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Experimental food supplementation affects the physical development, behaviour and survival of Little Owl *Athene noctua* nestlings

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In birds, energy supply during growth is a major predictor of the fledglings' physical condition and survival prospects. Differential quantity and quality of fledglings produced under varying nestling food supplies are likely to affect the number of offspring that recruit into the breeding population. However, the underlying mechanisms and associated consequences are still poorly known. Using a partial cross-fostering and food supplementation experiment, we estimated the effect of variation in food supply during growth on nestling survival and fledgling phenotypic traits of Little Owls *Athene noctua*. Survival to fledging was much higher in food-supplemented nestlings (98.6%) than in control nestlings (82.4%). Furthermore, supplemented nestlings were on average 8.9 g heavier and were more likely to develop subcutaneous fat deposits (99.4 vs. 73.7% of treatment and control nestlings, respectively). Supplemented nestlings also had on average longer wings than control nestlings, but tarsi and culmen did not differ significantly. Furthermore, experimentally supplemented fledglings struggled more when handled and emerged sooner from tonic immobility than control fledglings. The irises of supplemented fledglings were less intensely coloured. The experimentally induced changes in nestling development probably affect individual performance beyond fledging. Nestlings from orchard-dominated habitats were larger than those from habitats dominated by arable land. As nestling food supply is largely determined by natural food availability, we conclude that habitat quality affects Little Owl productivity and offspring quality, and ultimately, population dynamics.

Keywords: eye coloration, fledgling behaviour, fledgling phenotype, nestling energy supply, nestling growth, nestling survival, partial cross-fostering, reproductive success.

Energy supply during growth is a major determinant of an individual's physical condition and future reproductive prospects (Lindström 1999). In altricial birds, nestling food supply is determined by natural food availability and parental decisions (Eldegard & Sonerud 2010). The resulting energy flow to the nest affects nestling survival (e.g. Wiehn & Korpimäki 1997, Gonzáles *et al.* 2006, Thorup *et al.* 2010, Wellicome *et al.* 2013) and phenotypic characteristics at fledging. Specifically, augmented energy supply increases fledgling body mass (e.g. Naef-Daenzer & Keller 1999, Hipkiss *et al.* 2002, Santangeli *et al.* 2012) and enhances feather growth (e.g. Berthold 1976, Granbom & Smith 2006) as well as skeletal growth (e.g. Richner 1992, Granbom & Smith 2006, Wellicome *et al.* 2013). In turn, phenotypic traits potentially affect the future performance of independent birds. For example, fledging body mass is a major determinant of post-fledging survival, behavioural performance and recruitment into the breeding population (Verboven & Visser 1998, Naef-Daenzer *et al.* 2001, Naef-Daenzer & Grüebler 2008). Other phenotypic traits such as coloration and behavioural traits govern

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interactions with conspecifics and the environment, and may signal individual quality (Réale *et al.* 2007, Cote *et al.* 2010, Almasi *et al.* 2012, Wolf & Weissing 2012, Avilés & Parejo 2013). However, the relationships between ecological factors, the expression of phenotypic traits, their proximate influences on individual life-histories and the ultimate consequences on key parameters of population dynamics are poorly understood in most species (Benard & McCauley 2008, Fuller 2012). Understanding the consequences of differential food supply on nestling development is essential to understanding the full effects of natal habitat quality on patterns and processes at the population level.

The populations of many bird species closely associated with agricultural landscapes have declined throughout Europe (Donald *et al.* 2001). Habitat degradation in terms of availability of nesting sites and reduced food resources are believed to be the main causes of population declines (Pain & Pienkowski 1997). Modern agricultural landscapes are characterized by low structural heterogeneity and, consequently, low biodiversity (Pain & Pienkowski 1997). Therefore, agricultural changes may affect the reproductive performance via the mechanisms discussed above.

Table 1. Number of control and experimentally supplemented (i.e. treatment) broods across years. Broods involved in partial cross-fostering are listed separately from broods not involved in partial cross-fostering. The total number of nestlings exchanged in each year is given in the last column.

	Year	Control broods	Treatment broods	Exchanged nestlings
Cross-fostered	2010	14	14	54
	2011	14	14	50
Not cross-fostered	2009	13	0	–
	2010	4	0	–
	2011	16	3	–
	2012	16	16	–
Total		77	47	104

This study investigated the effect of food supply during growth on nestling survival and phenotypic development in the Little Owl *Athene noctua*. The Little Owl is a characteristic species of agricultural landscapes in Europe (van Nieuwenhuyse *et al.* 2008), with population declines throughout large parts of Europe over the past three decades (van Nieuwenhuyse *et al.* 2008). Reduced food availability as a consequence of habitat degradation is regarded as one of the main causes of poor reproductive success across Europe (van Nieuwenhuyse *et al.* 2008). However, the effects of food supply during the nestling phase on phenotypic development and nestling survival are unclear. Under the general assumption that an increase in food supply enhances nestling development and reduces sibling competition (Mock *et al.* 1990, Drummond 2001), we predicted that experimental food supplementation during the nestling phase would improve nestling survival and affect nestling phenotype and behaviour. Specifically, we predicted that food supplementation would increase body mass and fat deposition, enhance structural growth (i.e. skeleton and plumage) and affect individual behaviour.

METHODS

The study was conducted in a Little Owl sub-population of approximately 220 breeding pairs in southern Germany (Landkreis Ludwigsburg, Baden-Württemberg, 48°53′43″N, 9°11′45″E). Since 1988 the breeding success of the entire

Table 2. Sample sizes for the analyses of different parameters. The two numbers in the last column indicate the number of observations/number of individuals with unknown sex, respectively.

Analysed parameter	Observations	Individuals	Family history	Year	Sex unknown
Body mass	936	389	173	4	92/68
Wing	635	324	154	3	54/47
Tarsus	877	384	173	4	81/63
Culmen	875	385	173	4	82/64
Fat deposits	215	215	116	3	4/4
Countenance	199	199	111	3	1/1
Eye colour	179	179	99	3	1/1
Tonic immobility	231	231	129	3	1/1
Survival	414	414	181	4	93/93

Table 3. Parameter estimates (and 95% CrI of the posterior distribution) of the nine models run for the different response variables: (a) body condition (i.e. body mass and fat), (b) structural growth (wing, tarsus and culmen), (c) survival, (d) behaviour (countenance, tonic immobility) and (e) eye colour. For tonic immobility exp (coef), standard deviations (coef) and *P*-value are given for the fixed factors. Standard deviations are given for random effects. Significant fixed effects according to the 95% CrI are marked with asterisks. Orthogonal polynomials were constructed and all covariates were standardized (mean = 0, sd = 1) prior to analysis. Sample sizes are given in Table 2.

	(a) Body condition					
	Body mass			Fat		
Fixed factors	Estimate	95% CrI		Estimate	95% CrI	
Treatment	8.88[a]	6.04	11.81	4.13 [a]	2.02	6.26
Age	23.18 [a]	22.07	24.24	0.01	−0.67	0.67
Age^2	−9.68 [a]	−10.51	−8.84	–	–	–
Age^3	2.70 [a]	1.88	3.50	–	–	–
Hatching date	2.09 [a]	0.36	3.90	0.04	−0.89	0.98
Hatching date^2	−0.26	−2.13	1.62	1.12 [a]	0.02	2.22
Brood size	0.11	−1.51	1.74	−0.20	−1.07	0.66
Developmental rank	−2.08 [a]	−3.10	−1.07	−0.53	−1.18	0.12
Intercept	113.12 [a]	109.85	116.38	1.03	−1.38	3.38

Random factors	sd	sd
Year	3.13	1.90
Family history	10.31	2.69
Individual	4.88	–
Residuals	9.69	–

	(b) Structural growth									(c) Survival		
	Wing length			Tarsus length			Culmen length			Survival		
Fixed factors	Estimate	95% CrI		Estimate	95% CrI		Estimate	95% CrI		Estimate	95% CrI	
Treatment	4.35 [a]	3.00	5.68	0.39	−0.03	0.79	0.10	−0.01	0.23	2.72 [a]	1.31	4.11
Age	35.80 [a]	35.22	36.39	4.57 [a]	4.41	4.73	1.39 [a]	1.35	1.44	–	–	–
Age^2	−1.15 [a]	−1.65	−0.67	−1.68 [a]	−1.81	−1.54	−0.35 [a]	−0.39	−0.31	–	–	–
Age^3	−2.80 [a]	−3.25	−2.36	0.38 [a]	0.25	0.51	0.05 [a]	0.01	0.08	–	–	–
Hatching date	1.57 [a]	0.72	2.40	0.28 [a]	0.06	0.51	0.07 [a]	0.01	0.14	1.05 [a]	0.14	1.96
Hatching date^2	−0.82 [a]	−1.64	−0.01	−0.06	−0.29	0.17	−0.06	−0.13	0.01	0.60	−0.37	1.61
Brood size	0.68	−0.13	1.48	0.79 [a]	0.56	1.04	0.08 [a]	0.02	0.15	0.04	−0.46	0.56
Developmental rank	0.26	−0.21	0.74	−0.21 [a]	−0.37	−0.06	−0.01	−0.06	0.04	−0.77 [a]	−1.15	−0.39
Treatment × brood size	−2.34 [a]	−3.50	−1.14	−0.86 [a]	−1.25	−0.46	–	–	–	–	–	–
Treatment × hatching date	–	–	–	–	–	–	–	–	–	−2.25 [a]	−4.09	−0.48
Intercept	76.01 [a]	75.09	76.95	31.11 [a]	30.28	31.96	11.54 [a]	11.37	11.71	1.55 [a]	0.93	2.15

Random factors	sd	sd	sd	sd
Year	0.00	0.94	0.20	0.00
Family history	4.16	1.16	0.30	2.19
Individual	1.76	0.52	0.27	–
Residuals	4.17	1.66	0.47	–

(continued)

Table 3. *(continued)*

	(d) Behaviour						(e) Eye colour		
	Countenance			Tonic immobility			Eye colour		
Fixed factors	Estimate	95% CrI		Exp (coef)	se (coef)	*P*	Estimate	95% CrI	
Treatment	1.33 [a]	0.24	2.44	1.49 [a]	0.19	0.035	−1.11 [a]	−2.23	−0.00
Age	0.54 [a]	0.04	1.03	0.99	0.11	0.910	0.08	−0.47	0.61
Hatching date	−0.23	−0.75	0.30	1.25 [a]	0.10	0.021	0.60 [a]	0.00	1.21
Hatching date^2	0.19	−0.31	0.70	1.10	0.10	0.310	−0.44	−1.03	0.16
Brood size	−0.40	−1.03	0.19	0.91	0.11	0.390	0.06	−0.53	0.64
Developmental rank	0.54 [a]	0.02	1.03	1.05	0.08	0.590	−0.37	−0.86	0.11
Intercept	0.51	−1.12	2.21	–	–	–	1.58 [a]	0.55	2.64
Random factors	sd			sd			sd		
Year	1.31			0.02			0.50		
Family history	1.67			0.47			1.68		
Experimenter	–			0.39			–		

[a] Significant.

sub-population has been monitored (H. Keil unpubl. data). For this study, 124 broods with 414 hatchlings were closely monitored during the four breeding seasons from 2009 to 2012.

Experimental design

To quantify the effects of variation in food supply we conducted a partial cross-fostering and food supplementation field experiment. Partial cross-fostering allowed supplementing only some nestlings from the same family. Thus, intrinsic factors acting on survival and phenotypic development could be statistically controlled for. Two synchronous broods were denoted as partner broods. One of the two was randomly assigned to experimental treatment (i.e. food supplementation). Depending on brood size, one or two nestlings of similar age and body weight were exchanged among partner broods, keeping brood sizes constant. Exchange took place at *c.* 14 days of age. Conservation concerns prohibited earlier manipulation of nestlings. Food supplementation started after exchange. All broods were visited every second day for 36 days. Thus, visits continued beyond fledging at *c.* 30 days of age, when nestlings leave the nestbox for the first time. After fledging, juvenile Little Owls stay within close proximity of and regularly return to the nestbox. During visits a total of 480 g of dead laboratory mice per nestling was deposited inside the nestbox of supplemented broods, 20 g per visit and nestling for the first six visits, 30 g per visit and nestling thereafter. Food supplementation represented a *c.* 40% increase in food compared with natural food supply (Juillard 1984).

This study aimed to determine the effects of nestling food supply while controlling for potential confounding effects, hence the use of cross-fostering to control for intrinsic factors. In 2010 and 2011, a total of 56 broods were subjected to the full experiment (i.e. partial cross-fostering and food supplementation, Table 1). In addition, 68 broods not subjected to partial cross-fostering were included better to estimate the effect of food supplementation (49 control broods and 19 supplemented broods, Table 1). No treatment was performed in 2009.

Data collection

The hatching date of each nestling was determined using developmental illustrations (van Nieuwenhuyse *et al.* 2008) and the equations given in Juillard (1979). Owlets were measured at the ages of 13.7 ± 5.57 days (mean ± 1 sd, n = 267, hereafter first measurement), 21.5 ± 3.99 days (n = 345, second measurement) and 29.4 ± 3.29 days (n = 307, third measurement). To minimize disturbances across the population, the first measurement was only taken in broods handled anyway during partial cross-fostering. Plastic colour rings were used to identify individuals prior to ringing. At each measurement body mass (to the nearest 0.1 g), and the lengths of the wing and of the ninth primary (to the nearest 0.5 mm), as well as of the tarsus and the culmen (to the nearest 0.1 mm) were recorded.

At the third measurement, feather samples were obtained for genetic sex determination of the nestlings, and the presence or absence of fat deposits under the wing was recorded. Growth conditions may also affect appearance (e.g. eye colour) and behaviour. Both have the potential to affect later life-history stages (Bortolotti *et al.* 2003, Duckworth & Badyaev 2007, Duckworth 2008, Guillemain *et al.* 2012). Thus, we recorded eye coloration (i.e. pale or intense as compared with reference photographs) as one trait of individual appearance that has potential signalling functions (Guillemain *et al.* 2012). Additionally, we recorded the owlets' behaviour during handling (i.e. passive or struggling) and conducted a tonic immobility (TI) test (Forkman *et al.* 2007). To induce TI, an owlet was held on its back for 10 s. Upon release, time until righting of the animal was recorded. If this time was less than 10 s in three consecutive trials, TI was classified as 'not induced'. The TI test was terminated 290 s after release. Individuals that failed to get up during the

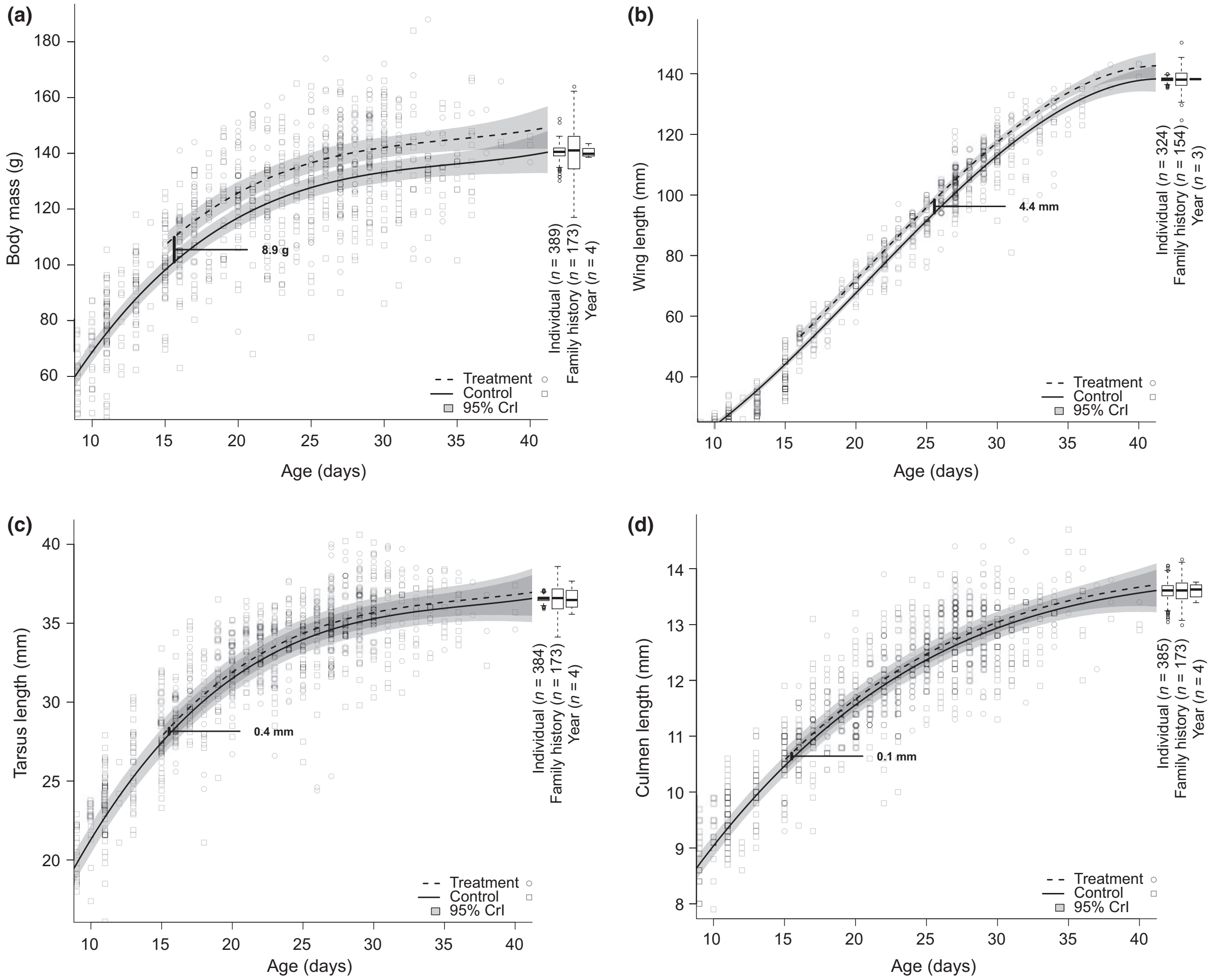

Figure 1. Growth curves of food-supplemented (dashed line) and control (solid line) nestlings for (a) body mass, (b) wing length, (c) tarsus length and (d) culmen length. Open circles and squares represent measurements of food-supplemented and control owlets, respectively. Ninety-five per cent CrI from the posterior distribution are shaded in grey. Boxplots represent model outputs for the random effects. Sample sizes are given in Table 2.

experiment were assigned a TI time of 290 s ($n = 34$).

Hatching asynchrony within a brood leads to a developmental gradient among siblings (van Nieuwenhuyse *et al.* 2008). When food is limited, this gradient benefits early-hatched nestlings in sibling competition (Mock 1985). To include this developmental gradient, a developmental rank was assigned to each nestling according to the estimated hatching date. The oldest nestlings in a brood were assigned a rank of zero. Nest mates were assigned the same rank if the estimated age difference was < 2 days. Because the developmental rank of a nestling may change during the nestling phase (e.g. when a sibling dies), the developmental rank was adjusted at each measurement.

Each measurement was assigned a label 'family history' combining the individual's original family and its family at measurement. For example, the first measurement of a nestling that hatched in family 'A' but was later exchanged to family 'B' was labelled 'A A'. The second and third measurements of the same nestling (i.e. after the exchange) were labelled 'A B'. Thus, this factor accounted for the exchange, representing the original as well as the foster family.

Due to missing data, sample sizes differed between analyses of dependent variables (Table 2).

Analysis of morphological parameters

The effect of food supplementation on morphological parameters (i.e. body mass, length of wing, tarsus and culmen) was analysed using linear mixed effects models with the package arm (Gelman & Su 2013) in the statistical software R 3.0.2 64-bit (R Core Team 2013). Morphological parameters were separately modelled as a function of nestling age with treatment as a fixed factor. The maximal model included fixed effects to control for the developmental rank, for brood size at measurement and for hatching date. Polynomial factors were included for age (cubic) and hatching date (quadratic) to approximate the non-linear nature of nestling growth and environmental conditions throughout the year. The individual and family history were included as random factors to account for individual dependency and individual history, respectively. Year was also included as a random factor. The maximal model further included two-way interactions between food supplementation and fixed factors. Backwards model selection according to the Bayesian Information Criterion (BIC) was used to remove non-significant interactions (Burnham & Anderson 2002). To facilitate model convergence, orthogonal polynomials of age and hatching date were used and all covariates were standardized (mean = 0, sd = 1). Residuals of the maximal model were inspected to confirm model assumptions. Ninety-five per cent Credible intervals (CrI) for all fixed and random factors were obtained from the simulated posterior distribution (5000 simulations). As some individuals were not sexed (Table 2), the effect of sex as a fixed effect was determined *post-hoc* on a reduced dataset (using the CrI from 5000 simulations).

Analysis of binomial variables

Binomial response variables (i.e. presence/absence of fat deposits under the wing, passive/struggling behaviour during handling, pale/intense eye coloration) were analysed with generalized linear mixed-effects models assuming a binomial distribution of the residuals with the package arm (Gelman & Su 2013) in R 3.0.2 64-bit (R Core Team 2013). The analytical approach was identical to the analysis of morphological parameters, but the maximal model was adjusted to the specific characteristics of the data. As only a single measurement per individual was taken, no random factor for the individual was included. Datasets were reduced to measurements at ages from 25 to 30 days. As a consequence, age was only included as a linear fixed factor.

Analysis of nestling survival

Nestling survival was defined as survival from hatching to day 30 post-hatching. Analysis of nestling survival was identical to the analysis of binomial variables, except that nestling age was excluded from the analysis. As animals were only sexed shortly before fledging (i.e. after nestling mortality has occurred), no *post-hoc* test for the effect of sex on survival was conducted.

Analysis of tonic immobility

Factors affecting the duration of TI were analysed using Cox proportional hazards models with random effect with the package coxme (Therneau 2012) in R 3.0.2 64-bit (R Core Team 2013). The analytical approach was identical to the analysis of morphological parameters (i.e. backwards model selection according to the BIC, use of orthogonal polynomials and standardization of covariates). The maximal model was based on the maximal model of binomial variables. As the experimenter has a marked effect on the TI test (Forkman *et al.* 2007), the experimenter was included as a random effect in the maximal model. Model interferences were based on the score test statistics at a significance level of 0.05.

Effects of environmental factors

Nestling food supply is ultimately driven by natural food availability. Thus, experimental food supplementation simulates an improvement in habitat quality in terms of food availability. As the availability of food varies among territories and over time, we expected nestling survival and development to vary in relation to environmental factors that are linked to natural food sources. Based on the models used to calculate the effect of food supplementation, we performed a *post-hoc* test for the interaction between food supplementation and the random effect year, and for differences between broods reared in different habitats as a fixed effect (i.e. habitats dominated by arable land vs. orchard-dominated habitats).

The 95% CrI of the posterior distribution (5000 simulations) was used to determine the significance of fixed factors. Because the CrI does not allow for significance testing of random effects, the BIC was used to test the treatment–year interactions (Burnham & Anderson 2002).

RESULTS

Body condition

On average, food-supplemented nestlings were 8.9 g (CrI = 6.0–11.8 g) heavier than control nestlings (Table 3a, Fig. 1a). Compared with the average body mass of control nestlings between ages of 26 and 35 days (133.6 g, $n = 200$) this corresponded to an increase of 6.7%. In addition, virtually all supplemented nestlings had subcutaneous fat deposits under the wing ($P_{treatment} = 0.994$, CrI = 0.955–0.999; Fig. 2a), whereas fat was observed in fewer than three-quarters of the control nestlings ($P_{control} = 0.737$, CrI = 0.201–0.967; Fig. 2a).

Factors other than experimental treatment substantially affected the body condition (taken as body mass and fat deposits, both integral parts of body condition) of Little Owl fledglings. The growth curve of body mass showed the typical rapid growth during the nestling phase reaching an asymptote around fledging at *c.* 30 days (Fig. 1a). Furthermore, body condition varied with hatching date. Nestlings hatched late in the breeding season grew heavier than nestlings hatched early in the season (Table 3a). In contrast, nestlings hatched early and late in the breeding season were more likely to have subcutaneous fat stores than birds hatched mid-season (Table 3a). Within broods, the developmental rank of the owlets also affected body mass. Lower-ranked (i.e. earlier-hatched) nest mates grew heavier than higher-ranked nest mates (Fig. 3a). Brood size had no effect on body

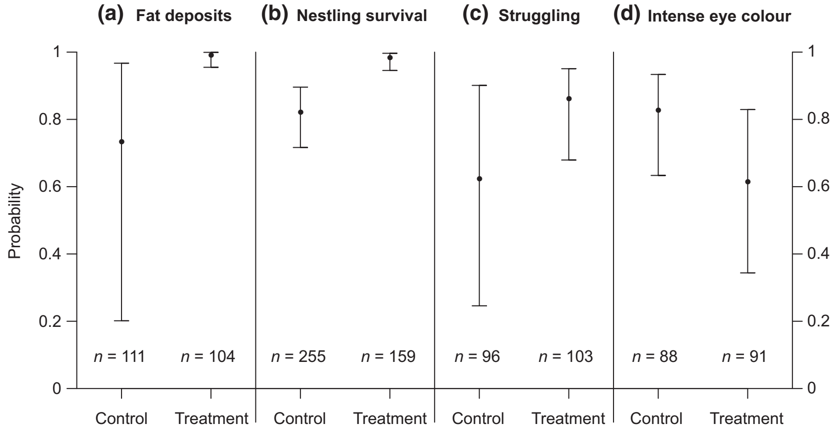

Figure 2. Effects of food supplementation on the probability of (a) having subcutaneous fat deposits under the wing, (b) surviving the nestling phase, (c) struggling when handled and (d) having intense eye coloration in Little Owl nestlings. Error bars indicate the 95% CrI of the posterior distribution.

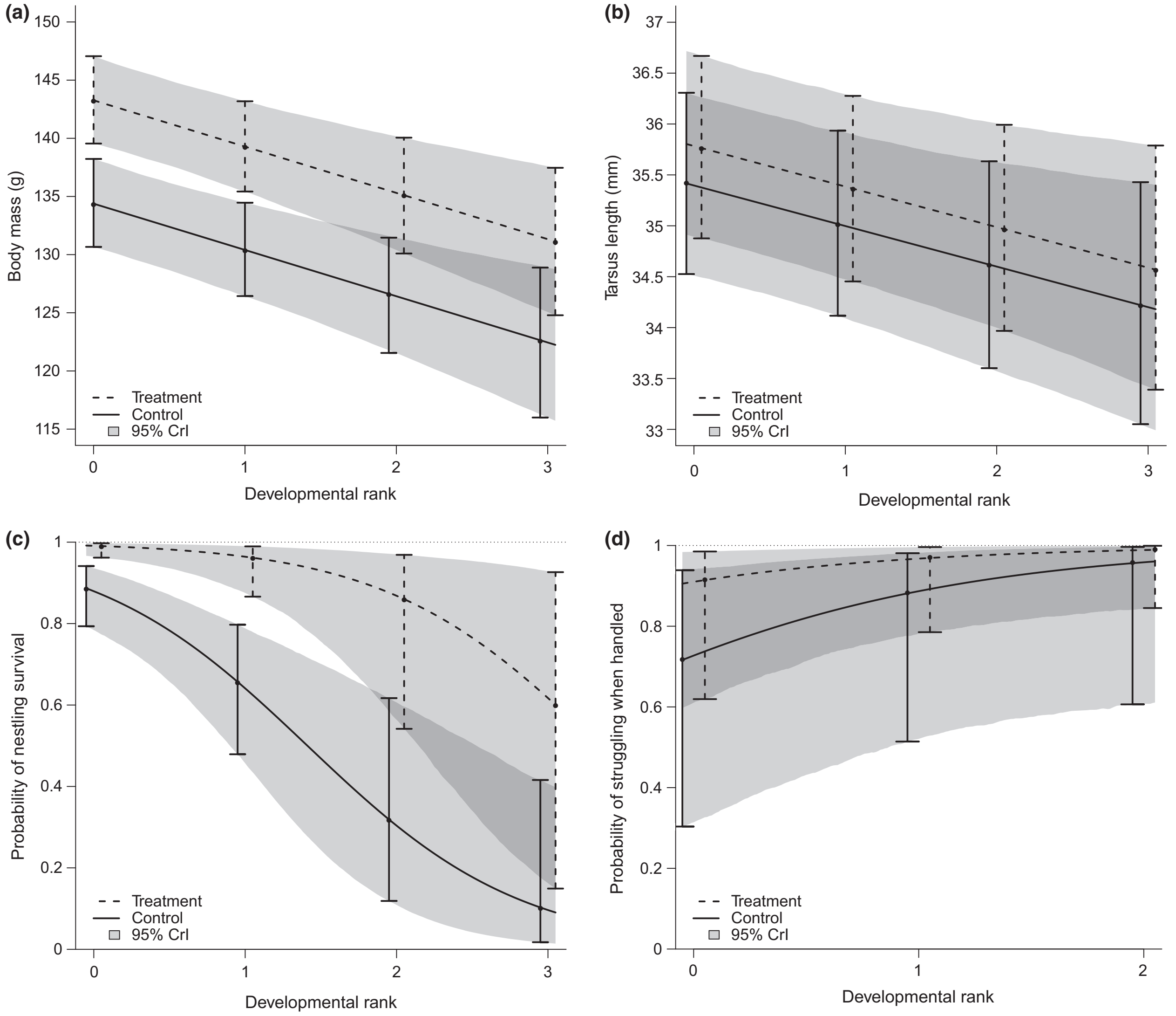

Figure 3. Effect of developmental rank and experimental treatment on nestling (a) body mass, (b) tarsus length, (c) survival probability and (d) the probability of struggling when handled. A developmental rank of zero represents the first-hatched Little Owl within a family. The shown effects were predicted for 30-day-old nestlings. The dashed and solid lines represent the food-supplemented and control group, respectively. Ninety-five per cent CrI from the posterior distribution are shaded in grey. Sample sizes are given in Table 2.

condition (Table 3a). Model selection under the BIC revealed no significant interaction and the *post-hoc* tests revealed no effect of sex on body condition (Table 4).

Structural growth

On average, the wings of supplemented nestlings were 4.4 mm (CrI = 3.0–5.7 mm) longer than those of control birds (Table 3b, Fig. 1b). Compared with the average wing length of control nestlings between ages of 26 and 35 days (110.1 mm, $n = 131$) this corresponded to an increase of 4.0%. The increase of 0.4 mm (CrI = −0.03 to 0.79 mm) in tarsus length and 0.1 mm (CrI = −0.01 to 0.22 mm) in culmen length due to food supplementation was not significant (Table 3b, Fig. 1c, d). Compared with the average tarsus and culmen length of control nestlings between ages of 26 and 35 days (tarsus: 35.0 mm, $n = 182$; culmen: 12.8 mm, $n = 171$) this corresponded to an increase of 1.1 and 0.8%, respectively.

Table 4. *Post-hoc* tests for sex. Estimates for the factor sex (males), degrees of freedom (df), the 95% CrI from the posterior distribution, ΔBIC ($\text{BIC}_{\text{without sex}} - \text{BIC}_{\text{with sex}}$) and sample sizes are given. ΔBIC > 2 is considered significant. For tonic immobility, the exp (coeff) is given instead of the estimate.

Response variable	Estimate	df	95% CrI		ΔBIC	Observations	Individuals	Background	Year
Body mass	0.78	1	−1.02	2.56	−6.03	844	321	164	4
Wing	0.47	1	−0.52	1.44	−5.48	581	277	150	3
Tarsus	−0.03	1	−0.34	0.28	−6.64	796	321	164	4
Culmen	0.10	1	−0.00	0.20	−2.72	793	321	164	4
Fat deposits	−0.05	1	−1.09	1.01	−5.60	211	211	116	3
Countenance	−0.28	1	−1.19	0.62	−4.96	198	198	111	3
Tonic immobility	1.15	1	–	–	−4.53	230	230	128	3
Eye colour	0.28	1	−0.62	1.17	−4.88	178	178	98	3

Structural growth was also related to factors other than food supplementation. As with body mass, the pattern of structural growth was primarily related to nestling age. Structural growth was most pronounced during the nestling period and approached an asymptote after 30 days for tarsus and culmen, somewhat later for wing (Fig. 1b–d). Furthermore, advancing breeding season positively affected structural growth (Table 3b). Little Owls hatching at the end of May grew approximately 8 mm longer wings, 1 mm longer tarsi and 0.5 mm longer culmen than those hatching at the end of April. For wing growth, this seasonal effect decreased towards the end of the breeding season (Table 3b). In addition, lower-ranked (i.e. earlier-hatched) nest mates grew longer tarsi than higher-ranked nest mates (Fig. 3b) and larger broods produced larger nestlings in terms of skeletal growth (Table 3b). The effect of food supplementation on the size of wing and tarsus was more pronounced in small broods than in large broods (Table 3b). *Post-hoc* tests revealed no effect of sex on structural growth (Table 4).

Nestling survival

Almost all supplemented nestlings survived the first 30 days after hatching ($P_{treatment} = 0.986$, CrI = 0.945–0.997). In contrast, 17.6% of the control owlets died as nestlings ($P_{control} = 0.824$, CrI = 0.717–0.896; Fig. 2b). Survival of non-supplemented nestlings improved with advancing breeding season and thus the effect of food supplementation declined with advancing season (Fig. 4). Within control broods, nestling survival was strongly related to the developmental rank. Within broods of large hatching asynchrony, the first-hatched (i.e. lowest-ranked) nestlings had a nine-fold higher survival probability compared with last-hatched nestlings (Fig. 3c). Food supplementation virtually eliminated this effect. This indicates that the youngest nest mates within a brood profited most from experimental food supplementation. Brood size had no significant effect on nestling survival (Table 3c).

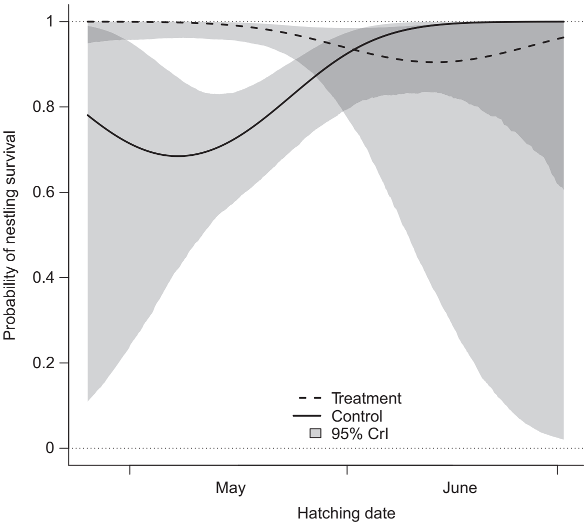

Figure 4. Effects of hatching date and experimental treatment on Little Owl nestling survival. The dashed and solid lines represent the food-supplemented and control group, respectively. Ninety-five per cent CrI from the posterior distribution is shaded in grey. Sample sizes are given in Table 2.

Behavioural traits

Compared with control nestlings, a higher proportion of supplemented nestlings struggled when handled ($P_{treatment} = 0.864$, CrI = 0.680–0.951; $P_{control} = 0.626$, CrI = 0.246–0.901; Fig. 2c). Supplemented nestlings also emerged on average 14.8 s sooner from tonic immobility ($P = 0.035$).

Behavioural traits were also related to factors other than experimental food supplementation. The probability of a nestling struggling during handling increased with nestling age (Table 3d) and with increasing developmental rank (Table 3d, Fig. 3d). Furthermore, time to emergence from TI decreased over the course of the breeding season (Table 3d). Brood size did not affect countenance during handling or TI time (Table 3d). For behavioural traits, model selection under the BIC revealed no significant interaction and the *post-hoc* tests for sex were not significant (Table 4).

Eye coloration

Compared with control nestlings, a significantly lower proportion of supplemented nestlings had intensively coloured eyes ($P_{treatment} = 0.617$, CrI = 0.344–0.829; $P_{control} = 0.830$, CrI = 0.633–0.934; Fig. 2d). Hatching date was the only factor besides experimental treatment affecting eye coloration (Table 3e). The probability that nestlings had intensively coloured eyes increased over the course of the season. Model selection under the BIC revealed no significant interaction for eye coloration and the *post-hoc* test revealed no effect of sex on eye coloration (Table 4).

Effects of environmental factors

For all dependent variables the interactions between food supplementation and year were not significant according to the BIC ($\Delta\text{BIC} = \text{BIC}_{\text{without interaction}} - \text{BIC}_{\text{with interaction}}$ for TI = 0.00, ΔBIC for all other tested variables < -10; $\Delta\text{BIC} > 2$ is considered significant).

Across the entire dataset and controlling for experimental effects, Little Owl nestlings growing up in orchard-dominated habitats were significantly heavier (4.94 g, CrI = 0.09–10.09 g) and larger in terms of wing length (2.55 mm, CrI = 0.18–4.83 mm) and tarsus length (0.77 mm, CrI = 0.15–1.41 mm) than were nestlings growing up in habitats dominated by arable land.

DISCUSSION

Food supplementation experiments have frequently been used to determine the effects of energy supply during different phases of the reproductive cycle (Martin 1987) and often show that sufficient food supply during early development is crucial for the fledgling's physical condition and survival prospects (e.g. Dewey & Kennedy 2001, Thorup *et al.* 2010, Wellicome *et al.* 2013). In our study, experimental food supplementation strongly increased Little Owl survival from hatching to fledging and profoundly altered the nestlings' physical and behavioural development. Supplemented nestlings accumulated substantial subcutaneous fat stores, and fledged heavier and with longer wings. Their eyes were paler, potentially affecting the signalling function of eyes (e.g. Guillemain *et al.* 2012). Supplemented nestlings also struggled more when handled and emerged faster from tonic immobility. Thus, increasing nestling food supply substantially affected two major aspects of reproductive success: the survival of nestlings to fledging and the quality of fledglings.

The clear effects of the flow of energy to Little Owl broods are ultimately driven by natural variation in food availability. Our results highlight that control broods grew below the maximal physiological rate, suggesting that parents did not fully compensate for environmental variation in food availability. Thus, spatial and temporal variation in food availability is expected to cause spatial and temporal variation in offspring survival and quality. Rodents are a major food source of Little Owls in Central Europe (van Nieuwenhuyse *et al.* 2008). Orchards, extensive grassland, pasture and edge structures sustain high rodent densities, whereas mechanically cultivated fields are virtually rodent-free (Apolloni 2013). The results of this study match this pattern; Little Owl territories encompassing a large proportion of orchards and grassland produced larger fledglings (in terms of body mass, wing and tarsus) compared with territories dominated by arable land. On the temporal scale, live trapping data from 2010–2012 (M. Perrig unpubl. data) indicated that rodent density increased in each year from spring to summer. This may explain the findings that Little Owl nestlings hatched in June were larger (in terms of body mass, wing, tarsus and culmen) and emerged earlier from TI than nestlings hatched in May. Also, June hatchlings were more likely to survive the nestling phase. We conclude that spatio-temporal patterns of natural food availability directly translate into variation in the productivity and offspring quality of Little Owl broods to an extent similar to the results of the experimental supplementation.

The flow of energy to nestlings is also mediated through parental performance and decisions. Parents face a trade-off in energy allocation between current and future reproduction (Eldegard & Sonerud 2010, Santangeli *et al.* 2012) and this may provide an explanation for control broods not developing at maximum rates. From a parental perspective, the related reduction in numbers and quality of the current brood may be outweighed by the long-term allocation of parental resources to subsequent reproductive attempts.

Hatching order and food supplementation

The hatching order within a brood also had marked effects on nestling survival and development. First-hatched nestlings grew larger (in terms of tarsal length) and heavier, struggled less when handled and had much better chances of surviving the nestling phase. The difference in physical development between the first- and the last-hatched owlet in a brood with high hatching asynchrony was even larger than the average effect size of the treatment. The survival rates of the highest-ranked owlets were particularly low and strongly increased with food supplementation. Accordingly, within broods, last-hatched nestlings profited most from food supplementation, which increased the overall fledging success of the brood. The likely mechanism behind improved survival of late-hatched nestlings is therefore that food supplementation reduced competition among siblings, which in turn mitigated brood reduction (for a review of the brood reduction hypothesis see Mock *et al.* 1990, Drummond 2001).

Effects beyond fledging

Although the consequences of increased nestling survival on reproductive success and, in turn, on demographic parameters are straightforward, whereas the effects of fledgling phenotypic traits on post-fledging performance and future reproductive success are not. There is evidence that some phenotypic traits at fledging influence the performance of independent birds. For example, body mass at fledging was correlated with post-fledging home-range size, short-term movements and survival, and with the potential to recruit into the breeding population (Simons & Martin 1990, Both *et al.* 1999, Naef-Daenzer *et al.* 2001, Monròs *et al.* 2002, Naef-Daenzer & Grüebler 2008). For other phenotypic traits the consequences are still controversial. For example, avian fat stores are important energy reserves, but also increase emergency take-off time (Witter & Cuthill 1993). Thus, fat stores may improve or decrease post-fledging survival.

CONCLUSIONS

Two main conclusions emerge from our results. First, variation in nestling food supply leads to differential nestling survival and differential physical and behavioural development. Therefore, the proximate effect of increasing the flow of energy to the brood is to increase the number of fledging offspring and alter their quality. These findings contribute to explaining the seasonal, annual and spatial variation in fecundity documented in Little Owl populations (van Nieuwenhuyse *et al.* 2008). Secondly, the results suggest that the cascade of mechanisms that links habitat characteristics to reproductive output and offspring quality may have further consequences for key parameters of demography. The trophic conditions during early development probably carry over to post-fledging survival, dispersal movements and recruitment. Consequently, full comprehension of the chain of mechanisms determining reproductive performance and offspring survival contributes to clarifying the ecological processes that cause the declines in farmland species such as the Little Owl. From a wider perspective, our results underline that agricultural impacts at spatial and trophic levels strongly affect the reproductive performance of species and thus the biodiversity of agricultural landscapes. Accordingly, conservation measures to optimize habitat quality and food resources may strongly support the productivity of local populations.

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