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Brood sex ratio in European Honey Buzzards *Pernis apivorus* is related to spring phenology

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In sexually size-dimorphic bird species, rearing costs of sons and daughters usually differ and may be important in the evolution of offspring sex ratio adjustment. Raptors have reversed sexual size dimorphism and the smaller males are sometimes found to be overrepresented in food-poor territories or years. As a raptor with small reversed sexual dimorphism (6% in body mass), the European Honey Buzzard *Pernis apivorus* is expected to show little or no brood sex ratio bias in relation to environmental conditions. We molecularly sexed 311 chicks of 195 broods in and around The Netherlands, during 1996–2014. We examined which environmental factors explained brood sex ratio variation best. Overall, sex ratio was not biased (all nests pooled: 50.8% females) but more females were produced in years when on average Honey Buzzards bred earlier (32% sex ratio change over a ten-day range in annual mean laying date). Within-year laying date variation, hatching order, abundance of wasp (Vespinae) nests (main food source) and summer weather did not explain sex ratio variation. In the Veluwe and Drenthe (1974–2014), Honey Buzzards laid eggs earlier when the spring was warmer, which resulted in a c. 9-day advance in laying date over 40 years. As warm spring weather was also a predictor of a higher density of wasp colonies, we expected female chicks to benefit more from growing up in wasp-rich years than males, if the sex ratio biases were adaptive. However, this differential growth benefit was not noticeable in chick body mass; chick body mass was best explained by negative effects of relative laying date (within a year) and hatching order. The potential benefit for female nestlings (compared to males) of growing up in years with warm springs, when egg laying occurs early and wasp colonies are more abundant, remains unknown.

Key words: adjustment, body condition, environment, food, laying date, long-lived, migratory, raptor, social wasps, temperature

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The sex of offspring is expected to influence the fitness of parents, when the costs and benefits of producing sons or daughters differ and depend on environmental or parental conditions (Trivers & Willard 1973). A mechanism by which parents can allocate sexes non-randomly, in accordance with these conditions, enables parents to maximize their fitness under varying circumstances and would be favoured by selection (Trivers & Willard 1973, Clutton-Brock 1986, Cockburn *et al.* 2002).

Animal offspring sex ratios have been found to vary with many different conditions: e.g. when one sex is rarer in the population, it is produced more (in the fish Atlantic Silverside *Menidia menidia*; Conover & van Voorhees 1990); when the father is attractive, more sons are produced (Pied Flycatchers *Ficedula hypoleuca*, Ellegren *et al.* 1996, Blue Tits *Cyanistes caeruleus*, Sheldon *et al.* 1999); when daughters help their parents to rear future broods, they are produced more on high-quality territories where this help pays off

(Seychelles Warblers *Acrocephalus sechellensis*, Komdeur 1996). In sexually size-dimorphic bird species, the larger sex is generally more costly to rear in terms of food demand (Anderson *et al.* 1993a, Krijgsveld *et al.* 1998, Vedder *et al.* 2005a, Magrath *et al.* 2007), which would favour food-dependent sex ratio adjustment (e.g. Bowers *et al.* 2015).

In raptors (order Accipitriformes), falcons (Falconiformes) and owls (Strigiformes), reversed sexual size dimorphism is common and females can weigh up to twice as much as males in the case of Eurasian Sparrowhawks *Accipiter nisus* (Cramp & Simmons 1980, Dunning 2007). In these orders, sex ratios have been found to vary with final brood size (e.g. Olsen & Cockburn 1991, Byholm *et al.* 2002a). This may be caused by chick mortality depending on initial brood size, brood sex ratio and different food demands of both sexes (Dijkstra *et al.* 1998, Vedder *et al.* 2005b). Further, males are sometimes found to be overrepresented among offspring in food-poor years (Brommer *et al.* 2003, Bijlsma 2018) or food-poor territories (Chakarov *et al.* 2015), a pattern that can also be found in eggs (Appleby *et al.* 1997). Lastly, brood sex ratio has often been reported to relate to laying date (e.g. Olsen & Cockburn 1991, Zijlstra *et al.* 1992, Bijlsma 1993, Daan *et al.* 1996, Byholm *et al.* 2002b), possibly selected for by sex-specific benefits of being born early (Dijkstra *et al.* 1990, Daan *et al.* 1996).

Because the eggs of raptors and owls hatch asynchronously within a clutch (Cramp & Simmons 1980), siblings differ in age, which results in a size hierarchy. First-hatched chicks can monopolise food items in the nest (Anderson *et al.* 1993b) and have best survival prospects (McDonald *et al.* 2005). This may explain why first-hatched and last-hatched chicks can show opposite seasonal trends in sex ratio, as in Eurasian Kestrels *Falco tinnunculus* (Dijkstra *et al.* 1990). On the other hand, a size hierarchy among siblings can be alleviated by producing males first and females last, as was found in Harris's Hawks *Parabuteo unicinctus* (Bednarz & Hayden 1991) and Eurasian Eagle Owls *Bubo bubo* (Penteriani *et al.* 2010) and which may increase overall fledging success.

Within the family of Accipitridae, the European Honey Buzzard *Pernis apivorus* is an exceptional species, having only slight sexual size dimorphism, with adult females weighing on average 6% more than males (Cramp & Simmons 1980). Unlike other raptors, it feeds mostly on insects during the breeding season (larvae of social wasps, mainly Common Wasp *Vespula vulgaris* and German Wasp *V. germanica* in our study areas); bumblebee *Bombus* spp. larvae, frogs and nestling birds are alternatives when wasp supply is scarce (Harmsen & Bijlsma 2014, van Diermen *et al.* 2015). In Honey Buzzards, chicks hatch asynchronously, two to five days apart (Bijlsma 1993, camera observations by R. Riem Vis and V.S. van Bergen in The Netherlands), allowing us to reliably study sex ratios over the laying sequence. Honey Buzzards lay small clutches of almost always two eggs (i.e. 95% of complete clutches, while 4% has one egg and 1% has three eggs, n = 399 in The Netherlands during 1996– 2021; data of Werkgroep Roofvogels Nederland, see also Bijlsma 1986, 1993, and for Britain, Roberts & Law 2014), allowing us to investigate environmental effects on sex ratio, without the confounding effects of clutch size variation. Given the low sexual size dimorphism in Honey Buzzards and because sibling aggression is limited (Wendland 1935, Ziesemer *et al.* 2021), sex ratios might not change the sibling size hierarchy to a large extent.

Based on the small sexual size dimorphism in Honey Buzzards, we expect no bias in overall brood sex ratio, and no adjustment of sex ratio to environmental conditions. We explore brood sex ratio variation in Honey Buzzard broods in relation to food abundance, spring and summer temperature and precipitation (as variables that may determine or predict food abundance) and timing of breeding. We disentangle possible causes of any (if present) sex ratio trends over laying date by separating between-year from within-year variation. To understand what may cause annual variation in laying date, we examine the influence of spring weather using a dataset that spans 41 years (1974–2014). To evaluate whether male and female nestlings grow differently in different circumstances, we examine how the size-corrected body mass of nestlings relates to environmental factors.

METHODS

Study areas and Honey Buzzard nests

We consider two datasets of mainly Dutch Honey Buzzard nests, thus within the normal breeding range of the Honey Buzzard in the western Palearctic (Bijlsma 1997a). Dataset A contained 221 nests, from the period 1996–2014, which were studied and sampled for sexing by volunteer bird ringers in deciduous, coniferous and mixed woodlands on sandy soils across The Netherlands and adjacent parts of Belgium and Germany (see Figure 1). Dataset B contained 213 nests, from a longer time span, which were studied by RGB in the Veluwe, central Netherlands (1974–2014, 52°02'N, 5°45'E) and Drenthe, northern Netherlands (1990–

Female Honey Buzzard shades smallest of two chicks against the glaring sun (>35°C). The previous day, the oldest chick weighed 450 g (wing 114 mm, male) and the youngest 64 g (wing 26 mm, female). Extreme food shortage (2013 was wasp-poor) resulted in retarded growth of the youngest: the real age difference was two days (17 vs. 15 days old) but the sizes suggested 11 days. Such retarded chicks eventually die in the nest, as did this one (photo RGB, Forestry of Smilde, 3 August 2013).

2014, 52°52'N, 6°17'E), two afforested areas about 100 km apart (Bijlsma *et al.* 2012). The two datasets overlapped by 38 nests. In both datasets, nests were searched for using methods described by Bijlsma (1997b) and Bijlsma *et al.* (2012). Dataset A was used for the analysis of factors explaining sex ratio and body mass, dataset B for the analysis of factors explaining laying date. For the composition and annual sample sizes of dataset A, see 'Molecular sexing of chicks'. Dataset B contained on average 5.2 ± SD 3.2 nests per year (range: 1–13), 4.0 ± 3.8 per surveyed year in the Veluwe (range: 0–13) and 1.8 ± 1.3 per surveyed year in Drenthe (range: 0–5).

For 207 nests in dataset A and 212 nests in dataset B, the laying date could be estimated, by subtracting 34 days incubation duration from the hatching date of the oldest chick (Bijlsma 1997b). Incubation duration is constant across years and clutch sizes (32–34 days; Bijlsma 1997b, Roberts & Law 2014). Hatching date was observed directly by nest visits in the first three days after hatching of the oldest chick (12 nests in dataset A, 24 nests in dataset B) or else, backdated from the age of the brood on the first date they were measured (209 nests in dataset A, 26.0 ± SD 8.9 days old, range: 4–43; 189 nests in dataset B, 22.5 ± 11.6 days old, range: 4–49). The age of the brood was derived from the maximum wing chord (flattened and straightened, henceforth wing length) of the oldest – or sole – chick in the nest, using growth curves from Bijlsma (1997b). Wing length of the oldest chick is a reliable measure for age, as it grows consistently except in the rare years with extreme wasp scarcity (Bijlsma 1997b, Bijlsma *et al.* 1997, van Manen *et al.* 2011, van Manen 2013). Hatching order was derived from the difference between siblings in wing length (Bijlsma 1997b, van Manen 2013). Clutch size was recorded during incubation or indirectly during a visit in the nestling stage. When determined indirectly, clutch size was assumed two when either two chicks had been recorded on the nest at any point (alive or dead) or one chick and an unhatched egg. When only one chick was recorded, and the nest had not been visited during incubation, clutch size remained unknown (see ‘Molecular sexing of chicks’ for sample sizes). Hatching order remained unknown when a nest held only a single chick (with or without an egg), except in cases where a chick of known order had died or fledged. Wing length and body mass (including crop mass) were used to estimate relative body mass (see ‘Statistical analyses’). If chicks were measured repeatedly (26 nests in dataset A), we included only the measurement of the last date on which all siblings were present. Thus, we assessed relative body mass (using dataset A) at a mean age of 29.4 ± SD 7.0 days (range: 9–48); chicks on average fledge at an age of 42 days (Bijlsma 1997b).

Weather data

Weather data were extracted from four stations of the Royal Netherlands Meteorological Institute (KNMI; www.knmi.nl) across The Netherlands, all from 1974 onwards: in the north (Eelde, 53°07'N, 06°35'E), centre (De Bilt, 52°06'N, 05°10'E), east (Twente, 52°16'N, 06°53'E) and south (Volkel, 51°39'N, 05°42'E; Figure 1). We defined spring as May and summer as June–August. Stations showed similar variation in mean spring temperature (all pairwise Pearson’s correlations: $r > 0.954$, $n = 41$, $P < 0.001$) and total spring precipitation (all pairwise correlations: $r > 0.418$, $n = 41$, $P < 0.007$) and mean summer temperature (all pairwise correlations: $r > 0.915$, $n = 41$, $P < 0.001$) and total summer precipitation (all pairwise correlations: $r > 0.619$, $n = 41$, $P < 0.001$). For further analysis, we took the average of the four stations, for each spring and summer separately, of the mean temperature and total amount of precipitation. We regard May as the pre-laying stage, as Honey Buzzards arrive at the breeding grounds in early May and initiate egg-laying between mid-May and mid-June, whereas June–August spans the incubation and chick-rearing stage (Bijlsma 1993, van Manen *et al.* 2011, van Diermen *et al.* 2014).

Because our study period spans decades, we report the changes in weather here using linear models. May temperatures increased during 1974–2014 ($\beta = 0.042$, $F_{1,39} = 4.982$, $P = 0.031$; Figure S1A), but not in 1996–2014 (i.e. when chicks were sexed, $\beta = -0.0004$, $F_{1,17} = 0.00004$, $P = 0.995$). Summer temperatures (June–August) have also increased since 1974 ($\beta = 0.0359$, $F_{1,39} = 8.966$, $P = 0.005$, Figure S1A), but not since 1996 ($\beta = 0.0169$, $F_{1,17} = 0.311$, $P = 0.584$). The total precipitation in May has not changed significantly since 1974 ($\beta = 0.454$, $F_{1,39} = 1.762$, $P = 0.192$; Figure S1B), nor since 1996 ($\beta = 1.123$, $F_{1,17} = 1.146$, $P = 0.299$). In June–August however, the total precipitation has increased since 1974 ($\beta = 1.9149$, $F_{1,39} = 7.237$, $P = 0.011$; Figure S1B), but not since 1996 ($\beta = 1.349$, $F_{1,17} = 0.339$, $P = 0.568$).

Food abundance

Prey remains found on nests were mostly combs of social wasps (95% of 6485 prey items collected in The Netherlands between 1971 and 2013; Harmsen & Bijlsma 2014), mainly Common Wasp and German Wasp. The importance of alternative prey, mostly amphibians and nestling birds, varied between years, regions and probably parents (males bringing frogs more often than females), as evident from cameras placed near nests (van Diermen *et al.* 2015). We consider wasp abundance a reliable measure of food abundance.

Wasp abundance was measured as an index: the number of wasp nests (alive and depredated) incidentally encountered on foot per 100 hours of field work by RGB in forests and fields of the Veluwe (100 km2) and Drenthe (45 km^2), during May–August in 1974–2014 (Bijlsma *et al.* 2012, Bijlsma 2018; see Berkvens

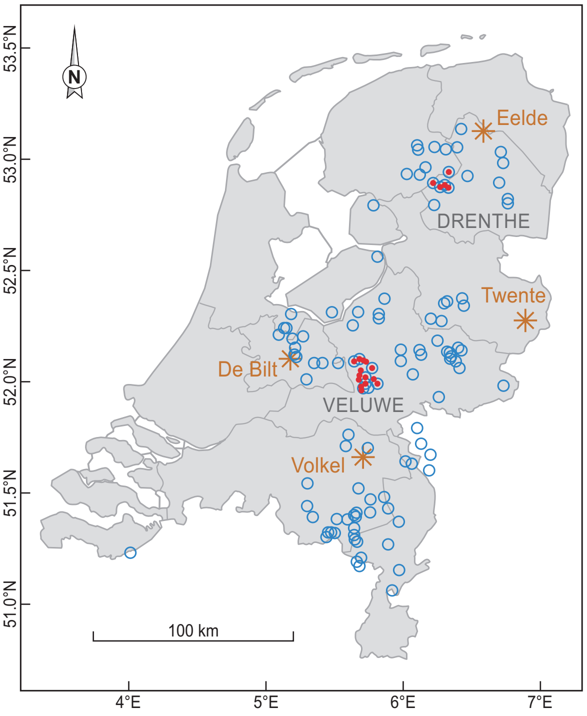

Figure 1. Locations of sampled Honey Buzzard nests (blue circles, dataset A, 1996–2014), the areas Veluwe (100 km^2) and Drenthe (45 km^2) where wasp abundance and Honey Buzzard nests were monitored (red dots, dataset B, 1974–2014) and KNMI weather stations (orange stars, Eelde, De Bilt, Twente, Volkel).

& van Diermen 2021 for habitat-related differences of encountering wasp nests). RGB did not specifically search for, but was continuously alert to, wasp nests during fieldwork. Data for both areas were combined in our index, but in 1974–1990 only the Veluwe was surveyed, in 2000–2004 only Drenthe, and both areas in 1991–1999 and 2005–2014. Per year, the average number of field hours in May–August was 650 ± SD 176 (range: 370–1114) and the average number of wasp nests found 69 ± 73 (range: 2–325). For years in which both areas were surveyed, the annual wasp nest abundance indices (henceforth wasp abundance) tended to be on average slightly higher in the Veluwe than in Drenthe, but not significantly (respectively, 9.9 vs. 7.5 nests per 100 hours, medians: 7.0 vs. 5.5, Wilcoxon signed rank test: $V = 139$, $P = 0.080$) and annual variations were very similar in both regions (Pearson's product moment correlation: $r = 0.92$, $n = 19$, $P < 0.001$) despite being about 100 km apart. Based on this, we assume that this index accurately describes annual variation in wasp abundance over a wider area. We further validated this assumption based on high positive correlations with several other measures of wasp abundance from northern Netherlands (Table S1; see also Bijlsma *et al.* 2012).

Molecular sexing of chicks

From each chick, one or two small growing feathers were taken from the mantle (333 chicks, 1996–2014) or a blood sample was taken from the brachial vein ($n = 44$, 1996–2001, 2003–2008, 2012–2014, only by RGB) and stored in 96% ethanol. DNA was extracted from the blood or from a finely cut base of a growing feather with the ammonium acetate method, following Richardson *et al.* (2001). DNA was not diluted before PCR. DNA samples were molecularly sexed using the method of Kahn *et al.* (1998). PCR reactions were carried out in 10 μl volume, containing 0.2 mM of each dNTP, 1× PCR-buffer (Roche diagnostics GmbH), 0.2U taq polymerase (Roche diagnostics GmbH), 3.0 mM MgCl2, 1.0 μM of each primer 1237L/1272H and 2.0 μl DNA . PCR program was as follows: 1 min 94°C, followed by 35 cycles of 30 s 94°C, 60 s 50°C and 60 s 72°C and a final extension of 2 min 72°C. PCR products were separated on 3.3% agarose gels and stained with ethidium bromide.

In total 377 chicks were sampled (on average 19.8 per year, range: 5–38) from 221 nests (average 11.6 per year, range: 3–22). Of these, 82%, i.e. 311 chicks could be sexed (on average 16.4 per year, range: 4–37). Sexing success was higher with blood samples (95%, 42/44) than feather samples (81%, 269/333). Failure was due to contamination and poor sample conservation. Most sexed chicks were sampled across The Netherlands (298, all years), few in Germany (9, in 1997 and 1999) and Belgium (4, in 2013 and 2014). Sexed chicks were from 195 nests (on average 10.3 per year, range: 3–21), of which the clutch size was two (168 nests), one (3 nests) or unknown (24 nests). Of the nests with two eggs, 165 hatched two chicks and three a single chick. Of the 165 nests with two chicks, we managed to sex both chicks in 116 nests and only one chick in 49 nests. In the latter case one sibling remained unsexed, because it had already fledged (7 nests), had died or disappeared before the sampling date (14 nests), or because the molecular sexing failed (28 nests). The sexed, remaining chick was in 29 nests the first-hatched chick, in 14 nests the second-hatched and in 6 nests of unknown order. In another 26 nests the sexing of all chicks had failed (13 nests with two and 13 with one chick).

Statistical analysis

To understand which factors influence wasp abundance, we took the natural logarithm of the wasp abundance index (for normalization) as the dependent variable in a set of linear models (LM). This model set contained five one-factor models (year as continuous variable, spring temperature, spring precipitation, summer temperature, summer precipitation), all ten two-factor additive combinations, a full additive model and a null-model. The full model had variance inflation factors (VIF) < 3, all other additive models < 2. The best model was defined based on AIC (Akaike 1973), corrected for small sample sizes (AICc), as the simplest model within 2 AICc from the model with lowest AICc.

To understand which factors influence laying date (dataset B), we created a set of linear mixed models, with a random intercept of year. This model set contained four one-factor models (year, spring temperature and spring precipitation, and area – Veluwe or Drenthe), all ten two- and three-factor additive combinations, a full additive model and a null model. All models had VIF < 2. The best model was again chosen based on AICc as described above.

To study which variables predicted the sex of nestlings (dataset A), we created a set of binomial Generalized Linear Models (GLM) with logit link functions. We could not include random effects of year and/or brood, because the number of observations per year and brood was too low, causing model singularity. However, we confirmed that the best model was also best when these two random effects were included (although resulting in singular models) and tested

Chicks of Honey Buzzard at 8 (left) and 6 days old (wings 35 and 23 mm, masses 159 and 103 g). May of 2021 was cold and the summer wasp-poor, yet the empty combs of Common Wasp (front) and German Wasp (left) suggest a good food supply on this nest (photo RGB, Dieverzand, 6 July 2021).

The same two chicks, now 33 and 31 days old. The developmental age difference, as visible from morphometrics, was still two days and both chicks were in good condition (wings 272 and 258 mm, masses 800 and 825 g). The latter is in line with the laying date (25 May) being a few days before the year's mean, 27 May (photo RGB, Dieverzand, 31 July 2021).

whether the effect in the best model still held when based on annual mean values. For further analysis, we decomposed a nest's laying date (i.e. absolute laying date, date as such) into the annual mean laying date of that year (including all nests sampled for sexing in that year) and the nest's deviation in days from that mean (i.e. within-year relative laying date). The set of models explaining sex (binary, 0: male or 1: female) consisted of nine one-factor models (chick order, spring temperature, spring precipitation, summer temperature, summer precipitation, absolute laying date, annual mean laying date, relative laying date, wasp abundance), two two-factor models (spring temperature + precipitation, summer temperature + precipitation) and ten models in which interactions with chick order were added to the above models, to see whether effects differed between the first- and second-hatched chick. We also included a null-model. To test whether the effect of laying date differed on the between- and within-year levels, we followed van de Pol & Wright (2009) using a model including absolute laying date and annual mean laying date, so that the latter indicates the difference between the effect of mean and relative laying date. We included only chicks for which all variables were known (272 chicks, 4–34 per year from 2–18 nests, including 46 nests with one and 113 nests with two chicks sexed) into our model set, to allow model performance comparison based on AICc as described above. The additive models had VIF < 2.

To study how chicks of both sexes grow in different environments, we examined variation in size-corrected body mass, i.e. mass divided by the expected body mass of a chick given its wing length (e.g. Green 2001). The expected body mass was calculated by a second order polynomial, based on wing length and its squared term, for males and females separately. We then examined variation in relative body mass using linear mixed models, including random intercepts for year and brood. The set of models consisted of the same 21 models as mentioned above for sex, plus a one-factor model (sex), and models where an interaction with sex was added to the eleven one- and two-factor models mentioned above for sex. The models including an interaction with sex are of special interest, as these test whether the sexes benefitted differently from certain rearing conditions. We also included a null-model. We excluded small chicks ($n = 7$, wing < 100 mm, body mass < 400 g) because they have not long experienced their rearing conditions and their crop mass would introduce too much noise. We included only chicks for which all variables were known (260 chicks, 4–34 per year from 2–18 nests, including 46 nests with one chick and 107 nests with two chicks sexed) and compared these models based on AICc as described above. The additive models had VIF < 2.

A full crop can be up to 75–80 gram in large chicks (Bijlsma 1997b) but was not recorded in all nests. A subset of chicks in the Veluwe and Drenthe where crop size was scored by RGB (on a three-point scale; Bijlsma 1997b) was analysed with a random effect of nest ID, to evaluate the possible confounding effect of crop size on body mass. Although relative body mass increased with crop score (LMM: $\beta = 0.064 \pm$ SE 0.017, $F_{1,39.2} = 14.108$, $P < 0.001$), this effect did not differ between the sexes (LMM: interaction crop × sex, $\beta = 0.005 \pm 0.025$, $F_{1,33.3} = 0.208$, $P = 0.836$). Crop score did not differ between first- and second-hatched chicks (LMM: $\beta_{\text{first-second}} = 0.035 \pm 0.072$, $F_{1,24.4} = 0.229$, $P = 0.636$) and did not vary with relative laying date (LMM: $\beta = -0.047 \pm 0.028$, $F_{1,33.2} = 2.863$, $P = 0.100$).

All statistical tests were performed in R (R Core Team 2017), using the package 'lme4' (Bates *et al.* 2015) and the package 'lmerTest' to calculate *P*-values and degrees of freedom for LMM with Satterthwaite's method (Kuznetsova *et al.* 2017).

RESULTS

Honey Buzzard laying date

Laying dates were similar in the different study areas. For the years 1996–2014, the laying date did not differ between nests monitored by RGB in Veluwe and Drenthe on the one hand ($n = 61$, laying date = 146.4 ± 4.85 (±SD), i.e. 26 May, range: 17 May – 11 June) and nests in the rest of our study area on the other hand ($n = 169$, 147.2 ± 7.09, i.e. 27 May, range 11 May – 25 June; Two-way ANOVA, $F_{1,210} = 0.788$, $P = 0.376$; see also Figure 3A) while correcting for significant between-year variation ($F_{18,210} = 1.904$, $P = 0.017$). Laying dates were also independent of the sexing success, as differences were only minor compared to the overall range of laying dates. In dataset A, the laying date differed near-significantly between nests in which all (113 nests with two chicks and 3 nests with one chick, laying date = 146.2 ± 7.05 (±SD), i.e. 26 May), one out of two ($n = 49$, laying date = 147.5 ± 6.08), none ($n = 23$, laying date = 149.7 ± 8.0) or one out of an unknown number ($n = 19$, laying date = 148.7 ± 4.04) of the eggs were successfully sexed at the chick stage (Two-way ANOVA, $F_{3,185} = 2.574$, $P = 0.055$; no significant pairwise differences), while correcting for significant variation between years ($F_{18,185} = 2.051$, $P = 0.009$).

The laying date of Honey Buzzards (dataset B, 1974–2014) was best explained by spring temperature alone (ΔAICc = 0.93, the simplest among six models with ΔAICc < 2, Table S2): Honey Buzzards laid earlier when spring was warmer (LMM: $\beta = -2.607 \pm 0.435$ (±SE) day per °C, $F_{1,36.1} = 35.868$, $P < 0.001$; Figure 2B). This is in line with the c. 9 days advance in laying date of Honey Buzzards over the last 40 years. Also corrected for the additive effects of spring temperature, precipitation and area, Honey Buzzards had advanced egg laying c. 7.5 days in 40 years (model: Year + T5 + P5 + Area, ΔAICc = 0.00, LMM: $\beta = -0.188 \pm 0.059$ (±SE), $F_{1,59.1} = 10.030$, $P = 0.001$), while the spring temperature effect was still similar ($\beta = -1.870 \pm 0.393$, $F_{1,32.0} = 22.648$, $P < 0.001$), spring precipitation had a delaying effect on laying date ($\beta = 0.071 \pm 0.021$, $F_{1,29.7} = 11.553$, $P = 0.002$; Figure 2C) and laying date did not differ between areas ($\beta_{\text{Veluwe-Drenthe}} = -0.316 \pm 1.122$, $F_{1,204.9} = 0.079$, $P = 0.779$). However, this more complex model did not perform substantially better than spring temperature alone.

Wasp abundance showed large annual variation (Figure 2A), which was best explained by spring temperature and spring precipitation. Both these one-factor models were regarded as best models, so we describe these effects based on their additive combination, the model that was ranked highest (Table S3). Wasps were more abundant in years with a higher spring temperature (LMM using log-transformed wasp abundance index: $\beta = 0.241 \pm 0.119$ (±SE) per °C, $F_{1,38} = 4.145$, $P = 0.049$; Figure 2B) and with less spring precipitation ($\beta = -0.013 \pm 0.007$ per mm, $F_{1,38} = 3.667$, $P = 0.063$, Figure 2C). Wasp abundance tended to show a decrease over the years (LMM, ΔAICc = 0.12; $\beta = -0.029 \pm 0.015$, $F_{1,38} = 3.549$, $P = 0.067$), when corrected for the effect of spring temperature ($\beta = 0.356 \pm 0.125$, $F_{1,38} = 8.162$, $P = 0.007$).

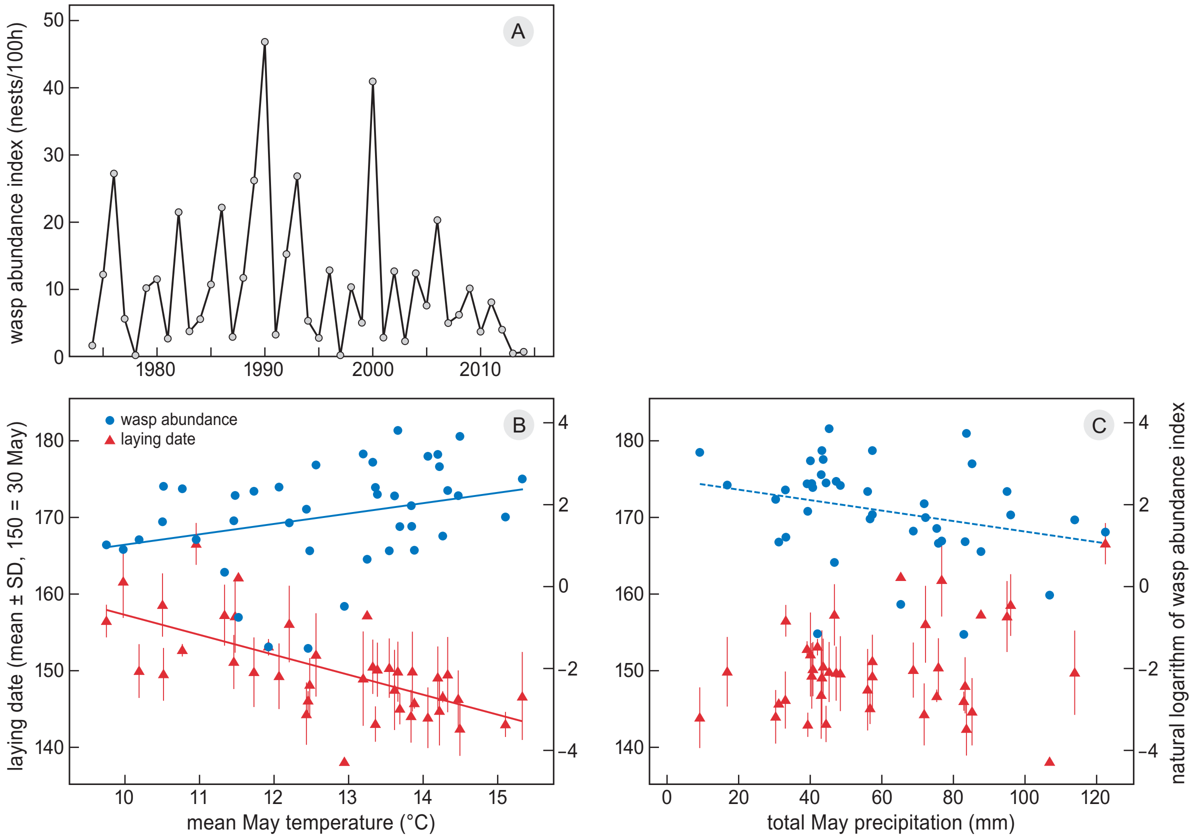

Figure 2. Honey Buzzard phenology and wasp abundance in relation to spring weather, in Veluwe and Drenthe, The Netherlands, in the years 1974–2014. (A) Annual variation in summer wasp abundance (number of nests found per 100 field hours). (B) When May was warmer, Honey Buzzards laid eggs earlier and wasps were more abundant. (C) When May was more rainy, wasps tended to be less abundant. In B and C, the regressions for wasp abundance were based on a linear model (including both spring temperature and precipitation) and that for laying date on a linear mixed model including spring temperature and a random intercept of year (see Results: Honey Buzzard laying date).

Nestling sex

In the whole dataset A (311 chicks), 50.8% of offspring were female, not deviating from parity (Exact Binomial Test: $P = 0.821$), while in the restricted dataset of chicks for which all environmental variables were known, 48.9% were female (not deviating from parity, 272 chicks, $P = 0.762$). Also, no overall sex bias was found in all broods of only one live chick (54.5% female, 44 chicks, $P = 0.652$), all completely sexed broods of two chicks (47.8% female, 232 chicks, $P = 0.555$), all first-hatched chicks (49.0% female, 147 chicks, $P = 0.869$) nor in all second-hatched chicks (50.4% female, 129 chicks, $P = 1.0$).

The sex of a nestling was most strongly associated with the annual mean laying date (Table 1): in years when Honey Buzzards on average laid eggs earlier, the proportion of females was higher (GLM: $\beta = -0.133 \pm 0.051$ (±SE), 95% CI: –0.235 to –0.034, $z = -2.599$, $P = 0.009$), showing a 32% change in sex ratio over a 10 day range in annual mean laying date (Figure 3B). No other model performed equally well (all $\Delta AICc > 2$). Within years, the relative laying date did not predict nestling sex ($\Delta AICc = 6.36$), and the effects of annual mean and within-year relative laying date were significantly different (GLM: $\beta_{mean}-\beta_{relative} = -0.119 \pm 0.056$ (±SE), $z = -2.114$, $P = 0.035$). The effect of mean laying date on nestling sex was similar when using annual means (including only 13 years with ≥10 sexed chicks per year; LM: $\beta = -0.0392 \pm 0.0135$ (±SE), $z = -2.908$, $P = 0.014$).

The overall frequencies of brood compositions of complete broods (all years combined) did not differ from those expected based on random sex allocation (24 FF broods, 31 FM, 32 MF and 29 MM; χ^2 test for given probabilities: $\chi^2_3 = 1.3103$, $P = 0.727$) and mixed broods occurred over the whole range of annual mean laying dates (Figure S2). The sex of single chicks on nests where a sibling had died (14 chicks) or where clutch size was unknown (24 chicks) did not vary with the annual mean laying date (ANOVA: $F_{1,36} = 0.707$, $P = 0.406$).

Nestling body mass

The expected body mass of nestlings, given their wing length, was described as follows, for males: body mass = 45.07 + 4.360 × wing – 0.0056 × wing^2, and for females: body mass = –201.8 + 7.017 × wing – 0.0110 × wing^2 (Figure 4A). Female body mass seemed more variable (at a given wing length) and females appeared to reach their asymptotic body mass at shorter wing lengths than males, even though the asymptotic mass is higher in females than in males (Figure 4A).

Variation in the relative mass of chicks (i.e. mass/expected mass given its wing length) was best explained by interacting effects of chick order and within-year relative laying date (Table 2). The relative body mass of second-hatched chicks was lower than that of first-hatched chicks (LMM: $\beta = -0.0351 \pm 0.0081$ (±SE), $F_{1,118.8} = 18.966$, $P < 0.001$) and decreased more steeply with relative laying date (0.0086 per day) than

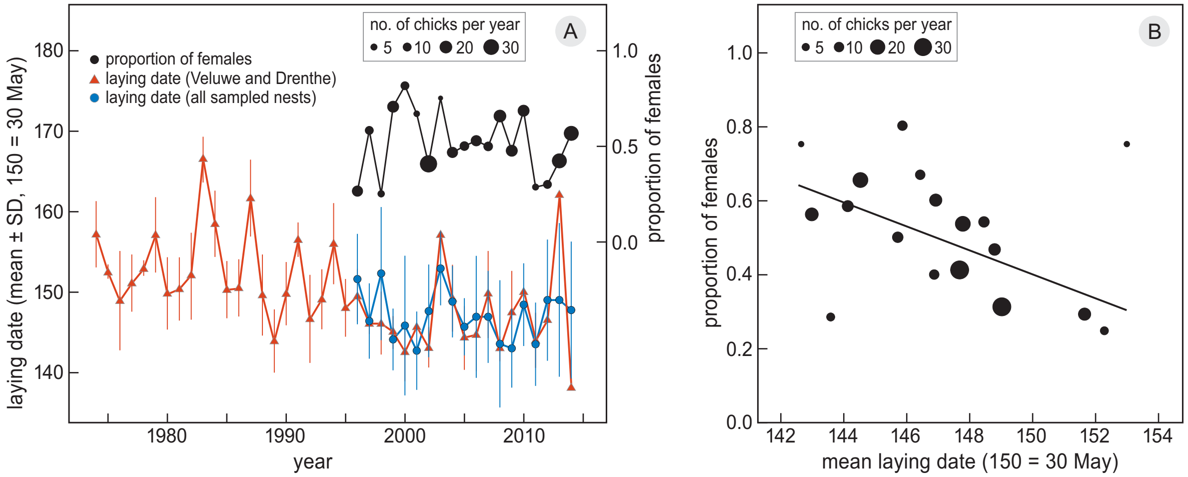

Figure 3. Relation between Honey Buzzard brood sex ratio and annual phenology. (A) In the Veluwe and Drenthe, The Netherlands, the laying date advanced during 1974–2014 by 0.19 day/year (red triangles, dataset B), while the laying dates did not differ significantly from the set of nests sampled for sexing during 1996–2014 (blue dots, dataset A). There was no brood sex ratio trend over the years (black dots, where dot area corresponds to sample size). (B) Annual mean laying date explained nestling sex best: more females were produced in years when Honey Buzzards on average laid eggs earlier. The line depicts a binomial model (GLM) using individual chicks, but annual means in sex ratio are shown for clarity.

that of first-hatched chicks (0.0033 per day; interaction LR×Order: $\beta = -0.0053 \pm 0.0013$, $F_{1,117.2} = 16.794$, $P < 0.001$, Figure 4B). Although a direct effect of food abundance on chick body mass was expected, the model with wasp abundance did not perform well in explaining relative body mass (ΔAICc = 9.25, LMM: $\beta = -0.0274 \pm 0.0078$, $F_{1,10.6} = 12.327$, $P = 0.005$; Figure S3). We found no support for a difference between the sexes in environmental effects on body mass (models including an interaction with sex: all AICc > 24.74).

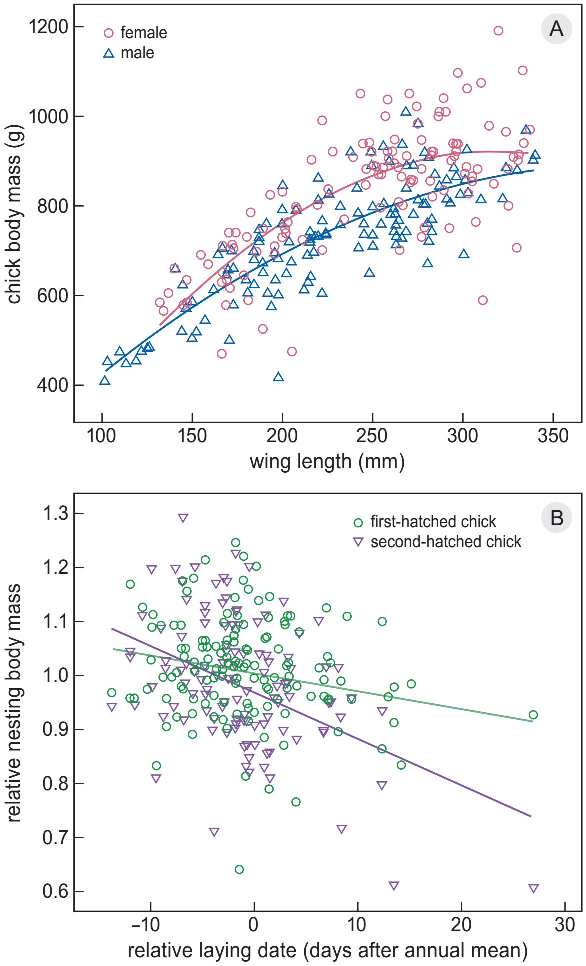

Figure 4. Body mass of Honey Buzzard nestlings in relation to their size and the environment. (A) The expected body mass of a chick, based on its wing length, was calculated for males and females separately using second order polynomials, including random intercepts of year and brood. (B) Relative chick body mass (calculated as: mass/expected mass) was best explained by interacting effects of within-year relative laying date (days relative to the annual mean) and chick order: chicks that hatched later within the season had a lower body mass and this effect was stronger for second- than first-hatched chicks.

Nestling sex was best predicted by annual mean laying date (Table 1), but we did not find this relationship for the relative mass of nestlings (Table 2; ΔAICc = 20.34).

DISCUSSION

As expected based on the small reversed sexual size dimorphism in Honey Buzzards, we did not find a bias in overall nestling sex ratio, nor a relation of nestling sex with environmental variables. However, we did find a strong relationship between nestling sex and the annual mean laying date: in years when Honey Buzzards on average laid eggs early, relatively more females were produced. We regard it likely that this sex ratio was already present in eggs and did not result from sex-specific chick mortality. First, a higher mortality of females in late years is conceivable (starvation of the

Table 1. Summary of performance of candidate models (ranked according to AICc) explaining nestling sex in Honey Buzzards. Models are binomial GLMs on individual nestlings. LM: annual mean laying date, LR: relative laying date, LA: absolute laying date, Order: chick hatching sequence (first or second), T5: spring temperature (May), P5: spring precipitation (May), T678: summer temperature (Jun–Aug), P678: summer precipitation, W: natural logarithm of wasp abundance index. In cases we report the interaction, also the main effects were included. Number of parameters in the model is given by *K*.

Model	*K*	ΔAICc	AICc weight
LM	2	0.00	0.49
Order × LM	4	3.81	0.07
T5	2	3.83	0.07
LA	2	4.20	0.06
T5 + P5	3	4.53	0.05
T678	2	4.61	0.05
1	1	4.97	0.04
P5	2	5.85	0.03
LR	2	6.36	0.02
P678	2	6.58	0.02
T678 + P678	3	6.61	0.02
W	2	6.94	0.02
Order	2	6.95	0.02
Order × T5	4	7.54	0.01
Order × LA	4	8.19	0.01
Order × T678	4	8.53	0.01
Order × P678	4	8.56	0.01
Order × W	4	9.54	0.00
Order × P5	4	9.85	0.00
Order × T678 + Order × P678	6	9.93	0.00
Order × LR	4	10.17	0.00
Order × T5 + Order × P5	6	10.31	0.00

larger sex), but a higher mortality of males in early years is not (the smaller sex is expected to survive in food-rich years). Second, if within-brood competition would lead to sex-specific mortality, mixed-sex broods would be particularly underrepresented, but this was not the case. Third, sibling aggression in Honey Buzzards is weak (Wendland 1935, Ziesemer *et al.* 2021), chick mortality is generally modest (Bijlsma 1993, Van Manen *et al.* 2011, Roberts & Law 2014), and nest predation – a major cause of mortality among Honey Buzzard chicks – usually results in total, not partial, brood loss and is unlikely to be sex-specific (see previous references and Bijlsma 2004, van Diermen *et al.* 2014, Bijlsma 2018).

Table 2. Summary of performance of candidate models (ranked according to AICc) explaining relative body mass of Honey Buzzard nestlings. Models are linear mixed models (including random intercepts of year and brood) on individual nestlings, with relative body mass being the relative deviation from the expected body mass of a chick based on its wing length (see curves in Figure 4A). LM: annual mean laying date, LR: relative laying date, LA: absolute laying date, Sex: male or female, Order: chick hatching sequence (first or second), T5: spring temperature (May), P5: spring precipitation (May), T678: summer temperature (Jun–Aug), P678: summer precipitation, W: natural logarithm of wasp abundance index. In cases we report the interaction, also the main effects were included. Number of parameters in the model is given by *K*.

Model	*K*	ΔAICc	AICc weight
Order × LR	7	0.00	0.73
LR	5	3.52	0.13
LA	5	4.81	0.07
Order × LA	7	5.03	0.06
W	5	9.25	0.01
1	4	9.39	0.01
Order	5	9.64	0.01
T678	5	16.03	0.00
Order × W	7	17.33	0.00
S	5	18.75	0.00
T5	5	18.91	0.00
LM	5	20.34	0.00
P5	5	23.72	0.00
S × W	7	24.74	0.00
Order × T678	7	24.90	0.00
S × LR	7	25.81	0.00
P678	5	26.21	0.00
S × Order	7	26.67	0.00
S × LA	7	27.22	0.00
Order × T5	7	29.02	0.00
Order × LM	7	32.19	0.00
T678 + P678	6	32.49	0.00
S × T678	7	33.25	0.00
T5 + P5	6	33.27	0.00
S × T5	7	35.97	0.00
Order × P5	7	40.15	0.00
S × LM	7	40.84	0.00
Order × P678	7	43.91	0.00
S × P5	7	46.65	0.00
S × P678	7	52.50	0.00
Order × T678 + Order × P678	9	58.77	0.00
Order × T5 + Order × P5	9	59.56	0.00
S × T5 + S × P5	9	63.86	0.00
S × T678 + S × P678	9	66.78	0.00

In other bird species, brood sex ratio has also been found to be related to the timing of breeding, but that concerned patterns in laying date on the individual level, namely absolute laying date (i.e. date as such; Dijkstra *et al.* 1990, Olsen & Cockburn 1991, Zijlstra *et al.* 1992, Bijlsma 1993, Daan *et al.* 1996, Byholm *et al.* 2002b, Brommer *et al.* 2003, Chakarov *et al.* 2015) or relative laying date (i.e. within-year variation relative to the year's mean; e.g. Arroyo 2002, Bowers *et al.* 2015). In contrast, for Honey Buzzards, we showed that sex ratio was related to laying date on the annual level (i.e. population-wide timing), and not the individual level. As the sample size was small in many years, we combined all years to test for an effect of individual timing (i.e. relative laying date within a year) on nestling sex, assuming that this effect was similar in all years. However, we could not reliably test whether the effect of relative laying date on nestling sex differed between early and late years.

Whereas the absolute and relative laying date reflect the timing of individual pairs, the annual mean laying date reflects the timing of the whole population in that year, which depends on the general circumstances of the year. The general circumstances that the mean laying date reflects are probably to a large extent related to spring conditions, as laying date variation was explained by spring temperature. Spring weather – temperature and precipitation – was an important predictor of wasp abundance (see also Nadolski 2013, Lester *et al.* 2017). If the sex ratio biases in Honey Buzzard broods are adaptive, they suggest that female nestlings, which are overrepresented in years when Honey Buzzards lay eggs early, might benefit more from growing up in wasp-rich years than males do. However, the brood sex ratio was not directly related to wasp abundance itself. This has two possible explanations. First, Honey Buzzards have incomplete information about the upcoming wasp abundance at the time of egg-laying, and could only rely on cues predicting the wasp abundance. This incomplete information may lead to suboptimal decisions if summer conditions affect the wasp abundance to a large extent. However, we did not find important effects of summer weather on wasp abundance (see also Nadolski 2013, Lester *et al.* 2017). Second, we did not measure wasp abundance for each nest separately, but in two large areas in central and northern Netherlands. Thus, we could not test for a fine-scale relationship of laying date and sex ratio with wasp abundance; however, we regard this to be an unlikely explanation, as wasp abundance was correlated between areas and with other wasp abundance measures in other locations. More importantly, the home ranges of Honey Buzzards are large (tens of km^2) and overlap between pairs, although they are largely exclusive between partners (van Manen *et al.* 2011, van Diermen *et al.* 2015). Lastly, if fine-scale processes were important in determining brood sex ratio, we would expect to find a relationship of sex ratio with laying date on the individual level (i.e. absolute or relative laying date), instead of with population-wide annual mean laying date.

The fact that nestling sex was not explained best by spring weather itself, could indicate that Honey Buzzards may use cues, other than spring temperature or precipitation, which we have not included in our analysis but do predict wasp abundance. These cues may already be present during spring stopover in Europe, as Honey Buzzard laying dates are correlated with arrival dates, in particular of the female (with ten days between arrival and laying; fifteen days for males), which in turn depend partly on stopover duration in Europe (as derived from data obtained via Honey Buzzards equipped with a GPS-datalogger; van Manen *et al.* 2011, W. van Manen pers. comm.). However, again, if individual stopover duration influenced brood sex ratio, the relative laying date (rather than the annual mean laying date) should explain sex ratio variation.

In the case of Honey Buzzards, the potential benefit for female nestlings (compared to males) in years when egg laying occurs early, with an abundance of wasp colonies, after warm, dry springs, is yet unknown. We did not find a larger benefit for female nestlings in terms of relative body mass. Relative body mass was best explained by relative laying date and chick hatching order (see also van Manen *et al.* 2011, van Diermen *et al.* 2014, 2015). This relationship of nestling body mass with the timing of individual nests suggests that nests do differ in their environmental conditions or parental performance. This contrasts with the sex ratio which did not show a relationship with individual timing, but only with population timing.

The potential benefit for female nestlings may be in a component during growth that we have not measured (e.g. hormone or immune status; Frauendorf *et al.* 2021), or may occur only after fledging. Perhaps females may benefit more than males in terms of post-fledging survival, if the survival of females is more influenced by environmental conditions than the survival of males. In some raptors and owls, post-fledging survival has been found to differ between the sexes (Kenward *et al.* 1999, Wiens *et al.* 2006), whereas other studies did not find a sexual difference (McIntyre *et al.* 2004, Sunde 2005). In general, post-fledging survival was higher when food was more abundant and when chicks had a higher body mass (Todd *et al.* 2003, Wiens *et al.* 2006, Naef-Daenzer & Grüebler 2016), but whether such effects are stronger in females than males seems yet unexplored. Alternatively, females may benefit more than males if their future breeding performance is more strongly influenced by environmental conditions during growth than that of males. In Tawny Owls *Strix aluco*, food-rich territories produced relatively more female than male chicks, and growing up under food-rich conditions also increased the future reproductive output of females more than that of males (Appleby *et al.* 1997). The mechanism for a potential breeding benefit – if any – for female Honey Buzzard nestlings is unclear. In Honey Buzzards, a benefit of growing up in wasp-rich years would have to carry over multiple years, as juveniles stay in Africa for at least their first summer (Strandberg *et al.* 2012) and do not start breeding before their third year (Bijlsma 2014, Roberts & Law 2014, Riem Vis *et al.* 2019). Perhaps this time period until first breeding is shortened more in females than in males, when growing up in food-rich years. Such a differential benefit explained the adaptive value of seasonal sex ratio biases in Eurasian Kestrels, but in Eurasian Kestrels this benefit occurred over a short time span, namely in the probability to start breeding as a yearling (Dijkstra *et al.* 1990). For Honey Buzzards, the interval between fledging and first breeding is several years longer, during which much can happen that is not related to conditions at fledging.

The overall sex ratio among nestlings was found to be balanced, at least during our study period of 1996–2014, when earlier breeding following warmer springs had become the norm. In previous decades, notably the 1970s and 1980s, laying was decidedly later. Given the female bias among nestlings in the earliest breeding seasons within the period of 1996– 2014, it makes one wonder whether the brood sex ratio was more male-biased in the previous tens of years when laying started much later. Our study into brood sex ratio started just too late to cover the step-change in timing of breeding of Honey Buzzards. It shows that a study can never be long enough.

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REFERENCES

Akaike H. 1973. Information theory and an extension of the maximum likelihood principle. In: Petrov B.N. & Csáki F. (eds) Proceedings of the 2nd International Symposium on Information Theory, Budapest, 1972, pp. 267–281.

Anderson D.J., Reeve J., Gomez J.E.M., Weathers W.W., Hutson S., Cunningham H.V. & Bird D.M. 1993a. Sexual size dimorphism and food requirements of nesting birds. Can. J. Zool. 71: 2541–2545.

Anderson D.J., Budde C., Apanius V., Gomez J.E.M., Bird D.M. & Weathers W.W. 1993b. Prey size influences female competitive dominance in nestling American kestrels (*Falco sparverius*). Ecology 74: 367–376.

Appleby B.M., Petty S.J., Blakey J.K., Rainey P. & MacDonald D.W. 1997. Does variation of sex ratio enhance reproductive success of offspring in Tawny Owls (*Strix aluco*)? Proc. R. Soc. Lond. B. 264: 1111–1116.

Arroyo B.E. 2002. Fledgling sex ratio variation and future reproduction probability in Montagu's Harrier, *Circus pygargus*. Behav. Ecol. Sociobiol. 52: 109–116.

Bates D., Mächler M., Bolker B.M. & Walker S.C. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67: 1–48. doi:10.18637/jss.v067.i01

Bednarz J.C. & Hayden T.J. 1991. Skewed brood sex ratio and sex-biased hatching sequence in Harris's Hawks. Am. Nat. 137: 116–132.

Berkvens M. & van Diermen J. 2021. Ground nests of wasps: what defines the chance of predation by Honey Buzzards *Pernis apivorus*? Natuur.oriolus 87: 1–17 (in Dutch with English summary)

Bijlsma R.G. 1986. Occurrence and breeding biology of the Honey Buzzard *Pernis apivorus* on the SW-Veluwe and in the SE-Achterhoek. Limosa 59: 61–66 (in Dutch with English summary)

Bijlsma R.G. 1993. Ecologische atlas van de Nederlandse roofvogels. Schuyt & Co., Haarlem.

Bijlsma R.G. 1997a. Honey Buzzard. In: Hagemeijer W.J.M. & Blair M.J. (eds) The EBCC atlas of European breeding birds. Their distribution and abundance. T & AD Poyser, London.

Bijlsma R.G. 1997b. Handleiding veldonderzoek Roofvogels. KNNV Uitgeverij, Utrecht.

Bijlsma R.G. 2004. What is the predation risk for European Honey-buzzards *Pernis apivorus* in Dutch forests inhabited by food-stressed Northern Goshawks *Accipiter gentilis*? Takkeling 12: 185–197 (in Dutch with English summary)

Bijlsma R.G. 2014. From cradle to grave: natal dispersal and premature death of a female Honey Buzzard *Pernis apivorus*. Takkeling 22: 200–207 (in Dutch with English summary)

Bijlsma R.G. 2018. Trends and breeding performance of raptors in The Netherlands in 2017. Takkeling 26: 5–47 (in Dutch with English summary)

Bijlsma R.G., van Manen M. & Ottens H.J. 1997. Growth of starving Honey Buzzards *Pernis apivorus*. Takkeling 5(3): 20–30 (in Dutch with English summary)

Bijlsma R.G., Vermeulen M., Hemerik L. & Klok C. 2012. Demography of European Honey Buzzards *Pernis apivorus*. Ardea 100: 163–177.

Bowers E.K., Thompson C.F. & Sakaluk S.K. 2015. Persistent sex-by-environment effects on offspring fitness and sex-ratio adjustment in a wild bird population. J. Anim. Ecol. 84: 473–486.

Brommer J.E., Karell P., Pihlaja T., Painter J.N., Primmer C.R. & Pietiäinen H. 2003. Ural owl sex allocation and parental investment under poor food conditions. Oecologia 137: 140–147.

Byholm P., Ranta E., Kaitala V., Lindén H., Saurola P. & Wikman M. 2002a. Resource availability and goshawk offspring sex ratio variation: a large-scale ecological phenomenon. J. Anim. Ecol. 71: 994–1001.

Byholm P., Brommer J.E. & Saurola P. 2002b. Scale and seasonal sex-ratio trends in northern goshawk *Accipiter gentilis* broods. J. Avian Biol. 33: 399–406.

Chakarov N., Pauli M., Mueller A-K., Potiek A., Grünkorn T., Dijkstra C. & Krüger O. 2015. Territory quality and plumage morph predict offspring sex ratio variation in a raptor. PLoS One 10: e0138295.

Clutton-Brock T.H. 1986. Sex ratio variation in birds. Ibis 128: 317–329.

Cockburn A., Legge S. & Double M.C. 2002. Sex ratios in birds and mammals: can the hypotheses be disentangled? In: Hrdy I.C.W. Sex ratios. Concepts and research methods: 266–286. Cambridge University Press, New York.

Conover D.O. & Van Voorhees D.A. 1990. Evolution of a balanced sex ratio by frequency-dependent selection in a fish. Science 250: 1556–1558.

Cramp S. & Simmons K.E.L. (eds) 1980. The birds of the Western Palearctic, Vol. II. Oxford University Press, Oxford.

Daan S., Dijkstra C. & Weissing F.J. 1996. An evolutionary explanation for seasonal trends in avian sex ratios. Behav. Ecol. 7: 426–430.

Dijkstra C., Daan S. & Buker J.B. 1990. Adaptive seasonal variation in the sex ratio of Kestrel broods. Funct. Ecol. 4: 143–147.

Dijkstra C., Daan S. & Pen I. 1998. Fledging sex ratios in relation to brood size in size-dimorphic altricial birds. Behav. Ecol. 9: 287–296.

Dunning Jr. J.B. 2007. CRC Handbook of avian body masses, second edition. CRC Press, USA.

Ellegren H., Gustafsson L. & Sheldon B.C. 1996. Sex ratio adjustment in relation to paternal attractiveness in a wild bird population. PNAS 93: 11723–11728.

Frauendorf M., Allen A.M., Verhulst S., Jongejans E., Ens B.J., van der Kolk H.J., de Kroon H., Nienhuis J. & van de Pol M. 2021. Conceptualizing and quantifying body condition using structural equation modelling: A user guide. J. Anim. Ecol. 90: 2478–2496.

Green A.J. 2001. Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82: 1473–1483.

Harmsen M. & Bijlsma R.G. 2014. Honey Buzzard *Pernis apivorus* depredates nest of *Vespa crabro*, or: social wasps in the diet of Honey buzzards in The Netherlands. Takkeling 22: 100–106 (in Dutch with English summary)

Kahn N.W., St. John J. & Quinn T. 1998. Chromosome-specific intron size differences in the avian CHD gene provide an efficient method for sex identification in birds. Auk 115: 1074–1078.

Kenward R.E., Marcström V. & Karlbom M. 1999. Demographic estimates from radio-tagging: models of age-specific survival and breeding in the goshawk. J. Anim. Ecol. 68: 1020–1033.

Komdeur J. 1996. Facultative sex ratio bias in the offspring of Seychelles warblers. Proc. Royal Soc. B. 263: 661–666.

Krijgsveld K.L., Daan S., Dijkstra C. & Visser G.H. 1998. Energy requirements for growth in relation to sexual size dimorphism in marsh harrier *Circus aeruginosus* nestlings. Physiol. Zool. 71: 693–702.

Kuznetsova A., Brockhoff P.B. & Christensen R.H.B. 2017. lmerTest package: Tests in linear mixed effects models. J. Stat. Softw. 82: 1–26. doi:10.18637/jss.v082.i13

Lester P.J., Haywood J., Archer M.E. & Shortall C.R. 2017. The long-term population dynamics of common wasps in their native and invaded range. J. Anim. Ecol. 86: 337–347.

Magrath M.J.L., van Lieshout E., Pen I., Visser G.H. & Komdeur J. 2007. Estimating expenditure on male and female offspring in a sexually size-dimorphic bird: a comparison of different methods. J. Anim. Ecol. 76: 1169–1180.

McDonald P.G., Olsen P.D. & Cockburn A. 2005. Sex allocation and nestling survival in a dimorphic raptor: does size matter? Behav. Ecol. 16: 922–930.

McIntyre C.L. 2004. Golden Eagles in Denali National Park and Preserve: Productivity and survival in relation to landscape characteristics of nesting territories. PhD thesis, Oregon State University, USA.

Nadolski J. 2013. Phenology of European Hornet, *Vespa crabro* L. and Saxon Wasps, *Dolichovespula saxonica* Fabr. (Hymenoptera: Vespidae) and the influence of the weather on the reproductive success of wasps societies in urban conditions. Sociobiol. 60: 477–483.

Naef-Daenzer B. & Grüebler M.U. 2016. Post-fledging survival of altricial birds: Ecological determinants and adaptation. J. Field Ornithol. 87: 227–250.

Olsen P.D. & Cockburn A. 1991. Female-biased sex allocation in peregrine falcons and other raptors. Behav. Ecol. Sociobiol. 28: 417–423.

Penteriani V. *et al.* & Talavera O.M. 2010. Sex allocation from an owl perspective: clutch order could determine brood sex to reduce sibling aggression in the Eagle Owl *Bubo bubo*. Ornis Fennica 87: 135–143.

R Core Team 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org

Richardson D.S., Jury F.L., Blaakmeer K., Komdeur J. & Burke T. 2001. Parentage assignment and extra-group paternity in a cooperative breeder: the Seychelles warbler (*Acrocephalus sechellensis*). Mol. Ecol. 10: 2263–2273.

Riem Vis R., van Bergen V.S. & Brinkgreve J. 2019. Summer nests of Honey Buzzards *Pernis apivorus* and their subsequent use, with notes on breeding and natal dispersal. Takkeling 27: 100–116 (in Dutch with English summary)

Roberts S.J. & Law C. 2014. Honey-buzzards in Britain. British Birds 107: 668–691.

Sheldon B.C., Andersson S., Griffith S.C., Örnborg J. & Sendecka J. 1999. Ultraviolet colour variation influences blue tit sex ratios. Nature 402: 874–877.

Strandberg R., Hake M., Klaassen R.H.G. & Alerstam T. 2012. Movements of immature European Honey Buzzards *Pernis apivorus* in tropical Africa. Ardea 100: 157–162.

Sunde P. 2005. Predators control post-fledging mortality in Tawny Owls, *Strix aluco*. Oikos 110: 461–472.

Todd L.D., Poulin R.G., Wellicome T.I. & Brigham R.M. 2003. Post-fledging survival of burrowing owls in Saskatchewan. J. Wildl. Manage. 67: 512–519.

Trivers R.L. & Willard D.E. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179: 90–92.

van de Pol M., & Wright J. 2009. A simple method for distinguishing within- versus between-subject effects using mixed models. Anim. Behav. 77: 753–758.

van Diermen J., van Rijn S. & van Manen W. 2014. Wespendief in Kempen-Broek & Het Groene Woud, Jaarbericht 2014. ARK-Natuurontwikkeling, Laag-Keppel.

van Diermen J., van Manen W. & van Rijn S. 2015. Wespendief in Het Groene Woud en Kempen-Broek, onderzoek 2013–15. ARK Natuurontwikkeling, Nijmegen.

van Manen W. 2013. Biology of Honey Buzzards *Pernis apivorus* in the primaeval forest of Białowieża, Poland. Takkeling 21: 101–126 (in Dutch with English summary)

van Manen W., van Diermen J., van Rijn S. & van Geneijgen P. 2011. Ecology of Honey Buzzard in the Veluwe Natura 2000 site (central NL) during 2008–2010, population level, breeding biology, habitat use and food. Natura 2000 report Province of Gelderland, Arnhem/Treetop Foundation, Assen. (in Dutch with English summary)

Vedder O., Dekker A.L., Visser G.H. & Dijkstra C. 2005a. Sex-specific energy requirements in nestlings of an extremely sexually size dimorphic bird, the European sparrowhawk (*Accipiter nisus*). Behav. Ecol. Sociobiol. 58: 429–436.

Vedder O., Dijkstra C., Dekker A.L., Waasdorp S. & Visser G.H. 2005b. Sex-specific nestling body mass in relation to brood sex composition in the Eurasian Sparrowhawk *Accipiter nisus*. Ardea 93: 179–187.

Wendland V. 1935. Der Wespenbussard (*Pernis apivorus* L.). J. Ornithol. 83: 88–104.

Wiens J.D., Noon B.R. & Reynolds R.T. 2006. Post-fledging survival of northern goshawks: the importance of prey abundance, weather, and dispersal. Ecol. Applic. 16: 406–418.

Ziesemer F., Schlüter M. & Grünkorn T. 2021. Video-Beobachtungen an Horsten des Wespenbussards *Pernis apivorus* in Schleswig-Holstein. Corax 24: 369–380.

Zijlstra M., Daan S. & Bruinenberg-Rinsma J. 1992. Seasonal variation in the sex ratio of Marsh Harrier *Circus aeruginosus* broods. Funct. Ecol. 6: 553–559.

SAMENVATTING

Bij vogelsoorten waarvan de twee geslachten in lichaamsgrootte verschillen, zullen de kosten van het grootbrengen van dochters en zonen verschillend zijn. Vanuit evolutionair oogpunt bekeken, is het voordelig als de vogels kunnen beïnvloeden of ze zonen of dochters zullen voortbrengen. Bij roofvogels zijn de vrouwtjes groter dan de mannetjes. In een aantal gevallen is, zoals verwacht, gevonden dat in voedselarme jaren of territoria er relatief meer zonen dan dochters worden geproduceerd. Bij Wespendieven *Pernis apivorus* verschillen de geslachten maar weinig (6%) in gewicht. Wij verwachtten daarom dat er geen invloed is van omgevingsfactoren op de geslachtsverhouding van de jongen. In 1996–2014 hebben wij in Nederland van 311 jongen uit 195 broedsels uit Nederland en omgeving genetisch het geslacht bepaald en onderzocht welke omgevingsfactoren de geslachtsverhouding het beste konden verklaren. De totale geslachtsverhouding was niet scheef (50,8% vrouwtjes). In jaren waarin de vogels hun eieren vroeger in het seizoen legden, werden er echter meer dochters geboren (32% verandering in geslachtsverhouding over een bereik van tien dagen van de jaarlijkse gemiddelde legdatum). De relatieve legdatum binnen een jaar, de uitkomstvolgorde, het voorkomen van wespen (hoofdvoedsel) en het weer ’s zomers waren niet van invloed op de variatie in de geslachtsverhouding. Op de Veluwe en in Drenthe (1974–2014) legden de Wespendieven hun eieren vroeger in het seizoen als de maand mei warmer was. Dit resulteerde in een algehele vervroeging van de legdatum van ongeveer negen dagen in 40 jaar (parallel met stijging temperatuur in mei). Omdat een warme (en droge) meimaand doorgaans betekent dat er in dat jaar meer wespennesten zijn, verwachtten we dat dochters meer van wespenrijke jaren zouden profiteren dan zonen. Dat was echter niet terug te zien in het lichaamsgewicht, dat vooral afhing van de relatieve legdatum (negatief effect binnen een jaar) en de volgorde van het uitkomen (eerste jong is zwaarder dan het tweede). Het eventuele voordeel voor vrouwtjes (ten opzichte van mannetjes) om op te groeien in jaren met een warme meimaand, een vroege gemiddelde eilegdatum en veel wespen is vooralsnog onbekend.

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SUPPLEMENTARY MATERIAL

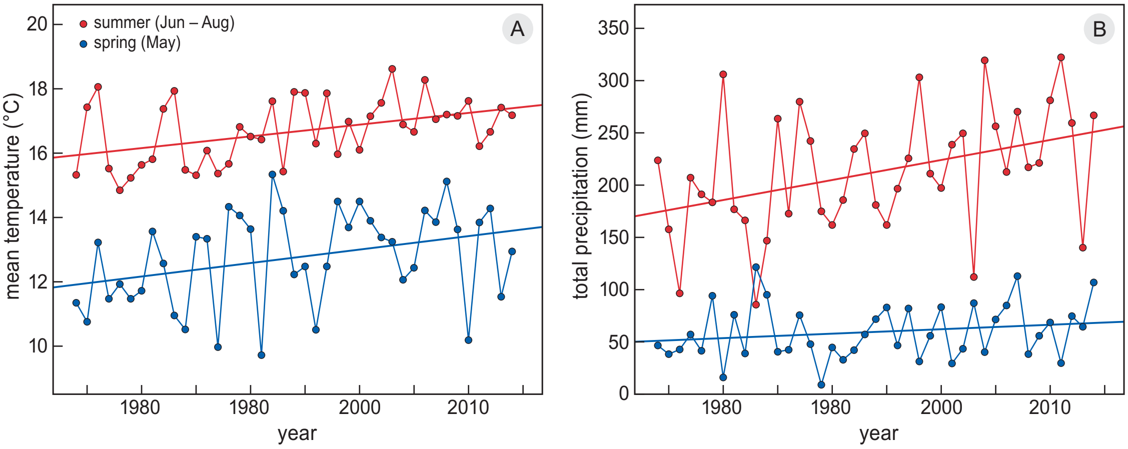

Figure S1. (A) Temperature and (B) precipitation trends in The Netherlands. Mean temperature was calculated from four KNMI weather stations across eastern Netherlands (see Figure 1) for spring (month of May) and summer (months June, July, August). Total precipitation was averaged over these four weather stations, for spring (month of May) and summer (months June, July, August). Data from www.knmi.nl. In both spring and summer, temperatures increased significantly between 1974 and 2014. During these years, precipitation increased significantly in summer, whereas spring precipitation did not change significantly.

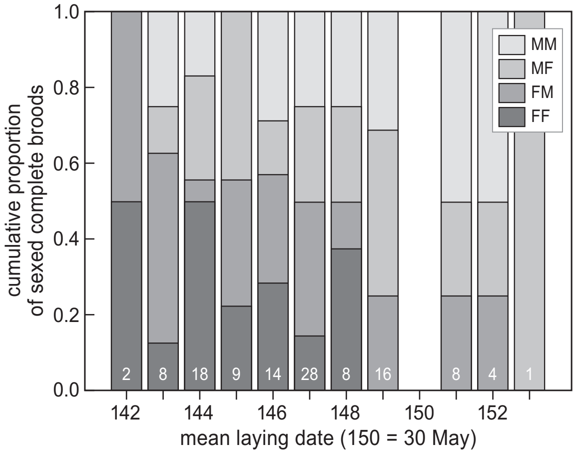

Figure S2. Sex compositions of Honey Buzzard broods in relation to annual phenology, during 1996–2014. Only completely sexed two-chick broods are included for the sex composition. Codes are e.g. MF: first-hatched chick is a male, second-hatched chick is a female. Sample sizes are shown at the bottom (number of broods).

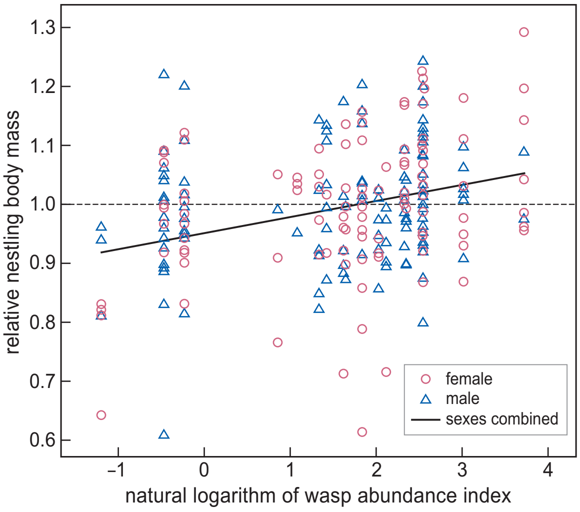

Figure S3. Relative body mass of Honey Buzzard nestling in relation to wasp abundance. Relative chick body mass (mass/expected mass based on chick wing length) was found to increase with wasp abundance (model 'W' in Table 2, ΔAICc = 9.25). The models included random intercepts of year and brood. The dotted line indicates the average mass, given a chick's wing length.

Table S1. Correlation of the wasp colony abundance index (1974–2014) with other measures of wasp abundance in the northern Netherlands. The different measures are sorted by their Pearson's product-moment correlation coefficient *r*. See Bijlsma *et al.* (2012) for correlations until the year 2005.

Wasp abundance measure	Habitat and location	Number of overlapping years with the wasp abundance index of Veluwe and Drenthe	Correlation (*r*) with wasp index of Veluwe and Drenthe, and significance	Observer
Annual visual impression of number of colonies in shed, expressed on relative scale as none (0), few (1), moderate numbers (2) and many (3).	Solitary house with shed in ecotone farmland/woodland Ravenswoud (52°58'15", 6°22'55"E)	9 (2006–2014)	0.9221 ($P < 0.001$)	Leo Zwarts
Absolute number of nests found on 2 transects (each 1 km length, observing a width of 5 metres left and right of transect), walked slowly in first week of August, specifically searching for active and depredated wasp colonies.	(1) Coniferous and mixed woodland with lane of *Fagus sylvatica*, Torenlaan, forestry of Smilde (52°53'31"N, 6°19'35"E); (2) Coniferous woodland (*Pinus sylvestris*, *Picea abies*), Grensweg, forestry of Smilde-Berkenheuvel (52°53'11"N, 6°18'12"E)	23 (1992–2014)	0.9172 ($P < 0.001$)	RGB
Annual absolute numbers of colonies on a solitary house.	Solitary wooden house with reed-thatched roof, in mixed forest along the edge of a small heath, Berkenheuvel (52°52'31"N, 6°16'27"E)	23 (1992–2014)	0.7679 ($P < 0.001$)	RGB
Annual visual impression of number of wasps visiting a greengrocer's shop, expressed on a relative scale as none (0), few (1), moderate numbers (2) and (very) many (3).	Shop on the edge of a small village (Diever; 52°51'21"N, 6°19'11"E).	23 (1992–2014)	0.7470 ($P < 0.001$)	Joop Kamp
Number of workers of social wasps recorded foraging during one minute in tree tops in July–August (between 07:15 and 17:45 summer time), within 2 m of the observer, in annually 6–32 coniferous trees.	Annually 6–32 coniferous trees across a wooded area of 45 km^2 in forestry of Smilde and Berkenheuvel (Centred location: 52°53'09"N, 6°20'31"E).	23 (1992–2014)	0.7189 ($P < 0.001$)	RGB

Table S2. Summary of performance of candidate models (ranked according to AICc) explaining laying date in Honey Buzzards. Models are linear mixed models (including a random intercept of year) explaining absolute laying date of individual nests. Number of parameters in the model is given by *K*. Year: linear trend of year, T5: May temperature, P5: May precipitation, Area: Drenthe or Veluwe. The best model was regarded as that with May temperature, i.e. the simplest model within 2 ΔAICc of the top model.

Model	*K*	ΔAICc	AICc weight
Year + T5 + P5 + Area	7	0.00	0.22
Year + T5 + P5	6	0.01	0.22
T5 + Area	5	0.78	0.15
T5	4	0.93	0.14
Year + T5 + Area	6	1.52	0.10
Year + T5	5	1.70	0.10
T5 + P5 + Area	6	3.52	0.04
T5 + P5	5	4.58	0.02
Year + P5 + Area	6	16.20	0.00
Year + P5	5	16.27	0.00
Year + Area	5	19.86	0.00
Year	4	20.03	0.00
Area	4	24.00	0.00
1	3	24.62	0.00
P5 + Area	5	25.88	0.00
P5	4	27.29	0.00

Table S3. Summary of performance of candidate models (ranked according to AICc) explaining variation in wasp abundance. Models are linear models explaining the natural logarithm of the wasp abundance index (number of wasp nests encountered per 100 field hours in Veluwe and Drenthe). Number of parameters in the model is given by *K*. T5: May temperature, P5: May precipitation, T678: summer temperature (Jun–Aug), P678: summer precipitation. Year: linear trend of year.

Model	*K*	ΔAICc	AICc weight
T5 + P5	4	0.00	0.25
T5 + Year	4	0.12	0.24
T5	3	1.32	0.13
P5	3	1.78	0.10
T5 + T678	4	3.39	0.05
T5 + P678	4	3.63	0.04
P5 + Year	4	3.98	0.03
P5 + P678	4	4.01	0.03
1	2	4.14	0.03
P5 + T678	4	4.18	0.03
Year	3	5.63	0.02
T5 + P5 + T678 + P678 + Year	7	5.91	0.01
T678	3	6.42	0.01
P678	3	6.47	0.01
P678 + Year	4	7.89	0.01
T678 + Year	4	8.07	0.00
T678 + P678	4	8.88	0.00