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Abstract

Parental care is highly variable, reflecting that parents make flexible decisions about how much care to provide in response to variation in the cost and/or benefit of care. Handicapping has traditionally been used as a tool for increasing the energetic cost of care, thereby inducing a reduction in care by handicapped parents. However, recent evidence shows that handicapped parents sometimes provide more care, suggesting that handicapping can trigger terminal investment. Here, we investigate responses to different levels of handicapping in the burying beetle *Nicrophorus vespilloides* by comparing handicapped female parents fitted with a wide range of handicaps, as well as control females without a handicap. We found that handicapped females spent more time provisioning food and less time being absent from the crypt than control females, whilst there was no effect of the level of handicapping among handicapped females. We found no effect of handicapping on larval begging behavior, larval performance (mean larval mass and brood size at dispersal), or female investment in future reproduction (i.e., weight gain whilst breeding and life span after breeding). Our findings provide no support for the widely held assumption that handicapping simply increases the cost of care. Instead, our results are consistent with the suggestion that handicapping triggers terminal investment by suppressing the condition of parents below the threshold at which terminal investment is triggered.

Keywords: cost of care; *Nicrophorus vespilloides*; parental decision; reproductive trade-off; terminal investment.

Introduction

Parental care encompasses any parental trait that enhances the survival and/or growth of a parent's offspring, often at a cost to the parent's ability to invest in other current or future offspring (Clutton-Brock 1991; Royle et al. 2012). Parental care is highly variable (Clutton-Brock 1991; Royle et al. 2012), reflecting that parents make flexible decisions about how much care to provide due to variation in the cost of care to themselves and/or the benefit to their offspring (Royle et al. 2014; Ratz and Smiseth 2018). For example, parents are expected to provide less care should there be an increase in the cost of care, as shown by handicapping experiments on birds and insects (Wright and Cuthill 1989; Harrison et al. 2009; Suzuki and Nagano 2009). Handicapping experiments are used to study negotiation between parents in birds with biparental care (Harrison et al. 2009), and their rationale is to increase the energetic cost of providing care at a given level by attaching a lead weight to the base of the handicapped parent's tail feathers (Wright and Cuthill 1989) or by clipping some of its flight feathers (Slagsvold and Lifjeld 1988; 1990). Most such experiments find that handicapped parents provide less care than control parents (e.g. Wright and Cuthill 1989; Harrison et al. 2009), confirming that parents plastically reduce the amount of care they provide when the cost of care increases. However, a recent study on the burying beetle *Nicrophorus vespilloides* found that handicapped females provided *more* care than control females (Ratz and Smiseth 2018). This finding contradicts the implicit assumption that handicapping simply increases the cost of care. In light of this, there is now a need to improve our understanding of how parents respond to handicapping given its important role in the study of parental care.

One potential explanation for why handicapped parents sometimes provide more care than control parents is that handicapping can trigger a shift towards greater investment in current reproduction (Ratz and Smiseth 2018), often referred to as terminal investment (Williams 1966; Clutton-Brock 1984). Theory suggests that terminal investment is triggered when an individual's condition deteriorates below a certain threshold value, thereby reducing its future survival prospects (Duffield et al. 2017). Handicapping could trigger terminal investment if it suppresses the parent's condition below this threshold value by, for example, reducing its foraging ability or increasing its energy expenditure. Thus, current evidence suggests that handicapping might influence the parent's behavior either by increasing the energetic cost of care or by triggering terminal investment. We note that these two effects are not mutually exclusive, as handicapping could both increase the cost of care and trigger terminal investment. If so, we might expect more complex responses to handicapping that are determined by a combination of whether or not the handicap suppresses the parent's condition below the threshold triggering terminal investment and the extent to which the handicap increases the energetic cost of care. As outlined below, in order to advance our understanding of the effects of handicapping, we now need novel experimental designs that monitor how caring parents respond to different levels of handicapping.

In this study, we investigated how female parents responded to different levels of handicapping in a burying beetle. Burying beetles of the genus *Nicrophorus* are ideal study systems to explore this issue because they show highly elaborate forms of parental care, including provisioning of pre-digested carrion to the larvae and depositing antimicrobial secretions to preserve the small vertebrate carcass used for breeding as a food source throughout larval development (Scott 1998). Furthermore, these beetles have been subject to handicapping experiments, showing that handicapped parents either provide less care, as reported in studies on *Nicrophorus quadripunctatus* and *N. orbicollis* (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016), or more care, as reported in *N. vespilloides* (Ratz and Smiseth 2018). One potential explanation for why studies have reported contrasting effects of handicapping is that these studies used different levels of handicapping. For example, studies showing that handicapped parents provide less care used larger weights that were about 40–50% of a parent's body mass (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016), whereas the study reporting that handicapped parents provide more care used smaller weights that were about 20–30% of a parent's body mass (Ratz and Smiseth 2018). Although this pattern suggests that parents provide more care in response to a relatively small handicap but less care in response to a relatively large handicap, there is now a need for experimental work monitoring how parents respond to different levels of handicapping within a single species.

Our aim was to investigate how single female parents respond to different levels of handicapping in the burying beetle *N. vespilloides*. We handicapped females by attaching a small weight to their pronotum (Suzuki and Nagano 2009). The weights weighed 0.037–0.242g, corresponding to 11–103% of a female's body mass. We also included a control treatment, where females were not fitted with a weight but otherwise were handled in the same way as handicapped females. Prior work shows that females respond by providing more care when fitted with a 0.05g weight (Ratz and Smiseth 2018), suggesting that the threshold triggering terminal investment is below this level of handicapping. We then tested for subsequent effects on the amount of care provided by females (i.e., time spent provisioning food and maintaining the carcass) during the period where females provide direct care for larvae, as well as on offspring performance (i.e., mean larval mass, number of larvae at dispersal and larval begging behavior) and female investment in future reproduction (i.e., weight change whilst breeding and life span after breeding).

If handicapping primarily increased the cost of care, we predicted that females should provide progressively less care as the level of handicapping increased (Figure 1a). Furthermore, offspring performance should gradually decline as the level of handicapping increases, and females should pay a progressively higher cost in terms of their investment in future reproduction. Conversely, if handicapping primarily triggered terminal investment, we predicted that the effects of the level of handicapping should be discontinuous with handicapped females providing more care than control females provided that the handicap suppressed the parent's condition below the threshold value (Figure 1b). Below this threshold, handicapped parents should provide as much care as control parents. Above the threshold, handicapped parents should provide more care than control parents, but the former should provide the same level of care regardless of the level of handicapping (Figure 1b). Furthermore, offspring performance should be higher, whilst female investment in future reproduction should be lower, above the threshold than below. Finally, if handicapping both elevates the cost of care and triggers terminal investment, we predicted that the effects of the level of handicapping should be discontinuous with a marked increase in care by handicapped parents at the threshold value (Figure 1c). However, above this threshold, handicapped parents should provide progressively less care as the level of handicapping increases. Furthermore, offspring performance and female investment in future reproduction should gradually decline with the level of handicapping above the threshold.

Materials and methods

Source and rearing of experimental beetles

The beetles used in this experiment came from a laboratory stock population originating from beetles collected at Corstorphine Hill Local Nature Reserve and Hermitage of Braid and Blackford Hill Local Nature Reserve, Edinburgh, UK. Non-breeding adult beetles were housed in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil. All beetles were fed organic beef twice a week and maintained under a constant temperature (20°C) and a 16:8h light:dark photoperiod.

Experimental design and procedures

We manipulated the level of handicapping by attaching a non-toxic fishing weight (Dinsmores, Aldridge, UK and DGT, Shirley, UK) to the pronotum of caring females (see below for further details). The weights used in our experiment weighed 0.037–0.242g, corresponding to 11–103% of the initial body mass of females. We used this range to ensure that our handicaps overlapped the range used in prior work on this species (20–30%; Ratz and Smiseth 2018) and on *N. quadripunctatus* and *N. orbicollis* (40–50%; Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016). We also included weights that went beyond this range used to ensure that our handicaps were large enough to induce a potential increase in the energetic cost of care. Our design included a control treatment, where females were not fitted with a weight but were otherwise handled and treated in the same way as handicapped females. In this experiment, we focused on the response of a single parent to exclude potential compensatory responses by its partner. We did this given that our aim was to establish whether handicapping increases the cost of care, triggers terminal investment, or both. We specifically focused on single female parents because females provide more parental care than males in this species (Eggert et al. 1998; Rauter and Moore 2004) and because the experimental removal of the male has no effect on offspring fitness under laboratory conditions (Smiseth et al. 2005).

We began the experiment by pairing females and males at random, transferring each pair into a larger plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass (Livefoods Direct, Sheffield, UK) of a standardized size (14.68 –19.98g). One day before the expected date of hatching (i.e., two days after the beginning of egg laying), we randomly assigned each female to the handicapping or the control treatment (i.e., no weight; hereafter referred to as 0g). Although the nominal mass of the weights was categorical (0.05g, 0.10g or 0.20g), there was considerable variation in the mass of weights within each category (range, mean ± SE for

0.05g, 0.10g and 0.20g weights, respectively: 0.0370–0.0757g, 0.0544 ± 0.0017g; 0.0716–0.1241g, 0.0959 ± 0.0019; 0.1702–0.2423g, 0.1988 ± 0.0026). We weighed all females before and after subjecting them to the handicapping treatment, using the difference in mass as a measure of the mass of the handicap provided to each female. We attached the weight to the pronotum of each handicapped female using instant-adhesive glue (Suzuki & Nagano, 2009; Creighton et al., 2015; Suzuki, 2016; Ratz and Smiseth 2018). Before attaching the weight, we gently scraped the surface of the apex of the pronotum using fine sandpaper (P600). We did so to remove impurities, thereby improving adhesion of the weight. We treated females assigned to the control treatment in the same way as handicapped females (i.e., we weighed them before and after handling, handled them, and scraped the surface of, and applied glue to, their pronotum), except that no weight was attached to their pronotum. For further details on the handicapping procedure, we refer to Ratz and Smiseth (2018).

Once handicapped females had been fitted with a weight and control females had been handled, we moved them together with their mouse carcass to a fresh container filled with moist soil. We did this to separate females from their eggs, thereby allowing us to provide them with standardized experimental broods. Once the larvae started hatching, we collected them in a temporary holding container, using them to generate experimental broods comprised of 10 same-aged larvae of mixed maternal origin (Smiseth et al. 2007a). For practical reasons, we allocated females broods comprising some larvae that were their own and some that were foreign. It is unlikely that this would influence our results as there is no evidence that females differentiate between their own and foreign larvae in this species. Instead, females have a temporal kin discrimination mechanism whereby they kill any larvae arriving on the carcass before their own eggs would have hatched (Müller and Eggert 1990). Thus, to avoid infanticide, we ensured that we only provided females with an experimental brood once their own eggs had hatched. We used experimental rather than natural broods in this experiment to control for potential confounding effects due to variation in the number of larvae in the brood and the age of the brood, both of which are known to influence the amount of care provided by females in *N. vespilloides* (Smiseth et al. 2003; Smiseth et al. 2007a; 2007b). We removed male parents at the same time as we moved females to a fresh container.

We recorded data on the amount of care provided by handicapped and control females 24 h (±15 min) after we placed the larvae on the carcass. This time point corresponds to the peak in time spent providing care towards larvae in this species (Smiseth et al. 2003). We collected behavioral data using instantaneous sampling every 1 min for 30 min under red light, in accordance with established protocols (e.g. Smiseth and Moore 2002, 2004a; Ratz and Smiseth 2018). Although the 30 min sampling period is a relatively small part of the period when females provide direct care for the larvae (larvae become nutritionally independent 72 h after hatching), there are positive correlations between different measures of parental care in *N. vespilloides* (Andrews et al. 2017), and the amount of time spent providing care 24h after hatching is positively correlated with the time at which the parents desert the brood (Pilakouta, N., Hanlon, B. & Smiseth, P.T., personal communication). Thus, our sampling period is representative of the total amount of care provided by females. At each scan, we recorded whether the female was engaged in the following behaviors: *provisioning food*, defined as when there was mouth-to-mouth contact between the female and at least one larva, *maintaining the carcass*, defined as when the female was excavating the soil around the carcass or coating the carcass with secretions or *absent from the crypt*, defined as when the female was away from the crypt (i.e., the depression surrounding the carcass). We conducted the behavioural observations blind with respect to treatments as far as this was practically possible. The observations were blind for the different levels of handicapping, as it was not possible for the observer to identify the size of the handicap in the dim light conditions of the observation conducted. However, it was not possible to keep the control treatment (i.e., 0g) blind, as the observer could tell whether females had been provided with a weight or not.

At the same time as we recorded data on the amount of care provided by females, we also recorded data on larval begging to test for potential effects of handicapping on larval behavior. In burying beetles, larval begging is tactile and begging larvae raise their bodies towards the female and touch the female with their legs (Smiseth and Moore 2002). Larval begging only occurs when the parent is in close contact with the larvae, defined as a distance less than or equal to the width of the female's pronotum (Rauter and Moore 1999; Smiseth and Moore 2002). At each scan, we counted the number of larvae that were begging. We calculated the average proportion of time spent begging per larva in the brood as $B = (\Sigma b/n)/p$, where Σb is the cumulative number of begging events during the 30-min observation period, n is the brood size at the time of observation and p is the number of scans during which the female was near the larvae. This metric provides a measure of larval begging that is largely independent of variation in female behavior towards the larvae (Smiseth and Moore 2004b).

At the time of larval dispersal from the carcass, which normally takes place about 5 days after hatching, we recorded the number of surviving larvae in the brood and weighed the brood. We did this to test for potential effects of handicapping on offspring performance. We calculated mean larval mass by dividing the total brood mass by the number of surviving larvae in the brood. In this species, body size is a key determinant of an individual's reproductive success and adult body size is highly correlated with larval mass at dispersal (Otronen 1988; Safryn and Scott 2000). At the time of larval dispersal, we also removed the weights from the female's pronotum by gently twisting the weight or lifting it off using soft forceps. We removed the weights at this time to obtain information on the potential fitness cost of handicapping during the period when females provided care for their larvae. We then recorded the post-breeding body mass of each female, which we used to calculate the female's weight change whilst breeding as the difference between post- and pre-breeding body mass. Finally, we recorded female life span after breeding. To this end, we moved all females into individual containers and we then checked each container twice a week and recorded the date of death for each female.

We set up 137 pairs in total in the course of this experiment. We excluded 3 females that did not lay any eggs, 11 females whose eggs that did not hatch and 3 females for which the weight of the handicap was recorded incorrectly, yielding the following final sample sizes for female parental behavior, larval begging, mean larval mass at dispersal and female weight change: control females (0g weight: N = 30), and handicapped females (0.037–0.242g: N = 90). We further excluded two females from our analyses on brood size at dispersal because the number of larvae was uncertain, yielding the following final sample sizes for brood size: control females (N = 29), and handicapped females (N = 89). For female life span, we excluded 35 females for the reasons stated above and because we could not remove their weights, yielding the following final sample sizes for this trait: control females (N = 28), and handicapped females (N = 67).

Statistical analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team 2019). Behavioral traits were recorded as the total number of scans out of a maximum of 30 scans and were therefore analyzed assuming a binomial error structure. Given that our data on time spent provisioning food, maintaining the carcass and absent from the crypt by females showed over-dispersion and minor zero-inflation, we analyzed these data using a Bayesian approach with the *MCMC_{GLMM}* R package (Hadfield 2010), fitting the models with a binomial error structure using “multinomial2” and a flat improper prior. We analyzed data on offspring performance and female investment in current and future reproduction using general linear models with a Gaussian error structure for normally distributed traits (mean larval mass at dispersal and female weight change), and using generalized linear models with a binomial error structure for larval begging and a Poisson error structure for other traits representing count data (female life span and brood size at dispersal).

Given that our main aim was to test for an overall effect of the level of handicapping on our traits of interest and given the considerable variation in mass of fishing weights (see above for further details), we treated handicapping as a continuous linear predictor, including a quadratic term to test for possible non-linear effects of handicapping. We included the initial weight of the female at the time of treatment as a predictor in the models to account for potential variation among different-sized females in their response to the level of handicapping. We also included brood size at the time of observation as a covariate in the model on female parental behavior, and we included brood size at dispersal in the model on female weight change because brood size influences food provisioning in this species (e.g. Smiseth et al. 2007a; Ratz and Smiseth 2018). Finally, we included female weight change as a covariate in the model on female lifespan given that prior work shows that life span is positively correlated with weight change (Gray et al. 2018). Parameter estimates for the Bayesian model are given as posterior means ± 95% CIs of 1499 samples ran for 1.5×10^6 iterations with a thinning interval of 1.0×10^3 and a burn-in of 1.0×10^3.

Results

Female parental behavior

Handicapping had a positive linear effect on the amount of time females spent provisioning food to the brood, whilst there was a negative effect of the quadratic term of handicapping (Figure 2a; Table 1). Visual inspection of confidence intervals suggests that handicapped females spent more time provisioning food than control females, but that there was no effect of the level of handicapping among handicapped females (Figure 2a). This interpretation is supported by posthoc tests, showing that handicapped females spent more time provisioning food than control females (estimate = 1.129, lower 95% = 0.416, upper 95% = 1.940, P_{MCMC} = 0.001) and that there was no effect of the level of handicapping when restricting the analysis to handicapped females (estimate = 18.4, lower 95% = –15.07, upper 95% = 50.9, P_{MCMC} = 0.278). Handicapping had a negative linear effect on the amount time females were absent from the crypt, and there was a positive effect of the quadratic term of handicapping (Figure 2b, Table 1). Visual inspection suggests that control females were more likely to abandon the brood temporarily than handicapped females, whilst there was no effect of the level of handicapping among handicapped females (Figure 2b). This interpretation is supported by posthoc tests, showing that handicapped females spent less time being absent than control females (estimate = –6.510, lower 95% = –10.6, upper 95% = –2.000, P_{MCMC} = 0.001) and that there was no effect of the level of handicapping when restricting the analysis to handicapped females (estimate = –184.7, lower 95% = –451.1, upper 95% = 65.1929, P_{MCMC} = 0.108). There was no linear effect of handicapping and no effect of the quadratic term on time spent maintaining the carcass (Table 1).

There was no effect of brood size at the time of observation on time spent provisioning food (estimate = 0.136, lower 95% = –0.026, upper 95% = 0.288, P_{MCMC} = 0.092), time spent absent from the crypt (estimate = 0.036, lower 95% = – 0.882, upper 95% = 0.973, $\mathrm{P}_{\mathrm{MCMC}}$ = 0.925), or time spent maintaining the carcass (estimate = 0.108, lower 95% = – 0.070, upper 95% = 0.282, $\mathrm{P}_{\mathrm{MCMC}}$ = 0.235). Likewise, there was no effect of the initial weight of females on time spent provisioning food (estimate = – 4.63, lower 95% = – 10.4, upper 95% = 1.84, $\mathrm{P}_{\mathrm{MCMC}}$ = 0.111), time spent absent from the crypt (estimate = 22.6, lower 95% = – 18.8, upper 95% = 65.3, $\mathrm{P}_{\mathrm{MCMC}}$ = 0.273), or time spent maintaining the carcass (estimate = 4.25, lower 95% = – 2.69, upper 95% = 11.0, $\mathrm{P}_{\mathrm{MCMC}}$ = 0.272).

Offspring performance

There were no effects of either the linear or the quadratic terms of handicapping on larval begging (Table 2). Likewise, there were no effects of the linear or the quadratic terms of handicapping on mean larval mass at dispersal (Table 2) or brood size at dispersal (Table 2). Thus, there was no evidence that larvae spent less time begging in response to handicapping of their female parent even though handicapped females spent more time provisioning food, and there was no evidence that handicapping of the female affected on offspring performance. There was no effect of the initial weight of females on larval begging (estimate = – 4.40, SE = 7.49, z = – 0.588, P = 0.557), mean larval mass (estimate = – 0.070, SE = 0.051, t = – 1.38, P = 0.171), or brood size (estimate = –0.340, SE = 2.28, t = – 0.149, P = 0.882).

Female investment in current and future reproduction

There were no effects of the linear or quadratic terms of handicapping on female weight change whilst breeding (Table 2) or female life span after breeding (Table 2). Likewise, brood size at dispersal had no effect on female relative weight change (estimate = – 0.412, SE = 0.519, t = –0.795, P = 0.429). The initial weight of females had no effect on female relative weight change (estimate = 25.4, SE = 28.7, t = 0.886, P = 0.378), but it had a significant positive effect on female life span with heavier females living for longer (estimate = 0.823, SE = 0.240, z = 3.43, P = 0.001). Finally, female weight change had no effect on female life span (estimate = –0.0003, SE = 0.0009, z = –0.300, P = 0.764).

Discussion

Here, we tested for effects of different levels of handicapping on the amount of care provided by female parents, the performance of their offspring and female investment towards current reproduction in the burying beetle *N. vespilloides*. At the time point in larval development corresponding to the peak in parental care, handicapped females spent more time provisioning food to the brood and less time being away from the crypt than control females. This finding confirms evidence from a recent study on *N. vespilloides* reporting that handicapped females provide more care than control females (Ratz and Smiseth 2018). We found no evidence of females providing less care as the level of handicapping increased. Furthermore, there was no evidence that handicapping influenced time spent maintaining the carcass by females, larval begging behavior, larval performance (i.e., mean larvae size at dispersal and larval survival until dispersal), or female investment in current and future reproduction (i.e., weight change over the reproductive attempt or female life span after breeding). Below, we provide a more detailed discussion of our results and their implications for our understanding of how handicapping affects parental care decisions.

Our main finding was that handicapped females spent more time provisioning food than control females, but that there was no effect of the level of handicapping among handicapped females. The first finding is consistent with prior work on this species showing that handicapped females spend more time provisioning food (Ratz and Smiseth 2018). Handicapped females are predicted to provide more care than control females if handicapping suppresses the female's condition below the threshold value triggering terminal investment (Duffield et al. 2017). Thus, our results provide further evidence that handicapping can trigger terminal investment and suggest that even the smaller handicaps used in our experiment were sufficient to suppress the female's condition below the threshold value. The second finding (i.e., that there was no effect of the level of handicapping among handicapped females) is consistent with what we predicted if handicapping primarily induced a shift towards greater investment in current reproduction (Figure 1b). In contrast, if handicapping both induced such a shift and increased the energetic cost of care, we predicted that handicapped females should provide progressively less care as the level of handicapping increased (Figure 1c). One potential explanation for why we found no evidence that handicapped females provided less care as the level of handicapping increased is that our handicaps were too small to increase the energetic cost of care. This explanation seems unlikely given that our experiment included handicaps that were substantially larger than those used in prior studies on burying beetles reporting that handicapped females provided less care than control females (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016), Thus, our results have important implications for our understanding of handicapping by confirming that its effects on parental behavior cannot be explained simply as a consequence of an increase in the energetic cost of providing a given level care, as implicitly assumed in prior handicapping experiments (Ratz and Smiseth 2018).

An alternative explanation for why handicapped females provide more care than control females is that handicapping might have a differential effect on activities associated with different modes of locomotion. For example, in burying beetles, females walk whilst caring for their current brood, whilst they fly whilst searching for carcasses for use in future reproductive attempts (Scott 1998). Increasing the level of handicapping might trigger a shift towards greater investment in current reproduction if handicapping has a greater impact on the energetic cost of flight than on the energetic cost of walking. There is some support for this suggestion from prior work on the burying beetle *N. quadripunctatus* indicating that handicapped females cease flying but continue walking (Nagano and Suzuki 2009). Handicapping may have limited impact on walking in these beetles given that females have been reported to move vertebrate carcasses weighing up to 30g (i.e., objects weighing over 100 times more than the largest handicaps used in our experiment) for several meters (Scott

1998). Thus, our results may reflect that handicapping in burying beetles may have a greater impact on the cost of locating a new carcass required for initiating a future reproductive attempt than on the cost of providing care in the current reproductive attempt.

Our finding that handicapped females provided more care than control females contrasts with prior handicapping experiments on birds (e.g. Wright and Cuthill 1989; Harrison et al. 2009) and other species of burying beetles (*N. quadripunctatus*: Suzuki and Nagano 2009; Suzuki 2016; *N. orbicollis*: Creighton et al. 2015) reporting that handicapped parents provide less care than controls. One potential explanation for why our results differ from those of prior studies is that handicapping primarily increases the cost of care in birds and other species of burying beetles, whilst it primarily triggers a shift towards greater investment in current reproduction in our study species. For example, in altricial birds, parents fly continuously between the nest and the foraging sites in the surrounding environment to provision their nestlings with arthropods or other sources of food. Thus, we might expect handicapping to have greater impact on the energetic cost of care in birds than in our study species. Although this suggestion might explain why our results differ from prior studies on birds, it seems unlikely that it accounts for the difference between our study species and other species of burying beetles. The reason for this is that all burying beetles breed on carcasses of small vertebrates and that, in all species, parents walk rather than fly whilst caring for their larvae. Instead, the different results from studies on different species of burying beetles might reflect differences in their life histories. For example, a recent study shows that larval survival is more dependent on parental care in *N. orbicollis* than in *N. vespilloides* (Capodeanu-Nägler et al. 2016). Thus, there may be differences between species of burying beetles with respect to the returns on investment in current reproduction. Alternatively, there might be differences in the availability of resources for investment in future reproduction between different species. If so, this might lead to interspecific variation in the trade-off between current and future reproduction. Currently, relatively little is know about differences between species of burying beetles with respect to availability of resources and the trade-off between current and future reproduction. Thus, obtaining such information should now be a priority to help explaining why studies on different species of burying beetles sometimes find somewhat different results.

One potential explanation for why our results differ from those of prior studies on burying beetles is that females may respond differentially to handicapping depending on whether they are assisted by a male partner or not. In our study, as well as in the prior study reporting that handicapped females provided more care than controls (Ratz and Smiseth 2018), handicapped and control females reared their brood on their own without assistance from a male partner. In contrast, handicapped and control females reared their brood with the assistance from a male partner in studies reporting that handicapped females provided less care than controls (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016). Thus, handicapped females might provide less care when assisted by a male partner, whilst they provide more care when rearing the brood on their own. Such a differential response to handicapping might be expected if the presence of a male partner buffers against any negative effects on offspring should females provide care. If so, handicapped females could reduce their contribution towards care without harming their offspring's fitness when assisted by a male partner, whilst this would not be the case when rearing the brood on their own. Thus, there is now a need for studies that investigate whether female burying beetles respond differentially to handicapping depending on whether they are assisted by a male partner or not.

We found that handicapped females spent less time being absent from the crypt than control females. Currently, little is known about why breeding females temporarily leave the crypt in this species, but potential explanations are that females do so to explore the surrounding area for signs of conspecific intruders and/or predators. Thus, our results suggest that handicapped females are less inclined to explore the surrounding area than control females. An alternative explanation is that handicapped females remained within the crypt simply as a consequence of reduced mobility. However, if this was the case, we should also expect handicapped females to spend less time provisioning food than control females given that this behavior also requires mobility. Thus, given that we found that handicapped females spent more time provisioning food, this explanation seems unlikely (Figure 2). Our study highlights that there is a need to investigate why breeding females temporarily leave the crypt in this species.

We found no evidence that handicapping affected larval begging behavior, larval performance (i.e., mean larval mass or larval survival until dispersal), or female investment in current and future reproduction (i.e., weight change over reproduction and life span after reproduction). These findings are surprising given that handicapped females spent more time provisioning food towards larvae than control females. Prior work shows that larval begging in *N. vespilloides* reflects larval hunger state (Smiseth and Moore 2004a) and that larvae grow to a larger size when receiving more care from female parents (Andrews et al. 2017). Thus, we might expect larvae reared by handicapped females to be less hungry, therefore spending less time begging, and to grow to be a larger size than larvae reared by control females. One potential explanation for why we found no such effects is that the quality of care (e.g., nutritional quality of pre-digested carrion transferred to larvae via mouth-to-mouth contact) was lower in handicapped females than in control females. If so, larvae might receive a similar amount of care regardless of whether they are reared by handicapped or control females. An alternative explanation is that handicapping had a differential effect at different times of the larvae’s development. Our results show that handicapped females spent more time providing care at the time point in larval development corresponding to the peak in parental care (i.e., 24h after hatching) than control females. Given that we recorded effects on female parental behavior at a single time point, and we cannot rule out the possibility that handicapped females provided less care either earlier or later in development. Finally, we found that handicapping had no effect on female weight change during breeding or female life span. These results contrast with those of most studies on birds, showing that handicapped females lose more weight than control females (e.g. Markman et al. 1995; Slagsvold and Lifjeld 1990; Sanz et al. 2000). As discussed above, the energetic cost of care might be relatively high in birds, in which case we might expect handicapped females to lose more weight than controls. In contrast, the energetic cost of care might be relatively low in burying beetles. There is also evidence that parents forage from the carcass whilst breeding (Pilakouta et al. 2016), which may allow handicapped females to compensate for the energetic cost of handicapping by consuming more food from the carcass (Ratz and Smiseth 2018).

Our study adds to our understanding of the terminal investment hypothesis; that is, the suggestion that parents should increase their investment in reproduction during their final reproductive attempt (Williams 1966, Hirschfield and Tinkle 1975, Clutton-Brock 1984). Traditionally, the terminal investment hypothesis has focused on increases in investment in reproduction with age (Clutton-Brock 1984), but its rationale applies to any factor that suppresses the condition of parents below a certain threshold that reduces their prospects for future reproduction. Indeed, there is mounting evidence that terminal investment is triggered by a range of factors other than age, including immune challenges (e.g., Podmokła et al. 2014), intraspecific competition (e.g., Rebar and Greenfield 2017) and predation risk (e.g., Knight et al. 2000). Thus, our results suggest that handicapping can be added to the list of factors that can induce terminal investment by suppressing the parent's condition. We suggest that handicapping would provide a useful tool for studying terminal investment as it provides a simple experimental tool for suppressing an individual's condition. Given that handicaps can be removed, such experiments could be used to establish whether individuals reverse their decisions to invest more in current reproduction should their condition improve at a later stage.

In conclusion, we found that handicapped females spent more time providing care than control females, possibly reflecting that handicapping suppresses the condition of females below the threshold triggering terminal investment (Duffield et al. 2017). Our results have important implications for our understanding of the effects of handicapping, which is a key experimental tool used by behavioral ecologists to study negotiation between parents in species with biparental care (Harrison et al. 2009). Such studies are based on the assumption that handicapping primarily increases the energetic cost of care, and our results show that this is not necessarily the case. This conclusion emphasizes that handicapping experiments can lead to different outcomes in different species, presumably reflecting differences in the modes of locomotion of caring parents, differences in life histories, and/or differential responses depending on the presence or absence of a partner. Thus, we encourage further handicapping experiments across a variety of different taxa and social contexts.

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Table 1: Effects of handicapping (linear and quadratic terms) on time spent provisioning food, maintaining the carcass and being absent from crypt by females. Values were obtained from Bayesian GLMs using *MCMCglmm*. The sample sizes were 30 for control females (i.e., 0g weight) and 90 handicapped females (i.e., 0.037–0.242g weight), respectively.

	Handicapping				Handicapping^2			
	Estimate	Lower 95%	Upper 95%	P_{MCMC}	Estimate	Lower 95%	Upper 95%	P_{MCMC}
Provisioning food	19.4	4.83	33.2	**0.004**	– 66.1	– 125.3	1.42	**0.033**
Absent from the crypt	–134.1	– 238.6	–49.6	**<0.0001**	491.0	83.5	919.7	**0.008**
Maintenance of carcass	15.2	– 1.18	30.7	0.056	–47.5	–124.4	18.8	0.192

Table 2: Effects of handicapping (linear and quadratic terms) on larval begging behaviour, larval performance (mean larval mass and brood size) and female investment in current and future reproduction (female weight change and female life span). Values were obtained from GLMs. The sample sizes for larval begging, mean larval mass and female weight change were 30 for control females (i.e., 0g weight) and 90 for handicapped females (i.e., 0.037–0.242g weight), respectively. The sample sizes for brood size were 29 for control and 118 for handicapped females, and the sample sizes for female life span were 28 for control and 67 for handicapped females.

	Handicapping				Handicapping^2			
	Estimate	SE	t/z-value	*P-value*	Estimate	SE	t/z-value	*P-value*
Larval begging	2.17	18.1	0.120	0.904	–3.70	79.5	–0.047	0.963
Mean larval mass	0.051	0.116	0.444	0.658	–0.235	0.519	–0.454	0.651
Brood size	8.94	4.89	1.827	0.070	-35.4	22.3	–1.59	0.115
Female weight change	11.6	63.5	0.182	0.856	132.9	292.3	0.454	0.651
Female life span	0.334	0.526	0.635	0.526	–2.01	2.42	–0.830	0.406

Figures

Figure 1: Predicted effects of the level of handicapping on the amount of care provided by parents. If handicapping primarily elevates the cost of care, parents should provide progressively less care as the level of handicapping increases (a). If handicapping primarily triggers terminal investment, the effects of the level of handicapping should be discontinuous with a marked increase in care by handicapped parents at the threshold value (b). Handicapped parents should provide as much care as control parents below this threshold, whilst they should provide more care than control parents above the threshold. Handicapped parents should provide the same level of care regardless of the level of handicapping above the threshold. If handicapping both elevates the cost of care and triggers terminal investment, the effects of the level of handicapping should also be discontinuous with a marked increase in care by handicapped parents at the threshold value (c). However, in this case, handicapped parents should provide progressively less care as the level of handicapping increases above the threshold.

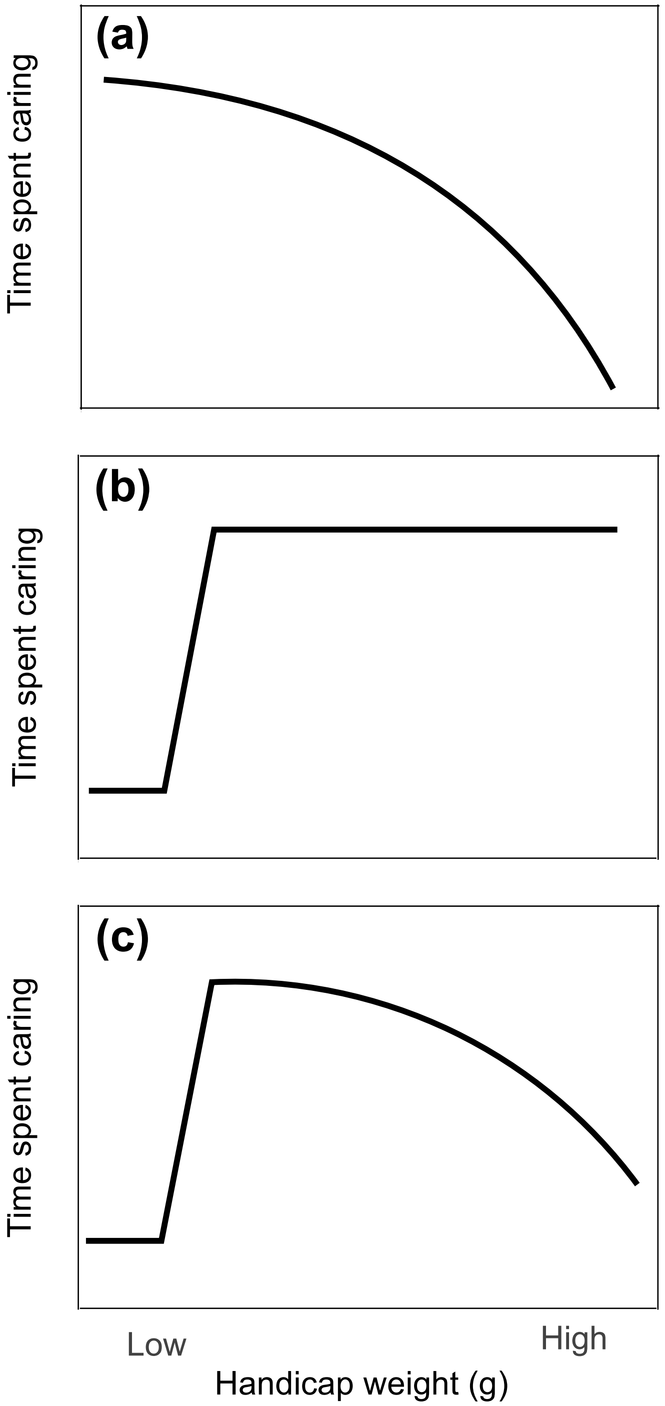
(a)
Time spent caring
(b)
Time spent caring
(c)
Time spent caring
Low
High
Handicap weight (g)

Figure 2: Effects of the level of handicapping on the proportion of time spent provisioning by the female (a) and time absent from the crypt (b). Proportions represent the total time spent provisioning or absent from the crypt during the 30-min observation period, divided by 30. The black lines represent polynomial regression lines (± 95% confidence intervals) from GLMs assuming a binomial error structure.