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Life history and convervation of Elliptio crassidens from the Blue River, Indiana.

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LIFE HISTORY AND CONSERVATION OF *Elliptio crassidens* FROM THE BLUE RIVER, INDIANA

By

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B.A., Butler University, 1997

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ABSTRACT

LIFE HISTORY AND CONSERVATION OF *Elliptio crassidens*
FROM THE BLUE RIVER, INDIANA

Cassandra L. Hauswald

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This study assessed life history components for the elephantear freshwater mussel, *Elliptio crassidens* (Lamarck, 1819). The main focus of this study was to determine the suitability of various fish species as a host for *E. crassidens* and to determine the population status in terms of age structure, recruitment and reproduction of *E. crassidens* from the Blue River drainage of south-central Indiana. General observations on the life history of *E. crassidens* were made: brooding conditions for release of larvae, larval behavior, and larval shell dimensions. Ages of *E. crassidens* shells from the Blue River were also determined. A Geographic Information System (GIS) analysis compared overlap in distribution between *E. crassidens* and potential fish hosts. This analysis demonstrated that percentage overlap between the ranges of *E. crassidens* and potential fish hosts was not a sound indicator of host fish suitability. However, several potential host species were identified and others were eliminated from consideration.

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INTRODUCTION

The freshwater mussel, *Elliptio crassidens* or elephantear, is a relatively large, long-lived mussel of the Mississippi River and Gulf Coast basins (NatureServe 2009). The elephantear is typically dark in color and older individuals develop a scaly, sloughing periostacum. This species is a short-term brooder, releasing larvae in late spring (Watters et al. 2009). Elephantear is a large river mussel that adapts to life in smaller tributaries of large rivers with fluvial conditions (Ahlstedt and McDonough 1993).

Freshwater mussels are large, filter-feeding invertebrates that inhabit the benthos of many streams, rivers, and lakes. They are protected by a calcareous shell that forms best when the mineral content of the waters in which they occur is high (Smith 2001). Thus, the limestone regions of the United States and Canada have historically supported a greater number of bivalve species than are found anywhere else in the world (McMahon and Bogan 2001, Smith 2001). Specifically, the Mississippi River basin supports the greatest diversity of unionids on Earth (Coker et al. 1921, van der Schalie and van der Schalie 1950, Neves et al. 1997).

Of North America's 297 freshwater mussel species, 70% are listed as rare, threatened, or endangered (Williams et al. 1993) and as such are considered the most critically endangered group of organisms in North America (Bogan 1993, Neves 1993, O'Dee and Watters 2000, Strayer et al. 2004). The freshwater mussel fauna of the

Mississippi River drainage has declined alarmingly in diversity, with over half of the mussel species occurring in the Mississippi River drainage estimated to be increasingly vulnerable to extinction (NatureServe 2009). This drainage historically harbored over 100 species, 19 of which are now extinct.

Equally alarming are the once widespread and common species that are declining rangewide, but are still represented by enough populations to be considered relatively stable (Cummings and Mayer 1997, Watters 1995). Such is the case for *Elliptio crassidens* (common name = elephantear, Turgeon et al. 1998), a common freshwater mussel of the Mississippi River and Mobile Basin drainages (Cummings and Mayer 1992, NatureServe 2009). *Elliptio crassidens* is ranked as a G5 species by NatureServe (2009), which represents a stable conservation status. Surveys for *E. crassidens* indicate that it is not reproducing in the majority of rivers where it naturally occurs (Gangloff 2003, Miller and Payne 2000a).

Reasons for the decline of freshwater mussels, and for many other freshwater animals, include historic and current stresses to freshwater systems. The most significant stresses to freshwater mussels are urbanization and the resulting sedimentation and habitat alteration, navigational improvements that alter natural flows, point and non-point sources of pollution and overharvest from the pearl button industry (Brim Box and Mossa 1999, Richter et al. 1997). Urbanization is a current threat to freshwater mussels in the Mississippi and Gulf Coast drainage basins (Gillies et al. 2002) while the historic loss of forest cover to agricultural pursuits continues to stress freshwater mussels. These perturbations combine to increase pulse flows due to increased channelization, which in turn increases shear stress on mussel beds (Richter et al. 1997, Peck and Smart 1986).

Impoundment of interior rivers within the United States for improved navigation is both a current and historic threat to mussels as impoundments alter habitat, fish movement, and associated fish-mussel interactions (Watters 1996, Vaughn and Taylor 1999). The pearl button industry no longer presents the greatest threat to North America's mussel fauna. At the height of the pearl button industry's prime, over 55,000 tons of shells per year were harvested in the Mississippi River basin. The industry collapsed in the early 1940's, but was followed by harvests for the cultured pearl industry, which resulted in a much lower harvest rate of fewer than 3,100 tons/ year during the mid-1990's (Neves 1999). This industry so seriously depleted many stocks (Claassen 1994) that the follow-up stresses associated with urbanization and impoundments compounded the threat and resulted in the decline of many species.

Freshwater mussel harvesting today is a regulated industry that targets a different subset of mussel species for the cultured and freshwater pearl trade. Instead of targeting mussel species that have uniform-shaped, large shells, the industry now seeks shells that boast colorful nacre and/or form good material for making blanks. Many states, such as Indiana, are not allowing new permits for mussel collecting, but states like Tennessee allocated 262 permits in 2007, generating $130,824 in revenue for that state (Hubbs 2009). Commercial harvest, although minimal, could threaten a number of species in harvestable water bodies, but control over the timing and harvestable segments can minimize the threat to population integrity.

In the calm before the storm of pre-World War II America, biologists were calling not only for the conservation of freshwater mussels, but also for their propagation to ameliorate the ravages of overharvest in one of the country's most valuable natural resources (Coker et al. 1921). Their thoughts ring just as true today as the status of freshwater mussels is just as dire (Hubbs 2009), although the threats have changed. The fact that mussels still persist at all in rivers large and small throughout the eastern United States demonstrates their resilience.

Freshwater mussels partition their habitat based upon flow and substrate, which also predicts what their fish host may be and thus a particular species' niche. Freshwater mussels typically exist in a gradient from upstream to downstream. Headwater-type mussel species utilize fish adapted to that environment. Hosts and species assemblages change as stream size increases and as stream habitats change. Greater mussel diversity occurs in the main channel as large river mussels utilize large river fishes, such as freshwater drum, *Aplodinotus grunniens*, and catfish species, *Ictalurus* spp. Shifts in mussel communities exist along a transitional gradient based upon flow and substrate type. A mix of medium- and large-river mussels often form a transitional community type as medium-river species give way to large-river species on a gradient from upstream to downstream. Such is the case in Indiana's Blue River from approximately river mile 43 to 60 where the mussel diversity is represented by 18 co-occurring species, with *E. crassidens* being the sixth most abundant species as of 2003 (Sietman and Hauswald 2004). Considered a large river mussel, *E. crassidens*, is also represented in lower order streams, like the Blue River, by locally abundant populations.

The Blue River in southern Indiana is a fourth order, spring-fed tributary of the Ohio River (Figure 1). The river and its watershed are biologically rich, with a variety of aquatic, terrestrial, and subterranean communities that support several globally rare plant and animal species, including; the spotted darter (*Etheostoma maculatum*), hellbender

(*Cryptobranchus alleganiensis)*, and Short's goldenrod (*Solidago shortii;* NatureServe 2009*)*. The mussel community in the lower one-third of the river is considered unique in its composition of large and medium-river species. This mix of species is experiencing a shift towards a more homogenous community structure of medium-river mussels and lack of big river mussel species. This is occurring despite the excellent water quality in the stream and apparently suitable habitat quality (Weilbaker et al. 1985).

Native unionid mussels have a unique reproductive strategy which may be contributing to the poor recruitment some of these species are experiencing in the Blue River as well as similar streams throughout the Mississippi River basin. Glochidia develop in the gill chambers of a mussel and are stored until the water reaches the proper temperature for gravid females to release the larval mussels (Kat 1984, Watters and O'Dee 2000, Smith 2001). Although some mussels are considered generalists in their host fish selection, others are very host-specific, obligate parasites (Yeager and Saylor 1995, Keller and Ruessler 1997, O'Dee and Watters 2000). Species that are rare and/or have never been abundant most likely have a narrower group of hosts or even a single host (Hoggarth 1992, Watters 1996, Haag and Warren 1997).

Fertilization in freshwater mussels occurs when males release sperm into the water column, which is subsequently filtered by the females. Mussels can filter approximately 34 liters of water per day (Allen 1914) and females in close proximity to males releasing sperm are likely to siphon water containing sperm into the incurrent aperture. Fertilization is successful when the spermatozoa travel to the incurrent siphon of the female mussel, and by ciliary action the sperm is moved to the suprabranchial chamber where the female has released oocyctes. Upon encountering the oocytes, sperm may penetrate, resulting in a fertilized ovum, which being heavier than water, will drop into the water tubes of the female mussel. Unfertilized oocytes may also drop into the water tubes where they have more time to encounter spermatozoa. Unfertilized eggs may be a signal that the spermatozoa taken in were not sufficient for fertilization of all the oocytes.

In 1948 Matteson reported on the reproductive biology of *Elliptio complanatus*. He found through his study of this species in Michigan that males release spermatozoa in late April through late May. In *E. complanatus* the entire outer gills act as a marsupium and the incidence of abortion among females of *E. complanatus* is high compared to other species. The conglutinate is best described as a cobweb-like structure and has not adapted to mimic a prey item that would attract a fish. The time required for the development of the young of *E. complanatus* from fertilization to mature glochidia is roughly one month and the glochidia do not gain in size during the parasitic stage. Brooding in *E. crassidens* is assumed to be similar to *E. complanatus* as both species in the genus are characterized as holding their larvae in the outer gills only and the larvae are expressed in similar fashion.

Freshwater mussels are categorized by the length of time in which they brood their larvae. In general, mussels are termed short-term brooders, tachytictic, if the release of larvae occurs within a few weeks after the fertilization process. With these species, fertilization occurs in early spring with glochidial release in late spring or early summer. Mussels are considered long-term brooders (bradytictic) if the female mussel broods larvae over winter after being fertilized in the fall (Neves and Widlak 1988). Presumably, the long-term brooders invest more time in brooding larvae that can be released over a much longer time period than the short-term brooders. The broad application of short versus long term brooder, however, does not strictly refer to the season in which larvae are released. Short-term brooders typically release glochidia in late spring. Short-term brooders, such as *Elliptio crassidens,* release larvae in late spring when the water temperature warms (Watters et al. 2009). The time of gravidity varies across the species' range from north to south as a function of water temperature making it difficult to broadly state that *E. crassidens* in an Indiana stream is gravid at the same time as *E. crassidens* in an Alabama stream.

There are four varieties of brooding strategies that occur in freshwater mussels. These include a broadcast release of glochidia, which does not serve to directly attract a fish. A second type of brooding entails the release of a conglutinate, or mass of glochidia. The appearance of the conglutinate can vary greatly between species as different mussel species have adapted to attract different fish hosts (Haag and Staton 2003, Barnhart et al. 2008). Some mussels dispel one or more conglutinates, which is then ingested by the fish. The other two brooding strategies involve the use of a "lure" which attracts fish to the mussel for the expulsion of glochidia in either a broadcast or conglutinate release. In certain mussel species, larvae are hooked, which is an adaptation allowing those species to attach directly to fin tissue. As they pass over the gills of the host, the larvae of the conglutinate clasp onto the tissue (Helfrich et al. 1997). If the fish is a suitable host the gill tissues will encapsulate the glochidia, which will remain on the host for several weeks to several months depending upon the water temperature.

Mussels are sedentary creatures aside from their larval stage when they parasitize fish. These fish act as a vector to transport them to other locations within or between river systems. In general, mussels have co-evolved in similar habitat with their fish hosts (Haag and Warren 1998). The release of glochidia is timed to occur when the host is present and most likely to encounter the larvae (Chamberlain 1934). Thus, the release of larvae in early spring may be an adaptation to coincide with the spring spawning run of migratory fish, while those mussels that release larvae in late summer may capitalize on aggregations of local fishes in low water pools.

Most freshwater mussel larvae are parasitic upon specific aquatic, vertebrate hosts, typically fish, although instances of amphibian hosts have been documented (Barnhart et al. 1998). This host "specificity" likely has more to do with fish immunity to certain mussel species than with a larval mussel's preference for a particular fish species (Watters 1992).

Little growth of the larval shell occurs on the fish host for most species, rather, this is a time when the larvae take in nutrients from the host and develop a second adductor muscle as well as other internal tissues (Smith 2001). Thus, mussel larvae appear translucent before they encounter a host and opaque after the parasitic phase of their lifecycle.

Just as critical as encountering the proper fish host is the water quality of the stream where the juvenile mussel is deposited. Along with the importance of a high mineral content, as filter feeders, mussels also require water containing high amounts of organic matter (Vaughn and Hakencamp 2001). Systems degraded by high nitrogen levels may have experienced shifts from beneficial to non-beneficial algae. Such shifts essentially starve juvenile mussels as they waste energy filtering copious amounts of algae from which they can derive no energy (Patterson et al. 1999).

Dam construction and river management have changed the movement patterns of fish throughout the main stem Ohio River and into tributary streams (Watters 1996). Shifts in mussel species diversity in the Blue River may therefore be due to a change in fish host presence or abundance in the river, particularly during the periods of the year when larval mussels attach. This community-level response is likely exacerbated by the aging populations of large river mussel species that have not experienced successful recruitment for many years due to the absence of host fishes.

The fish host represents a complex variable in the tenuous lifecycle of a freshwater mussel, but an equally important aspect of mussel ecology is the substrate in which the mussel embeds (Kat 1982). Mussels have partitioned their habitat by occupying different substrate types and varying flow regimes. *Elliptio crassidens* is an adaptable species that occupies a range of habitats, from mud, sand, or fine gravel in large rivers where the flow is not rapid, to medium-sized rivers with a greater current that presents more gravel and cobble substrates (Golladay et al. 2003).

A critical stage in the freshwater mussel's lifecycle is the point when the juvenile mussel is shed from its host. At this stage, the juvenile is so small that the current may be able to carry it to suitable substrate. The juvenile mussel needs to begin feeding soon after excystment or it will starve to death. Falling on a bottom of bedrock, for instance, will allow the juvenile to begin feeding, but it must encounter suitable substrate in which to extend its foot for anchoring. Conversely, being deposited onto a thick layer of fine sediment may make the prospect of feeding difficult as the young mussel may only be filtering silt, also leading to starvation (Scruggs 1960, Ellis 1936).

While substrate type can often predict what species will occur, there are more variables involved in the existence of freshwater mussels in a particular area than substrate alone; including water depth and velocity (Bartsch et al. 2009, Strayer 1993). The stability of the substrate is also a great predictor of mussel presence (Strayer and Ralley 1993). Due to their sedentary nature, freshwater mussels cannot move significant distances to avoid being buried by silt or being scoured away in a rain event. Recent mussel declines can often be attributed to urbanization that has increased run-off leading to mussel bed instability (Brim Box and Mossa 1999)

Very little literature is devoted to *E. crassidens*, supporting the need for a basic life history compilation. Historically, *E. crassidens* was not economically important for the pearl button industry because of its colored nacre. Much of the early research on life history and specifically on reproductive strategies of freshwater mussels was focused on those species that had economic value (Ortmann 1912, Coker et al. 1921).

In later years, the research has shifted to threatened and endangered species so that once again *E. crassidens* has been overlooked (Yeager and Saylor 1995, Jones and Neves 2002). This species enjoys a widespread distribution, but is only abundant locally (Cummings and Mayer 1992, Williams et al. 2008). More recent surveys demonstrate the alarming decline in this animal, though its local abundance in discrete patches has kept it off of most state watch lists.

A number of external factors interact to affect the success of maturation in a freshwater mussel. Teasing apart the life history of *E. crassidens* into its basic parts is necessary to assess its conservation needs and to find solutions for the sources of stress on each life history component.

Hypotheses

This research assessed the potential for host-limitation versus reproductive limitation to see if loss of host fish is a cause of *E. crassidens'* low abundance and skewed population size structure in the Blue River. This was accomplished by examining *E. crassidens* from this river for reproductive viability and then infecting various fish species with glochidia from *E. crassidens*. Gravid *E. crassidens* from the Tennessee River were utilized as duplicates on some of the fish host studies.

The goal of this research was to use laboratory inoculations to determine fish hosts for *E. crassidens*. In addition, observations were made on the size of individuals collected from the Blue River, Indiana and thin-sectioning techniques were utilized to determine the age class of mussels collected in the Blue River. Observations on reproductive timing and glochidia size and behavior were made as well. Finally, an analysis of overlap between *E. crassidens* distribution and various fish species' distribution was performed to predict which species might be suitable as fish hosts for *E. crassidens*.

This research tested two hypotheses. 1) that the individuals of *Elliptio crassidens* in the Blue River, Indiana are senescent. 2) that the host fish for *Elliptio crassidens* is absent in the Blue River.

These experiments addressed the apparent lack of recruitment of juvenile *E. crassidens* by establishing whether the Blue River population is too old to be reproductively viable as well as by determining if any of the fish species present in the river can act as suitable hosts for larval *E. crassidens*. It is hoped that this research might lead to means of increasing the *E. crassidens* population in the Blue River.

MATERIALS AND METHODS

Hypothesis 1: The individuals of *Elliptio crassidens* in the Blue River, Indiana are senescent.

Brooding Period and Release of Larvae

Thirty-five *E. crassidens* were collected from the Blue River on June 30 and July 3 of 2006. The timing of collections was based upon the expectation that this species is a short-term brooder, gravid from early June to mid-July (Parmalee and Bogan 1998). Collections were made using a combination of snorkeling and SCUBA assisted diving. Permits were obtained from the Indiana Division of Fish and Wildlife for the collection of *E. crassidens* and the transport of this species from Indiana to the Center for Mollusk Conservation in Frankfort, KY.

Mussels were collected in the area of Stagestop Campground, (site 1) on June 30 and upstream of the intersection of Indiana Highway 62 and Highway 4-62 on July 3 (site 2, Figures 1, 2 & 3). Both locations are contained within Harrison-Crawford State Forest. For transport to Kentucky, mussels were submerged in 25 gallon coolers filled with water from the Blue River. Mussels were transported in aerated coolers filled with water from the collection sites.

Elliptio crassidens collected from the Blue River were held in two concrete tanks, which received a gravity-fed flow from waters of the Elkhorn Creek. The tanks were filled with a mixture of 6-20 millimeter gravel mixed with sand. The tanks were housed within a cinder block hatchery facility with approximately 50 other holding tanks containing various mussel species. Lighting was a mixture of natural light from windows and fluorescent light that was used during normal working hours. To account for any lack of natural light, a Sun Gro lighting system mimics natural sunlight conditions directly above the tanks. Room temperature in this facility was 22.2 °C. Continuous river flow kept water temperatures in tanks similar to outside water temperatures.

Mussel tanks were flushed daily to prevent silting in the tanks as the flow arrangement was not rapid enough to purge the tanks of silt while delivering adequate nutrients. Flushing was achieved by increasing the flow in the tank and gently sweeping the shells with a soft bristle brush to remove fine sediment that had accumulated. Prior to flushing the tanks, animals and substrate were checked for conglutinates and/or signs of distress, such as gaping shells signaling increased respiration. Seasonally related turbid river conditions in the Elkhorn River, the water source, made this process a requisite during periods of high flow.

Housing the mussels in a laboratory setting with flow-through tanks at natural temperatures allowed more frequent observations of individuals and allowed larvae to be collected from the elephantear. Larvae were extracted from the gills of gravid females by flushing water across the lamellae of the gills when glochidia appear fully charged and ready to be released. Larvae were tested for viability by introducing a saline solution to a

Petri dish with a subset of larvae. If larval valves snapped closed in response to the solution they were considered viable (Jones and Neves 2002).

The collected *E. crassidens* were monitored in the laboratory on a daily basis as the tanks were cleaned. When conglutinates were first noted in the tanks the water temperature and date were recorded. Conglutinates were then examined under a microscope to check for viability and larval maturation. Two individuals from the Blue River released larvae while two individuals from the Tennessee River also released larvae. Only gravid females from Tennessee River were brought into the laboratory in late March 2007, while individuals from were not gravid when collected in July 2006. Ten larvae from the Blue River were measured under an inverted microscope (Nikon T100, 400x) to determine height, width and hinge length.

<u>Age and Growth</u>

Thin sections were made from the left valve of 19 individuals. No animals were sacrificed; rather, 6 animals that did not survive in the laboratory were thin-sectioned. Additionally, 13 fresh dead shells were collected in the vicinity of known *E. crassidens* beds in the Blue River.

Shells of the Blue River *E. crassidens* that perished in the laboratory as well as fresh dead individuals from the Blue River were aged using annual growth ring counts. This was accomplished by sectioning one valve per shell into a manageable cross-section with a diamond-impregnated blade. This half of the valve was then glued with epoxy onto a frosted cover slide. After the shell was affixed to the slide another slice was made. The thin section of shell is approximately 300 microns thick. The section is then viewed under a microscope and annual growth rings are counted (Haag and Commens-Carson 2008, Rypel et al. 2008).

Hypothesis 2: The host fish for *Elliptio crassidens* is absent in the Blue River.

Host Suitability

Twenty one fish species were infested with larvae from *E. crassidens* (Table 1). Fish infestation occurred on April 24 and April 26, 2007. All fish were infested with larvae from the Blue River as a goal of this research was to obtain juveniles for re-introduction into the Blue River system. Additional larvae from Tennessee River *E. crassidens* populations were collected in the field during various mussel surveys. Only gravid females from the Tennessee River were collected and then transported back to the laboratory to be held in quarantine. Tennessee River larvae were used as duplicates on certain fish species (Table 2).

Fish were treated with a 150-200 mg per liter solution of 99.5% tricaine methanosulfonate (MS222), to prepare them for inoculation with glochidia. A powder form of MS222 was added into a cooler containing 370 liters of water to create a bath for larger fish to be anesthetized while a smaller plastic bucket containing one liter of water was used for smaller fishes. After each fish was anesthetized, which usually occurred within one minute of exposure, it was removed from the solution for introduction of larvae to the left gill. This technique required a minimum of two people, one to hold the fish with the left gill open and another to pipette an estimated 100 larvae onto the gill. Care was taken to keep the pipette from touching the gill surface so the larvae would not close prematurely, which could prevent sufficient exposure to the potential host fish gills.

The fish handler took care in reapplying Stress Coat ™, a synthetic slime coating that minimizes damage to fish scales, to their hands between handling each fish. Fish were placed in their holding tanks with no interim time spent in a recovery tank of any type.

Fish were placed in tanks proportionate to their size. Duplicate individuals of the small, easy-to-accommodate cyprinids were utilized. Fish such as sauger, *Sander canadensis* (Griffin and Smith 1834) that are easily agitated and large species like drum, *Aplodinotus grunniens* (Rafinesque 1819), were held in opaque, 20 gallon Rubbermaid totes. Gizzard shad were held in a large, 25-gallon rectangular tank that allowed them ample room to swim with a mesh cover over the top to minimize disturbance. All tanks were on a re-circulating system with a pre-established biological filtration system. Well water was added to the system as necessary, which was usually every 2 to 3 days. Temperature was recorded each time the tanks and screens were examined for juveniles.

Screens of 150 μm were placed on the outlet of each tank at day 8 to capture any mature juveniles that were being sloughed. This size screen was chosen because the mean dimensions of the larvae were 164 micrometers long by 149 micrometers wide. This size screen was deemed ample for capturing the larvae without capturing too much of the tank debris upon cleaning the tanks in search of juveniles (Khym and Layzer 2000 Yeager and Saylor 1995). When the screens were added to the tanks, the water flow was decreased to prevent the screens from becoming clogged with debris from the tank. Screens and tanks were checked every other day from day 15 to day 26.

Small fish holding tanks were checked by first transferring the fish to a holding bucket and then washing the entire contents of the tank into a 150 μm screen. The contents of the filter screen were rinsed into a Petri dish. Before examining the contents of the Petri dish, the tank was refilled with well water and the fish were returned to their respective tanks where the screen was replaced and the water flow was resumed. Fish were returned to their tanks within five minutes.

For large tanks, the bottom and sides of the tanks were siphoned into a 5-gallon bucket. The contents of the 5-gallon bucket as well as the screen were then filtered as before through a 150 μm screen which was then rinsed into a Petri dish for examination.

The contents of the Petri dish were thoroughly examined under an inverted microscope (Nikon T100, 400x). Results of each filtered sample were recorded. In addition to checking the tanks and screens every other day, tanks were monitored by staff at the Center for Freshwater Mollusk Conservation for irregularities, including dead fish. When dead fish were encountered the gills were immediately examined for any signs of encysted larvae and the results were recorded.

<u>Host Distribution Analysis</u>

Of the 31 recognized species in the *Elliptio* genus (Turgeon et al. 1998), only seven have any reported attempts at identification of their fish host. Of the two Interior Basin species, *Elliptio crassidens* and *Elliptio dilatata* (Rafinesque 1820), only reports of natural infestations are noted, none of which verified successful transformation into mature juveniles. *Elliptio crassidens* is reported to have been naturally infested on the skipjack herring based upon gill tissue examinations of wild-captured fish in the family Clupeidae: *Alosa* chrysochloris (Rafinesque 1820) (Fuller 1974, Ortmann 1914).

Given that percids and centrarchids dominate the list of successful laboratory transformations, these fish families were utilized in this study. Cyprinids were also used as other *Elliptio* species use this fish family as hosts. Twenty-one species of fish were utilized for laboratory infestations. The skipjack herring, *Alosa chrysochloris*, was an obvious choice for laboratory testing of fish host suitability (Ortmann 1914, Fuller 1974). The fish species chosen for this study were representative of fish families in and near *E. crassidens* populations and/or common fish in the Mississippi River drainage. All fish for this experiment came from Kentucky waters, though all species occur at the Blue River study site (See Table 3).

Fish distributions were determined using the Atlas of North American Freshwater Fishes (Lee et al. 1980), along with NatureServe data. From this data, a Geographic Information System (GIS) shape file was digitized for a subset of fish species utilized in this study. An *E. crassidens* distribution shape file was also created based upon NatureServe data and mussel collections at the Illinois Natural History Survey and Ohio State University's mussel database. A discrepancy does exist in the data between fish and mussels in that fish distribution is on a finer scale, the 8-digit Hydrologic Unit Code (HUC) and the mussel data is coarser at the 6-digit HUC. The result is that *E. crassidens* is depicted as more broadly represented in a larger watershed, while a fish species is categorized and thus shown in smaller, more detailed watersheds only.

An overlap of distribution was created between selected fish species and *E. crassidens*. In addition to overlays created for a subset of fishes tested, an overlay for fishes that are suspected candidates for further fish host studies was also created.

RESULTS

Hypothesis 1: The individuals of *Elliptio crassidens* in the Blue River, Indiana are senescent.

Collection and Observation

The glochidia of *E. crassidens* were active, observed by their behavior of opening and closing continuously with slight pause in between their efforts. In the case of *E. crassidens*, when a sub-sample of larvae was exposed to a salt solution, their behavior involved snapping closed.

The average dimensions of the larvae measured in this study were 164 μm total length (TL) x 149 μm total width (TW) with an average hinge length of 54 μm (see Table 4 and Figure 4). Being a short-term breeder, and thus investing less time in developing larvae, *E. crassidens* larvae are small in comparison to other *Elliptio* larvae (Hoggarth 1999, O'Brien et al. 2003).

While the conglutinate produced by *E. crassidens* does not appear to be mimicking a particular food source, the mass could appear similar to a grub or worm. Watters (2009) describes the conglutinate as simple and non-elastic while Ortmann (1912) describes the mass of glochidia as leaf-shaped. It was estimated that 60 to 70% of the conglutinate was composed of viable glochidia while the remainder represented undeveloped eggs. The conglutinate remained as a collective packet, but any abrasion on the conglutinate could have exposed the larvae. Other species that exhibit a conglutinate appear dependent on the shredding action of a predatory fish to release larvae (Watters 1999).

When examined, females were observed to have the outer gills charged with larvae. The gills were tan in color, but became creamy white when the female was gravid. In two cases, when larvae were not yet mature, I waited for glochidia to develop and be expelled, but this never occurred. Presumably, the larvae were reabsorbed.

Age and Growth of Adults

In the Blue River, the average size of individuals was 124 mm total length by 86 mm in height by 57 mm in width (Table 5). Shell sectioning revealed that animals in this size class were an average of 56 years of age (range = 45 to 72) (Figure 5). It is known that these individuals are reproductively viable as shown by individuals in the lab releasing viable glochidia.

Hypothesis 2: The host fish for *Elliptio crassidens* is absent in the Blue River.

Fish Infestation

None of the 20 fish species (Tables 1 and 2) infested with *E. crassidens* larvae resulted in a successful transformation into a juvenile mussel. No viable glochidia were collected from the tanks during cleaning. The only fish to perish during this experiment were cyprinids that were duplicates.

Correlation of Distributions

Elliptio crassidens Distribution

In this study, *E. crassidens* were collected from the Blue River, a fourth order tributary stream of the Ohio River. Most *E. crassidens* in the Ohio River are found in sediment ranging from mud to fine gravels (Cummings and Mayer 1992). In Illinois rivers, they are typically found in a bottom matrix of stones and coarse gravel in swift current no less than 1.8 meters in depth (Parmalee 1967). Individuals of *E. crassidens* in the Blue River occupy the heads of riffles where the substrate is a mix of gravel and cobble and the water depth is typically 0.9 to 1.2 meters at most (Figure 2 and 3). Williams and Shuster (1989) found *E. crassidens* occurring in waters with a depth of 3.0 to 4.6 meters with a substrate of coarse sand and gravel. Corresponding to this habitat shift, the individuals in the Blue River are large for the species, heavily inflated, and thick-shelled. All of these adaptations allow *E. crassidens* to thrive in the swifter current at the head of riffles (Smith 2001). Presumably, the large size and shell thickness of *E. crassidens* in the Blue River is due to the high organic content and the high mineral content of the water. Ortmann (1920) found a similar correlation in stream systems of the upper Ohio River.

Elliptio crassidens is adaptable to impoundments as demonstrated by its abundance in Wheeler Reservoir, Alabama (Ahlstedt and McDonough 1993) as well as its abundance in the tail waters of Guntersville Lake, Alabama (Garner 1997). The canal between Kentucky and Barkley Lake represents another lentic site (Paukert and Fisher 2001) where *E. crassidens* is reproducing.

Elliptio crassidens had a historic distribution throughout the Mississippi, Alabama and Apalachicola River basins (Parmalee and Bogan 1998). It occupies large rivers and their tributaries in portions of the following states: Alabama, Florida, Georgia, Indiana, Illinois, Kentucky, Louisiana, Michigan, Minnesota, Missouri, Ohio, Pennsylvania, Tennessee, West Virginia, and Wisconsin. The species status varies from abundant to rare widely across its range (see Table 6).

Archaeological digs along the Tennessee, Tombigbee, Alabama, Etowah, and Chattahoochee Rivers have reported *E. crassidens* shells. Alabama boasts the largest number of river systems containing *E. crassidens*.

In Kentucky, *E. crassidens* was documented in the Cumberland basin in 1911 as the second most abundant species in the Cumberland River (Wilson and Clark 1914). In Neel and Allen's 1964 survey, *E. crassidens* was considered rare in the tributary streams of the Cumberland River, but it became common to abundant as the Cumberland River approached the Tennessee line. Neel and Allen refer to *E. crassidens'* high abundance in the Cumberland River below the falls and comment on its significance as a valued pearl mussel, sharing that distinction with *Lampsilis ovata* (Say, 1817). Cicerello et al. (1991) summarized the literature on *E. crassidens* in Kentucky, and concluded that the mussel is found occasionally in the lower Cumberland and Tennessee Rivers, but avoids the reservoirs of Kentucky and Barkley Lakes. This is in contrast to its current status of being present and reproducing in the canal between Barkley and Kentucky Lakes, a lentic system, presumably in response to its fish host being present in sufficient numbers (see Table 7). It is sporadic in the upper Green, upper Cumberland, and Big Sandy Rivers of

Kentucky while it is rare in the lower Green River, Tygarts Creek, Kinniconick Creek and Little Sandy River.

Williams and Schuster's 1989 comparison of the mussel fauna in the Ohio River from Catlettsburg, Kentucky to Cairo, Illinois during the time period of 1967-1968 and 1982 reveals an interesting shift in the number of *E. crassidens* in this stretch of the Ohio River. In 1967, *E. crassidens* represented 5.9% of the total number of mussels collected in this stretch of the Ohio River system. In 1982, its numbers had increased to represent 12.1% of the total number of mussels taken, or the second most abundant species in this stretch of the Ohio River.

Taylor (1980) studied the Ohio River mussels from Greenup, Kentucky north to the Ohio's origin at Pittsburgh, Pennsylvania and recovered no fresh dead specimens of *E. crassidens* though many sub-fossilized shells were counted in 1979. Taylor reported that *E. crassidens* was, however, a major constituent of shell middens in this section of the Ohio River.

In the Tombigbee River of Mississippi, *E. crassidens* was reported in 1906, 1939, and 1974. Additionally, excavations of pre-historic sites (500 B.C. to 1650 A.D) on the Tombigbee and its tributaries, Lubbub Creek and Tibbee River, found *E. crassidens* shells that accounted for 3 to 26 percent of the total number of shells depending upon the site. Jones et al. (2005) in examining museum records for Mississippi, found *E. crassidens* occupying the Big Black, the Tennessee and the Mississippi River. *Elliptio crassidens* has also been found in the following Gulf Coastal drainages: Lake Ponchartrain and the Pearl, Pascagoula, and Tombigbee Rivers (Jones et al. 2005).

In the Meramec River, Missouri, Buchanan (1980) reports *E. crassidens* as occupying the mid-river downstream nearly to the mouth of the river's confluence with the Mississippi. The species has also been reported in Missouri from the Castor River in 1994 (The Ohio State University 2009).

Starrett (1971) summarizes the findings of the Illinois River mussel fauna from 1870 to 1969. *Elliptio crassidens* was present in the 1870 to 1900 and 1906 to 1912 surveys, but was absent during the 1966 to 1969 survey. This large river is comparable to the Wabash where *E. crassidens* still persists (Fisher 2006). The disappearance of *Elliptio crassidens* from the Illinois River somewhere between 1913 and 1930 may have been due to navigation and other related perturbations. Parmalee (1967) lists the elephantear in the Kingston Lake middens (1000-1400 A.D) alongside the Illinois River, 24.1 kilometers southwest of Peoria, Illinois. In 1874 this species was considered abundant in the upper watershed area of the river as reported by Calkins (1874). Danglade (1914) found examples of the species in many sites on the lower Illinois River, but says the elephantear made up a small percentage of the mussel beds. Parmalee reports in 1967 that in Illinois *E. crassidens* is found in the lower Ohio and Wabash Rivers as well as in the Mississippi River above St. Louis. Interestingly, he also reports that *E. crassidens* was present in a 1957 survey of the Illinois River, but absent 10 years later.

In Minnesota *E. crassidens* was historically found in the Minnesota, Mississippi, Vermillion, and St. Croix rivers. Today it is only found at the mouth of the St. Croix River (Sietman 2009). In neighboring Wisconsin there are reports of *E. crassidens* from the St. Croix and Mississippi River systems. *Elliptio crassidens* has also been found in the Eau Claire River of the Chippewa drainage as well as the Des Plaines and Upper Fox rivers of the Lower Illinois drainage (Mathiak 1979). The species is listed as critically imperiled in Wisconsin.

Ohio lists *E. crassidens* as endangered in the state. It occurs in Big Darby Creek, but is rare. *Elliptio crassidens* also occurs in portions of the Ohio River bordering the state of Ohio. In West Virginia *E. crassidens* occurs in tributaries of the Ohio River; Elk River, the Kanawha River and Twelve Pole Creek (The Ohio State University 2009). *Elliptio crassidens* has been extirpated from Pennsylvania, but once occupied the Ohio and Allegheny rivers in western Pennsylvania (Spoo 2008).

In Georgia, *E. crassidens* is known from the Apalachicola, Alabama and Middle Tennessee river basins. *Elliptio crassidens* is currently listed as stable in Georgia based upon its local abundance in the Apalachicola, Chipola, and Flint River main stem (Brim Box and Williams 2000). *Elliptio crassidens* is also found in Florida within the Apalachicola and Choctawhatchee-Escambia drainages. Specifically, the species occupies the Lower Choctawhatchee, Yellow, Escambia, Chipola, and Perdido Rivers.

In Indiana, *E. crassidens* is found in the Ohio River tributaries. Daniels (1903) lists the species as occurring in the Ohio, Wabash, and Tippecanoe Rivers. The Blue River is not listed as a locality for *E. crassidens* in the 1903 report, although other mussel species are listed for the Blue River Goodrich and van der Schalie (1944) listed *E. crassidens* as occurring in the larger rivers that drain to the Ohio and Weilbaker et al. (1985) reported *E. crassidens* from the Blue River.

Elliptio crassidens historically occurred in most medium and large rivers of Tennessee. It is now restricted to the Cumberland and Tennessee Rivers and their large tributaries, including; the Elk, Duck, Big South Fork, Cumberland and Holston rivers (Parmalee and Bogan 1998). The species is also still present in west Tennessee's Hatchie River (Starnes and Bogan 1988). Prior to 1960, *E. crassidens* was also present in the Emory, French Broad, Hiwasee, Sequatchie, Obey, and Red rivers.

<u>Geographic Information System Analysis of Distribution</u>

The distribution of *E. crassidens* is shown in Figure 7. Figures 8 through 15 represent the overlap in distribution between selected fish species and the range of *E. crassidens*. Fish species were chosen based upon their similarity in ranges and/or habitat preferences. The percentage overlap between the ranges of *A. chrysochloris* and that of *E. crassidens* was analyzed as a reference to compare the other selected species. By dividing the area of the shared range between *E. crassidens* and the fish species by the entire range of *E. crassidens* a percentage of overlap in range was divided. The percentage overlap in historic range between the two species is 40% (Figure 8) while that of the green sunfish *Lepomis cyanellus* (Rafinesque 1819) and *E. crassidens* is 91% (Figure 9). A *Lepomis* species was utilized in the fish host experiment and should have yielded at least marginal success at transforming larvae if the genus were a host. *Lepomis cyanellus* represents a species that is ubiquitous across the range of *E. crassidens* as well as across substrate types. Five *Lepomis* species occur in the Blue River, also suggesting that this genus is not suitable as a fish host for *E. crassidens*.

Fish Distribution

The longnose gar *Lepisosteus osseus* (Linnaeus 1758) occurs across 87% of the range of *E. crassidens* (Figure 10). This genus was not examined in fish host experiments due to the difficulty of housing this species. This high percentage of overlap in ranges suggests this species should be considered for future fish host studies, possibly *in situ*. *Lepisosteus osseus* has been recorded from the Blue River.

The emerald shiner *Notropis atherinoides* (Rafinesque 1818) is another ubiquitous species that occurs in the Blue River as well as across 72% of the range of *E. crassidens* (Figure 11). Two species of *Notropis* were tested for suitability as host for *E. crassidens* larvae with no success. The striped shiner *Luxilus chrysocephalus* (Rafinesque 1820) is present and represents 72% (Figure 12) of the range of *E. crassidens*. This species, like *N. atherinoides*, is not a likely candidate for *E. crassidens*. If these or other widespread species were a suitable host it is likely that *E. crassidens* would not be disappearing from most Interior Basin waterways.

The percent overlap does not appear to be a good indicator of host fish suitability. While the silver chub *Macrhybopsis storeriana*, (Kirtland 1845), the bigmouth buffalo *Ictiobus cyprinellus* (Cuvier and Valenciennes 1844), and the paddlefish *Polyodon spathula* (Walbaum 1792) do not constitute as high a percentage of the range of *E. crassidens*, (Figures 13, 14, and 15), these species prefer a sandy substrate in large rivers, which fits *E. crassidens* habitat. *M. storeriana* and *I. cyprinellus* are both noted from the Blue River while *P. spathula* is not. These fish were not tested as a fish host, but should be considered in future studies based upon their habitat preferences.

Figure 16 represents an alternative or corrected approach to the method used to determine fish ranges in Figure 8 to 15. This correction matches the coarser watershed scale in which the range of *E. crassidens* is depicted. In Figures 8 through 15, the fish ranges were mapped in eight-digit hydrologic unit codes (HUCS). The distribution of *Elliptio crassidens*, however, is mapped at the six-digit HUC scale. Since fish can and do move up and downstream in watersheds it is a safe assumption that they occur throughout a larger watershed and not just in the location where they are documented. Figure 16 thus represents a much closer, 91%, overlap in range with *E. crassidens*. This approach involves extrapolation, but does make the percent overlap at least a potentially better indicator of fish host suitability.

DISCUSSION

While the conglutinates in this study were located on the substrate around the female mussel or in some cases resting on her shell, any significant water current could suspend the conglutinate in the water column. The conglutinate of *E. crassidens* is white and opaque. The conglutinate of *E. dilatata* is described as white and lanceolate (Utterback 1915) while the conglutinate of *E. arca* varies from a thick mucus to a true conglutinates lacking a distinct shape (Haag and Warren 2003).

An inference from the active snapping behavior of *E. crassidens* larvae paired with the non-descript appearance of the conglutinate is that their strategy for finding a host is based upon chance encounters. The behavior of the larvae indicates that their energy is expended in the form of an active snapping reflex.

The larvae of *E. crassidens* do not resemble any particular food item that certain fish species may seek. Instead, the conglutinate appears amorphous and loosely held together by a gelatinous membrane. The structure of the conglutinate suggests that *E. crassidens'* suitable fish host might be a grazer feeding in the pelagic zone. *Alosa chrysochloris* is a pelagic feeder and the structure *of E. crassidens'* conglutinate supports this as a potential fish host. Other pelagic feeders that could be fish hosts include; paddlefish, *Polyodon spathula*, emerald shiner, *Notropis atherinoides*, and striped shiner, *Luxilus chrysocephalus*. The structure of the conglutinate also supports the negative fish host results for piscivorous and benthic feeders such as green sunfish, *Lepomis cyanellus,* silver chub, *Macrhybopsis storeriana,* sauger, *Sander canadensis* and drum, *Aplodinotus grunniens*.

Individuals from the Blue River and from the Tennessee River released larvae within 0.2 degrees Celsius of one another. The individuals from the two different river systems were held in separate containers so individuals releasing glochidia could be identified. Since *E. crassidens* releases larvae in mid-spring when the water temperatures are warming, the parasitic phase should be complete in less than 20 days at which time the juvenile mussel is released from the fish gill or fin tissue and the sedentary phase of the mussel begins. The parasitic phase of another co-occurring *Elliptio* species, *E. complanatus* is 18 days (Matteson 1948).

A number of factors could be limiting *E. crassidens* reproductive efforts including: degraded water quality, an altered flow regime, sedimentation, low population numbers, old age, or a lack of suitable fish host(s). Each of these factors was considered when determining this species' current status. Water quality and quantity would presumably affect the common mussel species of the Blue River equally. A 2003 survey (Sietman and Hauswald 2004) of mussels in the Blue River (Table 8) found three other mussel populations, including: *Obliquaria reflexa*, the pimpleback *Quadrula pustulosa* (Lea 1831) and the rainbow mussel *Villosa iris* (Lea 1829) were recruiting juveniles. *Obliquaria reflexa* and *Q. pustulosa* co-occurred with *E. crassidens,* suggesting that general water quality conditions are not limiting reproduction and recruitment.

The species demonstrating recruitment in the Blue River are generally of the medium river varieties. The Blue River has several species demonstrating recent recruitment as evidenced in a 2008 survey. These include: the threeridge (*Amblema plicata*), purple wartyback (*Cyclonaias tuberculata*), wavy-rayed lampmussel (*Lampsilis fasciola*), fluted shell (*Lasmigona costata*), fragile papershell (*Leptodea fragilis*), pink heelsplitter (*Potamilus alatus*), pimpleback (*Quadrula pustulosa*), and pistolgrip (*Tritogonia verrucosa*). Note from Table 8, however, that many large river mussel species were historically present in the system, but are now either extinct from the system or non-reproducing, as is the case for *E. crassidens*. Of the 44 native species documented from the river, seven are considered large river mussels with four of these species now present in the Blue River. The river system's disconnection appears to be between the Blue River and its Ohio River confluence in regard to movement of large river fishes upstream into the Blue River during the spawning and brooding period of large river mussel species.

In the Blue River, *E. crassidens* is found in a mixed substrate of large cobble and boulders within a sandy substrate at the head of riffles. Little sedimentation or silting was found in the areas where *E. crassidens* is most abundant. Given the adoption of no-till farming in the watershed in the 1980's, sedimentation is viewed as a historic, but declining, threat to mussel recruitment in the Blue River. This can partially explain missing cohorts in the river, but is not a limitation to current mussel recruitment. In addition, *E. crassidens* is classified as a large river mussel that prefers sandy substrates. It is reasonable to expect that *E. crassidens* would be more tolerant of sedimentation than smaller, headwater-type mussel species.

The elephantear occurs in the Blue River entirely within the confines of Harrison-Crawford State Forest, from approximately river mile seven to river mile nineteen.

Stream banks are stabilized by riparian vegetation, although heavy rain events within the watershed, especially in the Corydon area, can cause uncharacteristic flashiness within the river, which may be leading to bank erosion downstream of Harrison Spring.

The lifecycle of *E. crassidens,* as with all freshwater mussels, involves males releasing sperm into the water column so that females will filter that water and allow fertilization to take place. Studies have shown that successful fertilization requires a minimum of 10 individuals per square meter in other *Elliptio* species (Downing et al. 1993). The most densely populated *E. crassidens* beds in the Blue River meet this minimum threshold. The question of whether *E. crassidens* individuals in the Blue River were too senescent to reproduce was resolved by gathering 38 individuals into a single laboratory holding area where they were spaced less than 0.5 meters apart. Two individuals of *Elliptio crassidens* released viable larvae in April 2007, eliminating the possibility that all *E. crassidens* in the Blue River were too old to reproduce.

While some individual *E. crassidens* from the Blue River are reproductively viable, it is possible that the population may have never been self-supporting. The Ohio River population of *E. crassidens* could have been the source of newly recruited individuals to the Blue River. Thus, an examination of fish hosts is a logical step in determining whether *E. crassidens* in smaller streams can be self-sustaining.

In April 2007, *E. crassidens* was monitored in the laboratory for gravidity by examining the gills for presence of glochidia packets. The presence of larvae confirmed that *E. crassidens* from the Blue River are still reproductively viable. Larvae from these mussels were used to infest 21 fish species (Tables 1 and 2). However, no positive fish host identifications were made as no *E. crassidens* larvae were successfully transformed into juvenile mussels on the fish species utilized.

Based on the literature reviewed, *E. crassidens* has a wide distribution, but is often locally abundant. Its present status is difficult to assess given that it is a large constituent of select surveys, but the age class structure suggests that there is limited recruitment in many areas. Exceptions do occur below large dams, such as those on the Tennessee River. *Elliptio crassidens* is thriving in reservoirs, thus fish hosts must be occurring in these areas.

Elliptio crassidens is valued in the cultured pearl industry to seed freshwater pearls that will be tinted a pink or purple color. Hubbard (1953) reported that musseling was in full swing on the Ohio River near Leavenworth with one harvester collecting 3,000 pounds of mussels a week bringing $50/ton (Williams and Schuster 1989). *Elliptio crassidens* source population in the Blue River may originate to the mid-fifties. This time period certainly corresponds to the average age of individuals that are found in the Blue River. The locations of the abundant *E. crassidens* beds correspond with easily accessible areas to state highway 62, all in close proximity to Leavenworth (10 miles), which was a hub of activity for the musseling industry (Lund 1995).

In 2003, *E. crassidens* were the sixth most abundant mussel in the Blue River in terms of numbers, but no juvenile mussels of this species were found (Table 9; Sietman and Hauswald 2004). The skipjack herring, *Alosa chrysochloris*, has been reported as the obligate larval host (Howard 1914, Fuller 1974). Skipjack herring are not as common in the Blue River as in the past (Eigenmann and Beeson 1894, Gerking 1945, Clay and Carter 1962, Janisch 1972, Baker and Forsyth 1986, Carnahan 2000). Furthermore, elephantear populations are patchy in distribution (Cummings and Mayer 1992) and in the upper Ohio River the population is depressed and is not experiencing high juvenile recruitment (Miller and Payne 2000a). The absence of *E. crassidens* juveniles in the Blue River could be explained by the absence of *A. chrysochloris*, but it is unknown whether other fish species may also be suitable hosts. *Alosa chrysochloris* is present in the Cannellton pool of the Ohio River that connects to the Blue River, but only represents 2.2% of the species composition (ORSANCO 2007).

The timing of seasonal high and low flows along with temperatures could also affect the co-occurrence of *A. chrysochloris* with brooding *E. crassidens*. Successful recruitment of the ebonyshell *Fusconaia ebena* (Lea 1831) in the Ohio River was related to flow stage during the brooding period (Miller and Payne 2000b).

In contrast, the threehorn wartyback, *Obliquaria reflexa*, is not abundant in the Blue River, only seven individuals were found in the 2003 survey, but one of those seven was a juvenile less than 3 years of age, which may indicate that this species is successfully recruiting in the river (Sietman and Hauswald 2004). The observation of juvenile recruitment in this species and others in the Blue River does suggest that the water quality is not a limiting factor to reproduction of mussels in the river. Species in the Blue River demonstrating recent recruitment include the threeridge *Amblema plicata* (Say 1817) the purple wartyback *Cyclonaias tuberculata* (Rafinesque 1820), the wavyrayed lampmussel *Lampsilis fasciola* (Rafinesque 1820), the fatmucket *Lampsilis siliquoidea* (Barnes 1823), the flutedshell *Lasmigona costata* (Rafinesque 1820), *Quadrula pustulosa*, and *Villosa iris* (Sietman and Hauswald 2004).

Dams are built for recreation opportunities, navigation, power, and/or flood control. A consequence of dams is the modification of in-stream habitats. The substrate upstream of a dam becomes more homogenous as the depth of the river or stream increases. Due to reduced current at greater depths, silt and sediments accumulate. This accumulation smothers available mussel habitat. Water depth affects water temperature as depth increases the water column is thermally stratified.

The portion of a stream below a dam is disturbed, but this disturbance is more closely tied to the purpose of the dam. For example, a flood control dam can dewater a downstream segment of the water body during periods of low flow while a power-producing dam can drastically change the flow and temperature regime. Scouring and substrate instability are common habitat disturbances resulting from dams.

Along with a distinct change in habitat availability, a shift in mussel diversity also occurs resulting both from a habitat change and from the geographic barrier presented by a dam structure. While some dams may be navigable during high flows, many more pose a permanent physical barrier for species migration and gene flow. Mussel diversity upstream and downstream of a dam may change for different reasons. Upstream of a dam, the composition of a mussel community shifts from riffle-run type species to pool type species. The shift in species is also determined by the type of fish that can migrate and/or survive upstream of a dam. For example, a mussel requiring a riffle-dwelling fish host that is trapped upstream of a dam where riffle habitat is destroyed will be eliminated upstream of the dam where that fish species no longer survives.

The converse of the fish habitat being destroyed upstream of the dam is the mussel habitat that is often destroyed many miles downstream of a dam resulting from instability in the substrate as well as changes to the physical characteristics of the water. Using the previous example of riffle-dwelling fish species, in the downstream example those fish may be able to survive, but the temperature of the water released from the dam may be too cold or too swift to support a sustainable mussel population. The intricate relationship between mussels and fish hosts makes the actual cause and effect of depauperate communities of each species inextricable.

High-lift dams prevent annual upstream migrations of *A. chrysochloris* (Fuller 1974). Ten such dams, constructed during this century, occur between the Blue River study sites and the confluence of the Ohio River with the Mississippi River. It is likely that numbers of this species in the Ohio River have declined as a result of the reduction in skipjack herring. A study of *Fusconaia ebena* in the upper Mississippi River found that the decline in this species coincided with the prevention of skipjack herring moving upstream of Lock and Dam 19 in Keokuk, Iowa (Coker 1930, Kelner and Sietman 2000). Figure 6 presents a compilation of *E. crassidens* locations from surveys conducted by the Indiana Department of Natural Resources (Fisher 2006). All location points, shown as circles on the map, represent findings from 1980 to present. The key denotes "Live" events as those recording living species, "FD" as freshly dead individuals, "WD" as weathered dead shells; and "SF" as sub-fossilized shells. The black circles shown along the southern edge of Indiana (Figure 6) represent collections from the Ohio River made by Williams and Shuster in 1989 and Clarke in 1994 (Clarke 1995).

Williams Dam is the largest dam on the East Fork of the White River and is the only dam on that river that inhibits fish movement upstream (Figure 6). It might be possible for a few fish to breach it, but only during about the largest 10 floods on record, when the dam is completely underwater. *Alosa chrysochloris* is no longer found above Williams Dam, though other likely host candidates do occur above and below this dam, including; *Lepomis cyanellus, Lepisosteus osseus, Notropis atherinoides, Ictiobus cyprinellus*, and *Luxilis* sp. (Fisher, personal communication). The upstream-most black dot on the East Fork of the White River (Figure 6) represents the location of Williams Dam.

The historic versus current distribution of *E. crassidens* represents a change in the network of freshwater streams draining a significant portion of the eastern United States. While *E. crassidens* does not appear to be highly susceptible to pollution, given its abundance during the post-depression era of land clearing and associated high soil loss from agricultural practices, the modification of large free-flowing rivers has altered the large river habitat such that *E. crassidens* cannot effectively recruit offspring.

The plight of freshwater mussels in the Mississippi river drainage can be directly linked to navigational channels and their degree of flexibility in accommodating ecosystem functions in tandem with economic necessity. A program from the 1929 dedication of the Ohio River's completed canalization summed up the purely economical drivers that began the alteration of not only the Ohio River, but its numerous tributary streams calling the Ohio River in its natural state one of the world's great rivers. Almost a century later ecological function remains disconnected from economics. Such ideologies must be softened if the 70% of imperiled freshwater mussels in the United States are going to rebound to self-sustaining populations.

The Blue River enters the Ohio River at river mile 660. McAlpine Lock and Dam is the nearest upstream dam, at river mile 604.4. Cannelton Lock and Dam is downstream of the Blue River at river mile 720.7. McAlpine Lock and Dam opened to navigation in 1961, updating dam 41, which was originally installed in 1911. Cannelton Lock and Dam opened in 1975, replacing dams 43 to 45, which were opened in 1921, 1926, and 1927, respectively. Both McAlpine and Cannelton have two lock chambers. While all of these dams maintained a 9-foot navigation channel, updates to the early lock and dam system allowed for increased distance between locks and increased lock length, which allowed longer tows to efficiently clear the dams (United States Army Corps of Engineers 1979).

The median age of *E. crassidens* collected in the Blue River is 56 years. An important note here is that only shells of *E. crassidens* that perished in the laboratory or were found as freshly dead individuals in the Blue River were used for the thin-sectioning analysis. While no *E. crassidens* were found in the 2003 or 2008 surveys with smaller shell dimensions than those currently held under laboratory conditions, some of the smaller shells held in the laboratory were not thin-sectioned. No animals have been sacrificed in this study as their rarity in the Blue River precludes this action. The age cohort may be slightly skewed, but the median age correlated with the shell dimensions reflects the trend of shell dimensions in the Blue River population.

The depth of the Ohio River was significantly altered in the 1920s with installation of the new high-lift dams. This perturbation to the river's ecosystem function would reflect itself in *E. crassidens* over eighty years of age being found in the Blue River. The Cannelton Lock and Dam opened to river traffic in 1975, but construction on this structure began ten years previous in 1965. However, the median age of 56 years shows that the majority of reproduction occurred in the mid-1950s.

Elliptio crassidens is a large river mussel with an estimated maximum life span of 60 to 80 years. The fact that only one individual older than 55 years is found in the thin-sectioning sample is not surprising. What is alarming is the absence of a young cohort of *E. crassidens*. The absence of young mussels is coincident with the construction of the "improved" Cannelton Lock and Dam system that not only created a deeper Ohio River pool between Cannelton and the McAlpine Lock and Dam, but also served as a greater barrier to fish migration. If the fish host for *E. crassidens* is a migratory species, like skipjack herring (*A. chrysochloris*), the higher dam may have prevented the mass migration of these fish. The blocked migration of skipjack herring and the change in habitat on the upper Mississippi River by hydroelectric dams has been implicated in the eradication of the ebony shell, *Fusconaia ebena*, above the dams (Fuller 1974, 1980).

The lower the Blue River is slackwater for approximately 13 miles upstream of its confluence with the Ohio because of the influence from the Cannellton Lock and Dam. This habitat has not been surveyed for mussels, but likely contains those species that are mud and silt-tolerant. While *E. crassidens* may not occur in this stretch of the river, the backwater habitat is not considered to be a deterrent to upstream fish migration.

Aside from geographic limits to their upstream migration, temperature and habitat changes in the lake-like pools between the dams may also alter temperature sensitive migrations. Any one of these factors could have halted recruitment in *E. crassidens* in the Blue River, but it is more likely a combination of factors that have caused a failure in the reproductive success of the Blue River population.

CONCLUSION

The difficulty in determining a fish host for this species, as well as the unpredictability in finding larvae to use in fish host tests helps to explain the lack of literature on host species of *E. crassidens*. The information presented herein should provide a helpful starting point for future investigations into fish host studies for the elephantear.

The skipjack herring, *Alosa chrysochloris,* spawns from May to June in the Upper Mississippi drainage. This spawning period roughly mimics the larval release of *E. crassidens* larvae. Water temperature is the likely signal to *A. chrysochloris* to begin their spring spawning run upstream in large rivers and into tributaries. The operation of high lift dams on the Ohio and Upper Mississippi Rivers modifies habitat and associated water quality parameters, such as water temperature. If *A. chrysochloris* is the fish host for *E. crassidens* and it is not co-occurring with this mussel when it releases larvae then no recruitment can occur. The *E. crassidens* population in the Blue River has proven reproductively viable in a laboratory setting. Mussel species adapted to medium-river fish species are reproducing successfully in the Blue River with 11 species demonstrating recruitment within the past 10 years. These two factors, the reproductive viability of elephant ear from the Blue River and the recruitment of certain mussel species, indicate a break in the life cycle of this and other large river mussel species in the river. The reduction in population size of *A. chrysochloris* in the Ohio River and the interrupted migration patterns of those that do persist could be a possible implication in the lack of reproduction in the Blue River *E. crassidens* population as well as those in other streams of the Interior Basin.

Alosa alabamae is also a large river clupeid that has been entirely eradicated from the Ohio River as a result of dams (Pearson and Krumholz 1984, Pearson and Pearson 1989). While this is not the only potential host for *E. crassidens*, it is quite conceivable that *A. chrysochloris* and *A. alabamae* could both be the fish hosts that allowed the wide distribution of this mussel before navigational improvements on the Ohio River began in the mid-1950's.

RECOMMENDATIONS

Further research coupled with applied conservation is necessary to ensure the continued survival of *E. crassidens*. In the laboratory, improvements in fish host studies should be made while in the field possibilities exist for improving habitat both for *E. crassidens* and fish populations. The following is a list of action items that are a natural continuation of this initial study.

1. The fish host of the elephantear needs to be identified in a laboratory setting. Using the fish host results from this study along with the species list for the Kentucky Lake canal, where the elephantear is recruiting, should improve the chances of determining potential fish hosts for *Elliptio crassidens*. While overlap in range did not appear to be a good predictor of fish host potential, these overlap maps can be used as a guide to eliminate non-hosts in further trials. The value in these maps may be the apparent lack of overlap or low percentage overlap that elucidates the low probability of mussels encountering the correct fish.

2. Improve methods for holding *Alosa chrysochloris* and other big river species should be undertaken as a research project of its own. In particular, capturing young of the year for big river fishes would provide relatively small individuals that may lend themselves to a laboratory setting. It may be important to collect fish during cold

temperatures; such as late fall, and explore holding methods for the large river fishes to be held in more naturalized holding facilities.

3. Consider habitat alteration that improves fish movement. Further conservation efforts for *Elliptio crassidens* include measures that are good for freshwater aquatic systems as a whole for improved connectivity and ecological function. These include dam removals and/or dam modifications, improved landuse practices and stocking of fish species that are hosts for threatened mussels.

Large scale dam removals are an unlikely occurrence in the Ohio River Basin. However, any efforts at minimizing the effects of dams by returning to more natural flow regimes and re-connecting habitats will positively impact the mussel and fish host interactions in large and small river systems. Installation of fish ladders at Locks and Dams are being conducted by the Army Corps of Engineers and should be promoted to improve habitat connection for large river fishes.

4. Improve water quality. Continued water quality improvements are warranted within the Blue River watershed to increase the likelihood that the system can support the elephantear and to make a case for re-introduction efforts in the future if necessary. Improvements in agricultural practices that reduce silt entering the river from stream bank erosion are necessary. In particular, reforestation of stream banks not only reduces direct erosion, but also shades and cools the stream to maintain cool water temperatures. The Blue River is a spring-fed river so a focus on re-vegetating sinkholes is also a step towards improved water quality. The Blue River remains a viable system because of its spring-fed nature and thus cool water temperatures as well as from the fact that over fifty-percent of the watershed is still in a natural vegetated condition. Efforts to keep forests

intact and to add acreage to the Conservation Reserve Program (CRP) will benefit the Blue River.

5. Introduce skipjack herring back into portions of their historic geographical range. Stocking of *Alosa chrysochloris* above Williams Dam on the White River in Indiana would be a beneficial research project. Sections of this upstream habitat are highly forested and appear intact from a floodplain connectivity perspective. Stocking skipjack herring and then monitoring those fish for encystment of glochidia of multiple mussel species would be beneficial. While stocking is considered a temporary fix, it does have the added bonus of benefitting anglers, which can make it a more attractive practice for state and federal entities.

Further exploration in the lower Blue River's confluence with the Ohio River is warranted. Little is understood of this backwater area's mussel fauna due to the water depth and a lack of specialized equipment employed in searching these types of Indiana waters. Though it is doubtful, it is unknown if the elephantear may be recruiting in this 13 mile section of the Blue River. The fish assemblage in these waters is also not well studied aside from a sport fish angle. Understanding the fish and mussel communities in this section of this stream would prove insightful. Additionally, studying the gills of fish occurring in the lower Blue River coincident with mussel spawning in the Ohio River could shed some light on whether elephantear and other mussel species are being transported from the Ohio River into the Blue River.

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APPENDIX

Figure 1. Study sites, Blue River, Indiana, showing locations of nearest Ohio River Locks and Dams.

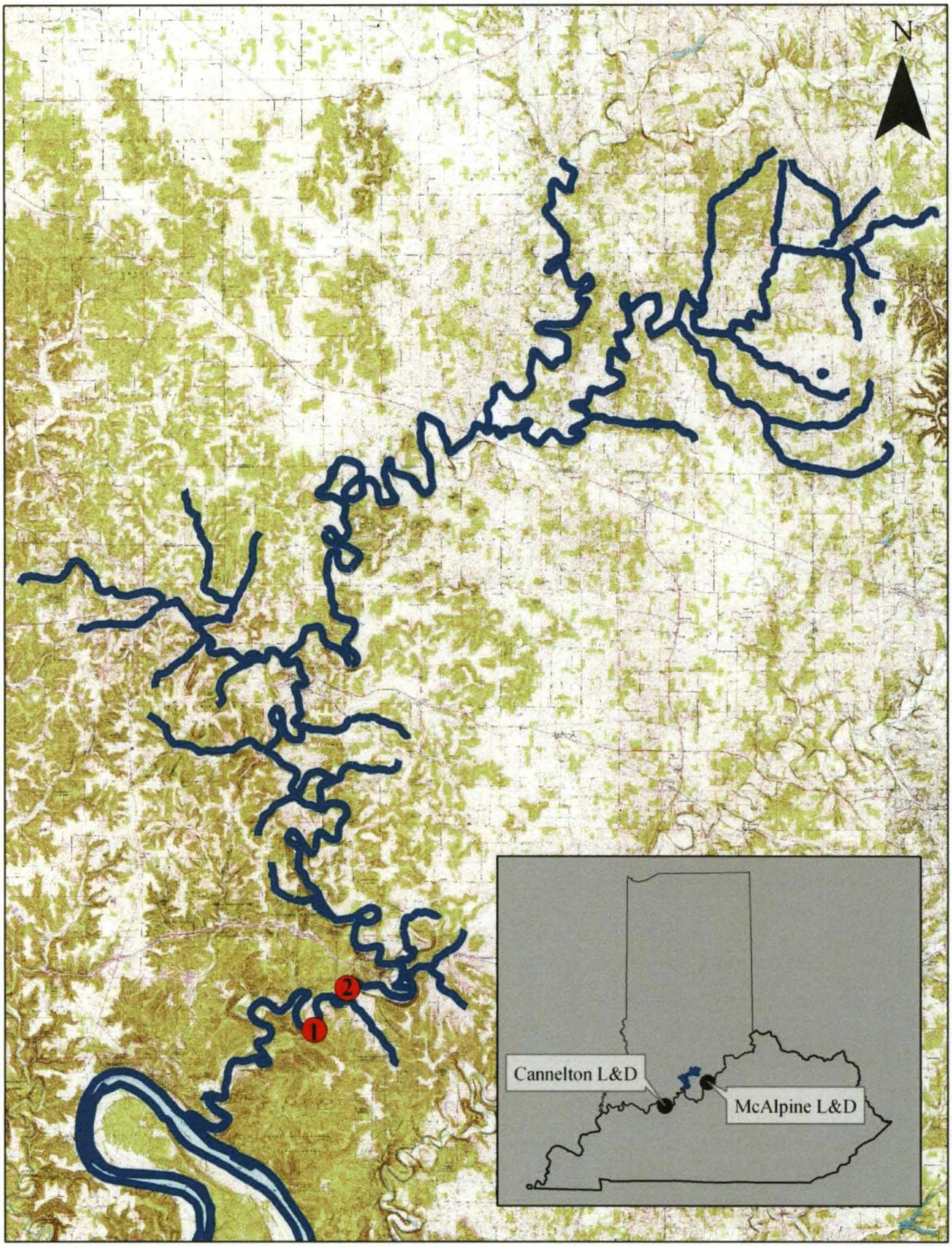


Figure 2. *Elliptio crassidens* Collection Site 1 – Stagestop Campground

Figure 3. *Elliptio crassidens* Collection Site 2 –Highway 4-62 bridge.

Figure 4. Glochidia of *Elliptio crassidens* (Baker 1928).

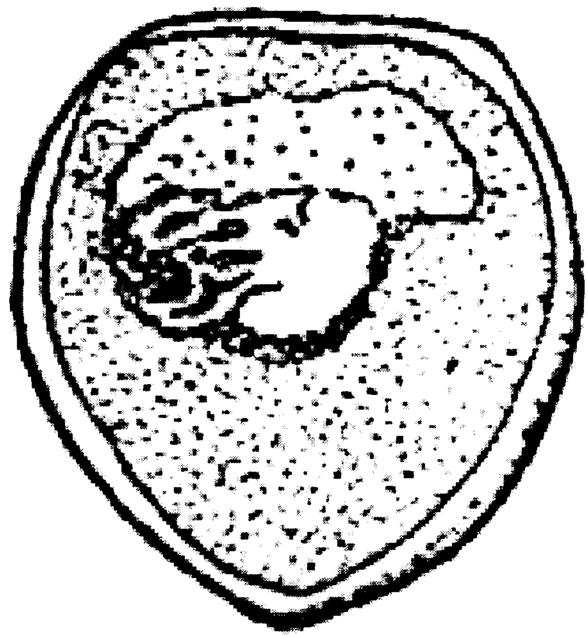


Table 1. Fish species utilized in laboratory infestations on *Elliptio crassidens* from the Blue River, Indiana (names follow those used by Nelson et al. 2004).

Fish Species	No. Fish Tested	Time (d)	No. Juveniles Recovered
Ambloplites rupestris	1	23	0
Amierus natalis	1	23	0
Aplodonitus grunniens	1	23	0
Cyprinella spiloptera	3	23	0
Dorosoma cepedianum	1	23	0
Erimystax amblops	1	23	0
Hybopsis dissimilis	1	23	0
Ictalurus punctatus	1	23	0
Lepomis macrochirus	1	23	0
Lythrurus fasciolaris	3	23	0
Micropterus punctulatus	1	23	0
Moxostoma erythrurum	1	23	0
Notropis buccatus	3	23	0
Notropis rubellus	2	23	0
Percina copelandi	2	23	0
Pimephales notatus	1	23	0
Pimephales promelas	2	23	0
Pomoxis sp.	1	23	0
Pylodictis olivaris	1	23	0
Sander canadensis	1	23	0

Table 2. Fish species utilized in laboratory infestations on *Elliptio crassidens* from Tennessee River, Tennessee (names follow those used by Nelson et al. 2004).

Fish Species	No. Fish Tested	Time (d)	No. Juveniles Recovered
Cyprinella spiloptera	3	24	0
Lythrurus fasciolaris	3	24	0
Micropterus punctulatus	1	23	0
Notropis buccatus	3	24	0
Notropis rubellus	2	24	0
Pimephales promelas	2	23	0
Pomoxis spp.	1	23	0

Table 3. Current and historic fish species from the Blue River, Indiana (Eigenmann and Beeson 1894, Gerking 1945, Janisch 1972, Baker and Forsyth 1986, Carnahan 2000).

Family	Scientific Name	Common Name
Anguillidae – Eels	*Anguilla rostrata*	American eel
Atherinidae - Silversides	*Labidesthes sicculus*	Brook silverside
Catostomidae - Suckers	*Carpiodes carpio*	River carpsucker
	Carpiodes cyprinus	Quillback
	Carpiodes velifer	Highfin carpsucker
	Catostomus commersonii	White sucker
	Erimyzon oblongus	Creek chub sucker
	Hypentelium nigricans	Northern hog sucker
	Ictiobus bubalus	Smallmouth buffalo
	Ictiobus cyprinellus	Bigmouth buffalo
	Minytrema melanops	Spotted sucker
	Moxostoma carinatum	River redhorse
	Moxostoma duquesnei	Black redhorse
	Moxostoma erythrurum	Golden redhorse
	Moxostoma macrolepidotum	Shorthead redhorse
Centrarchidae - Sunfishes	*Ambloplites rupestris*	Rock bass
	Lepomis cyanellus	Green sunfish
	Lepomis gulosus	Warmouth
	Lepomis macrochirus	Bluegill
	Lepomis megalotis	Longear sunfish
	Lepomis microlophus	Redear sunfish
	Micropterus dolomieu	Smallmouth bass
	Micropterus punctulatus	Spotted bass
	Micropterus salmoides	Largemouth bass
	Pomoxis annularis	White crappie
	Pomoxis nigromaculatus	Black crappie
Clupeidae - Shads, Herrings	*Alosa chrysochloris*	Skipjack herring
	Dorosoma cepedianum	Gizzard shad
	Dorosoma petenense	Threadfin shad
Cottidae - Sculpins	*Cottus carolinae*	Banded sculpin
Cyprinidae - Minnows, Shiners	*Campostoma anomalum*	Central stoneroller
	Carassius auratus	Goldfish
	Cyprinella spiloptera	Spotfin shiner
	Cyprinella whipplei	Steelcolor shiner
	Cyprinus carpio	Common carp
	Erimystax dissimilis	Streamline chub
	Hybopsis amblyops	Bigeye chub
	Luxilus chrysocephalus	Striped shiner

Cyprinidae - Minnows, Shiners	*Lythrurus fasciolaris*	Scarlet shiner
	Macrhybopsis storeriana	Silver chub
	Notemigonus crysoleucas	Golden shiner
	Notropis atherinoides	Emerald shiner
	Notropis blennius	River shiner
	Notropis boops	Bigeye shiner
	Notropis buccatus	Silverjaw minnow
	Notropis stramineus	Sand shiner
	Notropis photogenis	Silver shiner
	Notropis rubellus	Rosyface shiner
	Notropis volucellus	Mimic shiner
	Opsopoeodus emiliae	Pugnose minnow
	Phenacobius mirabilis	Suckermouth minnow
	Phoxinus erythrogaster	Southern redbelly dace
	Pimephales notatus	Bluntnose minnow
	Pimephales promelas	Fathead minnow
	Semotilus atromaculatus	Creek Chub
Esocidae - Pickerels, etc.	*Esox americanus*	Redfin Pickerel
Fundulidae - Topminnows, Killifish	*Fundulus notatus*	Blackstripe topminnow
Hiodontidae - Goldeye, Mooneye	*Hiodon tergisus*	Mooneye
Ictaluridae - Catfishes	*Ameiurus melas*	Black bullhead
	Ameiurus natalis	Yellow bullhead
	Ameiurus nebulosus	Brown bullhead
	Ictalurus punctatus	Channel Catfish
	Noturus flavus	Stonecat
	Noturus miurus	Brindled madtom
	Pylodictis olivaris	Flathead catfish
Lepisosteidae - Gars	*Lepisosteus osseus*	Longnose gar
Petromyzontidae - Lampreys	*Ichthyomyzon unicuspis*	Silver lamprey
Percichthyidae - Temperate basses	*Morone chrysops*	White bass
	Morone saxatilis	Striped bass
Percidae - Darters, Perches, Sauger	*Etheostoma blennioides*	Greenside darter
	Etheostoma caeruleum	Rainbow darter
	Etheostoma camurum	Bluebreast darter
	Etheostoma flabellare	Fantail darter
	Etheostoma maculatum	Spotted darter
	Etheostoma nigrum	Johnny darter
	Etheostoma spectabile	Orangethroat darter
	Etheostoma variatum	Variegate darter

Percidae - Darters, Perches, Sauger	*Etheostoma zonale*	Banded darter
	Percina caprodes	Logperch
	Percina phoxocephala	Slenderhead darter
	Percina sciera	Dusky darter
	Sander canadensis	Sauger
Sciaenidae - Drums	*Aplodinotus grunniens*	Freshwater drum

Table 4. Dimensions (micrometers) of larval Elliptio crassidens from the Blue River, Indiana; 2007.

	N	Height (mm)	Length (mm)	Hinge Length (mm)
		162.50	150.00	75.00
		162.50	150.00	50.00
		159.40	150.00	50.00
		175.00	143.75	50.00
		168.75	143.75	56.25
		162.50	150.00	50.00
		162.50	156.25	56.25
		162.50	150.00	50.00
		159.40	150.00	50.00
		162.50	150.00	56.25
		163.76	149.38	54.38
Mean	**10**	**180.13**	**164.31**	**59.81**
SD		**4.46**	**3.37**	**7.42**

Table 5. Shell dimensions (millimeters) for *Elliptio crassidens* collected from the Blue River, Indiana; 2006. N=35.

	Length	Height	Width
	93.04	90.18	58.71
	95.53	88.86	59.99
	96.40	86.83	61.87
	106.77	80.86	53.43
	108.61	75.84	51.22
	110.92	82.75	51.32
	111.20	77.42	54.29
	113.25	80.93	55.96
	114.52	80.27	53.12
	117.85	78.37	57.18
	119.63	87.76	56.98
	119.91	87.26	69.04
	120.47	84.02	61.41
	121.17	79.01	54.19
	121.40	89.89	62.13
	122.94	83.22	54.21
	123.12	82.00	54.93
	124.44	92.97	62.80
	125.70	88.98	49.46
	127.37	88.33	60.07
	127.68	84.56	51.75
	133.29	84.93	51.72
	133.37	86.55	59.08
	134.04	78.67	51.64
	134.05	91.83	54.74
	135.39	94.65	59.16
	135.42	91.18	64.93
	135.58	92.24	60.64
	137.84	93.30	58.31
	138.53	98.47	66.78
	140.67	85.56	56.87
	141.48	94.61	62.75
	141.70	92.22	56.16
	142.42	94.18	62.97
	143.38	95.24	58.95
Mean	**124.26**	**86.97**	**57.68**
SD	**13.85**	**5.91**	**4.78**

Figure 5. Age of *Elliptio crassidens* from the Blue River, Indiana that were utilized in thin-sectioning.

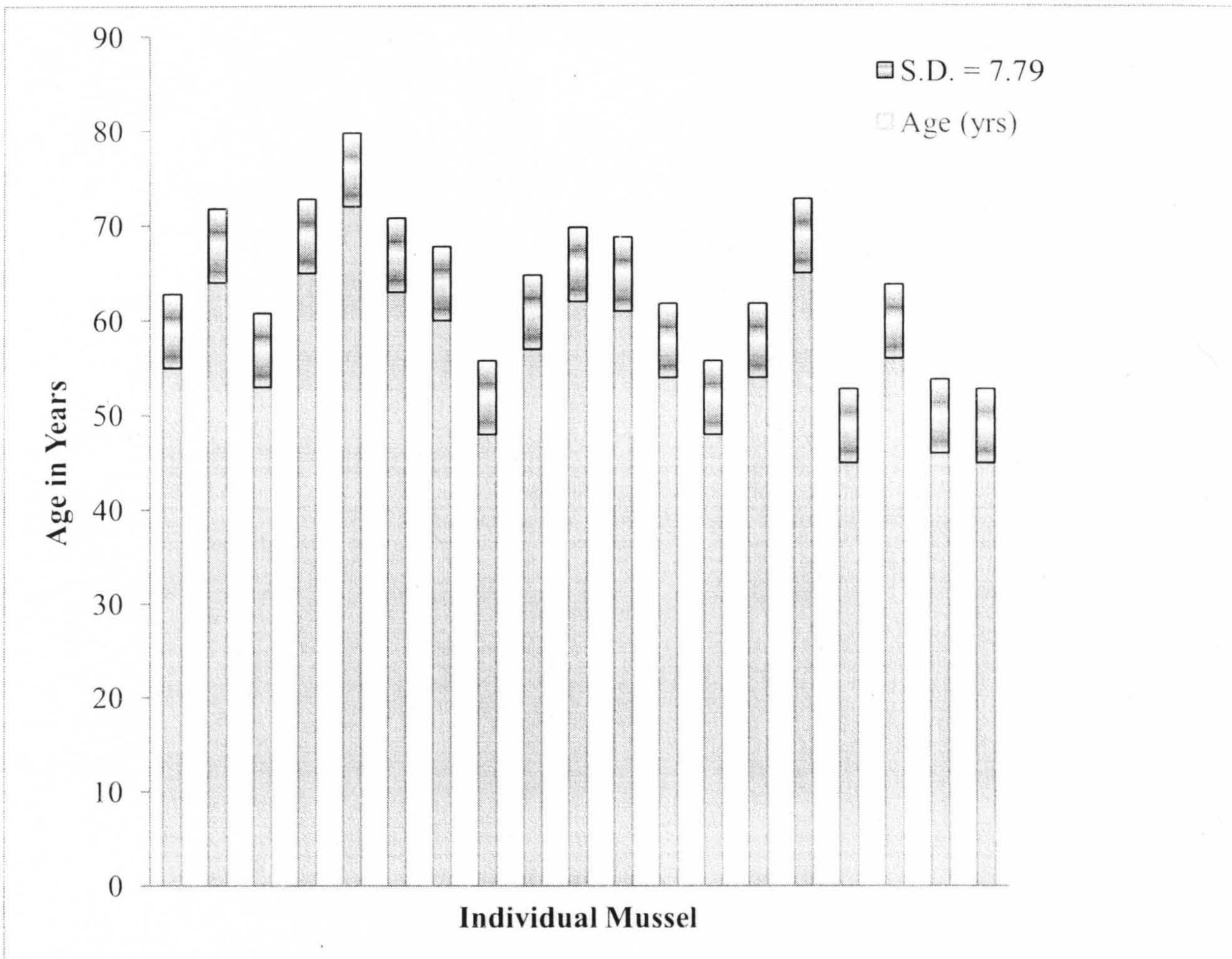


Figure 6. *Elliptio crassidens* distribution in Indiana from Fisher (personal communication). Points represent collections from 1980 to 1980 to present.

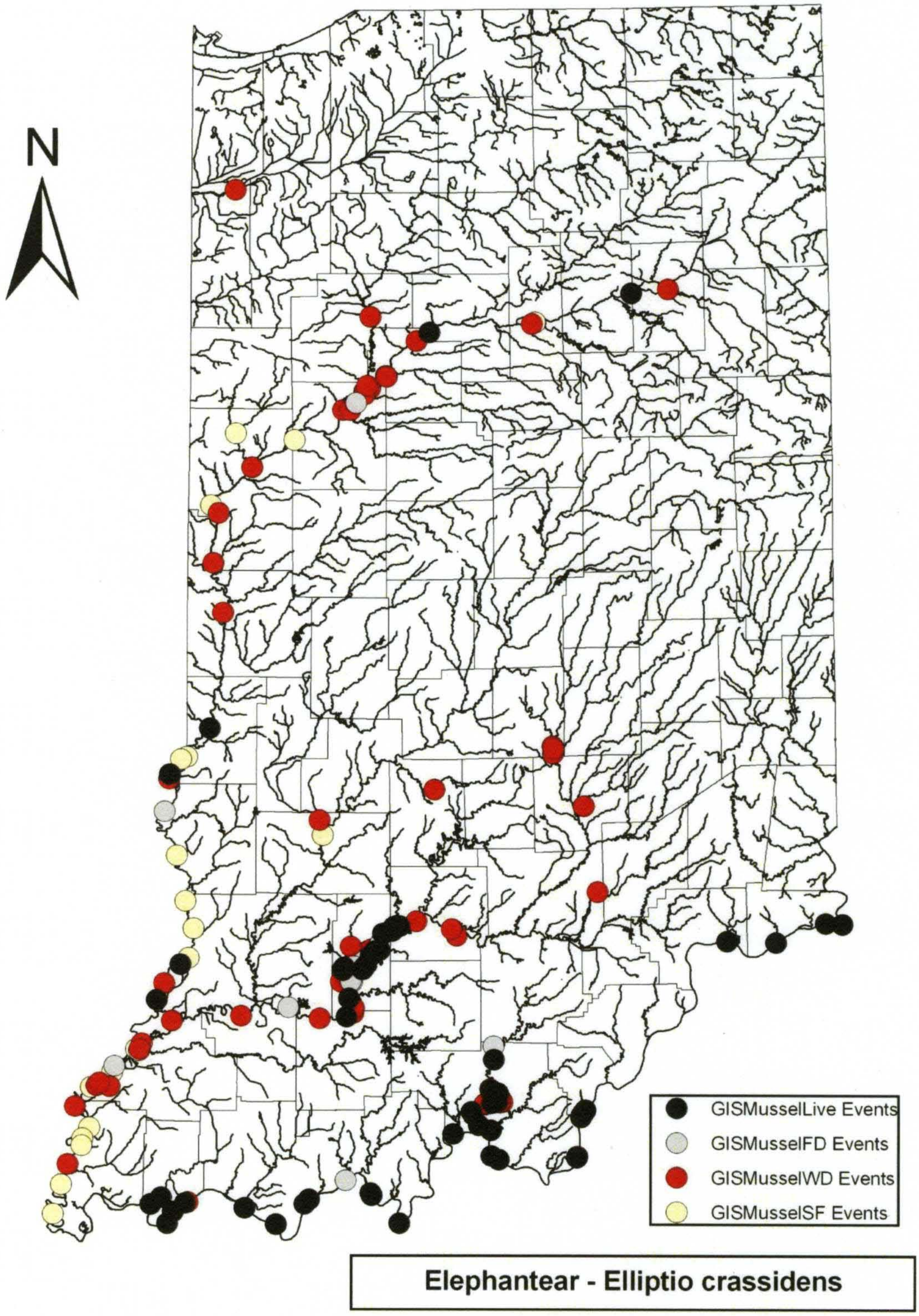


Table 6. Range-wide state status of *Elliptio crassidens* (NatureServe 2009).

State/Province	State/Province Status
Alabama	Secure/S5
Florida	Not Ranked/SNR
Georgia	Secure/S5
Illinois	Imperiled/S2
Iowa	Imperiled/S2
Kentucky	Presumed Extirpated/SX
Louisiana	Imperiled/S2
Minnesota	Critically Imperiled/S1
Mississippi	Apparently Secure/S4
Missouri	Critically Imperiled/S1
Ohio	Critically Imperiled/S1
Oklahoma	Possibly Extirpated/SH
Pennsylvania	Presumed Extirpated/SX
Tennessee	Secure/S5
Virginia	Critically Imperiled/S1
West Virginia	Imperiled/S2
Wisconsin	Critically Imperiled/S1
Ontario	Not ranked/SNR
Quebec	Imperiled/S2

Table 7. Fish species list for Kentucky and Barkley Lake canal, Kentucky (Rister 2007).

Scientific Name	Common Name
Alosa chrysochloris	Skipjack herring
Ameiurus melas	Black bullhead
Ameiurus natalis	Yellow bullhead
Catostomus commersonii	White sucker
Dorosoma cepedianum	Gizzard shad
Etheostoma smithi	Slabrock darter
Ictalurus furcatus	Blue catfish
Ictalurus punctatus	Channel catfish
Ictiobus spp.	Buffalo
Lepisosteus spp.	Gar
Lepomis cyanellus	Green sunfish
Lepomis gulosus	Warmouth
Lepomis macrochirus	Bluegill
Lepomis megalotis	Longear sunfish
Micropterus punctulatus	Spotted bass
Morone chrysops	White bass
Morone mississippiensis	Yellow bass
Notropis atherinoides	Emerald shiner
Percina caprodes	Logperch
Percina shumardi	River darter
Polyodon spathula	Paddlefish
Pomoxis spp.	Crappie
Pylodictis olivaris	Flathead catfish
Sander canadensis	Sauger

Table 8. Current and historic mussel species from the Blue River, Indiana 1903–2003 (Daniels 1903, Goodrich and van der Schalie 1944, Weilbaker et al. 1985, Baker and Forsyth 1986, Sietman and Hauswald 2004).

Species	Common	Global Status	Current(C)/ Historic(H)
Actinonaias ligamentina	mucket	G5	C
Alasmidonta marginata	elktoe	G4	H
Alasmidonta viridis	slippershell	G4/G5	C
Amblema plicata	threeridge	G5	C
Anodonta suborbiculata	flat floater	G5	C
Cyclonaias tuberculata	purple wartyback	G5	C
Elliptio crassidens	elephantear	G5	C
Elliptio dilatata	spike	G5	C
Epioblasma triquetra	snuffbox	G3	H
Fusconaia flava	Wabash pigtoe	G5	C
Lampsilis cardium	plain pocketbook	G5	C
Lampsilis fasciola	wavyrayed lampmussel	G4	C
Lampsilis siliquoidea	fatmucket	G5	C
Lampsilis teres	yellow sandshell	G5	H
Lasmigona complanata	white heelsplitter	G5	C
Lasmigona costata	flutedshell	G5	C
Leptodea fragilis	papershell	G5	C
Ligumia recta	black sandshell	G5	C
Ligumia subrostrata	pondmussel	G4G5	H
Megalonaias nervosa	washboard	G5	C
Obliquaria reflexa	threehorn wartyback	G5	C
Obovaria retusa	ring pink	G1	H
Obovaria subrotunda	round hickorynut	G4	H
Pleurobema clava	clubshell	G2	H
Pleurobema cordatum	Ohio pigtoe	G3	C
Pleurobema rubrum	pyramid pigtoe	G2	H
Pleurobema sintoxia	round pigtoe	G4	H
Potamilus alatus	pink heelsplitter	G5	C
Potamilus ohiensis	pink papershell	G5	C
Ptychobranchus fasciolaris	kidneyshell	G4G5	C
Pyganodon grandis	giant floater	G5	C
Quadrula metanevra	monkeyface	G4	H
Quadrula nodulata	wartyback	G4	H
Quadrula pustulosa	pimpleback	G5	C
Quadrula quadrula	mapleleaf	G5	H
Simpsonaias ambigua	salamander mussel	G3	C
Strophitus undulatus	creeper	G5	H
Toxolasma lividus	purple liliput	G2	H
Tritogonia verrucosa	pistolgrip	G4	C
Truncilla donaciformis	fawnsfoot	G5	C
Truncilla truncata	deertoe	G5	C
Villosa iris	rainbow	G5	C
Villosa lienosa	little spectaclecase	G5	H
Corbicula fluminea	Asian clam	G5-Exotic	

Table 9. Length frequency (%) distribution for *Elliptio crassidens* collected from the Blue River, southern Indiana (Sietman and Hauswald, 2004).

Length (mm)	101-110	111-120	121-130	131-140	141-150	(n)	Mean
% Collected Individuals	1.7	23.0	40.0	28.3	6.7	60	127.3

Figure 7. Total known distribution of *Elliptio crassidens* distribution based upon USGS 6-Digit Hydrologic Unit Code.

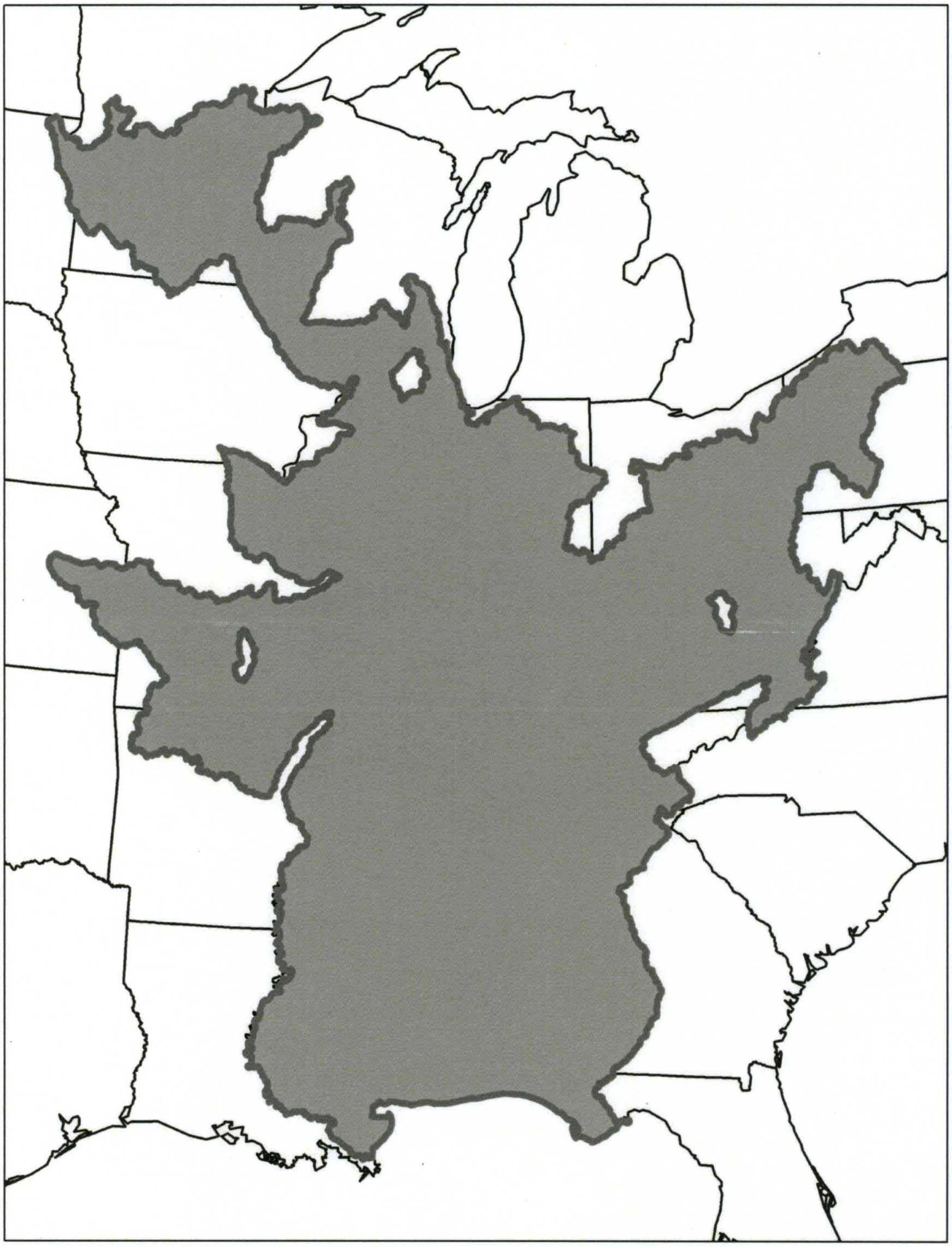


Figure 8. Current and historic distributional overlap between *Elliptio crassidens* and *Alosa chrysochloris*

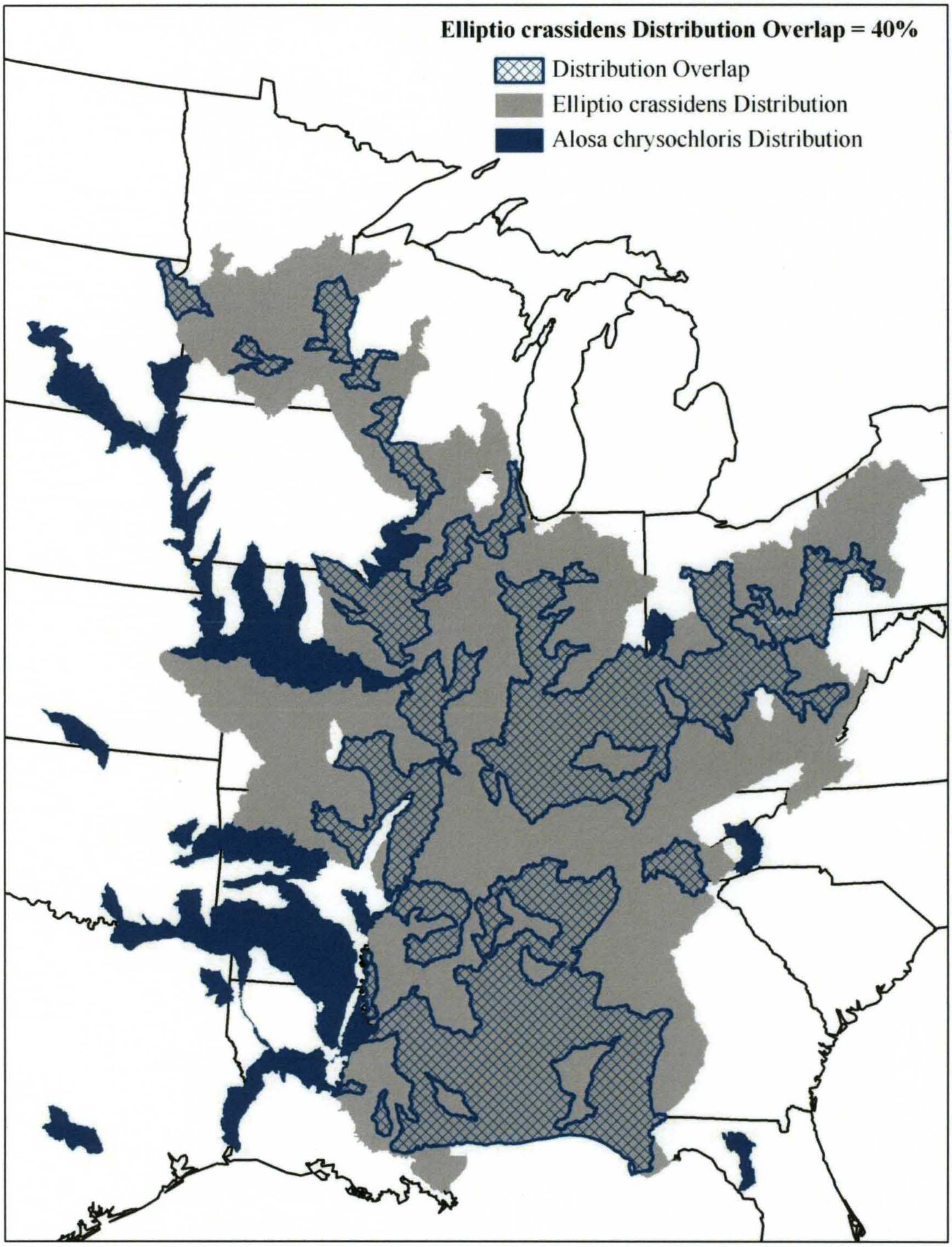


Figure 9. Current and historic distributional overlap between *Elliptio crassidens* and *Lepomis cyanellus*

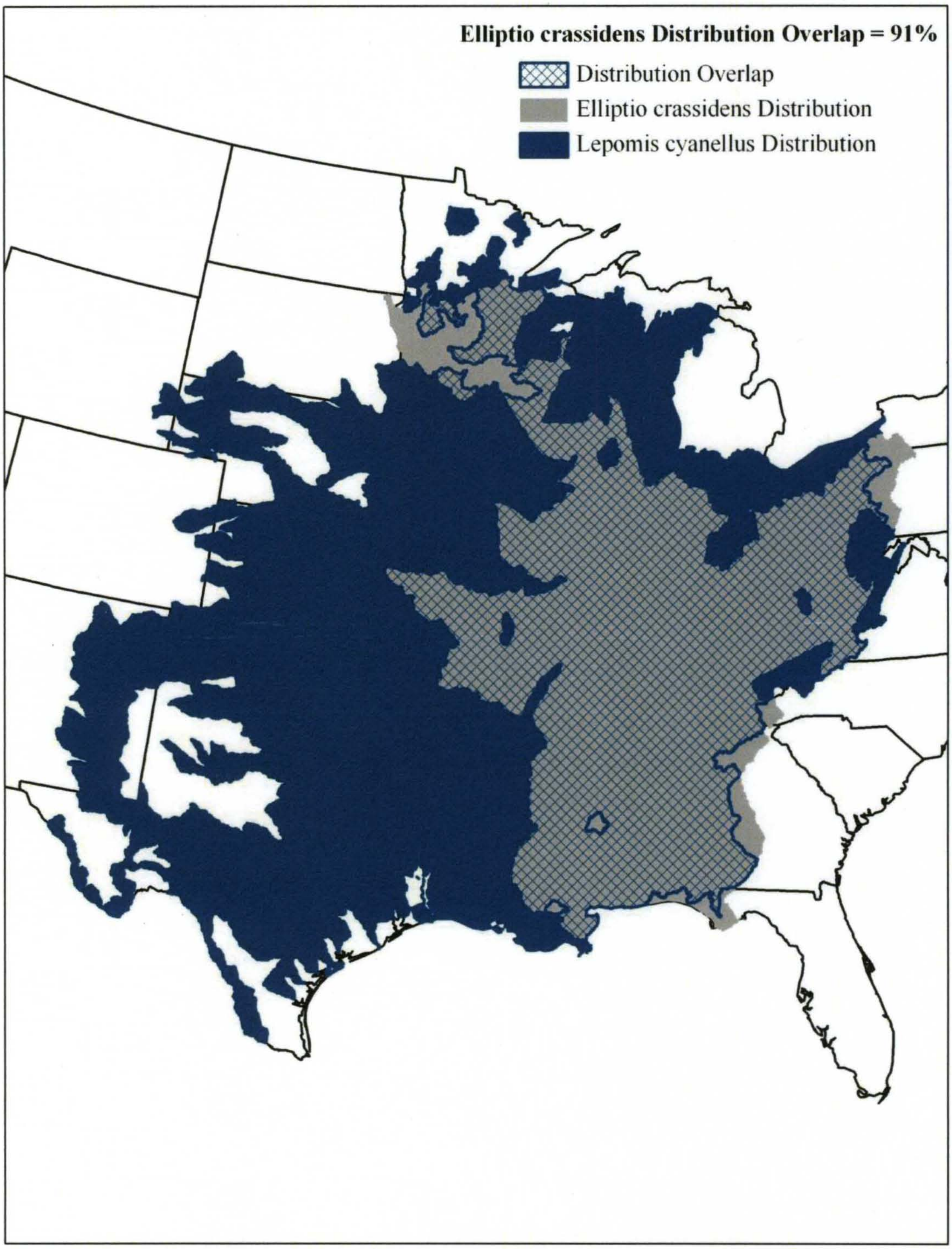


Figure 10. Current and historic distributional overlap between *Elliptio crassidens* and *Lepisosteus osseus*

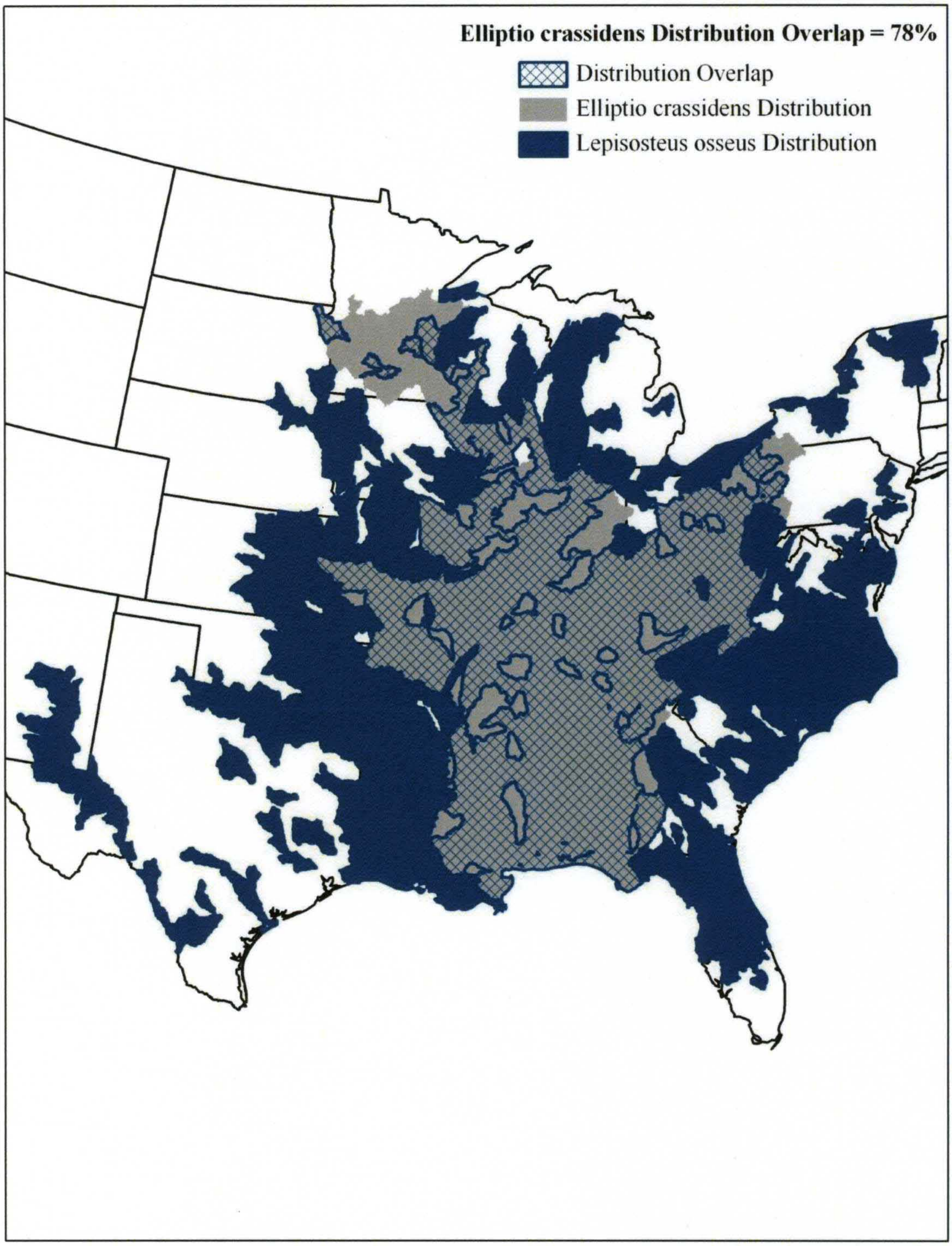


Figure 11. Current and historic distributional overlap between *Elliptio crassidens* and *Notropis atherinoides*

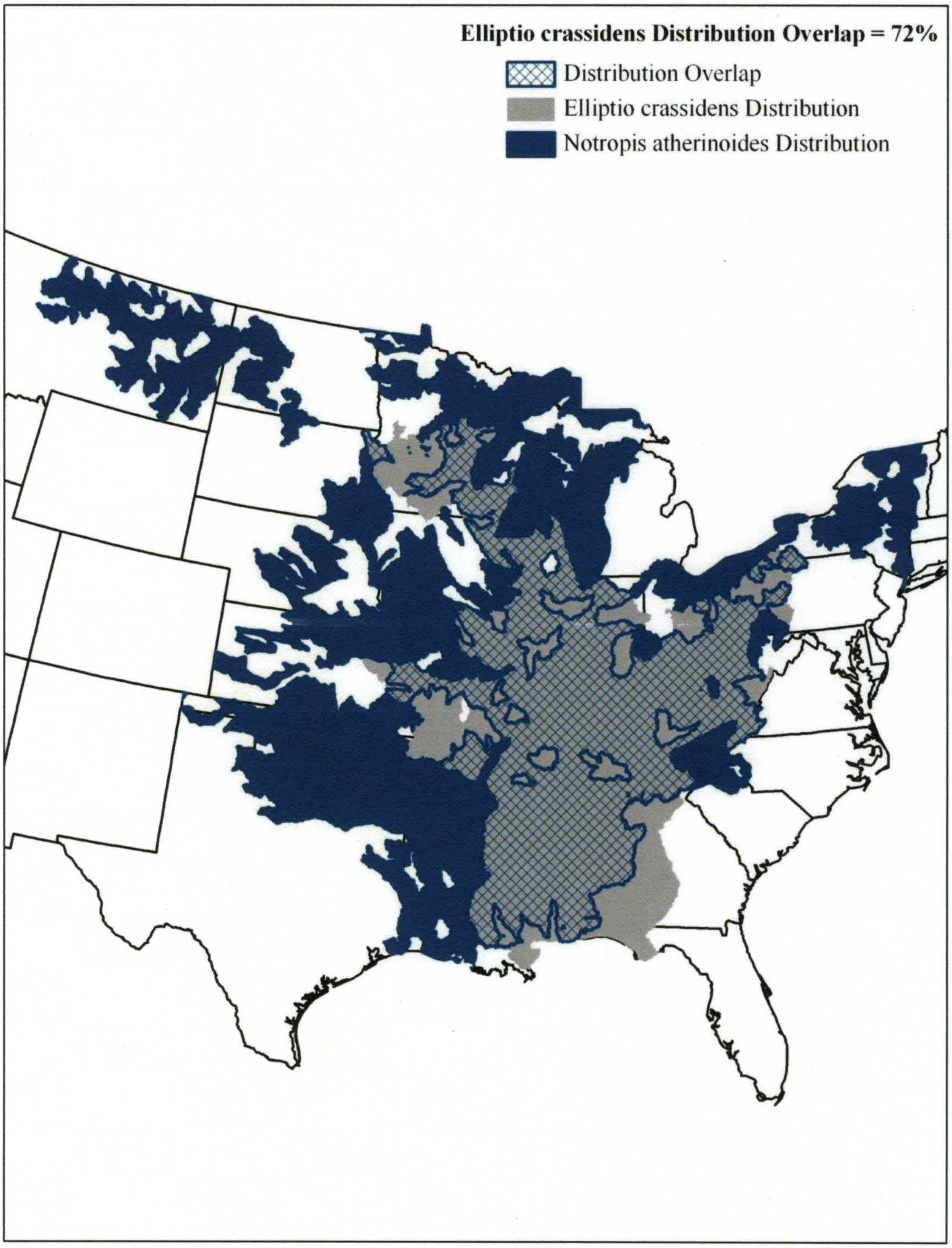


Figure 12. Current and historic distributional overlap between *Elliptio crassidens* and *Luxilus chrysocephalus*

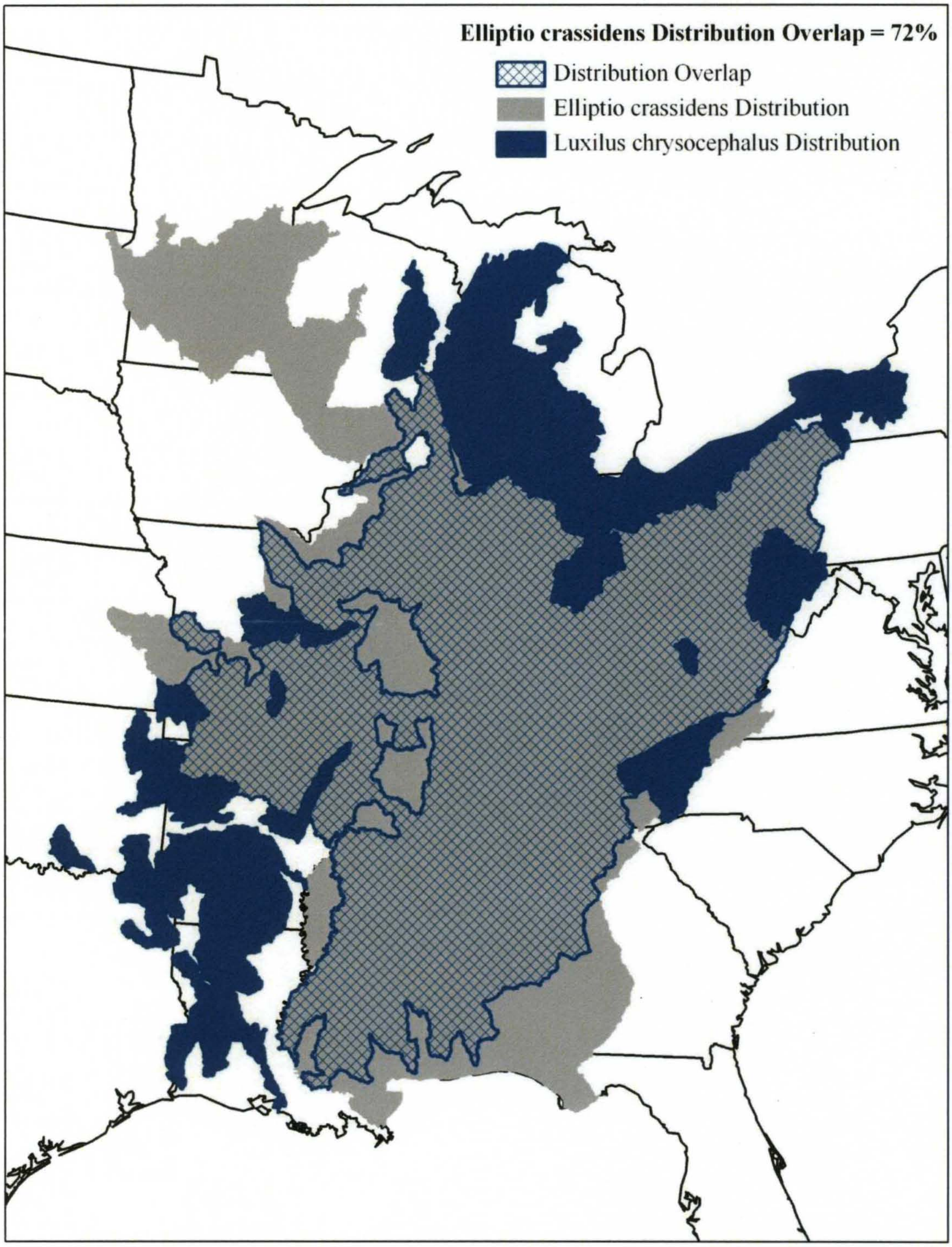


Figure 13. Current and historic distributional overlap between *Elliptio crassidens* and *Macrhybopsis storeriana*

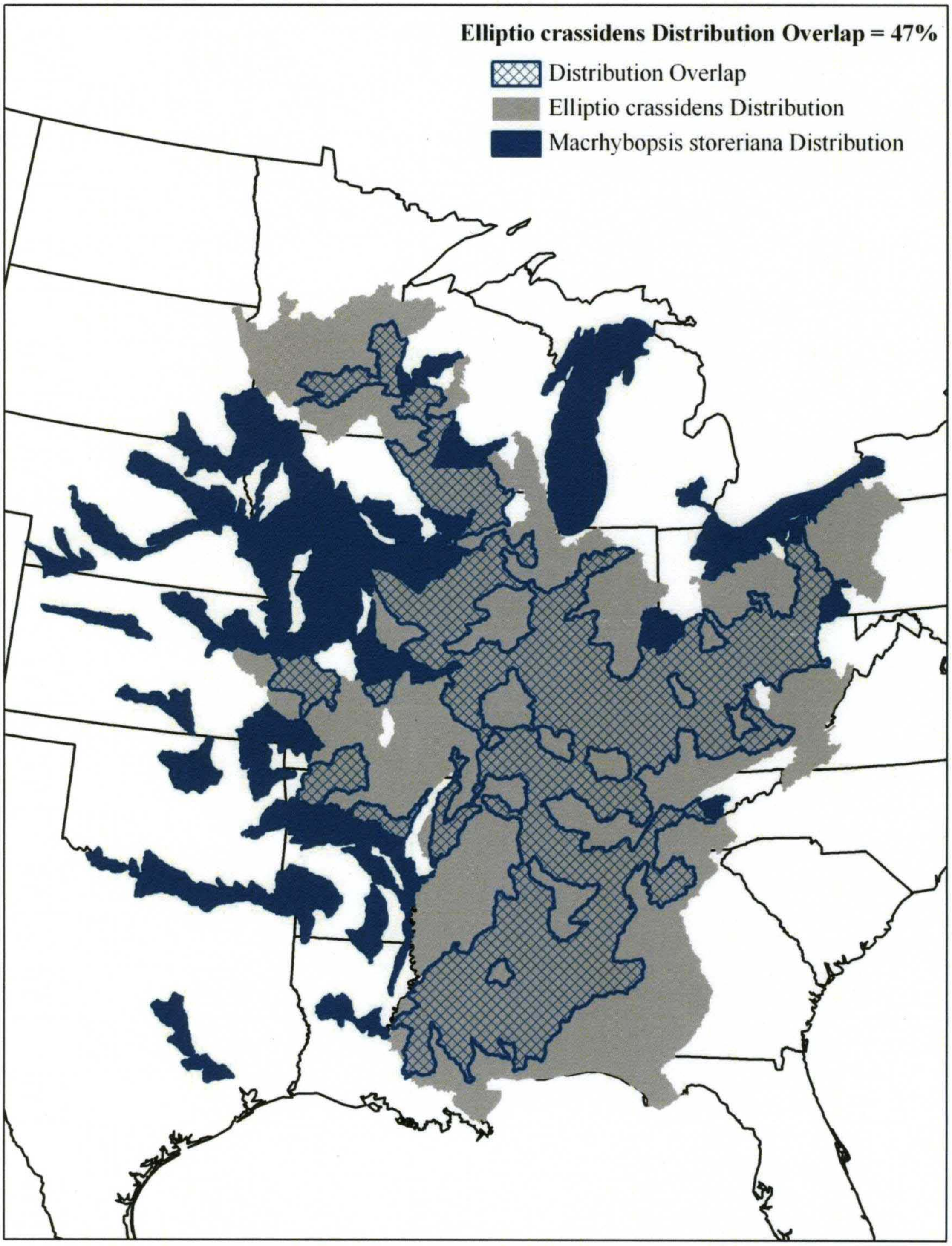


Figure 14. Current and historic distributional overlap between *Elliptio crassidens* and *Ictiobus cyprinellus*

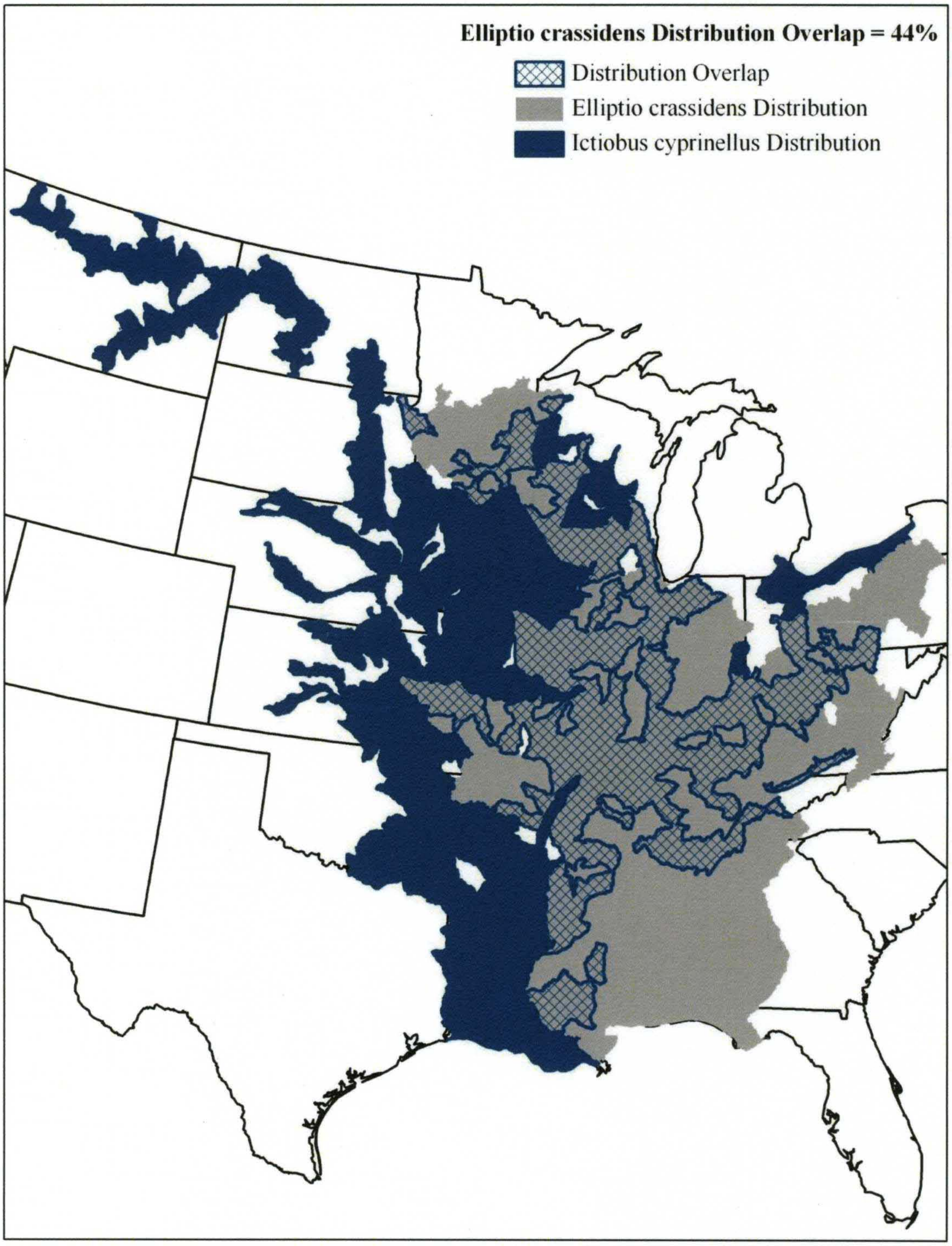


Figure 15. Current and historic distributional overlap between *Elliptio crassidens* and *Polyodon spathula*

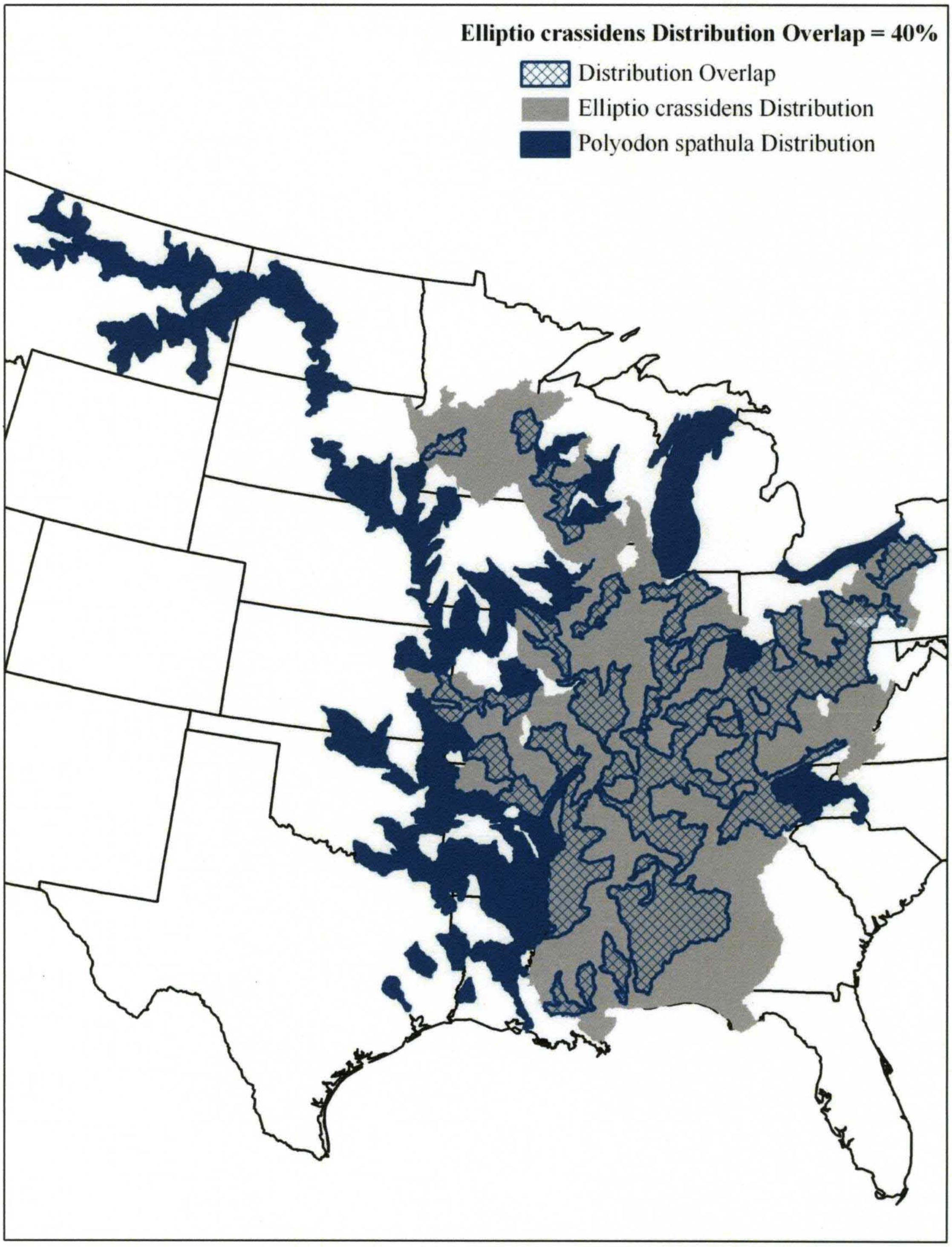
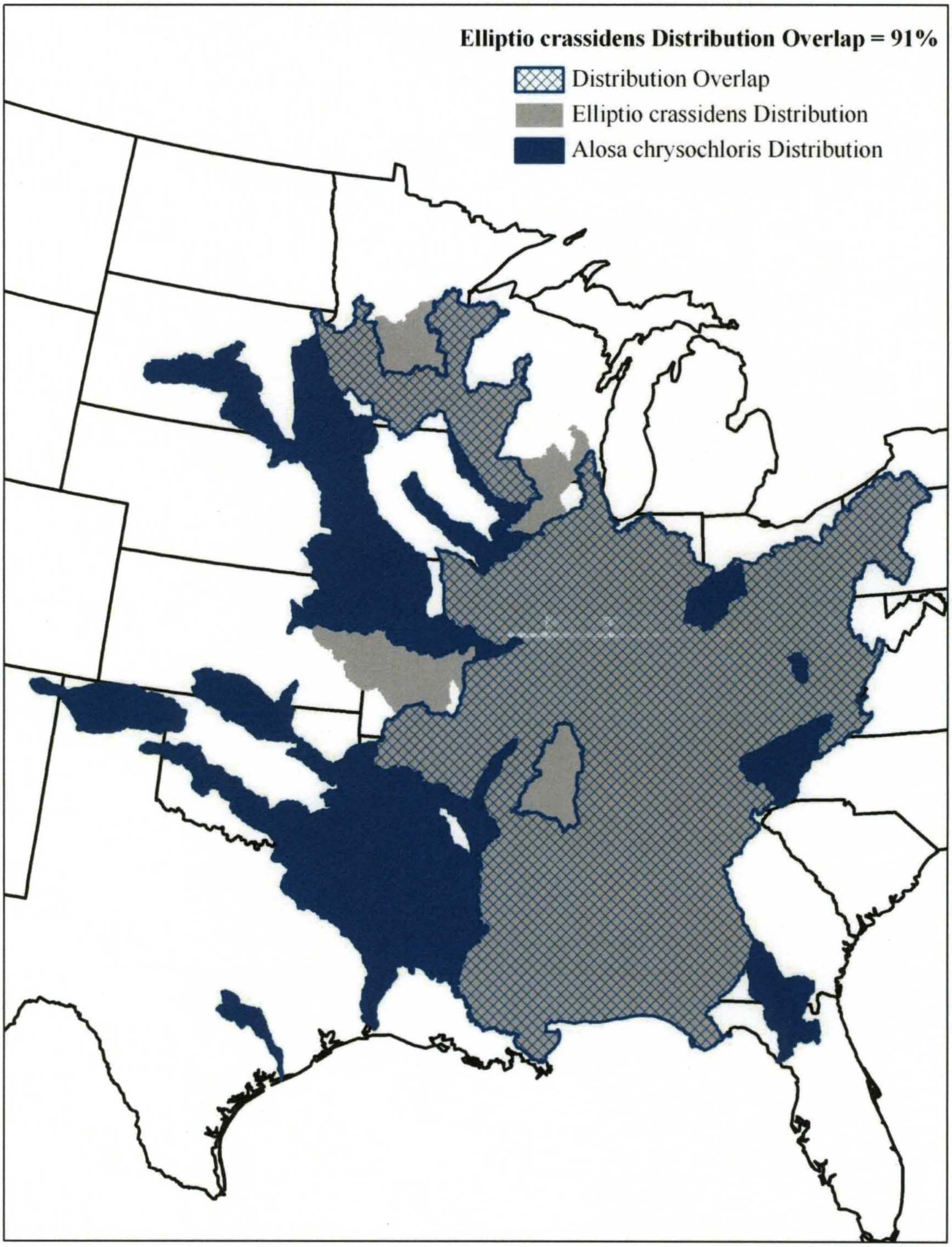


Figure 16. Corrected current and historic distributional overlap between *Elliptio crassidens* and *Alosa chrysochloris*



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