



# The significance of seed food in chick development re-evaluated by tracking day-to-day dietary variation in the nestlings of a granivorous passerine

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The dietary adjustment of nestlings of granivorous birds to a seed diet and the different morphological characteristics of ingested food have rarely been examined in natural conditions. It has been suggested that the provision of cereal grains to nestlings of some seed-eating bird species in modern agroecosystems is the result of poor food conditions after agricultural intensification. We analysed the abundance of invertebrate prey in the main foraging habitat of parent birds, daily changes (from hatching to fledging) in the efficiency of cereal seed digestion, and the dietary characteristics, diet composition and prey type delivered to nestlings of the Yellowhammer *Emberiza citrinella*. Analysis of faecal sacs from nests located in breeding habitat with an abundant invertebrate fauna revealed no relationship between the proportion of cereal seeds in the diet of nestlings and the food supply in the main foraging sites of the parents. Neonate nestlings (1 day old) exclusively received weakly chitinized invertebrate prey (arachnids and flies), but from the second day of life the nestlings were fed a variety of highly chitinized invertebrate prey, the percentage biomass of which did not change for the remainder of the nesting period. Cereal grains started to be delivered to 3-day-old nestlings and were already efficiently digested, and the percentage biomass of this food type increased progressively with nestling age. We suggest that the provisioning of cereal grains to nestlings is not forced by external factors, such as modern agricultural intensification; rather, it is an intentional behaviour of parent birds aimed at achieving physiological adjustment to seed food in the early stages of ontogeny.

**Keywords:** agricultural intensification, cereal grains, dietary adjustment, invertebrate prey, nestling development, seed diet, Yellowhammer.

Determining the source of differences in diet, food composition or prey characteristics is essential for acquiring an understanding of the adaptability and/or trade-off between predators and the suitability of the environment they inhabit. This is especially important in changeable or periodic habitats like agroecosystems, where resources are severely affected by human activities (Wilson *et al.*

1999, Hart *et al.* 2006, Holland *et al.* 2006, Pearce-Higgins 2010).

The dietary adjustment of nestling granivorous birds to a seed diet and the different morphological characteristics of ingested prey have rarely been examined in natural conditions, although these could provide significant insights into both the physiological/nutritional ecology of growing nestlings and the adaptability of a species to changing food resources in periodic environments (e.g. Díaz 1996, Wilson *et al.* 1999, Holland *et al.* 2006,

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Gil-Delgado *et al.* 2009, Pearce-Higgins 2010, Douglas *et al.* 2012). It has been suggested that the provision of cereal grains to nestlings of some seed-eating bird species living in modern agroecosystems, such as some European buntings (*Emberizidae*), is the result of poor food conditions owing to shortages of animal prey, the availability of which has decreased as a result of agricultural intensification (Brickle & Harper 1999, Morris *et al.* 2001, 2005, Hart *et al.* 2006, Douglas *et al.* 2012). Therefore, it has been suggested that cereal crops can provide an additional source of nestling food in the form of partially ripe cereal grain (Stoate *et al.* 1998, Morris *et al.* 2001, 2005, Douglas *et al.* 2012). Consequently, cereal grains have been considered to be a secondary or sub-optimal food source for nestlings of some bunting species, only exploited by parent birds when the preferred invertebrate prey items are unavailable, for example in poor weather conditions (Evans *et al.* 1997, Morris *et al.* 2001, MacLeod *et al.* 2005, Douglas *et al.* 2012).

Prior to the agricultural intensification of the latter part of the 20th century, cereal grains were rare or absent in the nestling diet of these birds (Morris *et al.* 2001). It is possible that the exploitation of grain for bunting nestlings may have increased as invertebrate communities on arable land have been diminished by agricultural intensification (Morris *et al.* 2001, Douglas *et al.* 2012). More importantly, however, in suggesting that cereal grains are a sub-optimal food source, we may be neglecting a key aspect of the nutritional physiology and ecology of growing nestlings of granivorous species in the context of their natural switch from an animal diet to a seed diet, as a potential explanation of the high proportion of cereal grains in a nestling's diet (Díaz 1996). In general, the diet of granivorous nestlings gradually changes during their growth, from mostly insects to mostly seeds (Wilson *et al.* 1999, Holland *et al.* 2006). Furthermore, experimental studies have shown that nestlings of House Sparrows *Passer domesticus*, which have a nestling diet similar to that of buntings and an apparent switch to a seed diet at the age of 3 days, grow normally throughout their development on either high protein/carbohydrate-free or lower protein/carbohydrate-containing diets. Moreover, ontogenetic increases in the activity of intestinal carbohydrases are partly induced by high dietary carbohydrate and are partly programmed independently of diet (Brzęk *et al.* 2009, 2011).

Many herbivorous animals selectively eat unripe fruit or seeds, and some preferentially eat new tissues growing from germinating seeds (Glück 1985, Díaz 1996, Valera *et al.* 1997, White 2011). In particular, unripe cereal seeds may constitute an additional source of soluble amino acids, which are concentrated in grains during this phase of plant development (Allen & Hume 1997). This behaviour gives access to otherwise limited or unavailable amino acids, which are necessary to sustain the successful production and growth of young. For the same reason, the diet of breeding females and neonates of many presumed strictly herbivorous animals is supplemented with animal protein (Glück 1985, Díaz 1996, White 2011). However, because these foods are often only consumed for limited periods, and make up only a small proportion of the total diet, they are usually dismissed as unimportant to the animals' nutrition. It is suggested that actively looking for such food may well reveal it to be far more common and important to the successful breeding of herbivores than has been thought in the past (White 2011). Moreover, previous studies have shown that in some species the ability to digest seeds (in terms of the percentage of broken seeds present in faeces) may increase as the nestlings grow, but this does not appear to be universal across all granivorous bird species (Green *et al.* 1987). This is due to seed processing, such as the de-husking of seeds (Díaz 1996), as well as to a more diversified diet consisting of plant and animal fractions differing in their proportions and digestibility, which presumably also affects seed digestion or the presence of intact seeds in bird droppings (Valera *et al.* 1997, Orłowski *et al.* 2016).

Our objective was to re-assess the contribution of various prey groups, including cereal grains, in the diet of the nestlings of a European bunting, the Yellowhammer *Emberiza citrinella*, by tracking the day-to-day dietary variation and efficiency of seed digestion over the period from hatching to fledging. A large number of papers have described the dietary composition, including the cereal grain provisioning of nestlings, of the Yellowhammer (Stoate *et al.* 1998, Morris *et al.* 2001, 2005, MacLeod *et al.* 2005, Hart *et al.* 2006, Douglas *et al.* 2012). However, to the best of our knowledge, no one has yet undertaken a detailed analysis of the prey items in terms of their individual mass and the proportion of plant material delivered throughout the brood-rearing period with respect to the consecutive days of a nestling's life.

Importantly, all the recent dietary studies have consistently shown that cereal grains are the sole plant component in the diet of nestling Yellowhammers. Thus, the lack of other seed types in diet samples is the norm in Yellowhammer nestlings. We test two expectations. First, if plant food appears in the diet of nestling Yellowhammers, the invertebrate resources around nests will be fewer, i.e. we expect a negative relationship between the invertebrate food supply and the amount of plant food delivered; and if nestlings receive seeds, these should be considerably better digested as the nestlings grow older. Secondly, we expect that the composition of invertebrate prey delivered to nestlings will change as they grow, mostly as a result of a decrease in dietary specialization, an increase in dietary diversity and the growing contribution of more highly chitinized invertebrate prey.

## METHODS

This study was undertaken in a heterogeneous agricultural area of the Sudeten Foreland (200–250 m a.s.l.) in south-western Poland (a full description of the site is given in Supporting Information Appendix S1). The study area (c. 400 km<sup>2</sup>) is characterized by a low-intensity arable farming system, with small, privately owned fields (0.1 ha up to several hectares) and a network of linear, semi-natural field margins (Wuczyński *et al.* 2011, 2014). The area matches High Nature Value Farmland Type 2 (Paracchini *et al.* 2007). Within the study area, crop fields are the main foraging habitat of the Yellowhammers, and the field margins, especially the sides of the well-vegetated ditches, are their main nesting sites (Appendix S1). The area seems to be a good habitat for breeding Yellowhammers, and the species occurs in high densities of 4.2 pairs/10 ha during the breeding season (data collected in six 50-ha study plots, 2003–2005; Wuczyński 2016). The density in linear habitat features was 0.9 pairs/km in field margins with various types of vegetation or 1.7 pairs/km in shrubby field margins (Wuczyński *et al.* 2011). In comparison, the breeding density of Yellowhammers at the landscape scale in other regions of Poland is much lower, not exceeding 1 pair/10 ha (Tryjanowski *et al.* 2009), and is strongly dependent on the presence of woody boundary vegetation (Bradbury *et al.* 2000, Whittingham *et al.* 2005, Tryjanowski *et al.* 2009, Dunn *et al.* 2015). Thus, our Yellowhammer population

most likely formed a good test population for examining the seed component in the diet. The Yellowhammer population in Poland declined significantly between 2000 and 2013 (Chodkiewicz *et al.* 2013), which in general mirrors the negative population trend of the species across Europe (BirdLife International 2016).

## Dietary analysis

Faecal sacs were collected when handling and ringing nestlings at the nests over three breeding seasons, 2006–2008. The nestlings from which the faecal sacs were sampled were aged on the basis of biometric measurements and stage of feather development (A. Wuczyński unpubl. data). The nestlings were aged in 12 classes between day 1 (day of hatching) and day 12 on the basis of the oldest nestling in the nest. Yellowhammer nestlings hatch synchronously in 1 day, although some discrepancies (up to c. 1 day of age) between siblings within the same brood are possible.

Over the three breeding seasons, we collected 930 faecal sacs from 56 different broods. In each study year, the target nests were distributed in various parts of the study area to avoid the inclusion of broods from the same parental birds. Also, within-season repeatability of broods was avoided. Hence, given the relatively large study area (c. 400 km<sup>2</sup>) and the high density of birds, we assumed that all broods (and brood-assigned faecal samples) represented different, spatially separated pairs of Yellowhammers. The numbers of broods/faecal sacs collected in consecutive years were as follows: 2006 (14/145), 2007 (23/406) and 2008 (19/427). As some faecal sacs were collected from the same broods on consecutive days, the dietary data for these broods were treated separately in various age categories in the subsequent analysis. Our sampling dates were between 26 April and 17 August. The average daily temperature (°C) and total precipitation (mm) for June–July was 19.9/99.4 (in 2006), 18.5/188.8 (in 2007) and 18.0/94.0 (in 2008) – these data were obtained from the weather station in the village of Sieniawka, situated in the central part of the study area (50°47'N, 16°47'E).

Before analysis, the droppings were crushed and separated in Petri dishes. The food components in the individual droppings were identified to the finest possible taxonomic level. Faecal analysis was performed using a binocular microscope at 40× magnification.

### Invertebrate abundance at foraging sites of adult Yellowhammers

During the brood-rearing period, we observed adult foraging trips from 29 nests. Based on 681 foraging flights, we found that Yellowhammers preferentially collected food in boundary features, oil-seed rape fields, woodland and spring cereals, whereas maize plantations, winter cereals and fallow land were avoided (Wuczyński & Grzesiak 2007; Appendices S1 and S2).

A detailed survey of the abundance of the invertebrate fauna was conducted around 23 of these nests, for which we identified 81 different sites at which adult Yellowhammers foraged (two to three sites around each nest). Using a vacuum sampling technique (biocenometer – samples were taken by covering 0.25 m<sup>2</sup> of the ground with a bottomless cage; Ryszkowski & Karg 1977), we took five samples from each of these sites on one date at the same time as the parent Yellowhammers were provisioning their nestlings. The timing of invertebrate sampling was between 3 May and 28 July; these dates were all within c. 2 weeks of the sampling dates of faecal samples of nestlings. The dietary data for each nest and concurrent invertebrate samples represented at least three age classes of nestlings present in a particular nest. Overall, we took vacuum samples in five main types of habitats utilized as foraging sites by adult Yellowhammers: boundary features ( $n = 85$  samples), cereal crops (155), oil-seed rape (70), other crops (50) and fallow land (25). We gathered a total of 385 invertebrate samples: around four nests (60 samples) in 2006, 12 nests (201 samples) in 2007 and seven nests (120 samples) in 2008 (Appendix S1). The sampled invertebrates were frozen and then identified to the finest possible taxonomic level. The individual dry mass of particular invertebrate species/taxa was taken from Karg (1989). A full list of invertebrates recorded is available on request from the authors. The number and dry mass of invertebrates per square metre were calculated for each site. In the subsequent analysis, the vacuum-sampled invertebrates were classified into nine major groups (Arachnida, Coleoptera, Homoptera, Orthoptera, Lepidoptera, Heteroptera, Diptera, Hymenoptera and other invertebrates; Appendix S1) and the samples from different foraging habitats around one particular nest were pooled.

### Assessment of the plant fraction in the diet and the efficiency of cereal seed digestion

The content of cereal grains (the sole type of plant food in our study; see Results) was estimated per individual faecal sac. This estimate was obtained using three different methods: (1) a visual estimate of the percentage volume of cereal grains; (2) weighing the remains of cereals present in an individual faecal sac; and (3) an approximate quantification of the amount of consumed seeds based on the presence of the remains of cereal grains; only the results of the last-mentioned analysis were combined with the animal fraction in the diet in the subsequent analysis.

The percentage volume of cereal grains has been visually estimated in previous dietary studies on nestling Yellowhammers (Stoate *et al.* 1998, MacLeod *et al.* 2005, Douglas *et al.* 2012). In those studies, the area of cereal remains were estimated under a microscope in grid squares (10 × 10 mm) and calculated as the percentage of the sample area across a Petri dish rounded to the nearest 5%. This assessment was conducted for all the droppings analysed ( $n = 930$ ).

The weighing method was applied to a smaller sample of droppings ( $n = 335$ ), representing each consecutive age class of nestlings. The main aim of this assessment was to evaluate whether the degree of digestion of cereal seeds varied with nestling age. Initially, using microscopic examination, we carefully separated all the visible remains of cereal grains present in a dropping; these were then weighed to an accuracy of 1 mg. We assumed that the total mass of cereal grain remains recovered from one dropping was the percentage mass of one entire cereal seed ingested by a nestling and was therefore an indirect index of the digestion rate. As the cereal seeds fed to the nestlings by adult Yellowhammers were in various stages of maturation (A. Wuczyński unpubl. data; Supporting Information Fig. S1), the weight of cereal grain remains per individual dropping was expressed as the percentage mass of one cereal seed in the milky or mature stage; both represented the potential minimum and maximum mass of one cereal seed ingested. Owing to the high degree of digestibility of cereal grains, we were unable to identify them to species level. However, given the predominance of wheat and barley crops in our study area and our observations of the Yellowhammer behaviour (while they were

foraging both on spilt ungerminated grains and on not fully mature cereal seeds directly from spikelets), and also the similarity of wheat seeds to those of triticale (J. Czarnecka pers. obs.), we presumed that all these three species of cereals were represented in the droppings. Consequently, for the mass of one mature wheat/triticale/barley seed we used the following values: 43.8 mg (average: 47.3/40.4/43.6 mg for these three species, respectively; data of seed weights obtained from the local cereal grain producer; Danko Co., Choryń, Poland) for mature grains, and 21.3 mg for grains at the milky stage, given that the mass of a cereal seed in the milky stage is 46.6% of the mass of a mature cereal seed (Zečević *et al.* 2007). Lastly, we estimated, having removed the seed content, that the mass of the husk of one wheat grain was *c.* 8% (i.e. 3.5 mg) of the total mass of one seed (J. Karg unpubl. data).

The third method involved the approximate quantification of the amount of consumed seeds based on the presence of cereal grain remains. Because of the high frequency of cereal grains in droppings and their large individual mass relative to that of invertebrate prey items (see Results), we decided to pool the data relating to the seed fraction of the diet with the animal fraction in the diet. To quantify approximately the seed fraction in the diet, we applied the rule used in reconstructing avian diets whereby the presence of some hard-to-quantify prey item is taken to be the conventional basis for the calculation of one prey item per individual pellet or faecal sample (Sutherland 2004, Resano-Mayor *et al.* 2014). Hence, we assumed each dropping containing cereal seed remains to correspond to one cereal (wheat/triticale/barley) grain, and to calculate the biomass calculation we assumed the mass of one mature cereal grain to be 43.8 mg (see above). This approach probably represents the minimum amount of almost-mature cereal grains ingested, i.e. spilt grains or seeds gleaned at around harvest time (from late June onwards). However, in the case of the lighter, immature cereal grains in the milk stage, this could be an overestimate.

### Assessment of the invertebrate fraction in the diet

For each faecal sac, the numbers of prey items representing particular invertebrate taxa (uncorrected in terms of potential bias due to the better digestibility of some soft-bodied prey groups; e.g.

Pulido & Diaz 1994, Moreby & Stoate 2000, 2001) were established from the numbers of observed fragments of chitinous parts, chiefly the elytra (for different families and genera of Coleoptera, Homoptera or Heteroptera), wings (in the case of Diptera, Hymenoptera), mouthparts (most of the orders) and other preserved organs (e.g. limbs, petiolus, clypeus, mandible). When determining the number of prey items belonging to a particular taxon, a rule summing the different chitinous parts to the level of one individual was applied: two or more different fragments of chitinous parts (e.g. head, mandibles, six legs and other parts in the case of ants) from one dropping were treated as belonging to the same individual of a given species (Orłowski & Karg 2011). The mass of prey was calculated as dry weight (mg d.w.); these values were obtained from detailed measurements of insect weights based on the analysis of 479 087 individuals of different insect taxa (after Karg 1989).

As the diet of nestlings, especially the youngest ones, can differ from that of older individuals with respect to the chitin content of the prey delivered, we categorized the identified prey into four groups (three of them representing the animal fraction of the diet and one representing the plant fraction of the diet) using our previous classification of invertebrate prey taxa of insectivorous nestlings (Orłowski *et al.* 2015). These were soft-bodied prey (weakly chitinized such as Arachnida, larvae), intermediately chitinized (Diptera, Plecoptera, Hymenoptera, Heteroptera and Orthoptera), heavily chitinized prey (such as adult Coleoptera and small snail shells), and cereal grains (based on their frequency of occurrence assuming one cereal grain per dropping where this item was present; see above).

### Statistical analysis

In line with our previous approaches to faecal analysis using a multi-dimensional description of the diets of insectivorous nestlings (Orłowski & Karg 2013, Orłowski *et al.* 2014b, 2015), we determined eight principal dietary characteristics for each dropping: frequency of occurrence, dietary diversity, the total number of all/animal prey items, the total biomass of all/animal prey and the average weight of all/animal prey. We also determined the composition of the diet expressed as the number and biomass of six major food types consumed (class/order of various invertebrates:

Arachnida, Diptera/Hymenoptera, Coleoptera, Lepidoptera and other animal prey, as well as cereal grains) and groups of prey with different chitin/cereal grain content. We separately analysed the data for animal prey and all prey, the latter category including values representing animal (various invertebrate prey items) and plant (cereal grains) items. To perform the statistical analysis, six major food types were selected, representing taxonomically and morphologically related taxa: Arachnida, Coleoptera, Hemiptera (Heteroptera + Homoptera), Lepidoptera (primarily caterpillars), Diptera/Hymenoptera and other invertebrates (Orthoptera, Mollusca, Diplopoda and unidentified insects).

The key question was whether the proportion of cereal grains in the diet of nestlings depended on the abundance of invertebrates present in the adult Yellowhammer main foraging habitats. In this analysis, we used the average proportion of cereal grains in droppings ( $n = 559$ ) per brood ( $n = 23$ ) determined by approximately quantifying the amount of consumed seeds in terms of the presence of cereal grain remains (third method; see above). The abundance of invertebrates was expressed as the average density of all invertebrates per  $1 \text{ m}^2$  across all the main foraging habitats around one individual nest (Appendix S2). Then we used Pearson's correlation coefficient to assess the relationship between the proportion of cereal grains in the diet and the density of all invertebrates determined for the same nests.

The analysis of the selectivity of invertebrate prey in relation to their abundance around nests was based on a comparison of the percentage composition of the six main invertebrate components (Arachnida, Diptera/Hymenoptera, Coleoptera, Hemiptera, Lepidoptera and all other invertebrates) measured at the main foraging sites of adult Yellowhammers and diet samples of nestlings from 23 nests. To calculate the dietary composition we used 559 faecal sacs from nestlings in at least three different age classes (Appendix S2). We used MANOVA to assess the difference in percentage composition across the six major invertebrate components of the diet samples compared with that of the samples collected at the main foraging sites. The paired *t*-test was used to compare the percentage of particular invertebrate groups in diet samples with that in the main foraging habitats around the nests (Appendix S2).

The next step in our analysis was to assess differences in the main dietary characteristics, including division of the diet into major food types and four groups of prey with differing chitin/cereal grain content, among nestlings in 12 age classes (1–12 days old). The differences in the percentage volume of cereal grains and main dietary characteristics between nestlings in the 12 daily age classes were tested using MANOVA with a nested design; the independent variables were the age of nestlings and the individual brood identity (nested within the age of nestlings). MANOVA was applied to assess differences in dietary composition (expressed as the six major food types and the four groups of prey with differing chitin/cereal grain content) between nestlings of various ages, also taking into account brood identity nested within the age of nestlings. Additionally, the Spearman rank correlation coefficient was used to assess the directionality of changes (decrease/increase) with chick age in days in terms of the contribution of the percentage biomass of these food types in 1- to 3-day-old nestlings and in all nestlings. Because of the limited number of categories of age (days), the exact variant of the two-sided significance test for Spearman's coefficients was computed (Mundry & Fischer 1998), using STATXACT 11, a software for small-sample categorical and non-parametric data (Cytel Inc., Cambridge, MA, USA).

Before the analysis, some data were log-transformed to improve the normality of the distribution and also to equalize variance and the linearity between the response and explanatory variables, so as to meet the assumptions of linear modelling. All percentage data were arcsine-transformed before analysis. The statistical analyses were done in STATISTICA 7.0 (StatSoft 2006) and EXCEL software. A probability of  $P < 0.05$  was considered statistically significant.

## RESULTS

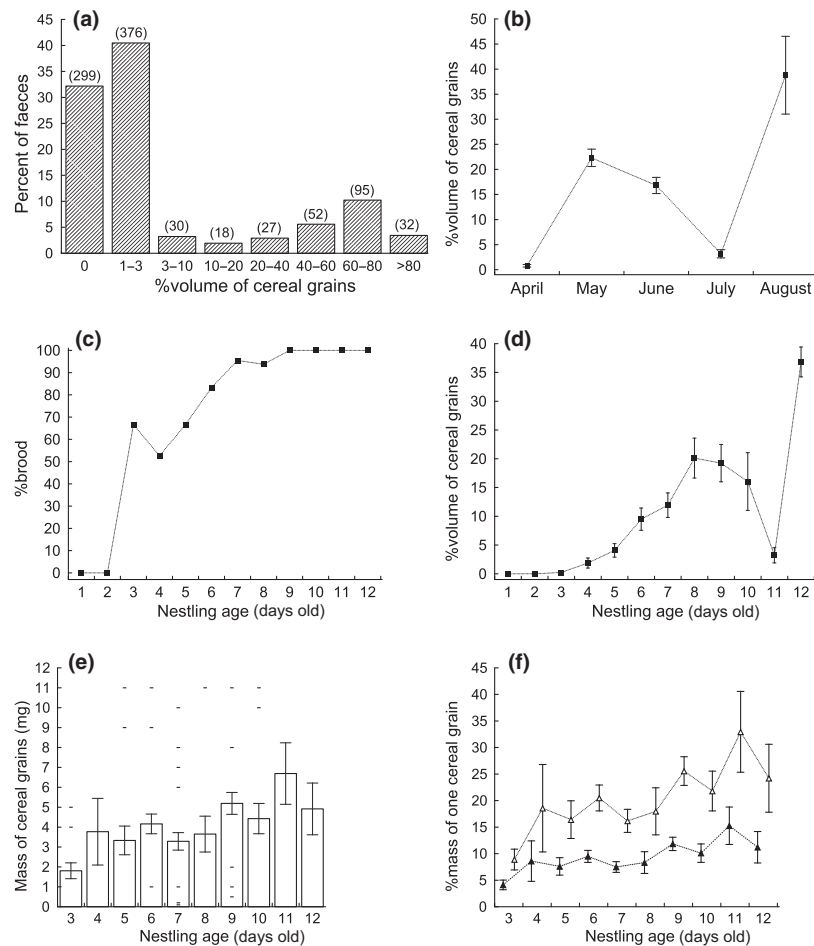
Analysis of invertebrate fauna from 385 vacuum samples yielded 3391 invertebrate items in total, both the number and the biomass of which varied significantly between the five types of habitats used (i.e. boundary features, cereal crops, oil-seed rape, other crops and fallow land) by adult Yellowhammers to collect food for nestlings (Appendix S1). The faecal samples produced a total of 6709 invertebrate prey items representing

110 different taxa, and plant material representing the remains of cereal grains (Appendix S2). Graphical presentations of the dietary composition, treating the prey groups in greater detail, are given in Appendix S3.

### Significance and efficiency of cereal seed digestion

On average, the remains of cereal grains were found in 71.5% of broods in the consecutive age classes and in 67.9% (631/930) of the faecal sacs

(Fig. 1a–d). Cereal grains began to be delivered to 3-day-old nestlings (Fig. 1c–f), but the percentage volume of grains was highest in August (Fig. 1b). A detailed assessment of the weight of cereal grain remains, recovered from smaller samples of droppings in which these remains were present ( $n = 335$  droppings), showed that on average there were 3.98 mg (95% CL 3.45–4.50 mg) of such remains per individual faecal sac (Fig. 1e), which corresponds closely with the mass of one seed husk (3.5 mg). Only in four out of 335 droppings were partly undigested larger portions of cereal



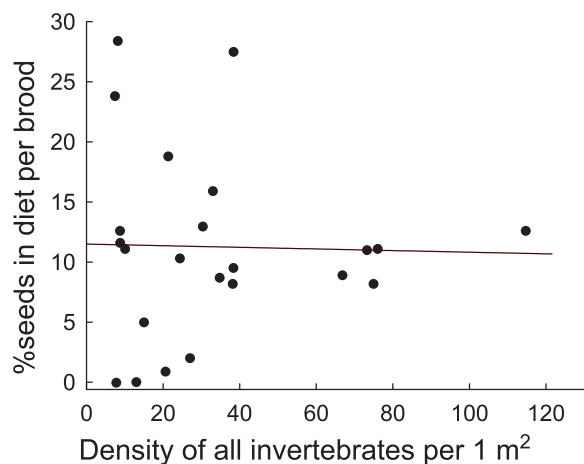
**Figure 1.** Cereal seed component (a–e) and assessment of cereal seed digestion efficiency (f) in nestling Yellowhammers *Emberiza citrinella* (average  $\pm 1$  se depicted on b and d–f): overall visual estimate of the percentage volume of cereal remains (a) and by month (b) in all faecal samples ( $n = 930$ ) without division by age; the number of faecal sac samples per month: April (39), May (319), June (367), July (172) and August (31). Frequency of occurrence per brood (c) and percentage volume (d) of cereal remains in consecutive age classes; the numbers of brood/faecal samples analysed in 12 consecutive age classes: 1 day old (4/11), 2 days old (5/17), 3 days old (9/43), 4 days old (19/87), 5 days old (21/101), 6 days old (24/121), 7 days old (22/127), 8 days old (16/78), 9 days old (8/70), 10 days old (5/24), 11 days old (4/32) and 12 days old (9/219). Total mass of remains of cereal grains (e), also expressed as %mass of one cereal seed (f) in the milky stage [20.3 mg ( $\Delta$ )] and the mature stage [43.8 mg ( $\blacktriangle$ )] per faecal sample; the dashes denote extreme values. The sample size for subsequent age classes (e–f): 3/13 (days old/number of droppings), 4/24, 5/36, 6/74, 7/57, 8/53, 9/39, 10/14, 11/13 and 12/12.

seeds present (weighing *c.* half of one mature cereal grain); in all the other droppings only cereal husks were preserved (Fig. 1e). The data obtained on the mass of cereal seed remains were used to assess the efficiency with which cereal seeds were digested. This showed that on average the mass of all cereal grain remains recovered from an individual dropping constituted only 9% (95% CL 8–10%) of the mass of one mature cereal grain (i.e. the average mass of one wheat/triticale/barley seed in the milky and mature stage was 20.3 and 43.8 mg, respectively) or 20% (95% CL 8–10%) of the mass of one cereal seed in the milky stage (Fig. 1f). We found a weak but statistically significant positive relationship between nestling age and the mass of cereal grain remains recovered from droppings (Fig. 1e), and also between the age of nestlings and the percentage mass of cereal grain remains in the mature and milky stages (Fig. 1f; Spearman's coefficient,  $r_s = 0.228$ ,  $P < 0.001$ ,  $n = 335$ , in each case).

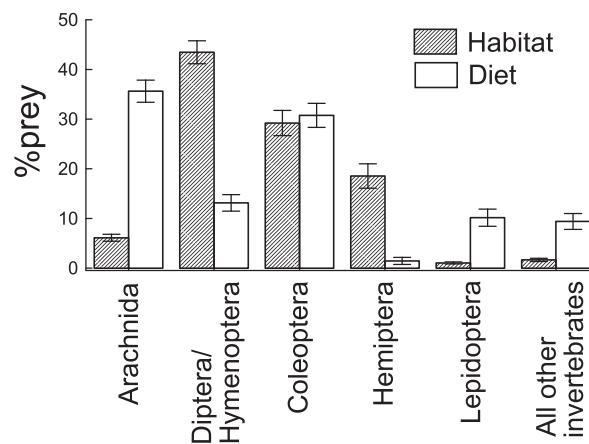
### Selectivity of prey in relation to their abundance in the foraging habitat

The proportion of cereal seeds in the diet of broods from 23 nests (around which we assessed the abundance of invertebrates) was not correlated with the total density of invertebrates present in the main foraging habitats of adult Yellowhammers (Pearson's coefficient,  $r = -0.03$ ,  $P = 0.901$ ,  $n = 23$ ; Fig. 2).

The percentage composition of the six major invertebrate components of the diet samples compared with that of the vacuum samples collected at the main foraging sites around the same 23 nests varied significantly (MANOVA, Wilk's  $\lambda = 0.105$ ,  $F_{6,39} = 55.57$ ,  $P < 0.001$ ; Fig. 3), suggesting that parent Yellowhammers did not select prey at random. Parent Yellowhammers preferentially chose to feed their nestlings on three prey groups and the percentage of each of these was significantly higher in the diet samples than in the utilized habitats: Arachnida (the proportion of which was on average *c.* 6-fold higher in the diet than in the vacuum samples), Lepidoptera (10-fold higher) and all other invertebrates (6-fold higher; paired *t*-test,  $df = 22$ ,  $P < 0.0001$ , in each case; Fig. 3). In contrast, the percentages of two other invertebrate components were significantly lower in the diet samples compared with those from the main foraging habitats: Diptera/Hymenoptera



**Figure 2.** Relationship between the average percentage of cereal grains in the diet and the average density of all invertebrates present in the main foraging habitats of adult Yellowhammers (see Table S1 for more details). Data obtained from 23 different broods in which droppings were collected from nestlings in at least three different age classes.



**Figure 3.** Comparison of the percentage composition of major invertebrate components in the diet of nestling Yellowhammers with that of vacuum samples taken from the foraging sites (pooled across various habitats) around the same 23 Yellowhammer nests (see Table S3 for details).

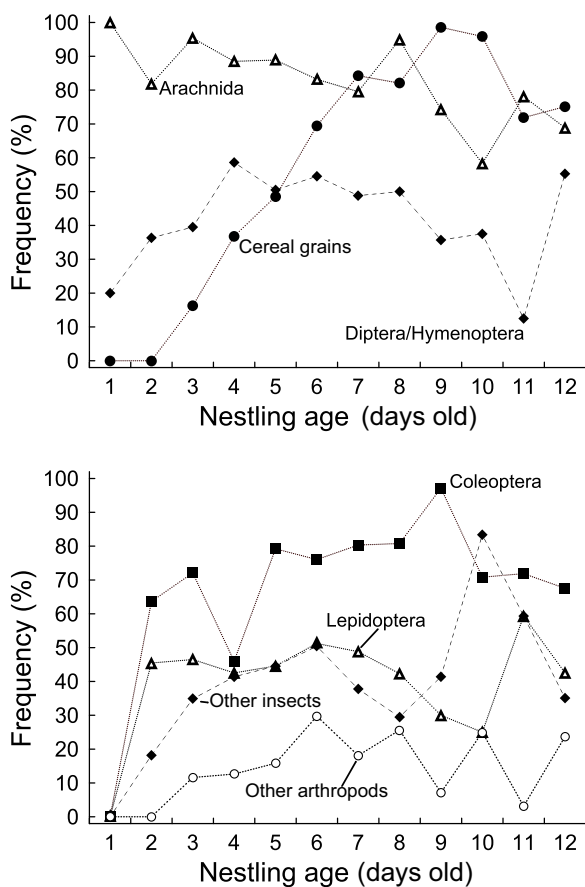
Hymenoptera (on average 4-fold lower in the diet than in the vacuum samples) and Hemiptera (13-fold lower; paired *t*-test,  $P < 0.0001$ , in both cases). Only the percentage of Coleoptera (the main invertebrate type available; Fig. S4) was similar in the diet and habitat samples (paired *t*-test,  $P = 0.582$ ; Fig. 3).



### Diet of nestlings in relation to their age

Neonate nestlings (1 day old) exclusively received weakly chitinized invertebrate prey (arachnids and flies), but from the second day of life the nestlings were fed a variety of highly chitinized invertebrate prey (Fig. 4), the percentage biomass of which did not change for the remainder of the nestling period (Appendix S3).

The seven main dietary characteristics – dietary diversity, the total number of all/animal prey items, the total biomass of all/animal prey and the average weight of all/animal prey – showed marked and statistically significant differences associated with brood identity and nestling age (Fig. 5; Supporting Information Table S3). Generally, all the dietary characteristics showed an apparent increase between the 1st and 6th day of nestling life, and the highest gain at this time, nearly



**Figure 4.** Frequency of occurrence of seven major prey groups identified in 930 faecal sacs of nestling Yellowhammers *Emberiza citrinella* by age (days old); to aid presentation, the different prey groups are spread over two figures.

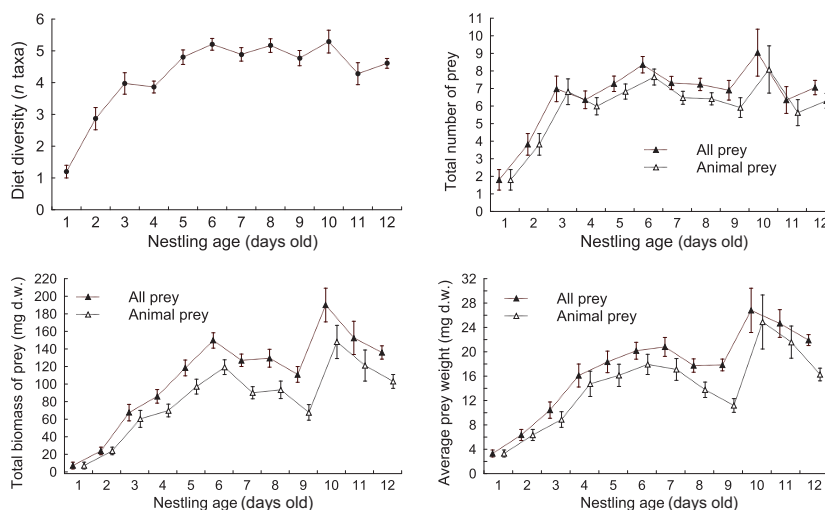
21-fold, was displayed by the total biomass of all prey (Fig. 5).

MANOVA was applied to assess the differences in dietary composition expressed as four dietary variables (each representing the six major food types: Arachnida, Diptera/Hymenoptera, Coleoptera, Lepidoptera, cereal grains and other animal prey), i.e. number of prey, total biomass of prey, percentage of prey and percentage of prey biomass, and showed marked differences between individual broods (nested within the age category) and between nestlings in various age classes (Table 1). Although the differences were highly significant for both effects, much higher Wilk's lambdas associated with age indicated that the effect of age far outweighed that of brood identity (Table 1). MANOVA also showed significant differences in the four analogous dietary variables expressing the contribution of cereal seeds and chitin content in the prey (Appendix S3). Logistic regression further supported the effect of age on diet composition (Appendix S3).

The percentage volume of cereal grains increased significantly with nestling age controlling for brood identity (two-way ANOVA,  $R^2 = 0.683$ ,  $F_{144,774} = 11.59$ ,  $P < 0.0001$ ), and varied significantly between months across the 12 age classes (two-way ANOVA,  $R^2 = 0.348$ ,  $F_{42,878} = 11.16$ ,  $P < 0.0001$ ) (Fig. 1d). Similarly, the percentage biomass of cereal grains (calculated on the basis of their frequency of occurrence: third method) progressively increased (up to 30–50% of the total biomass consumed) in the diet of 9- to 12-day-old nestlings. The percentage biomass of cereal grains in faeces was significantly and positively correlated with age, both in 1- to 3-day-old nestlings and in all nestlings (Spearman's coefficient,  $r_s = 0.221$  and  $0.343$ ,  $P = 0.046$  and  $< 0.001$ , respectively).

### DISCUSSION

Revealing a synthetic and multi-dimensional picture of dietary changes in nestlings of the Yellowhammer, a granivorous passerine and flagship species of European farmlands, our study has characterized the mixed plant–animal diet throughout their nesting period. The regular occurrence and high cereal seed digestion efficiency over the entire brood-rearing period, and the surprisingly large proportion of cereal seeds in the diet of Yellowhammer nestlings, were of particular importance. Hence, we were unable to confirm our first



**Figure 5.** The seven principal dietary characteristics (average  $\pm 1$  se) of nestling Yellowhammers *Emberiza citrinella* by age (days old). All prey = animal + plant (cereal grain) material. The values for plant material were calculated based on the frequency of occurrence/individual mass of one cereal grain.

**Table 1.** Results of MANOVA testing the effect of age (days old) and brood identity (nested within age) on dietary composition expressed as the number, percentage of prey, total biomass and percentage of prey biomass representing the six major food types (Arachnida, Diptera/Hymenoptera, Coleoptera, Lepidoptera, cereal grains and other animal prey; see Figs S5 and S6) identified in faeces of Yellowhammer *Emberiza citrinella* nestlings.

Source of variation	Brood identity (age)					Age				
	Wilk's $\lambda$	df			F	P	Wilk's $\lambda$	df		
		H <sub>0</sub>	Error					H <sub>0</sub>	Error	F
Number of prey	0.015	798	4661.5	6.00	< 0.0001	0.479	66	4157.7	5.60	< 0.0001
Percentage of prey	0.036	798	4661.5	4.26	< 0.0001	0.489	66	4157.7	8.76	< 0.0001
Total biomass of prey	0.019	798	4661.5	5.47	< 0.0001	0.488	66	4157.7	9.05	< 0.0001
Percentage of prey biomass	0.044	798	4661.5	3.88	< 0.0001	0.463	66	4157.7	9.42	< 0.0001

expectation: nestlings received a large amount of plant food and the proportion of cereal seeds in their diet was independent of the invertebrate food supply in the parent Yellowhammer main foraging sites, an observation previously reported in other studies on this species (MacLeod *et al.* 2005, Douglas *et al.* 2012).

Our study is the first to analyse concurrently the number and biomass of consumed plant and animal items. In earlier dietary studies of European buntings, cereal grains were analysed separately, only the percentage volume/frequency occurrence of this kind of food being considered. In this respect, our findings are consistent with previous results, which showed a progressive increase in the proportion of cereal grains with nestling age

(MacLeod *et al.* 2005, Douglas *et al.* 2012). Most notably, we obtained our results by quantifying the amount of consumed seeds based on the presence of cereal grain remains, which presumably represents the minimum amount of cereal seeds ingested when they are in near-complete maturation stages, i.e. spilt grains (Fig. S1) or around harvest time (from late June). For lighter immature cereal seeds at the milky stage, however, this figure could have been overestimated. On the other hand, as our analysis of cereal seed digestibility showed, the (low) mass of cereal grain remains present in one faecal sac corresponds closely to the mass of a single seed husk (i.e. 3.5 mg; Fig. 1e–f). Hence, this ultimately justifies the simultaneous analysis of plant and animal fractions in the diet,

despite certain limitations concerning the quality and/or quantity of cereal seeds consumed. However, owing to the availability of spilt (i.e. ungerminated) cereal seeds in the study area (Fig. S1), nestling Yellowhammers were presumably fed such seeds over the entire breeding period. Moreover, early nestlings (3–5 days old) presumably received fewer cereal grains because of the large size of this food type, as the average weight of one invertebrate item supplied to 1- to 3-day-old nestlings was < 10 mg dry weight (Fig. 5), i.e. less than a quarter of one cereal grain. Our analysis may also indicate that older nestlings in particular consumed considerably more cereal grains: the biomass of a single invertebrate prey item (weighing up to c. 140 mg d.w. per dropping; Fig. 5) is equivalent to at least three cereal seeds. This suggests a disproportionately higher number/biomass of consumed cereal grains, especially in nestlings older than 6–7 days.

Nestlings digested cereal grains sufficiently well from the beginning of their provisioning to their third day of life. This may be indicative of the early development of the transition to granivory, i.e. the mechanical breakdown of seeds and the activity of digestive enzymes responsible for seed digestion. For instance, chicks of the herbivorous Red-legged Partridge *Alectoris rufa* and Grey Partridge *Perdix perdix* (both showing a progressive increase in the proportion of plant material in the diet from c. 20% 1 day after hatching) aged between 1 and 25 days old were able to break down from 85% to c. 95% of all seeds of wild grasses during their passage through the digestive tract (Green *et al.* 1987). The high efficiency of seed digestion in nestling Yellowhammers, as indicated by the low mass of cereal grain remains recovered from droppings in nestlings aged 3 days and older (Fig. 1e–f), compared with both species of partridges, was presumably due to seed size (smaller seeds in partridge chicks might pass intact more easily than large cereal grains through the digestive tract) and the variable contribution of invertebrates in their diets, as well as the size of these birds. In particular, the large cereal grains provisioned in parallel with highly chitinized insects (such as Coleoptera; from the age of 2 days; Fig. 5) might be indicative of the early development of the ability to digest both chitin (reflecting the high percentage of highly chitinized invertebrate prey) and plant/seed food in Yellowhammer nestlings (see Appendix S4 for further

interpretations of the results). Alternatively, it is possible that parent Yellowhammers deliver to their nestlings cereal seeds with different degrees of pre-treatment, i.e. de-husked seeds for younger nestlings (Díaz 1996). However, to resolve this question, further studies need to assess the quality, quantity and species of cereal seeds sampled directly from Yellowhammer nestlings immediately after their provision by parents (e.g. by collaring nestlings), which would allow unbiased samples to be obtained (*sensu* Valera *et al.* 1997).

In comparison with nestlings of specialist species with a seed-only diet or with no apparent dietary switch from animals/invertebrates to seeds (*sensu* Wiens & Johnston 1977), as in some cardueline/estrildid finches (e.g. Morton & Davies 1983, Allen & Hume 1997, Valera *et al.* 2005, Moorcroft *et al.* 2006, Gil-Delgado *et al.* 2009, see Appendix S4), the variety of invertebrate prey, along with its high biomass and the apparent early switch from an animal to a seed diet reported here in Yellowhammer nestlings, suggests that in this bunting (and most likely in other related species), regulatory mechanisms adjust the activity of digestive enzymes to changing diet type, as described previously in House Sparrows (Brzęk *et al.* 2009, 2010, 2011). Although the diet of adult granivores can be restricted to seeds (mostly during autumn and winter), nestlings of these birds appear to represent a continuum from specialist/obligatory seed-eaters to species with a mixed invertebrate/seed diet or even an animal diet (as in the Chaffinch *Fringilla coelebs* or Tree Sparrow *Passer montanus*; Newton 1967, Field *et al.* 2008). Consequently, the nestlings of Yellowhammers, as well as those of other bunting species, are placed in the middle of such a continuum. Cereal grains are also the much preferred food type and constitute the staple diet of adult Yellowhammers during the autumn–winter period (Perkins *et al.* 2007, Orłowski *et al.* 2014a). Therefore, we conclude that the provisioning of cereal grains to nestling Yellowhammers is an intentional behaviour on the part of the parent birds, which aims to achieve the physiological adjustment enabling nestlings to switch from animal/invertebrate to seed food in the early stages of ontogeny.

Another important point is that the density of invertebrates in the foraging habitats of adult Yellowhammers in our study area determined by vacuum sampling was c. 20- to 30-fold higher than the data reported by MacLeod *et al.* (2005), who

sampled with a sweep net. This large difference may reflect the efficiency of the invertebrate sampling technique rather than the real difference in abundance of invertebrates in the two study areas. However, for an unequivocal biological interpretation of invertebrate abundance in the environment and selectivity of particular invertebrate groups by ground-foraging insectivorous birds, vacuum sampling is much more appropriate, the results of which represent a real, or at least a less biased, measure of invertebrate abundance (Ryszkowski & Karg 1977, Green 1984), which has clear implications for studies of the food supply of these birds.

Our study has shown that nestling Yellowhammers are fed a variety of invertebrate prey and plant material, the latter being restricted to basically one food type – cereal grains (presumably of two/three cereal species). Although wild plants/weeds are widespread in the study area (i.e. in semi-natural habitats like fallow land, abandoned grassland and road verges), the lack of wild plant seeds in the nestling diet indicates the strong dependence of foraging/feeding parent Yellowhammers on cereal cultivation (Morris *et al.* 2001, Wuczyński & Grzesiak 2007, Douglas *et al.* 2010, Dunn *et al.* 2010). This implies that cereal grains are not a secondary food resorted to in the absence of superior (invertebrate) prey, as has been suggested in some populations, where farming intensification may be driving population declines. However, ultimately, an assessment of the proportion of cereal grains on survival (in the nest and post-fledging) would better answer this question. An assessment of the seed fraction is thus desirable in order to acquire the full picture of the nestling food composition in birds with a mixed plant–animal diet, i.e. most granivorous species. In view of the plentiful invertebrate fauna observed in the study area, our Yellowhammer population is unlikely to be limited by food availability. Therefore, this population is likely to be a good model for other dietary studies, especially when compared with declining populations on intensive farmland. As during nestling provisioning Yellowhammers forage predominantly on the ground, selecting open patches with or without low vegetation, the maintenance of diversifying crop structure (accessibility to non-sown open patches like tractor ruts) and non-cropped habitats (field margins, road verges or dirt roads) is essential for this granivorous species.

Our study clearly shows that the composition and quality of the diet varies significantly with nestling age. Therefore, comparisons and general descriptions of dietary variations between various population/studies of nestlings of individual granivorous bird species without considering their age may be biased. This implies the need for balanced samples and/or the cautious drawing of inferences in cases when samples differ in age. Further studies focused on the nutritional/dietary ecology, eco-physiology and digestive abilities of the nestlings of granivorous birds breeding in farmland and linking agricultural/land-use with ongoing food supply/dietary variations in these and other bird guilds are needed.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Mosaic agricultural landscape of the Sudeten Foreland, SW Poland, with wild vegetation growing on various field borders, such as mid-field ditches, which are the main breeding habitat of Yellowhammers (a); open micro-plot ( $c. 1 \text{ m}^2$ ) with low, undersized cereals and wild vegetation growing in a field of winter cereals where Yellowhammers often forage during the brood rearing period (b); spilt/ungerminated cereal grains on a spring barley field (c); not fully mature spikelet of barley with grains partly eaten by Yellowhammers (d); adult Yellowhammer foraging on ungerminated grains in a field of spring cereals (e); a quick-trap method of vacuum sampling in the study area with a biocenometer (samples taken by covering  $0.25 \text{ m}^2$  of the ground with a bottomless cage; Ryszkowski & Karg 1977). (All photographs: A. Wuczyński).

**Figure S2.** Jacobs' habitat selectivity index of adult Yellowhammers during the brood rearing period in an agricultural landscape in the Sudeten Foreland determined for 681 foraging flights from 29 different nests (from Wuczyński & Grzesiak 2007).

**Figure S3.** Density (a) and biomass (b) of major invertebrate taxa in the main foraging sites of adult Yellowhammers during the brood rearing period based on 385 vacuum samples taken in the five main types of foraging habitat: boundary features ( $n = 85$  samples), cereal crops (155), oil-seed rape (70), other crops (50), fallow land (25) located around 23 different nests.

**Figure S4.** General comparison of total percentages of number (a) and biomass (b) of eight major invertebrate groups from the habitat (based on all 385 vacuum samples; 3391 sampled invertebrates) and diet (6709 invertebrate items from 930 faecal sacs; cf. Table S2 for the list of prey) of nestling Yellowhammers *Emberiza citrinella*.

**Figure S5.** The average ( $\pm$ se) number and biomass of major food types (class/orders of invertebrates and cereal grains) identified in faeces of nestling Yellowhammers *Emberiza citrinella* vs. age (days old).

**Figure S6.** The average ( $\pm$ se) percentage number and percentage biomass of major food types (class/orders of invertebrates and cereal grains) identified in faeces of nestling Yellowhammers *Emberiza citrinella* vs. age (days old). Note the values for cereal grains presented in Fig. S7.

**Figure S7.** The average ( $\pm$ se) number, biomass, percentage number and percentage biomass of four types of prey in relation to chitin content (soft-bodied prey, intermediately chitinized and heavily chitinized) and cereal grains identified in faeces of nestling Yellowhammers *Emberiza citrinella* vs. age (days old).

**Table S1.** Comparison of the percentage composition of the major invertebrate groups in the vacuum samples ( $n = 385$  samples) at the main foraging sites (hab) (i.e. pooled data across five habitat types: boundary features, cereal crops, oil-seed rape, other crops and fallow land) and the diet of nestling Yellowhammers ( $n = 559$  faecal sacs from nestlings from at least three different age classes) specified for 23 individual nests; the last two digits in # nest denote the sampling year.

**Table S2.** Percentage (%) composition and characteristics of prey in the diet of nestling Yel-

lowhammers *Emberiza citrinella* on consecutive days of life (1–12 days old) based on the analysis of 930 faecal samples from south-western Poland, 2006–2008.

**Table S3.** Results of ANOVA (type III ss) testing the effect of age (days old) and brood identity (nested within age) on the main dietary characteristics (diversity, number, prey mass and total biomass; see Fig. 5) identified in faeces of Yellowhammer *Emberiza citrinella* nestlings.

**Table S4.** Results of MANOVA testing the effect of age (days old) and brood identity (nested within age) on dietary composition expressed the contribution of seeds and chitin content in prey (weakly, intermediately and heavily chitinized; see Fig. S7) in faeces of Yellowhammer *Emberiza citrinella* nestlings.

**Table S5.** Average values of different dietary variables/food categories per faecal sample across all analysed faeces ( $n = 930$ ) of nestling Yellowhammers *Emberiza citrinella* aged between 1 and 12 days old from south-western Poland, 2006–2008.

**Appendix S1.** Invertebrate prey abundance and biomass at sites where food for nestlings was collected.

**Appendix S2.** Comparison between the composition of the major invertebrate groups in the vacuum samples at the main foraging sites of parent birds and in the diet of nestling Yellowhammers.

**Appendix S3.** Diet composition of nestling Yellowhammers.

**Appendix S4.** Profitability of cereal seed provision to nestling Yellowhammers: further interpretations of the results.