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Body condition variation in kestrel (*Falco tinnunculus*) nestlings in relation to breeding conditions

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Abstract The body condition index (i.e., body mass corrected for age or size differences) is commonly used to investigate offspring condition in nestling birds. The body condition index reflects different parameters related to the general nutritional state of nestlings and may predict survival prospects. Since conditions experienced during the growth period can affect the fitness of nestlings in adulthood, we investigated proximate and ultimate factors underlying body condition index variation in kestrel (*Falco tinnunculus*) nestlings in a 9-year field study and we carried out two cross-fostering experiments to disentangle the origin (genetics plus maternal effects) and rearing (environment effect) components of body condition index variation. In total, we sampled 2,065 nestlings from 464 broods and used 121 nestlings from 24 broods in the cross-fostering experiments. We found that nestlings from larger broods had higher body condition index than nestlings from smaller ones, but this pattern did not emerge in two of the 9 years of study; nestlings born later in the breeding season had lower body condition index in some years but not in others; the decrease of body condition index over the breeding season emerged in all but three-chick broods; males and females did not differ neither in body condition index nor in the covariation between body mass and wing length, while this result was limited to one of the nine field study years; the annual mean value

of body condition index did not covary with the total rainfall; both the origin and rearing components explained body condition index variation, but their relative contributions varied from a year to another. Overall, these results suggest that the brood size is not a good predictor of body condition index; the rule “nesting early in the season is better” is less general than previously thought; the body condition index may contain origin variance, whose expression may be modulated by environmental conditions.

Keywords Birds · Brood size · Genetic variance · Hatching date · Heritability · Reproductive success

Introduction

Conditions experienced during growth and development affect the individual’s life-history trajectory, a variant of the life-history pattern (Lindström 1999; Metcalfe and Monaghan 2001; Monaghan 2008). Long-term consequences of conditions experienced during early life on the adult phenotype have been documented in various bird and mammal species (Lindström 1999; Metcalfe and Monaghan 2001; Monaghan 2008). While nestling birds can cope effectively with non-optimal early conditions, the compensatory response may determine a variety of costs, such as physiological or structural, whose detrimental effects may emerge later in adulthood (e.g., Lindström 1999; Metcalfe and Monaghan 2001; Nowicki and Searcy 2005; Fisher et al. 2006; Monaghan 2008). Therefore, it is necessary to identify the factors that explain condition variation during growth and development of chicks.

The body condition index, i.e., body mass corrected for age or body size differences, is commonly used to quantify energy reserves in birds. Indeed, the body condition index is primarily a good predictor of body energy reserves, such as fat or proteins (Conway et al. 1994; Ardia 2005, 2006), accumulated during the nestling phase. For example, in American kestrel (*Falco sparverius*)

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nestlings the body condition index is positively related to blood albumin levels, which indicate the amount of proteins in the diet and the nutritional condition of the chick (Ardia 2006).

There is also evidence that body condition index may mirror other relevant health parameters. For example, in American kestrel nestlings body condition index was negatively related to plasma stress hormone levels (Sockman and Schwabl 2001). Also, Alonso-Alvarez and Tella (2001) found a non-linear effect of body condition on T-cell-mediated immunity in captive yellow-legged gulls (*Larus cachinnans*). While this evidence, the body condition index seems not to be a general index of the health status. In fact, several studies found negative or a lack of relationships between some physiological parameters and body condition index. Moreover, these relationships may also change in the same species between years. For example, one study on wild nestling white storks (*Ciconia ciconia*) showed that a significant relationship between body condition index and T-cell-mediated immune response may emerge in some years but not in others (Jovani et al. 2004). Another study on Eurasian kestrel nestlings showed that body condition index did not covary with circulating hydroperoxides (marker of oxidative damage) nor with serum antioxidant capacity in a 1-year study (Costantini et al. 2006). Finally, a study on barn swallow (*Hirundo rustica*) nestlings found a weak correlation between body condition index and haematocrit (Cuervo et al. 2007).

While the body condition index may not always mirror other health-related physiological parameters of the bird, the nutritional status of the bird is still an important factor for its future life because it may affect its future survival perspectives (Merilä 1996; de Kogel 1997; Merilä and Svensson 1997), as well as the adult phenotype (Metcalf and Monaghan 2001; Monaghan 2008). Moreover, depending on the nutritional status of the individual, androgen-dependent signals may act as indicators of body condition itself or indicators of immune system quality (Pérez-Rodríguez et al. 2006).

Environmental components, such as food availability or sibling competition, can explain most body condition index variation. However, several cross-fostering studies have indicated a role of the either origin or genetic components, as well. For example, it was shown in cross-fostering experiments that factors, such as the amount and quality of received food, are major determinants of offspring body condition index in the collared flycatcher *Ficedula albicollis* (Merilä 1996). However, the same study concluded that there are also inherited differences in body condition index, which are translated into offspring viability and survival prospects.

These results also suggested that the effects of nest of origin on body condition index may vary with environmental conditions. Therefore, disentangling the origin and rearing effects on body condition is important to identify the factors underlying variation in this fitness trait.

In this study, we sought to investigate proximate and ultimate factors underlying body condition index

variation in nestlings of the Eurasian kestrel (*Falco tinnunculus*), a small open-country monogamous raptor widespread throughout the Palaearctic, Afrotropical, and Oriental Regions (Cramp and Simmons 1980; Village 1990). We sampled 464 broods to evaluate which factors best explain the body condition index variation over a 9-year field study and we carried out two cross-fostering experiments to define the relative contribution of the nest of origin (genetic background plus maternal effects) and nest of rearing (environment) to body condition index.

With the advance of the breeding season, environmental conditions get worse (e.g., Cavé 1968; Daan and Dijkstra 1982; Tolonen and Korpimäki 1995) and therefore we would expect nestlings born later in the season to be in worse body condition. However, in the late season, brood sizes are smaller and this could mitigate negative effects of lack of food. Therefore, we would expect smaller brood sizes not to be always in worse body condition. It is evident that these predictions are associated to some extent with each other. Therefore, we have also included in the model two-way interactions to try to disentangle the patterns of covariation between body condition index and brood size and hatching date, respectively, and to see if such patterns are consistent between years.

The size of subcutaneous fat reserves is moderately heritable in broiler chickens (Becker et al. 1984) and there is evidence that the ability to assimilate and store energy is also affected by the genetic background (Pym and Solvyns 1979; Borowsky and Kallman 1993). Moreover, a significant contribution of nest of origin to body condition index was found in several bird species, whose magnitude, however, differed between years (Merilä 1996; Merilä et al. 1999; Tella et al. 2000b). Therefore, we would expect the contribution of the origin component to body condition index to emerge and to differ between years.

Materials and methods

Study area and data collection

The field study was carried out from 1999 to 2007 in a 1,200-km² area around Rome. In total, we collected data from 2,065 nestlings (229.4 nestlings/year; 464 broods, 168 nest-boxes; Table 1). Reproductive data were collected from nest-boxes attached to the pylons of utility lines of two local electric power companies. In a previous work, we showed that body condition index (i.e., body mass corrected for wing length; higher values meaning higher energy stores) is not affected by the magnetic fields produced by the current flowing through the conductor (Costantini et al. 2007a). Nests were mainly located in cultivated (cereal fields) and uncultivated areas (fallow fields and pastures). Nest-boxes were visited each year from the end of March until mid-July to assess occupation and to record clutch size, hatching

Table 1 Descriptive statistics for body mass (grams), wing length (mm), hatching date (days from January 1st) and body condition (residuals) measured in a Mediterranean population of Eurasian kestrels during the 1999–2007 breeding seasons

Year	Brood size	<i>N</i> nest-boxes	<i>N</i> nestlings	Body mass	Wing length	Hatching date	Body condition
1999	3	7	21	199.9 ± 8.9	134.5 ± 12.2	147.0 ± 3.3	+1.5 ± 7.4
	4	17	68	194.3 ± 4.6	121.5 ± 5.1	146.8 ± 1.5	+6.0 ± 3.3
	5	21	105	196.5 ± 3.7	125.6 ± 4.6	136.1 ± 0.7	+5.0 ± 2.8
	6	8	48	214.7 ± 3.8	139.9 ± 5.4	131.5 ± 0.9	+12.1 ± 3.3
Total		53	242	199.8 ± 2.4	128.0 ± 2.9	139.1 ± 0.7	+6.4 ± 1.8
2000	3	3	9	162.9 ± 9.3	78.9 ± 6.0	154.3 ± 3.2	+7.6 ± 6.4
	4	8	32	202.3 ± 4.0	141.4 ± 6.4	148.8 ± 1.8	-1.4 ± 5.6
	5	16	80	211.8 ± 2.2	133.6 ± 3.9	140.4 ± 1.2	+14.1 ± 3.1
	6	3	18	163.3 ± 15.2	99.2 ± 14.4	137.7 ± 2.2	-7.8 ± 6.7
Total		30	139	200.2 ± 3.0	127.4 ± 3.6	142.9 ± 0.9	+7.3 ± 2.5
2001	3	24	72	181.6 ± 4.7	101.0 ± 4.4	144.9 ± 1.7	+9.1 ± 3.4
	4	38	152	189.7 ± 3.8	111.6 ± 3.6	141.1 ± 1.3	+9.0 ± 1.9
	5	35	175	187.4 ± 3.2	112.3 ± 3.4	138.4 ± 1.0	+6.1 ± 1.9
	6	12	71	200.8 ± 5.0	130.5 ± 5.0	136.7 ± 1.5	+5.5 ± 2.9
Total		109	470	189.3 ± 2.0	113.1 ± 2.0	140.0 ± 0.7	+7.4 ± 1.2
2002	3	12	36	192.0 ± 6.6	147.0 ± 8.1	144.8 ± 2.8	-16.0 ± 4.0
	4	23	92	190.3 ± 4.1	128.4 ± 4.8	141.5 ± 1.2	-3.4 ± 2.9
	5	18	90	191.2 ± 4.1	126.9 ± 4.5	139.9 ± 0.9	-1.3 ± 2.7
	6	5	30	202.8 ± 6.5	136.8 ± 7.7	127.0 ± 1.7	+2.6 ± 5.9
Total		58	248	192.4 ± 2.4	131.6 ± 2.8	139.7 ± 0.8	-3.8 ± 1.7
2003	3	6	18	180.1 ± 9.9	139.9 ± 12.3	146.0 ± 2.1	-22.5 ± 4.2
	4	25	100	176.6 ± 4.4	112.1 ± 5.0	137.1 ± 1.1	-4.5 ± 3.2
	5	32	160	176.7 ± 4.2	112.8 ± 4.2	133.8 ± 0.9	-4.9 ± 2.7
	6	17	102	147.4 ± 5.5	76.7 ± 3.9	135.7 ± 0.9	-6.2 ± 2.9
Total		80	380	169.0 ± 2.7	104.2 ± 2.7	135.8 ± 0.5	-5.9 ± 1.6
2004	3	7	21	147.2 ± 14.3	71.7 ± 8.5	147.7 ± 2.8	-2.6 ± 8.2
	4	19	75	151.6 ± 5.8	82.5 ± 4.4	148.4 ± 1.2	-6.5 ± 3.0
	5	24	119	161.4 ± 4.8	90.7 ± 4.0	142.4 ± 1.2	-3.1 ± 2.3
	6	2	12	175.5 ± 16.3	101.8 ± 16.6	129.5 ± 0.2	+2.4 ± 5.3
Total		52	227	157.6 ± 3.5	86.8 ± 2.8	144.2 ± 0.8	-3.9 ± 1.8
2005	3	2	6	180.8 ± 10.3	164.3 ± 4.7	151.0 ± 8.1	-40.7 ± 7.1
	4	6	24	199.6 ± 3.9	160.0 ± 4.1	152.5 ± 1.9	-18.6 ± 3.6
	5	6	29	187.9 ± 4.9	124.5 ± 6.1	134.4 ± 1.8	-2.1 ± 3.6
	6	2	11	200.5 ± 6.1	143.4 ± 8.2	143.5 ± 5.0	-4.7 ± 8.4
Total		16	70	193.3 ± 2.8	143.0 ± 3.7	143.4 ± 1.7	-11.6 ± 2.8
2006	3	8	24	201.7 ± 9.7	139.4 ± 9.1	137.8 ± 1.6	-0.5 ± 4.1
	4	11	44	195.4 ± 4.5	138.8 ± 4.2	142.9 ± 1.3	-6.3 ± 3.8
	5	10	50	198.2 ± 4.5	143.4 ± 3.8	140.6 ± 1.3	-7.1 ± 3.1
	6	4	24	222.7 ± 4.2	161.9 ± 2.5	138.3 ± 0.8	+3.0 ± 3.8
Total		33	142	202.1 ± 2.8	144.4 ± 2.5	140.4 ± 0.7	-4.0 ± 1.9
2007	3	4	12	172.7 ± 17.2	126.8 ± 18.6	144.5 ± 5.0	-19.7 ± 6.1
	4	12	48	184.9 ± 5.9	119.7 ± 4.9	139.3 ± 1.5	-2.1 ± 4.2
	5	14	69	194.0 ± 4.6	135.3 ± 4.3	137.7 ± 0.5	-5.0 ± 2.5
	6	3	18	187.1 ± 9.6	113.4 ± 11.4	131.0 ± 0.2	+5.0 ± 2.8
Total		33	147	188.4 ± 3.4	126.9 ± 3.3	137.9 ± 0.7	-4.0 ± 2.0

Values are shown as mean ± SE

date, brood size and fledging success. For each nestling, we measured body mass (grams; within-measurer repeatability, $r = 0.999$, $N = 158$, $P < 0.001$; Lessells and Boag 1987) and wing length (maximum chord, mm; within-measurer repeatability, $r = 0.997$, $N = 158$, $P < 0.001$; Lessells and Boag 1987) (Table 1). To determine sex differences in body condition index, we sexed 124 nestlings in 2003 (34 broods). We used the molecular methods described in Fridolfsson and Ellegren (1999). Hatching dates were determined by visiting the nests in the pre-hatching period or by a growth curve (error: ±1 day).

Total rainfalls during the nestling period (May–June) were used as an index of the environmental conditions (Table 2). Data on total rainfalls were purchased from

two meteorological stations, Porta Maggiore and Tor Bella Monaca (<http://www.romameteo.it>), which were the only meteorological stations located into our study area that recorded data on rainfalls over all the study period. An average value of rainfalls for both stations was included in statistical analyses.

To disentangle the nest of origin and nest of rearing components of body condition index variation, we carried out a cross-fostering experiment in 2003 (16 broods; 82 nestlings) and another in 2004 (eight broods; 39 nestlings). The selected broods were matched for brood size, hatching date, and habitat. Two or three nestlings were cross-fostered, depending on the brood size. The nestlings of the matched nests having comparable wing length and body mass were cross-fostered when the

oldest chick in the brood was 7 days old (the age of the other chicks in the brood ranged from 4 to 7 days; kestrel nestlings fledge around 28–31 days old). This timing assured that all chicks had hatched and avoided disturbance during the hatching period. Non-fostered and fostered chicks did not differ in pre-experiment values of body mass, wing length, and body condition index (see “Results”). The remaining nestlings within the brood were also handled to control for potential handling effects. Chicks of this age cannot be ringed, so they were individually marked with temporary plastic rings, which were subsequently changed with aluminium ones. Fifteen days later, we recorded the body mass and wing length of each nestling. Nest-boxes included in the 2003 cross-fostering experiment were different from those in 2004.

Statistical analyses

All analyses were performed with STATISTICA 7.0 (StatSoft 2004, Tulsa, OK, USA). General linear models with type III sums of squares were run to determine the factors accounting for body condition index variation over the 9-year field study.

Many nest-boxes appeared more than once in the data-set because they were monitored for consecutive

Table 2 Mean values of total rainfall recorded during the nestling period (May–June)

Year	Rainfall (mm)
1999	23.03
2000	14.23
2001	40.25
2002	50.23
2003	2.10
2004	40.10
2005	34.60
2006	11.60
2007	50.20

Table 3 Output of general linear models with type III sums of squares performed to determine the factors accounting for body condition index variation

Term	<i>df</i>	<i>F</i>	<i>P</i>
Intercept	1	111,933.6	< 0.001
Nest-box	167	5.8	< 0.001
Year	7	9.5	< 0.001
Brood size	1	5.0	0.027
Hatching date	1	2.0	0.18
Wing length	1	3001.8	< 0.001
Rainfall	1	0.3	0.64
Year × brood size	8	4.3	< 0.001
Year × hatching date	9	14.7	< 0.001
Brood size × hatching date	1	5.6	0.018
Error	1869		

Nest-box and year were both included as random factors
df Degrees of freedom

years. Thus, some pairs could have contributed more than one brood to the data-set. For this reason, nest-box ($N = 168$) was included as a random factor to control for pseudoreplication. Year was included as a random factor, while brood size, hatching date and rainfall were included as covariates. We also included the following interactions: brood size × year, brood size × hatching date, hatching date × year. Results of the full model are shown in Table 3.

In nestling birds, body mass is primarily a function of age. Therefore, we estimated the body condition index as body mass relative to wing length (index of age), according to other studies on kestrel nestlings (Tella et al. 2000a, 2000b; Costantini et al. 2006; Casagrande et al. 2007). In ecological studies, body condition index is often measured as the ratio between body mass and wing or tarsus length, or the residuals of a linear regression of body mass on wing or tarsus length. However, these methods have been criticized and some concerns have been raised (García-Berthou 2001; Green 2001). To overcome the concerns, we included body mass and wing length in the model as, respectively, dependent variable and covariate, as suggested by García-Berthou (2001).

It could be argued that tarsus length is a mostly invariant body part once full size has been attained, while wing length can vary with condition and environmental conditions. We did not measure tarsus length in our population. However, data of the second author collected from another Italian kestrel population show that correcting body mass for wing or tarsus length gives very similar values of body condition ($r = 0.70$, $n = 142$, $P < 0.001$). Therefore, we are very confident in our index of body condition.

Body mass did not meet the assumption of normality even after log-, square-root, or power transformation. We decided to include untransformed values in the model because of low values of kurtosis (mean ± SE: 0.42 ± 0.11). As suggested by Armitage and Berry (1996), parametric tests are sufficiently robust toward mild violations of the normality assumption (kurtosis is extreme if less than -1 or greater than 2 ; Chiarotti 2004), particularly for large data-sets, as in our case. Moreover, results did not change if transformed or untransformed values were used (data not shown).

To test the effect of sex in 2003, we ran a separate Mixed ANCOVA with Satterthwaite’s approximation of degrees of freedom, including nest as random factor, sex as fixed factor, brood size, and hatching date as covariates, and the sex × wing length interaction to determine if the relationship between body mass and wing length differed between males and females.

The effects of the nest of origin and of the nest of rearing on body condition index variation were estimated by a widely employed approach (e.g., Smith and Wettermark 1995; Merilä 1996), which we modified slightly, i.e., we performed a three-factor nested ANCOVA instead of a three-factor nested ANOVA. All

terms were included as random factors. The main factors were duplicate (a pair of nests matched for brood size and hatching date), nest of rearing and nest of origin (both nested within duplicate), while wing length was included as covariate, according to García-Berthou (2001). In the model we also included year as a random factor and all its two-way interactions, and the term duplicate was nested within year. The term duplicate accounts for environmental differences between pairs of nests (e.g., temporal variation). Within duplicates, the variation due to the nest of origin estimates variation attributable to genetic transmission ($\frac{1}{2}V_A$), but also includes a quarter of the dominance variance ($\frac{1}{4}V_D$) and pre-manipulation parental effects (V_P). The term nest of rearing estimates the effects of the common environment (V_{EC}), and error variance equals random environmental variation plus $\frac{1}{2}V_A + \frac{3}{4}V_D$ (Falconer and Mackay 1996; Merilä 1996).

Comparisons between non-fostered and fostered nestlings in pre-experiment values of body mass and wing length were carried out by ANOVA model (nest-box as a random factor), while of body condition index by ANCOVA model (nest-box as a random factor). One duplicate was excluded from the analyses for the 2003 cross-fostering because some chicks were not measured. Finally, an ANCOVA model with nest-box as a random factor was run to test for differences in effects of handling between non-fostered and fostered nestlings.

It could be argued that sexual dimorphism in the size of nestling kestrels may affect the results, since body condition is partly dependent on a measure of size, such as wing length. In our 2003 sample, males and females did not differ in wing length (our study: $F_{1,89} = 0.07$, $P = 0.80$). Similar results have been shown in a report on nestling kestrels from Dijkstra et al. (1990). However, several studies show that female kestrel nestlings are heavier than males even if there would be no difference in wing length (Dijkstra et al. 1990; Fargallo et al. 2002; Laaksonen et al. 2008). This might cause the distribution of the variable to be two-peaked. In our case, the overall distributions of body mass, wing length or of body mass residuals were not two-peaked (data not shown).

Results

Correlates of body condition index

Results of the full model are shown in Table 3. Briefly, we found significant variation between nest-boxes in the body condition index of nestlings. In general, nestlings from larger broods had higher body condition index than nestlings from smaller ones (Fig. 1), however, this pattern was not always observed. For example, in 2000 and 2001 three-chick broods had higher body condition index than six-chick ones (see Table 1). Nestlings born later in the breeding season had lower body condition index in some years but not in others. Also, the decrease of body condition index

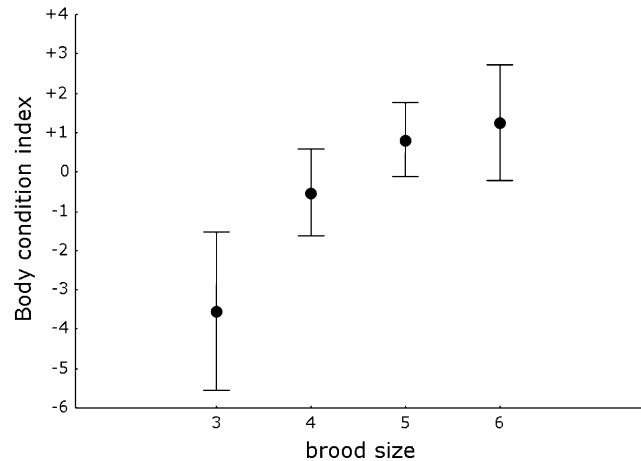


Fig. 1 Larger Eurasian kestrel broods had higher body condition index over a 9-year study period (2065 nestlings, 464 broods). Body condition index is shown as residuals of an ordinary least-squares regression of body mass onto wing length ($R^2 = 0.63$, $F_{1,2063} = 3501.7$, $P < 0.001$) to illustrate body mass values corrected for wing length variation. Values are shown as mean \pm SE

over the breeding season differed between brood sizes. Using a partial correlation (wing length and hatching date as independent variables and body mass as a dependent variable), we found that body condition index of nestlings significantly ($P < 0.01$) decreased over the breeding season in four- ($r = -0.17$), five- ($r = -0.22$) and six-chick ($r = -0.15$) broods, but not in three-chick ($r = -0.05$, $P = 0.48$) ones. The body condition index did not covary with the total rainfall ($r = -0.10$).

Similar results and conclusions were obtained including in the full model the residuals of a linear regression of body mass onto wing length (results not shown).

The variance of body condition index measured in 2003 was explained by nest ($P < 0.001$) and hatching date (negative covariation: $P = 0.021$), but not by sex ($F_{1,50.04} = 0.64$, $P = 0.43$) or brood size ($P = 0.49$). The pattern of covariation between body mass and wing length did not differ between the sexes (sex \times wing length: $P = 0.96$).

Environmental and origin components of body condition index

Pre-treatment values did not differ between non-fostered and fostered nestlings in both 2003 (body mass: $F_{1,55} = 1.06$, $P = 0.31$; wing length: $F_{1,55} = 3.81$, $P = 0.06$; body condition index: $F_{1,54} = 0.60$, $P = 0.44$) and 2004 (body mass: $F_{1,30} = 0.12$, $P = 0.73$; wing length: $F_{1,30} = 0.0003$, $P = 0.99$; body condition index: $F_{1,29} = 2.15$, $P = 0.15$). Finally, there was no effect of handling on post-experimental values of body condition index, as there was no difference between

non-fostered and fostered nestlings in either year (2003: $F_{1,64} = 0.73$, $P = 0.40$; 2004: $F_{1,29} = 0.65$, $P = 0.43$).

Given that in the full model we found a significant interaction between year and nest of origin ($F_{12,83} = 2.37$, $P = 0.011$), we run separate models for each year. The 2003 cross-fostering (eight duplicates, 16 broods) experiment showed that body condition index variation was significantly explained by the environmental component, with a lower contribution of nest of origin (Table 4). In contrast, the 2004 cross-fostering (four duplicates, eight broods) showed that body condition index variation was mostly explained by the nest of origin, with a small contribution of the environmental component (Table 4).

Discussion

The main results of our study were that: nestlings from larger broods had higher body condition index than nestlings from smaller ones, but this pattern did not emerge in two of the 9 study years; nestlings born later in the breeding season had lower body condition index in some years, but not in others; the decrease of body condition index over the breeding season emerged in all but three-chick broods; males and females did not differ neither in body condition index nor in the covariation between body mass and wing length, while this result was limited to one of the 9 field study years; the rainfall of the nestling period did not covary with the body condition index; both the origin and rearing components explained body condition index variation, but their relative contributions varied from a year to another.

Condition-related variables measured in nestlings may be affected by brood size, likely as a result of trade-off between sibling competition and the ability of parents to provide their offspring with food (Dijkstra et al. 1990; Mock and Parker 1997). Defining the relative importance of these factors was beyond the scope of the present study and our results do not allow us to disentangle their relative importance.

In general, studies on effects of brood size on health status of chicks show lack of effects (Tella et al. 2000a)

or a worse health status of chicks in larger brood sizes (e.g., Dijkstra et al. 1990; Saino et al. 1997; Fargallo et al. 2002; Martínez-Padilla 2006). We did find that larger broods showed higher body condition index across all but 2 study years. Moreover, all but three-chick broods showed a decrease of body condition index over the breeding season. These results were mainly due to three-chick broods, which showed very low body condition index values compared to other brood sizes. Three-chick broods were derived from clutches of three eggs or clutches that were originally larger but that failed to hatch completely or that had some chicks dying within the first days of life. This suggests that three-chick broods were, in general, of lower quality, perhaps because of low quality parents or a low quality nesting habitat, which are two non-mutually exclusive explanations. The significant interaction between brood size and hatching date also suggests that the body condition index could not necessarily be associated with brood size, but that it may be also linked to how early or late the parents start breeding. This point is also relevant in that environmental conditions may vary across the breeding season; therefore, rearing a same number of nestlings may be quite different whether this is done during a good or a bad season. These results also suggest that effects of brood size on nestling body condition index and health cannot be generalized to all species and to all nesting conditions. Factors, such as geographic region, inter-annual variation in food availability, nutrient composition of the diet, ability of parents to provide food to offspring, and habitat quality (e.g., food availability, levels and types of pollutants) might explain why the effects of brood size are inconsistent across studies.

The expected negative association between body condition index and hatching date did not always occur in the various years of the study period. Several studies on kestrels from non-Mediterranean areas showed that environmental conditions, such as food availability, get worse as the season progresses, causing a decline of clutch/brood or egg size or increased parental effort (Cavé 1968; Daan and Dijkstra 1982; Dijkstra et al. 1982; Beukeboom et al. 1988; Tolonen and Korpimäki 1995; Valkama et al. 2002). Early studies on kestrels also

Table 4 Results of a three-factor nested ANCOVA performed to test the effects of duplicate, nest of rearing and nest of origin (both nested within duplicate) on body condition variation in Eurasian kestrel nestlings in 2003 (16 broods, 82 nestlings) and 2004 (eight broods, 39 nestlings)

Variable	Source of variation	SS	df	MS	F	P	Variance (%)
2003	Duplicate	15019.76	7	2145.68	8.28	0.024	47.3
	Rearing (dupli)	1443.75	8	180.47	0.86	0.56	0.0
	Origin (dupli)	2250.19	8	281.27	1.36	0.23	3.9
	Residual	11770.13	57	206.49			48.8
2004	Duplicate	2077.10	3	692.37	0.76	0.56	0.0
	Rearing (dupli)	937.13	4	234.28	1.72	0.17	6.9
	Origin (dupli)	3295.42	4	823.86	6.68	0.0008	51.7
	Residual	3205.23	26	123.28			41.4

df Degrees of freedom, SS sum of squares, MS mean squares

emphasized a presumed advantage of birds breeding early in the season because they can produce larger and healthier broods than late ones, thereby achieving greater breeding success (Dijkstra 1988). Our results suggest that this pattern may not be a general rule in the Mediterranean region. One possible explanation is that kestrels in non-Mediterranean areas are quite specialist in preying on voles; however, in Mediterranean areas, kestrels are more generalist predators that may get access to other sources of food at the end of the breeding season (Costantini et al. 2005, 2007b). In light of these results and of recent studies arguing against an adaptive advantage of breeding earlier rather than later (Hendry and Day 2005; Casagrande et al. 2006), the rule “nesting early in the season is better” is not the only plausible.

The lack of covariation between spring rainfall and body condition index suggests that weather conditions during the nestling period did not affect growth of nestlings. However, the effect of rainfall could have been masked by an adaptive response of kestrels (e.g., change in maternal allocation or in gene expression) as suggested by cross-fostering experiments (see below). Rainfall may have different effects on birds' reproductive success, depending on its amount and timing. Negative effects of rainfall may emerge directly increasing chilling of nestlings (e.g., Newton 1978; Bradley et al. 1997). Conversely, indirect effects emerge because of a decrease in parents' hunting efficiency during heavy rainfall, so increasing the likelihood of starvation or loss of energy reserves of nestlings (e.g., Newton 1978; Dawson and Bortolotti 2000). Also, dry springs caused a low number of chicks of lesser kestrels (*Falco naumanni*) at successful nests and chicks fledging in poor body condition in a study in south-western Spain (Rodríguez and Bustamante 2003).

The results of cross-fostering experiments show that both the nest of origin (genetic plus maternal effects) and the nest of rearing (environment effects) may significantly contribute to body condition index variation in nestling kestrels, but to different extents according to the breeding season. This pattern could depend on environmental conditions, such as weather and food availability, which are known to affect the magnitude of genetic expression.

The 2 study years (2003 vs. 2004) did not differ in the fledging success (98 vs. 96.8%; χ^2 n.s.), neither in mean body condition index (−5.9 vs. −3.9; χ^2 n.s.), while total rainfalls during the nestling period were lower in 2003 than in 2004 (2.1 vs. 40.1 mm; $\chi^2 < 0.001$). These data suggest that environmental conditions could have modulated somehow either the genetic expression of body condition or the maternal investment in eggs, while this did not cause any differences in body condition between the 2 years. Such a scenario could explain the lack of correlation between body condition index and rainfall emerged from the overall sample. Further studies are, however, needed to test these hypotheses.

Two non-mutually exclusive explanations for the cross-fostering results are possible. First, variable environmental conditions may have caused different-maternal allocation between years. In fact, maternal effects, such as antioxidants and nutrients, can have a profound influence on offspring condition in a variety of vertebrates, from birds (Gil 2003; Young and Badyaev 2004) to mammals (Reinhold 2002; Weaver et al. 2007). Second, fitness traits may harbor high genetic variance, whose expression might be better in good breeding seasons or under good growth conditions (Merilä and Sheldon 1999). In our case, the magnitude of rainfall during the nestling period, which was not severe, may have increased prey availability and decreased thermal stress for nestlings.

One more point is warranted of consideration. Several studies showed that female kestrel nestlings are heavier than males (Dijkstra et al. 1990; Fargallo et al. 2002; Laaksonen et al. 2008). Such size differences are important in sibling competition and, therefore, this could be reflected on growth trajectories, body condition index or on other condition-related variables (Fargallo et al. 2002; Laaksonen et al. 2004; Martínez-Padilla et al. 2004). For example, kestrel male chicks showed lower body condition than females, but only in treatments suffering from food restrictions (Fargallo et al. 2002). We analyzed sex differences in body condition in 2003 only and we did not consider effect of sex, if any, in our cross-fostering experiment. Therefore, we cannot rule out the possibility that body size differences between sexes could have explained some of the variation observed, while it is impossible to say to what extent.

In conclusion, the results of our study suggest that the brood size is not a good predictor of body condition index; the rule “nesting early in the season is better” is less general than previously thought; the body condition index may contain origin variance, whose expression may be modulated by environmental conditions.

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