mu\text{g}$  Cu/kg shell may be present; however, the levels of copper are more accurately presented as below detectable limits by this procedure. Regardless of the accumulated metal, copper is found in low levels in the water column and uniformly distributed in the sediments and bivalve shells. This uniformity of copper throughout the Clinch River basin may be due to coal mining practices or pesticide use in the watershed. The levels at which copper is found in the sediments and bivalves is too low to have adverse effects on adult mussels (Jacobson 1990), but

juvenile mussels burrow and feed in the interstitial spaces of the sediment such that exposure to copper is maximized (Yeager et al. 1994). It is possible that the levels of copper identified in the sediments riverwide (20 ppm) may have adverse effects on juveniles and interrupt the mussel life cycle.

The results of this study confirm the findings of many previous studies (Ortmann 1918, Bates and Dennis 1978, Neves et al. 1980, Ahlstedt 1986, Stansbery et al. 1986, Dennis 1987, Bruenderman 1989, Dennis 1989, Neves and Odom 1989, Goudreau et al. 1993) that the mussel fauna in the upper Clinch River is in grave danger of extirpation. Unfortunately, this study falls short of identifying the sources of impairment creating inhospitable habitat for the sensitive mussel communities. Siltation was identified, erosion of stream banks, as a potential cause of decline at one site, and untreated sewage input as a source of enrichment at another (Table 5.1). It is likely that a combination of these factors and many others are contributing to mussel fauna decline. Intermittent toxicity due to agricultural and town runoff was not addressed in this study, but may be a large contributing factor in mussel decline. Such toxicity is difficult to determine due to its intermittent nature; however, at a rate of 36% to 52% annual adult mortality, identification and removal of the sources of impact is imperative.

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**Table 5.1: Summary of population demographics of freshwater mussels at 4 study sites in the upper Clinch River.**

<b>PARAMETER</b>	<b>SITE</b>	<b>SOURCE</b>
<b>Growth rate</b>	<b>Hackneys</b>	<b>sewage enrichment</b>
<b>Periods of recruitment</b>	<b>Slant</b>	<b>siltation</b>
<b>Adult mortality</b>	<b>Pounding Mill</b>	<b>?</b>
<b>Adult mortality</b>	<b>Hackneys</b>	<b>?</b>
<b>Adult mortality</b>	<b>Kyle's Ford</b>	<b>?</b>

**APPENDIX A**  
**SAS PROGRAMS**

Appendix A: SAS programs used for von Bertalanffy growth rate comparisons among 2 sites, 3 sites, and 4 sites.

Two site comparison

1 The SAS System 10:34 Wednesday, May 4, 1994

NOTE: Copyright(c) 1989 by SAS Institute Inc., Cary, NC USA.

NOTE: SAS (r) Proprietary Software Release 6.07 TS304

Licensed to VIRGINIA TECH COMPUTING, Site 0001798003.

NOTE: Running on IBM Model 3090 Serial Number 070649.

NOTE: AUTOEXEC processing beginning; file is VTSAS LINEMODE \* .

NOTE: AUTOEXEC processing completed.

```
1 *Dummy-variable method for 2 site comparison;
2 options linesize = 75 pagesize = 80 nocenter;
3 data one;
4 input z1 t l;
5 *z1 = 0 is dummy variable for site 1 data set;
6 *z1 = 1 is dummy variable for site 2 data set;
7 cards;
```

NOTE: The data set WORK.ONE has 374 observations and 3 variables.

```
382 ;
383 data one; set one;
384 if t > 18 then delete;
```

NOTE: The data set WORK.ONE has 328 observations and 3 variables.

```
385 proc sort; by z1 t;
```

NOTE: The data set WORK.ONE has 328 observations and 3 variables.

```
386 proc means noprint;
387 by z1 t; var l;
388 output out = two mean = l;
```

NOTE: The data set WORK.TWO has 36 observations and 5 variables.

```
389 proc print;
```

NOTE: The PROCEDURE PRINT printed page 1.

```
390 data two; set two;
```



```

6      *z1 = 1 z2 = 0 are dummy variables for site 2 data set;
7      *z1 = 0 z2 = 1 are dummy variables for site 3 data set;
8      cards;
NOTE: The data set WORK.ONE has 393 observations and 4 variables.

```

```

402     ;
403     data one; set one;
404     if t > 14 then delete;
NOTE: The data set WORK.ONE has 377 observations and 4 variables.

```

```

405     proc sort; by z1 z2 t;
NOTE: The data set WORK.ONE has 377 observations and 4 variables.

```

```

406     proc means noprint; by z1 z2 t;
407     var l;
408     output out = two mean = l;
NOTE: The data set WORK.TWO has 42 observations and 6 variables.

```

```

409     proc print;
NOTE: The PROCEDURE PRINT printed page 1.

```

```

410     data two; set two;
NOTE: The data set WORK.TWO has 42 observations and 6 variables.

```

```

411     proc nlin method = dud best = 20;
412     parameter linf = 55 k = 0.21 t0 = -.08;
*parameters linf, k, and t0 were estimated using fish.parm;
413     model l = linf*(1-exp(-k*(t-t0)));
414     title 'Small Model : No indicator variables';
NOTE: The PROCEDURE NLIN printed page 2.

```

```

415     proc nlin method = dud best = 20;
416     parameter linf = 55 k = 0.21 t0 = -.08
417     b0 = 0 b1 = 0 b2 = 0 c0 = 0 c1 = 0 c2 = 0;
*parameters b0, b1, b2, c0, c1, c2 begin iterations at 0;
4   1   8           m   o   d   e   l
l = (linf + b0*z1 + c0*z2)*(1-exp(-(k + b1*z1 + c1*z2)*(t-(t0 + b2*z1 + c2*z2))));
419     title 'FULL MODEL: with indicator variables (2 dummy for 3 parms)';
420     output out = one predicted = pl;
NOTE: The data set WORK.ONE has 42 observations and 7 variables.
NOTE: The PROCEDURE NLIN printed pages 3-5.

```

421 proc print; var z1 z2 t l pl;

NOTE: The PROCEDURE PRINT printed page 6.

NOTE: Viobuf storage reached 47 percent full.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414

### Four site comparison

1 The SAS System

10:38 Wednesday, May 4, 1994

NOTE: Copyright(c) 1989 by SAS Institute Inc., Cary, NC USA.

NOTE: SAS (r) Proprietary Software Release 6.07 TS304

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NOTE: Running on IBM Model 3090 Serial Number 070649.

NOTE: AUTOEXEC processing beginning; file is VTSAS LINEMODE \* .

NOTE: AUTOEXEC processing completed.

1 \*Dummy-variable method for 4 site comparisons;

2 options linesize = 75 pagesize = 80 nocenter;

3 data one;

4 input z1 z2 t l;

5 \*z1 = 0 z2 = 0 are dummy variables for site 1 data set;

6 \*z1 = 1 z2 = 0 are dummy variables for site 2 data set;

7 \*z1 = 0 z2 = 1 are dummy variables for site 3 data set;

8 \*z1 = 1 z2 = 1 are dummy variables for site 4 data set;

9 cards;

NOTE: The data set WORK.ONE has 455 observations and 4 variables.

465 ;

466 data one; set one;

467 if t > 11 then delete;

NOTE: The data set WORK.ONE has 401 observations and 4 variables.

468 proc sort; by z1 z2 t;

NOTE: The data set WORK.ONE has 401 observations and 4 variables.

469 proc means noprint; by z1 z2 t;

470 var l;

471 output out = two mean = l;

NOTE: The data set WORK.TWO has 44 observations and 6 variables.

472 proc print;

NOTE: The PROCEDURE PRINT printed page 1.

473 data two; set two;

NOTE: The data set WORK.TWO has 44 observations and 6 variables.

474 proc nlin method = dud best = 20;

475 parameter linf = 66 k = 0.22 t0 = -.49;

\*parameters linf, k, and t0 were estimated using fish.parm;

476 model l = linf\*(1-exp(-k\*(t-t0)));

477 title 'Small Model : No indicator variables';

NOTE: The PROCEDURE NLIN printed page 2.

478 proc nlin method = dud best = 20;

479 parameter linf = 66 k = 0.22 t0 = -.49

480 b0 = 0 b1 = 0 b2 = 0 c0 = 0 c1 = 0 c2 = 0;

\*parameters b0, b1, b2, c0, c1, c2, begin iterations at 0;

481 model

$l = (\text{linf} + b0*z1 + c0*z2) * (1 - \exp(-(k + b1*z1 + c1*z2) * (t - (t0 + b2*z1 + c2*z2)))));$

482 title 'FULL MODEL: with indicator variables (2 dummy for 3 parms)';

483 output out = one predicted = pl;

NOTE: The data set WORK.ONE has 44 observations and 7 variables.

NOTE: The PROCEDURE NLIN printed pages 3-7.

484 proc print; var z1 z2 t l pl;

NOTE: The PROCEDURE PRINT printed page 8.

NOTE: Viobuf storage reached 48 percent full.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414

**APPENDIX B**

**SHELL LENGTHS-AT-AGE**

Appendix B: Predicted shell lengths at age and observed shell lengths at age for each species at each site. Shaded areas indicate age classes not heavily depredated by muskrats.

*Medionidus conradicus* at Pounding Mill:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	10	7.01	1.98	8.08
2	0	11	16.56	3.87	16.81
3	3	14	25.72	3.73	23.85
4	14	15	30.96	3.94	29.51
5	22	12	34.41	4.28	34.06
6	20	10	36.58	4.57	37.73
7	16	8	40.09	3.15	40.68
8	25	12	42.16	2.90	43.05
9	3	5	44.65	2.18	44.96
10	7	8	45.97	1.45	46.50
11	3	6	47.49	1.53	47.74
12	2	5	48.99	0.59	48.73
13	4	5	49.50	1.44	49.53
14	1	2	50.98	1.03	50.18
15	0	0	---	---	50.70
16	1	1	50.33	0.00	51.12
17	0	0	---	---	51.45
18	0	0	---	---	51.72
19	0	0	---	---	51.94
20	0	0	---	---	52.12
21	0	0	---	---	52.26
22	0	0	---	---	52.37
23	0	0	---	---	52.46

24 1 1 53.65 0.00 52.54  
 122 125

*Medionidus conradicus* at Hackneys:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	10	12.01	5.35	11.65
2	0	12	20.70	3.63	20.46
3	2	13	26.42	3.97	27.56
4	16	17	33.55	5.37	33.42
5	31	20	38.45	4.70	38.24
6	16	11	42.92	4.31	42.22
7	13	10	46.60	3.24	45.50
8	5	5	47.31	3.67	48.21
9	10	9	50.48	2.37	50.44
10	6	7	51.48	2.79	52.28
11	5	6	53.31	2.74	53.79
12	6	7	55.24	2.72	55.04
13	4	5	56.04	3.03	56.07
14	3	4	58.39	1.94	56.92
15	4	3	56.82	4.63	57.62
16	4	3	56.31	3.20	58.20
17	1	1	61.81	0.00	58.67
18	6	4	57.73	3.67	59.06
19	0	0	---	---	59.39
20	0	0	---	---	59.65
21	0	0	---	---	59.87
22	0	0	---	---	60.05
23	0	0	---	---	60.20

24            0            0            0            60.33  
 132           147            ---            ---            ---

*Medionidus conradicus* at Kyle's Ford:

Age	N (in midden)	N backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	7	11.68	6.28	14.33
2	3	9	21.50	4.85	20.54
3	5	10	27.99	5.17	25.84
4	11	14	31.79	4.14	30.38
5	26	20	35.16	3.65	34.25
6	17	14	38.01	4.74	37.57
7	7	8	40.43	4.74	40.40
8	5	7	42.71	5.26	42.82
9	7	9	44.67	4.16	44.89
10	2	4	42.89	3.60	46.65
11	1	4	47.86	4.69	48.16
12	2	4	46.56	3.76	49.45
13	1	4	49.99	2.49	50.56
14	2	4	51.16	2.50	51.50
15	1	1	54.62	0.00	52.31
16	1	1	54.16	0.00	53.00
17	1	1	55.15	0.00	53.59
18	0	0	---	---	54.09
19	0	0	---	---	54.52
20	0	0	---	---	54.89
21	0	0	---	---	55.20
22	0	0	---	---	55.47
23	0	0	---	---	55.70

24 0 0 0 55.90  
 92 121 --- ---

*Villosa iris* at Pounding Mill:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	14	7.16	1.97	6.08
2	0	15	13.92	3.02	14.02
3	1	15	19.63	3.83	19.47
4	9	15	24.54	5.37	24.45
5	30	13	29.96	6.68	29.02
6	43	14	31.94	8.68	33.20
7	44	14	36.11	8.56	37.03
8	33	8	44.51	3.47	40.54
9	31	10	43.32	8.39	43.76
10	9	6	49.90	2.45	46.71
11	8	6	51.10	3.17	49.40
12	7	6	52.96	3.41	51.87
13	11	5	52.47	5.00	54.14
14	6	6	53.81	3.42	56.21
15	2	3	55.09	3.77	58.11
16	5	5	57.15	3.65	59.85
17	1	1	61.26	0.00	61.44
18	3	2	53.81	3.15	62.90
19	0	0	---	---	64.24
20	1	1	77.22	0.00	65.47
21	0	0	---	---	66.59
22	0	0	---	---	67.62
23	0	0	---	---	68.56

24	0	0	0	---	---	69.42
25	0	0	0	---	---	70.21
	244	159				

*Villosa iris* at Hackneys:

Age	N (in midden)	N backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	8	4.85	1.35	3.50
2	0	12	12.17	4.02	13.23
3	0	12	21.27	3.73	21.67
4	1	13	29.46	4.70	28.99
5	8	16	36.57	6.20	35.34
6	5	12	40.35	7.25	40.85
7	7	13	45.56	5.88	45.63
8	5	10	47.59	8.39	49.78
9	3	7	47.24	9.01	53.37
10	4	7	58.57	5.88	56.49
11	9	11	57.99	7.42	59.19
12	2	5	65.21	1.19	61.54
13	2	4	68.20	1.89	63.57
14	4	5	66.69	5.49	65.34
15	2	3	66.96	9.77	66.87
16	2	2	64.91	13.46	68.20
17	2	2	70.70	3.29	69.35
18	0	0	---	---	70.35
19	0	0	---	---	71.22
20	1	1	75.15	0.00	71.97
21	0	0	---	---	72.62
22	0	0	---	---	73.18

23	0	0	---	---	73.68
24	0	0	---	---	74.10
25	1	1	69.93	0.00	74.47
	58	144			

*Villosa iris* at Kyle's Ford:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	12	8.39	2.20	14.36
2	0	13	20.89	3.95	20.97
3	1	14	27.86	4.66	26.76
4	1	11	35.09	5.98	31.83
5	17	16	39.92	5.70	36.28
6	24	17	44.11	6.69	40.18
7	10	7	50.27	6.46	43.60
8	4	4	49.62	7.32	46.60
9	8	4	51.27	8.34	49.22
10	2	2	46.26	1.27	51.52
11	3	2	47.99	0.93	53.54
12	1	1	49.93	0.00	55.32
13	1	1	51.66	0.00	56.87
14	1	1	52.85	0.00	58.23
15	3	2	55.38	1.48	59.42
16	0	0	---	---	60.46
17	0	0	---	---	61.38
18	0	0	---	---	62.18
19	0	0	---	---	62.89
20	1	1	70.57	0.00	63.51
21	0	0	---	---	64.05

22	0	0	---	---	64.52
23	1	1	71.09	0.00	64.94
24	0	0	---	---	65.30
25	0	0	---	---	65.62
	78	109			

*Lampsilis fasciola (female)* at Pounding Mill:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	11	12.51	3.42	12.95
2	0	12	22.02	6.15	22.14
3	0	12	31.02	9.33	29.57
4	0	12	37.04	9.88	35.57
5	0	12	40.75	10.16	40.41
6	1	12	43.28	8.90	44.33
7	3	10	44.85	8.56	47.49
8	2	6	50.23	2.91	50.05
9	2	5	52.58	1.85	52.12
10	1	4	52.80	3.30	53.79
11	2	3	55.57	0.95	55.14
12	2	2	57.53	0.89	56.23
13	1	0	---	---	57.11
14	0	0	---	---	57.82
15	0	0	---	---	58.40
16	0	0	---	---	58.86
17	0	0	---	---	59.24
18	0	0	---	---	59.54
19	0	0	---	---	59.78
20	0	0	---	---	59.98

21	0	0	---	---	60.14
22	0	0	---	---	60.27
23	0	0	---	---	60.38
24	0	0	---	---	60.46
	14	101			

*Lampsilis fasciola (female)* at Hackneys:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	12	19.16	4.88	23.41
2	0	12	31.96	4.64	31.43
3	5	14	41.90	4.08	38.26
4	7	12	47.19	3.87	44.08
5	6	10	51.52	3.07	49.05
6	12	12	54.12	4.41	53.28
7	11	10	56.48	5.45	56.89
8	11	10	58.51	5.42	59.96
9	9	7	60.84	6.18	62.58
10	20	10	61.89	6.06	64.82
11	11	5	62.76	8.92	66.72
12	10	6	65.21	7.29	68.34
13	4	3	69.79	10.60	69.73
14	4	3	72.55	10.96	70.91
15	2	2	76.75	11.95	71.91
16	9	5	73.90	9.08	72.77
17	2	1	71.05	0.00	73.50
18	0	0	---	---	74.12
19	0	0	---	---	74.65
20	0	0	---	---	75.10

21	0	0	---	---	75.49
22	0	0	---	---	75.82
23	0	0	---	---	76.10
24	1	1	78.39	0.00	76.34
	124	135			

*Lampsilis fasciola (female)* at Slant:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	3	14.64	3.06	11.70
2	0	5	24.65	3.11	21.52
3	0	6	32.94	5.57	29.78
4	0	9	39.50	5.74	36.72
5	0	9	45.02	5.87	42.55
6	0	9	48.65	6.98	47.44
7	1	9	51.35	7.34	51.56
8	1	9	53.25	8.35	55.02
9	1	9	55.37	9.38	57.92
10	2	7	59.14	9.74	60.36
11	7	7	55.57	6.42	62.41
12	6	5	56.84	8.26	64.14
13	12	7	61.13	8.93	65.58
14	6	3	67.72	6.25	66.80
15	2	2	68.79	11.26	67.82
16	4	2	69.64	10.90	68.68
17	3	1	78.79	0.00	69.40
18	3	2	67.87	5.42	70.01
19	5	2	75.12	7.34	70.52
20	1	1	69.21	0.00	70.95

21	5	2	73.60	2.50	71.31
22	0	0	---	---	71.61
23	0	0	---	---	71.86
24	0	0	---	---	72.08
	59	109			

*Lampsilis fasciola* (female) at Kyle's Ford:

Age	N (in midden)	N backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	14	16.52	4.45	19.61
2	0	15	28.48	6.11	28.13
3	2	15	39.92	2.88	35.21
4	6	15	43.94	3.28	41.08
5	7	14	46.25	3.27	45.96
6	8	12	48.17	3.42	50.02
7	8	9	51.27	3.01	53.38
8	6	5	54.00	3.35	56.18
9	1	2	57.29	4.70	58.50
10	1	2	59.14	4.79	60.43
11	2	2	60.86	4.61	62.03
12	0	1	64.93	0.00	63.36
13	0	1	65.98	0.00	64.46
14	0	1	66.70	0.00	65.38
15	0	0	---	---	66.14
16	0	0	---	---	66.77
17	0	0	---	---	67.30
18	0	0	---	---	67.73
19	1	1	68.38	0.00	68.09
20	1	0	---	---	68.40

21 0 0 68.64  
 22 0 0 68.85  
 23 0 0 69.02  
 24 0 0 69.17

*Lampsilis fasciola* (male) at Pounding Mill:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	6	13.68	2.18	12.84
2	0	7	24.36	5.82	24.72
3	0	7	34.15	5.44	34.44
4	0	7	41.91	5.00	42.39
5	0	7	48.82	4.59	48.89
6	2	7	53.01	4.78	54.20
7	1	5	58.20	4.17	58.55
8	1	5	63.79	4.18	62.10
9	0	4	65.47	2.84	65.00
10	1	4	67.88	1.85	67.38
11	2	4	69.66	1.55	69.32
12	0	3	71.56	2.27	70.91
13	0	3	72.58	2.18	72.20
14	0	2	72.47	0.54	73.26
15	2	3	73.77	1.75	74.13
16	0	0	---	---	74.84
17	1	1	74.61	0.00	75.42
18	0	0	---	---	75.90
19	0	0	---	---	76.28
20	0	0	---	---	76.60

43 109

21 0 0 0 75 76.86

*Lampsilis fasciola* (male) at Hackneys:

Age	N (in midden)	N backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	14	15.92	5.16	17.36
2	0	16	27.14	5.62	28.55
3	5	20	37.32	6.52	37.78
4	9	18	43.27	5.62	45.38
5	15	17	51.88	4.19	51.63
6	18	13	57.88	2.12	56.79
7	10	9	61.83	1.96	61.03
8	9	9	65.70	1.77	64.53
9	3	5	68.66	1.90	67.41
10	8	8	69.41	2.17	69.78
11	19	11	72.39	3.04	71.74
12	8	5	71.20	3.54	73.35
13	5	3	74.14	1.83	74.67
14	2	1	77.10	0.00	75.77
15	9	4	77.40	4.18	76.67
16	3	1	75.36	0.00	77.41
17	2	1	77.19	0.00	78.02
18	0	0	---	---	78.52
19	0	0	---	---	78.93
20	4	2	77.96	10.51	79.28
21	2	1	81.96	0.00	79.57
	131	158			

*Lampsilis fasciola* (male) at Slant:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	1	17.94	0.00	19.19
2	0	6	28.27	3.47	27.47
3	0	6	37.73	3.87	34.74
4	0	7	44.45	4.66	41.14
5	0	11	46.44	5.62	46.76
6	0	11	50.94	6.83	51.69
7	1	11	55.25	7.24	56.03
8	1	11	58.66	7.08	59.84
9	1	9	61.28	8.10	63.19
10	2	7	66.04	6.24	66.13
11	4	9	68.52	5.50	68.72
12	5	10	70.24	5.07	70.99
13	5	9	72.80	5.31	72.98
14	3	8	76.47	4.29	74.74
15	1	4	77.46	3.14	76.28
16	2	3	80.23	3.33	77.63
17	2	4	81.46	4.72	78.82
18	1	2	82.05	5.18	79.87
19	2	3	79.92	1.90	80.79
20	0	0	---	---	81.60
21	2	2	78.76	1.94	82.30
32	1	1	85.34	0.00	86.20
	33	135			

*Lampsilis fasciola* (male) at Kyle's Ford:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	11	15.62	4.45	18.27
2	0	12	28.09	4.28	27.69
3	1	13	37.85	5.85	35.52
4	4	14	44.18	5.61	42.02
5	6	14	48.33	6.10	47.42
6	5	12	52.41	4.86	51.90
7	9	12	56.11	3.86	55.63
8	5	9	57.68	4.45	58.72
9	3	6	60.54	3.41	61.29
10	1	2	60.55	2.03	63.43
11	1	4	64.80	2.93	65.20
12	0	3	65.46	3.27	66.67
13	1	3	67.21	3.11	67.90
14	1	2	68.07	1.83	68.91
15	0	1	67.95	0.00	69.76
16	0	0	---	---	70.46
17	1	1	70.66	0.00	71.04
18	0	0	---	---	71.52
19	1	1	77.82	0.00	71.92
20	0	0	---	---	72.26
21	0	0	---	---	72.53
	39	120			

*Actinonaias pectorosa* at Hackneys:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	17	12.62	3.41	12.37
2	0	20	24.60	4.41	26.72
3	1	20	40.39	3.46	38.68
4	19	22	51.14	5.98	49.21
5	35	21	58.44	5.66	57.96
6	55	27	64.12	5.69	65.39
7	31	17	71.73	5.43	71.69
8	17	8	76.28	5.02	77.04
9	11	3	82.83	6.03	81.58
10	2	1	84.20	0.00	85.43
11	2	1	85.99	0.00	88.69
12	0	0	---	---	91.46
13	11	1	96.53	0.00	93.81
14	4	0	---	---	95.81
15	4	0	---	---	97.50
16	8	0	---	---	98.94
17	0	0	---	---	100.15
18	0	0	---	---	101.19
19	0	0	---	---	102.06
20	0	0	---	---	102.81
21	0	0	---	---	103.44
	200	158			

*Actinonaias pectorosa* at Slant:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	5	18.11	5.67	21.19
2	0	8	31.74	6.11	31.29
3	0	9	43.46	8.09	40.58
4	1	10	51.43	10.39	49.12
5	2	11	58.31	11.77	56.96
6	1	10	65.11	13.03	64.17
7	4	12	70.07	12.26	70.80
8	5	10	75.87	11.87	76.89
9	5	10	80.17	11.78	82.49
10	4	9	85.95	11.90	87.64
11	6	10	89.17	9.86	92.36
12	13	14	94.52	9.60	96.71
13	4	5	101.79	7.26	100.71
14	1	3	107.11	8.24	104.38
15	1	3	109.27	7.61	107.75
16	0	1	119.50	0.00	110.86
17	2	2	114.56	11.64	113.71
18	1	1	108.80	0.00	116.33
19	0	0	---	---	118.74
20	0	0	---	---	120.95
21	0	0	---	---	122.98
	50	133			

*Actinonaias pectorosa* at Kyle's Ford:

Age	N (in midden)	N backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	15	13.52	4.70	12.35
2	3	19	27.27	6.39	26.57
3	11	19	38.06	8.05	38.41
4	40	19	47.40	8.77	48.28
5	92	22	52.98	8.76	56.50
6	121	25	62.90	9.26	63.35
7	103	18	68.08	9.98	69.06
8	53	9	74.66	6.50	73.81
9	41	7	77.04	6.57	77.77
10	37	5	81.40	6.43	81.08
11	22	3	88.24	4.21	83.83
12	25	3	89.54	4.23	86.12
13	0	0	---	---	88.02
14	0	0	---	---	89.62
15	7	2	91.14	5.52	90.94
16	0	0	---	---	92.04
17	6	0	---	---	92.96
18	6	0	---	---	93.73
19	18	0	---	---	94.37
20	18	0	---	---	94.90
21	23	1	91.19	0.00	95.35
	626	167			

*Elliptio dilatata* at Hackneys:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	15	15.33	2.77	16.81
2	1	16	30.25	4.34	27.86
3	10	18	37.73	5.71	36.99
4	28	21	43.06	6.34	44.54
5	55	19	50.05	6.62	50.77
6	35	11	54.87	5.63	55.92
7	43	9	60.76	4.66	60.18
8	92	16	65.79	6.23	63.69
9	45	9	66.45	8.89	66.60
10	25	5	67.70	6.18	69.00
11	28	6	71.27	6.56	70.98
12	27	5	75.22	8.54	72.62
13	42	7	75.31	6.23	73.97
14	13	3	72.77	0.63	75.09
15	8	3	74.11	1.14	76.02
16	8	3	75.07	1.00	76.78
17	14	2	79.05	5.59	77.41
18	19	3	77.58	1.87	77.93
19	9	1	76.18	0.00	78.36
20	13	2	77.71	0.71	78.72
21	13	0	---	---	79.01
22	0	0	---	---	79.25
23	1	1	83.42	0.00	79.45
24	0	0	---	---	79.62
25	0	0	---	---	79.76

26	0	0	---	---	---	79.87
27	0	0	---	---	---	79.96
28	0	0	---	---	---	80.04
29	0	0	---	---	---	80.11
30	0	0	---	---	---	80.16
31	0	0	---	---	---	80.20
32	0	0	---	---	---	80.24
33	0	0	---	---	---	80.27
34	0	0	---	---	---	80.29
35	0	0	---	---	---	80.31
36	0	0	---	---	---	80.33
37	0	0	---	---	---	80.34
38	0	0	---	---	---	80.35
39	0	0	---	---	---	80.36
40	0	0	---	---	---	80.37
	529	175				

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*Elliptio dilatata* at Slant:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	8	15.39	2.74	9.18
2	0	10	22.86	4.97	17.49
3	0	10	28.35	5.50	25.03
4	0	11	34.15	5.82	31.85
5	0	13	41.57	6.31	38.04
6	0	12	44.65	6.80	43.65
7	1	13	48.55	6.85	48.73
8	3	14	51.83	6.71	53.34
9	7	13	56.81	5.04	57.51

10	10	12	60.79	5.16	61.29
11	9	8	64.28	5.72	64.72
12	10	8	67.15	5.77	67.83
13	14	10	66.55	6.91	70.64
14	6	5	71.47	8.46	73.19
15	11	6	73.29	7.86	75.50
16	12	6	76.94	6.11	77.60
17	8	4	79.64	7.20	79.50
18	15	7	80.18	7.04	81.22
19	10	5	83.82	6.62	82.77
20	15	7	85.41	6.00	84.19
21	8	3	84.13	7.98	85.47
22	0	0	---	---	86.63
23	0	0	---	---	87.68
24	3	1	83.70	0.00	88.63
25	0	0	---	---	89.49
26	0	0	---	---	90.28
27	0	0	---	---	90.98
28	4	1	103.32	0.00	91.63
29	4	2	98.41	2.86	92.21
30	8	1	87.85	0.00	92.74
31	2	1	88.28	0.00	93.22
32	2	1	89.53	0.00	93.65
33	0	0	---	---	94.04
34	0	0	---	---	94.40
35	5	2	88.82	5.42	94.72
36	0	0	---	---	95.01
37	1	1	100.83	0.00	95.28
38	0	0	---	---	95.52
39	0	0	---	---	95.74
40	1	1	103.07	0.00	95.93
48	4	1	95.08	0.00	96.97

61 1 1 97.63 0.00 97.60

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*Elliptio dilatata* at Kyle's Ford:

Age	N (in midden)	N backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	10	12.19	2.32	11.61
2	0	13	20.05	4.18	21.50
3	1	14	30.82	6.67	29.79
4	3	18	36.91	5.59	36.74
5	5	19	43.19	5.75	42.57
6	9	21	46.83	6.43	47.46
7	8	19	51.28	5.77	51.55
8	6	15	52.94	5.72	54.99
9	10	15	58.41	4.54	57.87
10	1	7	61.24	4.49	60.28
11	2	6	61.77	2.65	62.30
12	1	5	64.21	2.66	64.00
13	2	4	67.17	4.82	65.42
14	1	3	67.31	4.01	66.62
15	4	4	68.56	3.83	67.62
16	3	1	67.23	0.00	68.46
17	2	0	--	--	69.16
18	2	0	--	--	69.75
19	3	1	65.84	0.00	70.24
20	5	1	75.43	0.00	70.66
21	1	1	69.30	0.00	71.00
22	0	0	--	--	71.30
23	0	0	--	--	71.54

24	0	0	---	---	---	71.74
25	0	0	---	---	---	71.92
26	0	0	---	---	---	72.06
27	0	0	---	---	---	72.18
28	0	0	---	---	---	72.28
29	0	0	---	---	---	72.37
30	0	0	---	---	---	72.44
31	0	0	---	---	---	72.50
32	0	0	---	---	---	72.55
33	0	0	---	---	---	72.59
34	0	0	---	---	---	72.62
35	0	0	---	---	---	72.65
36	0	0	---	---	---	72.68
37	0	0	---	---	---	72.70
38	0	0	---	---	---	72.72
39	0	0	---	---	---	72.73
40	0	0	---	---	---	72.74
	69	177				

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*Fusconaia cor* at Hackneys:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	10	8.10	3.04	7.32
2	0	13	14.76	2.54	15.47
3	1	14	21.08	3.02	22.59
4	4	15	28.05	3.91	28.82
5	1	13	34.79	4.23	34.27
6	3	14	40.17	5.02	39.03
7	3	13	43.87	4.54	43.20

8	2	11	47.27	4.80	46.85
9	6	14	51.73	5.08	50.03
10	7	13	54.23	5.28	52.82
11	2	8	55.04	6.02	55.26
12	3	9	57.57	5.84	57.39
13	6	8	59.09	6.37	59.26
14	4	7	60.29	7.16	60.87
15	5	7	61.87	7.33	62.31
16	2	5	61.30	7.80	63.56
17	3	5	62.64	7.47	64.65
18	3	5	64.03	7.70	65.60
19	6	7	65.09	6.45	66.44
20	3	5	66.13	8.06	67.17
21	4	2	64.78	0.79	67.81
22	11	5	73.71	3.84	68.36
23	16	9	71.58	4.05	68.85
24	10	1	ERR	ERR	69.28
25	0	0	---	---	69.65
26	0	0	---	---	69.98
27	0	0	---	---	70.26
28	0	0	---	---	70.52
29	0	0	---	---	70.73
30	0	0	---	---	70.92
	105	213			

*Fusconaia cor* at Slant:

Age	N (in midden)	backmeasured & thin sectioned	Mean observed length	Std. dev. of observed length	von Bertalanffy predicted length
1	0	5	12.78	0.85	11.10

2	0	10	17.10	1.09	18.10
3	0	11	22.52	1.91	24.34
4	0	11	28.44	2.51	29.89
5	0	11	33.89	3.32	34.83
6	0	11	39.99	3.21	39.23
7	0	12	44.95	2.73	43.15
8	0	12	48.02	1.99	46.64
9	1	11	50.78	2.62	49.75
10	1	10	53.30	2.72	52.52
11	2	10	55.48	2.89	54.98
12	2	6	58.52	3.10	57.18
13	3	5	60.73	4.10	59.13
14	2	4	59.48	5.79	60.87
15	1	3	63.39	5.48	62.42
16	4	5	59.59	6.97	63.80
17	2	3	65.30	7.52	65.03
18	4	4	65.18	6.63	66.13
19	1	1	67.05	0.00	67.10
20	2	2	67.93	0.40	67.97
21	5	4	66.04	5.49	68.74
22	3	2	65.25	0.87	69.43
23	0	0	---	---	70.04
24	0	0	---	---	70.59
25	0	0	---	---	71.08
26	1	1	74.05	0.00	71.51
27	2	2	75.27	0.77	71.89
28	0	0	---	---	72.24
29	0	0	---	---	72.54
30	1	1	73.59	0.00	72.81
	37	157			

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Blacksburg, VA 24061  
(703) 231-9071

**Education:**

M.S. Biology -- June 1994. Virginia Polytechnic Institute & State University, Blacksburg, Virginia. Q.C.A. 3.9.

B.S. Biology (minor:chemistry) -- May 1990. Virginia Polytechnic Institute & State University, Blacksburg, Virginia.

**Recent employment:**

Graduate Research Assistantship -- Dept. of Biology, VPI&SU -- Jan. 1992 - Dec. 1992. Jan. 1994 -- Aug. 1994.

Graduate Teaching Assistantship -- Dept. of Biology, VPI&SU -- Jan. 1993 - Dec. 1993.

**Related experience:**

Laboratory

Freshwater and marine acute and chronic toxicity testing with various zooplankton, crustacean, and fish species

Sediment toxicity testing with zooplankton, insect, and mollusk species

Detoxification testing of molluscicide

USEPA Rapid Bioassessment Protocol

Atomic Absorption Spectrophotometer for heavy metal analysis

Quality Assurance/Quality Control standard operating procedures

Culturing of algae and zooplankton

Thin section aging technique of freshwater mussels

Water chemistry

Parametric and nonparametric statistical analysis

Field

Identification of freshwater mussels, fishes and aquatic insects

Electrofishing, seining, and trapping freshwater fishes

Quantitative and qualitative sampling of benthic macroinvertebrates --

Surbur, PIBS, HESS, D-frame kick net

Qualitative and quantitative bivalve sampling

Water quality sampling and assessment

Municipal and industrial point source discharge assessments -- thermal, fly ash, heavy ash, waste water  
Municipal and industrial nonpoint source runoff assessments -- agricultural, logging, coal mining  
Biofouling control of asiatic clam and zebra mussel

### Teaching

Instructed General and Principle Biology laboratories with 25-30 undergraduates  
Prepared lectures, designed, prepared, and graded quizzes, weekly assignments, and papers  
Designed and prepared freshwater ecology experiment for biology laboratory manual  
Received an overall rating of 3.8/4.0 from student evaluations  
Guest lecturer for Aquatic Ecotoxicology class

### Technical reports:

Cherry, D. S., M. G. Dobbs, J. C. Scott. 1994. Acute flow-through toxicity tests with Betz laboratories' Clam-trol CT-2. Inland Silverside (*Menidia beryllina*) 96-hour acute bioassay.

Cherry, D. S., M. G. Dobbs, J. C. Scott. 1994. Acute flow-through toxicity tests with Betz laboratories' Clam-trol CT-2. *Mysidopsis bahia* 96-hour acute bioassay.

Cherry, D. S., M. G. Dobbs, J. C. Scott. 1994. Acute flow-through toxicity tests with Betz laboratories' Clam-trol CT-2. Rainbow trout (*Oncorhynchus mykiss*) 96-hour acute bioassay.

Cherry, D. S., M. G. Dobbs, J. C. Scott. 1994. Acute flow-through toxicity tests with Betz laboratories' Clam-trol CT-2. Sheepshead minnow (*Cyprinodon variegatus*) 96-hour acute bioassay.

Cherry, D. S., M. G. Dobbs, A. Mikailoff, S. R. Lynde, J. R. Bidwell, M. M. Yeager, and J. C. Fischer [Scott]. 1992. Acute Toxicity and Chronic Impairment Testing of *Ceriodaphnia dubia*, and fathead minnow (*Pimephales promelas*) to Robins Air Force Ramp -- Phase II Effluent -- September 1992 tests.

**Invited seminars:**

Scott, J. C. 1994. Clinch River Power Plant Controversy. Aquatic Ecotoxicology class lecture. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Scott, J. C., R. J. Neves, D. S. Cherry. 1994. Anthropogenic effects on the freshwater mussel fauna of the upper Clinch River, Virginia to Tennessee. North American Benthological Society. Orlando, Florida.

**Other presentations and seminars:**

Scott, J. C. 1994. Population demographics of six freshwater mussel species (Bivalvia:Unionidae) in the upper Clinch River, Virginia and Tennessee. Departmental defense seminar. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Fischer [Scott], J. C., R. J. Neves, D. S. Cherry. 1993. Growth Rates and Age-Class Structures of Freshwater Mussels as an Indication of Population Stability in the Clinch River, Virginia to Tennessee. Society for Environmental Toxicity and Chemistry. Houston, Texas.

Fischer [Scott], J. C. 1993. Freshwater Mussels of the United States. Biology Laboratory Seminar. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Fischer [Scott], J. C., R. J. Neves, J. L. Farris and D. S. Cherry. 1993. Growth Rates and Age Class Structures of a Freshwater Mussel Species, *Lampsilis fasciola*, in the Clinch River, Virginia to Tennessee. Association of Southeastern Biologists. Virginia Beach, Virginia.

Fischer [Scott], J. C., J. L. Farris, R. J. Neves and D. S. Cherry. 1992. An Examination of Growth Rates of Three Freshwater Mussel Species in the Clinch River, Virginia to Tennessee. Upper Mississippi River Conservation Committee. St. Louis, Missouri.

Yeager, M. M., D. S. Cherry, J. C. Scott, J. H. Van Hassel. 1994. In-stream validation of the effects of intermittent sediment toxicity on recruitment of juvenile unionid mussels. Society for Environmental Toxicology and Chemistry. Denver, Colorado.

Church, G. W., D. S. Cherry, J. C. Fischer [Scott] and R. J. Neves. 1993. Mussel Community Health Assessment in the Clinch River, Virginia. North American Benthological Society. Calgary, Canada.

**Relevant curriculum:**

Animal Physiology	Aquatic Ecotoxicology
Aquatic Entomology	Aquatic Microbiology
Biometry	Cell Physiology
Environ. Animal Physiology	Fish Biology
Freshwater Ecology	Hazard Evaluation of Toxic Chemicals
Invertebrate Zoology	Microbiology
Principles of Ecology	Stream processes
Zooplankton Ecology	

**Professional Societies:**

Association of Southeastern Biologists (ASB)  
North American Benthological Society (NABS)  
Society for Environmental Toxicity and Chemistry (SETAC)

**Honors:**

Tuition Scholarship, VPI&SU -- 1992/93 and 1993/94  
Excellent rating by graduate student evaluation committee (1 of 2 masters students out of 40 total to receive excellent score)  
Walter B. Ellet Memorial Scholarship for Chemistry, VPI&SU -- 1987/88

  
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