

Sept 29, 1997

REPORT:

Fertilization success in freshwater mussels.

Project period: July 1, 1996- June 30, 1997

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Natural History Division

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## INTRODUCTION

This report describes an investigation of fertilization success in unionid mussels. The reproduction of unionids is of interest because of the endangered status of many species within this family. Of an original fauna of roughly 300 species in North America, at least 21 unionids are already extinct and 56 are federally classified as endangered. Many workers believe that over half of North American unionid species are in danger of extinction over the next 50 years (Bogan 1993, Biggins et al. 1995, Neves 1997).

One of the possible impediments to reproduction of rare mussel species is failure of fertilization of the eggs. Male mussels release milt into the water. Females retain their eggs within the marsupial gills and must filter sperm from the water in order for fertilization to occur. In still water, the dispersal of sperm by swimming must be limited by the small size of spermatozoa and by their limited energy reserves. In flowing water, sperm can move only downstream and their concentration in the water will quickly be diluted. In either case, if individuals of a species are rare and sparsely distributed, transfer of sperm among individuals may be unlikely, and eggs may not be fertilized.

Very few studies have attempted to test the hypothesis that mussel population density limits fertilization success. Downing et al. (1993) found that fertilization of *Elliptio complanata* was strongly correlated with population density in a lake environment. In contrast, Bauer (1987) asserted that fertilization of the stream-dwelling European pearl mussel, *Margaritifera margaritifera*, was unrelated to the upstream proximity of conspecifics, and suggested that isolated individuals might self-fertilize. Neves (1997) reported high rates of fertilization in rare and sparsely distributed unionids and also suggested that facultative hermaphroditism and self-fertilization may be widespread.

In the present study, I attempted to compare fertilization success among individual mussels that differed in their proximity to conspecifics in natural populations. The study was carried out at two sites in the Spring River in Lawrence County, Missouri. The species studied were *Elliptio dilatata* and *Fusconaia ozarkensis*, both of which belong to the unionid subfamily Ambleminae. Amblemine species were studied because the release of eggs from the marsupial gills can be induced by hypoxia. This response allows collection and examination of the developing embryos without sacrificing the adults.

I hypothesized that females which were far downstream from conspecifics would have lower fertilization rates than those with conspecifics nearby. However, the investigation showed that the eggs of *E. dilatata* were uniformly fertile regardless of position and population density, while *F. ozarkensis* always exhibited a large proportion of undeveloped eggs. As a result, the focus of the study shifted to an examination of sterile egg production and interpretation of the role of sterile eggs as a reproductive strategy. This report also summarizes observations on conglutinate structure, sex ratios, reproductive and developmental synchrony.

## METHODS

### Study sites

Descriptions of all sites examined are listed in Appendix 1. Ten sites were sampled qualitatively in order to determine if mussels were present and to determine if the sites were suitable for the study. Two of these sites, located on the Spring River, were chosen for quantitative sampling. Site #1 was 100 m downstream of the Highway 97 bridge, north of Stotts City, SE4 S14 T28N R28W, Lawrence County, Missouri. Site #2 was located approximately 8 miles upstream of Site #1 and 100 m upstream of a bridge just NE of the town of Hoberg, NE4 S11 T27N R27W, Lawrence County, Missouri.

Most of the mussels described in the report are those which were recovered during quantitative sampling in June and July, 1996 at Sites #1 and #2. In July 1997 another group of eight gravid *Fusconaia ozarkensis* was collected from the Spring River (Site #3) to augment measurements of the proportion of sterile eggs in the conglutinates of that species.

### Quantitative sampling

I sought to relate the percent fertilization of eggs to the proximity of conspecifics upstream of gravid individuals. In order to determine the upstream proximity of conspecifics, we sampled mussels quantitatively along linear transects parallel to water flow. Sampling began by qualitative searching to locate a gravid female. When a gravid female mussel was located, her position was marked and a linear transect was laid out upstream and parallel to the water flow. The path of the water current was traced by pouring a slurry of plaster into the water upstream and observing its movement downstream. The transect was then marked by placing white-painted bricks on the substrate at 1-m intervals. Flow rate was estimated by timing the drift of an orange along the length of the transect. Channel width and water depth were measured across midpoint of the transect. Water temperature was recorded at each site for several days using a Stowaway temperature logger (Onset Computer).

An area 1 m wide and up to 15 m long was quantitatively sampled along each transect. The substrate was removed from the sampled area in portions 1/2 m wide, 1/3 m long and at least 12 cm deep. Hands and garden hoes were used to rake the gravel into boxes lined with 1/4 inch mesh wire screen. Each box of substrate was then removed to shore and the contents examined carefully for mussels. After examination the substrate was returned to the excavation in the stream bed. As mussels were recovered they were placed in plastic bags which were marked to indicate their position in the transect.

### Measurements

Each individual was measured (length, height, and width) to the nearest mm, weighed to the nearest 0.1 gram, and engraved with an identification number on the right valve using a Dremel rotary tool. Superficial growth annuli were counted to estimate age. Each mussel was checked for gravidity by gently spreading the valves using a nasal speculum and visually examining the demibranchs. Gravid individuals were recognizable by the swollen appearance and opacity of the

outer demibranchs. Conglutinates were collected from gravid mussels by holding these individuals overnight in water in sealed Ziploc plastic sandwich bags. Conglutinates were generally released within about 12 hours, presumably in response to hypoxia. No mortality was associated with this treatment.

### Sex determination

The probable sex of non-gravid individuals was determined by measuring the number of ctenidial filaments per interlamellar septum. Oocyte-producing individuals (females and/or female hermaphrodites) have more closely-spaced interlamellar septa and therefore fewer ctenidial filaments per septum (Ortmann 1911). In order to count the filaments, a small portion of the left outer demibranch was removed from each individual with a sharp scissors and examined under magnification. Three interlamellar counts were averaged for each sample. Removing a portion of the outer demibranch did not appear to do serious harm. Mussels held in aquaria for several weeks after gill-clipping suffered less than 10% mortality. Most of the few mussels that died were those whose adductors were damaged during the operation. After the holding period the surviving mussels were returned to the site of capture.

### Conglutinates and eggs

Conglutinates were examined and photographed under 40X and 100X magnification. Embryo diameters were measured with an ocular micrometer. The embryos were classified on a descriptive scale of development (Table 1). When undeveloped eggs were present in significant numbers (i.e. more than 2% of the total), the proportion of unfertilized eggs was determined by counting at least 50 eggs in each of at least 5 conglutinates per individual. The conglutinates of *Fusconaia ozarkensis* were small enough to permit complete counts of eggs in each. Counts were made under a compound microscope. In some cases a video image of the microscope field of view was projected on a white board. Each egg in the field of view was marked on the white board as it was counted and classified. Conglutinates were preserved either in 70% ethanol or in 5% buffered formalin, both of which gave satisfactory preservation for light microscopy.

## RESULTS

### Quantitative sampling

A total of 49 square meters was quantitatively sampled; 17 square meters at site #1 and 32 square meters at site #2 (294 individual samples total, each with area of 1/6 m<sup>2</sup>). Six species of unionid mussels were recovered in the quantitative samples. These were *Elliptio dilatata*, *Venustaconcha ellipsiformis*, *Fusconaia ozarkensis*, *Pleurobema coccineum*, and *Alasmidonta viridis*. *Lampsilis rafinesqueana* was also present at site 1 but did not appear in quantitative samples.

## Population densities

The most abundant species collected at site #1 was *E. dilatata*, followed by *P. coccineum*, *F. ozarkensis*, and *V. ellipsiformis*. At site #2, which was further upstream, the most abundant species was *V. ellipsiformis*, followed by *E. dilatata*, *F. ozarkensis*, and *A. viridis*. Population density was calculated for each square meter quantitatively sampled (Figures 1, 2). On this basis, population densities for individual species ranged from zero to over 20 individuals/m<sup>2</sup> (Table 2, Figures 1,2).

## Sex ratios

Sex of individual mussels appeared to be reliably correlated with ctenidial filament counts. Filament counts of *Elliptio dilatata* were strongly bimodally distributed and all gravid individuals fell in the lower portion of the distribution of filament counts (Figure 3). Therefore, it appears justifiable to infer the sex of non-gravid individuals using these counts. The inferred sex ratios of all three species examined were fairly close to 50/50 (Table 3). The proportion of inferred females that were gravid averaged over 80% (Table 3).

## Embryonic stages and reproductive synchrony in *Elliptio dilatata*

The degree of reproductive synchrony in *E. dilatata* was assessed by comparing the developmental stage of embryos among and within individuals. 71.2% of individuals with female gill morphology carried embryos or glochidia. The most common developmental stages were 4-5 (gastrula and early glochidia with adductor visible). Only 4.8% of gravid *Elliptio* had cleavage stage embryos, and 14.3% had mature glochidia. There was some indication of a bimodal distribution of developmental stage among individuals, which might indicate two "cohorts" with somewhat offset timing of reproduction (Figure 4). However, without knowledge of the time necessary to complete each developmental "stage", it is possible that the more frequent stages are simply those which take longer to complete.

Embryos within an individual female were usually at a similar stage of development. Infrequently, a few undeveloped eggs were observed along with developing embryos, and some individuals were observed with both blastulae and gastrulae. However, no individuals were observed with more widely separated stages of development, e.g. both blastulae and glochidia. Thus, it appears that fertilization and development are well-synchronized among embryos in individual *Elliptio dilatata*.

Embryo diameter decreases during gastrulation and can be used as a measure of the progress of development. Figure 5 shows the frequency distributions of embryo diameters from seven individual *E. dilatata*. The individuals are presented in order of the degree of embryonic development, beginning with blastulae, through gastrulation, up to initial appearance of the adductor muscle. These histograms show that embryo diameter decreases during gastrulation and then increases with further development. During gastrulation, bimodal distributions of embryo diameter tend to occur, showing that some embryos are more advanced than others (i.e. #16 and

#46 in Figure 5). However, these embryos do not appear to differ greatly in timing of development, because it appears that gastrulation is a fairly rapid process (see below).

The conglomerates of *Elliptio* usually were comprised of two layers of eggs. The timing of egg deposition in these layers is not known. Both layers might be formed simultaneously or one layer might be completed before the other is begun. Interestingly, several conglomerates were observed in which the gastrula-stage embryos of one layer were uniformly of smaller size (i.e. somewhat more advanced development) than those in the adjacent layer. This observation may suggest that the eggs in one layer are fertilized and positioned before the adjacent layer is begun.

### Timing of development

Previous studies suggest that the development of amblemine glochidia generally requires from 3 to 6 weeks depending on species and water temperature (Weaver et al. 1991 and references therein). In the present study, one individual of *Elliptio dilatata* that was collected with stage 2 (cleavage) embryos released a few conglomerates periodically during the succeeding six days in the lab. These conglomerates were examined each day in order to observe the rate of embryonic development (Table 4). Over the course of 6 days embryonic development proceeded from the 2-4 cell cleavage embryos (stage 2) to embryos with adductors and hinge line evident but no ventral cleft (stage 5). At that point no further conglomerates were released, and the observations were terminated. It appears that the early development of the embryos is quite rapid, requiring only 6 days at 20 C to progress from cleavage to stage 5.

### Fertilization success

In *Elliptio dilatata*, eggs were uniformly and essentially completely fertilized in every individual examined. Undeveloped eggs were observed in only a few individuals, and the proportion of undeveloped eggs within an individual never exceeded about 1%. With essentially no variability in fertilization success, no test of correlation was possible between fertilization success and population density.

In contrast to *E. dilatata*, both *Fusconaia ozarkensis* and *Pleurobema coccineum* produced large numbers of undeveloped eggs along with the developing embryos. The proportion of undeveloped eggs varied among conglomerates within individuals, among individuals, and among species (Figures 6, 7). In *F. ozarkensis* the overall mean proportion of undeveloped eggs was 40% and varied among individuals from 15% to 51% (Figure 6). The proportion of sterile eggs was higher in *P. coccineum*. The estimates of the percent sterile eggs in the three individuals examined were 63.9%, 76.3%, and 89% (Figure 7).

### Description of conglomerates:

*Elliptio dilatata*: In this species, even immature conglomerates were fragile and fragmented during release, so that measurements of intact conglomerate dimensions were not possible. Eggs and embryos were white in color. The fragments of immature conglomerates were flattened, and usually 1-3 layers thick. The eggs of each layer were packed very regularly with each egg surrounded by six others. Mature conglomerates of *Elliptio* disintegrated before or during release,

and most of the mature glochidia observed were free of the egg membrane. Glochidia free of the egg membrane extended the larval thread. Larval threads and mucus suspended the glochidia within a loose mass.

*Fusconaia ozarkensis*: Both mature and immature conglomerates of *F. ozarkensis* were relatively solid and did not disintegrate after release. The conglomerates were spindle-shaped and the ova were not packed in distinct layers. The eggs and developing embryos of *F. ozarkensis* were usually pink to red in color. However, two of 14 gravid individuals examined had white eggs and embryos. Individuals of *F. flava* and *F. ebena* in Missouri and Arkansas likewise produce either red or white eggs (Barnhart, unpublished observations). Glochidia were relatively transparent, so that the color and opacity of conglomerates was a function of the color and relative proportion of sterile eggs, were opaque. The conglomerates of *F. ozarkensis* had average length of 6.1 mm and width of 1.1 mm. The average number of conglomerates released per individual was 238 (S.D. 35, n=8).

Eggs of *Fusconaia* containing glochidia ruptured easily. It was possible to release the mature glochidia by drawing the conglomerate in and out of a Pasteur pipette. This action approximates the action of host fish feeding on prey. The sterile eggs were durable and were not broken by this treatment, and the egg membranes were sufficiently tough that the conglomerate remained intact even when drawn rapidly and repeatedly through a narrow aperture. The mature glochidia each deployed a larval thread upon release from the egg. There was little tendency for the glochidia to clump and no mucus was evident.

*Pleurobema coccineum*: Only three gravid individuals of *P. coccineum* were examined in the study. The eggs and embryos were pale orange or salmon in color. The conglomerates were more fragile than those of *F. ozarkensis* and fragmented easily, so that it was difficult to ascertain number and dimensions. However, the conglomerates were clearly larger and fewer in number than those produced by similar-sized *F. ozarkensis*. The number of conglomerates released by one individual was approximately 42. The overall shape of intact individual conglomerates was a flattened elongate oval, with length and width roughly 11 mm and 3 mm. The conglomerates were layered, and the layers adhered in stacks that could sometimes be separated over most of their length. Occasionally conglomerates were paired at one end.

## DISCUSSION

Surprisingly few studies have addressed the relationship between population density and fertilization success in unionids. Downing et al. (1993) correlated fertilization rates in *Elliptio complanata* with population density in a lake environment. Most individuals had either very low or nearly complete fertilization success. Fertilization rates dropped below 50% when population density was below 18 individuals/m<sup>2</sup>, and fertilization failed completely at population densities below 10/m<sup>2</sup>. These population densities are much higher than those of *Elliptio dilatata* in the present study (Table 3).

Bauer (1987) tested fertilization success in an experimental population of the stream-dwelling European pearl mussel, *Margaritifera margaritifera*. Fifty-five individuals were removed

from a bed of approximately 5000 mussels and were placed upstream in a linear transect at 2m intervals. The author asserted that no mussels were present further upstream. During the subsequent year of study, these individuals showed similar fecundity, and the eggs developed with essentially complete fertilization regardless of position in the study area. Sex was determined by examination of gametes in fluid sampled from the gonads. Many individuals producing oocytes also contained sperm (i.e. were hermaphrodite). Bauer also found strong negative correlation between the proportion of hermaphrodites at a site and the number of mussels immediately upstream, and suggested that females may become hermaphrodite in response to lack of sperm or lack of chemical cues from conspecifics upstream.

Hermaphroditism and self-fertilization might explain the ability of many very rare and sparsely distributed unionid species to maintain high rates of fertilization (Neves 1997). However, the hypothesis of self-fertilization in isolated individuals has apparently not been tested directly. In the present study, fertilization was uniformly high in *E. dilatata*, even though the population density of in this study was low, averaging only 0.83/m<sup>2</sup> and 5.71/m<sup>2</sup> at the Hoberg and Stott City sites, respectively (Table 2, Figures 1, 2). Successful fertilization of stream-dwelling *Elliptio dilatata* occurred at population densities less than one tenth of that at which complete failure of fertilization was observed in lake-dwelling *Elliptio complanata* (Downing et al. 1991). At least two explanations are possible. First, the transport of sperm from upstream individuals may be sufficient to ensure fertilization even at low population densities. Second, individuals might self-fertilize, as suggested by Bauer (1987) for *Margaritifera* and by Neves (1997). Hermaphroditism has not yet been investigated in *E. dilatata*. Sex ratios in the present study do not appear to differ significantly from 50:50. Although lake-dwelling populations of *Elliptio complanata* reportedly exhibit a high proportion of gonadal hermaphroditism (Downing et al. 1989, Downing et al. 1993), these mussels apparently do not self-fertilize to compensate for low population density.

Interestingly, several authors have reported consistently high proportions of undeveloped eggs in certain species of unionids, apparently unrelated to population density (Table 5). Undeveloped eggs appear to be particularly common in species of the genus *Fusconaia*. In reference to *Fusconaia ebena*, Howard (1914) stated that "[I have found] many unfertilized eggs in the marsupia of these mussels. In the examination of hundreds of [individuals] from various points in the region I have found this state quite general." In reference to *Fusconaia flava*, Howard stated that: "The number of undeveloped eggs is surprisingly high, sometimes more than 75%". Likewise, in a more recent study, the average rate of fertilization of eggs of *Fusconaia edgariana* in the North Fork Holston River was only about 30% (Kitchel 1985).

These observations raise a significant question. Are undeveloped eggs in these species the result of pathology, or are they in fact a normal feature of reproduction? Many mussel species release cohesive masses of eggs, called conglutinates. Conglutinates appear to function as an attractant or bait for host fish. Host fish attempt to ingest conglutinates and thereby become infected with the parasitic glochidia larvae. Herein, I propose that sterile eggs are a normal feature of reproduction in many unionids, and that these sterile eggs enhance the attractiveness of conglutinates to host fish, thereby enhancing reproductive success.

In *Fusconaia edgariana*, conglomerates included 58-89% (average 70%) sterile eggs (Kitchel 1985). *Fusconaia masoni* conglomerates consist of about 30% sterile eggs (G. T. Watters, personal communication). However, not all *Fusconaia* species produce large numbers of sterile eggs. *Fusconaia barnesiana* produced less than 3% sterile eggs on average (Kitchel 1985). In *Fusconaia cuneolus*, 33 individuals had 1% or less sterile eggs, while 8 individuals had 3-5% sterile eggs (Bruenderman and Neves 1993).

Some authors have interpreted the presence of sterile eggs in *Fusconaia* species as a pathological condition that might result in reduced numbers of successfully encysted glochidia (Kitchel 1985). However, it is unlikely that a pathological condition would occur consistently in particular species at widely separated times and in widely separated localities. Rather, it seems likely that the production of sterile eggs is a normal feature of reproduction in these species. In order for such a condition to evolve, the reduction of the number of glochidia produced must have been offset by an increased probability of survival for the remaining glochidia.

It appears likely that sterile eggs enhance the probability that the remaining glochidia will attach to host fish. Sterile eggs probably attract host fish, which attempt to feed upon the conglomerates and thereby become infected with glochidia. The sterile eggs provide color and opacity to conglomerates and thereby render them more visible to hosts. Mature eggs bearing glochidia are transparent, whereas sterile eggs are opaque and often brightly colored. Although evidence is scant, it appears that sterile eggs may also make conglomerates more attractive to the host through taste and scent. Fishes generally feed avidly upon conglomerates (Barnhart, unpublished observations). When the proportion of sterile eggs is high, fishes may ignore the discomfort of glochidia attachment in return for the food reward of the sterile eggs.

Sterile eggs also appear to serve a structural role in conglomerates by making them more durable. In *Fusconaia ozarkensis*, fertile eggs are fragile and rupture easily. Glochidia can be dislodged from the eggs by sucking the conglomerates through the aperture of a Pasteur pipette. However, this treatment generally does not fragment the conglomerate, because the sterile eggs do not break easily and they cohere strongly. By remaining intact, the conglomerate may increase handling time by host fishes and increase the chances for glochidia attachment. In contrast, many conglomerates that lack sterile eggs are durable only when immature. For example, the mature conglomerates of *E. dilatata* disintegrate upon release, and the glochidia are released into the water suspended by mucus and their extended larval threads.

With respect to sterile eggs, amblymine mussels appear to exhibit two contrasting strategies for infecting host fish. Production of high proportions of sterile eggs may be advantageous in situations where host fish can be attracted to feed upon relatively durable conglomerates. This strategy may increase the individual probability of success of glochidia, and thereby compensate for the smaller numbers of glochidia produced. Species exhibiting this strategy include *Fusconaia ozarkensis*, *Fusconaia flava*, and *Cyprogenia aberti*. Alternatively, species such as *Elliptio dilatata* forego the production of sterile eggs and instead produce larger numbers of glochidia. These glochidia are broadcast, rather than packaged in durable conglomerates. This strategy might be more successful in situations where conglomerates are less visible or less attractive to host fishes.

The mechanism by which sterile eggs are produced is unknown. Processes during or after meiosis may produce ova that are incapable of development. Alternatively, the access of sperm to ova might be regulated to limit fertilization. Limited evidence seems to favor the former hypothesis. Sterile eggs are of similar size to fertile eggs. However, the contents of sterile eggs completely fill the vitelline membrane. In contrast, fertile eggs always exhibit a separation between the ovum or the developing embryo and the vitelline membrane. Preliminary observations indicate that both types of eggs are present simultaneously in the gonads of female *Fusconaia* (Barnhart, unpublished observations). Histological examinations and ultrastructural comparisons of sterile and fertile eggs may help to understand their structural and genetic differences.

The evolution and phylogenetic distribution of conglutinate structure and of sterile egg production are of interest. At this time, too few species have been examined to allow any broad generalizations. However, the phylogeny of unionids is currently being examined in several labs by molecular methods. With reliable phylogenies, it may soon be possible to reconstruct the evolution of reproductive strategies. The presence of sterile eggs in five of the seven species of *Fusconaia* that have been examined suggests that this feature may be primitive within the genus. Other genera which exhibit this characteristic include *Plethobasus*, *Pleurobema*, and *Cyprogenia* (see Table 5).

The variability of sterile egg production among individuals and populations may also provide clues to understanding the evolution of this reproductive strategy. Adaptations to local conditions, such as the locally predominate host species, might result in higher or lower proportions of sterile eggs to achieve optimal host infections. Variation of sterile egg production among individual *Fusconaia ebena* appears to result in contrasting mechanisms for host infection (Barnhart, unpublished observations). Conglutinates with high proportions of glochidia disintegrate upon release, so that large numbers of glochidia are released only loosely associated by mucus and larval threads. This "broadcast" strategy might favor indiscriminant infection of host fish. In contrast, conglutinates that contain small numbers of glochidia and large numbers of sterile eggs tend to remain intact upon release and would attract hosts that feed upon the conglutinates. This "bait" strategy produces fewer glochidia but could place higher proportions of the glochidia on host fish.

#### Management implications.

This study shows that fertilization of stream-dwelling *Elliptio dilatata* is not impaired by population density much lower than that which prevents fertilization of lake-dwelling *Elliptio complanata*. Further study is needed in order to understand whether failure of fertilization ever limits reproduction of stream-dwelling unionids. Recognition and understanding of the normal occurrence of sterile eggs in certain species is important so that this adaptation will not be confounded with instances where unfertilized eggs are the result of pollution or low population density.

The effects of population density and population distribution on reproduction must be understood in order to establish viable populations through relocation or through captive propagation and release (Neves 1997). Decisions must be made whether to concentrate individuals in small areas or to distribute them more broadly at lower densities. Although

preliminary, the present results support suggestions that successful fertilization of stream-dwelling unionids may not require high population density. Further study is needed, but the present evidence does suggest that it would not be advantageous, in this respect, to concentrate individuals beyond 1/m<sup>2</sup>. Conversely, stocking at low population densities may permit more sites to be stocked, perhaps improving the chances that some individuals will survive in the heterogeneous and unpredictable benthic environments of streams.

#### Dissemination of results

The results of this study have been reported at the 1997 Annual Meeting of the North American Benthological Society, the 1996 Missouri Forest, Fish and Wildlife Conference, the 1997 Kansas Mussel Workshop, and in the Triannual Unionid Report. A manuscript is in preparation for journal publication.

#### Acknowledgements

Andrew Roberts and Frank Ruisech were “full partners” in the field work and were largely responsible for the laborious task of counting conglomerates and eggs. I am also grateful to David Oesch and Brinnon Morris for help with field work and analyses, and to Elise Kitchel and Tom Watters for sharing unpublished data. Special thanks to Janet Sternburg, Dennis Figg, and to the Missouri Department of Conservation, Natural History Section, for support.

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Table 1. Staging scheme used to classify embryos.

1. unfertilized eggs
  2. cleavage, 2-32 cells
  3. blastula
  4. gastrula
  5. adductor visible, hinge line not visible  
adductor visible, hinge line visible
  6. ventral cleft visible
  7. glochidia within egg membranes
  8. live glochidia free of egg membranes
  9. dead glochidia free of egg membranes
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Table 2. Mean population densities per meter-square. Abbreviations indicate *Elliptio dilatata*, *Fusconaia ozarkensis*, *Pleurobema coccineum*, *Venustaconcha ellipsiformis*, *Alasmidonta viridis*, and *Corbicula fluminea*. Population densities are illustrated in Figures 1, 2.

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Site 1: Spring River at Stott City

<u>Species</u>	<u><i>E.d.</i></u>	<u><i>F.o.</i></u>	<u><i>P.c.</i></u>	<u><i>V.e.</i></u>	<u><i>A.v.</i></u>	<u><i>C.f.</i></u>
Mean (N/m <sup>2</sup> )	5.71	1.14	1.43	0.43	0	1.57
Standard deviation	2.430	1.676	1.134	0.535	0	1.397

Site 2: Spring River at Hoberg

<u>Species</u>	<u><i>E.d.</i></u>	<u><i>F.o.</i></u>	<u><i>P.c.</i></u>	<u><i>V.e.</i></u>	<u><i>A.v.</i></u>	<u><i>C.f.</i></u>
Mean (N/m <sup>2</sup> )	0.83	0.28	0	3.38	0.03	0
Standard deviation	1.071	0.455	0	4.686	0.186	0

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Table 3. Sex ratios inferred from ctenidial filament counts. The number and percentage of male and female individuals are indicated. "Gravid females" refers to the number and the percentage of female individuals that were gravid.

Species	Males	Females	Gravid females
<i>Elliptio dilatata</i>	50 (45.9%)	59 (54.1%)	42 (71.2%)
<i>Fusconaia ozarkensis</i>	10 (62.5%)	6 (37.5%)	5 (83.3%)
<i>Pleurobema coccineum</i>	7 (46.7%)	8 (53.3%)	7 (87.5%)

Table 4. Timing of early development of embryos from an individual *E. dilatata*. Temperature was approximately 20 C.

Hours elapsed	Predominant Stage (see Table 1)	Predominant condition of embryos
0	2	4 cell
5	2	8 cell
20	3	20-40 cell blastula
44	3	Blastula
68	4	Gastrula
116	5	Adductor and hinge line evident, no gape
140	5	Shells advanced nearly to edge

Table 5. Unionids exhibiting high proportions of undeveloped eggs in conglutinates

	Species	Source
1.	<i>Fusconaia ebena</i>	LeFevre & Curtis 1910, Howard 1914, Barnhart unpublished
2.	<i>Fusconaia flava</i>	Howard 1914, Barnhart unpublished
3.	<i>Fusconaia edgariana</i>	Kitchel, 1985
4.	<i>Fusconaia ozarkensis</i>	present study
5.	<i>Fusconaia masoni</i>	G.T. Watters, pers. comm.
6.	<i>Plethobasus cyphyus</i>	Lefevre & Curtis 1910
7.	<i>Pleurobema coccineum</i>	present study
8.	<i>Cyprogenia aberti</i>	Barnhart 1997

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## FIGURE LEGENDS

Figure 1. Frequency distribution of population densities at site #1. Bar height shows the percentage of meter-square quadrats that contained the indicated number of individuals. Total number of quadrats=17. Quadrats that lacked a species are not shown.

Figure 2. Frequency distribution of population densities at site #2. Bar height shows the percentage of meter-square quadrats that contained the indicated number of individuals. Total number of quadrats=32. Quadrats that lacked a species are not shown.

Figure 3. Distribution of ctenidial filament counts among gravid and non-gravid individuals of *Elliptio dilatata*. Female unionids have more frequent interlamellar septa, and therefore fewer filaments per septal interval. Filament counts of all gravid individuals fell in the lower portion of the bimodal distribution.

Figure 4. Frequency distribution of predominant embryo developmental stage among gravid individuals of *Elliptio dilatata*. These individuals were collected between July 9 and July 16, 1996. Staging scheme is presented in Table 1.

Figure 5. Frequency distributions of embryo diameters in seven individuals of *Elliptio dilatata*. The identification number of each individual and the approximate stage of embryonic development are indicated. The data are arranged from top to bottom in order of increasing development.

Figure 6. Frequency distribution of the proportion of sterile eggs in conglomerates from eight individual *Fusconaia ozarkensis* collected from site #10. Eighteen conglomerates were analyzed from each individual. All eggs in each conglomerate were counted and classified as to fertile or sterile.

Figure 7. Frequency distribution of the proportion of sterile eggs in conglomerates of *Pleurobema coccineum* from site #2. Five conglomerates were analyzed from each of three individuals. Approximately 50 eggs in each of three regions of each conglomerate were counted and classified as to fertile or sterile.

Figure 1

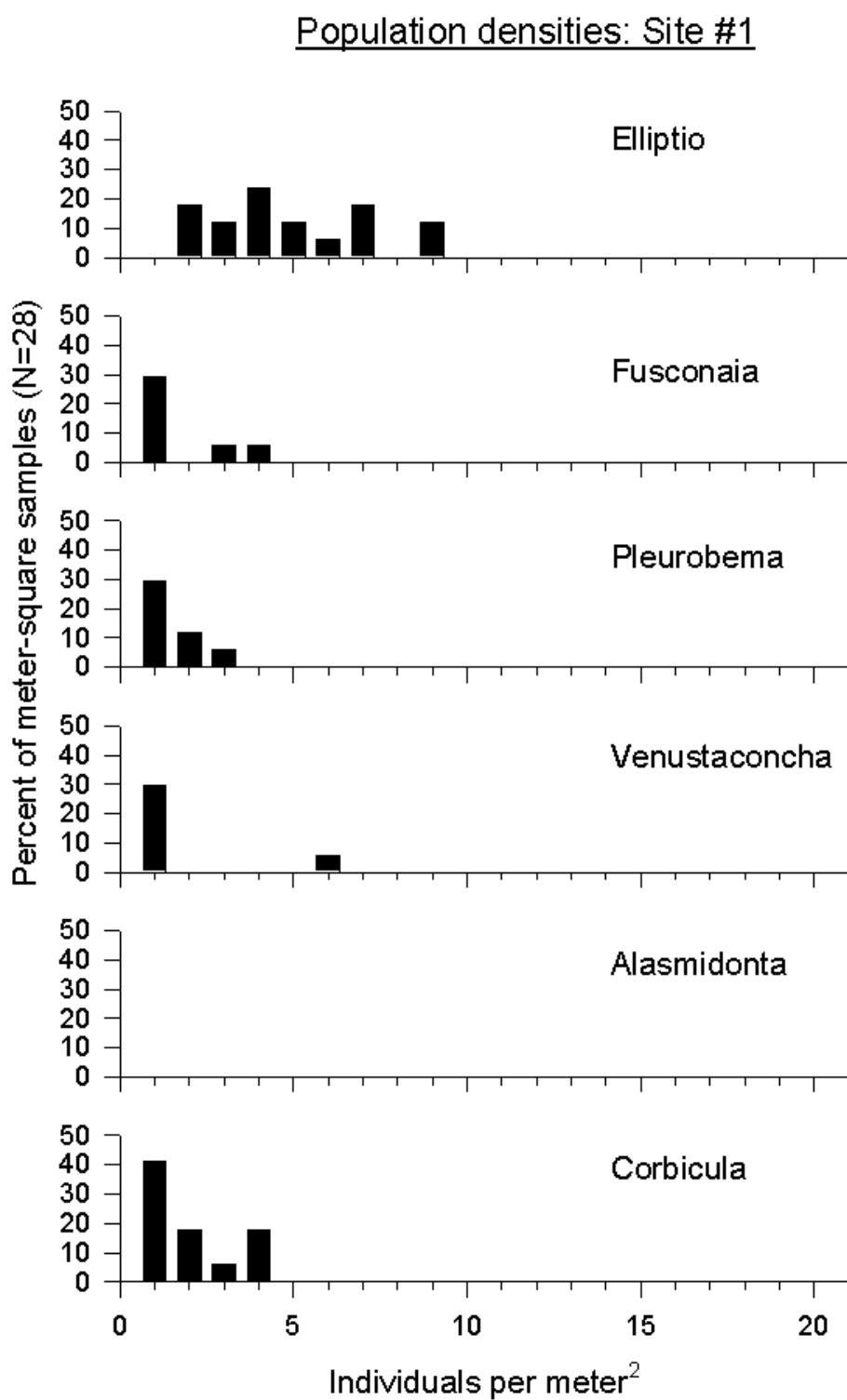


Figure 2

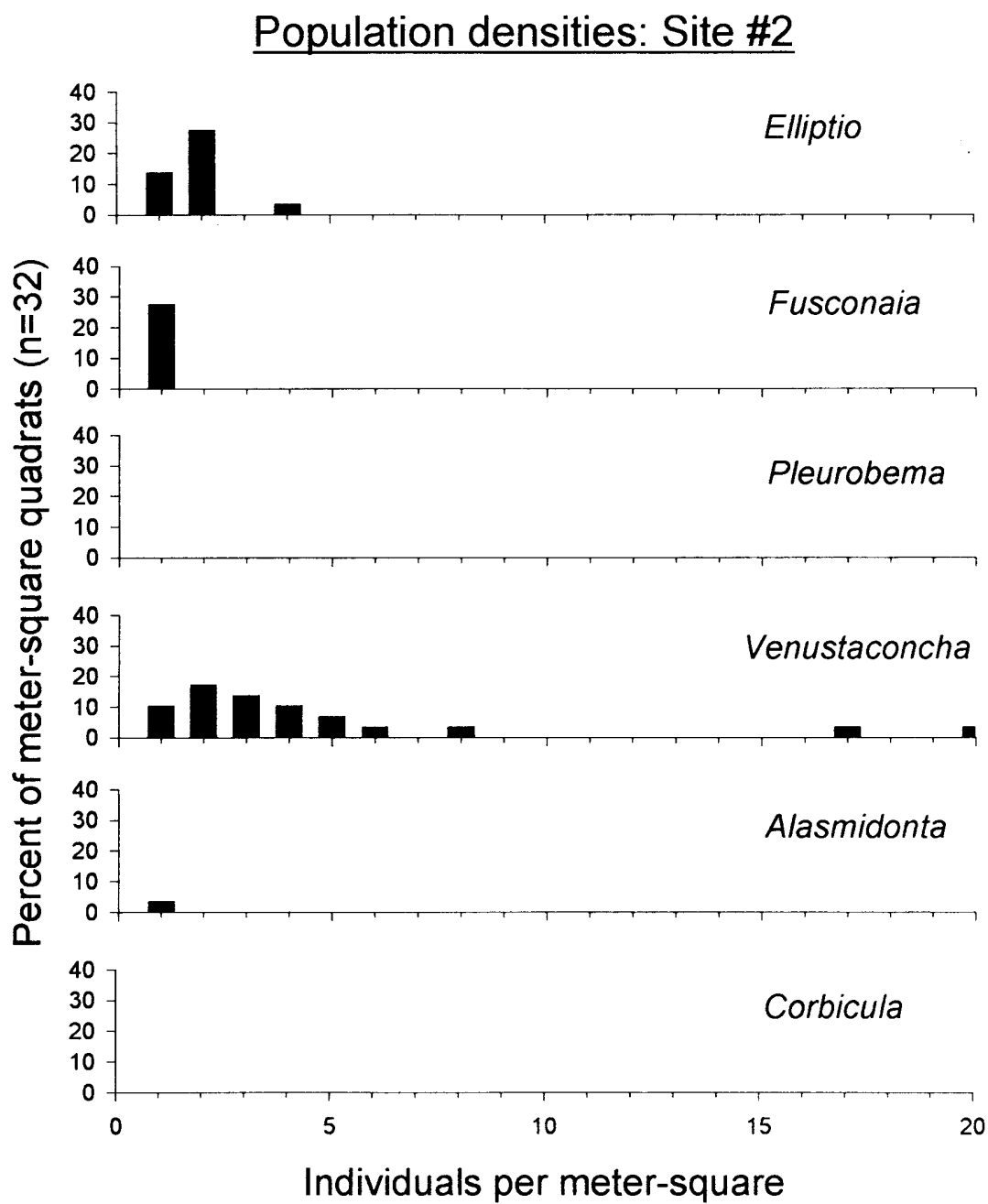


Figure 3

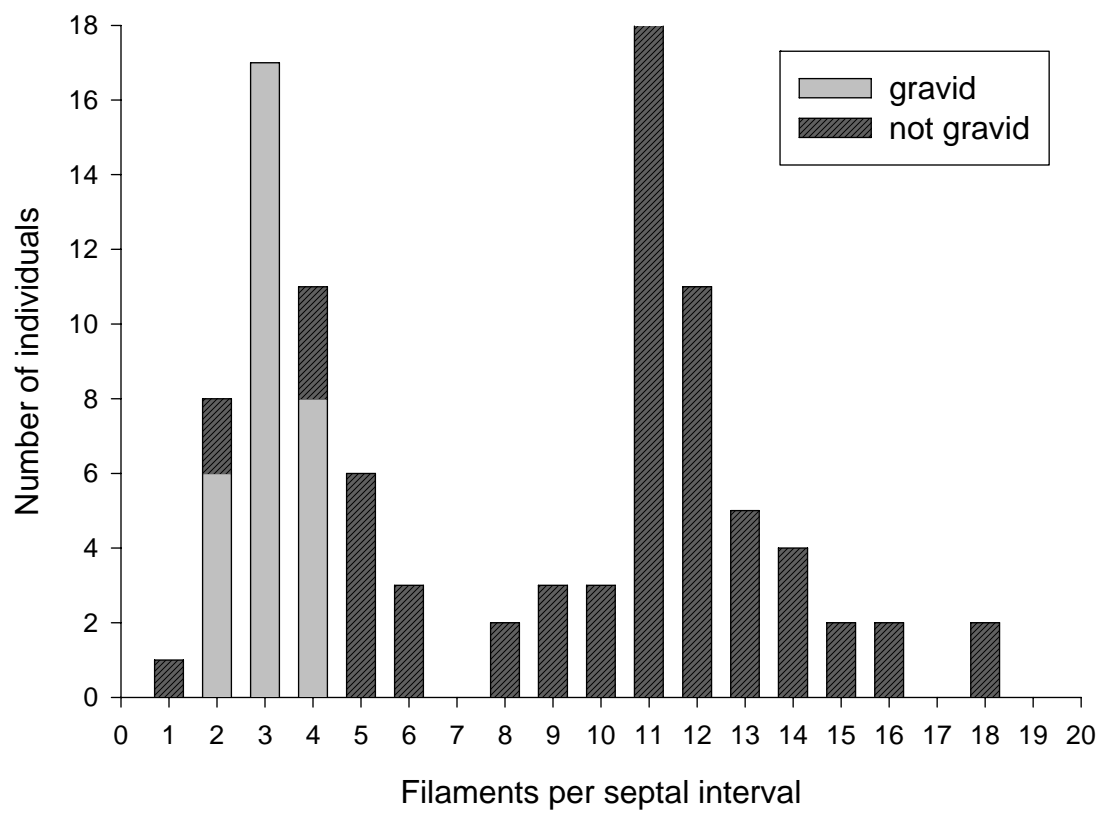


Figure 4

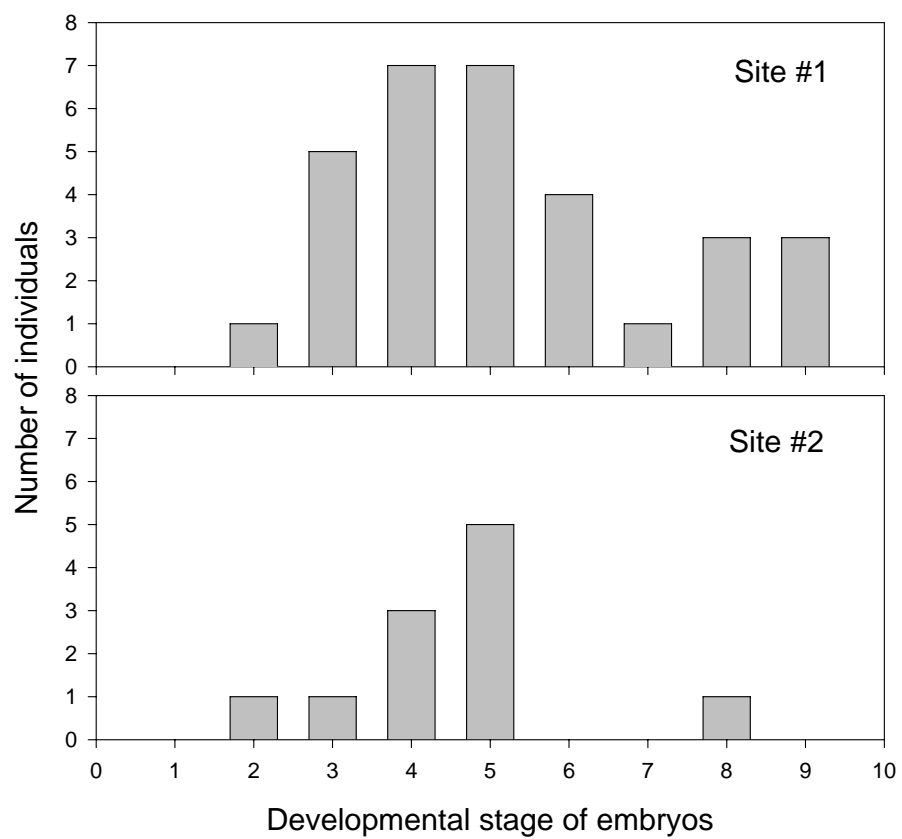


Figure 5

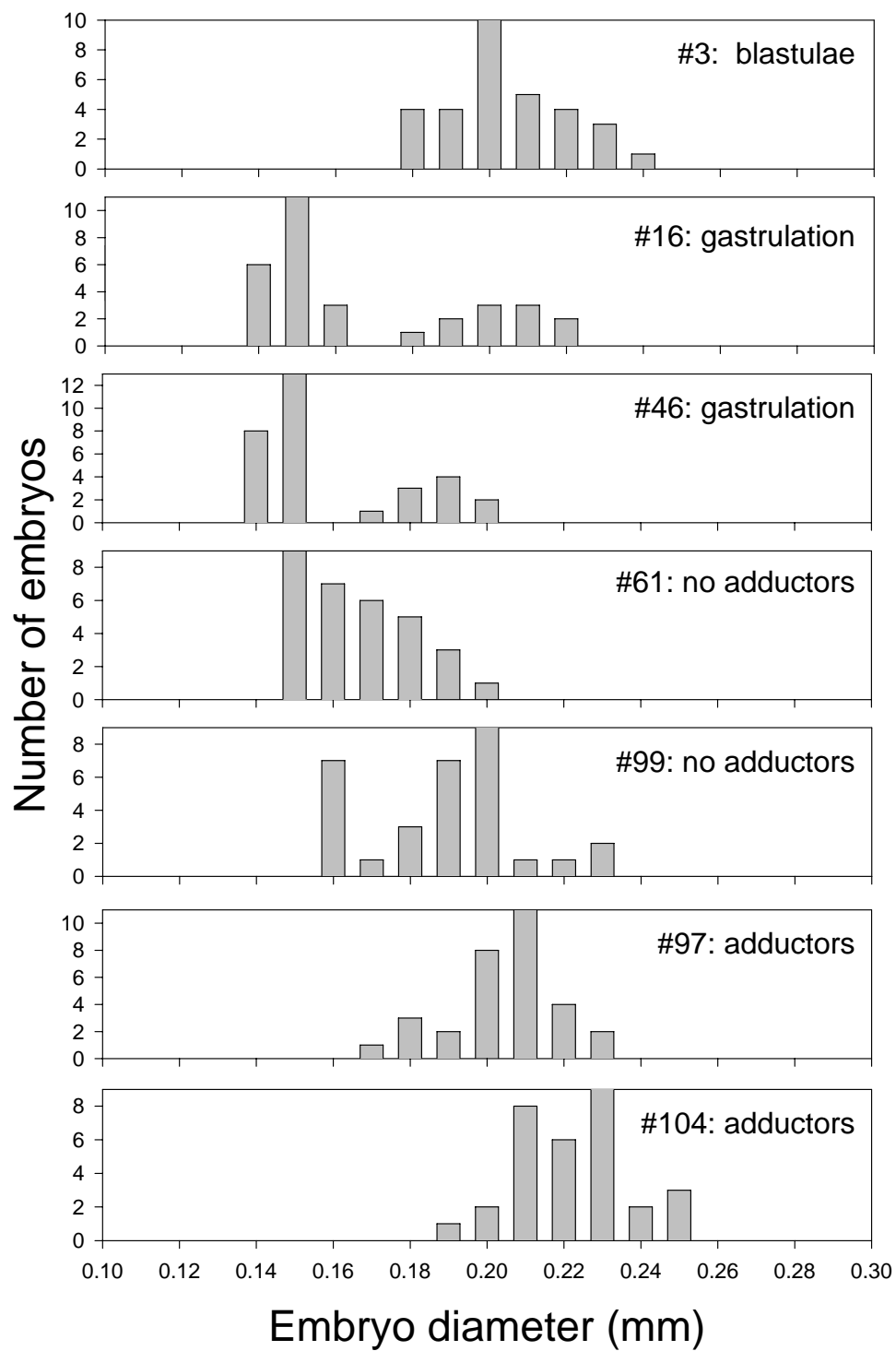


Figure 6

Frequency of sterile eggs in conglomerates of *Fusconaia ozarkensis* from the Spring River, Lawrence Co. MO

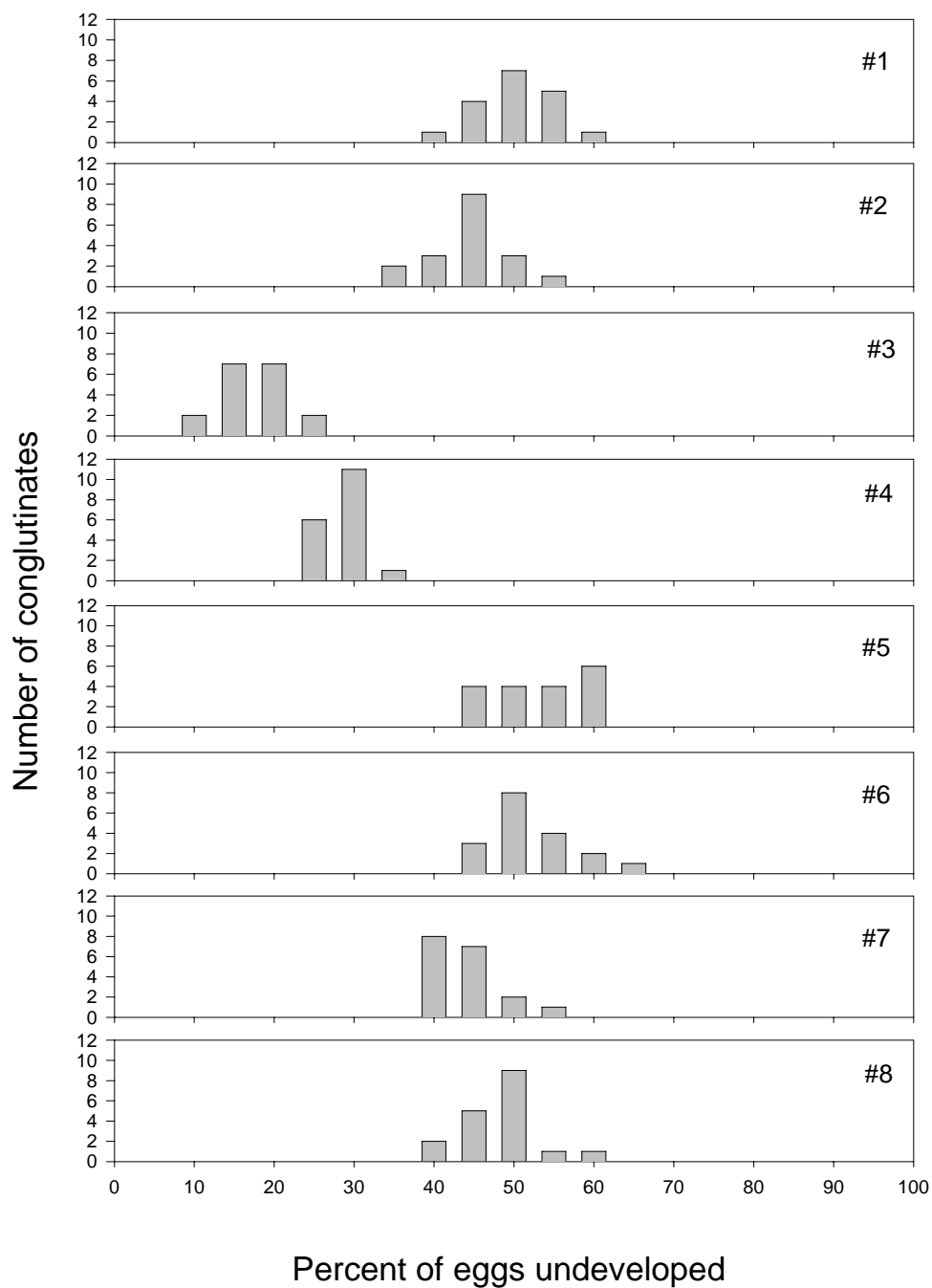
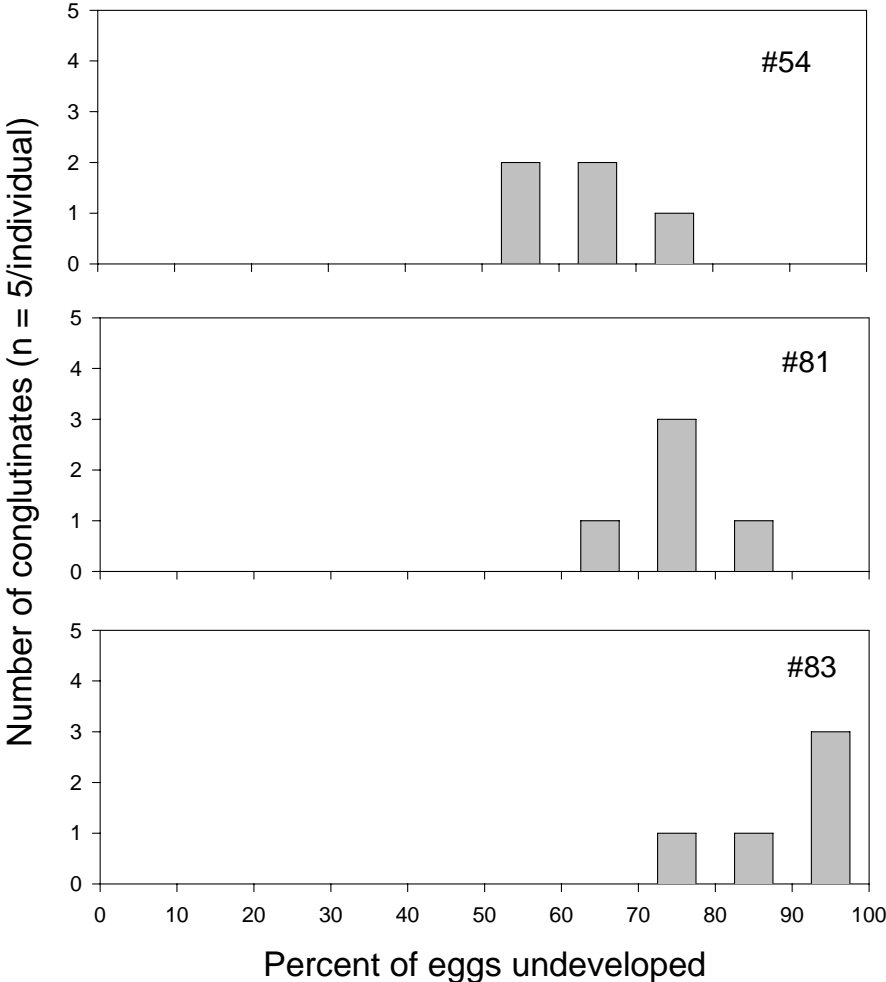


Figure 7

*Pleurobema coccineum*



## APPENDIX 1.

Sites searched for mussels in the course of this study. Quantitative sampling was carried out at site #1 and site #2.

1. Spring River at Highway 97 north of Stotts City, SE4 S14R28W T28N Lawrence County, Missouri. May 23, July 9, July 11, July 28, 1996. Species present live: *Elliptio dilatata*, *Venustaconcha ellipsiformis*, *Fusconaia ozarkensis*, *Pleurobema coccineum*.
2. Spring River above and below bridge northeast of Hoberg, NE4 S11 R27W T27N Lawrence County, Missouri. June 6, July 15, July 16, 1996. Species present live: *Venustaconcha ellipsiformis*, *Elliptio dilatata*, *Fusconaia ozarkensis*, *Lampsilis rafinesqueana*, *Alasmidonta viridis*.
3. Spring River NE of Highway H bridge, SW4 S2 R27W T27N Lawrence County, Missouri. July 18, 1997. Species present live: *Venustaconcha ellipsiformis*, *Elliptio dilatata*, *Fusconaia ozarkensis*.
4. Honey Creek above bridge northeast of Hoberg, SE4 S2 R27W T27N Lawrence County, Missouri. June 6, 1996. Three searched 45 minutes with view boxes, found no sign of unionids. Absence of unionids is interesting at this site, because it appears very similar to the confluent branch of Spring River that is immediately adjacent and has numerous mussels. A large sod farm is upstream of this site.
5. Turnback Creek at Highway K, S22 R26W T30N Dade County, Missouri. May 23, 1996. Two searched banks and shallow water for 30 minutes, saw no sign of unionids.
6. Turnback Creek, Antioch Bridge Access, S15 R26W T30N Dade County, Missouri. May 29, 1996. Two searched 2 hours with view boxes. Species present live: live *Elliptio dilatata* (5), and *Venustaconcha ellipsiformis* (1).
7. Turnback Creek at Fiddler's Ford Access, S33 R26W T31N Dade County, Missouri. Two searched banks and shallow water for 30 minutes, saw no live unionids. A few dead shells of *Elliptio dilatata*.
8. Turnback Creek at bridge SW4 S34 T29N R25W Greene County, Missouri. June 6, 1996. Three searched one hour with view boxes, found a few shell fragments only.
9. Clear Creek, north of Ash Grove at Phenix Wildlife Area, S4 T30N R24W Greene County, Missouri. May 23, 1996. Two searched 30 minutes, saw no sign of unionids.
10. Finley River at Greenbridge, NE4 S14 T27N R21W Christian County, Missouri. June 27, 1997. One snorkeled 30 minutes. Species present live: *Fusconaia ozarkensis* (5), *Lampsilis reeveiana* (1), *Corbicula fluminea*.