Recent studies have shown that the development of the immune system appears to be energetically costly and resource-limited (Klasing and Leshschinsky 1999, Lochmiller and Deerenberg 2000, Norris and Evans 2000, Alonso-Alvarez and Tella 2001, Martin et al. 2003, Bonneaud et al. 2003). During energetically stressful periods therefore, resources may be reallocated towards other costly functions (Sheldon and Verhulst 1996) such as growth (Birkhead et al. 1999, Hõrak et al. 1999, Soler et al. 2003, Love et al. 2005), given that it may be very

$P < 0.0001$) and final ($r = 0.90$, $P < 0.0001$) measurements was high, and we used mean values of the three measurements. Finally, in both years we performed 30 minute behavioral observations using spotting scopes of all nest boxes over three consecutive days when nestlings were aged 6–10 days, for assessment of parental provisioning rates. Provisioning rates were calculated per chick, per hour based on the mean brood size of the nest for the three day observation period.

Molecular sexing

Blood samples were collected from chicks near fledging (17 days of age) in heparinized collecting tubes by means of brachial vein puncture (80–100 μL), and a portion of this sample was transferred to a piece of filter paper and frozen at $-20°C$ for sex analysis. Collected carcasses of deceased nestlings and blood samples were analyzed using a polymerase chain reaction (PCR) amplification process based on techniques used by Griffiths et al. (1996). DNA was isolated from the red blood cell samples using Insta-gene matrix (Bio-Rad Laboratories, Hercules, California, Cat. No. 732-6030), and from the tissue samples using the Qiagen DNAeasy kit, both following manufacturers' protocols. PCR amplification was run using the P2 (5′-TCTGCATCGCTAAATCC-TTT) and C2 (5′-AGAAATCATTCCAGAAGTTCA) primer set, followed by digestion with HAE III (Vanderkist et al. 1998).

Statistical analyses

General linear mixed models (GLMM) were used to analyze sex and brood size effects on nestling immune response. Briefly, sex and brood size were included as factors, and nestling body mass was included as a covariate (where required); maternal identity and year were used as random factors. Non-significant interactions were backward eliminated and all tests were two-tailed with the significance level set at $P = 0.05$. Mortality analysis was performed with a Generalized Linear Model with binomial error distribution and a logit link function (Crawley 1993). Brood size and offspring sex were included in the model as categorical factors, and hatch mass as a covariate. The fit of this model is measured as a χ^2 statistic.

Results

Provisioning and growth rates

Behavioral observations revealed that parental feeding rates were higher in 2003 than in 2002 (2002: 3.09 ± 0.36 feeds/nestling/hour, 2003: 3.96 ± 0.23 feeds/nestling/hour,

$F_{1,52} = 4.09$, $P < 0.05$). Growth rates within the linear growth phase of starlings (hatch to 10 days of age; Ricklefs and Peters 1979) were also higher in 2003 than 2002 (2002: 5.68 ± 0.09 g/day, 2003: 5.98 ± 0.07 g/day, $F_{1,102} = 9.21$, $P < 0.005$). We found no significant effect

P $=0.003$; males: 73.11 ± 0.67 g, females: 69.41 ± 0.32 g),
we did not detect either a year (P $=$

sexually-selected differences (Folstad and Karter 1992, Westneat and Birkhead 1998, Saino et al. 1997b).

We did not observe a consistent relationship between nestling immune response and brood size which contrasts with results of recent studies, all of which report a consistent negative relationship between brood size and immune response in nestlings (Saino et al. 1997a, Hõrak et al. 1999, Naguib et al. 2004). The present study was undertaken across years rather than within one year or under controlled laboratory conditions as in previous studies. Since post-natal growth in avian nestlings requires substantial resources (Starck and Ricklefs 1998), inter-annual variation in resource variability should affect nestling immune response more than growth in relation to brood size. Ecological variation across years can have significant effects on a number of physiological processes (Piersma and Lindström 1997, Vézina and Williams 2003), and our results reinforce the need for multi-year studies in the 751.i40Piersbm45..7(6 66 0¨41.i40Pm4739.i6d(n)-765(a)70 -rtence)-340.9(in)-347.7(wild)-3 not possible in the present study. Ferslly, previous multi-0Pm4739.i6year studies have focused 6 66609.5(imm)23.5(un70 -rtence)-612.7(in about the development of immunity in juvenile passer-0Pm4739.i6ines, especislly post-fledging. Since immune func7(6 66256.9(has)]TJT* Sexual–size dimorphism and food requirements of nestling

Riedstra, B., Dijkstra, C. and Daan, S. 1998. Daily energy requirements of male and female marsh harrier nestlings. –