

**Effects of Water Flow, Distance, and Male Density on the Fertilization Success of
Freshwater Mussels**

by

Tyler Lee Mosley

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 4, 2012

Keywords: Freshwater mussel, density, reproduction, fertilization, gravity, flow

Copyright 2012 by Tyler Lee Mosley

Approved by

James Stoeckel, Chair, Assistant Professor of Fisheries and Allied Aquacultures
Brian Helms, Collections Manager, Biological Sciences
Elise Irwin, Associate Professor of Fisheries and Allied Aquacultures

Abstract

Previous studies suggest that fertilization of female freshwater mussels is dependent on the distance between mates and may fail at low mussel density. This has critical implications for conservation of sparse populations. This study assesses the fertilization success of two common species, *Lampsilis straminea* (Conrad, 1834) and *Fusconaia ebena* (Lea, 1831). We assessed fertilization success by using constructed stream channels and manipulating the distance from mates, presence of water flow, number of males, and number of females. Fertilization success was determined in three ways: proportion of gravid females per pen, fertilization efficiency, and glochidia per female. In *L. straminea*, water flow and distance from males did not have a significant effect on any measurements of fertilization success. When upstream females were present, fertilization success of distant (25m) females increased, compared to low fertilization success when upstream females were absent. Fertilization efficiency seemed to be “all or nothing” in both species with females being either barren or exhibiting >80% fertilization of brooded eggs. In *F. ebena*, results were unclear but did show fertilization failure at long distances (25m) with no flow. Overall, fertilization success of freshwater mussels seemed to be independent of distance from males up to 25 m, but dependent on some threshold of female density for optimal fertilization to occur at the farthest distance tested. Results suggest that freshwater mussels have a very efficient fertilization process and can exhibit high fertilization success at low population densities when populations are dominated by females.

Acknowledgments

This study was supported by grants from the U.S. Environmental Protection Agency and funds from the U.S. Forest Service – Southern Research Station. First, I would like to thank my advisor Dr. James Stoeckel for the opportunity to be a graduate student. His patience and guidance through the process has been invaluable to me. I would also like to thank the following people for helping with my research: My committee members, Dr. Brian Helms, and Dr. Elise Irwin for their contributions to the development, analysis, and writing of this thesis. Dr. Wendell Haag and Mickey Bland for their assistance in the laboratory, support, and funding from the Center for Bottomland Hardwoods Research. I am extremely grateful to the Crustacean and Molluscan Ecology Lab (CAMEL) members: Catlin Ames, Daniel Foree, Michael Hart, Andrew Gascho-Landis, and Brad Staton for their sweat, friendship, field assistance, and moral support during this journey. I am also tremendously grateful for my parents and grandparents, Tim and Teresa, and Earl and Ruby for their love and support before, during, and after my time as a graduate student. Lastly, a special thanks to my wife, Aimee, for providing her loving support and constant patience.

Table of Contents

Abstract.....	ii
Acknowledgments	iii
List of Tables	v
List of Figures.....	vi
List of Abbreviations	vii
Chapter 1: Reproductive success of <i>Lampsilis straminea</i> (Conrad, 1834) in relation to distance, male density, and water flow	1
Introduction	1
Methods	5
Results	10
Discussion	14
Chapter 2: Reproductive success of <i>Fusconaia ebena</i> (Lea, 1831) in relation to distance and water flow	21
Introduction	21
Methods.....	22
Results	27
Discussion	29
Chapter 3: Hurdles for the future conservation of freshwater mussels	32
Literature Cited	37

List of Tables

Table 1: Pen Assignments for experiment 1utilizing <i>L. straminea</i>	44
Table 2: Pen assignments for experiment 2 utilizing <i>L. straminea</i>	45
Table 3: Number of gravid females used from each pen to assess fertilization rates in <i>L. straminea</i>	46
Table 4: Number of gravid females used from each pen to assess fertilization rates in <i>L.</i> <i>straminea</i>	47
Table 5: Pen assignments for flow experiment using <i>F. ebena</i>	48
Table 6: Stages seen in Gill and Gonad Extractions in Figures 8 and 9.....	49
Table 7: Number of gravid females used from each pen to assess fertilization rates for <i>F. ebena</i>	50

List of Figures

Figure 1: Stream channel setup and pen placement in ponds for all experiments	51
Figure 2: Results from experiment 1: flow and no flow; with proportion of gravid females, fertilization efficiency, and mean number of glochidia per female	55
Figure 3: Number of glochidia per gravid female versus shell length from experiment 1: flow and no flow	53
Figure 4: Results from experiment 2: male density and female competition; no upstream females present (25 m only) including proportion of gravid females, fertilization efficiency, and mean number of glochidia per female	54
Figure 5: Number of glochidia per gravid female versus shell length from experiment 2: Male density and female competition	55
Figure 6: Results from experiment 2: male density and female competition; upstream females present (1, 10, 25 m) including proportion of gravid females, fertilization efficiency, and mean number of glochidia per female	56
Figure 7: Gamete and fertilization stages of <i>F. ebena</i>	57
Figure 8: <i>F. ebena</i> fertilization stage results from weekly gravidity checks in flow streams ...	58
Figure 9: <i>F. ebena</i> fertilization stage results from weekly gravidity checks in no flow streams	59
Figure 10: Results from <i>F. ebena</i> flow experiment; with proportion of gravid females, fertilization efficiency, and mean number of glochidia per female	60
Figure 11: Number of glochidia per gravid female versus shell length from <i>F. ebena</i> flow experiment	61

List of Abbreviations

hp	horse power
m	meter
L	length
W	width
sd	standard deviation
df	degrees of freedom
F	f-value
T	t-value
ANOVA	analysis of variance

Ch.1) Reproductive success of *Lampsilis straminea* (Conrad, 1834) in relation to distance, male density, and water flow

Introduction:

An Allee effect is defined as a positive relationship between any component of an individual's fitness and the number and / or density of its conspecifics (Stephens et al. 1999). Small populations experiencing Allee effects are at risk for going extinct since further reduction in population size or density may result in reduced individual fitness (Boukal and Berec 2002). Understanding the mechanisms that can lead to Allee effects in patchy populations is critical when designing effective conservation plans that allow populations to increase in both population growth rate and number (Deredec and Courchamp 2007). This concept is crucial for managers when re-introducing new populations into the wild or augmenting small existing populations.

Allee effects have been used as an important organizational framework in studies ranging from parasite-host relationships and spread of disease (Krkosek et al. 2012) to relationships between mate density and fertilization efficiency in plant and animal populations (Wagenius et al. 2007, Babcock and Keesing 1999). For example, in many marine invertebrates there are two strategies of gamete exchange: 1) Broadcast spawning where males and females release gametes into the water column for external fertilization and 2) Spermcasting where males release sperm while females retain eggs and fertilization is internal. In both strategies, fertilization efficiency is strongly affected by decreasing population densities. At low population densities, individual fitness declines due to the lack of mates within an effective fertilization distance. In broadcasting

species, fertilization success has been shown to increase with increasing male density (Levitan 1991). Water flow can decrease fertilization success of broadcasting species due to sperm dilution from water current (Pennington 1985). In contrast, declining male density and increased water flow have shown no adverse effects on fertilization success in spermcasting species (Phillipi et al. 2004, Serrao and Havenhand 2009). Spermcasting species are thought to be more efficient than broadcasting species because they require much lower concentrations of sperm to achieve high fertilization success (Pemberton et al. 2003). Spermcasting species release their sperm in packets containing thousands of spermatozoa so that even if a female collects only one packet, thousands of eggs may be fertilized (Yund 2000, Bishop and Pemberton 2005).

Similar to many marine invertebrates (e.g. corals, sponges, and surf clams) freshwater mussels (Family Unionidae) are spermcasters. Male unionid mussels (hereafter referred to as mussels) release their gametes into the water column, often in the form of spermatozeugmata, or sperm spheres (Lynn 1994, Waller and Lasee 1997, Ishibashi et al. 2000). Spermatozeugmata are packets of spermatozoa heads encased within a membrane with the tails of sperm exposed to the environment. Females take in sperm during normal siphoning activity (McMahon and Bogan 2001). Fertilization takes place inside the suprabranchial chamber, presumably when ova are being moved from the gonad to the marsupial, the portion of the gill where fertilized eggs will be held (Lillie 1901, Yokley 1972, Williams et al. 2008). After fertilization the embryos develop into parasitic larvae (glochidia) and are retained in the gills. Females can thus be classified as gravid based on the presence of swollen gills (Moles and Layzer 2008).

Competition can be defined as one individual interfering with another for the same limiting resource. Exploitative competition specifically involves indirect consumption of a limited resource that depletes the resource for others (Kreutzer and Lampert 1999). In marine broadcast spawners, there is competition directly between free spermatozoa in the water column to effectively fertilize eggs present (Levitan 1998). Removing sperm due to fertilization of an individual female's eggs may be considered direct competition between individual females. In spermcasting species, increasing the number of females present could result in exploitative competition for sperm between individual females from upstream to downstream in stream conditions. Female freshwater mussels could be subject to these conditions if sperm from upstream males becomes a limiting factor.

Freshwater mussel populations have been declining in North America over a long period of time; with an estimated 213 of 297 species considered threatened or endangered (Williams et al. 1992). As population densities decrease, there is a great need to understand the relationship between male-female distances and fertilization success. However, few studies have examined the process of fertilization in freshwater mussels. More commonly, research on freshwater mussels has focused on the events that happen after fertilization, as mussels transfer their parasitic glochidia to vertebrate hosts (e.g. Barnhart et al. 2008, Eads et al. 2010, Warren and Haag 2003, and others).

Results from the few studies directly examining fertilization efficiency in freshwater mussels are contradictory. Downing et al. 1993 examined the reproductive success of *Elliptio complanata* in a lentic system and found that densities of less than 10 mussels/ m² resulted in

substantial decreases in fertilization success, and often failure. Solitary females would become gravid only if they had the ability to self-fertilize. They suggested that sparse populations may have very low overall reproductive success, with successful reproduction limited to restricted areas with relatively high local densities. In contrast to the lentic study, Moles and Layzer (2008) examined reproductive success of *Actinonaias ligamentina* in a lotic system and found that fertilization rates were independent of local mussel density and suggested that females could be fertilized by distant males. Neither study directly measured fertilization distances, but taken together, they imply that effective fertilization distances may increase in the presence of moderate, directional currents rather than decreasing due to sperm dilution by water currents.

Lampsilis straminea (Southern Fatmucket) (Conrad, 1834) is endemic to the Gulf basins, and is typically found in lotic rather than lentic habitats suggesting some requirement for water flow (Williams et al. 2008). However, large populations have been observed in small streams that maintain water flow in normal to wet years but decrease to isolated pools in the summer and early fall of dry years (Mosley, pers. obs). Because *L. straminea* typically spawns during the late summer to early fall (Williams et al. 2008) it may be subjected to either lotic or lentic conditions during the spawning season depending on rainfall. Thus, effective fertilization distance of this species may vary with annual weather patterns.

In this study we used long (31 m), semi-natural, experimental streams to examine the relationship between fertilization distance and reproductive success in *L. straminea*. We expected that reproductive success of females would decline with increasing distance from males. Furthermore, we expected that while fertilization distances would increase in the

presence of current, fertilization success would decrease for distant females with decreasing male density and increasing proximal females. Therefore we wanted to test the following hypotheses:

- 1) Fertilization success of females declines with increasing distance from males more strongly in the absence of current.
- 2) Fertilization success of females declines with increasing distance from males more strongly when upstream males are rare.
- 3) Fertilization success of females declines with increasing distance from males more strongly when proximal females compete with distant females for sperm from upstream males.

Methods:

Lampsilis straminea were collected from Line Creek in Bullock County, Alabama and Opintlocco Creek in Macon County, Alabama between May and August in both 2010 and 2011. It was assumed that most glochidia from the previous season had been released, but females had yet to be fertilized during the current season. Animals were transported to the lab and sex was determined by external shell morphology. To verify gender identification, gametes were extracted from the gonads of a subset of individuals and examined under a microscope to identify sperm or eggs (Saha and Layzer 2008). These individuals were not used in this experiment as we were uncertain the effect that gamete extraction would have on subsequent fertilization. All animals were held in ponds of the same size as the experimental ponds prior to initiation of experiments. All female mussels (identified by external morphology) were checked

for gravidity 24 hours prior to experiments by prying apart the valves and inspecting for swollen gills. No females were found to be gravid prior to use in experiments.

Artificial Streams

Experiments were conducted in 0.1 ha (0.25 acre) experimental ponds at the South Auburn Fisheries Research Station (Auburn University). An artificial stream was created in each pond (one stream per pond) by installing a 31 m long baffle along one side of the pond and building an airlift at one end of the stream (Figure 1A). Two 3 hp blowers (Sweetwater, Aquatic Ecosystems, Apopka, FL, USA) supplied air to all of the airlifts. When the air was turned on the airlift generated a slow current in its respective stream. Flow was set to mimic low flow conditions typical of low gradient streams in the area.

Within each artificial stream, rectangular mussel pens (1m L x 2m W) were constructed of plastic netting (0.02 m² mesh size) extending 0.1 m above the substrate and 0.2 m below the substrate. Each corral held only males or females. A male pen was placed at the head of each stream, ~ 3 m downstream of the airlift. Female pens were placed at distances of 1 m, 10 m, and 25 m from the center of the male pen. Ponds were filled from a nearby stream (Odum Creek, Lee Co., AL) approximately 2 months before initiating experiments. Water quality parameters (pH, alkalinity (ppm), and calcium hardness (ppm)) were monitored biweekly.

Experiment 1: Flow and No Flow

In 2010, we conducted an experiment to examine the effect of flow on fertilization distances. Airlifts were turned on in two replicate “flow” streams, and left off in two replicate “no-flow” streams. Water velocity in flow and no-flow streams was measured using a flow

meter (Flo-mate 2000, Flow-tronic, Welkenreadt, Belgium) at five equidistant points along each stream 0.4 - 0.7 m below the surface and equidistant between the baffle and shoreline along the raceways.

Fifteen males were placed in the male pens of each stream, and ten females were placed in pens 1, 10, and 25 meters downstream of the males (Table 1). A control pond with the same raceway and airlift system as the treatment ponds contained only females and airlines were left turned off. This pond served as a check to see whether females could become gravid in the absence of males. Gravid females in the control pond would indicate either an ability to self-fertilize, or an ability to store sperm from a previous fertilization event. All mussels were placed in the pens on 17 August, and left undisturbed until 17 November at which time they were collected from the ponds and returned to the lab for analysis.

Laboratory analysis

At the end of the experiment, each female was pried open and examined for swollen gills – an indicator of gravidity (Haag and Staton 2003). Gills were removed from up to 5 gravid females in each pen and preserved in 75% ethanol for further analysis (Tables 2, 4). Fertilization success was assessed and quantified in three ways: proportion gravid females within a given pen, fertilization efficiency (proportion glochidia to unfertilized eggs per gravid female), and total number glochidia per gravid female

Contents were flushed out of preserved gills by placing a whole gill in a Petri dish, puncturing the gill along each water tubule with fine tip forceps, and flushing water over the gill. Gill contents were flushed into a beaker and diluted with tap water to 300 -2500 ml depending on

number of glochidia flushed from the gills. The resultant sample was mixed using a plunger to avoid creating a vortex inside the beaker. Three 1 ml subsamples were withdrawn from the homogenized sample and examined under a dissecting microscope at 20X magnification.

Glochidia and unfertilized eggs were counted in each subsample. If subsample counts were not within 10% of each other, a fourth subsample was withdrawn and counted. Percent fertilization for each gravid female examined was calculated as the number of glochidia divided by the total number of unfertilized eggs plus glochidia. Total number of glochidia for a given gravid female was estimated by the following formula:

$G = C * S/SB$ where

G = total glochidia per female

C = total glochidia counted in all subsamples

S = sample volume (ml)

SB = combined subsample volume (ml)

Fifteen non-gravid females were also examined using the same procedure to ensure that flaccid gills contained neither glochidia nor unfertilized eggs.

Experiment 2: Male density and female competition

In 2011, we conducted a second experiment to examine the effects of male density, and presence/absence of competing females on fertilization distances. To reduce the impact of collection on natural populations, we re-used 68 females and 51 males from the previous year's experiment. An additional 92 females and 17 males were collected from Line (Bullock Co., AL) and Opintlocco (Macon Co., AL) Creeks from May – July. All individuals were mixed and

randomly assigned to experimental treatments. Experimental design is shown in Table 2. Four “low male” streams were stocked with two males per pond. Also, four “high male” streams were stocked with 15 males per pond. Within each male density treatment, two replicate streams were stocked with 10 females at each pen (1, 10, and 25 meters) for a total of 30 females per stream. The other two replicate streams were stocked with 10 females at the 25 m pen, but no females at the 1 or 10 m pens. This allowed us to test the effect of low male density on long distance fertilization (25 m) in the presence and absence of upstream female competitors for sperm (Table 2)

Air was turned on for all streams regardless of treatment. Flow was measured in the same manner as for the previous experiment. Mussels were placed in the pens on 12 July and left undisturbed until 1 November when they were removed for laboratory analysis. Fertilization efficiency was assessed using the same methodology as in the previous experiment.

Statistical analysis

We used a repeated measures ANOVA to test for water quality differences between experimental ponds. We used a one-way ANOVA to test for differences in both male and female size between ponds. In order to determine whether fecundity (#/glochidia per female) increased with shell size in either experiment we used linear regression.

We used binary logistic regression to test for differences in the proportion of gravid females between female pens (1, 10 and 25 m away from male pen) in flow and no-flow streams. We then used Fisher’s exact test of proportions to test for differences in the proportion of gravid females between flow and no flow ponds at the farthest distance (25 m). We used a one-way

ANOVA followed by Tukey's test to test for differences in fertilization efficiency and the number of glochidia per gravid mussel between female pens (1, 10, and 25 m away from the male pen) in flow and non-flow streams. We then used a two – sample T test to test for differences in both fertilization efficiency and glochidia per gravid females between flow and non-flow ponds at the farthest distance (25 m) (Minitab 16, State College, PA, USA). All proportion data was arcsine square root transformed before analysis.

Experiment two was analyzed in the same manner as experiment one (see above) for low and high-male ponds.

Results:

Experiment 1: Flow vs. No Flow

Biweekly water quality values (pH, alkalinity, and calcium hardness) from August to November 2010 across all ponds averaged 8.04 ± 0.47 , 49.2 ± 15.8 ppm, and 39.6 ± 18.4 ppm respectively. Water quality did not differ significantly between flow and no flow streams for pH (d.f.=5, $F=0.86$, $p=0.138$), alkalinity (d.f.=5, $F=1.09$, $p=0.469$), or hardness (d.f.= 5, $F=0.29$, $p=0.980$). The average flow rate in replicate flow streams was 0.03 ± 0.01 sd m/s. The flow rate in replicate no-flow streams was not detectable (minimum detectable flow = 0.01 m/s).

Only one mussel, a control female, escaped from its pen during the course of the experiment. It was found along the edge of the pen at the end of the experimental period. None of the females in the control pond (males absent) exhibited swollen gills. Flushing of flaccid gills from 5 control females revealed no unfertilized eggs or glochidia. Survivorship across all pens and treatments was 98.5% (197/200). At the end of the experiment mortality was low with 2

males dying: one in a flow stream and one in a no-flow stream. One female in the 1 m pen of a flow stream also died. Average shell length did not differ between flow and no-flow streams for either males (d.f.=1, $F=0.00$, $p=0.990$) or females (d.f.=1, $F=1.78$, $p=0.089$)

The proportion of gravid females did not decline with increasing distance from males in either flow ($Z=-0.87$, $p=0.384$; Figure 2a) or no-flow ($Z=-1.01$, $p=0.314$; Figure 2b) streams. Proportion of gravid females at the farthest distance (25m) did not differ between flow and no-flow streams ($Z= -1.15$, $p= 0.301$) but the proportion of gravid females was more variable in no-flow ($60\% \pm 57$ sd%) compared to flow ($80\% \pm 14$ sd%) streams (Figure 2a,b). Fertilization efficiency of gravid females was high ($> 90\%$) for gravid females in all pens and did not change significantly with distance in either the flow (d.f.=2, $F=1.24$, $p= 0.306$; Figure 2c) or no-flow streams (d.f.=2, $F=2.45$, $p=0.109$; Figure 2d). Fertilization efficiency at the farthest distance (25 m) did not significantly differ (d.f.=1, $T=-2.5$, $p=0.243$) between flow and no-flow streams (Figure 2c, d). The average number of glochidia per gravid female did not decline with distance in either the flow (d.f.=27, $F=5.59$, $p=0.163$; Figure 2e) or no-flow streams (d.f.=23, $F=5.02$, $p=0.179$; Figure 2f). Number of glochidia per gravid female at the farthest distance (25 m) did not significantly differ (d.f.=1, $T=-1.03$, $p=0.492$) between the flow and no-flow streams (Figure 2e, f). Because we found no significant relationship between mussel shell length and number glochidia (Figure 3; d.f.=1, $F=1.16$, $p=0.286$), we report glochidial production as glochidia per female rather than glochidia as a function of length or mass.

Experiment 2: Male density and female competition

Two males escaped their pens during the course of Experiment 2. One was in a “two-male” pond containing only ten females (25 m pen). The second male was in a 15 male pond containing 30 females (1, 10, and 25 m pens). Neither of these males was recovered after the end of the experiment. Mortality across all pens and treatments was low (4.3%; 11/258) with 4 females dying in the 1 m pens, 1 female dying in a 10 m pen, and 4 females dying in the 25 m pens. The maximum number of female deaths in any given pen was 3 (1m pen in a low density with competition stream). Two females from the control pond were also found dead in their pen. None of the females in the control pond (males absent) exhibited swollen gills at the end of the experimental period. Flushing of flaccid gills from 5 control females revealed no unfertilized eggs or glochidia. The average flow rate across all replicate streams was 0.04 ± 0.01 sd m/s. Average shell length did not differ between treatments for either males (d.f.=7, $F=0.22$, $p=0.978$) or females (d.f.=7, $F=1.23$, $p=0.883$).

In the absence of competition from upstream females (i.e. no females present in either the 1 m or 10 m pens), the proportion of gravid females at 25 m declined significantly ($Z=3.40$, $p<0.0001$) in the low male streams (Fig. 4a). Because only one of the low male streams exhibited gravid females, we compared fertilization efficiency and number glochidia per gravid female between individual streams (two high-male streams and one low-male stream) rather than between treatments. Fertilization efficiency was high ($81.9 \% \pm 1.8$ sd %), but significantly lower (d.f.=1, $F=18.65$, $p=0.002$) in the single low-male stream than in either high-male stream ($95.9 \% \pm 3.7$ sd%; $99.1 \% \pm 0.85$ sd%; Fig 4b). Mean number glochidia per gravid female was

not significantly different between any of the three streams (d.f.=1, $F=0.63$, $p=0.447$; Fig. 4c). Because we found no significant relationship between shell length and number glochidia (d.f.=1, $F=0.00$, $p=0.988$) for all mussels used in experiment 2, we report glochidial production as glochidia per female rather than glochidia as a function of length or mass (Figure 5).

When competition from upstream females was present (i.e. females were present in the 1m and 10m and 25m pens), there was no significant decrease ($Z=1.21$, $p=0.146$) in proportion gravid females with distance in the high-male streams (Fig. 6a). However, in the low-male streams, the proportion of gravid females in the 1 m pen was significantly lower than in either the 10m ($Z=2.41$, $p=0.009$) or 25m ($Z=3.16$, $p=0.001$) pens (Fig 6b). In contrast to the streams where upstream females were absent (see previous section; Fig. 4a), proportion of gravid females did not significantly differ between the low and high-male streams at the farthest distance (25 m) when upstream females were present (d.f.=1, $T=0.92$, $p=0.238$; Fig 6a, b). Proportion of gravid females at 25 m was significantly higher ($Z=3.90$, $p<0.0001$) in low-male streams with competing upstream females (Fig 6b) compared to low-male streams where competing upstream females were absent (Fig. 4a). There was no decrease in fertilization efficiency with increasing distance in high-male (d.f.=2, $F=0.91$, $p=0.416$; Fig. 6c) or low-male (d.f.=2, $f=0.09$, $p=0.913$; Fig. 6d) streams. At the farthest distance (25m), fertilization efficiency did not differ significantly (d.f.=1, $T= 1.83$, $p=0.318$) between high and low-male streams (Fig. 6c, d). There was no significant decrease in mean number of glochidia per female with increasing distance in high male (d.f.=2, $F=0.64$, $p= 0.517$; Fig 6e) or low-male (d.f.=2, $F=0.41$, $p=0.666$; Fig. 6f) streams. Number of glochidia per gravid female at the farthest distance (25 m) did not

significantly differ (d.f.=1, $T=8.14$, $p=0.078$) between the high and low-male streams (Figure 6e, f).

Discussion:

Allee effects can affect populations when there is reduced individual fitness as population size decreases. Results from these experiments showed that *L. straminea* can exhibit high fertilization success even at low population densities and under conditions of low to no water flow. Thus, even at small population sizes this species is resistant to Allee effects arising from fertilization failure.

L. straminea seems to have a very efficient mechanism for long distance fertilization. At a low (0.03 m/s) current velocity, our maximum male-female distance of 25 m was not enough to cause any significant decrease in fertilization success. The proportion of gravid females remained high (> 75%) and fertilization efficiency remained > 90%. Even in the absence of measurable directional currents (no-flow streams), we observed a similar pattern. The proportion of gravid females did not decline significantly with distance, but did become more variable at 25 m, suggesting that we may have been approaching a limiting distance for some individuals under no flow conditions. In one replicate no-flow pond, 10/10 females became gravid at 25 m while in the other pond only 2/10 females became gravid.

Fertilization appeared to be “all or nothing” with females either barren or fully fertilized. Even in the pen with only 2/10 gravid females (25 m, no flow, replicate pond) gravid females exhibited > 90% fertilization of eggs in the gills. Because glochidia per gravid female did not decline with increasing distance, high fertilization efficiencies observed in this study were not

likely an artifact of unfertilized eggs in the gills being resorbed before animals were collected for analysis. If fertilization efficiency did in fact decline with distance and partially fertilized females were simply resorbing unfertilized eggs, we would have expected to see a decline in glochidia per gravid female with increasing distance. However, gravid females at 25 m produced just as many glochidia as females at shorter distances from the male pen. Thus, non-gravid females were more likely a result of a failure to load eggs into the gills for fertilization than a result of insufficient sperm in the water column. Similarly, the high rate of fertilization success was not likely an artifact of sperm storage or hermaphroditism. No females became gravid in our control ponds.

Bivalve spawning has been shown to be controlled by not only environmental conditions but also chemical signals associated with gametes (Galtsoff 1938, Krug et al. 2009). Chemical cues could play a role in stimulating female unionid mussels to transfer eggs from ovaries to the gills to be fertilized (spawning). It is possible that females differ in the concentration of sperm and/or associated male pheromones required to initiate spawning. In our study, evidence suggests that there was enough sperm to fertilize the majority of eggs in any female who spawned, regardless of male density or current. However, the lack of unfertilized eggs in the gills of non-gravid females suggests that not all females spawned. Females that were triggered to spawn became fully fertilized, but females that presumably “waited” for a higher sperm threshold to be surpassed exhibited total reproductive failure. Similar evidence and explanations have also been presented for *Ligumia subrostrata*, (Gascho Landis et al. 2012) where proportion of gravid females was inversely related to suspended solid concentrations, but fertilization

efficiency of gravid females did not decline with increasing suspended solids. Another explanation could be a “resting” phase in *L. straminea* similar to that found in *Actinonaias ligamentina* (Moles and Layzer 2008), though many females were used in both experiments of this study and found to be gravid in each year.

The production of spermatzeugmata, rather than release of free swimming sperm, may be a mechanism allowing for highly efficient fertilization across long distances. Although spermatzeugmata have not been reported for *L. straminea*, they have been reported for several other unionid mussel species (Trimble and Gaudin 1975, Lynn 1994, Waller and Lasee 1997). Spermatzeugmata may use the exposed tails of the spermatozoa to increase motility in the water, allowing them to actively swim to females (Falese et al. 2011) even in the absence of current. Sperm enclosed in spermatzeugmata have been shown to stay viable for up to 72 hours (Ishibashi et al. 2000), allowing time for downstream movement and uptake by distant females.

At low male densities (2 males/stream), in the absence of proximal females competing for sperm, there seemed to be a reduced capacity for long-distance fertilization. The proportion of gravid females at 25 m decreased relative to high male-density streams, with one low-male stream having total reproductive failure, and another having only limited fertilization success (20% gravid females). However, those few females that were fertilized in the low-male pond exhibited high fertilization efficiency (~80%) and did not exhibit smaller broods (average number glochidia per female) compared to the high-male ponds. Thus, the reduction in proportion of gravid females was again not likely the result of too few sperm for successful individual fertilization. We expected that fertilization success would be reduced even further

when upstream females were present and presumably competed with downstream females for sperm. However this was not the case. Proportion of gravid females increased, rather than decreased, with increasing distance from males. The proportion of gravid females in the 25 m pens was significantly higher when upstream (1m and 10 m) females were present compared to when they were absent. There was no indication of decreased fertilization success or glochidial production, suggesting that the presence of upstream females in combination with low male density (2 males/stream) did not result in sperm limitation. Taken together, these finding suggests that there was no competition between upstream and downstream females for sperm resources in these streams

It has been hypothesized that pheromones produced by ripe females (those with mature eggs in their ovaries) may attract spermatozeugmata (Barnhart and Roberts 1997). In our study, when males (2 males/stream) and females (in 25 m pen only) were at a low density, we saw a marked decrease in the proportion of gravid females. However, when male density remained low, but females were added to the 1 and 10 m pens, fertilization success increased significantly in the 25 m pen. Production of pheromones by ripe females could explain this pattern. When proximal (1m) females were absent, spermatozeugmata may not have been able to sense the ten ripe females that were 25 m downstream. However when females were present at all distances, spermatozeugmata may have been triggered to swarm downstream to the 1 m pen, then to the 10 m pen and then to the 25 m pen.

One problem with this scenario is that all streams in the second experiment were “flow” streams and pheromones would be expected to drift away from, rather than towards, upstream

spermatozeugmata. However, Dahl et al. (1998) showed that aquatic invertebrates can sense kairomones from downstream predators over short distances at low (~ 0.03 m/s) velocities, presumably due to upstream transport of kairomones by backflows and eddies. Current velocity was low (0.04 ± 0.01 m/s) in our streams. Thus spermatozeugmata may have been able to sense ripe, downstream females, although upstream transport of pheromones would have had to have occurred over longer distances than reported by Dahl et al. (1998). A more troublesome objection to this scenario is presented by the fact that at an average transport rate of 0.04 m/s, sperm spheres should have been transported 25 m downstream within 10.4 minutes, well within the 72 hr. survivorship window for spermatozeugmata reported by Ishibashi et al. (2000). Unless they were actively swimming upstream, spermatozeugmata should have been carried 25 m downstream to receptive females regardless of whether they sensed pheromones.

A similarly puzzling result at low male densities occurred in streams with females in all three pens. The proportion of gravid females increased, rather than decreased, with increasing distance from spawning males. Because only two males were present in these streams, it may be that spermatozeugmata were at first only narrowly distributed across the stream width, but became more evenly distributed across the stream width with increasing travel distance from males due to dilution by currents. This could explain why a higher proportion of females were fertilized in distal pens compared to proximal pens.

Conclusions and Implications

Mussel conservation and restoration efforts revolve around several questions: 1) What constitutes a “healthy” population size? 2) What population density threshold should trigger

conservation activities (repopulation or augmentation) and 3) How far apart should local population patches be from each other?

Defining healthy populations of freshwater mussels is difficult when considering all the density dependent factors that potentially affect population viability. In populations of many invertebrate species, Allee effects can arise when population density falls below a threshold beyond which fertilization efficiency and/or ability to locate mates declines. However, our study suggests that fertilization success is not strongly density dependent in freshwater mussels and is not likely to be improved by conservation programs focusing on population augmentation. If population augmentation is chosen as a conservation strategy to alleviate other density dependent factors that cause Allee effects, our study suggests that mussels can be spread out over a larger area, rather than densely stocked in a relatively small area of a target stream, and still be likely to exhibit high fertilization success. Consideration of sex ratios and spatial arrangement of subpopulations may be more important than local population densities *per se*.

Our research was focused on a mussel species that is endemic to the Mobile Basin and has not exhibited a precipitous decline (Williams et al. 2008). Future research is needed to determine whether our observed trends in fertilization success are consistent or variable among mussel species (particularly those that are declining) that varies in habitat (lentic vs. lotic), bedding tendencies (densely bedding species vs. non-bedding species) or other characteristics. It is possible that fertilization success is strongly density dependent in a subset of mussel species but not in others. Additional work focusing on spermatozeugmata swimming behavior, dilution patterns, and attraction to distant females under lotic and lentic conditions would be extremely

useful to more fully understanding the relationship between population density and fertilization success, and the conditions under which fertilization limitation is likely to lead to Allee effects. If fertilization success is not strongly density dependent for a majority of mussel species, future studies are needed to determine what specific density dependent pressures are likely to be alleviated when conservation efforts are focused on population augmentation via translocated or hatchery produced animals.

Ch. 2) Reproductive success of *Fusconaia ebena* (Lea, 1831) in relation to distance and water flow

Introduction:

Within North America, freshwater mussels are classified in five tribes within two subfamilies: Unioninae and Ambleminae. The tribe Anodontini is in the Unioninae; and the tribes Amblemini, Lampsilini, Pleurobemini, and Quadrulini are in the Ambleminae subfamily (Graf and Cummings 2007, Barnhart et al. 2008). Species in these tribes exhibit two types of general breeding strategies: long-term (bradytyctic) and short-term (tachytyctic) brooding (Ortmann 1919). The long-term brooders in subfamilies Anodontini and Lampsilini typically spawn in the summer months, hold their glochidia in the gills over winter, and release them in the following spring. Short-term brooders (tachytyctic) in the subfamilies Amblemini, Pleurobemini, and Quadrulini most often spawn in the spring and release glochidia in the summer of the same year (Coker et al. 1921, Williams et al. 2008).

In the previous chapter, I describe experiments to examine effects of flow and male density on fertilization efficiency of a freshwater mussel (*Lampsilis straminea*, Conrad, 1834) in the Lampsilini tribe. *Lampsilis straminea* is a long-term brooder that inhabits small to medium sized streams, is subject to lotic and lentic conditions (depending on rainfall) and typically does not form dense beds. In this chapter, I examine effects of flow on fertilization distance of *Fusconaia ebena*, a short term brooder in the Pleurobemini tribe.

Fusconaia ebena (Ebonyshell) (Lea, 1831) is a common, large-river species found throughout the Mississippi, Ohio, and Gulf basins (Williams et al. 2008). It is considered a

short-term brooder, being found gravid from April to September (Coker 1921, Williams et al. 2008, and others) and usually occurs in dense beds. Because this species typically spawns in the spring and inhabits large rivers, it is normally subjected to flowing conditions during the spawning season. The relationship between fertilization success, distance, and flow may be much different for mussel species that live in dense beds in large river systems as compared to non-bedding species that occur in small streams. Typical male-female distances should be shorter for bedding than non-bedding species, and large-river habitat is not typically subject to lentic conditions. Thus, species such as *F. ebena* may not have evolved spermatozuogmata capable of long-distance fertilization, particularly under conditions of no flow. However, short term brooders are much more difficult to work with in an experimental setting. They hold mature glochidia for a much shorter time period than long term brooders and often abort their fertilized eggs when disturbed or handled.

In this study, we repeat the flow experiment of chapter one using *F. ebena* as our model species and ask the following questions:

- 1) What effect does increasing distance between male and female mussels have on fertilization success?
- 2) What effect does water flow have on fertilization distance as compared to no flow?

Methods:

Experimental animals

Fusconaia ebena were collected from the Alabama River, in Wilcox County, Alabama near Camden. Mussels were collected in February 2011, after glochidia were released from the

previous season but prior to fertilization in the current season. Unlike *L. straminea*, we could not use shell morphology to determine gender because *F. ebena* is not sexually dimorphic. Instead, a gamete extraction procedure (Saha and Layzer 2008) was performed to check for mature gametes and identify gender of all individuals 2 weeks before placement in pens. Immediately prior to placement in pens, all female mussels were checked visually for gravidity by prying apart the valves and inspecting for swollen gills. All females were determined to be non-gravid before use in the experiment.

Experimental design

The experiment was conducted in 0.1 ha (0.25 acre) experimental ponds at the South Auburn Fisheries Research Station (Auburn University). An artificial stream was created in each pond (one stream per pond) by installing a 31 m long baffle approximately 4 m from one side of the pond and installing an airlift at one end of the stream (Figure 1). Two 3 hp blowers (Sweetwater, Aquatic Ecosystems, Apopka, FL, USA) supplied air to all of the experimental ponds. When the air was turned on, a slow current was generated in the appropriate stream.

Within each artificial stream, rectangular mussel pens (1m L x 2m W) were constructed of plastic netting (0.02 m² mesh size) extending 0.1 m above the substrate and 0.2 m below the substrate. A male pen was placed at the head of each stream, ~ 3 m downstream of the airlift. Female pens were placed at distances of 1 m, 10 m, and 25 m from the center of the male pen. Ponds were filled from a nearby stream (Odum Creek, Lee Co., AL) approximately 9 months before initiating experiments. Water quality parameters (pH, alkalinity, and calcium hardness) were monitored biweekly.

Airlifts were turned on in two streams, but left off in two other streams. Water velocity in flow and no-flow streams was measured using a flowmeter (Flo-mate 2000, Flow-tronic, Welkenreadt, Belgium) at five equidistant points along each stream 0.4 - 0.7 m below the surface and equidistant between the baffle and shoreline along the raceways.

Fifteen males were placed in the male pens of each stream, and ten females were placed in pens 1, 10, and 25 meters downstream of the males (Table 5). A control pond with an identical stream system was stocked with 20 females (no males) and the airlift turned on. Gravid females in the control pond would indicate either an ability to self-fertilize, or an ability to store sperm from a previous fertilization event.

Mussels were placed in the pens on 4 March. On 13 May we checked 12 females (3 from each 1 m pen) for gravidity by gently inserting a wedge between their valves and using one 18 gauge needle to extract a small amount of fluid from their gills and a separate needle to extract a small amount of fluid from the gonads (Saha and Layzer 2008). Gill and gonad extracts were checked under a microscope at 40X magnification for egg stage and fertilization and scored according to Table 6. Immature eggs that were observed had no albumin ring within each individual egg (Figure 7a). Mature eggs observed had fully developed yolks (dark, smooth spherical centers) inside of and albumin ring (Figure 7b) (Saha and Layzer 2008). Fertilized eggs had initiated cell division and multiple embryonic cells were visible within the egg membrane. Early stage glochidia were defined as embryos that had begun to assume a D-shape. Mature glochidia were defined as embryos with well-developed valves (Figure7c).

Because only one of twelve females examined on 13 May had fertilized eggs in her gills, we left the remainder of the mussels undisturbed until the following week. On 20 May we checked 8 females from the 1 m pen in a flow stream. Six of the eight females had early stage glochidia while two were still unfertilized. Believing the females were still in the early stages of fertilization, we left the remaining mussels undisturbed for another week. On May 27 we collected and performed gonad and gill extractions on all mussels from the experimental streams. Unfertilized mussels were returned to their original pens and reexamined on June 1 and June 8. On each sampling date, fertilized females were sacrificed and gills preserved in 75% ethanol for further analysis. Five control females were checked on both 13 May and 20 May and returned to their pen. Ten of the control females were collected on 27 May and the other ten control individuals were collected on 1 June.

Laboratory analysis

Fertilization success of gravid females was quantified in three ways: proportion gravid females within a given pen, proportion glochidia to unfertilized eggs per gravid female, proportion of glochidia to unfertilized eggs ($\text{glochidia} / (\text{glochidia} + \text{unfertilized eggs})$) per female, and total number glochidia per gravid female.

Contents were flushed out of preserved gills by placing a whole gill in a Petri dish, puncturing the gill along each water tubule with fine tip forceps, and flushing water over the gill. Gill contents were then flushed into a beaker and diluted to 1000 - 1500 ml with RO water. The resultant sample was mixed using a plunger to avoid creating a vortex inside the beaker. Three 1 ml subsamples were withdrawn from the homogenized sample and examined under a dissecting

microscope at 20X magnification. Glochidia and unfertilized eggs were counted in each subsample. If subsample counts were not within 10% of each other, a fourth subsample was withdrawn and counted. Percent fertilization for each gravid female examined was calculated as the number of glochidia divided by the total number of unfertilized eggs plus glochidia. Total number of glochidia for a given gravid female was estimated by the following formula:

$G = C * S/SB$ where

G = total glochidia per female

C = total glochidia counted in all subsamples

S = sample volume (ml)

SB = combined subsample volume (ml)

Non-gravid females (n=10) were also examined using the same procedure to ensure that flaccid gills contained neither glochidia nor unfertilized eggs.

Statistical Analysis

We used a repeated measures ANOVA was run to test for differences in water quality between all ponds. A one-way ANOVA test was used to test for differences in both males and female size between all ponds individually. In order to determine whether fecundity (#/glochidia per female) increased with shell size in either experiment we used linear regression. We used a one-way ANOVA to test for differences in both male and female size between ponds to determine if there were any differences in size as a factor that could possibly influence gamete production and fertilization success

Fertilization success was not statistically analyzed in this experiment for several reasons. First, due to the fact that these mussels were handled multiple times and had gill and gonad extractions on multiple (6) occasions; accurate fertilization and gravidity rates would not be consistent with methods used in the first chapter. Secondly, the control pond was compromised with a male and therefore we were unable to determine if any of these animals from the collected populations are able to self – fertilize or able to become hermaphroditic. Finally, and most importantly, only a total of 16 out of 120 female mussels in the treatment ponds ever became gravid during the entire experiment. Of those 16, 6 were observed on 20 May when only a subset of mussels were sampled and thus could not be included in the analysis. Results of this experiment are presented to serve as an aid for designing future studies with short term brooders, rather than to definitively answer the original hypotheses of this study.

Results:

Biweekly water quality values (pH, alkalinity, and calcium hardness) from March to June 2011 across all ponds averaged 8.25 ± 0.3 sd, 30 ± 15.1 sd, and 60 ± 12.2 sd respectively. Water quality did not differ significantly between flow and no-flow streams for pH ($p = d.f.=5, F=0.53, p=0.733$), alkalinity ($d.f.=5, F=0.38, p= 0.892$), and hardness ($d.f.=5, F=0.00, p= 0.999$). The average flow rate across both raceways was 0.03 ± 0.01 sd m/s. The flow rate in no-flow raceways was undetectable (minimum detectable flow = 0.01 m/s).

No mussels escaped from pens during the course of the experiment. Survivorship across all pens and treatments was 99.5% (199/200). The one death was a female from a pond with flow in the 1 m pen. Nine of nineteen females in the control pond exhibited swollen gills. Flushing of

gills from control females revealed glochidia (fertilized) and structural (non-fertilized) eggs. One male was found in the control pond. Shell length did not differ between flow and no-flow streams for either males (d.f.=1, $F=0.00$, $p=0.982$) or females (d.f.=1, $F=0.49$, $p=0.484$).

Of the 12 females (3 females from each 1 m pen) that were checked on May 13, only one was gravid, all non-gravid females were returned to the ponds. Five control females were checked and none were gravid, all were returned to their pen. On 20 May, we checked 8 females from a 1 m pen in a flow stream. Six of the eight females were found to be in the early stages of fertilization, and were returned to the ponds to mature. Five different control females were checked and none were gravid, all were returned to their pen. On 27 May, all females in the experimental ponds were checked; a total of ten females were found gravid, all gravid females were sacrificed and gills stored in ethanol. Six of the gravid females were from flow streams and four were found in the no-flow streams. Ten control females were checked and five were found to be gravid, all were taken out of the pen. On 1 June, all remaining females in the experimental ponds were checked; a total of six females were found gravid. All six were from flow streams, they were sacrificed and gills stored in ethanol, also 10 different control individuals were collected, four of nine females were found gravid. A male was also found in the control pen. On June 8 all remaining females in the ponds were checked and found to have immature eggs in the gonad and empty gills. The experiment was terminated at this point (Figures 8 and 9; Table 7).

F. ebena were able to fertilize females at the farthest downstream distance (25 m) in flow streams but there was complete fertilization failure at 25 m in streams with no flow (Figure 10). In one stream with no flow, complete fertilization failure was observed with no gravid females

seen over the entire experimental period. At the conclusion of the experiment, fertilization success was low across all distances and flow conditions. Across both treatments, the maximum proportion of gravid females in all pens was 0.3 (3 gravid females out of ten total females). (Figure 10 a and b). Overall fertilization efficiency was high with an average of $91\% \pm 8$ sd % across all gravid females (n=16) (Figure 10 c and d). We found no relationship between mussel shell length and number glochidia (d.f.=1, $F=1.39$, $p=0.260$), and report glochidia production as glochidia per female rather than a function of length or mass (Figure 11). The number of glochidia per mussel was variable (range = 7000 to 218000) among females (Figure 10 e and f).

Discussion:

We hypothesized that large-river species living in dense beds may exhibit a shorter effective fertilization distance than small stream species that tend to occur in a more scattered distribution, since spermatozeugmata need only travel shorter distances between spawning males and receptive females. Fertilization distances may be more strongly dependent on current in large-river species since they have evolved in a habitat where current is more stable than in small streams where reaches tend to pool up during summer months and exhibit little to no current.

In this study, *F. ebena*, a large river species that occurs in dense beds, were successful at fertilizing females at the farthest downstream distance (25 m) with flow. Though low numbers of fertilization success were shown across all treatments we did see fertilization failure (0/10) at 25 m with no flow. This could be a function of spermatozeugmata not being viable long enough to make it that distance with no assistance from current. This fertilization failure supports the idea that densely bedding riverine species have evolved to exhibit shorter effective fertilization

distances since they typically do not require long distances for successful fertilization. However, it is difficult to draw firm conclusions from this study since few females (e.g. maximum of 3 out of ten) became gravid in any given pen. Also, as in the previous experiment with *Lampsilis straminea*, fertilization efficiency and glochidial production was high amongst all gravid females regardless of treatment, indicating that fertilization was “all or nothing” and sperm was not limiting.

Multiple brood production has been shown in short – term brooders in the genus *Elliptio*, in which some species produce two and three clutches in a given brooding season (Price and Eads 2011). Because we assessed fertilization on multiple occasions, this experiment offered an opportunity to directly test whether *F. ebena* produce multiple broods. On 13 May, mussels were recovered from ponds and found to be in early stages of fertilization and were placed back in pond to mature for 7 days. On subsequent gravidity checks these females were never seen gravid again. This release of glochidia within 7 days may suggest that this species only spawns once per brooding season and quickly releases conglomerates into the water column. Alternately, it may be that handling induced mussels to abort their broods, a common phenomenon observed in short term brooders (Garner et al. 1999).

Non - sexually dimorphic species must be checked for sex by gamete extractions (Saha and Layzer 2008). All individuals in this experiment were checked in this same manner. One misidentification of a male in the control pond resulted in a failed control assessment. These individuals showed the highest fertilization success (9/19) out of any pen in the experiment, with one male presumably fertilizing nine females within a 1m x 2m space. This high fertilization

success was not likely to be due to hermaphroditism since all other individuals that were collected (n=199) during the course of the experiment were identified as the same gender as indicated in the beginning of the experiment. Fertilization studies may be feasible, but are very difficult to conduct with short-term brooders. Pond studies offer “natural” settings for the animals, but in this case, results may have been clearer in a more controlled environment such as a flume or tank, where mussels could be monitored for glochidial release but not disturbed. Additionally, since we observed glochidial release within 7 days, timing the collection of experimental animals to coincide with the short period of time that they retain a brood is crucial to understanding the actual fertilization success within the population being tested. Further testing in the lab utilizing individual flow-through pond water tanks would be essential to answering how long females retain broods, whether multiple broods per spawning season occur, and addressing the effects of handling on fertilization processes (abortion of embryos, delayed or premature releases, etc.). Using tanks would allow for constant monitoring of the release of glochidia from individual mussels without having to handle them while subjecting mussels to ambient light conditions and natural water conditions. If individuals were observed releasing conglomerates only once per brooding season, but having staggered release periods, collection of experimental individuals would have to occur only after individuals started releasing conglomerates to ensure that there was no interference in the fertilization process.

Ch. 3) Hurdles for the conservation of freshwater mussels

Over 70% of the almost 300 described species of freshwater mussels are considered to be endangered, threatened, or of special concern (Williams et al. 1992). The southeastern United States is a global hotspot for freshwater mussel diversity with 174 species known to have been present within the most diverse state - Alabama (Williams et al. 2008). However, most freshwater mussel populations in North America are in decline. In Alabama alone, 78% of native freshwater mussels are in some kind of peril including population decline, which includes both extirpation from the state and extinction. Forty one species are considered endangered and 7 species considered threatened. Additionally, in the past century about 28 species of mussels have become extinct (Mirarchi 2004).

Freshwater mussels tend to be found in patches or beds. Substrate stability and water movement have been cited as the most important factors that lead to these mussel beds in streams and rivers (Hartfield and Ebert 1986, McMahon and Bogan 2001). The water flow must be strong enough to wash away buildup of siltation around the animals, but weak enough so the substrate will remain stable, so the animals are not washed downstream (Vaughn and Taylor 1999). Where these requirements are met freshwater mussels can occur; typically in beds where multiple species are present, and in aggregate (Vaughn and Pyron 1995, Strayer et al. 1994).

The distribution patterns of freshwater mussels have been attributed to many variables both within the stream and surrounding watersheds. Hydrologic variables such as in-stream flow and stream channel geomorphology have been proposed as mechanisms that can predict where mussels may be present in a stream (Di Maio and Corkum 1995, Gangloff and Feminella

2007). Shear stress on the benthos of the stream channel is also another factor that has been suggested to contribute to mussel abundance/ presence in the stream, due to the lack of strength of juvenile mussels to withstand the stress on them from the water (Layzer and Madison 1995). Substrate stability at high flows can also be a determinant in where mussels are distributed in the stream channel (Allen and Vaughn 2009). Some minimum water movement may be required of riverine species to rid the immediate area of waste and replenish it with fresh nutrients for feeding (Steuer et al. 2008). All of these factors have been negatively affected by the increase in urbanization, or loss of forested land to agriculture in the Southeastern U.S., where the majority of freshwater mussel diversity is located (Gillies et al. 2003).

Declines in mussel populations have also been attributed to decreased reproduction and recruitment. For example, declines in reproduction and recruitment of freshwater mussels have been linked to the effects of altered flow regimes below hydroelectric dams (Hardison and Layzer 2001, Moles and Layzer 2008). The change in temperature from release of hypolimnetic water from the impoundments that dams create can alter reproductive cycles, most directly targeting the timing of gametogenesis and glochidia maturation (Layzer et al. 1993). Restrictions or complete loss of host fish have also been noted to have significant negative impact on the recruitment of freshwater mussels in regulated rivers, due to the fish's critical role in the freshwater mussel life cycle (Galibrath and Vaughn 2009, Watters 1996, Williams 1992).

Man-made chemicals in surface waters also can affect the reproductive timing and success of freshwater mussels. Pharmaceuticals, such as serotonin and fluoxetine, have both been shown to induce spawning in freshwater mussels. These chemicals are present in surface

waters close to wastewater treatment plants and may affect spawning timing and release of glochidia (Bringolf et al. 2010). These chemicals can also alter the mantle lure display behavior of Lampsiline mussels, interfering with glochidial transfer to fish and decreased recruitment (Cope et al. 2008, Bringolf et al. 2010). Multiple studies have shown that effluents from wastewater treatment facilities can cause negative responses in freshwater mussels which include altered gametogenesis (Gange et al. 2001, Gange et al. 2004).

Increasing the knowledge of how these animals reproduce is needed to provide the most current information to managers for the conservation of the group. Previous studies have suggested that the alteration of flowing water has had a negative effect on fertilization rates (Moles and Layzer 2008) but none of these studies have directly tested whether these issues significantly reduce the fertilization of female mussels in beds present in the rivers. The direct manipulation of water flow, density of mating individuals, and competition for gametes has not been experimentally tested in freshwater mussels.

From the results described in the previous chapters; freshwater mussels may be very efficient at fertilizing mates across large distances. Results from these studies suggest that fertilization success can be affected by factors such as female density, gamete availability, and distance from males. Fertilization success in long-term brooders, (Lampsilini) may not require high population densities, even under lentic conditions. Our findings indicate that population densities required for efficient fertilization of freshwater mussels may be smaller than first thought, indicating that Allee effects associated with fertilization effects may not play an important role in population extinctions (Downing et al. 1993). These populations may not

necessarily have to be densely aggregated to have high fertilization success, since we observed high fertilization success at long distances (25 m). Additionally, the presence of proximal females seemed to enhance, rather than limit, fertilization of downstream females.

There may be a critical female density threshold or spatial arrangement in order for distant females to be efficiently fertilized. If so, managers may need to consider female density and spatial arrangement more than overall population density when deciding when small populations are at risk of reproductive failure. Also, there may be mechanisms other than fertilization that would cause small populations of mussels to suffer from Allee effects. Several areas of the life cycle in the freshwater mussel can become density dependent bottlenecks. For example, some species of mussels display a lure to attract fish to strike, which is made from modified mantle flaps and other structures (Haag and Warren 1999). If displaying females are sparse, this might lead to a reduced host fish attraction to that area, reducing the chance that a fish would strike the female and become infested with glochidia. Also, if at low adult population densities there is concurrent decrease in the total number of newly metamorphosed juveniles on the stream bottom, the juveniles may be less able to absorb predation pressure by flatworms and other predators of juvenile freshwater mussels (Zimmerman et al. 2003)

To determine when and how sperm can be limiting, two simple alterations to this experimental design could be used. The first recommendation would be to increase the length of the raceway/stream channel and place females at further distances to see where fertilization success declines significantly. The second recommendation would be to increase the number of

females in the stream to find a tipping point where upstream females begin depleting the water column of sperm to the extent that downstream conspecifics become sperm limited.

Results from the short-term brooding species (Amblemini) are not as clear, and need to be further examined. In these dense bedding riverine species, as in *F. ebena*, distance from mating individuals may be a more important factor, as indicated by our observation of fertilization failure at long distances with no flow. This finding could be due to possible sperm viability issues, resulting from the adaptation of living in these dense beds and not needing long sperm viability to fertilize proximal mates.

Fertilization distance issues can play an important role in the conservation and repopulation of freshwater mussels in streams and rivers. Spatial arrangement and distance from possible mates can be an important factor when deciding if populations are at risk for reproductive failure and possible population decline. If the patterns observed in long-term brooding species hold true, and these animals are able to effectively fertilize at long distances; then conservation efforts focusing on augmentation and/or re-introductions can stock individuals over a larger area and in multiple patches within the stream channel rather than a smaller number of very dense patches. Also, if these small populations are not suffering from Allee effects, augmentation is not the conservation action needed. A more in-depth analysis must be conducted to determine whether population decline is due to other factors, such as host fish presence/absence, or water quality issues. More research is needed in this area, but these results are promising in that density dependent fertilization success may not be a major bottleneck for freshwater mussels.

Literature Cited

- Allen, D.C. and C.C. Vaughn. "Burrowing behavior of freshwater mussels in experimentally manipulated communities." *Journal of the North American Benthological Society*, 2009: 28(1) 93-100.
- Babcock, R. and J. Keesing. "Fertilization biology of the abalone *Haliotis laevis*: laboratory and field studies." *Canadian Journal of Fisheries and Aquatic Sciences*, 1999: 56(9): 1668-1678.
- Barnhart, M.C., and A. Roberts. "Reproduction and fish hosts of unioninids from the Ozark Uplifts." In *Conservation and Management of Freshwater Mussels II: Initiatives for the Future*, 16-20. Rock Island, IL., 1997.
- Barnhart, M.C., W.R. Haag, and W.N. Roston. "Adaptations to host infection and larval parasitism in Unionoida." *Journal of the North American Benthological Society*, 2008: 27 (2): 370 - 394.
- Bishop, J.D.D. and A.J. Pemberton. "The third way: spermcast mating in sessile marine invertebrates." *Sexual Selection and Mating Systems in Hermaphrodites at the annual meeting of the Society for Integrative and Comparative Biology*. San Diego, CA: Oxford University Press, 2005. 46:4 398-406.
- Boukal, D.S., and L. Berec. "Single-Species Models of the Allee Effect: Extinction Boundaries, Sex Ratios, and Mate Encounters." *Journal of Theoretical Biology*, 2002: 218(3): 375-394.
- Bringolf, R.B., R.B. Heltsley, T.J. Newton, C.B. Eads, S.J. Fraley, D. Shea, W.G. Cope. "Environmental Occurrence and Reproductive Effects of the Pharmaceutical Fluoxetine in Native Freshwater Mussels." *Environmental Toxicology and Chemistry*, 2010: 29(6):1311-1318.
- Coker, R.E., A.F. Shira, H.W. Clark, A.D. Howard. "Natural History and Propagation of freshwater mussels." *Bulletin of the United States Bureau of Fishes*, 1921: 37: 77-181.
- Cope, W.G., R.B. Bringolf, D.B. Buchwalter, T.J. Newton, C.G. Ingersoll, N. Wang, T. Anugspurger, F.J. Dwyer, M.C. Barnhart, R.J. Neves, and E. Hammer. "Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental

- contaminants." *Journal of the North American Benthological Society*, 2008: 27(2): 451-462.
- Dahl, J., P. Anders Nilsson and L.B. Pettersson. "Against the flow: chemical detection of downstream predators in running waters." *Proceedings of the Royal Society Biological Sciences*, 1998: 265: 1339-1344.
- Deredec, A., and F. Courchamp. "Importance of the Allee Effect for reintroductions." *Ecoscience*, 2007: 14(4): 440-451.
- Di Maio, J., and L.D. Corkum. "Relationship between the spatial distribution of freshwater mussels (Bivalvia: Unionidae) and the hydrological variability of rivers." *Canadian Journal of Zoology*, 1995: 73: 663-671.
- Downing, J.A., Y. Rochon, M. Perusse, and H. Harvey. "Spatial aggregation, body size, and reproductive success in the freshwater mussel, *Elliptio complanata*." *Journal of North American Benthological Society*, 1993: 12(2): 148-156.
- Eads, C.B., R.B. Bringolf, R.D. Greiner, A.E. Bogan, and J.F. Levine. "Fish Hosts of the Carolina Hellsplitter (*Lasmigona decorata*), a Federally Endangered Freshwater Mussel (Bivalvia: Unionidae)." *American Malacological Bulletin*, 2010: 28 (1-2): 151-158.
- Falese, L.E., M.P. Russell, and N.R. Dollahon. "Spermcasting of spermatozeugmata by the bivalves *Nutricula condusa* and *N. tantilla*." *Invertebrate Biology*, 2011: 130(4): 334-343.
- Galibraith, H.S. and C.C. Vaughn. "Temperature and food interact to influence gamete development in freshwater mussels." *Hydrobiologia*, 2009: (636) 1: 35-47.
- Galtsoff, P.S. "Physiology of Reproduction of *Ostrea virginica*: Stimulation of spawning in the female oyster." *The Biological Bulletin*, 1938: 75:286-307.
- Gange, F., C. Blaise, and J. Hellou. "Endocrine disruption and health effects of caged mussels, *Elliptio complanata*, placed downstream from a primary-treated municipal effluent plume for 1 year." *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 2004: 138(1): 33-44.
- Gange, F., C. Blaise, M. Salazar, S. Salazar, and P.D. Hansen. "Evaluation of estrogenic effects of municipal effluents to the freshwater mussel, *Elliptio complanata*." *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 2001: 128(2): 213-225.

- Gangloff, M.M. and J.W. Feminella. "Stream channel geomorphology influences mussel abundance in southern Appalachian streams, U.S.A." *Freshwater Biology*, 2007: 64-74.
- Garner, J.T., T.M. Haggerty, and R.F. Moflin. "Reproductive cycle of *Quadrula metanevra* (Bivalvia: Unionidae) in the Pickwick Dam tailwater of the Tennessee River." *American Midland Naturalist*, 1999: 141: 277-283.
- Gascho Landis, A.M., T.L. Mosley, W.R. Haag, and J.A. Stoeckel. "Effects of temperature and photoperiod on lure display and glochidial release in a freshwater mussel." *Freshwater Science*, 2012: 31(3): 775-786.
- Gillies, R.R., J. Brim Box, J. Skymanzik, and E.J. Rodemaker. "Effects of urbanization of the aquatic fauna of the Line Creek watershed, Atlanta - a satellite perspective." *Remote Sensing of Environment*, 2003: 86: 411-422.
- Graf, D.L. and K.S. Cummings. "Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida)." *Journal of Molluscan Studies*, 2007: 73: 291-314.
- Haag, W.R. and J.L. Stanton. "Variation in fecundity and other reproductive traits in freshwater mussels." *Freshwater Biology*, 2003: 48: 2118-2130.
- Haag, W.R. and M.L. Warren. "Mantle displays of freshwater mussels elicit attacks from fish." *Freshwater Biology*, 1999: 42:1 35-40.
- Hardison, B.S. and J. B. Layzer. "Relations between complex hydraulics and the localized distribution of mussels in three regulated rivers." *Regulated Rivers: Research and Management*, 2001: 17: 77-88.
- Hartfield, P.D. and D. Ebert. "The mussels of southwest Mississippi streams." *American Malacological Bulletin*, 1986: 4: 2-23.
- Ishibashi, R., A. Komaru, and T. Kondo. "Sperm Sphere in Unionid Mussels (Bivalvia: Unionidae)." *Zoological Science*, 2000: 947-950.
- Kreutzer, C. and W. Lampert. "Exploitative Competition in Differently Sized *Daphnia* Species: A Mechanistic Explanation." *Ecology*, 1999: 80:7 2348-2357.
- Krkosek, M., B.M. Connors, M.A. Lewis, and R. Poulin. "Allee Effects May Slow the Spread of Parasites in a Coastal Marine Ecosystem." *American Naturalist*, 2012.

- Krug, P.J., J.A. Riffell, and R.K. Zimmer. "Endogenous signaling pathways and chemical communication between sperm and egg." *The Journal of Experimental Biology*, 2009: 212: 1092-1100.
- Layzer, J.B. and L.M. Madison. "Microhabitat use by freshwater mussels and recommendations for determining their instream flow needs." *River Research and Applications*, 1995: 10(2-4): 329-345.
- Layzer, J.B., M.E. Gordon, and R.M. Anderson. "Mussels: The forgotten fauna of regulated rivers: A case study of the Caney Fork River." *Regulated Rivers: Research and Management*, 1993: 8: 63-71.
- Levitan, D.R. "Influence of body size and population density on fertilization success and reproductive output in a free-spawning invertebrate." *The Biological Bulletin*, 1991: 181: 261-268.
- Levitan, D.R. "Sexual Selection in External Fertilizers." In *Evolutionary Behavioral Ecology*, by D. and C. Fox Westneat, 365-378. New York: Oxford Press, 2012.
- Levitan, D.R. "Sperm Limitation, Gamete Competition, and Sexual Selection in External Fertilizers." In *Sperm Competition and Sexual Selection*, by T.R. and A.P. Moller Birkhead, 175-201. San Diego, CA: Academic Press, 1998.
- Levitan, D.R., M.A. Sewell, and F.S. Chia. "How Distribution and Abundance Influence Fertilization Success in the Sea Urchin *Strongylocentrotus Franciscanus*." *Ecology*, 1992: 73:1 248 - 254.
- Lillie, F.R. "The organization of the egg of *Unio*, based on a study of its maturation, fertilization, and cleavage." *Journal of Morphology*, 1901: 17: 227-292.
- Lynn, J.W. "The ultrastructure of the sperm and motile spermatozeugmata released from the freshwater mussel *Anodonta grandis* (Mollusca: Bivalvia: Unionidae)." *Canadian Journal of Zoology*, 1994: 72; 1452-1461.
- McMahon, R.F. and A.E. Bogan. *Mollusca: Bivalvia*. In Thorp, J.H., Covich, A.P. (Eds.), *Ecology and Classification of North American Freshwater Invertebrates*. San Diego: Academic Press, 2001.
- Mirarchi, R.E. *Imperiled Aquatic Mollusks and Fishes, Volume 2*. Tuscaloosa: University of Alabama Press, 2004.

- Moles, K.R. and J. B. Layzer. "Reproductive ecology of *Actinonaias ligamentina* (Bivalvia: Unionidae) in a regulated river." *Journal of the North American Benthological Society*, 2008: 27 (1): 212-222.
- Ortmann, A. E. "Monograph of the naiads of Pennsylvania. Part III: systematic account of the genera and species." *Memoirs of the Carnegie Museum*, 1919: 8: 1-384.
- Pemberton, A.J., R.N. Hughes, P.H. Manriquez, and J.D. Bishop. "Efficient utilization of very dilute aquatic sperm: sperm competition may be more likely than sperm limitation when eggs are retained." *Proceedings of the Royal Society Biological Sciences*, 2003: 270: S233 -S226.
- Pennington, J.T. "The Ecology of Fertilization of Echinoid eggs: The consequences of sperm dilution, adult aggregation, and synchronous spawning." *The Biological Bulletin*, 1985: 169: 417-430.
- Phillippi, A., E. Hamann, and P.O. Yund. "Fertilization in an egg-brooding colonial ascidian does not vary with population density." *The Biological Bulletin*, 2004: 206: 152-160.
- Price, J.E., and C.B. Eads. "Brooding Patterns in Three Freshwater Mussels of the Genus *Elliptio* in the Broad River in South Carolina." *American Malacological Bulletin*, 2011: (1-2) 121-126.
- Saha, S. and J.B. Layzer. "Evaluation of a nonlethal technique for determining sex of freshwater mussels." *Journal of the North American Benthological Society*, 2008: 24 (1): 84-89.
- Serrao, E.A., and J.N. Havenhand. "Fertilization Strategies." In *Marine Hard Bottom Communities: Patterns, Dynamics, and Change*, 149-164. Heidelberg: Springer, 2009.
- Stephens, P.A., W.J. Sutherland, and R.P. Freckleton. "What is an Allee Effect?" *Nordic Society Oikos*, 1999: 87(1): 185-190.
- Steuer, J.J., T.J. Newton, and S.J. Zigler. "Use of complex hydraulic variables to predict the distribution and density of unionids in a side channel of the Upper Mississippi River." *Hydrobiologia*, 2008: 610:67-82.
- Strayer, D.L., D.C. Hunter, L.C. Smith, and C.K. Borg. "Distribution, abundance, and roles of freshwater clams (Bivalvia, Unionidae) in the freshwater tidal Hudson River." *Freshwater Biology*, 1994: 31: 239-248.

- Trimble, J.J. and D. Gaudin. "Fine structure of the sperm of the freshwater clam, *Ligumia subostrata* (Say, 1983)." *Veliger*, 1975: 18: 34-36.
- Vaughn, C.C. and C. M. Taylor. "Impoundments and the Decline of Freshwater Mussels: a Case Study of an Extinction Gradient." *Conservation Biology*, 1999: 912-920.
- Vaughn, C.C. and M. Pyron. "Population ecology of the endangered Quachita Rock Pocketbook Mussel, *Arkansia wheeleri* (Bivalvia: Unionacea), in the Kimachi River, Oklahoma." *American Malacological Bulletin*, 1995: 11: 145-151.
- Wagenius, S., E. Lonsdorf, and C. Neuhauser. "Patch aging and the S-Allee effect: breeding system effect on the demographic response of plants to habitat fragmentation." *American Naturalist*, 2007: 169: 383-397.
- Waller, D. L. and B. A. Lasee. "External Morphology of Spermatozoa nad Spermatozeugmata of the Freshwater Mussel *Truncilla truncata* (Mollusca: Bivalvia: Unionidae)." *American Midland Naturalist*, 1997: 220-223.
- Warrern, W.R. Haag and M.L. "Host fishes and infection strategies of freshwater mussels in large Mobile Basin streams, USA." *Journal of the North American Benthological Society*, 2003: 22: 78-91.
- Watters, G.T. "Small dams as barriers to freshwater mussels (Mollusca: Pelecypoda: Unionidae) and their hosts." *Biological Conservation*, 1995: 75:79-85.
- Williams, J. D., M.L Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. "Conservation status of the freshwater mussels of the United States and Canada." *Fisheries*, 1992: 6-22.
- Williams, J.D., A.E. Bogan, and J.T. Garner. *Freshwater mussels of Alabama and the Mobile Basin in Georgia, Mississippi, and Tennessee*. Tuscaloosa: University of Alabama Press, 2008.
- Yokley, P, Jr. "Life History of *Pleruobema cordatum* (Rafinesque, 1820) (Bivalvia, Unionacea)." *Malacologia*, 1972: 11: 351-364.
- Yund, P.O. "How severe is sperm limitation in natural populations of marine free-spawners?" *Trends in Ecology and Evolution*, 2000: 15:1 10-13.

Zimmerman, L.L., R.J. Neves, and D.G. Smith. "Control of Predacious Flatworms
Macrostomum sp. in Culturing Juvenile Freshwater Mussels." *North American Journal of
Aquaculture*, 2003: 65:1 28-32.

Table 1: Pen assignments for experiment 1 utilizing *L. straminea*.

<i>Pond</i>	<i>Flow (Y/N)</i>	<i>Male</i>	<i>Female 1m</i>	<i>Female 10m</i>	<i>Female 25m</i>
1	Y	15	10	10	10
2	N	15	10	10	10
3	N	15	10	10	10
4	Y	15	10	10	10

Table 2: Pen assignments for experiment 2 utilizing *L. straminea*

<i>Pond</i>	<i>Male</i>	<i>Female 1m</i>	<i>Female 10m</i>	<i>Female 25m</i>
1	15	-	-	10
2	2	-	-	10
3	15	10	10	10
4	2	10	10	10
5	15	10	10	10
6	2	10	10	10
7	15	-	-	10
8	2	-	-	10

Table 3: Number of gravid females used from each pen of experiment 1 to assess fertilization rates in *L. straminea*.

Pond	Pen 1m	Pen 10m	Pen 25m
1	5	5	5
2	5	4	5
3	4	5	2
4	5	5	5

Table 4: Number of gravid females used from each pen of experiment 2 to assess fertilization rates in *L. straminea*.

Pond	Pen 1m	Pen 10m	Pen 25m
1	-	-	5
2	-	-	5
3	5	5	5
4	2	5	5
5	5	5	5
6	3	5	5
7	-	-	0
8	-	-	5

Table 5: Pen assignments for flow experiment using *F. ebena*

<i>Pond</i>	<i>Flow (Y/N)</i>	<i>Male</i>	<i>Female 1m</i>	<i>Female 10m</i>	<i>Female 25m</i>
1	Y	15	10	10	10
2	N	15	10	10	10
3	N	15	10	10	10
4	Y	15	10	10	10

Table 6: Stages seen in gill and gonad extractions in Figures 8 and 9

Rating	Gonad Extraction Stage	Gill Extraction Stage
0	-	Non swollen
0.5	Immature eggs	Empty
1	Mature eggs	Empty
2	Mature eggs	Mature eggs
3	Mature eggs	Fertilized eggs
4	Mature eggs	Early stage glochidia
5	Mature eggs	Glochidia
7	Empty	Empty
8	Empty	Immature eggs

Table 7: Number of gravid females used from each pen to assess fertilization rates for *F. ebena*.

<i>Pond</i>	<i>Pen 1m</i>	<i>Pen 10m</i>	<i>Pen 25m</i>
1	1	3	1
2	0	0	0
3	1	3	0
4	2	2	2



Figure 1a: Picture of experimental pond with airlift and artificial stream channel system in pond.
1b: Schematic of pen placement in pond in relation to the stream channel and airlift systems.

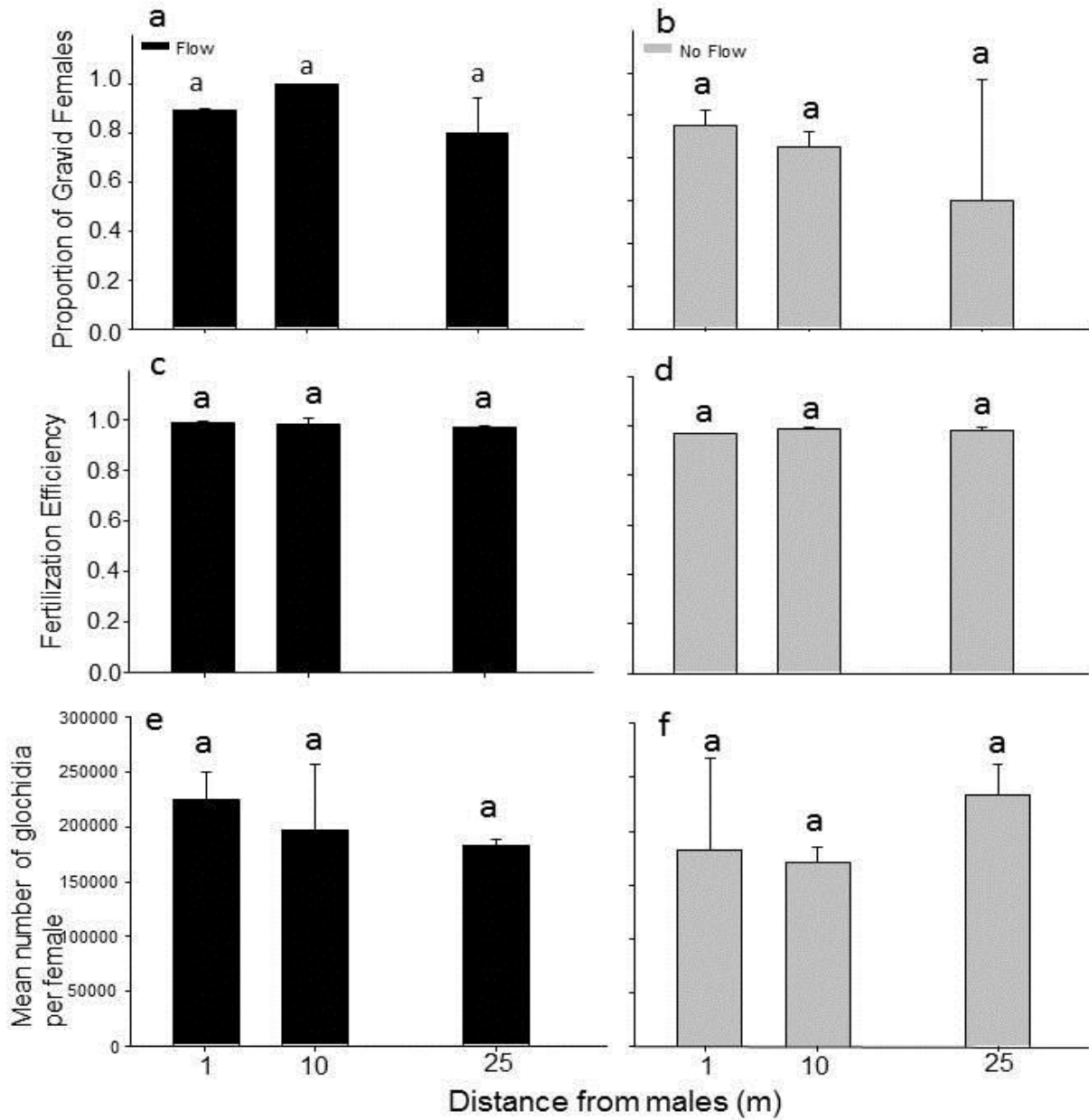


Figure 2: Proportion of gravid females in a) flow and b) no-flow streams. Percent fertilization in gravid mussels in c) flow and d) no-flow streams. Glochidia per gravid female in e) flow and f) no-flow streams at each of the three distances from spawning males. Error bars represent ± 1 sd. Letters above bars indicate whether significant differences were found.

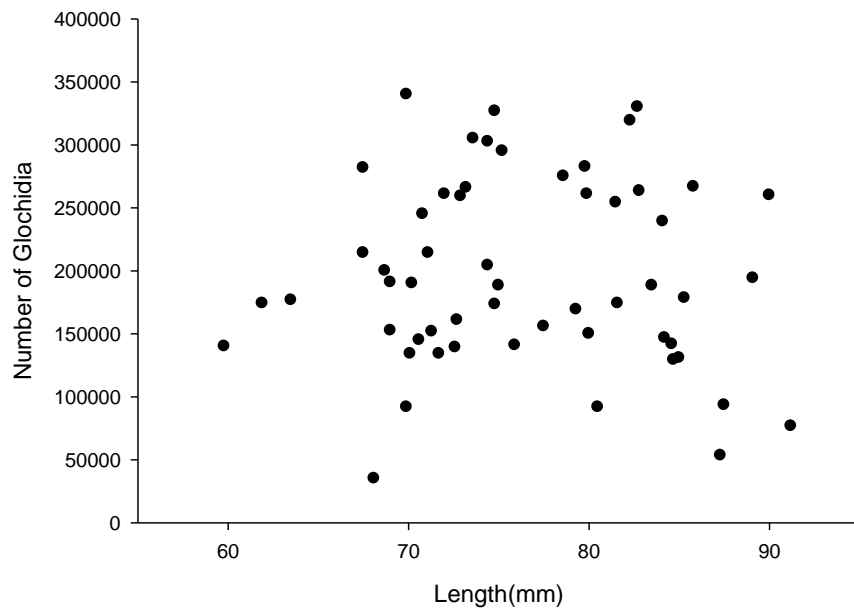


Figure 3: Number of glochidia per gravid female versus shell length in the flow experiment

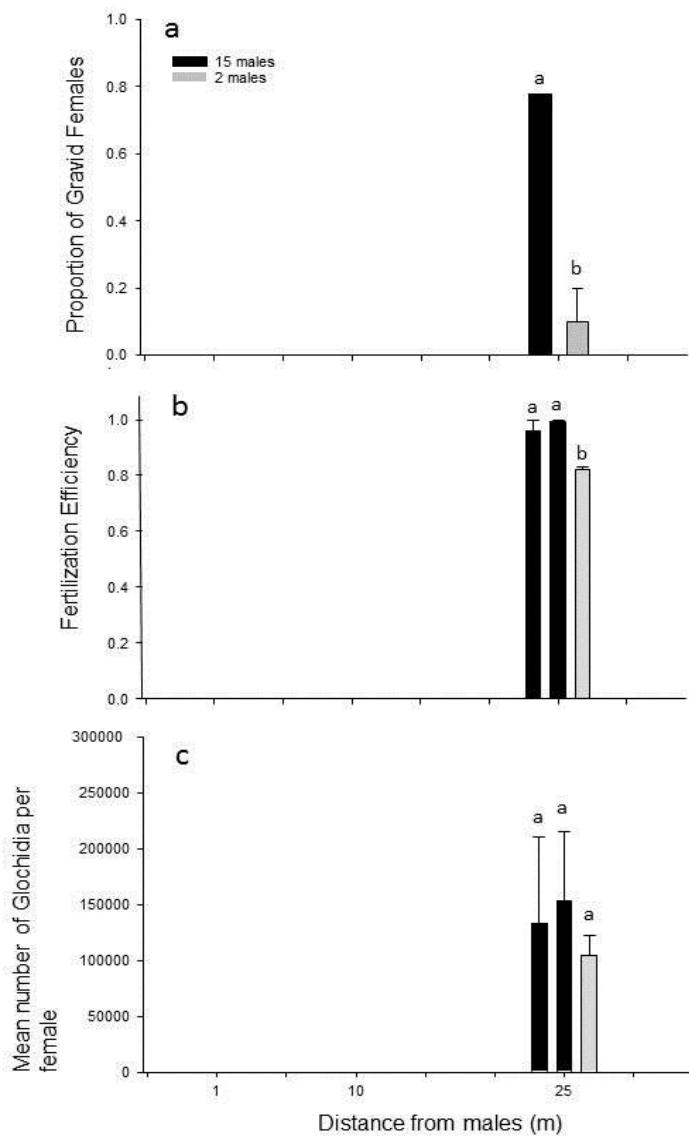


Figure 4a) Proportion of gravid females; b) percent fertilization of gravid females and c) glochidia per female at 25 m with no competition from upstream females. Each bar in panels b and c represents results from single ponds. Error bars represent ± 1 sd. Letters above bars indicate where significant differences were found.

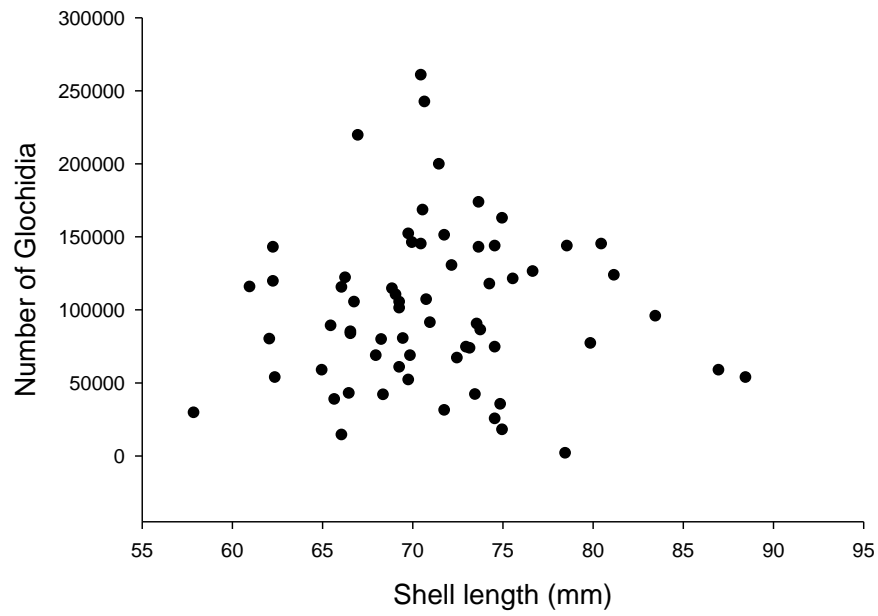


Figure 5: Number of glochidia per gravid female versus shell length in experiment two.

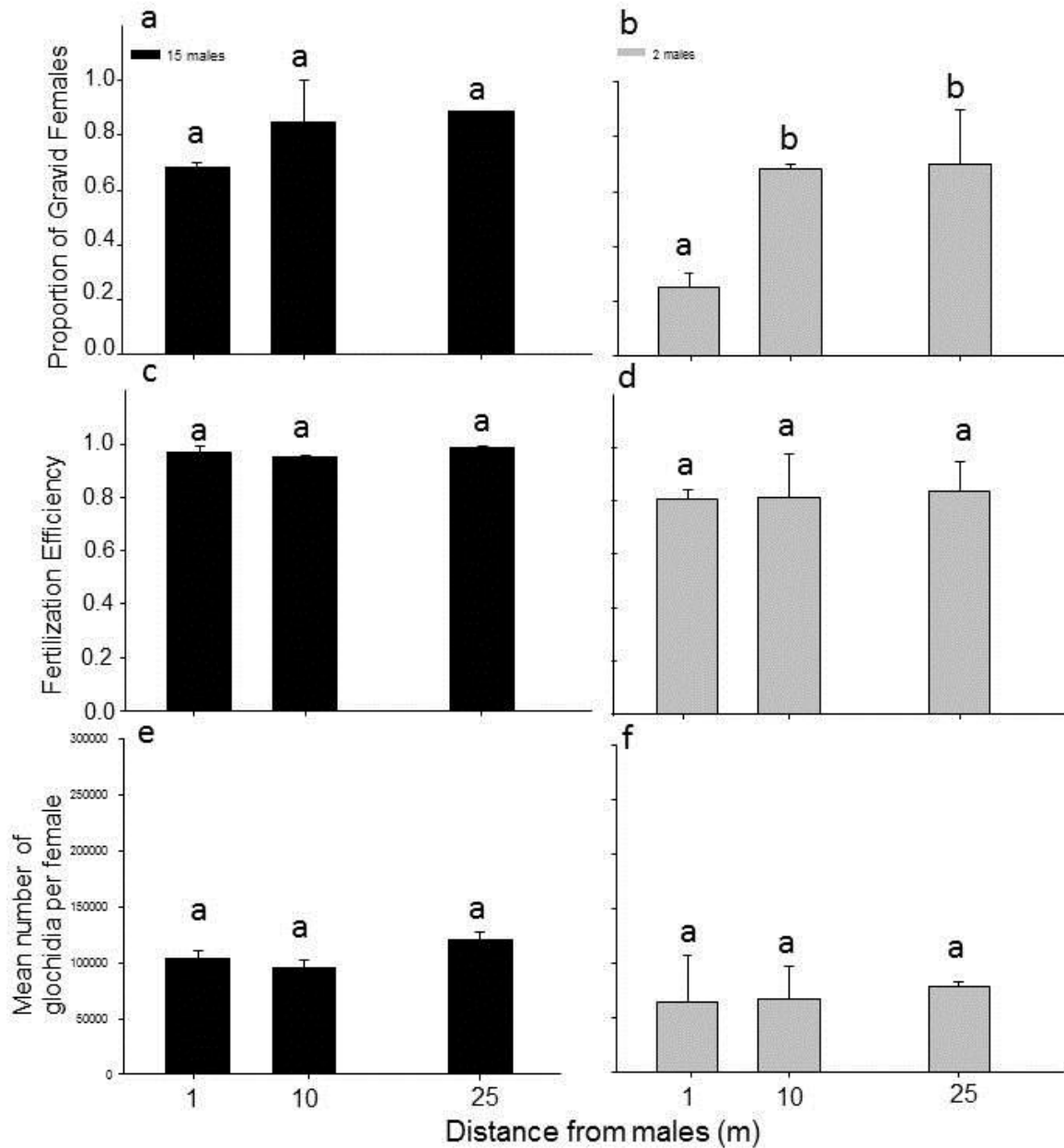


Figure 6: Proportion of gravid females in a) high and b) low-male streams; percent fertilization in gravid mussels in c) high and d) low-male streams; and glochidia per gravid female in e) high and f) low-male streams at each of the three distances from spawning males. Error bars represent ± 1 sd. Letters above bars indicate where significant differences were found.

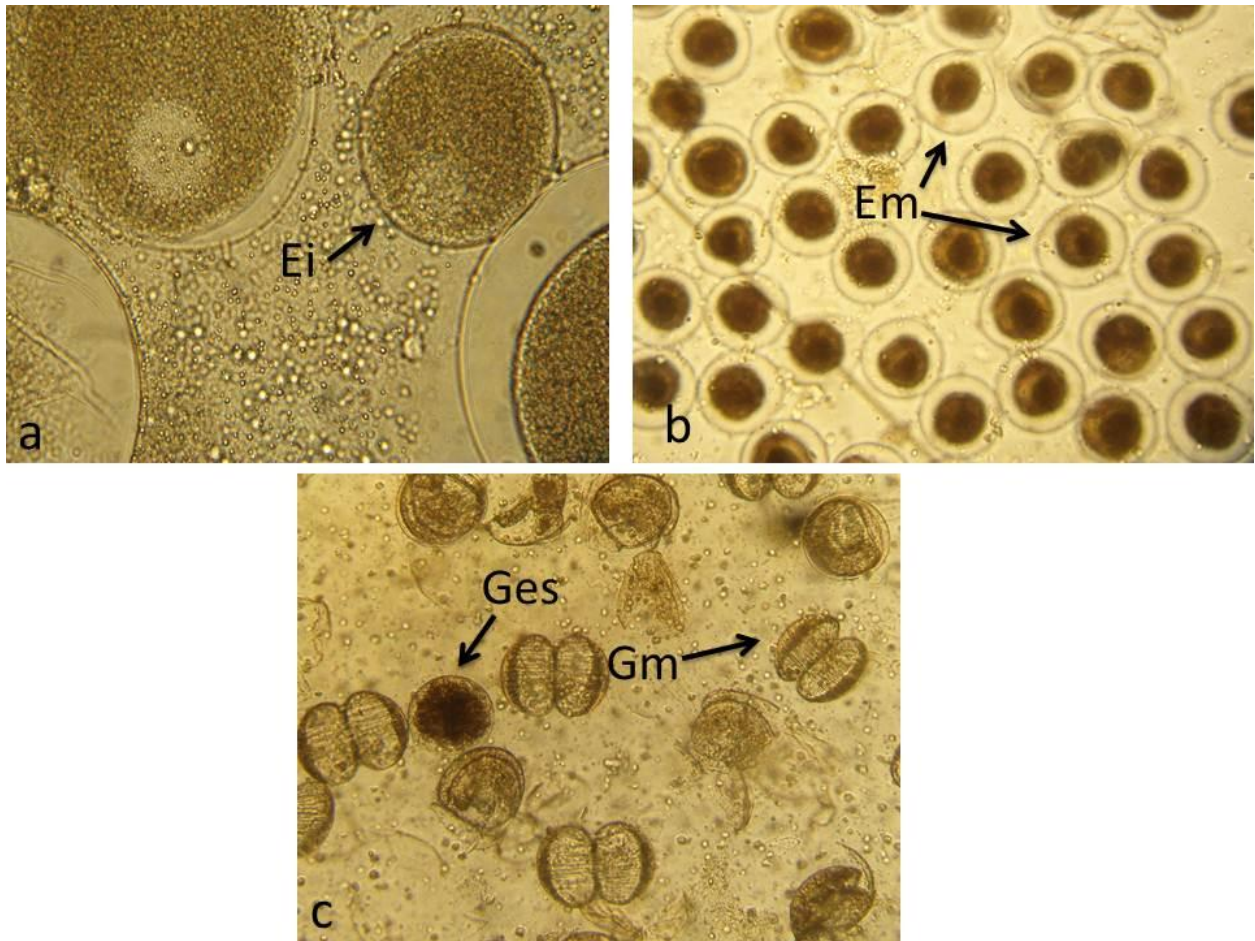


Figure 7: Gamete and fertilization stages of *F. ebena* a) Immature eggs (Ei) b) Mature eggs (Em) c) Early stage glochidia (Ges) and Mature glochidia (Gm)

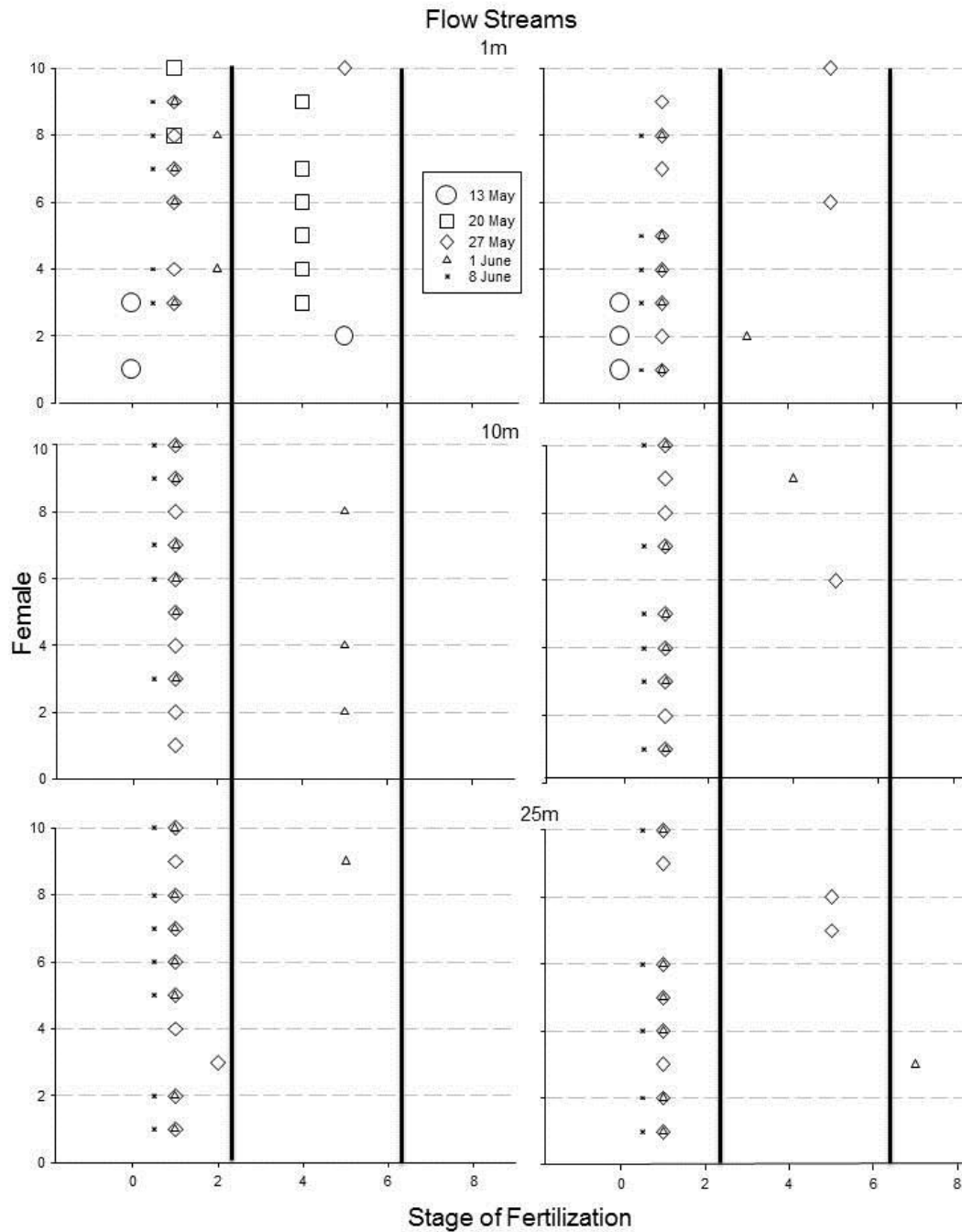


Figure 8: Results of weekly gravidity checks in flow streams at each distance. Each graph contains the results from a single pen of a single stream. Y-axis represents individual females. X-axis represents stage of fertilization from gill and gonad extractions (see table 6). Symbols decrease in size relative to later dates. Bold black lines bracket the stages where fertilization occurred.

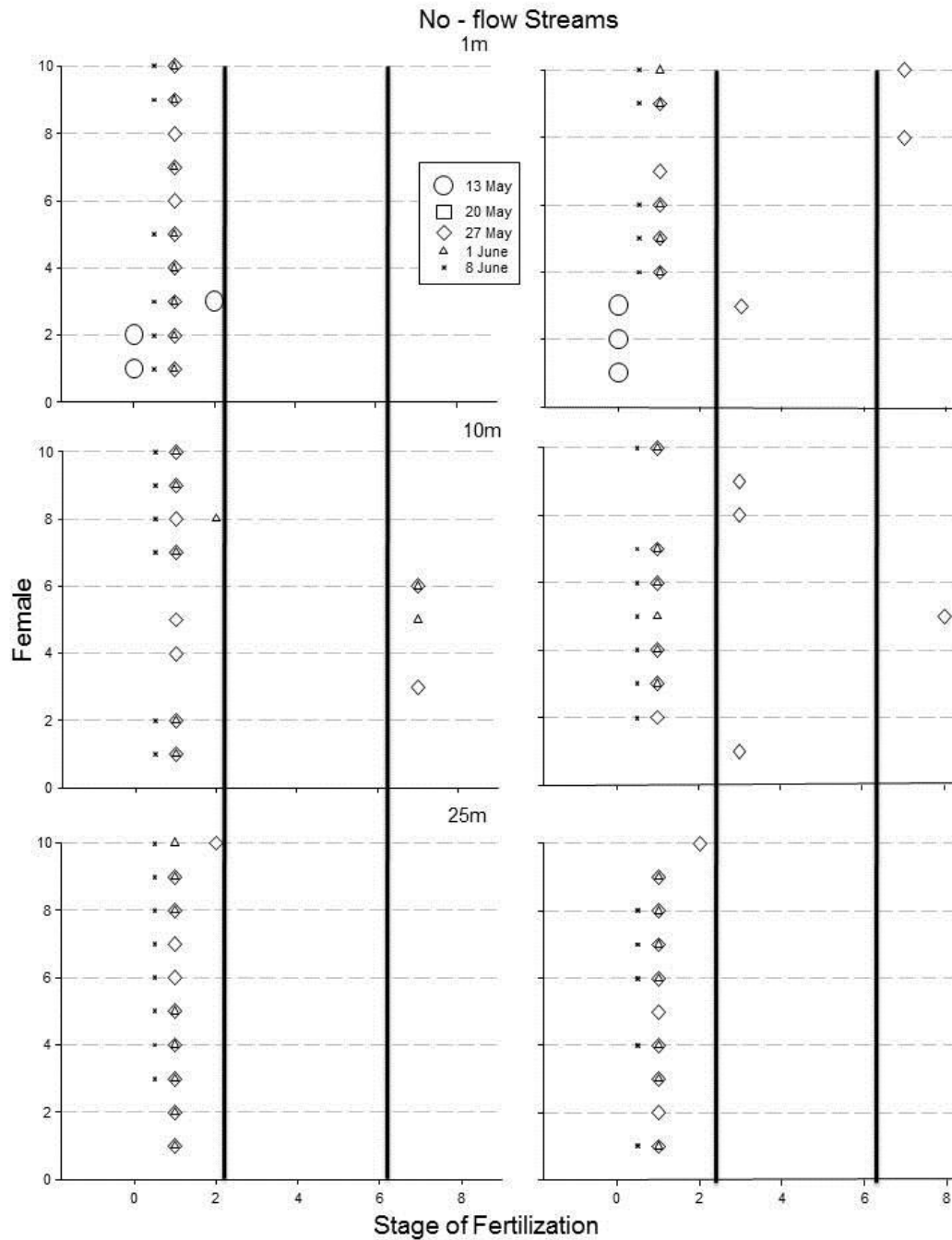


Figure 9: Results of weekly gravidity checks in no - flow streams at each distance. Y-axis represents individual females. X-axis represents stage of fertilization from gill and gonad extractions (see table 6). Symbols decrease in size relative to later dates. Bold black lines bracket the stages where fertilization occurred. Each graph contains the results from a single stream.

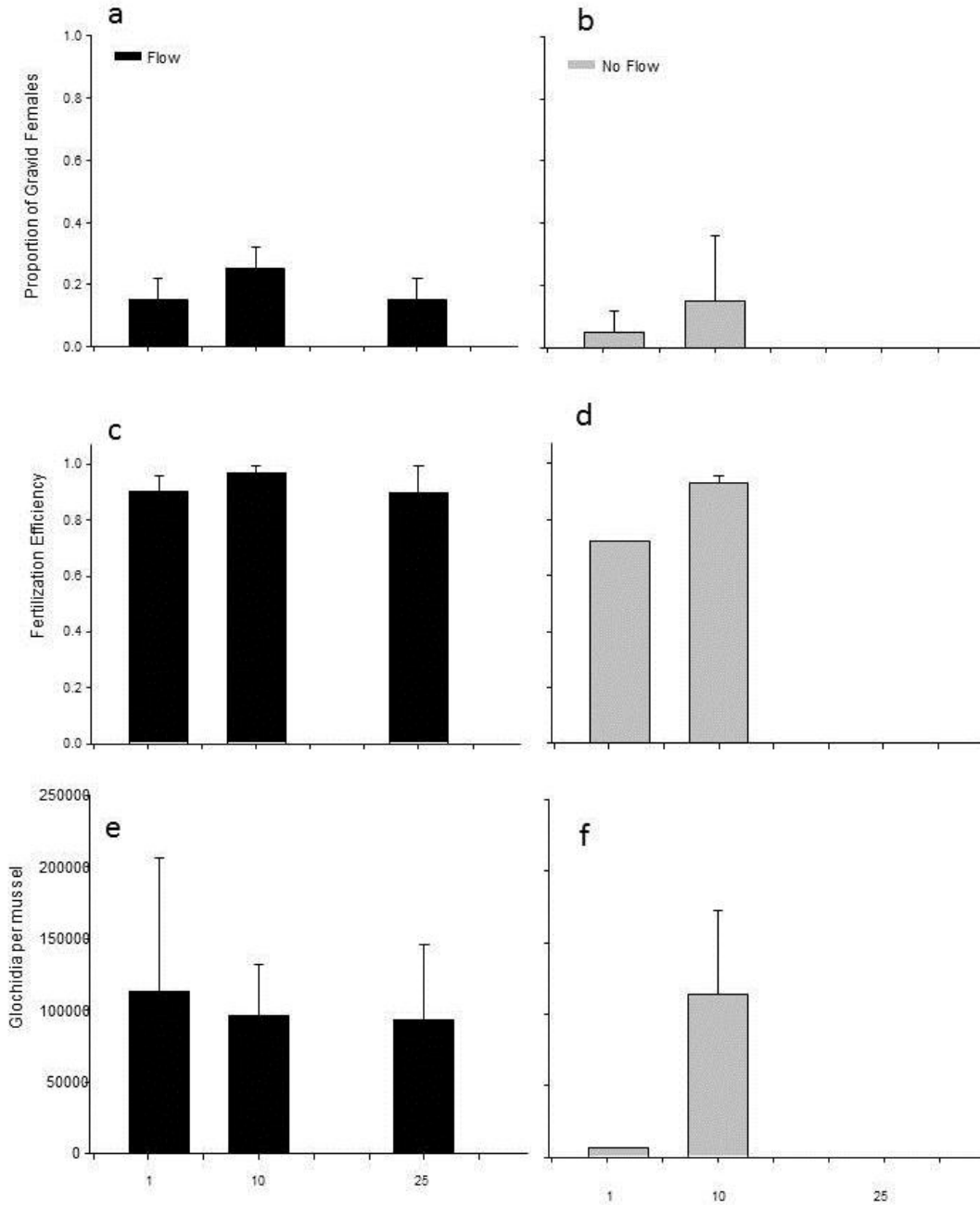


Figure 10: Proportion of gravid females in a) flow and b) no-flow streams; fertilization efficiency of gravid females in c) flow and d) no-flow streams. Glochidia per gravid female in e) flow and f) no-flow streams. Error bars represent ± 1 sd.

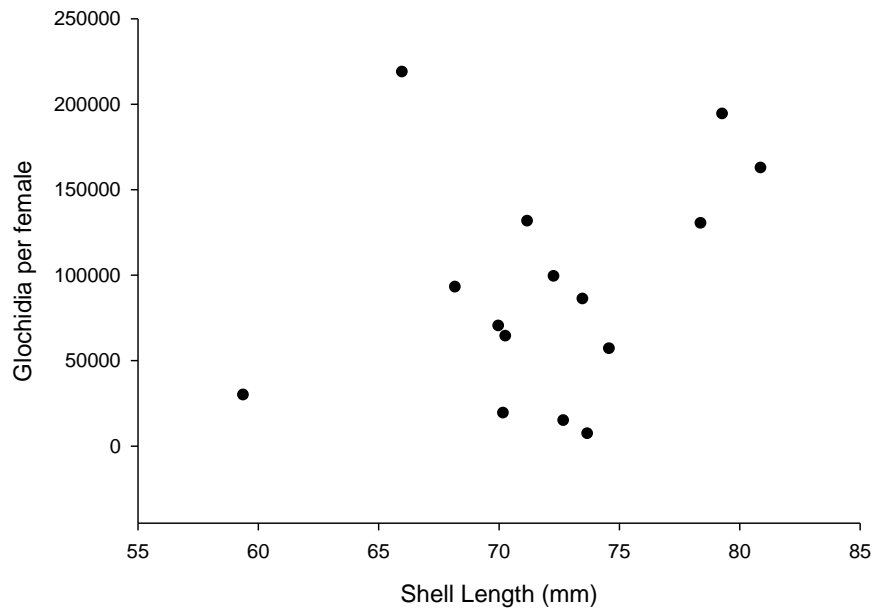


Figure 11: Glochidia per gravid female versus shell length