

Reproductive Success and Parental Care of *Nicrophorus investigator*

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ABSTRACT

For years the standard of care in burying beetles has been a male and female, bi-parental care, but is it really? The intent of this experiment was to measure the success of burying beetle larvae produced under four different parental conditions after carcass preparation; 50 pairs of *Nicrophorus investigator* were collected from the wild and randomly assigned treatments of both parents, solo female, solo male, and neither parent, imposed the day that larvae hatched. The time it took for each stage of development was recorded. Once the larvae reached the last instar and consumed the carcass they were counted and weighed.

The traditional pair of male and female parents was predicted to lead to the best larval growth (total brood mass, average larval mass, and larval number). The larvae without parents were not predicted to survive, and the uniparental (male or female parent only) were predicted to fall in between. The results found that broods with parents (at least one) did better than broods without attending adults (broods raised by solo females were just as successful as broods raised with both parents, and male and no parents were the poorest). Solo male parents produced significantly smaller broods, and broods without parents were able to survive without parental feeding. These results can give us more insight to the different behavior of male and female burying beetles.

INTRODUCTION

Burying beetles (Coleoptera: Silphidae: Nicrophorus) are a type of carrion beetle that feed and reproduce in the carcasses of small vertebrates. The chemo-sensors on the end of a burying beetle's antennae can sense the sulfur containing chemicals emitted by a carcass (Kalinova, 2009). Once they find a carcass they roll it into a ball, strip the fur or feathers, deposit anal secretions to prevent decay, and bury it to hide this rare resource from competitors. After the carcass is prepared the parents will lay the eggs six to ten centimeters away. Just before the eggs hatch the parents will regurgitate fluids in a depression on the carcass to make it softer for the hatching larvae to eat. After the larvae have hatched and are inside the carcass the parents will continue to maintain and protect the carcass while regurgitating food to the larvae. During this process the entire carcass is consumed by both the larvae and the parents. (Trumbo, 1990a)

The extended parental behavior of the burying beetle categorizes them as semi-social insects. They are in-between the extremely social insects, such as bees and ants, and extremely asocial insects, such as grasshoppers and butterflies. Burying beetles are also unique because they have the ability to hide a resource from the competition, in this case other burying beetles, flies, and ants. These behaviors of the parents are thought to give significant insight to the evolution of nesting behavior. (Trumbo, 2012)

With so much attention given to their parental care it leads to many questions, what selects for both parents to be so committed to the young? How critical is the regurgitation of food and other continued parental care after the carcass is prepared? Are males and female

equivalent in their ability to provide care to the larvae (post-hatching?). The costs incurred in parental care (investment) include both time and energy, so that the benefit (improved larval condition, faster development) must exceed these costs to be selected for. Since the earliest papers on different species of burying beetles, bi-parental care has been considered the standard for this insect (Eggert 1998, Rauter 2002, Smiseth 2007). The purpose of this experiment was to question the role of parents in the success of larvae after carcass preparation. This experiment was designed to extend preliminary research at Rocky Mountain Biological Lab in the summer of 2013, in which *Nicrophorus investigator* larvae were found to survive with only one, or even no parents at all. If the larvae without parents survived, it would lead to a new question. How successful are broods raised with two parents, solo female, solo male, or no parents at all?

Treatments were designed so that larvae were reared under four experimental conditions that spanned the range of parental care behaviors found in *Nicrophorus*: biparental, uniparental (male and female), and no parental attention. This will provide the necessary information on the relative fitness of each strategy, which may help to explain the different parental care behaviors of each sex.

Based on past studies, it was predicted that given the same amount of resource (mouse grams) the broods tended by both a male and female parent would be more successful (more than two larvae), and larger in size and number (total brood mass). With only one parent (male or female) a smaller percentage would be successful, or their growth would be reduced, and an even smaller percentage would survive and their development would be poorer. A second prediction is that bi-parental care speeds larval development, so that the advantage of

biparental care is to increase the parent's future reproduction, as opposed to the condition of the current brood.

METHODS

Field Sites

This experiment was conducted at Rocky Mountain Biological Laboratory field station, located at 2,900 m elevation in Gothic, Colorado. The beetles used for this experiment were trapped from the wild along both sides of Gothic Hwy just north of the Gothic Town site (See Figure 1).

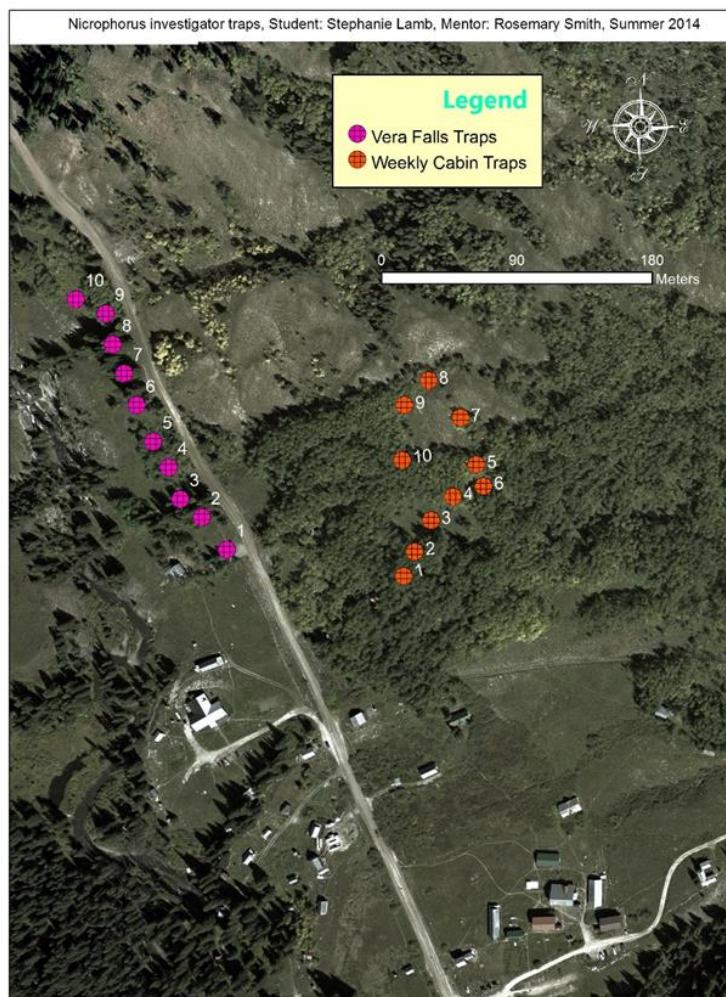


FIG. 1 Traps were set along east and west sides of Gothic Hwy

Traps were made of empty coffee cans filled with about 1/4 soil. Five drainage holes were punched in the bottom, and a raw chicken leg was placed inside on top of the soil. A screen with a ¼ inch hole punched in the middle was fitted to the top of the can to allow the beetles to crawl in. A rubber band was placed over the top of the can to hold the screen in place. Next the screen was pressed downward into a funnel shape to prevent the beetles from crawling out the same hole they entered. A chicken wire barrier was also wrapped around each can with an opening in the bottom to prevent predators, such as foxes and pine martens, from entering through the top. The can traps were attached to aspen trees by wrapping a wire around the trunk of the tree and through the chicken wire barrier that was wrapped around the can. They were attached to the tree trunks about 2 meters above ground level and about 20-30 meters apart.

Treatment Methods

As beetles were caught they were collected in plastic bins with small holes in the top for air, and then supplied with dampened paper towel, and raw chicken. The bins were then put into a bear proof cage outside the lab where they were exposed to ambient temperatures.

Once 50 males and females were caught they were sexed, measured, and matched by approximate same size. Out of the 50 pairs, 48 were randomly assigned to one of these four treatments (N=12 each): uniparental male, uniparental female, biparental, and no parents. Two pairs were left unassigned as extras in case a pair failed to prepare the carcass or produce fertilized eggs.

After assigning treatments, each pair was put into their own breeding container. The container was a clear, plastic tub (L 17 x W 13.5 x D 9 cm). Each tub was filled with sifted soil to a depth of 7 cm the day before and supplied with a freshly thawed mouse carcass earlier in the day. Since the size of the carcass can affect the size and number of larvae in a burying beetle brood (Scott, 1998, Smiseth 2014) lab mice were used to ensure each pair had a carcass with the same weight. The tubs were kept in an outdoor sheltered room exposed to ambient temperatures.

As the beetle pairs worked at different speeds burying and preparing the carcass, and laying their eggs, they were observed every 2-3 hours. Once the carcass was completely buried, we began to check for eggs. All broods took about 1 day to lay their eggs after burial, and 3-3.5 days to hatch. There was no difference from start of the experiment to the burial of the carcass (ANOVA: $F=0.07861$, $p=0.5081$). There was no difference between groups for the duration of eggs to larvae (ANOVA: $F=0.7114$, $p=0.5504$). There also was not a difference between groups from burial time to the time the beetles laid eggs (ANOVA: $F=0.09638$, $p=0.4183$). These similar starting conditions show that any differences in the experiment were due to the treatments.

Once the larvae were observed emerging from the eggs (about 5 days after placing the pairs in the tubs) parental care was manipulated. Both parents were taken away from 12 broods. The male was taken away from 12 broods, and the female was taken away from the third set of 12. For a control group. 12 broods were left with both male and female to raise the brood together. These four groups (both parents, solo male, solo female, and no parents at all) were assigned to each pair as the independent variable.

After the carcass was eaten and the larvae of a container had started to wander on the soil surface the brood in that container was counted and each individual larvae was weighed. After the larvae were weighed they were put into overwintering chambers near the same field sites they were trapped in. Once all broods had finished their carcass the data was entered into excel and imported into a JMP program.

Statistical Analysis

Parental treatments (labeled as both, solo female, solo male, and neither) were entered into JMP software as the independent variable followed by the four dependent response variable columns: 1) Total Brood Mass, 2) Average size of larvae, 3) Average number of larvae per brood, and 4) Duration of Larval feeding. Duration of larval feeding was measured as the duration of time from when larvae were first spotted on the carcass until the carcass was fully consumed and the larvae had burrowed back into the soil.

A one-way analysis of variance was also run on all four dependent variables using JMP software. For significant ANOVA results, post-hoc tests were performed to indicate which means were statistically different.

RESULTS

Of the 50 pairs started in this experiment, all but two buried carcasses and larvae hatched. Thus all treatments started with 12 replicates. Of these, there was an effect of treatment on larval success. All pairs (biparental) and uniparental female treatments reared at

least two surviving larvae. One of the uniparental male broods had only one larvae, but the remainder produced at least two. In the treatment in which both parents were removed (non-parental), the larvae all died in two broods, but in the remainder at least two survived.

Total Brood Mass

The total mass of each brood was calculated by summing the individual larvae weights in each brood. There was a significant effect of treatment on total brood mass (ANOVA: $F=5.815$, $P=0.002$) Figure 2. There was no significant difference in total brood mass between the bi-parental and the uniparental female treatments (ANOVA, post-hoc test: Tukey $p=0.964$). Both of these treatments had significantly higher total brood mass (g) than non-parental and the uniparental male (Tukey $p=.891$).

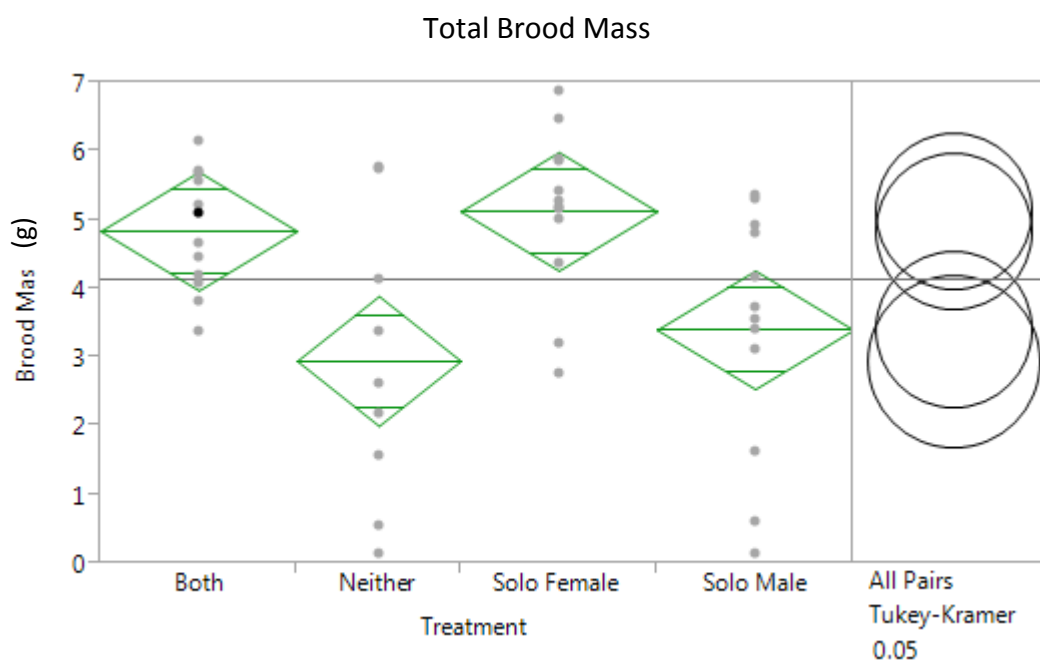
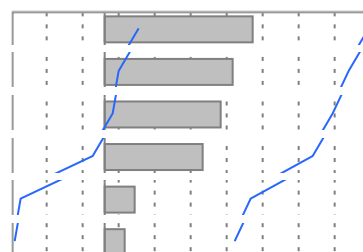


Fig 2

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	3	38.28359	12.7612	5.8153	0.0020
Error	42	92.16607	2.1944		
C. Total	45	130.44965			

Level		Mean
Solo Female	A	5.1277500
Both	A B	4.8404167
Solo Male	B C	3.4024167
Neither	C	2.9498000

Level	- Level	Difference	Std Err Dif	p-Value
Solo Female	Neither	2.177950	0.6342809	0.0071*
Both	Neither	1.890617	0.6342809	0.0237*
Solo Female	Solo Male	1.725333	0.6047631	0.0326*
Both	Solo Male	1.438000	0.6047631	0.0971
Solo Male	Neither	0.452617	0.6342809	0.8911
Solo Female	Both	0.287333	0.6047631	0.9642



Total brood mass consists of both the number and the mean size of larvae. Thus, it is interesting to conduct separate analyses on these two components to see which contributes to the differences observed in total brood mass.

Average Individual Larvae Weight in Each Brood

Individual larva mass was marginally affected by treatment (Figure 3). Larvae were slightly larger in two of the treatments (biparental, and uniparental female) than in either the uniparental male or the non-parental treatments (ANOVA, $F=2.86$, $p=.047$). A post-hoc Tukey test was applied there were not any differences between treatments (lowest $p=0.07$) A less conservative Students T-test was applied instead and reported a significantly larger larvae in the biparental, and uniparental female when compared to the non-parental treatment. Larvae from the uniparental male treatment were intermediate in size, and not significantly larger than non-parental (Student T—Test $p=.246$).

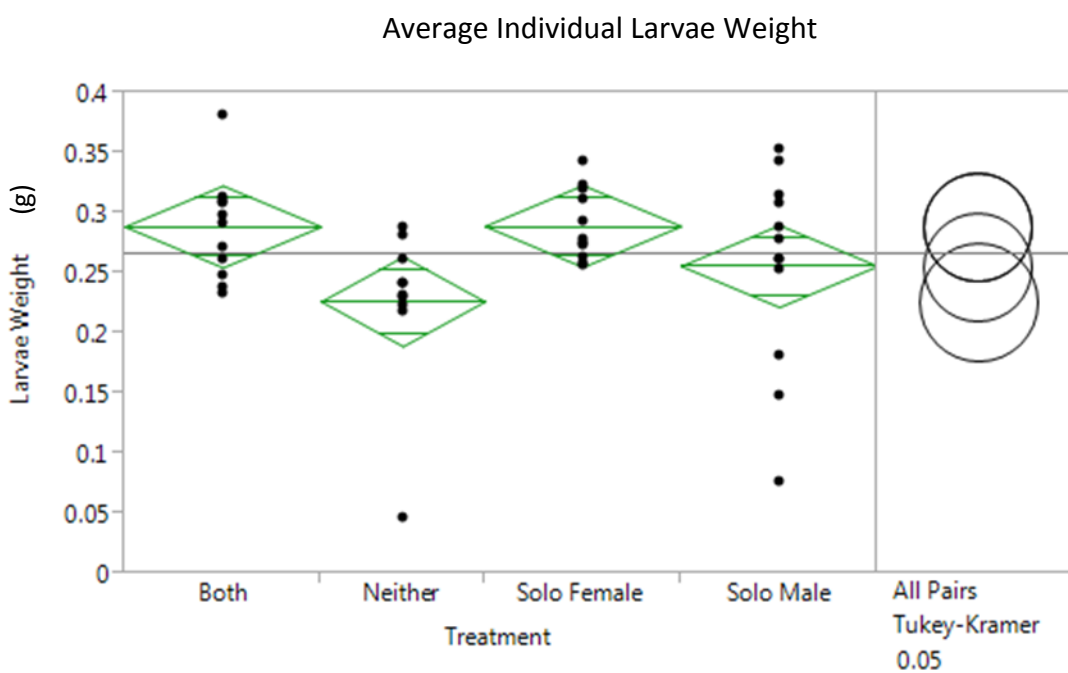
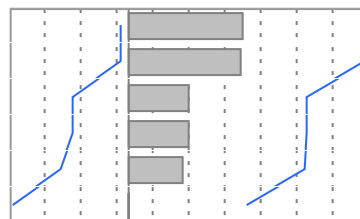


Fig 3 Individual larvae were weighed and all broods were averaged to the nearest hundredth gram.

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	3	0.02946046	0.009820	2.8692	0.0477*
Error	42	0.14374965	0.003423		
C. Total	45	0.17321011			

Level	- Level	Difference	Std Err Dif	p-Value
Solo Female	Neither	0.0626833	0.0250495	0.0741
Both	Neither	0.0622667	0.0250495	0.0769
Solo Female	Solo Male	0.0332500	0.0238838	0.5112
Both	Solo Male	0.0328333	0.0238838	0.5219
Solo Male	Neither	0.0294333	0.0250495	0.6457
Solo Female	Both	0.0004167	0.0238838	1.0000



Average Number of Larvae in Each Brood

There was a significant effect of parental treatment on brood size (larval number) and range (ANOVA, $F=5.480$, $p=0.0027$), Figure 4. Broods without parents produced significantly fewer larvae, and with greater range, than the biparental group and the uniparental females group.

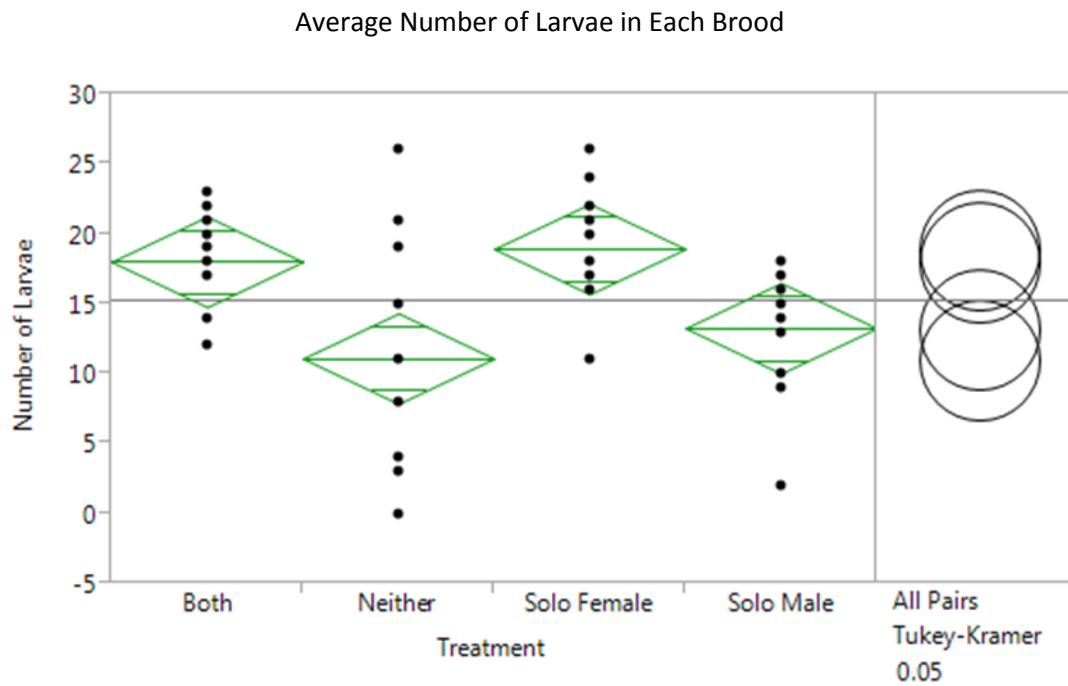


Fig 4

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	3	508.2292	169.410	5.4806	0.0027*
Error	44	1360.0833	30.911		
C. Total	47	1868.3125			

Ordered Differences Report								
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value		
Solo Female	Neither	7.833333	2.269764	1.77305	13.89362	0.0066*		
Both	Neither	6.916667	2.269764	0.85638	12.97695	0.0196*		
Solo Female	Solo Male	5.666667	2.269764	-0.39362	11.72695	0.0744		
Both	Solo Male	4.750000	2.269764	-1.31028	10.81028	0.1714		
Solo Male	Neither	2.166667	2.269764	-3.89362	8.22695	0.7756		
Solo Female	Both	0.916667	2.269764	-5.14362	6.97695	0.9774		

Level			Mean
Solo Female	A		18.916667
Both	A		18.000000
Solo Male	A	B	13.250000
Neither	B		11.083333

Duration of Larval Growth and Feeding

There were no significant difference in time to bury the carcass or to oviposit in the four treatments. As stated before in the methods section, all broods took about 1 day to lay their eggs after burial, and 3-3.5 days to hatch. This was expected, as no treatment had yet been imposed. However, once the larvae hatched and parental removals imposed, the length of the feeding stages and growth duration for each varied (Figure 5).

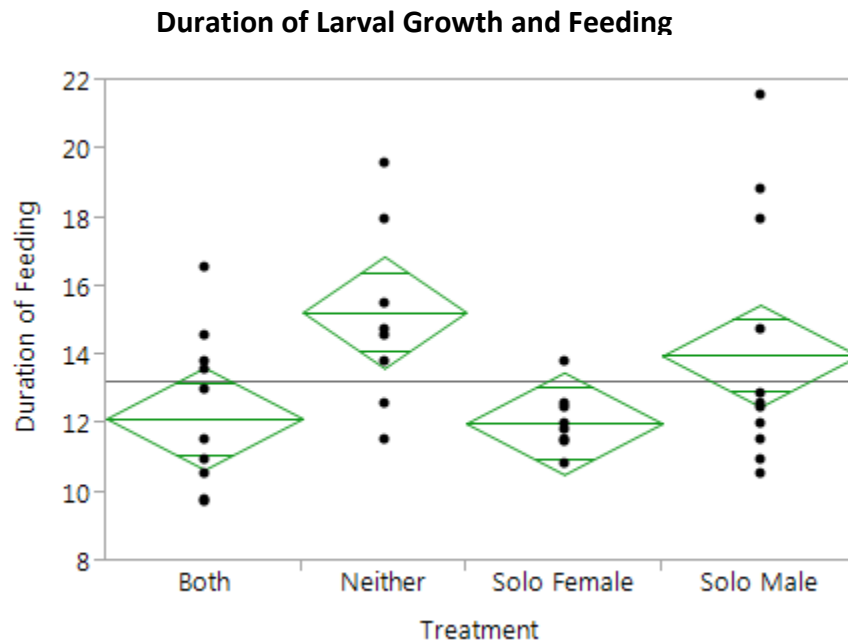


Fig 5

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	3	79.19655	26.3988	4.0607	0.0127*
Error	42	273.04406	6.5010		
C. Total	45	352.24061			

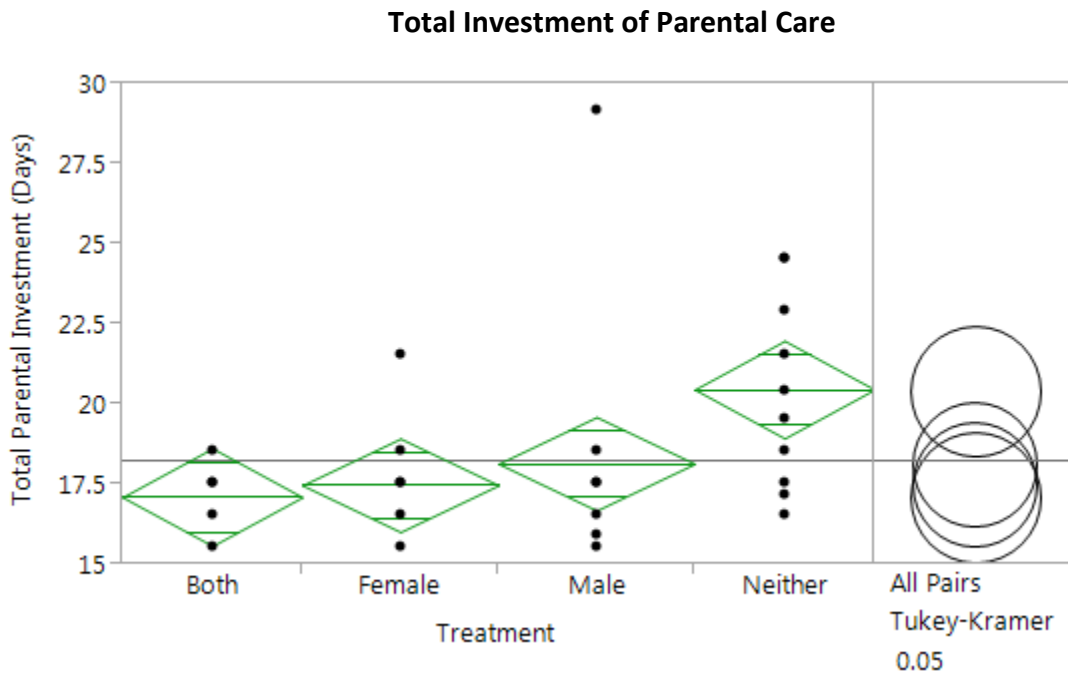
Level			Mean
Neither	A		15.256000
Solo Male	A	B	13.988333
Both	B		12.160833
Solo Female	B		12.018333

Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
Neither	Solo Female	3.237667	1.091723	0.31735	6.157979	0.0246*
Neither	Both	3.095167	1.091723	0.17485	6.015479	0.0341*
Solo Male	Solo Female	1.970000	1.040917	-0.81441	4.754409	0.2467
Solo Male	Both	1.827500	1.040917	-0.95691	4.611909	0.3087
Neither	Solo Male	1.267667	1.091723	-1.65265	4.187979	0.6542
Both	Solo Female	0.142500	1.040917	-2.64191	2.926909	0.9991

Total Investment of Parental Care

Total investment of parental care was measured in days, from the start of the experiment to the end of the feeding stage. Larvae without parents took the longest to develop and were most different from biparental broods ($p=0.0153$). Males measured close to the same amount of time as larvae without parents ($p=0.6542$). Solo females took almost the same amount of time as parents paired together ($p=0.9843$). Treatments had an effect on total investment of parental care ($F=4.0279$, $p=0.0132$).



Analysis of Variance

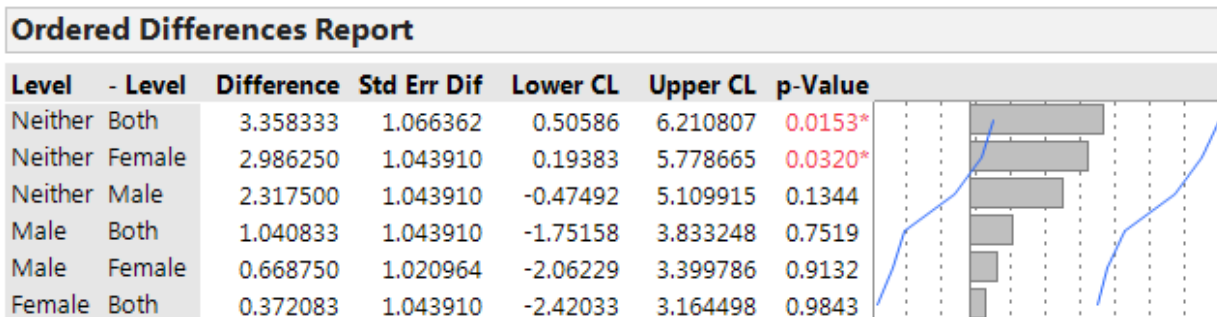
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	3	75.57486	25.1916	4.0279	0.0132*
Error	42	262.67667	6.2542		
C. Total	45	338.25153			

Level

Neither	A
Male	A B
Female	B
Both	B

Mean

Neither	20.458333
Male	18.140833
Female	17.472083
Both	17.100000



DISCUSSION

To review, the previous prediction hypothesized the success of larvae in each group in the following order:

Both Parents > Solo Female ≥ Solo Male > Neither

The prediction makes sense based on the standard bi-parental care that is published in journals, but the results above show a different story summarized by the equation:

Solo females = Both Parents ≥ Solo Male ≥ Neither

The prediction was not supported. Two results are of considerable interest: the success of the larvae in the non-parental treatment was unexpected, and it was also interesting to see that the larvae reared by uniparental females did equally as well as the “both parents” group.

Larvae Without Parents

This experiment also shows that the larvae do not need assistance after carcass preparation and are capable of growing and feeding on their own. Some broods were even similar in size to treatment groups with parents present.

Total investment of parental care took the longest for larvae without parents. They took the longest to develop and were most different from biparental broods ($p=.0153$). Solo females took almost the same amount of time as parents paired together ($p=0.9843$). Treatments had an effect on total investment of parental care ($F=4.0279$, $p=0.0132$).

Larvae without parents will survive, but will be significantly smaller and there will be less of them. This was the case even in the absence of intruders, which supports previous work indicating the importance of parental effort in the continued carcass maintenance and feeding of the early instars. There was also evidence that the brood number was more variable in the non-parental treatment, demonstrating the brood culling/matching to resource that presumably is carried out when by parents when they are present (Bartlett, 1987). There is also another interesting element to this variable. The bar graph shows a large range for the neither group, and much smaller ranges for the other three groups. This could be because burying beetle parents have been known to cull their young in order to match the brood size to the carcass size (Trumbo, 1990b). These results confirm and support what we know about this behavior.

The duration of feeding stage statistics are not far from expected and reasonable. The feeding stages appeared to vary during observations in the lab, but did not show an overall significant difference with a one-way analysis of variance. The order of differences table

reports a significant difference between the “neither” and “both” groups in which the broods without parents took much longer to grow than broods with both parents.

This result is expected since the parents could speed the process along using regurgitation. Studies have also shown larvae of other *Nicrophorus* species will allocate their own feeding schedules by begging, but are still able to eat on their own (Smiseth, 2003).

Overall, broods without either parent, had less larvae per brood, and had smaller larvae, but did develop. The fact that they did not require the parent’s regurgitation to survive confirms that factors other than feeding, such as carcass selection, maintenance, or protection, are more imperative to reproductive success and fitness. More experiments could be also be done to investigate the success of larvae without parents.

Larvae With Parents

Solo females are equally capable of raising a brood just as successful as broods with both parents. They are also capable of producing a significantly more successful brood than a solo male or the group without parents. Broods raised by Solo females were closer to the “both parents” group in every measured variable (closer than the solo males or larvae without parents).

The equal success of the solo female to both parents could be due to the absence of the male. If the male is not present to feed on the carcass the female has unlimited access to more nutritious parts of the carcass (Borncoraglio, 2012.) This is an unsurprising find that suggests there has been some selection for female only care which is more common than bi-parental care in the animal kingdom.

Males measured close to the same amount of time as larvae without parents ($p=0.6542$). It has also been discovered in other *Nicrophorus* species that the males spend more time guarding the carcass, rather than feeding larvae (Fetherston, 1990). Conspecific competition could also cause males to stay with the larvae longer (Koulinos, 2000), maybe the presence of so many other beetles nearby triggered this same response from males in this experiment. These previous studies could help explain why *Nicrophorus investigator* larvae took so much longer in the solo male treatment group.

Considerations for Future Research

There are a few things that should be taken into consideration should this experiment be repeated. The captivity of the small containers could be improved. A larger area so that parents can get away from young may be helpful in preventing injury to larvae. Some parents were starting to injure the larvae after the larvae had left the carcass (just before being weighed). This could affect the survival and weight of each larvae if the parents had not been monitored carefully. This experiment also did not include a natural scenario with competition or predators. Carcasses without parental protection could have been subject to maggots and parasites (nematodes) in the wild. This experiment was meant to focus on the feeding and carcass maintenance, but it would be interesting to introduce competitors as a new manipulative experiment. Beetles were also paired randomly. It would be challenging, but rewarding to design an experiment that compared the success of offspring from parents that had been matched randomly vs. having free choice of a mate. Maternal nutrition has been

found to affect both size and number of larvae in a brood in the closely related species *Nicrophorus defodiens* (Steiger 2007). *Nicrophorus vespilloides* was affected by competition with microbes in one study, and brood size was discovered to be a phenoplastic trait with *Nicrophorus orbicollis* in another (Creighton 2005, McLean 2014). It is possible that something similar is happening with these four treatments in *Nicrophorus investigator*.

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