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Asynchronous hatching in the burying beetle, *Nicrophorus quadripunctatus*, maxmizes parental fitness

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Running title

Hatching pattern and parental investment

Abstract

Life history theory predicts that natural selection favours parents that balance investment across offspring to maximise fitness. Theoretical studies have shown that the optimal level of parental investment from the offspring's perspective exceeds that of its parents, and the disparity between the two generates evolutionary conflict for the allocation of parental investment. In various species, the offspring hatch asynchronously. The age hierarchy of the offspring usually establishes competitive asymmetries within the brood, and determines the allocation of parental investment among offspring. However, it is not clear whether the allocation of parental investment determined by hatching pattern is optimal for parent or offspring. Here, we manipulated the hatching pattern of the burying beetle *Nicrophorus quadripunctatus* to demonstrate the influence of hatching pattern on the allocation of parental investment. We found that the total weight of a brood was largest in the group that mimicked the natural hatching pattern, with the offspring skewed towards early hatchers. This increases parental fitness. However, hatching patterns with more later hatchers had heavier individual offspring weights, which increases offspring fitness, but this hatching pattern is not observed in the wild. Thus, our study suggests that the natural hatching pattern optimises parental fitness, rather than offspring fitness.

Keywords: *Nicrophorus*; burying beetle; asynchronous hatching; allocation of parental investment; parent-offspring conflict

Introduction

Life history theory predicts that natural selection favours parents that balance investment across offspring to maxmise lifetime reproductive success (Williams, 1966; Trivers, 1972; Maynard-Smith, 1977; Westneat & Sargent, 1996). Parents allocate a tremendous amount of resources to their offspring (Clutton-Brock, 1991), known as parental investment. Theoretical studies show there is a conflict between parent and offspring over the allocation of parental investment called parent-offspring conflict (Trivers, 1974; Godfray & Johnstone, 2000). For example, in a monogamous sexually reproducing species (where the relatedness of parents and offspring is 0.5), individual offspring benefit twice as much as their parents from parental investment (Lazarus & Inglis, 1986). Thus, the optimal level of investment from the offspring's perspective exceeds that of its parents, and the disparity between the two generates evolutionary conflict. In general, parents are selected to optimally allocate parental investment among offspring. Offspring are selected to obtain more parental investment than the distribution of resources among offspring favoured by parents, and compete against their siblings for parental investment. As a result, parent-offspring conflict causes a co-evolutionary arms race of reciprocally acting traits in parent and offspring (Royle *et al.*, 2012).

In various classes, hatching of offspring occurs over an extended period of time, e.g. altricial birds (Magrath, 1990; Stoleson & Beissinger, 1995; Stenning, 1996), amphibians (Ryan & Plague, 2004), reptiles (While *et al.*, 2007), and insects (Nalepa, 1988; Smiseth *et al.*, 2006). This mode of hatching is called asynchronous hatching. The age hierarchy of the offspring caused by asynchronous hatching usually establishes competitive asymmetries within the brood, and older siblings obtain a larger amount of parental investment (Mock & Forbes, 1995; Forbes *et al.*, 1997; Mock & Parker, 1997; Forbes & Glassey, 2000; Hall *et al.*, 2010). Thus, hatching pattern strongly influences offspring fitness. Parental fitness is determined by the sum of the fitness of each individual offspring. Therefore, the hatching pattern also affects parental fitness by defining how parental investment is allocated among offspring. Hence, there may be conflict between parents and offspring over hatching pattern.

The burying beetle *Nicrophorus quadripunctatus* is one of the species in which hatching of offspring occurs asynchronously (Smiseth *et al.*, 2006; Takata *et al.*, 2013). The burying beetle, like other species of the same genus, uses the carcasses of small vertebrates as a food resource for their larvae. Eggs are laid in the soil near the carcass asynchronously and the larvae hatch asynchronously (Takata *et al.*, 2013). After hatching, the larvae crawl towards the carcass and obtain food by begging for pre-digested carrion from their parents or by self-feeding from the carcass (in *N. vespilloides*; Smiseth & Moore, 2002; Smiseth et al., 2003; in *N. quadripunctatus*; Takata, unpublished data). The larvae compete for parental food provisioning and early hatching larvae typically obtain more food and these individuals grow heavier than their siblings (Takata *et al.*, 2013). Smiseth *et al.* (2008) investigated the adaptive significance of asynchronous hatching in *N. vespilloides*. They established three types of broods with different hatching patterns: synchronous, moderately asynchronous and highly asynchronous; with a hatching span of 0, 24 and 48 hours, and showed that the hatching pattern affects larval survival rate, with moderately asynchronous hatching broods surviving better. However, it is not clear whether the allocation of parental investment, as determined by hatching pattern, is optimal for the parents or for the offspring in the burying beetle. In this study, we manipulated the hatching pattern to demonstrate the influence of hatching pattern on the allocation of parental investment to determine whether it is optimal for the parents or offspring. We predict that if hatching pattern in this species is optimal for the parents, the total combined offspring body weight, which is an indicator of the parent’s reproductive performance, will be maximised. Alternatively, if the hatching pattern is optimal for the offspring, each individual offspring’s body weight will be maximised.

Materials and methods

Collection and maintenance of the beetles

In September 2012, 200 adult *Nicrophorus quadripunctatus* Kraatz were collected in baited pitfall traps in Tokyo, Japan, and first-generation offspring were reared in the laboratory. The beetles were maintained individually in small transparent plastic cups (height 4 cm, diameter 6 cm) at 20 ± 1 °C under a 14:10 h light:dark cycle. After they emerged as adults, they were fed Turkestan cockroach (*Blatta lateralis*) three times a week. All males and females used in this experiment were sexually mature and ranged between 21 and 35 days of age.

General experimental procedure

Pairs of randomly selected, non-sibling, virgin male and female beetles were placed together in a plastic cup (height 8 cm, diameter 15 cm) with moist peat, and were provided with 15 ± 0.5 g of carcass. We provided chicken meat for breeding because we can accurately control the size of carcass. The beetles in the plastic cup were kept in a dark incubator at 20 ± 1 °C for 93 h. During this period, female beetles laid eggs in the soil near the carcass. Then, the female and the carcass were transferred to a second, new plastic cup with moist peat. The male beetles were removed from the original plastic cup at this stage because parental care by male parents has no effect on larval growth or survival under laboratory conditions (Smiseth *et al.*, 2005). The eggs were left to hatch in the original plastic cup. Then, hatching of larvae was checked at 12hourly intervals and newly hatched larvae were used for experiments.

To investigate the influence of hatching pattern on larval body weight and survival, 5 experimental groups with different hatching patterns were set up: one group with synchronous hatching (Group A) and four groups with asynchronous hatching (Groups B – E). In all experimental groups, 15 larvae were presented to a female parent after the female's own eggs started to hatch. We presented unrelated offspring to parents to experimentally manipulate the hatching pattern, some of which were not normally observed in *N. quadripunctatus.* However, the influence of this unnatural condition on our results is considered to be negligible, because previous studies showed that burying beetle parents provide care to the brood irrespective of hatching pattern (Smiseth *et al.*, 2008; Smiseth & Morgan, 2009). Furthermore, the beetles cannot directly recognise their genetic relationship to larvae, if the larvae are provided to the parent after the parent's own eggs are expected to hatch (Müller & Eggert, 1990).

Broods in group A were generated by placing all larvae simultaneously into the second plastic cup with the female. Broods for groups B – E were generated by placing early hatching larvae into the plastic cup, followed by middle hatching larvae at 12 hours later and late hatching larvae at 24 hours later. In each experimental group, different numbers of early, middle and late hatching larvae were added to simulate different hatching patterns (see Table 1 for details). Nine replicates were made for group A, 11 for group C and 10 of each of the groups B, D and E. All groups of asynchronous broods were well within the natural variation for hatching span (25 ± 12 h, mean ± SD) and brood size (number of hatching larvae: 22 ± 8, surviving larvae: 11 ± 4, mean ± SD) observed when *N. quadripunctatus* was reared on 15 g of chicken carcass in the laboratory (see Takata *et al.*, 2013). Group C (hatching skew: -0.27) best mimics the natural hatching pattern of the burying beetle (hatching skew: -0.15 ± 0.27, mean ± SD). Hatching skew is an index of the degree to which hatching was skewed towards the earlier part of hatching period (see Smiseth *et al.* (2008) for detailed information and the calculation of hatching skew). For the groups with asynchronous hatching, the hatching pattern in group B is the most skewed towards the earlier part of hatching period, and the degree of hatching skew of each group decreases in the following order: B, C, D, E.

To discriminate early, middle and late hatching larvae, each group of larvae were randomly marked by cutting the outer part of either the right or left hind or middle leg when larvae were 12 hours old. We confirmed in the pilot study that this treatment does not affect larval body weight or survival.

To measure growth, larvae on the carcass were weighed individually at 12 hour intervals until their dispersal from the carcass. Larval body weight normally peaks at 120 hours (Takata *et al.*, 2013), thus body weight at 120 hours was used as an index of larval quality. The number of larvae in each brood that survived to the age at which they dispersed from the carcass was used as an index of survival.

Statistical analysis

To investigate the influence of hatching pattern on offspring body weight and survival, we used a generalized linear mixed model (GLMM) with the lme4 package for R (Bates & Maechler, 2010). All GLMMs were conducted using R 2.12.1 GUI 1.35 (http://cran.r-project.org).

To investigate the differences in total combined offspring body weight among groups, total body weight of the whole brood combined at 120 hours was treated as a response variable assuming a Gaussian distribution, with experimental groups as an explanatory variable and brood identity as a random factor.

To investigate the differences in body weight of individual offspring among experimental groups, individual offspring body weight at 120 hours old, for early, middle and late hatching larvae, was treated as a response variable assuming a Gaussian distribution, with experimental groups as an explanatory variable and brood identity as a random factor.

To investigate the differences in survival rate among experimental groups, offspring survival, for early, middle and late hatching larvae, was treated as a response variable assuming a binomial distribution, with experimental groups as an explanatory variable and brood identity as a random factor. Since analyses were performed multiple times to assess differences between experimental groups, P-values were corrected for multiple comparisons using the Holm correction.

To investigate the effects of hatching skew on offspring body weight, individual offspring body mass at 120 hours old was treated as a response variable assuming a Gaussian distribution, with hatching skew as an explanatory variable and brood identity as a random factor. In this analysis, the effect of hatching skew on offspring body weight was analysed separately for early, middle and late hatching larvae.

To investigate the effects of hatching skew on offspring survival, offspring survival rate was treated as a response variable assuming a binomial distribution, with hatching skew as an explanatory variable and brood identity as a random factor. The effects of hatching skew on offspring survival were analysed separately for early, middle and late hatching larvae.

To investigate the effect of timing of hatching on offspring body weight, individual offspring body mass at 120 hours old was treated as a response variable assuming a Gaussian distribution, with the timing of hatching as an explanatory variable and brood identity as a random factor.

Finally, to investigate the effect of timing of hatching on offspring survival, offspring survival rate was treated as a response variable assuming a binomial distribution, with the timing of hatching as an explanatory variable and brood identity as a random factor.

Results

Differences in offspring body weight and survival among experimental groups

The total body weight of the whole brood combined was largest in group C (2457.9 ± 135.5 mg, mean ± SE) and E (2424.1 ± 163.9 mg), and heavier than group A (1949.5 ± 112.7 mg), B (2209.8 ± 238.9 mg) and D (2240.6 ± 221.5 mg) (Fig. 1), although no statistically significant difference in total body weight among groups was detected. Offspring in groups A (208.9 ± 5.9 mg, mean ± SE), C (208.3 ± 4.8 mg) and E (205.4 ± 4.5 mg) grew significantly better than offspring in groups B (187.4 ± 4.7 mg) and D (183.7 ± 5.2 mg) (Fig. 2; Table 2). Offspring in the asynchronous hatching groups B (0.79 ± 0.05, mean ± SE), C (0.77 ± 0.04), D (0.81 ± 0.04) and E (0.79 ± 0.04) had significantly higher survival than offspring in the synchronous hatching group A (0.61 ± 0.05 mg) (Fig. 3; Table 3).

The effect of hatching pattern on body weight and survival of early, middle and late hatching larvae

Hatching skew had a significant effect on the body weight of early (GLMM: estimate = 50.194, $F_{1, 219} = 17.779$, $P < 0.001$), middle (GLMM: estimate = 66.291, $F_{1, 161} = 26.095$, $P < 0.001$) and late hatching larvae (GLMM: estimate = 47.045, $F_{1, 96} = 10.748$, $P = 0.001$) (Fig. 4). Broods with more later hatchers have larger individual offspring weights.

Hatching skew had a significant effect on offspring survival rate in middle hatching offspring (GLMM: estimate = 3.681, $F_{1, 203} = 24.101$, $P < 0.001$), but not in late hatching offspring (GLMM: estimate = 1.242, $F_{1, 159} = 3.646$, $P = 0.058$) or early hatching offspring (GLMM: estimate = -0.506, $F_{1, 247} = 0.321$, $P = 0.572$) (Fig. 5).

Timing of hatching and offspring body weight and survival

Timing of hatching had a statistically significant effect on offspring body weight and survival rate (Table 4, Fig. 4, 5). Earlier hatching offspring had a significantly higher survival and heavier body weight than later hatching offspring in all groups.

Discussion

In this study, we investigated whether the hatching pattern is optimal for the parents or offspring by measuring individual offspring body weight and survival in groups of *N. quadripunctatus*. We found that the total weight of the brood combined is largest in group C, which mimicked the natural hatching pattern of *N. quadripunctatus*, and group E. Both groups had an absolute hatching skew of 0.27. We also found that early hatching larvae were larger in mass at the time of dispersal in all groups. The weight of individual larva in early, middle and late hatching phases increased as the number of larvae in the early hatching phase decreased. Consequently, individual offspring body weight was maximised in group E with least early hatching larvae. Our study demonstrated the influence of hatching pattern on fitness components of *N. quadripunctatus* parent and offspring.

Burying beetles have to locate a carcass for reproduction. Since carcasses are not abundant relative to the large number of individuals in the beetle population, beetles must compete among conspecific individuals for carcasses. Beetles with a larger individual body size are more likely to win the competition for resources (Wilson & Fudge, 1984; Bartlett & Ashworth, 1988; Otronen, 1988). The body size of an adult beetle is determined by the amount of nutrients ingested during the larval stage (Bartlett & Ashworth, 1988). Thus, heavy individual larvae are endowed with more reproductive value. In this study, the hatching pattern of group E produced the heaviest individual larvae in all three hatching phases. Each of the larger larvae would develop into adult beetles with greater body size and be more likely to possess a carcass for reproduction. This indicates that the hatching pattern of group E is optimal for offspring. However, the hatching pattern of group E has not been observed in wild populations, but those of group C have. Although improving larval fitness increases parental fitness, producing many larvae of moderate size increases parental fitness (Trivers, 1974; Godfray & Johnstone, 2000). This seems to favour the hatching pattern of group C with individual larvae of a moderate size. These results suggest the larvae have no power to manipulate their hatching pattern – it is decided by the parent.

Interestingly, the total weight of group E was similar to that in group C. Thus, there would be no conflict between parent and offspring over parental investment in group E. Natural selection could also favour this hatching pattern, though it has not been observed in *N. quadripunctatus*. This could be due to the fitness valley formed by group D, where the survival rate of the offspring was significantly lower, resulting in a smaller total weight of the brood compared to groups C and E. This fitness valley may be preventing the evolution of the hatching pattern seen in group E.

In this study, the early hatching larvae were heavier at dispersal in all groups. This indicates that these larvae receive more parental investment than late hatching larvae. Previous reports on burying beetles (Smiseth *et al.*, 2007; Takata *et al.*, 2013) and birds (Mock & Forbes, 1995; Forbes *et al.*, 1997; Mock & Parker, 1997; Forbes & Glassey, 2000; Hall *et al.*, 2010) also showed that early hatching individuals obtain more parental investment than late hatching ones. These results suggest that hatching pattern influences the allocation of parental investment among offspring, and sibling competition mediates it. Parents may be able to exploit sibling competition and adjust allocation of parental investment among siblings to acquire a higher fitness return by modulating hatching pattern.

Smiseth *et al.* (2008) did not find a significant influence of hatching skew on body weight or survival of offspring in other congeneric species of the burying beetle *N. vespilloides*, although they found that offspring survival is significantly affected by hatching spread which is the time elapsed between hatching of the first and the last larva from a given clutch. Their results are inconsistent with our study on *N. quadripunctatus*. This difference may be due to differences between the species, or due to the methodology used. They observed the effect of hatching skew on fitness using the natural hatching pattern. Hatching skew and hatching spread have some genetic basis (Smiseth *et al.*, 2008). Furthermore, hatching pattern has fitness components, as suggested in our study. Therefore, natural selection will favour an adaptive hatching pattern, which will soon become fixed in a population, resulting in low variation in hatching pattern. In our study, hatching skew was experimentally manipulated to large extent. This experimental set-up predisposed us to detect the effect of hatching skew on fitness.

In conclusion, we have shown that the natural hatching pattern of *N. quadripunctatus* maximises the total weight of the brood but not individual offspring body weight. Our results suggest that the hatching pattern is optimal for parent, rather than offspring. The natural hatching pattern of *N. quadripunctatus*, with the offspring skewed towards early hatchers (Takata *et al.*, 2013), is similar to that of altruistic birds (Magrath, 1990; Stoleson & Beissinger, 1995). We encourage further studies to investigate whether hatching pattern is optimal for the parents or offspring in other phylogenetically distinct species.

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Ethics declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

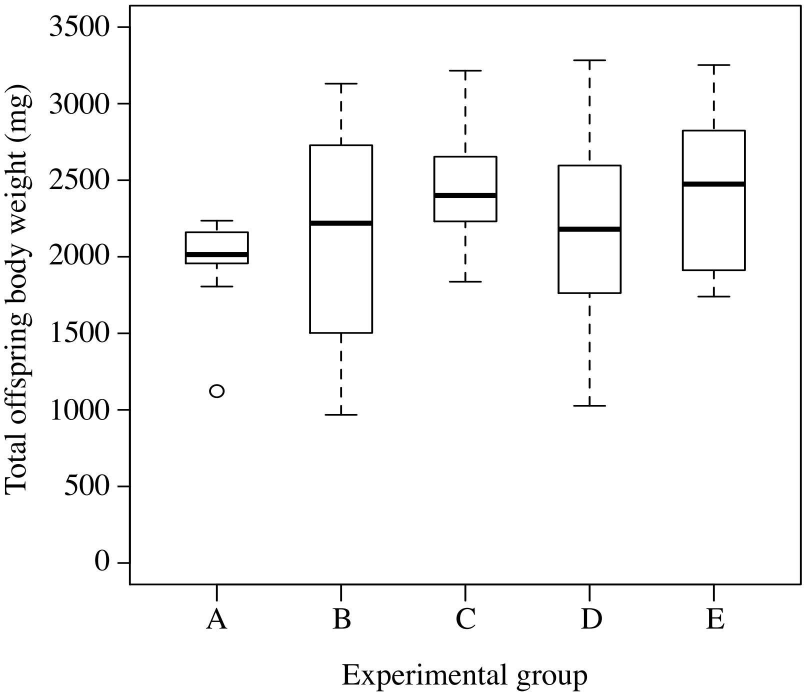

Figure 1. Hatching patterns and the total combined offspring body weight. Data are presented as mean ± SE. Sample sizes are 9 for group A, 11 for group C and 10 for groups B, D and E.

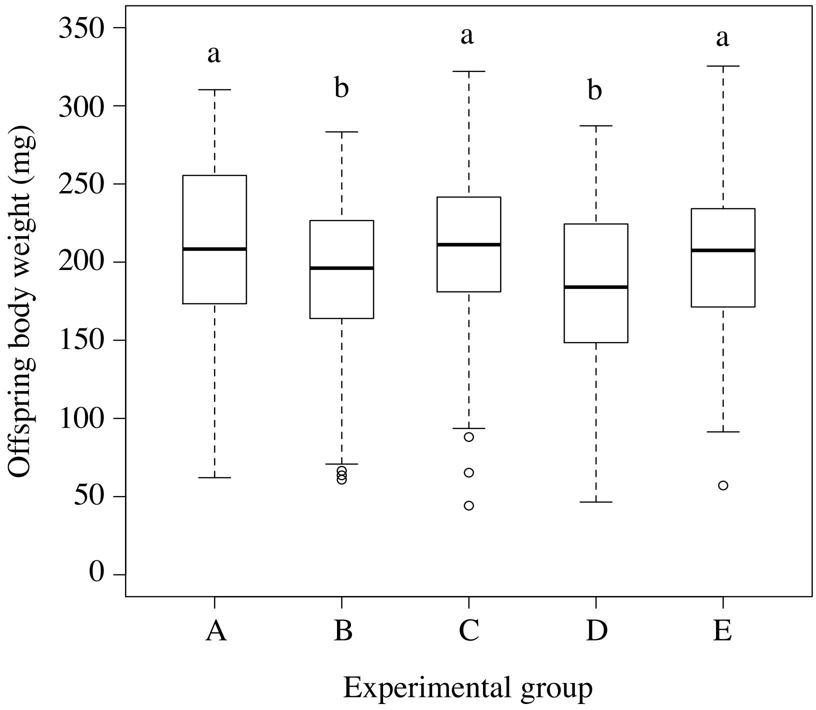

Figure 2. Hatching patterns and individual offspring body weight. Box plot showing the individual offspring body weight at 120 hours old in each experimental group. Different letters indicate those that are significantly different from each other. Significance levels were adjusted with Holm correction for multiple comparisons ($P < 0.05$).

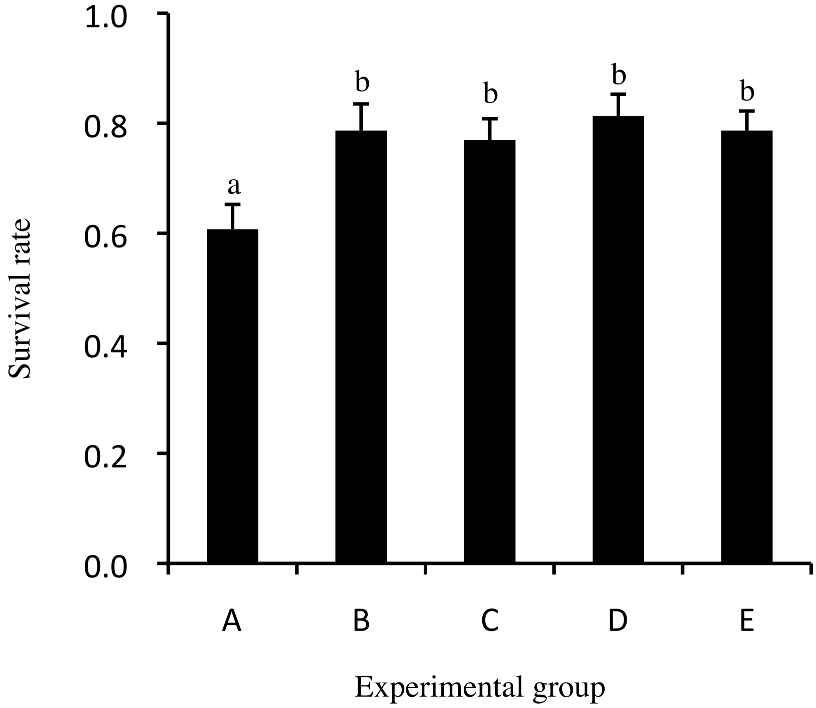

Figure 3. Hatching patterns and offspring survival rate. Data are presented as mean + SE. Different letters indicate those that are significantly different from each other. Significance levels were adjusted with Holm correction for multiple comparisons ($P < 0.05$). Sample sizes are 135 for group A, 165 for group C and 150 for groups B, D and E.

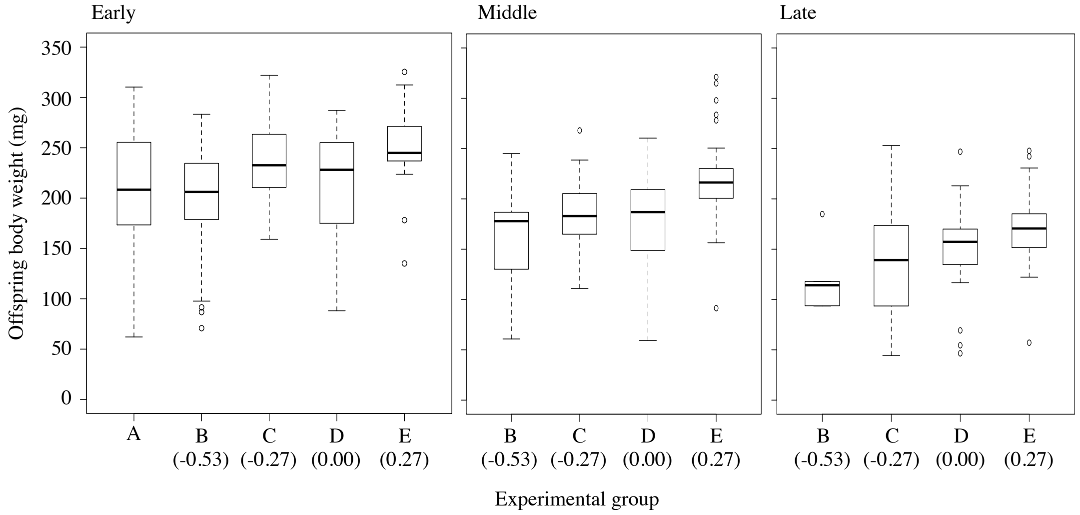

Figure 4. The effect of hatching skew on offspring body weight of early, middle and late hatching offspring. Box plot showing the offspring body weight at 120 hours old in each experimental group. Values in brackets denote hatching skew.

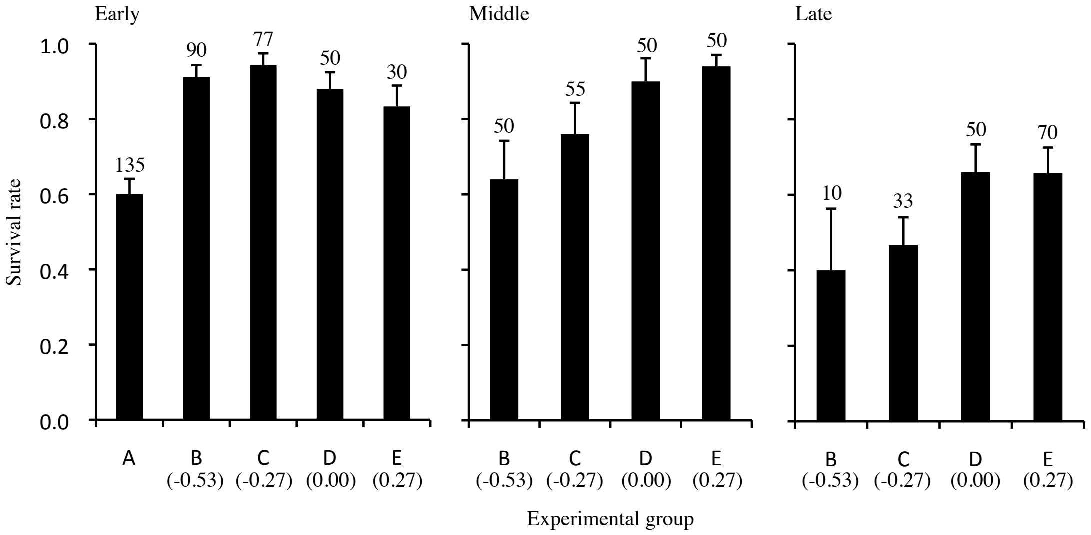

Figure 5. The effect of hatching skew on survival rate of early, middle and late hatching offspring. Data are presented as mean + SE. Numbers on the bars represent sample sizes. Values in brackets denote hatching skew.

Table 1. Experimental groups used to investigate the influence of hatching pattern on offspring body weight and survival.

Group	Early	Middle	Late	Hatching skew
A	15	0	0	-
B	9	5	1	-0.53
C	7	5	3	-0.27
D	5	5	5	0.00
E	3	5	7	0.27

The number of early, middle and late hatching offspring added in each experimental group and the hatching skew of each hatching pattern are shown. Group A exhibits synchronous hatching, while groups B – E exhibit asynchronous hatching. See Smiseth *et al.* (2008) for the calculation of hatching skew.

Table 2. GLMM testing the difference in the total body weight (mg) of the whole brood combined for the 10 pairwise comparisons between each experimental groups

	Group A	Group B	Group C	Group D
Group B	**0.028**			
Group C	0.939	**0.014**		
Group D	**0.016**	1.000	**0.006**	
Group E	1.000	**0.031**	1.000	**0.016**

Holm corrected p-values are shown. Values in **bold** indicate statistically significant results ($P < 0.05$). See Table 1 for details of experimental groups.

Table 3. GLMM testing the difference in offspring survival for the 10 pairwise comparisons between each experimental group units

	Group A	Group B	Group C	Group D
Group B	**0.035**			
Group C	**0.008**	1.000		
Group D	**0.001**	1.000	1.000	
Group E	**0.008**	1.000	1.000	1.000

Holm corrected P-values are shown. Values in **bold** indicate statistically significant results ($P < 0.05$). See Table 1 for details of experimental groups.

Table 4. GLMM testing for the effect of timing of hatching on individual offspring body weight and survival in each experimental group

Factor	Group	Early	Middle	Late	Estimate	df	*P*
Body weight (mg)	B	202.3 ± 5.5	162.6 ± 8.5	127.7 ± 19.8	-2.279	1, 93	**< 0.001**
	C	237.5 ± 4.2	182.4 ± 6.0	141.2 ± 15.4	-2.387	1, 93	**< 0.001**
	D	217.7 ± 9.0	173.5 ± 7.3	152.1 ± 7.3	-1.239	1, 72	**< 0.001**
	E	251.3 ± 7.8	216.9 ± 5.8	168.8 ± 4.7	-1.282	1, 88	**< 0.001**
Survival	B	0.91 ± 0.03	0.64 ± 0.10	0.40 ± 0.16	-62.610	1, 118	**< 0.001**
	C	0.94 ± 0.03	0.76 ± 0.08	0.47 ± 0.07	-48.320	1, 118	**< 0.001**
	D	0.88 ± 0.04	0.90 ± 0.06	0.66 ± 0.07	-51.930	1, 88	**0.002**
	E	0.83 ± 0.06	0.94 ± 0.03	0.66 ± 0.07	-52.710	1, 118	**< 0.001**

The mean ± SE is shown for offspring body weight and survival rate of early, middle and late hatching offspring in each experimental group. *P*-values in **bold** indicate that timing of hatching is statistically significant. See Table 1 for details of experimental groups.