Quantifying and identifying unionid larvae in drift and on fishes of the Sipsey River, Alabama

Final Report

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by

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Abstract

We examined patterns of abundance of mussel larvae (glochidia) in stream drift and on fishes at a diverse, large stream mussel community in the Sipsey River, Alabama. We used recently developed techniques for glochidial identification to evaluate seasonal patterns of glochidia abundance and composition in the drift and to identify glochidia species-host fish associations. Although we were not able to validate morphological identification of glochidia with molecular confirmation, our analysis indicated an average probability that glochidia were correctly identified to species of 83%. Glochidia from short-term brooding species (*Amblema plicata, Elliptio arca, Fusconaia cerina, Pleurobema decisum, Obliquaria reflexa,* and *Quadrula asperata*) were abundant from May to August and present through October but did not occur in drift at other times of the year. Long-term brooding species (*Lampsilis* spp., *Medionidus acutissimus*, *Obovaria unicolor*, and *Villosa* spp.) occurred in several short peaks in spring, summer, and fall, but were generally less abundant than short-term brooding species. We found glochidial transmission resulted in parasitization of only a small component of the total fish assemblage (9.2% of 1,988 fishes examined). Glochidia infestations were unevenly distributed among individuals, species, and families. This study suggests that in diverse mussel-fish communities, infestations on hosts are limited to specific taxonomic groups and guilds, the maintenance of which is vital to unionid conservation.

Introduction

Freshwater unionid mussels are distinguished as both one of the most imperiled groups of organisms worldwide and as a guild whose life histories and functional roles in ecosystems are not well understood (Bogan 1993, Vaughn and Hakenkamp 2001, Haag and Warren 2003). North America supports the highest unionid diversity in the world (Bogan 1993). Ninety-one percent of species ranges (269 of 297 currently recognized taxa) overlap at least one southeastern state, making the southeast an unparalleled center for bivalve biodiversity (Neves et al. 1997). At the same time, the greatest decline of endemic species is in the southeastern U.S., and believed to be the result of habitat destruction from pollution and modification of river channels, declines in host fish populations, and introduced species (Bogan 1993). Unfortunately, alterations of a majority of freshwater habitat occurred prior to our understanding of the ecosystem processes provided by intact freshwater mussel beds, which can dominate the biomass of bottom-dwelling organisms in North American rivers (Parmalee and Bogan 1998). Therefore, free-flowing watersheds with intact mussel populations represent an important information source for conservation management, as they may still reflect functional roles of unionid mussels in a community context (Haag and Warren 1998, Vaughn and Hakenkamp 2001, Vaughn et al. 2004).

For freshwater mussels, the larval stage is a critical period during which larvae (glochidia) must briefly parasitize suitable fish hosts in order to transform into free-living juveniles. Because most host relationships are determined from laboratory infection trials, little is known about how host use or infection strategies influence fish infestation patterns in the wild. Some field work has documented low rates of glochidial

transmission on putative fish hosts (4 to 14%), implying unionid reproductive success is determined in part by abundance of fish hosts (Neves and Widlak 1988, Holland-Bartels and Kammer 1989, Weiss and Layzer 1995). Information that does exist from field surveys is available for < 25% of North American bivalve species (Fuller 1974, Hoggarth 1992). However, these data are ambiguous if not linked to laboratory host trials confirming successful metamorphosis to the juvenile stage as glochidia can be rejected by a fish's immune response (Reuling 1919). Moreover, most field studies have either represented systems with low mussel diversity (Tedla and Fernando 1969, Stern 1978, Trdan 1981, Zaler and Neves 1982a, Threlfall 1986, Jansen and Hanson 1991), focused on a single species with distinctive morphology (Zale and Neves 1982b, Bruenderman and Neves 1993, Cummings and Mayer 1993, Gordon and Layzer 1993, Hove and Neves 1994, Baird 2000), or identified glochidia to genus, sub-family, or not at all (Neves and Widlak 1988, Holland-Bartels and Kammer 1989, Weaver et al. 1991, Weiss and Layzer 1995, Baird 2000). As far as we are aware, species-level patterns of glochidia occurrence in stream drift and on fishes in diverse mussel assemblages have not been accomplished.

The Sipsey River, Alabama, is useful for the study of mussel-host fish relationships because it is unregulated and harbors a diverse aquatic fauna with a restively intact mussel assemblage (McCullagh et al. 2002). Moreover, laboratory host fish infection trials have identified native fish species that glochidia use to successfully transform to juvenile life stages (Haag and Warren 1997, Haag and Warren 2003), which can be used in tandem with fish infestation patterns in the wild. The objectives of our study were to 1) quantify glochidia densities in drift samples, 2) quantify infestations of glochidia on fishes, and 3) identify glochidia in drift and on fishes using shell

morphology compared to DNA fingerprinting. The information we will provide from this investigation will assist in understanding the relationship between seasonal abundance of glochidia in stream drift and fish host use in a natural environmental setting. In addition, results of this research could provide an objective comparison of the use of morphological and molecular techniques to identify glochidia to species.

Materials and Methods-drift study

Field sampling

We established four permanent sampling points (replicates) in the river's thalweg 40.5 river km downstream from where fish sampling occurred (Fig. 1). On each sample date, we placed a single drift net with a detachable sample bucket (100 μm mesh size, net opening = 307 x 457 mm) at each sample point and collected stream drift for 30 min between 1000-1500 hours (see Neves and Widlak 1988). We calculated volume of water sampled (m^3) from measurements of water velocity and depth taken from the mouth of each net; we also measured water temperature in the study reach on each sample date. We stained drift samples in the field with phloxine B in order to differentiate glochidia from the sediment. Because most mussel species in the Sipsey River release glochidia in late spring and summer (Haag and Warren 2003), we sampled approximately weekly from May to August, and then twice monthly for the rest of the year; sampling commenced in May 2004 and concluded in April 2005.

Laboratory procedures and data analysis

We rinsed each sample over a 1 mm and a 100 μm sieve to remove debris, then preserved the material in 95% ethanol. We subsampled 16 % of the total volume of each sample, then counted and measured all glochidia in each subsample under a dissecting microscope equipped with a digital camera and video imaging software. We measured length, height, and hinge length of each glochidium to the nearest 1 μm (see Kennedy and Haag 2005). Each glochidium was then placed in individual cells filled with 100% ethanol, and frozen for subsequent genetic verification. Each glochidium was identified to species morphologically using discriminant functions of shell dimensions that had been determined previously (Kennedy & Haag 2005). On each date, we calculated mean glochidia abundance of all glochidia found in the four replicate samples and determined mean species abundances from the same replicate samples.

Results-drift study

Drift samples collected from May 2004 to April 2005 showed seasonal patterns of glochidia in drift of the Sipsey River, with a total of 1,457 individuals measured and identified. Total glochidia abundance was highest from May to early August; mean glochidia densities varied from 10/10 m^3 to 30/10 m^3 (Fig. 2). Peak glochidia releases occurred on May 28th (mean = 29/10 m^3) and August 8th, 2004 (mean = 30/10 m^3). Total glochidia densities were low from mid August to April, ranging from 0 to 5/10 m^3 (Figure 1). Water temperatures ranged from 10 ^{o}C on January 31, 2005 to 27 ^{o}C on July 15, 2004.

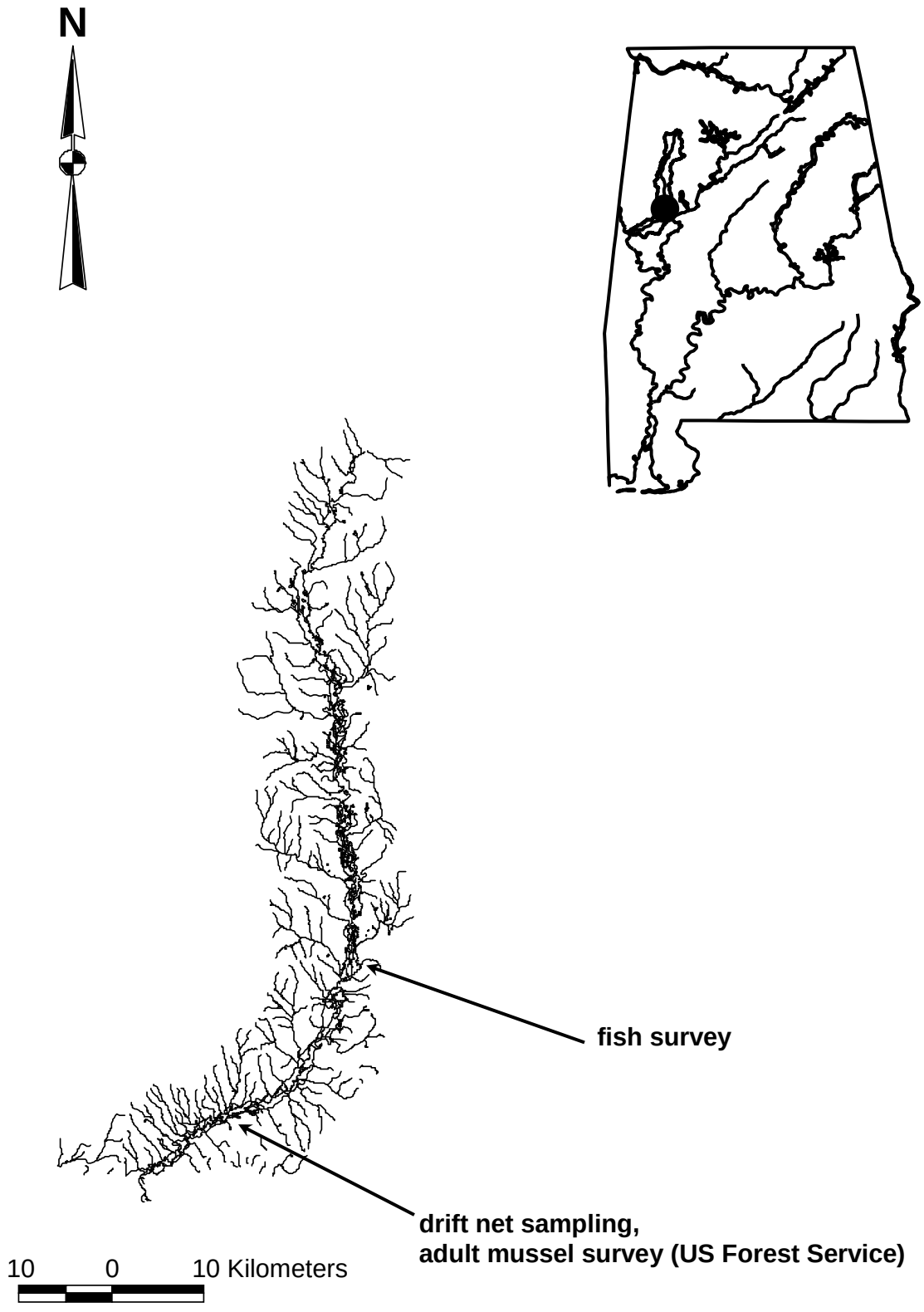

Figure 1. Sipsey River study site.

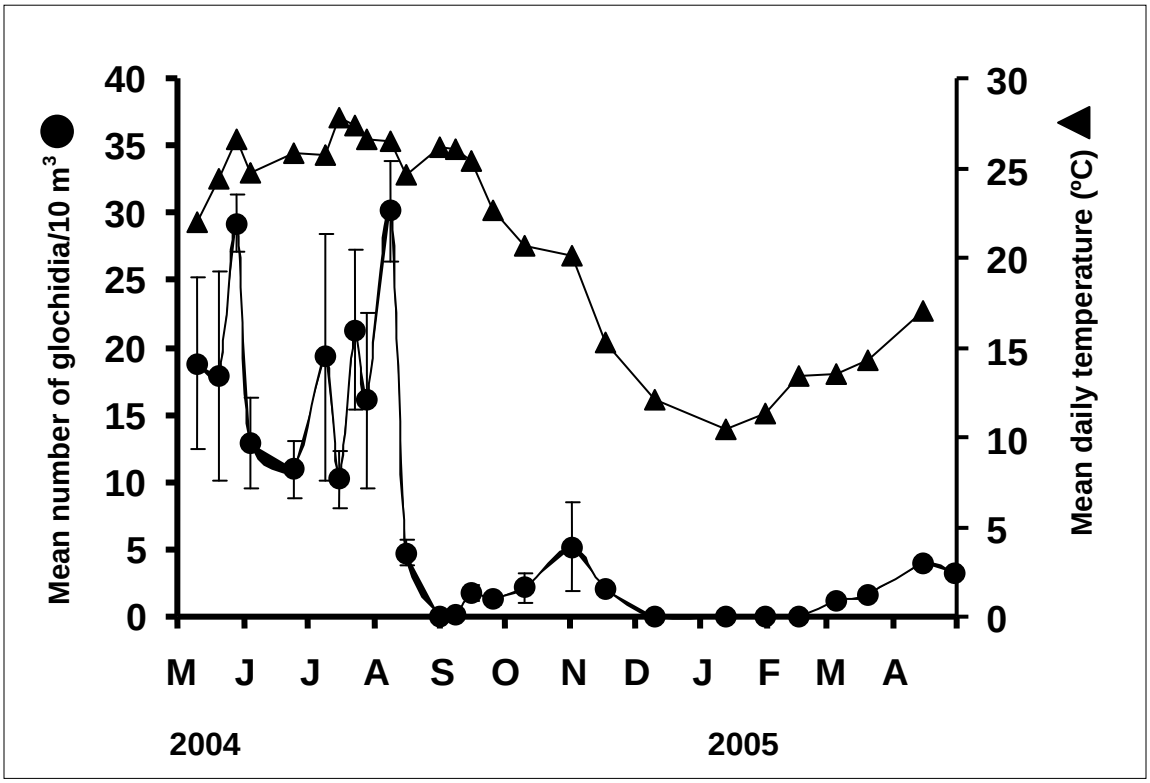

Figure 2. Total mean (± 1 standard deviation) number of glochidia / 10 m^3 water sampled and mean daily temperature.

A total of 16 species of glochidia were identified in drift samples; mean probability of correct species classifications ranged from a low of 60% (*Lampsilis straminea*) to a high of 96% (*Fusconaia cerina*), and averaged 83% (Table 1). Of the 16 species, 7 are considered short-term brooders and were present in drift from May to mid October; densities ranged from 0 to 86 glochidia/10 m^3, with the exception of *Quadrula asperata*, which was present on two other dates in fall and spring (Table 2). Three short-term brooding species were present only during May through July sampling: *Amblema plicata*, *Elliptio arca*, and *Quadrula rumphiana*. The additional 4 short-term brooders

were *Fusconaia cerina*, *Obliquaria reflexa*, *Pleurobema decisum*, and *Quadrula asperata*. *Fusconaia cerina* was the most prevalent species in drift, occurring from May to mid October, with peak densities in late July and early August sampling. O*bliquaria reflexa* occurred in drift from May to mid August and again on two dates in September. We found *Pleurobema decisum* in drift from May to mid August and on one date in September, with highest densities occurring in July and early August. We found *Quadrula asperata* in drift from May to early August and on one date in both November and April.

The remaining 9 species are all long term brooding species and were present in low densities throughout sampling, with the exception of mid August through September and December through January, when no glochidia were collected in drift (Table 3). Densities of long-term brooding species ranged from 0 to 11.1/ 10 m^3. *Hamiota perovalis*, *Potamilus purpuratus*, and *Villosa vibex* were present on 2 or fewer dates during spring and summer sampling. *Lampsilis ornata*, *Lampsilis teres*, and *Medionidus acutissimus* were the predominant long-term brooding species; *L. ornata* and *M. acutissimus* reached highest densities in November, and highest density of *L. teres* occurred in April.

Table 1. Glochidia species collected in drift of the Sipsey River, Alabama. Mean probabilities (in %) of correct species classifications (± 95% confidence limits; C.L.) were based on error rates of misclassifying glochidia using shell morphometrics (Kennedy & Haag 2005). Probabilities (p) were arcsine $\sqrt{p}$ transformed (p') before calculating statistics, then backward transformed $(\sin p')^2$ for data presentation.

Tribe	Species	No. glochidia identified	Prob. correct classification mean (95% C.L.)
Amblemini			
	Amblema plicata	140	63 (61-65)
Quadrulini			
	Quadrula asperata	121	94 (91-95)
	Quadrula rumphiana	2	97 (90-100)
Pleurobemini	*Elliptio arca*	130	74 (71-77)
	Fusconaia cerina	532	96 (96-97)
	Pleurobema decisum (1 & 2)	260	93 (91-95)
Lampsilini			
	Lampsilis ornata	28	68 (62-74)
	Hamiota perovalis	2	64 (49-77)
	Lampsilis straminea	11	60 (51-68)
	Lampsilis teres	47	89 (84-93)
	Medionidus acutissimus	58	82 (77-86)
	Obliquaria reflexa	78	68 (63-72)
	Obovaria unicolor	34	84 (78-90)
	Potamilus purpuratus	1	100
	Villosa lienosa	11	86 (78-93)
	Villosa vibex	2	70 (46-90)

Table 2. Densities (no./ 10 m^3) of short-term brooding species in drift of the Sipsey River, Alabama from May 2004 to April 2005. The two glochidia morphotypes of *Pleurobema decisum* were combined for this analysis.

	2004																2005	
Species	5/10	5/20	5/28	6/4	6/24	7/9	7/15	7/23	7/28	8/8	8/16	9/8	9/15	9/25	10/10	11/1	11/17	4/15	4/30
Amblema plicata	26.3	15.6	18.2	3.7	0.4	1.1	0.7	2.4	0.9										
Elliptio arca	14.2	19.3	15.9	3.2				1.4	6.3										
Fusconaia cerina	3.2	11.5	52.4	15.9	27.0	19.0	10.9	41.4	22.4	86.0	15.2	0.5	5.1	3.1	4.3				
Obliquaria reflexa	2.6	0.7	4.9	4.3	0.4	4.2	3.4	7.9	6.3	7.2	1.3		1.7	1.0					
Pleurobema decisum	11.6	7.4	11.0	12.2	7.5	35.9	10.2	17.9	13.9	20.9	2.5			1.0					
Quadrula asperata	6.8	10.4	6.1	7.4	5.2	6.3	2.0	4.7	7.6	2.4							0.9	2.2	
Quadrula rumphiana						1.1	0.7												

Table 3. Densities (no./ 10 m^3) of long-term brooding species in drift of the Sipsey River, Alabama from May 2004 to April 2005.

	2004																2005	
Species	5/10	5/20	5/28	6/4	6/24	7/9	7/15	7/23	7/28	8/8	8/16	9/8	9/15	9/25	10/10	11/1	11/17	4/15	4/30
Hamiota perovalis	0.5						0.7												
Lampsilis ornata	1.1	0.7	0.6		0.4	2.1	1.4								2.9	7.6	4.4	2.2	
Lampsilis straminea		0.4					2.7			0.8						2.3	0.9		1.2
Lampsilis teres	2.1	1.1	2.8	2.1	0.8	2.1	2.0	2.8	1.7	3.2								11.1	5.8
Medionidus acutissimus	2.6	2.9	1.1	2.7	0.4	4.2	3.4	2.4	1.7						1.4	9.9	1.8		3.5
Obovaria unicolor	1.1	0.4	3.3		1.2		2.7	3.3	3.4							0.8			2.3
Potamilus purpuratus						1.1													
Villosa lienosa	2.6	0.7	0.6		0.4			0.9											
Villosa vibex	0.5	0.4																	

Materials and Methods- glochidia infestations on fishes

Fish sampling

We sampled fishes monthly (every 21-47 days) between July 2003 and February 2005 from the Sipsey River (Fig. 1). We used a boat mounted electroshocking unit, hoop nets (with a 4' leads and 55mm mesh), minnow traps (6mm mesh), and a seine (2mm mesh) during every collection in order to obtain a representative sample of the fish assemblage. Immediately after capture, fishes were euthanized with an overdose of tricaine (MS -222, 0.32 g/l), transferred to ice, and temporarily stored in 10% buffered formalin in the laboratory. After ≥ 30 days of formalin storage, we identified, separated and stored all fishes into labeled jars filled with 69% ethanol and deposited them into the University of Alabama Ichthyological Collection (UAIC).

Laboratory protocol

We selected fish species for examination based on results of other regional field studies. We examined fishes externally using a 4x magnifying lens, then removed all gill arches, soaked them in a 5% potassium hydroxide solution for 1 to 5 minutes, and examined all gill filaments under a dissecting microscope. Upon finding a glochidium, we removed as much surrounding gill tissue as possible without damaging the shell, then placed all glochidia from each fish into separate well tray cells filled with 69% ethanol. We randomly selected glochidia to measure using a consistent subsampling protocol based on densities of encysted glochidia. We measured shell length, hinge length, and shell height of each glochidium to the nearest 1μm using a binocular microscope equipped with a digital camera and video imaging software. Each measured glochidium

was then placed in its own individual cell for subsequent molecular identification; we used shell measurements to identify each glochidium to putative species using discriminant function analysis described by Kennedy and Haag (2005).

Data analysis-monthly infestations

For fishes that we captured in sufficient quantities, we developed monthly glochidia parasite-host fish infestations patterns using proportions of glochidia species that comprised the total number of encysted glochidia. For each fish species or genus, we multiplied these proportions by percentage of infected individuals in order to scale figures relative to infestation levels. For simplicity, glochidia species comprising >5% of the total for any month were categorized by species, whereas glochidia species that consistently comprised <5% of the monthly total were grouped as 'other'.

Results-glochidia infestations on fishes

We found glochidia infestations were unevenly distributed among fish families, species, and individuals (Appendix A). Twenty-one of 49 species in 7 families were infected with glochidia; percentage of individuals infected with glochidia ranged from a high of 17% (Lepisosteidae) to a low of 3% (Fundulidae; Fig. 3). Of the 1,988 individuals examined, we found 3,045 encysted glochidia on 183 infected fishes (9.2% of total fishes examined), with one glochidium found on a fin of the weed shiner, *Notropis texanus*. Thirty-eight percent (n= 1,158) of all encysted glochidia were found on spotted gar (*Lepisosteus oculatus*).

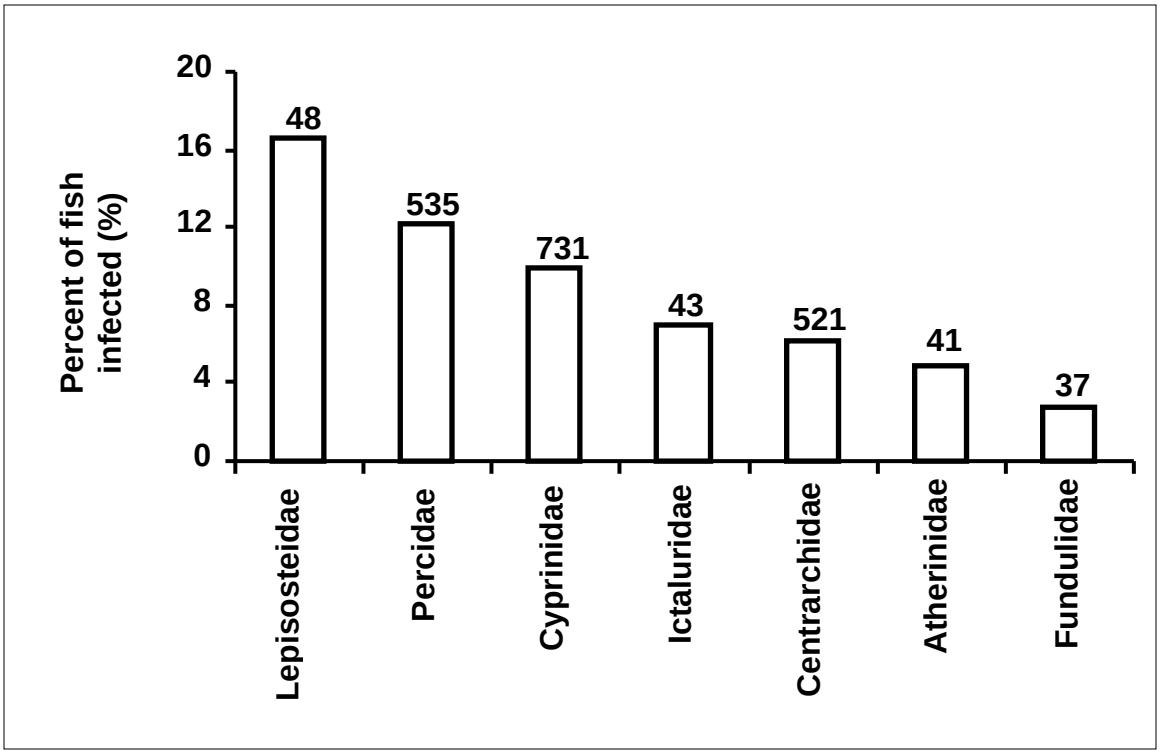

Figure 3. Percentage of fishes infected with glochidia by family. Number above each bar represents sample size.

We identified 37% of encysted glochidia (n= 1,115) to 18 species using shell morphometrics; mean probability of correct species classifications ranged from a low of 58% (*Amblema plicata*) to a high of 99% (*Pleurobema decisum* 1), and averaged 82% (Table 4). The three most abundant unionid species encysted on fishes were: *Obovaria unicolor* (24% of total glochidia identified), *Pleurobema decisum* 1 (13% of total), and *Lampsilis teres* (12% of total). These three unionids comprised nearly half (49%) of all identified glochidia found on fishes.

Table 4. Encysted glochidia identified to species using shell morphometrics (Kennedy & Haag 2005). The two morphotypes of *Pleurobema decisum* (1 & 2) are kept separate for this analysis. Probabilities (p) were arcsine $\sqrt{p}$ transformed (p') before calculating statistics, then backward transformed $(\sin p')^2$ for data presentation. Mean probabilities (in %) of correct species classifications (± 95% confidence limits; C.L.) were based on original misclassification error rates.

Tribe	Species	No. glochidia identified	Prob. correct classification mean (95% C.L.)
Amblemini			
	Amblema plicata	8	58 (39-75)
Quadrulini			
	Megalonaias nervosa	7	98 (90-100)
	Quadrula asperata	80	85 (81-89)
	Quadrula rumphiana	5	94 (84-100)
	Tritogonia verrucosa	1	51
Pleurobemini	*Elliptio arca*	9	71 (51-88)
	Fusconaia cerina	25	92 (85-97)
	Pleurobema decisum 1	141	99 (99-100)
	Pleurobema decisum 2	10	83 (70-94)
Lampsilini			
	Lampsilis ornata	133	69 (65-72)
	Hamiota perovalis	2	65 (20-97)
	Lampsilis straminea	37	68 (55-81)
	Lampsilis teres	134	81 (77-85)
	Medionidus acutissimus	113	79 (75-83)
	Obliquaria reflexa	101	66 (62-70)
	Obovaria unicolor	266	85 (83-87)
	Villosa lienosa	40	81 (76-86)
	Villosa vibex	3	80 (35-100)

Glochidia infestation patterns revealed temporal differences between fish species and genera (Figs. 4A-F). We found higher levels of fish infestations from April through October; *Cyprinella venusta* showed clearest temporal pattern, with individuals infected from April through August (Fig. 4A). Most *Lepisosteus oculatus* were infected from April until July (Fig. 4B), and *Micropterus* spp. were generally infected from June through October (Fig. 4C). We found lower incidence of infestations from December through February, but this was not always the case. For instance, we found *Lepomis* spp. were infected in January months and February 2004 (Fig. 4D). Both genera of darters were also infected during winter months (December through February); *Ammocrypta* spp. showing higher incidence of infection than *Etheostoma* spp. (Fig. 4 E & F).

Glochidia species richness differed for these putative fish hosts. For some fish species or genera, a majority of encysted glochidia were identified to a single species. We found 68% of encysted glochidia on *Cyprinella venusta* were *Pleurobema decisum* 1 (Fig. 4A), 42% of encysted glochidia on *Lepisosteus oculatus* were *Lampsilis ornata* (Fig. 4B), and 61% of encysted glochidia on *Ammocrypta* spp. were *Obovaria unicolor* (Fig. 4E). Other putative fish hosts did not show as distinct of a single-species association. Larvae of 5 different species of Lampsiline unionids were found encysted on *Micropterus* spp.; species proportions ranged from 5% to 34% of the total (Fig. 4C). We found *Lepomis* spp. harbored 15 unionid species; most (83% of total) were identified either as *Quadrula asperata* or a Lampsiline species (Fig. 4D). We identified all encysted glochidia on *Etheostoma* spp. as Lampsiline unionids, although species proportions were not noticeably skewed, and ranged from 11% for *Lampsilis ornata* to 28% for both *Obovaria unicolor* and *Medionidus acutissimus* (Fig. 4F).

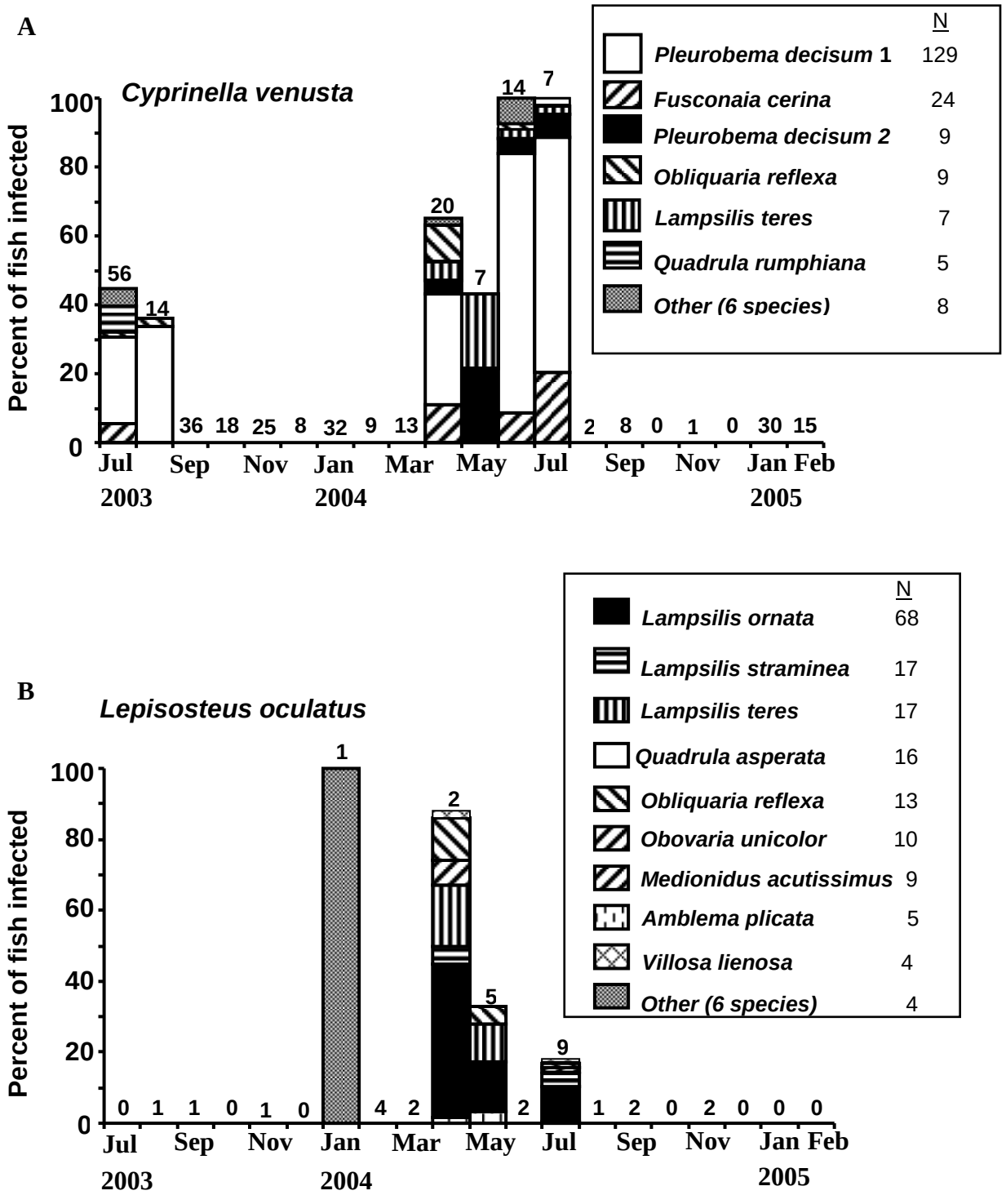

Figure 4. Monthly fish infestations (percent of fish examined) for A) blacktail shiner (*Cyprinella venusta*) and B) spotted gar (*Lepisosteus oculatus*). Number above each bar/month represents sample size.

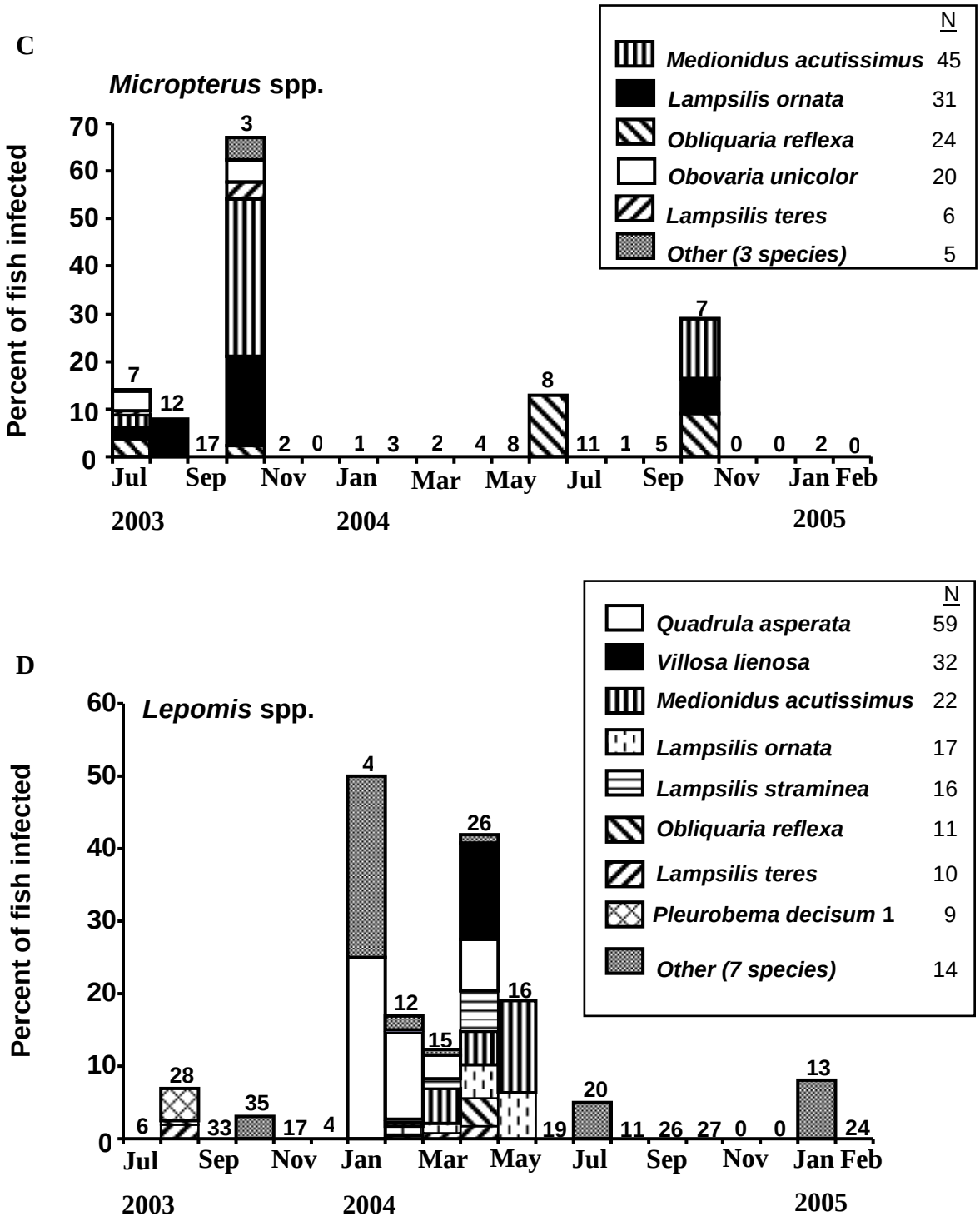

Figure 4. Monthly fish infestations (percent of fish examined) for C) *Micropterus* spp.: spotted bass (*M. punctulatus*), largemouth bass (*M. salmoides*) and D) *Lepomis* spp.: dollar sunfish (*L. marginatus*), redspotted sunfish (*L. miniatus*), bluegill (*L. macrochirus*). Number above each bar/month represents sample size.

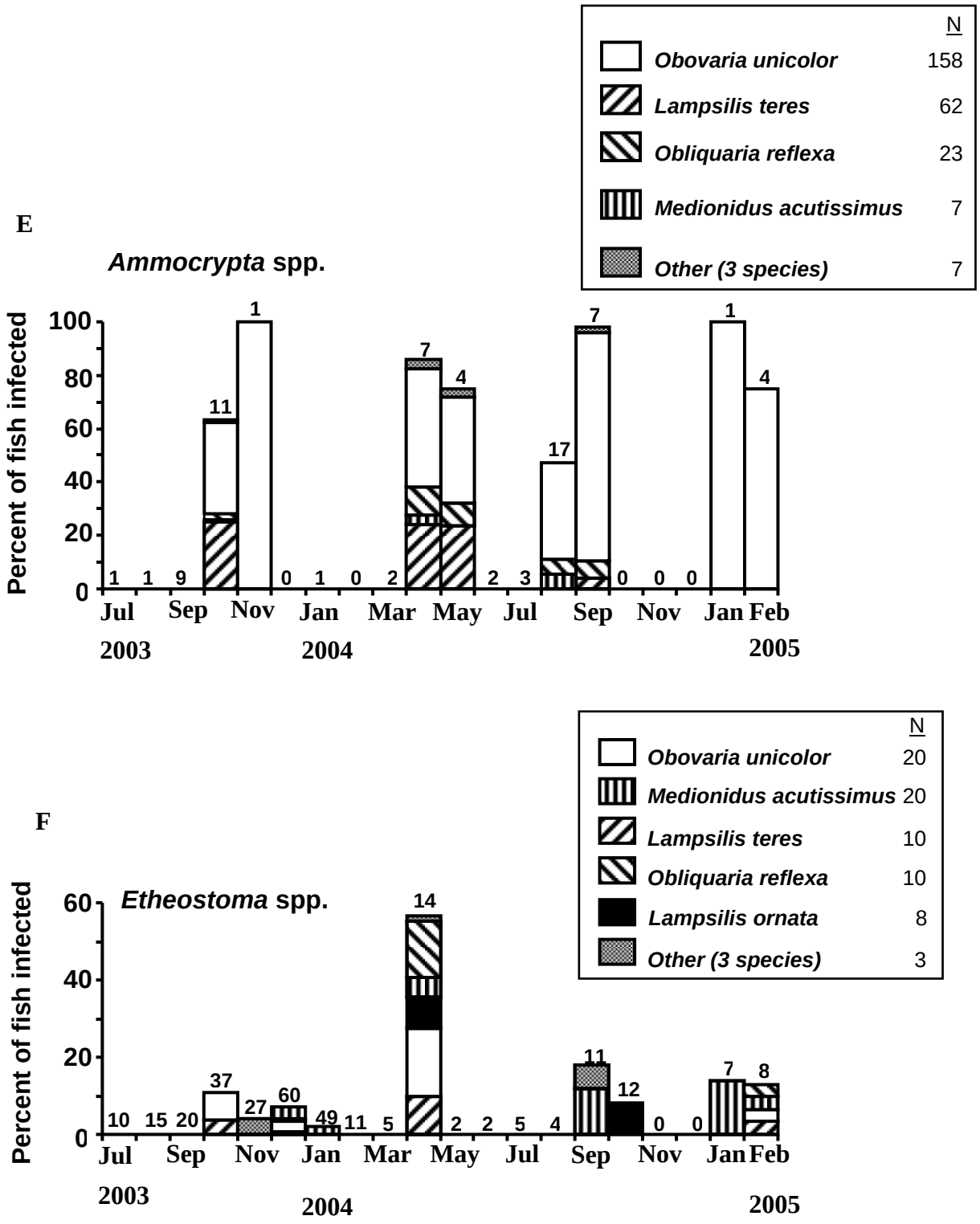

Figure 4. Monthly fish infestations (percent of fish examined) for E) *Ammocrypta* spp.: naked sand darter (*A. beani*), southern sand darter (*A. meridiana*) and F) *Etheostoma* spp.: speckled darter (*E. stigmaeum*), gulf darter (*E. swaini*), bluntnose darter (*E. chlorosoma*), johnny darter (*E. nigrum*), rock darter (*E. rupestre*). Number above each bar/month represents sample size.

Materials and Methods- molecular validation of glochidia species

The molecular portion of this study focused on identifying glochidia to species using restriction fragment length polymorphisms (RFLPs). Tissue from previously collected and identified adult mussels was used to create an RFLP library. A DNA reference library was created using tissue from identified adult mussels native to the Sipsey River as fingerprints to taxonomically identify glochidia found in drift samples. Adult specimens were collected and available from Scientific Collections at the University of Alabama. Genomic DNA was extracted from adult specimens and the 16S mitochondrial rDNA was amplified using universal primer pairs. The amplified DNA was cleaned and digested with 5 restriction enzymes to create species specific banding patterns (RFLP library). Unknown glochidia samples were analyzed in the same manner and compared to the RFLP library for molecular-level species identification.

Results-molecular validation of glochidia species

Total DNA was isolated from all adult mussel species and PCR amplification was successful for a portion of the specimens (Table 1). The twelve successful amplicons were digested and a partial RFLP library was constructed with three restriction enzymes (Table 1). The partial library was informative, but unable to be used for glochidia identification due to missing species.

Table 1. Separation of species by RFLP banding patterns; an 'x' indicates species identification/separation. Digests were run separately, with *Hae*III the first restriction enzyme digest and TaqI the last. Species in grey denote unsuccessful amplifications.

		Restriction Enzyme		
Tribe	Species	*Hae*III	*Fok*I	*Taq*I
Anodontini	*Pyganodon grandis*			
Amblemini	*Amblema plicata*		x	
Quadrulini	*Megalonaias nervosa*			
	Quadrula asperata			
	Quadrula rumphiana			x
	Tritogonia verrucosa			
Pleurobemini	*Elliptio arca*	x		
	Fusconaia cerina			
	Pleurobema decisum			x
Lampsilini	*Leptodea fragilis*			
	Lampsilis ornata			x
	Hamiota perovalis			
	Lampsilis straminea	x		
	Lampsilis teres	x		
	Medionidus acutissimus		x	
	Obliquaria reflexa			
	Potamilus purpuratus	x		
	Villosa lienosa	x		
	Villosa vibex	x		

PCR amplification for 8 of 20 species was unsuccessful with fresh, frozen, and preserved specimens. Various techniques were used, including: changes in thermalcycler conditions, reaction mixtures, TAQ, DNA concentrations, specimen types, and inhibitor removals (such as bovine serum albumin). We did not use other primers due to time limitations. Universal primer sequences are thought to amplify all organisms within a particular group (i.e. phylum, order, etc). However, recent studies have suggested that "universal" primers may be unsuccessful for the amplification of every species (Farris et al., unpublished). The primers used in this study were universal mitochondrial primers, but they did not provide data for all species. The construction of an RFLP library from known mussel specimens was unsuccessful due to limitations of "universal" primers.

Discussion

Drift net study

The seasonal timing of glochidia release within the Sipsey River was similar to previous studies in other streams. Short-term brooders are generally reported releasing glochidia from mid to late summer (e.g. Neves and Widlak 1988, Weaver et al.1991, Bruenderman and Neves 1993) and all Sipsey River short-term brooders were collected in drift during summer months. Two species, *Fusconaia cerina* and *Pleurobema decisum*, were present during fall sampling as well. Glochidia from long-term brooding species are present in drift year round in low densities (Neves and Widlak 1988) and 9 long-term brooding species were present in the Sipsey River throughout the sampling year in low densities, with the exception of late summer and winter. It is likely that most long-term brooding species spawn from late summer to early fall in the Sipsey River.

Short-term brooders released glochidia for a longer duration in the Sipsey River than previous reports from other streams, and no glochidia from long-term brooders were found in drift during the winter, as earlier studies have found. This indicates other factors besides time of year affects mussel releases of glochidia. Numerous studies have indicated a correlation between a threshold temperature and the onset of glochidia release in freshwater mussels (Chamberlain 1934, Young and Williams 1984, Kondo 1993, and Watters and O'Dee 1998). Because short-term brooders release glochidia during summer, it is probable that the water must reach a certain warm water threshold that brings about the cue for the release of their glochidia. Water temperatures at the study site were higher than 20 ^{o}C from May through mid October. This indicates that if a warm water cue for release of glochidia in short-term brooders exists, it is likely that it is at or

above 20 ^{o}C, and may explain the earlier and more protracted period of glochidial release for short-term brooders in the Sipsey River. Since glochidia of long-term brooders were not present when water temperatures were below 13 ^{o}C in this study, there may be a cold water threshold for these species as well.

Glochidia infestation patterns on fishes

Our results are the first demonstration of species-specific parasite-host associations in a diverse riverine ecosystem. We found glochidial transmission resulted in highly skewed parasite loads on individual fishes, where only 9.2% of the 1,988 fishes we examined were infected with glochidia. Infestations were also unevenly distributed among 21 species in 7 families. Contrary to other studies which typically found cyprinid species carried most of encysted glochidia, we found lepisosteid and percid species were more heavily infected. More than one-third of all encysted glochidia were carried by spotted gar. This species has not been identified as a host in previous field or laboratory investigations, although its congener, the longnose gar, was found to carry glochidia in a Kentucky river drainage (Weiss and Layzer 1995). Although we did not examine catostomids for infestations, our results are unlikely to change with their inclusion since this family has not been identified as a major host source for glochidia. Besides, we collected low numbers of most catostomid species, so trends in incidence of infestations would not be possible to develop.

All of the 3 unionid species (*Obovaria unicolor*, *Pleurobema decisum*, and *Lampsilis teres*) that comprised nearly half of the encysted glochidia we identified using shell morphometrics exhibited some degree of species-specific host fish associations.

Both *O. unicolor* and *L. teres* comprised >85% of total encysted glochidia found on *Ammocrypta beani* and *A. meridiana*. Both glochidial morphotypes of *Pleurobema decisum* comprised >72% of total encysted glochidia found on *Cyprinella venusta*. Other unionid species exhibited even more distinctive species-specific associations (*Fusconaia cerina*, *Quadrula rumphiana-Cyprinella venusta*; *Amblema plicata-Lepisosteus oculatus*), while other species were found ubiquitously on fishes (*Obliquaria reflexa*).

Seasonal trends in incidence of infections indicated higher fish infestations from April through October and lowest levels from December through February.

Molecular validation of glochidia species

Confirmation of glochidial species identifications with molecular techniques was not possible to perform. We plan to address potential misclassifications of glochidia in subsequent publications (Culp et al., in prep. ; Kennedy et al., in prep) by excluding individual glochidia with a low probability of correct classification using a bootstrapping approach.

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Kennedy, TB and WR Haag. 2005. Using morphometrics to identify glochidia from a diverse freshwater mussel community. Journal of the North American Benthological Society 24(4):880-889.

ORAL PRESENTATIONS

North American Benthological Society. 2006. **Kennedy, TB**, WR Haag and AC Benke. Occurrence of parasitic freshwater mussel larvae on host fishes in a diverse southeastern river, USA. Anchorage, Alaska (Abstract published in Bulletin of the N. American Benth. Soc. 23:110).

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POSTER PRESENTATIONS

University of Alabama, Undergraduate Research, Creative Activity Competition. 2007. **AB McGraw**, TB Kennedy and AC Benke. Temporal infestation of *Pleurobema decisum* glochidia on the host fish *Cyprinella venusta* in an unregulated southeastern USA river.

University of Alabama, Undergraduate Research, Creative Activity Competition. 2005. **JC Phillips** and TB Kennedy. Tag, you're it! The freshwater mussel parasite-fish host relationship in a southeastern watershed.

Appendix A. Encysted glochidia on fishes sampled from the Sipsey River, July 2003-February 2005.

			Number of fish		Distribution on infected fish			
Family	**Genus**	**species**	**examined**	**infected**	**Mean**	**Max**	**Total**	**% of glochidia**
Lepisosteidae	*Lepisosteus*	*oculatus*	33	8	145	605	1158	38.0
	Lepisosteus	*osseus*	15	0	0	0	0	
Clupeidae	*Dorosoma*	*cepedianum*	7	0	0	0	0	
Cyprinidae	*Cyprinella*	*venusta*[1]	316	67	5	42	335	11.0
	Notropis	*texanus*	196	4	1	1	4	0.1
	Hybopsis	*winchelli*[1]	22	1	-	1	1	<0.1
	Lythrurus	*bellus*	74	0	0	0	0	
	Pimephales	*vigilax*	46	0	0	0	0	
	Opsopoeodus	*emiliae*	6	0	0	0	0	
	Notropis	*ammophilus*	65	0	0	0	0	
	Notropis	*stilbius*	4	0	0	0	0	
	Macrhybopsis	*storeriana*	2	0	0	0	0	
Ictaluridae	*Noturus*	*funebris*	11	3	2	3	6	0.2
	Ameiurus	*melas*	1	0	0	0	0	
	Ictalurus	*punctatus*	14	0	0	0	0	
	Noturus	*leptacanthus*	13	0	0	0	0	
	Pylodictis	*olivaris*	4	0	0	0	0	
Aphredoderidae	*Aphredoderus*	*sayanus*	2	0	0	0	0	
Atherinidae	*Labidesthes*	*sicculus*	41	2	-	1	2	0.1
Fundulidae	*Fundulus*	*olivaceus*[2]	37	1	-	1	1	<0.1
Poeciliidae	*Gambusia*	*affinis*	12	0	0	0	0	
Centrarchidae	*Micropterus*	*salmoides*[1]	59	3	134	399	401	13.2
	Lepomis	*megalotis*[2]	111	9	39	186	348	11.4
	Micropterus	*punctulatus*[2]	35	4	40	87	160	5.3
	Lepomis	*marginatus*	48	4	21	58	82	2.7
	Lepomis	*miniatus*	25	5	4	11	22	0.7
	Lepomis	*macrochirus*[2]	152	7	2	4	11	0.4
	Ambloplites	*ariommus*	26	0	0	0	0	
	Lepomis	*cyanellus*	7	0	0	0	0	
	Lepomis	*gulosus*	20	0	0	0	0	
	Lepomis	*microlophus*	10	0	0	0	0	
	Pomoxis	*annularis*	9	0	0	0	0	
	Pomoxis	*nigromaculatus*	19	0	0	0	0	
Percidae	*Ammocrypta*	*beani*[1]	44	25	13	38	334	11.0
	Ammocrypta	*meridiana*[1]	26	12	8	24	97	3.2
	Etheostoma	*stigmaeum*[1]	152	17	4	16	61	2.0

			Number of fish		Distribution on infected fish			
Family	**Genus**	**species**	**examined**	**infected**	**Mean**	**Max**	**Total**	**% of glochidia**
	Etheostoma	*swaini*[1]	116	5	2	7	12	0.4
	Etheostoma	*chlorosoma*	28	1	-	3	3	0.1
	Etheostoma	*nigrum*[1]	7	2	-	1	2	0.1
	Percina	*maculata*	20	1	-	2	2	0.1
	Etheostoma	*rupestre*[1]	34	2	-	2	4	0.1
	Etheostoma	*histrio*	10	0	0	0	0	
	Etheostoma	*lachneri*	6	0	0	0	0	
	Etheostoma	*parvipinne*	2	0	0	0	0	
	Etheostoma	*proeliare*	17	0	0	0	0	
	Percina	*nigrofasciata*	55	0	0	0	0	
	Percina	*vigil*	14	0	0	0	0	
	Percina	*kathae*	4	0	0	0	0	
Sciaenidae	*Aplodinotus*	*grunniens*	11	0	0	0	0	
Number of species examined:			49					
Number of individuals examined:			1988					
Percentage of individuals infected:			9.2					
Total number of encysted glochidia:							3046	

[1]Identified as suitable or marginal host in laboratory trials using native species of the Sipsey River, Mobile Basin (Haag and Warren 2003).

[2]Identified as suitable or marginal host in laboratory trials using native species of the Black Warrior River, Mobile Basin (Haag and Warren 1997).