Final Report

Life History and Population Biology of the State Special Concern Ouachita Creekshell, *Villosa arkansasensis (I. Lea 1862)*

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This is the final report to the Arkansas Game and Fish Commission on the Life History and Population Biology of the state special concern Ouachita creekshell, *Villosa arkansasensis* (Lea 1862). The information within this report belongs to both the principle investigators and the Arkansas Game and Fish Commission and should not be used in publication without the consent of both parties.

The purpose of this research is to:

1. Initiate studies of the status of the Ouachita creekshell mussel (*Villosa arkansasensis*) within its known range to include its relative abundance, population demographics, and habitat use;

2. Identify the suitable host fish for the Ouachita creekshell mussel (*Villosa arkansasensis*) to include timing of reproduction and identification of host fish.

EXECUTIVE SUMMARY

Freshwater mussels (Mollusca: Unionoidea) are important components of aquatic ecosystems and are considered among the most taxonomically diverse groups in North America with 300+ recognized species. They are also among the most imperiled species in North America with approximately 72% of freshwater mussel species considered threatened, endangered, or of special concern. A number of factors threaten population size and recruitment of freshwater mussels including: 1) the construction of impoundments, 2) the introduction of exotic species, and 3) stream channelization, and 4) a number of other permanent chemical and physical habitat changes. Freshwater mussel assemblages are also affected by changes in fish community composition, which can reduce host fish contact with the glochidia, thus reducing glochidia survival.

Villosa arkansasensis, the Ouachita creekshell, is categorized as an S2 species in Arkansas meaning it is a species of special concern that is very rare, typically between 5 and 20 estimated occurrences or with many individuals in few occurrences, often susceptible to becoming extirpated. This species is endemic to the streams of the Ouachita Mountains of Arkansas and Oklahoma.

The purpose of this research is to: 1) initiate studies of the status of the Ouachita creekshell mussel (*V. arkansasensis*) within its known range; 2) conduct assessments of mussel beds and surrounding habitat quality using a systematic assessment technique for physical stream and surrounding watershed structure; and 3) compare identified fish hosts distributions to determine potential areas of mussel habitat or potential interactions between identified fish host and the target mussel species.

In general, relative numbers of *V. arkansasensis* collected by Harris and Gordon (1988) were similar to the current survey. Catch per unit effort for this survey was below 0.5 *V. arkansasensis* per hour for most stations, and below 2.5 mussels per hour for all mussel species. Species richness for this survey was slightly lower for the Ouachita River drainage compared to Harris and Gordon (1988), while similar to each other in the Saline River drainage. In 2006, C. L. Davidson and W. R. Posey II conducted new surveys in the Middle Fork Saline River and Alum Fork Saline River resulting in one site on the MFSR with one live *V. arkansasensis* individual and three sites on the AFSR yielding a total of 21 live *V. arkansasensis* individuals.

U. S. EPA RBP habitat assessment values of *V. arkansasensis* survey sites in the Saline and Ouachita Rivers ranged between suboptimal to optimal with 13 of 16 Saline River sites ranking optimal and 6 of 7 Ouachita River sites ranking optimal.

Based on mark and recapture data, late summer (July and August) and early autumn (September) appear to be the optimal time to survey *V. arkansasensis* with winter sampling being secondary. The relatively high number of marks with relatively low number of recaptures suggests that density and population sizes of *V. arkansasensis* are higher than predicted from qualitative surveys. Overall male to female ratio at Site LPS026 was 2.0 with males always outnumbering females on all sampling dates. Overall male to female ratio at Site LPS050 was 0.7, with more males being observed only for the autumn 2004 sampling date. The overall male to female ratio for Site LPS102 was 0.8 with autumn 2004 having a ratio of 1.0, but winter 2004/2005 had a female biased ratio of 0.5, and summer 2005 had a male bias of 1.5. Mark and recapture comparisons of within site pair-wise seasonal differences of mean length size frequency distributions and cumulative distribution frequencies did not show conclusive patterns,

as means were mostly similar and, except for a few cases, cumulative distribution frequencies were similar.

For the *V. arkansasensis* host suitability trials, the first round was completed in spring 2005 resulting in 19 transformations on three host species. Based on the first round of trials, the primary host appears to be the shadow bass (*Ambloplites ariommus*) with secondary hosts being the Creole darter (*Etheostoma collettei*) and green sunfish (*Lepomis cyanellus).* The second round of propagation was completed in spring 2006 and performed on individuals from both the Ouachita and Saline River drainages. This trial resulted in 33 transformations from three host species; 30 transformations on Saline River fish and three transformations on Ouachita River fish. Successful host fish species for the spring 2006 trial included the Creole darter (*E. collettei*) and greenside darter (*E. blennioides*). Thus, *V. arkansasensis* appears to use fish from two fish families: Centrarchidae and Percidae.

Laboratory determination of suitable host fish should be considered a preliminary determination of the functional host fish for mussels. Even though a fish may be determined as suitable, it does not mean that the fish and mussel interact reproductively in the stream. With this cautionary statement, determining a suitable host can still provide insight into possible host - symbiote relationships and be used as a potential management tool. Using the results of the host suitability trials, examination of distributional patterns of suitable host fish for *V. arkansasensis* reveals a common biogeographical pattern amongst the fishes. *Etheostoma collettei* is a Red River drainage endemic, while *E. blennioides* and *A. ariommus* each have disjunct populations in the Interior Highlands.

Because the status survey of this study indicated lower numbers of *V. arkansasensis* individuals and fewer locations, populations should be monitored in the future at 5 to 10 year intervals to follow trends. It is suggested, that due to the ability to detect this species being optimal in late summer or early autumn, that future status surveys or monitoring should be conducted in late summer or early autumn.

While current habitat quality was suboptimal to optimal at survey sites, the Saline and Ouachita watershed land use is changing at a rapid rate, especially in the Saline watershed which is being converted from forested to urban / suburban land use. Furthermore, forested areas in the watersheds are widely harvested. Other associated uses within the watersheds, such as water removal for irrigation and drinking water, pose a potential threat to water quantity for both freshwater mussels and their host fish. Thus, detailed studies on stream impacts from water development projects need to be conducted and best management practices implemented in order to protect the stream ecosystems.

Mark and recapture studies should be continued to estimate population sizes and follow population trends at the three sites of this study. Continued mark and recapture sampling will provide insight into the reproductive biology, age and growth, and size structure which will provide valuable information for managing this species of concern.

Finally, managing the suitable host fish of this species will aid in the management of the mussel species. While suitable host are not guaranteed to be definitive host for mussels, they represent physiologically compatible hosts and provide the mussel with the best chance of completing its life cycle. Therefore, the distribution and abundance of this Ouachita River drainage endemic mussel is suspected to have followed the biogeographical pattern of its potential host fish. The consequence of mussel distribution being tied to the distribution of host fish is that the management for the suitable host fish is paramount in order to conserve and manage the mussel of interest. The fact that the relative abundance of most suitable *V.*

arkansasensis host fish is fairly low indicates that managing the suitable host fish and by default their habitat is a priority in mussel conservation for this Ouachita Mountains Ecoregion endemic.

INTRODUCTION

Freshwater mussels (Mollusca: Unionoidea) are important components of aquatic ecosystems and are considered among the most taxonomically diverse groups in North America with 300+ recognized species (Turgeon *et al*. 1998). They are also among the most imperiled species in North America (Neves 1991) with approximately 72% of freshwater mussel species considered threatened, endangered, or of special concern (Williams *et al.* 1993).

Taxonomy

Villosa arkansasensis belongs to the Subfamily Lampsilinae along with the species of other genera such as *Cyprogenia, Ellipsaria, Epioblasma, Lampsilis,* and *Potamilus*. There is currently debate on the classification of *Villosa* species. Watters has preliminarily analyzed the "Virtual *Villosa*" using conchology and zoogeography to divide the existing genus *Villosa* into six different "genera" grouped as type *Villosa*, Genus A, Genus B, Genus C, Genus D and Genus E. *Villosa arkansasensis* is included in Genus D with *V. constricta* and *V. choctawensis.* To verify these divisions, genetic analysis is in progress (www.biosci.ohio-state.edu/~molluscs).

Life History and Ecology

Reproduction. The determination of fish hosts plays a very important role in freshwater mussel conservation, because most mussels are obligate parasites on fish and cannot complete their life cycle if the correct host is unavailable (Eckert 2003). Freshwater mussels begin their life cycles in the larval (glochidial) stage, and size and number of glochidia per brood vary among species (Bauer 1994). Bauer (1994) found no relationship between shell size of the species and size of the glochidia, but did find a positive correlation between female size and fertility, and a negative correlation between glochidia size and fertility. Larger females tend to produce more glochidia, however in females that produce larger glochidia, smaller numbers are

produced. Bauer (1994) also found relationships between glochidia morphology and size as well as fish host ecology and development. Hooked glochidia tend to be larger than unhooked, and species that rely on fish hosts utilizing running water tend to be smaller than those that utilize standing water. Additionally, glochidia size increases as host fish range increases, and larger glochidia tend to have a shorter developmental period than smaller glochidia, however the length of breeding season appeared to have no effect.

Once glochidia are produced, they are released from the female at which point they must parasitize the gill filaments and/or fins of host fish where they will be transformed into juveniles (Parmalee and Bogan 1998). This transformation occurs in two stages with first the digestion of the larval adductor mussel and then the development of the juveniles' anatomical structures (Watters, unpublished manuscript). Once transformation occurs, juvenile mussels are sloughed from the host fish and begin life independently.

The reproductive strategies of mussels vary greatly depending on the species. Until recently it was believed that mussels were broadcast spawners releasing large quantities of glochidia into the water in hopes of contact with a suitable host fish. This method however is actually very rare, and most mussels tend to be more host specific. In mussels that are host specific, two methods are used for presenting glochidia to a host fish. One method is typically found in lampsiline species and involves modifications to the mantle tissue displayed as moving lures mimicking fish or invertebrates (http://unionid.missouristate.edu) that are typically prey items for the host fish. The display of *Villosa arkansasensis* can be viewed at http://www.clt.astate.edu/achristian/Video/MOV01155.MPG. The second presentation strategy involves production of glochidia packets that mimic worms and insect larvae (Haag and Warren 2000; Haag *et al*. 1995; Watters 2006). Some species even go as far as combining these

conglutinates to form one superconglutinate tethered to the mussel by a strand of mucus and dead eggs (Watters 2006) (http://www.biosci.ohio-state.edu/~molluscs/OSUM2). In mussels that are host generalists, females often produce a mucous like web of glochidia that entangle the fish as they swim through it and the glochidia are able to encyst on a wide variety of species (Haag and Warren 1998; Strayer *et al*. 2004).

If the glochidia attach to a non-suitable host, they are sloughed before transformation occurs. This is attributed to the natural immunity of the non-host fish to the species of mussel (Fustish and Millemann 1978; Karna and Millemann 1978; Waller and Mitchell 1989). In addition to natural immunity, mussel species must also compete with the acquired immunity of some hosts. This acquired immunity may come as a result of a fish being submitted to multiple glochidia exposures of a specific mussel species (Bauer 1987; Bauer and Vogel 1987; Watters and O'Dee 1996). In many cases, however, there is a lack of suitable hosts available for encystment, which may lead to the decline of mussel populations.

There are typically two brooding chronology types in mussels: 1) bradytictic or long-term brooders over-winter glochidia in gill pouches until spring or summer; and 2) tachytictic or short-term brooders typically infest fish during the late summer to early autumn and fall off within a few weeks. However, a third strategy termed host over-winterers may exist. Bradytictic species allow glochidia the longest developmental time in the female with the least amount of time for growth as a juvenile before winter. Tachytictic species allow the shortest developmental time in the female with the most time for growth to occur on the fish host with limited dispersal (equal to that of bradytictic species) Host over-winterers allow the least amount of developmental time in the female, the most time attached to the fish host with greater potential for long range dispersal. However, it is also the most dangerous for the glochidia because there

is limited protection and a higher chance of being knocked off or of the host fish dying (Watters 2006).

Adult stage. Species survival is dependant upon recruitment of juveniles. A 15-year study of *Fusconaia ebena* in the lower Ohio River indicated the population was dominated by two recruitment classes (Payne and Miller 2000). Recruitment in all other years was minimal or non-existent. The study originated in 1983 with 71 percent of the population represented by a single size class (1981). This group of individuals was almost completely missing when the survey was repeated in 1998. The second recruitment class was reported in 1992 and comprised 85% of the population. The domination of a few recruitment classes in a population can be related to favorable conditions during the early life stages (Payne and Miller 2000).

Increased shell size results in thicker, denser shells (Kesler and Bailey 1993). Many species show sexual dimorphism where the more competitive sex is larger, typically males. In freshwater mussel species, the females typically exhibit sexual dimorphism related to reproductive life history traits (Hastie *et al*. 2000). Lampsiline female mussels (such as *Villosa arkansasensis*) usually have a truncated and expanded ventral margin of the shell, providing more room for brooding young.

Mussels have generally been considered sessile organisms, but several recent studies have reported both vertical and horizontal movement. These movements are most often related to changes in temperature and day length. The height of the shell above the substrate for *Lampsilis siliquoidea* was found to correlate with day length (Perles *et al*. 2003). *Elliptio complanata* has been observed to burrow below the substrate during winter months, returning to the surface around February (Balfour and Smock 1995), and this may be related to reproductive period (Ayers 1984, cited in Balfour and Smock 1995). *Villosa arkansasensis* has been observed

to migrate vertically, returning to the surface when the weather warms as they prepare to discharge glochidia. Studies have shown that mussels orient their siphons upstream, parallel to flow. Orientations have also been shown to vary between rivers (DiMaio and Corkum 1997).

A mussel bed is defined differently by various researchers depending on the question being addressed. Commercial quality (*i.e*. sufficient size, quantity, and quality of mussels to support commercial harvest) mussel beds in Arkansas have been defined as an area that contains $\geq$10 mussel/m² (Harris *et al*. 1993, Christian and Harris 2004) and quantitative population estimates for discrete mussel beds have been performed in most drainages within Arkansas (Rust 1993, Christian 1995, Posey 1997, Davidson 1997).

Nutrient Cycling. Freshwater bivalves play an important role in nutrient cycling and several recent studies have shown that excretory products from bivalves should provide readily useable resources for phytoplankton (James 1987, Lauritsen and Mozley 1989, Vaughn and Hakenkamp 2001). Christian *et al*. (2004) stated that dense populations of mussels may have an effect on concentrations of suspended fine particulate organic matter (FPOM) and are therefore a very important factor in aquatic food webs. Lauritsen (1986) stated that filter-feeding bivalves remove suspended particles from surrounding water and that laboratory experiments using estuarine bivalves indicate the capability of processing up to 2L per hour per individual. Due to their high abundance in freshwater systems, *Corbicula fluminea* appear to have the greatest potential for improving water quality by seston removal. Nichols and Garling (2000) suggested that mussels might use bacteria as a significant source of C and N, and this was observed in a small stream study in Ohio with two species of mussels (Christian *et al*. 2004). It is also believed that while bacteria are the primary source of C, they cannot support mussel growth alone (Strayer *et al*. 2004), but rather they may supplement other food sources, provide growth

factors, and in some small headwater streams, they may be the primary food source where phytoplankton is limited.

Measuring digestive fluid enzymes may be a useful way to determine trophic position of many organisms including mussels. Christian *et al*. (2004) found that activity rates of mussels were higher in late summer and autumn and that ^{13}C was lower in mussels than FPOM in the summer and autumn months suggesting that mussels either used a subset of FPOM, another food resource, or had low turnover rates. Although juveniles consume bacteria as one of their primary food sources, studies have failed to show that the addition of bacteria to algal diets has an added effect on growth (Gatenby *et al*. 1997).

Habitats. Morris and Corkum (1996) reported that mussel assemblages vary with habitat type, specifically grassy and forested riparian zones. Riparian buffer zones can be native vegetation or areas of planted vegetation (Dillaha *et al*. 1989) that act to limit the introduction of potentially harmful materials into the aquatic environment (Castelle *et al*. 1994). These buffer zones may be grass strips reaching into the river and creating sediment traps along the rivers edge or forested areas that shade the river from sunlight. Increased riparian zones reduce the amount of sediment clogging the stream, minimizing its affects on the mussels that inhabit the area. This is important because sedimentation can clog the mussel gills leading to death (Morris and Corkum 1996). Morris and Corkum (1996) noted an increase of *Pyganodon grandis* in areas with grassy riparian zones, probably due to the filtering capacity of the system and the high sediment and flow tolerances of the species (Clarke 1981). Morris and Corkum (1996) also reported that ammonium concentrations in the grassy rivers were 17 times higher than in forested rivers. These studies have shown that mussels inhabit a variety of habitats and that macro- and microhabitat usage varies between not only species but drainage as well.

Threats

Allan and Flecker (1993) proposed six factors of critical importance in lotic systems with habitat loss and degradation at the top followed by the spread of exotic species, overexploitation, secondary extinctions, chemical and organic pollution, and climate change. Although habitat degradation is considered the primary factor in the decline of mussel species (Williams *et al.* 1993, Neves 1993), a number of factors threaten population size and recruitment of freshwater mussels including: 1) the construction of impoundments, 2) the introduction of exotic species, and 3) channelization, and 4) a number of other permanent chemical and physical habitat changes. Freshwater mussel assemblages are also affected by changes in fish community composition, which can reduce host fish contact with the glochidia, thus reducing glochidia survival (Neves 1993).

Habitat Degradation. Williams *et al*. (1993) stated that the single most important cause of decline is the destruction of habitat, and that while habitat destruction continues, increased populations of non-native mollusks decimate the remaining native populations. Many unionaceans have limited ranges that are slowly diminishing, yet the factors that control the distribution and abundance of these animals are poorly known (Strayer and Ralley 1993). Zoogeography (van der Schalie 1945, van der Schalie and van der Schalie 1950, Strayer and Ralley 1993), gross organic pollution (Stansbery 1970, Fuller 1974, McMahon 1991, Strayer and Ralley 1993) and habitat characteristics such as stream size, gradient, current speed, and water depth (Ortmann 1919, Baker 1928, Clarke and Berg 1959, Clarke 1981a, Strayer and Ralley 1993) influence freshwater mussel distributions. Although there is a tremendous amount of supporting literature, these concepts are based largely on “informal impressions rather than on

critical measurements", and the few tests that have attempted to define unionacean habitat requirements have had mixed results (Strayer and Ralley 1993).

Impoundments. One of the most devastating impacts on mussel populations is the presence of impoundments to the river systems in which the mussels and their host fish live. Host fish are essential in the life cycle of most mussels. As a result of this requirement, mussel distribution patterns have been linked to fish migration patterns (Watters 1996). Watters (1996) stated that the distribution of some species is limited to the area of stream below the dam. Of the species studied, none were found to occur above the dam.

Vaughn and Taylor (1999) investigated the effects of impoundments on mussel assemblages and found that the assemblages were greatly impacted directly below the dam. Their study evaluated two different reaches of the Little River, OK, one directly below the Pine Creek Reservoir and the second below the confluence with Mountain Fork River. The reach below Pine Creek Reservoir had a high number of dead shells directly below the dam, but no live mussels, suggesting that population numbers were high before the dam was constructed. Mussel assemblages did not recover until 20 km downstream of the dam. At that point, only common mussel species (*Amblema plicata, Fusconaia flava, and Quadrula pustulosa)* were found, and more rare species only occurred at high species richness sites. *Arkansia wheeleri,* a federally endangered species, only occurred at the site farthest from the impoundment.

Impoundments not only affect recruitment and distribution of mussels, but result in increased sedimentation that has been shown to affect mussel feeding and lower their metabolism (Aldridge *et al*. 1987). River systems have natural high and low flow periods, however impoundments alter the natural pattern, causing extreme high and low periods at the "wrong" time. Mussels are very vulnerable to these fluctuations because they are slow moving

individuals that can't adjust quickly to changing water depths and velocities. The increased water velocity also displaces juveniles that have not yet been able to burrow into the substrate (Layzer and Madison 1995). High water velocities change sediment patterns, erode banks, and deposit newly eroded and scoured sediment on mussel beds smothering the mussels, sometimes killing entire assemblages. Sediment scouring also affects reintroduction directly below the dam (Layzer and Gordon 1993). Vaughn and Taylor (1999) found that for mussel assemblages to recover from the effects of impoundments, a considerable distance of river is necessary. Mussel populations are highly sensitive to changes in the environment, and therefore provide insight on the link between hydrology, geomorphology, and biology (Howard and Cuffey 2003). Howard and Cuffey (2003) found that some of the variability of mussel distribution patterns was related to the physical constraints of flow conditions and channel character, and that mussels occurred in areas where the chance of displacement during high flow was low. In this study, they found mussels to be primarily located in pools, completely absent in riffles, and as few as 3.5% located in runs, showing a preference to areas where the possibility of dislocation was minimal. In general, macrohabitat type appeared to be a determining factor of mussel presence, while microhabitat type did not with the exception of *Margaritifera falcata*, which showed a preference to substrate type. Howard and Cuffey's (2003) findings were consistent with those of Strayer (1999), which suggested that mussel beds typically occur in flow refuges where shear stress and the likelihood of displacement during flooding is low. While Howard and Cuffey (2003) showed some species such as *M. falcata* prefer pools, Vannote and Minshall (1982) suggest that runs are better habitats for mussels due to increased rate of seston transport and lack of seasonal scouring and sedimentation.

Fish Host Availability. Neves (1993) stated that host fish specificity may be another factor leading to the decline of mussel species. Mussels that rely on only one or two species of fish for reproductive success are more vulnerable to declines in suitable host fish populations than those mussels that are capable of using a number of fish species. The requirement for the host fish stage of the life cycle via the encounter between glochidia and host fish makes population recruitment difficult in perfect circumstances. The loss of habitat and decline in host fish species further reduces chances of successful reproduction and recruitment for freshwater mussels. The increased difficulty in polluted waters or those waters with altered temperature regimes below impoundments can lead to increased recruitment failure (Neves 1993).

Exotic Species. *Corbicula fluminea* was first introduced to the west coast of the United States in the late 1800's. It has since populated streams across the U. S. where it competes with freshwater mussels for habitat and food sources. *Corbicula fluminea* inhabits both lentic and lotic areas, but they require well-oxygenated sediment and are therefore found near the shore in lentic systems (McMahon 1983). *Corbicula fluminea* is not tolerant of extremely cold temperatures, and when temperatures fall below 2°C for long periods of time, the entire population may die, being replaced by dispersal of juveniles from upstream (Rodgers *et al.* 1979).

Chemical and Physical Habitat Changes. A combination of long-term water contamination and siltation can lead to the decline in mussel populations. Contaminations by heavy metals, chlorine, and ammonia have been shown to be related to industry and agriculture (Rand and Petrocelli 1985, Neves 1993). Farris *et al*. (1994) used *C. fluminea* and *Mudalia dilatata* to test the affects of zinc on cellulolytic activity of mollusks in both laboratory and field settings, finding a decline in cellulolytic activity for *C. fluminea* after 20 days of exposure and

for *M. dilatata* after 10 days of exposure. They also noticed metal uptake and variation in cellulolytic activity varied with season. In their experiment, snails showed the inability to depurate Zn within the first 10 days, while clams had a more delayed response occurring after Day 20, suggesting that snails were more suitable for use in short-term stress tests while clams were better indicators of long-term effects.

Siltation related to agriculture, mining, and other stream-use practices degrades water quality, clogs the gills of mussels, reduces feeding efficiency and growth, and eventually causes death (Ellis 1936, Kat 1982, Neves 1993, Bogan 1993). Siltation affects mussels in several different ways, both indirectly and directly (Brim Box and Mossa 1999). It can block interstitial spaces, preventing nutrient flow to mussels buried in the substrate (Gordon *et al*. 1992). Finer sediments may remain suspended, blocking light (Davies-Colley *et al.* 1992), preventing photosynthesis by algae, and eliminating a food source for the mussels. It can clog mussel gills preventing feeding and respiration (Ellis 1936, Kat 1982). Increased sedimentation in Chester Morse Lake and Lake Sammamish (western Washington, USA) may be related to clear-cutting and urbanization (Birch *et al*.1980). Increased sedimentation has been documented in several watersheds where agricultural practices, urbanization, and logging have increased, devastating freshwater mussels that inhabit these systems.

Predation. Predation has been found to have a negative effect on prey populations in many systems for years. Streams, however, do not necessarily hold true to this pattern. While a number of studies have shown a strong negative effect of predation on benthic prey, there are some that show little or no effect (Wooster 1994).

Currently, muskrats are believed to be the most common predator of freshwater mussels; however it is likely that other mammal species utilize mussels as a food source. Neves and

Odom (1989) found that muskrats preyed on mussels and *C. fluminea* most of the year with the highest predation rates occurring in areas with the highest mussel populations. Hanson *et al.* (1989) found that predation rates changed with season. The highest consumption rate was found to be during autumn (September through November) with an average of 350 mussels being taken, followed by an average of 120 being taken between April 21 and April 28, and the fewest amount being taken from ice-out to mid-summer at an average of 50 mussels. Along with variation in predation rates between seasons, there is also evidence supporting size and species selection of mussels by muskrats (Watters 1994). Neves and Odom (1989) compared middens in the North Fork Holston River, Virginia and found that five of eight species were found in the same abundance in the middens as they were in the bed during quadrat sampling. Muskrats seem to choose younger, smaller individuals, perhaps because older, larger individuals are too cumbersome to bring to the surface. The cost of choosing older, larger individuals may outweigh the benefit because the energy expended to open the shell and retrieve the mussel tissue may be much greater than the nutrition gained from consumption. The exact reason for these preferences has not yet been determined (Watters 1994).

Villosa arkansasensis (Ouachita creekshell)

Villosa arkansasensis (Lea 1852) is a headwater species that is known from 30+ sites in Arkansas, 29 of which occur in the Caddo, Little Missouri, Ouachita, and Saline rivers of the Ouachita Drainage system (Johnson 1980, Harris *et al*. 1997, Christian and Harris 2004). *Villosa arkansasensis* has also been reported to occur in small numbers at a few sites in the Poteau River (Harris 1994, Vaughn and Spooner 2004) and the headwaters of the Fourche La Fave River (Harris 2001) in Arkansas.

Villosa arkansasensis, like most lampsiline mussels, is a sexually dimorphic species. The male is ovate, while the female has a truncated and expanded posterior end and ventral margin. This modification provides room for the developing young (Figure 1).

Villosa arkansasensis is listed as an Arkansas state species of special concern that is endemic to the streams of the Ouachita Mountains of Arkansas and Oklahoma (Harris *et al*. 1997, Harris 1999). Little is known regarding the macro- and micro-habitat preferences of *V. arkansasensis*, and to date, no host fish species have been identified.

As of yet, little is known of the reproductive life history of *Villosa arkansasensis,* but like other *Villosa* species, it is expected to be bradytictic. Recent observations also suggest that like other *Villosa* species, *V. arkansasensis* produces a lure modified from mantle tissue. Displays that have been observed in a laboratory setting show the use of synchronous papillae movements where the foot creates a wave action of the mantle to attract potential host fish. The display is quick and is likely to be missed unless closely observed.

The objectives of this study were: 1) to initiate studies of the status of the Ouachita creekshell mussel (*Villosa arkansasensis*) within its known range to include relative abundance, population demographics, and habitat use, and 2) to identify the suitable host fish for the Ouachita creekshell mussel (*Villosa arkansasensis*) to include timing of reproduction and identification of host fish.

MATERIALS AND METHODS

Study Area

Study sites included 23 localities on the Saline and Ouachita rivers located in the Ouachita Mountains Ecoregion of western Arkansas (Figure 2; Table 1). Harris and Gordon (1988) visited all but four of these sites in the original status survey for *Lampsilis powellii*

(Arkansas fatmucket) conducted during 1987-1988. In the Saline River drainage, five sites are located on the main stem of the Saline River (SR), four on the Alum Fork Saline River (AFSR), seven on the Middle Fork Saline River (MFSR), and one on the North Fork Saline River (NFSR) in Grant and Saline counties, Arkansas. In the Ouachita River drainage, five sites are located on the main stem of the Ouachita River (OR), eight on the South Fork Ouachita River (SFOR), and one on the North Fork Ouachita River (NFOR) in Polk and Montgomery counties, Arkansas.

The SR is the longest free flowing river in the state, traversing 288 km from the confluence of the AFSR and NFSR until it flows into the OR (Figure 2). The AFSR is 71 km in length flowing from above Lake Winona to the confluence with the NFSR. The NFSR is 82 km long flowing from Hamilton to the confluence with the AFSR.

The water quality of the SR is designated as Class A, and the Arkansas Department of Environmental Quality (ADEQ) has designated it as an ecologically sensitive and extraordinary resource water body (ERW). A stream is given a Class A designation if it is determined to be suitable for drinking and all other uses (Arkansas Pollution Control and Ecology 2001). ERWs are defined as a water body in which its beneficial use is a combination of the chemical, physical and biological characteristics of a water body and its watershed which is characterized by scenic beauty, aesthetics, scientific values, broad scope recreation potential, and intangible social values.

The Ouachita River is 974 km long and flows through Arkansas and Louisiana (Figure 2 – Arkansas portion only). The upper reaches of the Ouachita River originate near Acorn, AR from which the river continues for 113 km until it reaches the backwaters of Lake Ouachita, which was impounded in 1952. Its channel is described as narrow with a series of rapids and quiet pools. The water quality of the Ouachita River is classified as primary contact recreation

(Arkansas Pollution Control and Ecology 2001) Both the Saline and Ouachita rivers possess the Outstanding Remarkable Values (ORV) of Scenery (S), Recreation (R), Geology (G), Fish (F), and Wildlife (W) (Arkansas Pollution Control and Ecology 2001)

OBJECTIVE 1

Status Survey Methods. Status surveys were conducted at 23 sites primarily where previous surveys (Harris and Gordon (1988) located populations of *Villosa arkansasensis from 2003 to 2005* (Table 1). Surveys were conducted using snorkeling techniques and hookah diving where necessary (Davidson and Clem 2002). Timed searches by one to four surveyors within the areas of suitable habitat were used to determine species distribution and abundance, and to obtain data regarding sex and age distribution of *V. arkansasensis* and other species present. Timed searches were chosen over quadrat methods because they are considered to be more effective for detecting rare species (Strayer *et al.* 1997, Metcalfe-Smith *et al.* 2000) which makes quadrat sampling ineffective. Results of timed searches are reported as number of mussels per person per hour. The amount of time spent searching a given area varied from 45 - 105 minutes and was dependent upon mussel density. This amount of time has been reported as sufficient to detect sparse populations in small streams (Strayer *et al.* 1997). All survey sites were photographed and waypoints were recorded as latitude and longitude using geographic positioning system (GPS) receivers.

Mussels located during the survey were placed in nylon mesh bags for transport to the shore. While on shore, all mussels were identified to species using Harris and Gordon (1990), Oesch (1995), and Parmalee and Bogan (1998) for reference. Listed mussel species, including *Lampsilis powellii*, were recorded as male or female (whenever possible), and measured to the nearest 0.1 mm for length (maximum anterior-to-posterior measurement), width (maximum

lateral measurement), and height (maximum dorso-ventral measurement) using dial calipers. Female *V. arkansasensis* were also checked for gravidity. All *V. arkansasensis* were assigned a unique number that was etched on the left valve using a cordless Dremel® tool. Numbers were assigned at each site beginning with 01. Mussels were then returned to their respective collection sites and properly re-positioned as they were found with respect to substrate type, depth, and direction of flow. Additionally, relic valves were noted and collected. Voucher specimens of non-federally protected species were taken from each watershed. The relative abundance of the target species *V. arkansasensis*, along with non-target mussels was determined at the 23 previously defined sites and reported as individuals per person hour per unit area. Because *V. arkansasensis* is sexually dimorphic, sex ratios of populations were able to be determined.

Status Survey Habitat Characterization and Assessment. Habitat assessment for *V. arkansasensis* was based on modifications of the EPA Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Barbour *et al.* 1999) and methods of Harris (1994). U.S. EPA RBP places 13 habitat parameters in one of four condition categories based on values assigned ranging from 20 to 0. Values of 20 – 16 are optimal, 15 – 11 are suboptimal, 10 - 6 are marginal, and 5 - 0 are poor. A total score is derived from the sum of all values given resulting in the placement into a total condition category. A site is placed in the total condition category of optimal if the sum of all values is between 200 - 152, in suboptimal for totals between 153 -101, marginal for totals between 100 - 36, and poor for totals between 35 - 0.

Characterization of physical habitat was also completed at each site using Basin Area Stream Surveys (BASS) (Clingenpeel and Cochran 1992). Flow rate, substrate type, and surrounding bank and vegetation characteristics were also measured (Harris 1994). In addition,

10 randomly selected gravelometer measurements were taken within riffles at each site along with measurements of total size and portion embedded of these randomly selected rocks.

Quarterly Mark and Recapture Sampling. Four sites (LPS027, LPS050, LPS102, and LPS105) were chosen, two in the upper Saline River drainage and two in the upper Ouachita River drainage, to conduct quarterly mark and recapture studies to follow population structure and demographics. However, the number of *V. arkansasensis* individuals at LPS105 was very low, thus non-informative for the mark and recapture sampling analysis. Sampling dates for these included sampling events included summer 2003 (03) or summer 2004 (04), autumn 2004 (04), winter 2004/2005 (04/05), and summer 2005 (05). Sampling protocol consisted of setting up a centerline transect as the center of an X, Y coordinate system for each site. Snorkeling or SCUBA was used to visually search a consistent area for each site for one person hour, starting on the downstream end of the search area and proceeding upstream. No substrate excavation was utilized during the search. Once an individual was observed, a survey flag was placed in the substrate to mark the location. The X, Y location of the individual was recorded, the sex and reproductive status (gravid, not gravid female) determined, and the length, height, and width measured. For each new capture, a unique identification code was assigned and etched on the right valve using a Dremel™ tool. The individual was then returned to its exact location.

Sex ratios were determined by dividing the number of males by the number of females, with values >1 indicating a male bias and values <1 indicating a female bias. Sex ratios were determined for each site by sampling date, and an overall site sex ratio was determined by summing the total number of males and dividing it by the total number of females for all sample dates.

Wilcoxon and Kolmogorov-Smirnov statistics were calculated for seasonal pair-wise comparisons within each site of mean length and the cumulative distribution frequency (CDF) of the lengths. The Wilcoxon test is a non-parametric means test that is appropriate for non-normally distributed data, which is typically the case for freshwater mussel size frequency data. The Kolmogorov-Smirnov test is used to compare the CDF of two data sets. Simply, it determines if the shape of the curve is similar between two datasets, independent of the total sample size for each data set.

OBJECTIVE 2

Fish Host Suitability

Gravid Mussel Collection. Gravid females were identified by carefully spreading the valves using a modified nasal speculum and inspecting the gills. A female was deemed gravid if the gill marsupia were found inflated with glochidia (Harris and Gordon 1990). Gravid female mussels were collected and transported in aerated coolers filled with river water to the U.S. Fish and Wildlife Service National Fish Hatchery (MSNFH) in Mammoth Spring, AR. Upon arrival at the hatchery, the mussels were held in an aerated, flow-through aquarium containing a 2.5 cm layer of medium sized gravel for substrate. Mussels were allowed to acclimate to the hatchery environment for one week prior to host trials. Females were held no longer than six weeks and they were returned to the sites from which they were collected.

Fish Collection. Fish were collected using seining and electro-fishing techniques following those methods described by Reynolds (1996). For electro-fishing, a Honda 350EX 3000-watt continuous peak backpack shocker pulse generator with 10-amp maximum output regulated by Coffelt Manufacturing Inc. Mark 10 cps (cycles per second) variable pulsator unit was used to stun fish for subsequent collection. A species list of potential fish hosts was

compiled using Robison and Buchanan (1988). Taxonomic keys were used to validate field identification of fish species (Robison and Buchanan 1988, Page and Burr 1991, Pflieger 1997). Fish were inspected for field encysted glochidia prior to infestation and returned to the site whenever glochidia were evident. Potential host fish that were not field encysted were transported to MSNFH in an aerated tank filled with river water at ambient conditions.

Once at the hatchery, potential host fish were held in aerated, continuous-flow 20-L aquaria for a period of one week prior to glochidia infection (Zale and Neves 1982, Rogers *et al.* 2001). During this time period, fish were fed a diet of night crawlers and fairy shrimp. All fish used for host suitability were inspected for parasites and disease before being used in trials. Upon fish mortality or trial completion, all fish were fixed in 10% formalin, preserved in 70% ethanol, and their gills were inspected for encysted glochidia.

Fish for Saline River *V. arkansasensis* host suitability trials were collected in March 2005 from the AFSR (LPS027). The 2006 host fish were collected in March 2006 from the Irons Fork Ouachita River (LPS102) for Ouachita River *V. arkansasensis* females, and darters (Percidae) only were collected from the AFSR (LPS027) for Saline River *V. arkansasensis* females.

Experimental Facility Setup. Host suitability trials took place at the MSNFH. The holding/experimental facility consisted of a number of 20-L glass aquaria and an Aquatic Habitat® system, consisting of 5.5-L and 11.4-L plastic aquaria. All tanks were set up for continuous flow through of water, and in addition, 20-L aquaria were supplied with constant aeration. The water supply was drawn directly from Mammoth Spring (water temperature range 15 - 18°C) in 2004 - 2006. Fish and mussels were exposed to ambient diel light patterns due to the presence windows at the facility.

Determination of Reproductive Timing. Female *V. arkansasensis* were visually inspected in the field on a monthly basis from August 2004 to July 2006 for gills filled with glochidia to determine the period of gravidity and glochidia release.

Glochidia Collection and Infestation Glochidia were obtained from gravid females utilizing a non-invasive technique. A sterile, 5-mL syringe with 20-gauge needle filled with synthetic water (hardness = 100 mg/L) was carefully inserted between the female's spread valves. Water was flushed gently across the gills to remove glochidia from the marsupium and then drained into a gridded glass Petri dish.

Glochidia were considered mature if valves were open and free of any embryonic case. A sub-sample of obtained glochidia was tested for viability by subjection to a concentrated sodium chloride solution A drop of concentrated sodium chloride was added to the mature glochidia, and when the glochidia were observed snapping shut, they were considered viable and suitable for testing (Coker *et al.* 1921, Zale and Neves 1982).

Glochidia were introduced to the fish following the bucket aeration technique outlined in and Hove and Neves (1994) and Hove *et al.* (2000). Fish were exposed to glochidia for a period of 20 minutes.

Upon mortality or upon completion of the trial, all fish used during the host suitability trials were preserved in a 10% formalin solution. A trial was considered completed when no sloughed glochidia or juveniles were collected for a period of four weeks and the fish showed no signs of encystment upon inspection of the gills.

***Villosa arkansasensis* Transformed Juvenile Collection.** Parasitized fish were isolated in 20-L flow-through monitoring tanks and siphoned three times weekly and checked for juvenile transformation until inspected fish were no longer parasitized. Siphonate from the

larger tanks were collected and filtered through a 300 µm Nitex® mesh to remove larger sized organic fractions followed by 105 µm Nitex® mesh to retain juveniles for inspection, and the smaller tanks were filtered through the 105 µm Nitex® only. The contents of the siphon were inspected using a dissecting microscope fitted with a polarizing lens for efficient identification of transformed juveniles. Juvenile mussels were placed in a holding tank until the host trial was completed at which point they were transported back to site where the females were collected and released.

Suitable Host Fish Distributions and Relative Abundance

Based on the results of the fish host suitability trials, the host fish distributions and relative abundance in the Ouachita and Saline rivers were compared. Comparisons were based on data reported in Robison and Buchanan (1988) and collected by Arkansas Department of Environmental Quality (available on their web site at http://www.adeq.state.ar.us/water/data_fish/fish.asp) utilizing a variety of sampling methods such as seines, electro-fishing, gill nets, and combinations of these methods. The distribution and relative distribution of the fish was compared to determine the potential impact fish distributions and abundance have on mussel distributions due to their life history requirements.

RESULTS

OBJECTIVE 1

Villosa arkansasensis Status Survey

Total *Villosa arkansasensis* collected in the current survey was significantly lower (n = 70) than reported by Harris and Gordon (1988) (n=98) (Wilcoxon Signed Rank Test, W=0.892569; p=0.0424). This study had 13 sites with fewer individuals, three sites with more

individuals, and one site with the same number of *V. arkansasensis* individuals as Harris and Gordon (1988) (Figure 3).

Catch per unit effort for this survey was <0.5 *V. arkansasensis* per hour for most stations, and <2.5 mussels per hour for all mussel species (Figure 4). Species richness for this survey was slightly lower for the OR compared to Harris and Gordon (1988), while similar to each other in the SR (Figure 5).

Size frequency data of OR and SR *V. arkansasensis* indicates medium to large individual size ranges, with no individuals <30 mm observed (Figure 6). Comparison of *V. arkansasensis* mean lengths indicated the SR population (x = 56.6 mm) was significantly larger than the OR population (x = 39.8 mm) (Wilcoxon Rank Sums Test, DF = 71,31; p=0.0084). Furthermore, there was a significant difference in the CDF between the two rivers (Kolmogorov-Smirnov test, D=0.2930; p =0.039). The estimated mean SR CDF was 42.1 mm (±1.36 95% CI) while the OR CDF was 38.0 mm (±3.47 95% CI). Both the Wilcoxon and Kolmogorov-Smirnov tests indicated that the size structure between the two rivers is different, with the SR consisting of larger individuals.

In 2006, C. L. Davidson and W. R. Posey II conducted new surveys in the MFSR and AFSR. Their survey resulted in one site on the MFSR with one live *V. arkansasensis* individual and three sites on the AFSR yielding a total of 21 live *V. arkansasensis* individuals (Table 2).

***Villosa arkansasensis* Habitat Characterization and Assessment**

Based on USEPA RBP habitat assessment, habitat quality in the SR and OR ranges from suboptimal to optimal (Table 3). Three of 16 SR sites were ranked suboptimal and one of seven OR sites were ranked suboptimal. Metrics that consistently influenced low scores include

embeddedness, velocity / depth regime, and sediment deposition (Table 4). Low bank stability, low bank vegetation, and narrow riparian width values also influenced two sites (Table 4).

In terms of habitat selection, SR *V. arkansasensis* individuals were found in glides, runs, and pools in equal frequency (Figure 7). Meanwhile, OR individuals were found equally in glides and runs. Overall, analysis suggests that *V. arkansasensis* prefer glides and runs over pools and riffles.

***Villosa arkansasensis* Mark and Recapture Sites**

LPS026. During the Autumn 04 sampling, nine individuals were collected, while three individuals were collected in Summer 05. Based on this observation, autumn was the optimal time to collect individuals at this site (Figure 8). Of the 12 individuals marked at this site, none were recaptured, representing a 0.0% recapture rate. Overall male to female ratio at this site was 2.0 with males outnumbering females on all sampling dates (Table 5).

Individuals captured in Summer 05 were somewhat larger than those found in Autumn 04 with mean lengths of 39.4 (±1.8 SE) and 35.4 (±1.0 SE) mm, respectively. However, there was no significant difference in means (Wilcoxon S=29, Z=1.66702, p=0.0955). No Kolmogorov-Smirnov test was conducted for CDF because sample sizes were below 10 individuals, but the curves did show a slight shift to a larger size for Summer 05 (Figure 8).

LPS050. A total of 37, 7, 14, and 51 individuals were collected during summer 03, autumn 04, winter 04/05, and summer 05, respectively. Summer appears to be the optimal time to collect individuals at this site (Figure 9). Of the 74 individuals marked at this site, only one recapture was observed representing a 1.3% recapture rate. Individual #2 was marked in autumn 04 and recaptured in summer 05. Overall male to female ratio at this site was 0.7 with more

males observed only for the autumn 04 sampling date (Table 5). Winter 04/05 was highly skewed towards females, while summer 05 had only slightly more females.

Mean length was similar for the summer 03 and autumn 04 sampling dates (Wilcoxon S=161.5, Z=0.1123, Z=0.9106), with means of 43.6 mm (±1.0 SE) and 43.9 mm (±1.7 SE), respectively. No CDF analysis was calculated due to sample size <10 for the autumn 04 sampling date.

Mean length was slightly, but not statistically significantly, larger for the summer 03 versus winter 04/05 sampling dates (Wilcoxon S=161.5, Z=0.1123, Z=0.9106), with means of 43.6 mm (±1.0 SE) and 40.4 mm (±0.8 SE), respectively. The Kolmogorov-Smirnov test comparing CDF of the two sampling dates was significantly different (D=0.4073, p=0.049), with Summer 03 having a larger CDF curve than winter 04/05.

Mean length for Summer 03 was similar to summer 05, with means of 43.6 mm (±1.0 SE) and 43.7 mm (±0.7 SE), respectively (Wilcoxon S=163.1, Z=-.12681, p=0.8991). The Kolmogorov-Smirnov test comparing CDF of the two sampling dates was not significantly different (D=0.1553, p=0.642), indicating similar CDF.

Mean length was significantly larger for autumn 04 compared to winter 04/05 with means of 43.9 mm (±1.7 SE) and 40.4 mm (±0.8 SE), respectively (Wilcoxon S=100, Z=1.67970, p= 0.0930). No CDF analysis was calculated due to samples size <10 for autumn 04.

Mean length was similar for autumn 04 and summer 05 (Wilcoxon S=203.5, Z=-0.05968, Z=0.9524) with means of 43.9 mm (±1.7 SE) and 43.7 mm (±0.7 SE), respectively. No CDF analysis was calculated due to samples size <10 for autumn 04.

Mean length was significantly larger for summer 05 than winter 04/05 with means of 43.7 mm (±0.6 SE) and 40.4 mm (±0.8 SE), respectively (Wilcoxon S=299.5, Z=-2.58524, p=0.0097).

LPS102. A total of 8, 4, 13, and 12 individuals were collected at LPS102 on sampling dates summer 04, autumn 04, winter 04/05, and summer 05, respectively (Figure 10). Similar numbers of individuals were found at all times except for autumn 04. Of the 28 individuals marked at this site, only one recapture was observed representing a 3.5% recapture rate. Individual #23 was marked in winter 04/05 and recaptured in summer 05. The overall male to female ratio for this site was 0.8 with autumn 04 having a ratio of 1.0 (Table 5). Winter 04/05 had a female biased ratio of 0.5, while summer 05 had a male bias of 1.5.

Mean lengths were similar for summer 04 and autumn 04, with means of 39.0 mm (±2.4 SE) and 40.0 mm (±2.1 SE), respectively (Wilcoxon S=23, Z=-0.42780), p=0.6689). No CDF analysis was conducted because sample sizes were <10 individuals for each of the dates.

Mean length for summer 04 was slightly larger, but not statistically significant, versus winter 04/05 with means of 39.0 mm (±2.4 SE) and 36.0 mm (±1.5 SE), respectively (Wilcoxon S=104, Z=1.12252, p=0.2616). No CDF analysis was conducted because sample size was <10 individuals for summer 04.

Mean length for summer 04 was slightly larger, but not statistically significant, when compared to summer 05 with means of 39.0 mm (±2.4 SE) and 36.8 mm (±1.2 SE), respectively (Wilcoxon S=100.5, Z=1.23582, p=0.2165). No CDF analysis was conducted because sample size was <10 individuals for Summer 04.

Mean length was slightly larger, but not statistically significant, for autumn 04 versus winter 04/05 with means of 39.0 mm (±2.4 SE) and 36.1 mm (±1.5 SE), respectively (Wilcoxon

S=44, Z=0.84973, Z=0.3955). No CDF analysis was conducted due to samples size <10 for autumn 04.

Mean length was slightly larger, but not statistically significant, for autumn 04 versus summer 05,with means of 39.0 mm (±2.4 SE) and 36.8 mm (±1.2 SE), respectively (Wilcoxon S=41, Z=0.78882, Z=0.4302). No CDF analysis was performed due to samples size being <10 for autumn 04.

Mean length was similar for the winter 04/05 and summer 05 sampling dates, with means of 36.1 mm (±1.5 SE) and 36.8 mm (±1.2 SE), respectively (Wilcoxon S=162, Z=0.29916, p=0.7648). There was no difference between the CDF of winter 04/05 and summer 05 (Kolmogorov-Smirnov D=0.3615, p=0.366).

OBJECTIVE 2

***Villosa arkansasensis* Reproduction and Host Suitability**

Gravid *V. arkansasensis* females were observed from October 2004 thru July 2005. August 2005 was the first month gravid females were not present. After two years of monitoring reproductive stages, it seems certain that *V. arkansasensis* is a long-term brooder, holding glochidia in the fall and over winter until release in the spring.

The first round of host suitability trials was completed spring 2005 resulting in 19 transformations on three host species (Tables 6 and 7). For the first round of propagation, fecundity was estimated at ~15,000 glochidia per female. Glochidia from the first trial were measured and larvae were determined to be approximately 250 μm long x 200 μm wide. The primary host appeared to be the shadow bass (*Ambloplites ariommus*) with the Creole darter (*Etheostoma collettei*) and green sunfish (*Lepomis cyanellus)* as secondary hosts. Fish used for propagation of LPS050 mussels included: *A. ariommus, Campostoma anomalum,*

Chaenobryttus gulosus, E. blennioides, E. collettei, E. nigrum, E. whipplei, E. zonale, Fundulus olivaceus, Hypentelium nigricans, Ichthyomyzon gagei, Labidesthes sicculus, L. cyanellus, L. megalotis, Micropterus dolomieu, Notropis atherinoides, N. boops, Noturus nocturnus, Percina caprodes, and Pimephales notatus.

The second round of propagation was completed spring 2006 with a slightly later start date allowing glochidia more time to mature in hopes of having better transformation success. This round of trials was performed on individuals from both the Ouachita and Saline River drainages and resulted in 33 transformations from three host species (Tables 6 and 7), 30 transformations from the SR and three transformations from the OR. Fecundity for the second round of propagation was estimated at ~35-47,000 glochidia per female with transformation success rates of 0.002% and 0.066% for the Ouachita and Saline River drainages, respectively. Successful host fish species for the spring 2006 trial included: Creole darter (*E. collettei*) and greenside darter (*E. blennioides*). Fish used for propagation from the AFSR included: *E. blennioides, E. collettei, E. zonale, E. whipplei, Noturus lachneri, and N. nocturnus.* For the Ouachita River drainage, potential fish host included: *Ambloplites ariommus, Campostoma anomalum, Chaenobryttus gulosus, E. blennioides, E. radiosum, E. whipplei, Lepomis cyanellus, L. megalotis, Micropterus salmoides, Notropis boops, and Pimephales notatus.*

***Villosa arkansasensis* Suitable Host Fish Distribution**

Ambloplites ariommus Viosca populations in Arkansas are disjunct from the larger core distribution of populations in the Gulf Coastal Plain of Louisiana, Mississippi, Alabama and Georgia (Robison and Buchanan 1988). Distribution in Arkansas includes the Red, Ouachita, Arkansas, Illinois, Little Red, Strawberry, Spring, Black and St. Francis river drainages. *Ambloplites ariommus* typically inhabits clear mountain streams of moderate to high gradient

above the Fall Line. The disjunct Arkansas distribution for *A. ariommus* overlaps with the distribution of *Villosa arkansasensis.*

Lepomis cyanellus Rafinesque distribution in Arkansas is statewide and contiguous with its North American distribution throughout the Interior Basin (Robison and Buchanan 1988). The green sunfish is a habitat generalist that can be found in almost all types of aquatic habitat in Arkansas.

Etheostoma blennioides Rafinesque distribution in Arkansas is disjunct from other populations in the upper Mississippi and Ohio River drainages (Robison and Buchanan 1988). In Arkansas, the greenside darter is abundant in the Ozark Uplands, and common in the uplands of the Ouachita River drainage. The greenside darter is found in riffles over gravel and rubble bottoms in small to medium size streams with moderate to swift current and low turbidity. There are four subspecies of the greenside darter but only one, *E. b. newmanii*, occurs in Arkansas (Robison and Buchanan 1988). This subspecies is believed to have two races, one that is found in the Little Red, Arkansas, and Ouachita drainages, and the other that is distributed on the St. Francis, Current, Black, and upper White river drainages (Robison and Buchanan 1988).

Etheostoma collettei Birdsong and Knapp occurs in Arkansas, Oklahoma, and Louisiana. It is most abundant in southern Arkansas in the eastern Saline, Ouachita, Caddo, Little Missouri, Cossatot and Rolling Fork rivers. The Creole darter can be found in headwaters to small streams in swift current over a gravel bottom or in rocky chutes with heavy submergent vegetation. Taxonomically or morphologically, Saline and Ouachita River populations differ in cheek scalation: Saline River populations have naked cheeks, while Ouachita River populations have scaled cheeks.

Villosa arkansasensis Suitable Host Fish Relative Abundance

Ambloplites ariommus relative abundance in the Ouachita River drainage ranged from 0 to 1.4 % of all fish collected at nine sites (Table 6). In the Saline River drainage, relative abundance of the shadow bass ranged from 0.1 to 0.4% of all fish collected at nine sites (Table 7).

Lepomis cyanellus relative abundance in the Ouachita River drainage ranged from 0.8 to 15.9 % of all fish collected at nine sites (Table 6). In the Saline River drainage, relative abundance of the green sunfish ranged from 0.0 to 3.8% of all fish collected at nine sites (Table 7).

Etheostoma blennioides relative abundance in the Ouachita River drainage ranged from 0 to 5.3 % of all fish collected at nine sites (Table 6). In the Saline River drainage, relative abundance of the shadow bass ranged from1.2 to 10.6% of all fish collected at nine sites (Table 7).

Etheostoma collettei relative abundance in the Ouachita River drainage ranged from 0 to 0.6 % of all fish collected at nine sites (Table 6). In the Saline River drainage, relative abundance of the shadow bass ranged from 0.3 to 4.1% of all fish collected at nine sites (Table 7).

DISCUSSION

OBJECTIVE 1

Villosa arkansasensis Status Survey

Villosa arkansasensis (Lea 1852) is a headwater species that is known from 30+ sites in Arkansas, 29 of which occur in the Caddo, Little Missouri, Ouachita, and Saline rivers of the Ouachita Drainage system (Johnson 1980, Harris *et al*. 1997, Christian and Harris 2004). *Villosa arkansasensis* has also been reported to occur in small numbers at a few sites in the

Poteau River (Harris 1994, Vaughn and Spooner 2004) and the headwaters of the Fourche La Fave River (Harris 2001) in Arkansas.

A number of the sites have seen a decrease in population size since surveys in 1987-1988 by Harris and Gordon (1988). Harris and Gordon (1988) listed 18 sites where live *V. arkansasensis* were present with a total of 98 individuals observed. The 2003-2004 study (Farris et al. 2005) found eight sites with a total of 70 living *V. arkansasensis*, and numbers are based on a one time search. During this survey, only three sites (LPS050, LPS027 and LPS102) had an increase in specimens observed in comparison with Harris and Gordon (1988). Site LPS050 had the largest number of *V. arkansasensis* for both surveys. Harris and Gordon (1988) reported seven sites with single observations of *V. arkansasensis* and two sites with only two observations, indicating that there were already low numbers of individuals at these sites, and the probability of detecting *V. arkansasensis* was going to be low. On the other hand, quarterly sampling at four of the original 18 sites resulted in >150 individuals, with maximum observations occurring in late August through early October. Because most sampling for this survey was conducted in June and July, and Harris and Gordon (1988) conducted a majority of sampling in September and October, it is possible that the present survey missed maximum detection probability dates whereas Harris and Gordon (1988) surveyed during the best time of detection. In fact, during periods of reproduction, females were observed to burrow into the substrate and are more difficult to locate. Conducting the quarterly sampling allowed for a more intensive study covering all seasons.

Species richness on the Saline River increased at only 50% of the sites from 1988 to 2003-04, while almost all of the Ouachita River sites showed an increase. These findings could be attributed to changes in habitat as well as time of year in which the survey was completed.

The Davidson and Posey 2006 survey of the Middle and Alum Forks of the Saline River showed a relatively low species richness with only three sites having diversity >10 species. Among the 24 sites surveyed, they found 521 individuals, 22 of which were *V. arkansasensis*. The difference in site identifications makes it difficult to determine if these are new *V. arkansasensis* sites or if they are the same sites that were surveyed during 2003-04.

***Villosa arkansasensis* Habitat Characterization and Assessment**

Villosa arkansasensis was found in a variety of habitats between drainages, but the common characteristic is the use of areas consisting primarily of small cobble, gravel and sand. In the Saline River, *V. arkansasensis* was found evenly distributed between glides, runs and pools and substrate consisting of large cobble, boulders and bedrock as well as the smaller substrates previously mentioned, but the majority of the mussels were located in the interstitial spaces between large rocks. *Villosa arkansasensis* at OR sites were located primarily in glides and runs and were absent in pools. These sites have a much lower density of large cobble and boulders and almost no bedrock, and in these areas, the streams were dominated by much finer substrate including large quantities of fine particulate organic matter (FPOM).

As for the habitat assessment, none of the sites scores indicated drastic impairment in terms of general habitat quality as all scores were suboptimal to optimal, with no marginal or poor rankings. However, historical measurements of habitat quality are not available to gauge if habitat scores have changed over time. Furthermore, US EPA Rapid Bioassessment Protocols were designed to measure fish and general aquatic macroinvertebrate habitat and not specifically designed for mussels in general or the potential specific habitat requirements of the target mussel species. Further habitat monitoring, be it assessment or characterization through such protocols such as BASS, is suggested.

Villosa arkansasensis **Mark and Recapture Study**

Based on mark and recapture data, late summer (July and August) and early autumn (September) appear to be the optimal time to survey *V. arkansasensis*, with winter sampling secondary. Two factors that may explain this observation include water levels and clarity and reproductive timing. In late spring and early summer, water levels are usually higher, but receding from rain events, and water clarity is reduced due to run off. By mid to late summer, water levels typically have dropped to base flows and both water levels and clarity are influenced by episodic rain events. Late summer to early autumn water levels are typically at annual low base flows and rain events are typically not as frequent, thus providing for low, clear water and the resultant improved visibility. Observation of reproductive events for *V. arkansasensis* suggests that females release their glochidia some time in late winter to early spring (March to April). Based on observations of gravid females, fertilization must occur some time in late summer to early autumn, at which time both males and females should migrate to the surface to release and capture sperm, respectively. Winter sampling may also yield more observations; however, there seems to be a sexual bias for more females being observed than males at this time. Water temperatures are cool, and depth and clarity may be unpredictable due to more frequent rain events. The observation of a female bias in the winter may be the result of females preparing to release their glochidia on host fish during late winter or early spring.

The low number of recaptures suggests that density and population sizes of *V. arkansasensis* are higher than would be predicted from qualitative surveys. Rogers *et al*. (2001) reported a 3.9% recapture rate for 312 marked tan riffle shell individuals over a 3-year period, which is similar to recapture rates in this study. In the near future, population estimates will be

determined using methods referenced in Rogers *et al*. (2001) which were used by Schumacher and Eschmeyer (1943) on several fish species in Tennessee lakes and ponds.

Like the size frequency distribution illustrated for the status survey, individuals <30 mm length were not observed at the mark and recapture study sites. This may be a concern, as the size frequency distribution is unimodal, suggesting a lack of recruitment into the population. However, unimodal size frequency distributions are typical of smaller species, while multimodal size frequency distributions are more typical of large species (Christian *et al*. 2005). It is possible that because of *V. arkansasensis* small size, individuals grow quickly to the 30 mm size class with slow growth thereafter due to becoming sexually mature and investing energy into reproduction rather than growth. Additionally, *V. arkansasensis* may be dependent on specific environmental conditions that occur infrequently in order to have successful reproductive events. It has been shown in Ohio River *Fusconaia ebena* that successful cohorts recruit every five to seven years (Payne and Miller 2000).

Comparisons of within site, pair-wise seasonal differences of mean length frequency distributions and CDF did not show conclusive patterns, as means were mostly similar and except for a few cases, CDF were also similar. This suggests that growth rate or change in population demographics is low and probably influenced by the differences in number of individuals captured and sex bias observed.

OBJECTIVE 2

Villosa arkansasensis Reproductive Timing and Host Fish Suitability

Because gravid *V. arkansasensis* females were observed from October thru July over two years of monitoring reproductive stages, it seems certain that *V. arkansasensis* is a long-term brooder, holding glochidia in the fall and over winter until release in the spring. Based on two

rounds of trials, suitable host fish for *V. arkansasensis* are the shadow bass (*Ambloplites ariommus*), the green sunfish (*Lepomis cyanellus),* the Creole darter (*Etheostoma collettei*), and the greenside darter (*E. blennioides*). Thus, *V. arkansasensis* appears to use fish from two fish families: Centrarchidae and Percidae.

Suitable Host Fish Distributions and Relative Abundance

Laboratory determination of suitable host fish should be considered a preliminary determination of the functional host fish for mussels. Even though a fish may be determined as suitable, it does not mean that the fish and mussel interact reproductively in the stream. With this cautionary statement, determining a suitable host can still provide insight into possible host - symbiote relationships and be used as a potential management tool.

Using the results of the host suitability trials, examination of distributional patterns of suitable host fish for *V. arkansasensis* leads to the potential of a common biogeographical pattern amongst the fishes. *Etheostoma collettei* is a Red River drainage endemic, while *E. blennioides* and *Ambloplites ariommus* each have disjunct populations in the Interior Highlands (Robison and Buchanan 1988).

Mayden (1985) suggested that Ouachita Mountain region endemic fish, crayfish, and salamanders, including *Etheostoma*, occurring in the Kiamichi, Little, and Ouachita rivers once shared a common drainage basin not shared with the Red River or any other present day drainage. He further states that the continuous distributions of these taxa were later dissected by drainage alterations which resulted in evolution of species with more restricted highland ranges. In the case of the *Ambloplites* distributions, main channel dispersal of fishes in this region may have occurred, followed by large scale extinction in intervening areas.

Therefore, the distribution and abundance of this endemic mussel is suspected to have followed the biogeographical pattern of its potential host fish. The consequence of mussel distribution being tied to the distribution of host fish is that the management for the suitable host fish is paramount in order to conserve and manage the mussel of interest. The fact that relative abundance of most suitable host fish for *Villosa arkansasensis* is fairly low indicates that managing the suitable host fish and by default its habitat is a priority in mussel conservation for Ouachita River drainage endemics.

MANAGEMENT AND RESEARCH RECOMMENDATIONS

Because the status survey portion of this study indicated lower numbers of individuals and fewer locations, populations should be monitored in the future at 5 to 10 year intervals to follow trends. Future status surveys or monitoring should be conducted during the period of maximum detestability for *Villosa arkansasensis*, late summer or early autumn.

While current habitat quality was suboptimal to optimal at survey sites, the Saline and Ouachita watershed land uses is changing at a rapid rate, especially the Saline watershed which is being converted from forested to urban / suburban land use. Furthermore, forested areas in the watersheds are widely harvested. Other associated uses for the watersheds such as water removal for irrigation and drinking water, pose a potential threat to water quantity for both freshwater mussels and their host fish. Thus, detailed studies on stream impacts from water development projects need to be conducted and best management practices implemented in order to protect the stream ecosystems.

Mark and recapture studies should be continued to follow population trends at the three sites of this study and can be used to estimate population sizes. Continued mark and recapture

sampling will provide insight into the reproductive biology, age and growth, and size structure which will provide valuable information for managing this species of concern.

Finally, managing the suitable host fish will aid in the management *Villosa arkansasensis*. While suitable host are not guaranteed to be definitive host for mussels, they represent physiologically compatible hosts and provide the mussel with the best chance of completing its life cycle.

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Figure 1. Female (top) and male (bottom) *Villosa arkansasensis*.

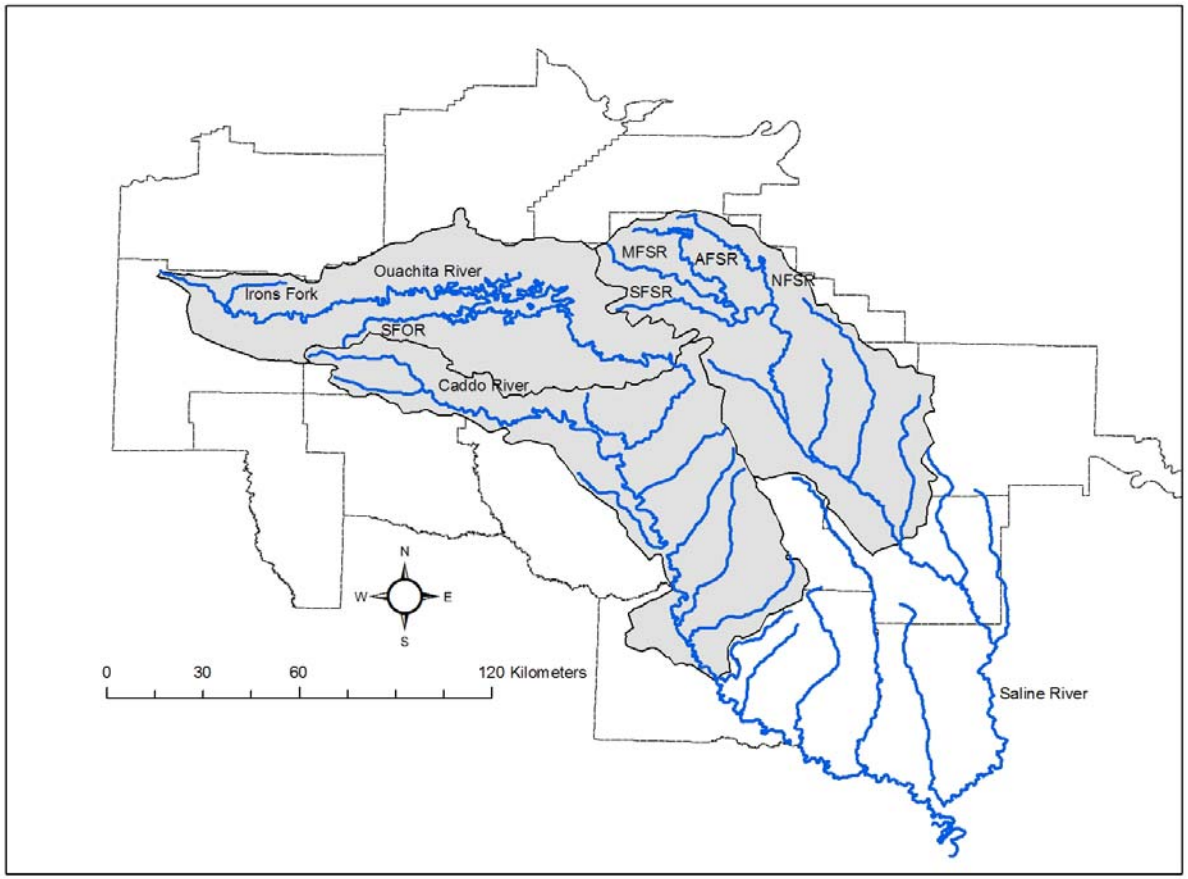

Figure 2. The Upper Saline and Ouachita drainage in which the 2002 to 2005 *Villosa arkansasensis* status survey was conducted. Tributaries sampled included: Saline River [North Fork Saline (NFSR), Alum Fork Saline (AFSR), Middle Fork Saline (MFSR), South Fork Saline (SFSR)]; Ouachita River [Irons Fork, South Fork (SROR), and Caddo].

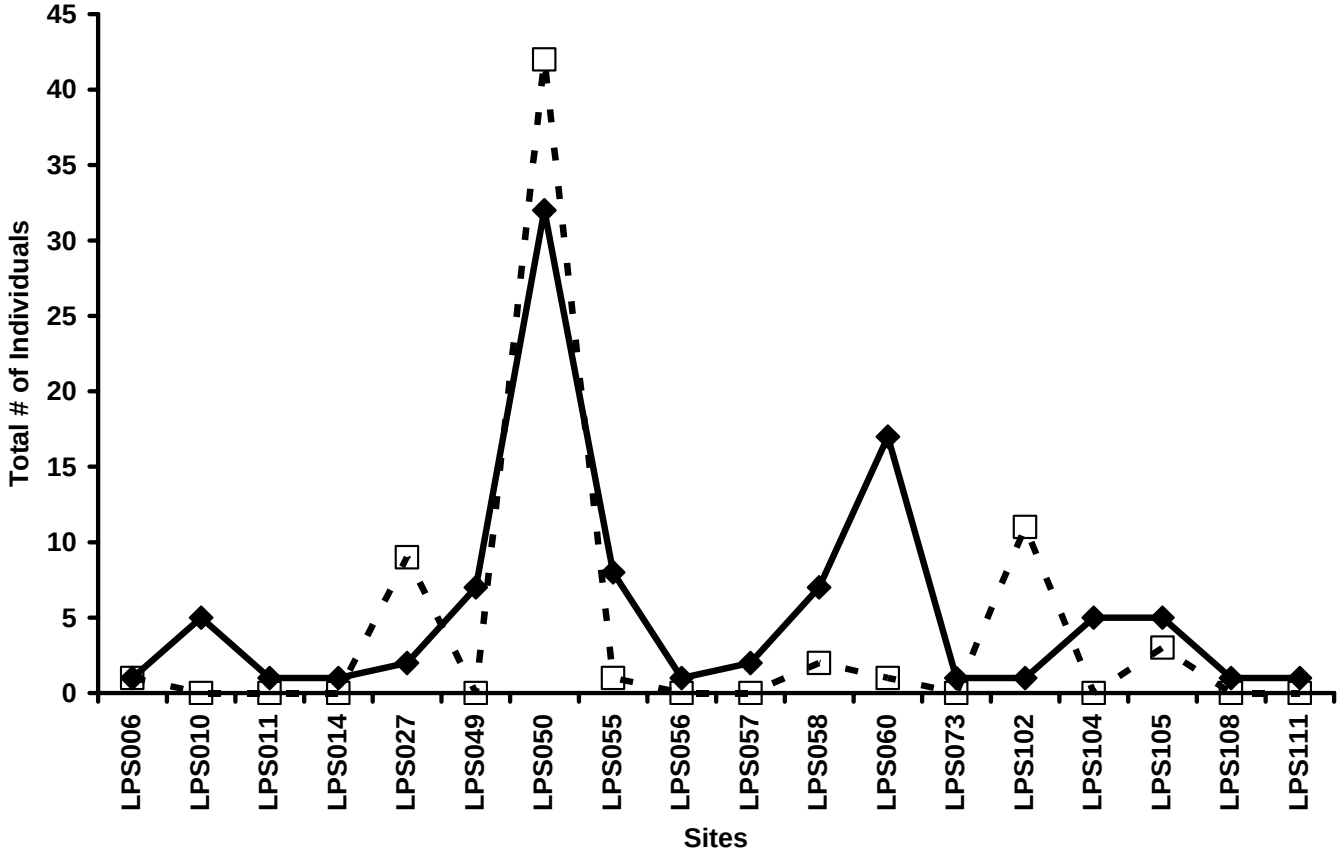

Figure 3. Comparison of *Villosa arkansasensis* abundance between Harris and Gordon (1988) (solid line, diamond) and 2003-04 status survey data (dashed line, square).

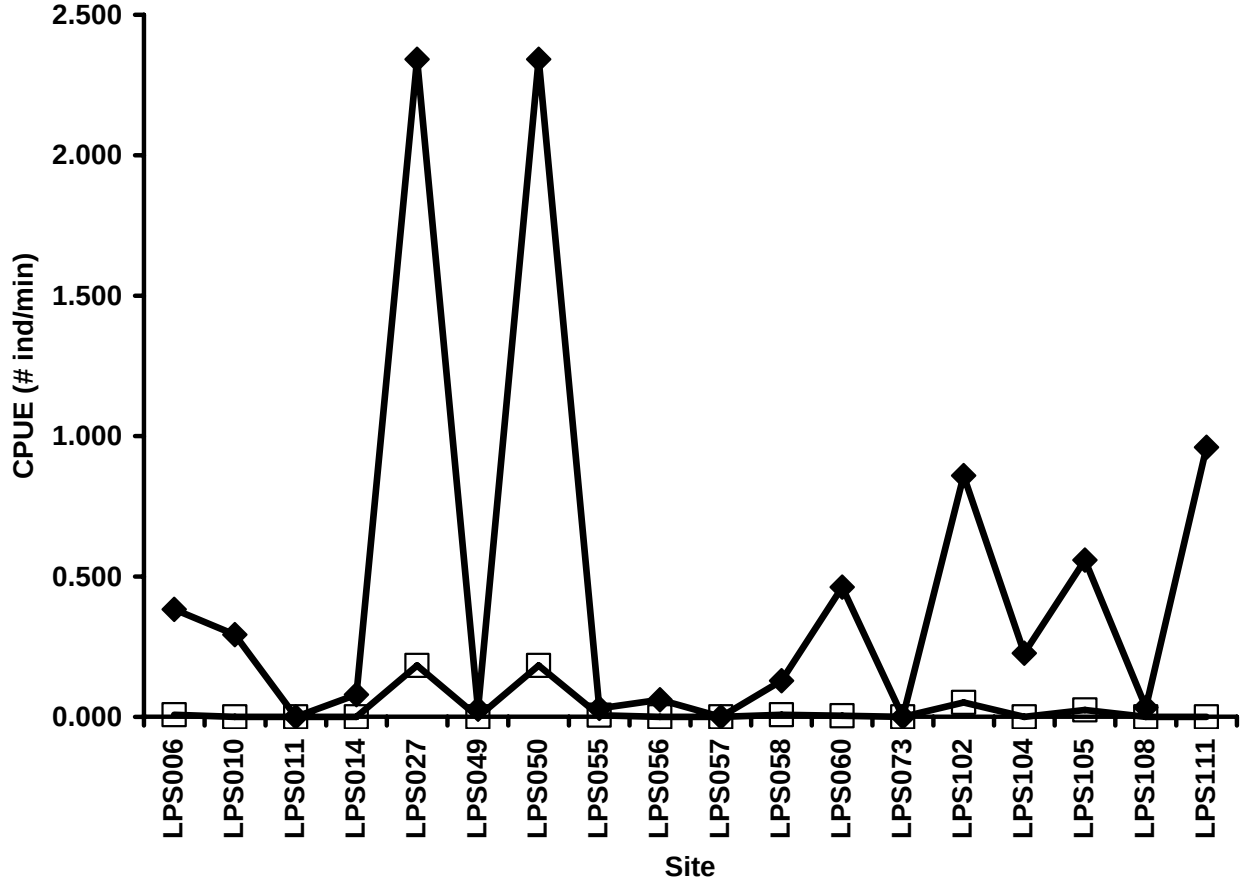

Figure 4. Catch per unit effort (CPUE) for 2003-04 status survey for *Villosa arkansasensis* (clear square) vs. all species present (black diamond).

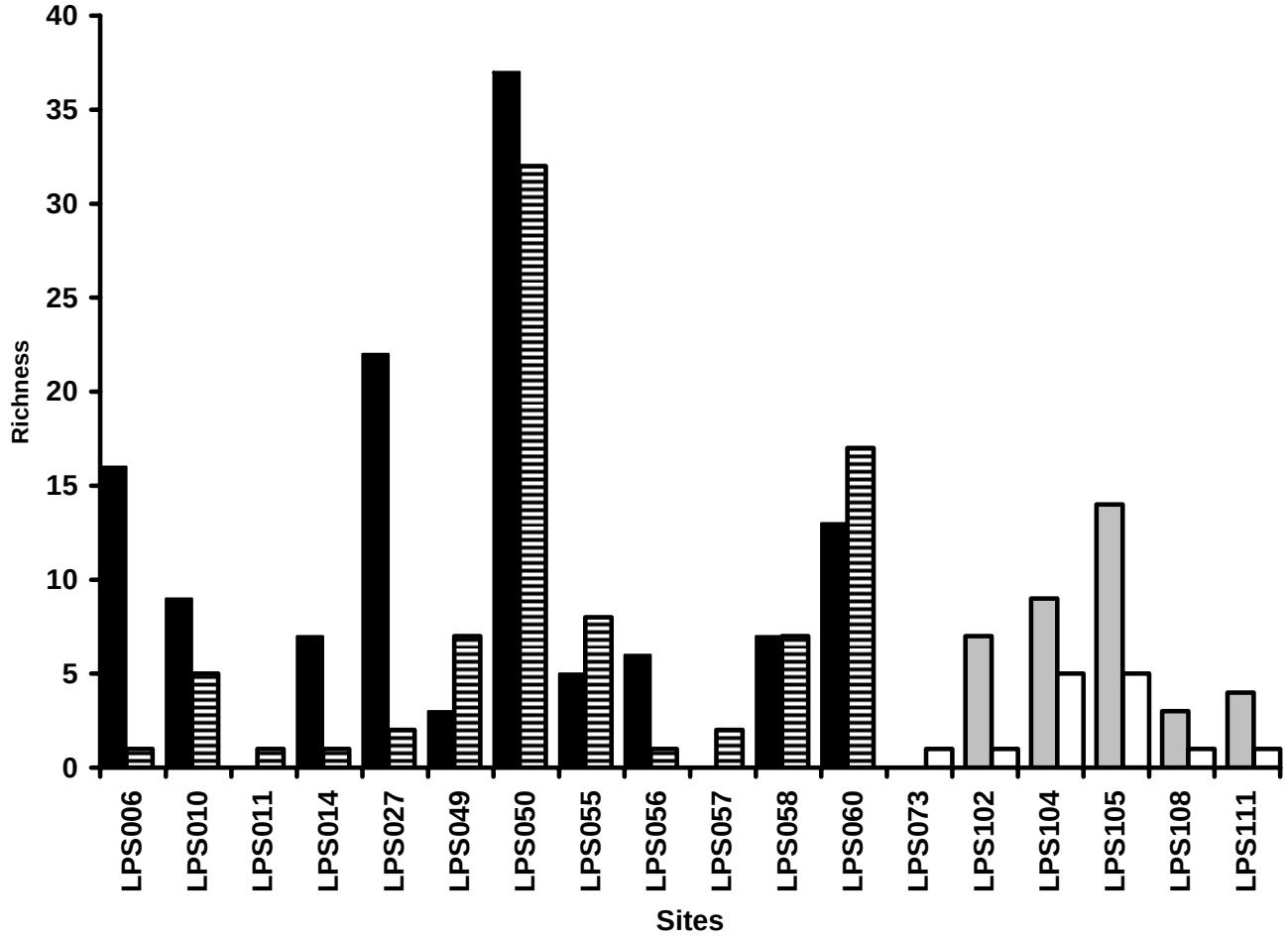

Figure 5. Mussel species richness of Harris and Gordon (1988) vs. 2003-04 status survey data for Saline River 2003-04 (black), Saline River 1988 (black stripe), Ouachita River 2003-04 (gray), and Ouachita River 1988 (white).

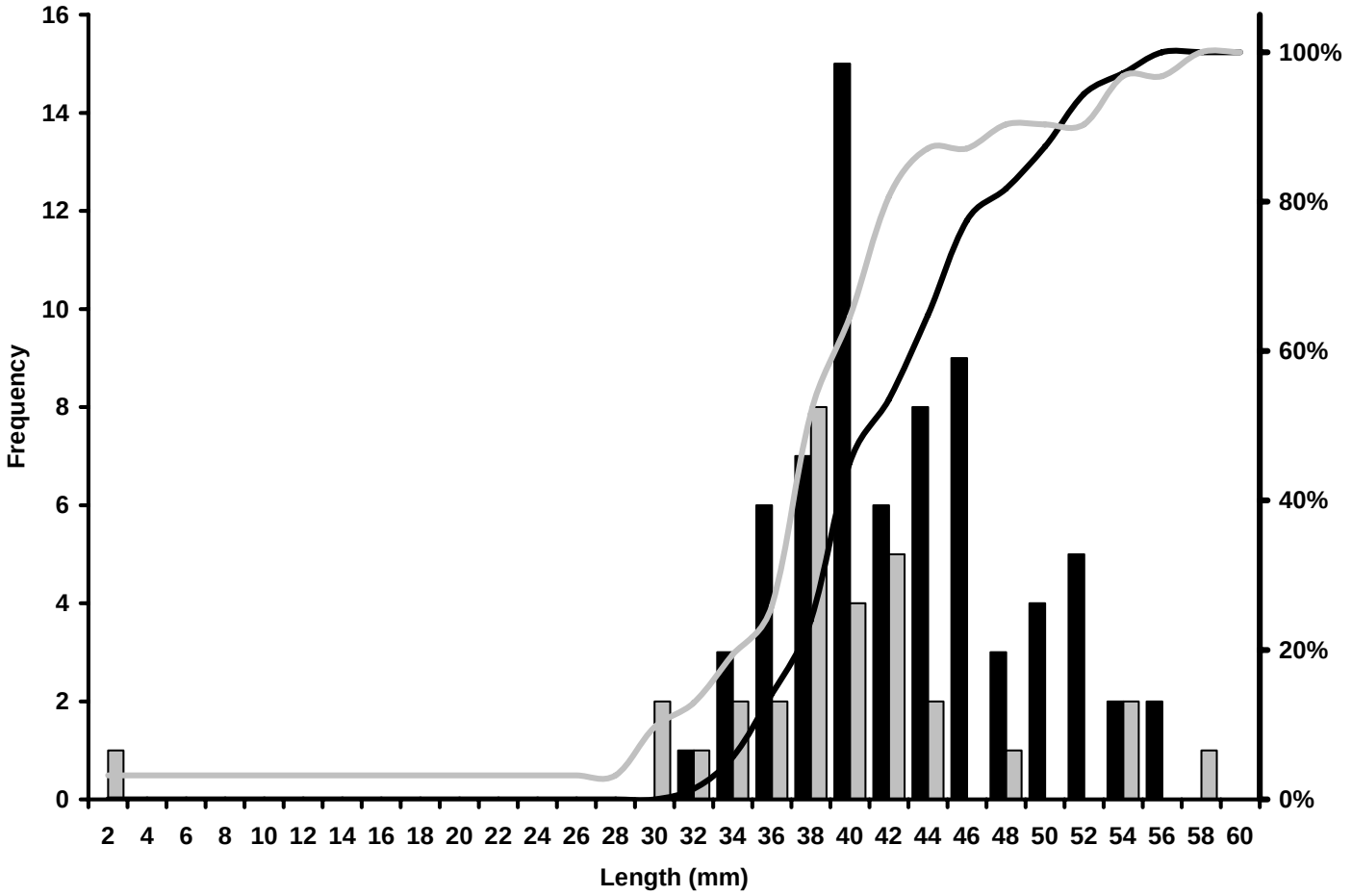

Figure 6. Size frequency distributions (bars) and cumulative distribution frequency (lines) for *Villosa arkansasensis* from 13 sites of the 2003-05 status survey in the Saline River (black bars and line) and Ouachita River (gray bars and line).

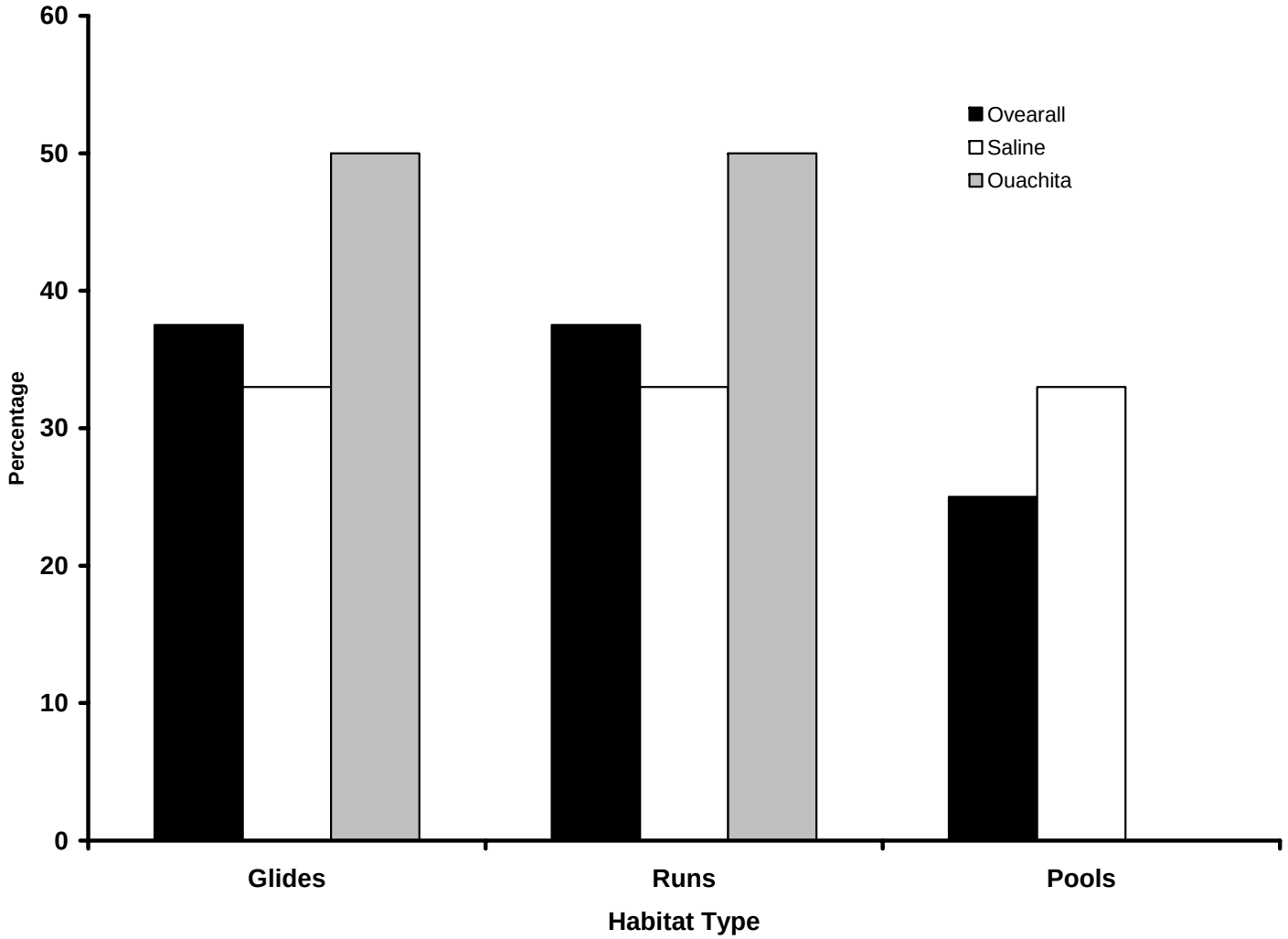

Figure 7. Percentage of *Villosa arkansasensis* occurrences in glides, runs, and pools in the Saline River (clear bar), the Ouachita River (gray bar), and in both rivers combined (black bar).

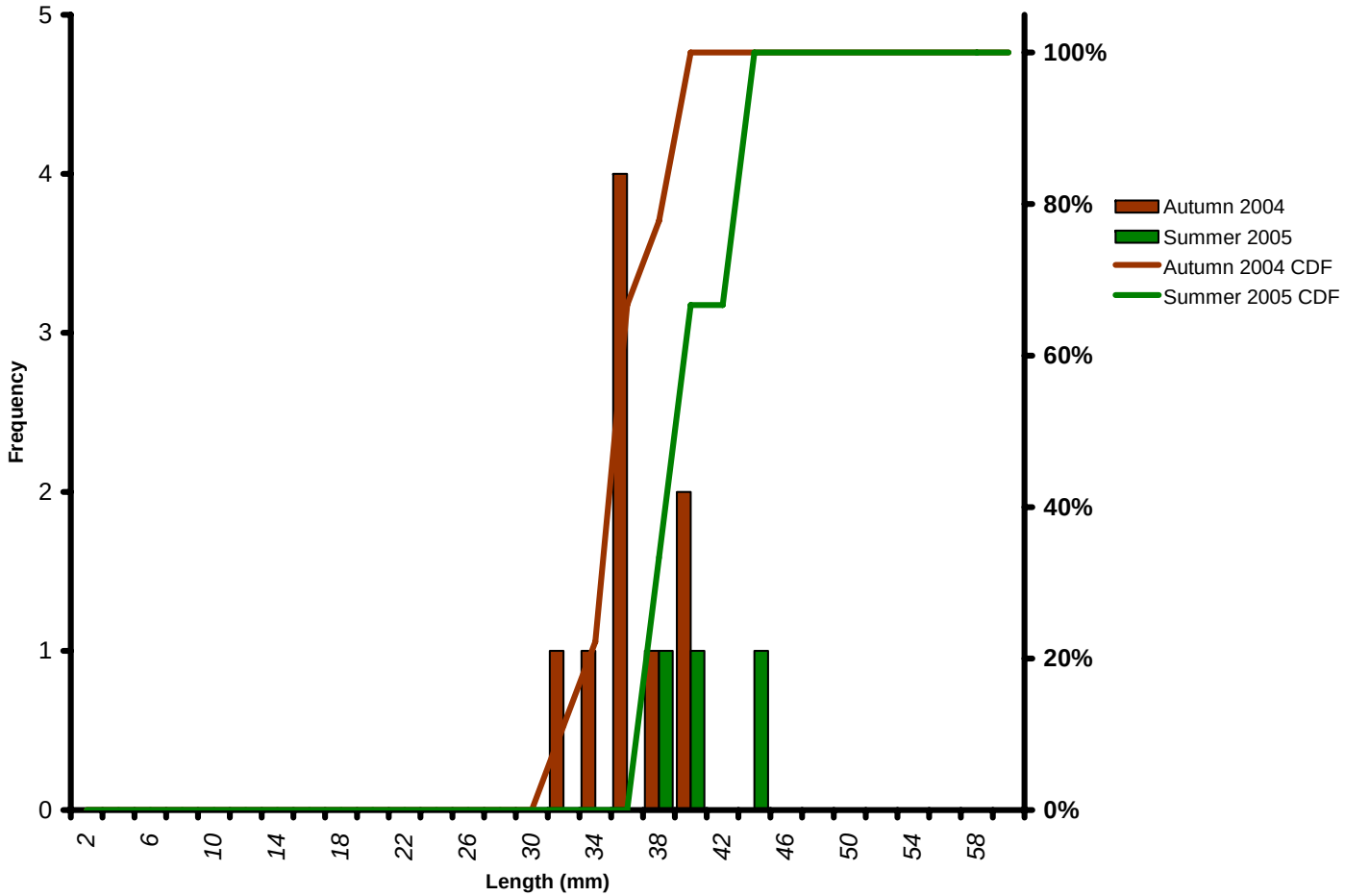

Figure 8. Length frequency histogram (bars) and cumulative distribution frequency (lines) of *V. arkansasensis* LPS027 mark and recapture data from autumn 2004 and summer 2005.

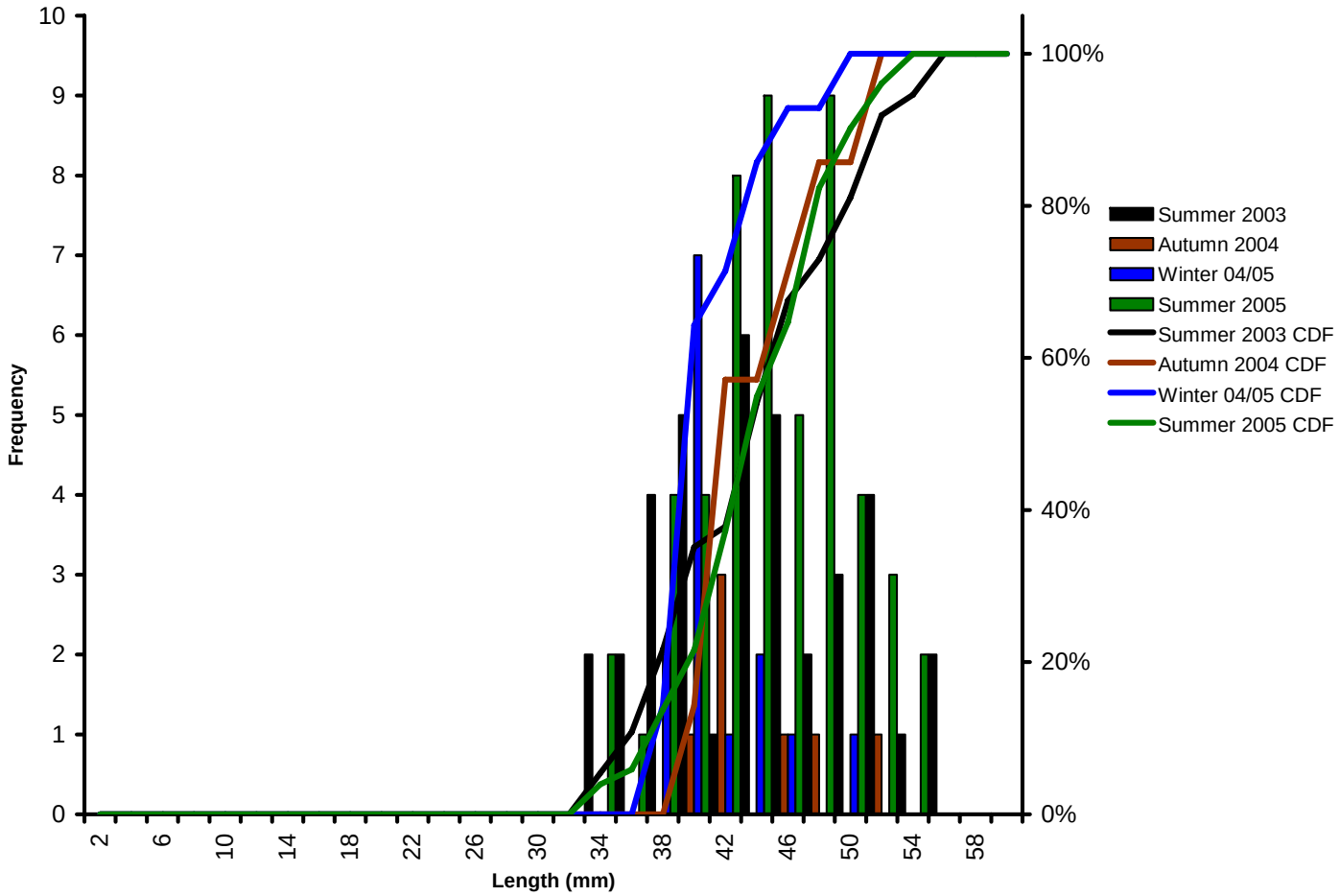

Figure 9. Length frequency histogram (bars) and cumulative distribution frequency (lines) of *V. arkansasensis* LPS050 mark and recapture data from summer 2003, autumn 2004, winter 04/05, and summer 2005.

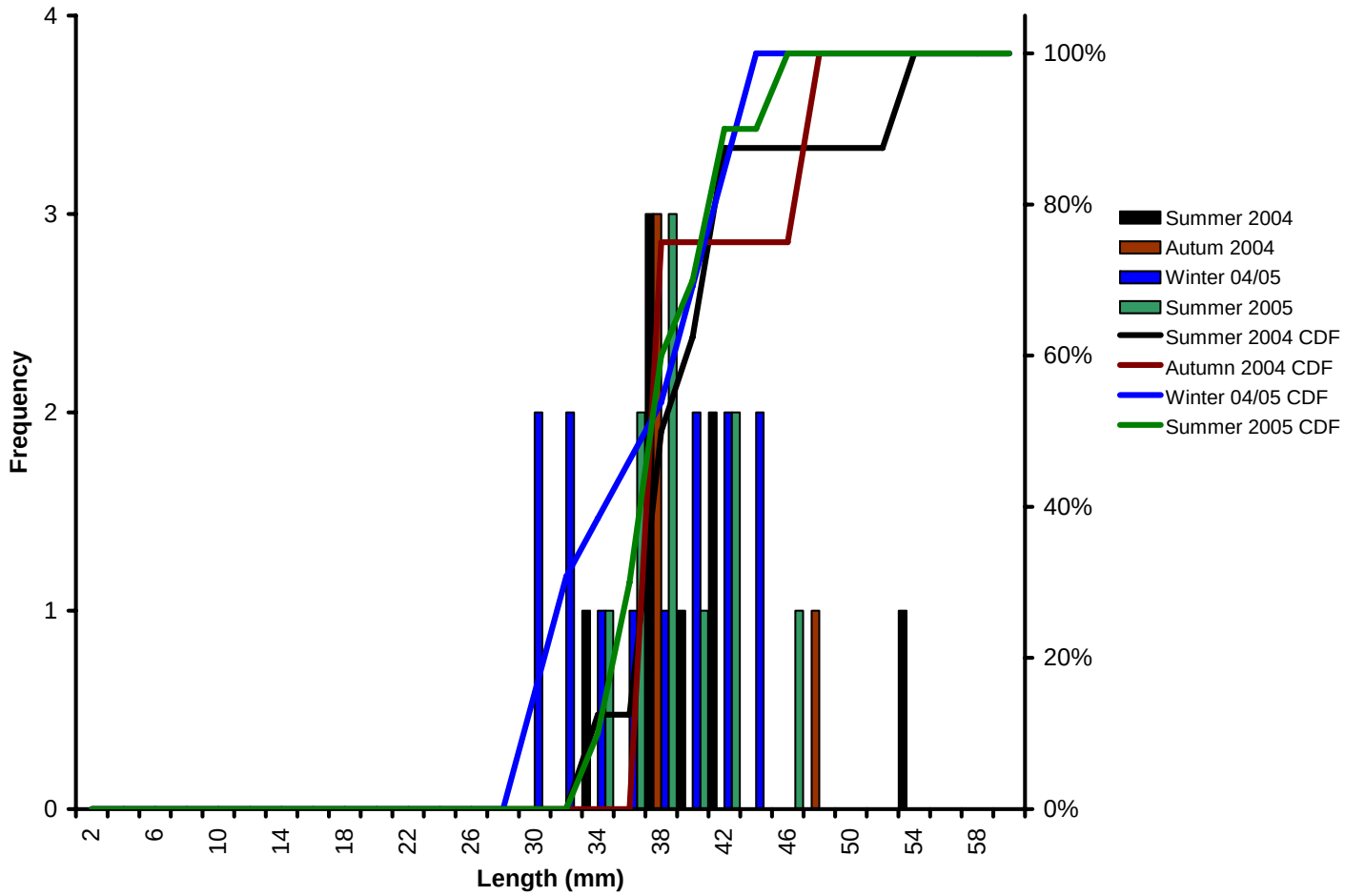

Figure 10. Length frequency histogram (bars) and cumulative distribution frequency (lines) of *V. arkansasensis* LPS102 mark and recapture data from summer 2004, autumn 2004, winter 04/05, and summer 2005.

Table 1. *Villosa arkansasensis* status survey site codes (Harris and Gordon 1988), GPS coordinates (Decimal degrees; NAD 83) USGS Quadrat, County, Township, Range, Section, and corner information for 2003 – 04 survey sites.

Site	GPS coordinates	Quadrat	County	T	R	S	Corner
Saline River							
LPS006	N 34.5871	Benton	Saline	2S	15W	4	NE1/4
	W 092.6049						NE1/4
LPS010	N 34.53831	Benton	Saline	2S	15W	22	NW1/4
	W 092.60365						SW1/4
LPS011	N 34.5289	Benton	Saline	2S	15W	27	SE1/4
	W 092.5962						NE1/4
LPS014	N 34.492894	Tull	Saline	3S	15W	1	NW1/4
	W 092.572364						SW1/4
LPS026	N 34.6729	Lonsdale NE	Saline	1S	17W	2	SW1/4
	W 092.7990						NW1/4
LPS027	N 34.6728	Lonsdale NE	Saline	1N	17W	32	NW1/4
	W 092.7988						SW1/4
LPS048	N 34.6380	Lonsdale NE	Saline	1N	17W	18	SW1/4
	W 092.7662						SE1/4
LPS049	N 34.74650	Goosepond	Saline	1N	18W	12	SW1/4
	W 092.87682						NW1/4
LPS050	N 34.7706	Paron SW	Saline	2N	18W	36	SW1/4
	W 092.8766						SW1/4
LPS055	N 34.71244	Jessiville	Garland	1N	19W	28	NW1/4
	W 093.03527						NE1/4
LPS056	N 34.71890	Goosepond	Garland	1N	19W	23	SE1/4
	W 092.99228						NW1/4
LPS057	N 34.70293	Goosepond	Garland	1N	19W	25	SW1/4
	W 092.98640						NW1/4
LPS058	N 34.6804	Goosepond	Saline	1S	18W	3	NW1/4
	W 092.9245						NW1/4
LPS060	N 34.6720	Goosepond	Saline	1S	18W	1	NW1/4
	W 092.8895						SW1/4
LPS061	N 34.6662	Lonsdale NE	Saline	1S	17W	5	SW1/4
	W 092.8532						SW1/4
LPS066	N 34.70558	Lake Norrell	Saline	1N	15W	30	NW1/4
	W 092.6546						NW1/4
Ouachita River							
LPS073	N 34.60646	Lonsdale	Garland	2S	17W	3	SE1/4
	W 094.17195						SE1/4
LPS093	N 34.55978	Mt. Ida	Montgomery	2S	26W	24	NE1/4
	W 093.71745						SE1/4
LPS102	N 34.61661	Mena	Polk	1S	29W	31	SW1/4
	W 094.13941						Middle
LPS104	N 34.56378	Board Camp	Polk	2S	29W	23	NW1/4
	W 094.06514						SE1/4
LPS105	N 34.5744	Pine Ridge	Polk	2S	28W	16	
	W 094.0029						
LPS108	N 34.6135	Mt. Ida	Montgomery	1S	26W	35	SE1/4
	W 093.7462						NW1/4
LPS111	N 34.6106	Mt. Ida	Montgomery	1S	26W	36	SE1/4
	W 093.7265						NW1/4

Table 2. Species diversity and abundance for the Middle Fork Saline River (MFSR) and Alum Fork Saline River (AFSR) 2006 (Data provided by C. L. Davidson and W. R. Posey, personal communication).

Site	**Stream Name**	**No. Species**	**No. Individuals**	**No. *Villosa arkansasensis***
MFSA05150601	MFSR	1	6	0
MFSA05150602	MFSR	7	381	0
MFSA05160601	MFSR	7	26	0
MFSA05170601	MFSR	3	5	0
MFSA05180603	MFSR	7	21	0
MFSA05180602	MFSR	4	5	0
MFSA05180601	MFSR	5	13	0
MFSA05170603	MFSR	8	27	0
MFSA05170602	MFSR	12	30	1
AFSA06300614	AFSR	17	81	0
AFSA06300613	AFSR	8	15	0
AFSA06300612	AFSR	3	3	0
AFSA06300611	AFSR	2	6	0
AFSA06290610	AFSR	5	61	0
AFSA06290609	AFSR	9	66	0
AFSA06290608	AFSR	5	24	5
AFSA06290607	AFSR	7	65	6
AFSA06290606B	AFSR	5	33	10
AFSA06280606	AFSR	1	2	0
AFSA06270605	AFSR	7	11	0
AFSA06270604	AFSR	12	128	0
AFSA06270603	AFSR	3	7	0
AFSA06270602	AFSR	4	7	0
AFSA06270601	*AFSR*	*3*	*12*	*0*
Totals			**521**	**22**

Table 3. U.S. EPA Rapid Bioassessment Protocol total scores and condition category for status survey sites on the Saline, Ouachita, and Caddo rivers from March – October 2003.

Site	Total Score	Condition Category
AFSR01	180	Optimal
AFSR02	166	Optimal
AFSR03	182	Optimal
AFSR04	174	Optimal
MFSR01	175	Optimal
MFSR02	168	Optimal
MFSR03	168	Optimal
MFSR04	175	Optimal
MFSR05	163	Optimal
MFSR06	157	Optimal
NFSR01	155	Suboptimal
SR01	176	Optimal
SR02	148	Suboptimal
SR03	152	Suboptimal
SR04	145	Suboptimal
SR05	175	Optimal
NFOR01	158	Optimal
SFOR01	160	Optimal
SFOR02	165	Optimal
SFOR03	160	Optimal
SFOR04	158	Optimal
SFOR05	163	Optimal
SFOR06	157	Optimal
SFOR07	130	Suboptimal
SFOR08	149	Suboptimal
OR01	145	Suboptimal
OR02	173	Optimal
OR03	157	Optimal
OR04	167	Optimal
OR05	157	Optimal
CR01	170	Optimal
CR02	164	Optimal

Table 4. EPA Habitat results for the Saline and Ouachita River status survey sites LPS006-LPS111 for the 2003-2005 field seasons.

Site	Epifaunal Substrate	Emb.	Velocity /Depth regime	Sed. Dep	Channel		Freq. of riffles (or bends)	Bank Stability		Vegetation		Riparian Width		Total Score	Classification
					Flow Status	Alteration		Right Bank	Left Bank	Right Bank	Left Bank	Right Bank	Left Bank		
Saline River															
LPS006	16	17	19	19	19	18	19	8	9	8	8	9	7	169	Optimal
LPS010	12	15	13	18	17	18	16	7	7	7	5	9	8	152	Optimal
LPS011	20	18	10	11	9	19	13	9	6	9	8	10	10	142	Sub-optimal
LPS014	19	8	15	19	19	19	13	10	10	10	10	9	7	168	Optimal
LPS026	18	18	16	18	14	13	16	8	9	9	9	9	9	157	Optimal
LPS027	16	16	10	16	9	18	16	10	8	9	9	9	9	146	Sub-optimal
LPS048	17	17	10	18	11	18	15	9	9	9	9	9	9	160	Optimal
LPS049	13	16	9	18	15	19	19	9	9	9	9	9	9	163	Optimal
LPS050	17	19	18	19	16	17	11	9	9	10	10	9	10	164	Optimal
LPS055	19	11	14	14	18	19	16	7	7	6	7	5	5	148	Sub-optimal
LPS056	15	18	14	17	14	19	14	9	9	9	9	9	8	164	Optimal
LPS057	15	14	14	13	15	19	18	9	9	9	9	9	9	162	Optimal
LPS058	16	17	10	19	19	19	17	10	10	10	10	9	9	166	Optimal
LPS060	18	16	17	18	15	19	14	9	9	9	9	8	7	161	Optimal
LPS061	18	17	17	17	14	18	15	8	9	9	9	9	9	169	Optimal
LPS066	17	17	16	18	13	17	15	9	9	9	9	9	9	167	Optimal
Ouachita River															
LPS073	19	7	15	13	18	19	19	6	6	5	5	7	7	146	Sub-optimal
LPS093	18	14	15	17	13	18	17	8	8	9	9	9	9	164	Optimal
LPS102	17	11	15	18	16	18	19	9	9	9	9	9	9	168	Optimal
LPS104	18	17	15	16	15	19	13	9	9	9	9	9	8	166	Optimal
LPS105	17	18	10	16	20	19	9	10	10	10	10	4	4	153	Optimal
LPS108	18	16	15	14	10	19	19	10	9	10	10	10	10	170	Optimal
LPS111	19	18	15	19	8	19	17	10	10	10	10	8	8	171	Optimal

Table 5. Individual sampling date and overall male to female ratios for the three mark and recapture study sites for three sampling dates.

Site	Season	Males	Females	Ratio
LPS026	Autumn 04	5	4	1.3
	Winter 04/05	0	0	0.0
	Summer 05	3	0	3:0
	Overall	8	4	2.0
LPS050	Autumn 04	4	3	1.3
	Winter 04/05	2	14	0.1
	Summer 05	24	27	0.9
	Overall	30	44	0.7
LPS102	Autumn 04	2	2	1.0
	Winter 04/05	5	10	0.5
	Summer 05	6	4	1.5
	Overall	13	16	0.8

Table 6. Host suitability rank (based on percent juvenile excystment success) and relative abundance (RA) of Ouachita River drainage fish that were found to be suitable host for *Villosa arkansasensis*. OR = Ouachita River, IF = Irons Fork, SFOR = South Fork Ouachita River, and LMR = Little Missouri River. Data obtained from Arkansas Department of Environmental Quality (http://www.adeq.state.ar.us/water/data_fish/fish.asp).

Host Fish		OR at Cove Creek (1992)		IF above Iron Fork Lake (1998)		SFOR 6.5 NW Black Springs (1999)		SROR W. Mt Ida (1983)		Cossatot River (1985)		Caddo River above Hwy 84 (1985)		Caddo River below Hwy 84 (1986)		LMR above Al Pike Campground (1983)		LMR at Crater of Diamonds state Park (1987)	
	V. arkansasensis rank	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)
Ambloplites ariommus	1	7	1.0			1	0.2	6	0.8	1	0.2	6	0.3	2	0.5	5	1.4	1	0.2
Lepomis cyanellus	4	24	3.5	57	15.9	6	1.1	30	4.0	10	1.7	27	1.2	3	0.8	10	2.8	5	0.8
Etheostoma blennioides	3	27	4.0			20	3.6	35	4.6			83	3.7	1	0.3	19	5.3	17	2.54
Etheostoma collettei	2	4	0.6															4	0.6

Table 7. Host suitability rank (based on percent juvenile excystment success) and relative abundance (RA) of Saline River drainage fish that were found to be suitable host for *Villosa arkansasensis*. NFSR = North Fork Saline River, AFSR = Alum Fork Saline River, MFSR = South Fork Saline River, and SR = Saline River. Data obtained from Arkansas Department of Environmental Quality (http://www.adeq.state.ar.us/water/data_fish/fish.asp).

Host Fish		NFSR 1 mile NE of Bland (1996)		AFSR at HWY 9 (1996)		MFSR at Goosepond Road (1996)		MFSR at Vance Road (2003)		MFSR NE of Hot Springs Village (2003)		MFSR at Goose Pond Road (2003)		MFSR at Talley Cemetery Road (2003)		SFSR 2.5 miles S of Owensville (1996)		SR upstream of Depot Creek (1985)	
	V. arkansasensis rank	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)	Number	RA (% of Total)
Ambloplites ariommus	1	1	0.1	1	0.1	7	0.4	3	0.1	4	0.1	7	0.4	5	0.3	1	0.1	2	0.1
Lepomis cyanellus	4	37	2.1	32	2.6	31	1.6	44	1.6	58	1.9	61	3.4	70	3.8	19	1.4		
Etheostoma blennioides	3	43	2.4	76	6.1	140	7.4	105	3.9	147	4.8	152	8.4	22	1.2	36	2.6	152	10.6
Etheostoma collettei	2	6	0.3	36	3.0	34	1.8	26	1.0	30	1.0	74	4.1	32	1.7	27	1.9	7	0.5