

Sperm retention in female *Nicrophorus investigator*

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Abstract: Burying beetles (*Nicrophorus*, Coleoptera: Silphidae) invest a great deal of time in parental care. The distinctive biparental care includes the burial, preparation, and defense of a small invertebrate carcass and regurgitation of pre-digested carrion for the young. While typically a male and female participate in such behaviors, it has been observed that a female can raise a brood on her own, therefore indicating females have the capability to retain sperm. A previous study on *Nicrophorus vespilloides* has shown the retention rate to diminish within the third week after the initial mating. This study focuses on the sperm retention rate of female *Nicrophorus investigator* and has shown females are able to retain sperm through the 31st day of retention after the initial mating. The results have implications with regard to both male and female behaviors associated with sperm retention and raising the young.

Introduction

Parental care studies show there is variance in the level of parental involvement, whether communal nesting, biparental care, uniparental care, or early desertion before any care is provided, and it fluctuates with relation to the trade-off between current and future reproductive success (Webb et al. 2002). Many avian communities invest in communal care under the concept of helpers. Communal breeding can be defined as “a social system...defined primarily by the regular involvement of helpers in the feeding and care of the young” where helpers can be defined as “a bird which assists in the nesting of an individual other than its mate, or feeds or otherwise attends a bird of whatever age which is neither its mate nor its dependent offspring” (Brown 1978). This definition can be extended to any communal care system in which the individuals all care for one other regardless of kinship. Biparental care incorporates the care of both the male and female when raising the young. It is fairly uncommon because males typically have the first opportunity to leave the young and seek additional mates when they are “‘emancipated’ from the care of young during gestation and lactation” (Gubernick and Teferi 2000). However, some species do partake in biparental care, and studies indicate the importance

of monogamy may have evolved over time as the additional care from the male increases offspring survival to an extent great enough to outweigh the costs of lost mating opportunities (Gubernick and Teferi 2000). In other cases, only one parent, typically the female, will raise the young in a form of uniparental care, resulting from the second parent deciding to desert before providing any care at all for the offspring (Webb et al. 2002). Finally, some offspring are left with no parental care in instances such as sea turtles where the eggs are laid, buried, and the young are left to survive on their own once they hatch. A current desertion model suggests that offspring are deserted a particular age and time. The average length of parental care in a population is determined with consideration given to chance of remating within a current breeding season, and the number of breeding attempts made is determined by these patterns of care and desertion (Webb et al. 2002).

Burying beetles (*Nicrophorus*, Coleoptera: Silphidae) exhibit a variety of rare and unique habits when raising offspring. Though unusual for many species, they partake in biparental care in which both the male and female interact cooperatively in the rearing process, beginning as the two search independently for the small vertebrate carcasses on which the larvae will be reared. They encounter a carcass using sensitive chemosensors and, if necessary, they move it to a suitable location for burial (Scott 1998). Once a carcass is in the correct location, the male and female both work to prepare and bury the carcass and hollow out a cavity in which the young will feed on predigested carrion (Eggert and Müller 1997). In order to prepare the carcass, the beetles remove all hair, roll the carcass into a ball, and ensure the carcass is both moist and mold-free through the depositing of anal secretions, which delay the rate of decomposition. The final chamber is buried on average at about 10 cm beneath the surface, though it may be as deep as 60 cm into the ground, and eggs are then laid in the nearby soil (Scott 1998). When the larvae hatch,

they crawl to the carcass, gather around the predigested carrion, and both the male and female will participate in feeding the young through regurgitation (Suzuki 2004). While tending to the young, both parents also work to defend the carcass and larvae from invading individuals of their own or another species (Müller et al. 2007). Females tend to have more direct contact when caring for the larvae and spend more time provisioning whereas the males adjust to the necessity of care providing based on the female and spend the remainder of the time defending the brood site (Smiseth and Moore 2004). One study showed that the survival and mass of the larvae is significantly higher in broods receiving biparental care throughout the development cycle (Eggert et al. 1998), although not all studies have found this. Extended parental care is thought to be due in part to the unpredictability of carcass availability. As a carcass is also valuable to other taxa, the beetles respond accordingly by treating each reproductive event as their only opportunity to breed, therefore investing a great deal of time in attempting to maximize their individual fitness level with that particular brood (Scott 1998).

Interestingly, a uniparental care system can also exist, as a female can store sperm in a spermatheca and raise a brood on her own (Eggert and Müller 2000). In this situation a male could mate with a female away from a carcass or during an initial brood care event and have future offspring without providing any care. The only documented study within the genus *Nicrophorus* found that the sperm stored in the spermatheca of female *Nicrophorus vespilloides* became inviable after two to three weeks (Eggert and Müller 1992). In our study, we describe the location and morphology of the spermatheca and assess the sperm retention rate in female *Nicrophorus investigator* using the presence of offspring as a measure of sperm viability. The results provide additional insight into the related behaviors of both males and females in the

process of parental care, focusing on understanding the duration of male parental care and alternative mating tactics.

Materials and Methods

Nicrophorus investigator overwinter in the soil as prepupae and emerge as adults the following summer. For this study, I trapped over 200 adults between June 22, 2009 and July 12, 2009 from three field sites at the Rocky Mountain Biological Laboratory, RMBL, Gunnison County, Colorado. In order to capture the beetles, metal cans filled with dirt and baited with a piece of raw chicken were used. At the Vera Falls Meadow site, I buried five metal cans into the ground such that the opening at the top of the can was level with the ground. A metal screen with a beetle-sized hole was then spread across the can and secured with a large rubber band. The screen was fixed in a concave fashion, which allowed the beetles to enter the trap but prevented them from leaving as their natural instinct to escape it to crawl up and the entrance hole is located at the lowest point of the screen. To prevent bait-robbing by fox and other carnivorous animals, a .3 m x .3 m square quarter-inch hardware cloth was nailed over the top of the can and reinforced by rocks at each of the four corners. A similar set-up was used at the Murray lab site with the additional placement of two-foot tall 5.08 cm x 10.16 cm metal fencing supported by stakes surrounding each of the five cans. In the aspen grove behind Weekley Cabin, 22 cans were placed in an oval throughout the grove and secured to trees approximately one and a half meters above the ground. The cans were prepared in the same fashion as the cans in the other two sites and were secured to the trees using baling wire.

At 8 AM on July 13, 2009 I selected 140 wild-caught females and 140 wild-caught males at random and they were held for a 24-hour period in separate containers. Each female was placed individually in a 9 cm x 13 cm x 17 cm plastic storage bin containing raw chicken as a

food source, and the males were held communally in a larger bin, also with raw chicken. The following day, July 14th, at 8 AM, I placed one male with each female. At 45-minute intervals, I observed the pairs for interactive behaviors including proximity, activity, mounting, and mating. I took 18 observations from 8 AM to 9:45 PM. At 10 PM I then combined the beetles into five large storage bins, each containing 28 beetle pairs to ensure all females were mated. After an additional 19 hours and nine observations, the males were removed from the containers. Moist paper towels and food were provided to the females and they were housed in the RMBL animal room with indirect window light and ambient temperature.

After ten days of retention, ten females were selected at random, two from each of the five tubs on the morning of the 11th day. I recorded the mass, length of pronotum, and length of elytra of each of the ten females and placed them individually into a 9 cm x 13 cm x 17 cm plastic storage bin filled with approximately 6 cm of soil and a thawed white lab mouse weighing between 20-21.99g. The presence of the mouse stimulated the females to lay their eggs. I observed the females a minimum of five times daily to determine the stage of carcass burial, the egg laying activity, and the hatching success. Live larvae indicated viable sperm was present in the spermatheca. This process was repeated, each time with ten new females randomly selected, on the 13th, 15th, 17th, 19th, 21st, and 25th days after hatching, at which point the summer session ended. I was able to make observations through the carcass burial of the sixth group, those beginning at day 21. My observations of the carcass burial of day 25 and egg laying activity and hatching success of day 21 and 25 individuals were continued, at intervals of much less frequency (three times per day), by Ellen Gurton, another member of my lab. Members of Dr. Rosemary Smith's lab continued the experiment with the remaining females for the 31st, 35th, and 40th days. A second group of mated females were held from August until mid-September and

were then given a mouse at the 45th, 48th, 51st, and 56th day after mating, stimulating the egg-laying process. Burial and larval observation data were not collected for the 31st-56th day trials. Instead, brood success was measured as at least one viable larvae.

In order to confirm that the fertility of the female does not decrease due to age or captivity, a control group was used for comparison. I randomly selected an additional 18 wild-caught females and held them separately in the RMBL animal room in a sixth plastic container identical to those holding the experimental females. On the tenth day after the initial experimental mating, three females were selected at random from the control containers and paired with three randomly selected wild-caught males. I recorded the mass, length of pronotum, and length of elytra of each individual and each pair was placed in a plastic container along with soil and a white lab mouse between 20-21.99g, under the same conditions as the experimental females. The process was repeated on the 16^h, 21st, 27th, 40th, and 56th day after the original mating to confirm female fertility. I observed the mating behavior, carcass burial, egg laying, and offspring viability for a minimum of five times daily. I was able to make observations through the hatching of day 10 and day 16 individuals and through the carcass burial of day 21 individuals. Ellen Gurton continued with my observations (three times per day) through the hatching of the day 21 individuals. Burial and larval observation data were not collected for the 27th-56th day trials. Instead, brood success was measured as at least one viable larvae.

Finally, after the experiment I dissected all females who did not produce viable offspring or lay eggs to check the contents of their spermatheca. To prevent the killing of any live sperm through the use of freezing or ethanol, I decapitated the females and cut off the abdomen segment of their body. Under a dissecting microscope, the terga of the abdomen was carefully removed by cutting slits along the lateral ridges toward the anus and carefully peeling the terga

away from the abdominal tissue in the direction of the anus. I then extracted the most distal abdominal tergite and exposed the hindgut and female genitalia by cutting a small slit along one side of the lateral ridge, and peeling aside the terga while pulling the segment away from the remainder of the stationary abdomen. This gradually exposed the digestive tract and two pale yellow bursas. The spermatheca was located nestled between the two bursas (Figure 1). While the bursas are slightly yellow in color, the spermatheca is a translucent pearly white. It is the only organ to retain its shape when squeezed with the fine forceps, due to the associated sclerotized pump, and is typically bean-shaped and compact, though slightly elongated with the presence of live sperm. Once extracted from between the bursa, the spermatheca was placed on a slide and a wet mount was created. The slide was viewed on a Zeiss microscope under 400x magnification. The sperm was visible as fine silver threads clustered heavily around the perimeter of the pressed spermatheca. Though dead, and therefore immobile, when present in the infertile females, when compared to a recently mated female, the sperm was alive and moved in a whip-like fashion.

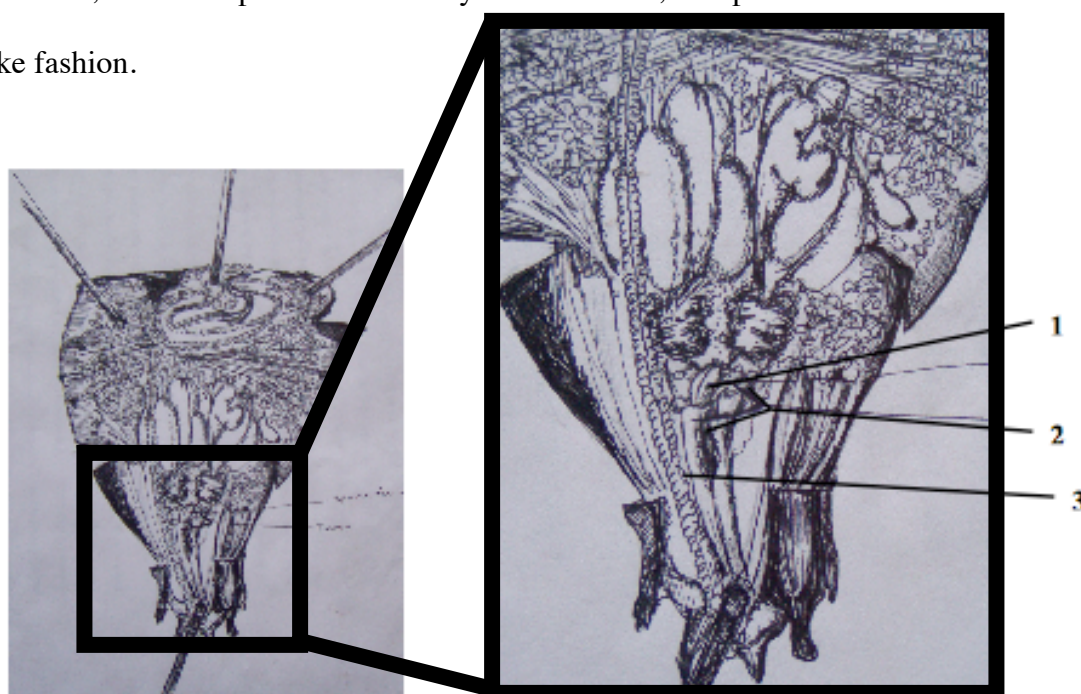


Figure 1. Female *Nicrophorus* abdomen. Dorsal-ventral view. Magnified 10x. Line 1 indicates the spermatheca. Line 2 indicates the bursas. Line 3 indicates the digestive tract.

Results

There was no significant difference in mass, elytron length, or pronotum length for the females within the experimental or control trials, or between experimental and control trials. Any variation among the values was not due to time passed since initial mating (Table 1).

In the experimental trials when all trials are included, there is a slight increase in length of time passed until burial and hatching as days past initial mating increase. However, if the seventh group is excluded, the time until burial remains fairly constant over the course of the experimental trials. If the sixth and seventh groups are excluded, the time until hatching declines as time past initial mating increases (Figure 1).

In the control trials when all trials are included, there is a decrease in time until burial and an increase in time until hatching as time past the initial experimental mating increases. If the third group is excluded from time until hatching, there is a decrease in time until hatching as time past the initial experimental mating increases (Figure 2).

When comparing the experimental to control trials, when group seven of the experimental trials are excluded, the time until burial for the experimental groups remains constant while the control group time until burial decreases (Figure 3). When the sixth and seventh experimental groups are excluded and the third control group is excluded, there is a similar decrease in time until hatching for both experimental and control designs (Figure 4).

The proportion of females capable of producing a brood (= retain viable sperm) remained high (>90%) for 31 days after mating (Figure 5). After 35 days, only 40% of females were able to produce viable offspring, and (in the later experiment) no females were able to produce viable offspring by day 48 after the initial mating. In comparison the control females (fresh sperm) also had high brood success up to 30 days, with a decline to 50% brood production after 40 days of

being held in captivity (Fig 5). Although there were significant differences in mean brood weights among the broods produced in trial (ANOVA, $F=5.7383$, (59,7); $p<0.001$), this was not related to the time since mating (Figure 6).

Discussion

Adult body size does not influence female egg production, but it does limit reproductive success, as smaller individuals are less frequently able to secure and maintain a carcass for their brood (Scott 1998). Because there was no competition for carcass resources in this experiment, and because size does not influence the egg production, the slight variation among individual sizes are not statistically significant nor are they possible factors for variation in brood production over time.

For the experimental trials, my observations terminated before the viewing of the seventh group burial time. This may explain the differences observed in the charts. When the seventh group is excluded, the time until burial remains fairly constant, but when Ellen's observations of the seventh group are included, there is an increase in time until burial. This discrepancy may be a result of frequency in observation made. I made observations at many points throughout the day, and as a result it is likely that I more closely pinpointed the time of burial. Many critical hours may have passed from the actual time of burial until the time of the observation made by Ellen, and this would explain the increase seen when her observations are included. I was also unable to observe the hatching of the sixth and seventh groups. When these groups are included, hatching time remains relatively constant. When groups six and seven are excluded, there is a decline in time until hatching. This again may be a variation in observation frequency.

In the control trials, I was able to see the burial of all three groups. However, I did not observe the hatching of the third group. As with the experimental trials, the inconsistency in results whether the third group is included or excluded is likely due to the difference between my and Ellen's observations.

To maintain a level of constancy, only the trials that included my observations and excluded Ellen's were used to compare the experimental to control groups. This representation showed that over time, the burial time in the control groups decreased. When two parents are present and time passed original mating is greater, the two beetles are able to bury the carcass at a much faster rate than the solo females of the experimental groups. Hatching time between the two groups maintains a steady decline in both the experimental and control groups and is not affected by the time passed since the initial experimental mating. The decline in hatching rate may be due to better identification of hatching time when observing, an increase in outdoor temperature as it became later in the season, or perhaps the smaller time frame between which hatching time and pupal encasement for overwintering must occur. The factor of greatest influence could be determined in future experiments. Multiple individuals could make observations of the same groups to account for greater accuracy in pinpointing the hatching time. Hatching times could be observed early in the season through late season, recording temperature changes as the season progresses, to monitor influence of temperature and time until the necessary pupal encasement to determine if these are contributing factors. Further studies must be carried out to better understand the role of these aspects in hatching time.

These data show female *Nicrophorus investigator* can retain sperm for 31 days and still produce viable offspring before there is a noticeable decline. All control females were able to produce viable offspring on the 27th day after the initial experimental mating and by the 40th day

half of the control females were still able to produce viable offspring. Note that the small number of control females used in each trial may have influenced the drop in retention rate at the 40th day.

My expectation was that fertility would decline in 2-3 weeks and as such, I provided females with mice at day 11 post-mating and continued to day 30, thinking this would encompass a period of declining fertility. However, my data clearly show that females retain fertile for much longer than expected (at least 30 days). This experiment generates the necessity to repeat the trials with larger numbers of mated and control females held for at least 25 days before attempting to lay eggs.

Although the premise of this experiment was that females are storing sperm (as is what I observed in the spermatheca), it is possible that females may store fertilized eggs. Thus, the phenomena would not sperm storage but rather fertilized egg retention. Further studies would be needed to determine which of these two possibilities is occurring.

The duration of sperm storage has implications for the breeding and parental care behaviors. When the beetles partake in biparental care, the adults remain with the offspring until they complete the third instar stage of development and leave the carcass to pupate. Mating and egg fertilization take place during the carcass burial process. Eggs are laid soon after burial and hatch within days. The growth rate of larvae depends solely on the carcass resource prepared by the parents, and the young larvae require parental feeding. As a result, the parents will attend the brood and carcass for about 12 to 18 days until the carcass is fully consumed and the larvae distribute to pupate (Smith et al. 2000).

My results support the hypothesis that the continued male presence may in fact be for purposes other than carcass defense and maintenance (Jenkins et al. 2000). Given that the

females are able to sufficiently retain sperm for 31 days, by the time she has the opportunity to leave in search of a new carcass, the sperm still retained within the spermatheca from the father of the current brood will be in the process of degradation. If the male deserts early, he would only be guaranteed one successful brood. However, in remaining over the full course of the larval development, he can ensure the carcass is tended to such that the offspring are able to survive, but also continue to mate with the female. In doing so, he increases the likelihood that she raises a second brood in the current mating season with his sperm should she find another carcass before the end of the season. As she goes in search of new carrion, he leaves to seek both another carcass and second mate of the season. Because the females are able to retain sperm for such an extended period of time, they can rear a second brood in the same season without finding a second mate. As a result, the males have the potential of fathering as many as three broods within one season while only providing the parental care to two broods. This sperm retention process increases male fitness through producing a greater number of offspring while expending fewer resources and less energy in a given season. The duality of the beetles participating in both biparental and uniparental care systems ultimately results in the greatest number of offspring at the lowest expense under the premise of extensive sperm retention of 31 days by the female *Nicrophorus investigator*.

Literature Cited

- Brown, Jerram L. 1978. Avian Communal Breeding Systems. *Annual Review of Ecology and Systematics*. **9**: 123-155.
- Eggert, A. -K. and J. K. Müller. 1992. Joint breeding in female burying beetles. *Behav. Ecol. Sociobiol.* **31**: 237-242.

- Eggert, A. -K. and J. K. Müller. 1997. Biparental care and social evolution in burying beetles: lessons from the larder. *The Evolution of Social Behavior in Insects and Arachnids* (J. Choe, B. Crespi, eds.): 216-236.
- Eggert, A. -K., M. Reinking and J. K. Müller. 1998. Parental care improves offspring survival and growth in burying beetles. *Anim. Behav.* **55**: 97- 107.
- Eggert, A. -K. and J. K. Müller. 2000. Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*). *Behav. Ecol.* **11**: 357-366.
- Gubernick, David J. and Taye Teferi. 2000. Adaptive Significance of male parental care in a monogamous animal. *Proc. R. Soc. Lond.* **267**: 147-150.
- Jenkins, E. V., C. Morris, and S. Blackman. 2000. Delayed benefits of paternal care in the burying beetle *Nicrophorus vespilloides*. *Animal Behavior*, 60:443-451.
- Müller, J. K., V. Braunisch, W. Hwang and A. -K. Eggert. 2007. Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. *Behav. Ecol.* **18**: 196-203.
- Scott, M. P. 1998. The ecology and behavior of burying beetles. *Annu. Rev. Entomol.* **43**: 596-618.
- Smiseth, P. T. and A. J. Moore. 2004. Behavioral dynamics between caring males and females in a beetle with facultative biparental care. *Behav. Ecol.* **15**: 621-628.
- Smith, Rosemary J., Amy Hines, Stephanie Richmond, Melissa Merrick, Allison Drew, and Rachelle Fargo. 2000. Altitudinal Variation in Body Size and Population Density of *Nicrophorus investigator* (Coleoptera: Silphidae). *Environmental Entomology*. **29**: 290-298.

- Suzuki, S. 2004. Brood size reduction in *Nicrophorus vespilloides* after usurpation of carrion from *Nicrophorus quadripunctatus* (Coleoptera: Silphidae). *Entomological Science*. **7**: 207-210.
- Webb, James N., Tamás Székely, Alasdair I. Houston, and John M. McNamara. 2002. A theoretical analysis of the energetic costs and consequences of parental care decisions. *Phil. Trans. R. Soc. Lond.* **357**: 331-340.

Experimental Trials							
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Days Past Initial Mating	11	13	15	17	19	21	25
Average Female Wt (g)	0.353	0.353	0.357	0.375	0.309	0.366	0.347
Standard Deviation Female Wt	0.086	0.100	0.118	0.089	0.100	0.085	0.075
SEM Female Wt	0.027	0.032	0.037	0.028	0.031	0.027	0.024
Average Female Ely (mm)	9.59	9.50	9.51	9.73	9.07	9.79	8.39
Standard Deviation Female Ely	0.85	1.09	1.26	0.91	1.16	0.89	1.02
SEM Female Ely	0.27	0.35	0.40	0.29	0.37	0.28	0.32
Average Female Pro (mm)	5.57	5.47	5.43	5.64	5.22	5.66	5.53
Standard Deviation Female Pro	0.47	0.59	0.65	0.48	0.60	0.47	0.53
SEM Female Pro	0.15	0.19	0.21	0.15	0.19	0.15	0.17
Control Trials							
	Group 1	Group 2	Group 3				
Days Past Initial Mating	11	16	21				
Average Female Wt (g)	0.327	0.445	0.446				
Standard Deviation Female Wt	0.107	0.130	0.070				
SEM Female Wt	0.061	0.075	0.040				
Average Female Ely (mm)	9.12	10.43	10.46				
Standard Deviation Female Ely	1.07	1.02	0.75				
SEM Female Ely	0.62	0.59	0.44				
Average Female Pro (mm)	5.38	6.01	5.95				
Standard Deviation Female Pro	0.63	0.59	0.30				
SEM Female Pro	0.37	0.34	0.17				

Table 1 Average, mean standard deviation, and standard error of mean of the mass, elytron (ely) length, and pronotum (pro) length in experimental and control trial females

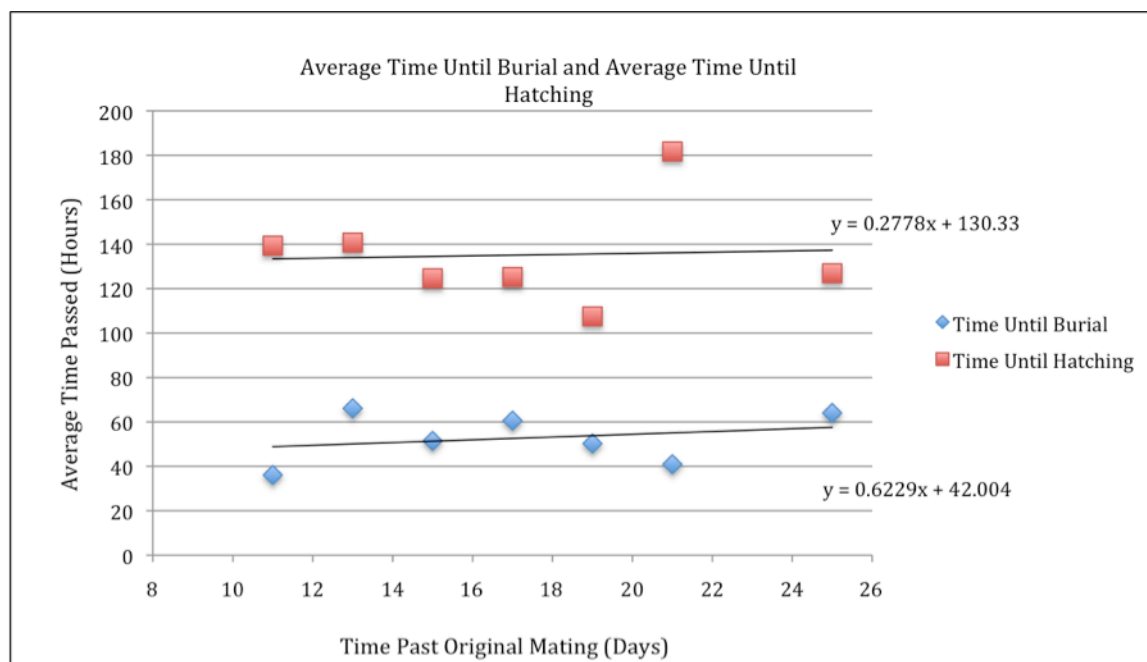
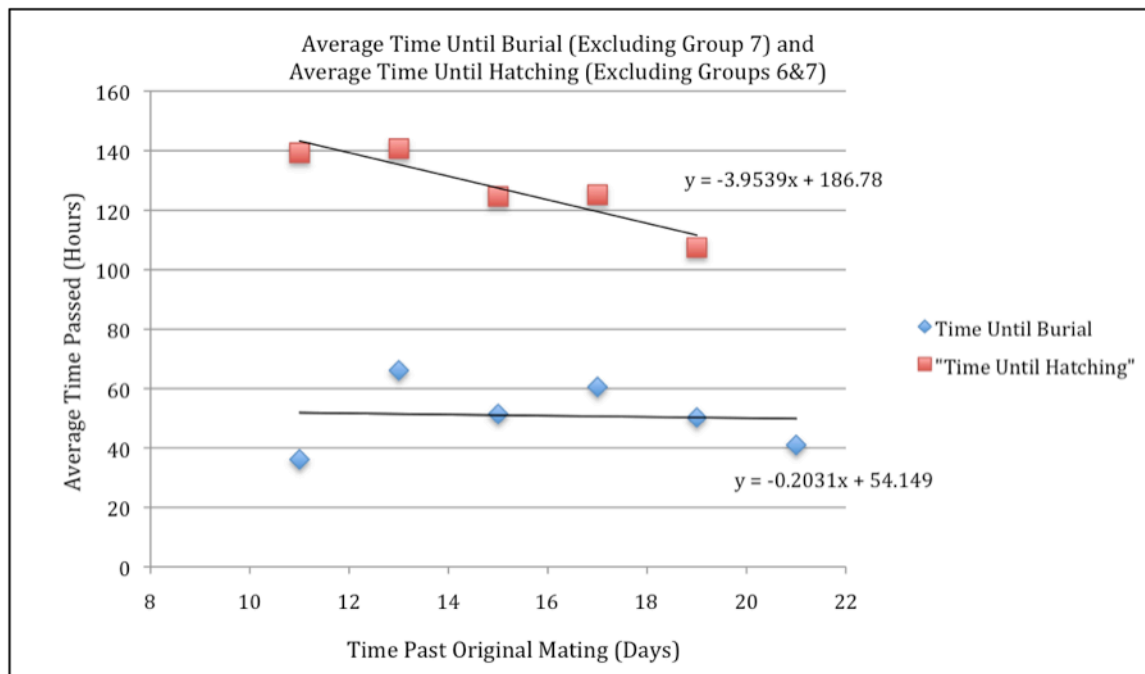


Figure 1 Average time until burial and hatching in experimental trials

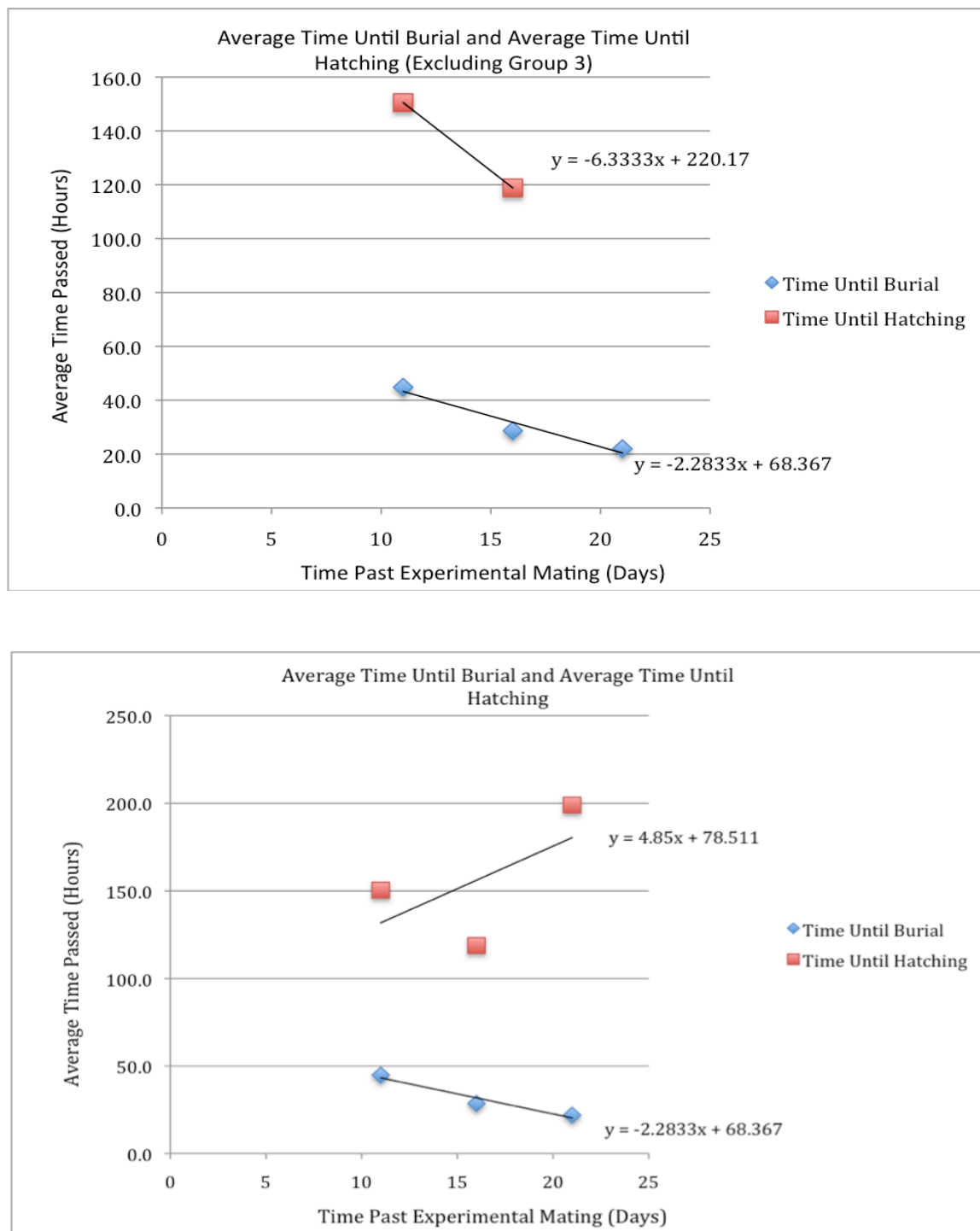


Figure 2 Average time until burial and hatching in control trials

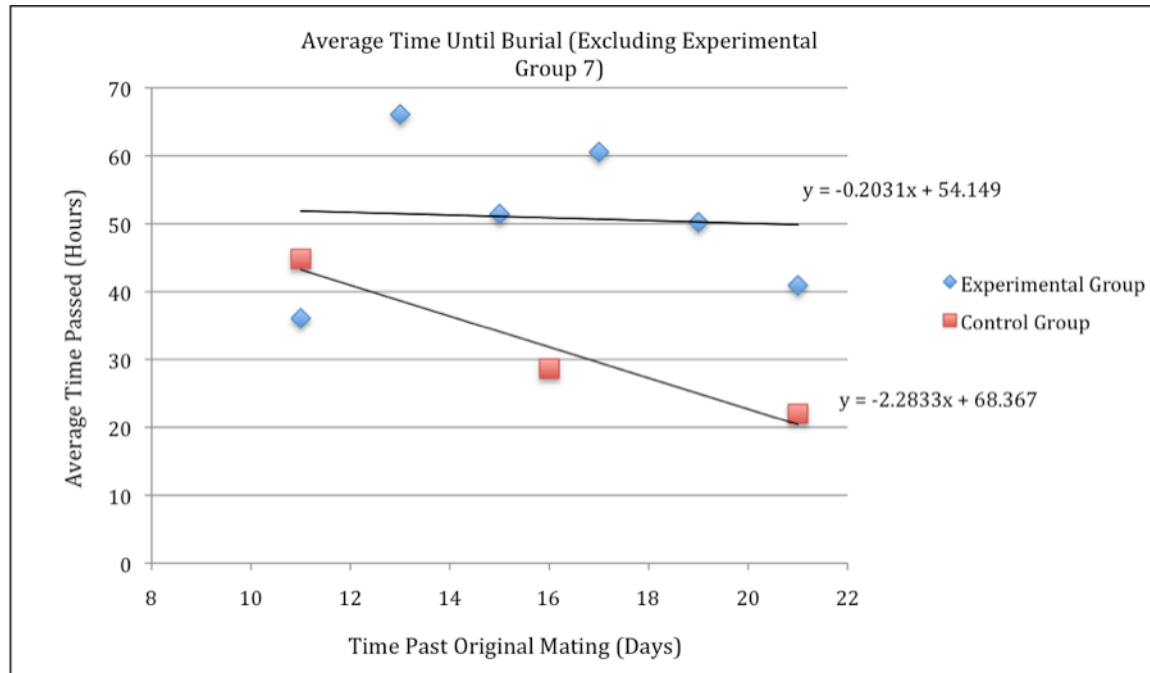


Figure 3 Average time until burial in experimental vs. control trials

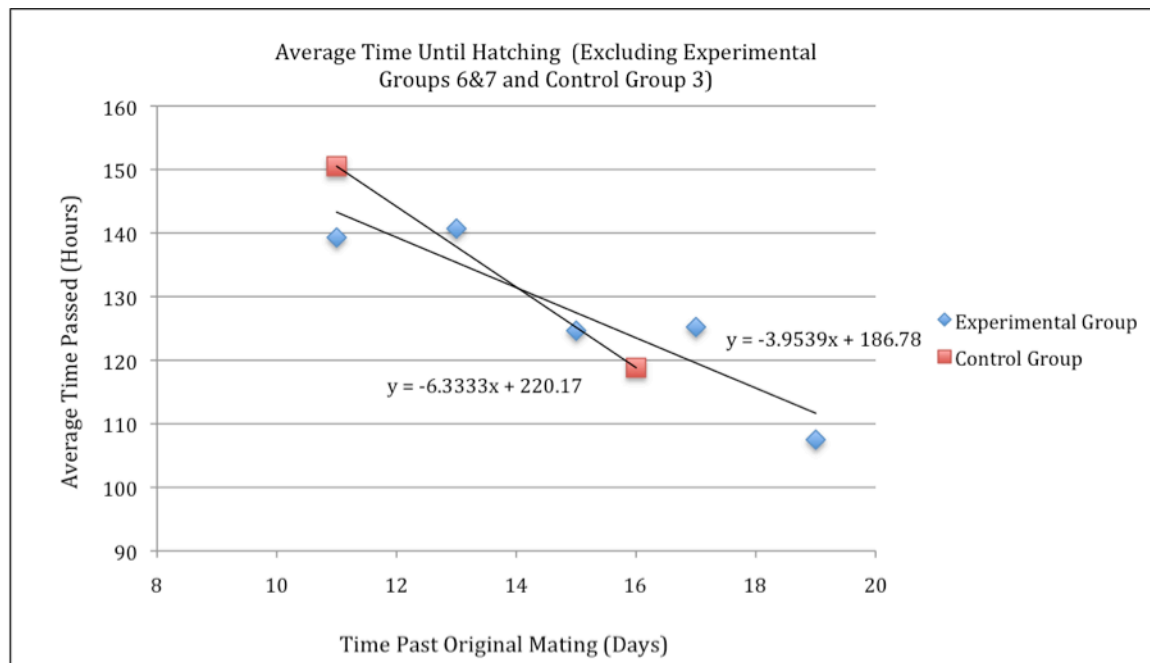


Figure 4 Average time until hatching in experimental vs. control trials

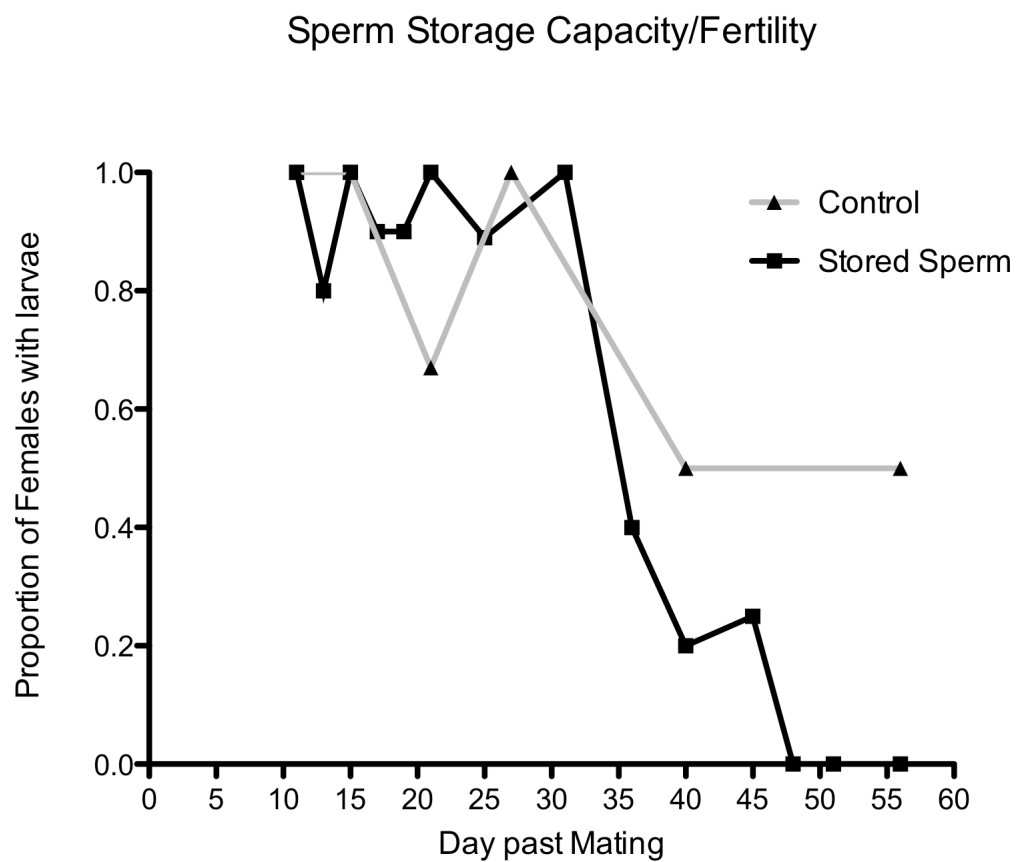
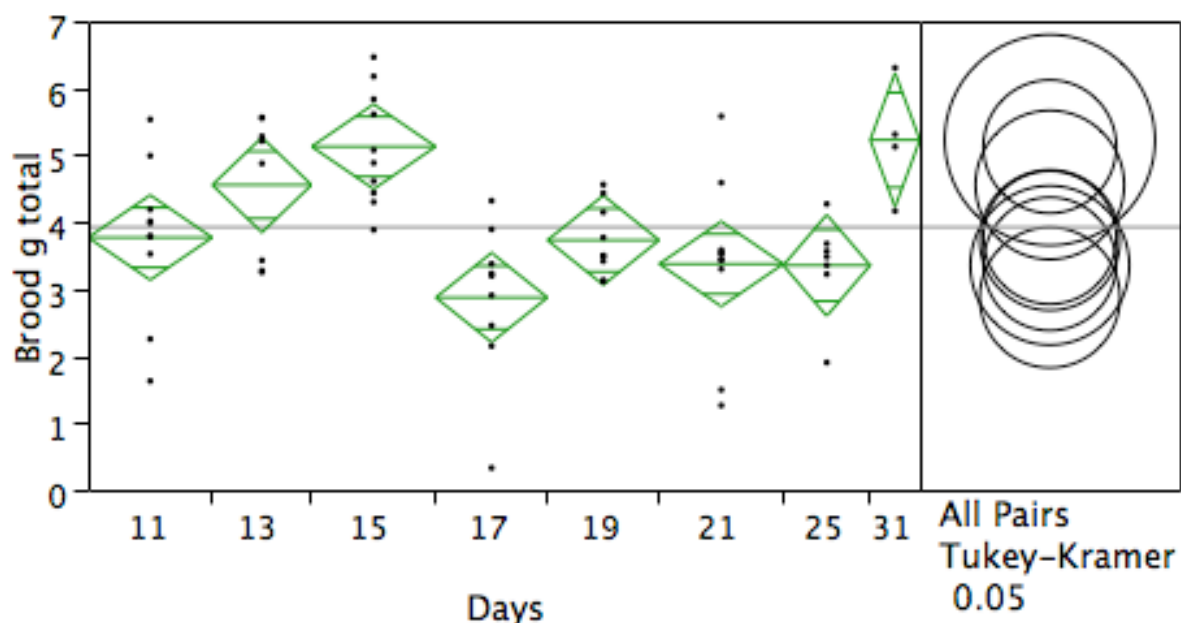


Figure 5 Proportion of females producing larvae by days past initial mating



Summary of Fit

R^2	0.405052
Adjusted R^2	0.334465
Root Mean Square Error	1.001033
Mean of Response	3.923508
Observations (or Sum Wgts)	67

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Days	7	40.251435	5.75020	5.7383	<.0001
Error	59	59.121969	1.00207		
C. Total	66	99.373403			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
11	10	3.76800	0.31655	3.1346	4.4014
13	8	4.55350	0.35392	3.8453	5.2617
15	10	5.12610	0.31655	4.4927	5.7595
17	9	2.87178	0.33368	2.2041	3.5395
19	9	3.72856	0.33368	3.0609	4.3962
21	10	3.37360	0.31655	2.7402	4.0070
25	7	3.35272	0.37835	2.5956	4.1098
31	4	5.22450	0.50052	4.2230	6.2260

Std Error uses a pooled estimate of error variance

Figure 6 One-way ANOVA of the total brood weight by days of sperm retention