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# Parental care and cooperation in the burying beetle *Nicrophorus* *vespilloides*

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## Lay Summary

Parents must choose how much time and effort to spend caring for their current offspring at the expense of future offspring or their own condition. These decisions are influenced by factors that alter the costs and benefits of providing care. If cooperating to provide care with a partner, a parent may also consider the costs and benefits its partner faces since this is likely to influence its contribution towards caring for their joint offspring. In this thesis, I expand on our current understanding of how parents choose what level of care to provide by exploring the effect of four factors: the number of offspring they cared for in a previous breeding attempt, food deprivation, the presence of intruders, and synchrony in female and male caring behaviour. My study species was the burying beetle *Nicrophorus vespilloides*, which breeds on the carcasses of small animals and provides parental care for its offspring. I found that females spent the same amount of time providing care regardless of the number of larvae they cared for during a previous breeding attempt. I also found that females and males maintained the amount of time they spent providing care in the presence or absence of intruders. In contrast, parents altered the level of care they provided in response to food deprivation and synchrony in the onset of care by the female and male. Overall, my findings highlight that the level of care parents provide is dynamic and is affected by a wide range of factors.

## Abstract

In species that provide parental care, individuals must choose how to split their resources between caring for their current offspring and investing in their own reproductive potential. These decisions are made based on factors that shift the balance of costs and benefits associated with allocating resources to current or future reproduction. For parents providing uniparental care such factors relate to the value of the current brood and the likelihood of future reproduction. Females and males that cooperate to provide biparental care, must also consider factors that may influence the contribution of their partner. In this thesis, I explore what affects the level of care parents provide for their offspring and how females and males that provide biparental care balance their relative contribution in the burying beetle *Nicrophorus vespilloides*. I focus on four factors: previous reproductive allocation, nutritional state, social environment, and synchrony in the onset of care. First, I found that females provided the same level of care to a subsequent brood regardless of previous reproductive allocation and resource access, which suggests that neither affected future ability to provide care. Next, I found that females adjusted their level of care in response to both their own nutritional state and that of their partner and that these decisions were independent of their partner's contribution, while males only responded to the contribution of their partner. Then, I found that parents provided a similar level of care regardless of the presence of female or male intruders. Finally, I found that males provided more care when the female and male started providing care asynchronously in comparison to when they started synchronously while females provided a similar level of care regardless.



# 1 General Introduction

In species that provide parental care, parents must balance allocating resources to their current offspring and their own future reproductive potential (Williams, 1966b; Clutton-Brock, 1991). This is because allocating energy and time to current reproduction draws resources away from somatic maintenance as well as incurring mating opportunity costs (Williams, 1966b). As such, parents make strategic decisions about how much care to provide based on factors that affect the costs and benefits associated with providing care for the current brood and shift their allocation of resources between current and future reproduction accordingly.

The level of care a parent provides is determined by factors that influence the value of the current brood and the likely future reproductive potential of the parent (Gross & Sargent, 1985; Winkler, 1987; Dale *et al.*, 1996). For example, individuals that are not likely to survive much longer may provide more care than healthy and young individuals since their future reproductive potential is low (Williams, 1966a; Clutton-Brock, 1991; Creighton *et al.*, 2009; Klug & Bonsall, 2010). Furthermore, parents may increase the amount of care they provide to large broods (Wright & Cuthill, 1990; Clutton-Brock, 1991; Parejo & Danchin, 2006; Magrath *et al.*, 2007; Sahm *et al.*, 2023). Such increased allocation in current reproduction may limit the level of care a parent can provide during future reproduction. It is often suggested that parents face a 'cost of reproduction' and may produce fewer or worse quality offspring during subsequent breeding attempts due to the trade-off between allocating resources to current and future reproduction (Williams, 1966b). Empirical evidence for a trade-off is mixed with some studies providing support but others finding no relationship or even a positive relationship between current and future

reproduction (Santos & Nakagawa, 2012). There are several explanations for these unexpected relationships. For example, some suggest that there are higher quality individuals that can allocate more towards both current and future reproduction in comparison to poorer quality individuals (van Noordwijk & de Jong, 1986). Others suggest that the sample size, and resulting statistical power, is often too low to detect any effect of manipulating reproductive allocation (Graves, 1991). In species where there is a trade-off, little is known about the mechanisms facilitating the reduction in offspring quality or quantity.

If the female and male cooperate to provide biparental care, parents may also adjust the level of care they provide in response to the contribution of their partner. This is because sexual conflict arises when parents cooperate to provide biparental care for their joint offspring since the benefit of producing numerous and high-quality offspring is shared between the female and the male but the time, energy and resource cost associated with providing care is paid separately (Houston *et al.*, 2005; Lessells, 2012). As a result, parents are predicted to be under selection pressure to shift as much of the caring responsibility as possible onto their partner to minimise their costs whilst also ensuring offspring quality and survival (Parker, 1985). Consequently, parents are inclined to modify their contribution in accordance with factors that influence both the costs and benefits their partner incurs in providing care, as well as their own.

The majority of work investigating how parents cooperate to provide biparental care has focused on how females and males alter their contribution in response to the contribution of their partner. Current theoretical literature suggests that there are three ways in which a parent may respond to the contribution of their partner. Firstly negotiation, which involves a parent responding to a decrease in its

partner's contribution by increasing the level of care it provides to incompletely compensate (McNamara *et al.*, 1999). Secondly matching, by which an individual adjusts its contribution to mirror that of its partner such that if there is a decrease in the level of care its partner is providing, it will respond by also decreasing the level of care it provides (Johnstone & Hinde, 2006). Finally, sealed-bid decisions are when a parent decides how much care to provide independently of its partner's contribution (Houston & Davies, 1985). Empirical evidence provides support for all three mechanisms across various species (Schwagmeyer *et al.*, 2002; Hinde, 2006; Matthey & Smiseth, 2015; Pilakouta *et al.*, 2015; Lendvai *et al.*, 2018). In birds, the most studied taxonomic group, negotiation is considered to be predominant (Harrison *et al.*, 2009). There is a lack of investigation beyond this into whether individuals alter the level of care they provide directly in response to factors that may cause differences in the costs and benefits associated with providing care which cause the observed differences in the contribution of their partner such as state, social environment or synchrony in behaviour.

The state of the female and male may affect their relative contribution to providing care but has received less attention than how parents respond to the contribution of their partner. I define state as an attribute which is likely to affect an individual's contribution towards care, such as nutritional status, body size, inbreeding status, infection status, or age. An individual's state could be expected to affect how much care it provides since it may influence the costs and benefits associated with providing care (Smiseth, 2017). As such, individuals may adjust the level of care they provide in response to their own state. For example, in a study that investigated the effect of body condition on biparental cooperation in cichlids, males in poor condition provided less care than males in good condition (Steinegger &

Taborsky, 2007). In addition, individuals are also likely to alter their contribution in response to the state of their partner since state may act as an indicator of the ability of the partner to contribute towards providing parental care. For example, in the same study as above, females paired with a male in poor condition provided more care than those paired with a male in good condition (Steinegger & Taborsky, 2007). As a result, the relative contribution of females and males to providing care for their joint offspring is likely to change depending on state. This idea opposes the key assumption of theoretical models of negotiation that parents are unable to directly respond to the state of their partner and so they make decisions about how much care to provide indirectly based on the contribution of their partner (McNamara *et al.*, 1999; McNamara & Wolf, 2022). Recent work has shown that parents adjust the level of care they provide in response to state independently to how they respond to the contribution of their partner supporting that the two mechanisms are not mutually exclusive and that parents can respond directly to the state of their partner (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). These studies have focused on body size and inbreeding status which are both permanent states (Steinegger & Taborsky, 2007; Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015); however, temporary states, such as nutritional status, are yet to be explored (but see Martínez-Flores *et al.*, 2024).

The social environment within which parents provide care for their offspring is another factor that is likely to affect the relative contribution of females and males. Here I define social environment as the presence or absence of conspecifics within the immediate vicinity of the breeding pair, excluding their current brood. The social environment is likely to affect the costs and benefits associated with providing care since it may provide information about access to resources, the possibility of future reproduction or the relative value of the current brood and so we expect parents to

alter the level of care they provide as the social environment changes. If the social environment affects the costs and benefits associated with providing care differently for female and male parents, there may also be a shift in the relative contribution of females and males towards caring for offspring. Research exploring how the social environment influences parental care has mainly focused on how males respond to extra-pair copulations. Theoretical work predicts that males will provide less care in the presence of an extra-pair male if their presence is associated with reduced likelihood of paternity to avoid providing care to unrelated offspring (Westneat & Sherman, 1993; Kokko, 1999; Sheldon, 2002) with mixed support from empirical studies (Sheldon & Ellegren, 1998; Peterson *et al.*, 2001; Hunt & Simmons, 2002; Alonzo & Heckman, 2009; Suter *et al.*, 2009; Bose *et al.*, 2016; Ball *et al.*, 2017). Despite this, little is known about how the relative contribution of females and males to care may shift in response to a variety of social environments beyond the presence of an extra-pair male.

Finally, synchrony in parental behaviour has also been highlighted as an important factor in how parents coordinate their contributions towards caring for joint offspring (Griffith, 2019; Prior, 2020). It is well established that parents adjust the level of care they provide in response to the contribution of their partner and that not only the amount of care but also the timing of care is important in biparental cooperation. For example, behavioural synchrony in food provisioning to offspring has been shown to have a positive effect on breeding success in zebra finches (Mariette & Griffith, 2012) and long-tailed finches (Rooij & Griffith, 2013). This is thought to be due to synchrony in parental behaviour increasing the efficiency of feeding or a reduction in the predation of chicks by avoiding drawing unnecessary attention to the nest. Despite this, there is no information about whether parents

adjust their behaviour in response to synchrony in the onset of care which is likely to influence the relative contribution of females and males to early stages of care such as nest building or incubation.

In this thesis, I expand the current understanding of which factors may affect the level of care parents provide for their offspring and the relative contribution of females and males when cooperating to provide biparental care. Here I focus on four factors: previous reproductive allocation, state, social environment, and synchrony in the onset of care.

### 1.1 The study system: *Nicrophorus vespilloides*

The study species I used to investigate what might affect the level of care females and males provide was the burying beetle, *Nicrophorus vespilloides* (Figure 1.1). *Nicrophorus vespilloides* are common throughout the UK and mainland Europe (Scott, 1998; Lane *et al.*, 2020). In the wild, they can feed on other small invertebrates but their main food source is carrion (Steiger *et al.*, 2007a). They breed on the carcasses of small vertebrates such as birds or rodents which serve as the main food source of both parents and offspring (Scott, 1998). Parents typically provide biparental care (Eggert *et al.*, 1998b; Scott, 1998) but in the absence of a partner as a result of desertion, death or, in the case of females, mating before finding a carcass, they will provide uniparental care (Smiseth *et al.* 2005; Muller *et al.* 2007). Pre-hatching care involves preparing the carcass by stripping it of any hair or feathers, rolling it into a ball and burying it underground (Scott, 1998; Trumbo & Robinson, 2008; Royle *et al.*, 2013). The carcass is then maintained by spreading antimicrobial secretions on the surface of the carcass to prevent microbial

competition (Hoback *et al.*, 2004; Rozen *et al.*, 2008; Cotter & Kilner, 2010; Duarte *et al.*, 2021). The female then lays eggs in the surrounding soil and when the larvae hatch, approximately three days later, they aggregate in a crater in the carcass created by the parents (Eggert & Müller, 1997; Smiseth *et al.*, 2006). The female and male then provide post-hatching care which involves food provisioning the larvae with pre-digested carrion, continuing to maintain the condition of the carcass and guarding the brood against potential threats (Eggert *et al.*, 1998b; Scott, 1998). Although both parents are capable of contributing to every aspect of care in the absence of their partner (Jenkins *et al.*, 2000; Smiseth *et al.*, 2005), females tend to spend more time food provisioning to the larvae (Eggert *et al.*, 1998b; Smiseth & Moore, 2002; Smiseth *et al.*, 2005; Walling *et al.*, 2008) whilst males spend more time guarding and maintaining the carcass (Fetherston *et al.*, 1990; Smiseth *et al.*, 2005). Males also tend to desert the breeding attempt before the female (Bartlett & Ashworth, 1988; Müller *et al.*, 2007). Approximately 5 days later, the larvae disperse from the carcass and into the surrounding soil to pupate. They then eclose into adult beetles after 3 weeks and are sexually mature approximately 10 days later (Eggert & Müller, 1997).

The availability of the carcasses that the beetles breed on are thought to be the main limiting factor on their reproductive success. Given they are a valuable and scarce resource, it seems unlikely burying beetles have the opportunity to breed multiple times in the wild. Despite this, previous studies have demonstrated that *N. vespilloides*, and the closely related *N. orbicollis*, are capable of breeding multiple times and that parents alter the level of care they provide in response to factors that shift the costs and benefits associated with providing care for the current brood which may indicate consideration of a future breeding attempt (Jenkins *et al.*, 2000;

Creighton *et al.*, 2009; Ward *et al.*, 2009; Billman *et al.*, 2014). This may also differ between females and males since males are capable of mating opportunistically in the absence of a carcass while females are not (Eggert, 1992). This being said, there is a last male precedent in sperm competition in *N. vespilloides* (Müller & Eggert, 1989; Hopwood *et al.*, 2015; House *et al.*, 2007) so it is unlikely that such opportunist mating greatly affects a male's reproductive output.



**Figure 1.1** - *N. vespilloides* parents providing post-hatching care for their offspring. Photo credit: Tom Ratz.

## 1.2 Parental care and cooperation in *N. vespilloides*

Burying beetles are an excellent study species to explore how different factors affect parental care and cooperation. Firstly, parents can cooperate to provide biparental care (Eggert *et al.*, 1998b; Scott, 1998) or uniparental care can be provided by a single female or male after mate loss or if a female is using stored sperm (Smiseth *et al.*, 2005; Muller *et al.*, 2007) so either a pair cooperating to



provide care or a single parent can be studied. Secondly, while larvae are capable for self-feeding, previous work has shown that parental care greatly increase growth and survival in broods (Eggert *et al.*, 1998b; Lock *et al.*, 2004; Andrews *et al.*, 2017). Thirdly, parental care is flexible in this species so females and males can adjust the level of care they provide based on factors that alter the costs and benefits associated with providing care themselves and for their partner (Creighton *et al.*, 2015; Matthey & Smiseth, 2015; Pilakouta *et al.*, 2015; Richardson & Smiseth, 2019b; Ratz *et al.*, 2021; Sahm *et al.*, 2023). Finally, it is relatively easy to manipulate a variety of factors which may influence the level of care parents provide in a laboratory setting. Reproductive allocation can be manipulated by altering the size of the brood a parent is caring for since parents spend more time providing care to larger broods than smaller broods (Smiseth & Moore, 2002). Food deprivation can be used to alter the nutritional state of females and males before breeding (Steiger *et al.* 2007; Hopwood *et al.*, 2013; Richardson and Smiseth 2019). The presence of intruders can be simulated without causing injury, displacement or death to the host parents by introducing dead conspecifics which females and males respond to similarly to how they would live intruders (Steiger *et al.*, 2009; Steiger & Müller, 2010; Paquet *et al.*, 2017; Ratz *et al.*, 2022). Furthermore, altering the timing of when females and males arrive at the carcass can be used to manipulate synchrony in the onset of care given a parent will start pre-hatching care such as stripping the carcass of fur or feathers regardless of the presence of a partner.

### 1.3 Aims

In this thesis, I explore how parents make decisions about reproductive allocation and how parents that provide biparental care balance their relative

contribution to caring for joint offspring. I focus on how a variety of factors (previous reproductive allocation, nutritional state, social environment, and synchrony in the onset of care) influence the level of care parents provide and how this subsequently affects offspring performance in both uniparental and biparental care settings.

## Chapter 2: Access to resources buffers against effects of current reproduction on future ability to provide care in a burying beetle

In this chapter, I explore how previous reproductive allocation and resource access affect the level of parental care females provide in a subsequent breeding attempt. Previous work has shown that increased allocation to current reproduction can lead to future broods being smaller or of worse quality (Nur, 1988; Parejo & Danchin, 2006; Martin & Festa-Bianchet, 2010). What is unclear is the mechanism leading to this relationship. Here, I tested whether a decrease in ability to provide parental care as a result of increased allocation to current reproduction is the mechanism facilitating such a decrease in reproductive success during subsequent breeding attempts.

## Chapter 3: Flexible females: nutritional state influences biparental cooperation in a burying beetle

Theoretical models of negotiation assume that parents are unable to assess the state of their partner directly and instead do so indirectly based on the contribution of their partner and adjust their contribution accordingly (McNamara *et al.*, 1999; McNamara & Wolf, 2022). Recent work has challenged this assumption by showing that parents can directly respond to permanent states such as body size and inbreeding status (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). Temporary and reversible states may have different implications for cooperation than permanent states since there is the opportunity for an individual in a worse state to recover. In

this chapter, I test whether parents adjust their relative contribution to care dependent on their own nutritional state (a temporary and reversible state), that of their partner or the interaction between the two.

#### Chapter 4: Intraspecific intruders and parental cooperation in a burying beetle

In this chapter, I investigated whether females and males adjust their relative contribution to providing care for their joint offspring in the presence or absence of intraspecific intruders. The social environment may affect how parents cooperate since it is likely to alter the costs and benefits associated with providing care. Both the female and male suffer a cost if an intruder is successful since it will kill their current brood. However, the parent of the opposite sex to the intruder may have the opportunity to remate with the intruder and produce a replacement brood. Given that the sex of the intruder likely affects the costs and benefits associated with providing care differently for females and males, I tested if any response to an intruder was dependent on the sex of the intruder, the presence of a partner intruder, or the interaction between the two.

#### Chapter 5: Synchrony in onset of parental care affects post-hatching cooperation and offspring performance

It is well established that parents adjust the level of care they provide in response to the contribution of their partner. If parents must balance allocating time and energy to different stages of the breeding attempt (pre-hatching and post-hatching care) they may adjust the level of care they provide in response to not only their partner's contribution to the current stage of care but also previous stages. Synchrony in the onset of parental care is likely to alter the parents' relative contributions to pre-hatching care and, as a result, impact how females and males balance their relative contribution to post-hatching care. In this chapter, I test

whether females and males adjust their relative contribution to post-hatching care depending on synchrony in the onset of care by manipulating parental arrival time at the carcass. Additionally, I investigated how any adjustments in female and male parental behaviour affected offspring performance.

## 2 Access to resources buffers against effects of current reproduction on future ability to provide care in a burying beetle

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### Abstract

Studies investigating the trade-off between current and future reproduction often find that increased allocation to current reproduction is associated with a reduction in the number or quality of future offspring. In species that provide parental care, this effect on future offspring may be mediated through a reduced future ability to provide care. Here, we test this idea in the burying beetle *Nicrophorus vespilloides*, a species in which parents shift the cost of reproduction towards future offspring and provide elaborate parental care. We manipulated brood size to alter the costs females experienced in association with current reproduction and measured the level of parental care during a subsequent breeding attempt. Given that these beetles breed on carcasses of small vertebrates, it is important to consider confounding effects due to benefits associated with resource access during breeding. We therefore manipulated access to carrion and measured the level of parental care during a subsequent breeding attempt. We found that females provided the same level of care regardless of previous brood size and resource

access, suggesting that neither affected future ability to provide care. This may reflect that parents feed on carrion during breeding, which may buffer against any costs of previous breeding attempts. Our results show that increased allocation to current reproduction is not necessarily associated with a reduction in future ability to provide care. Nevertheless, this may reflect unique aspects of our study system, and we encourage future work on systems where parents do not have access to a rich resource during breeding.

## 2.1 Introduction

The trade-off between current and future reproduction, often referred to as the 'cost of reproduction', is a central hypothesis to life history theory (Williams, 1966b). Allocation to current reproduction diverts resources from somatic maintenance, which, in turn, may reduce future reproductive potential. The cost of reproduction is often studied experimentally by altering parental allocation to current reproduction and then measuring its effect on future breeding attempts. This is usually achieved by manipulating brood size (Lessells, 1986; Koivula *et al.*, 2003; Parejo & Danchin, 2006), but can also involve manipulating the physiology of mothers (Oksanen *et al.*, 2002; Cox & Calsbeek, 2010) or the reproductive environment (Creighton *et al.*, 2009). Currently, evidence for such a trade-off is mixed with some studies supporting the hypothesis but others finding either no relationship or a positive relationship between current and future reproduction (Santos & Nakagawa, 2012). Within studies reporting evidence for a cost of reproduction, this cost can manifest in different ways. In some species, the cost to future reproduction is paid through a reduction in the parents' own survival, for example, as a result of increased susceptibility to predation (Veasey *et al.*, 2001) or parasitism (Alt *et al.*, 2015). Meanwhile, in other species, the

cost is shifted towards future offspring as indicated by a reduction in the number or quality of offspring produced in subsequent breeding attempts (Nur, 1988; Parejo & Danchin, 2006; Martin & Festa-Bianchet, 2010). In such species, little attention has been given to how the parents are able to shift the cost of reproduction onto future offspring and this therefore remains poorly understood.

In species where parents care for their offspring, parents may shift the cost of reproduction onto future offspring if increased allocation to reproduction is associated with a reduction in future ability to provide care. Parents that allocate more towards current reproduction may reduce the level of care they provide during subsequent breeding attempts either because they cannot allocate as much towards care as a result of higher investment in current reproduction, or because they strategically allocate less towards care in order to maintain their own condition and facilitate future breeding opportunities. For example, there is observational evidence that bighorn sheep (*Ovis canadensis*) ewes reduce the energy they allocate to their lambs via lactation and instead prioritise their own body mass when resources are limited (Martin & Festa-Bianchet, 2010). By doing so, ewes increase their own over-winter survival at the expense of that of their lambs. Such reduction in parental care may provide a general mechanism for the commonly observed reduction in number or quality of future offspring associated with increased allocation to current reproduction in species with parental care. However, little is known about this mechanism and there is now a need for more studies, including those based on an experimental approach, to investigate whether an increase in current reproduction is associated with a reduction in future ability to provide care.

We tested this idea by manipulating reproductive allocation to a current breeding attempt and measuring the effect on the level of care provided in a

subsequent breeding attempt by females of the burying beetle *Nicrophorus vespilloides*. Beetles in the genus *Nicrophorus* are well suited to test this idea because there is evidence for a cost of reproduction in *N. vespilloides* (Jenkins *et al.*, 2000; Ward *et al.*, 2009) and the closely related *N. orbicollis* (Creighton *et al.*, 2009; Billman *et al.*, 2014). Studies show that parents shift this cost towards future offspring as parents reduce the size and mass of future broods in response to an increase in allocation to current reproduction induced by manipulating male assistance in parental care (Jenkins *et al.*, 2000), brood size (Ward *et al.*, 2009) or carcass size (Creighton *et al.*, 2009; Billman *et al.*, 2014). *Nicrophorus vespilloides* breeds on a small vertebrate carcass that serves as a food source for caring parents and their offspring. Females (sometimes assisted by a male) provide elaborate parental care for their offspring by preparing the carcass, provisioning pre-digested carrion to the offspring, maintaining the carcass by spreading antimicrobials onto it, and guarding the offspring and carcass against conspecifics (Eggert *et al.*, 1998b; Scott, 1998). Larvae benefit from parental care in terms of increased growth and survival (Eggert *et al.*, 1998b; Lock *et al.*, 2004; Andrews *et al.*, 2017). Although previous work provides clear evidence that increased allocation is associated with a reduction in the size and quality of future broods, it is unknown whether this is due to a reduction in the level of parental care towards future broods.

Our aim was to test whether a reduction in the parent's future ability to provide care due to increased allocation to a current breeding attempt is the mechanism mediating the observed shift in the cost of reproduction towards future broods. We altered female allocation to current reproduction by manipulating whether or not females had the opportunity to breed and, if they did, the size of the brood they cared for. We then measured effects on the level of post-hatching care provided in a



future breeding attempt. If increasing the cost of reproduction in an initial breeding attempt affected a female's ability to provide care in a future breeding attempt, we predict that breeding females that had cared for a large brood would provide the lowest level of care during the subsequent breeding attempt, that non-breeding females would provide the highest level of care, whilst breeding females that had cared for a small brood would provide an intermediate level of care. In *N. vespilloides*, parents have access to a nutrient-rich carcass that they feed on during reproduction, which often leads to an increase in mass over a breeding attempt (Creighton *et al.*, 2009; Pilakouta *et al.*, 2016; Richardson *et al.*, 2019, 2020; Richardson & Smiseth, 2019a). Thus, when studying potential effects associated with a cost of reproduction, it is important to consider confounding effects due to potential benefits associated with gaining access to resources during breeding. Accordingly, we investigated whether prior access to a mouse carcass affected future post-hatching parental care. We therefore included two control groups. First, non-breeding females that had no prior carcass access and did not produce a brood and, therefore, had neither the benefits of carcass access nor the costs of reproducing. Second, non-breeding females that had access to a carcass prior to their first breeding attempt but did not produce a brood and, therefore, had the benefits of carcass access but suffered none of the costs of caring for larvae. If the benefit associated with carcass access in an initial breeding attempt affected a female's ability to provide care in a future breeding attempt, we predict that breeding and non-breeding females that had had prior access to a carcass would provide more care than non-breeding females that did not have such access. Finally, we tested for combined effects of the cost of reproduction and the benefit of carcass access on the level of post-hatching care females provided during a subsequent

breeding attempt. If there were such combined effects, we predicted that the pattern observed would be intermediate between those described above for what we predicted if there were effects due to the cost of reproduction or the benefit of carcass access.

## 2.2 Methods

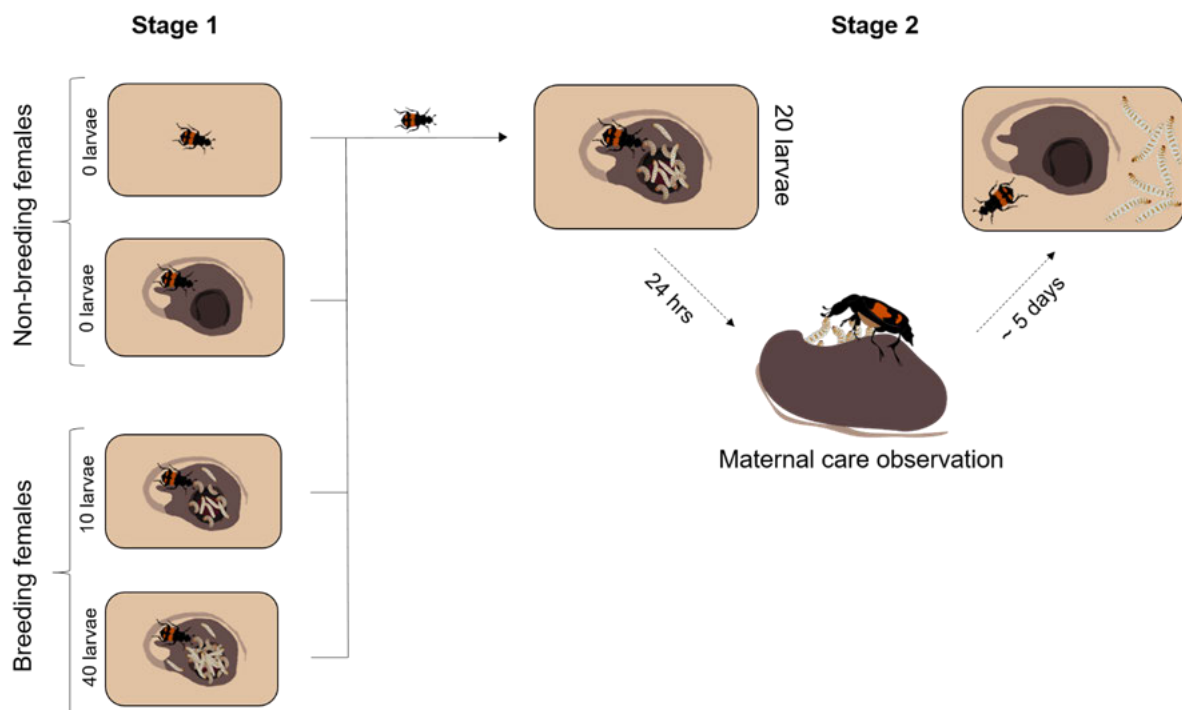
### 2.2.1 General methodology

We used beetles from an outbred laboratory population maintained at the University of Edinburgh. All beetles in the stock population were originally collected in Edinburgh, UK and the population was kept at 20 °C under a 16:8 h light:dark cycle. We housed non-breeding adults individually in clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil and fed them raw organic beef twice a week.

### 2.2.2 Experimental design

The experiment was conducted across two stages. In stage one, we randomly assigned unmated females to one of the following four treatments (Figure 2.1): breeding females allocated a large brood of 40 larvae (n = 32), breeding females allocated a small brood of 10 larvae (n = 32), non-breeding females provided with access to a mouse carcass (n = 30) or non-breeding females not provided with access to a mouse carcass (n = 30). We chose these brood sizes to represent a low and high level of reproductive allocation, respectively, since they are approximately half and double the average brood size for *N. vespilloides* (21 larvae; Smiseth & Moore, 2002) and fall well within the natural range for this species (2 – 45 larvae; Smiseth & Moore, 2002). We included the treatment of non-breeding females provided with access to a carcass to control for any benefits associated with carcass

access. The extent of the benefit associated with carcass access is unclear because *N. vespilloides* feeding habits in the absence of a carcass in the wild are unknown. However, they are suspected to feed on other insects as well as carrion, and carcasses would be of comparatively high nutritional value. Carcass access also stimulates mated and unmated females to lay fertilised or unfertilised eggs, respectively, and to provide pre-hatching parental care (i.e., burying and maintaining the carcass). It is not possible experimentally to separate the benefit of feeding from the carcass from the costs of egg laying and pre-hatching care. However, the cost of egg laying and providing pre-hatching care is known to be minimal (undetectable in Ward *et al.*, 2009). Accordingly, we expected the net effects of carcass access to be beneficial.



**Figure 2.1** - The experimental design used to investigate whether the costs of increased reproductive allocation and benefits of carcass access (manipulated during stage one) affect the level of care female provide during stage two.

In stage two, all females from stage one were provided with a carcass to initiate breeding and all females were allocated the same, standardised brood size (20 larvae; Figure 2.1). We used a standardised brood size to control for potential confounding effects due to variation in clutch size or brood size, which might affect the amount of care provided by females. This design allowed us to test whether the observed shift in the cost of reproduction towards future broods was mediated by a reduction in the parent's future ability to provide care whilst controlling for any potential benefit associated with carcass access. We note that this design excludes the possibility to test how treatment during stage one would affect the natural broods produced by the females during stage two. However, prior work on this species has established that increased allocation is associated with a reduction in the size and quality of future broods in *N. vespilloides* (Jenkins *et al.*, 2000; Ward *et al.*, 2009). If the observed shift in the cost of reproduction towards future offspring was mediated by a reduction in the parent's future ability to provide care, this would be detected as a reduction in the amount of care provided during stage two in this descending order: breeding females that had cared for a large brood during stage one, breeding females that had cared for a small brood, non-breeding females that had not had access to a carcass. If there was a benefit associated with carcass access, and this benefit affected the parent's future ability to provide care, this would be detected as an increase in the amount of care provided during stage two by non-breeding females that had had access to a carcass during stage one compared to non-breeding females that had not had access to a carcass.

In stage one of the experiment, breeding females were weighed and then paired with an unrelated male from the stock population. We transferred each pair into a clear plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil

containing a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (20 – 25 g;  $M \pm SE = 22.57 \pm 0.19$  g) to initiate mating. After 48 h, when the eggs had been laid but before the larvae had begun hatching, we moved the female and her carcass into a new container lined with fresh moist soil. At this point, we discarded the male since males sometimes assist with parental care, in which case the male might absorb some of the cost of reproduction if present. In addition, male involvement in providing care is highly variable (Smiseth & Moore, 2002) and so allowing the male to assist would generate variation in the extent to which males assisted. Therefore, we removed the male to make it easier to detect any cost of reproduction to the female. Removing the male has also been shown to have no effect on female caring behaviour or offspring fitness in *N. vespilloides* (Smiseth *et al.*, 2005). We then allocated breeding females either a small (10 larvae) or a large (40 larvae) foster brood which consisted of newly hatched larvae from at least two different mothers. We only allocated a foster brood to a female after her own eggs had started hatching since females use temporal kin recognition and kill larvae that arrive at the carcass before their own eggs have begun to hatch (Müller & Eggert, 1990). We left the females undisturbed to care for their brood until the larvae dispersed from the carcass approximately five days later upon which we recorded average larval mass, the proportion of larvae that survived to dispersal and female mass.

Non-breeding females were also weighed before being transferred into clear plastic containers (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil. Non-breeding females provided with access to a carcass were given a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (20–25 g;  $M \pm SE = 22.53 \pm 0.24$  g). Being provided with a carcass initiated pre-hatching parental care

(preparing the carcass for larvae) and the laying of unfertilised eggs. Non-breeding females without access to a carcass were fed raw organic beef twice a week (approximately 0.3 g) to ensure they did not starve. Unlike the presence of a carcass, the presence of beef did not initiate egg laying or pre-hatching care. We handled non-breeding females an equal number of times as we handled the breeding females described above. Non-breeding females that were provided access to a carcass had access to it for the same period as the breeding females. We then weighed the females at the end of stage one before they were transferred into individual clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil and left undisturbed for 24 h before proceeding to stage two.

In stage two, we weighed all females used in stage one before pairing them with an unrelated male from the stock population. We then transferred each pair into a clear plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil containing a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (20–25 g;  $M \pm SE = 22.63 \pm 0.12$  g). We allowed pairs to mate for 48 h after which the male was removed. We removed the male to control for potential confounding effects due to variation in the contribution of males towards caring for offspring as described above for stage one of the experiment. At this point, we recorded the number of eggs each female laid by counting the number of eggs visible through the bottom of the clear container. We chose this method to avoid handling and thereby potentially damaging the eggs. Furthermore, it has been shown that the visible number of eggs is strongly correlated with the actual clutch size when the container is lined with a thin layer of soil as they were here (Monteith *et al.*, 2012). We then allocated each female a foster brood consisting of 20 larvae using the same methods as in stage one. We conducted behavioural observations 24 h ( $\pm$

10 min) after we allocated females a foster brood, which is when parents of this species provide the highest level of care (Smiseth *et al.*, 2003). We did the behavioural observations under red light using instantaneous sampling every minute for 30 minutes consistent with established protocols (Smiseth & Moore, 2002). We then left the females to care for their brood until the larvae dispersed from the carcass approximately five days later. At the time of dispersal, we recorded average larval mass and the proportion of larvae that survived to dispersal. We weighed the females for a second time after the larvae had dispersed to estimate mass change over stage two.

After stage two, we transferred all females into individual clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil, maintained them using the same protocol applied to the stock population, and checked them twice a week until death. We did this to record their lifespan as the number of days from eclosion to the day the female was found dead.

### 2.2.3 Statistical Analyses

All statistical analyses were conducted using R version 3.6.1 (R Core Team, 2021) with the packages *car* (Fox & Weisberg, 2019), *MASS* (Venables & Ripley, 2022), and *glmmTMB* (Brooks *et al.*, 2017). We used zero-inflated binomial models in our analyses on time spent provisioning food to larvae since the data for this behaviour showed minor zero inflation. We used binomial models in our analysis on carcass maintenance and larval survival and linear models to analyse female mass change and mean larval mass at dispersal. In all these models, we included observation level as a random effect to account for over-dispersion (Harrison, 2015). We used a negative binomial model to analyse data on number of eggs laid during

stage two and Cox's proportional hazards to analyse data on female lifespan. All models included female treatment during stage one as a fixed effect with four levels (breeding and caring for a large brood, breeding and caring for a small brood, non-breeding and having access to a carcass, non-breeding and not having access to a carcass). We ran pairwise comparisons using a Tukey's test with the Bonferroni correction whenever treatment had a significant effect.

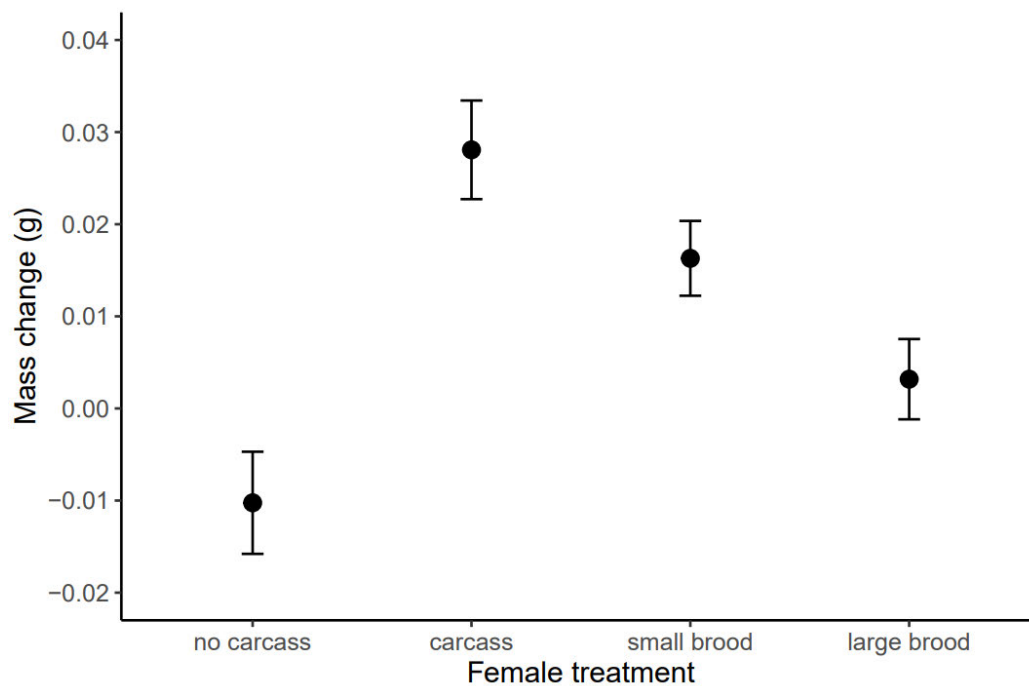
## 2.3 Results

### 2.3.1 Female mass change and offspring performance in stage one

Increased reproductive allocation negatively affected female mass change during stage one. Non-breeding females that had access to a carcass during stage one gained more mass than breeding females that cared for a large brood (estimate  $\pm$  SE =  $0.025 \pm 0.007$  g,  $z = 3.641$ ,  $P = 0.002$ ; Figure 2.2), while breeding females that cared for a small brood gained an intermediate amount of mass midway between breeding females that cared for a large brood (estimate  $\pm$  SE =  $0.013 \pm 0.007$  g,  $z = 1.950$ ,  $P = 0.321$ ; Figure 2.2) and non-breeding females that had access to a carcass (estimate  $\pm$  SE =  $0.012 \pm 0.007$  g,  $z = 1.723$ ,  $P = 0.525$ ; Figure 2.2). The benefit of carcass access was also evident from effects on female mass change during stage one since non-breeding females with access to a carcass gained more mass than non-breeding females that had no access to a carcass (estimate  $\pm$  SE =  $0.038 \pm 0.007$  g,  $z = 5.516$ ,  $P < 0.001$ ; Figure 2.2). There was no difference in mass change between breeding females that cared for a large brood and non-breeding females that had no access to a carcass (estimate  $\pm$  SE =  $-0.013 \pm 0.007$  g,  $z = -1.963$ ,  $P = 0.312$ ; Figure 2.2) and non-breeding females that had no access to a carcass gained less mass than breeding females that cared for a small brood



(estimate  $\pm$  SE =  $-0.027 \pm 0.007$  g,  $z = -3.881$ ,  $P = 0.001$ ; Figure 2.2). Thus, our results may suggest that the benefit associated with gaining access to a carcass cancels out or even exceeds the cost of reproduction.



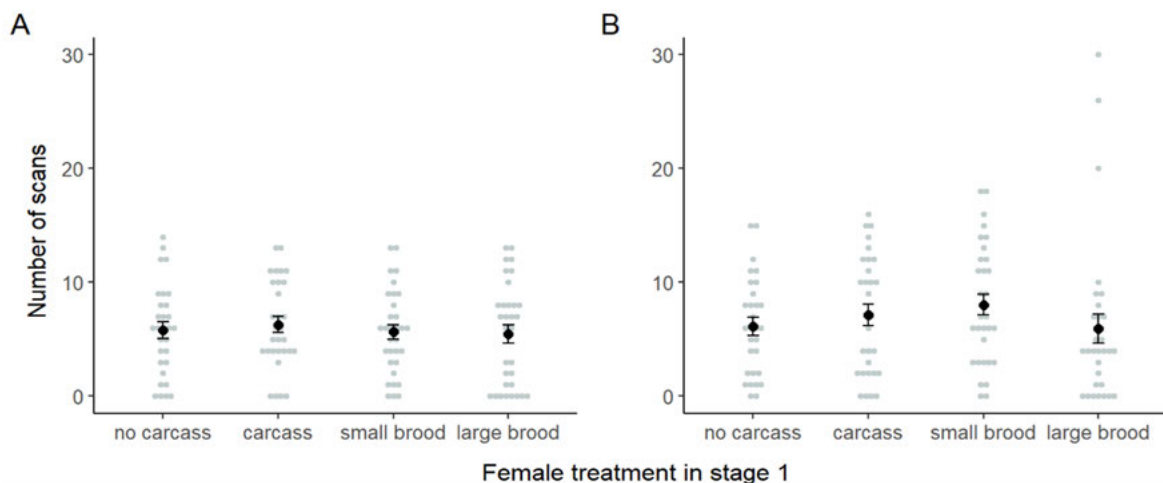
**Figure 2.2** - Mean mass change (g)  $\pm$  SE during stage one of non-breeding females not provided with access to a carcass (no carcass), non-breeding females provided with access to a carcass (carcass), breeding females allocated a small brood (small brood), and breeding females allocated a large brood (large brood).

The average mass per larvae at dispersal was higher in small broods than in large broods ( $\chi^2 = 0.01$ ,  $df = 1$ ,  $P = 0.002$ ) but there was no difference in larval survival between small and large broods ( $\chi^2 = 1.07$ ,  $df = 1$ ,  $P = 0.301$ ).

### 2.3.2 Time spent providing parental care in stage two

There was no evidence that the cost of increased reproductive allocation or the benefit of carcass access during stage one affected the amount of care provided by females during stage two of the experiment (Figure 2.3). There was no difference

in the number of scans during the observations that females from different treatment groups spent provisioning food to larvae ( $\chi^2 = 1.72$ ,  $df = 3$ ,  $P = 0.632$ ; Figure 2.3) or maintaining the carcass ( $\chi^2 = 3.35$ ,  $df = 3$ ,  $P = 0.340$ ; Figure 2.3). We note that we measured female caring behaviour in a single 30-minute observation and so we may not have detected a change in the distribution of care across the breeding attempt as a whole.

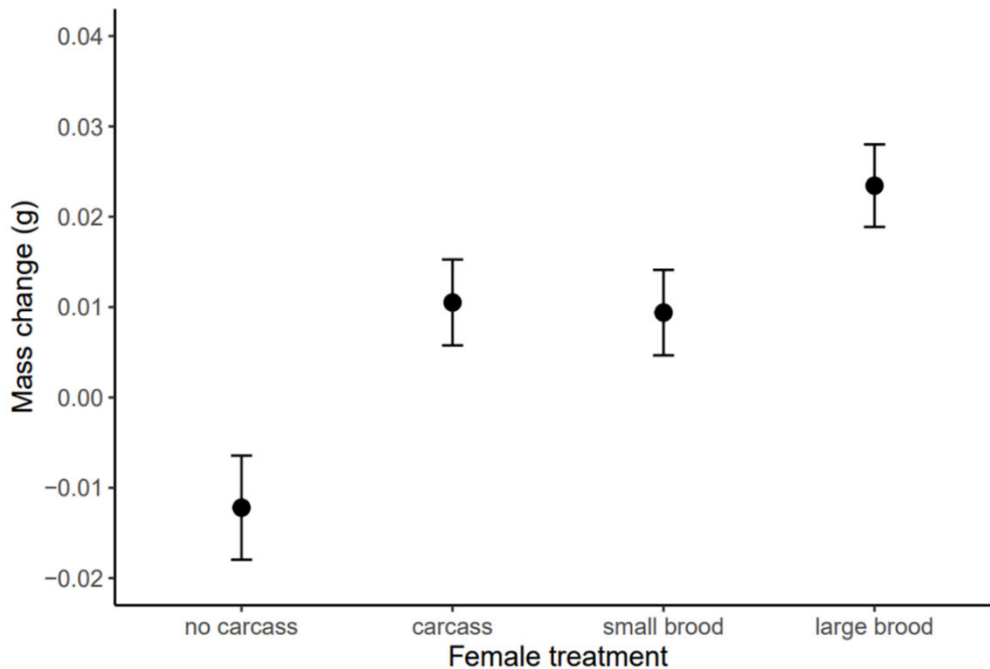


**Figure 2.3** - The number of scans during stage two observations in which the female was provisioning food to larvae (A) or maintaining the carcass (B) by females that, during stage one, were non-breeding and not provided with access to a carcass (no carcass), non-breeding and provided with access to a carcass (carcass), breeding and allocated a small brood (small brood), and breeding and allocated a large brood (large brood). Grey circles represent individual data, black circles and bars represent means  $\pm$  SE.

### 2.3.3 Female mass change, egg-laying and offspring performance in stage two

Non-breeding females that had no access to a carcass during stage one gained less mass in stage two than breeding females that had cared for a large brood (estimate  $\pm$  SE =  $-0.036 \pm 0.007$  g,  $t = -5.082$ ,  $P < 0.001$ ; Figure 2.4), breeding females that had cared for a small brood (estimate  $\pm$  SE =  $-0.022 \pm 0.007$  g,  $t = -3.079$ ,  $P = 0.016$ ; Figure 2.4) and non-breeding females that had access to a carcass

during stage one (estimate  $\pm$  SE =  $0.023 \pm 0.007$  g,  $t = 3.187$ ,  $P = 0.011$ ; Figure 2.4). Thus, females that benefitted from having had access to a carcass during stage one gained more mass during stage two. However, there was no evidence that the cost of caring for larvae in stage one affected female mass gain in a future breeding attempt since there was no difference in mass change in stage two between breeding females that had previously cared for a small brood or a large brood during stage one (estimate  $\pm$  SE =  $-0.014 \pm 0.007$  g,  $t = -2.037$ ,  $P = 0.263$ ; Figure 2.4). There was also no difference in mass gain between non-breeding females that previously had access to a carcass and breeding females that previously cared for a small brood (estimate  $\pm$  SE =  $0.001 \pm 0.007$  g,  $t = 0.160$ ,  $P = 0.999$ ; Figure 2.4) or a large brood (estimate  $\pm$  SE =  $-0.013 \pm 0.007$  g,  $t = 1.844$ ,  $P = 0.406$ ; Figure 2.4), further supporting that the cost of caring for larvae in stage one did not affect female mass change during stage two.



**Figure 2.4** - Mean mass change (g)  $\pm$  SE during stage two of females that, during stage one, were non-breeding and not provided with access to a carcass (no carcass), non-breeding and provided with access to a carcass (carcass), breeding and allocated a small brood (small brood), and breeding and allocated a large brood (large brood).

There was no evidence that the cost of increased allocation or the benefit of carcass access during stage one affected the number of eggs females laid during stage two since there was no difference in the number of eggs laid by females from the different treatment groups ( $\chi^2 = 3.946$ ,  $df = 3$ ,  $P = 0.267$ ).

There was no evidence that the cost of increased reproductive allocation in stage one affected offspring performance in stage two since there was no difference in larval survival or average larval mass at dispersal in broods reared by breeding females that had previously cared for a large brood or a small brood during stage one (Table 2.1). There was also no evidence for a benefit associated with access to a carcass during stage one since there was no difference in larval survival or

average larval mass at dispersal in broods reared by non-breeding females that had or did not have access to a carcass during stage one (Table 2.1). Nevertheless, there were some unexpected effects of our treatment of females during stage one on offspring performance during stage two. Non-breeding females that had no access to a carcass in stage one produced broods with a higher average larval mass than breeding females that previously cared for a small brood (Table 2.1). Additionally, broods reared by breeding females that had cared for a large brood during stage one had higher larval survival than broods reared by non-breeding females that had access to a carcass during stage one (Table 2.1).

**Table 2.1** - Pairwise comparisons between treatments for offspring performance at dispersal in stage two. Statistically significant P values (<0.05) are shown in bold.

	Proportional larval survival				Average mass per larvae (g)			
	Estimate	SE	z	P	Estimate	SE	z	P
Small – large	-0.40	0.29	-1.39	0.985	-0.00	0.00	-0.77	1.000
No carcass – large	-0.45	0.29	-1.52	0.768	0.01	0.01	2.13	0.210
Carcass – large	-0.91	0.29	-3.15	<b>0.010</b>	-0.00	0.01	-0.37	1.000
No carcass – small	-0.04	0.29	-0.15	1.000	0.01	0.01	2.89	<b>0.027</b>
Carcass – small	-0.51	0.28	-1.80	0.432	0.00	0.01	0.39	1.000
Carcass – no carcass	-0.47	0.29	-1.63	0.620	-0.01	0.01	-2.46	0.092

### 2.3.4 Female lifespan

There was no difference in lifespan between breeding females that cared for a large brood and breeding females that cared for a small brood (Table 2.2) or non-breeding females that had access to a carcass during stage one (Table 2.2). There was also no difference in lifespan between breeding females that cared for a small

brood during stage one and non-breeding females that had access to a carcass during stage one (Table 2.2). As such, there was no evidence that the cost of caring for a larger brood during stage one had an impact on female lifespan. Furthermore, there was no difference in lifespan between non-breeding females that did not have access to a carcass during stage one and those that did (Table 2.2). This finding suggests that the benefit of having access to a carcass during stage one had no effect on female lifespan. However, non-breeding females that had no access to a carcass during stage one had shorter lifespans than breeding females that cared for a small brood during stage one (Table 2.2) suggesting that the benefit of having had access to a carcass exceeded the cost of rearing a small brood. There was no difference in lifespan between non-breeding females that had no access to a carcass and breeding females that cared for a large brood (Table 2.2) suggesting the cost of rearing a large brood was cancelled out by the benefit of having access to a carcass.

**Table 2.2** - Pairwise comparisons between treatments for female lifespan. Statistically significant P values (<0.05) are shown in bold.

	HR	95% CI		P
		Lower	Upper	
Large - small	1.496	0.747	2.995	0.755
Large - carcass	1.257	0.632	2.504	0.999
Small - carcass	1.881	0.948	3.736	0.090
No carcass - carcass	1.126	0.565	2.248	0.999
No carcass – small	2.119	1.048	4.289	<b>0.029</b>
No carcass - large	1.418	0.715	2.809	0.999

## 2.4 Discussion

We found no detectable effect of brood size during a previous reproductive attempt on the amount of care breeding females provided during a subsequent breeding attempt. Thus, our study provided no support for the suggestion that females shift the cost of reproduction as a whole or the cost of caring for larvae specifically onto future offspring by providing less care during future breeding attempts. Furthermore, we found that breeding and non-breeding females that had had prior access to a carcass provided a similar amount of care as non-breeding females that did not have such access. Therefore, there was no evidence that the benefit of carcass access affected the level of post-hatching care females provided in a subsequent breeding attempt. Our results derive from an experimental design in which we manipulated the cost of reproduction using a well-established methodology (brood size manipulation). We are confident that our treatment in stage one had the intended effect on maternal allocation towards current reproduction since we found that breeding females that had cared for a large brood gained less mass than non-breeding females that had access to a carcass during stage one, while breeding females that had cared for a small brood gained an intermediate amount of mass. Our design also accounted for potential confounding effects due to the benefit associated with access to carrion by manipulating carcass access during stage one. This benefit was evident since non-breeding females with access to a carcass gained more mass than non-breeding females without access to a carcass. Finally, we measured effects on parental food provisioning to larvae and maintenance of the carcass, which are the most predominant parental care behaviours in this species and that are known to impact larval survival and growth (Smiseth & Moore, 2002;

Andrews *et al.*, 2017). In sum, we have confidence in our finding that *N. vespilloides* females provided similar levels of post-hatching parental care regardless of any previous costs associated with reproduction and benefits associated with carcass access.

Our main finding was that breeding females that had cared for a large brood provided a similar amount of care during a subsequent breeding attempt as breeding females that had cared for a small brood and non-breeding females. Thus, there was no support for the suggestion that a reduction in future ability to provide care is the mechanism allowing parents to shift the cost of reproduction onto future offspring as reported in prior work on beetles in the genus *Nicrophorus* (Jenkins *et al.*, 2000; Creighton *et al.*, 2009; Ward *et al.*, 2009; Billman *et al.*, 2014). There are several potential explanations for the lack of a difference in the amount of care provided by breeding females during stage two of the experiment. First, this could be due to a lack of response to brood size manipulation during stage one. This may be the case if females allocated a large brood during stage one do not provide more care to maintain their own condition for future breeding opportunities, or if females always work at capacity to provide the highest level of care, in which case they would have little room to escalate the level of care if allocated a large brood. There may appear to be some evidence for this suggestion since average larval mass during stage one was higher in small broods than in large broods. However, we argue that this explanation is unlikely given there is good evidence from prior studies on *N. vespilloides* showing that females provide more care when caring for an enlarged brood (Ratz and Smiseth, 2018; Richardson *et al.*, 2019; Smiseth *et al.*, 2007; Wang *et al.*, 2021). This suggests that breeding females allocated a large brood during stage one would have spent more time providing care than breeding females



allocated a small brood. Instead, the finding that average larval mass was higher in small broods than in large broods may simply reflect that there is a trade-off between number and size of offspring (Smiseth *et al.*, 2014; Richardson & Smiseth, 2019a).

Second, the lack of difference in the level of post-hatching care provided during stage two may reflect the nature of the resources used for breeding by *N. vespilloides*. This species breeds on carcasses of small vertebrates that are used as a food source for both parents and offspring (Scott, 1998). This means that females may gain a benefit associated with feeding from the carcass acquired for breeding. We anticipated such a benefit during both stages of our experiment. In stage one, this benefit was available to breeding females and non-breeding females provided with a carcass, but not to non-breeding females not provided with a carcass. Meanwhile, in stage two of our experiment, this benefit was available to all females since we provided all females with a carcass to initiate breeding. Thus, we anticipated seeing effects if the amount of care provided during stage two was affected by carcass access during both stages. Our findings suggest that this was not the case, possibly reflecting that carcass access during stage two had a much greater impact on the females' ability to provide post-hatching care than either the cost of reproduction or the benefit of carcass access during stage one. In other words, our results suggest that the carcass access associated with reproduction may buffer against any detrimental effects of costs or benefits due to increased allocation to reproduction or carcass access during previous breeding attempts on future post-hatching parental care.

Our study focused on the amount of care provided by females to larvae, which occurs after the female has had several days to feed and potentially recover from any reduction in condition due to previous allocation. In light of this, it may be

important to consider whether the trait in question manifests before or after parents have had access to a carcass when investigating the potential costs of reproduction in *N. vespilloides*. For example, whilst we found no effect of current allocation on the level of future post-hatching care females provide, there is evidence that increased allocation to current reproduction reduces a female's future ability to compete for a carcass (Richardson *et al.*, 2020). This differential effect on the ability to compete for a carcass and the ability to provide care for larvae may reflect that competition over carcass possession happens before either party has had a chance to feed on the carcass (Safryn & Scott, 2000), whilst care for larvae happens after females have fed from the carcass. This idea could be investigated by testing the effect of previous reproductive allocation on a trait that could be measured both before and after parents have fed on a carcass during a subsequent breeding attempt such as the ability to defend the carcass from an intruder. Similar results may be expected in other capital breeders that breed on resources obtained prior to reproduction and where parents use these as a food source. In contrast, different results might be expected for income breeders, such as many birds, where parents provision their offspring continuously throughout development with food obtained from the surrounding environment. In the latter species, increased allocation to current reproduction is likely to negatively affect the parents' future ability to provide parental care since parents face a trade-off between provisioning food to their offspring or consuming food for self-maintenance. We encourage future studies investigating whether shifting the cost of reproduction towards future offspring is mediated through a reduced future ability to provide care in species with a range of breeding strategies.

Given our finding that females with different levels of previous reproductive allocation provided the same level of care towards their offspring, we must consider alternative mechanisms for the finding that parents shift the cost of reproduction towards future offspring (Jenkins *et al.*, 2000; Creighton *et al.*, 2009; Ward *et al.*, 2009; Billman *et al.*, 2014). One explanation is that greater allocation to current reproduction may affect other aspects of future parental care. In *N. vespilloides*, allocation to current reproduction may affect future egg laying behaviour since, like competitive ability, egg laying occurs soon after the discovery of the carcass and when females may have had limited opportunities to recover from any reduction in condition due to previous allocation. Although we found no effect of previous reproductive allocation on the number of eggs females laid during stage two, there may be effects on the pattern of laying or the size of the eggs females produce in a subsequent breeding attempt. For example, females that allocate more to current reproduction may produce smaller eggs or delay the onset of egg laying in future breeding attempts (Mäenpää & Smiseth, 2017). In other species, alternative mechanisms include other behavioural traits, such as a reduction in ability to compete for resources necessary for reproduction (Fokkema *et al.*, 2016) or physiological traits such as a reduction in ornamentation quality limiting breeding opportunities (Siefferman & Hill, 2005). We encourage future work on a variety of taxa investigating alternative mechanisms that could cause increased allocation to current reproduction to result in the often-observed reduction in future fecundity.

There were some unexpected results from our experiment. Firstly, females that were previously provided access to a carcass (regardless of whether they were breeding or not) gained more mass when provided with a second carcass than females that were not previously provided with a carcass. This finding does not fit

our predictions for the effects of the cost of increased allocation or the benefit of carcass access during stage one. Instead, this result suggests that females that previously had access to a carcass responded by shifting towards greater allocation on future reproduction since mass gain during breeding is a proxy for allocation to future reproduction in *N. vespilloides* (Billman *et al.*, 2014; Creighton *et al.*, 2009). This shift may reflect that females responded to the presence of carcasses as an environmental cue about future breeding opportunities. Carcasses are normally a rare resource (Scott, 1998), and coming across two in quick succession may provide females with a cue that they find themselves in a resource-rich environment. In support of this suggestion, a previous study on *N. vespilloides* found that the quality of the carcass used for a breeding attempt influences reproductive investment in a subsequent breeding attempt, potentially by providing information about the resources available (Billman *et al.*, 2014).

Secondly, breeding females that were allocated a small brood in stage one produced broods with a lower average larval mass in stage two than non-breeding females that did not have access to a carcass in stage one. This finding appears to contrast with previous studies, which found that increased allocation to current reproduction is associated with a reduction in the size and mass of future broods (Jenkins *et al.*, 2000; Creighton *et al.*, 2009; Ward *et al.*, 2009; Billman *et al.*, 2014). However, we note that we provided females with foster broods of a standardised size during stage two. This was an important aspect of our design as it allowed us to control for any potential confounding effects on female behaviour due to variation in brood size during stage two. In contrast, prior studies manipulated aspects of reproductive allocation during a current breeding attempt and then allowed individuals to raise their natural brood during a subsequent breeding attempt or

throughout multiple breeding attempts for the rest of their lifetime. Our results are therefore not comparable with those of prior studies. We also found that breeding females that were allocated a small brood in stage one had a longer lifespan than non-breeding females that were not provided access to a carcass in stage one. This may appear to contrast with the results of Creighton *et al.*, (2009), which showed non-reproducing females to live longer than reproducing females. However, Creighton *et al.* (2009) compared the lifespan of reproducing females and females that never reproduced, whereas we compared females that did not reproduce initially but were allowed to reproduce during stage two with females that reproduced across both stages.

Why did breeding females allocated a small brood in stage one produce broods with a lower average larval mass in stage two and have a longer lifespan than non-breeding females without access to a carcass in stage one? We suggest that these results reflect the combined effects of breeding females shifting towards greater allocation in future reproduction, breeding females caring for a small brood in stage one suffering a lower cost of caring for larvae than breeding females caring for a large brood, and non-breeding females with access to a carcass responding to this treatment as a failed breeding attempt. As discussed above, breeding females may have shifted towards greater allocation in future reproduction if the presence of carcasses acts as an environmental cue about abundant future breeding opportunities. However, this alone cannot explain our finding since we would also expect breeding females that cared for a large brood and non-breeding females with access to a carcass to respond similarly, which was not the case. We therefore suggest that breeding females caring for a small brood suffered a lower cost of caring for larvae than breeding females caring for a large brood. Finally, we suggest

that non-breeding females with access to a carcass responded differently from breeding females given that they produced no larvae during stage one, and therefore may have perceived this as a failed breeding attempt.

In conclusion, our study shows that females maintain the level of parental care they provide regardless of any costs associated with previous reproductive allocation or any benefits associated with prior resource access. This is likely a result of the breeding strategy of *N. vespilloides* since they have access to a fixed food source during breeding which may facilitate their recovery from any costs associated with previous reproductive allocation. Our findings highlight the need for more work exploring the potential mechanisms that allow parents to shift the cost of current reproduction towards future offspring in species with a variety of life-history strategies.

### 3 Flexible females: nutritional state influences biparental cooperation in a burying beetle

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#### Abstract

In species that provide biparental care, there is sexual conflict between parents over how much each should contribute towards caring for their joint offspring. Theoretical models for the resolution of this conflict through behavioural negotiation between parents assume that parents cannot assess their partner's state directly but do so indirectly by monitoring their partner's contribution. Here, we test whether parents can assess their partner's state directly by investigating the effect of nutritional state on cooperation between parents in the burying beetle *Nicrophorus vespilloides*. We used a two-by-two factorial design, in which a well-fed or food-deprived female was paired with a well-fed or food-deprived male. We found that females adjusted their level of care in response to both their own nutritional state and that of their partner and that these decisions were independent of their partner's contribution. We found no evidence that males responded directly to nutritional state. Males instead responded indirectly based on the contribution of their partner. Our results suggest that parents are able to assess the state of their partner, in contrast to what has been assumed, and that these assessments play an important role in the mediation of sexual conflict between caring parents.

### 3.1 Introduction

There is sexual conflict between parents that provide biparental care since the benefits of care depend on the combined effort of the two parents, whereas the costs to future reproduction are paid individually (Houston *et al.*, 2005; Lessells, 2012). Parents are therefore under selection to shift as much of the workload as possible onto their partner (Parker, 1985). Theoretical models have identified several mechanisms by which this conflict may be resolved: negotiation, matching or a sealed-bid decision. Negotiation involves a parent responding to a decrease in the amount of care contributed by its partner by increasing its own contribution but only such that it incompletely compensates for the partner's decrease (McNamara *et al.*, 1999). Matching involves a parent responding to a change in its partner's contribution by increasing when its partner increases or decreasing when its partner decreases its contribution (Johnstone & Hinde, 2006). A sealed-bid decision is when a parent makes an initial decision about how much care to provide that is independent of its partner's contribution (Houston & Davies, 1985). There is empirical evidence supporting negotiation (Harrison *et al.*, 2009; Pilakouta *et al.*, 2015), matching (Hinde, 2006; Lendvai *et al.*, 2018) and sealed-bid decisions (Schwagmeyer *et al.*, 2002; Matthey & Smiseth, 2015; Pilakouta *et al.*, 2015) across different taxa; however, in birds, the most studied taxonomic group, negotiation is thought to be the predominant mechanism for cooperation (Harrison *et al.*, 2009).

Variation in the state of the parents may play an important role in determining how sexual conflict over care is resolved. Here we refer to 'state' as any attribute of an individual, such as its nutritional status, body size, inbreeding status, infection status, or age, which is likely to affect its contribution towards care and/or its



partner's contribution. A focal parent is likely to adjust its contribution based on its own state since variation in state may influence the cost and/or benefit functions associated with providing care (Smiseth, 2017). The focal parent may also adjust its contribution based on its partner's state since this may provide information about the likely future contribution of its partner and/or the potential value of the joint brood. Theoretical models for negotiation as a mechanism of conflict resolution assume that a focal parent cannot assess its partner's state directly and that it does so indirectly by monitoring its contribution (McNamara *et al.*, 1999; McNamara & Wolf, 2022). These models emphasize that variation in the state of parents plays a key role in promoting the evolution of negotiation as a behavioural mechanism of conflict resolution when the focal parent cannot assess its partner's state directly. Meanwhile, more recent research has shown that a focal parent can respond directly to variation in multiple states of its partner, and that it also independently responds to its partner's contribution (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). Thus, there is a need for more work exploring the role of state in negotiation in particular, and the mediation of sexual conflict between parents in general.

Here, we investigate the effects of a temporary and reversible state; that is, nutritional state, on the dynamics of biparental cooperation. Prior studies investigating the effects of state on biparental cooperation have focused on permanent states, such as inbreeding status and adult body size, which remain constant throughout adulthood. For a permanent state, we expect a focal parent to adjust its contribution to its own state and that of its partner since state affects ability to provide care. In support of this, previous studies have found that individuals adjust the amount of care they contribute based on their own body size and that of their partner (Pilakouta *et al.*, 2015) and the inbreeding status of their partner (Mattey &

Smiseth, 2015). In contrast, we might expect different dynamics for a temporary and reversible state, such as being malnourished or infected with a pathogen, given that a focal individual may improve its state by reducing its own contribution to care. Thus, for temporary and reversible states, biparental care may facilitate recovery by allowing a malnourished or infected individual to invest more in its own recovery, thereby shifting a greater amount of the workload over to its partner.

We tested this idea by manipulating the nutritional state of females and males of the burying beetle *Nicrophorus vespilloides* and measuring the effect on how a focal parent responded to its own state and that of its partner. Beetles in the genus *Nicrophorus* are well suited to test this idea. Firstly, parents provide elaborate biparental care and the level of care parents provide is flexible. *Nicrophorus vespilloides* breeds on a small vertebrate carcass that serves as a joint food source for both parents and their offspring. Female and male parents provide care for their offspring, including preparing and maintaining the carcass by spreading antimicrobials onto it, provisioning pre-digested carrion to their offspring, and guarding their offspring against conspecific intruders (Eggert *et al.*, 1998b; Scott, 1998). Although both parents are capable of all activities, females tend to spend more time food provisioning to the larvae (Eggert *et al.*, 1998b; Smiseth & Moore, 2002; Smiseth *et al.*, 2005) while males spend more time maintaining the carcass (Smiseth *et al.*, 2005). Secondly, it is relatively straightforward to manipulate an individual's nutritional state by simply subjecting them to food deprivation for seven days before breeding. Prior work also shows that food-deprived parents feed more during breeding than well-fed parents (Keppner *et al.*, 2018) and that females respond to their own nutritional state since food-deprived females spend less time maintaining the carcass and provisioning food to their larvae in comparison to well-

fed females (Richardson & Smiseth, 2019a). However, there is no information on the effects of nutritional state on the dynamics of biparental cooperation.

Our aim was to test whether parents adjust the level of care they provide based on the nutritional state of their partner and whether this was conditional upon their own nutritional state. To meet this aim, we used a two-by-two factorial design where a well-fed or food-deprived female was paired with a well-fed or food-deprived male. We predict that a food-deprived focal parent will decrease the level of care it provides when paired with a well-fed partner since its partner is able to compensate for a reduction in care due to the poor state of the focal parent. In contrast, a food-deprived focal parent may not be able to decrease the level of care it provides when paired with a food-deprived partner since its partner is less able to compensate. We also predict that the partner of a food-deprived parent will incompletely compensate for the expected lower level of care provided by the food-deprived parent, and that food-deprived individuals would provide less care than well-fed individuals since food-deprived individuals are likely to pay a higher cost of providing care (Richardson & Smiseth, 2019a). To determine whether a focal parent assessed its partner's state directly or indirectly by monitoring its partner's contribution, we added the partner's contribution to the model to test whether it accounted for any observed effects of the partner's state on the focal parent. We tested if food-deprived individuals prioritize improving their own nutritional state when compared to well-fed parents as a means to recover from any potential costs of food deprivation to their ability to invest in future reproduction. We predict that food-deprived individuals would consume more carrion and thus gain more mass during breeding than well-fed individuals. Finally, we tested for effects of nutritional state on the size and quality of the joint brood. We expect that the broods of food-deprived parents will be smaller

and of worse quality since we expect food-deprived parents to provide less care and to consume more carrion (a joint food source) which would reduce the amount of food available for the larvae.

## 3.2 Methods

### 3.2.1 General methodology

We used beetles from an outbred laboratory population originally collected in Edinburgh, UK and maintained at the University of Edinburgh. We housed all adults in the stock population individually in clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil and fed them raw organic beef twice a week. The stock population was kept at 20 °C under a 16:8 h light:dark cycle.

### 3.2.2 Experimental design

We used a two-by-two factorial design with the following treatments: a food-deprived female paired with a food-deprived male (n = 30), a food-deprived female paired with a well-fed male (n = 30), a well-fed female paired with a food-deprived male (n = 32), and a well-fed female paired with a well-fed male (n = 31). All individuals used in the experiment were at least 10 days post-eclosion to ensure they had reached sexual maturity and that feeding treatment had no effect on the rate of maturation. We weighed all individuals before assigning them to one of the four treatments in our experiment. We used established protocols to produce well-fed and food-deprived females and males. Food-deprived individuals were not fed during the 7-day treatment whereas well-fed individuals were fed twice with organic beef (approximately 0.3 g) during this period. This level of food deprivation was chosen since it leads to a significant drop in weight without causing an increase in mortality

(Richardson & Smiseth, 2019b; a). After seven days, we weighed all individuals to measure their post-treatment and pre-breeding mass. We used this to calculate weight change during the treatment period and confirm that our food deprivation treatment had the intended effect on nutritional state (see 3.3 Results).

Immediately after weighing, we paired up males and females at random, taking care to avoid mating between close relatives. We transferred each pair into a clear plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil. Each pair was provided with a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (15 - 20 g;  $M \pm SE = 18.47 \pm 0.11$  g) to initiate breeding. After 48 h, when the eggs had been laid but before the larvae had begun hatching, we moved the female, the male and their carcass into a new clear plastic container (17 cm × 12 cm × 6 cm) lined with fresh moist soil. We allocated each pair a foster brood consisting of 20 newly hatched larvae from at least two different mothers. We chose this brood size since it is close to the average brood size for *N. vespilloides* (21 larvae; Smiseth and Moore 2002). We used a standardized brood size to control for potential confounding effects due to variation in brood size. Such confounding effects might arise because our treatment might affect the number of eggs laid (Steiger *et al.*, 2007) and because brood size is known to affect the amount of care provided by parents (Smiseth & Moore, 2002). We allocated a foster brood to a pair only after their eggs had started hatching since parents use temporal kin recognition and so would kill larvae that arrive at the carcass before their own eggs started hatching (Müller & Eggert, 1990).

We conducted behavioural observations 24 h ( $\pm 15$  min) after we allocated pairs a foster brood since this is when parents provide the highest level of care in this species (Smiseth *et al.*, 2003). We did the observations under red light using

instantaneous sampling of female and male behavior every minute for 30 min consistent with established protocols (Smiseth & Moore, 2002). At each scan, we recorded whether females and males were providing direct care, indirect care or consuming carrion. We used number of scans as a proxy for the amount of time an individual spent providing care or consuming carrion. Direct care included provisioning food to the larvae (mouth-to-mouth contact between the parent and at least one larvae) and grooming the larvae. Indirect care included maintaining the carcass (spreading antimicrobial secretions onto its surface or modifying the position of the carcass) and guarding the brood and carcass against competitors or predators (standing still on the carcass facing away from the brood). After the observations, we left the pairs to care for their brood until the larvae dispersed from the carcass approximately five days later, upon which we recorded average larval mass and the proportion of larvae that survived to dispersal as measures of offspring performance, and female and male post-breeding mass to allow us to calculate individual mass change during breeding.

### 3.2.3 Statistical Analysis

All statistical analyses were conducted using R version 3.6.1 (R Core Team, 2021) with the packages *car* (Fox & Weisberg, 2019), *MASS* (Venables & Ripley, 2022), and *glmmTMB* (Brooks *et al.*, 2017). We used zero-inflated binomial models in our analyses on the amount of time spent providing direct care by females and males since the data for this behaviour showed minor zero inflation. We used binomial models in our analysis on time spent providing indirect care and consuming carrion by females and males, and larval survival to dispersal. In all these models, we included observation level as a random effect to account for over-dispersion (Harrison, 2015). We used linear models for data on female and male mass change

during the food deprivation treatment, female and male mass change during breeding, and mean larval mass at dispersal.

To determine whether the focal parent responded directly to the nutritional state of its partner or indirectly to its partner's contribution, we compared models where we included and excluded the amount of time spent providing direct or indirect care or consuming carrion by the partner as a factor (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). If including this factor reduced or removed any effect of the partner's nutritional state on the amount of time spent providing direct or indirect care or consuming carrion by the focal parent, we interpreted this as evidence that the focal parent responded indirectly to the contribution of its partner, as expected by theoretical models of negotiation. However, if including this factor did not negate the effect of partner's nutritional state on the amount of time spent providing direct or indirect care or consuming carrion by the focal parent, we interpreted this as evidence that the focal parent responded directly to the nutritional state of its partner. We note that our data on the responses of the focal parent to its partner's contribution are correlational and that we therefore cannot demonstrate a causal relationship between the contributions of females and males to parental care.

### 3.3 Results

#### 3.3.1 Effects of food deprivation on male and female mass change

Food-deprived females and males lost more mass during the treatment period than well-fed females and males (females: estimate =  $-0.052 \pm 0.005$  g,  $t = -11.11$ ,  $P < 0.001$ , males: estimate =  $-0.035 \pm 0.004$  g,  $t = -7.96$ ,  $P < 0.001$ ). This confirms that the food deprivation treatment had the intended effect of altering an individual's

nutritional state. There was no difference between female and male mass change during the food deprivation treatment (estimate =  $0.009 \pm 0.004$  g,  $t = 1.63$ ,  $P = 0.105$ ).

### 3.3.2 Effects of nutritional state on parental care and cooperation

There was a significant effect of the interaction between a female's nutritional state and the nutritional state of its partner on the amount of time spent providing direct care (Table 3.1). The estimate of this interaction effect was positive (Table 3.1), indicating that food-deprived females responded to being paired with a food-deprived male, rather than well-fed male, by more strongly increasing the amount of time they spent providing direct care than did well-fed females. Indeed, visual inspection of Figure 3.1 shows that food-deprived females spent more time providing direct care when paired with a food-deprived male than when paired with a well-fed male, while well-fed females provided similar levels of care regardless of whether they were paired with food-deprived or well-fed males. Thus, females responded to the nutritional state of their partner, but any such response was conditional upon the female's own nutritional state. There was also a significant main effect of female nutritional state on the amount of time spent providing direct care by females (Table 3.1, Figure 3.1). This finding is due to the interaction described above. There was no significant effect of the interaction between a male's nutritional state and the nutritional state of its partner on the amount of time spent providing direct care (Table 3.1, Figure 3.1). There was no main effect of the partner's nutritional state on the amount of time females and males spent providing direct care (Table 4.1, Figure 3.1). There was also no main effect of male nutritional state on the amount of time spent providing direct care by males (Table 3.1, Figure 3.1).



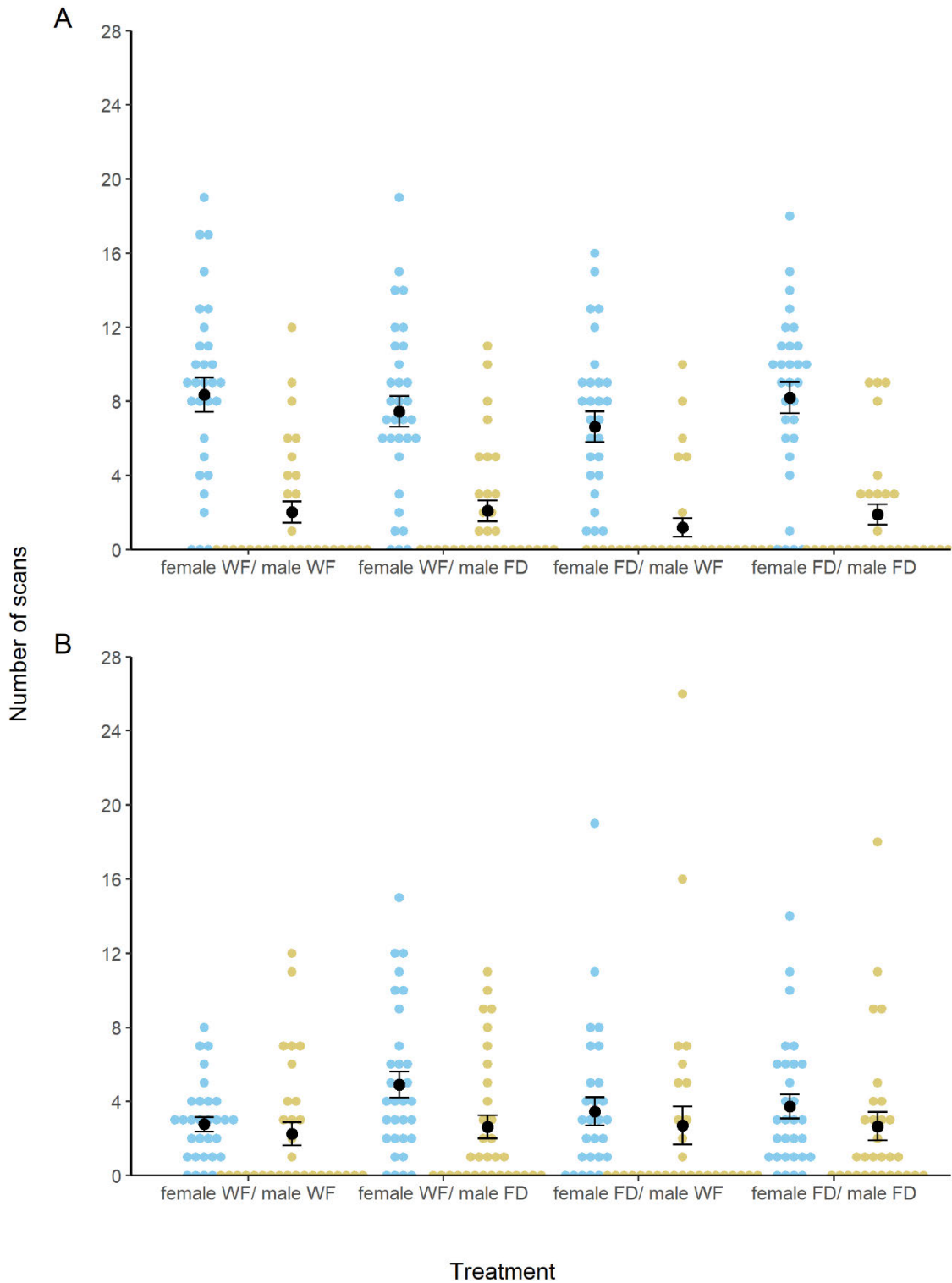
There was no effect of the interaction between a female's nutritional state and the nutritional state of its partner on the amount of time females spent providing indirect care; however, females provided more indirect care when paired with a food-deprived male (Table 3.1, Figure 3.1). Thus, females altered the amount of indirect care they provided in response to their partner's nutritional state but did so regardless of their own nutritional state. There was no significant effect of the interaction between a male's nutritional state and the nutritional state of its partner on the amount of time spent providing direct care (Table 3.1, Figure 3.1). There was no main effect of the partner's nutritional state on the amount of time males spent providing indirect care (Table 3.1, Figure 3.1). There was also no main effect of focal parent nutritional state on the amount of time spent providing indirect care by females or males (Table 3.1, Figure 3.1).

The partner's contribution was also a predictor of female care suggesting that females responded to the contribution of their partner. Females spent less time providing direct care as the amount of time males spent providing direct care increased (Table 3.1). Including or excluding the partner's contribution did not alter the effect of the interaction between the focal parent's nutritional state and the nutritional state of the partner on the amount of direct care provided by females (Table 3.1). This suggests that females responded independently to the state and contribution of their partner. Partner contribution was a predictor of male care, suggesting that males also responded to the contribution of their partner. Males spent less time providing direct care as the amount of time females spent providing direct care increased (Table 3.1). There was no relationship between the partner's contribution and the amount of time spent providing indirect care by females or males (Table 3.1). We note that we measured parental behaviour in a single 30

minute observation and so we may not have detected a change in the distribution of care across the breeding attempt as a whole.

**Table 3.1** - Summary of statistical tests for the effects of nutritional state on biparental cooperation over providing direct and indirect care excluding and including (\*) partner's contribution in the model. The reference category for the focal parent's nutritional state and the partner's nutritional state was 'well-fed'. Statistically significant P values (< 0.05) are shown in bold.

Behaviour	Focal parent's nutritional state				Partner's nutritional state				Interaction				Partner's contribution			
	Est	SE	z	P	Est	SE	z	P	Est	SE	z	P	Est	SE	z	P
Female direct	-0.41	0.19	-2.11	<b>0.035</b>	-0.24	0.19	-1.26	0.208	0.63	0.27	2.33	<b>0.020</b>				
Female direct*	-0.43	0.20	-2.12	<b>0.034</b>	-0.19	0.20	-0.98	0.325	0.60	0.28	2.13	<b>0.033</b>	-0.08	0.03	-2.42	<b>0.016</b>
Female indirect	0.11	0.31	0.35	0.724	0.65	0.29	2.22	<b>0.027</b>	-0.47	0.42	-1.12	0.262				
Female indirect*	0.11	0.31	0.35	0.723	0.65	0.29	2.22	<b>0.027</b>	-0.47	0.42	-1.12	0.262	-0.00	0.03	-0.10	0.917
Male direct	-0.23	0.34	-0.67	0.503	0.15	0.47	0.31	0.753	-0.07	0.58	-0.12	0.908				
Male direct*	-0.17	0.33	-0.54	0.593	0.10	0.46	0.22	0.827	-0.06	0.57	-0.11	0.912	-0.07	0.03	-2.38	<b>0.017</b>
Male indirect	0.44	0.66	0.66	0.507	-0.12	0.69	-0.18	0.859	0.16	0.94	0.17	0.865				
Male indirect*	0.45	0.67	0.68	0.498	-0.12	0.69	-0.17	0.865	0.15	0.95	0.16	0.876	-0.01	0.07	-0.14	0.887



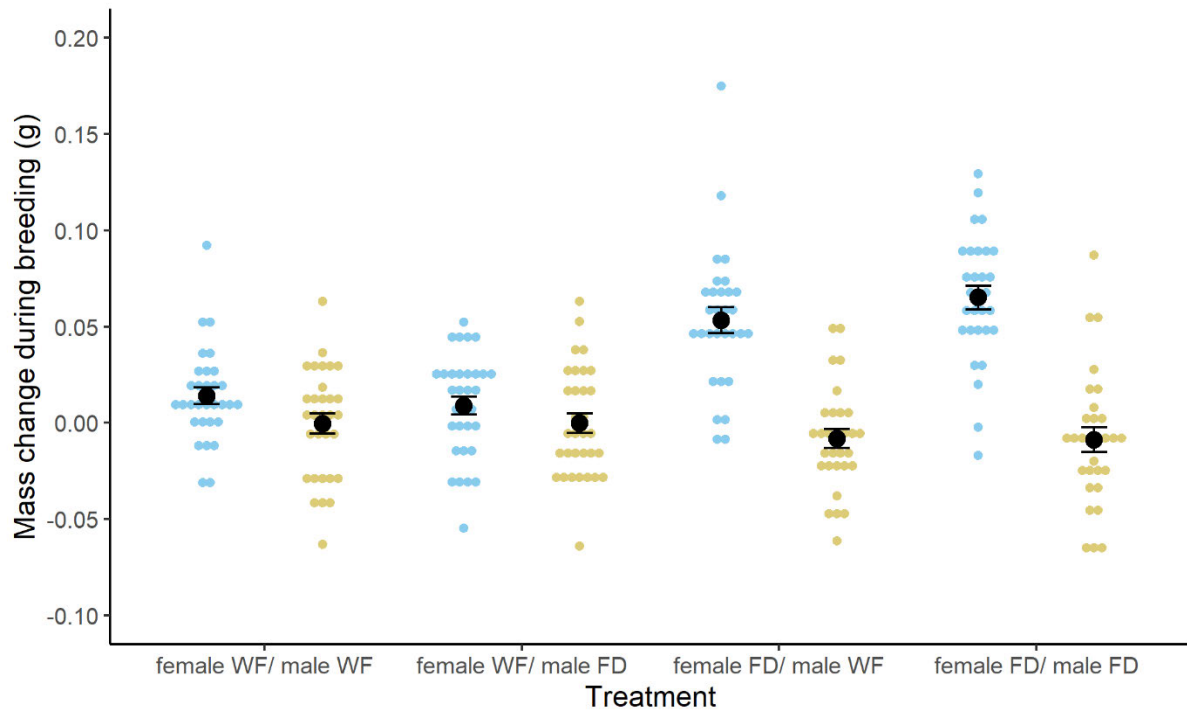
**Figure 3.1** - Comparison of the number of scans (out of 30) spent providing direct care (A) and indirect care (B) by well-fed (WF) or food-deprived (FD) females (blue points) and well-fed or food-deprived males (yellow points) caring for larvae during a 30 min behavioral observation (mean  $\pm$  SE).

### 3.3.3 Male and female mass change during breeding & time spent consuming carrion

In contrast to our predictions, there was no effect of the focal parent's nutritional state, the partner's nutritional state or the interaction between the two on the amount of time spent consuming carrion by females or males (Table 3.2). However, in line with our predictions, the female nutritional state affected female mass change during breeding with food-deprived females gaining more mass than well-fed females (Table 3.2, Figure 3.2). There was no effect of the male nutritional state on male mass change during breeding (Table 3.2). There was no effect of the interaction between the focal parent's nutritional state and the partner's nutritional state on the mass change of females or males during breeding (Table 3.2). There was also no effect of the partner's nutritional state on the mass change of females or males during breeding (Table 3.2).

**Table 3.2** - Summary of statistical tests for the effects of nutritional state on biparental cooperation over consumption excluding and including (\*) partner's contribution in the model and mass change during breeding. The reference category for the focal parent's nutritional state and the partner's nutritional state was 'well-fed'. Statistically significant P values (< 0.05) are shown in bold.

Behavior	Focal parent's nutritional state				Partner's nutritional state				Interaction				Partner's contribution			
	Est	SE	Test statistic	<i>P</i>	Est	SE	Test statistic	<i>P</i>	Est	SE	Test statistic	<i>P</i>	Est	SE	<i>z</i>	<i>P</i>
Female consumption	0.22	0.20	<i>z</i> = 1.10	0.273	-0.07	0.20	<i>z</i> = -0.36	0.716	-0.02	0.28	<i>z</i> = -0.05	0.957				
Female consumption*	0.22	0.20	<i>z</i> = 1.08	0.280	-0.09	0.20	<i>z</i> = -0.43	0.668	-0.00	0.28	<i>z</i> = -0.01	0.989	0.00	0.01	0.34	0.733
Female mass change	0.04	0.01	<i>t</i> = 5.13	<b>&lt; 0.001</b>	-0.01	0.01	<i>t</i> = -0.66	0.509	0.02	0.01	<i>t</i> = 1.54	0.125				
Male consumption	0.78	1.28	<i>z</i> = 0.61	0.541	-0.61	1.22	<i>z</i> = -0.50	0.616	0.67	1.81	<i>z</i> = 0.37	0.713				
Male consumption*	0.83	1.28	<i>z</i> = 0.65	0.514	-0.54	1.22	<i>z</i> = -0.44	0.657	0.64	1.79	<i>z</i> = 0.36	0.720	-0.08	0.08	-1.02	0.309
Male mass change	0.00	0.01	<i>t</i> = 0.11	0.915	-0.01	0.01	<i>t</i> = -1.48	0.141	-0.00	0.01	<i>t</i> = -0.25	0.802				



**Figure 3.2** - Comparison of the mass change during breeding by well-fed (WF) or food-deprived (FD) females (blue points) and well-fed or food-deprived males (yellow points) (mean  $\pm$  SE).

### 3.3.4 Offspring performance

Finally, we tested for effects of the parents' nutritional state on offspring performance upon dispersal. Average larval mass at dispersal was higher in broods cared for by food-deprived females (estimate =  $0.019 \pm 0.007$  g,  $t = 2.73$ ,  $P = 0.007$ ). There was no effect of male nutritional state (estimate =  $0.006 \pm 0.007$  g,  $t = 0.86$ ,  $P = 0.392$ ), and no effect of the interaction between female and male nutritional state (estimate =  $-0.016 \pm 0.010$  g,  $t = -1.668$ ,  $P = 0.098$ ) on average larval mass at dispersal. The proportion of larvae surviving to dispersal was not dependent on female nutritional state (estimate =  $0.060 \pm 0.236$  g,  $z = 0.25$ ,  $P = 0.801$ ), male

nutritional state (estimate =  $0.425 \pm 0.234$  g,  $z = 1.82$ ,  $P = 0.069$ ), or the interaction between the two (estimate =  $-0.159 \pm 0.334$  g,  $z = -0.48$ ,  $P = 0.634$ ).

### 3.4 Discussion

We found that females adjusted the amount of direct care they provided in response to the nutritional state of their partner and that this was conditional upon their own nutritional state. This interaction effect was likely driven by food-deprived females paired with well-fed males providing less care than food-deprived females paired with food-deprived males, while well-fed females provided similar levels of care regardless of whether they were paired with well-fed or food-deprived males. This finding is in line with our prediction that food-deprived individuals would reduce the amount of time they spend providing care if their partner is well-fed and is therefore capable of increasing its contribution. Reducing their contribution to care when paired with a well-fed male would enable food-deprived females to invest more into their own recovery whilst minimising any detrimental effects to the joint brood. In support of this suggestion, food-deprived females gained more mass during breeding than well-fed females, which we will discuss in greater detail below. We also found that the amount of time females spent providing direct care was affected by the partner's contribution. Females spent less time providing direct care when males spent more time providing direct care as predicted by negotiation models (McNamara *et al.*, 1999). The inclusion of partner's contribution in the model had little if any impact on the effect of the interaction or the partner's state on the amount of time females spent providing direct care. This suggests that females responded directly to the nutritional state of their partner, and that this response was independent of the response to the contribution of their partner.



Our results show that females adjust the amount of care they provide in response to the nutritional state of their partner and that they also independently respond to their partner's contribution. This is interesting for several reasons. Firstly, our results add to growing evidence that responses to partner's state and contribution are not mutually exclusive mechanisms for mediating conflict between caring parents (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). A potential explanation for why parents respond to their partner's state, as well as their partner's contribution, is that state and contribution may provide somewhat different information about the partner's expected future contribution to care (Pilakouta *et al.*, 2015). Alternatively, parents may respond to their partner's contribution to coordinate the distribution of parental care over time rather than to gain information about their partner's expected contribution (Smiseth, 2019). Thus, using two complementary mechanisms may enable parents to make a more accurate assessment of their partner's expected contribution or the timing of their contribution and allow them to fine-tune their response. Secondly, our results are in contrast with theoretical models of negotiation, which assume that parents assess the state of their partner indirectly based on their partner's contribution (McNamara *et al.*, 1999). Currently, experiments focus on manipulating the contribution of a focal parent, typically via handicapping or mate removal, and then measuring any responses by its partner (Harrison *et al.*, 2009). These designs may produce evidence that is biased towards negotiation since they negate the opportunity to test whether parents directly assess and respond to the state of their partner. As such, we suggest that future experiments are designed to reflect that multiple mechanisms may be involved in resolving sexual conflict, including direct responses to state as shown here. In a laboratory environment, this could be achieved by manipulating and measuring the

effect of state, in addition to partner contribution, on parental behavior. In situations where manipulating state is impractical or unethical, including observational data on parental state in addition to data on partner contribution may also provide further insight. In sum, our findings highlight the need for more work exploring how multiple mechanisms may play a role in the dynamic balance between cooperation and conflict in species with biparental care.

We found a different pattern for indirect care by females in comparison to the results for direct care by females as discussed above. Females spent more time providing indirect care when paired with a food-deprived partner regardless of their own nutritional state and their partner's contribution. This result confirms that females adjust their contribution based on the state of their partner, although, in this case, this response was independent of their own state. The absence of an interaction effect between focal parent nutritional state and partner nutritional state is surprising given that we expected individuals to respond to their partner being food-deprived only if they were well-fed and, therefore, capable of increasing their contribution. We found that both food-deprived and well-fed females increased the amount of time they spent providing indirect care when paired with a food-deprived male. A potential explanation is that providing indirect care is less energetically costly to females than providing direct care, and so they were able to increase the amount of indirect care they provide in response to male state even when food-deprived. Multiple studies have explored the cost of increased brood size (a strong predictor of the amount of direct care females provide) (Ward *et al.*, 2009; Ratz & Smiseth, 2018), but there is no information on the cost of providing indirect care alone or a direct comparison between direct and indirect care. To explore this idea, future work could test the energetic cost of providing direct and indirect care,

potentially by simultaneously manipulating brood size and carcass size and measuring the effect on females.

Our results imply that females can assess the nutritional state of their partner, and add to evidence that parents in *N. vespilloides* can assess and respond to various states of their partner, such as their inbreeding status and body size, independently of the partner's contribution (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). This raises questions as to how females do so. Our study was not designed to investigate the potential mechanisms that could be involved. Nevertheless, based on prior work, we suggest that cuticular chemicals are likely candidates. There is good evidence that cuticular chemicals play an important role in partner recognition in *N. vespilloides* (Steiger *et al.*, 2007b; Keppner *et al.*, 2017) and there is also evidence that cuticular hydrocarbons are indicators of diet (Steiger *et al.*, 2007b; Fedina *et al.*, 2012) and health (Beani *et al.*, 2019) in *N. vespilloides* as well as other insects. Thus, it seems likely that malnutrition may alter an individual's cuticular chemical profile, thereby allowing its partner to judge its nutritional state. We suggest future work further investigates the potential role of chemical cues as a mechanism mediating how parents are able to assess various cryptic states of their partner.

We found that males adjusted the amount of care they provided in response to the contribution of their partner, but there was no evidence that males responded to their partner's nutritional state, their own nutritional state, or the interaction between the two. Instead, males decreased the time spent providing direct care as the amount of time females spent providing direct care increased, as predicted by negotiation models (McNamara *et al.*, 1999). One explanation for this result is that males are unable to directly respond to the state of their partner and instead rely on the contribution of their partner as a measure of their ability to provide care.

However, this seems unlikely given that females responded directly to the nutritional state of their partner, and that prior studies on *N. vespilloides* show that males respond to other components of their partner's state (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). Our results add to a growing list of sex differences in caring behaviour in *N. vespilloides* (Smiseth & Moore, 2002; Walling *et al.*, 2008; Georgiou-Shippi *et al.*, 2018). Currently, we have a poor understanding of what may be driving these differences, and this is an area that would warrant further work.

A main aim of this study was to investigate whether the effects of temporary states on the dynamics of biparental cooperation differ from those reported previously for permanent states (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). We expected differences given that a parent has the opportunity to improve its state by reducing its contribution towards providing care for temporary and reversible states but not for permanent states. As such, we expected food-deprived individuals to provide less care than those in a comparably poor permanent state since, in addition to their reduced ability to provide care, they also have the incentive to invest in their own state whilst their partner incompletely compensates. There are both similarities and differences between the effects of nutritional state and previously tested permanent states. We found that food-deprived females spent less time providing direct care when paired with a well-fed male. Similarly, a previous study found that small females spend more time providing direct care when paired with a small male (Pilakouta *et al.*, 2015). In both cases, females in a comparatively worse state only reduced the level of care they provided if their partner was in a better state and was able to compensate. In contrast, previous work found that inbred females spent the same amount of time providing direct care regardless of their partners' state but that outbred females provided more care when paired with an inbred male (Mattey &

Smiseth, 2015). The challenge with making comparisons across different components of state is that this requires some way to calibrate the impact of states on the individual's ability to provide care. For example, we cannot know whether our treatment of seven days of food deprivation is equivalent to being of a particular size difference or a particular difference in inbreeding coefficients. As such, it is difficult to make true comparisons of the impact of nutritional state and body size or inbreeding state on parental cooperation. To overcome this, future studies may use a standardized test, such as measuring some aspect of performance, to calibrate the effect of different states on individuals. This would allow more accurate comparisons of any differential effects of temporary and permanent states on parental cooperation.

Parental mass change over the breeding attempt provided some insight into whether food-deprived individuals used the breeding attempt as an opportunity to recover from being in a temporarily worse state. We found no effect of the interaction between female and male nutritional state on female or male mass change during breeding to support this suggestion. However, food-deprived females gained more mass during breeding than well-fed females. In contrast, Keppner *et al.* (2018) found that females paired with food-deprived males weighed less at the end of a breeding attempt than those paired with well-fed males. This difference is likely a result of the smaller carcass size (8.5 – 11.5 g) used in Keppner *et al.* (2018) than in our study (15 – 20 g), causing greater competition for limited resources among females and males in Keppner *et al.* (2018). Our results show that females recovered from food deprivation when breeding, but that this response was independent of any male assistance. This recovery as well as the lack of an interaction effect may reflect that *N. vespilloides* breeds on small vertebrate carcasses that provide a food source for

both parents and offspring (Scott, 1998). As such, food-deprived individuals may not require assistance from a partner to recover since they do not have to engage in costly foraging for food from the surrounding environment.

We suggested that whether a species is a capital breeder or an income breeder may be important when considering the effects of nutritional state on parental cooperation. As argued above for our study species, there may be no interaction effects of the focal individual's nutritional state and that of its partner in capital breeders that acquire resources before breeding. In contrast, we might expect such an interaction effect in income breeders, where parents obtain food to provision to their offspring from the surrounding environment. Previous studies that investigated the effect of food availability on parental behaviour in such species, including stitchbirds (Low *et al.*, 2012) and Palestine sunbirds (Markman *et al.*, 2002), have shown differences in parental provisioning rates dependent on manipulation of food availability. One avenue for expanding research in such species is to use two-by-two factorial designs where food availability is manipulated for females and males and then measuring the subsequent effect on parental cooperation. We encourage future work exploring the effect of state on parental cooperation in both capital breeders and income breeders.

Finally, we found no negative effect of parental food-deprivation on offspring performance. Instead, average larval mass at dispersal was higher in broods cared for by food-deprived females and there was no difference in the proportion of larvae surviving to dispersal in response to parental nutritional state. This finding contrasts with that of Keppner *et al.* (2018) who found no difference in average larval mass dependent on parental nutritional state. Our finding was unexpected given that we predicted that food-deprived parents would provide less care and feed more on the

carcass, which is the sole food source for parents and larvae, leading to reduced larval performance upon dispersal. In this species, larval mass at dispersal is a strong predictor of adult size (Lock *et al.*, 2004), which is an important determinant of adult fitness (Otronen, 1988). As a result, parents are likely under selection to compensate for any initial reduction of care as a result of poor nutritional state by increased levels of care later in the breeding attempt or the larvae may compensate by increased rates of self-feeding later in the breeding attempt. Our results suggest overcompensation with food-deprived females producing better quality broods than well-fed females. Our experimental design may have facilitated this since we used relatively large mouse carcasses to ensure that there was ample food for both parents and larvae to feed from without much competition. Thus, there might have been a different outcome, similar to that reported by Keppner *et al.* (2018), had we used a small carcass such that there was more intense competition over the shared resource.

In conclusion, our study shows that females respond to both their own and their partner's nutritional state when deciding how much care to contribute and that these decisions are independent of those made based on the contribution of their partner. In contrast, males responded only to the contribution of their partner rather than the nutritional state of their partner. Our findings highlight the need for more work investigating how multiple mechanisms play a role in the resolution of sexual conflict over parental care and what may be driving sex differences in these mechanisms.

## 4 Intraspecific intruders and parental cooperation in a burying beetle

### Abstract

In species with biparental care, there is sexual conflict between parents over the relative contribution of the female and male to caring for their joint offspring. The social environment may affect how parents cooperate since it is likely to alter the costs and benefits associated with providing care. Here, we test whether parents adjust their contribution to care in response to the presence of intraspecific intruders in the burying beetle *Nicrophorus vespilloides*. We expected parents to provide more care in the presence of intruders to speed up the development of offspring. Additionally, we expected the relative contribution of males and females to depend on the sex of the intruder given the opportunity to remate. We used a two-by-two factorial design, in which a breeding pair was exposed to the presence or absence of a female intruder and the presence or absence of a male intruder. Despite responding aggressively towards intruders, we found that parents provided a similar level of care regardless of the presence of female or male intruders. One explanation for why parents did not adjust their relative contribution to care is that the presence of intruders during the current breeding attempt may have acted as a cue not just about the risk to the current brood but also about the likely risk of intruders during future breeding attempts. If so, parents would not benefit from shifting their allocation of resources to future reproduction. Overall, our results show that the presence of intruders does not alter biparental cooperation; nevertheless, we encourage future work on systems where intruder presence is more variable.



## 4.1 Introduction

In some species, females and males cooperate to provide biparental care for their joint offspring. Such cooperation leads to sexual conflict over the level of care each parent should contribute since both benefit from their combined effort, yet each parent pays a personal cost due to the time and energy that it allocates towards providing care. Therefore, each parent should attempt to shift as much as possible of the caring responsibility onto their partner (Parker, 1985; Lessells, 2012). In general, we expect females and males to adjust their relative contribution in response to factors that alter the costs and benefits associated with providing care, such as their partner's contribution (Harrison *et al.*, 2009; Lessells, 2012) and their own state or that of their partner (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015; Lambert & Smiseth, 2024). The social environment, here defined as the presence or absence of conspecifics within the immediate vicinity of the breeding pair, excluding their current brood, is one such factor that may shape the costs and benefits of care, and thereby the relative contribution towards care, of the two parents. For example, theoretical work predicts that male parents should decrease the level of care they provide in the presence of extra-pair males if their presence is associated with a loss in paternity to avoid investing time and effort into providing care for unrelated offspring (Westneat & Sherman, 1993; Kokko, 1999; Sheldon, 2002). Furthermore, males are expected to decrease the level of care they provide in response to the presence of additional females in their territory if, by doing so, they can increase their reproductive success by securing an additional mate (Magrath & Komdeur, 2003). Despite clear predictions about a shift in the costs and benefits associated with providing care dependent on these particular aspects of the social environment, there is a lack of

empirical studies or a theoretical framework outlining how the social environment in general might affect the relative contributions of females and males in a pair to caring for their joint offspring.

Here we investigate the impact of the presence and absence of female and male intraspecific intruders on the relative contribution to care by females and males within a breeding pair. We focus on the presence and absence of female and male intraspecific intruders since, as explained below, female and male parents are likely to differ in how they respond towards a female and a male intruder. We refer to intruders as individuals in the immediate vicinity that may threaten to kill and replace a current breeding pair's brood with their own brood. If an intruder is successful, both parents would suffer a cost associated with losing their current brood. Parents may decrease the level of care they provide in the presence of an intruder to avoid investing resources if it is early on in the breeding attempt to avoid wasting resources. Alternatively, in the presence of intruders, parents may respond by increasing the level of care they provide to speed up offspring development. However, the parent of the opposite sex to the intruder may have the opportunity to remate with the intruder, and thereby produce a secondary replacement brood, whereas the same-sex parent will likely be displaced or killed. As such, the parent that is the same sex as the intruder would face a higher cost of a takeover by the intruder than the parent of the opposite sex to the intruder, which in turn may cause the female and male parents to alter their relative contribution to caring for the joint brood.

We tested whether the presence of female and male intruders altered the relative contribution of females and males towards care in the burying beetle *Nicrophorus vespilloides*. Beetles in the genus *Nicrophorus* are well suited to test

this idea. Firstly, they provide biparental care and both females and males adjust the amount of care they provide in response to factors that alter the costs and benefits associated with providing care, such as their partner's contribution (Rauter & Moore, 2004; Smiseth & Moore, 2004; Smiseth *et al.*, 2005; Creighton *et al.*, 2015) and their own state and their partner's state (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015; Lambert & Smiseth, 2024). *Nicrophorus vespilloides* breeds on a small vertebrate carcass that acts as a food source and nest for parents and their offspring (Scott, 1998). Both parents contribute to caring for their offspring by preparing the carcass, protecting the offspring and carcass from predators and intraspecific intruders, provisioning pre-digested carrion to the offspring, and maintaining the carcass by spreading antimicrobials onto the surface (Eggert *et al.*, 1998; Scott, 1998). Secondly, intraspecific intruders regularly interact with breeding pairs since there is fierce intraspecific competition over carcasses (Scott, 1998). If successful, an intruder will kill or displace the resident of the same sex as itself and kill the resident's offspring so that the carcass can be used for their own brood. The intruder may mate with the parent of the opposite sex to produce a smaller replacement brood (Scott, 1990). Thus, if the carcass is taken over by an intruder, there is a cost to both parents since the current brood will be lost but, the cost is greater to the parent of the same sex as the intruder.

Previous work on burying beetles, *Nicrophorus* spp., has investigated how the sex of an intruder and the sex of the resident affect reproductive and defensive behaviour. Males are known to increase the duration of care and mating frequency with their partner since there is a last male precedent in sperm competition in *N. vespilloides* (Müller & Eggert, 1989; Hopwood *et al.*, 2015; House *et al.*, 2007). Work on *N. pustulatus* showed that male intruders were more likely to take over a carcass

than female intruders, while female intruders posed a greater threat to a resident female's reproductive success (Trumbo & Valletta, 2007). Additionally, resident females were more defensive towards male but not female intruders when accompanied by a male partner (Trumbo, 2007). In *N. vespilloides*, work on defensive behaviour showed that resident females engage in more fights against intruders and were more successful in defending their larvae than resident males (Georgiou-Shippi *et al.*, 2018). The only study to investigate the effect of the presence of intruders on the level of post-hatching care a pair provides focused on how females and males responded to a pair of intruders versus no intruders rather than how they responded to the presence or absence of male and female intruders (Ratz *et al.*, 2022). This study found that females provided more indirect care when in the presence of a pair of intruders whereas males provided the same level of care regardless of the presence of intruders (Ratz *et al.*, 2022). Despite clear sex differences in how parents respond to intruders and the importance of intruder sex in defensive behaviour, it is unclear how intruder sex influences the relative contribution of females and males towards providing post-hatching parental care.

We aimed to test whether resident female and male parents adjust the level of care they provide in response to the presence of an intraspecific intruder and whether any response was dependent on the sex of the intruder in relation to the parent, the presence of a second intruder, or the interaction between the two. We used a two-by-two factorial design in which a breeding pair was exposed to the presence or absence of a female and a male intruder. Thus, our design included the following four treatments: neither a female or male intruder, a female intruder only, a male intruder only, or both a female and male intruder. The presence of an intraspecific intruder is a threat to the current brood since they will likely attempt to

kill the larvae and take over the carcass. Consequently, we expected parents to respond by increasing the amount of care they provided to either speed up the development of the larvae or to use up the carcass quickly. If the intruder were to kill the current brood, the resident may have a chance to breed and produce a smaller replacement brood if the intruder is of the opposite sex to them, while this option would not be available to the resident if the intruder is of the same sex. As such, we expected resident parents to respond more strongly towards an intruder of the same sex as themselves in terms of increasing the amount of time they spent providing care than towards an intruder of the opposite sex. Alternatively, the presence of a same-sex competitor might increase uncertainty of parentage since a female intruder may lay eggs and act as a brood parasite (Müller *et al.*, 2007) and a male intruder may attempt to mate with the resident female thus leading to sperm competition (Eggert, 1992; Müller *et al.*, 2007). If so, parents may provide less care in the presence of a same-sex intruder to avoid investing time and resources into unrelated larvae. By testing whether females and males adjusted the amount of time they spent providing care in the presence of both a female and male intruder, we were able to isolate whether any response to a same-sex intruder also depended on the presence of an intruder of the opposite sex. It is well established that parents adjust the amount of care they provide in response to the amount of care their partner provides (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015; Lambert & Smiseth, 2024). As such, to determine whether any difference in a parent's contribution to care was dependent on intruder presence or partner contribution, we added the partner's contribution to each model to test whether it accounted for any observed effects of intruder presence on parental behaviour.

## 4.2 Methods

### 4.2.1 General methodology

We used beetles from an outbred laboratory population maintained at the University of Edinburgh. All beetles in the stock population were originally collected in Edinburgh, UK and the population was kept at 20 °C under a 16:8 h light:dark cycle. We housed non-breeding adults individually in clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil and fed them raw organic beef twice a week. All beetles used in the study were mature adults (i.e. at least 10 days post-eclosion).

### 4.2.2 Experimental design

We used a two-by-two factorial design in which a breeding pair was exposed to the absence or presence of a female and male intruder. Our experiment therefore included the following treatments: both female and male intruders absent (n = 28); female intruder present but male intruder absent (n = 24), female intruder absent but male intruder present (n = 26) or both female and male intruders present (n = 27). We weighed all individuals before pairing up females and males at random, taking care to avoid mating between close relatives. We randomly allocated each pair to a treatment group. We transferred each pair into a clear plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil. Each pair was provided with a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardised size (20 – 25 g;  $M \pm SE = 22.92 \pm 0.13$  g) to initiate breeding. We chose this carcass size since it was big enough to ensure parents had excess resources to avoid parents needing to feed on the intruders. After 48 h, when the eggs had been laid but before the larvae had begun hatching, we moved the female, the male and their carcass into a new container lined with fresh moist soil to ensure that we could allocate each pair a

foster brood of a standardised size. We allocated the pair a foster brood consisting of 20 newly hatched larvae from at least two different mothers. We chose this brood size since it is close to the average for *N. vespilloides* (21 larvae; Smiseth & Moore, 2002). We used a standardised brood size to control for potential confounding effects due to variation in brood size since brood size is known to affect the amount of care parents provide (Smiseth & Moore, 2002). We only allocated a foster brood to a pair after their eggs had started hatching since parents of this species use temporal kin recognition and kill larvae that arrive at the carcass before their own eggs have begun to hatch (Müller & Eggert, 1990).

Upon allocating a foster brood we also introduced any intruders dependent on treatment group. We chose to use dead intruders to control for confounding effects on the response of the resident female and male due to variation in harm inflicted by intruders that would occur if we used alive individuals. Live beetles could cause injury to the breeding pair, act as brood parasites and lay their own eggs, sneak copulations, or displace the pair and take over the carcass. Furthermore, it has previously been shown that *N. vespilloides* respond viciously to dead intruders or body parts of dead intruders which suggests that they perceive them as a threat and respond similarly to how they would respond to a live intruder (Steiger *et al.*, 2009; Steiger & Müller, 2010; Paquet *et al.*, 2017; Ratz *et al.*, 2022). We acknowledge that in previous studies parents only responded to dead individuals as a threat for a short period of time (Steiger *et al.*, 2009; Steiger & Müller, 2010); however, given our aim of was to simulate the presence of an intruder or a pair of intruders in the environment rather than to present a constant threat or cause physical damage to the host parents, dead intruders were a suitable cue. To kill the intruders, we froze them for 45 min before removing them from the freezer and allowing them to defrost

for 15 min. Intruder(s) were then placed next to the mouse carcass at the same time as the foster brood was introduced.

We conducted behavioural observations 24 h ( $\pm$  15 min) after we allocated pairs a foster brood and introduced any intruders, which is when parents of this species provide the highest level of care (Smiseth *et al.*, 2003). We chose this timeframe since we aimed to measure any strategic response by females and males to the presence of intruders that may involve adjusting the level of care they provide. The 24 h delay allowed us to distinguish such strategic adjustments from an immediate panic response to discovering an intruder near the carcass which could be observed directly after the introduction of the intruders. We did the observations under red light using instantaneous sampling of female and male behaviour every minute for 30 min consistent with established protocols (Smiseth & Moore, 2002). We recorded the number of sampling points out of 30 during which females and males spent providing direct care, providing indirect care, being absent from the carcass and consuming carrion. We used number of scans as a proxy for the amount of time and individual spent providing care, absent from the carcass or consuming carrion. Direct care included provisioning food to larvae and grooming the larvae. Indirect care included maintaining the carcass and guarding the brood against intruders. After the observations, we left the pairs to care for their brood until the larvae dispersed from the carcass approximately five days later, upon which we recorded average larval mass and the number of larvae that survived to dispersal as measures of offspring performance, and female and male post-breeding mass which allowed us to calculate individual mass change during breeding.

To test how parents responded to the intruders we recorded whether or not there was any evidence of intruder damage at the time of the behavioural



observation and when the larvae had dispersed from the carcass (Ratz *et al.*, 2022). A score of 0 indicated that the intruder was completely undamaged and a score of 1 indicated any evidence of damage to the intruder.

#### 4.2.3 Statistical Analysis

All statistical analyses were conducted using R version 4.2.2 (R Core Team, 2022) with the packages *car* (Fox & Weisberg, 2019), *MASS* (Venables & Ripley, 2022), and *glmmTMB* (Brooks *et al.*, 2017). First, we analysed whether or not there was any evidence of damage to the intruders inflicted by the breeding pair as a factor with two levels (0 or 1) at the time of the observation and when the larvae had dispersed from the carcass using a binomial model including the sex of the intruder, whether or not a second intruder was present and a random effect of the breeding pair. We used a zero-inflated binomial model in our analyses on the amount of time spent absent from the carcass by males, the amount of time spent consuming carrion by males and females and the amount of time spent providing direct care by females and males since the data for these behaviours showed minor zero inflation. For the models used to analyse female and male consuming carrion, female direct care, and male absence from the carcass we included observation level as a random effect to account for over-dispersion (Harrison, 2015). We used binomial models in our analysis on time spent providing indirect care by females and males, time spent absent from the carcass by females and the number of larvae that survived to dispersal. In all these models, we included observation level as a random effect to account for over-dispersion. We used linear models for data on female and male mass change during breeding, and average larval mass at dispersal. All models included the presence or absence of a female intruder and the presence or absence of a male intruder as fixed effects with two levels (present or absent).

To determine whether a parent responded directly to intruder presence or indirectly to its partner's contribution, we compared models where we included and excluded the amount of time the partner spent providing direct care, indirect care, absent from the carcass or consuming carrion as a factor (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015). If including this factor reduced or removed any effect of intruder presence on the amount of time spent providing direct care, providing indirect care, absent from the carcass or consuming carrion, we interpreted this as evidence that the focal parent responded indirectly to the contribution of its partner. However, if including this factor did not negate the effect of intruder presence on the amount of time the focal parent spent providing direct care, providing indirect care, being absent from the carcass or consuming carrion, we interpreted this as evidence that the focal parent responded directly to intruder presence. We note that our data on the responses of parents to their partner's contribution are correlational and that we therefore cannot demonstrate a causal relationship between the contributions of females and males to parental care.

## 4.3 Results

### 4.3.1 Damage to intruders

We first tested for damage to intruders at the end of the observation to confirm that the resident parents reacted to any intruder present at the time when we measured parental care. Of the 105 intruders used in the experiment, only 9 were damaged at the time of observation, indicating a low level of aggression from residents towards the intruders at this stage. The level of damage at observation was the same for both female and male intruders (estimate =  $-1.12 \pm 0.81$  g,  $z = -1.39$ ,  $P$

= 0.166) and damage was consistent regardless of the additional presence of an intruder of the opposite sex (estimate =  $-0.22 \pm 0.85$  g,  $z = -0.26$ ,  $P = 0.794$ ).

Next we tested for damage to intruders at the time of larval dispersal from the carcass as an indication of the overall damage done to any intruder throughout the breeding attempt as a whole. Upon dispersal, of the 105 intruders used in the experiment, 88 were damaged indicating a higher level of aggression from the residents towards the intruders by this stage. The level of damage at dispersal was the same for both female and male intruders (estimate =  $-1.11 \pm 0.82$  g,  $z = -1.37$ ,  $P = 0.171$ ) and damage was consistent regardless of the additional presence of an intruder of the opposite sex (estimate =  $-1.53 \pm 0.86$  g,  $z = -1.78$ ,  $P = 0.075$ ). Note that this could alternatively be a result of residents feeding on the intruders; however, we suggest this is less likely given the pairs were provided with a larger carcass so that there would be surplus food available.

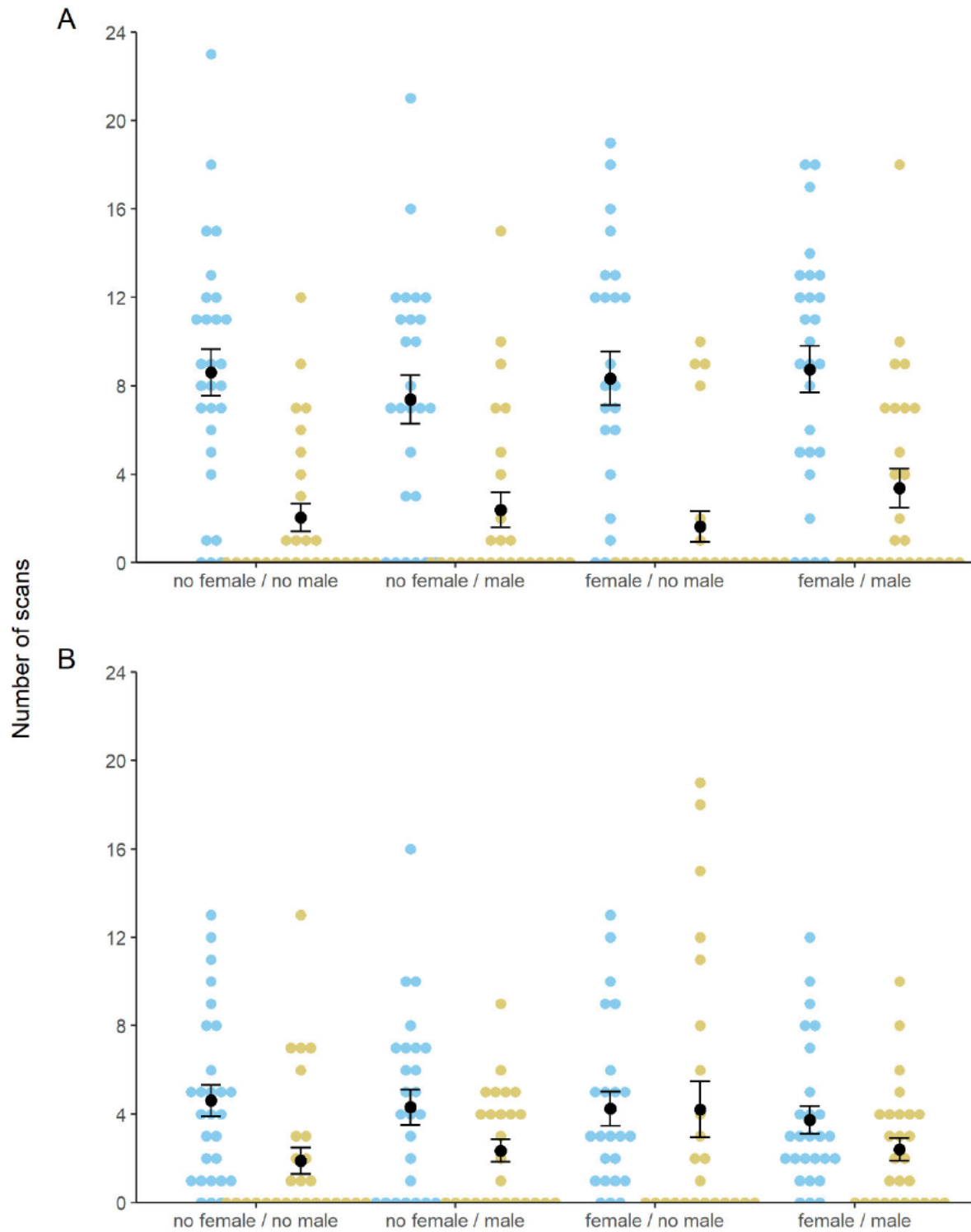
#### 4.3.2 Amount of parental care by female and male residents

There was no evidence that the presence of a female intruder, a male intruder or the interaction between the two affected the amount of time resident females spent providing direct care, providing indirect care or being absent from the carcass (Table 4.1, Figure 4.1). There was no difference in the amount of time resident males spent providing direct care, providing indirect care or being absent from the carcass dependent on the presence of a male intruder, a female intruder or the interaction between the two (Table 4.1, Figure 4.1). Thus, there was no support for our prediction that residents would change the amount of care they provided in response to the presence of intruders and that this response would be dependent on the sex of the intruder in relation to the parent and whether or not a second intruder was present or absent.

Males responded to female contribution, spending less time providing direct care as the amount of time females spent providing direct care increased (Table 4.1). Males also responded to the amount of time females spent absent from the carcass, spending less time at the carcass as females spent more time at the carcass (Table 4.1). There was no effect of the partner's contribution on the amount of time spent providing direct care by females, the amount of time spent absent from the carcass by females or the amount of time spent providing indirect care by females and males (Table 4.1). We note that we measured parental behaviour in a single 30-minute observation and so we may not have detected a change in the distribution of care across the breeding attempt as a whole.

**Table 4.1** - Summary of statistical tests for the effects of the presence of female and male intruders on biparental cooperation over providing direct and indirect care and absence from the carcass excluding and including (\*) partner's contribution in the model. The reference category for intruder presence was 'absent'. Statistically significant P values (< 0.05) are shown in bold.

Behaviour	Female intruder				Male intruder				Interaction				Partner's contribution			
	Est	SE	z	P	Est	SE	z	P	Est	SE	z	P	Est	SE	z	P
Female direct	0.05	0.23	0.23	0.819	0.01	0.23	0.03	0.975	0.05	0.32	0.152	0.879				
Female direct*	0.04	0.22	0.19	0.850	-0.01	0.23	-0.06	0.955	0.14	0.33	0.42	0.672	-0.04	0.02	-1.46	0.144
Female indirect	-0.12	0.33	-0.37	0.713	-0.16	0.32	-0.50	0.616	0.04	0.46	0.06	0.932				
Female indirect*	-0.06	0.33	-0.17	0.865	-0.15	0.32	-0.48	0.632	-0.01	0.46	-0.02	0.981	-0.03	0.03	-1.07	0.285
Female absence	0.29	2.40	0.23	0.901	0.47	2.26	0.21	0.839	-0.59	3.27	-0.18	0.856				
Female absence*	0.16	2.44	0.07	0.947	0.54	2.36	0.23	0.819	-0.94	3.37	-0.28	0.780	-0.08	0.07	-1.07	0.284
Male direct	0.39	0.23	1.67	0.095	0.21	0.20	1.03	0.301	-0.21	0.30	-0.70	0.482				
Male direct*	0.40	0.24	1.70	0.089	0.14	0.21	0.69	0.491	-0.10	0.30	-0.33	0.744	-0.03	0.01	-2.31	<b>0.021</b>
Male indirect	0.92	0.63	1.47	0.141	0.63	0.62	1.02	0.307	-0.80	0.87	-0.92	0.357				
Male indirect*	0.91	0.63	1.45	0.147	0.62	0.62	0.01	0.314	-0.79	0.87	-0.91	0.362	-0.01	0.06	-0.22	0.830
Male absence	0.01	1.95	0.00	0.997	0.28	1.85	0.15	0.878	-3.89	3.50	-1.11	0.267				
Male absence*	-0.09	1.90	-0.05	0.964	0.63	1.72	0.37	0.714	-1.42	2.85	-0.50	0.618	-0.60	0.09	-6.30	<b>&lt; 0.001</b>



**Figure 4.1** - Comparison of the number of scans (out of 30) spent providing direct care (A) and indirect care (B) by females (blue points) and males (yellow points) caring for larvae during a 30 min behavioural observation (mean  $\pm$  SE) dependent on the presence of intruders.

#### 4.3.3 Female and male mass change during breeding & time spent consuming carrion

Resident females lost mass during breeding in the presence of a female intruder despite no effect of the presence or absence of a female intruder on the amount of time resident females spent consuming carrion (Table 4.2, Figure 4.2). Resident females spent more time consuming carrion in the presence of a male intruder. However, there was no difference in female mass change during the breeding attempt in the presence or absence of a male intruder (Table 4.2, Figure 4.2). There was also no difference in female mass change or time spent consuming carrion dependent on an interaction effect of the presence or absence of a female and male intruder (Table 4.2, Figure 4.2). Thus, resident females responded to the presence of a male intruder and the presence of a female intruder, but neither response was conditional upon the presence of a second intruder.

Resident males spent less time consuming carrion in the presence of a male intruder; however, there was no difference in male mass change during breeding dependent on the presence or absence of a male intruder (Table 4.2, Figure 4.2). There was no difference in resident male mass change or time spent consuming carrion in the presence of a female intruder (Table 4.2, Figure 4.2). There was also no difference in resident male mass change or time spent consuming carrion dependent on an interaction effect of the presence or absence of a male and female intruder (Table 4.2, Figure 4.2). Thus, resident males responded to the presence of a male intruder and the response was not conditional upon the presence of a female intruder.

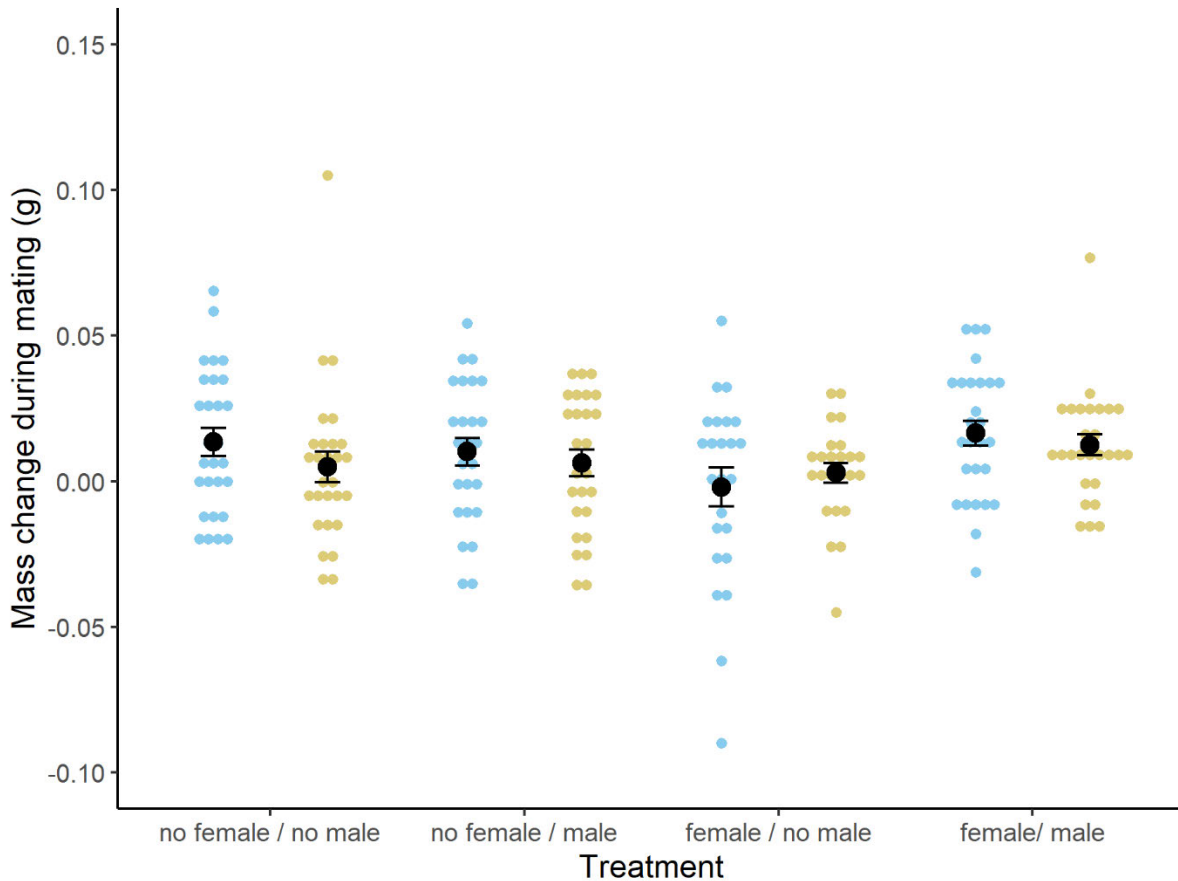
The amount of time females spent consuming carrion was a predictor of the amount of time males spent consuming carrion. Males spent less time consuming

carrion as the amount of time females spent consuming carrion increased (Table 4.2). Including or excluding the partner's behaviour did not alter the effect of the presence of a male intruder on the amount of time males spent consuming carrion (Table 4.2). This suggests that males responded independently to the presence of an intruder and female behaviour. There was no effect of the amount of time males spent consuming carrion on the amount of time females spent consuming carrion (Table 4.2).



**Table 4.2** - Summary of statistical tests for the effects of the presence of female and male intruders on biparental cooperation over consumption excluding and including (\*) partner's behaviour in the model and mass change during breeding. The reference category for intruder presence was 'absent'. Statistically significant P values (< 0.05) are shown in bold.

Behavior	Female intruder				Male intruder				Interaction				Partner's behaviour			
	Est	SE	Test statistic	<i>P</i>	Est	SE	Test statistic	<i>P</i>	Est	SE	Test statistic	<i>P</i>	Est	SE	<i>z</i>	<i>P</i>
Female consumption	0.10	0.23	<i>z</i> = 0.42	0.673	0.60	0.22	<i>z</i> = 2.64	<b>0.008</b>	-0.53	0.32	<i>z</i> = -1.65	0.098				
Female consumption*	0.03	0.23	<i>z</i> = 0.15	0.883	0.61	0.22	<i>z</i> = 2.74	<b>0.006</b>	-0.50	0.32	<i>z</i> = -1.56	0.119	0.03	0.02	1.48	0.139
Female mass change	-0.02	0.01	<i>t</i> = -2.10	<b>0.038</b>	-0.00	0.01	<i>t</i> = -0.54	0.589	0.02	0.01	<i>t</i> = -1.93	0.056				
Male consumption	-0.51	0.42	<i>z</i> = -1.20	0.229	-0.76	0.38	<i>z</i> = -2.01	<b>0.044</b>	0.84	0.55	<i>z</i> = 1.53	0.127				
Male consumption*	-0.45	0.40	<i>z</i> = -1.11	0.267	-0.81	0.36	<i>z</i> = 2.25	<b>0.025</b>	0.85	0.52	<i>z</i> = 1.62	0.105	-0.08	0.04	-2.10	<b>0.035</b>
Male mass change	0.00	0.01	<i>t</i> = 0.23	0.819	0.00	0.01	<i>t</i> = 0.34	0.734	0.00	0.01	<i>t</i> = 0.40	0.693				



**Figure 4.2** - Comparison of the mass change during breeding by females (blue points) and males (yellow points) dependent on the presence of intruders (mean  $\pm$  SE).

#### 4.3.4 Offspring performance

There was no difference in the number of larvae that survived to dispersal dependent on the presence of a female intruder (estimate =  $0.119 \pm 0.238$  g,  $z = 0.50$ ,  $P = 0.617$ ), a male intruder (estimate =  $0.044 \pm 0.232$  g,  $z = 0.19$ ,  $P = 0.849$ ), or the interaction between the two (estimate =  $-0.246 \pm 0.333$  g,  $z = -0.74$ ,  $P = 0.461$ ). There was also no difference in average larval mass at dispersal dependent on the presence of a female intruder (estimate =  $-0.006 \pm 0.006$  g,  $t = -0.05$ ,  $P = 0.298$ ), a male intruder (estimate =  $0.001 \pm 0.006$  g,  $t = 0.20$ ,  $P = 0.843$ ), or the interaction between the two (estimate =  $0.007 \pm 0.008$  g,  $t = 0.85$ ,  $P = 0.397$ ).

## 4.4 Discussion

We found no evidence that the presence or absence of a female intruder affected the amount of care provided by either females or males and, likewise, no evidence that the presence or absence of a male intruder affected the amount of care provided by females or males. As such, there was no evidence that a female resident increased its contribution to care more strongly in response to the presence of female intruders and no evidence that a male resident increased its contribution to care more strongly in response to the presence of male intruders. There are several potential explanations for why female and male parents did not adjust their contribution to care in response to the presence of female or male intruders. Firstly, this could be due to the treatment used in our experiment not having the intended effect. We introduced dead beetles to simulate the presence of female and male intruders, and one explanation is that the breeding pair may not have perceived the dead beetles as intruders and a potential threat to their brood. This explanation seems unlikely given that prior studies on *N. vespilloides* have established that resident female and male parents respond aggressively towards dead intruders near a carcass in a manner that is comparable to aggression directed towards a live intruder (Steiger *et al.*, 2009; Steiger & Müller, 2010; Paquet *et al.*, 2017; Ratz *et al.*, 2022). While the time scale of exposure was much shorter in (Steiger *et al.*, 2009; Steiger & Müller, 2010), the aim of introducing dead intruders in our study was to suggest the presence of intruders in the environment rather than to simulate continuous exposure so even an initial reaction by the host parents would achieve that aim. Furthermore, we found that over 80% of the intruders introduced during our experiment showed evidence of severe damage inflicted by the resident parents by

the time the larvae had dispersed from the carcass. This finding suggests that parents did perceive and respond to the dead intruders as a potential threat. Secondly, the lack of response could be due to the timing of when we introduced the intruders. We added intruders 24 h ahead of the time at which we did the behavioural observations, which means that the resident parents may have effectively neutralised the threat by the time of the parental care observations. This explanation also seems unlikely given that most of the damage to the intruders was inflicted in the interval between when we had conducted the observation and when the larvae dispersed from the carcass. This could have been a result of residents feeding on the intruders as the carcass depleted; however, given pairs were supplied with a large carcass to ensure resource availability, this seems unlikely. Instead, this may suggest the parents still perceived the intruders as a threat at the time of the behavioural observation. Furthermore, a previous study that introduced intruders 24 h before doing behavioural observations reported a response to the presence of intruders during their observations (Ratz *et al.*, 2022). As such, we have confidence that our experimental design had the intended effect and in our finding that *N. vespilloides* females and males provide the same level of care regardless of the presence of intraspecific intruders.

We expected resident parents to respond to the presence of intruders by increasing the amount of care they provided to either speed up the development of the larvae or to use up the carcass quickly. One potential explanation for the lack of difference in the level of post-hatching care provided by females and males is that increasing care does not affect the development time of the larvae or the attractiveness of the carcass. This is unlikely since previous studies have shown that post-hatching care leads to increased larval growth (Eggert *et al.*, 1998a).

Furthermore, in the closely related *Nicrophorus orbicollis*, takeover rate by intruders rapidly decreased 3 days after carcass burial, potentially as a result of resource depletion (Robertson, 1993). An alternative explanation is that the presence of intruders during the current breeding attempt also is a cue about a greater potential risk of intruders being present during future breeding attempts. Our prediction is based on the implicit assumption that parents benefit by shifting their allocation of resources towards caring for the current brood at the expense of future broods. This assumption is likely to be met if the presence of intruders is a cue about an immediate threat to the brood and that an increase in parental care has a greater impact on the current brood than on future broods. If the threat of intruders was consistent across breeding attempts, parents would not benefit from shifting their allocation of resources to future reproduction since any increased costs or benefits to providing care associated with intruder presence would be similar. Consequently, females and males would be expected to maintain the level of care they provide during the current breeding attempt regardless of the presence of intruders as we observe here. A similar argument is often suggested as an explanation for why males are not necessarily expected to reduce the level of parental care they provide in response to increased uncertainty of parentage (Maynard Smith, 1978; Westneat & Sherman, 1993). If paternity is consistently low across all breeding attempts, there would be no benefit to males from shifting the allocation of resources from the current breeding attempt to future breeding attempts, and uncertainty of paternity would not affect the level of male paternal care. As such, when evaluating the effect of social environment on biparental care it is important to consider that cues about the costs and benefits of care in a current breeding attempt may also act as cues about the expected costs and benefits of care in future breeding attempts.

Although there is theory for how the presence of additional males may impact the costs and benefits of paternal care by increasing the risk of sperm competition, there is no general theoretical framework outlining how the social environment might affect the relative contributions of females and males towards care for their joint offspring. We expect females and males to adjust their relative contribution to care in response to the social environment only if it affects the costs and benefits associated with providing care differently for females and males. When the social environment causes either the costs or benefits to increase equally for the female and male, we would expect their relative contribution to remain the same while the overall level of care increases or decreases accordingly. For example, if the presence of a brood parasite increases parental uncertainty equally for the female and the male, we would expect them both to reduce the level of care they provide but for the proportion of care each contributes to remain constant. In contrast, when the social environment affects the costs or benefits associated with providing care differently for females and males, we would expect their relative contributions to change. This has begun to be explored in terms of the response of females and males to paternal uncertainty and extra-pair fertilisations (Westneat & Sherman, 1993; Kokko, 1999; Sheldon, 2002; Suter *et al.*, 2009); however, similar principles have yet to be expanded to other aspects of social environment. Our results also highlight that for the social environment to influence the relative contribution of females and males, there must be an opportunity for future breeding attempts where the social environment will be different. In sum, we would expect a change in social environment to affect the relative contribution of parents when the costs and benefits associated with providing care are affected differently for females and males and when there is likely to be variation in the social environment during subsequent

breeding attempts. We encourage future work investigating this relationship further to form a general theoretical framework for the effect of social environment on parental care and cooperation.

Our finding that females and males provide similar levels of care in the presence and absence of intraspecific intruders contrasts with previous studies showing that parents alter the level of care they provide in response to intruders (Georgiou-Shippi *et al.*, 2018; Ratz *et al.*, 2022). Ratz *et al.* (2022) found that females spent more time on indirect care (i.e., maintaining the carcass) in the presence of a pair of intruders than when no intruders were present whereas we saw no difference between these groups. Given that our study included some of the same experimental treatments as those used in Ratz *et al.* (2022), we compared effect sizes and confidence intervals between our study and Ratz *et al.* (2022). Ratz *et al.* (2022) reported an effect size of 1.31 scans on indirect care with a 95% confidence interval of 0.177 - 2.443 scans which overlaps with the 95% confidence interval of our study (- 0.381 - 0.861 scans). Thus, there was no evidence of a statistical difference between the results of Ratz *et al.* (2022) and our results, which suggests that further work is needed to determine whether females spend more time on indirect care in the presence of a pair of intruders in comparison to when no intruders are present or not. Meanwhile, Georgiou-Shippi *et al.* (2018) found that female residents successfully defended their brood and engaged in more fights against intruders than male residents and that residents, regardless of their sex, spent more time provisioning food to larvae in the presence of a female intruder than in the presence of a male intruder. This study used an experimental design based on single parents only and therefore did not include the same treatment groups as those used in our study. Nevertheless, the contrast between the results of Georgiou-Shippi

*et al.* (2018) and our results suggests that females and males may alter the level of care they provide in response to intruders in the uniparental care setting of Georgiou-Shippi *et al.* (2018) but not in the biparental care setting of our study. A potential reason for this is that a pair is more likely to be able to successfully defend a brood against an intruder than a single individual breeding on their own. Indeed, previous work has shown that, in *Nicrophorus* spp., parents are better at dealing with competition in pairs than individually (Trumbo, 1994; Suzuki, 2013) and so the threat of an intruder may not be as prominent if the carcass is occupied by a pair. Thus, future work may compare the responses of male and female parents in uniparental and biparental care settings.

We found that females and males altered their carrion consumption in response to the presence of intruders. Females spent more time consuming carrion in the presence than in the absence of a male intruder. In contrast, males spent less time consuming carrion in the presence than in the absence of a male intruder. Given that carrion consumption is thought to indicate investment in somatic maintenance, these results may appear to suggest that females invest more in future reproduction in the presence of a male intruder while males invest less in future reproduction in the presence of a potential competitor. However, we urge caution in accepting this explanation since the presence or absence of a male or female intruder had no effects on female or male mass change across the breeding attempt as a whole or on offspring performance. The discrepancy between the findings for the amount of time females and males spent consuming carrion and female and male mass change across the breeding attempt as a whole might suggest that parents responded to the presence of an intruder by shifting the timing of when they fed for themselves rather than adjusting the amount of carrion they ate in total



throughout the breeding attempt. They may do so to gain a competitive advantage over the intruders by improving their own condition early in the breeding attempt. To test this, it would be interesting to measure changes in parental behaviour at multiple time points across the breeding attempt in response to the presence of intraspecific intruders.

We found evidence that both females and males responded to the behaviour of their partner. Males spent less time providing direct care as the amount of time females spent providing direct care increased. They also spent more time absent from the carcass as females spent less time absent from the carcass. These findings are in line with negotiation models for biparental cooperation that predict a parent will respond to their partner decreasing the level of care they provide by increasing the level of care they provide but only to incompletely compensate for the loss in care (McNamara *et al.*, 1999; Houston *et al.*, 2005). Our results are also consistent with previous studies on *N. vespilloides* showing that each parent adjusts the amount of care it provides in response to the contribution of their partner (Smiseth & Moore, 2004; Smiseth *et al.*, 2005; Matthey & Smiseth, 2015; Pilakouta *et al.*, 2015; Lambert & Smiseth, 2024).

In conclusion, the presence or absence of intraspecific intruders did not affect the relative contribution of females and males towards providing care for their joint offspring. This is likely because the presence of intruders during the current breeding attempt acts as a cue that intruders are also likely to be present during future breeding attempts so the costs and benefits associated with providing care remain constant. Our findings highlight the need for more empirical work investigating the effect of a variety of aspects of social environment on the relative contribution of parents to providing care to isolate whether females and males alter their

contribution dependent on sex-specific costs and benefits to inform a general theoretical framework.

# 5 Synchrony in onset of parental care affects post-hatching cooperation and offspring performance in a burying beetle

## Abstract

When cooperating to provide biparental care, each parent must balance its relative contribution to care with that of its partner. Prior work shows that each parent adjusts its contribution to care based on partner contribution, state and environmental conditions. Here, we investigate the effect of synchrony in the onset of parental care. Such synchrony is likely to alter the parents' relative contributions to pre-hatching care and, consequently, impact their relative contributions to post-hatching care. To test this idea, we manipulated synchrony in the onset of care in the burying beetle *Nicrophorus vespilloides* using a two-by-two factorial design with the following treatments: both parents arrived synchronously at a fresh carcass, the parents arrived asynchronously such that the female arrived at a fresh carcass and the male arrived 24 hours later, the parents arrived asynchronously such that the male arrived at a fresh carcass and the female arrived 24 hours later, or both parents arrived synchronously at a 24 hour old carcass. We found that males provided more indirect care when the parents arrived asynchronously at the carcass in comparison to when they arrived synchronously. Despite this, larval survival to dispersal was lower in broods cared for by parents that arrived asynchronously. Our results show that asynchrony in the onset of parental care alters the parents' relative contribution to post-hatching care, possibly by disrupting synchrony in the reproductive

physiology of the female and the male. We encourage future work linking synchrony in reproductive behaviour with synchrony in reproductive physiology.

## 5.1 Introduction

Biparental care refers to two parents working together to care for their shared offspring, spanning behaviours from nest building to food provisioning (Clutton-Brock, 1991; Royle *et al.*, 2012). This is a source of conflict since the cost of care relates to each parent's personal effort whereas the benefit of care depends on the combined efforts of both parents (Houston *et al.*, 2005; Lessells, 2012). As a result, caring parents are under selective pressure to shift as much of the burden of care onto their partner as possible (Parker, 1985). Biparental cooperation usually involves some degree of coordination whereby each parent adjusts its own contribution relative to the contribution of its partner (Harrison *et al.*, 2009; Lessells, 2012). This may involve negotiation, where a parent incompletely compensates for a decrease in its partner's contribution (McNamara *et al.*, 1999) or matching, where a parent mirrors any increase or decrease in the contribution of its partner (Johnstone & Hinde, 2006). In addition, each parent may adjust its contribution based on assessments of its own state and that of its partner, for example by responding to body size (Pilakouta *et al.*, 2015), inbreeding status (Mattey & Smiseth, 2015) or nutritional status (Lambert & Smiseth, 2024). Finally, each parent may also respond to environmental conditions such as fluctuations in climate (Vincze *et al.*, 2017), resource availability (Barbasch *et al.*, 2021) and the presence of predators (Fontaine & Martin, 2006) and adjust its investment in parental care accordingly.

Although we have a good understanding of how partner contribution, parental state and environmental conditions influence each parent's contribution to care, it is less understood how synchrony in the onset of parental care may influence biparental cooperation. Synchrony in the onset of care may matter when parents must allocate their time and energy budget between forms of care provided at different stages of the breeding attempt. For example, parents may provide pre-hatching care including nest building or any other forms of preparation for breeding, and post-hatching care in the form of food-provisioning or defending the offspring from predators. Thus, each parent must adjust its own contribution to care based on its partner's current contribution as well as based on its own contribution and its partner's contribution to care at earlier stages of the breeding attempt. Prior experiments investigating how parents respond to the contribution of their partner tend to focus on post-hatching care only, manipulating the contribution of the partner via handicapping or mate removal during the post-hatching stage of breeding (Harrison *et al.*, 2009). Such designs negate the opportunity to investigate whether parents also adjust the level of post-hatching care they provide in response to their own and their partner's contribution to care during the pre-hatching stage. Parents may alter their contribution to post-hatching care in response to their own and their partner's contribution to pre-hatching care because pre-hatching care can be energetically costly and time consuming. Consequently, if an individual spends more time providing pre-hatching care than their partner, they may be less able to contribute to post-hatching care. In response, their partner may provide more post-hatching care to compensate for any reduction. As such, it seems likely that an individual's contribution to the early stages of breeding may affect how parents cooperate to provide care during subsequent stages.

The burying beetle *Nicrophorus vespilloides* is well suited to test whether females and males adjust their contribution to post-hatching care in response to their own and their partner's contribution to pre-hatching care. Firstly, parents cooperate to provide biparental care across multiple stages of a breeding attempt. They breed on small vertebrate carcasses which they use as a resource for their offspring. On arrival at a carcass, both the female and the male contribute to pre-hatching care by preparing the carcass, including stripping it of fur or feathers, coating it in antimicrobials, and burying it to avoid detection (Eggert *et al.*, 1998b; Scott, 1998). Parents continue to cooperate to provide post-hatching care, including food provisioning to the larvae, guarding the larvae, and maintaining the condition of the carcass, until their larvae disperse (Eggert *et al.*, 1998b; Scott, 1998). Secondly, both females and males adjust the amount of care they provide in response to their partner's contribution (Smiseth & Moore, 2004; Smiseth *et al.*, 2005; Matthey & Smiseth, 2015; Pilakouta *et al.*, 2015; Lambert & Smiseth, 2024). Thirdly, it is relatively easy to manipulate an individual's contribution to pre-hatching care by altering arrival time at the carcass. Burying beetles are highly specialised in detecting carcasses through chemosensors in their antenna, and they are often among the first species to appear after an animal's death (Scott, 1998). Previous work has shown that, in *Nicrophorus ssp.*, parents are better at defending a carcass in a pair rather than individually (Trumbo, 1994; Suzuki, 2013) so it would be beneficial for a female and male to arrive synchronously. Despite this, it is unlikely that parents arrive at the carcass at exactly the same time. Since carcasses are rare and valuable, the parent who arrives first will start preparing the carcass regardless of the presence of a partner because pre-hatching care not only prepares the

carcass for a brood, it also conceals the carcass from other scavengers and prevents microbial competition.

Here, we tested whether females and males adjust their relative contribution to post-hatching care depending on synchrony in the onset of parental care by manipulating parental arrival time at the carcass. We used a two-by-two factorial design in which each parent arrived at either a fresh carcass or 24 h later. Thus, the four treatments were as follows: the parents arrived synchronously such that both the female and the male arrived at a fresh carcass, the parents arrived asynchronously such that the female arrived at a fresh carcass while the male arrived 24 h later, the parents arrived asynchronously such that the male arrived at a fresh carcass while the female arrived 24 h later, and the parents arrived synchronously such that both the female and male arrived at a 24 h old carcass. We predicted that asynchrony in arrival would impact the relative contribution to post-hatching care. The rationale for this is that the parent that arrived first would perform more pre-hatching care, and, as a result, would provide less post-hatching care, while the partner that arrived late would perform less pre-hatching care but that it, in line with negotiation models, would provide more post-hatching care to incompletely compensate for its partner's reduced contribution to post-hatching care. For example, when the female arrived at a fresh carcass while the male arrived 24 h later, we predicted the female to provide less, and the male to provide more, post-hatching care in comparison to when both arrived at the carcass synchronously. Conversely, when the male arrived at a fresh carcass while the female arrived 24 h later, we predicted the male to provide less, and the female to provide more, post-hatching care. Given previous studies show difference in the sensitivity to cues between females and males, we did not expect exactly the same levels of

compensation (Georgiou-Shippi *et al.*, 2018; Keppner *et al.*, 2018; Lambert & Smiseth, 2024). We included treatments where both parents arrived synchronously at a fresh carcass and a 24 h old carcass because this would allow us to separate between effects due to synchrony in the onset of parental care and effects due to the state of the carcass. We also tested for effects of parental arrival time on offspring performance; that is, the number of larvae surviving to dispersal and average larval mass. We expected offspring to perform worse when parents arrived asynchronously than when parents arrived synchronously since we expected lower overall levels of care when parents arrived asynchronously due to incomplete compensation.

## 5.2 Methods

### 5.2.1 General methodology

We used beetles from an outbred laboratory population originally collected in Edinburgh, UK, and maintained at the University of Edinburgh. We housed all adults in the stock population individually in clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil and fed them raw organic beef twice a week. The stock population was kept at 20 °C under a 16:8 h light:dark cycle. All individuals used in the experiment were at least 10 days post-eclosion to ensure they had reached sexual maturity.

### 5.2.2 Experimental design

We used a two-by-two factorial design with the following treatments: the parents arrived synchronously such that both the female and the male arrived at a fresh carcass (n = 28), the parents arrived asynchronously such that the female arrived at a fresh carcass and the male arrived 24 h later (n = 28), the parents



arrived asynchronously such that the male arrived at a fresh carcass and the female arrived 24 h later ( $n = 28$ ) or the parents arrived synchronously such that both the female and the male arrived at a 24 h old carcass ( $n = 28$ ). We chose a 24 h interval to induce asynchrony in the time of arrival since this interval was likely long enough for an early parent to substantially begin pre-hatching care. We also wanted to avoid the risk of females starting to lay unfertilised eggs in treatments where females arrived before males, which they might do approximately 48 h after finding a fresh carcass. We did this since laying unfertilised eggs may be energetically costly and thereby impact post-hatching care or offspring performance.

We weighed all individuals before assigning them to one of the four treatments in our experiment. Immediately after weighing, we paired up males and females at random, taking care to avoid mating between close relatives. We transferred individuals that were assigned to arrive at a fresh carcass into a clear plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil containing a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (20 - 25 g;  $M \pm SE = 22.71 \pm 0.13$  g). Individuals assigned to arrive later were then placed into the box 24 h after the mouse carcass had been left in the box at ambient room temperature. After the eggs had been laid (approximately 48 h after both the female and male were in the container with the mouse carcass together), but before the larvae had begun hatching, we moved the female, the male and their carcass into a new clear plastic container (17 cm × 12 cm × 6 cm) lined with fresh moist soil. We allocated each pair a foster brood consisting of 20 newly hatched larvae derived from eggs laid by at least two different females. We chose this brood size since it is close to the average brood size for *N. vespilloides* (21 larvae; Smiseth & Moore 2002). We used a standardized brood size to control for potential confounding effects due to

variation in brood size. Such confounding effects might arise because our treatment might affect the number of eggs laid and because brood size is known to affect the amount of care provided by parents (Smiseth & Moore, 2002). We allocated a foster brood to a pair only after their eggs had started hatching since parents use temporal kin recognition and so would kill larvae that arrive at the carcass before their own eggs begin to hatch (Müller & Eggert, 1990).

We conducted behavioural observations 24 h ( $\pm$  15 min) after we had allocated the foster brood since this coincides with the time at which parents provide the highest level of post-hatching care in this species (Smiseth *et al.*, 2003). We did the observations using instantaneous sampling of female and male behaviour every 60 s for 30 min, consistent with established protocols (Smiseth & Moore, 2002). We recorded the number of sampling points during which females and males spent providing direct post-hatching care (direct care) and indirect post-hatching care (indirect care). Direct care was defined as parents provisioning food to the larvae and grooming the larvae. Indirect care was defined as maintaining the carcass and guarding the brood and carcass against competitors or predators. We then left the pairs to care for their brood until the larvae dispersed from the carcass approximately five days later. At this point, we recorded the number of larvae that survived to dispersal and the average larval mass of the brood, and female and male post-breeding mass to allow us to calculate mass change during breeding.

### 5.2.3 Statistical Analysis

All statistical analyses were conducted using R version 4.3.2 (R Core Team, 2022) with the packages *car* (Fox & Weisberg, 2019), *MASS* (Venables & Ripley, 2022), and *glmmTMB* (Brooks *et al.*, 2017). We used a zero-inflated binomial model in our analyses on the amount of time spent providing direct care by males and

indirect care by females and males since the data for these behaviours showed minor zero inflation. We also included an observation level random effect in the model analysing female indirect care to account for over-dispersion (Harrison, 2015). We used a binomial model including an observation level random effect in our analyses on the amount of time spent providing direct care by females and the number of larvae that survived to dispersal. We used linear models for data on female and male mass change during breeding, and average larval mass at dispersal. All models included female treatment and male treatment as fixed effects with two levels (arrived at a fresh carcass and arrived at a 24 h old carcass). We ran pairwise comparisons using a Tukey's test whenever treatment had a significant effect.

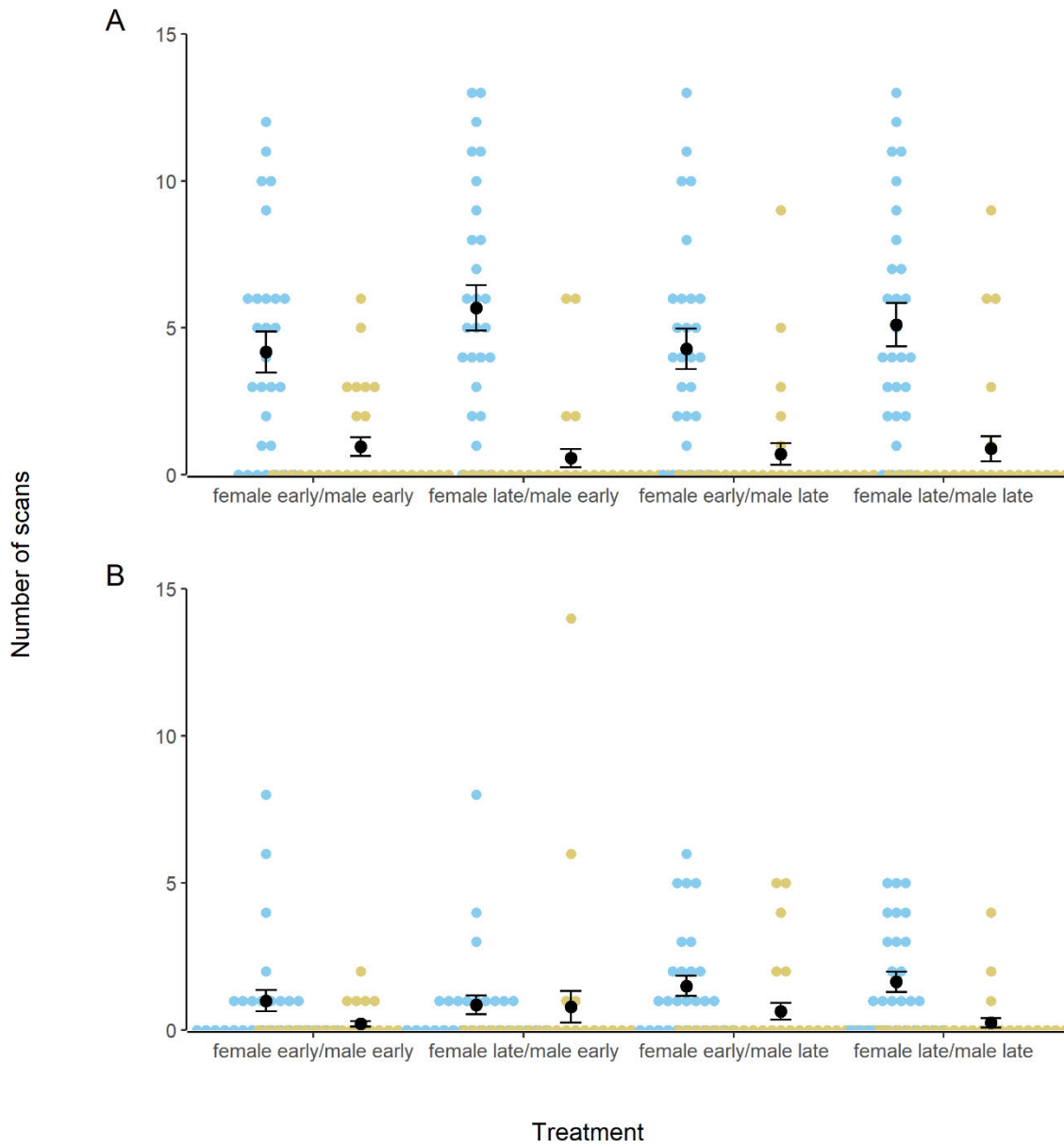
To determine whether a parent responded directly to synchrony in time of arrival at the carcass or indirectly to its partner's contribution, we compared models where we included and excluded the amount of time the partner spent providing direct care and indirect care as a factor (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015; Lambert & Smiseth, 2024). If including this factor reduced or removed any effect of arrival time on the amount of time spent providing direct care or providing indirect care, we interpreted this as evidence that the parent responded indirectly to the contribution of its partner. However, if including this factor did not negate any effect of arrival time on the amount of time the parent spent providing direct or indirect care, we interpreted this as evidence that the focal parent responded directly to arrival time. We note that our data on the responses of parents to their partner's contribution are correlational and that we therefore cannot demonstrate a causal relationship between the contributions of females and males to parental care.

## 5.3 Results

### 5.3.1 Effects of arrival time on post-hatching parental care

Asynchrony in the time of arrival at the carcass, detected as an effect of the interaction between the time of arrival for female and male parents, had a significant effect on the amount of time spent providing post-hatching care by males (Table 5.1). As predicted, males provided more post-hatching care when the female arrived at a fresh carcass while the male arrived 24 h later as compared to when both arrived at a fresh carcass (Tukey's post hoc test,  $P = 0.033$ ). However, in contrast to what we predicted, males also provided more post-hatching care when the male arrived at a fresh carcass while the female arrived 24 h later as compared to when both arrived at a fresh carcass (Tukey's post hoc test,  $P = 0.001$ ). There was also a significant main effect of the time of arrival of both female and male parents since males provided more indirect care when either the female or the male arrived at a fresh carcass (Table 5.1, Figure 5.1). These findings reflect the interaction described above with males providing more indirect care when parents arrived asynchronously at the carcass. There was no effect of female indirect care on male indirect care (Table 5.1), suggesting that the response described above to arrival time did not result from a response by males to variation in female behaviour. The amount of time males spent providing direct care was consistent regardless of the interaction between female arrival time and male arrival time, female arrival time, or male arrival time (Table 5.1, Figure 5.1). Males provided less direct care as females provided more direct care, which suggests that males adjusted their level of direct care to the amount of direct care provided by the females (Table 5.1).

In contrast to our predictions, the amount of time females spent providing direct and indirect care was consistent regardless of the interaction between female arrival time and male arrival time, female arrival time, or male arrival time (Table 5.1, Figure 5.1). The contribution by the partner was a significant predictor of female direct care with females providing less direct care as males provided more direct care (Table 5.1). There was no effect of male indirect care on female indirect care (Table 5.1). We note that we measured parental behaviour in a single 30-minute observation and so we may not have detected a change in the distribution of care across the breeding attempt as a whole.



**Figure 5.1** - Comparison of the number of scans (out of 30) spent providing direct care (A) and indirect care (B) by females (blue points) and males (yellow points) caring for larvae during a 30 min behavioural observation (mean  $\pm$  SE) dependent on female and male arrival time at the carcass.

**Table 5.1** - Summary of statistical tests for the effects of time of arrival on bi-parental cooperation over providing direct and indirect care excluding and including (\*) partner's contribution in the model. The reference category for the female arrival time and male arrival time was 'at a fresh carcass'. Statistically significant P values (< 0.05) are shown in bold.

Behaviour	Female arrival time				Male arrival time				Interaction				Partner's contribution			
	Est	SE	z	P	Est	SE	z	P	Est	SE	z	P	Est	SE	z	P
Female direct	0.45	0.31	1.49	0.137	0.06	0.31	0.20	0.845	-0.21	0.43	-0.48	0.634				
Female direct*	0.35	0.28	1.24	0.215	-0.00	0.29	-0.01	0.995	-0.08	0.40	-0.19	0.848	-0.21	0.06	-3.49	<b>&lt; 0.001</b>
Female indirect	-0.13	0.48	-0.27	0.788	0.64	0.45	1.41	0.160	0.25	0.64	0.39	0.694				
Female indirect*	0.38	0.30	1.28	0.200	0.04	0.27	0.15	0.885	-0.25	0.37	-0.68	0.495	-0.09	0.06	-0.80	0.072
Male direct	0.19	0.35	0.54	0.593	0.19	0.33	0.60	0.551	0.09	0.49	0.18	0.858				
Male direct*	0.05	0.36	0.14	0.890	-0.16	0.35	-0.47	0.641	0.58	0.52	1.12	0.263	-0.25	0.08	-3.27	<b>0.001</b>
Male indirect	2.01	0.54	3.75	<b>&lt; 0.001</b>	1.49	0.55	2.73	<b>0.006</b>	-2.71	0.79	-3.41	<b>&lt; 0.001</b>				
Male indirect*	1.94	0.56	3.44	<b>&lt; 0.001</b>	1.50	0.55	2.72	<b>0.006</b>	-2.50	0.93	2.70	<b>0.007</b>	-0.05	0.13	-0.41	0.684

### 5.3.2 Effects of arrival time on female and male mass change during breeding

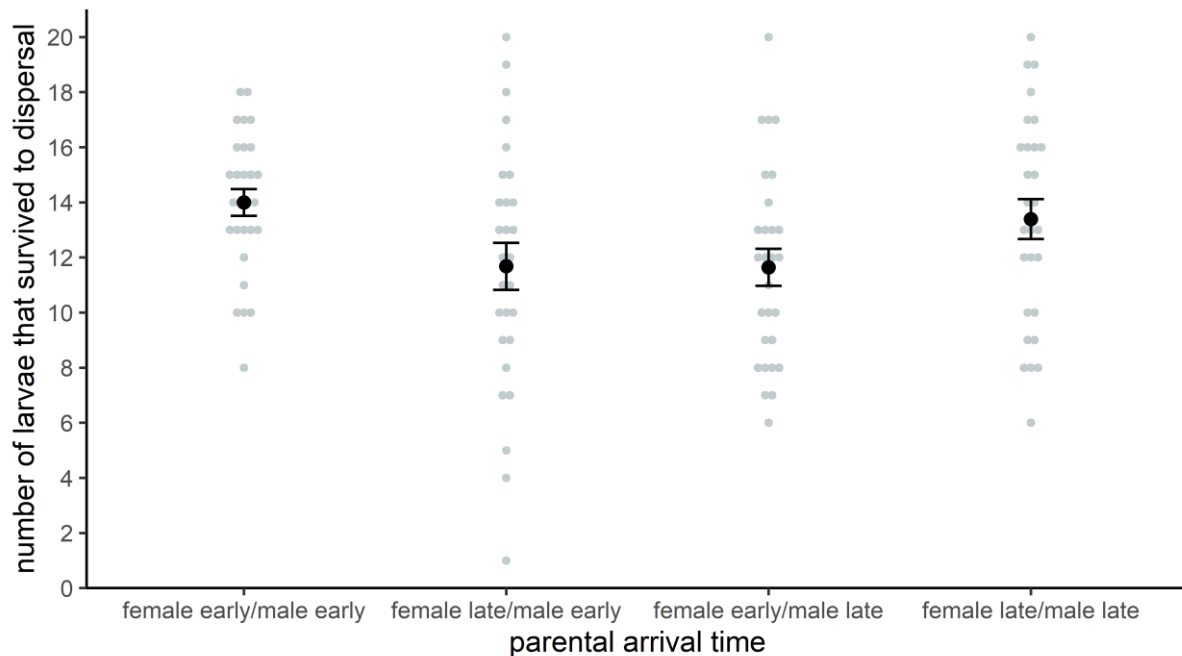
There was no effect of the interaction between the arrival time of female and male parents on the mass change during breeding of either females (estimate =  $0.003 \pm 0.010$  g,  $t = 0.26$ ,  $P = 0.799$ ) or males (estimate =  $- 0.003 \pm 0.012$  g,  $t = - 0.24$ ,  $P = 0.812$ ). Parental mass change during breeding was also consistent regardless of the arrival time of the female (females: estimate =  $- 0.002 \pm 0.007$  g,  $t = - 0.33$ ,  $P = 0.745$ ; males: estimate =  $- 0.002 \pm 0.009$  g,  $t = - 0.22$ ,  $P = 0.829$ ) or the male (females: estimate =  $0.003 \pm 0.007$  g,  $t = 0.38$ ,  $P = 0.702$ ; males: estimate =  $0.007 \pm 0.009$  g,  $t = 0.84$ ,  $P = 0.406$ ).

### 5.3.3 Effects of arrival time on offspring performance

Asynchrony in the time of arrival at the carcass, detected as a significant effect of the interaction between female and male arrival time, affected the number of larvae surviving from brood allocation to dispersal (estimate =  $0.952 \pm 0.331$ ,  $t = 2.87$ ,  $P = 0.004$ ; Figure 5.2). This interaction appeared to be driven by the number of larvae surviving to dispersal being higher when parents arrived synchronously at the carcass (i.e., both early or both late) in comparison to when parents arrived asynchronously (i.e., the female arrived at a fresh carcass and the male arrived 24 h later or the male arrived at a fresh carcass and the female arrived 24 h later) (Figure 5.2). However, the post-hoc pairwise comparisons showed no significant differences between any groups. There was a main effect of both female and male arrival time on the number of larvae that survived to dispersal - fewer larvae survived in broods cared for by females that arrived late (estimate =  $- 0.527 \pm 0.235$ ,  $t = - 2.25$ ,  $P = 0.025$ ) and in brood cared for by males that arrived late (estimate =  $- 0.539 \pm 0.234$ ,  $t = - 2.31$ ,  $P = 0.021$ ), which can be explained by the interaction effect described above. In contrast, average larval mass was consistent regardless of female arrival



time (estimate =  $-0.007 \pm 0.007$  g,  $t = -1.00$ ,  $P = 0.320$ ), male arrival time (estimate =  $-0.007 \pm 0.007$  g,  $t = -1.08$ ,  $P = 0.282$ ), or the interaction between the two (estimate =  $0.015 \pm 0.010$ g,  $t = 1.58$ ,  $P = 0.118$ ).



**Figure 5.2** - The number of larvae that survived from brood allocation to dispersal (mean  $\pm$  SE) dependent on parental arrival time at the carcass.

## 5.4 Discussion

We found that males spent more time providing indirect care when the parents arrived asynchronously on the carcass; that is, when one parent arrived 24 h after the other. We predicted the female to provide less post-hatching care, and the male to provide more post-hatching care when the female arrived at a fresh carcass while the male arrived 24 h later, as compared to when both arrived at the carcass synchronously. Conversely, we predicted the male to provide less post-hatching care, and the female to provide more post-hatching care, when the male arrived at a

fresh carcass while the female arrived 24 h later. The rationale for this was that, whenever one parent arrived earlier than the other, the first parent to arrive would spend more time providing pre-hatching care and so would be expected to provide less post-hatching care, prompting the latter to compensate incompletely. Instead, we found that males provided more post-hatching care both when they arrived first and when the female arrived first. Given that males would have provided more pre-hatching care when they arrived first and less when the female arrived first, it is unlikely that males adjusted their contribution to post-hatching care based on their contribution to pre-hatching care. Furthermore, females provided the same level of post-hatching care regardless of whether parents arrived synchronously or asynchronously at the carcass. Thus, our findings contradict our prediction that parents would provide less post-hatching care if they arrived before their partner. An alternative explanation for why males provided more care when either parent arrived late to the carcass is that the carcass was in a worse state of degradation than if both parents arrived at a fresh carcass since only one parent was initially available to prepare the carcass. Although both parents engage in all roles involved in post-hatching care, we might expect carcass degradation to have a greater impact on male care than on female care since males spend more time on indirect care (Smiseth *et al.*, 2005) whilst females spend more time on direct care for the larvae (Eggert *et al.*, 1998b; Smiseth *et al.*, 2005; Rozen *et al.*, 2008; Arce *et al.*, 2012). Instead, we found that males provided more indirect care when either the female or the male arrived late at the carcass, but not when both arrived late. As such, it seems more likely that males responded to the asynchrony in arrival time per se rather than the state of degradation of the carcass.

One potential explanation for the finding that males responded to the asynchrony in arrival time, regardless of whether the male or the female parent arrived first, is that asynchrony in the time of arrival somehow disrupted coordination in the timing of the reproductive physiology of the female and the male. Physiological and hormonal mechanisms regulate the initiation and maintenance of reproductive behaviours (Sockman *et al.*, 2000; Sharp & Blache, 2003; Miller *et al.*, 2009). If the synchrony of such mechanisms is disrupted, this may impact the subsequent coordination of parental care within the pair. For example, if the onset of the physiological and hormonal mechanisms that facilitate and regulate reproduction depends on arrival time at the carcass, asynchrony in arrival time could have led to a lack of coordination of these processes in the female and male, consequently affecting the coordination of care. In insects, juvenile hormones, which are essential for development and reproduction, are a likely candidate. Our experiment was not designed to assess female and male hormonal changes in response to the time of arrival at the carcass. Nevertheless, previous endocrinological studies on *Nicrophorus* spp. show that there is a spike in the level of juvenile hormones being released in females and males upon the discovery of a carcass (Scott & Panaitof, 2004; Trumbo & Robinson, 2008). Accordingly, when the female and male arrived at the carcass asynchronously, the release of juvenile hormones may have peaked at different times in the female and the male. Given the importance of juvenile hormones in aligning mating and parental care in *N. vespilloides* (Engel *et al.*, 2016), mismatches in the timing of juvenile hormone release could disrupt the coordination of parental care. Such a disruption may also have led to reduced offspring performance. Indeed, the number of larvae that survived to dispersal was higher in broods cared for by parents that arrived at the carcass synchronously compared to

those cared for by parents that arrived at the carcass asynchronously, which we discuss in greater detail below. In response, males may have increased the level of indirect care they provided in an attempt to compensate for a decrease in offspring performance when parents arrived at the carcass asynchronously.

We found no evidence that females altered the level of care they provided in response to the arrival time of females or males. This also contradicts our prediction that parents would provide less care if they arrived before their partner since we found that females provided the same level of care when they arrived before their partner, after their partner, and at the same time as their partner. One explanation is that females typically spend more time providing care than males during a breeding attempt and so may be working closer to capacity (Smiseth & Moore, 2004). If this were the case, females may have been less able to adjust the level of care they provided in response to parental arrival time. This seems unlikely given that prior studies on *N. vespilloides* have shown that females adjust the level of care they provide in response to other stimuli (Mattey & Smiseth, 2015; Pilakouta *et al.*, 2015; Lambert & Smiseth, 2024). Instead, our results add to a growing list of differences in caring behaviour between females and males in *N. vespilloides* (Smiseth & Moore, 2004; Walling *et al.*, 2008; Georgiou-Shippi *et al.*, 2018; Lambert & Smiseth, 2024). Currently, we do not know what causes these sex differences, due to a lack of understanding of differences in the costs and benefits of care for females and males. The benefits are expected to be similar for females and males given both are capable of providing care to improve the survival and quality of the brood. Additionally, there is both sperm competition and intraspecific brood parasitism in this species, so any increased costs of providing care associated with uncertainty in parentage may be similar for females and males. Males can attract and mate with

females away from a carcass, and thereby achieve some reproductive success without having access to a carcass, whereas females must have access to a carcass to reproduce so males may suffer a higher opportunity cost for providing care. However, if the costs associated with providing care were higher for males than females, we would have expected females to increase the level of care they provided in response to reduced offspring quality as a result of asynchrony in parental arrival time rather than males as observed here.

We also found that asynchrony in the time of arrival of females and males at the carcass affected offspring performance. The number of larvae that survived to dispersal was higher when parents arrived synchronously at the carcass compared to when they arrived asynchronously. This finding contradicts our prediction that offspring performance would be lower when parents arrived asynchronously as a result of a reduction in the overall level of care being provided associated with a reduction by the parent arriving first and incomplete compensation by its partner. Instead, offspring performance was lower when the female and male arrived asynchronously despite an increase in male care when the female and male arrived asynchronously. This result may provide further evidence for the idea that asynchronous arrival at the carcass may disrupt the coordination of the reproductive physiology of the female and the male. If asynchronous arrival at the carcass causes a mismatch in the release of juvenile hormones that results in a lack of coordination in providing parental care, as suggested above, this may affect the efficiency or quality of care being provided and, in turn, reduce offspring performance. To confirm this, it would be interesting to measure the release of juvenile hormones throughout the breeding attempt in response to the synchrony of female and male discovery of the carcass.

Our results are in line with research that shows synchrony in reproductive physiology and parental behaviour plays an important role in breeding success (Griffith, 2019; Prior, 2020). For example, in greylag geese, pairs with more coordinated patterns of yearly testosterone release have higher reproductive success (Hirschenhauser, 2012) and in great tits, pairs with more similar baseline corticosterone levels also have higher reproductive success (Ouyang *et al.*, 2014). Furthermore, behavioural synchrony in food provisioning to offspring has been shown to have a positive effect on breeding success in birds such as zebra finches (Mariette & Griffith, 2012) and long-tailed finches (Roosij & Griffith, 2013), potentially as a result of increased efficiency or a reduction in predation risk by reducing the conspicuousness of a nest. Here, we show another aspect of behavioural synchrony, the onset of breeding and parental care, plays an important role in parental cooperation and offspring performance. This may not be the case in other systems with biparental care that respond to other cues to coordinate reproductive physiology and, subsequently, caring behaviours. In *N. vespilloides*, the onset of care is directly linked with the discovery of a carcass which stimulates endocrinological changes. While access to resources required for breeding may be important in synchronising reproductive physiology in our study species, other cues such as seasonal changes, the presence of mates or courtship behaviour may play a role in coordinating the timing of reproductive physiology in other species. For example, many species continue courtship behaviours even after mate choice and pair formation displays (Baker & Baker, 1988; Johnson *et al.*, 2000; Dalziell & Welbergen, 2022). It is currently unclear why individuals engage in post-mating courtship (Eberhard, 2009). Suggestions include advertising male quality to other nearby females, and increasing male paternity through sperm competition, and cryptic female choice (Johnson *et al.*,

2000). Alternatively, post-copulatory displays may act as a means to ensure coordination and prevent any detrimental effects of mismatches in the timing of changes in reproductive physiology. Future studies may clarify the link between the synchrony of reproductive behaviours such as parental care and synchrony in reproductive physiology by manipulating one aspect and measuring any effect on the other.

In conclusion, synchrony in the onset of parental care influenced both paternal care and offspring performance. This may be because arrival time influences reproductive physiology and, as a result, the ability of the female and male to coordinate their contributions towards caring for their joint offspring. Our findings highlight the need for more work investigating the role of synchrony in both reproductive physiology and behaviour with the aim of understanding the link between the two.

## 6 General Discussion

Parents adjust the level of care they provide to their offspring in response to factors that are likely to affect the costs and benefits associated with allocating time and resources towards current reproduction (Williams, 1966b; Clutton-Brock, 1991). If they provide biparental care, the female and male must coordinate their cooperation so parents may adjust the level of care they provide in response to factors that influence the costs and benefits their partner faces, which may indicate their partner's likely future contribution, in addition to the costs and benefits associated with providing care themselves (Houston *et al.*, 2005; Lessells, 2012). In this thesis, I built upon the current understanding of how different factors may alter the costs and benefits associated with providing care and how parents respond accordingly in both uniparental care (Chapter 2) and biparental care (Chapter 3 - 5) settings using the burying beetle *N. vespilloides*.

I started by testing whether females that cared for a larger brood during a previous breeding attempt adjusted the level of care they provided to their current brood (Chapter 2). Previous studies investigating the trade-off between current and future reproduction often report that increased allocation to current reproduction is associated with a reduction in the number or quality of future offspring (Nur, 1988; Parejo & Danchin, 2006; Ward *et al.*, 2009; Martin & Festa-Bianchet, 2010). The aim was to assess whether parents shift any cost of increased allocation to current reproduction onto future offspring as a result of a reduced future ability to provide care. I found that females provided the same level of parental care regardless of previous reproductive allocation or resource access. This suggests that increased allocation to current reproduction is not necessarily associated with a reduction in a



female's future ability to provide care. I concluded that this likely reflects that *N. vespilloides* breeds on a nutrient-rich carcass which may allow females to recover from any costs associated with caring for a large brood during a previous breeding attempt. Future work may build on this by testing the effect of previous reproductive allocation on future ability to provide parental care in income breeders that do not have access to a fixed food source during breeding. Furthermore, my results highlight the need for more work exploring the potential mechanisms that allow parents to shift the cost of current reproduction onto future offspring, such as producing worse quality eggs or parents being less able to compete for resources to food provision offspring.

I then considered factors that may influence the level of care females and males provide when cooperating with a partner. First, I found that females adjusted the level of care they provided when paired with a food-deprived male depending on their own nutritional state (Chapter 3). Food-deprived females paired with food-deprived males provided more care than food-deprived females paired with well-fed males. Females and males also adjusted the amount of time they spent providing care depending on the contribution of their partner. My results contradict a key assumption of negotiation models which is that parents only adjust the level of care they provide in response to their partner's contribution because they are unable to directly assess and respond to the state of their partner (McNamara *et al.*, 1999; McNamara & Wolf, 2022). Instead, I found that parents adjust the level of care they provide in response to both the state of their partner directly and the contribution of their partner independently. The experimental design of future empirical studies could test for this by manipulating or observing both individual state and partner contribution when studying the relative contribution of females and males towards

providing care for their joint offspring. Theoretical models may also incorporate this by acknowledging a parent's response to the state of their partner, in addition but independently to partner contribution, plays an important role in the mediation of sexual conflict.

Second, I found that parents did not alter the level of care they provided when in the presence or absence of female or male intraspecific intruders (Chapter 4). This was surprising since I expected the presence of an intruder to affect the costs and benefits associated with providing care differently for female and male parents depending on the sex of the intruder. This is because any intruder threatens to take over the carcass and kill the current brood, whilst an opposite-sex intruder also offers a parent the opportunity to remate and produce a smaller replacement brood (Scott, 1998). As such, I expected both parents to increase the level of care they provided in the presence of intruders to speed up the development of the larvae or to use up the carcass more quickly but to a lesser extent if the intruder was of the opposite sex. A potential explanation for why I did not find what I expected is that this prediction assumes that there will be variance in the intruder presence across breeding attempts since it is only worth a parent altering their contribution if the costs or benefits associated with providing care are likely to change during future reproduction. Instead, parents may maintain the level of care they provide in the presence of intruders, as seen here, if the threat of intruders is likely to be consistent across breeding attempts. To expand on this, future studies may investigate the effect of a range of social environments, such as the presence of intraspecific brood parasites or helpers, on biparental cooperation to work towards forming a general theoretical framework.

Finally, I found that males provided more care when the onset of care was asynchronous for females and males, that is when one parent arrived earlier at the carcass and subsequently started providing care earlier than its partner (Chapter 5). This contradicted my prediction that the parent that arrived first at the carcass would perform the majority of pre-hatching care and, subsequently, less post-hatching care, while its partner that arrived later would contribute less to pre-hatching care but more to post-hatching care to incompletely compensate. Instead, my results may reflect a complex relationship between synchrony in the onset of care, the level of care parents provide and offspring performance. Asynchrony in the onset of parental care may have disrupted the coordination of the reproductive physiology within the pair, and subsequently the coordination of care, causing a reduction in offspring performance. The male may then have responded to this by increasing the amount of time it spent providing care in an attempt to compensate. Synchrony in the onset of parental care is directly related to carcass discovery in *N. vespilloides* since they rely on carcasses for breeding and females will only lay eggs in the presence of a carcass. As such, asynchrony in the onset of care may not have the same implications for species that rely on other cues to coordinate reproductive physiology such as seasonal changes. Future studies may expand on this by investigating the link between synchrony in reproductive physiology and behaviour by manipulating an aspect of physiology, for example, the timing of juvenile hormone release in *N. vespilloides*, and measuring the effect on the coordination of behaviours such as parental care.

Chapters 3 – 5 focused on biparental care whilst chapter 2 tested the effect of previous reproductive allocation on a female's future ability to provide care in a uniparental setting. If I were to manipulate previous reproductive allocation and

measure the effect on the level of care a female and male within a pair provides, instead of a single female, I would expect previous reproductive allocation to have little effect on the relative contribution of the female and male. This is because both parents would be able to feed from the carcass to recover from any costs associated with previous reproductive allocation, as seen with single females, which would allow them to maintain the level of care they provide. This prediction relies on the assumption that the carcass is large enough for both parents to feed from sufficiently. If resources were limited, I would expect both parents to not fully recover from any costs associated with previous reproductive allocation, making them less able to provide care as a result. To assess this, future work could explore any effect of previous reproductive allocation and resource access on the level of care females and males provide using a factorial design.

When taken together, my work on biparental cooperation (Chapter 3 – 5) has shown that parents rely on factors beyond the contribution of their partner, such as individual state, the social environment and synchrony in reproductive behaviour, to determine how much care to provide and that such factors influence parental cooperation in different ways. For example, I found no effect of the presence or absence of intruders on the relative contribution of females and males (Chapter 4) but parents did alter the level of care they provided in response to nutritional state (Chapter 3) and synchrony in the onset of care (Chapter 5). This may be because an individual's response to social environment during a current breeding attempt depends on how they perceive the likely future social environment as described above, whereas nutritional state and synchrony in the onset of care may more directly limit a parent's ability to provide care during the current breeding attempt. There were also sex differences in how parents responded to the different factors.

Females, but not males, adjusted the level of care they provided in response to nutritional state whilst males, but not females, adjusted the level of care they provided in response to synchrony in the onset of care. It is unclear why there may be sex differences in these responses since there is a lack of understanding of differences in the costs and benefits associated with providing care for female and male *N. vespilloides*. This is because the benefits of providing care are thought to be similar given both females and males are capable of providing care to improve offspring performance (Eggert *et al.*, 1998b; Smiseth *et al.*, 2005). Furthermore, the costs associated with uncertainty in parentage faced by females and males are also comparable given there is both intraspecific brood parasitism and sperm competition (Müller *et al.*, 2007). The only clear difference in cost associated with providing care is that males can mate with females in the absence of a carcass whereas females need access to a carcass to reproduce so males may face a higher opportunity cost associated with providing care than females (Eggert, 1992). However, this does not explain the sex differences in how parents respond to nutritional state and synchrony in the onset of care since males would face a higher opportunity cost in both scenarios.

Future studies investigating biparental cooperation should aim to clarify how and why changes in individual state, the social environment, or synchrony in reproductive behaviour alter the costs and benefits of providing care differently for females and males to better understand how the relative contribution of females and males may shift in response to a variety of factors. Furthermore, in a natural setting, these factors are likely to vary simultaneously and interact with each other. For example, food-deprived parents may respond more aggressively to the presence of intruders than well-fed parents since they are more reliant on the carcass as a food

source. Subsequently, future studies may involve manipulating multiple components of state or social environment simultaneously to better understand how the interaction of such factors may affect biparental cooperation.

## Concluding remarks

In this thesis, I investigated how different factors influence the level of care *N. vespilloides* parents provide to their offspring and how females and males balance their contributions when cooperating to provide biparental care. I found that parents maintained the level of care they provided regardless of previous reproductive allocation suggesting this is not the mechanism that allows parents to shift any cost of reproduction onto their offspring. Furthermore, I found evidence that parents respond to factors beyond the contribution of their partner to determine how much care to provide in a biparental care setting. For example, parents adjusted their relative contribution to care in response to their partner's nutritional status and synchrony in the onset of care. Finally, I expanded the understanding of how social environment influences biparental cooperation to encourage the formation of a general theoretical framework. In conclusion, the evidence reported here shows how previously unexplored factors may influence the costs and benefits of providing care, and how parents adjust their contribution in response.

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## Appendix: Journal articles arising from this thesis

## RESEARCH ARTICLE

# Access to resources buffers against effects of current reproduction on future ability to provide care in a burying beetle

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## Abstract

Studies investigating the trade-off between current and future reproduction often find that increased allocation to current reproduction is associated with a reduction in the number or quality of future offspring. In species that provide parental care, this effect on future offspring may be mediated through a reduced future ability to provide care. Here, we test this idea in the burying beetle *Nicrophorus vespilloides*, a species in which parents shift the cost of reproduction toward future offspring and provide elaborate parental care. We manipulated brood size to alter the costs females experienced in association with current reproduction and measured the level of parental care during a subsequent breeding attempt. Given that these beetles breed on carcasses of small vertebrates, it is important to consider confounding effects due to benefits associated with resource access during breeding. We, therefore, manipulated access to carrion and measured the level of parental care during a subsequent breeding attempt. We found that females provided the same level of care regardless of previous brood size and resource access, suggesting that neither affected future ability to provide care. This may reflect that parents feed on carrion during breeding, which may buffer against any costs of previous breeding attempts. Our results show that increased allocation to current reproduction is not necessarily associated with a reduction in future ability to provide care. Nevertheless, this may reflect unique aspects of our study system, and we encourage future work on systems where parents do not have access to a rich resource during breeding.

## KEYWORDS

cost of reproduction, life-history trade-offs, *Nicrophorus vespilloides*, parental care, reproductive allocation

## TAXONOMY CLASSIFICATION

Behavioural ecology

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## 1 | INTRODUCTION

The trade-off between current and future reproduction, often referred to as the “cost of reproduction,” is a central hypothesis to life-history theory (Williams, 1966). Allocation to current reproduction diverts resources from somatic maintenance, which, in turn, may reduce future reproductive potential. The cost of reproduction is often studied experimentally by altering parental allocation to current reproduction and then measuring its effect on future breeding attempts. This is usually achieved by manipulating brood size (Koivula et al., 2003; Lessells, 1986; Parejo & Danchin, 2006), but can also involve manipulating the physiology of mothers (Cox & Calsbeek, 2010; Oksanen et al., 2002) or the reproductive environment (Creighton et al., 2009). Currently, evidence for such a trade-off is mixed with some studies supporting the hypothesis but others finding either no relationship or a positive relationship between current and future reproduction (Santos & Nakagawa, 2012). Within studies reporting evidence for a cost of reproduction, this cost can manifest in different ways. In some species, the cost to future reproduction is paid through a reduction in the parents' own survival, for example, as a result of increased susceptibility to predation (Veasey et al., 2001) or parasitism (Alt et al., 2015). Meanwhile, in other species, the cost is shifted toward future offspring as indicated by a reduction in the number or quality of offspring produced in subsequent breeding attempts (Martin & Festa-Bianchet, 2010; Nur, 1988; Parejo & Danchin, 2006). In such species, little attention has been given to how the parents are able to shift the cost of reproduction onto future offspring and this therefore remains poorly understood.

In species where parents care for their offspring, parents may shift the cost of reproduction onto future offspring if increased allocation to reproduction is associated with a reduction in future ability to provide care. Parents that allocate more toward current reproduction may reduce the level of care they provide during subsequent breeding attempts either because they cannot allocate as much toward care as a result of higher investment in current reproduction, or because they strategically allocate less toward care in order to maintain their own condition and facilitate future breeding opportunities. For example, there is observational evidence that bighorn sheep (*Ovis canadensis*) ewes reduce the energy they allocate to their lambs via lactation and instead prioritize their own body mass when resources are limited (Martin & Festa-Bianchet, 2010). By doing so, ewes increase their own over-winter survival at the expense of that of their lambs. Such reduction in parental care may provide a general mechanism for the commonly observed reduction in number or quality of future offspring associated with increased allocation to current reproduction in species with parental care. However, little is known about this mechanism and there is now a need for more studies, including those based on an experimental approach, to investigate whether an increase in current reproduction is associated with a reduction in future ability to provide care.

We tested this idea by manipulating reproductive allocation to a current breeding attempt and measuring the effect on the level of care provided in a subsequent breeding attempt by females of the burying beetle *Nicrophorus vespilloides*. Beetles in the genus *Nicrophorus* are well suited to test this idea because there is evidence for a cost of reproduction in *N. vespilloides* (Jenkins et al., 2000; Ward et al., 2009) and the closely related *N. orbicollis* (Billman et al., 2014; Creighton et al., 2009). Studies show that parents shift this cost toward future offspring as parents reduce the size and mass of future broods in response to an increase in allocation to current reproduction induced by manipulating male assistance in parental care (Jenkins et al., 2000), brood size (Ward et al., 2009) or carcass size (Billman et al., 2014; Creighton et al., 2009). *Nicrophorus vespilloides* breeds on a small vertebrate carcass that serves as a food source for caring parents and their offspring. Females (sometimes assisted by a male) provide elaborate parental care for their offspring by preparing the carcass, provisioning pre-digested carrion to the offspring, maintaining the carcass by spreading antimicrobials onto it, and guarding the offspring and carcass against conspecifics (Eggert et al., 1998; Scott, 1998). Larvae benefit from parental care in terms of increased growth and survival (Andrews et al., 2017; Eggert et al., 1998; Lock et al., 2004). Although previous work provides clear evidence that increased allocation is associated with a reduction in the size and quality of future broods, it remains unknown whether this is due to a reduction in the level of parental care toward future broods.

Our aim was to test whether a reduction in the parent's future ability to provide care due to increased allocation to a current breeding attempt is the mechanism mediating the observed shift in the cost of reproduction toward future broods. We altered female allocation to current reproduction by manipulating whether or not females had the opportunity to breed and, if they did, the size of the brood they cared for. We then measured effects on the level of post-hatching care provided in a future breeding attempt. If increasing the cost of reproduction in an initial breeding attempt affected a female's ability to provide care in a future breeding attempt, we predict that breeding females that had cared for a large brood would provide the lowest level of care during the subsequent breeding attempt, that non-breeding females would provide the highest level of care, while breeding females that had cared for a small brood would provide an intermediate level of care. In *N. vespilloides*, parents have access to a nutrient-rich carcass that they feed on during reproduction, which often leads to an increase in mass over a breeding attempt (Creighton et al., 2009; Pilakouta et al., 2016; Richardson et al., 2019, 2020; Richardson & Smiseth, 2019). Thus, when studying potential effects associated with a cost of reproduction, it is important to consider confounding effects due to potential benefits associated with gaining access to resources during breeding. Accordingly, we investigated whether prior access to a mouse carcass affected future post-hatching parental care. We, therefore, included two control groups. First, non-breeding females that had no prior carcass access and did not produce a brood and, therefore, had neither the benefits of carcass access nor the costs of reproducing. Second, non-breeding females that had access

to a carcass prior to their first breeding attempt but did not produce a brood and, therefore, had the benefits of carcass access but suffered none of the costs of caring for larvae. If the benefit associated with carcass access in an initial breeding attempt affected a female's ability to provide care in a future breeding attempt, we predict that breeding and non-breeding females that had prior access to a carcass would provide more care than non-breeding females that did not have such access. Finally, we tested for combined effects of the cost of reproduction and the benefit of carcass access on the level of post-hatching care females provided during a subsequent breeding attempt. If there were such combined effects, we predicted that the pattern observed would be intermediate between those described above for what we predicted if there were effects due to the cost of reproduction or the benefit of carcass access.

## 2 | MATERIALS AND METHODS

### 2.1 | General methodology

We used beetles from an outbred laboratory population maintained at the University of Edinburgh. All beetles in the stock population were originally collected in Edinburgh, UK and the population was kept at 20°C under a 16:8 h light: dark cycle. We housed non-breeding adults individually in clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil and fed them raw organic beef twice a week.

### 2.2 | Experimental design

The experiment was conducted across two stages. In stage one, we randomly assigned unmated females to one of the following four treatments (Figure 1): breeding females allocated a large brood of 40 larvae ( $n = 32$ ), breeding females allocated a small brood of 10 larvae ( $n = 32$ ), non-breeding females provided with access to a mouse carcass ( $n = 30$ ) or non-breeding females not provided with access to a mouse carcass ( $n = 30$ ). We chose these brood sizes to represent a low and high level of reproductive allocation, respectively, since they are approximately half and double the average brood size for *N. vespilloides* (21 larvae; Smiseth & Moore, 2002) and fall well within the natural range for this species (2–45 larvae; Smiseth & Moore, 2002). We included the treatment of non-breeding females provided with access to a carcass to control for any benefits associated with carcass access. The extent of the benefit associated with carcass access is unclear because *N. vespilloides* feeding habits in the absence of a carcass in the wild are unknown. However, they are suspected to feed on other insects as well as carrion, and carcasses would be of comparatively high nutritional value. Carcass access also stimulates mated and unmated females to lay fertilized or unfertilized eggs, respectively, and to provide pre-hatching parental care (i.e., burying and maintaining the carcass). It is not possible experimentally to separate the benefit of feeding from the carcass from the costs of egg laying and pre-hatching care. However, the cost of egg laying and providing pre-hatching care is known to be minimal

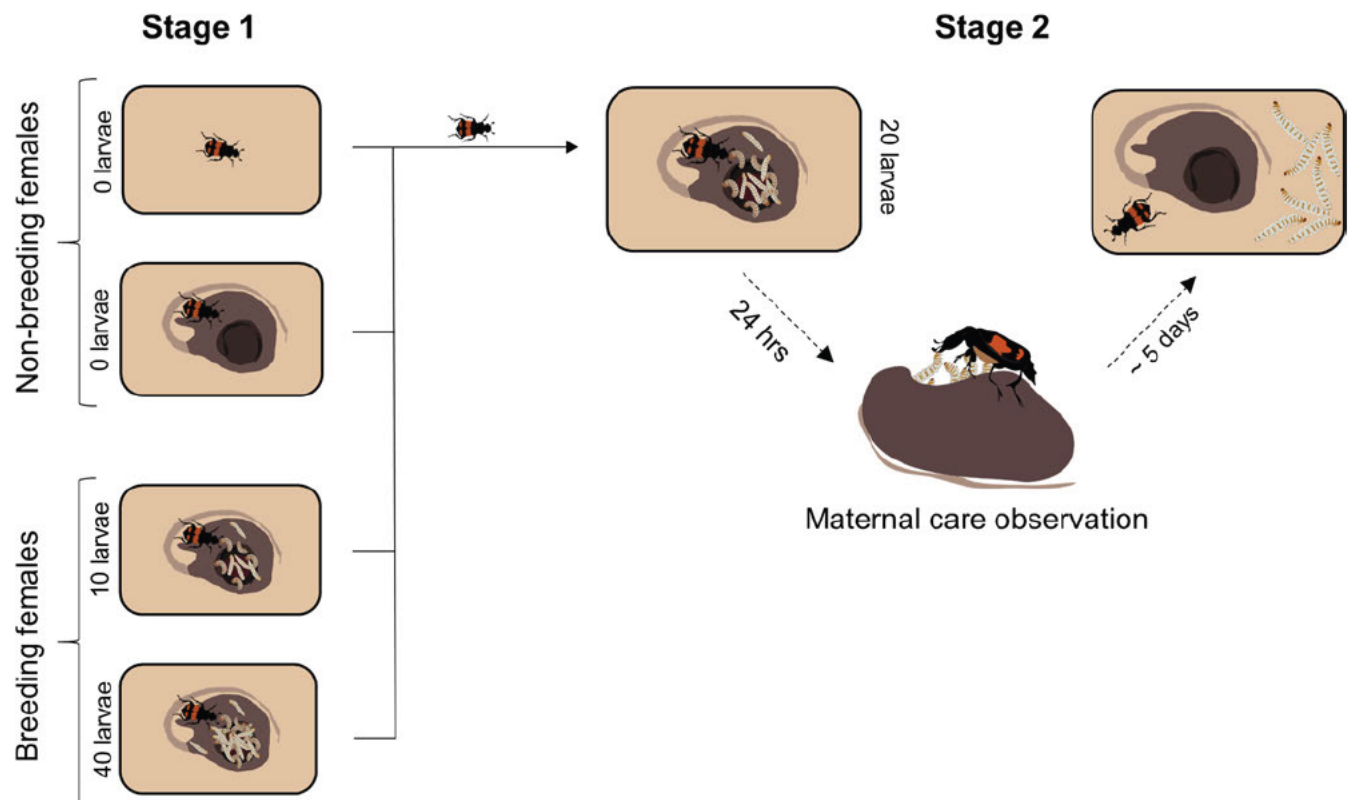


FIGURE 1 The experimental design used to investigate whether the cost of increased reproductive allocation and benefit of carcass access (manipulated during stage one) affect the level of care females provide during stage two.



(undetectable in Ward et al., 2009). Accordingly, we expected the net effects of carcass access to be beneficial.

In stage two, all females from stage one were provided with a carcass to initiate breeding and all females were allocated the same, standardized brood size (20 larvae; Figure 1). We used a standardized brood size to control for potential confounding effects due to variation in clutch size or brood size, which might affect the amount of care provided by females. This design allowed us to test whether the observed shift in the cost of reproduction toward future broods was mediated by a reduction in the parent's future ability to provide care while controlling for any potential benefit associated with carcass access. We note that this design excludes the possibility to test how treatment during stage one would affect the natural broods produced by the females during stage two. However, prior work on this species has established that increased allocation is associated with a reduction in the size and quality of future broods in *N. vespilloides* (Jenkins et al., 2000; Ward et al., 2009). If the observed shift in the cost of reproduction toward future offspring was mediated by a reduction in the parent's future ability to provide care, this would be detected as a reduction in the amount of care provided during stage two in this descending order: breeding females that had cared for a large brood during stage one, breeding females that had cared for a small brood, non-breeding females that had not had access to a carcass. If there was a benefit associated with carcass access, and this benefit affected the parent's future ability to provide care, this would be detected as an increase in the amount of care provided during stage two by non-breeding females that had access to a carcass during stage one compared to non-breeding females that had not had access to a carcass.

In stage one of the experiment, breeding females were weighed and then paired with an unrelated male from the stock population. We transferred each pair into a clear plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil containing a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (20–25 g;  $M \pm SE = 22.57 \pm 0.19$  g) to initiate mating. After 48 h, when the eggs had been laid but before the larvae had begun hatching, we moved the female and her carcass into a new container lined with fresh moist soil. At this point, we discarded the male since males sometimes assist with parental care, in which case the male might absorb some of the cost of reproduction if present. In addition, male involvement in providing care is highly variable (Smiseth & Moore, 2002) and so allowing the male to assist would generate variation in the extent to which males assisted. Therefore, we removed the male to make it easier to detect any cost of reproduction to the female. Removing the male has also been shown to have no effect on female caring behavior or offspring fitness in *N. vespilloides* (Smiseth et al., 2005). We then allocated breeding females either a small (10 larvae) or a large (40 larvae) foster brood which consisted of newly hatched larvae from at least two different mothers. We only allocated a foster brood to a female after her own eggs had started hatching since females use temporal kin recognition and kill larvae that arrive at the carcass before their own eggs have begun to hatch (Müller & Eggert, 1990). We left the females undisturbed

to care for their brood until the larvae dispersed from the carcass approximately 5 days later upon which we recorded average larval mass, the proportion of larvae that survived to dispersal and female mass.

Non-breeding females were also weighed before being transferred into clear plastic containers (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil. Non-breeding females provided with access to a carcass were given a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (20–25 g;  $M \pm SE = 22.53 \pm 0.24$  g). Being provided with a carcass initiated pre-hatching parental care (preparing the carcass for larvae) and the laying of unfertilized eggs. Non-breeding females without access to a carcass were fed raw organic beef twice a week (approximately 0.3 g) to ensure they did not starve. Unlike the presence of a carcass, the presence of beef did not initiate egg laying or pre-hatching care. We handled non-breeding females an equal number of times as we handled the breeding females described above. Non-breeding females that were provided access to a carcass had access to it for the same period as the breeding females. We then weighed the females at the end of stage one before they were transferred into individual clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil and left undisturbed for 24 hours before proceeding to stage two.

In stage two, we weighed all females used in stage one before pairing them with an unrelated male from the stock population. We then transferred each pair into a clear plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil containing a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (20–25 g;  $M \pm SE = 22.63 \pm 0.12$  g). We allowed pairs to mate for 48 h after which the male was removed. We removed the male to control for potential confounding effects due to variation in the contribution of males toward caring for offspring as described above for stage one of the experiment. At this point, we recorded the number of eggs each female laid by counting the number of eggs visible through the bottom of the clear container. We chose this method to avoid handling and thereby potentially damaging the eggs. Furthermore, it has been shown that the visible number of eggs is strongly correlated with the actual clutch size when the container is lined with a thin layer of soil as they were here (Monteith et al., 2012). We then allocated each female a foster brood consisting of 20 larvae using the same methods as in stage one. We conducted behavioral observations 24 h ( $\pm 10$  min) after we allocated females a foster brood, which is when parents of this species provide the highest level of care (Smiseth et al., 2003). We did the behavioral observations under red light using instantaneous sampling every minute for 30 min consistent with established protocols (Smiseth & Moore, 2002). We then left the females to care for their brood until the larvae dispersed from the carcass approximately 5 days later. At the time of dispersal, we recorded average larval mass and the proportion of larvae that survived to dispersal. We weighed the females for a second time after the larvae had dispersed to estimate mass change over stage two.

After stage two, we transferred all females into individual clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil,

maintained them using the same protocol applied to the stock population, and checked them twice a week until death. We did this to record their lifespan as the number of days from eclosion to the day the female was found dead.

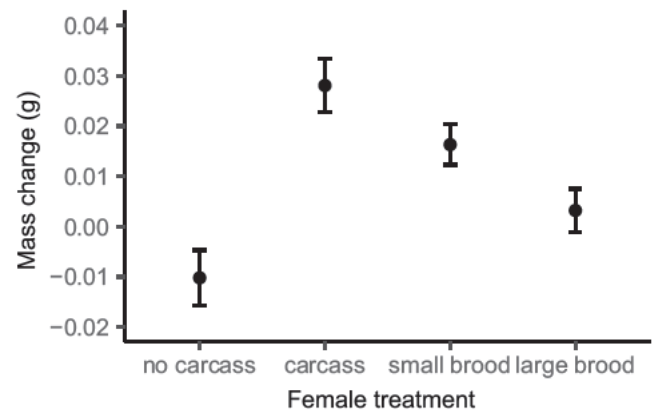
## 2.3 | Statistical analyses

All statistical analyses were conducted using R version 3.6.1 (R Core Team, 2021) with the packages *car* (Fox & Weisberg, 2019), *MASS* (Venables & Ripley, 2022), and *glmmTMB* (Brooks et al., 2017). We used zero-inflated binomial models in our analyses on time spent provisioning food to larvae since the data for this behavior showed minor zero inflation. We used binomial models in our analysis on carcass maintenance and larval survival and linear models to analyze female mass change and mean larval mass at dispersal. In all these models, we included observation level as a random effect to account for over-dispersion (Harrison, 2015). We used a negative binomial model to analyze data on number of eggs laid during stage two and Cox's proportional hazards to analyze data on female lifespan. All models included female treatment during stage one as a fixed effect with four levels (breeding and caring for a large brood, breeding and caring for a small brood, non-breeding and having access to a carcass, non-breeding and not having access to a carcass). We ran pairwise comparisons using a Tukey's test with the Bonferroni correction whenever treatment had a significant effect.

## 3 | RESULTS

### 3.1 | Female mass change and offspring performance in stage one

Increased reproductive allocation negatively affected female mass change during stage one. Non-breeding females that had access to a carcass during stage one gained more mass than breeding females that cared for a large brood (estimate  $\pm$  SE = 0.025  $\pm$  0.007 g,  $Z = 3.641$ ,  $p = .002$ ; Figure 2), while breeding females that cared for a small brood gained an intermediate amount of mass midway between breeding females that cared for a large brood (estimate  $\pm$  SE = 0.013  $\pm$  0.007 g,  $Z = 1.950$ ,  $p = .321$ ; Figure 2) and non-breeding females that had access to a carcass (estimate  $\pm$  SE = 0.012  $\pm$  0.007 g,  $Z = 1.723$ ,  $p = .525$ ; Figure 2). The benefit of carcass access was also evident from effects on female mass change during stage one since non-breeding females with access to a carcass gained more mass than non-breeding females that had no access to a carcass (estimate  $\pm$  SE = 0.038  $\pm$  0.007 g,  $Z = 5.516$ ,  $p < .001$ ; Figure 2). There was no difference in mass change between breeding females that cared for a large brood and non-breeding females that had no access to a carcass (estimate  $\pm$  SE = -0.013  $\pm$  0.007 g,  $Z = -1.963$ ,  $p = .312$ ; Figure 2) and non-breeding females that had no access to a carcass gained less mass than breeding females that cared for a small brood (estimate  $\pm$  SE = -0.027  $\pm$  0.007 g,  $Z = -3.881$ ,



**FIGURE 2** Mean mass change (g)  $\pm$  SE during stage one of non-breeding females not provided with access to a carcass (no carcass), non-breeding females provided with access to a carcass (carcass), breeding females allocated a small brood (small brood), and breeding females allocated a large brood (large brood).

$p = .001$ ; Figure 2). Thus, our results may suggest that the benefit associated with gaining access to a carcass cancels out or even exceeds the cost of reproduction.

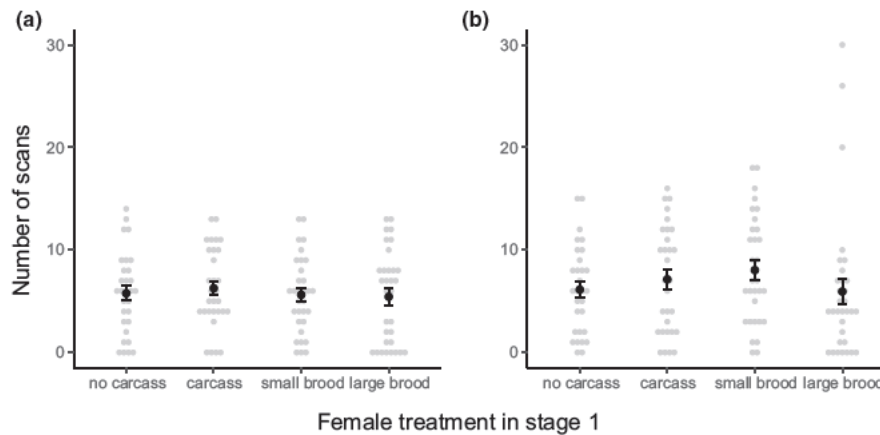
The average mass per larvae at dispersal was higher in small broods than in large broods ( $\chi^2 = 0.01$ ,  $df = 1$ ,  $p = .002$ ) but there was no difference in larval survival between small and large broods ( $\chi^2 = 1.07$ ,  $df = 1$ ,  $p = .301$ ).

### 3.2 | Time spent providing parental care in stage two

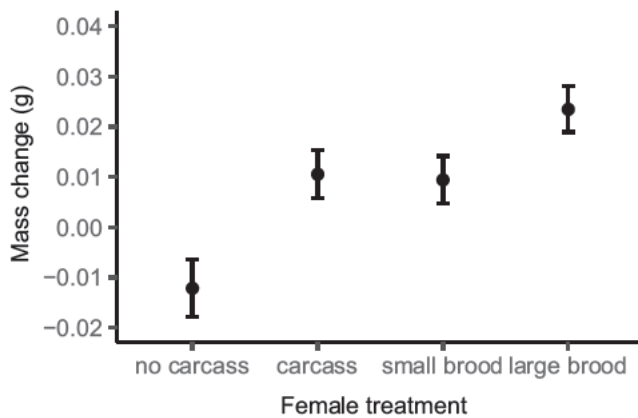
There was no evidence that the cost of increased reproductive allocation or the benefit of carcass access during stage one affected the amount of care provided by females during stage two of the experiment (Figure 3). There was no difference in the number of scans during the observations that females from different treatment groups spent provisioning food to larvae ( $\chi^2 = 1.72$ ,  $df = 3$ ,  $p = .632$ ; Figure 3) or maintaining the carcass ( $\chi^2 = 3.35$ ,  $df = 3$ ,  $p = .340$ ; Figure 3).

### 3.3 | Female mass change, egg-laying and offspring performance in stage two

Non-breeding females that had no access to a carcass during stage one gained less mass in stage two than breeding females that had cared for a large brood (estimate  $\pm$  SE = -0.036  $\pm$  0.007 g,  $t = -5.082$ ,  $p < .001$ ; Figure 4), breeding females that had cared for a small brood (estimate  $\pm$  SE = -0.022  $\pm$  0.007 g,  $t = -3.079$ ,  $p = .016$ ; Figure 4) and non-breeding females that had access to a carcass during stage one (estimate  $\pm$  SE = 0.023  $\pm$  0.007 g,  $t = 3.187$ ,  $p = .011$ ; Figure 4). Thus, females that benefitted from having had access to a carcass during stage one gained more mass during stage two. However, there was no evidence that the cost of caring for larvae in stage



**FIGURE 3** The number of scans during stage two observations in which the female was provisioning food to larvae (a) or maintaining the carcass (b) by females that, during stage one, were non-breeding and not provided with access to a carcass (no carcass), non-breeding and provided with access to a carcass (carcass), breeding and allocated a small brood (small brood), and breeding and allocated a large brood (large brood). Gray circles represent individual data, black circles and bars represent means  $\pm$  SE.



**FIGURE 4** Mean mass change (g)  $\pm$  SE during stage two of females that, during stage one, were non-breeding and not provided with access to a carcass (no carcass), non-breeding and provided with access to a carcass (carcass), breeding and allocated a small brood (small brood), and breeding and allocated a large brood (large brood).

one affected female mass gain in a future breeding attempt since there was no difference in mass change in stage two between breeding females that had previously cared for a small brood or a large brood during stage one (estimate  $\pm$  SE =  $-0.014 \pm 0.007$  g,  $t = -2.037$ ,  $p = .263$ ; Figure 4). There was also no difference in mass gain between non-breeding females that previously had access to a carcass and breeding females that previously cared for a small brood (estimate  $\pm$  SE =  $0.001 \pm 0.007$  g,  $t = 0.160$ ,  $p = .999$ ; Figure 4) or a large brood (estimate  $\pm$  SE =  $-0.013 \pm 0.007$  g,  $t = -1.844$ ,  $p = .406$ ; Figure 4), further supporting that the cost of caring for larvae in stage one did not affect female mass change during stage two.

There was no evidence that the cost of increased allocation or the benefit of carcass access during stage one affected the number of eggs females laid during stage two since there was no difference in the number of eggs laid by females from the different treatment groups ( $\chi^2 = 3.946$ ,  $df = 3$ ,  $p = .267$ ).

There was no evidence that the cost of increased reproductive allocation in stage one affected offspring performance in stage two since there was no difference in larval survival or average larval mass at dispersal in broods reared by breeding females that had previously cared for a large brood or a small brood during stage one (Table 1). There was also no evidence for a benefit associated with access to a carcass during stage one since there was no difference in larval survival or average larval mass at dispersal in broods reared by non-breeding females that had or did not have access to a carcass during stage one (Table 1). Nevertheless, there were some unexpected effects of our treatment of females during stage one on offspring performance during stage two. Non-breeding females that had no access to a carcass in stage one produced broods with a higher average larval mass than breeding females that previously cared for a small brood (Table 1). Additionally, broods reared by breeding females that had cared for a large brood during stage one had higher larval survival than broods reared by non-breeding females that had access to a carcass during stage one (Table 1).

### 3.4 | Female lifespan

There was no difference in lifespan between breeding females that cared for a large brood and breeding females that cared for a small brood (Table 2) or non-breeding females that had access to a carcass during stage one (Table 2). There was also no difference in lifespan between breeding females that cared for a small brood during stage one and non-breeding females that had access to a carcass during stage one (Table 2). As such, there was no evidence that the cost of caring for a larger brood during stage one had an impact on female lifespan. Furthermore, there was no difference in lifespan between non-breeding females that did not have access to a carcass during stage one and those that did (Table 2). This finding suggests that the benefit of having access to a carcass during stage one had no effect on female lifespan. However, non-breeding females that



TABLE 1 Pairwise comparisons between treatments for offspring performance at dispersal in stage two.

	Proportional larval survival				Average mass per larvae (g)			
	Estimate	SE	Z	p	Estimate	SE	Z	p
Small - large	-0.403	0.289	-1.391	.985	-0.003	0.004	-0.772	1.000
No carcass - large	-0.446	0.293	-1.522	.768	0.010	0.005	2.133	.210
Carcass - large	-0.911	0.289	-3.151	<b>.010</b>	-0.002	0.005	-0.367	1.000
No carcass - small	-0.043	0.286	-0.150	1.000	0.013	0.005	2.893	<b>.027</b>
Carcass - small	-0.508	0.282	-1.799	.432	0.002	0.005	0.393	1.000
Carcass - no carcass	-0.465	0.286	-1.629	.620	-0.011	0.005	-2.461	.092

Note: Statistically significant *p* values (<.05) are shown in bold.

TABLE 2 Pairwise comparisons between treatments for female lifespan.

	HR	95% CI		<i>p</i>
		Lower	Upper	
Large - small	1.496	0.747	2.995	.755
Large - carcass	1.257	0.632	2.504	.999
Small - carcass	1.881	0.948	3.736	.090
No carcass - carcass	1.126	0.565	2.248	.999
No carcass - small	2.119	1.048	4.289	<b>.029</b>
No carcass - large	1.418	0.715	2.809	.999

Note: Statistically significant *p* values (<.05) are shown in bold.

had no access to a carcass during stage one had shorter lifespans than breeding females that cared for a small brood during stage one (Table 2) suggesting that the benefit of having had access to a carcass exceeded the cost of rearing a small brood. There was no difference in lifespan between non-breeding females that had no access to a carcass and breeding females that cared for a large brood (Table 2) suggesting the cost of rearing a large brood was canceled out by the benefit of having access to a carcass.

## 4 | DISCUSSION

We found that breeding females that had cared for a large brood provided a similar amount of care during a subsequent breeding attempt as breeding females that had cared for a small brood and non-breeding females. Thus, our study provided no support for the suggestion that females shift the cost of reproduction as a whole, or the cost of caring for larvae specifically, onto future offspring by providing less care during future breeding attempts. Furthermore, we found that breeding and non-breeding females that had prior access to a carcass provided a similar amount of care as non-breeding females that did not have such access. Therefore, there was no evidence that the benefit of carcass access affected the level of post-hatching care females provided in a subsequent breeding attempt. Our results derive from an experimental design in which we

manipulated the cost of reproduction using well-established methodology (brood size manipulation). We are confident that our treatment had the intended effect since we found that breeding females that had cared for a large brood gained less mass than non-breeding females that had access to a carcass during stage one, while breeding females that had cared for a small brood gained an intermediate amount of mass. Our design also accounted for potential confounding effects due to the benefit associated with access to carrion by manipulating carcass access during stage one. This benefit was evident since non-breeding females with access to a carcass gained more mass than non-breeding females without access to a carcass. Finally, we measured effects on parental food provisioning to larvae and maintenance of the carcass, which are the most predominant parental care behaviors in this species and that are known to impact larval survival and growth (Andrews et al., 2017; Smiseth & Moore, 2002). In sum, we have confidence in our finding that *N. vespilloides* females provided similar levels of post-hatching parental care regardless of any previous costs associated with reproduction and benefits associated with carcass access.

Our main finding was that breeding females that had cared for a large brood provided a similar amount of care during a subsequent breeding attempt as breeding females that had cared for a small brood and non-breeding females. Thus, there was no support for the suggestion that a reduction in future ability to provide care is the mechanism allowing parents to shift the cost of reproduction onto future offspring as reported in prior work on beetles in the genus *Nicrophorus* (Billman et al., 2014; Creighton et al., 2009; Jenkins et al., 2000; Ward et al., 2009). There are several potential explanations for the lack of a difference in the amount of care provided by breeding females during stage two of the experiment. First, this could be due to a lack of response to brood size manipulation during stage one. This may be the case if females allocated a large brood during stage one do not to provide more care to maintain their own condition for future breeding opportunities, or if females always work at capacity to provide the highest level of care, in which case they would have little room to escalate the level of care if allocated a large brood. There may appear to be some evidence for this suggestion since average larval mass during stage one was higher in small broods than in large broods. However, we argue that this explanation is unlikely given there is good evidence from prior studies on



*N. vespilloides* showing that females provide more care when caring for an enlarged brood (Ratz & Smiseth, 2018; Richardson et al., 2019; Smiseth et al., 2007; Wang et al., 2021). This suggests that breeding females allocated a large brood during stage one would have spent more time providing care than breeding females allocated a small brood. Instead, the finding that average larval mass was higher in small broods than in large broods may simply reflect that there is a trade-off between number and size of offspring (Richardson & Smiseth, 2019; Smiseth et al., 2014).

Second, the lack of difference in the level of post-hatching care provided during stage two may reflect the nature of the resources used for breeding by *N. vespilloides*. This species breeds on carcasses of small vertebrates that are used as a food source for both parents and offspring (Scott, 1998). This means that females may gain a benefit associated with feeding from the carcass acquired for breeding. We anticipated such a benefit during both stages of our experiment. In stage one, this benefit was available to breeding females and non-breeding females provided with a carcass, but not to non-breeding females not provided with a carcass. Meanwhile, in stage two of our experiment, this benefit was available to all females since we provided all females with a carcass to initiate breeding. Thus, we anticipated seeing effects if the amount of care provided during stage two was affected by carcass access during both stages. Our findings suggest that this was not the case, possibly reflecting that carcass access during stage two had a much greater impact on the females' ability to provide post-hatching care than either the cost of reproduction or the benefit of carcass access during stage one. In other words, our results suggest that the carcass access associated with reproduction may buffer against any detrimental effects of costs or benefits due to increased allocation to reproduction or carcass access during previous breeding attempts on future post-hatching parental care.

Our study focused on the amount of care provided by females to larvae, which occurs after the female has had several days to feed and potentially recover from any reduction in condition due to previous allocation. In light of this, it may be important to consider whether the trait in question manifests before or after parents have had access to a carcass when investigating the potential costs of reproduction in *N. vespilloides*. For example while we found no effect of current allocation on the level of future post-hatching care females provide, there is evidence that increased allocation to current reproduction reduces a female's future ability to compete for a carcass (Richardson et al., 2020). This differential effect on the ability to compete for a carcass and the ability to provide care for larvae may reflect that competition over carcass possession happens before either party has had a chance to feed on the carcass (Safryn & Scott, 2000), while care for larvae happens after females have fed from the carcass. This idea could be investigated by testing the effect of previous reproductive allocation on a trait that could be measured both before and after parents have fed on a carcass during a subsequent breeding attempt such as the ability to defend the carcass from an intruder. Similar results may be expected in other capital breeders that breed on resources obtained prior to reproduction and where parents use these

as a food source. In contrast, different results might be expected for income breeders, such as many birds, where parents provision their offspring continuously throughout development with food obtained from the surrounding environment. In the latter species, increased allocation to current reproduction is likely to negatively affect the parents' future ability to provide parental care since parents face a trade-off between provisioning food to their offspring or consuming food for self-maintenance. We encourage future studies investigating whether shifting the cost of reproduction toward future offspring is mediated through a reduced future ability to provide care in species with a range of breeding strategies.

Given our finding that females with different levels of previous reproductive allocation provided the same level of care toward their offspring, we must consider alternative mechanisms for the finding that parents shift the cost of reproduction toward future offspring (Billman et al., 2014; Creighton et al., 2009; Jenkins et al., 2000; Ward et al., 2009). One explanation is that greater allocation to current reproduction may affect other aspects of future parental care. In *N. vespilloides*, allocation to current reproduction may affect future egg laying behavior since, like competitive ability, egg laying occurs soon after the discovery of the carcass and when females may have had limited opportunities to recover from any reduction in condition due to previous allocation. Although we found no effect of previous reproductive allocation on the number of eggs females laid during stage two, there may be effects on the pattern of laying or the size of the eggs females produce in a subsequent breeding attempt. For example, females that allocate more to current reproduction may produce smaller eggs or delay the onset of egg laying in future breeding attempts (Mäenpää & Smiseth, 2017). In other species, alternative mechanisms include other behavioral traits, such as a reduction in ability to compete for resources necessary for reproduction (Fokkema et al., 2016) or physiological traits such as a reduction in ornamentation quality limiting breeding opportunities (Siefferman & Hill, 2005). We encourage future work on a variety of taxa investigating alternative mechanisms that could cause increased allocation to current reproduction to result in the often-observed reduction in future fecundity.

There were some unexpected results from our experiment. First, females that were previously provided access to a carcass (regardless of whether they were breeding or not) gained more mass when provided with a second carcass than females that were not previously provided with a carcass. This finding does not fit our predictions for the effects of the cost of increased allocation or the benefit of carcass access during stage one. Instead, this result suggests that females that previously had access to a carcass responded by shifting toward greater allocation on future reproduction since mass gain during breeding is a proxy for allocation to future reproduction in *N. vespilloides* (Billman et al., 2014; Creighton et al., 2009). This shift may reflect that females responded to the presence of carcasses as an environmental cue about future breeding opportunities. Carcasses are normally a rare resource (Scott, 1998), and coming across two in quick succession may provide females with a cue that they find

themselves in a resource-rich environment. In support of this suggestion, a previous study on *N. vespilloides* found that the quality of the carcass used for a breeding attempt influences reproductive investment in a subsequent breeding attempt, potentially by providing information about the resources available (Billman et al., 2014).

Second, breeding females that were allocated a small brood in stage one produced broods with a lower average larval mass in stage two than non-breeding females that did not have access to a carcass in stage one. This finding appears to contrast with previous studies, which found that increased allocation to current reproduction is associated with a reduction in the size and mass of future broods (Billman et al., 2014; Creighton et al., 2009; Jenkins et al., 2000; Ward et al., 2009). However, we note that we provided females with foster broods of a standardized size during stage two. This was an important aspect of our design as it allowed us to control for any potential confounding effects on female behavior due to variation in brood size during stage two. In contrast, prior studies manipulated aspects of reproductive allocation during a current breeding attempt and then allowed individuals to raise their natural brood during a subsequent breeding attempt or throughout multiple breeding attempts for the rest of their lifetime. Our results are therefore not comparable with those of prior studies. We also found that breeding females that were allocated a small brood in stage one had a longer lifespan than non-breeding females that were not provided access to a carcass in stage one. This may appear to contrast with the results of Creighton et al. (2009), which showed non-reproducing females to live longer than reproducing females. However, Creighton et al. (2009) compared the lifespan of reproducing females and females that never reproduced, whereas we compared females that did not reproduce initially but were allowed to reproduce during stage two with females that reproduced across both stages.

Why did breeding females allocated a small brood in stage one produce broods with a lower average larval mass in stage two and have a longer lifespan than non-breeding females without access to a carcass in stage one? We suggest that these results reflect the combined effects of breeding females shifting toward greater allocation in future reproduction, breeding females caring for a small brood in stage one suffering a lower cost of caring for larvae than breeding females caring for a large brood, and non-breeding females with access to a carcass responding to this treatment as a failed breeding attempt. As discussed above, breeding females may have shifted toward greater allocation in future reproduction if the presence of carcasses acts as an environmental cue about abundant future breeding opportunities. However, this alone cannot explain our finding since we would also expect breeding females that cared for a large brood and non-breeding females with access to a carcass to respond similarly, which was not the case. We therefore suggest that breeding females caring for a small brood suffered a lower cost of caring for larvae than breeding females caring for a large brood. Finally, we suggest that non-breeding females with access to a carcass responded differently from breeding females given that they produced no larvae during stage one, and therefore may have perceived this as a failed breeding attempt.

In conclusion, our study shows that females maintain the level of parental care they provide regardless of any costs associated with previous reproductive allocation or any benefits associated with prior resource access. This is likely a result of the breeding strategy of *N. vespilloides* since they have access to a fixed food source during breeding which may facilitate their recovery from any costs associated with previous reproductive allocation. Our findings highlight the need for more work exploring the potential mechanisms that allow parents to shift the cost of current reproduction toward future offspring in species with a variety of life-history strategies.

#### AUTHOR CONTRIBUTIONS

**Per T. Smiseth:** Conceptualization (supporting); formal analysis (supporting); methodology (supporting); supervision (lead); writing – review and editing (equal). **Georgia A. Lambert:** Conceptualization (lead); data curation (lead); formal analysis (lead); methodology (lead); writing – original draft (lead); writing – review and editing (equal).

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#### CONFLICT OF INTEREST

None.

#### OPEN RESEARCH BADGES



This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at <https://doi.org/10.5061/dryad.gtht76hqc>.

#### DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.gtht76hqc>.

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Original Article

# Flexible females: nutritional state influences biparental cooperation in a burying beetle

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In species that provide biparental care, there is a sexual conflict between parents over how much each should contribute toward caring for their joint offspring. Theoretical models for the resolution of this conflict through behavioral negotiation between parents assume that parents cannot assess their partner's state directly but do so indirectly by monitoring their partner's contribution. Here, we test whether parents can assess their partner's state directly by investigating the effect of nutritional state on cooperation between parents in the burying beetle *Nicrophorus vespilloides*. We used a two-by-two factorial design, in which a well-fed or food-deprived female was paired with a well-fed or food-deprived male. We found that females adjusted their level of care in response to both their own nutritional state and that of their partner and that these decisions were independent of their partner's contribution. We found no evidence that males responded directly to the nutritional state. Males instead responded indirectly based on the contribution of their partner. Our results suggest that parents are able to assess the state of their partner, in contrast to what has been assumed, and that these assessments play an important role in the mediation of sexual conflict between caring parents.

**Key words:** cooperation, nutritional state, *Nicrophorus vespilloides*, parental care, sexual conflict.

## INTRODUCTION

There is sexual conflict between parents who provide biparental care since the benefits of care depend on the combined effort of the two parents, whereas the costs of future reproduction are paid individually (Houston et al. 2005; Lessells 2012). Parents are, therefore, under selection to shift as much of the workload as possible onto their partner (Parker 1985). Theoretical models have identified several mechanisms by which this conflict may be resolved: negotiation, matching, or a sealed-bid decision. Negotiation involves a parent responding to a decrease in the amount of care contributed by its partner by increasing its own contribution but only such that it incompletely compensates for the partner's decrease (McNamara et al. 1999). Matching involves a parent responding to a change in its partner's contribution by increasing when its partner increases or decreasing when its partner decreases its contribution (Johnstone and Hinde 2006). A sealed-bid decision is when a parent makes an initial decision about how much care to provide that is independent of its partner's contribution (Houston and Davies 1985). There is empirical evidence supporting negotiation (Harrison et al. 2009; Pilakouta et al. 2015), matching (Hinde 2006; Lendvai et al. 2018), and sealed-bid decisions (Schwagmeyer et al. 2002; Matthey and Smiseth 2015; Pilakouta et al. 2015) across different taxa; however,

in birds, the most studied taxonomic group, negotiation is thought to be the predominant mechanism for cooperation (Harrison et al. 2009).

Variations in the state of the parents may play an important role in determining how sexual conflict over care is resolved. Here, we refer to “state” as any attribute of an individual, such as its nutritional status, body size, inbreeding status, infection status, or age, which is likely to affect its contribution toward care and/or its partner's contribution. A focal parent is likely to adjust its contribution based on its own state since variation in state may influence the cost and/or benefit functions associated with providing care (Smiseth 2017). The focal parent may also adjust its contribution based on its partner's state since this may provide information about the likely future contribution of its partner and/or the potential value of the joint brood. Theoretical models for negotiation as a mechanism of conflict resolution assume that a focal parent cannot assess its partner's state directly and that it does so indirectly by monitoring its contribution (McNamara et al. 1999; McNamara and Wolf 2022). These models emphasize that variation in the state of parents plays a key role in promoting the evolution of negotiation as a behavioral mechanism of conflict resolution when the focal parent cannot assess its partner's state directly. Meanwhile, more recent research has shown that a focal parent can respond directly to variation in multiple states of its partner and that it also independently responds to its partner's contribution (Matthey and

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Smiseth 2015; Pilakouta et al. 2015). Thus, there is a need for more work exploring the role of state in negotiation in particular, and the mediation of sexual conflict between parents in general.

Here, we investigate the effects of a temporary and reversible state, that is, nutritional state, on the dynamics of biparental cooperation. Prior studies investigating the effects of state on biparental cooperation have focused on permanent states, such as inbreeding status and adult body size, which remain constant throughout adulthood. For a permanent state, we expect a focal parent to adjust its contribution to its own state and that of its partner since state affects the ability to provide care. In support of this, previous studies have found that individuals adjust the amount of care they contribute based on their own body size and that of their partner (Pilakouta et al. 2015) and the inbreeding status of their partner (Mattey and Smiseth 2015). In contrast, we might expect different dynamics for a temporary and reversible state, such as being malnourished or infected with a pathogen, given that a focal individual may improve its state by reducing its own contribution to care. Thus, for temporary and reversible states, biparental care may facilitate recovery by allowing a malnourished or infected individual to invest more in its own recovery, thereby shifting a greater amount of the workload over to its partner.

We tested this idea by manipulating the nutritional state of females and males of the burying beetle *Nicrophorus vespilloides* and measuring the effect on how a focal parent responded to its own state and that of its partner. Beetles in the genus *Nicrophorus* are well suited to test this idea. Firstly, parents provide biparental care, and the level of care parents provide is flexible. *Nicrophorus vespilloides* breeds on a small vertebrate carcass that serves as a joint food source for both parents and their offspring. Female and male parents provide care for their offspring, including preparing and maintaining the carcass by spreading antimicrobials onto it, provisioning pre-digested carrion to their offspring, and guarding their offspring against conspecific intruders (Eggert et al. 1998; Scott 1998). Although both parents are capable of all activities, females tend to spend more time food provisioning to the larvae (Eggert et al. 1998; Smiseth and Moore 2002; Smiseth et al. 2005), while males spend more time maintaining the carcass (Smiseth et al. 2005). Secondly, it is relatively straightforward to manipulate an individual's nutritional state by simply subjecting them to food deprivation for 7 days before breeding. Prior work also shows that food-deprived parents feed more during breeding than well-fed parents (Keppner et al. 2018) and that females respond to their own nutritional state since food-deprived females spend less time maintaining the carcass and provisioning food to their larvae in comparison to well-fed females (Richardson and Smiseth 2019a). However, there is no information on the effects of nutritional state on the dynamics of biparental cooperation.

Our aim was to test whether parents adjust the level of care they provide based on the nutritional state of their partner and whether this was conditional upon their own nutritional state. To meet this aim, we used a two-by-two factorial design where a well-fed or food-deprived female was paired with a well-fed or food-deprived male. We predict that a food-deprived focal parent will decrease the level of care it provides when paired with a well-fed partner since its partner is able to compensate for a reduction in care due to the poor state of the focal parent. In contrast, a food-deprived focal parent may not be able to decrease the level of care it provides when paired with a food-deprived partner since its partner is less able to compensate. We also predict that the partner of a food-deprived parent will incompletely compensate for the expected

lower level of care provided by the food-deprived parent and that food-deprived individuals would provide less care than well-fed individuals since food-deprived individuals are likely to pay a higher cost of providing care (Richardson and Smiseth 2019a). To determine whether a focal parent assessed its partner's state directly or indirectly by monitoring its partner's contribution, we added the partner's contribution to the model to test whether it accounted for any observed effects of the partner's state on the focal parent. We tested if food-deprived individuals prioritize improving their own nutritional state when compared to well-fed parents as a means to recover from any potential costs of food deprivation to their ability to invest in future reproduction. We predict that food-deprived individuals would consume more carrion and thus gain more mass during breeding than well-fed individuals. Finally, we tested for the effects of nutritional state on the size and quality of the joint brood. We expect that the broods of food-deprived parents will be smaller and of worse quality since we expect food-deprived parents to provide less care and to consume more carrion (a joint food source), which would reduce the amount of food available for the larvae.

## METHODS

### General methodology

We used beetles from an outbred laboratory population originally collected in Edinburgh, UK and maintained at the University of Edinburgh. We housed all adults in the stock population individually in clear plastic containers (12 cm × 8 cm × 2 cm) lined with moist soil and fed them raw organic beef twice a week. The stock population was kept at 20 °C under a 16:8 h light:dark cycle.

### Experimental design

We used a two-by-two factorial design with the following treatments: a food-deprived female paired with a food-deprived male ( $n = 30$ ), a food-deprived female paired with a well-fed male ( $n = 30$ ), a well-fed female paired with a food-deprived male ( $n = 32$ ), and a well-fed female paired with a well-fed male ( $n = 31$ ). All individuals used in the experiment were at least 10 days post-eclosion to ensure they had reached sexual maturity and that feeding treatment had no effect on the rate of maturation. We weighed all individuals before assigning them to one of the four treatments in our experiment. We used established protocols to produce well-fed and food-deprived females and males. Food-deprived individuals were not fed during the 7-day treatment, whereas well-fed individuals were fed twice with organic beef (approximately 0.3 g) during this period. This level of food deprivation was chosen since it leads to a significant drop in weight without causing an increase in mortality (Richardson and Smiseth 2019a, 2019b). After 7 days, we weighed all individuals to measure their post-treatment and pre-breeding mass. We used this to calculate weight change during the treatment period and confirm that our food deprivation treatment had the intended effect on the nutritional state (see Results).

Immediately after weighing, we paired up males and females at random, taking care to avoid mating between close relatives. We transferred each pair into a clear plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil. Each pair was provided with a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (15–20 g;  $M \pm SE = 18.47 \pm 0.11$  g) to initiate breeding. After 48 h, when the eggs had been laid but before the larvae had begun hatching, we moved the female, the male, and their carcass into a new clear plastic container (17 cm ×

12 cm × 6 cm) lined with fresh moist soil. We allocated each pair a foster brood consisting of 20 newly hatched larvae from at least two different mothers. We chose this brood size since it is close to the average brood size for *N. vespilloides* (21 larvae; Smiseth and Moore 2002). We used a standardized brood size to control for potential confounding effects due to variation in brood size. Such confounding effects might arise because our treatment might affect the number of eggs laid (Steiger et al. 2007) and because brood size is known to affect the amount of care provided by parents (Smiseth and Moore 2002). We allocated a foster brood to a pair only after their eggs had started hatching since parents use temporal kin recognition and so would kill larvae that arrive at the carcass before their own eggs started hatching (Müller and Eggert 1990).

We conducted behavioral observations 24 h ( $\pm$  15 min) after we allocated pairs a foster brood since this is when parents provide the highest level of care in this species (Smiseth et al. 2003). We did the observations under red light using instantaneous sampling of female and male behavior every minute for 30 min consistent with established protocols (Smiseth and Moore 2002). At each scan, we recorded whether females and males were providing direct care, indirect care, or consuming carrion. We used the number of scans as a proxy for the amount of time an individual spent providing care or consuming carrion. Direct care included provisioning food to the larvae (mouth-to-mouth contact between the parent and at least one larvae) and grooming the larvae. Indirect care included maintaining the carcass (spreading antimicrobial secretions onto its surface or modifying the position of the carcass) and guarding the brood and carcass against competitors or predators (standing still on the carcass facing away from the brood). After the observations, we left the pairs to care for their brood until the larvae dispersed from the carcass approximately 5 days later, upon which we recorded average larval mass and the proportion of larvae that survived to dispersal as measures of offspring performance, and female and male post-breeding mass to allow us to calculate individual mass change during breeding.

### Statistical analysis

All statistical analyses were conducted using R version 3.6.1 (R Core Team 2021) with the packages *car* (Fox and Weisberg 2019), *MASS* (Venables and Ripley 2022), and *glmmTMB* (Brooks et al. 2017). We used zero-inflated binomial models in our analyses on the amount of time spent providing direct care by females and males since the data for this behavior showed minor zero inflation. We used binomial models in our analysis on time spent providing indirect care and consuming carrion by females and males and larval survival to dispersal. In all these models, we included observation level as a random effect to account for over-dispersion (Harrison 2015). We used linear models for data on female and male mass change during the food deprivation treatment, female and male mass change during breeding, and mean larval mass at dispersal.

To determine whether the focal parent responded directly to the nutritional state of its partner or indirectly to its partner's contribution, we compared models where we included and excluded the amount of time spent providing direct or indirect care or consuming carrion by the partner as a factor (Mattey and Smiseth 2015; Pilakouta et al. 2015). If including this factor reduced or removed any effect of the partner's nutritional state on the amount of time spent providing direct or indirect care or consuming carrion by the focal parent, we interpreted this as evidence that the focal parent responded indirectly to the contribution of its partner,

as expected by theoretical models of negotiation. However, if including this factor did not negate the effect of the partner's nutritional state on the amount of time spent providing direct or indirect care or consuming carrion by the focal parent, we interpreted this as evidence that the focal parent responded directly to the nutritional state of its partner. We note that our data on the responses of the focal parent to its partner's contribution are correlational and that we therefore cannot demonstrate a causal relationship between the contributions of females and males to parental care.

## RESULTS

### Effects of food deprivation on male and female mass change

Food-deprived females and males lost more mass during the treatment period than well-fed females and males (females: estimate =  $-0.052 \pm 0.005$  g,  $t = -11.11$ ,  $P < 0.001$ , males: estimate =  $-0.035 \pm 0.004$  g,  $t = -7.96$ ,  $P < 0.001$ ). This confirms that the food deprivation treatment had the intended effect of altering an individual's nutritional state. There was no difference between female and male mass change during the food deprivation treatment (estimate =  $0.009 \pm 0.004$  g,  $t = 1.63$ ,  $P = 0.105$ ).

### Effects of nutritional state on parental care and cooperation

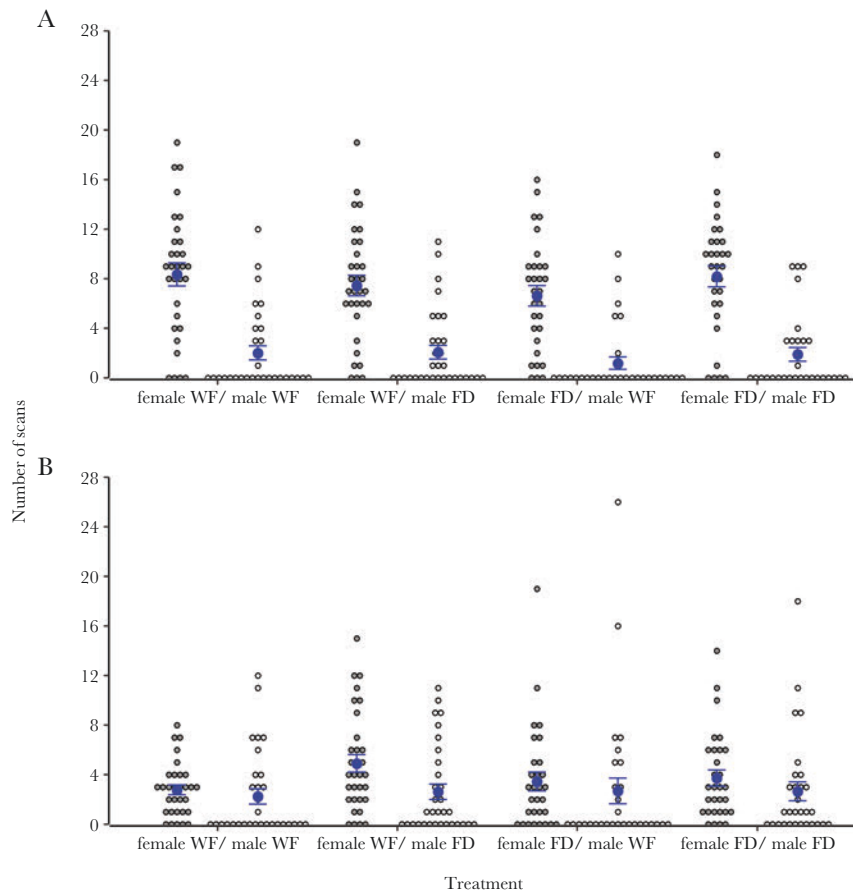
There was a significant effect of the interaction between a female's nutritional state and the nutritional state of its partner on the amount of time spent providing direct care (Table 1). The estimate of this interaction effect was positive (Table 1), indicating that food-deprived females responded to being paired with a food-deprived male, rather than well-fed male, by more strongly increasing the amount of time they spent providing direct care than did well-fed females. Indeed, visual inspection of Figure 1 shows that food-deprived females spent more time providing direct care when paired with a food-derived male than when paired with a well-fed male, while well-fed females provided similar levels of care regardless of whether they were paired with food-deprived or well-fed males. Thus, females responded to the nutritional state of their partner, but any such response was conditional upon the female's own nutritional state. There was also a significant main effect of female nutritional state on the amount of time spent providing direct care by females (Table 1, Figure 1). This finding is due to the interaction described above. There was no significant effect of the interaction between a male's nutritional state and the nutritional state of its partner on the amount of time spent providing direct care (Table 1, Figure 1). There was no main effect of the partner's nutritional state on the amount of time females and males spent providing direct care (Table 1, Figure 1). There was also no main effect of male nutritional state on the amount of time spent providing direct care by males (Table 1, Figure 1).

There was no effect of the interaction between a female's nutritional state and the nutritional state of its partner on the amount of time females spent providing indirect care; however, females provided more indirect care when paired with a food-deprived male (Table 1, Figure 1). Thus, females altered the amount of indirect care they provided in response to their partner's nutritional state but did so regardless of their own nutritional state. There was no significant effect of the interaction between a male's nutritional state and the nutritional state of its partner on the amount of time

**Table 1**

**Summary of statistical tests for the effects of nutritional state on bi-parental cooperation over providing direct and indirect care excluding and including (\*) partner's contribution in the model. The reference category for the focal parent's nutritional state and the partner's nutritional state was "well-fed." Statistically significant *P* values (<0.05) are shown in bold.**

Behavior	Focal parent's nutritional state				Partner's nutritional state				Interaction				Partner's contribution			
	Est	SE	<i>z</i>	<i>P</i>	Est	SE	<i>z</i>	<i>P</i>	Est	SE	<i>z</i>	<i>P</i>	Est	SE	<i>z</i>	<i>P</i>
Female direct	-0.41	0.19	-2.11	<b>0.035</b>	-0.24	0.19	-1.26	0.208	0.63	0.27	2.33	<b>0.020</b>	-0.08	0.03	-2.42	<b>0.016</b>
Female direct*	-0.43	0.20	-2.12	<b>0.034</b>	-0.19	0.20	-0.98	0.325	0.60	0.28	2.13	<b>0.033</b>	-0.08	0.03	-2.42	<b>0.016</b>
Female indirect	0.11	0.31	0.35	0.724	0.65	0.29	2.22	<b>0.027</b>	-0.47	0.42	-1.12	0.262	-0.00	0.03	-0.10	0.917
Female indirect*	0.11	0.31	0.35	0.723	0.65	0.29	2.22	<b>0.027</b>	-0.47	0.42	-1.12	0.262	-0.00	0.03	-0.10	0.917
Male direct	-0.23	0.34	-0.67	0.503	0.15	0.47	0.31	0.753	-0.07	0.58	-0.12	0.908	-0.07	0.03	-2.38	<b>0.017</b>
Male direct*	-0.17	0.33	-0.54	0.593	0.10	0.46	0.22	0.827	-0.06	0.57	-0.11	0.912	-0.07	0.03	-2.38	<b>0.017</b>
Male indirect	0.44	0.66	0.66	0.507	-0.12	0.69	-0.18	0.859	0.16	0.94	0.17	0.865	-0.01	0.07	-0.14	0.887
Male indirect*	0.45	0.67	0.68	0.498	-0.12	0.69	-0.17	0.865	0.15	0.95	0.16	0.876	-0.01	0.07	-0.14	0.887

**Figure 1**

Comparison of the number of scans (out of 30) spent providing direct care (A) and indirect care (B) by well-fed (WF) or food-deprived (FD) females (gray points) and well-fed or food-deprived males (white points) caring for larvae during a 30-min behavioral observation (mean  $\pm$  SE).

spent providing direct care (Table 1, Figure 1). There was no main effect of the partner's nutritional state on the amount of time males spent providing indirect care (Table 1, Figure 1). There was also no main effect of focal parent nutritional state on the amount of time spent providing indirect care by females or males (Table 1, Figure 1).

The partner's contribution was also a predictor of female care, suggesting that females responded to the contribution of their partner. Females spent less time providing direct care as the amount of time males spent providing direct care increased (Table 1). Including or excluding the partner's contribution did not alter the effect of the interaction between the focal parent's



nutritional state and the nutritional state of the partner on the amount of direct care provided by females (Table 1). This suggests that females responded independently to the state and contribution of their partner. Partner contribution was a predictor of male care, suggesting that males also responded to the contribution of their partner. Males spent less time providing direct care as the amount of time females spent providing direct care increased (Table 1). There was no relationship between the partner's contribution and the amount of time spent providing indirect care by females or males (Table 1).

### Male and female mass change during breeding and time spent consuming carrion

In contrast to our predictions, there was no effect of the focal parent's nutritional state, the partner's nutritional state or the interaction between the two on the amount of time spent consuming carrion by females or males (Table 2). However, in line with our predictions, the female nutritional state affected female mass change during breeding with food-deprived females gaining more mass than well-fed females (Table 2, Figure 2). There was no effect of the male nutritional state on male mass change during breeding (Table 2). There was no effect of the interaction between the focal parent's nutritional state and the partner's nutritional state on the mass change of females or males during breeding (Table 2). There was also no effect of the partner's nutritional state on the mass change of females or males during breeding (Table 2).

### Offspring performance

Finally, we tested for the effects of the parents' nutritional state on offspring performance upon dispersal. Average larval mass at dispersal was higher in broods cared for by food-deprived females (estimate =  $0.019 \pm 0.007$  g,  $t = 2.73$ ,  $P = 0.007$ ). There was no effect of male nutritional state (estimate =  $0.006 \pm 0.007$  g,  $t = 0.86$ ,  $P = 0.392$ ), and no effect of the interaction between female and male nutritional state (estimate =  $-0.016 \pm 0.010$  g,  $t = -1.668$ ,  $P = 0.098$ ) on average larval mass at dispersal. The proportion of larvae surviving to dispersal was not dependent on female nutritional state (estimate =  $0.060 \pm 0.236$  g,  $z = 0.25$ ,  $P = 0.801$ ), male nutritional state (estimate =  $0.425 \pm 0.234$  g,  $z = 1.82$ ,  $P = 0.069$ ),

or the interaction between the two (estimate =  $-0.159 \pm 0.334$  g,  $z = -0.48$ ,  $P = 0.634$ ).

## DISCUSSION

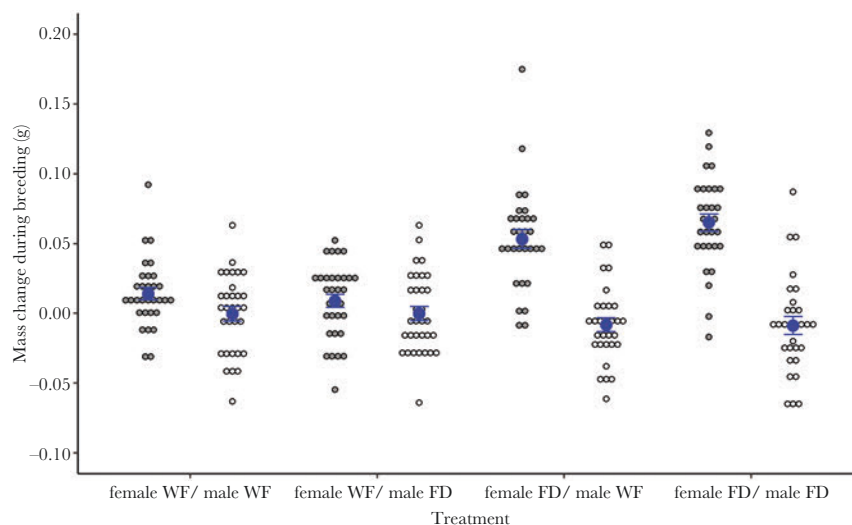
We found that females adjusted the amount of direct care they provided in response to the nutritional state of their partner and that this was conditional upon their own nutritional state. This interaction effect was likely driven by food-deprived females paired with well-fed males providing less care than food-deprived females paired with food-deprived males, while well-fed females provided similar levels of care regardless of whether they were paired with well-fed or food-deprived males. This finding is in line with our prediction that food-deprived individuals would reduce the amount of time they spend providing care if their partner is well-fed and is therefore capable of increasing its contribution. Reducing their contribution to care when paired with a well-fed male would enable food-deprived females to invest more into their own recovery whilst minimizing any detrimental effects to the joint brood. In support of this suggestion, food-deprived females gained more mass during breeding than well-fed females, which we will discuss in greater detail below. We also found that the amount of time females spent providing direct care was affected by the partner's contribution. Females spent less time providing direct care when males spent more time providing direct care as predicted by negotiation models (McNamara et al. 1999). The inclusion of partner's contribution in the model had little, if any, impact on the effect of the interaction or the partner's state on the amount of time females spent providing direct care. This suggests that females responded directly to the nutritional state of their partner and that this response was independent of the response to the contribution of their partner.

Our results show that females adjust the amount of care they provide in response to the nutritional state of their partner and that they also independently respond to their partner's contribution. This is interesting for several reasons. Firstly, our results add to growing evidence that responses to partner's state and contribution are not mutually exclusive mechanisms for mediating conflict between caring parents (Mattey and Smiseth 2015; Pilakouta et al. 2015). A potential explanation for why parents respond to their partner's state, as well as their partner's contribution, is that

**Table 2**

**Summary of statistical tests for the effects of nutritional state on bi-parental cooperation over consumption excluding and including (\*) partner's contribution in the model and mass change during breeding. The reference category for the focal parent's nutritional state and the partner's nutritional state was "well-fed." Statistically significant  $P$  values ( $<0.05$ ) are shown in bold.**

Behavior	Focal parent's nutritional state				Partner's nutritional state				Interaction				Partner's contribution			
	Est	SE	Test statistic	$P$	Est	SE	Test statistic	$P$	Est	SE	Test statistic	$P$	Est	SE	$z$	$P$
Female consumption	0.22	0.20	$z = 1.10$	0.273	-0.07	0.20	$z = -0.36$	0.716	-0.02	0.28	$z = -0.05$	0.957				
Female consumption*	0.22	0.20	$z = 1.08$	0.280	-0.09	0.20	$z = -0.43$	0.668	-0.00	0.28	$z = -0.01$	0.989	0.00	0.01	0.34	0.733
Female mass change	0.04	0.01	$t = 5.13$	<b>&lt; 0.001</b>	-0.01	0.01	$t = -0.66$	0.509	0.02	0.01	$t = 1.54$	0.125				
Male consumption	0.78	1.28	$z = 0.61$	0.541	-0.61	1.22	$z = -0.50$	0.616	0.67	1.81	$z = 0.37$	0.713				
Male consumption*	0.83	1.28	$z = 0.65$	0.514	-0.54	1.22	$z = -0.44$	0.657	0.64	1.79	$z = 0.36$	0.720	-0.08	0.08	-1.02	0.309
Male mass change	0.00	0.01	$t = 0.11$	0.915	-0.01	0.01	$t = -1.48$	0.141	-0.00	0.01	$t = -0.25$	0.802				



**Figure 2**

Comparison of the mass change during breeding by well-fed (WF) or food-deprived (FD) females (gray points) and well-fed or food-deprived males (white points) (mean  $\pm$  SE).

state and contribution may provide somewhat different information about the partner's expected future contribution to care (Pilakouta et al. 2015). Alternatively, parents may respond to their partner's contribution to coordinate the distribution of parental care over time rather than to gain information about their partner's expected contribution (Smiseth 2019). Thus, using two complementary mechanisms may enable parents to make a more accurate assessment of their partner's expected contribution or the timing of their contribution and allow them to fine-tune their response. Secondly, our results are in contrast with theoretical models of negotiation, which assume that parents assess the state of their partner indirectly based on their partner's contribution (McNamara et al. 1999). Currently, experiments focus on manipulating the contribution of a focal parent, typically via handicapping or mate removal, and then measuring any responses by its partner (Harrison et al. 2009). These designs may produce evidence that is biased toward negotiation since they negate the opportunity to test whether parents directly assess and respond to the state of their partner. As such, we suggest that future experiments are designed to reflect that multiple mechanisms may be involved in resolving sexual conflict, including direct responses to state as shown here. In a laboratory environment, this could be achieved by manipulating and measuring the effect of state, in addition to partner contribution, on parental behavior. In situations where manipulating state is impractical or unethical, including observational data on parental state in addition to data on partner contribution may also provide further insight. In sum, our findings highlight the need for more work exploring how multiple mechanisms may play a role in the dynamic balance between cooperation and conflict in species with biparental care.

We found a different pattern for indirect care by females in comparison to the results for direct care by females as discussed above. Females spent more time providing indirect care when paired with a food-deprived partner, regardless of their own nutritional state and their partner's contribution. This result confirms that females adjust their contribution based on the state of their partner, although, in this case, this response was independent of their own state. The absence of an interaction effect between the focal parent nutritional

state and partner nutritional state is surprising given that we expected individuals to respond to their partner being food-deprived only if they were well-fed and, therefore, capable of increasing their contribution. We found that both food-deprived and well-fed females increased the amount of time they spent providing indirect care when paired with a food-deprived male. A potential explanation is that providing indirect care is less energetically costly to females than providing direct care, and so they were able to increase the amount of indirect care they provide in response to male state even when food-deprived. Multiple studies have explored the cost of increased brood size (a strong predictor of the amount of direct care females provide) (Ward et al. 2009; Ratz and Smiseth 2018), but there is no information on the cost of providing indirect care alone or a direct comparison between direct and indirect care. To explore this idea, future work could test the energetic cost of providing direct and indirect care, potentially by simultaneously manipulating brood size and carcass size and measuring the effect on females.

Our results imply that females can assess the nutritional state of their partner and add to evidence that parents in *N. vespilloides* can assess and respond to various states of their partner, such as their inbreeding status and body size, independently of the partner's contribution (Mattey and Smiseth 2015; Pilakouta et al. 2015). This raises questions as to how females do so. Our study was not designed to investigate the potential mechanisms that could be involved. Nevertheless, based on prior work, we suggest that cuticular chemicals are likely candidates. There is good evidence that cuticular chemicals play an important role in partner recognition in *N. vespilloides* (Steiger et al. 2007; Keppner et al. 2017), and there is also evidence that cuticular hydrocarbons are indicators of diet (Steiger et al. 2007; Fedina et al. 2012) and health (Beani et al. 2019) in *N. vespilloides* as well as other insects. Thus, it seems likely that malnutrition may alter an individual's cuticular chemical profile, thereby allowing its partner to judge its nutritional state. We suggest future work further investigates the potential role of chemical cues as a mechanism mediating how parents are able to assess various cryptic states of their partner.

We found that males adjusted the amount of care they provided in response to the contribution of their partner, but there was no evidence that males responded to their partner's nutritional state, their own nutritional state, or the interaction between the two. Instead, males decreased the time spent providing direct care as the amount of time females spent providing direct care increased, as predicted by negotiation models (McNamara et al. 1999). One explanation for this result is that males are unable to directly respond to the state of their partner and instead rely on the contribution of their partner as a measure of their ability to provide care. However, this seems unlikely given that females responded directly to the nutritional state of their partner, and that prior studies on *N. vespilloides* show that males respond to other components of their partner's state (Mattey and Smiseth 2015; Pilakouta et al. 2015). Our results add to a growing list of sex differences in caring behavior in *N. vespilloides* (Smiseth and Moore 2004; Walling et al. 2008; Shippi et al. 2018). Currently, we have a poor understanding of what may be driving these differences, and this is an area that would warrant further work.

The main aim of this study was to investigate whether the effects of temporary states on the dynamics of biparental cooperation differ from those reported previously for permanent states (Mattey and Smiseth 2015; Pilakouta et al. 2015). We expected differences given that a parent has the opportunity to improve its state by reducing its contribution toward providing care for temporary and reversible states but not for permanent states. As such, we expected food-deprived individuals to provide less care than those in a comparably poor permanent state since, in addition to their reduced ability to provide care, they also have the incentive to invest in their own state whilst their partner incompletely compensates. There are both similarities and differences between the effects of nutritional state and previously tested permanent states. We found that food-deprived females spent less time providing direct care when paired with a well-fed male. Similarly, a previous study found that small females spend more time providing direct care when paired with a small male (Pilakouta et al. 2015). In both cases, females in a comparatively worse state only reduced the level of care they provided if their partner was in a better state and was able to compensate. In contrast, previous work found that inbred females spent the same amount of time providing direct care regardless of their partners' state but that outbred females provided more care when paired with an inbred male (Mattey and Smiseth 2015). The challenge with making comparisons across different components of state is that this requires some way to calibrate the impact of states on the individual's ability to provide care. For example, we cannot know whether our treatment of 7 days of food deprivation is equivalent to being of a particular size difference or a particular difference in inbreeding coefficients. As such, it is difficult to make true comparisons of the impact of nutritional state and body size or inbreeding state on parental cooperation. To overcome this, future studies may use a standardized test, such as measuring some aspect of performance, to calibrate the effect of different states on individuals. This would allow more accurate comparisons of any differential effects of temporary and permanent states on parental cooperation.

Parental mass change over the breeding attempt provided some insight into whether food-deprived individuals used the breeding attempt as an opportunity to recover from being in a temporarily worse state. We found no effect of the interaction between female and male nutritional state on female or male mass change during

breeding to support this suggestion. However, food-deprived females gained more mass during breeding than well-fed females. In contrast, Keppner et al. (2018) found that females paired with food-deprived males weighed less at the end of a breeding attempt than those paired with well-fed males. This difference is likely a result of the smaller carcass size (8.5–11.5 g) used in Keppner et al. (2018) than in our study (15–20 g), causing greater competition for limited resources among females and males in Keppner et al. (2018). Our results show that females recovered from food deprivation when breeding but that this response was independent of any male assistance. This recovery as well as the lack of an interaction effect may reflect that *N. vespilloides* breeds on small vertebrate carcasses that provide a food source for both parents and offspring (Scott 1998). As such, food-deprived individuals may not require assistance from a partner to recover since they do not have to engage in costly foraging for food from the surrounding environment.

We suggested that whether a species is a capital breeder or an income breeder may be important when considering the effects of nutritional state on parental cooperation. As argued above for our study species, there may be no interaction effects of the focal individual's nutritional state and that of its partner in capital breeders that acquire resources before breeding. In contrast, we might expect such an interaction effect in income breeders, where parents obtain food to provision to their offspring from the surrounding environment. Previous studies that investigated the effect of food availability on parental behavior in such species, including stitchbirds (Low et al. 2012) and Palestine sunbirds (Markman et al. 2002), have shown differences in parental provisioning rates dependent on manipulation of food availability. One avenue for expanding research in such species is to use two-by-two factorial designs where food availability is manipulated for females and males and then measuring the subsequent effect on parental cooperation. We encourage future work exploring the effect of state on parental cooperation in both capital breeders and income breeders.

Finally, we found no negative effect of parental food deprivation on offspring performance. Instead, average larval mass at dispersal was higher in broods cared for by food-deprived females, and there was no difference in the proportion of larvae surviving to dispersal in response to parental nutritional state. This finding contrasts with that of Keppner et al. (2018), who found no difference in average larval mass dependent on parental nutritional state. Our finding was unexpected given that we predicted that food-deprived parents would provide less care and feed more on the carcass, which is the sole food source for parents and larvae, leading to reduced larval performance upon dispersal. In this species, larval mass at dispersal is a strong predictor of adult size (Lock et al. 2004), which is an important determinant of adult fitness (Otronen 1988). As a result, parents are likely under selection to compensate for any initial reduction of care as a result of poor nutritional state by increased levels of care later in the breeding attempt, or the larvae may compensate by increased rates of self-feeding later in the breeding attempt. Our results suggest overcompensation with food-deprived females producing better quality broods than well-fed females. Our experimental design may have facilitated this since we used relatively large mouse carcasses to ensure that there was ample food for both parents and larvae to feed from without much competition. Thus, there might have been a different outcome, similar to that reported by Keppner et al. (2018), had we used a small carcass such that there was a more intense competition over the shared resource.

In conclusion, our study shows that females respond to both their own and their partner's nutritional state when deciding how much care to contribute and that these decisions are independent of those made based on the contribution of their partner. In contrast, males responded only to the contribution of their partner rather than the nutritional state of their partner. Our findings highlight the need for more work investigating how multiple mechanisms play a role in the resolution of sexual conflict over parental care and what may be driving sex differences in these mechanisms.

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## AUTHOR CONTRIBUTIONS

Georgia Lambert (Conceptualization [Lead], Data curation [Lead], Formal analysis [Lead], Methodology [Lead], Writing—original draft [Lead], Writing—review & editing [Equal]), and Per Smiseth (Conceptualization [Supporting], Formal analysis [Supporting], Methodology [Supporting], Supervision [Lead], Writing—review & editing [Equal])

## CONFLICT OF INTEREST

None declared.

## DATA AVAILABILITY

Analyses reported in this article can be reproduced using the data provided by Lambert and Smiseth (2024).

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