Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*

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Abstract

Parental care is highly variable, reflecting that parents make flexible decisions in response to variation in the cost of care to themselves and the benefit to their offspring. Much of the evidence that parents respond to such variation derives from handicapping and brood size manipulations, the separate effects of which are well understood. However, little is known about their joint effects. Here, we fill this gap by conducting a joint handicapping and brood size manipulation in the burying beetle *Nicrophorus vespilloides*. We handicapped half of the females by attaching a lead weight to their pronotum, leaving the remaining females as controls. We also manipulated brood size by providing each female with 5, 20 or 40 larvae. In contrast to what we predicted, handicapped females spent *more* time provisioning food than controls. We also found that handicapped females spent more time consuming carrion. Furthermore, handicapped females spent a similar amount of time consuming carrion regardless of brood size, whereas controls spent more time consuming carrion as brood increased. Females spent more time provisioning food towards larger broods, and females were more likely to engage in carrion consumption when caring for larger broods. We conclude that females respond to both handicapping and brood size manipulations, but these responses are largely independent of each other. Overall, our results suggest that handicapping might lead to a higher investment into current reproduction and that it might be associated with compensatory responses that negate the detrimental impact of higher cost of care in handicapped parents.

Introduction

Parental care is defined as any parental trait that has evolved to enhance the survival and/or growth of the parent's offspring, often at cost to the parent's own fitness (Royle *et al.*, 2012). Typical forms of care include protection against predators and other environmental hazards, and provisioning of food or other resources after hatching or birth (Smiseth *et al.*, 2012). In many species, parental care is highly variable, reflecting that parents make flexible decisions about how much care to provide in response to variation in the cost of care to

themselves and the benefit of care to their offspring (Alonso-alvarez & Velando, 2012; Royle *et al.*, 2014). In general, parents are expected to provide less care when the cost of care is higher and provide more care when the benefit of care is higher (Grodzinski & Johnstone, 2012). Much of the experimental evidence for these two predictions derives from handicapping and brood size manipulations, respectively. For example, handicapping experiments in birds and insects (based on attachments of weights or feather clipping) show that handicapped parents decrease their care, presumably because handicapping elevates the cost of care to parents (Wright & Cuthill, 1989; Harrison *et al.*, 2009; Suzuki & Nagano, 2009). Likewise, brood size manipulations in birds, fishes and insects show that parents usually provide more care towards enlarged broods, presumably because the benefit of care is higher, while parents provide less care towards reduced broods,

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presumably because the benefit of care is lower (e.g. Ridgway, 1989; Sanz, 1997; Rauter & Moore, 2004; Smiseth *et al.*, 2007). Thus, handicapping and brood size manipulations have been instrumental in providing experimental evidence showing that variation in the cost and benefit of care are key determinants of how parents make flexible decisions regarding how much care to provide for their offspring.

Although we have a good understanding of the separate effects of handicapping and brood size manipulations on the amount of care provided by parents, little is known about their joint effects. Despite the lack of formal theory, we can derive predictions from simple graphical models based on assumptions about how handicapping and brood size manipulations influence the cost and benefit functions of care (Fig. 1). These functions describe the effect that specific levels of parental care have on parental and offspring fitness, respectively (Smiseth, 2017). The cost function is assumed either to increase at an accelerating rate or to be linear. In either case, if handicapping increases the cost of care, handicapped parents are predicted to reduce their level of care (Fig. 1), as reported for birds (Wright & Cuthill, 1989; Harrison *et al.*, 2009) and insects (Suzuki & Nagano, 2009). Meanwhile, the benefit function is assumed to increase at a decelerating rate to reach an asymptote above which any further increase in care has no effect on offspring fitness (Trivers, 1974; Royle *et al.*, 2012). The benefit function describes the fitness effect on an individual offspring. Thus, in order to derive the indirect benefit function to the parent, we need to account for both the coefficient of relatedness between the parent and its offspring and the number of offspring in the brood (Fig. 1). If brood size enlargement increases the benefit of care, parents should increase their care towards enlarged broods (Fig. 1), as reported for fishes (e.g. Ridgway, 1989), birds (Sanz, 1997) and insects (e.g. Rauter & Moore, 2004; Smiseth *et al.*, 2007). Furthermore, this model predicts no interaction effect (or one that is too small to be detected) if handicapping leads to only minor divergence in the cost function at higher levels of care (Fig. 1a,b). On the other hand, it predicts an interaction effect if handicapping leads to a greater divergence in the cost function at higher levels of care (Fig. 1c,d). These predictions have never before been tested empirically, and here, we address this gap by conducting a joint handicapping and brood size manipulation experiment in the burying beetle *Nicrophorus vespilloides*.

Burying beetles of the genus *Nicrophorus* are ideal for studying the joint effects of handicapping and brood size as prior studies show that parents respond to both treatments (handicapping: Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Suzuki, 2016; brood size manipulations: Rauter & Moore, 2004; Smiseth *et al.*, 2007). These beetles breed on carcasses of small vertebrates that serve as the sole food source for the brood during larval development (Eggert *et al.*, 1998; Scott, 1998). Larvae can obtain resources by either feeding directly from the carcass or begging for predigested carrion from the parents (Smiseth & Moore, 2002; Smiseth *et al.*, 2003). In *N. vespilloides*, begging reflects the offspring's nutritional need (Smiseth & Moore, 2004b) and is costly to the offspring in terms of increased risk of filial cannibalism (Andrews & Smiseth, 2013). Prior work on *N. vespilloides* and *Nicrophorus orbicollis* shows that parents respond to brood size manipulations by increasing their food provisioning rate towards larger broods (Rauter & Moore, 2004; Smiseth *et al.*, 2007). Moreover, prior work on *Nicrophorus quadripunctatus* and *N. orbicollis* shows that handicapped parents provide less care than control parents (Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Suzuki, 2016). Although the reduction in parental care by handicapped parents is generally attributed to an increase in the cost of care, this response may also be caused by deteriorating condition of handicapped parents (Pilakouta *et al.*, 2015) or by stress induced by handicapping. Regardless of how handicapping leads to a reduction in parental care, there is no information on the joint effects of handicapping and brood size manipulations on the amount of care provided by parents.

Our main aim was to examine joint effects of handicapping and brood size on the overall level of care provided by females and on female weight change during breeding. The latter is used as a proxy for how much females consume from the carcass to invest into their future reproduction (Creighton *et al.*, 2009; Billman *et al.*, 2014). We expect an effect of the interaction between handicapping and brood size only if handicapping leads to a greater divergence in the cost function at higher levels of care (Fig. 1d). We predict main effects of handicapping and brood size, reflecting that weighted females provide less care to the brood than control females and that females provide more care to larger broods than to smaller ones. We predict an effect of the interaction between handicapping and brood size and main effects of handicapping and brood size on the amount of time spent provisioning food by parents. The reason for this is that this form of parental care is directed towards individual offspring within the brood (unlike other forms of care, such as carcass maintenance). We also predict that handicapping and an increase in brood size would be associated with a greater loss in weight of females, reflecting that weighted females pay a greater cost from their investment into the current brood and that larger broods require more care. Our second main aim was to test for subsequent consequences of handicapping and brood size on offspring begging and offspring performance. We predict that handicapping of females would lead to an increase in larval begging and have a detrimental impact on larval fitness given that weighted females would spend less time provisioning food to the brood. Similarly, we
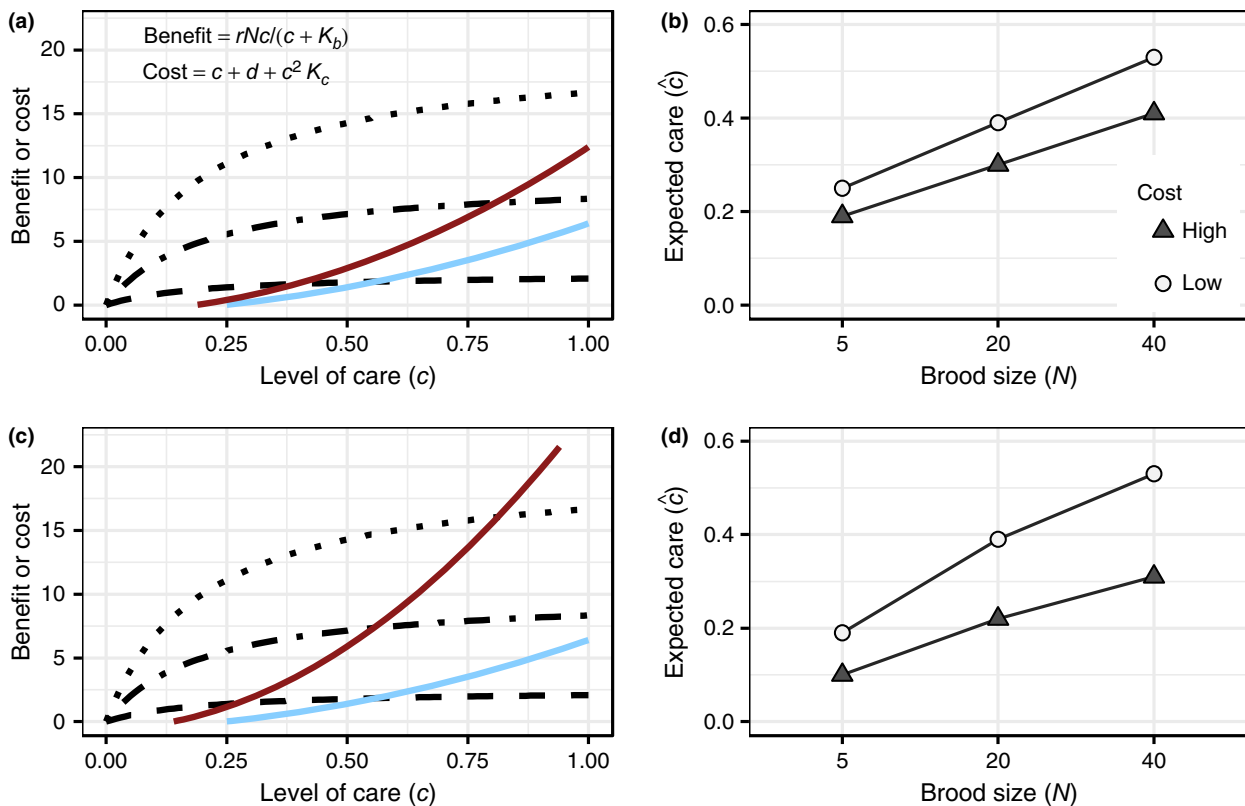
Fig. 1 Direct cost and indirect benefit functions of parental care in relation to the level of care (a, c). The cost functions (blue and red lines) increase at an accelerating rate, and the benefit functions (black lines) increase at a decelerating rate when a parent increases its level of parental care c. The specific cost and benefit returns to a parent depend on the coefficient of relatedness between the parent and its offspring (here, $r = 0.5$), the brood size N, the intercept b of the cost function, and the shapes k_c and k_b of the cost and benefit functions, respectively (adapted from Kölliker et al., 2010). The indirect benefit of care to the parent increases with the number of offspring in the brood, which in this case varies between 5 (dashed line), 20 (dashed-dotted line) or 40 offspring (dotted line). The direct cost of care to the parent may be relatively low (blue line, $k_{c\text{-}Low} = 6$) or high (red line), depending on whether females are handicapped or not. Handicapping may affect the slope of the cost function, shown here as the divergence in functions (red lines) at higher levels of care, here with $k_{c\text{-}High} = 10$ (a) and $k_{c\text{-}High} = 24$ (c). This model predicts that parents should provide less care when the cost of care is higher and the benefit of care is lower. The model also predicts that there should be an effect of the interaction between the cost and benefit of care if handicapping leads to a greater divergence in the cost function at higher levels of care (d). On the other hand, there may be no such an effect (or it may not be detectable) if handicapping leads to a minor divergence in the cost function at higher levels of care (b).

predict that an increase in brood size would lead to an increase in larval begging and have a detrimental impact on larval performance given that larger broods should be associated with more intense sibling competition (Smiseth et al., 2007).

Materials and methods

Beetle husbandry

We used the second to the fifth generation of beetles from a laboratory population of outbred beetles descending from a population collected in Corstorphine Hill, Edinburgh, UK. Adult beetles were kept individually in transparent plastic containers ($12 \times 8 \times 2$ cm) filled with moist soil. The laboratory conditions were kept constant throughout the experiment; that is, the beetles were kept at 20 °C and under a 16 : 8 h light:-dark photoperiod. Nonbreeding beetles were fed small pieces of beef twice a week.

Experimental design

We used a 2×3 factorial design to examine effects of handicapping of the female parent (weighted or control females) as one factor and brood size (5, 20 or 40 larvae) as the other factor. Previous work has found that weighted (i.e. handicapped) parents reduce their amount of parental care in the closely related *N. quadripunctatus* (Suzuki & Nagano, 2009) and

N. orbicollis (Creighton *et al.*, 2015). Meanwhile, brood size manipulations on *N. vespilloides* and *N. orbicollis* show that parents provide more care towards larger broods (Rauter & Moore, 2004; Smiseth *et al.*, 2007). In this experiment, we chose brood sizes of 5, 20 and 40 larvae as treatment levels reflecting that broods range in size from 2 to 47 larvae under laboratory conditions with a mean brood size of 21 larvae (Smiseth & Moore, 2002).

We selected an initial number of 231 virgin females for use in the experiment. At the start of the experiment, each female was paired with an unrelated virgin male. The pair was placed in a larger plastic container (17 × 12 × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass of a standardized size (22.31 ± 0.002 g; range: 20.45–23.51 g; Livefoods Direct, Sheffield, UK). Containers were checked for the presence of eggs the following days, and egg-laying date was recorded as the day where the first eggs were laid. Females were randomly assigned to a handicapping treatment (weighted or control) 1 day before the expected hatching date. At this stage, we moved females and their mouse carcasses into new boxes filled with fresh soil. We did this to separate females from their eggs, such that the larvae hatching from the eggs could be used to generate experimental broods of different sizes (Smiseth *et al.*, 2007). At this time, we also removed males because males often desert the brood before hatching and the presence or absence of males in *N. vespilloides* has no detectable impact on offspring fitness under laboratory conditions (Smiseth *et al.*, 2005). As soon as the eggs hatched, we randomly allocated each female a brood of newly hatched unrelated offspring made up of either 5, 20 or 40 larvae. We only allocated a female with an experimental brood once her own eggs had hatched given that parents will kill any larvae that emerge on the carcass before their own eggs have hatched (Müller & Eggert, 1990).

In parallel with the experimental females used in the experiments, we set up a total of 485 pairs of nonexperimental parents. These parents produced foster larvae that were used to generate the experimental foster broods. The foster broods were always of mixed maternity, which allowed us to eliminate any potential prenatal maternal effects associated with our handicapping treatments that can have had confounding effects on offspring and parental behaviours (Paquet *et al.*, 2015).

Handicapping procedure

To test the effects of handicapping on parental care, we weighted breeding females in the gap between the end of egg laying and the beginning of hatching. In this species, this gap occurs during the 2 days following the beginning of egg laying (Müller & Eggert, 1990). For weighted (handicapped) females, we attached a small lead weight to the pronotum of the female using instant-adhesive glue, as described in previous studies on the closely related *N. quadripunctatus* (Suzuki & Nagano, 2009; Suzuki, 2016) and *N. orbicollis* (Creighton *et al.*, 2015). In both species, handicapping reduced mobility of adult beetles and affected parental care behaviours by reducing the frequency of direct and indirect care (Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Suzuki, 2016). In our study, the mass of the weight together with the glue (0.06 ± 0.0008 g) represented approximately 20% of the initial female body mass ($n = 116$, 0.30 ± 0.004 g) measured shortly before handicapping. During the course of the experiment, we noticed that sizeable amounts of dirt were accumulating around the weight due to the digging behaviour of the burying beetles. This formed a lump on the pronotum and induced handicapped females to carry a total mass (i.e. lead weight + dirt) of approximately 30% their initial body mass (mean ± SE mass of dirt: 0.014 ± 0.0013 g). We had a control treatment of females that were of a similar body mass to the experimental females ($n = 101$, 0.30 ± 0.005 g). The control females were treated in the same way as the experimental females (i.e. these beetles were handled and disturbed), except that they had no weight attached to them.

Among the initial 231 experimental broods, 41 were excluded from the analysis for the following reasons: females lost their weights ($n = 12$) or died ($n = 3$) before the behavioural observations, females could not be allocated a foster brood ($n = 4$), females failed to produce eggs ($n = 6$), no eggs hatched from the clutch ($n = 9$), or eggs hatched before females were handicapped ($n = 7$). In addition to this, 11 broods were included in the behavioural analysis but excluded from analyses on fitness-related traits because the females had lost their weights or died between the time of observation and the time of larval dispersal. The final sample sizes for the different treatment groups were as follows for the behavioural traits measured 1 day after hatching (n_{d1}) and the fitness traits measured at larval dispersal (n_{disp}): control females with brood size of five larvae: $n_{d1} = n_{disp} = 29$; control females with a brood size of 20 larvae: $n_{d1} = n_{disp} = 29$; control females with a brood size of 40 larvae: $n_{d1} = n_{disp} = 34$; weighted females with a brood size of five larvae: $n_{d1} = 33$ and $n_{disp} = 29$; weighted females with a brood size of 20 larvae: $n_{d1} = 35$ and $n_{disp} = 31$; and weighted females with a brood size of 40 larvae: $n_{d1} = 30$ and $n_{disp} = 27$.

Female and offspring behaviours

We recorded parental and larval behaviours 24 h (±15 min) after the larvae were placed on the carcass, as this stage corresponds to the period when there is a peak in female food provisioning (Smiseth *et al.*, 2003, 2007). Behavioural observations were performed under

red light using instantaneous sampling every 1 min for 30 min. Both parental and larval behaviours were simultaneously observed and scored following methods described in previous studies (e.g. Smiseth & Moore, 2002, 2004a, b). To summarize briefly, we recorded the occurrence of parental food provisioning as the number of scans where there was mouth-to-mouth contacts with larvae, carcass maintenance as the number of scans where the female was spreading secretions on the surface of the carcass or excavating the crypt (i.e. the depression in the soil surrounding the carcass), and carrion consumption as the number of scans where female was feeding within the crater (i.e. the opening on the top of the carcass).

At each scan, we also recorded the number of larvae that were begging. We considered a larva to be begging when it raised its head towards the female while waving its legs or when it touched the female with its legs (Smiseth & Moore, 2002). We then calculated the average proportion time spent begging per larva in the brood as $B = (\Sigma b/n)/l$, where Σb is the total number of begging events during an observation session, n is the number of larvae in the brood at the time of observation, and l is the number of scans for which the female was near the larvae (Smiseth et al., 2003). We included the latter because larvae only beg when the parent is in close vicinity (i.e. less than or equal to the female's pronotum width; Rauter & Moore, 1999; Smiseth & Moore, 2002; Smiseth et al., 2007). Thus, this measure of begging is largely independent of the female's behaviour towards the larvae (Smiseth & Moore, 2004a).

Female weight change and offspring performance

To assess the consequences of handicapping and brood size on how much females consume from the carcass to invest in future reproduction, we measured the relative change in mass of females over the reproductive period. We estimated female weight change as the difference between the female's initial weight on the day preceding the hatching of her eggs and her final weight at the time of larval dispersal. We also tested for effects of handicapping and brood size on two measures of offspring performance: larval survival until dispersal and mean larval mass at dispersal. We measured effects on larval mass at dispersal because it determines adult body size, which in turn is known to be a major determinant of competitive ability and breeding success as adult in Nicrophorus species (Otronen, 1988; Safryn & Scott, 2000).

Statistical analyses

All statistical analyses were conducted using R v 3.3.3 (R Development Core Team, 2011) loaded with the packages car (Fox & Weisberg, 2017), MASS (Ripley et al., 2017), aod (Lesnoff & Lancelot, 2012) and MCMCglmm (Hadfield, 2010). Given that the behavioural traits in our experiment were count data bounded between 0 and 30 scans, we analysed the data using a binomial error distribution. We used general linear models for traits with a Gaussian distribution (female relative mass change and larval body mass at dispersal) and generalized linear models with a quasi-binomial distribution for traits that represent binary or count data with an upper limit (larval survival rate and larval begging). We used Bayesian generalized linear models fitted with a binomial error distribution to analyse food provisioning to the brood and carcass maintenance, whereas we used a Bayesian zero-inflated binomial model for carrion consumption to control for overdispersion and zero inflation. All Bayesian models were run using flat improper priors. We present parameter estimates for the Bayesian models as posterior means with 95% credible intervals of 2600 samples ran for 5.2×10^5 iterations with a thinning interval of 200 and a burn-in of 6×10^4. Outputs from the Bayesian zero-inflated binomial model allow us to test both the probability that females engaged into carrion consumption and, when consuming carrion at least once, how much time (i.e. number of scans) females spent consuming carrion during the observation period. All models included female handicapping treatment (control or weighted) and brood size (5, 20 or 40 larvae) and the interaction between them as fixed effects. Brood size was treated as a categorical predictor in the general linear and generalized linear models, whereas it had to be treated as a continuous predictor in the Bayesian models. In the general linear and generalized linear models, we used post hoc contrasts whenever handicapping and/or brood size had a significant effect on the variable of interest to test for differences between each treatment group or brood size category. In these tests, we used the Bonferroni correction for multiple testing.

Results

Female parental behaviour and weight change

There was no evidence of an effect of the interaction between handicapping and brood size on any of the two female parental behaviours (i.e. food provisioning and carcass maintenance) (Table 1; Fig. 2a,b) or on female weight change during the breeding attempt (Table 2; Fig. 2d). However, there was an effect of this interaction on the amount of time spent consuming carrion by females (Count model; Table 1). This interaction effect reflected that control females spent more time consuming carrion as brood increased, whereas weighted females spent a similar amount of time at this behaviour regardless of brood size (Fig. 2c).

Handicapping had a significant effect on the amount of time spent provisioning food to the brood and consuming carrion (Table 1). Contrary to what we

Table 1 Effects of the interaction between handicapping (weighted or control females) and brood size (5, 20 or 40 larvae) and the main effects of handicapping and brood size on female parental behaviours, that is food provisioning, carcass maintenance and female carrion consumption. Values are obtained from Bayesian GLMs using *MCMCglmm*.

	Interaction				Handicapping (weighted vs. control)				Brood size (continuous)			
	Mean	l-95%	u-95%	P_{MCMC}	Mean	l-95%	u-95%	P_{MCMC}	Mean	l-95%	u-95%	P_{MCMC}
Food provisioning	−0.011	−0.035	0.014	0.368	1.00	0.340	1.74	**0.002**	0.062	0.043	0.080	**< 0.0005**
Carcass maintenance	0.005	−0.021	0.028	0.699	−0.296	−0.949	0.330	0.380	0.015	−0.003	0.031	0.098
Carrion consumption												
Binary model	0.056	−0.050	0.174	0.239	−1.94	−3.66	−0.309	**0.023**	−0.094	−0.181	−0.028	**< 0.0005**
Count model	−0.023	−0.046	−0.001	**0.039**	0.856	0.249	1.45	**0.007**	0.008	−0.009	0.024	0.377

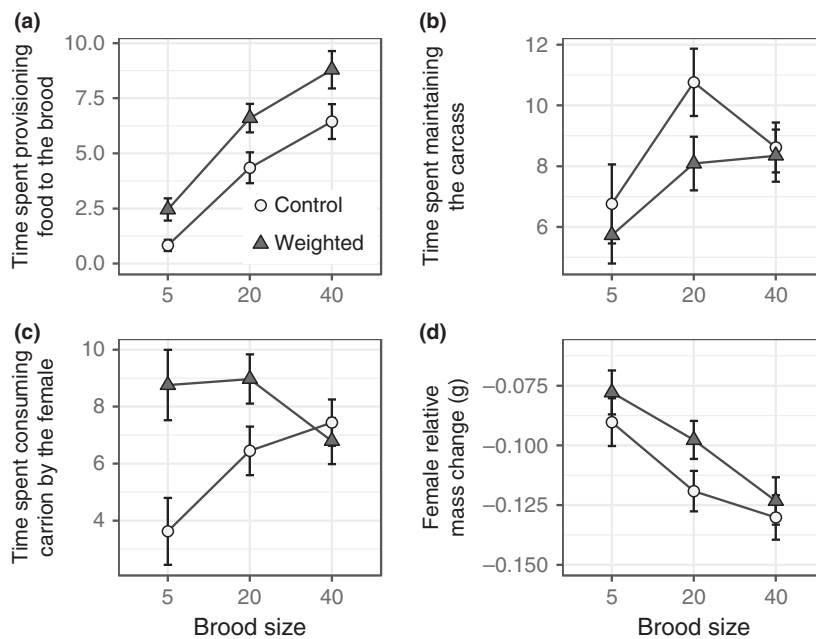Statistically significant *P* values (< 0.05) are shown in boldface.

Fig. 2 Effects of handicapping (weighted or control females) and brood size manipulation (5, 10 or 20 larvae) on the time spent (number of scans) by the female provisioning food to the brood (a), maintaining the carcass (b), consuming carrion (c) and on female weight change over the reproductive attempt (d). Mean ± SE.

Table 2 Effects of the interaction between handicapping (weighted or control females) and brood size (5, 20 or 40 larvae) and the main effects of handicapping and brood size on female mass change, larval begging and offspring performance (larval survival and mean larval mass). Values obtained from general linear models (female mass change and mean larval mass) and generalized linear models (larval begging and larval survival).

	Interaction			Handicapping			Brood size		
	LR χ^2	d.f.	P	LR χ^2	d.f.	P	LR χ^2	d.f.	P
Female mass change	0.645	2	0.724	3.34	1	0.067	21.2	2	**< 0.0001**
Larval begging	1.15	2	0.535	0.006	1	0.938	21.3	2	**< 0.0001**
Larval survival	2.34	2	0.310	0.016	1	0.899	12.6	2	**0.002**
Mean larval mass	5.17	2	0.075	0.029	1	0.864	167	2	**< 0.0001**

LR, likelihood ratio.
Statistically significant *P* values (< 0.05) are shown in boldface.

predicted, weighted females spent *more* time provisioning food to the brood than control females (Table 1; Fig. 2a). Weighted females were also more likely to

engage in carrion consumption and spent more time consuming carrion overall (Table 1; Fig. 2c). There was no evidence that handicapping had an effect on carcass

maintenance or female weight change (Tables 1 and 2; Fig. 2b,d).

Brood size had a significant effect on the amount of time spent provisioning food to the brood, the probability that females engaged in carrion consumption, as well as on female weight change (Tables 1 and 2; Fig. 2). Females spent more time provisioning food towards larger broods (Table 1, Fig. 2a). Likewise, females were more likely to engage in carrion consumption when caring for larger brood (Binary model; Table 1). Finally, females lost more weight when caring for broods of 20 than for broods of five larvae (Contrast 20 vs. 5 larvae: Estimate $= -0.024$, SE $= 0.009$, $z = -2.67$, $P = 0.02$), but lost a similar amount of weight when caring for broods of 20 and 40 larvae (Contrast 40 vs. 20 larvae: Estimate $= -0.018$, SE $= 0.009$, $z = -1.98$, $P = 0.14$). There was no effect of brood size on the amount of time spent maintaining the carcass (Table 1; Fig. 2b).

Offspring begging and performance

There was no effect of the interaction between handicapping and brood size on the average amount of time spent begging by the larvae, larval survival or mean larval mass at the time of dispersal (Table 2; Fig. 3). Likewise, there were no effects of handicapping on larval begging, larval survival or mean larval mass (Table 2). However, there was an effect of brood size on larval begging, larval survival and mean larval mass (Table 2; Fig. 3a–c). Larvae spent more time begging in broods of 20 or 40 larvae than in broods of five larvae (Contrast 20 vs. 5 larvae: Estimate $= 0.778$, SE $= 0.183$, $z = 4.25$, $P < 0.0001$; Contrast 40 vs. 5 larvae: Estimate $= 0.471$, SE $= 0.191$, $z = 2.47$, $P = 0.041$). Likewise, larval survival and mean larval mass were higher in broods of 20 compared to broods of five larvae (Contrast 20 vs. 5 larvae: Estimate $= 0.579$, SE $= 0.232$, $z = -2.50$, $P = 0.038$ and Estimate $= 0.012$, SE $= 0.004$, $z = 2.70$, $P = 0.02$, respectively) or 40 larvae (Contrast 40 vs. 20 larvae: Estimate $= -0.757$, SE $= 0.224$, $z = -3.38$, $P = 0.002$ and Estimate $= -0.052$, SE $= 0.004$, $z = -12.22$, $P < 0.0001$, respectively).

Discussion

The main aim of our study was to investigate effects of the interaction between handicapping and brood size on parental care and offspring performance in the burying beetle *N. vespilloides*. Assuming that handicapping increases the cost of care whereas brood size enlargement increases the benefit of care, we expected such interaction effects if handicapping leads to a greater divergence in the cost function at higher levels of care (Fig. 1d). We found no evidence for the presence of such an interaction effect on female parental behaviours (food provisioning and carcass maintenance),
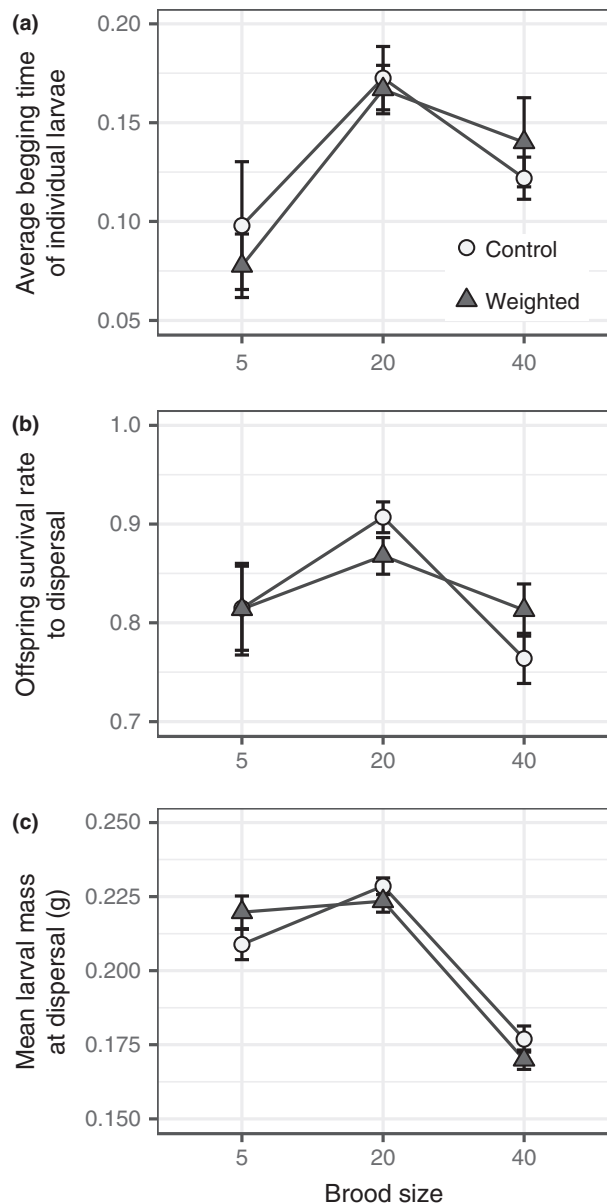
Fig. 3 Effects of handicapping (weighted or control females) and brood size manipulation (5, 10 or 20 larvae) on the average time spent begging by individual larvae in the brood (a), larval survival to dispersal (b) and mean larval mass at dispersal (c). Mean ± SE.

suggesting that these assumptions were not met in our study. Currently, we have little empirical information on the shape of the cost and benefit functions, and obtaining empirical estimates of these functions should now be a priority to guide future theoretical and empirical work in this field (Smiseth, 2017). However, there was an effect of this interaction on female carrion consumption, reflecting that control females consumed more carrion as brood size increased, whereas weighted

females consumed a similar amount of carrion regardless of brood size. This finding suggests that weighted females may compensate for the negative effects of handicapping by consuming more food. Moreover, brood size had an effect on most traits; that is, increasing female food provisioning and female probability to engage in carrion consumption, reducing female weight change during breeding, increasing larval begging and decreasing larval performance (larval survival and mean larval mass). In contrast, we found that handicapping had an effect on two female parental behaviours only; that is, increased carrion consumption and, contrary to what we predicted, *increased* time provisioning food to the brood. These results imply that handicapping can lead to an increase in parental care, suggesting that the effects of handicapping on parental care may be more complex than has been assumed in prior work using such experimental designs. Below, we provide a more in-depth discussion of our results and their implications for our understanding of flexible parental care.

A surprising finding of our study was that weighted females spent more time provisioning food than control females. This finding contradicts the widely held assumption that handicapping causes a reduction in parental care by increasing the cost of care. Handicapping experiments are traditionally used to study negotiation between parents in birds with biparental care, and their rationale is to increase the flight cost of care to the handicapped parent, thereby forcing it to reduce its contribution towards care (Harrison *et al.*, 2009). Such experiments are based on several types of handicapping treatments, including attachment of lead weights (e.g. Wright & Cuthill, 1989), clipping of flight feathers (Slagsvold & Lifjeld, 1988) and hormone manipulation (Hegner & Wingfield, 1987b). There is good evidence that handicapped parents provide less care than control parents regardless of which handicapping treatment is used (Harrison *et al.*, 2009). More recently, handicapping based on attachment of lead weights has been used to study negotiation between parents in two species of burying beetle, *N. quadripunctatus* and *N. orbicollis*, and these studies show that, as in birds, weighted females provide less care than control females (Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Suzuki, 2016). The opposite effects of handicapping on parental care reported in studies on *Nicrophorus* species might reflect differences in the level of handicapping as our weights were of 20–30% relative to body mass of the beetles, whereas studies in *N. quadripunctatus* (Suzuki & Nagano, 2009; Suzuki, 2016) and *N. orbicollis* (Creighton *et al.*, 2015) used weights of 40% and about 50%, respectively. As we discuss in greater detail below, handicapping may not only increase the cost of care, but also impact upon parental decisions through its effect on the parent's state (Pilakouta *et al.*, 2015). For example, the relatively

minor handicaps used in our study might have a greater impact on the parent's state than its costs of care, whereas the relatively major handicaps used in previous work might have greater impact on the cost of care. An alternative explanation is that these differences reflect species-specific response to handicapping due to divergent life-histories.

As hinted at above, handicapping may alter parental decisions about how much care to provide if it causes a decline in the parent's state (i.e. its condition, energy reserves or stress level; Pilakouta *et al.*, 2015). This in turn may lead to a reduction in parental care by weighted parents given that a decline in the parent's state should be associated with lower resources for investment in parental care and other priorities. Why then did we find that weighted females provided *more* care? One potential explanation for this finding is that weighted females responded to a decline in their state by shifting their investment towards their current brood at the expense of future reproduction. The terminal investment hypothesis predicts that parents should increase their investment into current reproduction when their prospects of future reproduction are lower (Clutton-Brock, 1984).

We would expect an increase in care by weighted females if this shift towards current investment more than outweighs the impact of the higher cost of care. There is some evidence for terminal investment from prior studies on species within the genus *Nicrophorus*. For example, in *N. vespilloides*, immune-challenged parents, which face higher risks of death from pathogens, increase their investment into current reproduction (Cotter *et al.*, 2010; Reavey *et al.*, 2015). Likewise, inbred males, which have a shortened lifespan, invest more into current reproduction and are more likely to risk injury in fights with conspecific competitors (Richardson & Smiseth, 2017). Finally, there is evidence that investment into current reproduction increases with the age of the female parent in *N. orbicollis* as predicted by the terminal investment hypothesis (Creighton *et al.*, 2009). Thus, if handicapping leads to terminal investment, we might have expected weighted females to gain less weight during breeding, as this trait is used as a proxy for investment in future reproduction (Creighton *et al.*, 2009; Billman *et al.*, 2014). We found no evidence that weighted females lost more weight during the breeding period than control females, suggesting that our results provide no overall support for terminal investment triggered by handicapping. However, as argued below, the lack of evidence for terminal investment based on data on female weight gain might reflect that handicapping also causes an increase in female food consumption.

We found that weighted females consumed a similar amount of carrion regardless of brood size, whereas control females consumed more carrion as brood size increased. In *N. vespilloides*, parents consume carrion

partly to provision food in the form of predigested carrion to their larvae and partly to replenish their own energy reserves (Mattey & Smiseth, 2015). Thus, our results suggest that control females increased their carrion consumption with brood size (Fig. 2c) to match the increase in food provisioning towards larger broods (Fig. 2a). In contrast, weighted females consumed a similar amount of carrion regardless of the brood size (Fig. 2c), presumably reflecting that these females adjusted their carrion consumption based on their own state rather than the brood size. Thus, control females consumed more carrion when they spent more time provisioning food to the brood, while there was no association between carrion consumption and food provisioning for weighted females. This finding also indicates that handicapping might trigger a compensatory response, whereby weighted females attempt to counteract the detrimental effects of handicapping due to an increase in the cost of care by increasing their energy reserves. For example, if handicapping increases the energetic cost of care, females might reduce this cost by building greater energy reserves. In *N. vespilloides*, it is relatively straightforward for females to increase their energy reserves as they can simply consume more from the carcass that is used for breeding (Boncoraglio & Kilner, 2012; Pilakouta *et al.*, 2016). If females increase their energy reserves to reduce the energetic cost of care, this may mask the expected effect of terminal investment on female mass gain.

As predicted, females provided more care and lost more weight when caring for larger broods. Meanwhile, we found that larvae in medium-sized broods spent more time begging, gained more weight and had higher survival than larvae in either small or large broods. These results are consistent with findings from previous work showing that parents tend to provide more care as brood size increases in insects, including *N. vespilloides* (e.g. Rauter & Moore, 2004; Smiseth *et al.*, 2007), fishes (e.g. Ridgway, 1989) and birds (e.g. Hegner & Wingfield, 1987a; Sanz, 1997). Thus, our results are in line with the prediction that females provide more care when the indirect benefit of care is higher due to an increase in the number of offspring in the brood (Fig. 1). The finding that females lost more weight when caring for larger broods is likely to reflect that larger broods require more care from females and that it is more costly for parents to care for such broods. Finally, the fact that larvae performed best in broods of intermediate size suggests that larval growth and survival are higher in broods closer to the average size in this species (i.e. 21 larvae; Smiseth & Moore, 2002). This finding may reflect a balance between sibling competition and sibling cooperation (Forbes, 2007; Falk *et al.*, 2014; Schrader *et al.*, 2015), whereby individual offspring in small broods benefit from the presence of other siblings through cooperative begging whereas individual offspring in large broods pay a cost in terms of increased competition (Johnstone, 2004). To sum up, our results confirm that variation in the benefit of care influences female decisions about how much care to provide to the current brood and how much resources to invest into current vs. future reproduction.

Parental care is a highly variable trait (Royle *et al.*, 2012), and this variation reflects that parents make flexible decisions about how much care to provide in response to variation in the cost and benefit of care. Here, we show that parents respond to both handicapping and brood size and that these responses are largely independent of each other. In our experiment, females appear to respond more strongly to variation in brood size than to handicapping, which might reflect that brood size manipulations have a greater impact on the benefit of care compared to the impact of handicapping on the cost of care. Furthermore, weighted females spent more time provisioning food to the brood and consuming carrion than control females. This finding supports the view that parents may respond to handicapping by increasing their investment into the current brood at the expense of investment in future reproduction and/or by increasing their energy reserves to compensate for the increased energetic cost of care. We suggest that future work on parental care based on handicapping should consider that this treatment may not only affect the cost of care, but that it may also lead to an increase in investment into current reproduction and compensatory responses that counteract the increased cost of care.

Acknowledgments

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