

# Dynamic terminal investment in male burying beetles

Paige A. Farchmin, Anne-Katrin Eggert, Kristin R. Duffield, Scott K. Sakaluk\*

*Behavior, Ecology, Evolution and Systematics Section, School of Biological Sciences, Illinois State University, Normal, IL, U.S.A.*

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\* Correspondence and present address: S. K. Sakaluk, Behavior, Ecology, Evolution & Systematics Section, 4120 Biological Sciences, Illinois State University, Normal, IL 61720-4120, U.S.A.

*E-mail address:* [sksakal@ilstu.edu](mailto:sksakal@ilstu.edu) (S. K. Sakaluk).

The dynamic terminal investment threshold model posits that the propensity of an individual to terminally invest in response to an immediate survival threat, such as an infection, depends on other factors that alter an individual's residual reproductive value. Here, we explore the potential for dynamic terminal investment in burying beetles, insects that inter small vertebrate carcasses as the sole source of food for their offspring and that exhibit extensive biparental care. We injected males at two different ages with heat-killed bacteria and measured their reproductive output, predicting that immune-challenged males would show a longer period of parental care, consume less of the carcass and produce a greater number of larvae in the current reproductive attempt compared with control males. We further predicted that terminal investment would be more evident in older males than in younger ones. Males challenged with heat-killed bacteria as virgins prior to their first reproductive attempt showed no evidence of terminal investment, whereas these same individuals when challenged at a later age as reproductively experienced breeders in a subsequent reproductive attempt showed increased reproductive output. Older, immune-challenged individuals gained less mass during the time on the carcass than control males, suggesting that this terminal investment was subsidized, at least in part, by males refraining from eating as much of the carcass as they might have otherwise done in the absence of an immune challenge, leaving more carrion for their offspring to consume at the expense of their own maintenance and future reproduction. Because it seems likely that an individual's residual reproductive value decreases with both increasing age and reproductive experience, the context-specific terminal investment shown by immune-challenged males in the current study aligns with theory.

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In response to an infection, animals with a functional immune system typically respond by upregulating their immunity to ward off the threat to their survival, but the energetic expenditure this requires often comes at a cost to their current or future reproduction (Lawniczak, Barnes, Linklater, Boone, Wigby, & Chapman, 2007; Sheldon & Verhulst, 1996; Zera & Harshman, 2001; Zuk & Stoehr, 2002). Occasionally, however, individuals respond to an infectious agent not by upregulating their immunity, but by increasing instead their investment in reproduction despite the increased mortality risk this entails. The latter response, known as terminal investment, occurs when the perceived fitness returns from future reproduction that might accrue through increased investment in immunity are less than the immediate benefits of current reproduction, as might occur when an infection threat is particularly severe (Clutton-Brock, 1984; Duffield, Bowers, Sakaluk, & Sadd, 2017; Williams, 1966).

Superficially, it might appear that the conventional tactic of trading off reproduction with immunity versus terminally investing in reproduction represent alternative strategies in responding to an infection threat, but a recent review of the terminal investment literature suggests that they represent two ends of a continuum of reproductive allocation. Duffield et al. (2017) showed how the propensity to shift to a terminal investment strategy might depend on other intrinsic and extrinsic factors, such as age or diet, that alter an individual's perceived residual reproductive value beyond the threat eliciting terminal investment, resulting in a shifting or dynamic threshold on the decision to terminally invest. Evidence of a dynamic terminal investment threshold is relatively scarce because it requires experimental manipulation of both the intensity of an infection threat (or some other threat to survival) and the residual reproductive value of experimental subjects. Duffield et al. (2018) provided the first direct empirical test of the dynamic terminal investment threshold in male decorated crickets, *Gryllodes sigillatus*, a species in which much of a male's reproductive effort is devoted to the production of calling song, stereotypic acoustic signals that function to attract sexually receptive females (Ketola, Kortet, & Kotiaho, 2007). Duffield et al. (2018) experimentally induced an immune response in some males by injecting them with heat-killed bacteria, thereby simulating an infection threat without the attendant costs of a live infection. By varying the intensity of the infection threat in males of different ages, they were able to document the existence of a dynamic terminal investment

threshold: males increased their calling effort at the highest threat levels, but this response was observed only in older males.

Burying beetles (genus *Nicrophorus*), insects that inter small vertebrate carcasses as the sole source of food for their offspring (Eggert & Müller, 1997; Pukowski, 1933; Scott, 1998), represent an ideal system with which to seek evidence of a dynamic terminal investment threshold. They reproduce in an environment of decaying carrion suffused with microbes (Arce, Johnston, Smiseth, & Rozen, 2012; Wang & Rozen, 2018), one demanding of a robust immunity. Moreover, both males and females jointly expend considerable time and effort in rearing their offspring (Eggert & Müller, 1997). Hence, reproduction and immunity likely often compete for limited energy reserves, establishing the potential for differential reproduction allocation contingent on whether parents trade off reproduction for increased immune function or opt instead to terminally invest. A male and female that succeed in locating a carcass and securing it against intraspecific competitors, first strip the carcass of its fur or feathers, before rounding it into a ball and burying it. Females lay their eggs in the soil near the carrion ball, from which larvae hatch and make their way to the carcass where they reside in a shallow depression created when the parents chew a hole through the integument of the carcass (Eggert & Müller, 1997; Pukowski, 1933; Scott, 1998). In most cases, the mouthparts of the larvae are not sufficiently sclerotized or serrated to feed directly on the carrion (Benowitz, Sparks, McKinney, Moore, & Moore, 2018), which are instead fed regurgitated carrion by their parents who respond to specialized begging behaviours of the larvae (Eggert, Reinking, & Müller, 1998; Pukowski, 1933; Rauter & Moore, 1999; Smiseth & Moore, 2002). Parents remain with the larvae throughout their development, feeding them and protecting them from other burying beetles, as well as other potential predators (Eggert & Müller, 1997; Scott, 1998). The female typically leaves the carcass when the larvae disperse to pupate in the surrounding soil, whereas the male typically leaves a day or two earlier; the exact sex difference in the timing of departure varies according to species (Scott & Traniello, 1990; Trumbo, 1991).

The immune system of burying beetles comprises both cellular and humoral components; the former typically entails the encapsulation of macroparasites, whereas the latter involves the production of antibacterial substances, such as lysozyme (Gillespie, Kanost, & Tenczek, 1997;

Lawniczak et al., 2007; Söderhall & Cerenius, 1998). In addition to these facets of personal immunity, burying beetles exhibit a form of social immunity that is promoted by transmission of antimicrobial substances and beneficial microbes. Parental beetles smear oral and anal secretions containing antimicrobial substances and beneficial microbes on the carcass to aid in its preservation (Arce et al., 2012; Cotter & Kilner, 2010; Duarte, Welch, Swannack, Wagner, & Kilner, 2018; Shukla et al., 2018). In *Nicrophorus orbicollis*, adult immunity is upregulated during the period of parental care, as manifest by an enhanced encapsulation response (Steiger, Gershman, Pettinger, Eggert, & Sakaluk, 2011). This response is not simply a function of the proximity of carrion, as subordinate individuals that had encountered the carcass, but had been evicted by the dominant pair, showed no comparable increase in immunity (Steiger et al., 2011; Steiger, Gershman, Pettinger, Eggert, & Sakaluk, 2012). Personal immunity is also upregulated during reproduction in *Nicrophorus vespilloides* (Reavey, Warnock, Garbett, & Cotter, 2015), but experimental wounding of females (a form of immune challenge) during a reproductive attempt results in reduced reproductive output (Reavey, Warnock, Vogel, & Cotter, 2014). Thus, it would seem that, under some circumstances at least, reproduction may trade off with immunity (Reavey et al., 2014).

The first evidence of terminal investment in burying beetles comes from studies of age-related differences in reproductive effort in female *N. orbicollis*. Creighton, Heflin, and Belk (2009) showed that older females produce larger broods and consume less of the carcass (thereby leaving more food for their offspring) than younger females. Similarly, Trumbo (2009) demonstrated that older females were more adept than younger females in taking over carcasses controlled by rivals (controlling for the effect of previous breeding experience), and that older females were more likely to initiate a brood. More recent work has shown that previous reproductive experience, in addition to age, can influence female reproductive effort. Billman, Creighton, and Belk (2014) showed that an experimental switch in carcass quality across successive broods alone is sufficient to evoke terminal investment. Females first breeding on a low-quality carcass before being switched to a high-quality carcass in a subsequent reproductive bout produced more young relative to control females, whereas the reverse was true for females first breeding on a high-quality carcass before being switched to a low-quality one.

That an immune challenge might similarly evoke a terminal investment in burying beetles was first shown in *N. vespilloides* injected with dead bacterial cells (*Micrococcus lysodeikticus*). Irrespective of age, immune-challenged females produced heavier broods than control females, and consumed less of the carcass than control females, as evidenced by differences in mass lost over the course of the breeding bout (Cotter, Ward, & Kilner, 2010). A later study showed that females injected with live bacteria (*Bacillus subtilis*) similarly produced heavier broods than control females, indicative of terminal investment (Reavey, Silva, & Cotter, 2015).

Although the propensity for female burying beetles to terminally invest in specific contexts is well established, the inclination for males to do so is unknown, even though males, like females, devote a significant portion of their reproductive effort to parental care. We do know, however, that males have weaker immune responses than females and may be under weaker selection for increased longevity than females (Steiger et al., 2011, 2012), circumstances that may render terminal investment less likely in males. On the other hand, males exhibit considerable plasticity in reproductive behaviour and parental care in other contexts (Eggert, 1992; Luzar, Schweizer, Sakaluk, & Steiger, 2017; Mulrey, Eggert, & Sakaluk, 2015; Royle & Hopwood, 2017; Sakaluk & Müller, 2008), so that it would be surprising if they did not retain some plasticity with respect to their reproductive allocation in the face of a survival threat. Here, we investigate the potential for dynamic terminal investment in male burying beetles, *Nicrophorus marginatus*, in two experiments in which we injected males at two different ages with heat-killed bacteria and measured their reproductive output. We predicted that terminally investing males would (1) show a longer period of parental care, as measured by the time at which the male abandoned the brood, (2) consume less of the carcass, as evidenced by mass gained during a reproductive bout, and (3) produce a greater number of larvae in the current reproductive attempt compared with control males. We further predicted that if terminal investment is influenced by an individual's intrinsic reproductive residual value, it would be more evident in older males than in younger ones.

## METHODS

## *Experimental Animals*

Adult *N. marginatus* were collected in baited pitfall traps at Banner Marsh (40.5397°N, 89.8644°W), which lies alongside the Illinois River about 40 km southwest of Peoria, Illinois, U.S.A. Field-caught individuals were brought back to the laboratory and maintained individually in plastic deli containers (0.5 litre) with moist peat as a substrate. The beetles were fed slivers of Purina Friskies (Nestle Purina, St Louis, MO, U.S.A.) wet cat food twice weekly.

Field-caught beetles were paired randomly for breeding shortly after capture. Each pair was placed in a clean container with a previously frozen, thawed mouse carcass. When the beetles were active on the carcass, the beetles and their carcass were transferred to another container with moist peat to bury the carcass, mate and lay eggs. The containers were transferred to a darkroom once the carcass was buried and checked daily. Larvae that dispersed from the carcass were removed from the carcass and placed into containers with about 4 cm of packed clean moist peat to pupate, with a maximum of 10 larvae to a container. These containers were kept in a dark incubator at 19 °C until adults eclosed. The newly emerged beetles were sexed and placed in a new container with about 2 cm of loose moist peat, either individually or with one same-sex sibling, and maintained in an incubator on a 16:8 h light:dark cycle at 20 °C. These individuals were fed Friskies wet cat food twice weekly, and the peat in their containers replaced every other week.

Once sexually mature, F1 adults were randomly mated with unrelated individuals of the opposite sex to create an F2 population as described above, which was used for our experiments. Larvae and newly emerged adults were maintained as described above for the F1 generation.

## *Experiment 1*

The beetles used in this experiment were sexually mature adults, approximately 3 weeks of age posteclosion. Forty virgin males were each randomly paired with an unrelated virgin female and randomly assigned to either a control or an experimental treatment. Experimental males were cold-anaesthetized on ice for 2–3 min, before being injected with 2 µl of heat-killed *Escherichia coli* ( $5 \times$



10<sup>8</sup>/ml) in Ringer's saline. The injection site was an intersegmental membrane on the beetle's dorsum underneath the elytra, where overall sclerotization is less pronounced than elsewhere on the beetles' body. Although we made no attempt to determine whether a challenge of this magnitude elicits an immune response, an identical concentration of heat-killed *E. coli* was sufficient to elicit both an immune response and terminal investment in crickets, *Gryllodes sigillatus* (Duffield et al., 2018; Duffield, Hampton, Houslay, Hunt, Sadd, & Sakaluk, 2019; Duffield, Hunt, Rapkin, Sadd, & Sakaluk, 2015), and injection of a different bacterial species, *Micrococcus lysodeikticus*, in females of the congeneric burying beetle, *N. vespilloides* has been shown to result in the upregulation of antibacterial activity (Cotter et al., 2010). Control males were similarly handled and cold-anaesthetized, but were not sham-injected, as previous studies have revealed that wounding alone can elicit an immune response in burying beetles (e.g. Cotter, Littlefair, Grantham, & Kilner, 2013; Reavy et al., 2014).

Males and females were weighed to the nearest 1 mg on a microbalance (Mettler PE 360). After males had received their respective treatments, they were each placed in a 0.5-litre plastic deli container containing their assigned female and a thawed mouse carcass, ranging in mass from 20.3 to 24.4 g, as measured using a Denver Instrument XE Series Model 400 electronic balance (mean mass  $\pm$  SE = 22.3  $\pm$  0.2 g). After about 15–30 min in these smaller containers, during which time we could confirm that the male and female had encountered each other and discovered the carcass, we gently transferred the carcass, along with the beetles clinging to it, to a much larger plastic pail (diameter = 20.5 cm, height = 13.3 cm) filled with moistened peat moss to approximately 5 cm below the rim and covered with a clear plastic lid to allow light penetration. Each pair was placed on top of the peat in their respective pail in the afternoon during their normal activity period. All of the carcasses were buried by the following morning, at which point, the tops of the pails were covered with 23  $\times$  23 cm square sections of laminated particle board such that the beetles were held in the dark and left undisturbed during the initial preparation of the carcass and egg laying. Then, each pail was placed into a larger clear plastic storage tub (length = 38.5 cm, width = 26.8 cm, height = 16.6 cm) equipped with a tight-fitting lid. The storage tubs containing their respective pails and experimental pairs were housed in a room maintained at 21–22 °C on an LD 16:8 h cycle.

Three days later, we rearranged the laminated squares covering the top of the pails by pushing them towards one side of the pail, which created a small opening about 2 cm wide at its widest point on the other side of the pail, and secured them to the pail in that position. At the opening, we taped a piece of paper towel over the rim of the pail in such a way that it extended down to the surface of the peat inside the pail, serving as an easy exit path that allowed the beetles to leave the pail at any time of their own volition, as would be the case in nature when adults abandon a depleted carcass at the end of a reproductive bout. We also placed moistened paper towels on the bottom of the storage tub to prevent desiccation of any beetles exiting the pail and falling into the tub below. Twice daily, at 0830 hours and 2030 hours, respectively, over the next few weeks, we checked all tubs to determine whether either the male or female had left the brood. Upon recovering any adult, we recorded the time and day it left the brood, weighed it a second time, fed it and placed it into a smaller container with peat for use in a second experiment (described below).

After both adults had left the brood, we carefully sifted through the peat to recover, weigh and count any larvae reared on the carcass. Three pairs failed to reproduce (two experimental and one control), and one male (experimental) escaped early in the experiment; these replicates were excluded from further analysis, leaving us with 17 experimental pairs and 19 control pairs.

### *Experiment 2*

In a subsequent experiment, half of the control males from the previous experiment were assigned to the experimental treatment and half to the control treatment, whereas half of the experimental males were assigned to the experimental treatment and half to the control treatment, and then given the opportunity to produce a second brood. This second experiment was, thus, a fully crossed, repeated-measures design with three main effects: treatment prior to the first brood, treatment prior to the second brood, and brood (the repeated factor). At the start of the second experiment, all subjects were reproductively experienced, having produced an initial brood, and were 60–70 days of adult age.

Treatments applied in experiment 2 were identical to those applied in experiment 1, and pairs

were allowed to reproduce according to the same housing arrangement and experimental protocol used in the first experiment. Carcasses given to experimental pairs ranged in mass from 17.2 to 27.9 g (mean mass  $\pm$  SE =  $21.4 \pm 0.7$  g).

Of the 36 males that successfully reproduced in experiment 1, six died between experiment 1 and experiment 2 (two control and four experimental); in addition, one pair failed to reproduce in the second experiment, and was also excluded from further analysis. This left us with a total of 29 pairs distributed across the four treatment groups as follows (first treatment/second treatment): C/C = 7, C/E = 9, E/C = 7, E/E = 6).

### *Statistical Analyses*

We used SAS statistical software (version 9.4; SAS Institute, Cary, NC, U.S.A.) and all tests were two tailed ( $\alpha = 0.05$ ). All means are reported  $\pm$  1 SE.

For experiment 1, we used general linear models in PROC GLM to evaluate the effects of treatment on (1) the length of time the male remained on the carcass before abandoning the brood, (2) the postbrood mass of the male, including pretreatment mass as a covariate, as a proxy for the amount of carrion consumed by the male, (3) the number of larvae produced as a measure of a male's reproductive success, including carcass mass as a covariate, and (4) mean larval mass, including carcass mass as a covariate.

For experiment 2, we conducted a repeated-measures analysis in PROC MIXED to assess the effect of treatment, brood (first or second brood), and their interaction on (1) the length of time the male remained on the carcass before abandoning the brood, (2) male mass gain (the difference in pre- and post-treatments weights of the males for each reproductive bout), (3) the number of larvae produced on the carcass and (4) mean larval mass. In the latter two analyses, we accounted for the influence of carcass mass by including the carcass\*age interaction for each treatment, assuming that any effect of carcass mass on reproductive output would be manifest only in the current brood, but not in a previous or later brood (but see Creighton et al., 2009). We used Satterthwaite's degrees-of-freedom approximation, which can result in noninteger denominator degrees of freedom, and an

autoregressive covariance structure, as it provided the best model fit of the appropriate covariance structures available.

## RESULTS

### *Experiment 1*

There were no significant effects of the experimental immune challenge on the length of time males remained on the carcass before abandoning the brood, the mass gained by the male, the number of larvae produced, or mean larval mass (Table 1). Carcass mass had no significant effect on reproductive output (Table 1).

### *Experiment 2*

Brood had a significant effect on the length of time males remained on the carcass ( $F_{1,25} = 20.53$ ,  $P < 0.0001$ ), but no other factor had a significant influence (all  $P > 0.05$ ). Specifically, the length of time males remained on the carcass during the first brood ( $6.93 \pm 0.39$  days) was significantly longer than the time they remained on the carcass during the second brood ( $5.03 \pm 0.39$  days).

There was also a significant effect of brood on the mass gained by males during their residency on the carcass ( $F_{1,25} = 7.56$ ,  $P = 0.01$ ; Fig. 1), and a significant interaction between time and the treatment males received prior to the second brood ( $F_{1,25} = 5.33$ ,  $P = 0.029$ ; Fig. 1). Specifically, males assigned to the control treatment prior to the second brood showed a significant increase in mass gained during the second brood compared with the first ( $F_{1,25} = 12.62$ ,  $P = 0.0015$ ), whereas the mass gained by males assigned to the experimental treatment prior to the second brood was similar across the two broods ( $F_{1,25} = 0.10$ ,  $P = 0.75$ ). To assess the possibility that treatment effects on male mass gain may have been influenced by compensatory carrion consumption by females, we also evaluated mass gained by females using the same repeated-measures model; however, neither female

age, male treatment prior to the first or second broods, or any of the higher-order interactions were statistically significant (all  $P > 0.05$ ).

There was a significant three-way interaction between treatment prior to the first brood, treatment prior to the second brood and brood in their effect on the number of larvae produced ( $F_{1,23} = 5.99$ ,  $P = 0.022$ ). There was also a significant interaction between the mass of the carcass in the second brood and brood ( $F_{1,23} = 7.71$ ,  $P = 0.0027$ ). When considering first the effect of the treatment prior to the first brood, pairwise comparisons revealed a modest increase in the number of larvae produced across successive broods, although this was statistically significant only for the control treatment (control:  $F_{1,23} = 4.43$ ,  $P = 0.046$ ; experimental:  $F_{1,23} = 3.04$ ,  $P = 0.094$ ; Fig. 2). When considering the effect of the treatment prior to the second brood, however, immune-challenged males showed a significant increase of nearly 33% in the number of larvae produced in the second brood relative to the first ( $F_{1,23} = 10.31$ ,  $P = 0.0039$ ), whereas control males showed no such difference ( $F_{1,23} = 0.38$ ,  $P = 0.54$ ; Fig. 2). The mass of the carcass provided in the first brood had no effect on the number of larvae produced in the first brood ( $F_{1,37.2} = 0.34$ ,  $P = 0.73$ ), and, not surprisingly, it had no effect on the number of larvae produced in the second ( $F_{1,37.2} = 0.92$ ,  $P = 0.36$ ). However, the number of larvae produced in the second brood increased with the mass of the carcass provided in the second brood (parameter estimate  $\pm$  SE =  $1.26 \pm 0.38$ ;  $F_{1,37.2} = 3.31$ ,  $P = 0.0021$ ). The mass of the carcass provided during the second brood could not, of course, have had any influence on the number of larvae produced in the first, and this was confirmed by the absence of a significant effect ( $F_{1,37.2} = -0.23$ ,  $P = 0.82$ ). The differential effect of carcass mass on the number of larvae produced across successive broods is likely due to the difference in the range in mass of carcasses offered to breeding pairs, which was much narrower in the first brood (4.1 g) compared with the second (10.7 g).

Neither brood, nor treatment prior to the first and second broods, nor their interaction had any significant effect on mean larval mass.

## DISCUSSION

The results of our study are broadly consistent with the dynamic terminal investment threshold model (Duffield et al., 2017), which posits that the propensity of an individual to terminally invest in response to an immediate survival threat, such as an infection, depends on other intrinsic or extrinsic factors that alter an individual's residual reproductive value. Male burying beetles challenged with heat-killed bacteria as virgins prior to their first reproductive attempt showed no evidence of terminal investment, whereas these same individuals when challenged at a later age as reproductively experienced breeders in a subsequent reproductive attempt exhibited increased reproductive output. These results parallel similar findings in crickets, in which older, immune-challenged males increased their calling effort in response to a graded increase in simulated infection threat, whereas younger males did not (Duffield et al., 2018).

Additional independent support for terminal investment in these older, immune-challenged individuals is evidenced by the fact that they gained less mass during the time on the carcass than control males. This suggests that this terminal investment was subsidized, at least in part, by males refraining from eating as much of the carcass as they might have otherwise done in the absence of an immune challenge. In support of this interpretation, Pilakouta, Richardson, & Smiseth (2016) showed that actual time spent feeding on the carcass is positively associated with increased mass gain. Although some of the difference in mass gain between control and immune-challenged males could be due to the energy expended in mounting an immune response, we know of no study that has shown that upregulation of immunity per se results in a measurable loss of mass in burying beetles. Moreover, our results align with other studies showing that parental mass gain is negatively related to brood size (e.g. Billman et al., 2014; Cotter et al., 2010; Creighton et al., 2009), irrespective of age, and in all of these cases, this has been interpreted as increased investment in the current brood.

If, as we have argued above, immune-challenged males curtail their consumption of the carcass, this would leave more carrion for their offspring to consume, but at the expense of their own maintenance and future reproduction. Indeed, male *N. vespilloides* given the opportunity to breed (and hence, feed on a carcass) subsequently attracted more females in the field via pheromone emission than control males (Chemnitz, Fujan, Bagrii, Ayasse, & Steiger, 2017; Chemnitz, Fujan, Winkelmann, & Steiger, 2018). This suggests that older males who refrain from feeding on the carcass might not be

able to sustain the same level of sexual signalling as less prudent males, and thereby pay a penalty in the form of decreased future mating success.

Although older, immune-challenged males gained less mass during their time on the carcass than control males, no such effect was observed in the females paired with these males, and, thus, it seems unlikely that treatment effects on the number of larvae produced resulted from alterations in female carcass consumption. We cannot, however, entirely rule out the possibility that differences in reproductive output across immune treatments might have been due to indirect effects on females, if, for example, females paired with immune-challenged males increased the quality of their parental care. In theory, we could have removed the female immediately after oviposition to eliminate this possibility, but this might have disrupted the normal coevolved pattern of biparental care (Smith, Creighton, & Belk, 2015). In burying beetles, uniparental male care is extremely rare under natural conditions, occurring in about 3% of naturally occurring broods in *N. vespilloides* (Eggert, 1992) and not at all in a study of three North American species (Scott & Traniello, 1990), whereas uniparental female care is quite common. Laboratory studies have shown that experimentally widowed males greatly increase the frequency and duration of brood care behaviour beyond levels normally observed (Fetherston, Scott, & Traniello, 1994; Rauter & Moore, 2004). Moreover, mate-removal experiments in *N. orbicollis* have shown that although culling of excess larvae by females is proportional to carcass mass, uniparental males overcull larvae across all carcass sizes, behaviour that seems maladaptive (Smith et al., 2015). Collectively, these observations suggest that it would be difficult to extrapolate inferences drawn from observations of uniparental males to the normal breeding environment.

Although males in the present study showed evidence of shifting terminal investment in response to an immune challenge as a function of age, whether female burying beetles do so is less clear. In *N. vespilloides*, immune-challenged females of varying age increased the mass of their broods relative to controls, but this response was not contingent on female age, as the age  $\times$  treatment interaction was not significant (Cotter et al., 2010). Thus, females appear to terminally invest in response to a survival threat, but their propensity to do so seems to be independent of their current intrinsic residual reproductive value. Similarly, female *N. vespilloides* handicapped by the attachment

of a small weight spent more time provisioning their offspring than control females, but the level of provisioning among handicapped females was not influenced by the severity of the handicap (Ratz, Nichol, & Smiseth, 2020). Older female *N. orbicollis* produced larger broods and consumed less of the carcass than younger females (Creighton et al., 2009) and even after controlling for previous breeding experience, older females were more highly motivated to compete for carcasses and initiate a brood than younger females (Trumbo, 2009). These latter findings are consistent with an age-specific propensity for terminal investment.

In the current study, males, irrespective of immune treatment, remained with the carcass longer in the first brood than in the second, a result seemingly at odds with age-specific terminal investment. However, males produced more larvae in their second broods than in their first (Fig. 2), and if the male's departure from the carcass is cued to the amount of carrion remaining, then the earlier departure of males in second broods may simply reflect the more rapid depletion of the carcass by larger broods.

Although immune-challenged male *N. marginatus* breeding for the first time showed no evidence of terminal investment, they also showed no evidence of a trade-off between reproduction and immunity, because there was no difference in the number of larvae produced or mean larval mass of control and immune-challenged males (Table 1). This result seems puzzling because if, as we had assumed, injection of heat-killed bacteria results in the upregulation of male immunity, this should have come at the cost of a reduced reproductive output. It may be that the injection failed to elicit an immune response in virgin males, but this seems unlikely, given that treatment effects consistent with terminal investment were documented in the second experiment. We note, however, that the difference in mass gained by control and immune-challenged males bordered on statistical significance ( $P = 0.088$ ; Table 1), and was in exactly the opposite direction as observed in the second experiment, as immune-challenged virgin males gained more mass than control males (an average of 12 mg, adjusting for pretreatment mass). Thus, it seems that immune-challenged males consumed more of the carcass than control males, seemingly prioritizing maintenance over reproduction, a trend consistent with a trade-off.

The differential reproductive allocation of immune-challenged males across successive



broods in experiment 2 could be due to age-specific terminal investment, but it could also be attributed to a difference in reproductive experience, as age and reproductive experience were perfectly confounded in this experiment. Evidence for both effects has been documented in female burying beetles. Older female *N. orbicollis* exhibit increased reproductive output compared with younger females (Creighton et al., 2009; Trumbo, 2009), whereas the opposite pattern was observed in *N. vespilloides* (Cotter et al., 2010). The quality of a carcass experienced during one breeding event can influence the degree of reproductive effort exhibited in a subsequent breeding attempt irrespective of female age (Billman et al., 2014). There can even be an interaction between age and previous breeding experience: female *N. vespilloides* exhibited a reproductive decline with the number of previous broods produced, but this effect was observed only in older females (Cotter et al., 2010). Regardless, it seems likely that an individual's residual reproductive value decreases with both an increase in age and an increase in breeding experience, and so the context-specific terminal investment shown by immune-challenged males in the current study aligns with theory (Duffield et al., 2017).

In conclusion, terminal investment in immune-challenged burying beetles was evidenced by a decrease in consumption of the resource critical to larval development (i.e. carrion) and an increase in the number of larvae produced. However, the strategy of terminal investment in male burying beetles was expressed only in older males with previous reproductive experience; thus, it appears that the threshold for terminal investment may vary in accordance with a decrease in an individual's residual reproductive value. Whether this pattern of dynamic terminal investment can be generalized to males of other burying beetle species remains uncertain. The extent to which larvae rely on parental care, specifically provisioning, varies considerably across species; in *Nicrophorus vespilloides*, for example, larvae are nutritionally independent of their parents, whereas in *N. orbicollis*, larvae are wholly reliant on parental feeding (Capodeanu-Nägler et al., 2016; Capodeanu-Nägler, Eggert, Vogel, Sakaluk, & Steiger, 2018; Capodeanu-Nägler, Prang et al., 2018). Some of the interspecific variation observed in female reproductive effort as a function of age and immune status could be due to diversification of offspring begging and parental provisioning among species, and the same could be true of males. We might predict, for example, that age-specific terminal investment would be less likely to occur in

species in which larvae rely more heavily on parental care (Duffield et al., 2017). Our understanding of terminal investment in burying beetles could be furthered by using a comparative approach that takes into account the divergent co-adaptation that has been documented among species with varying degrees of offspring dependency on parental care (Capodeanu-Nägler, Ruiz de la Torre, Eggert, Sakaluk, & Steiger, 2018).

## **Data Archiving**

The raw data are archived in the Mendeley Data Repository:

<http://dx.doi.org/10.17632/g2w9fwsp22.2>

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## **References**

- Arce, A.N., Johnston, P.R., Smiseth, P.T., & Rozen, D.E. (2012). Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology*, 25, 930–937.
- Benowitz, K.M., Sparks, M.E., McKinney, E.C., Moore, P.J., & Moore, A.J. (2018). Variation in mandible development and its relationship to dependence on parents across burying beetles. *Ecology and Evolution*, 8, 12832–12840.
- Billman, E.J., Creighton, J.C., & Belk, M.C. (2014). Prior experience affects allocation to current

- reproduction in a burying beetle. *Behavioral Ecology*, 25, 813–818.
- Capodeanu-Nägler, A., Eggert, A.-K., Vogel, H., Sakaluk, S.K., & Steiger, S. (2018). Species divergence in offspring begging and parental provisioning is linked to nutritional dependency of larval burying beetles. *Behavioral Ecology*, 29, 42–50.
- Capodeanu-Nägler, A., Keppner, E.M., Vogel, H., Ayasse, M., Eggert, A.-K., Sakaluk, S.K., et al. (2016). From facultative to obligatory parental care: Interspecific variation in offspring dependency on post-hatching care in burying beetles. *Scientific Reports*, 6, 29323.
- Capodeanu-Nägler, A., Prang, M.A., Trumbo, S.T., Vogel, H., Eggert, A.-K., Sakaluk, S.K., et al. (2018). Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Frontiers in Zoology*, 15, 33.
- Capodeanu-Nägler, A., Ruiz de la Torre, E., Eggert, A.-K., Sakaluk, S.K., & Steiger, S. (2018). Divergent co-evolutionary trajectories in parent–offspring interactions and discrimination against brood parasites revealed by interspecific cross-fostering. *Royal Society Open Science*, 5, 180189.
- Chemnitz, J., Fujan, I.S., Bagrii, N., Ayasse, M., & Steiger, S. (2017). Staying with the young enhances the fathers' attractiveness in burying beetles. *Evolution*, 71, 985–994.
- Chemnitz, J., Fujan, I.S., Winkelmann, C., & Steiger, S. (2018). Why are males more attractive after brood care? Proximate causes of enhanced sex pheromone emission in a burying beetle. *Physiological Entomology*, 43, 120–128.
- Clutton-Brock, T.H. (1984). Reproductive effort and terminal investment in iteroparous animals. *American Naturalist*, 123, 212–229.
- Cotter, S.C., & Kilner, R.M. (2010). Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*. *Journal of Animal Ecology*, 79, 35–43.
- Cotter S.C., Littlefair, J.E., Grantham, P.J., & Kilner, R.M. (2013). A direct physiological trade-off between personal and social immunity. *Journal of Animal Ecology*, 84, 846–853.
- Cotter, S.C., Ward, R.J.S., & Kilner, R.M. (2010). Age-specific reproductive investment in female burying beetles: Independent effects of state and risk of death. *Functional Ecology*, 25, 652–660.

- Creighton, J.C., Heflin, N.D., & Belk, M.C. (2009). Cost of reproduction, resource quality, and terminal investment in a burying beetle. *American Naturalist*, 174, 673–684.
- Duarte, A., Welch, M., Swannack, C., Wagner, J., & Kilner, R.M. (2018). Strategies for managing rival bacterial communities: Lessons from burying beetles. *Journal of Animal Ecology*, 87, 414–427.
- Duffield, K.R., Bowers, E.K., Sakaluk, S.K., & Sadd, B.M. (2017). A dynamic threshold model for terminal investment. *Behavioral Ecology and Sociobiology*, 71, 185.
- Duffield, K.R., Hampton, K.J., Houslay, T.M., Hunt, J., Rapkin, J., Sakaluk, S.K., et al. (2018). Age-dependent variation in the terminal investment threshold in male crickets. *Evolution*, 72, 578–589.
- Duffield, K.R., Hampton, K.J., Houslay, T.M., Hunt, J., Sadd, B.M., & Sakaluk, S.K. (2019). Inbreeding alters context-dependent reproductive effort and immunity in male crickets. *Journal of Evolutionary Biology*, 32, 731–741.
- Duffield, K.R., Hunt, J., Rapkin, J., Sadd, B.M., & Sakaluk, S.K. (2015). Terminal investment in the gustatory appeal of nuptial food gifts in crickets. *Journal of Evolutionary Biology*, 28, 1872–1881.
- Eggert, A.-K. (1992). Alternative male mate-finding tactics in burying beetles. *Behavioral Ecology*, 3, 243–254.
- Eggert, A.-K., & Müller, J.K. (1997). Biparental care and social evolution in burying beetles: Lessons from the larder. In J. C. Choe & B. J. Crespi (Eds.), *The evolution of social behavior in insects and arachnids* (pp. 216–236). Cambridge, U.K.: Cambridge University Press.
- Eggert, A.-K., Reinking, M., & Müller, J.K. (1998). Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour*, 55, 97–107.
- Fetherston, I.A., Scott, M.P., & Traniello, J.F.A. (1994). Behavioural compensation for mate loss in the burying beetle *Nicrophorus orbicollis*. *Animal Behaviour*, 47, 777–785.
- Gillespie, J.P., Kanost, M.R., & Trenczek, T. (1997). Biological mediators of insect immunity. *Annual Review of Entomology*, 42, 611–643.
- Ketola, T., Kortet, R., & Kotiaho, J.S. (2007). Testing theories of sexual selection in decorated

- crickets (*Gryllodes sigillatus*). *Evolutionary Ecology Research*, 9, 869–885.
- Lawniczak, M.K.N., Barnes, A.I., Linklater, J.R., Boone, J.M., Wigby, S., & Chapman, T. (2007). Mating and immunity in invertebrates. *Trends in Ecology & Evolution*, 22, 48–55.
- Luzar, A.B., Schweizer, R., Sakaluk, S.K., & Steiger, S. (2017). Access to a carcass, but not mating opportunities, influences paternal care in burying beetles. *Behavioral Ecology and Sociobiology*, 71, 7.
- Mulrey, T.E.P., Eggert, A.-K., & Sakaluk, S.K. (2015). Switching tactics: Phenotypic plasticity in the alternative mate-finding tactics of burying beetles. *Animal Behaviour*, 108, 175–182.
- Pilakouta, N., Richardson, J., & Smiseth, P.T. (2016). If you eat, I eat: Resolution of sexual conflict over consumption from a shared resource. *Animal Behaviour*, 111, 175–180.
- Pukowski, E. (1933). Ökologische Untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und Ökologie der Tiere*, 27, 518–586.
- Ratz, T., Nichol, T.W., & Smiseth, P.T. (2020). Parental responses to increasing levels of handicapping in a burying beetle. *Behavioral Ecology*, 31(1), 73–80.  
doi:10.1093/beheco/arz157.
- Rauter, C.M., & Moore, A.J. (1999). Do honest signalling models of offspring solicitation apply to insects? *Proceedings of the Royal Society of London B: Biological Sciences*, 266, 1691–1696.
- Rauter, C.M., & Moore, A.J. (2004). Time constraints and trade-offs among parental care behaviours: Effects of brood size, sex and loss of mate. *Animal Behaviour*, 68, 695–702.
- Reavey, C.E., Silva, F.W.S., & Cotter, S.C. (2015). Bacterial infection increases reproductive investment in burying beetles. *Insects*, 6, 926–942.
- Reavey, C.E., Warnock, N.D., Garbett, A.P., & Cotter, S.C. (2015). Aging in personal and social immunity: Do immune traits senesce at the same rate? *Ecology and Evolution*, 5, 4365–4375.
- Reavey, C.E., Warnock, N.D., Vogel, H., & Cotter, S.C. (2014). Trade-offs between personal immunity and reproduction in the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology*, 25, 415–423.
- Royle, N.J., & Hopwood, P.E. (2017). Covetable corpses and plastic beetles: The socioecological behavior of burying beetles. *Advances in the Study of Behavior*, 49, 101–146.

- Sakaluk, S.K., & Müller, J.K. (2008). Risk of sperm competition mediates copulation duration, but not paternity, of male burying beetles. *Journal of Insect Behavior*, 21, 153–163.
- Scott, M.P. (1998). The ecology and behavior of burying beetles. *Annual Review of Entomology*, 43, 595–618.
- Scott, M.P., & Traniello, J.F.A. (1990). Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (*Nicrophorus* spp.) *Animal Behaviour*, 39, 274–283.
- Sheldon, B.C., & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution*, 11, 317–321.
- Shukla, S.P., Plata, C., Reichelt, M., Steiger, S., Heckel, D.G., Kaltenpoth, M., et al. (2018). Microbiome-assisted carrion preservation aids larval development in a burying beetle. *Proceedings of the National Academy of Sciences of the United States of America*, 115, 11274–11279.
- Smiseth, P. T., & Moore, A. J. (2002). Does resource availability affect offspring begging and parental provisioning in a partially begging species? *Animal Behaviour*, 63, 577–585.
- Smith, A.N., Creighton, J.C., & Belk, M.C. (2015). Differences in patterns of reproductive allocation between the sexes in *Nicrophorus orbicollis*. *PLoS One*, 10, e0143762.
- Söderhäll, K., & Cerenius, L. (1998). Role of the prophenoloxidase-activating system in invertebrate immunity. *Current Opinion in Immunology*, 10, 23–28.
- Steiger, S., Gershman, S.N., Pettinger, A.M., Eggert, A.-K., & Sakaluk, S.K. (2011). Sex differences in immunity and rapid upregulation of immune defense during parental care in the burying beetle, *Nicrophorus orbicollis*. *Functional Ecology*, 25, 1368–1378.
- Steiger, S., Gershman, S.N., Pettinger, A.M., Eggert, A.-K., & Sakaluk, S.K. (2012). Dominance status and sex influence nutritional state and immunity in burying beetles *Nicrophorus orbicollis*. *Behavioral Ecology*, 23, 1126–1132.
- Trumbo, S.T. (1991). Reproductive benefits and the duration of paternal care in a biparental burying beetle, *Necrophorus orbicollis*. *Behaviour*, 117, 82–105.
- Trumbo, S.T. (2009). Age-related reproductive performance in the parental burying beetle,

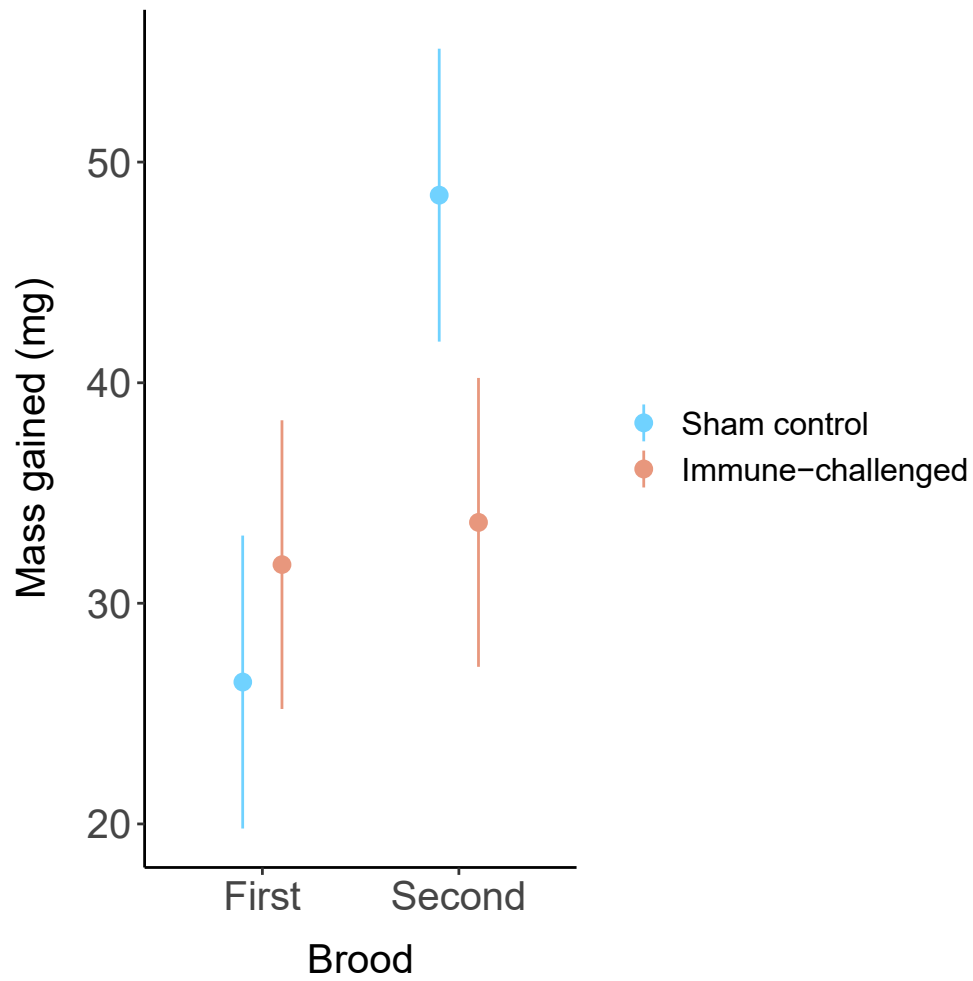
- Nicrophorus orbicollis*. *Behavioral Ecology*, 20, 951–956.
- Wang, Y., & Rozen, D.E. (2018). Gut microbiota in the burying beetle, *Nicrophorus vespilloides*, provide colonization resistance against larval bacterial pathogens. *Ecology and Evolution*, 8, 1646–1654.
- Williams, G.C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *American Naturalist*, 100, 687–690.
- Zera, A.J., & Harshman, L.G. (2001). The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics*, 32, 95–126.
- Zuk, M., & Stoehr, A.M. (2002). Immune defense and host life history. *American Naturalist*, 160(Suppl.), S9–S22.

**Table 1**

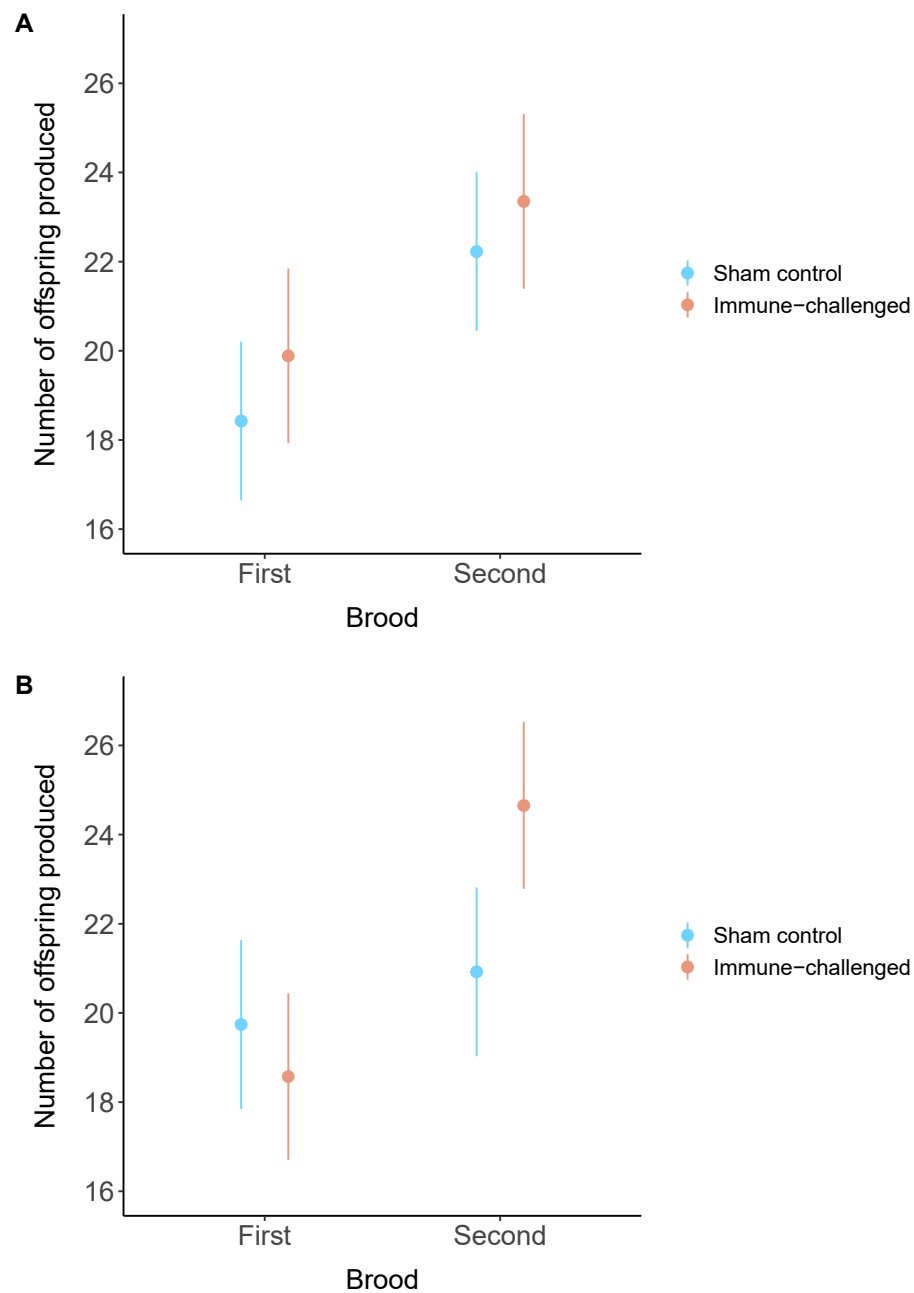
Experiment 1: effects of experimental immune challenge on male reproductive investment in the initial brood

| Effect                               | <i>F</i> | <i>df</i> | <i>P</i> |
|--------------------------------------|----------|-----------|----------|
| <b>Time on carcass</b>               |          |           |          |
| Treatment                            | 0.33     | 1, 34     | 0.572    |
| <b>Post-treatment male body mass</b> |          |           |          |
| Treatment                            | 3.09     | 1, 33     | 0.088    |
| Pretreatment mass                    | 952.49   | 1, 33     | <0.0001  |
| <b>Number of larvae</b>              |          |           |          |
| Treatment                            | 0.55     | 1, 33     | 0.462    |
| Carcass mass                         | 1.34     | 1, 33     | 0.255    |
| <b>Mean larval mass</b>              |          |           |          |
| Treatment                            | 0.14     | 1, 33     | 0.712    |
| Carcass mass                         | 0.00     | 1, 33     | 0.949    |





**Figure 1.** Effect of immune challenge applied prior to the second brood on mass gained by males during their time on the carcass. Centroids are least-squares means  $\pm$  SE.



**Figure 2.** Effect of immune challenge on number of offspring produced. (a) Applied prior to the first brood. (b) Applied prior to the second brood. Centroids are least-squares means  $\pm$  SE.