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## Parental responses to increasing levels of handicapping in a burying beetle

**Citation for published version:**

Ratz, T, Nicol, T & Smiseth, PT 2019, 'Parental responses to increasing levels of handicapping in a burying beetle', *Behavioral Ecology*. <https://doi.org/10.1093/beheco/arz157>

**Digital Object Identifier (DOI):**

[10.1093/beheco/arz157](https://doi.org/10.1093/beheco/arz157)

**Link:**

[Link to publication record in Edinburgh Research Explorer](#)

**Document Version:**

Peer reviewed version

**Published In:**

Behavioral Ecology

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# Parental responses to increasing levels of handicapping in

## 2 a burying beetle

4 Manuscript information:

Abstract: 221 words; main text (not including references and legends): 6234 words; 2 figures,

6 and 39 references

8 **Running title:** Handicapping and parental care in a burying beetle

10

## Abstract

12 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  
14 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  
16 handicapped parents sometimes provide more care, suggesting that handicapping can trigger  
terminal investment. Here, we investigate responses to different levels of handicapping in the  
18 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  
20 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  
22 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  
24 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  
26 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  
28 terminal investment is triggered.

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

## 32 **Introduction**

34 Parental care encompasses any parental trait that enhances the survival and/or growth of a  
36 parent's offspring, often at a cost to the parent's ability to invest in other current or future  
38 offspring (Clutton-Brock 1991; Royle et al. 2012). Parental care is highly variable (Clutton-  
40 Brock 1991; Royle et al. 2012), reflecting that parents make flexible decisions about how  
42 much care to provide due to variation in the cost of care to themselves and/or the benefit to  
44 their offspring (Royle et al. 2014; Ratz and Smiseth 2018). For example, parents are expected  
46 to provide less care should there be an increase in the cost of care, as shown by handicapping  
48 experiments on birds and insects (Wright and Cuthill 1989; Harrison et al. 2009; Suzuki and  
50 Nagano 2009). Handicapping experiments are used to study negotiation between parents in  
52 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  
58 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,  
60 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  
62 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  
64 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  
66 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  
68 of the effects of handicapping, we now need novel experimental designs that monitor how  
caring parents respond to different levels of handicapping.

70 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  
72 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  
74 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  
76 handicapping experiments, showing that handicapped parents either provide less care, as  
reported in studies on *Nicrophorus quadripunctatus* and *N. orbicollis* (Suzuki and Nagano  
78 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  
80 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

82 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  
84 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  
86 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  
88 a single species.

Our aim was to investigate how single female parents respond to different levels of  
90 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–  
92 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  
94 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  
96 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  
98 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  
100 dispersal and larval begging behavior) and female investment in future reproduction (i.e.,  
weight change whilst breeding and life span after breeding).

102 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).  
104 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  
106 future reproduction. Conversely, if handicapping primarily triggered terminal investment, we

108 predicted that the effects of the level of handicapping should be discontinuous with  
110 handicapped females providing more care than control females provided that the handicap  
112 suppressed the parent's condition below the threshold value (Figure 1b). Below this threshold,  
114 handicapped parents should provide as much care as control parents. Above the threshold,  
116 handicapped parents should provide more care than control parents, but the former should  
118 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.

## 122 **Materials and methods**

### *Source and rearing of experimental beetles*

124 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  
126 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  
128 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.

130

### *Experimental design and procedures*

132 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  
134 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  
136 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  
138 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  
140 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  
142 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  
144 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  
146 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  
148 conditions (Smiseth et al. 2005).

We began the experiment by pairing females and males at random, transferring each  
150 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  
152 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  
154 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  
156 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,  $0.0544 \pm 0.0017$ g; 0.0716–  
158 0.1241g,  $0.0959 \pm 0.0019$ ; 0.1702–0.2423g,  $0.1988 \pm 0.0026$ ). We weighed all females before  
and after subjecting them to the handicapping treatment, using the difference in mass as a  
160 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;  
162 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  
164 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  
166 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  
168 the handicapping procedure, we refer to Ratz and Smiseth (2018).

Once handicapped females had been fitted with a weight and control females had been  
170 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  
172 them with standardized experimental broods. Once the larvae started hatching, we collected  
them in a temporary holding container, using them to generate experimental broods  
174 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  
176 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.  
178 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).  
180 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

182 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  
184 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.

186 We recorded data on the amount of care provided by handicapped and control females  
24 h ( $\pm 15$  min) after we placed the larvae on the carcass. This time point corresponds to the  
188 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  
190 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  
192 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  
194 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  
196 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  
198 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  
200 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  
202 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  
204 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  
206 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.

208           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  
210 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  
212 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  
214 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  $B =$   
216  $(\Sigma b/n)/p$ , where  $\Sigma b$  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  
218 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  
220 (Smiseth and Moore 2004b).

          At the time of larval dispersal from the carcass, which normally takes place about 5  
222 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  
224 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  
226 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  
228 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  
230 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

232 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  
234 individual containers and we then checked each container twice a week and recorded the date  
of death for each female.

236 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  
238 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  
240 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  
242 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  
244 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  
246 handicapped females (N = 67).

## 248 *Statistical analysis*

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team  
250 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  
252 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  
254 approach with the *MCMCGLMM* R package (Hadfield 2010), fitting the models with a binomial  
error structure using “multinomial2” and a flat improper prior. We analyzed data on offspring  
256 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  
258 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  
260 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  
262 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  
264 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  
266 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  
268 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.  
270 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  
272 positively correlated with weight change (Gray et al. 2018). Parameter estimates for the  
Bayesian model are given as posterior means  $\pm$  95% CIs of 1499 samples ran for  $1.5 \times 10^6$   
274 iterations with a thinning interval of  $1.0 \times 10^3$  and a burn-in of  $1.0 \times 10^3$ .

## 276 **Results**

### *Female parental behavior*

278 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  
280 (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

282 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  
284 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  
286 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  
288 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  
290 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  
292 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  
294 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,  $P_{MCMC}$   
296 = 0.108). There was no linear effect of handicapping and no effect of the quadratic term on  
time spent maintaining the carcass (Table 1).

298 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  
300 absent from the crypt (estimate = 0.036, lower 95% = -0.882, upper 95% = 0.973,  $P_{MCMC} =$   
0.925), or time spent maintaining the carcass (estimate = 0.108, lower 95% = -0.070, upper  
302 95% = 0.282,  $P_{MCMC} = 0.235$ ). 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,  
304  $P_{MCMC} = 0.111$ ), time spent absent from the crypt (estimate = 22.6, lower 95% = -18.8, upper  
95% = 65.3,  $P_{MCMC} = 0.273$ ), or time spent maintaining the carcass (estimate = 4.25, lower  
306 95% = -2.69, upper 95% = 11.0,  $P_{MCMC} = 0.272$ ).

308 ***Offspring performance***

There were no effects of either the linear or the quadratic terms of handicapping on larval  
310 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).  
312 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,  
314 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 =  
316 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,  $t = -0.149$ ,  $P = 0.882$ ).

318

320 ***Female investment in current and future reproduction***

There were no effects of the linear or quadratic terms of handicapping on female weight  
322 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 =  
324 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  
326 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  
328 span (estimate = -0.0003, SE = 0.0009,  $z = -0.300$ ,  $P = 0.764$ ).

330 **Discussion**

Here, we tested for effects of different levels of handicapping on the amount of care provided  
332 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  
334 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  
336 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  
338 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  
340 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  
342 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  
344 handicapping affects parental care decisions.

Our main finding was that handicapped females spent more time provisioning food  
346 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  
348 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  
350 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  
352 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  
354 (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

356 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  
358 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  
360 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  
362 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  
364 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  
366 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  
368 and Smiseth 2018).

An alternative explanation for why handicapped females provide more care than  
370 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  
372 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  
374 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  
376 suggestion from prior work on the burying beetle *N. quadripunctatus* indicating that  
handicapped females cease flying but continue walking (Nagano and Suzuki 2009).  
378 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  
380 100 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  
382 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.

384 Our finding that handicapped females provided more care than control females  
contrasts with prior handicapping experiments on birds (e.g. Wright and Cuthill 1989;  
386 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  
388 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  
390 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  
392 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  
394 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  
396 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  
398 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  
400 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  
402 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  
404 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

406 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  
408 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  
410 results.

One potential explanation for why our results differ from those of prior studies on  
412 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  
414 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  
416 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  
418 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  
420 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  
422 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  
424 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  
426 differentially to handicapping depending on whether they are assisted by a male partner or  
not.

428 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  
430 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  
432 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  
434 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  
436 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  
438 highlights that there is a need to investigate why breeding females temporarily leave the crypt  
in this species.

440 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  
442 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  
444 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  
446 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  
448 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.,  
450 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  
452 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  
454 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

456 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  
458 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  
460 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  
462 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  
464 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  
466 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).

468 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  
470 reproductive attempt (Williams 1966, Hirschfield and Tinkle 1975, Clutton-Brock 1984).  
Traditionally, the terminal investment hypothesis has focused on increases in investment in  
472 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  
474 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.  
476 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  
478 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  
480 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  
482 decisions to invest more in current reproduction should their condition improve at a later  
stage.

484 In conclusion, we found that handicapped females spent more time providing care than  
control females, possibly reflecting that handicapping suppresses the condition of females  
486 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  
488 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  
490 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  
492 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  
494 depending on the presence or absence of a partner. Thus, we encourage further handicapping  
experiments across a variety of different taxa and social contexts.

496

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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 <sup>2</sup>			
	Estimate	Lower 95%	Upper 95%	<i>P</i> <sub>MCMC</sub>	Estimate	Lower 95%	Upper 95%	<i>P</i> <sub>MCMC</sub>
Provisioning food	19.4	4.83	33.2	<b>0.004</b>	– 66.1	– 125.3	1.42	<b>0.033</b>
Absent from the crypt	–134.1	– 238.6	–49.6	<b>&lt;0.0001</b>	491.0	83.5	919.7	<b>0.008</b>
Maintenance of carcass	15.2	– 1.18	30.7	0.056	–47.5	–124.4	18.8	0.192

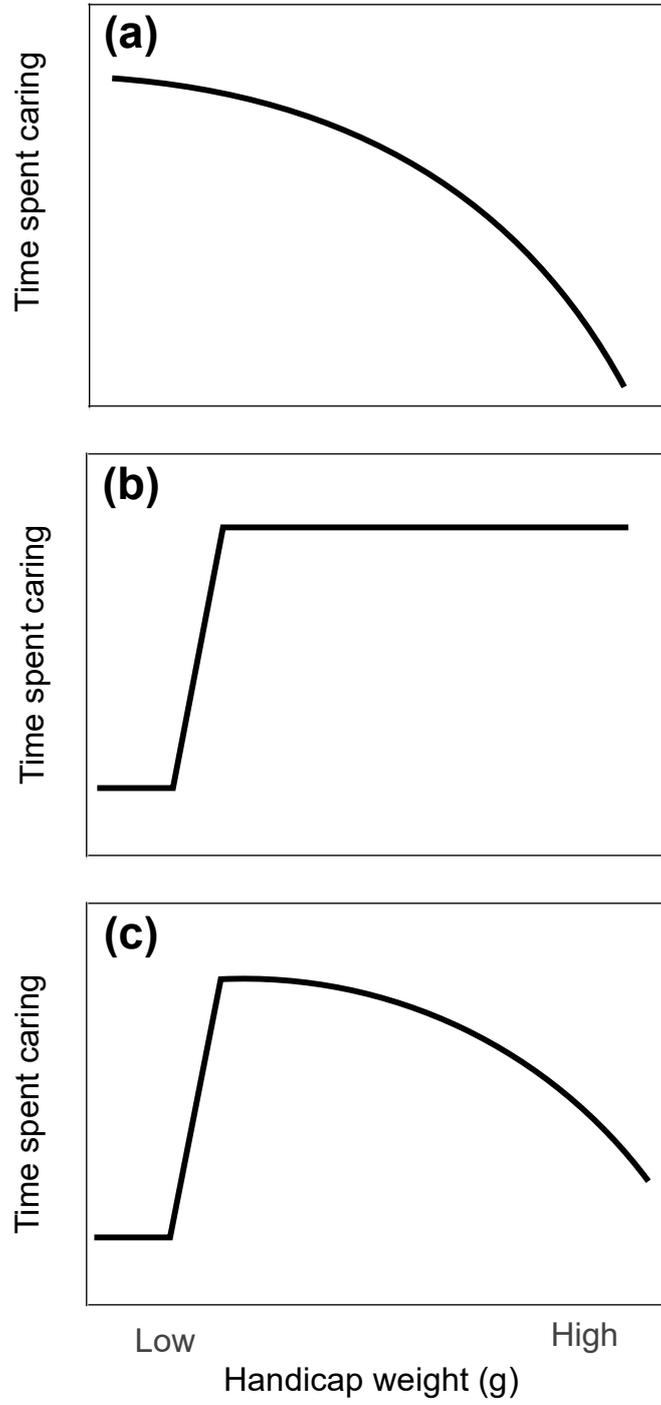
**Table 2:** Effects of handicapping (linear and quadratic terms) on larval begging behaviour, larval performance (mean larval mass and brood size) and female investment in current and future reproduction (female weight change and female life span). Values were obtained from GLMs. The sample sizes for 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 <sup>2</sup>			
	Estimate	SE	t/z-value	<i>P</i> -value	Estimate	SE	t/z-value	<i>P</i> -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.



**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 ( $\pm$  95% confidence intervals) from GLMs assuming a binomial error structure.

