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Egg size, begging behaviour and offspring fitness in *Nicrophorus vespilloides*

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Egg size reflects the amount of energy that female parents have invested in their offspring prior to hatching, and is thus often used as a proxy for prehatching investment. According to life history theory, prehatching investment, in turn, is at a trade-off with posthatching investment, as the amount of resources allocated at the prehatching stage diminishes the resources available at the posthatching stage. As small eggs have smaller energy reserves than large eggs, the offspring originating from small eggs may have higher hunger levels, and thus beg more, offering the parents information about the need for more posthatching care. However, little is known about the relationship between egg size and begging behaviour, and the fitness correlates of the two. In this study, we directly investigated the association between these two traits in the burying beetle *Nicrophorus vespilloides*. Concurrently, we examined the effects of egg size on two components of offspring fitness: offspring growth and survival. We found no association between egg size and offspring begging behaviour. Egg size did, however, show a mostly positive, albeit indirect, association with offspring fitness traits (development time, size and survival). Therefore, an increase in egg size does have an impact on offspring fitness, but this impact is not mediated through offspring begging. To our knowledge, this is the first time the relationship between egg size and begging behaviour has been investigated directly.

Keywords: Begging, egg size, fitness traits, *Nicrophorus*, prehatching investment

Word count: 7193

Egg size is a trait determined by parental phenotypes (Bernardo, 1996; Christians, 2002), in combination with the environment (Bize, Roulin, & Richner, 2002; Bonduriansky & Head, 2007; Hegyi, Rosivall, & Török, 2006; Helfenstein, Berthouly, Tanner, Karadas, & Richner, 2008), which generally has positive effects on offspring fitness across a wide range of taxa, including insects, fish, birds and amphibians (e.g. Christians, 2002; Fox, Thakar, & Mousseau, 1997; Kaplan, 1992; Krist, 2011; Taborsky, 2006; Williams, 1994). Egg size has often been used as a proxy for the amount of energy that female parents have invested in their progeny before hatching (Bernardo, 1996; Christians, 2002). Females can adjust this prehatching investment based on the environmental conditions they experience and/or anticipate (Fox et al., 1997; Kaplan, 1992; Taborsky, 2006; Vijendravarma, Narasimha, & Kawecki, 2010). Increased egg size benefits the early development of the offspring and improves their survival prospects, especially under adverse environmental conditions (Benton & Grant, 1999; Fox et al., 1997). In species with parental care, the parents may also alter the fitness of their offspring through posthatching investment, such as guarding or provisioning nutrition to their young (Clutton-Brock, 1991), which may affect the costs and benefits of allocating resources to the offspring at the prehatching stage. For example, it has been suggested that the prehatching investment of the female, in the form of egg size, may alter the posthatching investment of her partner (Paquet & Smiseth, 2016), and have implications for resources available for her future reproduction, thus resulting in differences in the female's own level of posthatching care (Lock, Smiseth, Moore, & Moore, 2007). The amount of posthatching care provided by both parents, in turn, may have different optimal levels for maximizing the fitness of the parents and of the offspring (Trivers, 1974). Due to this, egg size is a central trait for multiple life history trade-offs, including trade-offs between the number and size of offspring, and prehatching and posthatching investment; as well as for family conflicts such as sibling competition, parent-offspring conflict and sexual conflict. While egg size has been shown to have effects that suggest an important role for these trade-offs and conflicts, the reported effects have been variable (reviewed in

Bernardo, 1996; Christians, 2002; Krist, 2011), and the mechanisms behind how egg size relates to the investment decisions within the family are largely unknown.

One potential but unexplored mechanism through which egg size may influence the posthatching investment of the parents is through offspring behaviour. Given that small eggs have small energy reserves (Christians, 2002; Krist, 2011), offspring hatching from small eggs may beg at higher levels than offspring hatching from large eggs, because the former have higher hunger levels. Although no studies have directly tested for this association, some indirect evidence exists: For example, a study exploring the effects of reducing the amount of albumen in yellow-legged gull eggs found a negative correlation between egg size and begging rates of chicks in untreated control eggs (Alquati et al., 2007). Additionally, begging rates in birds often vary with hatching order (Gilby, Sorato, & Griffith, 2012; Müller, Boonen, Groothuis, & Eens, 2010; Rice et al., 2013; Soley, Siefferman, Navara, & Hill, 2011) and brood size (e.g. Helfenstein et al., 2008), which in turn are often correlated with egg size (Parsons, 1976; Slagsvold, Sandvik, Rofstad, Lorentsen, & Husby, 1984; Vinuela, 1997). Aside from the lack of experiments directly investigating the association between egg size per se and begging behaviour, the scarce literature on the topic is taxonomically heavily biased towards birds. This is a potential limitation, as the effects of large yolky eggs of birds may not be fully comparable to the small eggs of insects and some fishes (Bernardo, 1996), some of which also exhibit begging behaviour (fish: Noakes, 1979; Noakes & Barlow, 1973, insects: Mas & Kölliker, 2008). Thus this taxonomic bias may ignore the intricate differences between taxonomic groups with different types of posthatching care. To attain a more complete picture of the role of egg size in the within family conflicts and trade-offs, it is important to consider the influence of egg size on offspring behaviour directly, in a wide range of taxonomic groups, including non-avian systems.

Empirical evidence suggests that females lay larger eggs in adverse environmental conditions (Fox et al., 1997; Kaplan, 1992; Taborsky, 2006; Vijendravarma et al., 2010). However, the benefits from increased prehatching investment can be modified through parental care, which is also thought to have evolved to buffer against these adverse environmental conditions (Royle, Smiseth, & Kölliker, 2012). Congruently, a recent theoretical model suggests that a reduction in egg size is favoured when any effects of the reduction can be compensated for through posthatching parental care (Savage, Russell, & Johnstone, 2015). Due to their positive effects on offspring survival and growth, both egg size and parental care have been studied extensively (Bernardo, 1996; Clutton-Brock, 1991; Fox & Czesak, 2000; Royle et al., 2012; Smith & Fretwell, 1974), while the coevolution between the two has been discussed less frequently. Previous studies indicate that the effects of egg size on offspring growth and survival are much smaller than those of posthatching care (Monteith, Andrews, & Smiseth, 2012; Ricklefs, 1984), and often only detectable shortly after hatching (Williams, 1994). If the different energy reserves available for the offspring in different-sized eggs, however, are accurately reflected by offspring begging behaviour, the information transferred through begging can be classed as an honest signal of need (Kilner & Johnstone, 1997; Mock, Dugas, & Strickler, 2011), accurately informing the parent of the offsprings' resource requirements. Variation in egg size would thus provide a potential mechanism for adjusting the amount of parental care necessary to reach the parent's optimal level of allocation. Thus, the large effects of posthatching care on offspring growth and survival (Monteith et al., 2012; Ricklefs, 1984) may ultimately depend on prehatching investment in egg size.

We investigated the relationship between egg size and offspring begging behaviour in the burying beetle *Nicrophorus vespilloides*. Like all members of the genus *Nicrophorus*, these beetles utilize carcasses of small vertebrates, which they bury underground and use as a nest and a source of nutrition for their offspring (Scott, 1998). After mating, the females lay up to 60 eggs in the soil around the carcass (Scott, 1998). After hatching, the larvae crawl into the carcass, and one or both parents provide elaborate posthatching parental care for them. The larvae beg for food from their parents by touching the parent with their legs, and the parent responds to this behaviour by regurgitating predigested carrion for them (Rauter & Moore, 1999). The larvae are also capable of self-feeding, and their ability to do so improves over the course of their development (Smiseth, Darwell, & Moore, 2003). We measured egg size within clutches originating from parents of different sizes, picked a subset of larvae from each clutch and gave them to a foster female parent of a standardized age and size, and measured the begging behaviour of these broods. Thus, we directly explored the effect of egg size on begging behaviour, while controlling for the effects of posthatching parental behaviour. Should the energy reserves of small eggs be smaller than those of large eggs, we would expect to see a negative association between egg size and begging behaviour. We also measured the growth and survival of the offspring to estimate the importance of egg size for offspring fitness, in order to verify the predicted fitness benefits of larger energy reserves of large eggs. To our knowledge, this is the first time the relationship between egg size and begging behaviour has been explored directly, and thus our experiment presents an important addition to the literature on pre- and posthatching investment in a non-avian species.

METHODS

Origin and Husbandry of the Beetles

The beetles used in the experiments originated from an outbred laboratory population maintained at The University of Edinburgh, UK. The population descended from beetles trapped in Craiglockhart Hill in Edinburgh (UK) and in Warmond (Netherlands). All beetles were housed individually in transparent plastic containers (12 x 8 x 2 cm) filled with moist soil, and kept under constant light at 20°C. Nonbreeding beetles were fed small pieces of organic beef twice a week. For breeding, pairs of males and females, verifiably unrelated up to the granparent generation, were selected randomly and placed in a plastic container (17 x 12 x 6 cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (supplied by Livefoods Direct, Sheffield, UK).

Egg Trait Measurements

To capture the full scope of egg size variation, we paired beetles of known sizes to partners of corresponding sizes, in order to use the body size of the parents to produce eggs from either the small or the large end of the size distribution. Offspring size, including egg size, correlates with the size of the mother in *N. vespilloides* (Steiger, 2013), and some evidence suggests that the size of the father also has an effect in other insects (for example, Davis & Landolt, 2012). Thus, we measured the length of the pronotum of adult beetles with a Mitutoyo absolute digimatic caliper (1 - 150 mm) prior to the start of the experiment. The length of the pronotum is a good measure for size, as it does not change with time elapsed since last feeding, and is highly repeatable (Beeler, Rauter, & Moore, 1999). After the measurements, we divided the beetles into rough categories, classifying the individuals above the 75% quartile range as large (range: 4.61 - 4.87 mm), and those below the 25% quartile as small (range: 2.62 - 4.15 mm), leaving the rest of the beetles classified as medium-sized (range: 4.16 - 4.59 mm). We then selected pairs of nonsibling virgin male and female beetles within each size class to be mated together, with the expectation that the small pairs would produce small eggs, and the large pairs would produce large ones. A total of 203 matings were conducted, out of which 48, 128 and 27 were in the small, medium and large categories, respectively. The ages of the beetles and the amount of resources available were taken into account, as all beetles were mated 20 (±7) days after they eclosed as adults and given a mouse carcass of a standardized size (range 21 - 26 g).

The pairs of beetles were given 60 h to mate, prepare the mouse, and for the female to lay eggs in the soil. The parents were then removed from the boxes where eggs had been laid, and all medium-sized females and their respective mouse carcasses were moved to empty containers filled with moist soil. All males and the females of the small and large category were discarded. We only used the medium-sized females as foster parents, to control for the confounding effects of female size on offspring growth at the posthatching care stage, as female size has been shown to correlate with the amount of care given (Steiger, 2013). Males were discarded, as male care is highly variable, and has no detactable effects on the survival or growth of the offspring produced (Eggert, Reinking, & Müller, 1998; Smiseth, Dawson, Varley, & Moore, 2005). The majority of the eggs laid in the soil are situated at the bottom of the containers, and are visible through the transparent plastic. By counting the number of eggs visible at the bottom of the containers, we acquired an estimate for the total number of eggs laid, which correlates strongly with the actual clutch size (Monteith et al., 2012). After the eggs were counted, we scanned the egg boxes with a Canon CanoScan 9000F Mark II flatbed scanner, and the digital images were then used to measure the egg size.

We measured the eggs using Image J image processing program (Schneider, Rasband, & Eliceiri, 2012). Only eggs that were laying flat against the surface of the box were measured, avoiding tilted eggs or those that were only partially visible in the soil to prevent inaccurate measures. We measured the length and width of each egg (mean ± SD, 24.2 ± 8.3 eggs per brood), from the outermost tips of each specimen, using 300% magnification. We then calculated the volume V for each egg using the equation $V = (1/6)\Pi w^2 L$, where w is the width and L the length of the egg (Berrigan, 1991). A brood mean was then calculated from each egg measured in a brood as it is not possible to mark individual eggs to allow individual identification later during the experiment.

Behaviour Measurements

The egg boxes were checked for newly hatched larvae six times each day, and at hatching the development time from egg to hatching for each brood was recorded. After hatching, we picked 10 larvae from each brood, weighed them with a digital scale (Ohaus Pioneer, with an accuracy of 0.1 mg) to attain their initial brood masses, and gave them to a foster female. Only females whose own eggs had started hatching were selected as fosters to avoid filial cannibalism (Müller & Eggert, 1990). The foster female was then allowed to raise the larvae undisturbed for 24 h. We recorded larval begging 24 h (± 15 min) after the broods had been given to the females. This timing coincides with the peak in larval begging behaviour (Smiseth et al., 2003). The female was removed 30 min before starting the observation in order to prepare her for her use as a stimulus for recording larval begging. We killed the female by placing her in a -20°C freezer for 20 min, after which she was left to thaw for another 5 min before we pinned her onto a small plastic container (12 x 8 x 2 cm) lined with a moist paper towel. Larvae continue to beg for at least 2 h when presented with a dead parent (Smiseth, Andrews, Brown, & Prentice, 2010; Smiseth & Parker, 2008), and begin begging in response to chemical stimuli from the parent (Smiseth et al., 2010), and only beg in the presence of a parent (Rauter & Moore, 1999). As such, using a dead parent as a stimulus provides a good proxy for measuring larval begging behaviour in a way that is independent of the parent's behaviour towards the larvae. The females were pinned in a position mimicking that of a parent regurgitating food. Once the female had been pinned, we removed the larvae from the mouse carcass, and placed them next to the stimulus. We waited 5 min before starting the observations to give the larvae time to settle.

We recorded larval begging using instantaneous recording every 1 min during a 30 min observation period according to established protocols (Smiseth et al., 2010). The proportion of time spent begging by each larva in the brood, B, was calculated as $B = (b/l)/30$, where b is the total number of begging events during the 30 min observation period, and l is the mean number of larvae near the adult during each scan (i.e., within 0.8 cm diameter from the pin). Thus, we attained a measure for mean begging in a brood, comparable to our measure for egg volume. Not all broods that were initially set up could be used to attain behaviour data, partially due to hatching failure and time limitations. The total sample size amounted to 97 broods, with the majority of the broods originating from the pairings between parents in the medium sized class ($N = 21$, 57 and 19, for the small, medium and large, respectively).

Larval Growth and Mortality

To test for associations between egg size and fitness related traits of the offspring, we recorded larval mortality and growth for all broods after the behaviour observations. After the observations, the larvae were weighed, counted, and returned to the carcass to complete their development until the entire brood had dispersed from the carcass into the surrounding soil. Larval development time from hatching to dispersal was recorded for each brood. They received no parental care for the rest of their development, as the foster females had been killed. Removing the parent after 24 h also allowed us to homogenize the duration of care for each brood, thus limiting the effects of parental care to those due to variation in the levels of care provided at the peak time of need, and maximising the impact of care in the form of food provisioning - the behaviour most related to begging. We checked the boxes daily to record the timing of dispersal or death of all larvae in the brood. At dispersal we counted the number of larvae surviving, to account for mortality during the juvenile period, and weighed the broods once more.

Statistical Analyses

All analyses were conducted with R version 3.3.3 (R Core Team, 2017). To first analyse the effectiveness of the experimental treatment of parental size classes, we used linear models (lm) to compare the effects of parental size classes (small, medium, or large) on egg traits (egg volume, clutch size, egg development time). We then conducted Tukey's honest significance tests to attain pairwise comparisons between all size classes to determine whether the three parental size classes were different from one another. In the analysis of egg development time, we additionally added egg volume to the model as a covariate, to also compare the differences in development time due to egg volume itself. As the eggs in the treatment groups did not differ sufficiently from one another (see Results), we used egg volume as a continuous trait in further analysis.

In subsequent analyses, we used linear mixed effects models (package lme4; Bates, Maechler, Bolker, & Walker, 2014) for traits with gaussian error distribution (larval size, larval development time) and generalized linear mixed effects models (package glmmADMB; Fournier et al., 2012; Skaug, Fournier, Bolker, Magnuson, & Nielsen, 2014) for traits with negative binomial (larval mortality) or beta error distribution (larval begging). To account for any potential parental effects arising from limiting the size of both the biological mother and her partner within the size classes, we included the size class of the biological parents of the eggs as a random variable in all models hereafter. The specific structures for models for each trait analysed is described below. Within each model, we calculated a variance inflation factor (VIF) for each covariate to ensure that there were no problems with collinearity. When the variance inflation factor for a variable was >2, we redesigned the model by excluding any correlated effects. Only the models with no collinearity problems are presented below. After the initial models were defined, non-significant (P>0.05) terms were removed based on ANOVA's comparing the maximum likelihood estimates of the nested models in order to attain parsimonious models.

Larval Behaviour

In analysing the proportion of time spent begging by each larva within a brood, we assigned egg volume and the size of the larvae at observation time as fixed factors. This allowed us to test for the effects of initial offspring size, and also the effect of offspring size at the time of the observation. We also added the mass of the mouse as a covariate in the analysis, as the amount of food available is likely to influence offspring begging.

Larval Size and Mortality

We analysed the effect of egg volume on larval size at three different time points in juvenile development (hatching, 24 h after hatching, dispersal). As larvae at hatching were on average 68 ± 8 (mean ± SD) times smaller than larvae at dispersal, we rescaled the size measures at each time point to vary in the scale of 0-1, in order to focus our analysis on the shape of the relationship between egg volume and larval size, rather than the known differences between sizes of the larvae. We assigned larval size as the response variable, and the timepoint (hatching, 24 h after hatching, dispersal) as a fixed factor in the model, and the brood identity as a random effect to account for repeated measurements taken from each brood. To test for the effect of egg volume on larval size, we treated egg volume as a covariate. To test whether the effect of egg volume changed over the course of the development, we also added an interaction between timepoint and egg volume in the model, as a significant interaction would show us the differences between the steepness of the slope of egg volume at these timepoints in the development. The relationships between the original values for larval sizes at different timepoints were also examined with Pearson's correlations between the three size measures.

We also analysed larval development time from newly hatched larva to dispersal, and larval mortality from the start of the experiment to dispersal. In these models, we assigned egg volume and larval size at dispersal as covariates, to test for the effects of size at the beginning and at the end of the development on both traits. We also added the average begging rate of the brood in the model, as additional resources acquired through begging may speed up the development of the larvae, and potentially also affect survival. For analysis of larval development time, we also added egg development time in the model to examine the relationship between the two development rates. For the analysis of larval mortality, we added larval development time in the model to examine the effects of development time on survival of the larvae.

Ethical Note

All beetles used in this experiment were housed in conditions providing each individual with the space, nutrition and hygienic conditions required (see *Origin and Husbandry of the Beetles* above). The beetles that were killed for their use as stimulus in the experiment (N=97), as well as beetles discarded after they were removed from the experiment (N=309) were killed by freezing, ensuring the beetles died as quickly and painlessly as possible.

RESULTS

Predictors of Egg Traits

Parental size classes differed in the volume of eggs they produced (lm; $F_{2,122}$=12.28, P<0.001, Fig. 1a). However, the post-hoc pairwise comparisons showed that only the eggs produced by small parents were different from those produced by medium-sized parents (Tukey HSD; P<0.001) and large parents (Tukey HSD; P=0.020), while medium and large size classes produced eggs of equal size (Tukey HSD; P=0.369). Furthermore, relatively little variation in egg volume was explained by parental size classes altogether (R^2=0.17), suggesting that a substantial portion of variation in egg size remains unaccounted for by the experimental treatments. Similarly to egg volume, clutch size varied within different parental size classes (lm; $F_{2,197}$=5.61, P=0.004, Fig 1b). The post-hoc comparisons showed a similar pattern as egg size; clutches by small parents were smaller than both the medium-sized (Tukey HSD; P=0.014) and large parents (Tukey HSD; P=0.008), while the medium sized and large parents produced clutches of equal sizes (Tukey HSD; P=0.483). Parental size classes explained very little of the variation in clutch size (R^2=0.05). As suggested by their similar patterns, egg volume and clutch size were also positively correlated with one another (r_{123}=0.35, $P<0.001$). All in all, the parental size categories succeeded in creating eggs of different sizes, but did not result in clearly distinguishable categories, leading us to use egg volume as a continuous measure in the later analyses.

Parental size classes also significantly explained variation in egg development time (lm; $F_{2,115}$=3.29, P=0.041), possibly due to a negative trend indicating that eggs from larger parents developed slower (Fig. 1c). However, the post-hoc pairwise comparisons showed no differences between any of the size classes (Tukey HSD; Small-Medium: P=0.998, Small-Large: P=0.721, Medium-Large: P=0.535, Fig. 1c). Egg volume affected egg development time (lm; $F_{1,115}$=28.32, $P<0.001$), as eggs developed faster as egg size increased (slope ± SE = -0.80 ± 0.15).

Relationship between Egg Volume and Begging

There was a lot of variation in both egg volume and the average time spent begging by a larva in a brood (Fig. 2). Begging mostly occured at relatively low levels (mean ± SE; 12.4 ± 0.8 % of the time measured), comparable to other studies with a similar setting for measuring begging behaviour (Leigh & Smiseth, 2012; Mäenpää, Andrews, Collette, Leigh, & Smiseth, 2015; Smiseth et al., 2010). Egg volume did not have an effect on begging behaviour (GLMM; Par±SE = -0.51±0.39, z=-1.33, P=0.183, Fig. 2). Begging was also not affected by larval size at the time of the observation (GLMM; Par±SE = -12.09±26.29, z=-0.46, P=0.650). As such, we conclude that we found no evidence of a relationship between egg size and begging behaviour.

Effects of Egg Volume on Larval Size and Mortality

Egg volume determined larval size at hatching, but its direct effect on larval size disappeared towards the end of larval development as there was no detectable effect of egg volume either when larvae were 24h old or at dispersal (Table 1, Fig. 3). However, while the direct effect of egg volume disappeared quickly during development, it may still indirectly affect the size measures at later stages of juvenile development through its initial effect on size at hatching. In support of this, we found that size at hatching was correlated with size at dispersal (r_{90}=0.35, P<0.001), as well as size at 24 h (r_{95}=0.46, P<0.001), which in turn was also correlated with size at dispersal (r_{90}=0.42, P<0.001).

Larval development time (i.e. time to dispersal) was not associated with egg volume (Table 1). However, there was a relationship between larval development time and larval size at dispersal, with bigger larvae developing faster than smaller ones (Table 1). Larval development time was also positively affected by egg development time, as the faster the eggs developed, the faster the larvae reached dispersal (Table 1). Given that both development times were linked to size variables, our results show that large individuals were fast in their development both in egg phase and in the later juvenile phases.

Larval size at dispersal had the largest effect on larval mortality, with larger larvae having lower mortality during larval development (Table 1). Egg volume also had a small effect on mortality, as broods with larger eggs had a higher mortality from hatching to dispersal (Table 1). The disparity between the effects of the two size measures is likely to reflect developmental costs of fast growth of the larger eggs. This is also supported by the significant effect of larval development time on mortality: longer development time was associated with fewer deaths (Table 1).

DISCUSSION

We found no evidence of an association between egg size and larval begging behaviour in the burying beetle *Nicrophorus vespilloides.* Egg size did, however, in general have a positive impact on fitness-related traits of the offspring; more strongly at the beginning of the development with these effects subsiding towards the end of the juvenile period. In view of egg size being a central trait for multiple life history trade-offs and family conflicts, understanding the mechanisms through which the positive fitness effects of egg size are realized is important: It allows us to investigate how investment into pre- and posthatching care within the family are potentially determined, and thus how the said conflicts and trade-offs are resolved. Our findings support previous evidence for positive fitness effects of egg size (Bernardo, 1996; Christians, 2002; Krist, 2011), but suggest that the mechanism through which these effects are realized is not mediated through changes in offspring behaviour at least in this species. Instead, positive fitness effects of egg size can be caused by either intrinsic mechanisms within offspring, or the independent caring decisions made by the parent, but our results suggest that they are unlikely to be due to communication between the two.

We found a positive, albeit indirect, association between egg size and offspring fitness-related traits: larger eggs developed faster, and became larger larvae at hatching, which in turn had a positive effect on larval size at dispersal. Burying beetle larvae do not feed after they disperse from the carcass, and larval size at dispersal therefore determines adult size (Bartlett & Ashworth, 1988; Lock, Smiseth, & Moore, 2004). Adult body mass, in turn, is a good predictor of reproductive success, as it influences success in competition for access to carcasses used for breeding (Otronen, 1988). While the direct effects of egg volume disappear over the course of development, they may still have an indirect effect that cascades to size at dispersal and its fitness consequences. We also found that larval size at dispersal was negatively associated with larval mortality, indicating that large larvae had higher survival.

However, egg size directly affected mortality as the number of deaths in a brood increased with increasing egg volume. The disparate mortality effects of egg volume and larval size at dispersal are likely to be caused by physiological costs for growing fast, given that development time also negatively affected the number of larvae dying. Compensatory fast growth has been linked to oxidative stress, DNA damage, as well as the shortening of lifespan in multiple taxonomic groups from insects to mammals (e.g. De Block & Stoks, 2008; Lee, Monaghan, & Metcalfe, 2016; Stoks, De Block, & McPeek, 2006; Tarry-Adkins et al., 2013). Whether the benefits of growing fast and reaching a larger size at dispersal are likely to outweigh the costs depends on the environment experienced by the larvae. In the wild, the environment experienced by the beetles is characterized by high competition for carcasses (Otronen, 1988) and risk of brood paratisisim (Müller & Eggert, 1990), both of which indicate high levels of intraspecific competition, where fast growth may indeed provide competitive advantages despite the costs associated with it. On the whole, egg volume had a mainly positively connection to fitness-related traits. These findings agree with the direction of previous findings of a connection between egg size and fitness-related traits in multiple avian studies (reviewed in Krist, 2011).

Previous studies suggest that the effects of egg size on offspring growth and survival are small in comparison to the effects of posthatching parental care (Monteith et al., 2012; Ricklefs, 1984; Royle et al., 2012). Indeed, Monteith et al. (2012) found that the presence of a parent has a large, positive effect on offspring growth in *N. vespilloides*, while egg size has no detectable effects. While we found a positive, indirect influence of egg size on offspring fitness-related traits, this effect was largely mediated through traits other than egg size in itself (i.e. egg development time, larval size at hatching, larval size at dispersal). Therefore, in our data, the effect of egg size is indeed relatively weak at later juvenile stages, potentially reflecting that it is masked by stronger effects - such as those of posthatching parental care (Monteith et al., 2012). Importantly, because egg size was not associated with begging behaviour, there is no evidence that the amount of posthatching care is adjusted based on increased signaling of hunger in broods derived from small eggs, and thus begging is likely to be independent of egg size. However, it should be noted that we cannot rule out possible effects of egg volume on begging during the first few hours after hatching. In birds, egg components, such as maternal testosterone, have been shown to affect begging only early in the development (Schwabl, 1996), suggesting that our begging measurements may have been conducted too late to catch any initial differences. We conducted the observation at 24 h after hatching because this corresponds to the peak in larval begging, when larval need is at its highest (Smiseth et al., 2003). Should larvae originating from different-sized eggs beg differently, we expected these effects to be evident at the peak time of need in the period of larval dependency, especially as we measured begging as an average for broods of homogeneous egg sizes. Therefore, despite the potential effects of egg size on begging during the first few hours after hatching, any biologically important differences between broods would have been likely to be detectable at the peak time of need.

Our lack of evidence for an association between egg size and begging may indicate that egg size does not influence offspring need. If egg size is related to the offspring's energy reserves (Bernardo, 1996; Christians, 2002), we would expect smaller eggs to produce larvae with higher needs, as they are likely to be hungrier and also in a worse condition. Body condition may contribute to the needs of the offspring: In yellow-headed blackbirds, *Xanthocephalus xanthocephalus*, when hunger was controlled for, chicks begged at different levels based on their body condition. In the burying beetle, begging reflects larval hunger state, as starved larvae beg at higher levels than those that have been provided with ample amounts of food (Andrews & Smiseth, 2013; Rauter & Moore, 1999; Smiseth & Moore, 2004). However, as the larvae have the option of self-feeding, it is possible that they are capable of compensating for any differences in their needs by self-feeding unlike the offspring of avian species. Egg size may thus have negligible effects on the offspring's immediate needs. Contrary to our results on egg size, there is evidence that other egg traits, such as egg composition, laying order as well as the hormone levels associated with it, can affect offspring behaviour or performance in some species of birds and fish (Fuiman & Ojanguren, 2011; Gilby et al., 2012; Rice et al., 2013). These traits offer the offspring information about the environment they are experiencing (for example: laying order determines the competitive asymmetries within broods), which may be a stronger determinant for offspring needs than egg size is (Monteith et al., 2012; Ricklefs, 1984; Royle et al., 2012).

Unlike predictions based on life history theory (Stearns, 1992), clutch size and egg volume were not traded off against one another, but rather there was a positive association between clutch size and egg volume (Fig. 1a,b). Previous studies on the same species reported no association between the two traits (Andrews, Kruuk, & Smiseth, 2017; Monteith et al., 2012), contrasting our results. Traditionally, a positive association in size and number of offspring has been interpreted as a sign of individual heterogeneity in the fitness or quality of the parents, even though this interpretation and terminology associated with it has recently been questioned (Bergeron, Baeta, Pelletier, Réale, & Garant, 2011; Clancey & Byers, 2014; Lim, Senior, & Nakagawa, 2014; Wilson & Nussey, 2010). It is possible that the positive association in our data is due to the effects of maternal size and the benign laboratory environment. One determinant of the shape and strength of the relationship between size and number of offspring produced is total resource availability (van Noordwijk & de Jong, 1986). As the parents were not limited by resource availability, they may have been able to perform to their best ability, and thus the relationship shown here could have been due to the physiological maximum for both the size and number of offspring that a mother can produce, as determined by her size.

In conclusion, we provide the first direct exploration of the association between egg size and offspring begging behaviour. We found no association between these traits, indicating that prehatching investment into eggs does not translate into differences in begging behaviour of the offspring, and thus any changes in posthatching care cannot be explained through this mechanism. This lack of an association opens up new directions for exploring alternative pathways through which the often reported effects of egg size on offspring fitness, and their relationship with parental care, could function. Further research on a variety of taxa are needed to fully disentangle these mechanism, and thus to improve our understanding of how the maintenance of egg size polymorphism within populations has evolved (Bernardo, 1996; Christians, 2002; Krist, 2011).

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Table 1: The effects of egg volume on larval size, development time, and survival in *Nicrophorus vespilloides*.

Response	Factor	Coef	SE	df	t/z^1	P
Larval size	Timepoint					
	24 h	0.63	0.16	185	3.84	<0.001
	Dispersal	0.75	0.17	186	4.50	<0.001
	Egg volume	0.33	0.07	203	4.54	<0.001
	Timepoint: Egg volume					
	24 h: Egg volume	-0.31	0.08	185	-4.03	<0.001
	Dispersal: Egg volume	-0.38	0.08	187	-4.80	<0.001
Larval development time	Egg volume [2]	*-0.35*	*0.43*	*29*	*-0.81*	*0.423*
	Larval size (dispersal)	-15.62	4.76	83	-3.28	0.002
	Egg development time	0.59	0.21	83	2.90	0.005
Number of deaths	Egg volume	1.24	0.52	-	2.39	0.017
	Larval size (dispersal)	-26.31	6.36	-	-4.13	<0.001
	Larval development time	-0.38	0.13	-	-2.89	0.004

Estimates are derived from linear mixed effects model (lmer), with degrees of freedom and P-values attained through Satterwaithe approximation (larval size measures and development time), or from a generalized linear mixed effects model (glmmadmb; number of deaths).

[1] t-value for larval size traits and development time, z-value for the number of deaths.

[2] Parameters estimated from the last model containing the variable before it was dropped in the process of model simplification (values in italics).

FIGURE LEGENDS

Figure 1 The effect of parental size classes for egg traits in *Nicrophorus vespilloides.* Comparison of parental size class and (a) egg volume, (b) clutch size and (c) egg development time. Parental size class represents the small beetles (2.62 - 4.15 mm), medium sized beetles (4.16-4.59 mm) and large beetles (4.54 - 4.87 mm). Groupings of the post-hoc pairwise comparisons indicated in letters above each bar.

Figure 2 The association between egg volume and begging behaviour in *Nicrophorus vespilloides.* Begging is presented as percentage of time an average larva spent begging in a brood during the 30 minute instantanious scan sampling period, conducted as the larvae were 24 h old. Points represent the raw data, the solid black line is the predicted line derived from a generalized linear mixed effects model (glmmadmb), where the error structure was modelled with a beta distribution.

Figure 3 The effect of egg volume to larval size at (a) hatching, (b) 24 h after hatching and (c) dispersal in *Nicrophorus vespilloides.* Larval sizes presented at the same scale with one another, to allow comparisons of slopes disregarding the differences in scale brought on by growth. Points represent the raw data, the solid black lines are the predicted lines derived from a general linear mixed effects model (lmer).