REPRODUCTIVE BIOLOGY AND HOST REQUIREMENT DIFFERENCES AMONG ISOLATED POPULATIONS OF *CYPROGENIA ABERTI* (CONRAD, 1850)

A Thesis

Presented to

The Graduate College of

Southwest Missouri State University

In Partial Fulfillment

of the Requirements for the

Master of Science, Biology

By

Nathan L. Eckert

August 2003

REPRODUCTIVE BIOLOGY AND HOST REQUIREMENT DIFFERENCES AMONG ISOLATED POPULATIONS OF *CYPROGENIA ABERTI* (CONRAD, 1850)

Biology Department

Southwest Missouri State University, August 2003

Master of Science

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ABSTRACT

Cyprogenia aberti, the Western fanshell, is a rare and threatened pearly mussel endemic to the Interior Highlands of Eastern North America. Previous genetic analysis suggested that multiple species are present within this taxon. The present study sought phenotypic differences among genetically distinct populations in the upper Arkansas River system (Verdigris and Spring rivers), the St. Francis River, and the Ouachita River. Like other native mussels, the glochidia larvae of *Cyprogenia* are obligate parasites on particular species of host fish. Transformation success of glochidia was compared among 8 species of *Percina* and *Etheostoma*. The percentage of attached glochidia that transformed on individual fish ranged between 0 and 86%. Effective hosts (those that transformed a large proportion of attached glochidia) were always sympatric with the mussel population, and species with narrow geographic range were effective hosts only for sympatric mussel populations. However, two populations of a geographically widespread host species, the logperch, were effective hosts for each mussel population tested. The timing of glochidia and juvenile drop-off appeared to be related to the age or maturity of the glochidia. Glochidia size and shape differed among mussel populations. Conglutinate color, which is determined by the color of undeveloped eggs, varied within and among populations. Upper Arkansas mussels produced only white conglutinates. Ouachita and St. Francis mussels produced either red or brown conglutinates. The conglutinate color of individuals in the Ouachita and St. Francis populations correlated with two widely different mitochondrial genotypes present in both of these populations. Overall, the results demonstrate that all three mussel populations are distinct and they should be recognized and protected as such.

This abstract is approved as to form and content

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Dr. M. Chris Barnhart
Chairperson, Advisory Committee
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ACKNOWLEDGEMENTS

First, heartfelt thanks to my advisor Dr. Chris Barnhart. Without his insight and expertise, this project would have been much different. He helped me see things from a different perspective, and always challenged me to do more. I thoroughly enjoyed my time working in his lab.

I also want to recognize my committee members Dr. Dan Beckman and Dr. Mark McKnight. I enjoyed taking classes from each of them and their input during the editing process was much appreciated.

I must also thank the U.S. Fish and Wildlife Service, the SMSU Biology department, the SMSU graduate college and the Topping award fellowship for providing funding. Without the new equipment purchased with grant money, this research would have been very difficult.

I would like to thank the following individuals for helping me with field collecting and lab work. Dr. Chris Barnhart, Bryan Simmons, Shelley Good, Bob Brown, Ben Dodd, Sean Lynott, Bill Posey, Jeremy Myers, Todd Fobian, Dennis Whelan, Dave Ostendorf and Dave Herzog. I would also like to give special recognition to Ed Miller and Christian Hutson for all of the time they spent with me out in the field. Their help proved to be invaluable collecting animals in conditions that were often less than ideal.

For input and correspondence I wish to thank Dr. Jeanne Serb, Brian Obermeyer, Ryan Waters, Al Christian, Mark Smith, and Dr. Michael Hoggarth. They each took time to speak with me and also sent valuable information about *C. aberti*.

I must also thank a large group of people who collectively helped me get to this point, the first of which are my parents. Without their support I would not be here now. As a group I would like to thank all of the science teachers and professors that I have learned from over the years, I am grateful for their instruction. I would also like to thank a group of guys simple known as "the kids".

Finally, I would like to thank my wife Shelley for her enduring patience. She listened to my ideas, helped out at many various stages of the project (especially the statistical analysis), tolerated my long hours at the office and didn't complain too much when I diverted our road trips to try and find mussels.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Freshwater mussels (family Unionidae) are one of the most imperiled groups of organisms in the world. More than 70% of recognized species in North America are either endangered, threatened or of special concern (Williams et al. 1993). The greatest diversity of unionids in the world lies in the Mississippi river system, particularly in the southeastern United States (Neves et al. 1997). Unionids are threatened mostly by human activities. Channelization and impoundment of rivers, forest clearing, and increasing pollution have negatively impacted mussel assemblages by reducing suitable habitat, decreasing water quality, and altering native fish assemblages (Lefevre and Curtis 1912, Bogan 1993, Neves et al. 1997, Duncan 2002).

The most vulnerable stage of the Unionid life cycle is the larval stage. Larval mussels, called glochidia, are released from the female and must undergo a parasitic period on the gill filaments of a host fish before transforming into juveniles. Mussels are host specific and cannot complete their life cycle or sustain populations if the correct host species is not present in sufficient numbers. Glochidia that attach and transform must then leave the fish and land in suitable substrate in order to survive. These two restrictions lead to a bottleneck in the lifecycle of mussels. Only a small fraction of the total glochidia produced will ever reach a suitable host (Young and Williams 1984), and even fewer transform and reach substrate suitable for the growth from juvenile to adult.

Host requirements are known for only about one quarter of unionids, and many of those species should be restudied (Hoggarth 1992). Mussels vary in the degree of host specificity. Some species transform on multiple host species, while others may

transform on only one host species (Hoggarth 1992, Watters 1996, Haag and Warren 1997). The ability to use particular hosts can differ among closely related species of mussel (Riusech and Barnhart 2000) and a mussel species may transform optimally only on particular populations within a host species (Rogers et al. 2001). This co-evolution of mussels with sympatric hosts could be a factor leading to speciation (Graf 1997). It is important for conservation to determine host fish requirements for each mussel population, because propagation and restoration efforts will not be successful if appropriate host species are not present in the fish assemblage (Farzaad 1991).

The mussel host requirement is an important factor in dispersal and gene flow. The dispersal of juveniles upstream to new habitats is tied to the mobility of the host fish. Alleles that allow for transformation on a specific host fish can not be distributed to other populations when the host itself has limited mobility (Kat 1984, Berg et al. 1998). For example, mussels that utilize sedentary hosts such as darters have less chance for gene flow between isolated populations than mussels that utilize more mobile hosts such as drum or certain catfish. Clearly, these characteristics should be considered in any study seeking to distinguish among mussel populations for conservation purposes.

The conservation of freshwater mussels is impeded by inadequate and inaccurate understanding of their biological diversity and phylogenetic relationships. Species of freshwater mussels have historically been distinguished and defined by shell morphology alone, with little or no consideration given to physiological, ecological, or genetic characters. Studies show that shell morphology is not a reliable indicator of genetic similarities or differences among mussel populations (Lydeard et al. 1996, Hoeh and Gordon 1996). Recent genetic studies indicate that some genetically different

populations of freshwater mussels may not be reliably distinguished from one another by shell morphology (Roe and Lydeard 1998, King et al. 1999). For example, differences in the mitochondrial cytochrome oxidase (*cox1*) gene between two populations of one morphologically defined species, *Potamilus inflatus,* were greater than the difference between two other morphologically distinct species, *Potamilus purpuratus* and *Potamilus alatus* (Roe and Lydeard 1998). On the other hand, two species in different genera, the pink mucket (*Lampsilis abrupta*) and the mucket (*Actinonaias ligamentina*), showed no difference in cox1 sequence (Hsiu-Ping Liu, Pers. Comm). Finally, genetic analysis alone may fail to reveal critical aspects of biodiversity, such as host requirement. Therefore, in addition to genetic analysis, it is important to identify and investigate those physiological characteristics that are pertinent to management.

The genus *Cyprogenia* presently includes two species: the fanshell, *C. stegaria,* and the Western fanshell, *C. aberti. Cyprogenia stegaria* occurs in the Ohio, Cumberland, and Tennessee River systems, and *C. aberti* is found in the St. Francis, White, Ouachita and Arkansas River systems (Figure 1). *Cyprogenia stegaria* is federally endangered (Federal Register 1989). *Cyprogenia aberti* is listed as state endangered in Kansas and Missouri, and is a species of special concern in Arkansas. *Cyprogenia aberti* is believed to be extirpated from Oklahoma (Mather 1990). In a recent study of the mussel fauna of southeastern Kansas, *C. aberti* represented only 0.2% of all mussels sampled (Obermeyer et al. 1997).

Cyprogenia (fanshells) employ a unique reproductive strategy. *Cyprogenia* are bradytictic, meaning they begin brooding in the fall and release glochidia in the spring (Surber 1912, Ortmann 1919, Chamberlain 1934). Fanshells release their eggs as a

conglutinated wormlike strand (Lea 1834, Ortmann 1912, Lefevre and Curtis 1912). These conglutinates consist of a core of sterile eggs with only the outermost eggs containing viable glochidia (Barnhart 1997, Jones and Neves 2002; Figure 2). Conglutinates resemble annelid worms, a trait that attracts host fish [*Etheostoma flabellare*, the fantail darter, and *Percina caprodes*, the logperch for *C. aberti*, (Barnhart 1997), or *Percina roanoka,* the Roanoke darter, and *Etheostoma blennioides,* the greenside darter for *C. stegaria* (Jones and Neves 2002)] to attempt to consume them and encounter glochidia.

The colors of conglutinates vary among fanshell populations (Figure 3). Chamberlain (1934) observed *C. aberti* from the St. Francis River extruding red, worm-like conglutinates from mid-February to mid-March. Jones and Neves (2002) observed *C. stegaria* from the Clinch River with red conglutinates and Barnhart (1997) observed *C. aberti* from the Spring River (upper Arkansas River system) with white conglutinates.

The current taxonomic challenge to *C. aberti* was brought about by a phylogenetic analysis conducted by Jeanne Serb at the University of Alabama. Her analysis of portions of the mitochondrial *cox1* (529 bp) and *nad1* (597 bp) genes indicated that *C. aberti* is not a monophyletic group and may comprise 2 and possibly 5 distinct taxa (Serb and Obermeyer 2001, Serb 2003, Figure 4). In Serb's analysis, there are 2 major clades of fanshells, A and B. *Cyprogenia* from the Black River (White River system) are closely related to *C. stegaria* from the Clinch River in Tennessee, and form one clade within clade A. *Cyprogenia* from the upper Arkansas system also form one well-supported clade within clade A. A portion of the *Cyprogenia* from the Ouachita and St. Francis River systems form the final clade in clade A. *Cyprogenia* from the St.

Francis, White and Ouachita River systems form the two clades within Clade B. Because of this complexity more research is being conducted on both mitochondrial and nuclear DNA.

The present study is an investigation of reproductive characters in western populations of *Cyprogenia,* including those in the Verdigris and Spring rivers in the upper Arkansas River system, the St. Francis River, and Ouachita River. I compared the host requirements of these populations as well as the morphology of conglutinates and glochidia. Similarities and differences in host requirement can be used as evidence to aid in revising taxonomy within this genus, as well as in management decisions. These experiments test the prediction that fanshell populations will transform glochidia significantly better on sympatric host species than on allopatric host species.

METHODS

Identification and Names

Fish and mussel specimens used in this study were identified according to characters listed in Oesch (1984), Pflieger (1997), and Robison and Buchanan (1992). Voucher specimens were deposited with the Illinois Natural History Survey. In the following discussion, both scientific and common names will be used to refer to fish species according to Robins et al. (1991). The name "fanshell" will be used as a synonym for the genus *Cyprogenia*.

Mussel Collection and Care

Collection sites were located in 3 river systems, the upper Arkansas, St. Francis, and Ouachita (Figure 5, Table 1). Collections were made in the Verdigris River and Spring River in southeastern Kansas (upper Arkansas system), the St. Francis River in SE Missouri and the Ouachita River in central Arkansas. Gravid female *Cyprogenia aberti* were collected by hand while wading or snorkeling. Gravid females were recognized by gently opening the valves and observing the presence of inflated marsupial gills. After collection, mussels were transported in aerated coolers to SMSU where they were kept in plastic tubs filled with moderately hard synthetic fresh water which was made according to EPA formula (USEPA 1993). The water in each tub was changed every 2 weeks. Temperature was kept at 5º C to prevent discharge of conglutinates. The mussels were not fed during the study.

Fish Collection and Care

Fish collection sites were chosen based on availability of host fish and the absence of *C. aberti* in the immediate vicinity (Figure 5, Table 2). In most cases, the fish were collected using a backpack electrofishing unit and seine. Logperch were collected from the St. Francis River by trawling with a modified trawl (Herzog et. al, submitted). This method was extremely effective for collecting logperch, but the catch suffered a 50% mortality rate. At the time of collection, fish were treated with salt and acriflavin as a precautionary measure and then transported in aerated coolers to SMSU. Upon arrival in the lab fish were treated prophylactically with Kanamycin sulfate to prevent disease, and were acclimated to room temperature and housed in 20 gallon aquaria. Fish were fed live blackworms daily.

Species chosen for host testing included logperch (*Percina caprodes*), rainbow darter (*Etheostoma caeruleum*), slenderhead darter (*P. phoxocephala*), orangethroat darter (*E. spectabile squamosum*), greenside darter (*E. blennioides*), fantail darter (*E. flabellare*), banded darter (*E. zonale*) and the orangebelly darter (*E. radiosum*). Collection localities for each species are given in Table 2. These species were chosen based on high abundance at the sites and in some cases on preliminary tests that showed transformation.

Host Tests

Mussels were stimulated to release conglutinates by raising water temperature from 5 ºC to 20 ºC over approximately 7 hours. Each mussel began expelling conglutinates within a few hours of reaching room temperature. Three mussels from each site were used and a minimum of 3 conglutinates were collected from each.

Conglutinates were processed within hours of release. The length of each conglutinate was measured. Glochidia were then removed by shaking the conglutinate in 2 ml of water in the bulb of a disposable plastic pipette. The water was expelled and replaced frequently during this process to quickly remove dislodged glochidia before any damage occurred. Vigorous shaking for several minutes removed nearly all glochidia.

The number of glochidia per conglutinate was estimated as follows: The glochidia were suspended in 100-300 ml water by agitating with a pipette. While stirring, ten 20-microliter samples were removed with a volumetric pipette and placed as individual drops on polystyrene Petri plates. The glochidia in each drop were counted under magnification. The number per ml was multiplied by the total suspension volume to obtain the total number of glochidia. Glochidia from 3-6 conglutinates were pooled to obtain a similar number of glochidia from each mussel for inoculation of host fish. The viability of a portion of the glochidia was tested by observing the reaction to a salt solution, glochidia that closed were considered viable, while those glochidia that did not close were not counted towards the total viable glochidia (Table 3).

Each mussel population was tested using two host species from two rivers (Table 4). Host fish were separated into six groups, each group containing all four host species to be tested (Tables 5, 6). Each group was inoculated with glochidia from one mussel. Groups were placed in 3 L of water containing 18,000 glochidia for 15 min (experiment A) or for 11 min with 15,000 glochidia (experiments B & C). The fish were then placed in a clean aquarium for 10 minutes to rinse off stray glochidia, and then placed individually in 1-L tanks (see below).

Monitoring Transformation

Transformation was monitored in a specially modified AHAB® system (Aquatic Ecosystems, Inc.). Each fish was kept separately in a 1-L tank in a common recirculating system. Flow through each tank was maintained at approximately 1 L/min, and temperature was constant at 22ºC ± 1ºC . The system was modified so that water exiting each tank passed through filter cups with 125µm screen (Nitex®) that collected glochidia and juveniles. Each filter was checked every 2 days. Flow was increased to approximately 2 L/min for 30 minutes prior to checking the filters, in order to ensure that all glochidia or juveniles were removed from the tank. Each filter was rinsed into a glass dish and the catch was observed and counted under a dissecting microscope. The number of dead glochidia and transformed juveniles from each fish was recorded. Live juveniles were separated and either released at the site of origin or used in other experiments. One week after the final live juvenile was found each fish was removed from the system and the gill arches were examined for any remaining cysts. Standard length was recorded and the fish was preserved in ethanol.

The number of glochidia that attached, the number that transformed and the percentage transformation were calculated for each fish. ANOVA (GLM, Minitab 13.31) was conducted to test effects of individual mussel, mussel population, host species, and the interaction of mussel population and host species. Tukey's method was used to make paired comparisons among the combinations of mussel population and host species.

In order to compare the timing of drop-off among trials and experiments, the glochidia and juveniles that had dropped off at particular times (days 4,10,20 and 30) was determined for each fish. These numbers were later divided by the total number of

glochidia and juveniles recovered for that fish, and expressed as a percentage. Effects were tested by ANOVA followed by Tukey multiple comparisons.

Measurements

Shell dimensions of all gravid female mussels collected were measured using digital calipers. The total number of conglutinates produced was counted, as well as conglutinate length and total number of glochidia per conglutinate as mentioned above. These measurements were used to estimate fecundity.

Glochidia were collected from mussels at each location and measured to test for morphological differences among broods and populations. Glochidia were photographed with a digital camera and measured using a stage micrometer and ImageJ® (v1.29x, http://rsb.info.nih.gov/ij/index.html) image analysis software. Length and height of 10 glochidia from each of 6 mussels per population were measured for a total sample of 60 glochidia measured per population. Height, length and length/height were compared among individuals and populations using ANOVA.

RESULTS

Host Tests

The number of glochidia that attached and the number and percent of attached glochidia that transformed varied among the various combinations of host fish and mussel population (Tables 7, 8). The percent of glochidia that transformed was significantly affected by host fish species, and the interaction of host fish and mussel

population in both experiments (Tables 9, 10). No significant variation was associated with mussel population or individual mussel within population. However, in experiment A, there was a significant interaction between host and individual mussel (Table 9).

The number of glochidia attached was significantly affected by host fish species and the interaction of host fish and mussel population in both experiments (Tables 11, 12). In experiment A there was a significant effect of individual mussel within population.

The number of glochidia transformed was significantly affected by host fish species in both experiments (Tables 13, 14). In experiment B there was a significant effect of the interaction of host fish and mussel population (Table 14).

Paired comparisons were carried out for fixed factors that had significant effects in the ANOVA (Tables 9-14). In experiment A, a higher percentage and number of Verdigris than St. Francis glochidia transformed on Verdigris slenderhead darters, although a significantly lower number of Verdigris glochidia attached. Significantly more Verdigris than St. Francis glochidia attached to St. Francis logperch, but the number transformed and percent transformation did not differ significantly. Verdigris orangethroat darters were not a good host for either mussel population.

In experiment B, a higher percentage and number of Ouachita than Verdigris glochidia transformed on Ouachita orangebelly darters. A larger number of Verdigris glochidia attached to Verdigris logperch, but transformation was similar and high for both mussel populations. Verdigris slenderhead darters transformed a significantly higher percentage and number of Verdigris glochidia than of Ouachita glochidia. Banded darters from the Ouachita were not suitable hosts for either population.

Multiple comparisons of these mussel and host fish pairings showed that the slenderhead darter was the best host for Verdigris fanshell in experiment A, while no host was significantly better for St. Francis fanshell (Table 15). In experiment B, the *Percina* species (logperch and slenderhead darters) were better hosts for Verdigris fanshell than the *Etheostoma* species (orangebelly darter and banded darter), while the logperch and orangebelly darters were the best hosts for Ouachita fanshell (Table 15).

Transformation success also varied among individual host fish (Figures 6-9) Even within some pairings that generally showed poor transformation success, a few fish would transform high proportions of attached glochidia (Figures 7a, 8c, 9b). Likewise, some individuals of host species that had high average transformation success, transformed a low proportion (Figures 7a, 8b, 9a, 9d).

Transformation success was compared between experiments A and B. Verdigris glochidia were tested on slenderhead darter and logperch in both years of the study (experiment A and experiment B). Analysis of Variance indicated that transformation success was significantly lower on both host species in experiment A ($P<0.05$) than in experiment B.

Timing of Glochidia and Juvenile Drop-Off

Attached glochidia either dropped off without transforming or transformed to juveniles before dropping off. The temporal pattern of glochidia and juvenile drop-off was variable. Generally, glochidia drop-off peaked by day 2 and then declined, and was essentially complete after 10 days. Juvenile drop-off began at about day 16 (Figures 10-17).

In Experiment A, the shedding of glochidia was significantly affected by mussel population and host at Day 4, and by host and population*host interaction at Day 10 (Table 16). Verdigris glochidia were shed significantly faster than St. Francis glochidia from rainbow darters at Day 4 (Table 17).

In Experiment B, the shedding of glochidia was significantly affected by mussel population, host, and population*host interaction on Day 4, and by host and population*host interaction on Day 10 (Table 18). In both experiments, *Etheostoma* species shed Verdigris glochidia faster than *Percina* species by Day 4, and Day 10 (Tables 17, 19). Verdigris glochidia were shed significantly faster than Ouachita glochidia from orangebelly darters by Day 4 and Day 10 (Table 19).

Drop-off of juveniles occurred from 16-34 days during experiment A and 16-58 days during experiment B. No consistent or significant differences were seen among mussel populations in the timing of juvenile drop-off (Tables 20-23). Host fish and mussel population were the only factors that significantly affected juvenile drop-off timing. In experiment A, drop-off of Verdigris juveniles from rainbow darters slower than slenderhead darter or logperch juvenile drop-off. In experiment B, Ouachita juvenile drop-off was slower on orangebelly darters than the drop-off of Verdigris juveniles on banded darters, there was also a significant affect of population on Day 30 of experiment B, with Ouachita juveniles dropping off faster than Verdigris juveniles.

The shedding of Verdigris glochidia from slenderhead darters occurred more slowly during experiment A than experiment B at Day 4 and 10 (Table 24, Figure 18). There was no significant difference in glochidia drop-off rate from logperch at Day 4 or

10 (Table 24, Figure 19). Conversely, juvenile drop-off was faster in experiment A than B at Day 30 for both species (Table 24, Figures 18, 19).

Experiment C

Eight potential host species from two localities were infected with glochidia from a single Spring River mussel to test their ability to act as hosts (Table 25; Figure 20). High transformation success was seen on fantail darters (*E. flabellare*), and logperch. Percent of glochidia transformed was similar on logperch from two locations (Verdigris and Spring rivers). Banded sculpin (*Cottus carolinae*), slenderhead darter, greenside darter (*E. blennioides*), banded darter, stippled darter (*E. punctulatum*), and orangethroat darters were poor hosts.

Glochidia Measurements

Glochidia size and shape varied among different individual females as well as among mussel populations (Table 26). Glochidia from each population differed significantly in height. Ouachita glochidia were significantly longer than Verdigris and St. Francis (Table 26), and Verdigris glochidia were significantly more elongate than those from the other two populations (larger length/height ratio; Table 26).

Shell Dimensions, Conglutinate Number and Fecundity

Shell size of adult female mussels varied among populations (Table 27). Overall, Verdigris mussels were the largest and St. Francis mussels were smallest. These

differences were not tested statistically because of small sample size and varying age classes. In shape, Verdigris mussels were less inflated compared to those of the other populations (smaller width/length ratio; Table 27). Each of the 3 samples differed significantly in elongation (length/height ratio; Table 27).

The number of conglutinates observed in each mussel ranged from 9 to 67. The number of conglutinates per mussel was positively correlated with mussel size. The Verdigris mussels were largest and had the most conglutinates per mussel while the St. Francis mussels had the lowest number (Table 28).

Length of conglutinates varied within and among populations. Surprisingly, St. Francis mussels had the longest conglutinates, even though they had the smallest shell size. Ouachita mussels had the shortest conglutinates (Table 28).

The number of glochidia per conglutinate varied from 750 to 7560. Longer conglutinates tended to have more glochidia per conglutinate (Figure 21). St. Francis mussels had the highest number of glochidia per conglutinate, while Ouachita mussels had the lowest (Table 28).

Overall, fecundity estimates ranged from 19,656 to 311,751 offspring per female. Verdigris fanshell had a higher average estimated fecundity than either St. Francis or Ouachita mussels (Table 28).

Total number of water tubes was counted for two upper Arkansas fanshell of different sizes. The larger (L=76.2, H=61.2, W=35.1) had 80 water tubes (40 in each outer demibranch) while a smaller (L=71.0, H=52.4, W=29.3) contained only 48 water tubes (24 in each outer demibranch).

Conglutinate Color

Color of conglutinates varied among and within the three populations (Table 29, Figure 3). Each female produced only one color of conglutinate. All 15 upper Arkansas fanshell observed had white conglutinates. Three of 10 St. Francis females had red conglutinates while the rest had brown. Three of 7 Ouachita females had red conglutinates, while the rest had brown. Conglutinate color was determined primarily by the color of the undeveloped (sterile) eggs which made up the core of each conglutinate. Developed eggs containing glochidia appeared white (Figures 2, 3).

DISCUSSION

Factors Affecting Transformation Success

Unionid mussels are usually able to utilize only one or a few species of host fish (Hoggarth 1992, Watters 1996). Previous studies indicate that darters are hosts of *Cyprogenia*. Barnhart (1997) found that fantail darters and logperch are suitable hosts and that the banded sculpin is a marginal host for Spring River fanshell (upper Arkansas River system). Jones and Neves (2002) found 9 species to be potential hosts for Clinch River fanshell including mottled sculpin (*Cottus bairdi*), banded sculpin (*C. carolinae*), snubnose darter (*E. simoterum*), tangerine darter (*P. aurantiaca*), blotchside darter (*P. burtoni*), banded darter, greenside darter, logperch, and Roanoke darter (*P. roanoka*). Infection of goldfish with fanshell glochidia was noted by Chamberlain (1934), but

transformation was not mentioned. In the present study, common species of *Percina* and *Etheostoma* found at each study site were selected for testing.

In addition to host fish, other factors apparently affected transformation success during this study. Specifically, differences were observed between experiments and among individual mussels. Transformation was significantly lower in Experiment A than in Experiment B, including the results of the duplicate tests of Verdigris glochidia on slenderhead darters and logperch (Figures 18, 19). The lower transformation success in Experiment A is probably related to the age and condition of the glochidia used. Mussels for Experiment A were collected on January 19 (Verdigris) and February 15 (St. Francis), and the experiment began on June 18. The mussels had been kept for several months past the normal release date (February –March; Chamberlain 1934 and personal observations). Mussels for Experiment B were collected on February 4 – 11, and the experiment was started on March 7, when the mussels had been held only one month. The mussel used in Experiment C was collected on March 6, and the glochidia were used on May 20, with high transformation success (Figure 19). The prolonged storage of the glochidia for Experiment A might have reduced their ability to survive on the host fish and account for lower transformation success. The average initial viability of the glochidia, when tested with salt, was somewhat lower in Experiment A than the other two experiments (Table 3). It is also interesting that glochidia in experiments A and C transformed more quickly than those in Experiment B (see below).

Generally there was little variation in transformation success among glochidia from different individual females within populations. There was one exception, however. In experiment A, there was a significant interaction between host and individual mussel

(Table 9). Multiple comparisons showed that glochidia from Verdigris mussel #3 were significantly less successful than the other 2 Verdigris individuals on slenderhead darters (Table 30). There are two possible explanations for this difference. First, #3 may have possessed a genetic trait making it incompatible with the slenderhead darter. Alternatively, the glochidia may have been in poor condition. However, initial viability of the glochidia from this mussel was higher than the glochidia of the other two (Table 3). Interestingly, Spring River glochidia did not transform on slenderhead darters either (Table 25). These observations may argue that there is a genetic element in the upper Arkansas fanshell population that makes some mussels incompatible with slenderhead darters. That gene may be present at low frequency in the Verdigris fanshell population and higher frequency in the Spring River population.

Transformation success varied among the combinations of host species and mussel (Tables 8, 9) as well as between experiments. In order to compare the results, hosts were classified as good, fair, or poor for each mussel population based on the highest mean transformation success observed among the host-glochidia pairs in each experiment (Table 31). In each experiment, values of percent transformation ranging from the maximum observed to 2/3 maximum were classified as "good", from 2/3-1/3 maximum was classified as "fair", and from 1/3 maximum to zero was classified as "poor". Hosts were generally good or poor with only one pairing classified as fair. Most pairings produced at least a few juveniles. All 8 pairings classified as good were sympatric, while no allopatric pairings were good hosts.

Logperch were suitable hosts for each of the fanshell populations tested. The logperch is the largest darter in the interior highlands region and has the most extensive

range of any darter (Page 1983, Robinson and Buchanan 1992, Pflieger 1997). Logperch occur in every river system sampled for this study as well as the Ohio and upper Mississippi River systems (Page 1983). The logperch is most common over gravel and sand in medium-sized rivers but can be found in a variety of habitats from small, fast-flowing rocky streams to the margins of lakes and reservoirs. There are three recognized subspecies of the logperch (Morris and Page 1981), of which, only *P. c. fulvitaenia* was collected and used during this study.

The rainbow darter was a good host for sympatric St. Francis River fanshell, but a poor host for Verdigris River fanshell. The rainbow darter occurs in the upper Mississippi and Ohio River systems along with the White River system but is absent from the upper Arkansas system and the Ouachita (Pflieger 1997, Robison and Buchanan 1988, Kuehne and Barbour 1983). It is most often found in swift riffles over gravel in clear, high-gradient streams.

The slenderhead darter was a good host for the glochidia from 5 of 6 Verdigris River fanshells tested. However, the glochidia of one of six Verdigris females did poorly on this species (see above), and the slenderhead darter was not a suitable host for fanshells from the Spring River (Barnhart 1997, and experiment C in the present study). The slenderhead darter is a moderately sized darter that occurs in the upper Arkansas and Mississippi River systems, excluding the White and Ouachita River Systems (Page 1983). A single record in the St. Francis in Missouri has not been confirmed (Pflieger 1997). Preferred habitat includes medium gravel raceways and bedrock riffles with strong flow (Kuehne and Barbour 1983).

The orangethroat darter was a poor host for sympatric Verdigris, Spring, and St. Francis River fanshell. The orangethroat is superficially similar to the rainbow darter but tends to occupy slower and siltier habitat when it occurs syntopically with rainbow darters (Page 1983, Kuehne and Barbour 1983, Pflieger 1997, Robinson and Buchanan 1992). This species occurs from the upper Arkansas to the Ohio River system, not including the Ouachita River System (Page 1983). There are several described sub-species of orangethroat darter (Distler 1968, Ceas and Page 1997). The orangethroat tested during this study were *E. s. squamosum.*

Greenside darters from the Spring River were not hosts for Spring River fanshells. St. Francis greenside darters were also not hosts for St. Francis fanshells in a preliminary experiment. The greenside is the largest member of the genus *Etheostoma* and occurs from the Ohio and Tennessee River systems to the Spring River of the upper Arkansas (Kuehne and Barbour 1983). The greenside is found in the major river systems for this study, excluding the Verdigris River. Preferred habitat includes gravel or rock riffles of medium size rivers (Pflieger 1997). There are four subspecies of the greenside darter, of which only *E. b. newmannii* was collected and used during this study.

The banded darter was a poor host for fanshell from the Ouachita, Verdigris and Spring River. This darter has a wide distribution including the upper Mississippi, Ohio, Ouachita, White and upper Arkansas (Page 1983). Preferred habitat includes gravel or rock riffles with dense filamentous algae (Pflieger 1997). There are two subspecies of banded darter, only *E. z. zonale* was collected for this study.

The fantail darter was a good host for Spring River fanshell (Barnhart 1997, and experiment C). It was not tested with the other mussel populations. This species is found

from the Ohio, Tennessee, and upper Mississippi River systems to the upper Arkansas. It does not occur in the Ouachita River system (Robinson and Buchanan 1992). Suitable habitat includes shallow riffles away from swift current (Kuehne and Barbour 1983). There are at least three recognized subspecies of fantail darter. During this study only *E. f. lineolatum* (Pflieger 1997) were collected.

The orangebelly darter was a good host for Ouachita River fanshell. This species can only be found in the Red and Ouachita River systems (Kuehne and Barbour 1983). It most closely resembles the redfin darter, *E. whipplei* (Robinson and Buchanan 1992). Preferred habitat includes gravel riffles in small to medium streams. There are three recognized subspecies of orangebelly darter (Page 1983), of these only *E. r. radiosum* was tested during this study.

Only sympatric species were generally good hosts for each fanshell population. The only host compatible with all four mussel populations (logperch) is very widely distributed, but not always abundant. Logperch from the St. Francis River were good hosts both for Verdigris glochidia and for glochidia from the St. Francis. Likewise, logperch from the Verdigris River were good hosts for both Verdigris and Ouachita glochidia. These observations may indicate that the logperch is genetically homogenous across this range. Other broadly distributed darter species were not good hosts. Greenside, orangethroat and banded darters each occur at 2 or more of the mussel sites but were not good hosts for any population tested.

Only a few previous studies have investigated differences in transformation between closely related species or populations of a single species of unionid. (Riusech and Barnhart 2000, Rogers et. al 2001). Riusech and Barnhart (2000) placed glochidia

from different mussel populations on one host population. They found that the sympatric pairing of mussel and host fish transformed glochidia at a significantly higher rate. Rogers et. al (2001) inoculated two separate populations of host fish with the same mussel population and reported higher total transformation on sympatric than allopatric hosts.

Mechanisms that make a mussel compatible or incompatible with a host are poorly understood. Poor transformation of glochidia on a host fish may be due to innate or acquired responses of host fish. Immune reactions of fish to glochidia infections involve both specific (antibody) and non-specific responses. Perhaps glochidia surface antigens resemble host fish proteins, so that no immune (antibody) response is triggered. However, studies have shown that host fish acquired anti-glochidia antibodies several days after inoculation with glochidia (Meyers et. al 1980, Bauer and Vogel 1987, O'Connell and Neves 1999). Non-specific immune responses may also be partially responsible for lower transformation success on host fish. For example, eosinophils have been witnessed in cysts that surround the glochidia (Arey 1932).

Assuming that glochidia antigens influence host compatibility, natural selection should favor alleles that allow transformation to occur on those fish that are most likely to encounter the glochidia. There is no selective pressure to maintain compatibility with other species, including allopatric species, rare species, or those whose behavior does not bring them into contact with the glochidia. In the absence of selection pressure, alleles that maintain compatibility may be lost by genetic drift.

If host compatibility were determined simply by the presence or absence of a single allele, one might expect a bimodal distribution of transformation success in host

populations that possess that allele at moderate frequency. This was not the case (Figures 6-9). A range of transformation success was seen among individuals. Repeated measures on individual host fish are not practical, because of acquired immunity, but it appears that host compatibility is more complex than a simple yes or no.

The differences observed among populations suggest a lack of gene flow. Mussels depend on host fish for upstream movement. The hosts of fanshell are darters, which are small bodied fish that generally do not move long distances (Hill and Grossman 1987). Both host and the mussel populations are separated from other populations by long reaches of unsuitable habitat, now including reservoirs (Duncan 2002). In pre-settlement times, isolation and lack of gene flow may have resulted in the evolution of differences among populations, including those demonstrated in this study. In the present day, impoundment of rivers within the range of fanshell populations probably totally prevents gene exchange among populations as well as preventing recolonization of areas where the species may be extirpated. For example, every river within the upper Arkansas River system with a record of fanshell populations has been impounded (Fall, Verdigris, Spring, and Neosho).

Other Factors Affecting Number of Juveniles Transformed

In addition to percent transformed, the number of glochidia that attached to each fish and the number of juveniles that were produced were measured. The number of glochidia attached was significantly affected by host fish species and the interaction of host fish and mussel population in both experiments (Tables 11, 12). Variation in attachment among host fish can be expected, because the size of the host species varied

(Tables 7, 8). Larger fish ventilate more, thus encountering more glochidia, and have more gill surface area to which glochidia can attach. Fish were placed in tanks after a 10-15 min rinsing period. It is possible that glochidia were rejected even during this brief period by some innate mechanism.

Significant differences in total number transformed were observed, which was expected and not surprising. The number transformed depends heavily on the total number attached as well as percent transformation. These factors vary based on host fish and its' reaction to the glochidia as stated above.

Timing of Glochidia and Juvenile Drop-Off

The timing of the shedding of untransformed glochidia and transformed juveniles was affected by host species, mussel population, and the interaction of host and mussel. Rainbow darters shed Verdigris glochidia significantly faster than St. Francis glochidia (Table 17). Likewise, orangebelly darters shed Verdigris glochidia significantly faster than Ouachita glochidia (Table 19). That is, glochidia were cleared from the incompatible host more rapidly than the compatible host.

In experiment B, a higher proportion of Ouachita juveniles than Verdigris juveniles had dropped off by day 30 (Table 22). It could be that glochidia from fanshell at the Ouachita River had reached maturity at an earlier date. These sites are separated by approximately two hundred miles in latitude. Therefore, it is probable that water temperatures rise sooner in the Ouachita River system leading to earlier glochidia maturity.

The largest difference in drop-off timing was seen between experiments A and B (Table 24; Figures 18, 19). Glochidia were shed from fish faster during experiment B, but juvenile drop-off was faster during experiment A. This is probably due to the difference in condition of the glochidia. Timing of drop-off from logperch during experiment C resembled that of experiment A, without the high proportion of initial glochidia shedding (Figure 19). As discussed above, glochidia used in experiment A and C were older and may therefore have transformed more rapidly. Jones and Neves (2002) observed similar results in host tests with Clinch River fanshell. Early during the brooding season (November through February), they observed drop-off periods that often exceeded 60 days.

Glochidia Measurements

Dimensions of glochidia observed in this study were similar to those reported in previous studies (Table 26). However, previously published data are too few to allow statistical comparisons of size and shape among populations or species of fanshells. In the present study, significant difference in size and shape were observed among populations (Table 26). In particular, Verdigris glochidia were shorter in average height and significantly elongated in shape compared to Ouachita and St. Francis River glochidia. Ouachita and St. Francis River glochidia were similar in shape, but were significantly different in length and height. It should be noted that each population was represented by mussels from only a single site. More measurements should be made to determine whether differences are present among sites within populations.

Previously reported measurements of *Cyprogenia stegaria* glochidia are more similar to Ouachita glochidia than to the other two populations measured in the present study. Geographically speaking, the Ouachita River fanshell population lies roughly halfway between the Verdigris and Green River populations. Generally, the measurements show a gradient of glochidia height moving from East to West. Fanshell in the Green and Clinch rivers have glochidia heights of up to 185µm while maximum height measured in the St. Francis and Ouachita were 168µm and 166µm respectively, with the maximum observed value of 160µm for Verdigris glochidia.

Shell Dimensions and Fecundity

Fecundity is a measure of an individual's total reproductive potential or the total number of offspring a mussel produces in one year. Depending on species, age, and size, individual female mussels can release from 75,000 to 5 million glochidia in a single year (Coker et. al 1921, Kat 1984, Young and Williams 1984, Yeager and Neves 1986, Bauer 1988). Fanshell mussels have a relatively low fecundity when compared to other species. Fecundity of 4 Clinch River fanshell ranged from 22,357 to 63,459 (mean = 43,494; Jones and Neves 2002). Spring River fanshell fecundity ranging from 43,877 to 188,970 (mean = 93,923; Barnhart 1997). In the present study, fecundity estimates ranged from 19,656 to 311,751 offspring per female (Verdigris mean = 157,809, St. Francis mean = 63,182, Ouachita mean = 69,634).

There are several factors that affect fecundity of fanshell mussels. These are the number of conglutinates, length of the conglutinate, and the number of glochidia per conglutinate. The number of glochidia per conglutinate relates directly to the total

number of glochidia produced; fanshells tend to have a low number of conglutinates with several thousand glochidia each (Barnhart 1997, Jones and Neves 2002, Table 28 present study). One reason for low fecundity of fanshell mussels is the presence of sterile or unfertilized eggs. Barnhart (1997) found that fanshell conglutinates contained approximately 85% sterile eggs. It is presumed that the sterile eggs provide support for viable glochidia on the surface of the conglutinate, as well as enticing fish with a worm-like appearance.

The total number of conglutinates per mussel varied in this study. Not all water tubes contained conglutinates. St. Francis and Ouachita mussels collected February 15 and 4, respectively, did not have a conglutinate in each water tube. These collection dates are close to the time that Chamberlain (1934) first observed St. Francis River mussels releasing conglutinates. It is possible that mussels from the St. Francis and Ouachita rivers had already begun releasing conglutinates by their date of capture. It is also possible that these mussels only fill a portion of their water tubes with conglutinates, previous reports list fanshell with far fewer conglutinates than observed during this study (Ortmann 1912, Utterback 1915, Jones and Neves 2002).

Another factor in fecundity is the total length of the conglutinate. Longer conglutinates contain more eggs. In the present study, the mussels with the longest conglutinates were found to have the highest number of glochidia per conglutinate (Table 28, Figure 21).

Mussels from the upper Arkansas River system had the largest shells, the largest number of conglutinates, and the highest fecundity. Larger mussels have a larger number of conglutinates and thus greater fecundity (Bauer 1994, Baird 2000). During growth,

mussels add water tubes to the gills, thus increasing the number available to brood conglutinates. Interestingly, St. Francis River mussels were the smallest in shell length, but had the highest average conglutinate length and highest number of glochidia per conglutinate (Table 28). Nonetheless, the number of conglutinates and therefore the total number of glochidia recovered from these mussels was lowest of the 3 populations. Mussels from the Ouachita River had intermediate shell size, but produced the shortest conglutinates with the lowest number of glochidia per conglutinate (Table 28). One outlier with an average of 750 glochidia per conglutinate was also partly responsible for the intermediate fecundity in this population.

Conglutinate Color

Three different conglutinate (sterile egg) colors were noted in this study. Upper Arkansas fanshells produced only white conglutinates. Each individual mussel in the other two populations produced either red or brown conglutinates. Nearly all records of *C. stegaria* note mussels having red conglutinates (Sterki 1898; Ortmann 1912; Ortmann 1919; Jones and Neves 2002). However, Ortmann (1912) mentioned that he (and Sterki) had observed *Cyprogenia* in the Clinch River with white conglutinates. It is intriguing that two conglutinate colors have been reported in every location outside of the upper Arkansas River system.

Analysis of *Cyprogenia* mitochondrial gene sequences indicated that taxonomic revision of *C. aberti* may be necessary (Serb and Obermeyer 2001, Serb 2003). The analysis suggested that at least 2 and potentially 5 genetically distinct lineages are present currently within the accepted range of *C. aberti* (Figures 4, 22). The largest two

groupings are referred to as clade A and clade B. Remarkably, the St. Francis and the Ouachita populations include individuals from both Clade A and Clade B. The most intriguing discovery was that the two conglutinate color morphs were congruent with the phylogenetic analysis. Mussels that were observed in the present study were genotyped by Dr. Serb. Those individuals that had produced red conglutinates grouped in clade A, while mussels with brown conglutinates fell within clade B (Figure 22).

At least two interpretations of these observations are possible. First, there may be two species present in each of the St. Francis and Ouachita populations. Second, there may be two very different mitochondrial lineages present in a single species in each of these populations. Analysis of nuclear genes is needed to test these two hypotheses. More host studies are needed to determine if there is an ecological difference between mussels producing red and brown conglutinates.

Species Concepts

It is necessary to determine which species concept is the most practical for application to the conservation biology of freshwater mussels. Two classes of species concept have been recognized. The first focuses on reproductive communities while the second deals with evolutionary lineages (Sites and Crandall, 1997). The biological species concept (BSC) is the most prominent concept based upon reproductive communities. The BSC states that a species is a group of interbreeding natural populations that are reproductively isolated from other such populations (Mayr, 1996). However, reproductive isolation is usually inferred from morphology and not tested directly. In fact, it is impractical to test reproductive isolation directly in unionids. Even

if it could be shown that mussels from two different populations were capable of interbreeding, it would be very difficult to determine if the offspring were fertile, considering the long time period for a mussel to reach sexual maturity and the difficulty of maintaining juvenile mussels in a laboratory setting. The fanshell populations may be able to breed with each other when brought together. However, hybrid offspring might be incompatible with local hosts. For example, if Verdigris mussels were introduced to the Ouachita and interbred with the local population, the hybrid glochidia would probably be less able to transform on locally abundant hosts such as orangebelly darters and therefore be less likely to survive. For this reason these populations would be considered separate under the BSC.

The evolutionary species concept (ESC) is an example of a species concept based upon evolutionary lineages. The ESC states that a species is a group of organisms that maintains it's identity from other such groups through space and time, with its own independent evolutionary fate and historical tendencies (Wiley and Mayden, 2000). One problem with the evolutionary species is that it is impossible to determine if two isolated populations will maintain separate identities over time. The distinction of maintaining identity over space and time inherently includes the reproductive isolation that could not be tested under the BSC. The fanshell populations are physically isolated by many river miles. The Arkansas River valley separates the Ouachita populations from other populations. Channelization and impoundment of the Arkansas River has isolated upper Arkansas populations from others as well. Another isolating factor is that they employ darters as hosts which limits their potential to share genes with adjacent populations. The

combination of these factors indicates that these populations will maintain separate identities over space and time, a quality that makes them separate species under the ESC.

The phylogenetic species concept (PSC) states that a species is the least inclusive taxon recognized in a phylogenetic classification based on monophyly (Mishler and Brandon, 1987). The appeal of this concept is that phylogenetic relationships are testable and can be mapped out by analysis of nuclear or mitochondrial DNA sequences, as well as phenotypic characters. This concept makes no inference about the permanence of reproductive isolation. One criticism of this approach is that monophyly of mitochondria may not equate to monophyly of other character sets. The phylogenetic tree that has been constructed for *Cyprogenia* by Serb (2003, Figure 4) shows that fanshell from the upper Arkansas River system form one well-supported clade. This grouping is supported by differences in host requirement, glochidia shape, and conglutinate color found in this study. In that regard, fanshell from the upper Arkansas River can be recognized by unique character states and must be considered separate from the other fanshell populations under the PSC. Further study is needed to clarify the phylogeny and revise the classification of the other fanshell populations, but there will probably be justification for two or more other species of *Cyprogenia* under the PSC.

Another classification concept used in conservation and management is the Evolutionarily Significant Unit (ESU). An ESU is any population that (1) is substantially isolated from other conspecific population units and (2) represents an important component in the evolutionary legacy of the species (Federal Register, 1996). An ESU can be diagnosed based on the combination of genetic and ecological exchangeability (Crandall et al. 2000). Genetic exchangeability is the presence of gene flow between the

populations, while ecological exchangeability is based on whether or not the two populations can occupy the same ecological niche. Any group that can be shown to be unexchangeable with other groups, either genetically or ecologically, may be deemed an ESU. Fanshell populations meet these requirements. However, the ESU category is only recognized for vertebrate species under United States law (Federal Register, 1996).

In the case of fanshell mussels in Kansas, Missouri, and Arkansas, discussion about which species concept to apply is moot. The differences between Verdigris River fanshell and fanshell from other populations are so distinct that they are separate under any species concept. The physiological, morphological, ecological, and genetic differences discussed above lead to the conclusion that there are multiple *Cyprogenia* species west of the Mississippi River. The type population in the Verdigris River system should retain the name *C. aberti*. Further study of genetics and host requirements of populations in the Black River and populations east of the Mississippi are needed. All of the populations distinguished in this study should be protected as unique and irreplaceable entities that are inseparable from their respective host fish communities.

SUMMARY

1. Each of three populations of *Cyprogenia aberti* had different host requirements. Sympatric pairings of mussel and host had significantly better transformation success than allopatric pairings.

2. Logperch were a suitable host for glochidia of all fanshell populations tested, while slenderhead, fantail, rainbow, and orangebelly darters were suitable hosts only for their respective sympatric fanshell populations.
3. Conglutinate color differed among and within populations. Ouachita and St. Francis mussels produced either red or brown conglutinates. Conglutinate color in these populations correlated with two widely different mitochondrial genetic types. Mussels from the upper Arkansas system produced only white conglutinates.
4. Glochidia dimensions differed among populations, with Verdigris fanshell glochidia being smaller and more elongated than the larger St. Francis and Ouachita glochidia.
5. Age of glochidia had a significant effect on transformation timing and success. Propagation of fanshell mussels should take place between March and May for optimal efficiency.
6. Physiological, morphological, ecological, and genetic differences among populations indicate that multiple species of fanshell mussel occur west of the Mississippi River. *Cyprogenia* from the upper Arkansas River system should retain the name *C. aberti*, while taxonomic revision of other populations is necessary.

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TABLES

Table 1. Date and location of mussel collections.

Site #	Date	River	Locality
1.	1-19-02 2-11-03	Verdigris	(SW 1/4 Section 4 T31S R16E Montgomery Co. KS; UTM 15 263143E 4138004N Sycamore quad)
2.	2-15-02	St. Francis	Boy Scout Camp Lewallen (Wayne Co. MO; UTM 15 722800E 4123900N Patterson quad)
3.	2-4-03	Ouachita	Montgomery Co. AR; UTM 15 434359E 3828410N Mount Ida quad
4.	3-6-03	Spring	Near Hwy 96 bridge, Cherokee Co. KS; UTM 15 354339E 4115674N Crestline quad

Table 2. Date, species, and location of fish collections.

Site #	Date	Fish	River	Locality
5.	6-4-02 2-13-03	slenderhead orangethroat logperch	Elk	Montgomery Co. KS; UTM 15 240729E 4129347N Elk City quad
6.	5-30-02	rainbow	St. Francis	St. Francis Co. MO; UTM 15 723249E 4182590N Farmington quad
7.	6-15-02	logperch	St. Francis	Wayne Co. MO; UTM 15 721600E 4119100N Patterson quad
8.	2-4-03	orangebelly banded	Ouachita	Montgomery Co. AR; UTM 15 434359E 3828410N Mount Ida quad
9.	5-19-03	fantail orangethroat stippled banded	Spring	Lawrence Co. MO; 15 424789E 4103075N Mt Vernon quad
10.	5-19-03	banded sculpin logperch greenside	Spring	Lawrence Co. MO; UTM 15 420684E 4107952N Stotts City quad

Table 3. Viability and number of glochidia from each mussel used to inoculate host fish. Percent viable indicates the percentage of a subsample of glochidia that responded to salt solution by closing.

Experiment A			
Mussel	Percent viable	Number used	N fish inoculated
SF 1	96	18,840	20
SF 2	68	19,560	20
SF 3	93	19,080	20
VR 1	73	11,250	19
VR 2	72	18,270	19
VR 3	85	13,500	20
Experiment B			
OU 4	95	17,000	20
OU 5	92	16,050	20
OU 6	94	15,890	20
VR 4	94	15,000	20
VR 5	95	15,100	20
VR 6	95	14,700	20
Experiment C			
SP 1	79	14,350	20
SP 1	85	11,000	16

Table 4. Pairings of host fish and mussel populations in host experiments A and B

Fish (darter)- population	Mussel population		
	Verdigris	St. Francis	Ouachita
slenderhead –Verdigris	A & B	A	B
logperch - Verdigris	B	-	B
logperch - St. Francis	A	A	-
rainbow - St. Francis	A	A	-
orangethroat -Verdigris	A	A	-
banded – Ouachita	B	-	B
orangebelly – Ouachita	B	-	B

Table 5. Experiment A. Number and species of host fish inoculated with glochidia from St. Francis mussels (SF) and Verdigris mussels (VR).

Mussel ID	Number of host fish				
	logperch	slenderhead	rainbow	orangethroat	Total
SF #1	5	5	7	4	21
SF #2	4	5	7	3	19
SF #3	4	5	7	3	19
VR #1	5	5	7	4	21
VR #2	4	5	7	3	19
VR #3	4	5	7	3	19
Total	26	30	42	20	118

Table 6. Experiment B. Number of host fish inoculated with glochidia from Verdigris mussels (VR) and Ouachita mussels (OU).

Mussel ID	Number of host fish				
	logperch	slenderhead	banded	orangebelly	Total
VR #4	5	5	5	5	20
VR #5	5	5	5	5	20
VR #6	5	5	5	5	20
OU #4	5	5	5	5	20
OU #5	5	5	5	5	20
OU #6	5	5	5	5	20
Total	30	30	30	30	120

Table 7. Experiment A. Comparison of attachment and transformation of *Cyprogenia* glochidia from the St. Francis (SF) and Verdigris (VR) rivers on host fish from those rivers. Location, sample size, and average specimen standard length are given for each host fish species. Numbers are mean ± standard deviation. For each host species, the results from the SF and VR mussel populations were compared using Tukey tests and the P values are indicated. Statistically significant differences between mussel populations are indicated in bold.

Host fish (N) SL ± SD	Mussel	Attached	Transformed	% transformed
rainbow	SF	162 ± 66.7	15 ± 28.7	9 ± 12.8
SF (21)	VR	104 ± 49.7	1 ± 1.9	1 ± 1.3
42 ± 3.2		P = 0.912	0.855	0.089
orangethroat	SF	201 ± 125.8	2 ± 3.5	1 ± 2.4
VR (10)	VR	150 ± 63.5	1 ± 1.9	1 ± 1.4
42 ± 3.8		P = 0.995	> 0.999	> 0.999
logperch	SF	416 ± 283.8	53 ± 50.6	12 ± 8.9
SF (13)	VR	523 ± 269.5	51 ± 56.4	9 ± 8.9
92 ± 9.7		P = 0.612	0.999	0.990
slenderhead	SF	***381 ± 114.5***	13 ± 24.9	***4 ± 8.5***
VR (15)	VR	***210 ± 89.0***	48 ± 44.7	***23 ± 14.1***
56 ± 4.7		P = ***0.044***	0.077	***<0.001***

Table 8. Experiment B. Comparison of attachment and transformation of *Cyprogenia* glochidia from the Ouachita (OU) and Verdigris (VR) rivers on host fish. Location, sample size, and average specimen standard length are given for each host fish species. Numbers are mean ± standard deviation. For each host species, the results from the SF and VR mussel populations were compared using Tukey tests and the P values are indicated. Statistically significant differences between mussel populations are indicated in bold.

Host fish (N) SL ± SD	Mussel	Attached	Transformed	% transformed
orangebelly	OU	110 ± 39.9	***58 ± 423.0***	***48 ± 28.5***
OU (15)	VR	94 ± 47.2	***2 ± 2.4***	***1 ± 1.6***
45 ± 3.6		P = 0.992	***0.016***	***<0.001***
banded	OU	32 ± 11.2	2 ± 2.7	5 ± 9.5
OU (15)	VR	29 ± 15.5	1 ± 1.1	1 ± 2.5
40 ± 3.0		P = > 0.999	> 0.999	0.998
logperch	OU	***144 ± 69.7***	101 ± 68.6	65 ± 17.9
VR (15)	VR	***213 ± 96.1***	107 ± 55.4	52 ± 18.4
72 ± 9.6		P = ***0.017***	0.999	0.432
slenderhead	OU	190 ± 54.8	***20 ± 53.3***	***8 ± 16.2***
VR (15)	VR	172 ± 53.2	***94 ± 56.8***	***54 ± 23.5***
63 ± 5.5		P = 0.988	***<0.001***	***<0.001***

Table 9. ANOVA table for percent transformation. Experiment A. P = mussel population (2 populations: Verdigris and St. Francis), M = individual mussel (3 from each population), and H = host fish species (4 species tested). Statistically significant effects are indicated in bold.

Source	DF	Sequential SS	Adjusted SS	Adjusted MS	F	P
P	1	26.55	79.05	79.05	0.39	0.567
M(P)	4	971.86	813.95	203.49	1.57	0.243
H	***3***	***2337.14***	***2315.30***	***771.77***	***5.80***	***0.011***
P*H	***3***	***3251.03***	***3246.28***	***1082.09***	***8.14***	***0.003***
H*M(P)	***12***	***1597.91***	***1597.91***	***133.16***	***1.92***	***0.041***
Error	94	6520.26	6520.26	69.36		
Total	117	14704.75				

Table 10. ANOVA table for percent transformation. Experiment B. P = mussel population (2 populations: Verdigris and Ouachita), M = individual mussel (3 from each population), and H = host fish species (4 species tested). Statistically significant effects are indicated in bold.

Source	DF	Sequential SS	Adjusted SS	Adjusted MS	F	P
P	1	639.5	639.5	639.5	1.37	0.307
M(P)	4	1869.8	1869.8	467.4	1.55	0.251
H	***3***	***47121.0***	***47121.0***	***15707.0***	***51.97***	***<0.001***
P*H	***3***	***33105.2***	***33105.2***	***11035.1***	***36.51***	***<0.001***
H*M(P)	12	3626.7	3626.7	302.2	1.04	0.417
Error	96	27821.3	27821.3	289.8		
Total	119	114183.6				

Table 11. ANOVA table for number of glochidia attached. Experiment A. P = mussel population (2 populations: Verdigris and St. Francis), M = individual mussel (3 from each population), and H = host fish species (4 species tested). Statistically significant effects are indicated in bold.

Source	DF	Sequential SS	Adjusted SS	Adjusted MS	F	P
P	1	94608	45334	45334	0.50	0.517
M(P)	***4***	***346471***	***360950***	***90238***	***3.59***	***0.035***
H	***3***	***1920797***	***1935197***	***645066***	***25.34***	***<0.001***
P*H	***3***	***272190***	***284499***	***94833***	***3.73***	***0.042***
H*M(P)	12	305659	305659	25472	1.32	0.221
Error	94	1815462	1815462	19313		
Total	117	4755188				

Table 12. ANOVA table for number of glochidia attached. Experiment B. P = mussel population (2 populations: Verdigris and Ouachita), M = individual mussel (3 from each population), and H = host fish species (4 species tested). Statistically significant effects are indicated in bold.

Source	DF	Sequential SS	Adjusted SS	Adjusted MS	F	P
P	1	2017	2017	2017	0.42	0.553
M(P)	4	19269	19269	4817	2.99	0.063
H	***3***	***464198***	***464198***	***154733***	***95.96***	***<0.001***
P*H	***3***	***38251***	***38251***	***12750***	***7.91***	***0.004***
H*M(P)	12	19350	19350	1612	0.52	0.899
Error	96	299105	299105	3116		
Total	119	842190				

Table 13. ANOVA table for number transformed. Experiment A. P = mussel population (2 populations: Verdigris and St. Francis), M = individual mussel (3 from each population), and H = host fish species (4 species tested). Statistically significant effects are indicated in bold.

Source	DF	Sequential SS	Adjusted SS	Adjusted MS	F	P
P	1	163	751	751	0.42	0.553
M(P)	4	7763	7194	1799	1.23	0.348
H	***3***	***41360***	***39460***	***13153***	***8.83***	***0.002***
P*H	3	11145	11056	3685	2.47	0.111
H*M(P)	12	17887	17887	1491	1.45	0.158
Error	94	96757	96757	1029		
Total	117	175075				

Table 14. ANOVA table for number transformed. Experiment B. P = mussel population (2 populations: Verdigris and Ouachita), M = individual mussel (3 from each population), and H = host fish species (4 species tested). Statistically significant effects are indicated in bold.

Source	DF	Sequential SS	Adjusted SS	Adjusted MS	F	P
P	1	941	941	941	0.19	0.684
M(P)	4	19588	19588	4897	2.23	0.127
H	***3***	***173610***	***173610***	***57870***	***26.33***	***<0.001***
P*H	***3***	***63847***	***63847***	***21282***	***9.68***	***0.002***
H*M(P)	12	26375	26375	2198	1.21	0.285
Error	96	173920	173920	1812		
Total	119	458281				

Table 15. Multiple comparison of hosts within experiments A and B. Numbers are mean percent transformed ± standard deviation. Within a column, values having different superscripts are significantly different from one another ($P<0.05$).

Host fish	Experiment A		Experiment B	
	Verdigris	St. Francis	Verdigris	Ouachita
logperch	9 ± 8.9^{a}	12 ± 8.9^{a}	52 ± 18.4^{a}	65 ± 17.9^{a}
slenderhead	23 ± 14.1^{b}	4 ± 8.5^{a}	54 ± 23.5^{a}	8 ± 16.2^{b}
rainbow	1 ± 1.3^{a}	9 ± 12.8^{a}	--	--
orangethroat	1 ± 1.4^{a}	1 ± 2.4^{a}	--	--
orangebelly	--	--	1 ± 1.6^{b}	48 ± 28.5^{a}
banded	--	--	1 ± 2.5^{b}	5 ± 9.5^{b}

Table 16. ANOVA of glochidia drop-off timing between St. Francis and Verdigris fanshell during experiment A, data include percent of total glochidia dropped off by days 4 and 10 as a measure of drop-off timing between populations. Statistically significant effects are indicated in bold. P H M H*P=model

Day 4	Source	DF	Seq SS	Adj SS	Adj MS	F	P
	Pop	***1***	***10252.8***	***8723.2***	***8723.2***	***26.56***	***0.000***
	Host	***3***	***13943.1***	***14018.3***	***4672.8***	***14.23***	***0.000***
	Mussel	2	616.3	672.8	336.4	1.02	0.363
	Pop*Host	3	1276.2	1276.2	425.4	1.30	0.280
	Error	108	35470.6	35470.6	328.4		
	Total	117	61559.0				

Day 10	Source	DF	Seq SS	Adj SS	Adj MS	F	P
	Pop	1	166.69	134.08	134.08	2.83	0.095
	Host	***3***	***1580.19***	***1560.44***	***520.15***	***10.99***	***0.000***
	Mussel	2	15.20	17.45	8.73	0.18	0.832
	Pop*Host	***3***	***464.62***	***464.62***	***154.87***	***3.27***	***0.024***
	Error	108	5112.64	5112.64	47.34		
	Total	117	7339.33				

Table 17. Rate of glochidia drop-off from host fish during experiment A. Numbers are percent of the total untransformed glochidia that had dropped off by day 4 and day 10. Numbers are mean ± standard deviation. Significant differences between mussel populations on a host are shown in bold.

Host	Mussel population	Day 4	Day 10
orangethroat (Verdigris)	St. Francis	72 ± 23.9	94 ± 6.7
	Verdigris	96 ± 2.8	99 ± 0.6
rainbow (St. Francis)	St. Francis	***71 ± 21.7***	95 ± 8.1
	Verdigris	***95 ± 5.0***	99 ± 1.2
logperch (St. Francis)	St. Francis	55 ± 24.4	86 ± 8.6
	Verdigris	65 ± 19.9	90 ± 7.8
slenderhead (Verdigris)	St. Francis	57 ± 23.9	95 ± 10.2
	Verdigris	70 ± 8.2	90 ± 4.4

Table 18. ANOVA of glochidia drop-off timing between Ouachita and Verdigris fanshell during experiment B, data include percent of total drop-off of glochidia by days 4 and 10 as a measure of drop-off timing between populations. Statistically significant effects are indicated in bold. P H M H*P=model

Day 4	Source	DF	Seq SS	Adj SS	Adj MS	F	P
	Pop	***1***	***2003.7***	***2003.7***	***2003.7***	***17.44***	***0.014***
	Host	***3***	***9382.6***	***9382.6***	***3127.5***	***19.04***	***0.000***
	Mussel	2	459.6	459.6	114.9	0.70	0.594
	Pop*Host	***3***	***5886.0***	***5886.0***	***1962.0***	***11.94***	***0.000***
	Error	108	17742.4	17742.4	164.3		
	Total	117	35474.2				

Day 10	Source	DF	Seq SS	Adj SS	Adj MS	F	P
	Pop	1	170.69	170.69	170.69	6.27	0.066
	Host	***3***	***1256.41***	***1256.41***	***418.80***	***9.19***	***0.000***
	Mussel	2	108.88	108.88	27.22	0.60	0.665
	Pop*Host	***3***	***519.55***	***519.55***	***173.18***	***3.80***	***0.012***
	Error	108	4920.82	4920.82	45.56		
	Total	117	6976.36				

Table 19. Rate of glochidia drop-off from host fish during experiment B. Numbers are percent of the total untransformed glochidia that had dropped off by day 4 and day 10. Numbers are mean ± standard deviation (n=15). Significant differences between mussel populations on a host are shown in bold.

Host	Mussel population	Day 4	Day 10
orangebelly (Ouachita)	Ouachita	***55 ± 22.4***	***88 ± 11.6***
	Verdigris	***95 ± 6.7***	***99 ± 1.6***
banded (Ouachita)	Ouachita	92 ± 9.4	96 ± 6.4
	Verdigris	97 ± 6.2	99 ± 3.1
logperch (Verdigris)	Ouachita	73 ± 12.2	90 ± 7.1
	Verdigris	76 ± 21.4	91 ± 10.6
slenderhead (Verdigris)	Ouachita	97 ± 2.8	99 ± 1.1
	Verdigris	92 ± 3.8	98 ± 3.0

Table 20. ANOVA of juvenile drop-off timing between St. Francis and Verdigris fanshell during experiment A, data include percent of total juveniles that dropped off by days 20 and 30 as a measure of drop-off timing between populations. Statistically significant effects are indicated in bold. P H M H*P=model

Day 20	Source	DF	Seq SS	Adj SS	Adj MS	F	P
	Pop	1	1473	1128	1128	0.92	0.339
	Host	***3***	***17146***	***16794***	***5598***	***4.58***	***0.005***
	Mussel	2	5266	5378	2689	2.20	0.116
	Pop*Host	3	775	775	258	0.21	0.888
	Error	108	131908	131908	1221		
	Total	117	156568				

Day 30	Source	DF	Seq SS	Adj SS	Adj MS	F	P
	Pop	1	851	46	46	0.01	0.915
	Host	***3***	***98017***	***98333***	***32778***	***8.20***	***0.000***
	Mussel	2	8430	8806	4403	1.10	0.336
	Pop*Host	3	16626	16626	5542	1.39	0.251
	Error	108	431870	431870	3999		
	Total	117	555794				

Table 21. Rate of juvenile drop-off from host fish during experiment A. Numbers are mean ± standard deviation of the percent of the total transformed juveniles that had dropped off by day 20 or day 30. Trials of poor hosts (less than 1/3 of best observed transformation percentage) are in parentheses. No significant differences were observed.

Host	Mussel population	Day 20	Day 30
orangethroat	St. Francis	(3 ± 8.1)	(50 ± 52.7)
(Verdigris)	Verdigris	(0 ± 0)	(40 ± 51.6)
rainbow	St. Francis	15 ± 30.6	81 ± 40.2
(St. Francis)	Verdigris	(13 ± 33.2)	(47 ± 50.6)
logperch	St. Francis	20 ± 18.2	86 ± 36.3
(St. Francis)	Verdigris	16 ± 19.0	89 ± 28.2
slenderhead	St. Francis	(10 ± 16.5)	(80 ± 41.4)
(Verdigris)	Verdigris	5 ± 4.5	98 ± 3.1

Table 22. ANOVA of juvenile drop-off timing between Ouachita and Verdigris fanshell during experiment B, data include percent of total juveniles that had dropped off by day 20 and 30 as a measure of drop-off timing between populations. Statistically significant effects are indicated in bold. P H M H*P=model

Day 20	Source	DF	Seq SS	Adj SS	Adj MS	F	P
	Pop	1	12.1	12.1	12.1	0.13	0.734
	Host	3	1275.9	1275.9	425.3	1.27	0.287
	Mussel	2	365.4	365.4	91.4	0.27	0.894
	Pop*Host	3	662.4	662.4	220.8	0.66	0.577
	Error	108	36035.5	36035.5	333.7		
	Total	117	38351.3				

Day 30	Source	DF	Seq SS	Adj SS	Adj MS	F	P
	Pop	1	9850	9850	9850	7.05	0.057
	Host	***3***	***18111***	***18111***	***6037***	***4.66***	***0.004***
	Mussel	2	5587	5587	1397	1.08	0.371
	Pop*Host	3	2419	2419	806	0.62	0.602
	Error	108	140024	140024	1297		
	Total	117	175991				

Table 23. Rate of juvenile drop-off from host fish during experiment B. Numbers are mean ± standard deviation of the percent of the total transformed juveniles that had dropped off by day 20 or day 30. Trials of poor hosts (less than 1/3 of best observed transformation percentage) are in parentheses. No significant differences were observed.

Host	Mussel population	Day 20	Day 30
orangebelly (Ouachita)	Ouachita	10 ± 17.1	78 ± 28.2
	Verdigris	(21 ± 26.8)	(63 ± 43.5)
banded (Ouachita)	Ouachita	(17 ± 29.1)	(52 ± 50.7)
	Verdigris	(29 ± 17.2)	(86 ± 37.5)
logperch (Verdigris)	Ouachita	3 ± 3.8	56 ± 27.0
	Verdigris	8 ± 19.7	49 ± 30.8
slenderhead (Verdigris)	Ouachita	(1 ± 2.4)	(28 ± 36.9)
	Verdigris	2 ± 5.6	23 ± 26.4

Table 24. Comparison of drop-off of Verdigris shed glochidia and transformed juveniles on slenderhead darters and logperch used during both experiments A and B. Significant differences between experiments A and B are shown in bold.

Host	Exp.	Glochidia		Juveniles	
		Day 4	Day 10	Day 20	Day 30
slenderhead	A	***68 ± 8.2***	***90 ± 4.4***	5 ± 4.5	***98 ± 3.1***
slenderhead	B	***92 ± 3.8***	***98 ± 3.0***	2 ± 5.6	***23 ± 26.4***
logperch	A	65 ± 19.9	90 ± 7.8	16 ± 19.0	***89 ± 28.2***
logperch	B	76 ± 21.4	91 ± 10.6	11 ± 19.7	***49 ± 30.8***

Table 25. Experiment C. Host, number inoculated, location, number attached, number transformed, and percent transformation ± standard deviation for fish inoculated with Spring River mussel glochidia.

Host fish	N	River	Attached	Transformed	% transformed
slenderhead	5	Elk	85 ± 17.5	1 ± 1.3	1 ± 1.3
logperch	4	Elk	147 ± 42.3	65 ± 45.5	42 ± 23.0
logperch	2	Spring	230 ± 20. 9	99 ± 78.5	39 ± 13.5
fantail	5	Spring	42 ± 18.9	21 ± 10.4	50 ± 11.3
Banded sculpin	5	Spring	126 ±144. 5	1 ± 1.1	2 ± 1.7
orangethroat	5	Spring	58 ± 24.0	1 ± 2.6	5 ± 10.6
stippled	1	Spring	83	0	0
banded	2	Spring	36 ± 0.7	1 ± 0.7	1 ± 2.0
greenside	5	Spring	109 ± 35.1	0	0

Table 26. Length, height, and length/height ratio of glochidia of *Cyprogenia aberti* (C*a)* and *Cyprogenia stegaria* (*Cs*). Numbers are means ± standard deviation. Within a column, values having different superscripts are significantly different from one another (P<0.05). Other published measurements of *Cyprogenia* glochidia are included for comparison.

River	Sp	N	Length	Height	L/H ratio	Source
Verdigris	*Ca*	60	202.6 ± 5.10^{a}	$148.5 \pm .88^{a}$	1.37 ± 0.048^{a}	Present study
St. Francis	*Ca*	60	203.7 ± 6.54^{a}	154.2 ± 5.84^{b}	1.32 ± 0.044^{b}	Present study
Ouachita	*Ca*	60	209.1 ± 6.24^{b}	157.3 ± 4.68^{c}	1.33 ± 0.026^{b}	Present study
St. Francis	*Ca*	6	208 ± 7.03	154 ± 7.79	1.35	Hoggarth 1992
Green	*Cs*	3	206 ± 2.08	167 ± 3.06	1.23	Hoggarth 1992
Ohio	*Cs*	-	180	150	1.20	Ortmann1912
Cumberland	*Cs*	-	210	185	1.14	Surber 1912
Scioto	*Cs*	-	210	170	1.24	Sterki 1898

Table 27. Female mussel shell dimensions by population. Measurements given in millimeters. Sample size (N) is given for each population. Measurements that are significantly different from other populations are shown in bold.

	Verdigris (7)	St. Francis (7)	Ouachita (7)
Shell Length	75.7 ± 10.3	58.3 ± 1.7	71.7 ± 8.0
Shell Height	63.3 ± 8.0	45.6 ± 1.8	51.0 ± 6.1
Shell Width	32.7 ± 4.9	27.4 ± 2.8	34.2 ± 3.9
Width/length	***0.431 ± 0.02***	0.471 ± 0.03	0.478 ± 0.03
Length/height	***1.196 ± 0.04***	***1.308 ± 0.07***	***1.407 ± 0.06***

Table 28. Conglutinate measurements taken from each population. Sample size (N) is given for each population. Measurements given in millimeters. Fecundity is the product of the number of conglutinates and the number of glochidia per conglutinate.

Conglutinate	Verdigris (7)	St. Francis (4)	Ouachita (7)
N per mussel	33 ± 19.6	12 ± 1.7	24 ± 8.3
Length	53 ± 5.7	58 ± 13.1	44 ± 6.6
N glochidia/ conglutinate	4,011 ± 838	5,272 ± 2,306	2,803 ± 1,263
Fecundity	132,363 ± 101,441	63,182 ± 33,335	69,634 ± 51,651

Table 29. Number of mussels observed that produced white, brown or red conglutinates, versus population. Each mussel produced only one color of conglutinate.

Mussel population	Conglutinate color		
	White	Brown	Red
St. Francis	-	8	19**
Ouachita	-	4	3
Verdigris	10	-	-
Spring	4*	-	-

* Includes data from 3 mussels reported by Barnhart (1997)
** Includes historical record of 17 mussels from Chamberlain (1934)

Table 30. Comparison of percent transformation among individual Verdigris fanshell mussels used during experiment A. Numbers are mean ± standard deviation (N) where N is the number of host fish used in each trial. VR3 had significantly lower transformation success than VR1 or VR2 on slenderhead darters.

Host	Mussel Number		
	VR1	VR2	VR3
rainbow darter	0.5 ± 0.86 (7)	0.7 ± 0.98 (7)	1.4 ± 1.73 (7)
orangethroat darter	1.8 ± 2.36 (3)	0.1 ± 0.23 (3)	0.4 ± 0.74 (4)
logperch	9.8 ± 11.1 (4)	10.4 ± 11.63 (4)	6.2 ± 4.04 (4)
slenderhead darter	25.5 ± 6.56 (5)	35.0 ± 12.40 (5)	***7.3 ± 2.22 (5)***

Table 31. Range overlap and host suitability of fish species and fanshell populations. Hosts were classified as good, fair, or poor based on the highest transformation success observed in each experiment. Transformation success from maximum to 2/3 maximum=good, from 2/3-1/3 maximum=fair, and from 1/3 maximum to minimum= poor. Logperch and slenderhead were tested twice with Verdigris fanshells. Pairs that were not tested are also indicated**.** Good hosts were always sympatric, and allopatric species were always poor hosts.

Host fish	**Mussel populations**			
	Verdigris	**St. Francis**	**Ouachita**	**Spring**
logperch	sympatric fair, good	sympatric good	sympatric good	sympatric good
slenderhead	sympatric good, good	allopatric poor	allopatric poor	sympatric poor
rainbow	allopatric poor	sympatric good	allopatric not tested	allopatric not tested
orangethroat	sympatric poor	sympatric poor	allopatric not tested	sympatric poor
banded	sympatric poor	sympatric not tested	sympatric poor	sympatric poor
orangebelly	allopatric poor	allopatric not tested	sympatric good	allopatric not tested
fantail	sympatric not tested	sympatric not tested	allopatric not tested	sympatric good

FIGURES

Figure 1. Distribution of *Cyprogenia*. Rivers with records of *Cyprogenia* are shown within basins outlined in gray. Currently, all records East of the Mississippi River are classified as *Cyprogenia stegaria*, while records West of the Mississippi River are classified as *Cyprogenia aberti*.

Figure 2. Fanshell mussel conglutinates. The upper panel shows a female with two conglutinates partly extruded from the excurrent siphon. Notice the swollen "head" end of the conglutinates exiting the mussel first. The lower panel shows a conglutinate at higher magnification. The “head” end of the conglutinate at right consists primarily of undeveloped eggs. Light-colored areas consist mainly of eggs containing glochidia. A few dislodged glochidia are visible near the edge of the penny.

Figure 3. Conglutinates from three different females, showing the three colors observed in this study. Clockwise from top: white, brown, and red. Penny is shown for scale.

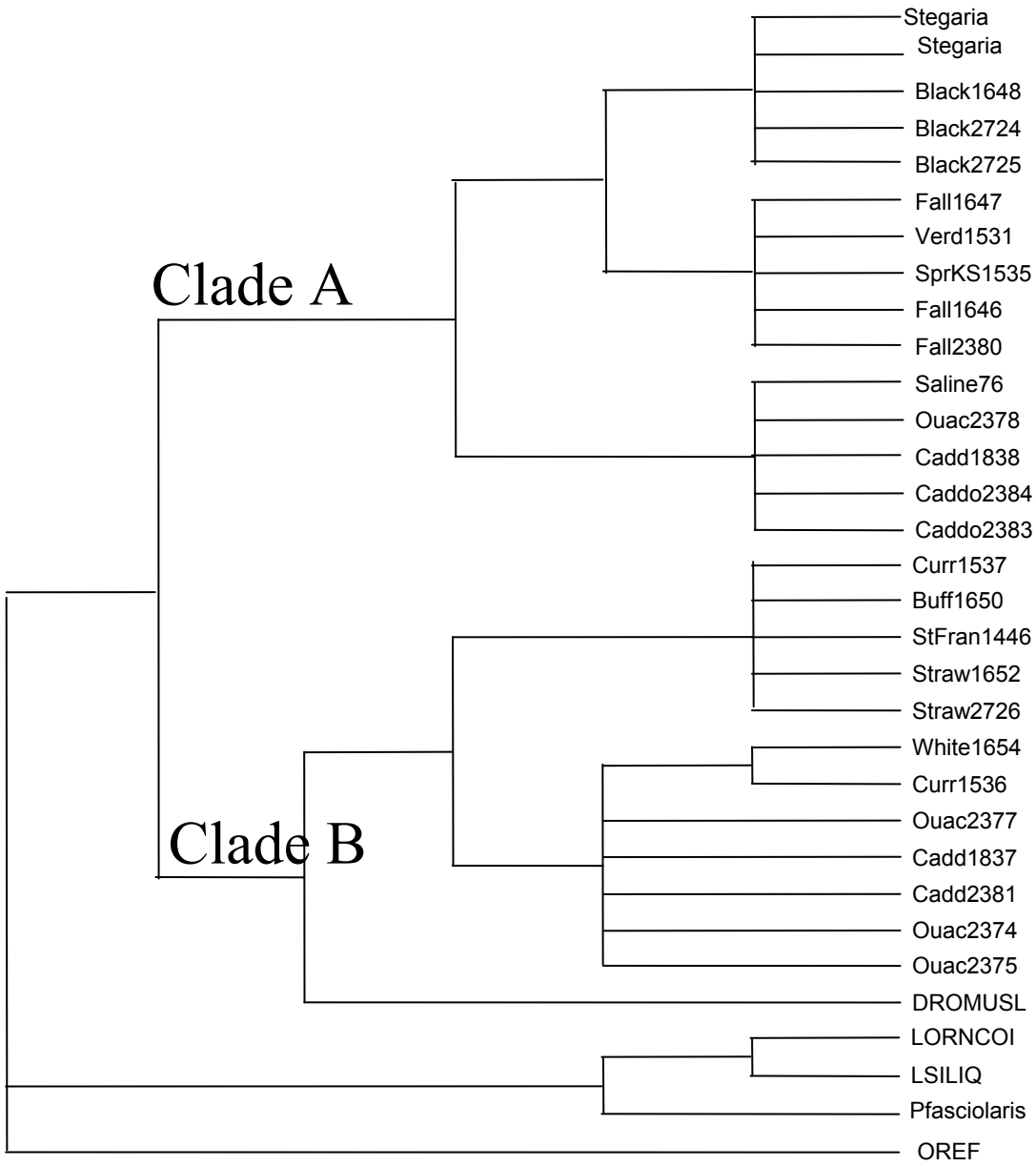

Figure 4. Phylogenetic analysis of *Cyprogenia* derived from sequence analysis of mitochondrial gene CO1 (Jeanne Serb, reproduced with permission). Specimen names at right indicate river of origin for *C. aberti* specimens: Black, Fall, Verdigris, Spring (upper Arkansas), Saline, Ouachita, Caddo, Current, Buffalo, St. Francis, Strawberry, and White. "Stegaria" = *Cyprogenia stegaria*.

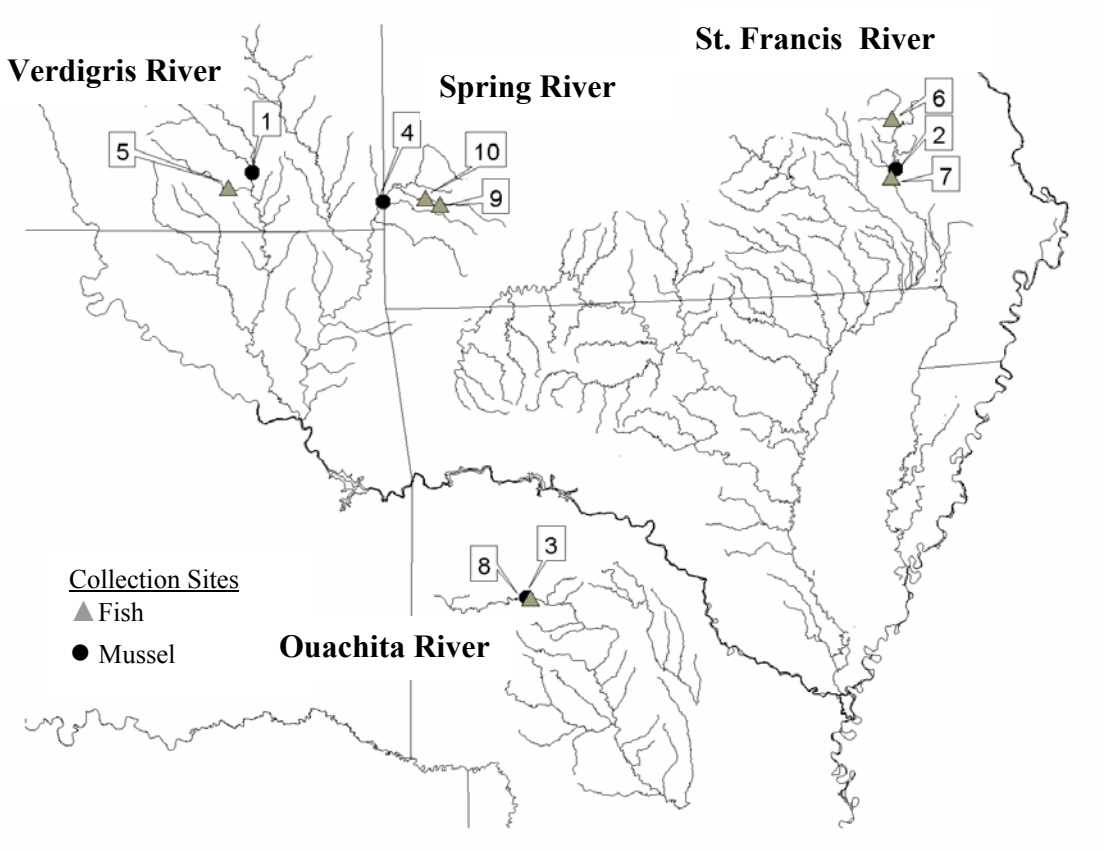

Figure 5. Location of fish and mussel collection sites. Site numbers correspond with Table 1 (mussel sites) and Table 2 (fish sites).

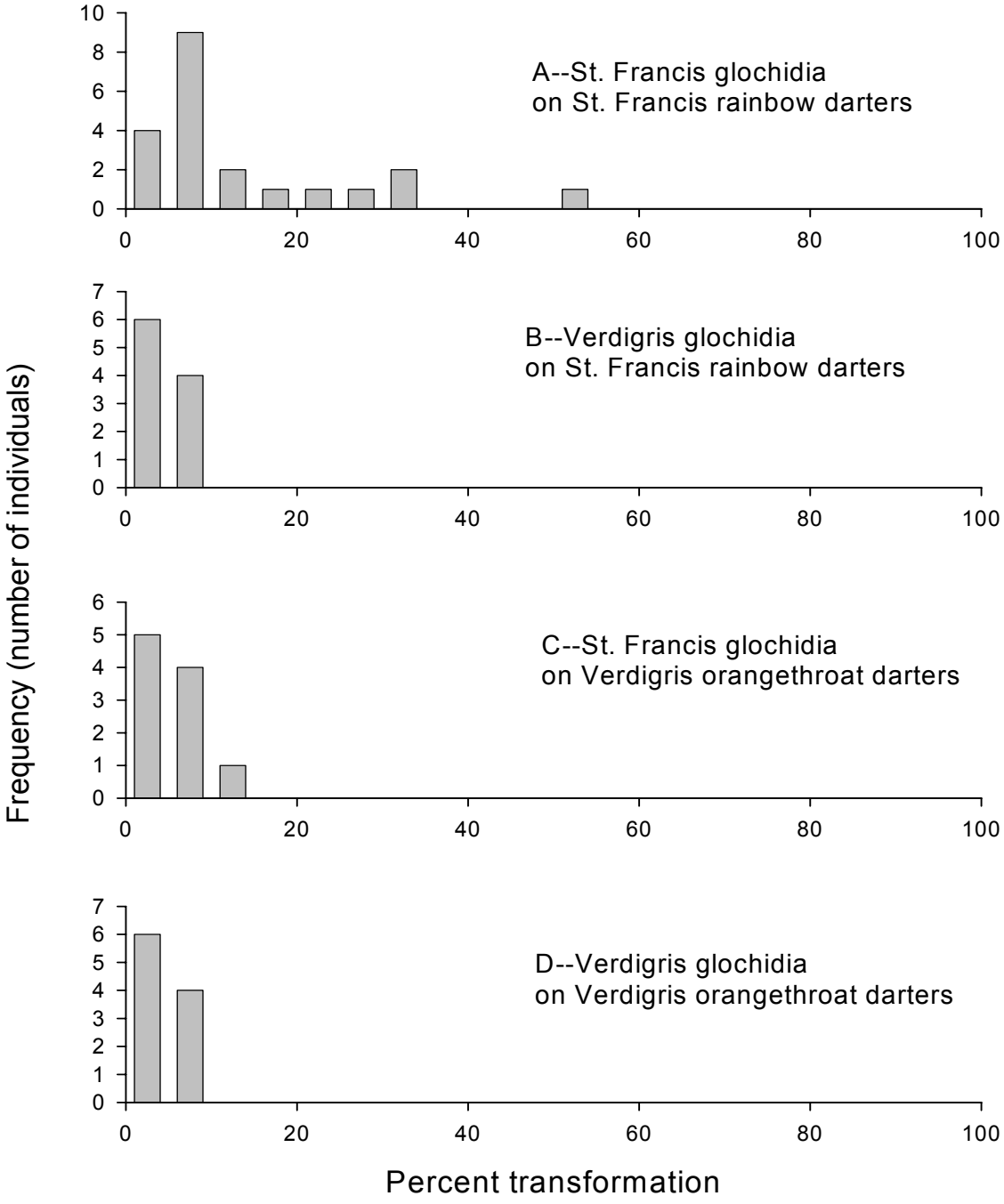

Figure 6. Experiment A, *Etheostoma*. Frequency distributions of percent transformation of glochidia on individual host fish.

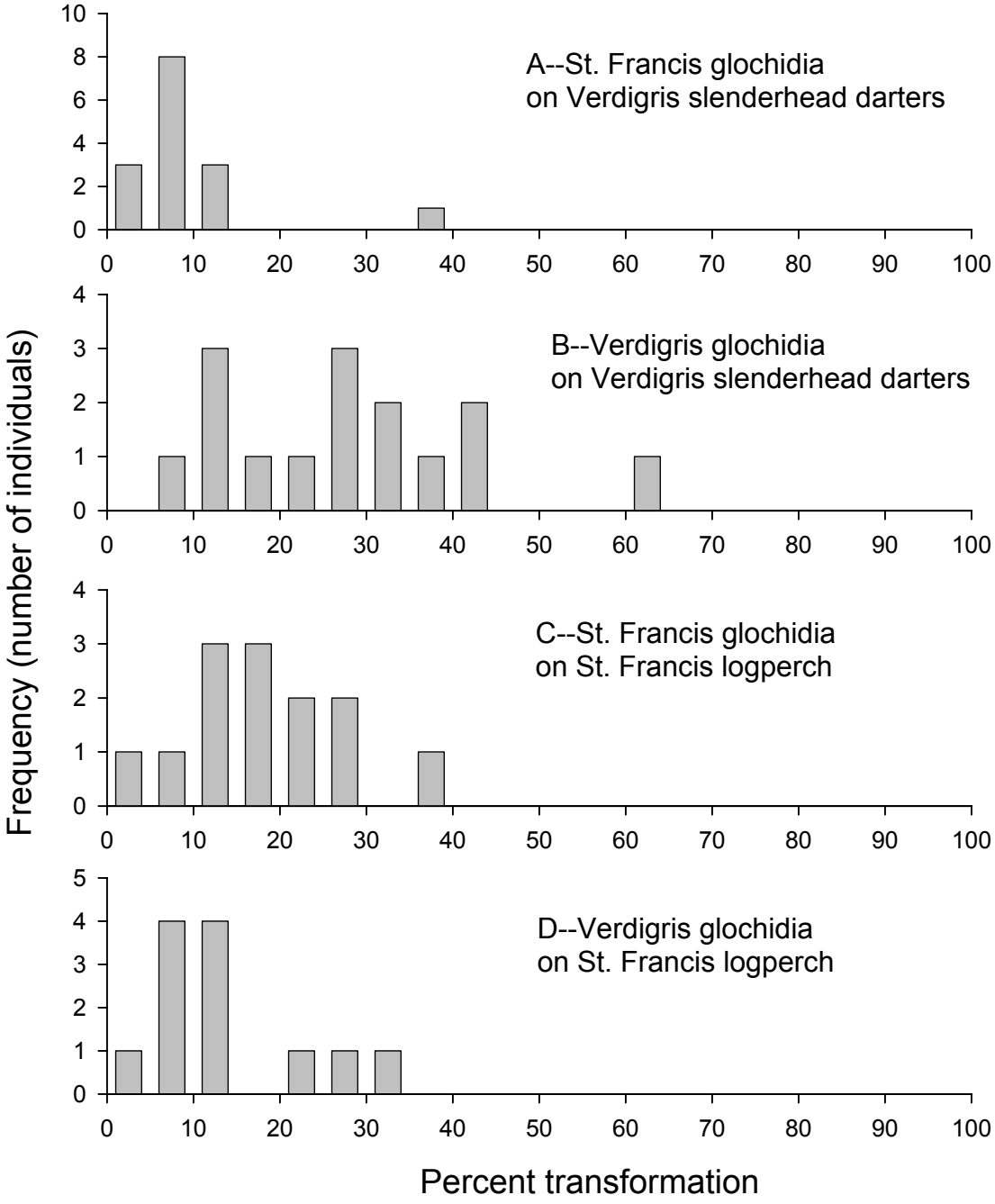

Figure 7. Experiment A, *Percina*. Frequency distributions of percent transformation of glochidia on individual host fish.

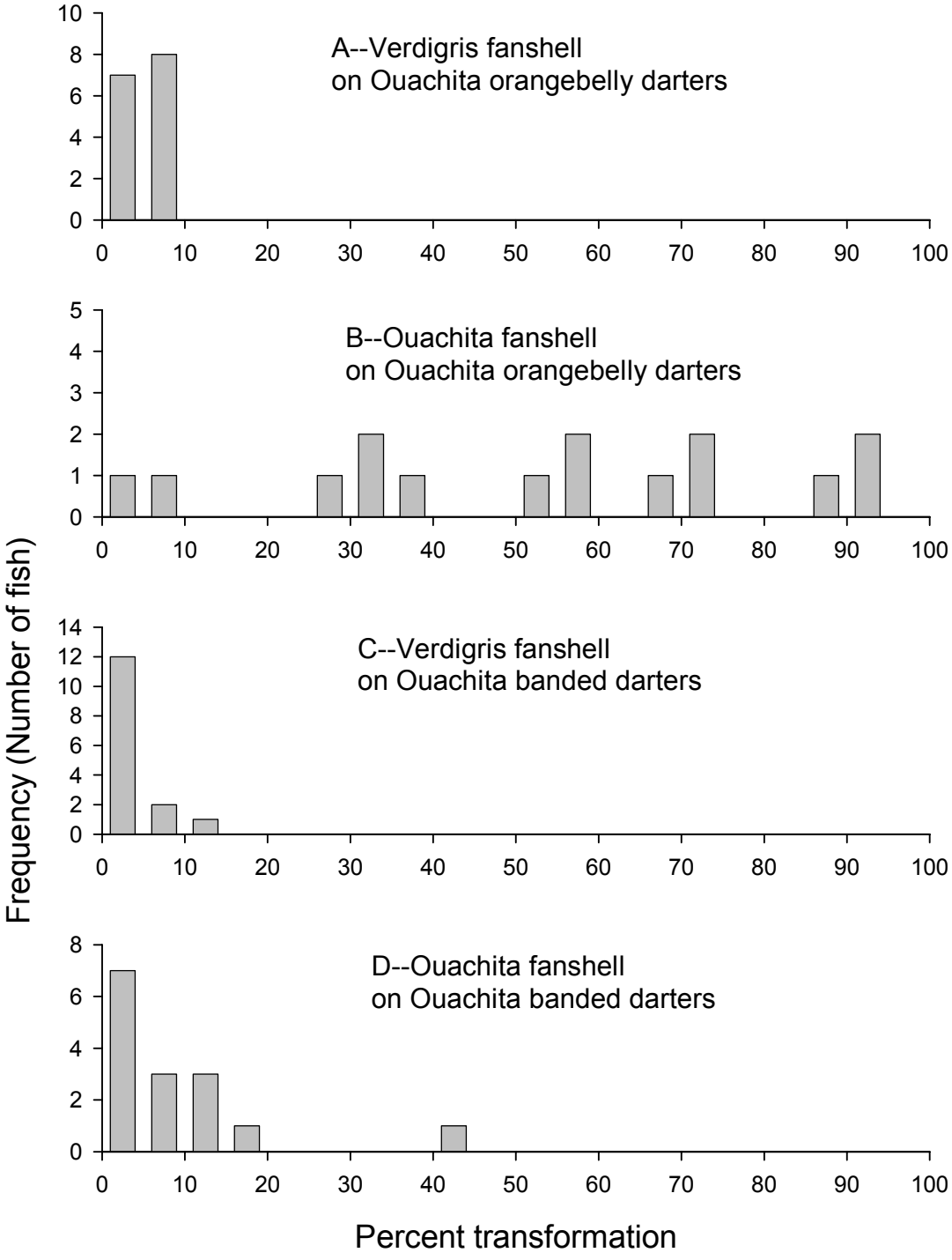

Figure 8. Experiment B, *Etheostoma*. Frequency distributions of percent transformation of glochidia on individual host fish.

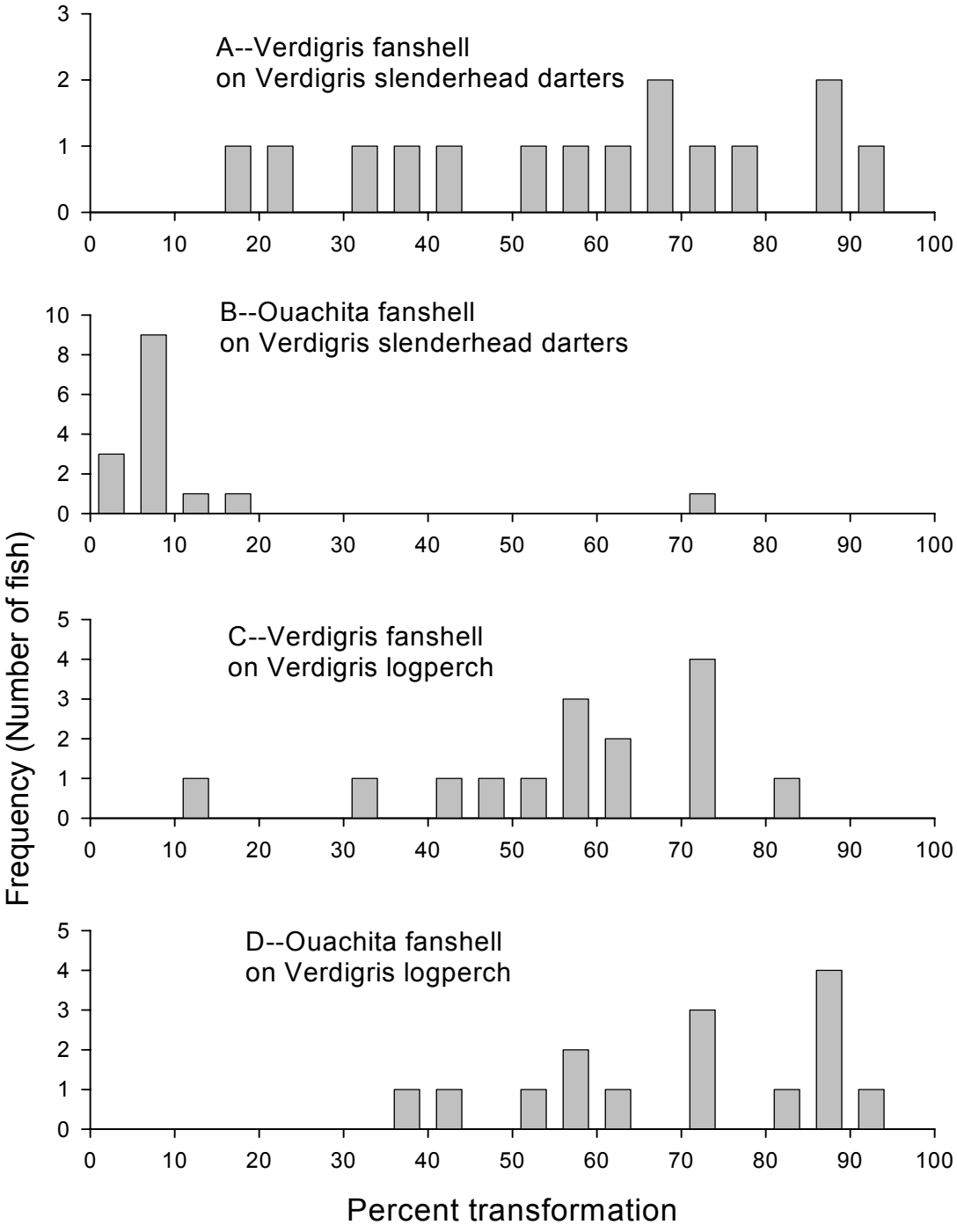

Figure 9. Percent Experiment B, *Percina*. Frequency distributions of percent transformation of glochidia on individual host fish.

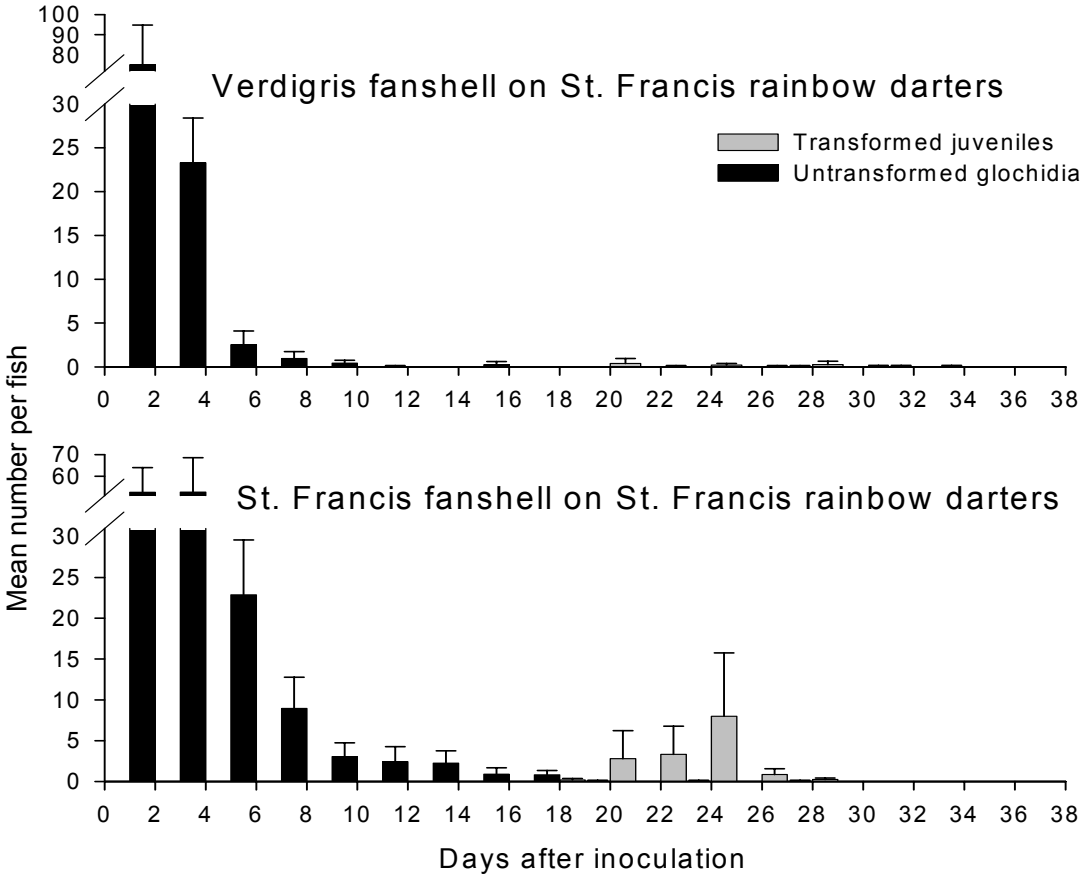

Figure 10. Time course of recovery of untransformed glochidia and transformed juveniles from rainbow darters during experiment A. Bars indicate mean and standard deviation.

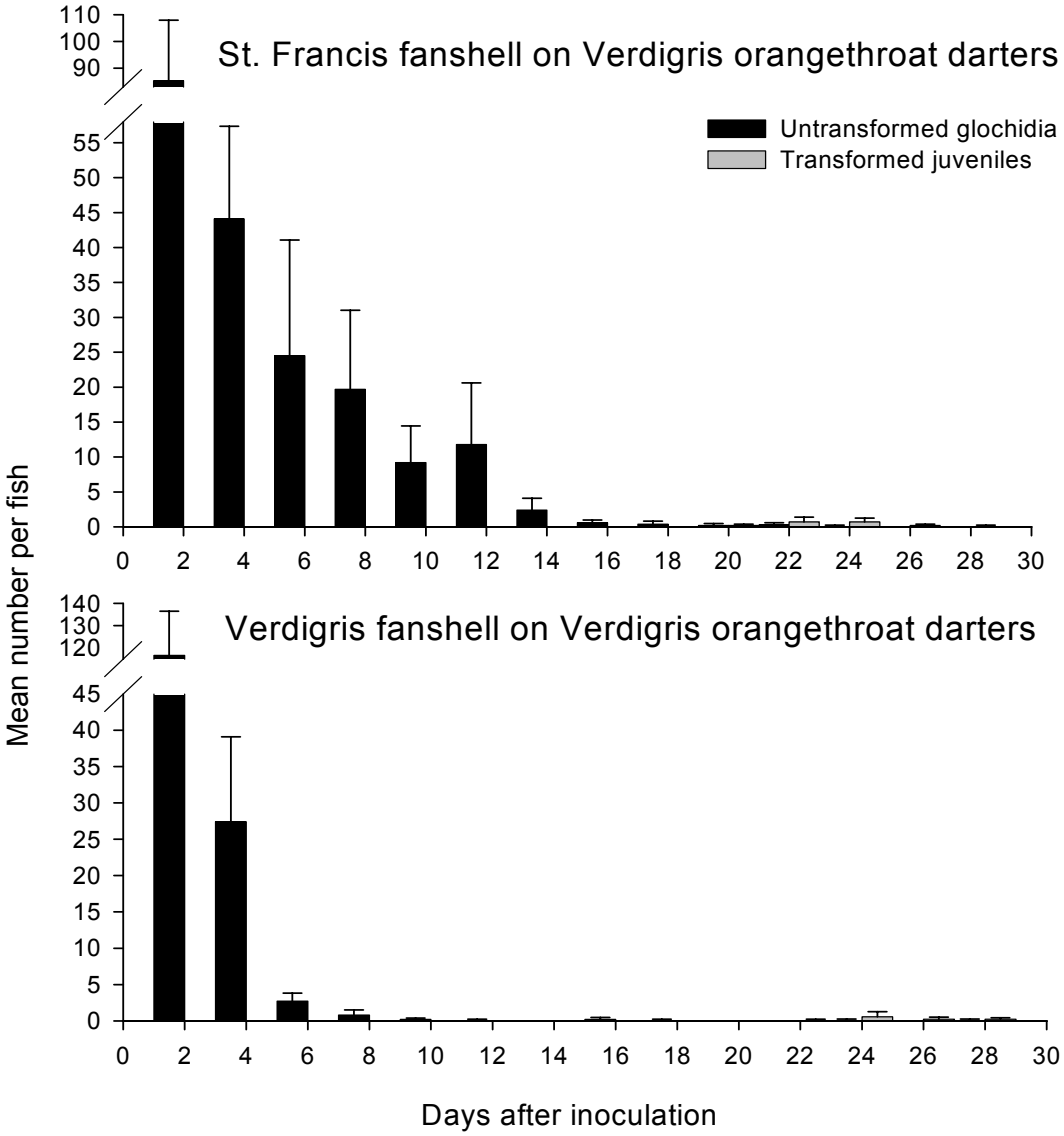

Figure 11. Time course of recovery of untransformed glochidia and transformed juveniles from orangethroat darters during experiment A. Bars indicate mean and standard deviation.

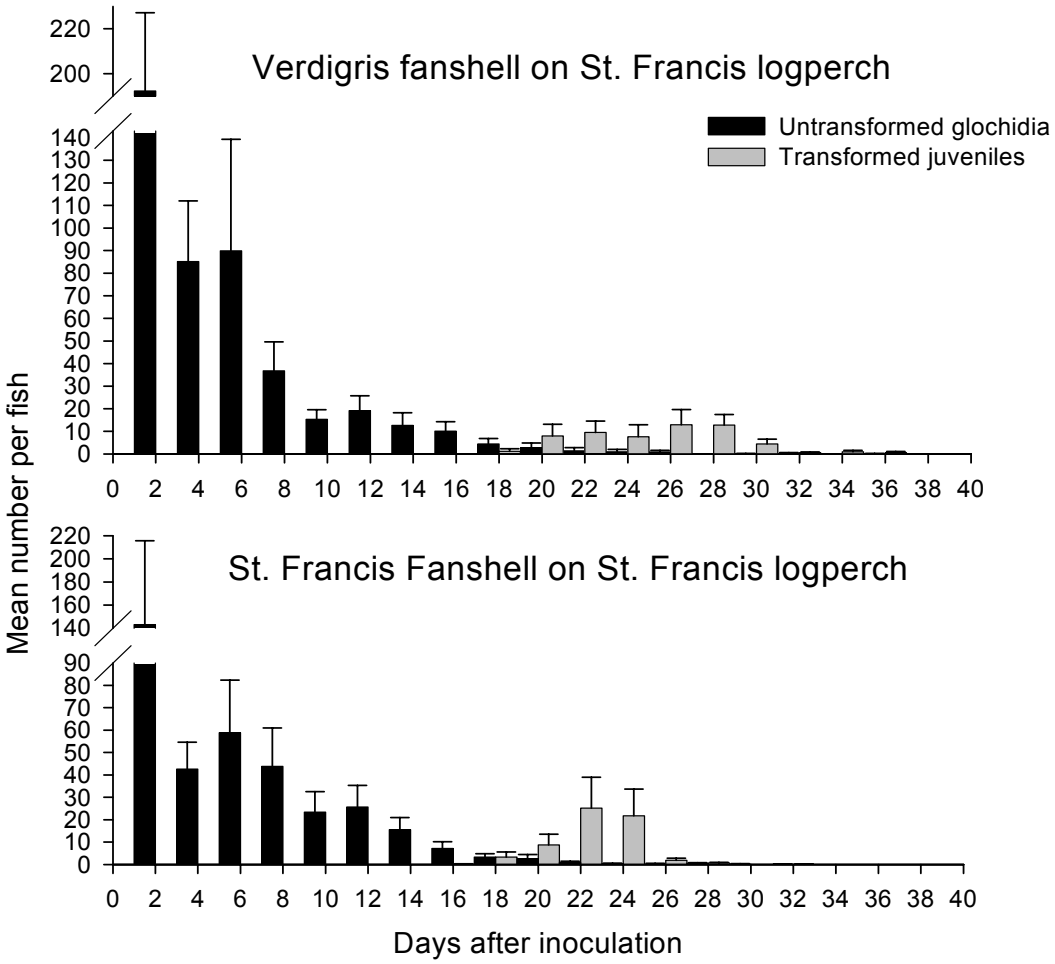

Figure 12. Time course of recovery of untransformed glochidia and transformed juveniles from logperch during experiment A. Bars indicate mean and standard deviation.

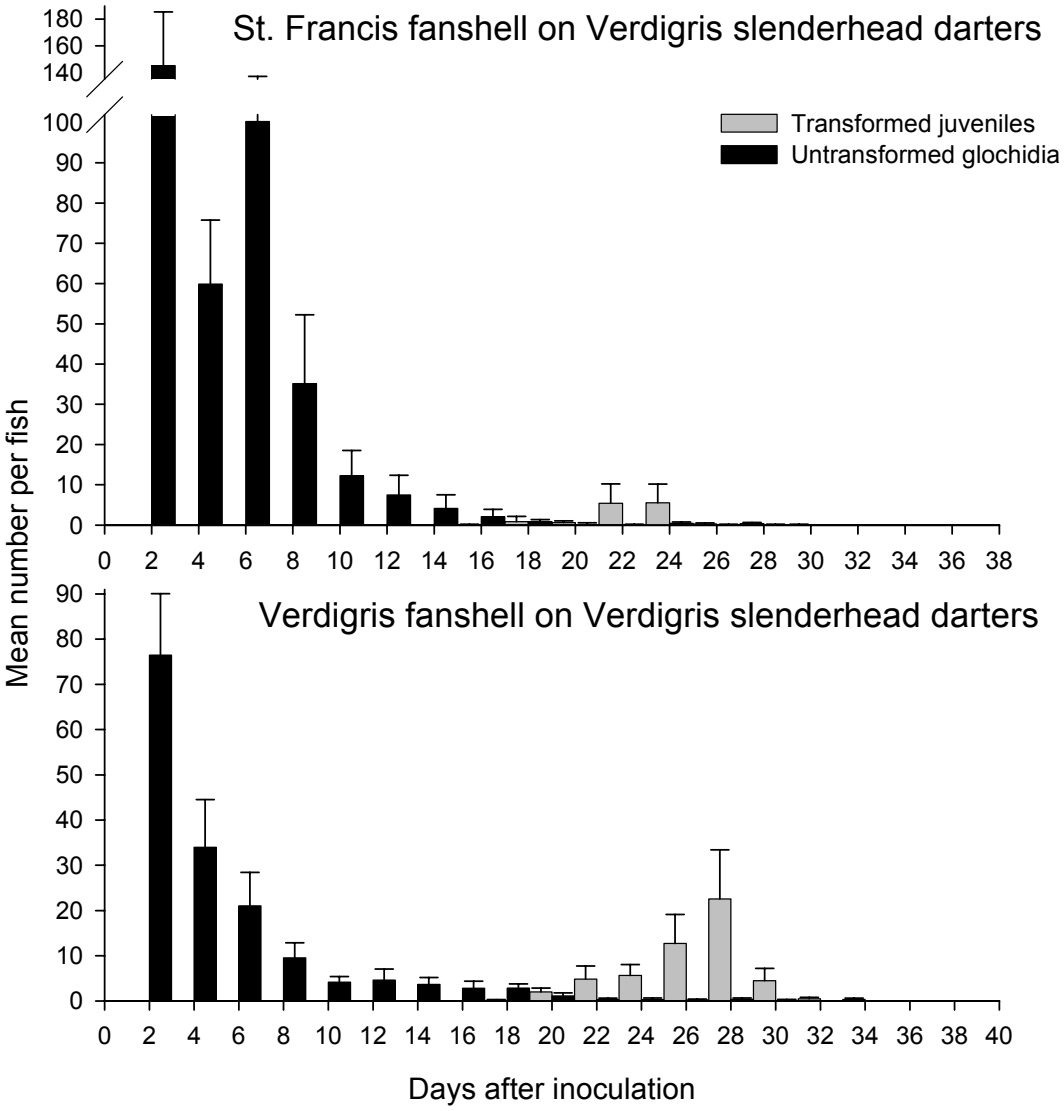

Figure 13. Time course of recovery of untransformed glochidia and transformed juveniles from slenderhead darters during experiment A. Bars indicate mean and standard deviation.

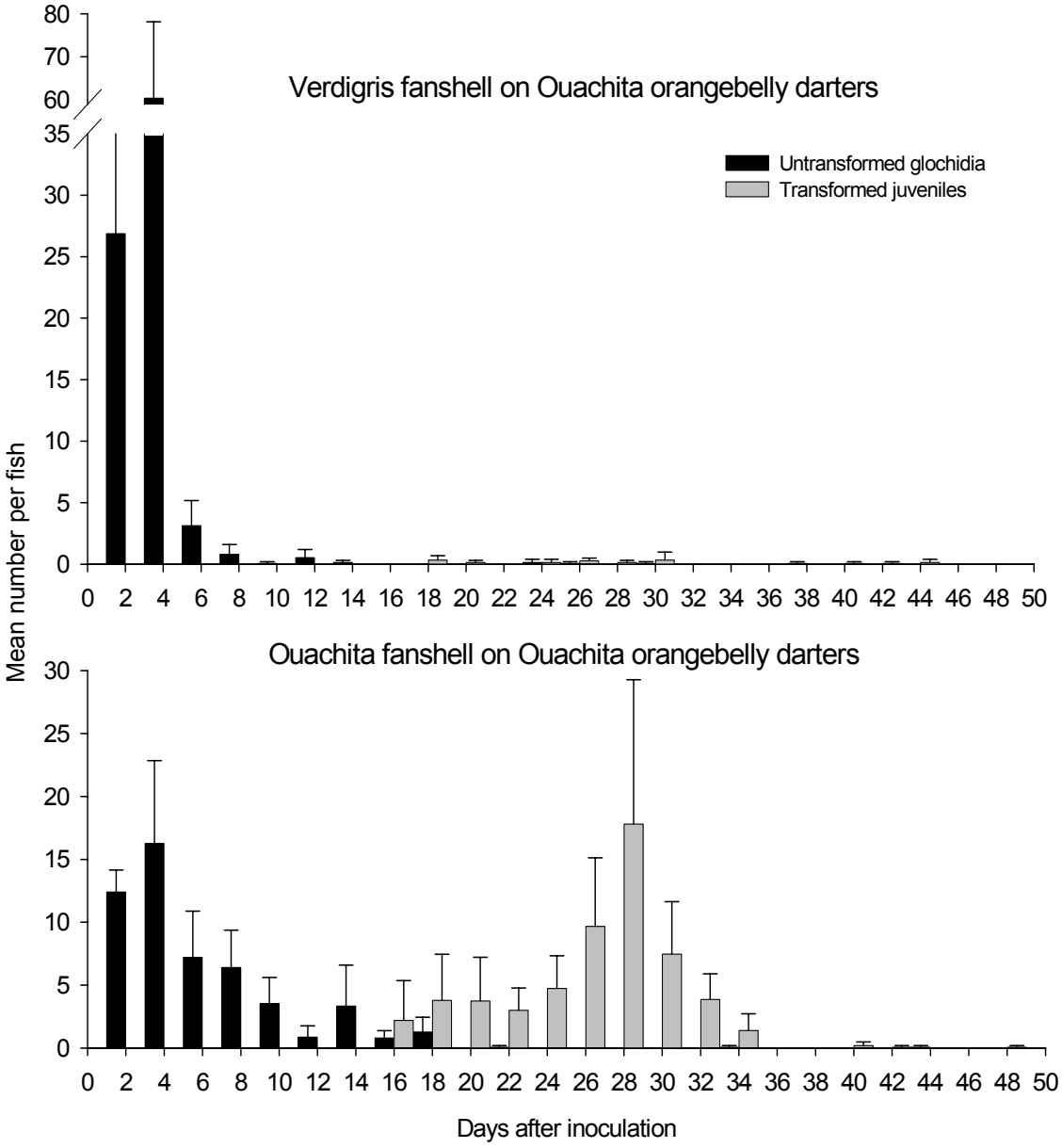

Figure 14. Time course of recovery of untransformed glochidia and transformed juveniles from orangebelly darters during experiment B. Bars indicate mean and standard deviation.

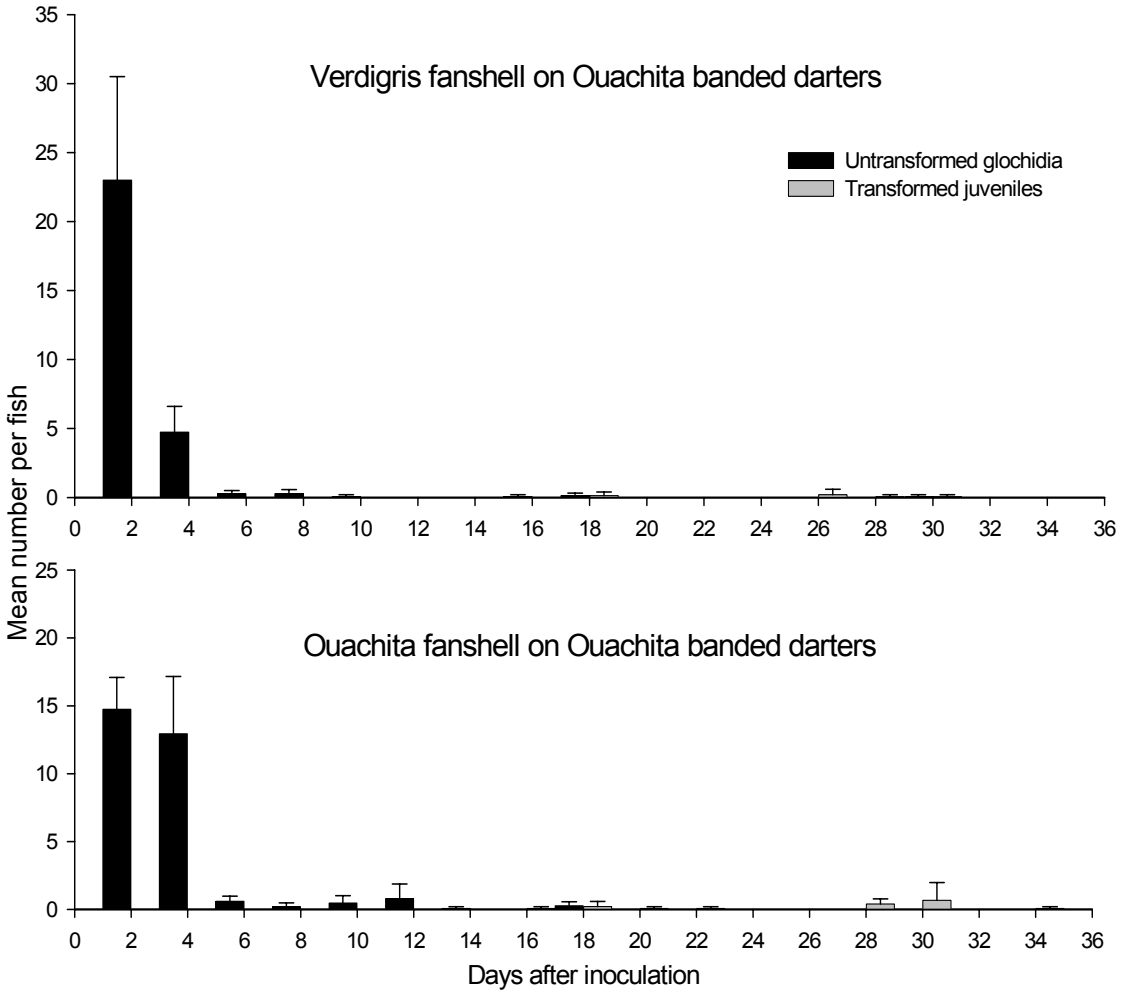

Figure 15. Time course of recovery of untransformed glochidia and transformed juveniles from banded darters during experiment B. Bars indicate mean and standard deviation.

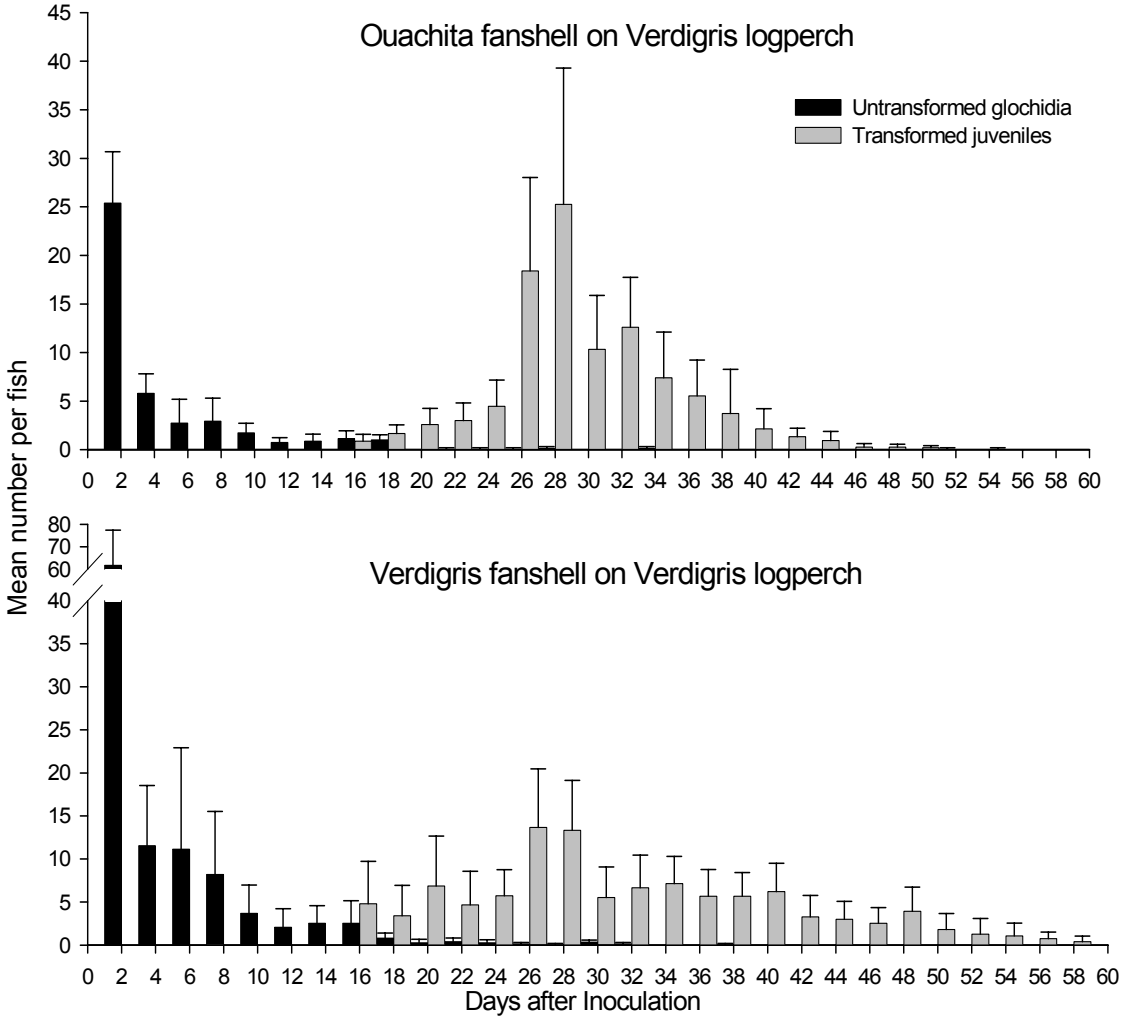

Figure 16. Time course of recovery of untransformed glochidia and transformed juveniles from logperch during experiment B. Bars indicate mean and standard deviation.

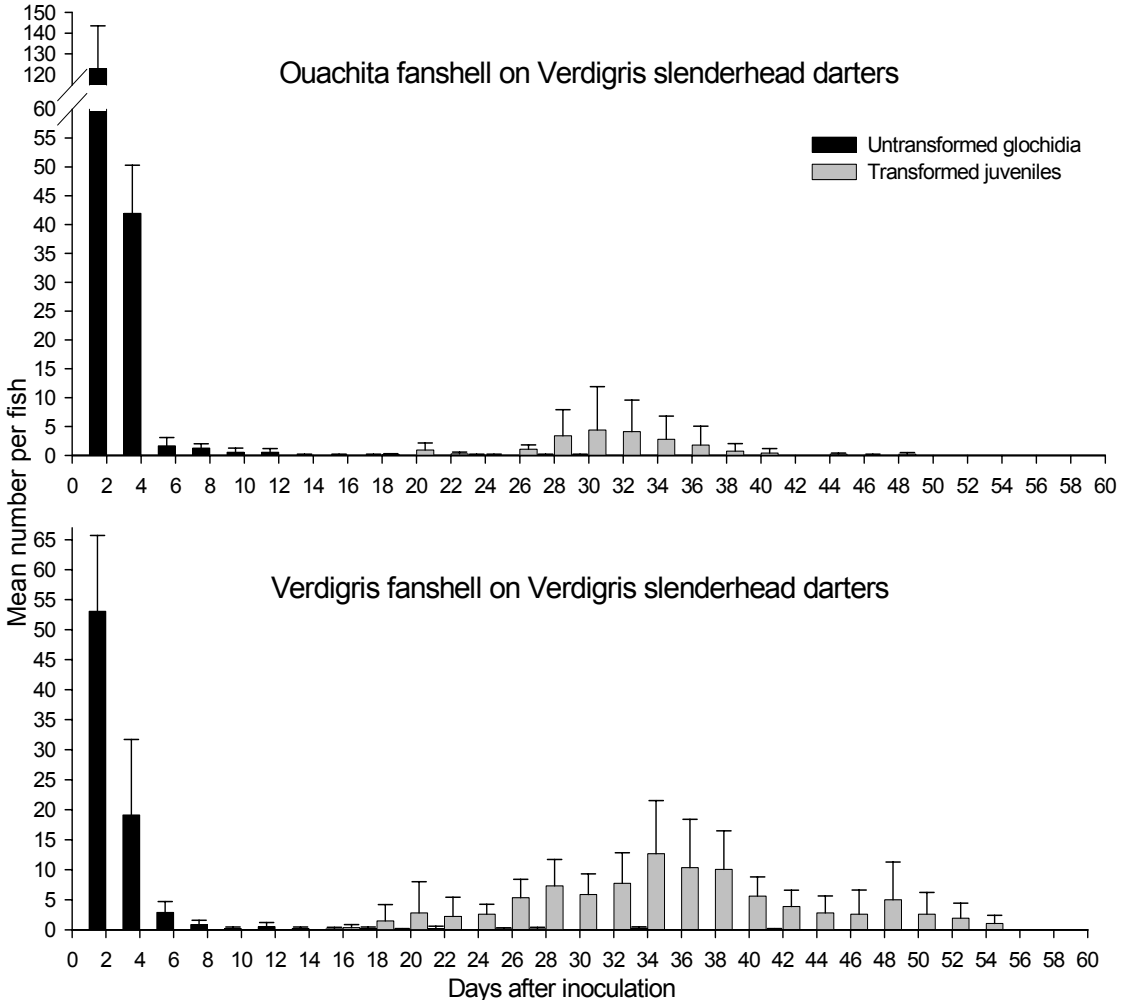

Figure 17. Time course of recovery of untransformed glochidia and transformed juveniles from slenderhead darters during experiment B. Bars indicate mean and standard deviation.

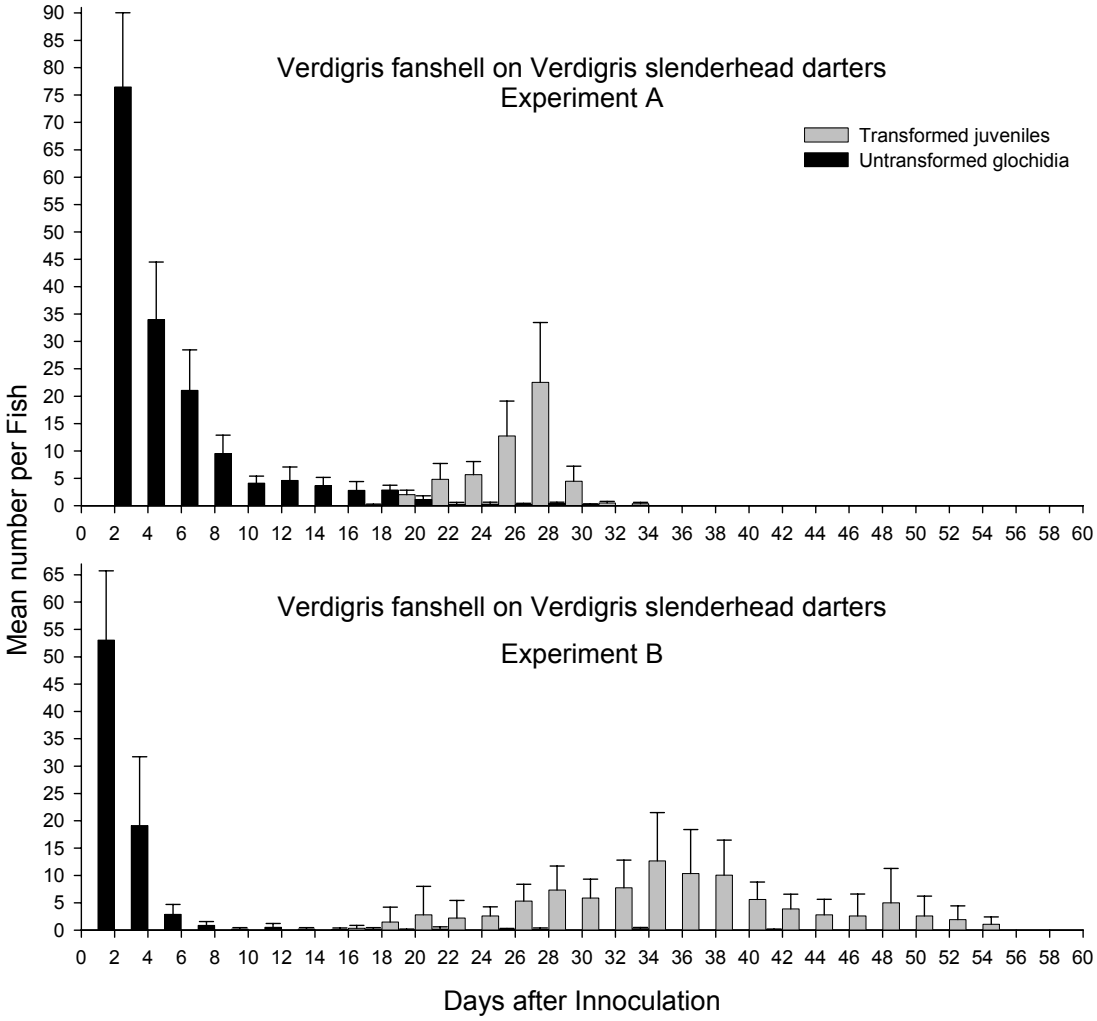

Figure 18. Comparison of the time course of recovery of untransformed glochidia and transformed juveniles for experiment A and B slenderhead darters inoculated with Verdigris fanshell. Bars indicate mean and standard deviation.

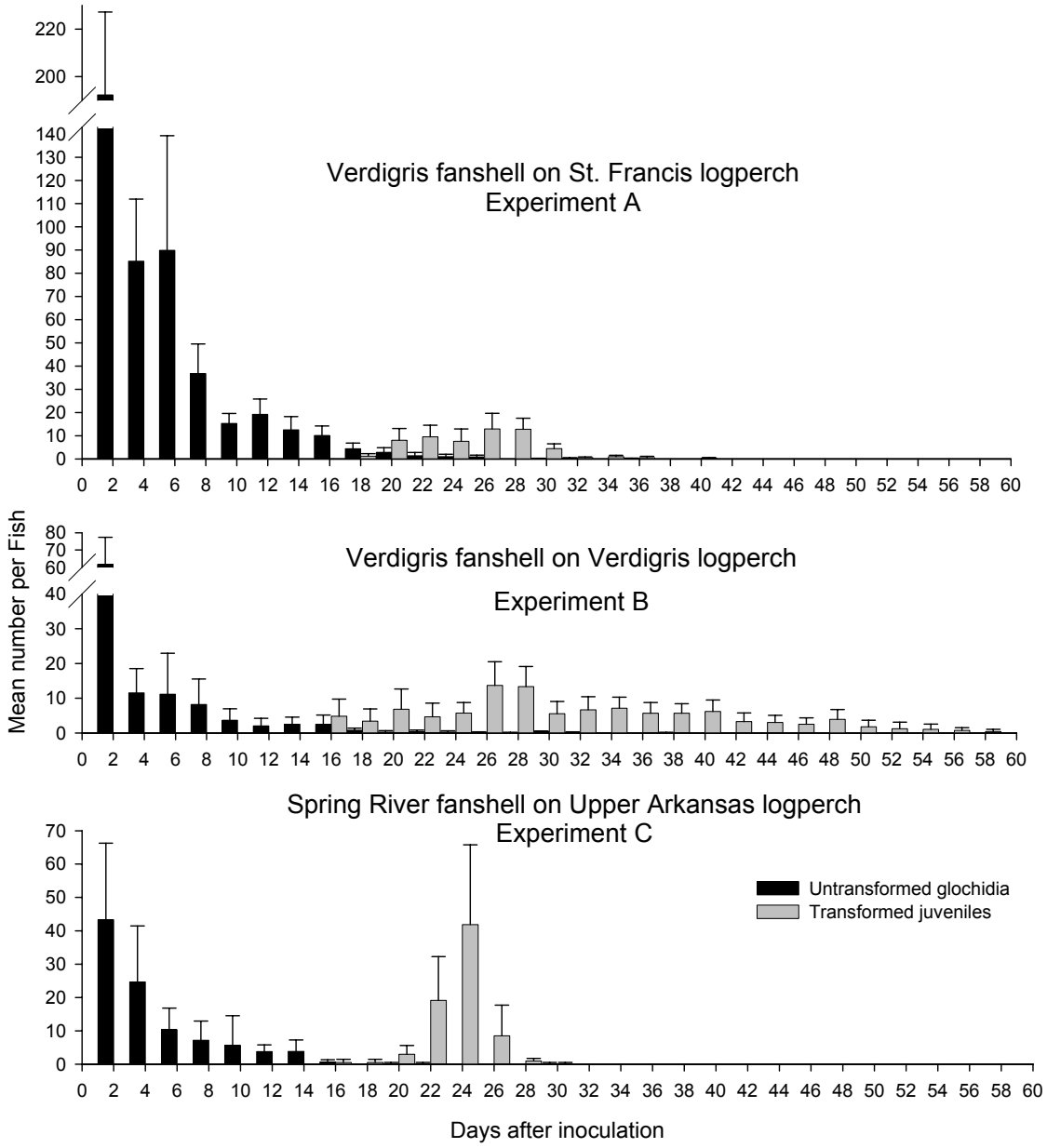

Figure 19. Time course of recovery of untransformed glochidia and transformed juveniles for logperch used in experiments A, B and C. Glochidia from experiment A were the oldest, while glochidia from experiment B were the youngest. Glochidia from experiment C were an intermediate age. Bars indicate mean and standard deviation.

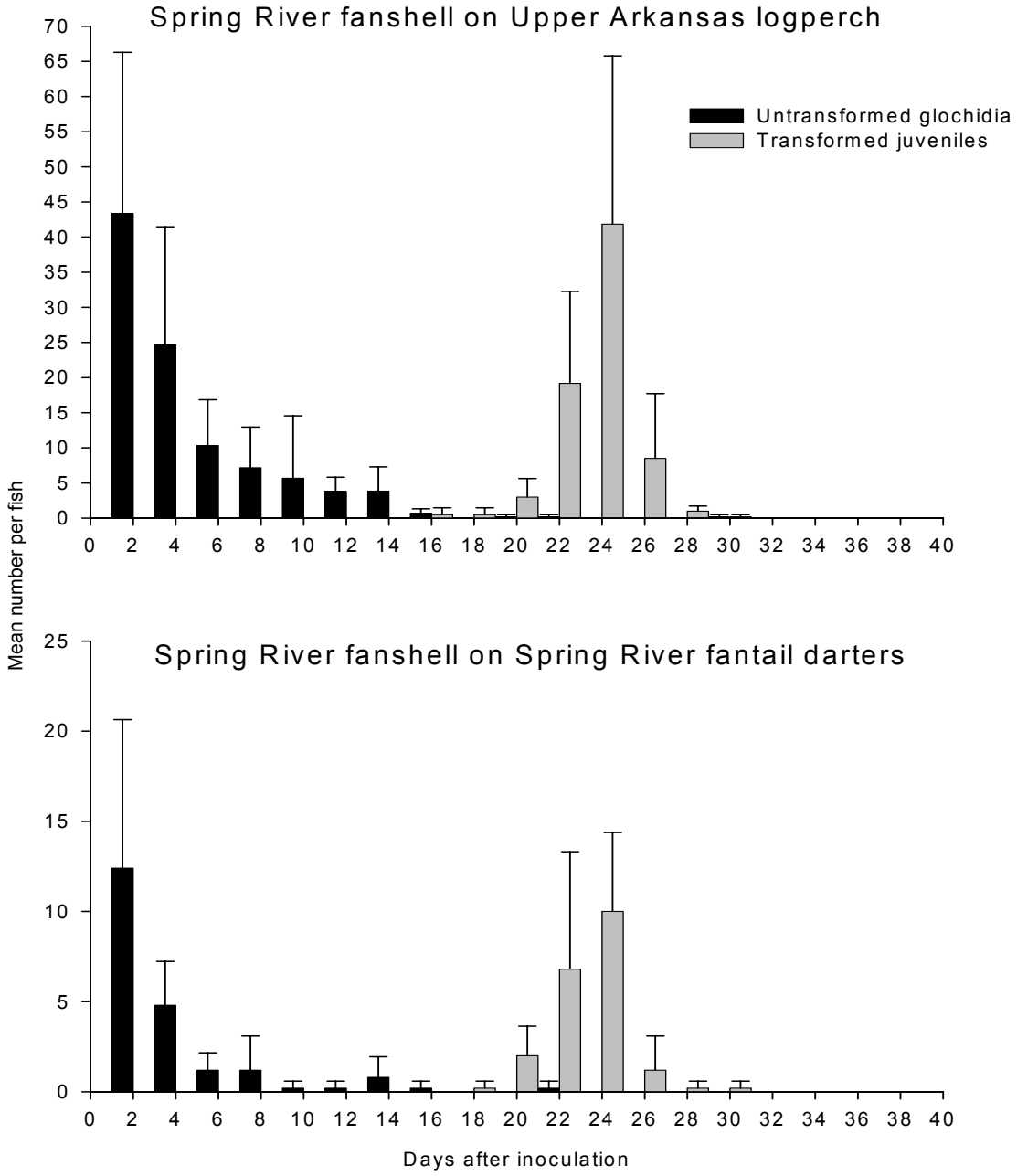

Figure 20. Time course of recovery of untransformed glochidia and transformed juveniles from logperch and fantail darters during experiment C. Bars indicate mean and standard deviation.

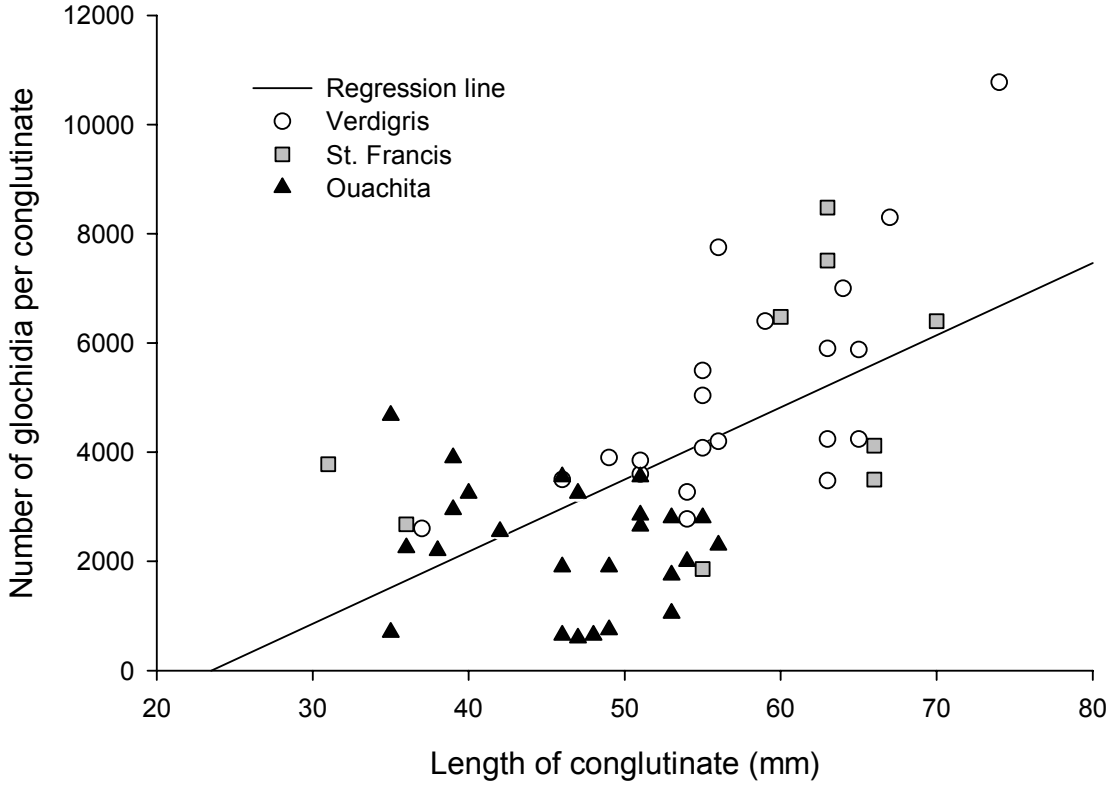

Figure 21. Plot of total glochidia per conglutinate vs. length conglutinate in millimeters for Verdigris, St. Francis, and Ouachita fanshell.

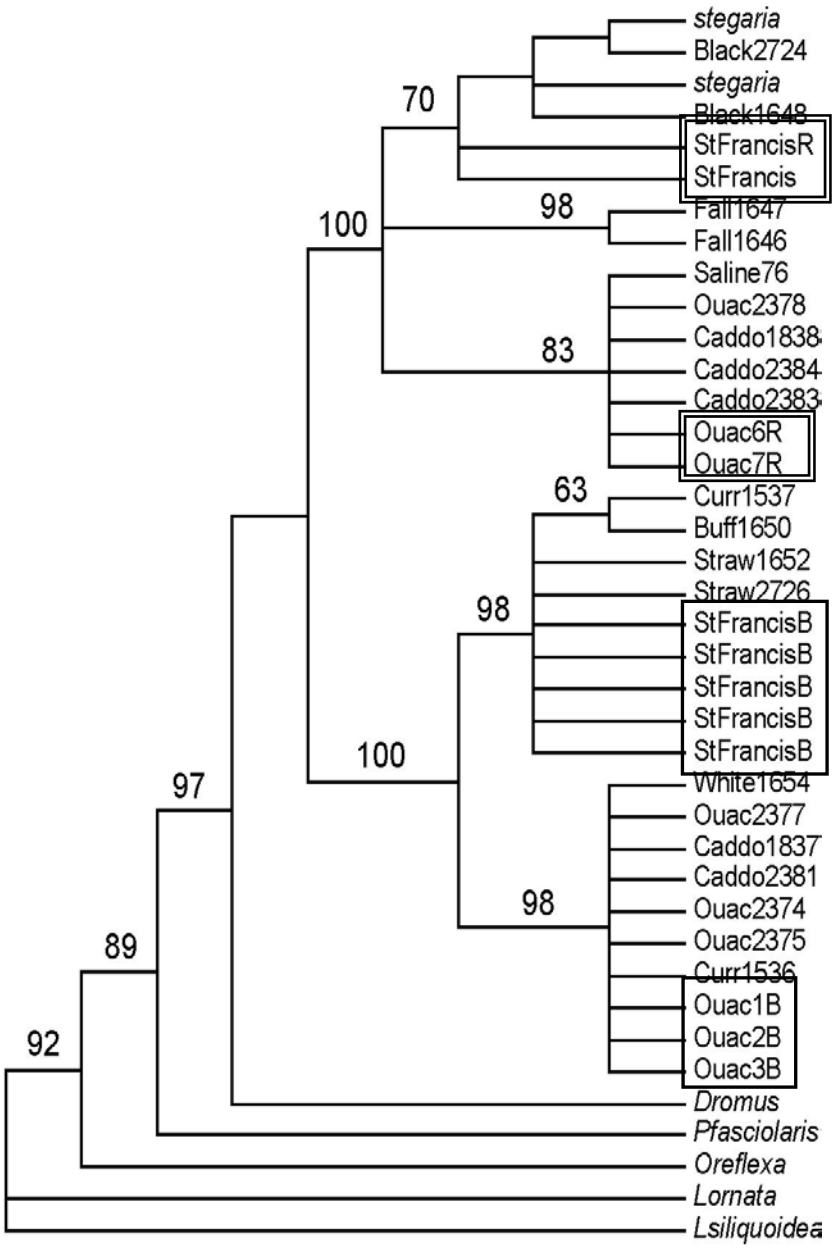

Figure 22. Correspondence of conglutinate color and mitochondrial genotype. The cladogram is derived from sequence analysis of a portion of the CO1 gene (Jeanne Serb, reproduced with permission). Specimen names at right indicate river of origin for *C. aberti* specimens: Black, St. Francis, Saline, Ouachita, Caddo, Current, Buffalo, Strawberry, and White. "Stegaria" = *Cyprogenia stegaria*. Boxes with double line indicate individual mussels that produced red conglutinates. Boxes with single line indicate individuals that produced brown conglutinates.