LIFE HISTORY OF THE SPECTACLECASE, *CUMBERLANDIA MONODONTA* SAY, 1829 (BIVALVIA, UNIONOIDEA, MARGARITIFERIDAE)

A Thesis

Presented to

The Graduate College of

Southwest Missouri State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Michael S. Baird

May 2000

LIFE HISTORY OF THE SPECTACLECASE, *CUMBERLANDIA MONODONTA* SAY, 1829 (BIVALVIA, UNIONOIDEA, MARGARITIFERIDAE)

Biology Department
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ABSTRACT

Cumberlandia monodonta is a distinctive species of freshwater mussel that was formerly widespread in the Mississippi River basin, but has suffered drastic decline. Reproductive biology and demography of *Cumberlandia* were investigated in the Gasconade and Meramec Rivers, Missouri, which appear to be the present stronghold of the species. During 1999, females were gravid with mature glochidia from early April to late May. No evidence of biannual reproduction was observed. Fecundity was $5.0 \times 10^6 \pm 2.38 \times 10^6$ (1.9×10^6 - 9.6×10^6) glochidia [mean ± st. dev. (range), n = 8]. Males as young as 5 years produced gametes, and females as young as 6. However, inferences from growth rate suggest that major investment in reproduction may not occur until after about 10 years of age. The sex ratio did not differ significantly from 50:50, and no hermaphroditic individuals were observed. Thirty species of fishes were tested as potential hosts by artificial infestation with glochidia, but no transformation was observed. Gills from 690 fish of 40 species were examined for natural infestation. One individual each of bigeye chub (*Notropis amblpos*) and shorthead redhorse (*Moxostoma macrolepidotum*) carried *Cumberlandia* glochidia. However, it is not yet known whether these species are suitable hosts.

Population density and age structure were investigated at 8 sites. Sites were delimited by the presence of *Cumberlandia* and ranged from 480-1800 m^2. Quadrats were placed using an adaptive design, excavated, and searched visually. Approximately 5% of total site area was searched. Over 6,000 live specimens were discovered and 2,880 were measured. The ages of 287 individuals were estimated by counting growth lines in the hinge ligament. Inferred growth rates based on these counts agreed with field measurements of growth, validating the assumption of annual growth lines. Ages estimated from growth lines ranged up to 56 years and were correlated with shell length (mm) as follows: age = (length * 15.4431) / (201.4524-length) (n = 278, $R^2 = 0.83$). This equation was used to infer age from shell length in the demographic samples. Inferred age distributions were similar in both rivers. The most abundant age classes were approximately 20-35 years. Among sites, population densities ranged from 1.2 to 12.8 (mean = 6.7) individuals per m^2, while local (i.e., single quadrat) densities ranged up to 120 individuals per m^2. Although a few young individuals were found at all sites, individuals less than 10 years old comprised only 13.2% of the sample. Therefore, it appears that these populations might be in decline, despite high population densities of adults.

This abstract is approved as to form and content

Chairman, Advisory Committee
Southwest Missouri State University

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Chairperson

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Member

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Member

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Associate Vice President for Academic Affairs and
Dean of the Graduate College

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Chris Barnhart, my advisor, mentor, and friend, without whom this thesis would not have been possible. His wisdom and guidance have made me a better biologist, while his patience (and sometimes tolerance) has helped me to become a better person. Thank you.

I would like to thank my committee members, Dr. John Havel and Dr. Dan Beckman, for their encouragement, suggestions, and criticisms regarding my research and the completion of this thesis. I would like to thank other faculty members, secretaries, and personnel for their technical and logistical support and help. You are all very appreciated.

This research could not have been completed without the help of many fellow graduate students and friends: Bryan Simmons, Frank Riusech, Dave Howlett, Christian Hutson, Lisa Solberg, Doug Dieterman, Brent Jamison, Dana Ramsey, Jennifer Moody-Weiss, and Jill Weggeman.

Finally, thanks to my family for their faith and support in my decisions, goals, and chosen career path, and to my dog “Muga” for keeping me sane and on my toes!

The US Fish and Wildlife Service, Missouri Department of Conservation, and the SMSU Biology Department and Graduate College, provided funding for this project.

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PREFACE

The unionoid mussels are a successful group of freshwater bivalves found in all types of inland waters throughout the world. Most species are lotic (i.e., river dwelling) but many species are well suited for lentic (i.e., pond or lake) environments. Bivalves are filter feeders and remove suspended algae and particulate organic matter (POM) from the water column. Mussels process large volumes of water and are therefore exposed to dissolved toxic substances such as heavy metals (Metcalfe-Smith and Green 1992, Imlay 1982). Bivalves are good biological indicators because of this intimate association with water quality, and are increasingly used in bioassays and biomonitoring (Doherty, et al. 1988, Warren et al. 1995). Bivalves are also important trophic links between suspended foods, such as phytoplankton and POM, and other organisms. For example, many fish species, such as freshwater drum *Aplodinotus grunniens*, blue catfish *Ictalurus furcatus*, and northern hog sucker *Hypentilium nigricans*, feed on freshwater bivalves and terrestrial mammals such as raccoon, muskrat, and otter also rely heavily on them as a food source. Live and dead shells of mussels increase surface area for periphyton and microorganism attachment and provide shelter for micro-invertebrates and small fishes.

Mussel declines – The Unionoidea are one of the most imperiled groups of animals in North America, with nearly 75% of recognized North American taxa considered to be extinct, rare and endangered, or of special concern (Williams et al. 1992, Stein and Flack 1997, NNMCC 1998). Population extirpations and declines of unionoids are largely due to human disturbances such as reservoir construction, channelization, deforestation, pollution, and altered flow regimes and alien fish introductions (NNMCC 1998). These impacts have damaged mussel populations directly and probably also indirectly by limiting or removing natural host fishes. Introduced non-native bivalves from the families Corbiculidae (i.e., Asiatic clams) and Dreissenidae (i.e., zebra mussels) may compete with native mussels for space and resources.

A national strategy for the conservation of the North American freshwater mussel fauna emphasizes the need for research on life histories, population dynamics, and density estimates of mussel populations (NNMCC 1998). Research of this nature is necessary for the conservation, management, propagation, and possible reintroduction of endangered native species.

Taxonomy and distribution - The superfamily Unionoidea includes the families Unionidae and Margaritiferidae. The Unionidae consists of three sub-families: Ambleminae, Lampsilinae, and Anodontinae, and the Margaritiferidae of two: Margaritiferinae and Cumberlandinae. These five subfamilies of unionoids comprise nearly 300 recognized species and sub species in North America (Turgeon et al. 1988). Early taxonomy of the Unionoidea was based largely on shell characters (Davis and Fuller 1981). Shell characters include shape, color, texture, patterns, beak height and sculpturing, cavity depth, nacre color, and dentition. Other classification systems incorporate soft anatomy, reproductive biology, and larval morphology. These characters include number, morphology, and location of marsupial demibranchs; location of developing larvae in the demibranchs; incubation period of the larvae; glochidial shell morphology; diaphragm morphology; and the presence or absence of a supra-anal opening (Ortmann 1911, Utterback 1915, Model 1964, Heard and Guckert 1970). More recently, classifications increasingly use genetic or molecular characters to distinguish and classify taxa (Davis and Fuller 1981, Lydeard et al. 1996).

Most workers consider the Margaritiferidae to be more primitive than the Unionidae (Ortmann 1911, Utterback 1915, Heard and Guckert 1970, Smith 1980, Bauer 1994). Characters presumed to be primitive include lack of true septa between the demibranch lamellae, lack of a supra-anal opening, tetragenous brooding (i.e., use all four gills to brood their young), and very small (~60 μm) semi-spherical, hookless glochidia (Utterback 1915, Howard 1915).

The Margaritiferidae range throughout portions of North America, Europe, and Asia (Smith 1988). Only five species occur in the continental United States. These are the eastern pearlshell mussel *Margaritifera margaritifera* (Linnaeus), western pearlshell mussel *Margaritifera falcata* (Gould), Louisiana pearlshell mussel *Margaritifera hembeli* (Conrad), Alabama pearlshell mussel *Margaritifera marriannae* (Johnson), and spectaclecase *Cumberlandia monodonta* (Say) (hereafter referred to as *Cumberlandia*).

Margaritifera margaritifera is distributed in the western and eastern parts of the United States, and is listed as a species of special concern in CT, MA, ME, NH, NY, PA, RI, VT (Williams et al. 1992). Its northeast distribution centers on New England, the Maritimes, and portions of the Adirondacks (Strayer and Jirka 1997). The conservation status of *Margaritifera falcata* in the United States is undetermined in AK, CA, ID, MT, NM, NV, OR, UT, WA, WY (Williams et al. 1992). *Margaritifera hembeli* is a threatened species and is restricted to 22 headwater streams within the Bayou Rapides, Bayou Rigolette, and Bayou Boeuf drainages in central Louisiana (Johnson and Brown 1998). *Margaritifera marriannae* was thought to be an isolated population of *M. hembeli* until Johnson (1983) separated them taxonomically according to differences in shell morphology. *Margaritifera marriannae* is only found in the state of Alabama, where it is endangered (Williams et al 1992).

Cumberlandia distribution and status – *Cumberlandia* is the only Margaritiferid presently found in the Mississippi River Basin, and it occurs nowhere else in the world. There are no detailed published data on the historic range of *Cumberlandia*. Most published distribution maps show a latitudinal range from Minnesota to the Gulf of Mexico, and a longitudinal range from the eastern borders of Nebraska and Kansas to Pennsylvania (Cummings and Mayer 1992, Oesch 1984, Parmalee and Bogan 1998). Historical collections of *Cumberlandia* have been made from the following rivers: the Rock, Kankakee, Des Plaines, Wabash, Ohio, and Mississippi Rivers, Illinois; the Wabash River, Indiana; the Tennessee River, Alabama; the Clinch, Cumberland, and Tennessee Rivers, Tennessee; the Upper Clinch River, Virginia; the Mississippi River, Iowa; the Green River, Kentucky; the St. Croix River, Minnesota/Wisconsin; and the Platte, Osage, Salt, Sac, Joachim, Borbeuse, Big, Meramec, Gasconade and Mississippi Rivers, Missouri (Cummings 1992, Oesch 1984, Parmalee and Bogan 1998, Natural Heritage Data Base [MDC], Roberts 1998, Buchanan 1980, Sue Bruenderman [MDC] personal communication, Goodrich and van der Schalie 1944, Stansbery 1966, Fuller 1978, Nelson and Freitag 1979, Travis Moore [MDC] personal communication).

There are no records of *Cumberlandia* from the Missouri River mainstem, nor any of its middle to upper tributaries, north or west of the Nebraska State border (Cummings 1992, Oesch 1984, Parmalee and Bogan 1998). Records in Nebraska are questionable (Burch 1973). Shells have been reported from a single location each in the Ouachita River, Arkansas, and upper Marais des Cygnes River, Kansas (Harris and Gordon 1987, Brian Obermeyer [Kansas Wildlife and Parks] personal communication). *Cumberlandia* is thought to be extirpated from Ohio and Indiana (Cummings 1992). The species has never been reported from Texas, Louisiana, or Pennsylvania (Bob Howells [Texas Game and Parks] and Kevin Cummings [Illinois Natural History Survey] personal communications). Only a small number of large populations are known from recent studies. Locations of these populations include: the Upper Clinch River in Virginia and Tennessee, the St. Croix River in Minnesota and Wisconsin, and the Osage, Gasconade and Meramec Rivers in Missouri (Sue Bruenderman [MDC] personal communication, Roberts 1998, Lee and Hove 1997, Michelle Steg [Virginia Tech] personal communication, Natural Heritage Data Base [MDC], Oesch 1984, Buchanan 1980).

Cumberlandia was reported in the Gasconade and Osage rivers, the Mississippi River, Northwest Missouri Lakes, and Platte River basin in northern Missouri (Utterback 1915). Later surveys by Oesch (1984) and Buchanan (1980) recovered individuals or small populations in Joachim Creek, and the Aux Vases, Salt, and Bourbeuse Rivers. Recent surveys have extended the range of *Cumberlandia* to include the Big River (a Meramec River tributary), and the Big Piney and Osage Fork Rivers (Gasconade River tributaries) (Natural History Data Base [MDC], Roberts 1998, Sue Bruenderman [MDC] personal communication, personal observation).

<u>Habitat accounts</u> - *Cumberlandia* is a riverine species, occupying medium to large rivers and streams. Utterback's (1915) mention of "Northwest Missouri Lakes" is problematic. In rivers *Cumberlandia* is usually found in dense beds, often in scattered patches or clusters, often with few or no other mussel species present. Most habitat accounts describe deep pools with moderate current and substrates dominated by large

cobbles and boulders. Stansbery (1966) gave perhaps the best general description for *Cumberlandia* habitat: "…it prefers a substrate among large rocks at the margin where swift current of the mainstream meets quieter water at the edges of pools."

Specific habitats vary. Researchers have collected *Cumberlandia* while diving or brailing in 16 to 40 feet of water (Nelson and Freitag 1979, Travis Moore [MDC] personal communication) and in slow current (i.e., 6.1 to 18.3 cm/second) (Buchanan 1980). Individuals have also been found in shallow (i.e., 2-300 cm) (personal observation) and highly turbulent or rushing water (Stansbery 1966, Fuller 1978, Parmalee 1967). In my experience, these mussels are strongly associated with large cobble and boulders. *Cumberlandia* have been found in silt, sand, firm mud, in vegetation, under large flat rocks, in bedrock crevices, between boulders, and in submerged root wads and tree stumps and riprap wing dams (Stansbery 1973, Buchanan 1980, Oesch 1984, Nelson and Freitag 1979, Travis Moore [MDC] personal communication).

<u>Species description</u> - *Cumberlandia* is easily identified. Its distinctive shape is reminiscent of its common name, spectaclecase. The shell is quite elongate, with rounded anterior and posterior ends. The anterior end is consistently taller (dorsal to ventral) than the posterior end. Individuals reach lengths of 180 mm or more (personal observation). Dorsal and ventral margins of adults are straight to moderately curved, and individuals are commonly subinflated ventrally near their center (Buchanan 1980, Oesch 1984). Beaks are raised slightly dorsal, but rarely appear above the hinge line. The shell is sculptured in the first year of growth. This sculpturing consists of strong ridges running parallel with the growth rests. Hinge ligaments are robust. The periostracum is

smooth without rays or other markings. Very young individuals are straw or caramel in color, turning dark brown to black with age (personal observation). Growth lines are quite distinct in young individuals, but become increasingly indistinct with age. Umbone regions are usually worn in adult individuals. Shells usually crack and flake easily if allowed to dry completely.

The internal shell surface of live and freshly dead *Cumberlandia* is also distinctive. Beak cavities are shallow with iridescent blue nacre in young individuals. On the anterior two-thirds, the nacre fades to white in older individuals, while the posterior third usually maintains its iridescence. The left anterior pseudocardinal tooth is lacking, hence the species name, *monodonta*. The right pseudocardinal is small and conical in shape. Lateral teeth are low and distinct in young individuals, becoming barely discernible in older animals.

Cumberlandia is similar in appearance to other margaritiferid species; however, none of these overlap its range. Unionid species that are superficially similar and are sometimes confused with *Cumberlandia* are the black-sandshell *Ligumia recta*, ladyfinger *Elliptio dilatata*, and salamander mussel *Simpsonaias ambigua*. The black-sandshell and ladyfinger can be distinguished from the spectaclecase by their heavier shell, lanceolate shape, smooth appearance, rayed epidermis (young only in the ladyfinger), well-developed teeth, and purple to peach-colored nacre. The salamander mussel reaches a maximum length of only 40-mm, so it can not be confused with adult *Cumberlandia*. A young *Cumberlandia* (less than 40-mm) is similar to an adult salamander mussel in lateral view. However, adult salamander mussels tend to be much more laterally inflated than a similar sized *Cumberlandia*, and will be much older, therefore having more growth lines. Also, their beaks are sharp with fine parallel looped sculpturing. *Cumberlandia* beaks are dull, and sculpturing consists of heavy ridges which run parallel to growth rests.

Very few studies have been directed at *Cumberlandia*. The available literature deals primarily with taxonomy (Utterback 1915; Burch 1973), anatomy (Smith 1980), or recent survey work (Nelson and Freitag 1978, Buchanan 1980 and 1994, Roberts 1998). The only published information on life history traits is contained in a few short communications (Howard 1915, van der Schalie 1966 and 1970, Gordon and Smith 1990, Lee and Hove 1997). An unpublished study of *Cumberlandia* in the St. Croix River investigated population size structure and seasonal timing of reproduction (David Heath [Minnesota Department of Natural Resources] personal communication).

Research on *Cumberlandia* is critically needed because of the troubled conservation status of this unique species. The spectaclecase is the only member of its genus and is one of only five Margaritiferid species found in North America. Although formerly widespread throughout the Mississippi drainage, it has declined dramatically throughout its range. *Cumberlandia* is currently considered to be threatened in Iowa, Illinois, Kentucky, Minnesota, Missouri, Tennessee, Virginia, and Wisconsin, and may already be extirpated from Alabama, Arkansas, Indiana and Ohio (Williams, et al 1992). Missouri appears to have the largest remaining populations, particularly in the Gasconade and Meramec Basins.

The purpose of the current study was to investigate life history characteristics that are important to the preservation of *Cumberlandia*. More specifically, the objectives of this study were to 1) determine reproductive periodicity and identify the host or hosts required for the completion of its life cycle, 2) determine the pattern of growth and develop a model to infer age from shell measurements, and 3) investigate the demography of several populations in the Gasconade and Meramec Rivers in Missouri. Chapter 1 will describe studies of reproduction of *Cumberlandia*, and Chapter 2 will describe age, growth, and population structure of populations in the Gasconade and Meramec Rivers.

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Chapter one

REPRODUCTIVE BIOLOGY OF THE SPECTACLECASE, *CUMBERLANDIA MONODONTA (SAY* 1829) IN THE MERAMEC AND GASCONADE RIVERS, MISSOURI

ABSTRACT

The reproductive biology of *Cumberlandia* was investigated using 278 individuals collected from 12 sites along the Gasconade and Meramec Rivers, Missouri. Males and females did not differ in external morphology, so sex was determined by gonad puncture. The observed sex ratio did not differ significantly from a 50:50 ratio. No hermaphroditic individuals were observed. Age at maturity was determined to be between 4 and 5 years for males, and 5 and 7 years for females. Eggs were observed in gills of 70% of females examined (n = 150) between September and December 1998, however females gravid with mature glochidia were only observed from 1 April to 24 May 1999. No evidence of biannual reproduction was observed. Conglutinates were pale, variable in size, and appeared branched and feather-like. The proportion of undeveloped eggs in conglutinates varied from zero to 100%. The number of glochidia produced was $5.0x10^6 \pm 2.38x10^6$ ($1.9x10^6$ - $9.6x10^6$) [mean ± st. dev. (range), n=8]. Thirty species of fishes were tested as potential hosts by artificial infestation with glochidia. No transformation was observed. Gills from 690 fish of 40 species were examined for natural glochidia infestations. One individual each of bigeye chub (*Notropis amblops*) and shorthead redhorse (*Moxostoma macrolepidotum*) carried *Cumberlandia* glochidia. It is not yet known whether these species are suitable hosts.

INTRODUCTION

North America contains the largest diversity of unionoid mussels of any continent. Two families of unionoids, the Unionidae and the Margaritiferidae, comprise nearly 300 recognized species and sub species in North America (Turgeon et al. 1988). Seventy-five percent of these taxa are thought to be extinct, rare and endangered, or of special concern, making the Unionoidea the most imperiled major taxon of animals in North America (National native mussel conservation committee (NNMCC) 1998, Stein and Flack 1997, Williams et al. 1992).

Among the many threatened North American unionoids, the spectaclecase *Cumberlandia* is unique in several respects, yet it has received very little attention from biologists. *Cumberlandia* is the only member of its genus and subfamily (Cumberlandinae) and is the only Margaritiferid species presently found in the Mississippi River drainage. Much of the reproductive biology of the species is poorly known, including breeding system (hermaphroditism vs. gonochorism), reproductive timing (long-term brooder vs. short-term brooder), fecundity, age at maturity, and the identity of the host species for the parasitic larval stage.

The unionoid life cycle is composed of four basic stages: the adult, fertilized egg, parasitic larva or glochidium, and juvenile mussel (Parmalee and Bogan 1998). Sexes are usually separate, although not always distinguishable externally. A few unionoids have been identified as hermaphroditic, but most species are gonochoristic (van der Schalie 1970, Heard 1975). The timing of reproduction in unionoids varies. Many species spawn in the fall, and release glochidia the following spring or summer. Other species spawn and release glochidia during the same spring or summer. Fecundity also varies, with the total number of glochidia produced by individual females ranging from tens of thousands to tens of millions (Bauer 1994). Eggs are held within spaces in the female's gills (marsupia). Males release sperm into the water, to be taken up by the female and fertilize the eggs, which develop into parasitic larvae termed glochidia. Glochidia must attach to a vertebrate host (usually a fish) for completion of the life cycle. During attachment, internal organs mature, but only a few species grow while encysted on their host (Bauer 1987a, Nezlin et al.1993).

Most unionoids are host-specific, and are able to utilize only one or a few host species. Many Lampsilines have evolved intricate adaptations to facilitate glochidia-host contact (e.g. hooked glochidia, mantle lures, and conglutinates) (e.g., Barnhart and Roberts 1996, Haag and Warren 1997, Hartfield and Hartfield 1996). However, Margaritiferids apparently generally lack these adaptations. Knowledge of host species is prerequisite for some interventions aimed at conserving unionoids, including captive propagation, and reintroduction. Hosts are frequently investigated by artificial inoculation with glochidia, but observation of transformation in laboratory conditions does not guarantee identification of ecologically relevant hosts. Therefore, it is also desirable to survey natural populations of mussels and their hosts and observe natural infestations (Michaelson and Neves 1995, Bauer 1987b, Smith 1976).

With the exception of surveys and anatomical work, very little is known about *Cumberlandia*. A few publications have described aspects of its reproductive period and pattern (Howard 1915, Gordon and Smith 1990, van der Schalie 1966 and 1970). A limited amount of additional information can be found in unpublished studies. Many other life history traits are still unknown, including its host organism, reproductive output, and age at sexual maturity. The goals of this study for *Cumberlandia* were to 1) describe sex ratios, occurrence of hermaphroditism, age at maturity, and fecundity ranges, and 2) determine reproductive timing and host organism(s) responsible for the completion of its life cycle.

MATERIALS AND METHODS

Study Areas - the study was carried out in the Gasconade and the Meramec rivers (**Figure 1**). Both are moderately large rivers and have similar drainage areas (5760 and 6368 square kilometers respectively) (Pflieger 1997). Both drain the northeastern portion of the Ozark Plateau. The Gasconade River flows northeast, 461 kilometers to its confluence with the Missouri River near the town of Gasconade, Missouri (Missouri Water Atlas, 1986). The Meramec River also flows northeast, and drains the area east of the Gasconade basin, 365 kilometers to its confluence with the Mississippi River, south of St. Louis, Missouri. Both rivers lie within the Ozark-Mississippi I Division of the Ozark Plateau which is characterized by high local relief, steep gradient streams, numerous springs, and clear surface water (Pflieger, 1997). Neither river has been impounded or channelized, and a total of 11 USGS gauging stations can be found along their mainstems, making stage and discharge information readily accessible.

Twelve sites with *Cumberlandia* populations were visited in the Gasconade and Meramec River basins **(Figure 1**). All but two of these sites were located on the Gasconade and Meramec River mainstems. The two exceptions were the Big River (a Meramec tributary) and the Big Piney River (a Gasconade tributary). All sites occurred on outside-river-bends, below limestone bluffs, and usually immediately downstream of a riffle or secondary channel. Large cobbles and boulders, with interspersed gravel and sand, dominated site substrates. Depth and current velocity was highly variable. See **Appendix A** for directions to collecting sites, and more detailed site descriptions.

Reproductive timing - *Cumberlandia* were collected in the Meramec and Gasconade rivers, Missouri at 12 sites on 22 dates between 8 September 1998 and 7 October 1999. A total of 384 (mean = 34 per site) animals were examined for sex and reproductive status (**Table 1**, **Figure 2**). Collections were made by snorkeling or diving using a surface air supply unit. The mussels were placed individually in bags in an insulated cooler with river water. Ice was added to the cooler during warm months. Mussels were transported to the lab and transferred to containers with aerated conditioned water. Some individuals were kept at room temperature (see below) but most were kept at 10 C. 285 individuals were measured (total length, width, height and weight) to the nearest 0.1 millimeter using dial calipers, weighed to the nearest 0.1 gram using an electronic field balance, and all were eventually sacrificed and sexed by gonad puncture (see below).

Fish used for laboratory host tests - Fish were obtained from field collections and hatcheries (**Table 2**). Collections were made with boat and backpack electrofishing gear, seines, cast nets, and hand-held dip nets. Fish were transported in insulated coolers with sufficient aeration. Stress related mortality was limited by the addition of marine salt and ice during warm-month collections, (i.e., June through September) (Murphy and Willis, 1996). Fish were transferred to 10 or 20-gallon aquaria, treated with Maracyn, and maintained at 18–20^{0} C until host tests were performed. Fish were fed commercial flake or pellet food, frozen brine shrimp, frozen bloodworms, or other fishes, depending on species.

Sex determination - *Cumberlandia* are not externally sexually dimorphic. Therefore, sex was determined by observing oocytes or spermatogonia in fluid obtained by gonad punctures. A nasal speculum was used to spread the shells apart, and a few microliters of fluid were then drawn from the gonad using a clean syringe and sterile # 23 needle. The fluid was then viewed under a light microscope at 100X magnification. In most individuals, only a single fluid sample was taken. However, in 11 individuals, gonad punctures were performed at 10 sites dispersed throughout the gonad, using a clean syringe for each site.

Fecundity- Fecundity was estimated in eight gravid females collected 1 and 2 April 1999 (**Table 3**). These individuals were kept in shallow water at room temperature in clean plastic containers, and were checked daily for expelled conglutinates (cohesive aggregates of eggs) (**Figure 2**). Conglutinates were preserved in 70% ethanol. Individuals were checked daily, until no more conglutinates were found for at least three weeks. All conglutinates from each individual were counted. A subset of these conglutinates from each individual (roughly 5%) was measured. The number of glochidia produced by each female was then estimated by dispersing all of the conglutinates from each female in a known volume of water, and counting the eggs and glochidia in subsamples of this volume. I used an Omni 2000 homogenizer to separate and suspend eggs and glochidia in 1000 milliliters of water. This treatment did not appear to rupture or fragment eggs or glochidia. To test this possibility, a single 1000-milliliter sample was homogenized at three 1-minute intervals, and four 47-microliter sub-samples were taken after each homogenization. The counts for each set of sub-samples were 153 ± 18.5, 181 ± 29.8, and 167 ± 22.6, respectively (mean ± standard deviation).

Direct counts were made from sub-samples taken from the suspensions. The suspension was stirred vigorously to suspend the glochidia, and a Gilson pipette was used to remove six 47-microliter samples for counting. Glochidia and embryos from each sample were counted at 40X magnification with a light microscope. Counts from each of the six samples were averaged, and this number was used to extrapolate total fecundity for each individual (**Table 3**).

Laboratory host infections - Potential host fish were inoculated with glochidia in the laboratory (**Table 2**). Glochidia were freed from conglutinates by vigorous pipetting in a clean dish of conditioned water. When available, glochidia from several individuals were used. Subsamples of glochidia were examined at 40 or 100X magnification with a light microscope. Viability was tested by adding salt crystals, which cause valve closure. In each case, over 98% of glochidia closed in response to saline.

Most test fishes were anesthetized with Finquel, and infected by pipetting glochidia directly onto the gills. Infected fish were immediately transferred to a recovery tank for ten minutes before being returned to the aquaria. Most fish were sacrificed at two weeks post-infection. The gills were dissected and examined under a compound microscope.

In some tests, fishes were infected by feeding on conglutinates that were pipetted into the aquaria. The fish were observed to ensure that each had consumed at least one fragment of conglutinate. In a few tests, fish were infected by swimming in a bath of conditioned water with viable glochidia. Glochidia were added to a 180 mm diameter glass bowl, with just enough conditioned water to enable test fish to swim upright. The bath was aerated with a pump and air stone to keep glochidia suspended in the water. Infection times varied from two to ten minutes. After infection, fishes were anesthetized, rinsed, and examined under a dissecting scope to make sure glochidia had attached to gills. All infected fish were anesthetized at two and four days post-infection and the gills were observed under a dissecting microscope for glochidial cysts.

Natural host infections - Fish were collected to assess natural infections with *Cumberlandia* (**Tables 5, 6**). Fishes were collected from within and around 2 known *Cumberlandia* beds with boat and backpack electrofishing gear and a seine. These collections were made a few weeks following the period when glochidia were thought to have been released (first week of April through the end of May). Up to approximately 25 individuals per species per drainage were captured. Fish were transported on ice to the lab, identified to species, and measured to total length (mm). Gills were dissected, labeled, and preserved in 90% ethanol until they could be examined for glochidia.

Observation of glochidia on preserved gills - *Cumberlandia* glochidia are very small (~60 μm). Therefore, gills were examined carefully to ensure that *Cumberlandia* glochidia were not overlooked. Gills from each fish were placed in 1 N NaOH in petri dishes. Sodium hydroxide hydrolyzed the tissue, enabling the glochidia to be observed more easily. Gills were left in NaOH for 30 minutes to several days, depending on size and amount of flesh on the arches. Any glochidia found were measured and identified by comparison with a reference collection of glochidia of known identity.

RESULTS

Sex determination and maturity - *Cumberlandia* shells were not sexually dimorphic. Two different allometric measures are shown in **Figures 4 and 5**. Of the 317 individuals that were examined by gonad puncture, 51.7% were male and 48.3% were female. These percentages did not differ significantly from 50:50. No hermaphroditic individuals were observed. That is, only sperm or ova, but not both, were seen in any one individual. The smallest three individuals in which sperm were observed were 44, 49, and 50 mm, and the smallest three with ova were 56, 74, and 76 mm. No gametes were seen in 12 individuals 9, 25, 29, 30, 25, 42, 40, 19, 14, 45, 41, 51 mm. Based on ages determined from the hinge ligament (chapter 2), these individuals were 1,2,3,3,2,4,4,2,1,4,4 and 5 years of age respectively.

Most gravid individuals released solid conglutinates, but in some individuals the eggs and glochidia did not cohere, and were released as a slurry of glochidia and small clumps of eggs, rather than conglutinates. There seemed, therefore, to be a range of conglutination. When conglutinates were well formed, they were pure to off-white in color, branched, and composed of mature glochidia, embryos, and undeveloped ova. Mean lengths and widths of conglutinates ranged from 10.02-15.78 and 2.35-3.70 mm respectively (**Table 3**, **Figure 2**).

Cumberlandia glochidia were subcircular in shape, and approximately 60 μm in both height and length. Their hinges measured approximately 40 μm in length (**Figure 3**). No hooks or grasping structures were observed. Measurements made by Howard (1915) agree with ours. These glochidia are apparently the smallest of any North

American unionoid (length = 6.05 ± 0.11μm, width = 6.00 ± 0.00μm, hinge length = 4.10 ± 0.22μm).

<u>Fecundity</u> - The number of conglutinates released per individual was 64.5 ± 13.9 (mean ± standard deviation, n=8) and ranged from 53-88. There was a positive correlation between number of conglutinates released and mass (**Figure 6**). Four of the eight individuals contained only ova, three individuals contained ova and glochidia, and one individual contained only glochidia. The percentage of ova in the three individuals that had both ova and glochidia were 9.5%, 22.4%, and 22.5%. Fecundity was calculated in two ways. If all ova and glochidia were included, regardless of stage of development, the mean ± standard deviation and range was $5.5 \times 10^6 \pm 2.47 \times 10^6$ (3.9×10^6 - 9.6×10^6). If only glochidia are counted, and only the individuals that had mature glochidia are included, the mean was $5.0 \times 10^6 \pm 2.38 \times 10^6$ (1.9×10^6 - 9.6×10^6).

<u>Laboratory host infections</u> - No transformation was observed in thirty-five tests on 26 potential host species. Seven families of fishes, including one exotic family (Gobiidae), and one amphibian family (Ambystomatidae) were tested (**Table 4**). Glochidia encysted on all species, but in all but four species, sloughed off by day three post-infection. Four species held encysted glochidia through day four post-infection: these were flathead catfish (*Pylodictus olivaris)*, channel catfish (*Ictalurus punctatus)*, longear sunfish (*Lepomis megalotis)*, and redear sunfish (*Lepomis microlophus*).

<u>Natural host infections</u> - Complete sets of gills from 690 individual fish (Gasconade 321, Meramec 369) were examined for the presence of glochidia or glochidial cysts. Ten families of fishes representing 40 species were examined (31 species from the Gasconade River, and 32 species from the Meramec River) (**Tables 5**

and 6). Two fishes collected from the Meramec River carried *Cumberlandia* glochidia: these were bigeye chub (*Notropis amblops)*, and short-head redhorse (*Moxostoma macrolepidotum)*. A single big-eye chub was found to have 17 *Cumberlandia* glochidia on its gills, and one short-head redhorse was found to have a single *Cumberlandia* glochidium on its gills (**Table 7**). Measurements from these recovered glochidia matched those of *Cumberlandia* voucher specimens. None of the 18 recovered *Cumberlandia* glochidia had noticeably grown while encysted. No *Cumberlandia* glochidia were recovered from fishes collected in the Gasconade River.

DISCUSSION

Sex ratio - The observed sex ratio (47:53 male:female) did not differ significantly from a 50:50 ratio (X^2 test). A 50:50 sex ratio is typical of *Margaritifera* (Hendelberg 1960, Bauer 1987d) and other unionoids (Yeager and Neves 1986, Weaver et al 1991). Although the shell shape of many unionoids is sexually dimorphic (Ortmann, 1911; Cummings and Mayer 1992; Oesch, 1984), this is not the case in *Cumberlandia* (Figures 4, 5). Even gravid females are difficult to identify externally, because the marsupial gills do not swell dramatically and are difficult to see through the shell gape. Monomorphic species can be sexed using soft anatomy (Ortmann, 1911; Heard and Guckert 1970, Heard, 1975), by viscera sectioning (Yokely, 1972; Smith, 1988; Yeager and Neves, 1986; Gordon and Smith 1990), or by gonad puncture (Bauer 1987d, Riusech 1999 thesis). Gonad puncture is particularly useful because it does not require sacrificing the individual.

Hermaphroditism - Observations of hermaphroditism in margaritiferids have been inconsistent. Smith (1988) found no evidence of hermaphroditism in 43 specimens of *M. hembeli*. No hermaphrodites were found among *M. Margaritifera* examined by Ortmann (1911) and Hendelberg (1960). However, van der Schalie (1970) reported 1 of 12 *M. Margaritifera* to be hermaphroditic. Bauer (1987d) examined 404 *M. Margaritifera* from eight rivers and found 47.5% were male, 20.8% were hermaphroditic, and 31.7% were female. Interestingly, Bauer noted change of sex (particularly females to hermaphrodites) in individuals removed from dense populations and relocated into areas devoid of mussels. Bauer (1987d) suggests that hermaphroditism may be rare in large, dense populations and more common in sparse populations, perhaps as an adaptive response.

In the present study, no hermaphrodites were observed. This result is consistent with previous studies of *Cumberlandia* (Howard 1915, Gordon and Smith 1990). Although van der Schalie (1966) reported that hermaphroditism might occur in *Cumberlandia*, a later, expanded, version of his paper did not report hermaphrodites in 12 *Cumberlandia* examined (van der Schalie 1970). Given the large number of individuals examined in the present study, hermaphroditism does not seem to be an important or common reproductive trait in this species.

Sexual maturity - Age at maturity varies among margaritiferids and other unionoideans. The age at maturity observed in this study of *Cumberlandia*, ~5-6 years (chapter 2), appears to be relatively young compared to other Margaritiferids. *Margaritifera hembeli* were sexually mature as small as 49-69 mm, and as young as estimated 6-9 years of age (Smith 1988). North American *Margaritifera margaritifera* males are thought to mature by age 7-8, and females by age 9 (Smith [University of Massachusetts] personal communication). European M. *Margaritifera* apparently mature at a much later age, approximately 20 years. This late maturity was associated with extremely long potential lifespan, exceeding 100 years (Bauer 1987d). Among unionids, *Venustaconcha pleasii* and *V. ellipsiformis* were inferred to mature at 4 years of age and seldom exceeded 15 years of age (Riusech 1999).

Reproductive timing - *Cumberlandia* appear to be short-term brooders that produce gametes and release glochidia mainly in the spring. In this study, *Cumberlandia* were examined in all but two months of one year (January and July 1999). Although most females (~70%) examined in September - November had a few eggs in their gills, no glochidia were observed during this time. All of 69 males examined during Sept - Nov contained mature spermatozoa. Gravid females, with laden gills and mature glochidia, were collected from the beginning of April through the end of May 1999. No gravid females were recovered before or after this period.

Other published data on *Cumberlandia* reproduction generally support my results. The earliest information is given by Lea (1842) who noted that 7 individuals, collected between mid September and mid November, did not have ova in the gills. A single individual collected on 2 May in the Mississippi River, near Moline, IL (similar latitude to my sites) bore both eggs and glochidia (Howard 1915). Both Howard (1915) and Gordon and Smith (1990) speculated that two broods were produced in a season. Gordon and Smith examined three specimens collected from the Meramec River, Missouri, on 27 October 1982, and inferred that they had recently spawned based upon spaces in their gonads. Other authors have repeated the inference of two broods (Oesch 1984, Parmalee and Bogan 1998). However, no evidence of biannual reproduction was observed in the present study.

Undeveloped eggs - Of the eight gravid *Cumberlandia* examined in detail, the proportion of undeveloped eggs varied from zero to 100%. Both Howard (1915) and Gordon and Smith (1990) made similar observations. Several explanations are possible. First of all, the undeveloped eggs may have been fertilized but not yet developed. The individuals that had ova but no glochidia might have simply been collected early in the brooding period. Second, some eggs may not have been fertilized. The observation that some individuals had mature glochidia as well as a fairly large proportion of undeveloped eggs is interesting. Ovulation may be a prolonged process (Gordon and Smith 1990). At least a few eggs are deposited into the gills in the fall and winter. These eggs may go unfertilized if males typically do not release sperm until spring. Alternatively, the supply of sperm may sometimes limit fertilization even in the spring, so that a proportion of the eggs go unfertilized. Third, many unionoids appear to normally produce a large number of structural or sterile eggs, which do not develop but are an important component of conglutinates (M. C. Barnhart, Southwest Missouri State University, unpublished observations).

Fecundity - Fecundity estimates for freshwater mussels range from tens of thousands to several million eggs per individual female (Bauer 1994, Neves 1993). The fecundity of *Cumberlandia* and other margaritiferids is impressive. In the present study, individual females released from 3 to 9 million glochidia (Table 3). Fecundity of the European pearl mussel, *M. margaritifera*, is similar (Bauer 1987d, 1994). Fecundity is positively related to body size and inversely related to glochidia size (Bauer 1994). The very large number of glochidia produced by *Cumberlandia* correlates with relatively large body size and with the extremely small size of the glochidia (60 x 60 µm), which apparently are the smallest of any North American unionoid. The glochidia of *M. margaritifera* measure 60 x 70 µm (Nezlin et al. 1993) to 60 x 80 µm (Smith 1976). Other species with very small glochidia include *Truncilla donaciformis* (60 x 63 µm) and *Leptodea fragilis* (70 x 95 µm) (Surber 1914).

Conglutinates - Many unionids release conglutinates, which are cohesive aggregations of eggs. Alternatively, glochidia may be free of the eggs when they are released. I could find no published literature on conglutinate production by *Margaritifera*. Conglutinates were often produced by *Cumberlandia* during this study. Other researchers have encountered conglutinates for this species as well (Lee and Hove 1997, Knudsen and Hove 1997). *Cumberlandia* conglutinates are pure to off-white in color and are composed of densely packed eggs. When eggs containing glochidia were present, undeveloped or immature eggs were in the minority. Conglutinates are quite variable in size due to breaking up upon release from the female. They have a branched, feathery shape, similar to branches of an evergreen tree (**Figure 2**). Previous descriptions of the conglutinates agree with our observations (Lee and Hove 1997, Knudsen and Hove 1997).

When conglutinates are released from the female, they are usually entrained in mucus. I have observed this in the laboratory. Lee and Hove (1997) describe this phenomenon, and it was videotaped by Mark Endris (Wisconsin Department of Natural Resources) in the lower St. Croix River. However, it is not yet understood whether *Cumberlandia* conglutinates are involved in host attraction. In other unionoid species, e.g. *Elliptio dilatata,* immature conglutinates are cohesive, while the mature glochidia are dispersed (i.e. not conglutinated) on release from the female. I have observed the release of loose glochidia and small fragments of conglutinate from some individuals of *Cumberlandia*. It is possible that solid conglutinates are not yet mature. Release of immature conglutinates can be induced by disturbance (personal observations).

<u>Fish host</u> - The host for *Cumberlandia* remains unknown. We have used similar methods to successfully transform several other species of mussels on suitable hosts. Therefore, it appears probable that we have not yet tested an appropriate host. In addition to the 35 potential host species tested in the present study, at least 20 other species have been tested by Hove et al. (1997) without success. Longer encystment times were observed in that study, exceeding 20 days on Iowa darter (*Etheostoma exile*), channel catfish (*Ictalurus punctatus*), longnose dace (*Rhinichthys cataractae*), northern redbelly dace (*Phoxinus eos*), and tiger salamander (*Ambystoma tigrinum*) (Hove et al. 1997). The significance of these longer encystment times, relative to the present study, is not clear.

Laboratory host tests may reveal potential hosts, but only observations of natural infestations can identify the ecologically relevant hosts. In addition to laboratory infestations, I attempted to identify potential hosts by collecting fishes that were naturally infected with *Cumberlandia* (cf. Smith 1976, Weaver et al 1991, Michaelson and Neves 1995, Roberts 1997). Two of 690 fish carried *Cumberlandia* glochidia: these were bigeye chub (*Notropis amblops),* and short-head redhorse (*Moxostoma macrolepidotum*). It is not known whether these fish are hosts, because transformation was not observed. Moreover, the encysted glochidia had not grown measurably. Mussel species with very small glochidia, including *M. margaritifera,* typically grow while encysted on their host (Nezlin et al. 1993, Bauer 1987a, and personal observation). Thus, the lack of growth further compromises interpretation of these observations. Moreover, big-eye chub apparently do not occur in the Gasconade River (Pflieger 1997, Matt Winston [MDC] personal communication). Therefore, even if it proves to be a host, bigeye chub is unlikely to be the sole host. Short-head redhorse are found in both the Gasconade and Meramec rivers.

Other margaritiferids use salmonids or madtoms (Ictaluridae) as hosts. Rainbow trout (*Salmo gairdnerii*) and brown trout (*Salmo trutta*) are hosts for *M. falcata* (Smith 1976). The brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) are hosts for *M. margaritifera* in Europe (Nezlin et al 1993, Bauer 1987c), while *Salmo fontinalis* is its natural host in Massachusetts (Smith 1976). In contrast, *M. hembeli*, uses the brown madtom *Noturus phaeus* (Johnson and Brown 1998). It is highly unlikely that *Cumberlandia* use a salmonid host since these fish are not native to the Mississippi River basin (Pflieger 1997). Three species of madtom are found in the Gasconade and Meramec rivers: the slender madtom (*Noturus exilis*), stonecat (*Noturus flavus*), and freckled madtom (*Noturus nocturnus*). In the present study, both slender madtoms and stonecats were tested unsuccessfully.

One of the difficulties in identifying the host for *Cumberlandia* is the great diversity of fish species occurring within its range. (**Appendix B**) lists the 90 fish species currently known to be shared by both the Gasconade and Meramec rivers, Missouri. The appendix also indicates the 58 species that are shared by these rivers and the St. Croix River in Minnesota, where *Cumberlandia* also occurs. Interestingly, the Black River system, which lacks *Cumberlandia,* also contains all of the fish species listed in Appendix A. Because *Cumberlandia* is relatively habitat-specific, its host or hosts may be habitat-specific also. Fishes that were commonly observed near beds included long-ear sunfish, smallmouth bass, largemouth bass, rainbow and/ or orangethroat darters, Missouri saddle darters, greenside darters, and northern hogsuckers.

Given the dramatic decline in abundance and range for this species, further research is needed to pinpoint its host organism. Identification of the host is essential to fully understand the life history strategy of *Cumberlandia*. Host identification would lend knowledge to the mode of dispersal, and help predict the location of desirable habitat and additional populations. Knowledge of the host may also help pinpoint reasons for declines. This information would ultimately be used in conserving and managing this species where it still occurs, and could potentially be used in propagation and reintroduction of *Cumberlandia* into native habitats throughout its range.

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Table 1. Chronological listing of *Cumberlandia* collections and reproductive status of females. N = 317. NG = females not gravid. Collection sites are labeled in Figure 1 and described in Appendix A.

Legal description	**County**	**Drainage**	**Site**	**Date**	**sex ratio (M:F)**	**n**	**Reproductive status**
T40NR01WS07	Franklin	Meramec	1	09-08-98	7:11	18	NG
T43NR4ES19	Jefferson	Meramec	2	09-09-98	11:8	19	NG
T36NR10WS13	Pulaski	Gasconade	6	09-29-98	9:11	20	NG
T42NR8WS15/16	Osage	Gasconade	7	10-27-98	9:6	20	NG
T40NR8WS8	Maries	Gasconade	8	10-27-98	7:11	20	NG
T36NR13WS22/23	Pulaski	Gasconade	9	10-29-98	10:10	20	NG
T43NR2ES20SE	Franklin	Meramec	3	11-17-98	8:12	20	NG
T39NR2WS15	Crawford	Meramec	4	11-17-98	10:10	20	NG
T36NR13WS22/23	Pulaski	Gasconade	9	12-30-98	3:6	10	NG
T39NR2WS15	Crawford	Meramec	4	02-25-99	4:3	7	NG
T36NR13WS22/23	Pulaski	Gasconade	9	02-25-99	2:6	8	NG
T36NR13WS22/23	Pulaski	Gasconade	9	04-01-99	3:7	10	Gravid
T43NR2ES20SE	Franklin	Meramec	3	04-02-99	5:5	12	Gravid
T36NR13WS22/23	Pulaski	Gasconade	9	05-03-99	N/A	10	Gravid
T39NR2WS15	Crawford	Meramec	4	05-03-99	N/A	10	Gravid
T39NR2WS15	Crawford	Meramec	4	05-19-99	N/A	17	Gravid
T36NR13WS22/23	Pulaski	Gasconade	9	05-19-99	N/A	12	Gravid
T43NR4ES19	Jefferson	Meramec	2	05-24-99	6:4	10	Gravid
T43NR2ES29SE	Franklin	Meramec	3	05-24-99	N/A	10	Gravid
T42NR8WS15/16	Osage	Gasconade	7	06-09-99	3:3	6	NG
T35NR14WS10/15	Laclede	Gasconade	10	08-09-99	N/A	8	NG
T35NR13WS19NW	Pulaski	Gasconade	11	08-12-99	5:5	15	NG
T36NR12WS6NE	Pulaski	Gasconade	12	08-26-99	17:15	36	NG
T40NR8WS8	Maries	Gasconade	8	09-09-99	22:10	34	NG
T43NR2ES20SE	Franklin	Meramec	3	09-15-99	14:3	18	NG
T39NR2WS15	Crawford	Meramec	4	09-23-99	1 male	1	NG
T40NR01WS07	Franklin	Meramec	1	10-04-99	4:3	7	NG
T40NR01WS06	Franklin	Meramec	5	10-07-99	4:4	9	NG

Table 2. Fishes collected for laboratory host tests on *Cumberlandia.*

Drainage/River Hatchery	County	Genus/species	Collection date	n
Chesapeake Hatchery	Lawrence	*Micropterus salmoides*	06-30-97	6
			04-23-98	4
			06-19-98	3
		Lepomis macrochirus	06-30-97	3
		Lepomis microlophus	06-30-97	4
		Stizostedion vitreum	06-19-97	4
Osage Catfisheries		*Ictalurus punctatus*	N/A	3
				3
Midwest Science Center Hatchery	Boone	*Pimephales promelas*	04-12-99	6
Missouri Drainage				
Missouri River	Boone	*Pylodictus olivaris*	10-02-98	7
Missouri River		*Ictalurus punctatus*	10-02-98	4
Missouri River		*Aplodinotus grunniens*	10-02-98	2
			08-18-97	8
White River Drainage				
James River	Greene	*Noturus flavater*	06-03-97	2
James River		*Noturus exilis*	04-11-98	4
James River		*Cottus ozarkae*	04-12-98	3
Pearson Cr.		*Cottus bairdi*	04-21-98	3
Pearson Cr.		*Cottus carolinae*	N/A	1
Pearson Cr.		*Etheostoma spectabile*	04-21-98	7
James River		*Etheostoma caeruleum*	04-11-98	4
Meramec Drainage				
Red Oak Cr.	Crawford	*Percina caprodes*	04-25-98	2
Red Oak Cr.		*Etheostoma tetrazonum*	4-25-98	1
Red Oak Cr.		*Etheostoma blennioides*	04-25-98	4
Red Oak Cr.		*Etheostoma caeruleum*	04-25-98	9
Red Oak Cr.		*Notropis chrysocephalis*	04-25-98	2
Red Oak Cr.		*Notropis zonatus*	04-25-98	3
Meramec R.		*Micropterus punctulatus*	04-02-99	2
Meramec R.		*Lepomis megalotis*	04-02-99	2

Table 3. Dimensions and fecundity of eight female *Cumberlandia*. The column labeled "Number of conglutinates" gives both the total number of conglutinates that were released, and in parentheses, the number that were measured. Conglutinate measurements are means ± standard deviation. Asterisk denotes samples consisting of ova only. Estimate in bold consisted of glochidia only.

Drainage	***Cumberlandia* measurements**			**Number of conglutinates**	**Conglutinate measurements**			**Estimated Number of ova and glochidia**
	Length (mm)	**Height (mm)**	**Mass (g)**		**Length (mm)**	**Width (mm)**	**Wet mass (g)**	
Gasconade	138.1	47.4	149.0	60 (12)	15.8 ± 4.19	3.7 ± 1.23	0.2 ± 0.06	**3,930,000** ± **533,147.5**
Gasconade	124.5	43.8	98.4	64 (13)	13.7 ± 2.56	2.5 ± 1.11	0.1 ± 0.03	*1,930,000 ± 276,895.6
Gasconade	148.4	49.4	145.0	60 (12)	10.0 ± 5.62	2.5 ± 0.87	0.1 ± 0.05	*5,730,000 ± 587,583.4
Gasconade	N/A	N/A	N/A	53 (12)	15.1 ± 3.43	3.0 ± 1.55	0.1 ± 0.09	*2,620,000 ± 221,522.0
Meramec	166.5	58.0	262.5	88 (22)	14.1 ± 3.88	2.4 ± 0.52	0.1 ± 0.04	*4,630,000 ± 655,754.2
Meramec	190.9	63.8	381.3	84 (22)	15.1 ± 3.68	2.7 ± 0.66	0.1 ± 0.06	9,570,000 ± 420,986.4
Meramec	139.0	53.0	118.5	54 (12)	12.3 ± 2.20	3.1 ± 1.24	0.1 ± 0.02	5,030,000 ± 834,303.0
Meramec	N/A	N/A	N/A	53 (12)	12.4 ± 2.91	3.0 ± 0.94	0.1 ± 0.10	6,300,000 ± 298,074.9

Table 4. *Cumberlandia* host tests, listed by family and species, with dates corresponding to: collection, artificial inoculation, days post-infection, and gill dissection. No transformation of glochidia to juvenile mussels was observed.

Family	Species	Locality	Collection date	n	Date infected	Day – 2 (cysts?)	Day – 4 (cysts?)	Date gills dissected	Success
Ictaluridae	*Pylodictus olivaris*	Missouri R. near Hartsburg, MO	10-2-98	4	4-1-99	Yes, few	Yes, few	4-15-99	No
				3	4-5-99	No	No	4-29-99	No
	Ictalurus punctatus	Missouri R. near Hartsburg, MO	10-2-98	4	4-1-99	Yes, few	Yes. few	4-15-99	No
	Ictalurus punctatus	Osage catfisheries	N/A	3	3-6-98	Yes	No	N/A	No
				3	4-23-98	Yes	No	N/A	No
	Noturus flavus	James River, Greene Co., MO	6-3-97	2	3-6-98	N/A	No	N/A	No
	Noturus exilis	James River, Greene Co., MO	4-11-98	4	4-23-98	Yes	No	N/A	No
			2-21-99	1	5-24-99	N/A	N/A	6-2-99	No
	Noturus exilis	Starks Cr., Hickory Co, MO	4-12-99	1	5-24-99	N/A	N/A	6-2-99	No
Cottidae	*Cottus carolinae*	N/A	N/A	1	4-5-99	No	No	4-15-99	No
	Cottus bairdi	Pearson Cr., Greene Co., MO	4-21-98	3	4-5-99	No	No	4-15-99	No
	Cottus ozarkae	James River, Greene Co., MO	4-12-98	3	4-23-98	N/A	No	N/A	No
Sciaenidae	*Aplodinotus grunniens*	Missouri R. near Hartsburg, MO	10-2-98	2	4-1-99	No	No	4-15-99	No
			8-18-97	4	3-6-98	No	No	N/A	No
				4	4-23-98	No	No	N/A	No
Percidae	*Etheostoma spectabile*	Pearson Cr., Greene Co., MO	4-21-98	4	4-1-99	No	No	4-15-99	No
				3	4-9-99	N/A	N/A	4-29-99	No
	Percina caprodes	Red Oak Cr.,	4-25-98	2	4-5-99	No	No	4-15-99	No
	Etheostoma tetrazonum	Red Oak Cr.,	4-25-98	1	4-5-99	No	No	4-15-99	No
				1	4-9-99	N/A	N/A	4-29-99	No
	Etheostoma blennioides	Red Oak Cr.,	4-25-98	4	4-5-99	No	No	4-15-99	No
	Etheostoma caeruleum	Red Oak Cr.,	4-25-98	9	4-9-99	N/A	N/A	4-29-99	No
	Etheostoma caeruleum	James River, Greene Co., MO	4-11-98	4	4-23-98	Fish died	Fish died	N/A	No
	Stizostedion vitreum	Chesapeake Hatchery	6-19-97	4	3-6-98	N/A	No	N/A	No
Centrarchidae	*Micropterus salmoides*	Chesapeake Hatchery	6-19-98	3	4-1-99	No	No	4-15-99	No
			6-30-97	6	3-6-98	No	No	N/A	No
			4-23-98	4	4-23-98	No	No	N/A	No
	Lepomis macrochirus	Chesapeake Hatchery	6-30-97	3	3-6-98	N/A	No	N/A	No
	Micropterus punctulatus	Meramec River,	4-2-99	2	4-5-99	No	No	N/A	No
	Lepomis megalotis	Meramec River,	4-2-99	2	4-5-99	Yes, few	Yes, few	4-29-99	No

Table 4. Continued.

	Lepomis megalotis	James R. Greene Co., MO	4-21-99	1	5-24-99	N/A	N/A	6-2-99	No
	Lepomis microlophus	Chesapeake Hatchery	6-30-97	4	4-23-98	Yes, many	Yes, many	N/A	No
Cyprinidae	*Notropis chrysocephalis*	Red Oak Cr.,	4-25-98	2	4-1-99	No	No	4-15-99	No
	Notropis zonatus	Red Oak Cr.,	4-25-98	3	4-1-98	No	No	4-15-99	No
	Pimephales promelas	Midwest Sci. Cntr., Columbia, MO	4-12-99	6	4-15-99	N/A	No	4-29-99	No
	Pimephalis promelas	Midwest Sci. Cntr., Columbia, MO	4-12-99	6	5-24-99	Yes, few	N/A	6-2-99	No
	Notropis cardinalis	Spring River, Verona, MO	7-22-97	4	4-23-98	N/A	No	N/A	No
	Notropis venustus	Black R., AR	5-21-99	4	5-24-99	Clean	N/A	6-2-99	No
	Campostoma oligolepis	Starks Cr. Hickory Co, MO	4-12-99	2	5-24-99	N/A	N/A	6-2-99	No
	Campostoma oligolepis	James R., Greene Co., MO	4-21-99	1	5-24-99	N/A	N/A	6-2-99	No
	Notropis nubilus	James R., Greene Co., MO	4-21-99	7	5-24-99	N/A	N/A	6-2-99	No
	Nocomis biguttatus	James R.Greene Co., MO	4-21-99	3	5-24-99	N/A	N/A	6-2-99	No
Catostomidae	*Hypentelium nigricans*	James R., Greene Co., MO	4-21-99	2	5-24-99	N/A	N/A	6-2-99	No
	Moxostoma duquesnei	James R., Greene Co., MO	4-21-99	1	5-24-99	N/A	N/A	6-2-99	No
Ambystomidae	*Ambystoma opacum*	Compton Hollow, Warren Co., MO	N/A	2	4-23-98	No	No	N/A	No
Gobiidae	*Neogobius melanostomus*	N/A		3	4-1-99	Yes, many	No	4-15-99	No

Table 5. Fishes collected 9 June 1999 from the Gasconade River to assess natural infections of *Cumberlandia.*

Family	Species	n	Size range (mm)	Mean length (mm)
Atherinidae	*Labidesthes sicculus*	1	83	83.0 ± 0.00
Catostomidae	*Moxostoma duquesnei*	15	124-351	207.0 ± 55.08
	Moxostoma erythrurum	28	102-453	224.4 ± 94.16
	Moxostoma carinatum	3	227-593	410.0 ± 258.80
	Moxostoma macrolepidotum	12	143-390	289.3 ± 91.00
	Ictiobus bubalus	12	137-485	351.3 ± 90.23
	Ictiobus cyprinellus	1	372	372.0 ± 0.00
	Carpiodes carpio	1	375	375.0 ± 0.00
	Carpiodes cyprinus	3	307-390	357.3 ± 44.23
	Carpiodes velifer	4	304-371	328.3 ± 29.41
	Hypentelium nigricans	4	111-265	159.5 ± 71.00
Centrarchidae	*Lepomis macrochirus*	18	64-184	119.0 ± 33.11
	Lepomis megalotis	25	84-186	130.5 ± 21.59
	Ambloplites rupestris	6	141-221	181.7 ± 33.64
	Micropterus salmoides	7	225-298	259.4 ± 24.07
	Micropterus punctulatus	11	196-367	276.2 ± 53.33
Clupeidae	*Dorosoma cepedianum*	14	230-364	303.3 ± 39.13
Cyprinidae	*Luxilus zonatus*	25	64-102	81.7 ± 9.20
	Cyprinella whipplei	11	62-87	73.6 ± 8.90
	Notropis rubellus	26	55-70	60.9 ± 3.93
	Campostoma oligolepis	23	70-106	88.7 ± 9.03
	Pimephales notatus	9	58-80	66.2 ± 8.41
Hiodontidae	*Hiodon alosoides*	1	294	294.0 ± 0.00
Ictaluridae	*Ictalurus punctatus*	14	246-545	456.1 ± 70.15
	Pylodictus olivaris	2	346-440	393.0 ± 66.47
Lepisosteidae	*Lepisosteus platostomus*	3	524-640	588.3 ± 59.03
	Lepisosteus osseus	1	405	405.0 ± 0.00
Percidae	*Percina caprodes*		125	125.0 ± 0.00
	Etheostoma blennioides	1	74	74.0 ± 0.00
	Etheostoma tetrazonum	26	52-65	59.0 ± 0.00
Scianidae	*Aplodinotus grunniens*	13	265-563	377.2 ± 102.11

Table 6. Fishes collected on 10 June 1999 from the Meramec River to assess natural infections of *Cumberlandia*.

Family	Species	n	Size range (mm)	Mean length (mm)
Catostomidae	*Moxostoma duquesnei*	30	122-382	291.7 ± 62.21
	Moxostoma erythrurum	12	284-385	349.8 ± 28.52
	Moxostoma carinatum	7	410-630	499.1 ± 72.06
	Moxostoma macrolepidotum	12	285-362	317.2 ± 26.51
	Ictiobus bubalus	10	247-402	288.0 ± 59.30
	Ictiobus cyprinellus	1	420	420.0 ±0.00
	Hypentelium nigricans	8	260-390	345.0 ± 45.61
Centrarchidae	*Lepomis macrochirus*	8	67-151	113.3 ± 35.05
	Lepomis megalotis	28	72-156	110.0 ± 24.94
	Ambloplites rupestris	1	145	145.0 ± 0.00
	Micropterus salmoides	2	255-319	287.0 ± 45.25
	Micropterus dolomieu	8	209-343	278.9 ± 48.26
Clupeidae	*Dorosoma cepedianum*	22	175-280	224.1 ± 31.04
Cottidae	*Cottus carolinae*	25	32-101	50.8 ± 16.85
Cyprinidae	*Luxilus zonatus*	25	65-106	82.7 ± 12.85
	Cyprinella whipplei	3	96-125	108.7 ± 14.84
	Notropis rubellus	16	57-70	62.3 ± 3.30
	Campostoma oligolepis	25	68-92	79.2 ± 5.57
	Pimephales notatus	11	53-84	67.0 ±10.56
	Notropis amblops	25	60-76	65.3 ± 3.66
	Notropis nubilus	11	60-68	64.0 ± 2.61
	Luxilus chrysocephalus	1	145	145.0 ± 0.00
	Notropis ludibundus	1	153	153.0 ± 0.00
	Notropis volucellus	21	58-70	64.8 ± 4.01
Ictaluridae	*Pylodictus olivaris*	1	310	310.0 ± 0.00
	Noturus exilis	11	57-91	71.4 ± 10.50
Lepisosteidae	*Lepisosteus osseus*	1	790	790.0 ± 0.00
Percidae	*Etheostoma blennioides*	2	63-66	64.5 ± 2.12
	Etheostoma tetrazonum	17	50-77	63.9 ± 8.98
	Etheostoma caruleum	11	47-63	52.0 ± 5.23
	Percina evides	2	66	66.0 ± 0.00
Sciaenidae	*Aplodinotus grunniens*	11	245-500	367.6 ± 89.40

Table 7. Glochidia recovered from fish collections listed by fish species and mussel family. Glochidia measurements are means (to the nearest 0.01 millimeter) ± standard deviation.

Fish species	Mussel family	n	Length (mm)	Height (mm)	Hinge (mm)
I. punctatus (n=11)	Lampsilinae	1	0.24	0.27	0.12
	Lampsilinae	4	0.24 ± 0.006	0.297 ± 0.006	0.095 ± 0.007
	Lampsilinae	1	0.28	0.31	0.09
	Lampsilinae	1	0.23	0.31	0.10
	Lampsilinae	1	0.28	0.32	0.12
	Lampsilinae	1	0.22	0.26	0.12
	Lampsilinae	6	0.28 ± 0.00	0.34 ± 0.00	0.13 ± 0.00
	Unknown	1	0.21	0.17	N/A
	Lampsilinae	1	0.23	0.29	0.09
	Lampsilinae	2	0.21 ± 0.00	0.29 ± 0.00	0.09 ± 0.00
	Lampsilinae	104	0.284 ± 0.008	0.341 ± 0.006	0.120 ± 0.005
	Lampsilinae	9	0.24 ± 0.00	0.29 ± 0.00	0.095 ± 0.00
M. macrolepidotum (n=3)	**Cumberlandia**	1	0.0625	0.0625	0.04
	Unknown	1	N/A	N/A	N/A
	Lampsilinae	1	0.22	0.26	0.11
M. erythrurum (n=4)	Unknown	1	N/A	N/A	N/A
	Ambleminae	1	0.23	0.23	0.14
	Unknown	1	0.225	0.295	0.10
	Ambleminae	1	0.15	0.14	0.12
	Unknown	1	N/A	N/A	N/A
M. duquesnei (n=2)	Lampsilinae	1	0.23	0.27	0.12
	Ambleminae	1	0.20	0.21	0.15
D. cepedianum (n=7)	Ambleminae	1	0.208	0.23	0.15
	Lampsilinae	1	0.23	0.28	0.12
	Unknown	1	N/A	N/A	N/A
	Ambleminae	1	0.215	0.225	0.15
	Ambleminae	1	0.16	0.16	0.13
	Ambleminae	3	0.20 ± 0.00	0.21 ± 0.00	0.15 ± 0.00
	Ambleminae	1	0.205	0.22	0.14
L. megalotis (n=4)	Ambleminae	16	0.221 ± 0.003	0.238 ± 0.005	0.139 ± 0.003
	Ambleminae	1	0.21	0.22	0.14
	Ambleminae	1	0.22	0.23	0.12
	Lampsilinae	8	0.175 ± 0.00	0.20 ± 0.00	0.10 ± 0.00
	Lampsilinae	1	0.215	0.28	0.095
L. machrochirus (n=2)	Lampsilinae	5	0.163 ± 0.003	0.185 ± 0.00	0.098 ± 0.003
	Ambleminae	6	0.18 ± 0.00	0.18 ± 0.00	0.10 ± 0.00
L. zonatus (n=4)	Unknown	1	N/A	N/A	N/A
	Unknown	1	N/A	N/A	N/A
	Ambleminae	1	0.16	0.12	0.14
	Ambleminae	1	0.165	0.17	0.14
A. grunniens (n=4)	Ambleminae	3	0.22 ± 0.00	0.24 ± 0.00	0.13 ± 0.00
	Ambleminae	77	0.306 ± 0.151	0.265 ± 0.120	0.15 ± 0.00
	Anodontinae	3	0.37 ± 0.00	0.37 ± 0.00	0.28 ± 0.00

Table 7. Continued

	Unknown	1	N/A	N/A	N/A
C. carpio (n=1)	Unknown	1	N/A	N/A	N/A
C. cyprinus (n=1)	Ambleminae	1	0.21	0.23	0.13
	Lampsilinae	1	0.21	0.23	0.10
H. alosoides (n=1)	*O. reflexa*	69	0.134 ± 0.011	0.091 ± 0.035	0.07 ± 0.000
I. bubalus (n=1)	Ambleminae	1	0.22	0.225	0.13
M. salmoides (n=1)	Lampsilinae	1	0.22	0.26	0.11
C. oligolepis (n=1)	Unknown	1	N/A	N/A	N/A
L. chrysocephalus (n=1)	Lampsilinae	1	0.28	0.35	0.15
N. amblops (n=1)	**Cumberlandia**	17	0.06 ± 0.00	0.065 ± 0.00	0.05 ± 0.00

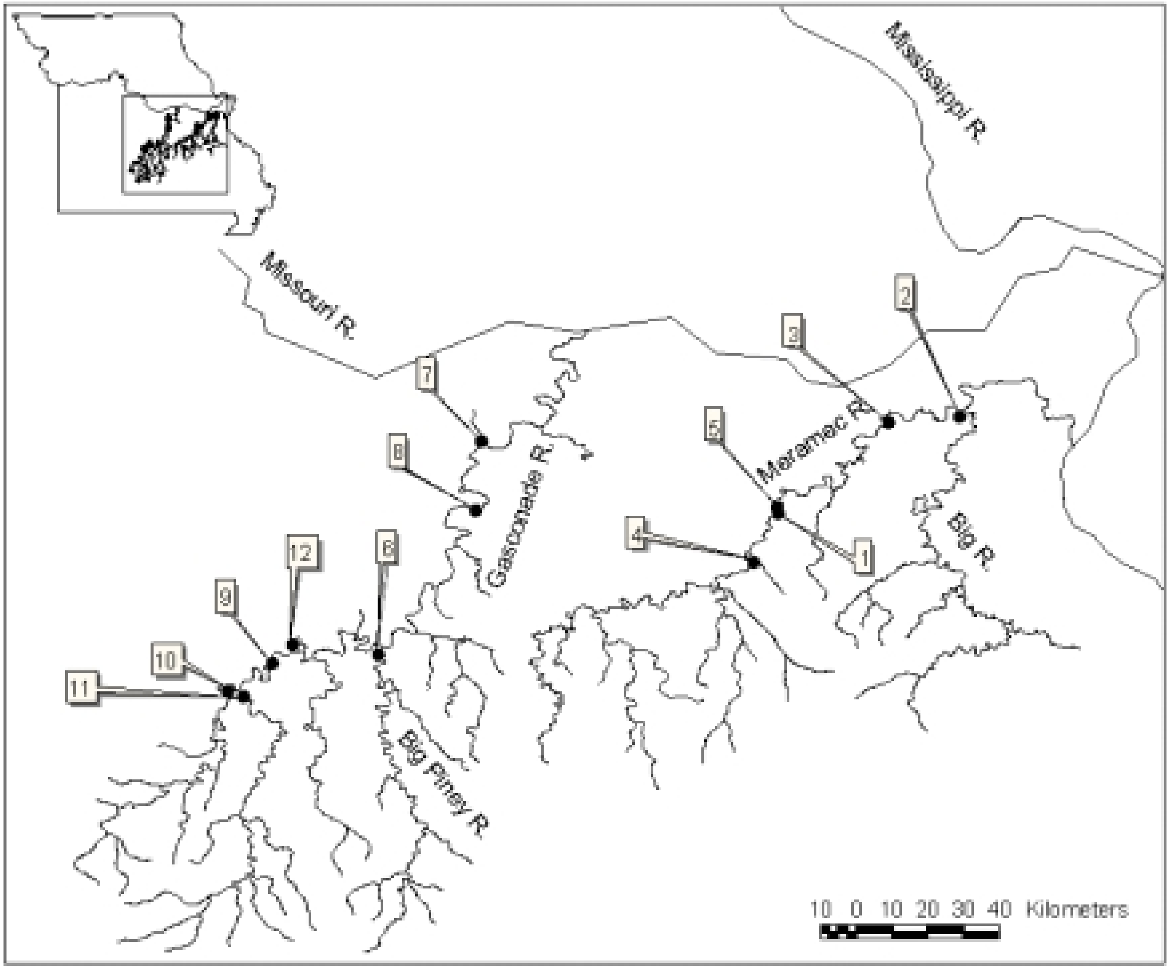

Figure 1. Map of the Gasconade and Meramec Rivers in Missouri showing locations where *Cumberlandia monodonta* were collected for this study. The collection sites are described by number in Appendix A.

Figure 2. Posterior end of gravid female *Cumberlandia*, with freshly released conglutinates.

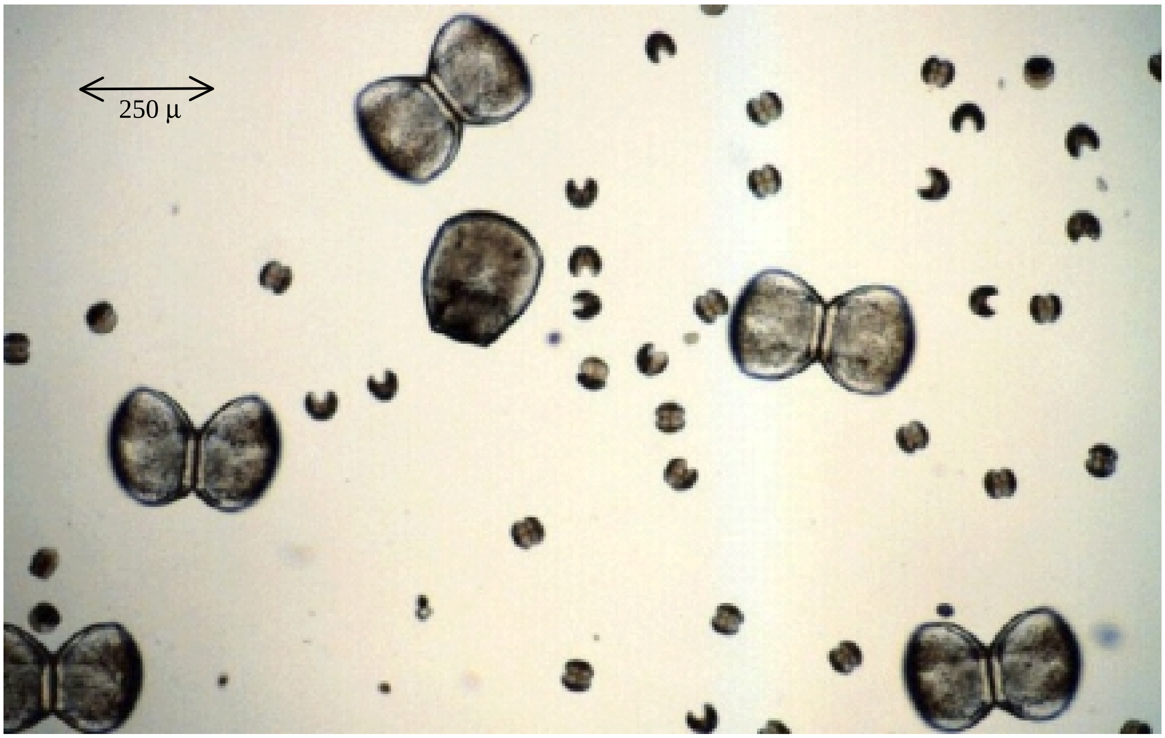

Figure 3. Glochidia of *Cumberlandia* (small) and *Lampsilis siliquoidea* (large). The glochidia of *Cumberlandia* average approximately 60 µm in length and are much smaller than those of typical unionids such as *Lampsilis*.

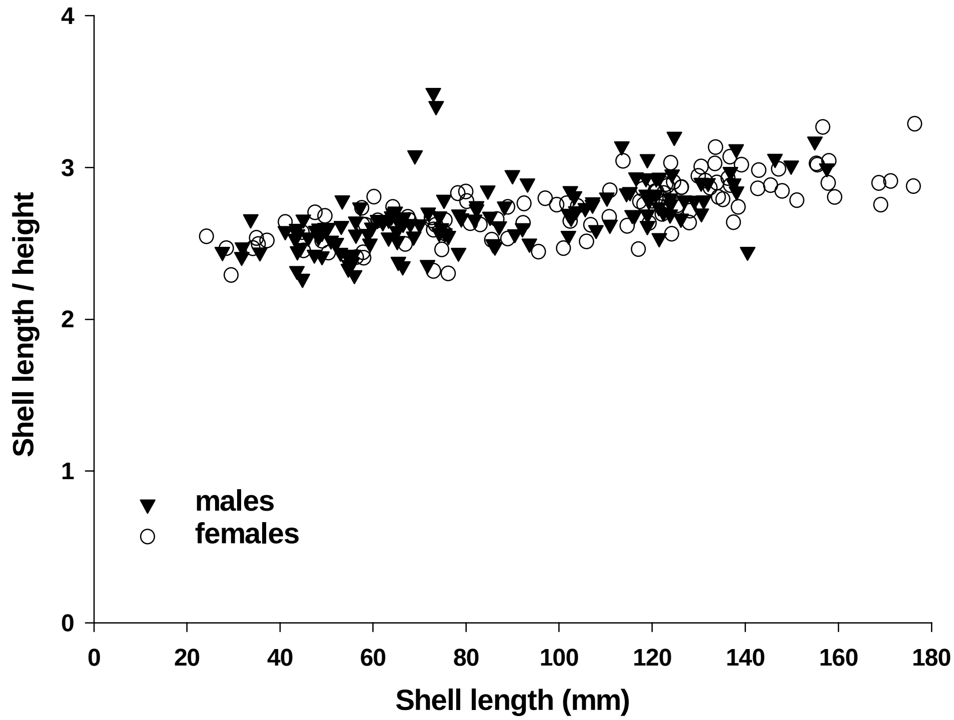

Figure 4. Ratio of shell length and height versus shell length for male and female *Cumberlandia***.** Shell shape elongated slightly with age. Males and females were similar. $R^2 = 0.38$. P value = <0.0001.

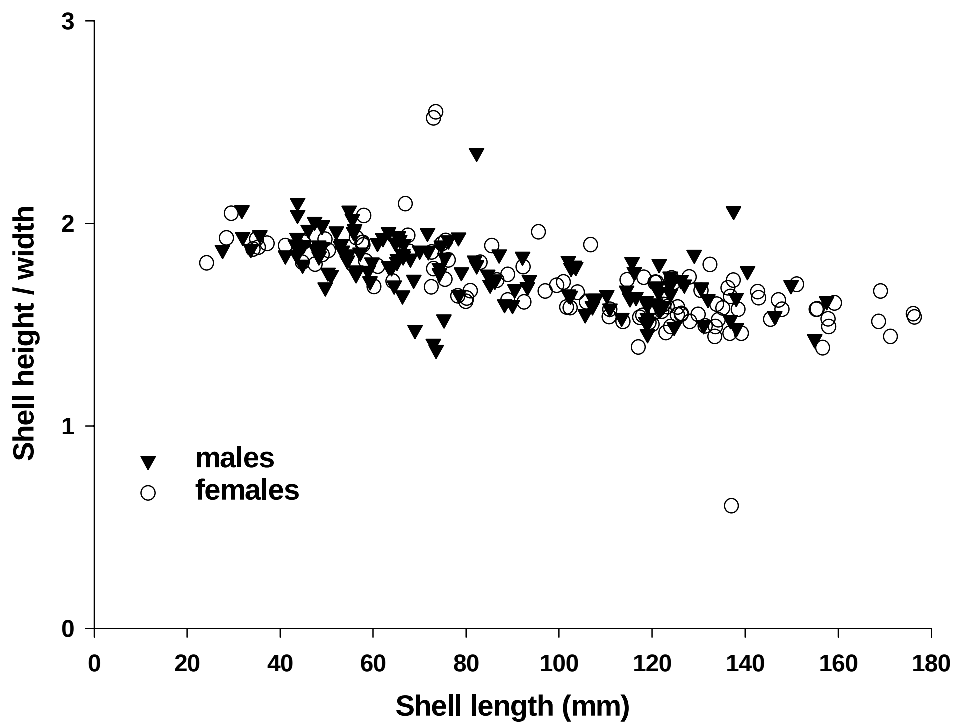

Figure 5. Ratio of shell height to width versus shell length for male and female *Cumberlandia*. Shell shape became more rounded in cross-section with increasing age. Males and females were similar. $R^2 = 0.42$. p value = < 0.0001.

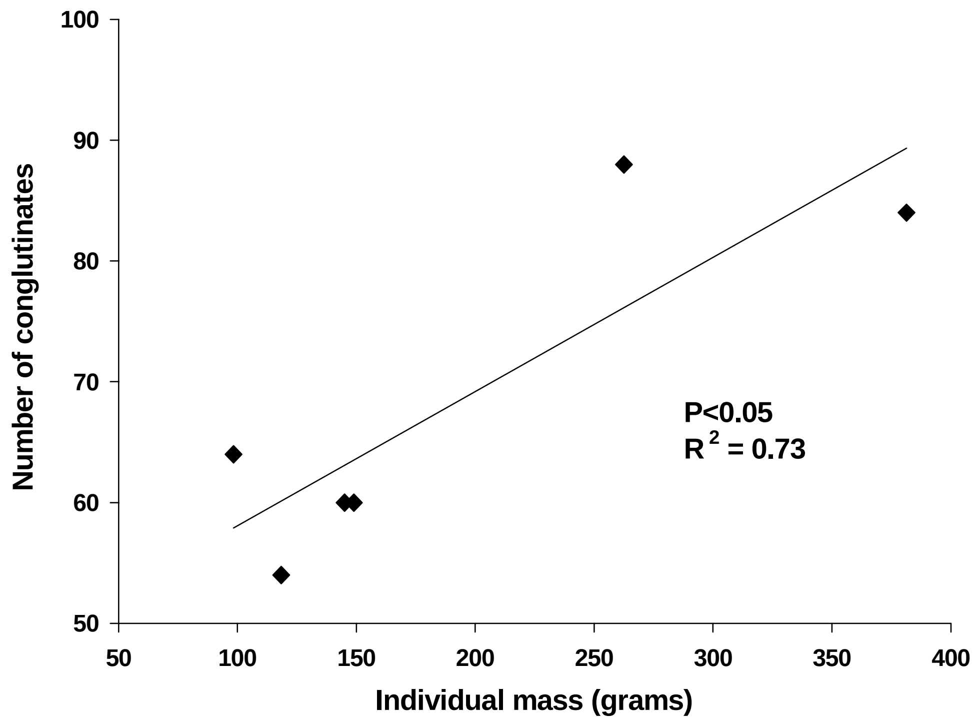

Figure 6. Number of conglutinates released versus individual mass. Larger individuals released greater numbers of conglutinates. N = 6.

Chapter two

AGE, GROWTH, AND POPULATION STRUCTURE OF THE SPECTACLECASE *CUMBERLANDIA MONODONTA*, (SAY 1829) IN THE MERAMEC AND GASCONADE RIVERS, MISSOURI

ABSTRACT

Demography of *Cumberlandia* was investigated at 8 sites in the Meramec and Gasconade rivers. Sites were delimited by the presence of *Cumberlandia*. Site area ranged from 480 to 1800 m^2. Water depth was 0.25-3.0 meters. Quadrats (¼ m^2) were placed using an adaptive cluster design, excavated to a depth of 15 cm by hand, and searched visually for mussels. Over 6,000 live specimens were discovered. Of these, 2,880 were measured (total shell length, height, width, hinge length, and wet weight). Approximately 23 individuals per site were sacrificed and their ages were estimated by counting growth lines in the hinge ligament. This method was validated by comparing inferred growth rates with growth measured directly during one year. Ages estimated from growth lines ranged from 1–56 years and were correlated with shell length. The length-age relationship was similar among sites and was described by the following equation: age = (length * 15.4431) / (201.4524-length) (n = 278, R^2 = 0.83). This equation was used to infer age (years) from shell length (mm) in the demographic samples. Inferred age distributions were similar in both rivers. The most abundant age classes were approximately 20-35 years. Among sites, population densities ranged from 1.2 to 12.8 (mean = 6.7) individuals per m^2, while local (i.e., single quadrat) densities ranged up to 120 individuals per m^2. Although a few young individuals were found at all sites, individuals less than 10 years old comprised only 13.2% of *Cumberlandia* sampled. Therefore, it appears that these populations might be in decline, despite high population densities of adults.

INTRODUCTION

Many species of unionoid mussels are in danger of extinction because of population extirpations and declines (Williams et al. 1992, Biggins et al.1995, Neves 1997). The effective conservation and management of threatened species depends largely on our knowledge of recruitment, age structure, growth rates, and mortality. These population characteristics are poorly understood for the majority of freshwater mussels, because statistically valid demographic data are lacking for most species. A national strategy for the conservation of the North American freshwater mussel fauna states that research on distribution and population dynamics is a high priority (NNMCC 1998).

Cumberlandia was formerly widespread throughout the Mississippi drainage but has declined dramatically throughout its range. *Cumberlandia* is currently considered to be threatened in Alabama, Arkansas, Iowa, Illinois, Kentucky, Minnesota, Missouri, Tennessee, Virginia, and Wisconsin, and may already be extirpated from Indiana and Ohio (Williams et al.1992). A few large populations still exist in the upper Clinch River in Virginia and Tennessee (Michelle Steg [Virginia Tech] personal communication), the St. Croix River in Minnesota and Wisconsin (Lee and Hove 1997), and the Osage, Gasconade and Meramec Rivers in Missouri, (Natural Heritage Data Base [MDC], Roberts 1998, Oesch 1984, Buchanan 1980 and 1994). The Gasconade and Meramec hold some of the largest remaining populations of *Cumberlandia*, which comprises 18-21% of the mussel fauna in these rivers (Buchanan 1994, Roberts 1998). The high densities of *Cumberlandia* found in these rivers create an ideal situation for obtaining demographic data for this threatened species.

Cumberlandia is a member of the family Margaritiferidae. Research on other Margaritiferids suggests that this family includes some of the longest-lived invertebrates known. For example, the freshwater pearl mussel, *Margaritifera margaritifera*, reaches ages of up to 132 years (Bauer 1992, Hendelberg 1960). Although Margaritiferids are still abundant in some localities, populations often consist mainly of old individuals. Such long-lived species may persist for decades even if reproduction is no longer occurring. Therefore, knowledge of population structure is crucial to determine the true conservation status of these populations (Bauer 1986).

Estimates of population density and age structure require 1) statistically valid methods for sampling and 2) a method for determining individual age. Like other Margaritiferids, *Cumberlandia* are highly clumped within their preferred habitats. Conventional sampling designs are inefficient when used to sample highly clumped populations. However, adaptive sampling can be used to sample such populations by focussing sampling effort in those areas which contain high densities of the target organism (Thompson and Seber 1996).

The age of bivalves can be estimated by counting growth lines on or within the shell (Chamberlain 1931, Bauer 1992, Metcalfe-Smith and Green 1992, Semenova et. al.1992, Bruenderman and Neves 1993, Johnson and Brown 1998). This approach is similar to the use of growth lines in ossified structures in vertebrates, or growth rings in woody plants (Guyette and Rabeni 1995). However, researchers agree that annual periodicity of growth lines is a hypothesis which needs to be examined and validated for each species separately (Beamish and McFarlane 1983, Neves and Moyer 1988).

The objectives of this study on *Cumberlandia* were to 1) develop and validate a technique to determine age of individuals, 2) develop an equation to predict age from shell length, and 3) describe the demography of eight populations in the Gasconade and Meramec Rivers, including population density and age structure.

MATERIALS AND METHODS

AGE AND GROWTH

Mussel collections - *Cumberlandia* were collected in the Gasconade and Meramec Rivers, Missouri at 12 sites (**Figure 1, Table 1, Appendix A**). Collections were made by snorkeling or diving using a surface air supply unit. Mussels were selected in order to obtain a large range of individual sizes at each site sampled for the purpose of developing growth curves. A total of 278 individuals (mean 23 per site) were measured (total length, width, height, and hinge ligament length) to the nearest 0.1 millimeter with dial calipers, weighed to the nearest 0.1 gram using an electronic field balance, sexed by gonad puncture (see chapter one), and aged by counting hinge ligament growth lines (**Figure 2**).

Age determination - Individual ages were inferred by counting growth lines in the hinge ligament (Hendelberg 1960). The hinge ligament is an elastic proteinaceous structure that connects the two mussel valves dorsally. The hinge ligaments were sectioned longitudinally using a razor blade set in a jig. After sectioning, the hinge ligaments were rinsed under a stream of water to remove fragments and debris. Ligaments were viewed under a dissecting microscope at 10–20X. It was important to view the ligament while wet, because dried ligaments did not show growth lines as clearly as wet ligaments. Growth lines were opaque and varied in width and contrast.

Wide (major) growth lines were usually separated by one or more thinner (minor) growth lines. Only major growth lines were counted. The assumption that these lines were annual was tested by comparing growth rates inferred from the age estimates with measured growth rates (see below). Major growth lines were counted starting at the posterior end of the ligament and working anterior. Many older shells had severe erosion on their hinge ligaments near the umbone. However, I could still discern the growth lines as impressions on the shell surface where the ligament had been attached. Thus, it was possible to complete counts even on eroded individuals.

In addition to age estimates from hinge ligaments, I also estimated age from external shell growth lines in 126 of the 278 individuals, in order to compare the two techniques. Only small individuals (i.e., less than 106 mm total length, or about 15 years old) were used for the comparison, because it was difficult to distinguish shell growth lines in older *Cumberlandia*. Shells were immersed in water and viewed with transmitted light to help visualize growth lines. Counts of shell growth lines and hinge ligament lines were made at different times to avoid bias.

<u>Growth curves</u> - The relationship between length and age (growth curve) was derived from the lengths and ages of 278 measured individuals. The relationship between log length and log age was approximately linear. Differences in this relationship among the 12 sites were tested with Analysis of Covariance (ANCOVA, Minitab version 11.0). There were no significant differences among sites or among drainages, so a single growth curve was calculated. The data were fitted using a two-parameter hyperbolic regression (Sigma-Plot, version 5.0).

Measurement of growth in the field by mark and recapture - Growth rates were examined by means of a mark-recapture study. A sample of 82 *Cumberlandia* were collected on 17-19 November 1998 and marked with adhesive tags. Each individual was brought to the surface, blotted, and allowed to air-dry for approximately ten minutes. A shallow depression was abraded near the posterior margin of the left valve of each shell, using a rotary tool and abrasive bit. The posterior end was chosen so that tags would be visible on the mussels in situ. Each mussel was allowed to dry for an additional five minutes before attaching tags. Red plastic bee-tags, 2-mm diameter, with white numbers (Almore Company, Portland, OR) were inserted into the depressions and attached with luting cement (GC Corporation Fuji I glass ionomer luting cement). The cement dried within two to three minutes. The tags were then sealed with Copal Cavity Varnish (Sultan Chemists, Inc.) to decrease wear on the numbers.

Each individual was measured (total length, width, height and weight) to the nearest 0.1 millimeter using dial calipers and to the nearest 0.1 gram using an electronic field balance. Individuals were then returned to their respective sites at eight locations (four at each site), that were chosen to facilitate subsequent recapture. These locations were upstream or adjacent to large boulders within the *Cumberlandia* beds. At some sites, bricks were painted fluorescent orange and placed near the sites to serve as markers. Maps were sketched to illustrate the location of each group and natural markers such as trees and boulders.

Marked individuals were recovered one year after tagging, and were measured and weighed with the same techniques and equipment that were used on the tagging date. Recaptured individuals were returned to the rivers after measurement.

Measurement of growth in the laboratory - Seven young individuals, each less than 55 mm total length, were maintained in the lab for three months (October 98 to January 99) to observe growth rates. Individuals were placed in sand substrate in a 180-mm diameter glass bowl, on the bottom of a recirculating rearing system. The water was kept at 23-25 °C, and mussels were fed a monoculture of algae (*Neochloris oleoabundans*) at approximately weekly intervals. After three months, individuals were measured again to the nearest millimeter with dial calipers to determine total shell length, width, and height.

POPULATION STRUCTURE

Quantitative sampling - Eight sites (four from each river) were sampled quantitatively to determine population density and age structure (**Figure 1, Table 1, Appendix A**). These sites were selected at random from twelve sites that were known from previous surveys to hold *Cumberlandia* (Natural Heritage Data Base [MDC], Roberts 1998, Buchanan 1994, Sue Bruenderman [MDC] personal communication). Therefore, the results of this study are representative only of habitats where *Cumberlandia* occur, and not all habitats within the rivers.

All *Cumberlandia* beds investigated were located on outside river bends, below bluff lines (**Appendix A**). Upstream boundaries were generally distinct, while downstream boundaries were less so. Laterally, beds seldom extended beyond the thalweg. Boundaries were delineated for each site during reconnoitering surveys. Each site was visually surveyed by snorkeling, examining substrate, and occasionally turning large rocks, beneath which *Cumberlandia* are often found. Site boundaries were established approximately ten meters beyond the position of the last live individual sighted.

Sites were of uniform width, paralleled the shoreline, and were 60-100 m long and 8-20 m wide (**Appendix A**). Each site was divided into 1-meter grids. Reference flags were placed on shore at ten-meter intervals. At each flag, transects perpendicular to the shoreline were marked with dive weights painted fluorescent orange. Specific plots within the site could then be located by measuring distances along the shore and transects using a surveying tape measure.

Adaptive sampling was carried out according to Thompson and Seber (1996). Sampling employed a grid of 1/4-m^2 units. The sampling design consisted of 24-30 initial units chosen at random within the grid. A random number table was used to select the initial sampling units within the grid. A consistent method of setting the 1/4m^2 quadrats into 1-m^2 grids was developed. For example, if a coordinate of (8,5) was chosen, the quadrat was placed so that its downstream side lay on the eight-meter transect, and its left side (when facing upstream) lay on the 4.5 meter transect.

A sampling criterion of 1-7 was chosen at each site, based on the general density of *Cumberlandia*. A larger criterion was chosen if the population density was high. If a sampled unit contained the critical number of *Cumberlandia*, then the adjacent units were also sampled. If neighboring units met the criterion, their adjacent units were sampled in turn. This process was continued until no more units meeting the criterion were encountered.

Sampling was carried out by two divers, using a surface air supply (Brownie Third Lung). Each diver placed quadrat frames made of 3/8” rebar according to the sampling design described above. Within these frames, the diver excavated the substrate to a depth of 15 cm by hand, and searched visually for mussels. All live native mussels encountered were collected and identified to species. From 167- 530 *Cumberlandia* were measured at each site (total length, width, height, and hinge ligament length) to the nearest 0.1 millimeter with dial calipers and weighed to the nearest 0.1 gram using an electronic field balance. Not all individuals recovered were measured. At sites with a large number of mussels, measurements (but not counts) were stopped after the first several hundred individuals recovered had been measured.

Ages of mussels from the demographic samples were inferred from shell lengths, using the relationship between length and age that was derived previously (above).

RESULTS

AGE AND GROWTH

The ages of individuals estimated from hinge growth lines ranged from 1–56 years. Age was correlated with both shell length and hinge length. The relationship between log length and log age was approximately linear for animals older than 3 years (**Figure 3**). The slope of this relationship was compared among sites to test differences in growth rate, which did not differ (ANCOVA, $p = 0.28$). Therefore, the age and length data from all sites were pooled and used to generate equations relating length and age. The most suitable growth model was a hyperbola of the form:

Age = (shell length * b)/(a – shell length). This model was fitted by regression to derive equations relating shell length (SL) and hinge length (HL) to age. The equations were:

Equation 1. $$Age = \frac{(SL \cdot 15.4431)}{(201.4524 - SL)}$$ (n = 278, $R^2 = 0.83$)

Equation 2. $$SL = 201.4524 \cdot \frac{Age}{(15.4431 + Age)}$$

Equation 3. $$Age = \frac{(HL \cdot 38.7291)}{(145.9095 - HL)}$$ (n = 278, R^2 = 0.84)

Equation 4. $$HL = 145.9095 \cdot \frac{Age}{(38.7291 + Age)}$$

The rate of growth of both shell length and hinge length decreased with increasing age (**Figures 4 and 5**). Male and female curves were similar (**Figure 6**). The ratio of hinge length to shell length increased with age. That is, older animals had longer hinges relative to shell length (**Figure 7**).

Demographic samples - The mean number of units sampled per site was 157 (range 53-268). The mean percent of the site area sampled was 5.0% (1.7-9.2%). The mean and variance of population density were calculated for each site according to Thompson and Seber (1996).

$\tilde{\mu}$ = estimated site population density (number of individuals per 1/4m^2).

N = number of units in the site.

n_1 = number of networks intersected by units of the initial random sample.

m_i = the number of units in the network containing unit i, minus edge units.

f_i = number of units in the initial sample that fall in the network containing unit i.

y_i = the number of individual mussels found in unit i.

w_i = mean y in network i.

$\hat{\text{var}}[\tilde{\mu}]$ = variance of site population density.

$\hat{\text{var}}[y_i]$ = variance of unit population density.

MI = Morisita's index of dispersion (a measure of dispersion which is independent of population density and sample size).

1. $$\tilde{\mu} = \frac{1}{n_1} \sum_{i=1}^{n_1} w_i$$

2. $$w_i = \frac{\sum_{i=1}^{m_i} y_i}{m_i}$$

3. $$\hat{\text{var}}[\tilde{\mu}] = \frac{N - n_i}{N n_i (n_i - 1)} \sum_{i=1}^{n_i} (w - \tilde{\mu})^2$$

4. $$\hat{\text{var}}[y_i] = \hat{\text{var}}[\tilde{\mu}] \sum_{i=1}^{n_i} m_i$$

5. $$MI = n \left[\frac{\sum x^2 - \sum x}{\left(\sum x\right) - \sum x} \right]$$

Population densities ranged among sites from 1.2 to 12.8 (mean = 6.7) individuals per m^2, while local (i.e., single quadrat) densities ranged up to 120 individuals per m^2. The mean estimated population size for the sampled sites was 7,122 individuals per site, and ranged from 933 – 22,697 (**Table 2**).

Populations were highly clumped, with the number of individuals per $1/4m^2$ quadrat ranging from 0 to 40. Morisita's index, a measure of dispersion, ranged from 0.55 – 7.45 among the sites, with mean = 4.64 (**Table 2**). Morisita's index tends to a value of 0 for random distributions, with higher values indicating clumping and lower values indicating more uniform distribution (Krebs 1989).

Ages of 2,880 individuals from the demographic samples were estimated from shell length (**Table 3**, **Figures 8, 9)**. The Gasconade and Meramec River populations had mean estimated ages of 25 and 32 years, respectively. The age distributions differed significantly between the two drainages (chi-square statistic = 116, df = 2, $p<0.001$), and among sites within the Gasconade river (chi-square statistic = 95.099, df = 6, $p<0.001$), and among sites within the Meramec river (chi-square = 278, df = 6, $p< 0.001$). The two sites with the largest proportion of juveniles and the most uniform age distributions were Gasconade site #8 (Paydown Conservation Access in Maries County) and Meramec site #3 (Fish Trap Rapids) (**Table 1, Appendix A**).

In general, few young individuals were recovered. Individuals less than 10 years of age comprised 10.4% of the Gasconade sample, and only 2.8% of the Meramec sample. Sites that contained the greatest proportion of sub-10 year old animals were Gasconade site 8 (17.4%) and site 12 (11.3%), and Meramec site 3 (10%) (**Table 2**).

<u>Field measurements of growth rate</u> - I recovered 49 of 82 tagged individuals (60%). The smallest individuals generally showed the greatest growth (**Figure 12**). Mean growth for all recovered individuals was 1.67 ± SD 2.39 mm and ranged from –0.4 to +13 mm. Height increased by 1.13 ± SD 1.15 mm and mass by 13.48 ± SD 10.78 grams. These data were compared to inferred growth rates (slope of the size vs. age curve) in order to validate age estimates (see discussion).

<u>Growth in the lab</u> - Six of seven small individuals (mean length 40 mm) held in an artificial rearing system for three months showed positive growth in shell length and height. Growth in shell length ranged from 0-0.45 mm (mean 0.30 ± SD 0.14) (**Table 4**). This growth was much less than under field conditions (see discussion).

DISCUSSION

Age determination - The ability to determine age of individuals is essential for demographic analysis of populations. Many workers have determined the age of bivalve molluscs by examining shell growth lines (Chamberlain 1931, Clark 1974, Day 1983, Riusech 1999). Growth lines may be visible on the shell surface, or the shell may be thin-sectioned to reveal growth lines in thick-shelled species. Neither method was satisfactory in *Cumberlandia*. Sectioning the shells was also impractical because they are thin. External shell growth lines were evident in young individuals but were difficult to distinguish in older individuals. Some workers have boiled the shells of *Margaritifera* in strong base to remove the periostracum, enabling growth lines to be more easily distinguished (Bauer 1992, Semenova 1992). I tried this technique with *Cumberlandia*, but the growth lines of old individuals were still ambiguous.

Growth lines on the ligament are less ambiguous to read than shell growth lines because they have higher contrast. However, similar to the shell, there are minor and major growth lines, so that a decision must be made as to which lines to count. I made counts of the broadest growth lines and assumed that the finer more numerous growth lines were the result of smaller cycles of growth rate (e.g., daily, monthly, etc.). The mechanism of formation of hinge ligament growth lines is unclear. However, they are hypothesized to result from changes in growth rate. The use of hinge growth lines for estimating age was developed for use with *M. margaritifera* (Wellman 1938 [in Hendelberg 1960], Hendelberg 1960, and Stober 1972). Hinge growth lines have apparently not been used to infer age of unionids other than Margaritiferids. Many unionids also show clear growth lines in the hinge (personal observations) and the method may prove useful in these species as well as margaritiferids.

Another interesting feature of the hinge ligament is the increase in the ratio of hinge/shell length with increasing age (**Figure 7**). This relationship provides another clue to age of individuals. That is, a shell with an unusually long ligament is likely to be old.

<u>Validation of aging method</u> - Growth lines are generally believed to result from changes in growth rate (Coker et al.1922, Chamberlain 1931, Lutz and Rhoads 1977, Downing et al.1992, Day 1983). Presumably, the largest changes in growth rate are seasonal and produce annual rings (Isely 1914, Day 1983). However, it is unclear whether all mussels form a single growth line each year, and this topic has been debated (Downing et.al.1992, Neves and Moyer 1988). Researchers agree that annual periodicity of growth lines is a hypothesis which needs to be examined and validated for each species (Neves and Moyer 1988, Beamish and McFarlane 1983).

There are at least 3 basic approaches to validating the annual nature of growth lines. These are 1) direct observation of annual growth lines in individuals marked and recaptured one or more years later (Kesler and Downing 1997), 2) use of a signature year, defined by some extraordinary event (Riusech 1999), and 3) comparison of growth rates inferred from growth lines with those observed from mark-recapture studies (Downing et.al.1992).

In the present study, growth rates inferred from age estimates were compared with growth rates measured from the mark-recapture study. The growth curve derived from the length and age measurements (equation 1) was used to predict growth rates as a function of length. These growth rates are equivalent to the slope of the growth curves (Δlength/Δage). The accuracy of these predicted growth rates is dependent upon the accuracy of the age measurements. For example, if ages were consistently underestimated, growth rate would be overestimated proportionately. Therefore, the accuracy of the predicted growth rates can be tested to validate the age estimates.

Measured annual growth rates derived from the mark- recapture study were compared with the predicted growth rates derived from the length – age curve (**Figure 12**). As expected, growth rate decreased with increasing individual size. Measured growth rates were variable, particularly in smaller individuals (**Tables 5 and 6**). This variability was probably not due to measurement imprecision. I tested the measurement precision by making 5 replicate length measurements of each of 6 individuals. The mean standard deviation of these 6 sets of measurements (0.237) was much less than the standard deviation of the residuals around the regression line of growth versus length (2.19). Measured growth versus shell length was fitted to a hyperbolic model using regression. Growth rate predicted from the length – age curve falls within the 95% confidence interval of this regression (**Figure 12**). The similarity between predicted and measured growth rate indicates that age estimates were accurate, if not precise. This comparison is apparently the first validation of age estimates from hinge ligament growth lines.

Ages estimated from hinge growth lines were also compared with ages estimated from external shell growth lines in young individuals. The two methods gave similar results (**Figure 13**).   Over 63% of the data agreed within 1 year, and 91% agreed within 3 years (**Figure 14**). Therefore, shell growth lines could be used to estimate ages of animals up to at least 15 years of age. This method may be advantageous, because shell growth lines can be counted without sacrificing the individual.

Modeling age and growth - The relationship between age and growth of animals is often modeled with an exponential equation that rises to a maximum, of the form: $L_t = L_\infty [1-^{e-K(t-to)}]$ where L_t is the length at time t, t_0 is the theoretical time when length is 0, and K is a constant that describes the growth rate. L_∞ is the asymptote or "length at infinite age". This model is commonly called a von Bertanlanfy curve. This model fits the data in the present study reasonably well. However, the model is not suitable for the purpose of inferring age from shell length, because it has an asymptote and therefore does not allow extrapolation. In the hyperbolic model, length continues to increase with age, which is more realistic. However, neither the von Bertalanfy nor the hyperbolic models accurately represent early growth (approximately 0-3 years), which is sigmoid in form (Riusech 1999).

The decrease in growth rate with age seen in this study is interesting, because the change may be caused by an increasing proportion of energy invested in reproduction after sexual maturity. Based upon observation of gametes in the gonads, *Cumberlandia* appear to mature at 4-5 years in males and 5-7 years in females (Chapter 1). However, the biggest change in growth rate appears to occur at 10-15 years of age (**Figure 4**). This observation may suggest that major investment in reproduction does not occur until after 10 years of age. Such late maturation would be comparable to that of *Margartifera* in Scotland, which reproduce at 12-13 years of age (Young and Williams 1984). In unionid mussels, the decline of growth rate (and presumably, sexual maturity) occurs much earlier, e.g. at 2-4 years in *Lampsilis* (Chamberlain 1931, Day 1983), and 4 years in *Venustaconcha* (Riusech 1999).

In the present study, growth rates were similar among sites and drainages. In contrast, Johnson and Brown (1998) and Bauer (1992) found significant differences in growth rates of *Margaritifera* among sites. The uniformity of growth rates in the present study presumably reflects similar conditions among the study sites.

The growth of seven individuals kept in the lab for 3 months (**Table 4**) was only about 15% of the rate predicted from the inferred growth curve (**Figure 4, 12**). The slow growth of these individuals was presumably due to inadequate nutrition. The algal monoculture (*Neochloris*) supplied as food generally does not seem to support normal growth of juvenile mussels (M. C. Barnhart, unpublished observations).

<u>Maximum age</u> - The family Margaritiferidae apparently includes some of the longest-lived freshwater invertebrates. Ages of *M. margaritifera* determined from growth lines sometimes exceed 100 years in Europe (Bauer 1992, Hendelberg 1960). The maximum ages of *Margaritifera hembeli*, estimated from size and growth rates, ranged among sites from 45-75 years (Johnson and Brown 1998). In the present study, ages of *Cumberlandia* estimated from hinge growth lines ranged up to 56 years. Some individuals recovered during demographic sampling were much larger than the oldest animals that were aged by examination of the hinge ligament, and may have been older. However, estimates of age from size have low precision in old individuals because of slow growth rates and the variability of size at age (**Figure 4**). A very large individual may simply be one that grew more rapidly than usual, rather than for a longer time.

Growth line counts may underestimate age if lines form at less than annual intervals. Negus (1966) found annual line formation in 77% of re-captured individuals of three species in the Thames River. Downing et al. (1992) found that lake-dwelling *Lampsilis siliquoidea*, and *Anodonta grandis* formed less than one annulus per year. They measured also growth rates of marked animals over 2-5 years, and suggested that these mussels might be much older than would be estimated from an assumption of annual growth lines. Kesler and Downing (1997) made similar observations of a large number of marked lake-dwelling *Elliptio complanata*. Clearly, it is important to validate the annual nature of growth lines. Riusech (1999) used a signature year, the 1993 flood, to validate annual formation of shell growth lines in two species of *Venustaconcha*, a stream-dwelling unionid. At 4 of 7 sites, these mussels showed significantly reduced growth in those parts of the shell that were inferred, from growth lines counts, to have formed in 1993.

Demographic sampling - The mean population density of *Cumberlandia* in this study was 6.72 / m^2, and ranged among sites from 1.2-12.8 per m^2. Local (i.e., ¼ m^2 quadrat) densities of up to 120 individuals per m^2 were observed. These population densities are very high compared to most unionid species in the same localities, or in other localities in these rivers. In my study, *Cumberlandia* accounted for 70-97% of mussels recovered at each site, and was 90% of all mussels recovered overall. These data reflect sites chosen for the presence of *Cumberlandia*. *Cumberlandia* was also the most abundant unionoid in a recent general survey of mussels in the Meramec River system, comprising 21% of all mussels recovered (Roberts 1998).

The distribution of *Cumberlandia* is highly clumped (**Table 2**). The mean network size was approximately 6 meters. The tendency of Margaritiferids to have aggregated distributions was reviewed and discussed briefly by Johnson and Brown (1998). The mechanism by which these animals achieve such distribution is unknown. Two possible mechanisms are 1) the juveniles may be dropped in particular habitats by the hosts, and 2) the juveniles may actively seek out aggregations of adults. Further study is needed to test these possibilities.

Researchers studying other margaritiferids have also noted high population densities (Young and Williams 1983, Bauer 1991, Lucey 1993, Johnson and Brown 1998). For example, mean population densities of *Margaritifera hembeli* in headwater streams in central Louisiana ranged from approximately 0.25-2.25/m^2 in 1 km stream reaches. Higher densities were obtained within smaller areas designated as beds, but the areas of the beds were not specified (Johnson and Brown 1998). A bed of *Margaritifera margaritifera* in Madison River, Montana, with area of 3,380 m^2, was estimated to have population density of 11.6 per m^2 (Stober 1972). Some reports also indicate high population densities for certain unionid species, for example, 32 individuals per m^2 for *Anodonta piscinalis* (Okland 1962) and 20 individuals per m^2 for *Elliptio complanata* (Kat 1982). Stern (1983) determined maximum densities of 60 mussels per m^2 for all species combined in the St. Croix River, Minnesota.

The age structure of populations differed significantly among sites and drainages. The two sites with the largest proportion of juveniles and the most uniform age distributions were Gasconade site #8 (Paydown Conservation Access in Maries County) and Meramec site #3 (Fish Trap Rapids). Fish Trap Rapids had the largest area (1800

m^2), the highest number of unionoid species present (17 species) and the largest population of *Cumberlandia* of all sites, with an estimated 22,697 individuals. However, the site at Paydown Access was not remarkable in terms of species diversity or population size.

The age distributions inferred in this study (**Figures 8-11**) are disturbing because of the relative lack of young individuals. Interestingly, another demographic study of *Cumberlandia* was carried out in the St. Croix River in 1989 (David Heath, Minnesota DNR, personal communication). These length data were analyzed using the growth curves derived in the present study. Interestingly, the St. Croix population showed length and inferred age distributions somewhat less skewed toward older individuals than the present study (**Figures 15, 16**). In a population with steady recruitment, young year classes should exceed or at least equal older year classes in abundance. There are several possibilities for the lack of young individuals in these data. Young individuals may: 1) have been overlooked during quantitative sampling, 2) be distributed more uniformly than the adults, or 3) be uncommon in these populations.

Very smaller (young) individuals are more difficult to recover by visual search than are larger individuals. This factor might account, in part, for the paucity of very small individuals in the sample. It is possible that the smallest age classes (0-4 years) were not sampled adequately, however, it is very unlikely that sampling would miss individuals larger than 40 mm (~5 years). The best evidence that individuals in the 4-10 year age classes were recovered is that these age classes were represented at some sites (Fish Trap Rapids).

A second possible explanation for the lack of young individuals in the samples assumes that these individuals are not distributed in the same contagious pattern as adults. If young individuals were more uniformly distributed, the adaptive sampling method used might underestimate their abundance, relative to the clumped older individuals. This would occur because adaptive sampling preferentially recovers the clumped individuals. However, this explanation appears unlikely, because young individuals were also rare in the initial, randomly placed quadrat samples.

The third possibility is that young individuals are, in fact, rare in these populations. Many recent studies on freshwater mussels report relatively low numbers of young individuals, and these results are generally interpreted as reflecting poor recruitment (Balfour and Smock 1995, Johnson and Brown 1988, Bauer 1988 and 1983, Kat 1982, Cawley 1982, Stober 1972). Recruitment may be irregular in some species, and may depend upon infrequent conditions that favor contact with the host fish as well as deposition of the juveniles in suitable habitat (Payne and Miller, in press). It appears that conditions for recruitment of *Cumberlandia* in the Gasconade and Meramec rivers have declined over the past 20-30 years. The causes for this apparent decline remain undetermined and require further study. Perhaps the abundance of the fish host has declined, but this possibility cannot be addressed until the host is determined. Further study of those few sites having relatively good recruitment patterns may help to reveal the factor or factors affecting the other sites. Clearly, these sites should also be protected from degradation.

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Table 1. Site locations for *Cumberlandia* age and growth collections and demographic sampling. See also, figure 1 and Appendix A. Sites in bold were sampled demographically.

Drainage	Site	Legal description	County
Meramec			
	1	T40NR01WS07	Franklin
	2	T43NR4ES19	Jefferson
	3	T43NR2ES20SE	Franklin
	4	T39NR2WS15	Crawford
	5	T40NR01WS06	Franklin
Gasconade			
	6	T36NR10WS13	Pulaski
	7	T42NR8WS15/16	Osage
	8	T40NR8WS8	Maries
	9	T36NR13WS22/23	Pulaski
	10	T35NR14WS10/15	Laclede
	11	T35NR13WS19NW	Pulaski
	12	T36NR12WS6NE	Pulaski

Table 2. Demographics of *Cumberlandia* at eight sites in the Gasconade and Meramec rivers. Site descriptions are given in Table 1 and Appendix A. N = number of *Cumberlandia* recovered. MI = Morisita's Index of dispersion.

Site	Site area (m^2)	Initial units	Total units	N	Population density (per 1/4m^2) Mean ± st. dev.	Estimated site population	MI	Percent young (<10 y)	Number of species present
Gasconade sites									
10	800	24	120	394	1.21± 0.042	3,857	14.57	1.81	2
11	600	24	115	324	0.90 ± 0.042	2,156	11.8	3.60	8
12	720	30	213	1003	1.66 ± 0.035	4,766	42.87	11.31	8
8	480	30	177	390	1.22 ± 0.034	2,333	20.83	17.41	7
Meramec sites									
3	1800	30	146	815	3.15 ± 0.034	22,697	17.41	10.00	17
4	900	30	268	1270	2.15 ± 0.040	7,753	41.88	0.00	3
1	800	30	53	19	0.29 ± 0.034	933	0.43	1.8	4
5	1080	30	162	879	2.89 ± 0.036	12,480	17.39	0.94	2

Table 3. Mean shell dimensions and inferred ages ± standard deviation for *Cumberlandia* recovered from adaptive sampling, by site. Ages were inferred from equation 1 (see materials and methods). Data were from Gasconade and Meramec combined. N = 2,880.

Site	**Length (mm)**	**Width (mm)**	**Height (mm)**	**Hinge length (mm)**	**Mass (g)**	**Age (years)**
Gasconade sites						
10	131.1 ± 19.15	27.5 ± 4.51	44.2 ± 6.32	67.2 ± 13.06	122.3 ± 44.93	31.8 ± 12.45
11	121.9 ± 20.00	27.2 ± 5.29	41.3 ± 6.44	62.2 ± 13.83	107.2 ± 42.41	25.7 ± 8.68
12	118.4 ± 28.49	25.3 ± 7.04	38.7 ± 8.72	60.1 ± 18.79	103.6 ± 8.72	25.4 ± 10.91
8	100.9 ± 29.01	22.9 ± 6.80	37.3 ± 8.81	51.9 ± 19.30	82.6 ± 8.81	21.3 ± 10.86
Meramec sites						
3	127.1 ± 32.64	28.1 ± 8.17	43.7 ± 9.18	59.95±23.00	141.1 ± 83.45	36.1 ± 25.24
4	129.6 ± 13.22	25.6 ± 3.51	41.0 ± 4.64	66.14±9.47	128.0 ± 44.84	29.7 ± 11.00
1	127.1 ± 13.95	26.8 ± 4.05	42.9 ± 4.38	65.49±10.09	122.4 ± 38.50	27.7 ± 7.75
5	136.0 ± 17.30	27.6 ± 4.23	45.7 ± 6.94	72.35±13.06	150.0 ± 53.08	35.6 ± 14.80

Table 4. Growth in three months by *Cumberlandia* juveniles (n = 7) in laboratory rearing system.

	Total length (mm)			Total height (mm)			Total width (mm)		
Individual	**10-27-98**	**1-27-99**	**growth**	**10-27-98**	**1-27-99**	**growth**	**10-27-98**	**1-27-99**	**growth**
1	48.55	48.65	0.1	19.20	20.10	0.90	10.30	11.20	0.90
2	54.50	54.75	0.25	20.55	21.15	0.60	10.85	11.80	0.95
3	28.95	29.40	0.45	11.60	11.95	0.35	6.05	6.25	0.20
4	27.50	27.90	0.40	11.80	12.20	0.40	5.65	5.90	0.25
5	45.90	45.90	N/A	18.70	18.70	N/A	9.40	9.40	N/A
6	45.35	45.75	0.40	18.45	19.10	0.65	9.45	9.45	N/A
7	31.25	31.45	0.2	12.20	12.40	0.20	6.40	6.45	0.05
Ave. growth			0.30±0.14			0.51±0.25			0.47±0.42

Table 5. Measurements of *Cumberlandia* marked and recaptured after 1 year in the Gasconade River (site 9). "X" indicates individuals were not recaptured.

Total length (mm)		Total height (mm)		Total weight (g)			
Mark date	**Recapture date**	**Mark date**	**Recapture date**	**Mark date**	**Recapture date**	**Growth**	**Remarks**
142.55	142.55	43.2	43.95	128.0	137.7	Y	
136.52	138.25	43.55	45.45	124.1	129.6	Y	
144.52	144.75	47.45	48.1	135.3	135.6	Y	No tag
130.0	X	42.52	X	110.9	X	N/A	
146.52	147.82	45.53	46.5	146.5	153.0	Y	
151.45	152.55	52.35	53.45	213.1	228.2	Y	
140.3	X	46.2	X	131.4	X	N/A	
126.1	127.3	41.25	42.3	80.6	90.0	Y	
128.55	129.45	39.45	41.3	121.6	114.3	Y	No tag
128.05	128.35	42.55	41.9	94.1	126.7	Y	
79.45	81.65	29.5	30.35	22.9	26.2	Y	
165.35	166.65	58.05	60.45	235.7	242.7	Y	
130.55	131.85	40.55	42.4	95.1	105.4	Y	
114.9	115.7	37.3	39.0	69.4	80.5	Y	
125.6	126.55	44.7	43.9	98.3	104.2	Y	
138.0	138.6	43.2	43.65	111.2	118.9	Y	
125.7	126.05	43.75	44.35	102.1	115.6	Y	
136.9	X	48.0	X	128.1	X	N/A	
138.8	138.8	45.95	45.35	127.4	146.5	N	
134.75	134.8	46.45	47.15	126.4	N/A	Y	dead
133.25	133.7	46.8	45.45	125.4	134.1	Y	
129.2	129.05	45.95	44.3	106.4	N/A	N	dead
110.0	111.0	38.75	39.5	68.4	77.4	Y	
116.7	116.9	38.0	38.4	69.9	74.4	Y	
162.75	163.15	53.85	52.35	196.2	215.3	Y	
136.05	135.65	42.7	42.4	123.8	N/A	N	dead
60.4	60.3	22.4	22.5	9.7	11.8	Y	
152.35	151.55	45.5	46.0	162.6	169.8	Y	
158.55	158.75	51.2	52.75	206.1	219.0	Y	
140.0	140.3	46.6	47.8	129.0	133.6	Y	
144.85	144.4	46.4	47.3	149.8	162.8	Y	
110.8	110.85	36.7	37.1	57.2	61.4	Y	
88.0	90.07	32.9	33.15	34.0	39.1	Y	
94.25	X	34.35	X	39.7	X	N/A	
73.85	75.65	26.75	27.9	16.9	19.3	Y	
76.05	76.4	27.1	27.55	19.8	21.1	Y	
67.35	X	24.0	X	13.6	X	N/A	
70.8	77.45	27.7	30.1	17.2	23.7	Y	
65.65	68.7	25.0	26.35	13.3	16.2	Y	

Table 6. Measurements of *Cumberlandia* marked and recaptured after 1 year in the Meramec River (site 3). "X" indicates individuals were not recaptured.

length (mm)		Total height (mm)		Total weight (g)			
Initial	Final	Initial	Final	Initial	Final	Growth	Remarks
63.95	X	26.45	X	15.2	X	N/A	
70.6	X	27.5	X	17.7	X	N/A	
83.6	X	35.45	X	44.7	X	N/A	
60.15	X	24.55	X	9.9	X	N/A	
62.35	X	26.25	X	13.1	X	N/A	
53.4	X	22.25	X	8.6	X	N/A	
64.25	X	26.85	X	15.8	X	N/A	
67.35	X	26.85	X	15.6	X	N/A	
76.4	89.85	30.9	36.65	23.6	42.2	Y	
87.9	X	36.7	X	38.4	X	N/A	
76	X	32.4	X	25.7	X	N/A	
96.65	X	36.85	X	53.7	X	N/A	
88.8	X	33.95	X	35.0	X	N/A	
91.45	X	36.7	X	44.9	X	N/A	
95.55	X	38.2	X	51.9	X	N/A	
98.55	102.1	40.4	41.55	59.6	74.5	Y	
107.05	114.75	40.15	42.05	66.1	86.8	Y	No tag
115.5	120.7	44.5	46.2	85.2	113.5	Y	
128	X	45.6	X	121.8	X	N/A	
133.6	X	47.6	X	112.6	X	N/A	
156.5	158.1	50.9	53.65	202.2	230.0	Y	
123.4	X	47.25	X	91.1	X	N/A	
139.6	141.55	49.0	50.85	173.9	200.3	Y	
154	155.3	52.8	54.0	205.4	229.8	Y	
132.65	132.95	51.15	53.75	158.4	150.3	Y	No tag
138.3	140.35	50.35	51.55	155.4	178.2	Y	
154.85	X	59.7	X	219.4	X	N/A	
135.95	X	45.9	X	140.4	X	N/A	
133.95	132.9	44.9	44.9	105.3	N/A	N	shrunk
131.55	X	51.75	X	137.8	X	N/A	
151.45	X	60.55	X	228.0	X	N/A	
147.0	147.0	54.3	55.5	199.8	229.0	Y	
158.6	159.6	55.6	56.55	245.3	276.0	Y	No tag
146.0	X	49.55	X	158.5	X	N/A	
133.95	136.5	47.45	49.75	159.8	186.9	Y	
145.65	149.3	53.5	55.35	187.1	217.9	Y	
147.45	149.9	51.5	52.7	174.9	203.1	Y	
153.05	155.6	49.45	51.3	216.4	249.9	Y	No tag
129.35	129.7	47.05	48.1	133.1	144.6	Y	
144.55	X	54.45	X	158.3	X	N/A	
139.0	141.4	53.45	54.9	168.2	195.6	Y	No tag
147.05	X	53.8	X	172.4	X	N/A	
142.05	X	48.2	X	167.0	X	N/A	
169.25	169.8	62.6	63.2	287.3	300.3	Y	

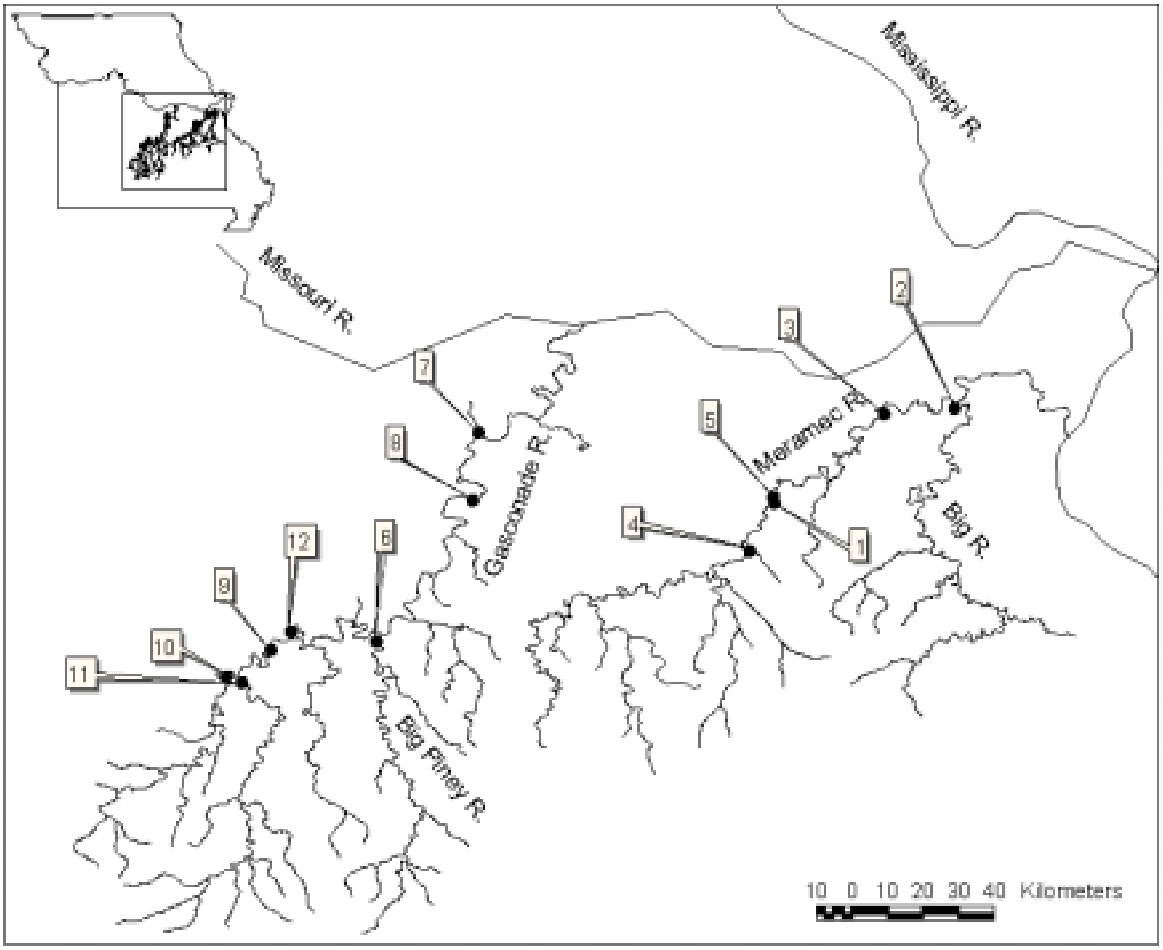

Figure 1. Map of the Gasconade and Meramec Rivers in Missouri, and locations where *Cumberlandia* were collected or quantitatively sampled. Numbers refer to site numbers (see appendix A).

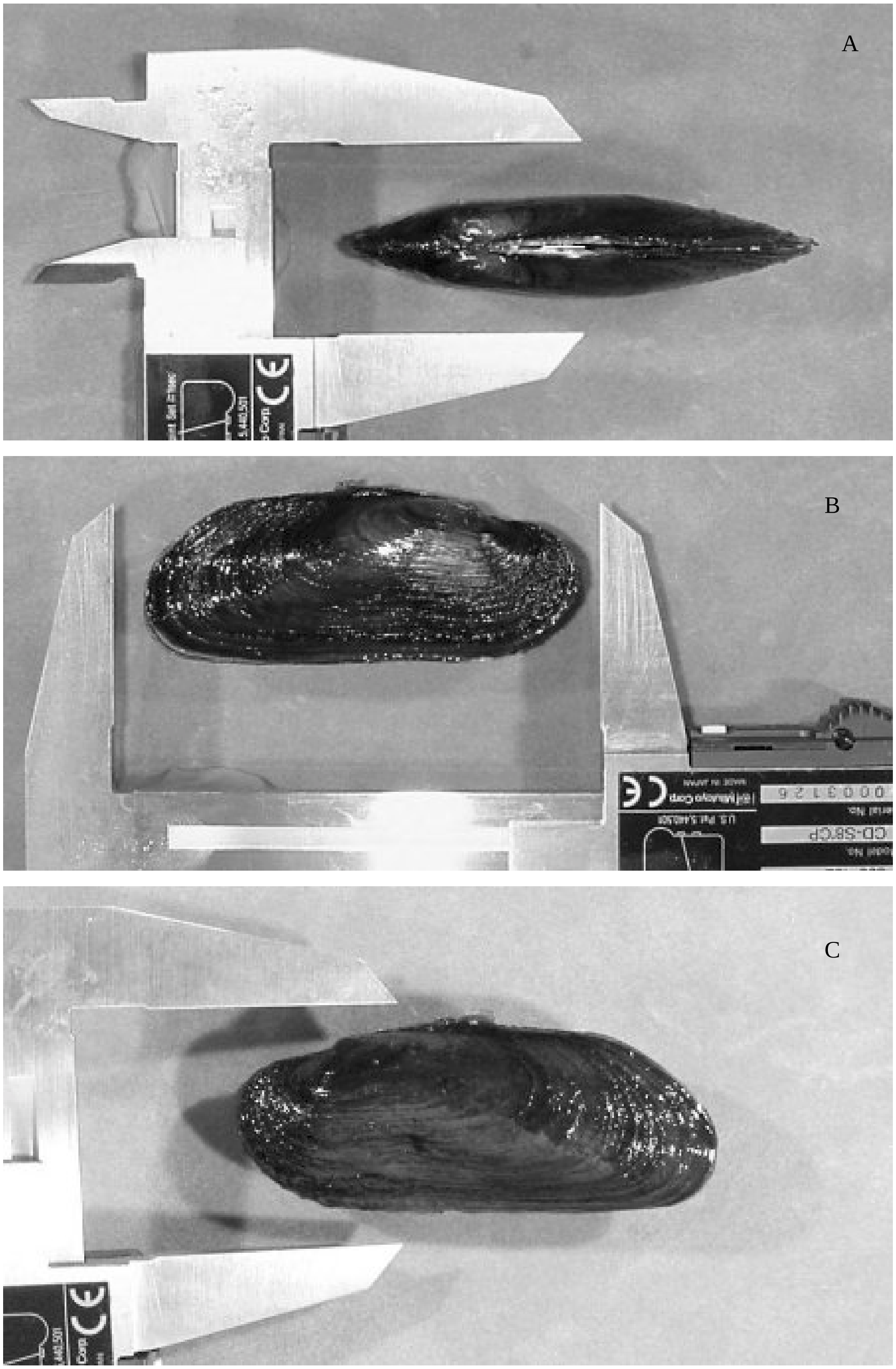

Figure 2. Dimensions of *Cumberlandia* (A, length; B, height; C, width).

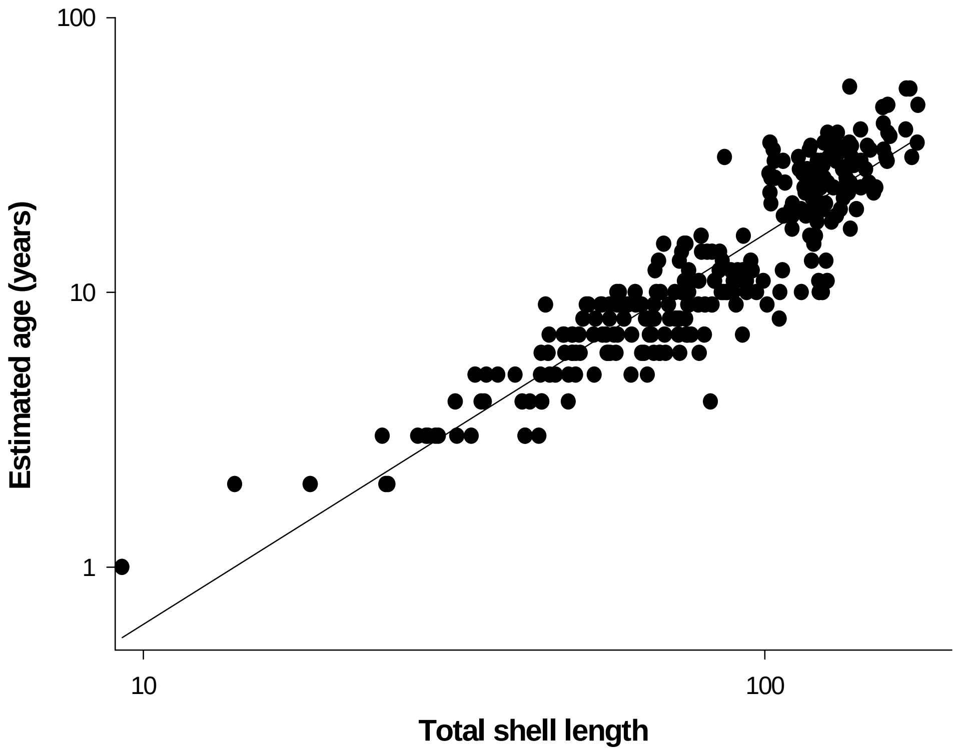

Figure 3. Relationship between age estimated from hinge growth lines and shell length. Note log axis. N = 278. Line was fitted by regression.

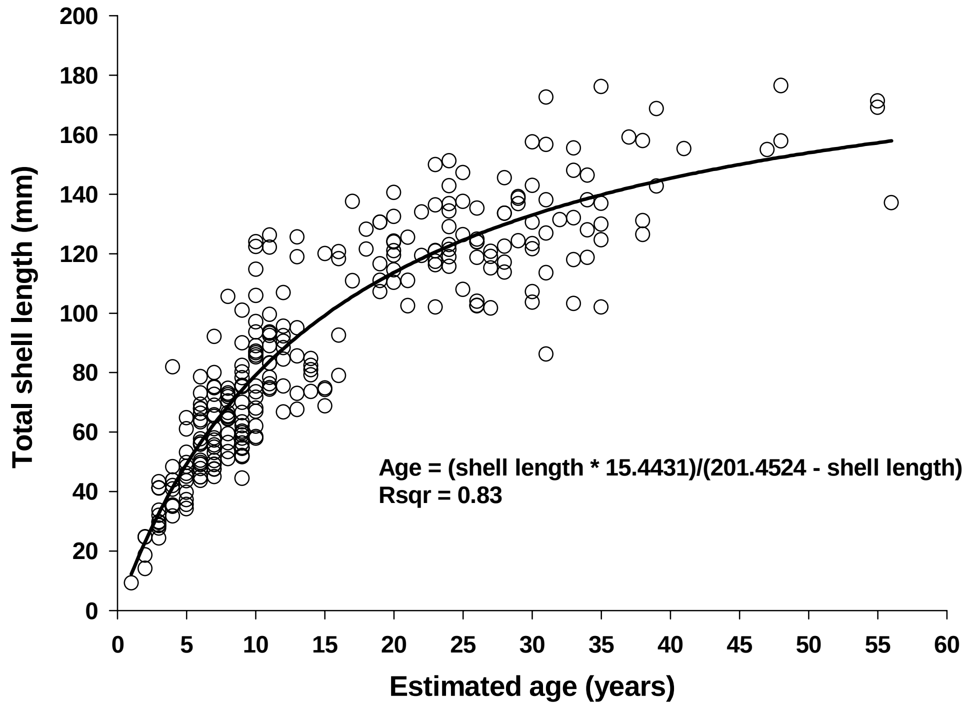

Figure 4. Shell length versus age estimated from hinge growth lines. Data from Gasconade and Meramec rivers were combined. Line was fitted by regression. N = 278.

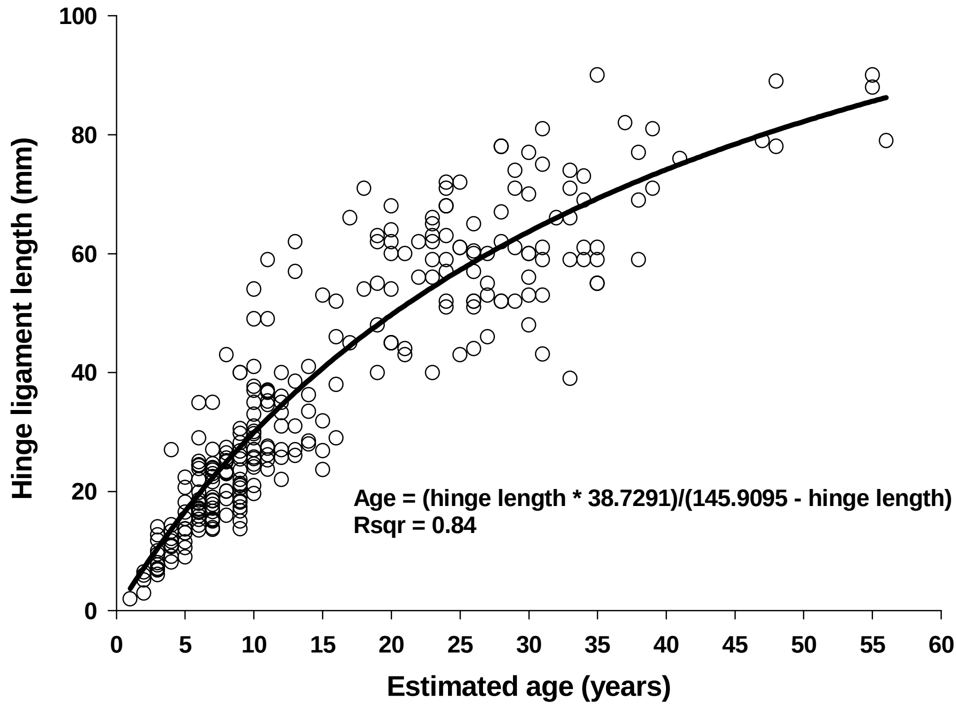

Figure 5. Hinge ligament length versus age estimated from hinge growth lines. Data were from the Gasconade and Meramec rivers combined. Line was fitted by regression. N = 278.

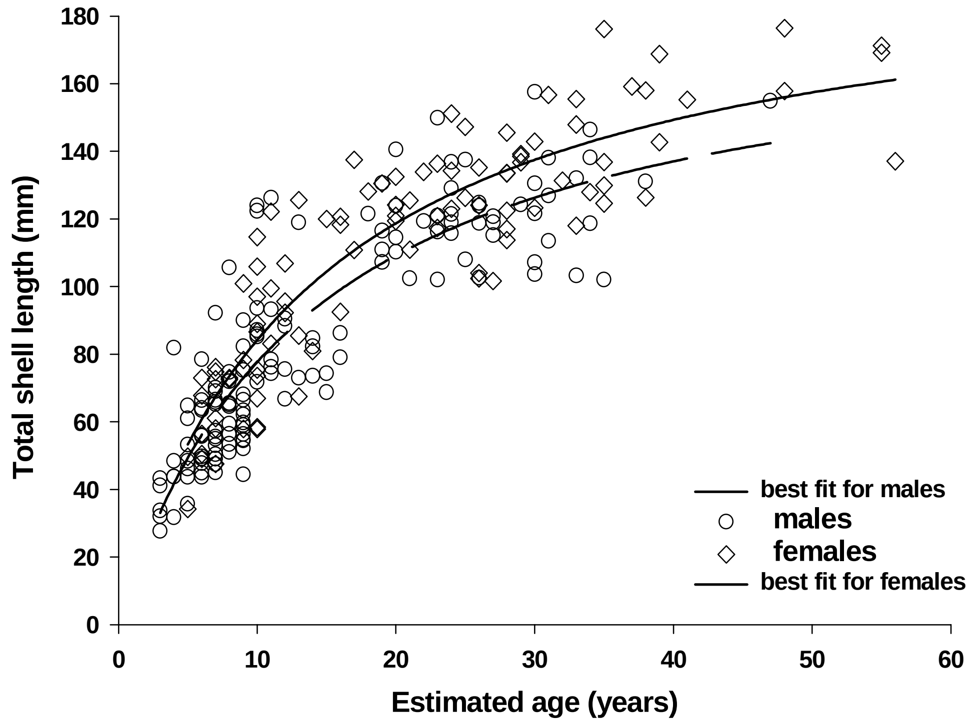

Figure 6. Length versus age of male and female *Cumberlandia* collected in the Gasconade and Meramec Rivers, Missouri. Lines are two-parameter hyperbola fitted by least-squares regression.

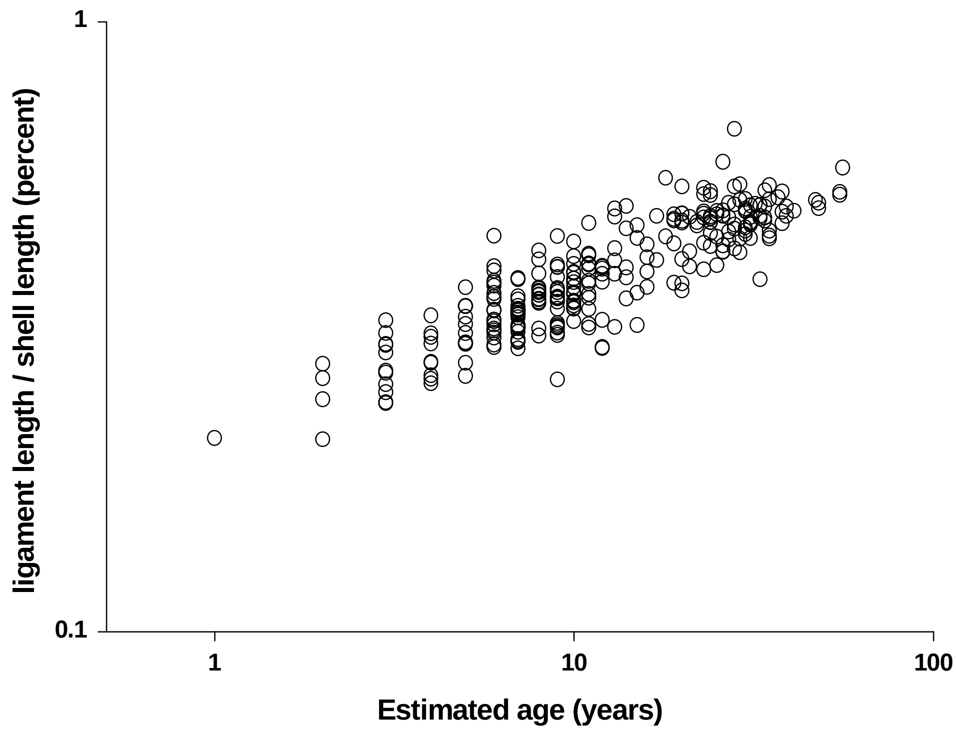

Figure 7. Ratio of hinge length to shell length vs. shell length. Data from Gasconade and Meramec rivers were combined. Note log axis. N = 278.

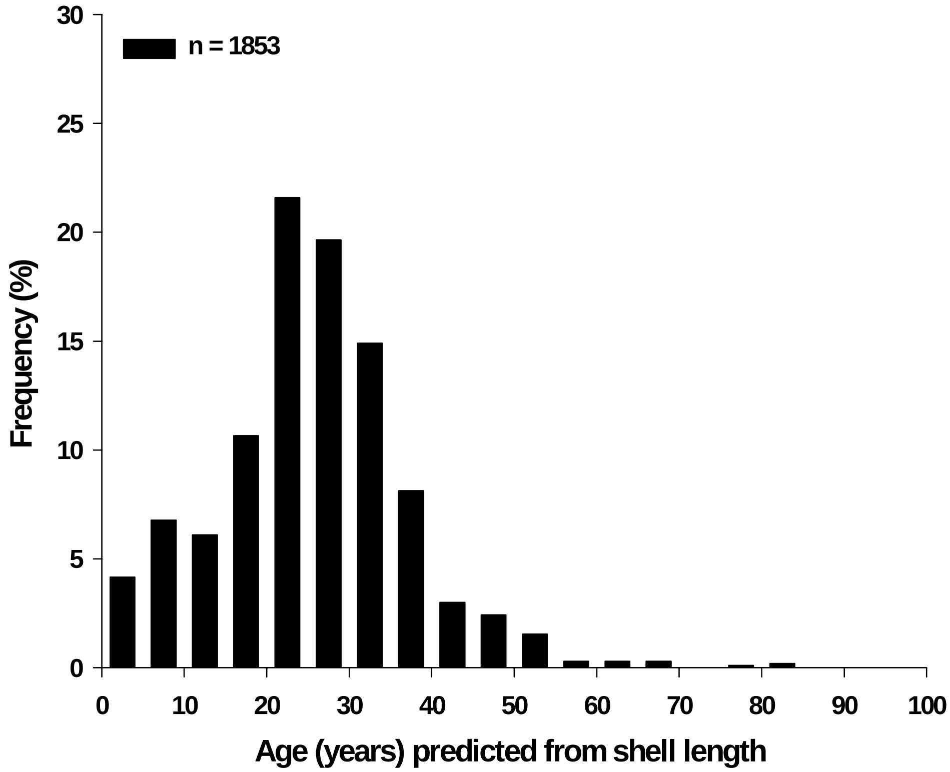

Figure 8. Age distribution of *Cumberlandia* in the Gasconade River. All sites were combined. Age was predicted from shell length. N = 1033.

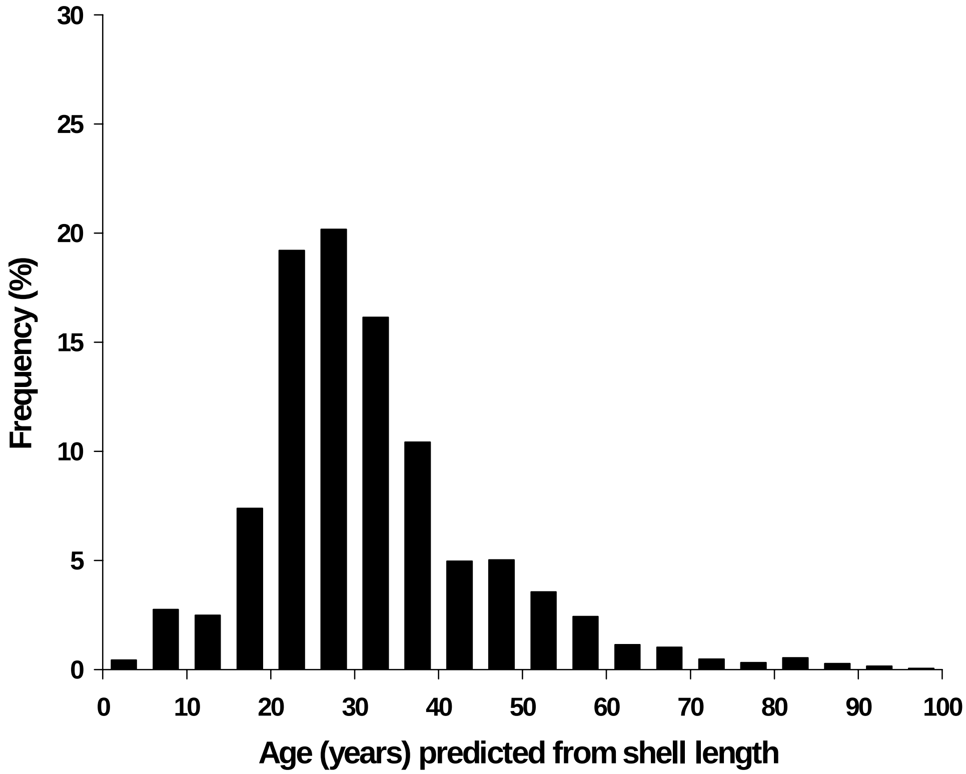

Figure 9. Age distribution of *Cumberlandia* in the Meramec River. All sites were combined. Age was predicted from shell length. N = 1853.

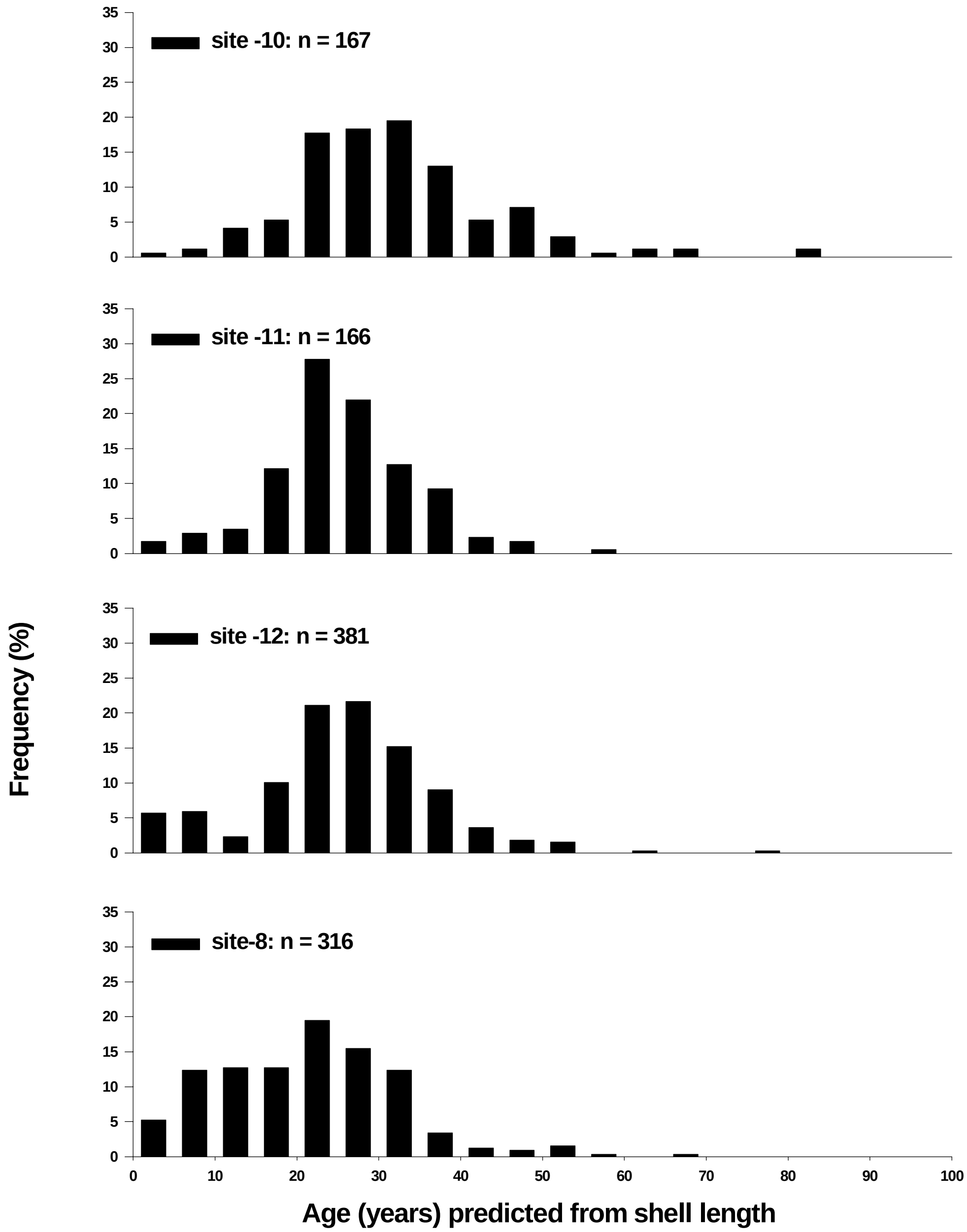

Figure 10. Age distributions of *Cumberlandia* at four sites in the Gasconade River. Age was predicted from shell length.

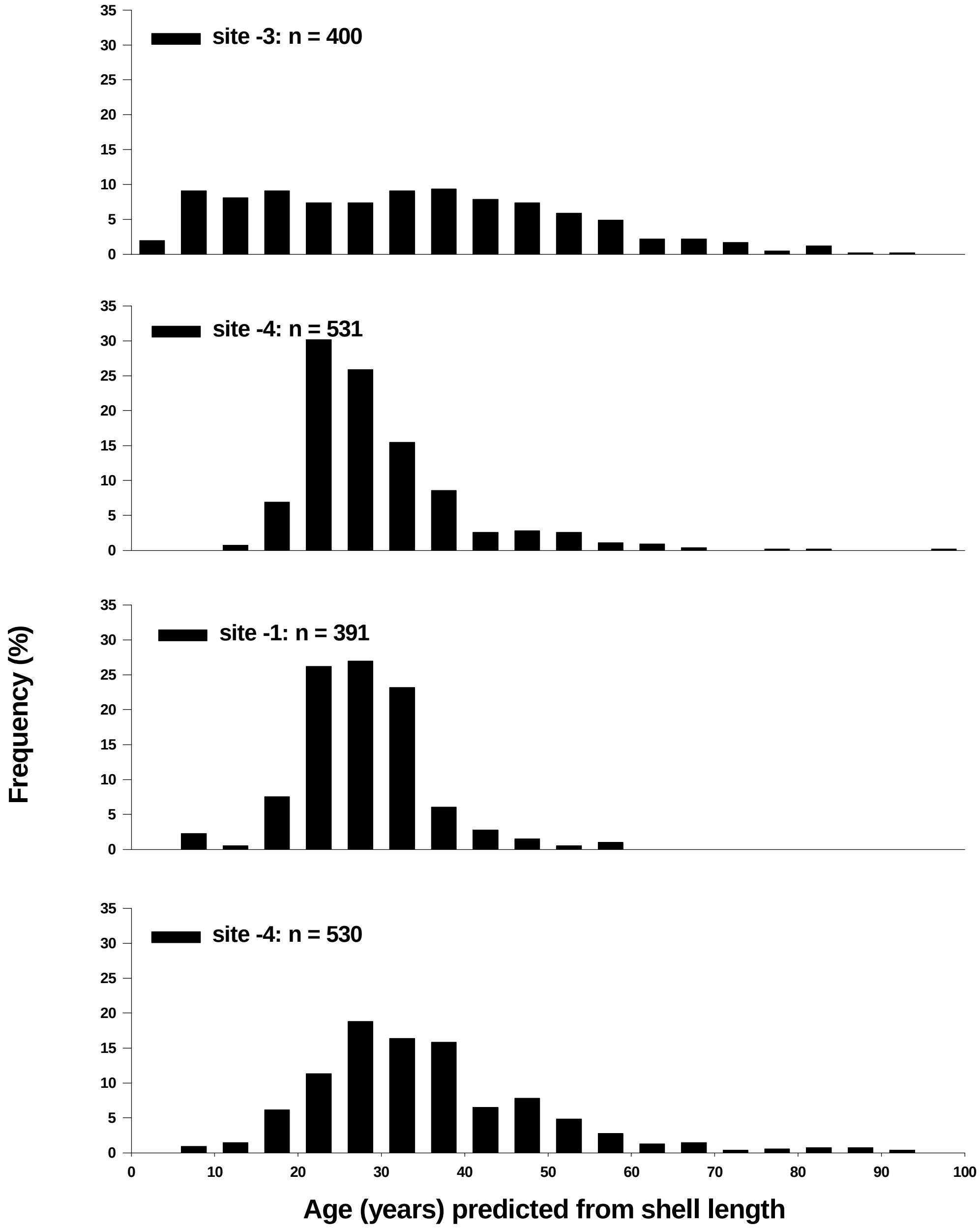

Figure 11. Age distributions of *Cumberlandia* at four sites in the Meramec River. Age was predicted from shell length.

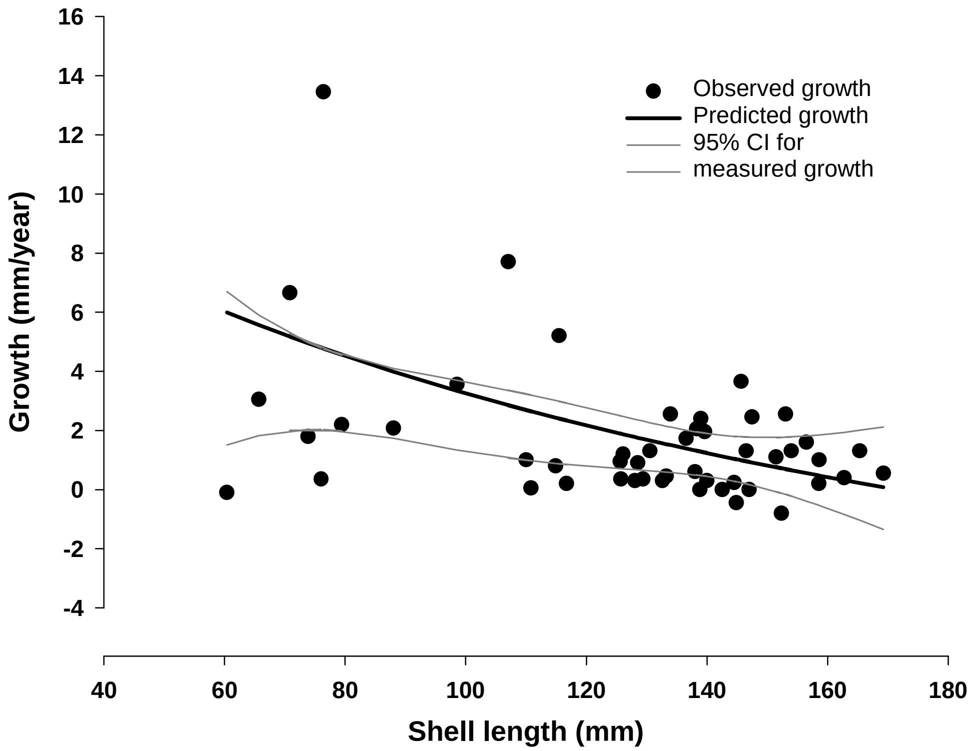

Figure 12. Comparison of predicted growth with observed annual growth. The line shown is the predicted growth based upon measurements of age and shell length of 278 individuals (see Figure 6). Symbols indicate growth measured directly in 49 individuals during 1 year in the field. The regression for these data was Growth= -9.51+ (17.20*229.39)/ (229.39+ length) (R2= 0.17). The 95% CI for this regression is indicated, and shows that the measured growth rates were similar to the prediction.

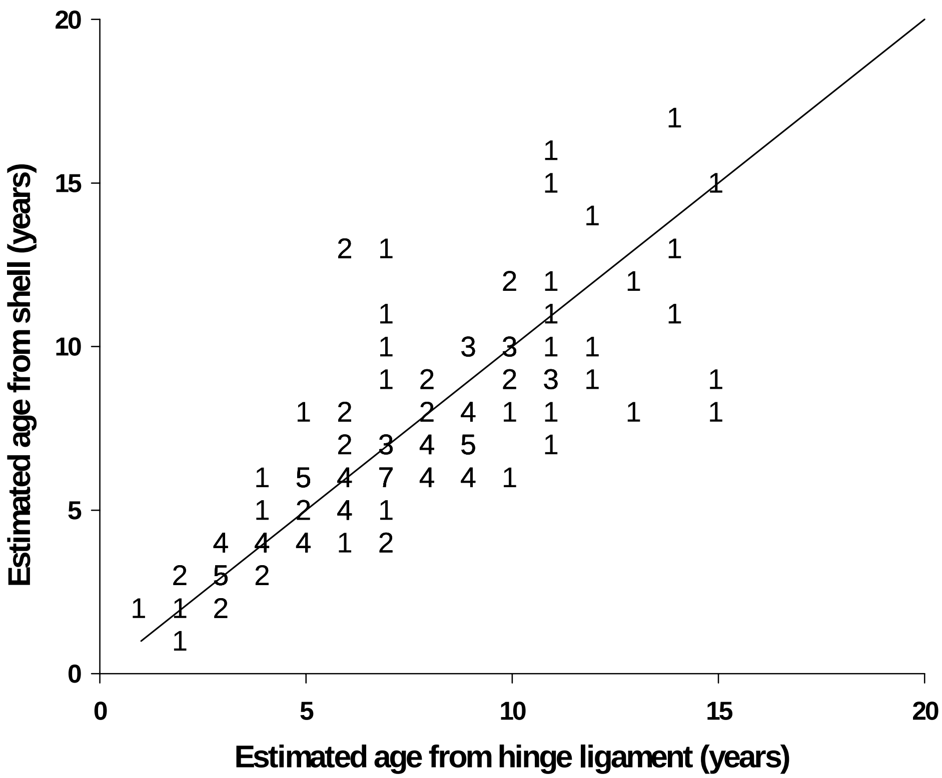

Figure 13. Comparison of ages estimated from shell growth lines and hinge ligament growth lines. The line is the line of identity. Numerals indicate the number of individuals at each point. Only young individuals were examined, because shell growth lines were difficult to distinguish in older individuals.

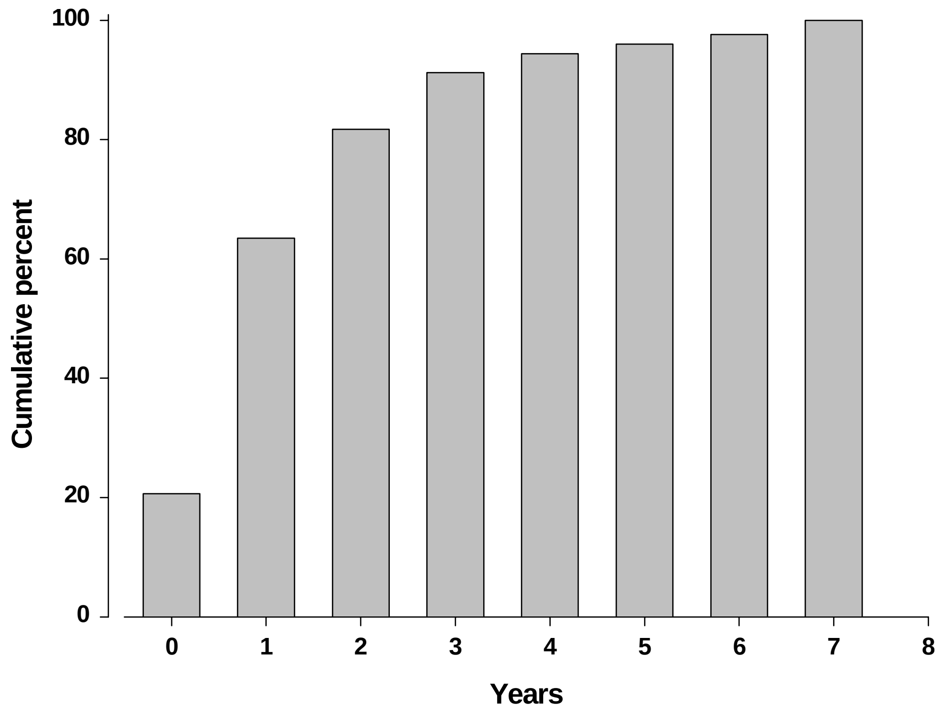

Figure 14. Agreement between ages estimated from shell growth lines and hinge ligament growth lines. Data are from Figure 15. Bars show the cumulative proportion of the data that differed by a value less than or equal to the specified number of years (0 = identity). N =126.

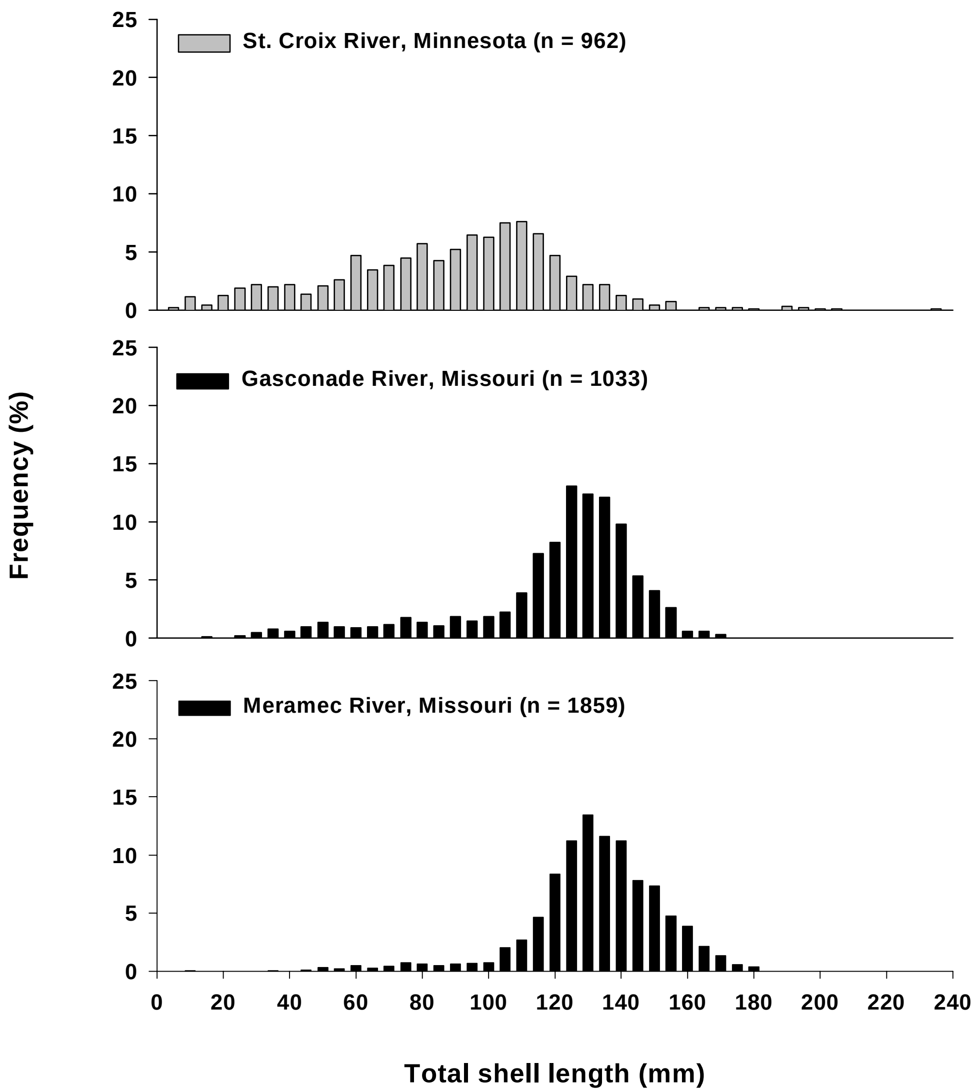

Figure 15. Length distributions of *Cumberlandia* in the St. Croix, Gasconade, and Meramec Rivers. Data for St. Croix River are from an unpublished study by David Heath [Minnesota DNR].

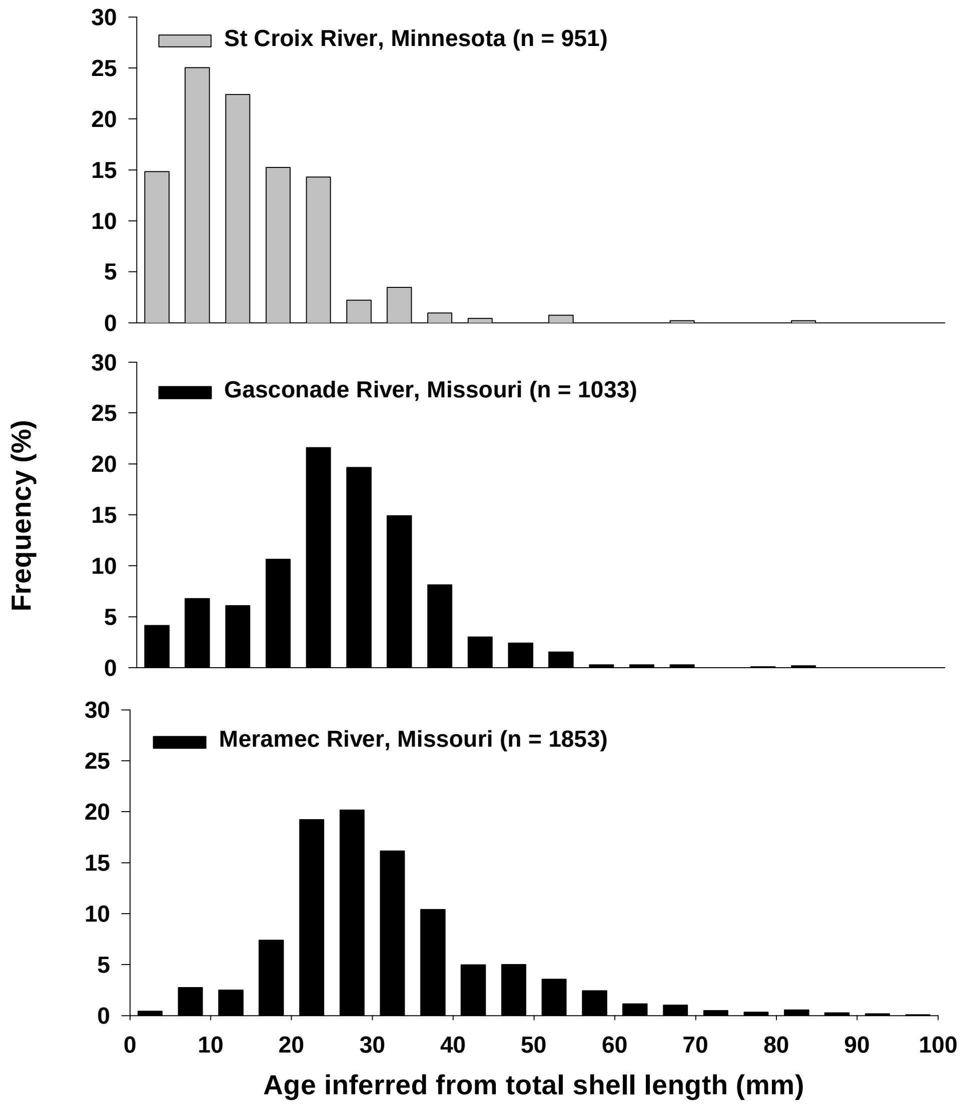

Figure 16. Age distributions of *Cumberlandia* in the St. Croix, Gasconade, and Meramec Rivers. Age was predicted from shell length. Data for St. Croix River are from an unpublished study by David Heath [Minnesota DNR].

APPENDIX A. Collection site descriptions.

MERAMEC RIVER (SITE 1) - Franklin County, Missouri, T40NR01WS07. From Sullivan, MO and I-44, go south on Highway 185 to Meramec State Park. Pass the visitor's center and go left at first intersection within the park. Follow this park road to the pay-booth near the main campground. After passing through the pay-booth, the road will meander to the right along the Meramec River. Follow the road until it ends in a small parking lot near a dumpster. There will be a foot trail from the parking lot leading to the river. Just upstream, there is a long island; about one-fourth of a mile downstream there is a bluff line. Across and downstream from the end of the foot trail is a riffle. The downstream portion of this riffle marks the upper boundary of the *Cumberlandia* site.

I visited this site on three occasions. One visit occurred on the 8 of September 1998 to collect individuals for age and growth studies, and the other two visits were 30 September 1999 and the 4 October 1999 to perform quantitative work. This is a shallow site with an average depth of 1 to 1.5 meters. There is good flow close to the riffle, and moderate flow throughout the site. Large cobble and boulders dominate the substrate. Sandy gravel with patches of rough bedrock are interspersed between the dominant substrates. During the quantitative work, the *Cumberlandia* bed was estimated to be approximately ten meters wide, starting five meters out from the far bank, by 80 meters in length. *Cumberlandia* were not sought beyond 100 meters downstream from the riffle.

MERAMEC RIVER (SITE 2) - Jefferson County, Missouri, T43NR4ES19. Meramec River Site two is actually on the Big River. From Eureka, MO and I-44, go south on Highway W. Highway W splits into W and FF. Stay on Highway W heading southeast. Within a mile and a half, the highway runs parallel to the Big River. To the right of the highway is a large bluff; to the left of the highway is a steep bank. As the bluff line comes to an end, there is a dirt driveway on the left-hand side of the road with enough room to park a vehicle or two. Getting into the river is always a problem, but the site begins approximately 50 meters downstream from the parking area. The *Cumberlandia* bed begins just downstream of the concrete stairs leading into the water on the near bank. The richest portion of the bed lies about 75 meters downstream from these stairs, and includes a diverse fauna of mussels, in addition to some beautiful *Cumberlandia*.

I visited this site on two occasions: 9 of September 1998 to collect individuals for age and growth studies, and 24 May 1999 to collect gravid females. This is a shallow site with an average depth of 0.75 meters. There is very good flow throughout the entire site. Large cobble and boulders dominate the substrate. Compacted sand, gravel, and small cobble are interspersed throughout the dominate substrates. Quantitative work was not performed at this site, and so rough estimates of *Cumberlandia* bed dimensions are not available.

MERAMEC RIVER (SITE 3) - Franklin County, Missouri, T43NR2ES20SE. From St. Clair, MO go east on I-44 about six miles to Highway O. Head east on Highway O approximately 4.5 miles and turn left on paved road before crossing the Meramec River. Stay on paved road until reaching RR 152. Take steep gravel road on the right side near RR 152, and follow until coming to a stop sign with a chain across the road. The owner of the entire river bottom, Mr. Sparks, lives in the house to the right of the stop sign. If home, they will let you through the chain across the road. Follow gravel road to the

Meramec River. Road will end just above a long gravel bar. The mussel bed is upstream of the rapids on your left, and across from the old concrete boat ramp on the near shore.

I visited this site on seven occasions: 17 November 1998 to collect individuals for age and growth studies, 2 April and 24 May 1999 in an attempt to collect gravid females, and 9,11,12, and 15 September 1999 to perform quantitative work.   This is a deep site with an average depth of 1.5 to 2.0 meters. There is very good flow throughout the entire site. The substrate is dominated by boulders. Compacted sand, gravel, and cobble are interspersed throughout the dominate substrates. There are only small patches of exposed bedrock at this site. During the quantitative work, the *Cumberlandia* bed was estimated to be approximately 20 meters wide by 90 meters in length. *Cumberlandia*, however, were found slightly further upstream and downstream of the delineated quantitative site.

MERAMEC RIVER (SITE 4) - Crawford County, Missouri, T39NR2WS15. The legal description is From Bourbon, MO and I-44, head southeast on Highway N for approximately seven miles. After crossing the Meramec River, turn left on Thicket-Ford Road. Follow this gravel road for about a mile and a quarter until the road meets the river on your left. There is a very steep trench below a culvert under the road from which you can access the river. The *Cumberlandia* bed begins about ten meters downstream from the culvert's trench.

I visited this site on seven occasions: 17 November 1998 to collect individuals for age and growth studies, 25 February, and the 3 and 19 May in an attempt to collect gravid females, and 16, 22, and 23 September 1999 to perform quantitative work. This is a moderately deep site with an average depth of 1.0 to 1.5 meters. There is very good flow throughout the entire site. Large smooth boulders and smooth bedrock dominate the substrate. Compacted sand, gravel, and cobble are interspersed throughout the dominate substrates. During the quantitative work, the *Cumberlandia* bed was estimated to be approximately 10 meters wide by 90 meters in length. *Cumberlandia*, however, were found slightly further upstream and downstream of the delineated quantitative site. *Cumberlandia* recruitment was low to non-existent at this site, and no other species of mussels were found.

MERAMEC RIVER (SITE 5) - Franklin County, Missouri, T40NR01WS06. Meramec River, quantitative site four is situated within Meramec State Park also. The legal description is From Sullivan, MO and I-44, go south on Highway 185 to Meramec State Park. Pass the visitor's center and go left at the first intersection within the park. Follow this park road to the pay-booth near the main campground. After passing through the pay-booth, take the last possible campsite road on your left and follow it to the last camp site, closest to the river. There is a foot trail, which will wind through the woods, parallel to a large bluff line. Stay on the main foot trail until it dead-ends at the river. A riprap embankment begins to your left on the near shore, and this marks the upstream boundary of the *Cumberlandia* bed.

I visited this site on three occasions: 5, 6, and 7 October 1999 to perform quantitative work. This is a deep site averaging 3.0 to 3.5 meters in depth. There is limited flow throughout the entire site. Large boulders and bedrock dominate the substrate. Many boulders are the size of vehicles. Compacted sand, gravel, and cobble are interspersed throughout the dominant substrates. Also, there are numerous large sunken trees throughout the site. During the quantitative work, the *Cumberlandia* bed was estimated to be approximately 12 meters wide by 90 meters in length. A few

Cumberlandia, however, were found slightly further downstream of the delineated quantitative site.

GASCONADE RIVER (SITE 6) - Pulaski County, Missouri, T36NR10WS13. Gasconade River site one is actually on the Big Piney River. From St. Roberts and I-44, head north on Highway 28 towards Dixon. Travel about two to three miles and look for an old abandoned grocery store on right hand side. This gravel road is not obvious. Follow road until it meets and parallels the river. *Cumberlandia* site extends along entire riprap bank on near side of river.

I only visited this site once: 29 September 1998, to collect individuals for age and growth studies. The average depth ranged from 1.0 to 3.0 meters. There is limited flow throughout most of the site, however, the deeper areas maintain good flow. Large boulders dominate the substrate. Many boulders are the size of vehicles. Compacted sand, gravel, and cobble are interspersed throughout the dominant substrates. Quantitative work was not performed at this site, and so rough estimates of *Cumberlandia* bed dimensions are not available.

GASCONADE RIVER (SITE 7) - Osage County, Missouri, T42NR8WS15/16. From Linn, MO and Highway 50, go south on Highway 89 approximately 5.5 miles to Rollins Ferry Access. From boat ramp, head downstream below large rock-bluff outcropping. There is a shoreline of natural rip-rap on your left, just downstream from the river bend. This natural rip-rap, and a house close to the river, marks the *Cumberlandia* site.

I visited this site twice: 27 October 1998 to collect individuals for age and growth studies, and 9 June 1999 to collect gravid females. The average depth ranged from 1.5 to 2.0 meters. There is good flow throughout the site. Large cobble and small boulders dominate the substrate. Compacted sand, gravel, and cobble are interspersed throughout the dominant substrates. There is little to no bedrock present. Quantitative work was not performed at this site, and so rough estimates of *Cumberlandia* bed dimensions are not available.

GASCONADE RIVER (SITE 8) - Maries County, Missouri, T40NR8WS8. From Vienna, MO go north on Highway 63 for 5.25 miles. Watch for the cantilever sign for Paydown Access. Take a right on gravel road before reaching Freeburg Towersite, and follow about seven miles to the Conservation department access. From boat access, head upstream for approximately 1.5 miles. You will travel along a long sweeping outside river bend, up through a long, narrower riffle area, and finally up a long shallow run. At this point, there will be pastureland to your left, the beginning of a bluff on your right, and a long pool ahead. The strong riffle leading into the pool from the left side marks the top boundary of the *Cumberlandia* site. Natural riprap along the right-hand side of the pool will help you find the site.

I visited this site on four occasions: 27 October 1998 to collect individuals for age and growth studies, and on the 1, 8, and 9 September 1999 to perform quantitative work. The average depth ranged from 1.5 to 2.5 meters. There is good flow throughout the site. Large cobble and boulders dominate the substrate. Compacted sand, gravel, and cobble are interspersed throughout the dominant substrates. There is little to no bedrock present. During the quantitative work, the *Cumberlandia* bed was estimated to be approximately 12 meters wide by 100 meters in length. A few *Cumberlandia*, however, were found slightly further upstream and downstream of the delineated quantitative site.

GASCONADE RIVER (SITE 9) - Pulaski County, Missouri, T36NR13WS22/23. From Buckhorn, MO and I-44, go west on I-44 three miles to Highway 7. Head north on Highway 7 for approximately six miles. Take a right on Rochester road and follow it until you cross the Gasconade River. Take a right on Riddle road, and follow road alongside steep bluff line. The *Cumberlandia* site is directly below the back entrance of the Cave Man Bar-B-Que resturant. Four large boulders project from the water on near shore.

I visited this site on six occasions: 29 October 1998 to collect individuals for age and growth studies, and 30 December 1998, 25 February, 1 April, 3 May, and 19 May 1999 to collect gravid females. This is a deep site averaging 2.5 to 3.0 meters in depth. There is usually moderate flow near shore and good flow down deep. Boulders dominate the substrate. Compacted sand, gravel, and cobble are interspersed throughout the dominant substrates. There is little to no bedrock present. Quantitative work was not performed at this site, and so rough estimates of *Cumberlandia* bed dimensions are not available.

GASCONADE RIVER (SITE 10) - Laclede County, Missouri, T35NR14WS10/15. From Lebanon, MO and I-44, go east on I-44 approximately 12 miles to the Gasconade River. The Department of Conservation's Hazelgreen Access is below the I-44 Bridge. From access, go downstream about three-fourths of a mile until you see a sharp bend in the river. This sharp bend creates a strong shallow riffle; the top of the *Cumberlandia* site starts where the river (i.e., riffle) begins to straighten out again.

I visited this site twice: 8 and 9 August 1999 to perform quantitative work. This is a shallow site, with depth averaging 0.75 meters, and thus flow is good throughout.

Large cobble and small boulders, each dominant substrate fairly well embedded. Compacted sand, gravel, and smaller cobble are interspersed throughout the dominant substrates. Bedrock was plentiful, especially beneath the smaller compacted substrates. During the quantitative work, the *Cumberlandia* bed was estimated to be approximately 10 meters wide by 80 meters in length. A few *Cumberlandia*, however, were found slightly further upstream and downstream of the delineated quantitative site.

GASCONADE RIVER (SITE 11) - Pulaski County, Missouri, T35NR13WS19NW. From Buckhorn, MO and I-44, go west on I-44 7.5 miles to route AB. Head southeast on route AB one-eighth of a mile and take the first rock road on your right. Follow to bridge over Gasconade River near campground. From bridge, go downstream approximately 1.0 mile. You will pass a series of four to five bends in the river, and come across a large macrophyte bed. The bed is so large that it stifles flow to your left and leaves only a narrow channel to your right. The top of the *Cumberlandia* site is near the bottom of the macrophyte bed, on the far side of the river (i.e., your left).

I visited this site twice: 11 and 12 August 1999 to perform quantitative work. This is a shallow site with an average depth of 0.5 meters. There is very good flow through the middle and lower sections, however, the macrophyte bed stifles flow near the top of the bed. Substrate is dominated by large cobble. Compacted sand and gravel are interspersed throughout the large cobble. Bedrock was patchy. During the quantitative work, the *Cumberlandia* bed was estimated to be approximately 8 meters wide by 75 meters in length. No *Cumberlandia* were found upstream or downstream of the delineated quantitative site.

GASCONADE RIVER (SITE 12) - Pulaski County, Missouri, T36NR12WS6NE. From Richland, MO go northeast on Highway 133 through Swedeborg. Approximately 1.75 miles east of Swedeborg, take a right on gravel road and follow to the Conservation Department's Schlicht Access. From access, go upstream approximately one mile to a large sweeping bend in the river. At the top of this bend is a strong riffle, which marks the top of the *Cumberlandia* site.

I visited this site on four occasions: 12, 16, 25 and 26 August 1999 to perform quantitative work. This is a shallow site with an average depth of 0.5 meters. Flow is very good throughout the site. Large cobble and small boulders dominate substrate. Compacted sand and gravel are interspersed throughout the dominant substrate. Bedrock was common. During the quantitative work, the *Cumberlandia* bed was estimated to be approximately 8 meters wide by 90 meters in length. Only occasional *Cumberlandia* were found downstream of the delineated quantitative site.

APPENDIX B. Fish species shared by the Gasconade and Meramec Rivers.

N=90 species. Species in bold are shared with the St. Croix River, Minnesota, where *Cumberlandia* is also found (N=58 species). Species with an asterisk were tested as hosts with lab infestations.

Alosa alabamae	**Alabama shad**
Ambloplites rupestris	**Rock bass**
Ameiurus melas	**Black bullhead**
Ameiurus natalis	Yellow bullhead
Anguilla rostrata	**American eel**
****Aplodinotus grunniens***	**Freshwater drum**
Campostoma oligolepis	**Largescale stoneroller**
Campostoma pullum	**Central stoneroller**
Carpiodes carpio	River carpsucker
Carpiodes cyprinus	**Quillback**
Carpiodes velifer	**Highfin carpsucker**
Catostomus commersonni	**White sucker**
****Cottus bairdi***	**Mottled sculpin**
**Cottus carolinae*	Banded sculpin
Cottus hypselurus	Ozark sculpin
Crystallaria asprella	**Crystal darter**
Cyprinella spiloptera	**Spotfin shiner**
Dorosoma cepedianum	**Gizzard shad**
**Etheostoma blennioides*	Greenside darter
****Etheostoma caeruleum***	**Rainbow darter**
****Etheostoma flabellare***	**Striped fantail darter**
****Etheostoma nigrum***	**Johnny darter**
Etheostoma punctulatum	Stippled darter
**Etheostoma s. spectabile*	Northern orangethroat dar
**Etheostoma tetrazonum*	Missouri saddled darter
Etheostoma zonale	Banded darter
Fundulus catenatus	Northern studfish
Fundulus olivaceous	Blackspotted topminnow
Fundulus sciadicus	Plains topminnow
Gambusia affinis	Western mosquitofish
Hiodon alosoides	**Goldeye**
Hiodon tergisus	**Mooneye**
Hybopsis x-punctata	Gravel chub
****Hypentelium nigricans***	**Northern hog sucker**
Ichthyomyzon castaneus	**Chestnut lamprey**
Ichthyomyzon fossor	**Northern brook lamprey**
****Ictalurus punctatus***	**Channel catfish**
Ictiobus bubalus	**Smallmouth buffalo**
Ictiobus cyprinellus	**Bigmouth buffalo**
Ictiobus niger	Black buffalo
Labidesthes sicculus	**Brook silverside**
Lepisosteus osseus	**Longnose gar**
Lepisosteus platostomus	**Shortnose gar**
****Lepomis cyanellus***	**Green sunfish**
Lepomis gulosus	**Warmouth**
Lepomis humilis	Orange spotted sunfish
****Lepomis macrochirus***	**Bluegill**
****Lepomis megalotis***	**Longear sunfish**
**Lepomis microlophus*	Redear sunfish
****Luxilus chrysocephalus***	**Striped shiner**
**Luxilus zonatus*	Bleeding shiner
Lythrurus u. umbratilis	Redfin shiner
Macrhybopsis storeriana	**Silver chub**
Micropterus dolomieu	**Smallmouth bass**
****Micropterus punctulatus***	**Spotted bass**
****Micropterus salmoides***	**Largemouth bass**
Minytrema melanops	**Spotted sucker**
Morone chrysops	**White bass**
Moxostoma anisurum	**Silver redhorse**
Moxostoma carinatum	**River redhorse**
**Moxostoma duquesnei*	Black redhorse
Moxostoma erythrurum	**Golden redhorse**
Moxostoma macrolepidotum	**Shorthead redhorse**
****Nocomis biguttatus***	**Hornyhead chub**
****Notemigonus crysoleucas***	**Golden shiner**
Notropis atherinoides	**Emerald shiner**
Notropis boops	Bigeye shiner
Notropis buchanani	Ghost shiner
Notropis greenei	Wedgespot shiner
Notropis ludibundus	Sand shiner
**Notropis nubilus*	Ozark minnow
Notropis rubellus	Rosyface shiner
****Notropis volucellus***	**Mimic shiner**
Notropis wickliffi	Channel shiner
**Noturus exilis*	Slender madtom
**Noturus flavus*	Stonecat
Noturus nocturnus	Freckled madtom
****Percina caprodes***	**Logperch**
Percina evides	**Gilt darter**
Percina phoxocephala	**Slenderhead darter**
Phenacobius mirabilis	Suckermouth minnow
Phoxinus erythrogaster	Southern redbelly dace
Pimephales notatus	**Bluntnose minnow**
****Pimephales promelas***	**Fathead minnow**
Pomoxis annularis	**White crappie**
Pomoxis nigromaculatus	**Black crappie**
****Pylodictis olivaris***	**Flathead catfish**
Semotilus atromaculatus	**Creek chub**
Stizostedion canadense	**Sauger**
****Stizostedion vitreum***	**Walleye**