

**DOES ENVIRONMENTAL VARIABILITY
EXPLAIN MALE PARENTAL CARE IN A BURYING BEETLE?**

by

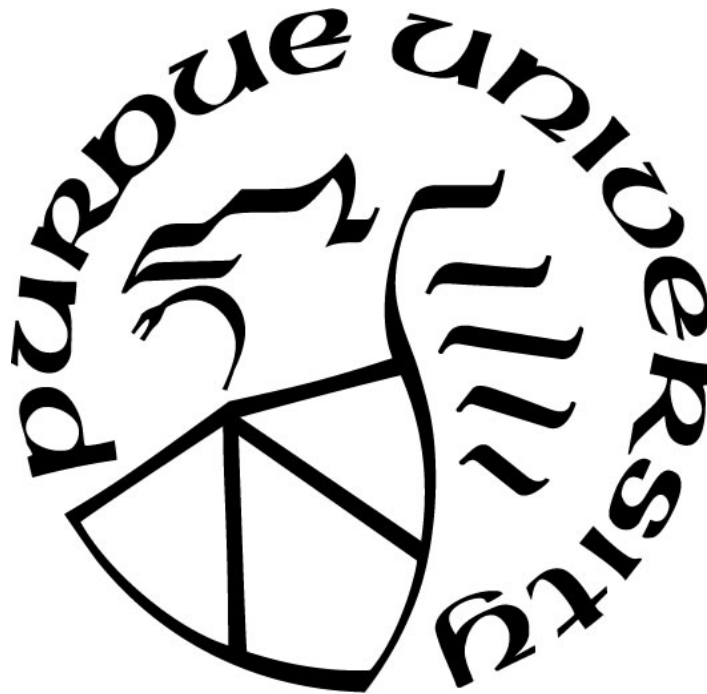
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ABSTRACT

Many animal species invest in extended parental care for their offspring. Parental care is costly, and natural selection favors investment strategies which maximize reproductive success. Biparental care is relatively rare, but when it does occur it has been found to increase success in terms of offspring survival and growth and in terms of future reproductive opportunities. In burying beetles (*Nicrophorus* spp.), both male and female participate in extended parental care. However, the fitness benefits of biparental care in burying beetles have been difficult to establish, with some studies reporting significantly smaller broods produced when both male and female are present. Variation in environmental conditions, such as temperature, is an important part of the context in which biparental care evolves. I hypothesize that biparental care acts as a buffer against environmental variation. This hypothesis predicts that biparental care will lead to greater reproductive success compared to uniparental care when temperature is increased during a reproductive attempt. I also tested the *load-lightening hypothesis*, which holds that biparental care benefits future reproduction by lowering the costs of reproduction. This predicts that the additional care by the other parent will allow females to rear higher quality second broods. I conducted a male removal experiment at two temperature treatments, using the species *Nicrophorus orbicollis*. I measured reproductive success during manipulated first brood and during second broods which females reared without a male, regardless of prior experience. I found that, contrary to my hypothesis, biparental care at the higher temperature resulted in reduced reproductive success compared to uniparental care. I found no effect of biparental care on the success of second broods. Instead, I found evidence of reproductive restraint associated with the higher temperature treatment in delayed egg-laying and increased feeding during second broods.

INTRODUCTION

Parental care includes any parental trait that increases offspring fitness (Smiseth 2019). Parental care can take numerous forms, ranging from basic traits such as provisioning an egg with nutrients to guarding and feeding offspring. Investment in offspring comes at a cost to a parent's ability to invest in other offspring, and natural selection favors investment strategies that maximize lifetime parental reproductive success (Trivers 1972; Magrath and Komdeur 2003).

Uniparental care, in which either the male or female provides care in the absence of the other, is the most common form of parental care (Smiseth 2019), and female-only care is predominant except in the bony fishes (Benun Sutton and Wilson 2019). Male care, including biparental care, is less common and can include both pre- and post-hatching care. Pre-hatching male care may consist of guarding females and nests, decreasing the threats of predation on vulnerable eggs and on the female, while also increasing his paternity by preventing the female from engaging in extra-pair copulations. Artiss and Martin (1995) found that male white-tailed ptarmigan, *Lagopus leucurus*, accompany females on pre-incubation foraging excursions to watch for predators. This added vigilance allows females to forage more, with likely fitness benefits to the female and her clutch. Male pre-hatching care may increase female fecundity directly, as when male katydids (*Conocephalus ictus*) give females high quality nuptial gifts (Ortíz-Jiménez and del Castillo 2015). However, this pre-hatching care has limited potential benefit to reproductive success, because clutch size is generally an exclusively female trait, influenced by how much post-hatching care the male is expected to provide (Smith and Härdling 2000; Stockley and Hobson 2016). Since post-hatching biparental care has greater potential to influence reproductive success, it has been the subject of much investigation.

A number of hypotheses have been proposed to explain the evolution of post-hatching biparental care. The *mutual benefit hypothesis* proposes that the fitness of both parents is greater when offspring are reared by both parents than when they are reared by either the male or female alone. Under this hypothesis, biparental care is expected when parents' chances of remating are low and the help of the additional parent increases reproductive success (Maynard Smith 1977; Robertson and Roitberg 1998; Pilakouta *et al.* 2018). This hypothesis predicts that, if one parent is experimentally removed, the fitness of the parents is reduced compared to controls where both are present. Support of this hypothesis has been found in a number of biparental species. For

example, Gubernick and Teferi (2000) found that the direct care provided by male California mice, *Peromyscus californicus*, such as huddling over pups and keeping them warm, is crucial to the fitness of the litter. Male removal was associated with decreased pup survival. Royle *et al.* (2006) found that biparental care in zebra finches (*Taeniopygia guttata*) creates more frequent feeding opportunities for nestlings, since both male and female regurgitate for their young. This increased feeding allows offspring to feed more frequently and grow faster.

The *load-lightening hypothesis* originating in cooperative breeding literature holds that spreading reproductive costs over multiple individuals leads to lower overall costs and greater lifetime reproductive success (Crick 1992; Scantlebury *et al.* 2002), and offers a plausible explanation for biparental care (West and Capellini 2016). This *load-lightening* predicts that cost reduction associated with biparental care may or may not have a benefit to the current brood, but may be seen in increased future reproduction. Nomi *et al.* (2018) found that male involvement with care in Japanese tits (*Parus minor*) does not increase fledgling success, but it does reduce reproductive costs to the female, seen in improved body condition. As a result, females were more likely to rear multiple broods. This effect may coincide with direct benefits, as seen in California mice, in which male care helps rear larger litters and also reduces the inter-birth recovery period of females, allowing females to rear more broods in a shorter period of time (Cantoni and Brown 1997).

An alternative hypothesis proposes that biparental care evolved as a buffer against environmental variability. This hypothesis predicts that male post-hatching care may show little or no benefit under benign conditions, but the additional care increases reproductive success under extreme environmental conditions. For example, Wynne-Edwards (1995) found that biparental care of the Djungarian dwarf hamster (*Phodopus campbelli*) is a facultative response to extreme cold that may not be favored under more benign temperatures. The presence of an additional parent allows coordination between them, reducing the time pups are unattended and at risk of cooling. Similarly, biparental care may allow reproduction under unfavorable conditions and, therefore, access to new niches. Brown *et al.* (2010) found that male care in the mimic poison frog (*Ranitomeya imitator*) provides a nutritional benefit to tadpoles. The male checks on the tadpoles and calls on the female to lay trophic eggs when the offspring are needing food, thereby allowing this species to utilize nutrient-poor small pools, a niche not available to related frog species lacking biparental care.

Burying beetles (*Nicrophorus* spp.) are unusual among insects in that both parents provide extensive parental care (Scott 1998). Burying beetles rear broods on small vertebrate carcasses, and parents engage in pre-hatching care such as carcass defense and preservation as well as post-hatching care such as brood defense, carcass maintenance, and offspring provisioning. Male presence before larvae hatch is important to secure the resource (Scott 1990) and his paternity of the offspring (Müller and Eggert 1989).

The benefit of the male's post-hatching care has been the subject of numerous studies on burying beetles. However, the results have been mixed. Benowitz and Moore (2016) found that biparental care produces more offspring than uniparental care, but there is more evidence that biparental care has no benefit in terms of brood size or quality (Bartlett 1988; Trumbo 1991; Müller *et al.* 1998) or even that it reduces reproductive success (Scott 1989; Smith *et al.* 2017). Smith *et al.* (2017) bred *N. orbicollis* with biparental care, uniparental care, or uniparental desertion strategies for both parents continuously until death and found that biparental care leads to lower lifetime fitness than both uniparental care and uniparental desertion strategies. These conflicting results do not provide strong support for the *mutual benefit hypothesis*. Jenkins *et al.* (2000) found evidence of a delayed benefit of biparental care, consistent with the *load-lightening hypothesis*, in the species *N. vespilloides*. Although there was no evidence of increased reproductive success for broods cared for by both parents, it was found that females were able to rear larger second broods with faster development. This would benefit the male from the previous brood because the female continues to store the sperm of past copulations, and he may sire offspring in her future broods regardless of his presence.

Variation in environmental conditions, such as temperature, is an important part of the context in which biparental care evolves, yet the reproductive success of biparental care under such variability has not been evaluated in burying beetles. Quinby *et al.* (2020) found that *N. orbicollis* females caring for their young in the absence of a male reared significantly fewer offspring at an unfavorably warm temperature (25°C), compared to a more benign temperature (20°C). This reduced success is likely due in part to thermoregulatory constraints on beetle activity (Merrick and Smith 2004) and the immunological demands of feeding on and preserving a carcass (Vogel *et al.* 2017). Such uniparental care, as opposed to no care or reduced care, has been found to buffer against the negative effects of ambient temperature in burying beetles (Grew *et al.* 2019; Benowitz *et al.* 2019). I hypothesize that biparental care has a similar

buffering effect against environmental variability, showing reproductive benefits of biparental care under unfavorable conditions.

I tested the effect of biparental care on reproductive success under environmental variability in the burying beetle *N. orbicollis*. I did this by conducting a male removal experiment while manipulating temperatures (either benign or unfavorably warm). I predicted that male presence would increase reproductive success at the higher temperature, as measured in terms of brood size, size of offspring, and rate of offspring development. I also tested possible effects of *load-lightening* by breeding females from all treatments a second time without a male and at a benign temperature. I predicted that biparental care in first broods would reduce the burden on the female, allowing her to invest more in their body condition, seen in greater relative mass gain compared to that of females in uniparental treatments. I further predicted that this improved body condition would affect future performance, seen in greater reproductive success during second broods, as measured by brood size, size of offspring, and rate of offspring development.

METHODS

Natural History of Burying Beetles

Burying beetles find and preserve small vertebrate carcasses, around which eggs are laid and which serve as food for their offspring. Burying beetles begin by burying the carcass, removing fur or feathers, rolling it into a ball, preserving the carcass with antimicrobial secretions, and defending it from conspecific and interspecific competitors (Scott 1998). After eggs hatch, one or both parents remain on the carcass to care for the growing larvae, which is important for larval growth and survival (Benowitz *et al.* 2016). Post-hatching parental care consists of continued defense and maintenance of the carcass and food regurgitation to larvae, all of which improve offspring survival and growth (Eggert *et al.* 1998). Parents also engage in filial cannibalism, which results in a positive correlation between carcass and brood size (Bartlett 1987). Broods that are too large tend to have smaller offspring (Scott 1989), and larger adult size is an important component of their ability to defend a carcass (Otronen 1988). Female and male *N. orbicollis* are capable of the full array of parental care, although females are involved more in the direct care of regurgitation, and males spend relatively more time guarding the brood (Fetherston *et al.* 1990). In most cases, the female will stay with the brood until larvae disperse and the resident male will remain with the carcass only until larvae reach the third instar stage, but males do care for longer periods of time in the event of female death or early desertion (Scott 1998). Because both male and female *N. orbicollis* have the potential to rear multiple broods, burying beetles maximize their lifetime reproductive success by balancing current parental investment with future reproductive opportunities (Creighton *et al.* 2009).

Laboratory Population

Nicrophorus orbicollis were collected near Big Falls, Wisconsin during June, 2018 and July, 2019 using pitfall traps baited with rotten chicken. In the laboratory, wild-caught male and female beetles were paired on 30-gram mouse carcasses to generate a laboratory population. The resulting offspring were housed individually in small plastic containers with moist paper towel and fed raw chicken liver *ad libitum* until they were used in the experiment. Experiments were conducted with genetically-unrelated males and females, each 20–30 days old (post-eclosion).

All individually-housed beetles and breeding pairs were kept on a 14:10 hour light:dark (L:D) cycle.

Experimental Design

The experiment consisted of four treatments (Table 1). Each treatment was a combination of two factors: temperature (21°C or 25°C) and parental care treatment (female-only or biparental). The benign temperature (21°C) was chosen based on previous laboratory studies of biparental care in this species (ex. Smith *et al.* 2017). The unfavorably warm temperature (25°C) was chosen based on Quinby *et al.* (2020), which found that *N. orbicollis* have lower reproductive success at this temperature.

Table 1. Factorial Design and Sample Sizes

	Post-hatching parental care treatment	
Treatment temperature during post-hatching care	Uniparental 21°C (n=16)	Biparental 21°C (n=16)
	Uniparental 25°C (n=17)	Biparental 25°C (n=17)

Broods in the Uniparental 21°C treatment were kept in an environmental chamber with an ambient temperature of 21°C throughout the breeding attempt, and males were removed the day prior to normal arrival of the offspring on the carcass. Broods in the Biparental 21°C treatment were kept in the 21°C environmental chamber throughout the breeding attempt, and males remained with the brood until they attempted to disperse. Broods in the Uniparental 25°C treatment were moved to an environmental chamber with an ambient temperature of 25°C, and the male was removed, both on the day prior to offspring normally arriving on the carcass. Broods in the Biparental 25°C treatment were moved to the 25°C environmental chamber on the day prior to offspring normally arriving on the carcass, and males remained with the brood until they attempted to disperse.

At the beginning of each breeding attempt, males and females were weighed and the width of their pronota measured using digital calipers. Each pair of beetles was randomly assigned to a treatment and placed into a plastic container (18 x 15 x 10 cm) filled half full with commercially purchased topsoil and on it placed a freshly thawed 30-gram mouse carcass. All broods were initially kept at 21°C. Broods were checked daily for progress. The treatment was

implemented on day four of the experiment, at which point larvae were expected to hatch shortly thereafter. All broods were removed from the environmental chamber and had the carcass overturned, and thus all treatments experienced similar disturbance. After hatching, larvae were counted and weighed on each of the first three days after arriving on the carcass. Upon completion of larval dispersal from the carcass, larvae were weighed and placed in a container with fresh soil for pupation and eclosion. The presence or absence of the parent(s) were recorded each day. Two successive absences from the carcass or surrounding area were considered indicative of dispersal. Deserting males were removed from the biparental treatments. Because females typically stay until larvae disperse, they were not removed until larvae dispersed, at which point females were housed individually at 21°C and fed raw chicken liver *ad libitum*.

After 2 days, the female was placed in a cylindrical plastic container (diameter 11 cm, height 8 cm) with moist paper towel, excess raw chicken liver, and an unmated male. This method was used by Jenkins *et al.* (2000) to account for any differences in sperm stored by the female resulting from different parental treatments. After approximately 24 hours with the male, the female was placed in a plastic container with a fresh 30-gram carcass. After this brood was completed, females were housed individually and fed raw chicken liver *ad libitum* until they died.

After offspring eclosed (usually 26–32 days after dispersing from the carcass), the adult offspring were measured and weighed. One male and female were randomly chosen from each newly-eclosed brood and housed individually with raw chicken liver *ad libitum* until they died.

Statistical Analysis

The experimental design incorporated two factors (male presence and temperature), each having two levels. This created a 2x2 factorial design, as seen in Table 1. All analyses were conducted using the R statistical software version 3.6.1 (R Core Team 2019). I used the “Anova” function in the “car” package (Fox and Weisberg 2019) and linear models to analyze the separate effects of parental care, temperature, and any interaction between the two factors on measures of reproductive success and parental investment. The Shapiro-Wilk test was used to determine normality, and generalized linear models were used in place of linear models for non-normal data. Mann-Whitney-Wilcoxon Tests were used when comparing male dispersal and male mass change between the two biparental treatments. When analyzing differences in offspring size,

subsamples of 2 newly eclosed offspring were randomly selected. This was done at the point of analysis, separate from the retention of two offspring for longevity analysis.

RESULTS

First-day larvae count, surviving offspring, newly eclosed offspring mass, surviving offspring, newly eclosed offspring mass, male mass change, and the female mass change (First Brood), were identified as having normal distributions. All other variables did not satisfy the assumption of normality.

First Brood

Number of offspring surviving to eclosion was used for a measure of brood size rather than the number of dispersing larvae. The average survival of offspring from dispersal to eclosion was $98 \pm 1\%$, and there were no differences across treatments ($p = 0.488$).

Treatment did not have an effect on the number of first instar larvae arriving on the carcass (Table 2). Temperature, presence of the male, and their interaction had a significant effect on brood mass at dispersal and number of surviving offspring (Table 2). The Biparental 25°C treatment produced significantly lighter broods with fewer surviving offspring than the other three treatments (Figure 1a). Treatment had no effect on individual offspring mass (Table 2; Figure 1b) or longevity (Table 2).

Table 2. Effect of Treatment on First Brood Offspring

	Temperature <i>P</i>	Male <i>P</i>	Interaction <i>P</i>
Larvae count, 1st day	0.398	0.175	0.476
Brood mass at dispersal	<0.001	<0.001	<0.001
Surviving offspring	<0.001	0.001	0.007
Newly eclosed offspring mass	0.701	0.210	0.616
Offspring longevity	0.485	0.455	0.237

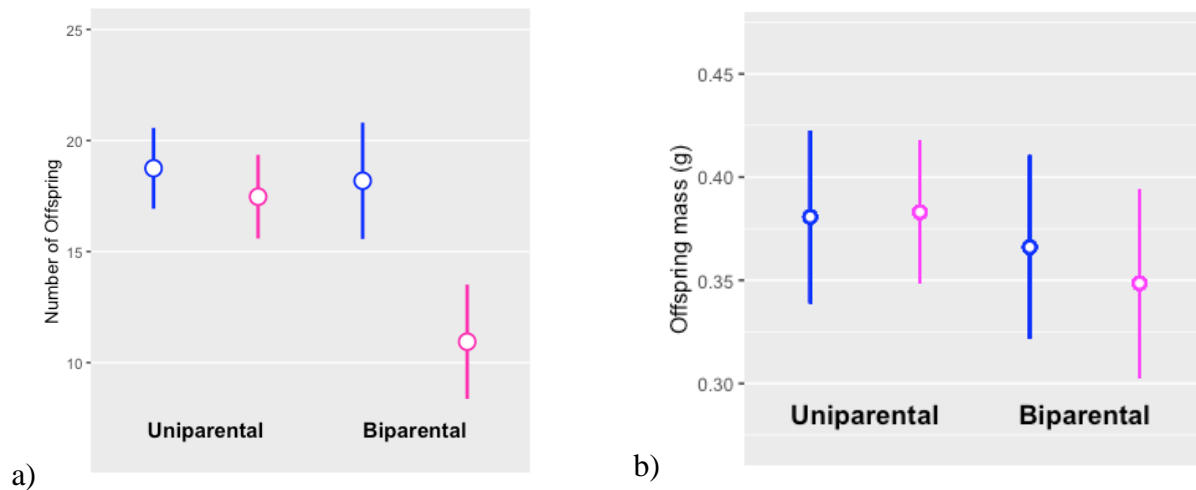


Figure 1. First Brood Reproductive Success a) Number of first brood offspring surviving to eclosion. Uniparental treatments are shown on the left of each graph, and biparental treatments are shown on the right. Blue represents the 21°C treatments, and pink represents 25°C treatments. b) First brood newly eclosed offspring mass.

Treatment had no effect on the time it took first brood larvae to arrive on the carcass (Table 3). Temperature, but not male presence, had an effect on first brood larval dispersal (Table 3). Broods kept at 25°C dispersed sooner than those kept at 21°C (Figure 2a). Correspondingly, females also dispersed sooner at 25°C, but there was no effect of male presence (Table 3; Figure 2b). Male dispersal was not affected by temperature treatment (Table 4). Female dispersal was not correlated with male dispersal at 21°C ($r_s = -0.07$, $p = 0.901$) or 25°C ($r_s = -0.01$, $p = 0.361$).

Treatment had no effect on time until eclosion for first brood offspring (Table 3).

Table 3. Effect of Treatment on First Brood Development and Female Dispersal

	Temperature <i>P</i>	Male <i>P</i>	Interaction <i>P</i>
Larval arrival	0.390	0.371	0.357
Larval dispersal	0.010	0.675	0.684
Eclosion	0.743	0.932	0.549
Female dispersal	<0.001	0.164	0.549

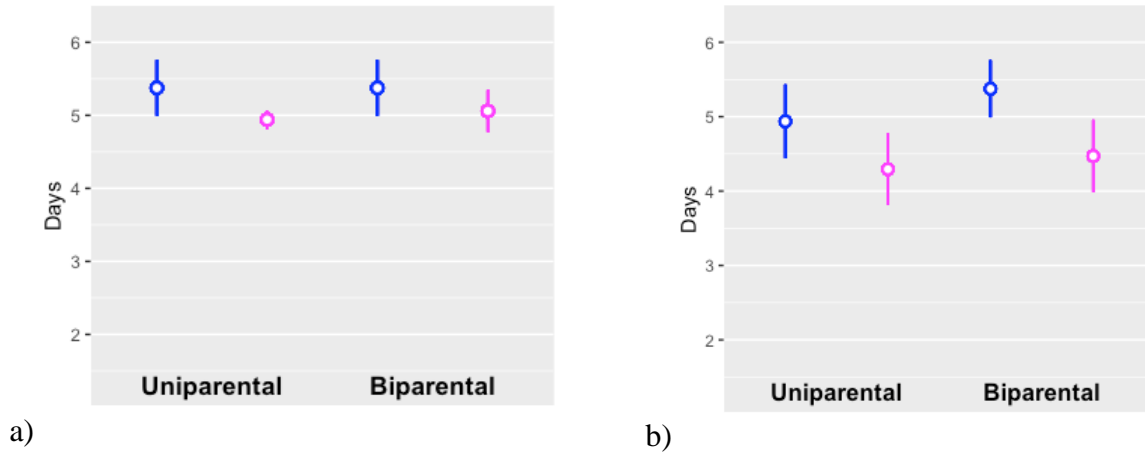


Figure 2. Dispersal from the First Brood a) Larval dispersal. Uniparental treatments are shown on the left of the graph, and biparental treatments are shown on the right. Blue represents the 21°C treatments, and pink represents 25°C treatments. b) Female dispersal.

Table 4. Effect of Temperature Treatment on Male Dispersal and Mass Change

	<i>P</i>
Male dispersal	0.600
Male mass Δ	0.958

Second Brood

Second broods were all conducted with only the female present and at a constant temperature of 21°C. Treatment effects refer to the first breeding attempt. Treatment did not have an effect on the number of first instar larvae arriving on the carcass (Table 5). Temperature, but not parental care treatment, had a significant effect on the total brood mass (Table 5). Heavier second broods followed 21°C treatments than those following 25°C treatments. Treatment did not have a significant effect on the number of second brood offspring surviving to eclosion (Table 5; Figure 3a), second brood individual offspring mass (Table 5; Figure 3b), or offspring longevity (Table 5).

Table 5. Effect of Treatment on Second Brood Offspring

	Temperature <i>P</i>	Male <i>P</i>	Interaction <i>P</i>
Larvae count, 1st day	0.243	0.826	0.942
Brood mass at dispersal	0.010	0.680	0.894
Surviving offspring	0.071	0.402	0.866
Newly eclosed offspring mass	0.083	0.441	0.567
Offspring longevity	0.238	0.562	0.159

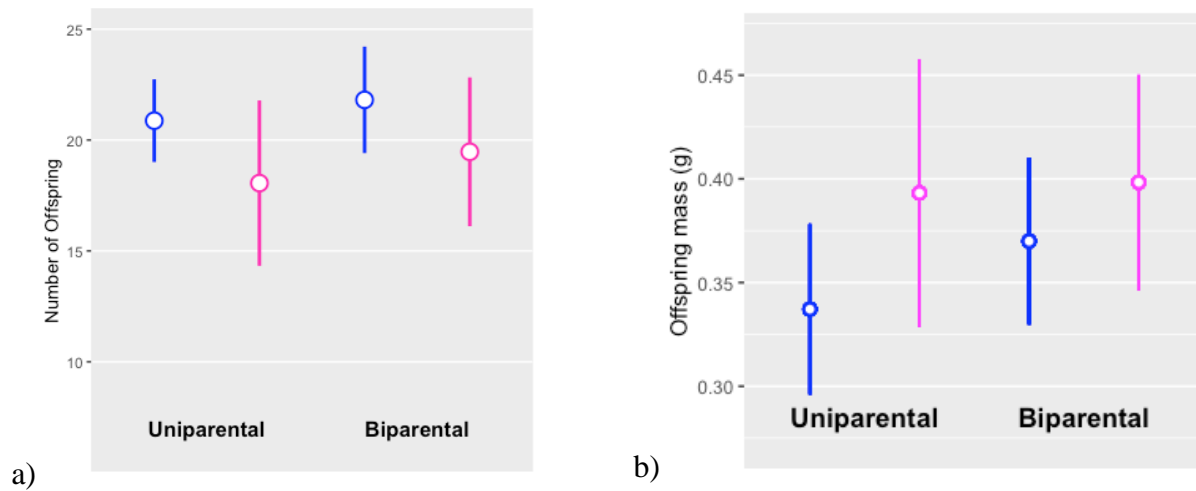
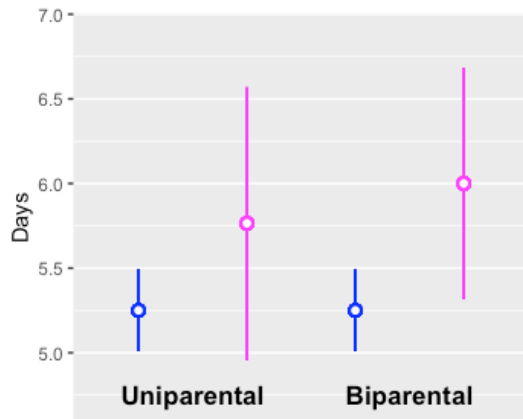


Figure 3. Second Brood Reproductive Success a) Number of second brood offspring surviving to eclosion. Uniparental treatments are shown on the left of each graph, and biparental treatments are shown on the right. Blue represents the 21°C treatments, and pink represents 25°C treatments. b) Second brood newly eclosed offspring mass.

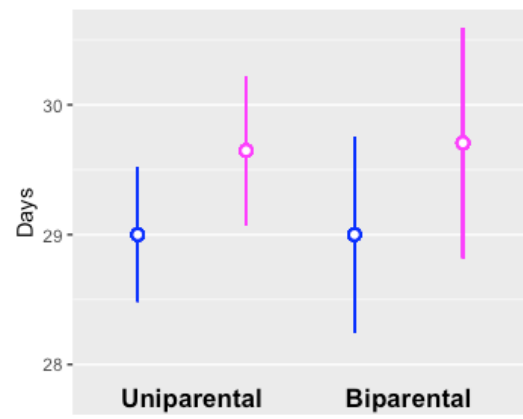
Temperature, but not parental care treatment, had a significant effect on the time it took second brood larvae to arrive on the carcass (Table 6). Broods following 25°C treatments took longer for larvae to arrive (Figure 4a). Treatment did not have an effect on the dispersal of larvae or females during second broods (Table 6). Temperature, but not parental care treatment, had an effect on time until eclosion for second brood offspring. (Table 6), with those belonging to 25°C treatments taking longer to eclose than those from 21°C treatments (Figure 4b). A positive correlation was found between offspring mass and time to eclosion ($r_s = 0.21$, $p < 0.001$), suggesting that larger offspring take longer to eclose. Therefore, the difference in time to eclosion may be explained by the difference in second brood offspring mass between temperature treatments (Figure 3b), although this difference was not significant (Table 5). Multiple linear regression with time to eclosion as the dependent variable and offspring mass and temperature treatment as the independent variables showed that only offspring mass was strongly correlated ($p < 0.001$) and not temperature treatment ($p = 0.165$).

Table 6. Effect of Treatment on Second Brood Development and Female Dispersal

	Temperature <i>P</i>	Male <i>P</i>	Interaction <i>P</i>
Larval arrival	0.018	0.650	0.660
Larval dispersal	0.878	0.291	0.305
Eclosion	0.040	0.927	0.929
Female dispersal	0.678	0.275	0.671



a)



b)

Figure 4. Larval Arrival and Time to Eclosion a) Time until larvae emerged on the carcass. Uniparental treatments are shown on the left of the graph, and biparental treatments are shown on the right. Blue represents the 21°C treatments, and pink represents 25°C treatments. b) Time until offspring eclosed.

Mass Change and Longevity

Treatment had no effect on female mass change during the first brood (Table 7). Male mass change was not affected by temperature treatment (Table 4). Temperature treatment, but not parental care treatment, had an effect on female mass change during the second brood (Table 7). Females who were subjected to 25°C treatments gained more mass during their second broods than those from the 21°C treatments (Figure 5). Female longevity was not affected by treatment (Table 7). Overall, females lived for 85 ± 5 days (all treatments).

Table 7. Effect of Treatment on Female Mass Change and Longevity

	Temperature <i>P</i>	Male <i>P</i>	Interaction <i>P</i>
Female mass Δ , First brood	0.856	0.747	0.425
Female mass Δ , Second brood	0.021	0.218	0.136
Female longevity	0.130	0.585	0.680

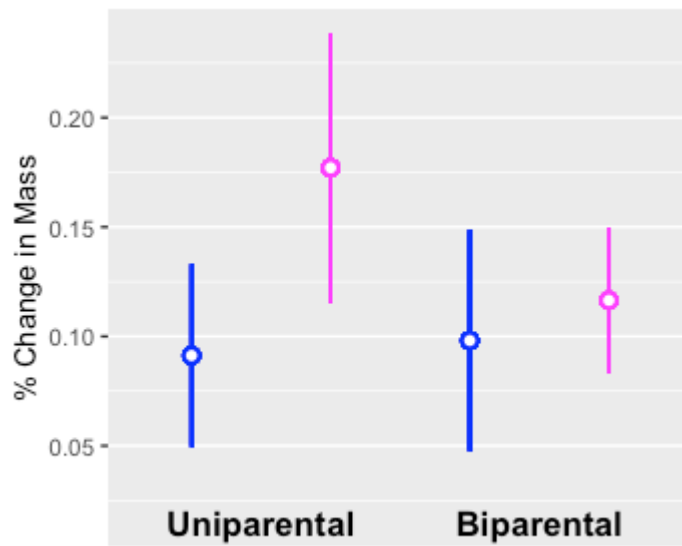


Figure 5. Female Percent Change in Mass During Second Brood. Uniparental treatments are shown on the left of the graph, and biparental treatments are shown on the right. Blue represents the 21°C treatments, and pink represents 25°C treatments.

DISCUSSION

In this study, I hypothesized that biparental care in burying beetles buffers against environmental variability. I tested this hypothesis with a male removal experiment at two temperature treatments (21°C and 25°C), and predicted that male presence would increase reproductive success in the unfavorably warm temperature. The reproductive success of first broods did not support the hypothesis that biparental care buffers against environmental variability. Contrary to the hypothesis, it was found that biparental care was actually associated with smaller broods at 25°C (Figure 1a). Additionally, male presence neither increased nor decreased fitness at 21°C (Figure 1a), consistent with numerous previous studies on burying beetles (Bartlett 1988; Trumbo 1991; Müller *et al.* 1998). I found no evidence that biparental care increased offspring quality under either temperature treatment. Parental care treatment had no significant effect in the first brood on the rate of development and eclosion (Table 3), offspring mass (Table 2; Figure 1b) or offspring longevity (Table 2).

The significant reduction in brood size associated with the Biparental 25°C treatment cannot be explained solely by the unfavorable temperature. No such reduction was seen in the Uniparental 25°C treatment (Figure 1a). Furthermore, such a response to temperature is unlikely to have adaptive value for females. Although caring for offspring at 25°C may be more costly than at 21°C, reducing brood size at the higher temperature would likely require greater investment by the female in the form of an increased contribution to social immunity. Larvae also produce antimicrobial secretions that help preserve the carcass, and it has been found that females are able to reduce their investment in carcass preservation by rearing larger broods (Duarte *et al.* 2016). Smith *et al.* (2015) found that uniparental rearing of smaller broods than normal for a given carcass size leads to lower fitness, and the authors suggested inability to maintain the carcass as a likely cause. In situations of biparental care, male burying beetles invest significantly less in their contribution to social immunity than their female counterparts (Cotter and Kilner 2010). For this reason, a reduction in brood size would be significantly less costly to the male in terms of current investment.

However, the final brood size of the Biparental 25°C treatment may not be the direct result of male culling, as there was no such reduction seen in the Biparental 21°C treatment (Figure 1a). Male *N. orbicollis* are less plastic in their culling behavior and cull more offspring

than females (Smith *et al.* 2015). Initial reduction in brood size may have different consequences for broods depending on the rate of decomposition of the carcass. If the male's contribution to social immunity does not compensate for the lost contribution of culled offspring, then the increased vulnerability of the carcass to spoilage may cause a reduction in larval survival that goes beyond filial cannibalism. This reduction in carcass quality may also explain why, despite the negative correlation expected between number of larvae and larvae size (Scott 1989), the reduced number of larvae in the first broods of the Biparental 25°C treatment did not coincide with an increase in the average offspring mass (Figure 1b). Additional research, including an ethogram of male and female behavior, should be conducted to determine the proximate cause of the brood reduction found in the Biparental 25°C treatment.

Research on burying beetle biparental care should also be conducted with a broader look at environmental variability. Two recent studies have shown that burying beetle parental care is important to buffering against the effects of colder temperatures. Grew *et al.* (2019) found that the facultative parental care exhibited by *N. vespilloides* assisted reproduction in cooler environments (15°C) but not benign (20°C) or warm ones (25°C). More offspring survived at the lower temperature with parental care than did without, but there was no difference in offspring survival at the other temperatures. Benowitz *et al.* (2019) found that the extended maternal care of *N. sayi* resulted in a decreased development time in a cooler environment (15°C). These studies were testing the benefits of uniparental care on relatively cold-tolerant species: *N. vespilloides* is a high-latitude species with virtually all its distribution north of 40°N (Sikes *et al.* 2016) and *N. sayi* is a species that breeds in spring around the Great Lakes (Wilson *et al.* 1984). My study species, *N. orbicollis*, is a relatively-low latitude species breeding in the central part of the summer (Wilson *et al.* 1984), but may also be expected to have similar buffering effects at colder temperatures. Furthermore, because of the local adaptation of *N. orbicollis* (Quinby *et al.* 2020), the northern population used in the present study may be more adapted to variation in the form of unfavorable cold than to unfavorable warmth, although my study did not include variation in the form of an unfavorably cold temperature.

I also tested possible effects of *load-lightening* by comparing the costs of the treatments to the female and comparing the reproductive success of second broods. I predicted that biparental care in first broods would lead to decreased costs to the female in both temperature treatments, as measured by her longevity and change in mass, and increased reproductive success

of females during these second broods, as measured by brood size, size of offspring, and rate of offspring development. Parental care treatment did not have a significant effect on the reproductive success of second broods, as measured by number and size of second brood offspring (Table 5; Figure 3a,b). Parental care treatment also did not have a significant effect on changes in female mass over the course of first or second broods, or on female longevity (Table 7).

Although there were no effects of biparental care on the second brood, effects of temperature were found. Larvae arrived on carcasses later in second broods following 25°C treatments (Figure 4a). Second broods following 25°C treatments were smaller (Figure 3a), and offspring took longer to eclose (Figure 4b). These temperature-related effects on second broods suggest that rearing broods at 25°C similar in size to those reared at 21°C (Figure 1a) was much more costly, and these costs negatively affected future reproduction. Body condition may be vulnerable to increased activity under extreme temperature (Merrick and Smith 2004) and the hazards of defending a carcass from spoilage (Vogel *et al.* 2017). Cotter *et al.* (2013) found evidence of a trade-off between personal immunity and social immunity in female burying beetles. Females investing heavily in carcass preservation would have dispersed in a more compromised state. However, I found no evidence of a temperature effect on body condition in terms of mass change during first broods (Table 7).

These results may suggest that females with prior experience of the higher temperature were exercising reproductive restraint during their second broods, investing less in this brood and more in improving their body condition. I found that females in the 25°C treatments gained more mass over the course of their second broods (Figure 5), perhaps by increased feeding prior to laying eggs. By recouping the cost of previous breeding at the higher temperature, females salvage future reproductive opportunity. Reproducing at 25°C may have a similar effect to over-investment in current reproduction by the female. Research on *N. orbicollis* has demonstrated that over-investing in current reproduction can lead to lower lifetime reproductive success. Creighton *et al.* (2009) manipulated the broods of *N. orbicollis* females to be abnormally large for the carcass size they were using, and found that females had lower mass gain, shorter lives with fewer reproductive attempts, and lower lifetime fitness than the controls. Therefore, salvaging future reproductive opportunity by rearing a smaller brood and feeding more after over-investing in the previous brood may maximize lifetime fitness.

The present study found no evidence that biparental care buffers against the effects of environmental variability. Neither brood fitness nor future reproductive success of the female was improved by the male's post-hatching care during the first brood under either temperature treatment. Instead, I found that when the higher temperature coincided with male presence, parents invested less in that brood. Both treatments in which females were subjected to the higher temperature during their first broods reduced their investment during their second broods. Biparental care of burying beetles should be studied under other forms of environmental variation, including lower temperatures, to determine whether male care provides any sort of buffer against these conditions.

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