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Life History Observations and Determination of Potential Host Fish Species for Chipola Slabshell, Elliptio chipolaensis

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Life History Observations and Determination of Potential Host Fish Species for Chipola Slabshell, *Elliptio chipolaensis*

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Lisa Preister

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I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

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Abstract

North America is home to nearly 300 species of freshwater mussels with approximately 80% being found in the southeastern United States. The Federally threatened species, *Elliptio chipolaensis* (Chipola slabshell), is considered an endemic species to the Chipola River Basin in the Florida panhandle. E. chipolaensis glochidia are released from the female in conglutinates The potential fish host species for *Elliptio chipolaensis* are the bluegill and redbreast sunfish. Sixty percent of the bluegill successfully transformed *E. chipolaensis* glochidia into juvenile mussels while 80% of the redbreast sunfish were successful.

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Introduction

The majority of North America's some 300 species of freshwater mussels belong to family Margaritiferidae or Unionidae (Fuller 1974, Brim Box and Williams 2000). Of all North American species, eighty percent are found in the southeastern United States (Brim Box and Williams 2000). However, mussel numbers are declining, both continentally and regionally, as threats to mussel populations are increasing. Within the United States, the U.S. Fish and Wildlife Service currently lists a total of 62 mussels as endangered and 8 species as threatened (USFWS 2005).

Knowledge of freshwater mussels is relatively limited. Around the turn of the Twentieth century there was a push for mussel research, driven by the pearl button industry (Coker et al 1921) and its interest in maximizing the propagation of mussels so that the shells could be used to make buttons. As the industry slowed with the use of plastic for buttons, the demand for mussel research dwindled (Fuller 1974). In the last several decades there has been resurgence in the investigation of mussels, emphasizing their role in ecological systems. Because freshwater mussels are an important part of benthic communities (Williams 1994), understanding their life histories and their relationships with other organisms and their environments is an important link in understanding the health of our streams and waterways. Mussels filter water and are an important food source for other invertebrate and vertebrate species. They are also important indicator species because they cannot quickly move away from a threat and are

susceptible to pollution. Thus, the composition of mussel communities in streams provides a direct indication of overall stream quality.

Silt, from agricultural practices, road construction and other erosion-causing development, is the most significant pollutant for aquatic systems (Clench and Turner 1956, Williams 1994). Because all unionid mussels are mucus-ciliary feeders, filtering phytoplankton and detritus from the water, excessive silt can smother mussels as well as their food sources. Even commonly occurring species are in potential peril from various environmental changes. Endemic species, occurring only in geographically restricted locations, are even more likely to quickly become extinct when their habitats are irreparably changed (Heard 1979). Detrimental human influences on fish communities are also a danger to mussels. Elimination of "pest" fish by the use of chemicals can kill off large numbers of mussels in the same body of water. Invasive fish species that out-compete native species can cause a loss of hosts for mussels. (See the discussion of mussel reproduction below.) This is of greatest concern for those mussel species specialized to parasitize only one or a few hosts (Fuller 1974). Endemic mussel species have a narrower habitat range and probably only one host fish species (Clench and Turner 1956). Loss of that host will result in a functionally extinct population of mussels. Even if there are reproducing individuals in a population, without a fish host to parasitize, the population is unable to survive.

Most freshwater mussels have an unusual method of reproduction (Figure 1). The male mussel releases sperm into the water column and nearby female mussels then draw

Figure 1 Generalized life cycle of unionid freshwater mussels (North Carolina Wildlife Resources Commission 2008).

water, containing sperm, into their gills through incurrent siphons (Figure 2). Eggs are fertilized and held in the demibranchs (Figure 3) or marsupial gills (Heard 1979). Fertilization in unionid mussels occurs in the outer two demibranchs (Coker et al 1921, Heard 1979). During the brooding period, fertilized eggs mature to become glochidia having two valves (shells) with the only developed internal organ being a single adductor muscle, enabling it to close its valves (Coker et al 1921).

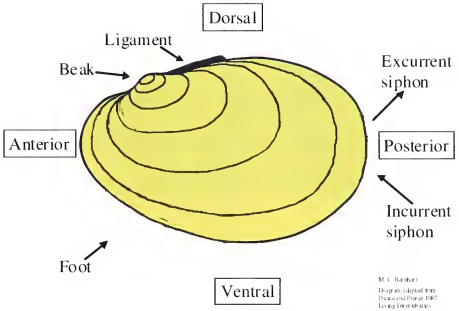

Figure 2 Exterior anatomy of a mussel (Barnhart 2005).

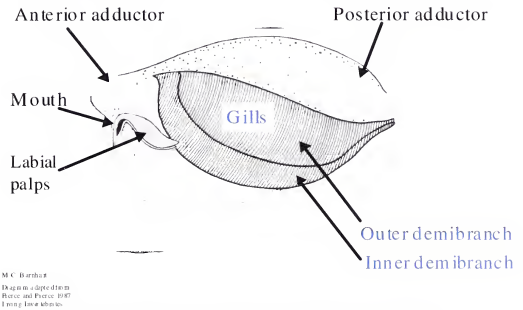

Figure 3 Interior anatomy of a mussel (Barnhart 2005).

The length of time that a mussel carries its glochidia varies from species to species. There are two types of breeders: bradytictic and tachytictic. Bradytictic breeders are long term breeders, carrying their glochidia in the marsupium over the winter months, releasing them in the spring or summer. Tachytictic breeders are short-

term breeders, meaning that they carry their glochidia for a short time, releasing them during that same spring or summer (Coker et al 1921, Burch 1973).

At the end of the brooding period, glochidia are released, through a variety of methods, into the water. They must attach as a parasite to a specific vertebrate host, most frequently a fish, in order to undergo transformation into a juvenile mussel. In some cases glochidia are released directly into the water column through the excurrent siphon. They may also be released in "packets" of glochidia, called conglutinates. Conglutinates often look like a food source to the host fish species. Some species of mussels also have a modified mantle extension that mimics a small fish, luring host fish in closer and increasing the chances that glochidia will successfully attach to a host. Glochidia attach to gills or fins, becoming encysted on the host, where they remain until transformation is complete (Coker et al 1921, Fuller 1974). During transformation, the fish tissue caught within the valves provides nutrients to the glochidia (Lefevre and Curtis 1912). It may take 24 to 48 hours for glochidia to be completely encysted (Coker et al 1921). If they attach to an unsuitable host, glochidia will slough off in a few days, suggesting that the development of species-specific fish lures has critical significance in survival of the mussel species.

Dispersal of juveniles within a stream system is a function of the host's range, as juvenile mussels will be carried within that range (Fuller 1974). After transformation, a juvenile mussel will excyst from its host and land on the substrate below. At this stage, a juvenile has two adductor muscles, rudimentary internal organs and a foot. It has not

grown considerably but is now able to live independently (Coker et al 1921). However, its survival depends upon a number of factors including: appropriate substrate, no predation, available food sources and suitable water chemistry. Unfortunately, little is known about ecological requirements of most unionid mussels (Williams 1994).

Research continues on the effect that glochidial infestation has on individual fish. It has long been believed that there is little harm done to the fish by the parasitic glochidia but that fish will develop immunity to infestation (Coker et al 1921), preventing glochidia from attaching to an individual host fish that had previously been successful in transforming glochidia to juvenile mussels.

Although the life history of *Elliptio chipolaensis* is poorly studied, comparison to other *Elliptio* species should suggest parallels to *E. chipolaensis*, based on similarities within the genus. In a study on fecundity, *E. arca* was found to brood young along the entire length of the outer pair of gills and to have eggs that were not bound to one another (Haag and Staton 2003). Coker et al (1921) noted that several *Elliptio* species were found to be tachytictic. It is expected that *E. chipolaensis* will have similar reproductive characteristics. In laboratory experiments, *E. pullata* and *E. buckleyi* both successfully transformed into juveniles on both largemouth bass and bluegill. In some cases, species in the same unionid genus have been determined to have similar hosts (Keller and Ruessler, 1997), suggesting that largemouth bass and bluegill may be candidate hosts for *E. chipolaensis*.

Table 1 Some *Elliptio* species and their known hosts.

Mussel	Host Fish	Common Name	Source
Elliptio pullata	*Micropterus salmoides*	Largemouth bass	Keller and
	Lepomis macrochirus	Bluegill	Ruessler 1997
E. buckleyi	*Micropterus salmoides*	Largemouth bass	Keller and
	Lepomis macrochirus	Bluegill	Ruessler 1997
E. fumata	*Perca flavescens* (Mitchell)	Yellow perch	Fuller 1974
	Lepomis cyanellus	Green sunfish	Brim Box and
	L. humilis	Orangespotted sunfish	Williams 2000
	Micropterus salmoides	Largemouth bass	
	Pomoxis annularis	White crappie	
	Fundulus diaphanus	Banded killifish	
E. crassidens	*Alosa chrysochloris*	Skipjack herring	Fuller 1974
E. dilatata	*Dorosoma cepedianum*	Gizzard shad	Fuller 1974
	Pylodictis olivaris	Flathead catfish	
	Pomoxis annularis	White crappie	
	P. nigromaculatus	Black crappie	
	Perca flavescens	Yellow perch	

Figure 4 *Elliptio chipolaensis*, Chipola slabshell.

Shell morphology of *E. chipolaensis* is described as having a chestnut to blackish brown colored periostracum with one to four concentric bands (Figure 4). The shell is small to medium (up to 85 mm in length), subelliptical in outline and has a slightly concave posterior slope. The nacre is salmon colored, most intense at the central area, fading to a bluish white. There is one corrugated pseudocardinal tooth in the right valve and two subequal pseudocardinal teeth in the left valve (Clench and Turner 1956, Burch 1973, Brim Box and Williams 2000).

E. chipolaensis has been considered endemic to the Chipola River in the Florida panhandle, but Brim Box and Williams extended its historic range (Figure 5) to include one site on Howard's Mill Creek, a tributary of the Chattahoochee River (2000). In their survey of the 17 historical sites, Brim Box and Williams found only a total of 12 *E. chipolaensis* at 4 of the sites (Figure 6). It has been found living in muddy sand with a moderate current (Heard 1979) as well as in silty sand or predominately sandy substrates (Brim Box and Williams 2000). Historically, *E. chipolaensis* has been considered to be a rare species (Clench and Turner 1956) and, in 1998, the U.S. Fish and Wildlife Service listed it as federally threatened. U.S. Fish and Wildlife's recovery plan for *E. chipolaensis* (as required under the Endangered Species Act) involves expanding our knowledge of this species' life history (USFWS 2003).

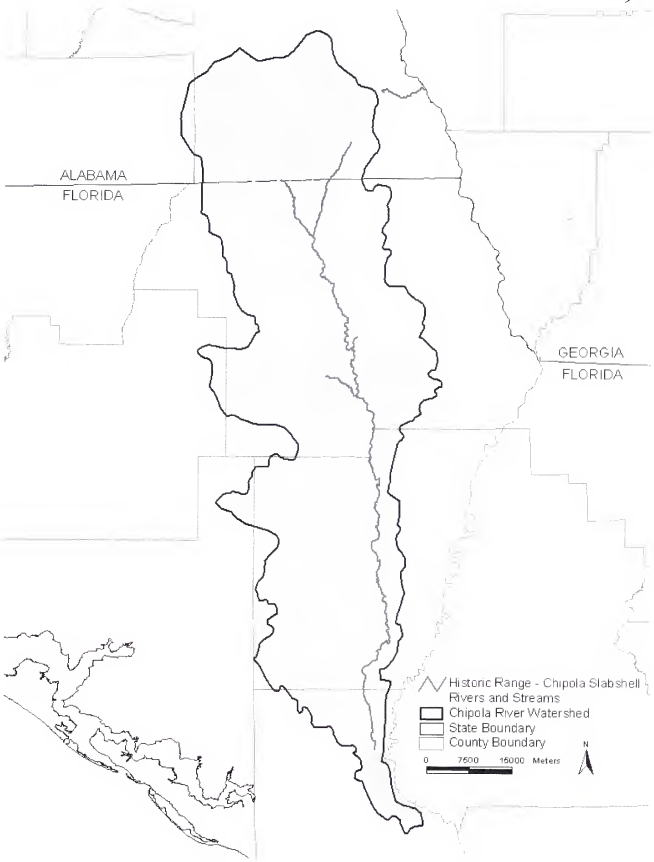

Figure 5 Historic Range of *Elliptio chipolaensis* (USFWS) based on data provided by Brim Box and Williams (2000).

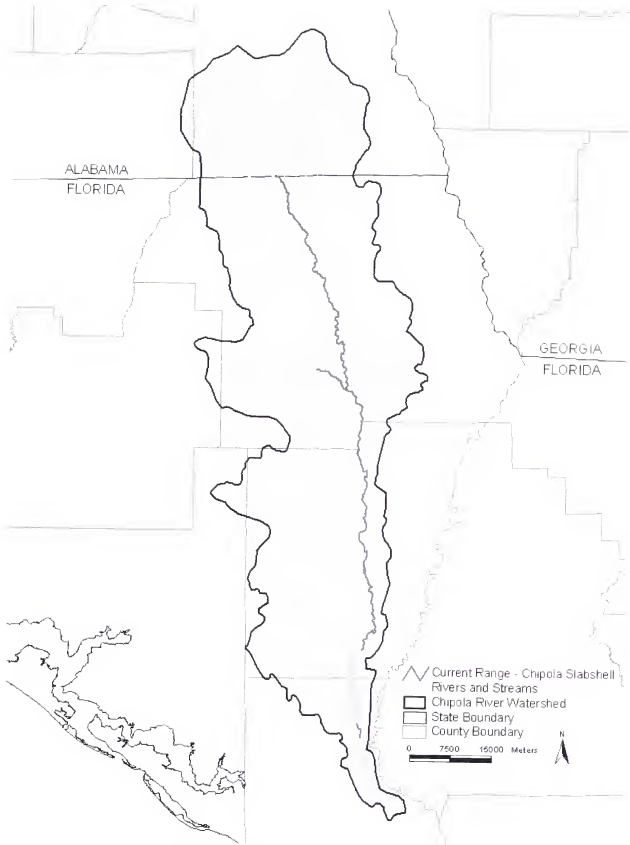

Figure 6 Current Range of *Elliptio chipolaensis* (USFWS) based on data provided by Brim Box and Williams (2000).

Objective

The primary objective of this study is to determine potential host fish species for the *Elliptio chipolaensis* by infecting fish species found in the Chipola River with the glochidia from the *E. chipolaensis*. Those species of fish that successfully transform glochidia into juvenile mussels can be considered potential hosts. A secondary objective of this study is to report any additional status or life history information observed for *Elliptio chipolaensis* and any other federally listed species discovered in the survey area.

Methods

Field research

The headwaters of the Chipola River originate in the southeast corner of Alabama, just north of the Florida border. It flows to the south-southeast, through Jackson, Calhoun and part of Gulf counties (Figures 5 & 6), until it joins the Apalachicola River. The Chipola River drainage basin covers an area of 1025 square miles (Florida Rivers Assessment 1990). It is a spring fed river and the fourth largest river in the Apalachicola-Chattahoochee-Flint (ACF) River Basin (Brim Box and Williams 2000).

Between March 2005 and August 2007, we conducted field surveys to locate populations of *Elliptio chipolaensis*. Survey teams consisted of myself, Carson Stringfellow (Columbus State University) and various students or other interested parties. Potential sites were identified using Brim Box and Williams' (2000) published list of reported *Elliptio chipolaensis* sites (Figure 7). Surveys were done using both timed and untimed tactile searches of suitable habitat within the stream (Strayer and Smith 2003). When conditions were favorable for snorkeling, this method was also used. During the first sampling season, all species found at each site were recorded. The number of sites accessed during 2005 was limited due to an unusually active hurricane season that caused the streams in the Chipola drainage system to flow at higher stages and be very turbid. During spring and summer of 2006, all species were recorded and any federally listed

species were measured for length using a digital caliper (Mitutoyo Digimatic Caliper, Series No. 500, +/- 0.02 mm) before being returned to the stream. To assist future research efforts regarding capture & release methods, listed species identified during the third season were measured for length and tagged with shellfish tags (Hallprint Pty. Ltd,. Holden Hill, SA, Australia, FPN, 8mm x 4mm) attached with cyanoacrylate glue prior to being returned to the stream (Lemarie et al 2000). Tag numbers were recorded and reported to USFWS office in Panama City, Florida. A total of nineteen sites were surveyed and seven sites were surveyed multiple times (Figure 8).

Table 2 List of surveyed sites.

COUNTY	*RIVER*	*LOCALITY*
Calhoun	Chipola River	at Hwy 274
Calhoun	Chipola River	downstream of Hwy 274 bridge
Gulf	Chipola River	
Gulf	Chipola River	at Chipola Cut Off
Gulf	Chipola River	at Chipola Park
Gulf	Chipola River	below Dead Lakes Dam
Gulf	Chipola River	downstream of Chipola cutoff
Gulf	Cypress Creek	southwest bank, near confluence with Chipola River
Jackson	Chipola River	at Hwy 167
Jackson	Chipola River	at Magnolia Road, below I-10, south of Marianna, FL
Jackson	Chipola River	at Peacock Bridge
Jackson	Cowarts Creek	at FL Hwy 2
Jackson	Dry Creek	at FL Hwy 73
Jackson	Dry Creek	at Iron Bridge Road
Jackson	Marshalls Creek	at FL Hwy 2
Jackson	Spring Creek	at FL Hwy 71, below Merritt's Mill Dam
Jackson	Spring Creek	at Turner Landing
Jackson	Spring Creek	below Turner Landing
Jackson	Waddell's Mill Creek	at Bump Nose Road

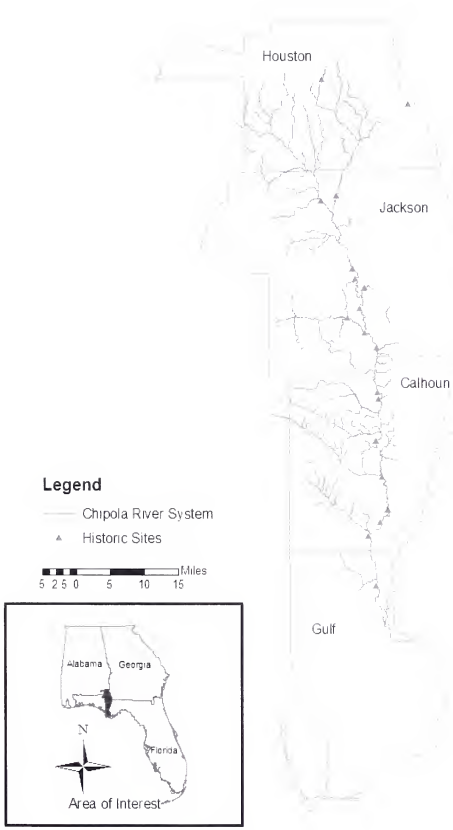

Figure 7 Map of historic sites for *Elliptio chipolaensis*.

Figure 8 Map of surveyed sites.

When *E.chipolaensis* were found, they were gently pried apart with fingers to expose the marsupial gills. Individuals with outer gills that appeared swollen and whitish in coloration were considered to be gravid. In June 2007, seven gravid females were transported to the laboratory to run fish host trials, three from Cowarts Creek at Highway 2 and four from the Chipola River below Peacock Bridge. In order to prevent premature abortion of glochidia, they were wrapped in wet burlap and placed on a plastic grate above a layer of ice in a thermal cooler (O'Brien 1997, Jason Wisnewski, Georgia Department of Natural Resources, Wildlife Division personal communication).

Laboratory research

The method of glochidia release was previously unknown, so gravid female *E. chipolaensis* were held in isolation, supplied with an algae food source and aeration stone, and allowed to release on their own. Many similar host fish studies have used a syringe to flush the glochidia from the gills of female mussels (O'Brien 1997, Haag et al 1999, Khym and Layzer 2000, Lima et al 2006). The period of fertilization and gravidity were also unknown for *E. chipolaensis* so in order to avoid flushing under-developed glochidia, the mussels were allowed to release naturally, giving glochidia the best chance to mature. This also provided an opportunity to determine exactly what method of release is used by *E. chipolaensis*.

At the time of release, conglutinates were removed from the tank with a pipette and viewed under a dissecting microscope. The releasing mussel, number of

conglutinates and date of release were logged for tracking purposes. If glochidia appeared to be mature, a subsample was subjected to a snap response test in which a small amount of NaCl is added to the subsample. If the glochidia are viable, they will exhibit snap response and close their valves (Coker et al 1921). Because *E. chipolaensis* is a federally protected species there was a limit to the number of individuals that could be used for this study. For this reason it was decided to use conglutinates for host fish infestation whenever they exhibited viability, without setting a minimum acceptable level of viability.

Figure 9 Opened and closed *E. chipolaensis* glochidia.

After determining that the glochidia exhibited a snap response (Figure 9), conglutinates were teased apart using a pipette and placed in a beaker containing approximately 2800 mL of water. An aeration stone was added to the beaker to keep the glochidia suspended in the water column. Generally, one species of fish was used for each conglutinate releasing event. For each species infestation, fish were placed, one at a

time, in the beaker containing the glochidia. Each fish was left in the suspension for 10 to 20 minutes, depending on the activity level of the fish species and the viability of the glochidia (Table 3). If the species was active in the water it was immersed for a shorter period of time. The blackbanded darters, however, did not move very much so they were left in the solution longer in order to maximize their exposure to the glochidia. The water containing the glochidia was checked periodically to determine if glochidia were still exhibiting a snap response. This was done by withdrawing a 1 ml subsample of the solution and checking it for viable glochidia using the NaCl test described previously.

Table 3 Fish species used in transformation trials.

Common Name	Scientific Name	# of Individuals	Time of exposure (minutes)
Bluegill	*Lepomis macrochirus*	10	10
Redbreast sunfish	*Lepomis auritus*	11	10
Clear chub	*Hybopsis spp.*	6	10
		4	20
Blackbanded darter	*Percina nigrofasciata*	11	20
Channel catfish	*Ictalurus punctatus*	2	20
Weed shiner	*Notropis texanus*	10	10
		2	20

Six species of fish were used to test for host determination (Table 3). Species were chosen based on their presence in the Chipola River (Appendix A) and availability when the gravid female *E. chipolaensis* were releasing conglutinates. Bluegill (*Lepomis macrochirus*) were obtained from the Warm Springs Fish Hatchery in Warm Springs, Georgia. The remaining species were collected from Hanahatchee Creek in Stewart County, Georgia and Ossahatchie Creek in Harris County, Georgia using a backpack electroshock fisher and minnow seine. These locations were selected because they are known to be devoid of mussel populations, in close proximity to the laboratory at

Columbus State University, and still located within the ACF River Basin. The wild caught individuals were transported to the laboratory in coolers and allowed to acclimate to aquarium conditions prior to being used in the transformation trials.

Once a fish had been immersed in the glochidia solution, it was placed in an isolation chamber with an aeration stone (Figure 10). The size of the isolation chamber varied between 1.5 L and 3L, depending on the size of the fish species. Isolation chambers were filled with previously conditioned tap water and fitted with a grate on the bottom that separated the fish from the bottom of the chamber. This was designed to prevent the fish from consuming anything that fell to the bottom of the chamber. The fish were fed a daily diet of commercial freeze-dried blood worms.

Figure 10 Bluegill being held in isolation chambers after infestation of glochidia.

During the first few days immediately following infestation, it was expected that any glochidia that dropped from the fish were not transformed, having simply been sloughed off without encysting. Initially, the isolation chambers were checked every other day for sloughed off glochidia. Water was poured off and filtered through an 100 micron filter. The fish were returned immediately to the isolation chamber that had been refilled with fresh conditioned water. Filters were viewed under a dissecting microscope and all glochidia were counted and recorded for each individual fish. After the early sloughing of glochidia there was a brief period during which no glochidia were found on the filters. Once glochidia were again discovered on the filter, the water in the isolation chamber was changed and filtered daily. This was continued until no glochidia were found on the filters for three consecutive days, after which time, filtering was done every other day until no glochidia had been found for two weeks.

Beginning on July 17, 2007, glochidia that were found on the filter were transferred to a petri dish with conditioned water to see if they had successfully transformed. An apparent transformation was determined by the subtle change in coloration, being more opaque than the original glochidia. A transformation was considered confirmed when the mussel showed active foot feeding when placed in the petri dish. This activity was documented with photographs and video (Appendix D).

In cases where a fish died prior to completion of the trial, it was fixed in 10% formalin and then preserved in 95% ethanol. Water from the isolation chamber of deceased individuals was filtered a final time to determine if any additional glochidia had

been shed. At the end of the study, the gills of all deceased fish were inspected under the dissecting microscope for the presence of encysted glochidia.

Any surviving fish or mussels were maintained in aquaria at Columbus State University for further observation or research. Permits issued by U. S. Fish and Wildlife Service and Florida Wildlife Commission prohibited the release of these individuals back into the stream system.

Results

Field research

Initially, species at each survey site were identified and recorded. Because the primary focus of the surveys was to locate populations of *Elliptio chipolaensis*, early surveys identified the different species and only their relative abundance or presence. During the first year of the study there were no *E. chipolaensis* located, prompting a thorough count of the species in subsequent years in order to provide data on the status of the mussels that are currently found at these sites. Between March 19, 2005 and August 20, 2007 nineteen sites were surveyed. Figures 11 thru 16 indicate the sites for each of the federally listed species located throughout the course of the study. Table 4 provides additional information on those sites and how many individuals of each listed species were identified. *E. chipolaensis* were identified at seven of the nineteen sites (Figure 12). Most sites had silty sand or sandy substrates but we also found *E. chipolaensis* in sandy gravel substrate. Appendix B lists all the species located at each surveyed site as well as the number of individuals when available.

A total of 168 *E. chipolaensis* were identified, of which 154 were measured for length. Length data were provided for an additional 219 *E. chipolaensis* by Michael Gangloff (Auburn University, personal communication) and Cianna Pender (Rhodes College, personal communication), both of whom were surveying independently in the Chipola River during the period of this study. *E. chipolaensis* varied in length from

21.98 mm to 80.14 mm, with the majority falling mid-range, between 40 and 60 mm (Figure 18).

Table 4 Endangered or threatened species located during field surveys.

COUNTY	RIVER	LOCALITY	SPECIES	Number of live individuals
Calhoun	Chipola River	at Hwy 274	*Hamiota subangulata*	1
Calhoun	Chipola River	downstream of Hwy 274 bridge	*Hamiota subangulata*	0 (1 dead)
Gulf	Chipola River		*Elliptio chipolaensis*	1
			Elliptoideus sloatianus	2
			Pleurobema pyriforme	5
Gulf	Chipola River	at Chipola Cut Off	*Amblema neislerii*	118
			Elliptio chipolaensis	9
Gulf	Chipola River	at Chipola Park	*Amblema neislerii*	2
			Pleurobema pyriforme	17
Gulf	Chipola River	below Dead Lakes Dam	*Amblema neislerii*	9
			Elliptio chipolaensis	56
Gulf	Chipola River	downstream of Chipola cutoff	*Amblema neislerii*	126
Jackson	Chipola River	at Hwy 167	*Hamiota subangulata*	2
			Pleurobema pyriforme	1
Jackson	Chipola River	at Magnolia Road, below I-10, south of Marianna, FL	*Elliptio chipolaensis*	23
			Hamiota subangulata	13
			Pleurobema pyriforme	1
Jackson	Chipola River	at Peacock Bridge	*Elliptio chipolaensis*	61
			Hamiota subangulata	8
Jackson	Cowarts Creek	at FL Hwy 2	*Elliptio chipolaensis*	13
			Hamiota subangulata	19
			Pleurobema pyriforme	8
Jackson	Dry Creek	at FL Hwy 73	*Elliptio chipolaensis*	5
			Hamiota subangulata	1
			Pleurobema pyriforme	55
Jackson	Dry Creek	at Iron Bridge Road	*Pleurobema pyriforme*	12
Jackson	Marshalls Creek	at FL Hwy 2	*Hamiota subangulata*	2
			Pleurobema pyriforme	4
Jackson	Waddell's Mill Creek	at Bump Nose Road	*Medionidus penicillatus*	COM
			Pleurobema pyriforme	COM

Figure 11 Map of sites where *Amblema neislerii* were located.

Figure 12 Map of sites where *Elliptio chipolaensis* were located.

Figure 13 Map of sites where *Elliptoideus sloatianus* were located.

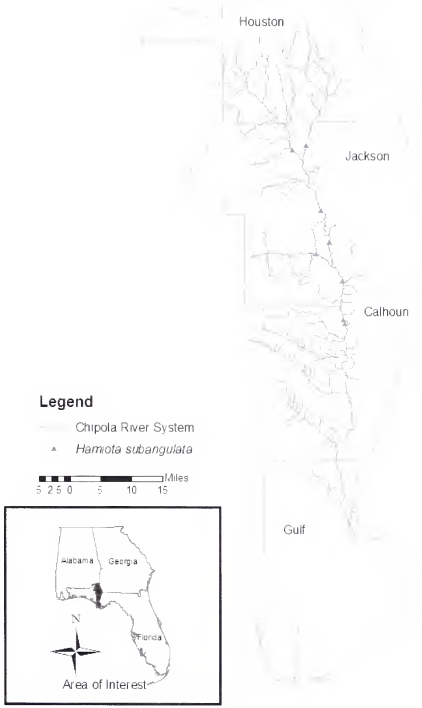

Figure 14 Map of sites where *Hamiota subangulata* were located.

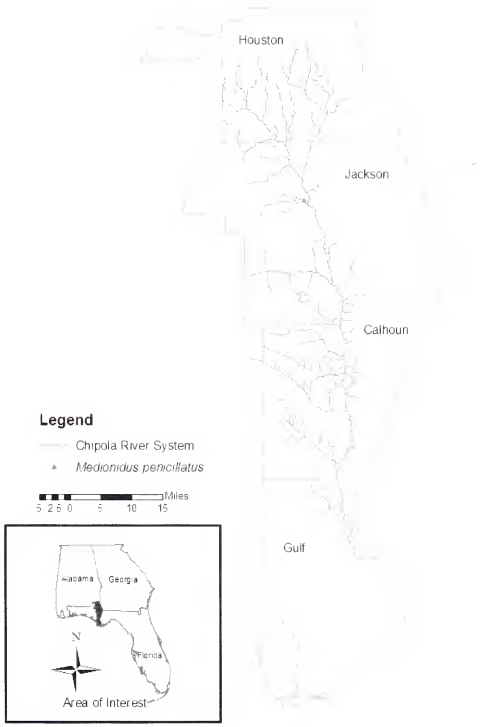

Figure 15 Map of sites where *Medionidus penicillatus* were located.

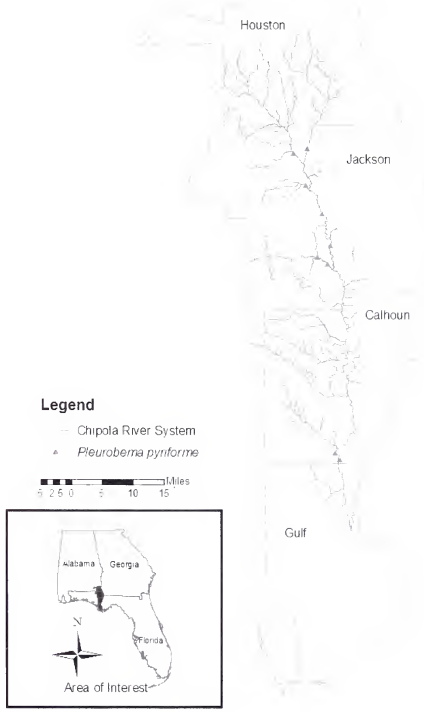

Figure 16 Map of sites where *Pleurobema pyriforme* were located.

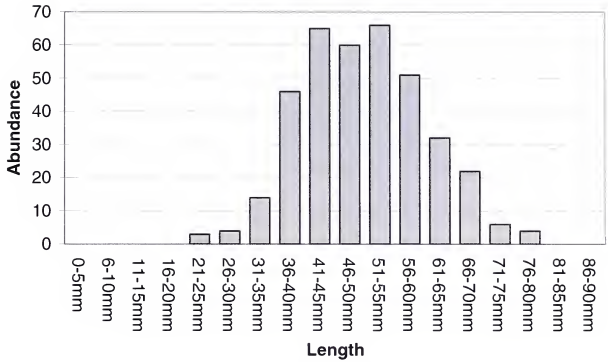

Figure 17 Population distribution of *E. chipolaensis* by length.

Prior reports indicated that gravid *E. chipolaensis* were recovered in June (Brim Box and Williams 2000). During this study, gravid females were found on July 8, 2006, June 9 and June 22, 2007. The gender of individuals was not determined, classified simply as gravid female or nongravid individuals. A follow up survey done in August 2007 found only nongravid *E. chipolaensis*, suggesting that the period of gravidity for *E. chipolaensis* is June and July. The time of spawning and fertilization, and thus brooding type, could not be determined. However, the location of the marsupial gills, which had previously been reported to be the outer demibranchs (Ortmann 1912, Brim Box and Williams 2000), was confirmed during this study.

Elliptio chipolaensis glochidia are released by the female in conglutinates (Figure 19). Conglutinates are approximately 13mm long and 3mm wide (Figure 20). Because conglutinates were released naturally and required for the infestation trials, counts of glochidia per conglutinate were not performed. Estimates can be made using the volume of water, the number of conglutinates, and the number of glochidia in a subsample of the glochidia solution but this method would not account for free floating glochidia released from the matrix during the conglutinate release that were inadvertently left in the mussels' isolation chambers.

Figure 18 *Elliptio chipolaensis* with expelled conglutinates.

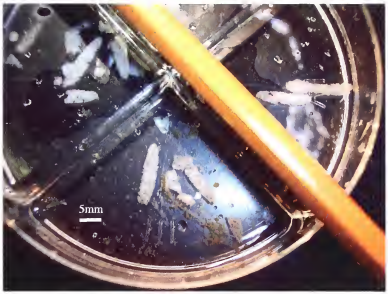

Figure 19 *Elliptio chipolaensis* conglutinates.

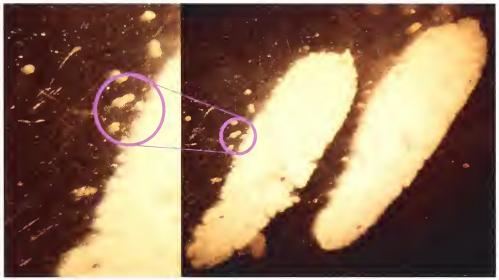

Figure 20 Close up of Elliptio chipolaensis conglutinates, highlighting the individual glochidia that were freed from the matrix.

Figure 21 shows conglutinates viewed under the dissecting microscope. The disturbance caused by pipette was usually enough to cause the glochidia to be released from the matrix that holds the eggs together in the conglutinate, as seen in highlighted portion of Figure 21. Some conglutinates were obviously released prior to full maturation of glochidia (Figure 22) while those that contained many viable glochidia still

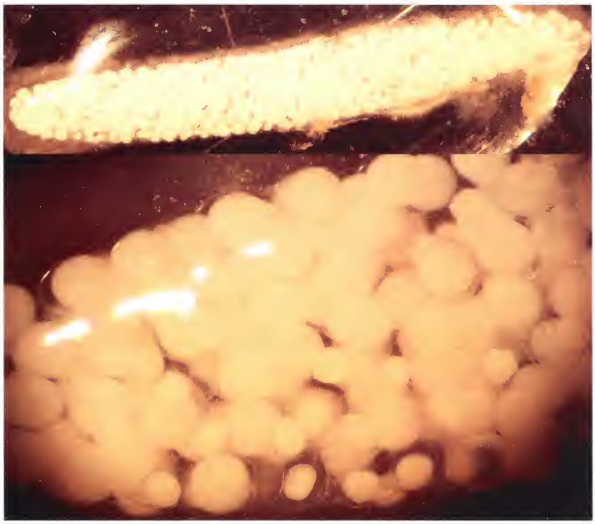

Figure 21 Prematurely released conglutinate with immature embryos and undeveloped eggs.

had a large number of undeveloped eggs (Figure 23). The undeveloped eggs may maintain structural stability of the conglutinate, serving to provide the shape that mimics the host fish's food source (Barnhart 2008).

E. chipolaensis glochidia were confirmed to be hookless (Ortmann 1912) when viewed under the dissecting microscope (Figure 24). Generally, hookless glochidia are found to attach to the fish host's gills, as opposed to hooked glochidia that attach outside the fish on areas like the fins or scales (Coker et al 1921). The snap response of the

Figure 22 Conglutinate showing mature glochidia (in green), immature embryo (in pink) and undeveloped eggs.

Figure 23 *E. chipolaensis* glochidia free of the conglutinate.

Elliptio chipolaensis glochidia was primarily triggered by the addition of NaCl. This differed from the activity observed by *E. crassidens* glochidia, which rapidly opened and closed while still contained within the matrix of the conglutinate. Video clips are included to exhibit this difference in glochidial activity.

Bluegill were infested on June 26, 2007 with the first confirmed transformation identified on July 17, 2007 and the last transformed juvenile identified on July 23, 2007. Transformation for these juvenile *E. chipolaensis* took between 29 and 35 days. In the case of the redbreast sunfish, infestation occurred on July 8, 2007 and transformed juveniles were identified between July 25, 2007 and August 6, 2007, or 17 to 29 days post infestation. Graphs of recovered mussels for each individual fish are included in Appendix C. Figure 25 illustrates the difference between apparent and confirmed transformations.

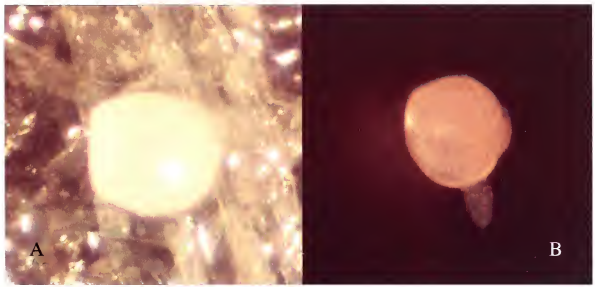

Figure 24 Excysted glochidia showing (A) apparent transformation and (B) confirmed transformation.

Table 5 Transformation data of glochidia on bluegill (*Lepomis macrochirus*).

Fish ID	# Mussels Recovered	# Apparent Transformation	% Apparent Transformation	# Confirmed Transformation	% Confirmed Transformation
BG_1	20	0	0	0	0
BG_2	36	4	11.11	2	5.56
BG_3	16	4	25	3	18.75
BG_4	48	9	18.75	1	2.08
BG_5	56	16	28.57	7	12.50
BG_6	16	1	6.25	1	6.25
BG_7	19	0	0	0	0
BG_8	60	0	0	0	0
BG_9	51	1	1.96	1	1.96
BG_10	19	0	0	0	0
TOTAL	341	35	10.26	15	4.40

Sixty percent of the bluegill showed apparent transformation and confirmed transformation of at least one juvenile *E. chipolaensis* (Table 5). O'Brien (1997) defined a host fish species as one in which (1) glochidia were successfully transformed on the species, (2) at least 30 percent of the individuals successfully transformed juvenile mussels, and (3) the species occurred in the same habitat as the mussel species. In this laboratory trial, bluegill have met the criteria and can be considered a potential host fish species.

Table 6 Transformation data of glochidia on redbreast sunfish (*Lepomis auritus*).

Fish ID	# Mussels Recovered	# Apparent Transformation	% Apparent Transformation	# Confirmed Transformation	% Confirmed Transformation
RB_15b	5	0	0	0	0
RB_16b	3	0	0	0	0
RB_17b	5	2	40	0	0
RB_19b	9	3	33.33	1	11.11
RB_21b	4	2	50	1	25
RB_23b	14	4	28.57	2	14.29
RB_25b	5	4	80	1	20
RB_29b	9	7	77.78	3	33.33
RB_35	9	5	55.56	2	22.22
RB_36b	16	12	75	1	6.25
RB_37b	12	5	41.67	1	8.33
TOTAL	91	44	48.35	12	13.19

In the case of redbreast sunfish, 80% of the fish showed apparent transformation of at least one juvenile *E. chipolaensis* while 72.73% of the fish showed confirmed transformation of at least one juvenile *E. chipolaensis* (Table 6). In this laboratory trial, redbreast sunfish have met O'Brien's criteria (1997) of at least 30% and can be considered a potential host fish species.

Because the glochidia were hookless, it was expected that they would attach to the gills of their host fish (Coker et al 1921). This was confirmed upon post-mortem inspection of the gills under the dissecting microscope. A single glochidum that had not excysted was found on the gills of a redbreast sunfish (Figure 26 A). Transformation of glochidia was successful on this individual (RB 19b) prior to its death. All remaining preserved gills showed no sign of encysted glochidia (Figure 26 B).

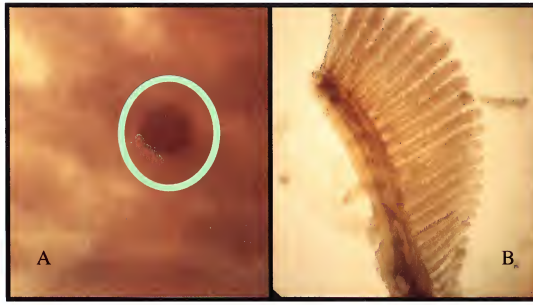

Figure 25 Preserved gills (A) with an encysted glochidia and (B) with no encysted glochidia.

All individuals of clear chubs (*Hybopsis spp.*), blackbanded darters (*Percina nigrofasciata*), channel catfish (*Ictalurus punctatus*), and weed shiners (*Notropis texanus*) showed no signs of encysting or transformation. These four species experienced an unexplained high rate of mortality within days of being infested but there was no evidence of encysted glochidia in the preserved gills, suggesting that infestation of glochidia was not the cause of death.

Any surviving juvenile mussels were monitored for several weeks to observe growth. Figure 27 show a juvenile fourteen days after encysting from the host fish. The glochidia shell appears to still be intact while a new shell has begun to grow. At the end of the study, juvenile mussels were added to an aquarium with the adult *E. chipolaensis* for future observation.

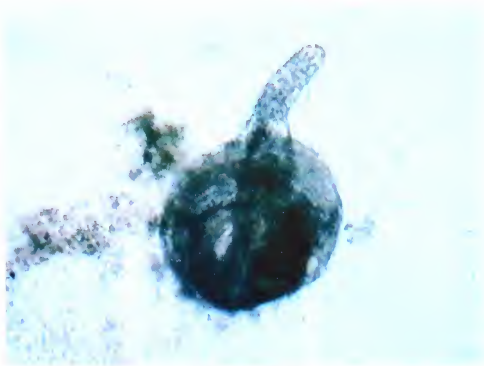

Figure 26 Juvenile mussel showing growth of shell after 14 days.

Preserved voucher specimens, conglutinates and glochidia of *E. chipolaensis* are currently stored at the College of Science, Columbus State University in Columbus, Georgia. Tissue samples, for DNA sequencing, and glochidia samples, for SEM photographs and sizing, were provided to James D. Williams, Florida Museum of Natural History, in Gainesville, Florida.

Discussion

At the beginning of this study, literature reviews found that very little research had been done regarding *Elliptio chipolaensis*. Based on surveys reported by Brim Box and Williams (2000), in which a total of only 12 mussels were found, there was great concern that the population had become even more rare than previously thought. While it was difficult to locate populations of *E. chipolaensis*, once one individual was found, we usually found several, suggesting that there were reproducing populations.

Mussels generally have a high rate of reproduction but a low rate of survival to juvenile and reproductive age. A true population distribution curve for this species would start with very high numbers of glochidia and slope sharply negative. The normal distribution for this species, shown in Figure 18, is artificial, most likely due to the tactile search methods used. Smaller (and thus younger) individuals were likely missed in tactile searches because they are more difficult to feel with fingers and because the juveniles may reside in a different region of the stream than the adults until they reach a size that is suitable for survival in the more vigorously flowing stream.

It is improbable that every *E. chipolaensis* was located during each of the surveys and, as such, we cannot draw conclusions about the entire population. The random tactile search method allow us to make generalizations based the individuals that were located. In surveys where gravid females were located, assuming that the sampled individuals represent the overall population at that site and time, we are able to determine the percent

of the population that was gravid (Table 7). This snapshot does not take into account how many were gravid earlier in the season and had already released their conglutinates or the number of gravid females that were not recovered, perhaps because they were deeper in the substrate or brooding their glochidia in a different area of the stream. It is possible that the earlier survey had a higher percent gravidity simply because fewer individuals had started to release at that point in the season. It would be beneficial to survey a single site in May, June, July and August to determine how the percent gravidity changes through the course of the brooding season. Repeating the surveys in subsequent years would supply information on consistency of gravidity. Because *E. chipolaensis* is not sexually dimorphic, field surveys do not give us accurate sex ratios for the population.

Table 7 Percent gravid females.

Date of Survey	# Gravid Females Collected	Total collected	Percent Gravidity
July 8, 2006	3	9	33.3%
June 9, 2007	3	6	50.0%
June 22, 2007	10	33	30.3%

While the outer demibranchs were confirmed as the brooding location of *E. chipolaensis*, it should be noted that the central section of the marsupial gills appeared to be more swollen than the anterior and posterior edges, perhaps indicating that only a portion of the outer demibranch is used for brooding or that maturation occurs earlier in the central section than the outer edges. Additional study is required to determine if

glochidia development occurs in stages. In this study, the risk of causing premature release of conglutinates precluded any additional tampering with the gravid females once they had been confirmed as gravid.

There appeared to be a pattern in which conglutinates were released by each gravid female. Each individual mussel would release a few conglutinates sporadically over the course of several days, sometimes containing mature glochidia but often not. Following the sporadic release, the mussel would then release a large number of conglutinates (Figure 19), containing mature glochidia. After the large release, a few more conglutinates may have been released but, in general, the mussel appeared to have emptied its marsupial demibranchs. The initial sporadic release was used in the laboratory to recognize that a mussel was nearing its large release allowing us to be prepared for the infestation of fish. While we were unable to determine if this same release timing occurred in situ, it allowed for some predictability of conglutinate release in the laboratory.

Initially, it was assumed that the transformed juveniles would have a significantly different appearance or shell morphology than the glochidia. However, a subtle change in the apparent opacity of the glochidia was eventually identified to be the best way to determine if the glochidia had transformed into a juvenile. This increased opacity is the result of the development of the foot and visceral mass within the valves. This change in appearance was not recognized as significant until July 17th, after some individuals excysted from bluegills had already been exhibiting this trait for several days. As a

result, the reported total of apparent and confirmed transformed individuals for bluegills is likely lower than it actually was, had this detail been identified sooner. Any transformed individuals that may have excysted several days earlier would also require that the transformation time be adjusted, starting sooner and extending the number of days that transformation occurred.

Comparing the transformation data for bluegill and redbreast sunfish (Appendix C), the graphs for the redbreast sunfish show a clear sloughing period, followed by inactivity and then excystment of transformed individuals. Repeating the host fish trials for the bluegill, knowing how similar the transformed juveniles look to the glochidia, might allow for identification of an inactive period for this species as well. It is possible that *E. chipolaensis* transform more quickly on bluegill but further trials are required to confirm this.

The original plan was to use wild caught fish from portions of the Chipola River Basin that did not have mussels present, in order to limit the number of variables introduced to the study. However, using the bluegill from the fish hatchery allowed us to be certain they had never come in contact with *Elliptio chipolaensis*, which could have increased the fish immunity to infestation. Hatchery fish had already been acclimated to aquaria so as to inflict less stress on them during infestation and isolation, ultimately resulting in lower mortality of the fish. Bluegill experienced no mortality during the course of the study. The remaining species were collected from streams in Muscogee County, Georgia. The convenience of being nearer to the campus laboratory as well as

the increased stress put on the fish by transporting them from the Chipola River, made this a logical choice. These species seemed to be well acclimated to aquaria prior to infestation. With the exception of redbreast sunfish, the wild-caught species did not survive infestation and isolation very well. While the cause of death is uncertain, individuals of those species that died did not have glochidia attached to their gills, suggesting that it was not the infestation of glochidia that caused death. Redbreast sunfish had a high survival rate with only one fish dying prior to completion of the study. Additional transformation studies should be performed on the species used in this study to expand the amount of data available for these species. Largemouth bass were not available at the time of conglutinate release so this species was not tested. This is still considered a possible host fish, due to the fact that it is the host fish for several other *Elliptio* species, and should be included in future transformation studies.

Glochidia used to infest bluegill on June 26, 2007 were still exhibiting snap responses to NaCl tests for viability on June 27 & 28. Glochidia used to infest black banded darters on July 5, 2007 were still showing some viability on July 7. However, glochidia used to infest clear chubs on June 28 showed no signs of viability on June 29. It is unclear why some glochidia were able to survive in suspension for 2 or 3 days while others did not survive more than a day. This may be related to any number of factors, including the health of the female mussel the glochidia were retrieved from, the health of glochidia upon conglutinate release, the release of a byproduct from the fish that caused glochidia die off, or simply a phenomenon found to occur only in the laboratory environment. Glochidia that survive longer in the water column have an increased

chance of coming in contact with the appropriate fish host and, therefore, the survivability of glochidia outside of the brooding demibranchs plays a role in successful reproduction. Additional research on this subject may be beneficial to the survival of this species.

Bluegill and redbreast sunfish are common species in the Chipola River and not likely a limiting factor to the survival of *E. chipolaensis*. Perhaps the scarcity of this species is due to its survival strategy. In order to successfully deliver viable glochidia to the appropriate host fish species, it has lowered the number of glochidia actually produced. Using unfertilized eggs to "create" the appearance of the conglutinate requires a trade off, producing fewer viable glochidia. But those glochidia that are produced are able to transform on more common fish species and, in the end, this allows the *E. chipolaensis* population to maintain a normal age distribution.

The Chipola River is categorized as a Class III water body by the Florida Department of Environmental Regulation and as such, is intended for recreation and propagation of fish and wildlife (FRA, 1990). It is also considered an Outstanding Florida Water (OFW), which means that it has been classified as worthy of special protection. State water quality regulation prohibits the permitting of discharge in an OFW that would decrease the water quality to a level below its quality when it was designated. It has been determined that 14.4 % of the total stream miles in the Chipola Drainage Basin are considered potentially impaired due to nutrients and conventional pollutants (Florida Department of Environmental Protection 2002).

In addition to the nutrient and pollution impairments in the Chipola River Basin, 7.2 billion gallons of water were used per day in Florida during 1995. This figure is projected to increase to nine billion by 2020. Sixty percent of the state's current water supply comes from aquifers, having a dramatic affect on the available groundwater (Hartnett 2000). Additionally, groundwater is used for agriculture, with Jackson county being the largest such user (FDEP 2002). This groundwater withdrawal has a direct impact on the springs and streams in the region. As water is artificially extracted from the aquifer, it becomes unavailable to provide recharge for the springs and streams. Even slight changes in stream discharges can change the balance of the ecosystem, altering stream habitats. This is the biggest threat to the survival of *E. chipolaensis*. Any damage to the Chipola River can potentially harm the *E. chipolaensis* population. As an endemic species, if *E. chipolaensis* disappear from the Chipola River, they become extinct. Conversely, any measure taken to protect the Chipola River and its surrounding water system will directly benefit *E. chipolaensis*. Conservation efforts, such as limiting water withdrawal and pollutant discharges, will help ensure the preservation of habitat for the *E. chipolaensis* and, consequentially, protect this species from extinction.

Conclusion

This study provided us with a great deal of information about *Elliptio chipolaensis* that, here to fore, had been unknown. We were able to identify several sites that support healthy populations of *E. chipolaensis* and provide this information to US Fish and Wildlife Service for future conservation efforts. Although there is no confirmation of the type of breeders, tachyditic or brachyditic, *E. chipolaensis* is gravid during the months of June and July. No evidence of a mantle lure was identified but rather glochidia are released in conglutinates that contain mature glochidia, immature embryos and, presumably, unfertilized eggs, resembling insect larva. *E. chipolaensis* glochidia are hookless and attach to the gills of their host fish. Successful transformation of glochidia occurred on bluegill and redbreast sunfish with transformation taking approximately 17 to 35 days. Upon excysting from the host fish, transformed juveniles have the same shape and general appearance as the glochidia.

Bluegill and redbreast sunfish successfully transformed glochidia into juvenile mussels in the laboratory setting and are potential fish hosts for *Elliptio chipolaensis*. Both species of fish are found in the Chipola River but in order to conclusively say that they are host fish species, *E. chipolaensis* glochidia must be found on these fish in the wild.

While the information obtained during this study is extremely valuable to the conservation of the *E. chipolaensis*, there is still much to be learned about this species. In

addition to repeating the host fish determination trials on the species used here, it is recommended that other fish species found in the Chipola River be tested for suitability. Other recommendations for research on the *E. chipolaensis* include further study on the percent of viable glochidia in a conglutinate, the use of structural eggs to maintain the conglutinate shape, the paternity of glochidia in a single gravid female, tag and release studies to monitor existing populations, determination of brooding type and method of fertilization, and the reproductive age of females.

Populations of *E. chipolaensis* are rare but the Chipola River is home to several sites where the species appear to be thriving. The Chipola River's designation as an Outstanding Florida Water not only affords protection to the drainage basin but to the *E. chipolaensis* as well. However, because *E. chipolaensis* has a seemingly low rate of reproduction, the population may not be able to recover if a serious pollution event were to occur in the Chipola River drainage basin. Maintaining and protecting the Chipola River, and its source tributaries and springs, goes hand in hand with the survival of the federally threatened *E. chipolaensis*.

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Appendix A

Fish of the Chipola River

This table shows the species composition of the upper Chipola River based on a survey done by Florida Fish and Wildlife Conservation (Bass, unpublished report). *Species names, as reported by the Florida FWC, added by author.

Species (*)	Common Name	Number	% No.
Lepisosteus oculatus	Spotted gar	3	0.34
Amia calva	Bowfin	2	0.23
Alosa chrysochloris	Skipjack herring	6	0.68
Dorosoma petenense	Threadfin shad	1	0.11
Esox niger	Chain pickerel	1	0.11
Hybopsis new species	Clear chub	1	0.11
Notropis emiliae	Pugnose minnow	3	0.34
Notropis petersoni	Coastal shiner	12	1.37
Notropis texanus	Weed shiner	112	12.76
Cyprinella venusta	Blacktail shiner	72	8.20
Carpiodes cyprinus	Quillback	1	0.11
Minytrema melanops	Spotted sucker	51	5.81
Moxostoma sp.	Greyfin redhorse	51	5.81
Ictalurus punctatus	Channel catfish	6	0.68
Noturus leptacanthus	Speckled madtom	1	0.11
Aphredoderus sayanus	Pirate perch	14	1.59
Labidesthes sicculus	Brook silverside	16	1.82
Ambloplites ariommus	Shadow bass	2	0.23
Lepomis auritus	Redbreast sunfish	193	21.98
Chaenobryttus gulosus	Warmouth	11	1.25
Lepomis macrochirus	Bluegill	116	13.21
Lepomis microlophus	Redear sunfish	101	11.50
Lepomis punctatus	Spotted sunfish	26	2.96
Micropterus cataractae	Shoal bass	6	0.68
Micropterus salmoides	Largemouth bass	25	2.85
Etheostoma swaini	Gulf darter	1	0.11
Percina nigrofasciata	Blackbanded darter	42	4.78
Mugil cephalus	Striped mullet	1	0.11
Trinectes maculatus	Hogchoker	1	0.11
Total:		878	100.00

Appendix B

Complete List of Survey Sites

Survey sites listing all species at each site and the number of individuals (P=present, TNC=too numerous to count, *=additional individuals were located but not counted, COM=common, VCOM=very common).

COUNTY	RIVER	LOCALITY	SPECIES	Number of live individuals
Calhoun	Chipola River	at Hwy 274	*Elliptio fumata*	8
			Elliptio crassidens	6
			Hamiota subangulata	1
			Villosa lienosa	12
			Villosa vibex	5
Calhoun	Chipola River	downstream of Hwy 274 bridge	*Elliptio fumata*	33
			Hamiota subangulata	0
			Toxolasma paulum	6
			Villosa lienosa	34
			Villosa villosa	7
Gulf	Chipola River		*Elliptio chipolaensis*	1
			Elliptio fumata	P*
			Elliptio crassidens	TNC*
			Elliptoideus sloatianus	2
			Pleurobema pyriforme	5
			Megalonaias nervosa	P*
			Villosa lienosa	P*
Gulf	Chipola River	at Chipola Cut Off	*Amblema neislerii*	118
			Anodonta heardi	1
			Elliptio chipolaensis	9
			Elliptio fumata/pullata	61
			Elliptio crassidens	20
			Glebula rotundata	42
			Lampsilis claibornesis	13
			Megalonaias nervosa	1
			Pyganodon cataracta	1
			Pyganodon grandis	1
			Quadrula infucata	6
			Toxolasma parvus	2
			Toxolasma paulum	0
			Utterbackia peggyae	1
			Villosa vibex	3
			Villosa villosa	2

Complete List of Survey Sites (continued)

COUNTY	RIVER	LOCALITY	SPECIES	Number of live individuals
Gulf	Chipola River	at Chipola Park	*Amblema neislerii*	2
			Elliptio fumata	119
			Glebula rotundata	1
			Megalonaias nervosa	14
			Pleurobema pyriforme	17
			Quadrula infucata	2
			Toxolasma paulum	50
			Utterbackia imbecillis	2
			Villosa lienosa	1
			Villosa vibex	3
			Villosa villosa	15
Gulf	Chipola River	below Dead Lakes Dam	*Amblema neislerii*	9
			Elliptio chipolaensis	56
			Elliptio fumata	293
			Elliptio crassidens	28
			Glebula rotundata	59
			Lampsilis floridensis	7
			Megalonaias nervosa	1
			Pyganodon cataracta	3
			Pyganodon grandis	8
			Quadrula infucata	2
			Toxolasma paulum	44
			Uniomerus columbensis	2
			Utterbackia imbecillis	4
			Utterbackia peggyae	6
			Villosa lienosa	6
			Villosa vibex	4
			Villosa villosa	9
Gulf	Chipola River	downstream of Chipola cutoff	*Amblema neislerii*	126
			Anodonta heardi	4
			Elliptio fumata/pullata	13
			Glebula rotundata	22
			Lampsilis floridensis	29
			Utterbackia peggyae	2
Gulf	Cypress Creek	southwest bank, near confluence with Chipola River	*Elliptio fumata*	34
			Uniomerus columbensis	9
Jackson	Chipola River	at Hwy 167	*Elliptio fumata*	55
			Elliptio crassidens	3
			Elliptio purpurella	2
			Hamiota subangulata	2
			Lampsilis claibornesis	2
			Pleurobema pyriforme	1
			Quadrula infucata	2
			Toxolasma paulum	9
			Villosa lienosa	34
			Villosa vibex	12
			Villosa villosa	2

Complete List of Survey Sites (continued)

COUNTY	RIVER	LOCALITY	SPECIES	Number of live individuals
Jackson	Chipola River	at Magnolia Road, below I-10, south of Marianna, FL	*Anodontes radiatus*	1
			Elliptio chipolaensis	23
			Elliptio fumata	100
			Elliptio fumata/pullata	97
			Elliptio crassidens	10
			Elliptio pullata	P*
			Elliptio purpurella	5
			Hamiota subangulata	13
			Lampsilis claibornesis	5
			Pleurobema pyriforme	1
			Quadrula infucata	3
			Toxolasma paulum	5
			Villosa lienosa	766*
			Villosa vibex	47*
			Villosa villosa	80*
Jackson	Chipola River	at Peacock Bridge	*Anodontes radiatus*	1
			Elliptio chipolaensis	61
			Elliptio fumata/pullata	74*
			Elliptio crassidens	175*
			Elliptio purpurella	15
			Hamiota subangulata	8
			Megalonaias nervosa	P
			Quadrula infucata	1*
			Toxolasma paulum	1
			Villosa lienosa	22*
			Villosa villosa	3
Jackson	Cowarts Creek	at FL Hwy 2	*Elliptio chipolaensis*	13
			Elliptio fumata	18
			Elliptio fumata/pullata	7
			Hamiota subangulata	19
			Pleurobema pyriforme	8
			Quadrula infucata	3
			Uniomerus columbensis	4
			Villosa lienosa	57
			Villosa vibex	9
			Villosa villosa	8
Jackson	Dry Creek	at FL Hwy 73	*Anodontes radiatus*	2
			Elliptio chipolaensis	5
			Elliptio fumata	171
			Elliptio fumata/pullata	285
			Elliptio crassidens	2
			Elliptio purpurella	26
			Hamiota subangulata	1
			Lampsilis claibornesis	5
			Pleurobema pyriforme	55
			Toxolasma paulum	13
			Uniomerus columbensis	21
			Villosa lienosa	69
			Villosa vibex	27
			Villosa villosa	30

Complete List of Survey Sites (continued)

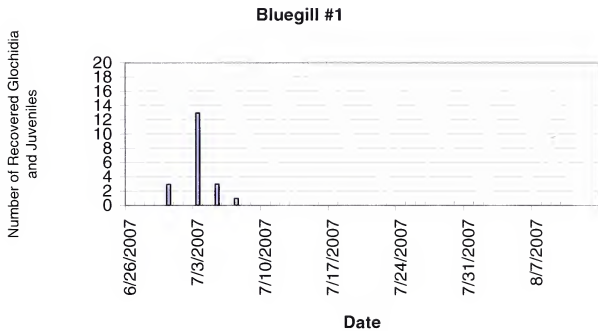
COUNTY	RIVER	LOCALITY	SPECIES	Number of live individuals
Jackson	Dry Creek	at Iron Bridge Road	*Elliptio fumata*	64
			Elliptio crassidens	2
			Elliptio purpurella	11
			Pleurobema pyriforme	12
			Villosa lienosa	6
			Villosa villosa	7
Jackson	Marshalls Creek	at FL Hwy 2	*Elliptio fumata*	8
			Hamiota subangulata	2
			Pleurobema pyriforme	4
			Quadrula infucata	9
			Uniomerus columbensis	1
			Villosa lienosa	17
			Villosa vibex	2
			Villosa villosa	1
Jackson	Spring Creek	at FL Hwy 71, below Merritt's Mill Dam	*Elliptio fumata*	315
			Elliptio crassidens	14
			Elliptio pullata	1
			Elliptio purpurella	1
			Elliptio spp.	16
			Toxolasma paulum	3
			Villosa lienosa	7
			Villosa vibex	1
Jackson	Spring Creek	at Turner Landing	*Elliptio buckleyi*	782
			Elliptio fumata	436
			Elliptio crassidens	8
			Elliptio purpurella	8
			Toxolasma paulum	1
			Uniomerus columbensis	63
			Villosa lienosa	46
			Villosa villosa	14
Jackson	Spring Creek	below Turner Landing	*Elliptio crassidens*	7
			Elliptio pullata	391
			Elliptio spp.	68
			Lampsilis claibornesis	1
			Toxolasma paulum	14
			Villosa lienosa	20
			Villosa vibex	12
Jackson	Waddell's Mill Creek	at Bump Nose Road	*Elliptio fumata*	VCOM
			Elliptio pullata	COM
			Lampsilis claibornesis	COM
			Medionidus penicillatus	COM
			Pleurobema pyriforme	COM
			Quadrula infucata	COM
			Villosa vibex	COM
			Villosa lienosa	COM
			Villosa villosa	COM

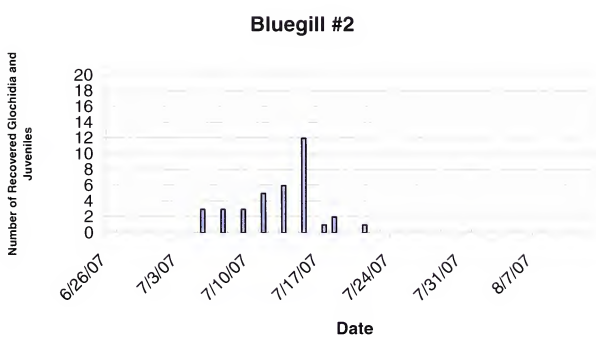
Appendix C

Transformation Data for Bluegill and Redbreast Sunfish

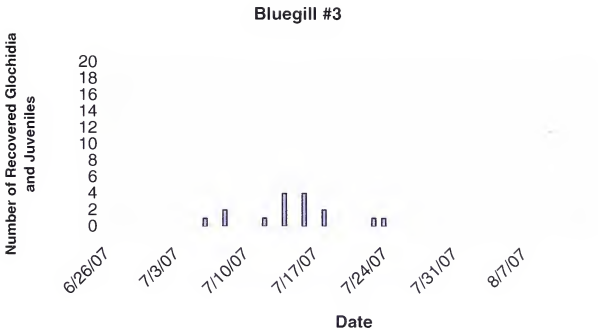
The following charts show the number of glochidia and juvenile mussels that were collected from the isolation chambers after the fish had been infested with glochidia. The redbreast sunfish show a distinct period of inactivity, during which time no mussels were recovered. The glochidia that were recovered earlier most likely did not attach and those recovered after the inactive period had either apparent or confirmed confirmation. A vertical black line on the graph indicates the day that the specific fish died.

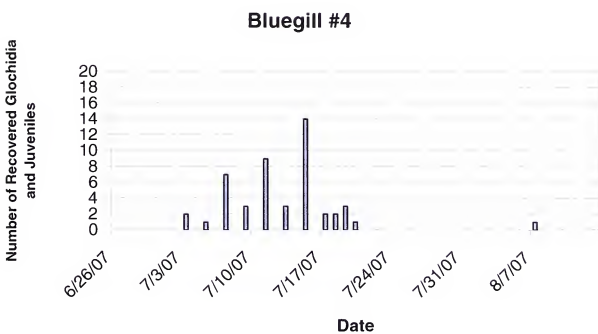
Transformation Data for Bluegill and Redbreast Sunfish (continued)



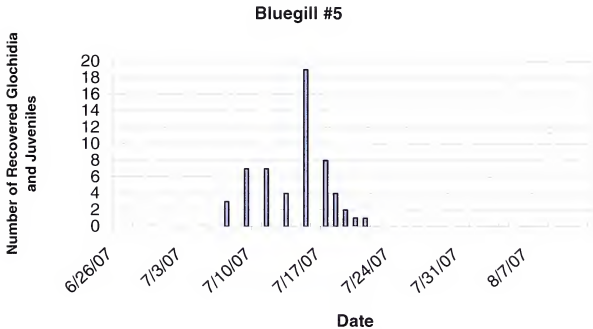


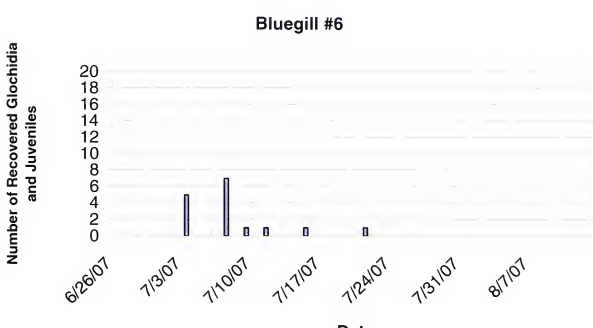
Transformation Data for Bluegill and Redbreast Sunfish (continued)



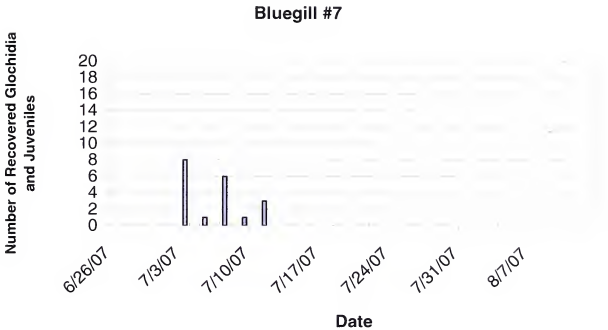


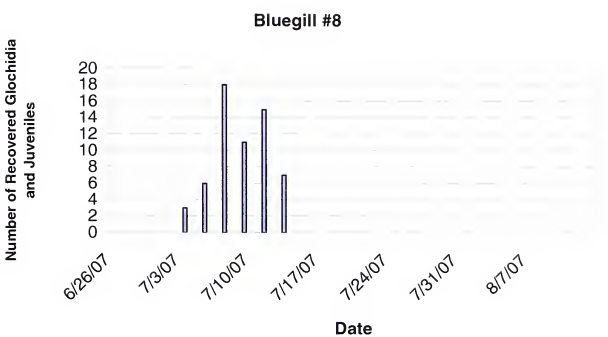
Transformation Data for Bluegill and Redbreast Sunfish (continued)



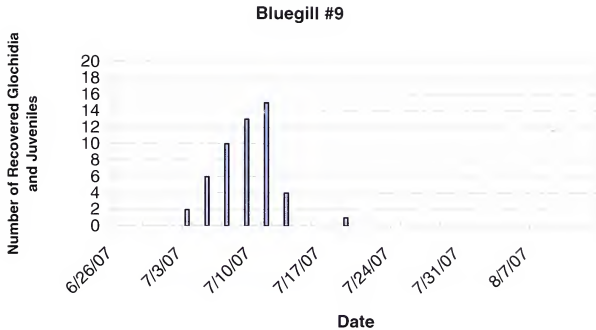


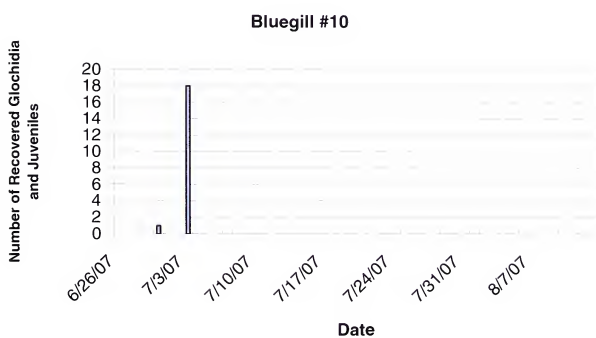
Transformation Data for Bluegill and Redbreast Sunfish (continued)



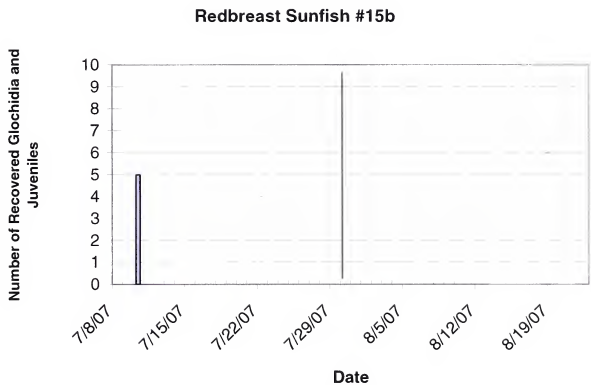


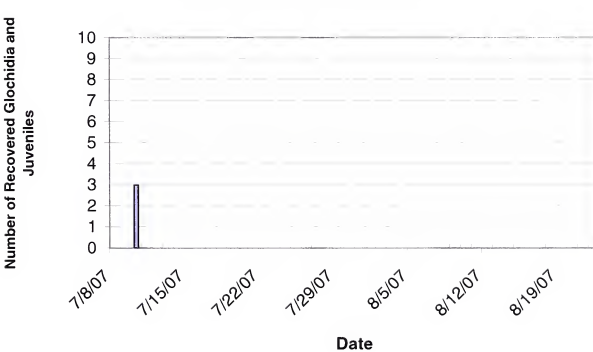
Transformation Data for Bluegill and Redbreast Sunfish (continued



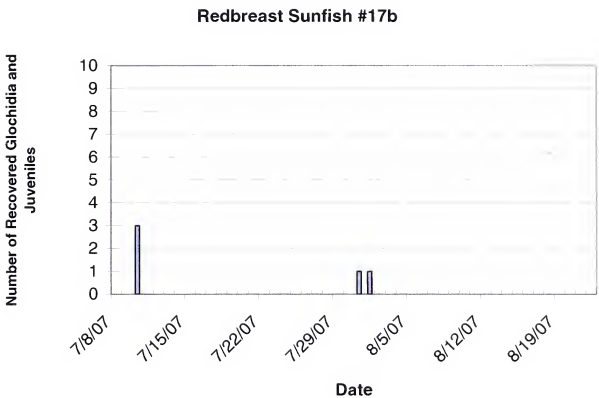


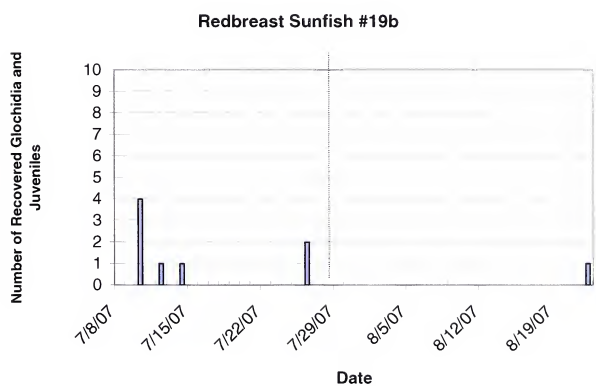
Transformation Data for Bluegill and Redbreast Sunfish (continued)



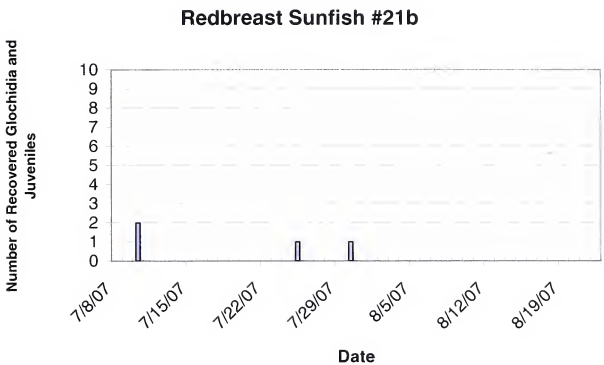


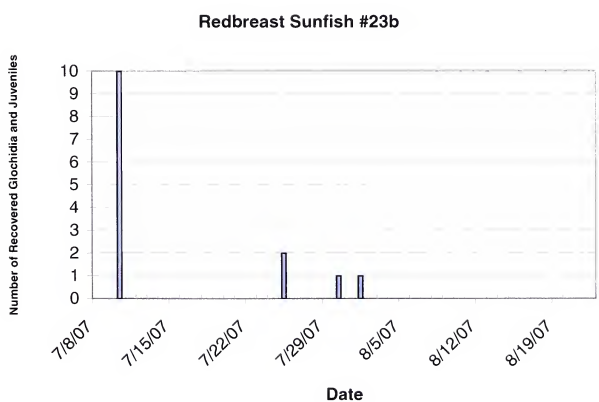
Transformation Data for Bluegill and Redbreast Sunfish (continued)



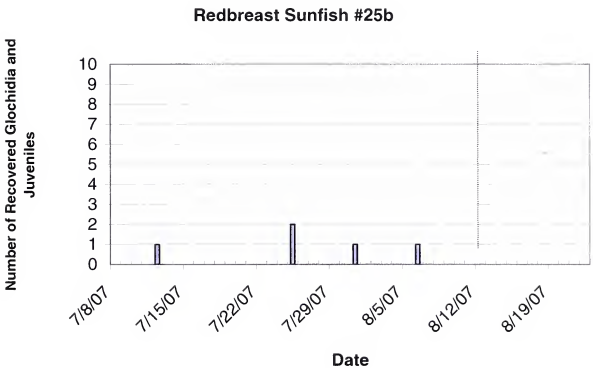


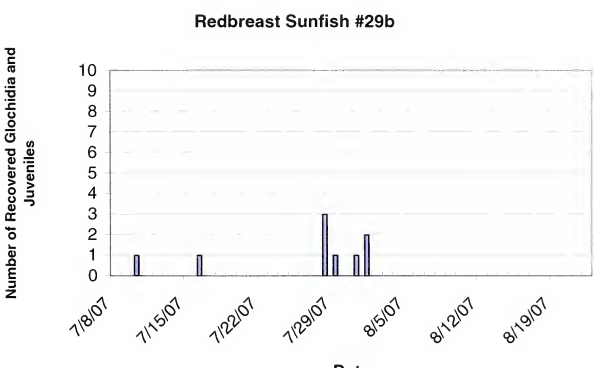
Transformation Data for Bluegill and Redbreast Sunfish (continued)



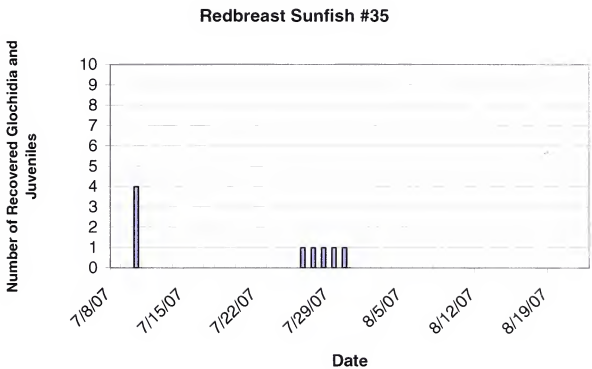


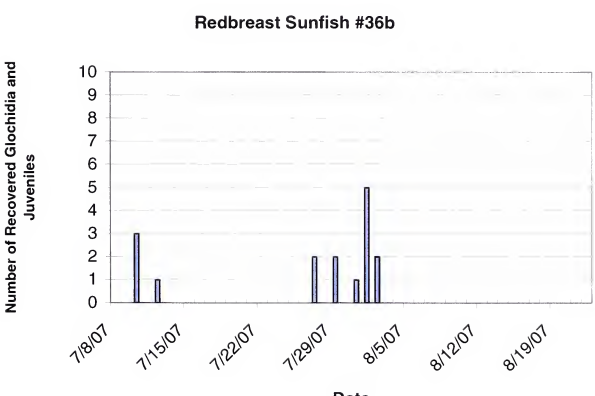
Transformation Data for Bluegill and Redbreast Sunfish (continued)



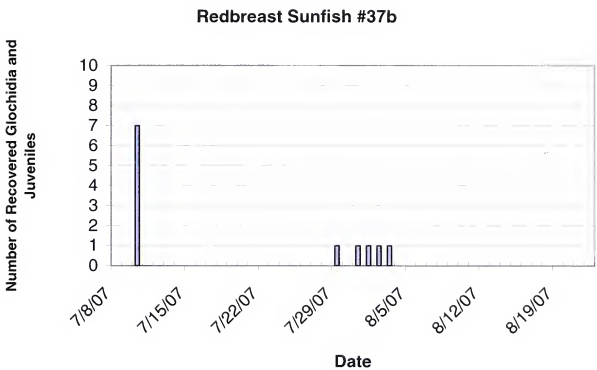


Transformation Data for Bluegill and Redbreast Sunfish (continued)





Transformation Data for Bluegill and Redbreast Sunfish (continued)



Appendix D

Additional Supplemental Media

The enclosed compact disc includes video clips of the following:

"Chipolaensis foot feeding & locomotion" shows a recently excysted transformed juvenile *Elliptio chipolaensis* foot feeding and moving around.

"Crassidens conglutinate" shows an *Elliptio crassidens* conglutinate, notably how many viable glochidia are present and how active they are while still contained in the matrix.

"*Elliptio chipolaensis*" is another clip of a juvenile mussel foot feeding.

"First confirmed chipolaensis foot feeding" is video of the first time that foot feeding was seen in the laboratory, confirming that the glochidia had successfully transformed.

The three clips with "glochidia viability test" show the glochidia responding to the addition of sodium chloride to their water, determining their viability.

"Redbreast sunfish #21b at 2 weeks" shows a juvenile *E. chipolaensis* after about two weeks of growth with new shells in addition to the glochidia shells.