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Sources and timing of calcium intake during reproduction in flycatchers

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Abstract Calcium availability may limit the reproductive output of birds and snail shells are considered to be the main source of calcium in many passerine species. This study of collared (*Ficedula albicollis*) and pied (*F. hypoleuca*) flycatchers evaluates calcium intake of a natural diet in Central Europe, and sex differences in the utilization of experimentally supplemented sources of calcium during the entire breeding period in aviary birds. The study provides the first evidence that successful reproduction of these species depends on the availability of woodlice (Isopoda) and millipedes (Diplopoda). Each of these two components provided about 3 times more calcium than the snail shells contained in a natural nestling diet. The breeding performance of aviary birds was poor when only snail shells and the fragments of eggshells were provided in food, i.e., irregular laying, smaller clutches, eggshell defects (25 of 53 eggs), and eggs dried-up during incubation. In contrast, no defective eggshell or dried-up eggs were found and the overall breeding performance increased 2–3 times when woodlice were added to the food. Females increased their intake of woodlice during both the pre-laying and laying periods, and both sexes did so during the nestling period. Both sexes took more woodlice in the evening than in the morning, independent of the nesting stage. Intake by females was low until 4 days before laying the first egg, then increased to the highest level, dropping immediately after laying the last egg. Intake of woodlice by both sexes increased steadily from hatching until the nestling age of about 10–12 days decreasing thereafter, which corresponds with the period of rapid skeletal growth. In contrast, the intake of mealworms increased until the nestling age of 13–14 days leveling off thereafter which corresponds with the growth curve of nestling body mass.

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Introduction

Decreased calcium availability might limit the reproductive output in birds (Simkis 1967; Ormerod et. al. 1991; Graveland et al. 1994; Wolf et al. 1998; Tilgar et al 2002). Birds need increased calcium intake mainly during two periods, those of egg-shell formation and nestling growth. The timing of calcium intake is well known in domestic fowl (Simkis 1967; Hughes and Wood-Gush 1971; Gilbert 1983), but few studies have been performed on wild birds. In passerines, which do not store calcium for egg formation in the medullary bone (Pahl et al. 1997), in contrast to chicken, geese, pigeons and waders (Simkis 1967; Gilbert 1983; Piersma et al. 1996), the calcium must be obtained on a daily basis. Thus, laying females in passerines need to search for calcium-rich materials.

Various calcium-rich items, such as snail shells, eggshells, bones and calcareous grit have been recorded as sources of calcium in birds (reviewed in Graveland 1990). Snail shells have been considered the main source of calcium for many passerine species. The importance of woodlice (Isopoda) and millipedes (Diplopoda) in this respect has already been mentioned (Davies 1977; Graveland and Van Gijzen 1994). A simple comparison of the known diet composition among passerine species outlines species-specific differences in the ability to use a particular source of calcium. Thus, tits (*Parus* spp.) and pipits (*Anthus* spp.) use mainly snail shells as a source of calcium (Graveland et al. 1994; Bureš and Weidinger 2000), in contrast to flycatchers (*Ficedula* spp.), wren (*Troglodytes troglodytes*) and robin (*Erithacus rubecula*), which often consume woodlice and millipedes (Bureš 1986; Krištín 1992).

Little is known about species-specific dependency on a particular source of calcium or the timing of demand for it (Bureš and Weidinger 2001; Dhondt and Hochachka 2001). Only a few studies have reported on changes in calcium intake during the entire period from pre-laying to incubation in passerines (Jones 1976; Schifferli 1977; Graveland and Berends 1997). To our knowledge, only one study provided data on total calcium intake during the nestling period (Bureš and Weidinger 2000), and no study has reported on the timing of calcium intake and sex-differences in the provisioning of calcium during the nestling period.

The collared (*Ficedula albicollis*) and pied (*F. hypoleuca*) flycatchers are small (11–14 g), migratory, hole nesting, insectivorous passerines, in which the female builds the nest and incubates the clutch, but both parents feed the nestlings. This paper reports on: (1) the importance of individual diet components as sources of calcium in natural populations of flycatchers; (2) the timing of utilization of various experimentally supplemented calcium-rich sources, during the entire breeding period in flycatchers kept in aviaries; and (3) the effects of these supplemented calcium-rich sources on breeding performance.

Materials and methods

Aviary experiments

The aviary experiments were conducted in the village Bohuňovice (49°38′N, 17°18′E, 230 m asl), central Moravia, Czech Republic, in 1997–2002. The birds used in the experiments were caught each spring in nest-boxes at the study plots located in lowland deciduous forest near Hynkov [7 km NW; collared flycatcher (CF)] and submontane beech forest near Malá Morávka [37 km N; pied flycatcher (PF)]. The birds were caught, measured and placed in experimental aviaries. All birds were ringed to avoid repeated use of the same individuals in subsequent years. Of the 41 pairs established, pair formation and breeding was followed in 29 cases: CF (5); PF (1); female CF + male PF (19); female PF + male CF (5). The mixed pairs were established for the purpose of inter-specific hybridization experiments, the results of which have been reported elsewhere (Veen et al. 2001). As no effect of species on food intake was detected (see below) both pure and mixed pairs were used in this study.

The aviaries (2×2×2 m) had double walls and a roof (net inside, wire outside) and were located among tall trees. Each aviary was equipped with one nest box (bottom area 140 cm^2), four branches about 1 m long, feeders, a watering place and a sufficient amount of dry grass (nest building material). During the first study period (1997–1999), only mealworms, small snail shells, pieces of hen eggshells and water were given ad libitum in separate feeders. Arthropods caught by sweep net in forest undergrowth were added at 0700 and 1900 hours on the first 3 days following hatching.

The nestboxes were checked daily at 0700 and 1900 hours. Newly laid eggs were marked, their length and width was measured to the nearest 0.1 mm, and egg volume was calculated using Hoyts (1979) formula. After hatching, the eggshells were collected from the aviaries, air dried and their thickness was measured at the blunt pole and along the egg side to 1 μm, using a micrometer screw gauge modified to accommodate curvature. Both the tarsus length (caliper, to 0.1 mm) and the body weight (Avinet, to 0.2 g) of the nestlings were measured on the day the first nestling fledged from the nestbox.

The feeding experiments were conducted during the second study period (2000–2002), when woodlice were added to the diet. A video camera was permanently focussed on the feeders in each aviary. The number of food items taken by both the male and female was video-recorded during one 45-min trial started either in the morning (0600 hours) or the evening (1800 hours) on alternate days. The trial was conducted daily from about 7 days before the start of laying until fledging, except for the incubation period, during which the trial was conducted daily in only five of the ten aviaries. Although food was available ad libitum throughout the day, all food components were supplied before the start of each trial. The taking of calcium rich components (woodlice, snail shells, eggshells) was recorded during all nesting stages while the taking of mealworms was recorded only during the nestling period. It was not possible to distinguish exactly between items consumed by parents or brought to nestlings from the video recordings. However, this is not critical for the aim of the present study. Hence, we use the term taking both to describe actual eating and the provisioning of nestlings.

After nesting, all adults (with young if breeding was successful) were transferred to the sites where they had been caught. All experimental procedures were approved and supervised by The Ethical Committee of Palacky University, Olomouc.

Analyses of calcium contents

Calcium contents in the eggshells and nestlings was measured by atomic absorption spectrophotometry and compared between aviary birds fed with a diet containing woodlice (2000–2002) and free-living collared flycatchers. Eggshell samples from two aviaries, one composite sample from all other aviaries, and one composite sample from free-living birds were analysed. Four fledglings (one per aviary) and three 15-day-old nestlings (one per nest box) from the study plot in the lowland forest were etherised, the digestive tract was removed and the total body calcium contents was measured.

Using data from former studies of flycatcher nestling diet in Moravia (Bureš 1986, 1995; Bureš and Stříteský 1996; Bureš and Horáčková 1998), we evaluated the importance of various diet components as calcium sources for nestling growth under natural conditions. The snails, woodlice and millipedes, collected during the above studies and stored in 70% ethanol, were dried and weighed. The total amount of calcium in the food was then calculated separately for each taxon, by multiplying the dry mass by the percentage of calcium contents. In these calculations, we used data on calcium content in woodlice (10.85% dry weight), millipedes (13.52%) and snail shells (33.13%) from the studies of Graveland and Van Gijzen (1994) and Graveland and Berends (1997). Other arthropods were omitted because they play a minor role as a calcium source in passerines (Graveland and Van Gijzen 1994; Bureš and Weidinger 2000).

Data analysis

The number of food items taken per experimental trial (count data) was analysed by fitting generalized linear mixed models (GLMM) with log link and Poisson error distribution (macro Glimmix, SAS Institute; Littell et al. 1996). The trials performed on individual birds throughout the nesting period do not represent independent observations. Moreover, because they formed pairs, individual birds do not represent independent units. To account for this hierarchical data structure, all mixed models contained a random effect of individual bird (lower level subject) nested within a random effect of pair (higher level subject). The reported tests of fixed effects are thus more conservative compared to an analysis assuming the independence of individual observations. The significance of the fixed effects was assessed by the Type III F-test with the denominator df estimated by the Satterthwaite method. The fixed effects included in the GLMM models as predictors of food intake were: an individuals sex, time of day (morning vs evening), day of the nesting stage (continuous) and brood size (continuous; only in models for the nestling period). We also examined the effect of species (CF vs PF) of the male and female within a pair, a quadratic effect of day and all two-way interactions. Both species effect and interactions were nonsignificant in all cases (P>0.2) and no systematic differences between species were found.

An effect of diet (with/without woodlice, i.e. the second vs the first study period) on individual egg fate (successful/failed) was analysed by fitting GLMM models with logit link and binomial error term. The random effect of nest (=pair) was included to account for the nonindependence of individual eggs within a nest. The effect of diet on the entire nest fate was analysed using the exact test for comparing two binomial proportions.

Clutch size, egg volume, eggshell thickness, nestling body weight and calcium contents in the eggshells and the nestlings of aviary birds were compared with those of free-living collared flycatchers in central Moravia (S. Bureš and M. Krist, unpublished data). The purpose of this comparison was to show that the breeding parameters of aviary birds fed on woodlice (2000–2002) were not widely deviant from natural values. No statistical tests were performed on these data, because of many confounding effects and possible inter-observer bias. An alternative way to test the growth of aviary nestlings was the parent-offspring comparison. The growth of the tarsus in flycatchers is known to be finished by the age of 13 days (Alatalo and Lundberg 1986). Hence, the mean tarsus length of nestlings was compared with that of parents using the paired *t*-test.

Results

Woodlice were about 5 (PF) and 10 (CF) times more abundant than snails in the natural nestling diet, and provided 2.6 (PF) and 3.4 (CF) times more calcium. A similar result was obtained in millipedes (Table 1).

The overall breeding performance of the aviary flycatchers was poor during the first study period (1997–1999), when only snails and eggshells were provided as a calcium source (Table 2). Birds deprived of woodlice laid significantly smaller clutches of eggs (mean difference 0.74 eggs; 95% CI: 0.11 to 1.38; t_{22}=2.42, P=0.024), commonly with shell defects. The defective eggs had either no, or a very thin, shell. These eggs were laid outside the nest boxes and were often found broken at the place of defecation (probably laid from the branch above). Laying was irregular, with recesses of up to 4 days and without any clear relationship between laying order and eggshell quality (Fig. 1). Females did not initiate incubation in two cases and left clutches after prolonged incubation (eggs were dried-up) in three cases. Desiccation was the main cause of hatching failure in incubated eggs with a developed shell (Table 2).

The breeding performance of aviary flycatchers was markedly better during the second study period (2000–2002) when woodlice were added to the diet (Table 2). Most eggs were laid from 0500 to 0700 hours (16 of 23 eggs in four clutches in 2000). Laying was regular (with a break of 1 day in three nests), all eggs had a well developed shell and the females incubated in all cases. No egg dried out during incubation; the causes of hatching failure (14 eggs) were infertility (12 eggs, including one complete clutch of six eggs), and a dead embryo (2 eggs). The egg and nestling characteristics of the aviary flycatchers fed on woodlice were comparable to those of free-living collared flycatchers in this area (Table 3). The aviary fledglings had similar tarsus lengths as their parents (mean difference: 0.05 mm; 95% CI: −0.78 to 0.69; paired t_7=0.34, P=0.741). The calcium contents of the eggshells and the total body calcium in the nestlings were not lower in the aviary than in free-living birds (Table 3).

Food intake experiments showed, that only 13 snails and no eggshell fragments were taken during the total 277 h of video recording (368 trials). Hence, these diet components were not analyzed in detail. Dead woodlice were avoided by flycatchers. Live woodlice were taken during the entire nesting period with two distinct peaks in females (egg-laying and nestling periods) and one peak in males (nestling period); both sexes took only a few woodlice during incubation (Fig. 2). Females took significantly more woodlice than males during both the pre-laying and laying periods, but not during the nestling period. Both sexes took significantly more woodlice in the evening than in the morning, independent of the nesting stage (Table 4). The intake of woodlice by females increased steadily during the week before laying, peaked during egg-laying (slightly decreased in the course of laying) and dropped abruptly after incubation began (Fig. 3, Table 4). After hatching, the intake of woodlice in both the female and male increased until the nestling age of 10–12 days, and then markedly decreased up to

Table 1 Calcium-rich components in the nestling diet of free-living flycatchers (*Ficedula* spp.) in Moravia. *n* number of food items in the diet samples

Diet component	Number *n*	Total dry mass (g)	Total calcium contents (g)[a]
Pied flycatcher (total *n*=2,094)			
Snail shells	5	0.040	0.013
Millipedes	12	0.266	0.036
Woodlice	27	0.309	0.034
Collared flycatcher (total *n*=2,528)			
Snail shells	7	0.055	0.018
Millipedes	18	0.398	0.054
Woodlice	74	0.576	0.062

[a] Calculated as the product of total dry mass and percentage of calcium contents (see Materials and methods)

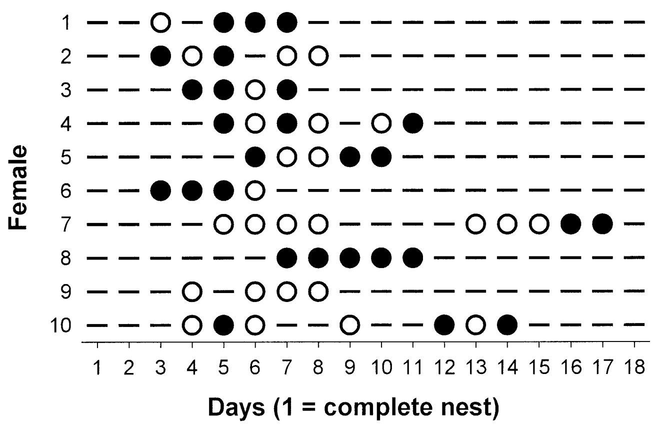

Fig. 1 Egg production by ten collared flycatcher (*Ficedula albicollis*) females kept in an aviary and fed a diet without woodlice (1997–1999). Eggs with developed shells (*filled circles*) and eggs with defective shells (*open circles*) are indicated

Table 2 Breeding performance of aviary flycatchers fed a diet with (2000–2002) or without (1997–1999) woodlice. Proportions (%*n*) were calculated from: (1) all eggs laid; (2) eggs with a developed shell incubated over the full incubation period; (3) unhatched eggs; and (4) all eggs laid, including all failures

Unit	Eggs					Nests				
Woodlice in diet	No		Yes		*P*	No		Yes		*P*
1 Defective eggshells	47.2	(53)	0.0	(83)	<0.001[a]	90.0	(10)	0.0	(15)	<0.001[a]
2 Unhatched eggs	52.2	(23)	17.7	(79)	0.044[b]	71.4	(7)	64.3	(14)	>0.9[a]
3 Desiccated eggs	91.7	(12)	0.0	(14)	<0.001[a]	100.0	(5)	0.0	(9)	<0.001[a]
4 Hatching success	20.8	(53)	82.3	(79)	<0.001[c]	40.0	(10)	92.9	(14)	0.019[a]
4 Total fledging success	20.8	(53)	57.0	(79)	0.045[d]	40.0	(10)	78.6	(14)	0.092[a]

[a] Exact test of difference between two proportions

[b, c, d] GLMM model (binomial error, logit link) with type of diet as a categorical predictor and nest as a random effect, denominator *df* estimated by the Satterthwaite method: [b] $F_{1,17}$=4.73; [c] $F_{1,20}$=15.04; [d] $F_{1,19}$=4.62

Table 3 Egg and nestling characteristics of aviary birds fed a diet containing woodlice (2000–2002) and free-living collared flycatchers. Values shown are mean±SE (*n*)

Variable	Aviary	Free-living
Clutch size	5.64±0.13 (14)	6.05±0.09 (38)
Egg volume (mm^3)[a]	1,668±39 (10)	1,649±32 (18)
Eggshell thickness at blunt pole (μm)[a]	68.30±0.83 (15)	73.65±0.79 (18)
Eggshell thickness at egg side (μm)[a]	69.87±1.42 (15)	74.45±1.03 (18)
Eggshell *Ca* contents (%)[b]	35.20±1.40 (3)	30.70 (1)
Eggshell *Mg* contents (%)[b]	0.18±0.02 (3)	0.16 (1)
Fledgling body mass (g)[a]	14.2±0.3 (11)	14.1±0.2 (43)
Fledgling total body *Ca* contents (mg)[c]	68.53±1.32 (4)	63.84±0.99 (3)

[a] *n* number of nests; the mean and SE were calculated from nest means

[b] *n* number of nests or composite samples

[c] *n* number of nestlings (one per nest)

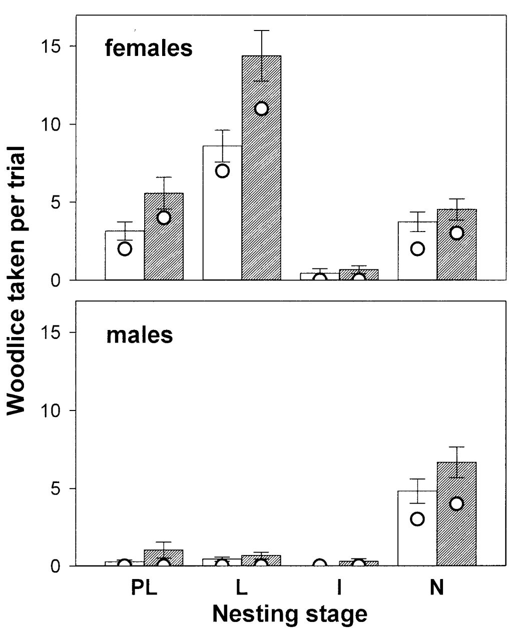

Fig. 2 Number of woodlice taken by aviary flycatchers per feeding trial during the pre-laying (*PL*), laying (*L*), incubation (*I*) and nestling (*N*) stage; with means±SE (*bars*) and medians (*circles*). *Open bars* morning trials, *hatched bars* evening trials

fledging (Figs. 4, 5, Table 4). In contrast to woodlice, the intake of mealworms increased after hatching until the nestling age of 13–14 days, and levelled off thereafter (Figs. 4, 5, Table 4). No effect of parent sex and time of day on the intake of mealworms was found.

Discussion

Calcium availability and breeding success

The studies of nestling diet in natural populations provide important data on feeding behaviour and the intake of nutrients. Such data are essential for the successful breeding of birds in captivity and should be considered when designing field or aviary experiments. The collared and pied flycatchers and their hybrids were successfully reared in captivity for the first time, which proved to be easy with the condition that a suitable calcium source (live woodlice) be provided in addition to ordinary food.

It seems that flycatchers recognize dead or immobile prey with difficulty (Löhrl 1976; Bureš 1986), and therefore did not take dead woodlice and eggshells, and snail shells only occasionally. Breeding the aviary flycatchers had very low success during the first study

Table 4 Results of feeding experiment with aviary flycatchers in 2000–2002. Fixed effect part of the GLMM models (Poisson error, log link) for individual nesting stages relating the number of food items taken (woodlice/mealworms) to the effect of sex, time of day (morning vs evening) and day of the respective nesting stage and brood size (continuous covariates). A quadratic effect of day is included in the models for the nestling stage

Nesting stage	Fixed effect (modelled level)	Estimate±SE	Type III test		
			DDF[a]	*F*	*P*
Number of woodlice					
Pre-laying	Sex (female)	2.071±0.408	16.4	25.75	0.001
	Daytime (evening)	0.545±0.138	128.0	15.69	0.001
	Day	0.309±0.044	130.0	49.42	0.001
Laying	Sex (female)	2.971±0.304	24.1	95.79	0.001
	Daytime (evening)	0.550±0.106	138.0	27.14	0.001
	Day	−0.070±0.032	139.0	4.91	0.028
Nestling	Sex (female)	−0.150±0.338	7.7	0.20	0.670
	Daytime (evening)	0.294±0.097	229.0	9.11	0.003
	Day	0.464±0.063	233.0	54.68	0.001
	Day^2	−0.023±0.004	231.0	42.86	0.001
	Brood size	0.426±0.133	163.0	10.30	0.002
Number of mealworms					
Nestling	Sex (female)	−0.181±0.279	6.7	0.42	0.537
	Daytime (evening)	0.019±0.058	115.0	0.11	0.743
	Day	0.219±0.039	118.0	31.97	0.001
	Day^2	−0.008±0.002	117.0	16.54	0.001
	Brood size	0.167±0.089	117.0	3.51	0.063

[a] Denominator *df* estimated by the Satterthwaite method; numerator *df*=1 for all models

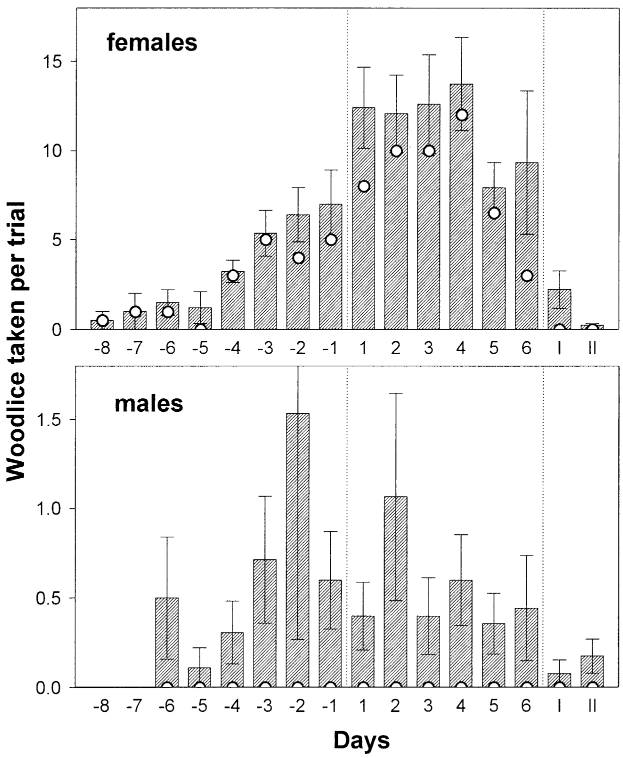

Fig. 3 Number of woodlice taken by aviary flycatchers per feeding trial during the pre-laying, laying and incubation stages (stages delimited by vertical dotted lines). Days are numbered relative to the start of laying (1st egg = day 1). The incubation stage is divided into 1st day after clutch completion (*I*) and all subsequent days (*II*); with means±SE (*bars*) and medians (*circles*). Note the different scale on the vertical axis for males and females

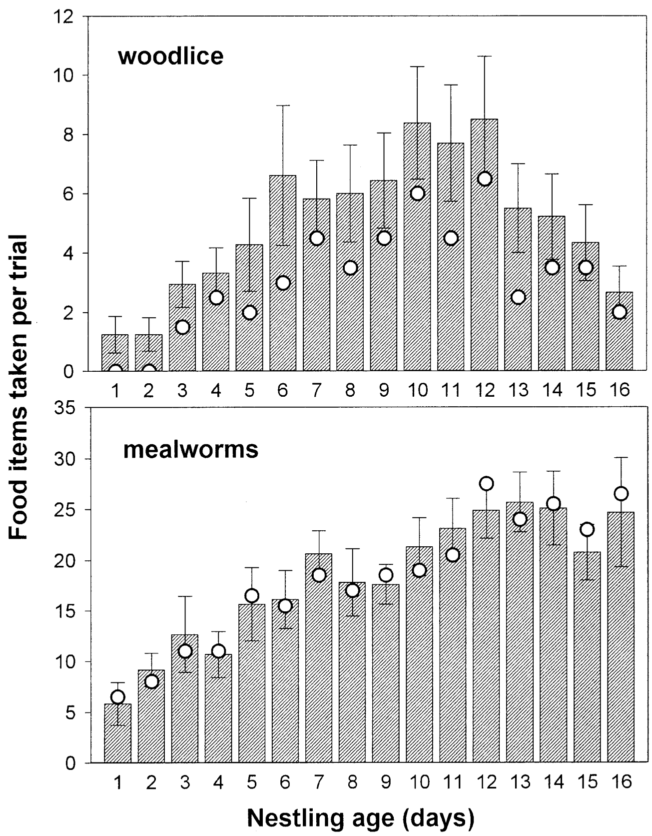

Fig. 4 Number of woodlice/mealworms taken by aviary flycatchers per feeding trial during the nestling period; with means±SE (*bars*) and medians (*circles*). Data for males and females are pooled because of the nonsignificant effect of sex (Table 4)

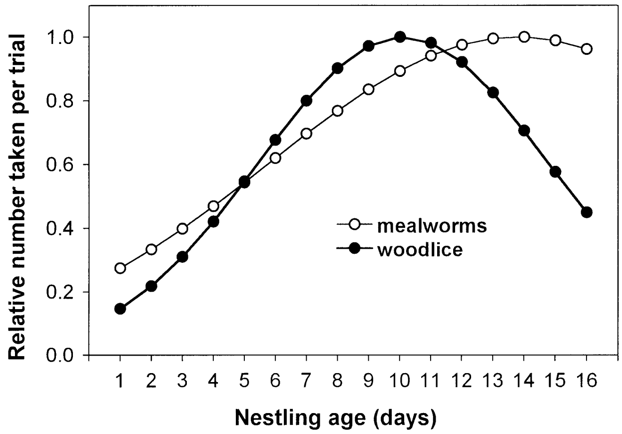

Fig. 5 The effect of nestling age on the number of woodlice/mealworms taken per feeding trial, estimated by the GLMM models (Table 4) from the raw data (Fig. 4). Predicted numbers are expressed as a proportion of the maximum value

period, when only snail shells and hen eggshells were provided in the diet. Eggshell defects were frequent, as previously recorded in pied flycatcher by Chek et al. (1993). The eggs without shells were never found in the nest box, but on the ground. The course of egg laying showed apparent interruptions, but it is possible that at least some eggs were laid without a shell and were not found among dry leaves on the ground, or were eaten by the female. All these observations correspond with those on tits (Graveland and Berends 1997). Nearly half of the eggs with a developed shell dried out during incubation. This is a known consequence of excessive evaporation from eggs with abnormal shell structure, as is known to occur in acidified areas (Drent and Woldendorp 1989).

The overall breeding performance of the aviary birds at least doubled during the second study period, when woodlice were provided in the diet. Neither the egg nor the nestling characteristics of these aviary birds were markedly different from those in natural populations. We did not apply statistical tests to this comparison (Table 3) because of the confounding effect of inter-specific hybridization on breeding performance in aviary mixed pairs. The proportion of unfertilized eggs in the aviaries (15.2%, n=79) was twice as high as in natural populations (7.3%; Král 1982). Apart from this, the reproductive success of all aviary birds could have been influenced by an unnaturally uniform diet. The nestling mortality was high in 2001; symptoms of intoxication (impaired growth and digestive problems) were recorded in two nests in which all the nestlings died. However, the causes of embryo and nestling mortality would require a more detailed study.

Temporal patterns of calcium intake

The results of the food intake experiment support the role of woodlice as the major source of calcium for flycatchers. Woodlice were preferred to other calcium-rich items by both parents during the entire breeding period. Females increased their intake of woodlice during the pre-laying and laying period, both sexes during the nestling period. Intake by females was low until 4 days before laying the first egg, then increased to the highest level, and dropped after laying the last egg. This pattern corresponds well with the intake of snail shells by tits (Graveland and Berends 1997).

In most bird species, eggshell formation takes place over night, and eggs are laid early in the morning. It has been suggested that birds generally increase their intake of calcium in the evening because they need to digest and immediately deposit calcium in the shells (Mongin and Sauveur 1974; Graveland and Berends 1997), but data from free-living birds are still scarce. Female flycatchers took more woodlice in the evening than in the morning during the egg laying period. As passerines do not store calcium in the medullary bone (Pahl et al. 1997), the fact that eggs were laid at daily intervals and mostly early in the morning means that the shells were formed in oviduct during the night with woodlice taken in the evening providing sufficient amounts of calcium. A maximum of calcium intake in the evening has also been observed in the house sparrow (*Passer domesticus*) (Schifferli 1977), domestic fowl (Gilbert 1983), the red-cockaded woodpecker (*Picoides borealis*) (Repasky et al. 1991) and the great tit (*Parus major*) (Graveland and Berends 1997). A new finding in our study is that the higher intake of calcium in the evening was not restricted to egg-laying females, but occurred in both sexes during the entire breeding period. No effect of daytime outside the laying period was found in tits (Graveland and Berends 1997). The effect found in flycatchers cannot be accounted for by a generally higher food intake in the evening, because the intake of mealworms did not differ between morning and evening trials.

The taking of woodlice increased steadily during the nestling period, until the nestling age of about 10–12 days and decreased thereafter. This temporal pattern corresponds with the period of rapid skeletal growth which at this age is finished in flycatchers (Alatalo and Lundberg 1986). We are not aware of any other study of calcium intake during nestling growth in passerines. In contrast to woodlice, the daily intake of mealworms increased during the nestling period until the nestling age of about 13–14 days and levelled off thereafter, which corresponds well with the growth curve of nestling body mass (Fig. 6).

Variation in utilized calcium sources

This study showed that woodlice and millipedes were the main source of calcium for flycatchers during the breeding period in Central Europe. Both woodlice and millipedes contained in the natural nestling diet provided about three times more calcium than did snail shells. This may explain why flycatchers are much less vulnerable to calcium deficiency in acidified areas than tits (Drent and Woldendorp 1989; Carlsson et at. 1991; Mänd and Tilgar

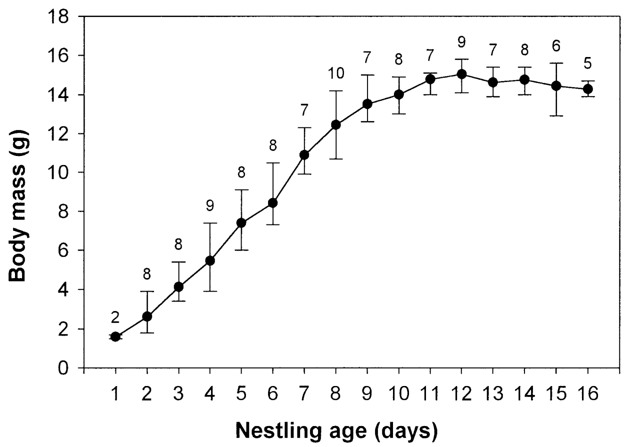

Fig. 6 The growth of body mass in free-living collared flycatcher nestlings; with means and ranges. The mean for each day was calculated as an unweighted mean from brood means (n=15 nests). The sample size for individual days is lower because the broods were usually weighed on alternate days

2003), in which calcium intake depends on the availability of snails (Graveland et al. 1994).

The available data suggest geographical variation in the feeding behaviour of flycatchers and/or their abilities to recognize and use different calcium sources. No eggshell and only a few snail shells were taken during our aviary experiments, whereas Tilgar et al. (1999) found that both snail shells and eggshells were often taken by pied flycatchers in Estonia. In pied flycatchers in Norway, the quality of the eggshell improved after the addition of crushed calcium carbonate to the diet (Chek et al. 1993). Taken together, our results support the suggestion of Mänd and Tilgar (2003) that the effect of calcium deficiency on birds can be both species- and population-specific. We propose that the reproductive success of flycatchers in Central Europe depends on the availability of woodlice and millipedes.

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References

Alatalo RV, Lundberg A (1986) Heritability and selection on tarsus length in the Pied Flycatcher (*Ficedula hypoleuca*). Evolution 40:574–583

Bureš S (1986) Composition of the diet and trophic ecology of the collared flycatcher (*Ficedula albicollis albicollis*) in three segments of groups of types of forest geobiocenoses in central Moravia (Czechoslovakia). Folia Zool 35:143–155

Bureš S (1995) Comparison of diet in collared flycatcher (*Ficedula albicollis*) and pied flycatcher (F*icedula hypoleuca*) nestlings in a hybrid zone. Folia Zool 44:247–253

Bureš S, Horáčková K (1998) Diet of pied flycatcher (*Ficedula hypoeuca*) nestlings in hybrid zone with collared flycatcher (*Ficedula albicollis*) in Moravia. Zprávy MOS 56:91–98

Bureš S, Stříteský J (1996) Diet of Collared Flycatcher (*Ficedula albicollis*) in a spruce forest. Zprávy MOS 54:75–78

Bureš S, Weidinger K (2000) Estimation of calcium intake by Meadow Pipit nestlings in an acidified area. J Avian Biol 31:426–429

Bureš S, Weidinger K (2001) Do pipits use experimentally supplemented rich sources of calcium more often in an acidified area? J Avian Biol 32:194–198

Carlsson H, Carlsson L, Wallin C, Wallin NE (1991) Great tits incubating empty nest cups. Ornis Svec 1:51–53

Chek AA, Lifjeld JT, Robertson RJ (1993) Captive study of copulation in the Pied Flycatcher *Ficedula hypoleuca*. Fauna Norv Ser C Cinclus 16:67–73

Davies NB (1977) Prey selection and the search strategy of the spotted flycatcher (*Muscicapa striata*): a field study on optimal foraging. Anim Behav 25:1016–1033

Dhondt AA, Hochachka WM (2001) Variations in calcium use by birds during the breeding season. Condor 103:592–598

Drent PJ, Woldendorp JW (1989) Acid rain and eggshells. Nature 339:431

Gilbert AB (1983) Calcium and reproductive function in the hen. Proc Nutr Soc 42:195–212

Graveland J (1990) Effects of acid precipitation on reproduction in birds. Experientia 46:962–970

Graveland J, Berends JE (1997) Timing of the calcium intake and effect of calcium deficiency on behaviour and egg laying in captive Great Tits, *Parus major*. Physiol Zool 70:74–84

Graveland J, Van Gijzen T (1994) Arthropods and seeds are not sufficient as calcium sources for shell formation and skeletal growth in passerines. Ardea 82:299–314

Graveland J, Van Der Wal R, Van Balen JH, Van Noordwijk AJ (1994) Poor reproduction in forest passerines from decline of snail abundance on acidified soils. Nature 368:446–448

Hoyt DF (1979) Practical methods of estimating volume and fresh weight of birds eggs. Auk 96:73–77

Hughes BO, Wood-Gush DGM (1971) A specific appetite for calcium in domestic chickens. Anim Behav 19:490–499

Jones PJ (1976) The utilization of calcareous grit by laying *Quelea quelea*. Ibis 118:575–576

Král M (1982) Beitrag zur Nistenbionomie des Halsbandgfliegen-schnäppers (*Ficedula albicollis* Temm.) im Nordmährischen Gesenke. Zprávy MOS 40:7–42

Krištín A (1992) Trophische Beziehungen zwischen Singvögeln und Wirbellosen im Eichen-Buchenwald zur Brutzeit. Orn Beob 89:157–169

Littell RC, Miliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, Cary, N.C.

Löhrl H (1976) Collared flycatcher. Br Birds 69:20–26

Mänd R, Tilgar V (2003) Does supplementary calcium reduce the cost of reproduction in the Pied Flycatcher *Ficedula hypoleuca* ? Ibis 145:67–77

Mongin P, Sauveur B (1974) Voluntary food and calcium intake by the laying hen. Br Poult Sci 15:349–359

Ormerod SJ, OHalloran J, Gribbin SD, Tyler SJ (1991) The ecology of Dippers *Cinclus cinclus* in relation to stream acidity in upland Wales: breeding performance, calcium physiology and nestling growth. J Appl Ecol 28:419–433

Pahl R, Winkler DW, Graveland J, Batterman BW (1997) Songbirds do not create long-term stores of calcium in their legs prior to laying: result from high-resolution radiography. Proc R Soc Lond B 264:239–244

Piersma T, Gudmundsson GA, Davidson NC, Morrison RIG (1996) Do arctic-breeding Red Knots (*Calidris canutus*) accumulate skeletal calcium before egg laying ? Can J Zool 74:2257–2261

Repasky RR, Blue RJ, Doerr PD (1991) Laying red-cockaded woodpeckers cache bone fragments. Condor 93:458–461

Schifferli L (1977) Bruchstücke von Schneckenhäuschen als Calciumquelle für die Bildung der Eischale beim Haussperling *Passer domesticus*. Orn Beob 74:71–74

Simkiss K (1967) Calcium in reproductive physiology. Chapman and Hall, New York

Tilgar V, Mänd R, Leivits A (1999) Effect of calcium availability and habitat quality on reproduction in Pied Flycatcher *Ficedula hypoleuca* and Great Tit *Parus major*. J Avian Biol 30:383–391

Tilgar V, Mänd R, Mägi M (2002) Calcium shortage as a constraint on reproduction in great tits *Parus major*: a field experiment. J Avian Biol 33:407–413

Veen T, Borge T, Griffith SC, Sätre GP, Bureš S, Gustafsson L, Sheldon BC (2001) Hybridization and adaptive mate choice in flycatchers. Nature 411:45–50

Wolf P, Bayer G, Wendler C, Kamphues J (1998) Mineral deficiency in pet birds. J Anim Physiol Anim Nutr 80:140–146