Master Thesis

Secondary brood sex ratio in little owls (*Athene noctua)*: patterns and determinants

Jolanda Humbel
March 2014

Supervised by:
Dr. Martin Grüebler, Swiss Ornithological Institute, Sempach
Dr. Beat Naef-Daenzer, Swiss Ornithological Institute, Sempach
Prof. Dr. Lukas Keller, Institute of Evolutionary Biology and Environmental Studies, University of Zurich

Abstract

In bird species with sexual size dimorphism, secondary brood sex ratio is often biased. Two general mechanisms can lead to this pattern. Either adaptive reproductive strategies result in a biased primary sex ratio or parental constraints or restraints result in a sex-biased nestling mortality. Using a big sample of sex determination data of little owl nestlings (*Athene noctua*) we examined the secondary brood sex ratio, sexual size dimorphism of nestlings, and factors affecting the survival of the owlets during the nestling period. Brood sex ratio in little owl broods was female-biased at fledging but not until the middle of the nestling period and it was female-biased at the end but not at the beginning of the breeding season. We found that female nestlings showed higher body mass than male nestlings of the same rank. A survival analysis corroborated that high-ranked (i.e. late-hatched) nestlings of low body mass showed reduced survival, resulting in a male-biased nestling mortality. We conclude that under limited food input to the nest the brood sex ratio of fledging juveniles is female-biased. This pattern of food-dependent secondary brood sex ratio can have important effects on the demographic parameters of endangered species.

Introduction

Biased sex ratios and their ultimate and proximate causes were in the focus of ecological interest since more than half a century (e.g. Fisher, 1930; Trivers & Willard, 1973; Hardy, 2002; Ferrer et al., 2009). Though many empirical studies tested the hypotheses of existing sex allocation theories, results are often equivocal and clear evidence for adaptive sex allocation strategies are rare (Hardy, 2002). In altricial birds, biased brood sex ratios at fledging can develop during three periods of the reproduction associated with different proximate mechanisms in birds. First, the sex ratio might be biased already in the pre-hatching period (primary sex ratio), i.e. females lay more eggs of one sex (Oigarden & Lifjeld, 2013). This is possible because females are the heterogametic sex in birds (e.g. Ellegren, 2000), and therefore both pre- (Ankney, 1982) and post-ovulatory (Komdeur et al., 2002) mechanisms of sex allocation exist. However, post-ovulatory mechanisms such as reabsorption of eggs of a specific sex are less probable because they are coupled with energy loss (Hipkiss, 2002; Komdeur & Pen, 2002). The mother can manipulate the sex of offspring in relation to the order of laying (Dijkstra et al., 1990; Badyaev et al., 2002). This may operate in the nestling phase as a basis for brood sex ratio adjustments to environmental conditions (Hasselquist & Kempenaers, 2002). Second, the hatchability of the sexes might be different (Alonso-Alvarez, 2006). This can result from pre-laying processes (i.e. differential female investment in eggs (Blount et al., 2002; Royle et al., 2003; Badyaev et al., 2006)) or from sex-specific sensitivity to differential breeding conditions (Cichon et al., 2005). Third, during the nestling period the mortality of one sex might be higher (Bradbury & Blakey, 1998; Kilner, 1998). Sex-specific mortality might be influenced through selective care by parents (Droge et al., 1991; Radford & Blakey, 2000; McDonald et al., 2005), non-random rank order of sexes within the brood (Badyaev et al., 2002), or sex dimorphism of the nestlings (e.g. Weatherhead & Teather, 1991; Oddie, 2000). In spite of the high diversity of possible proximate mechanisms, an evaluation of sex differences and brood sex ratios restricted to the nestling period can give deep insights into the complex ecology of sex-dimorphic species.

In a life-history context, parental investment into offspring of a specific sex depends on the costs and benefits of the investment to parents (Fisher, 1930; Trivers & Willard, 1973). Costs consist of parental expenditure of time and energy to rear offspring of a specific sex. Consequently, costs of reproduction should particularly differ between nestlings of different sexes in size-dimorphic species (Krijgsveld et al., 1998; Sheldon et al., 1998; Komdeur & Pen, 2002; Alonso-Alvarez, 2006). Costs and benefits of parental investment can also depend on environmental conditions (Hasselquist & Kempenaers, 2002). For example, if the parents breed in a high-quality habitat, they may have more offspring of the less dispersing sex because they benefit more from the local high-quality habitat (Romano et al., 2012). In contrast, if the ecological conditions in the breeding habitat are poor, the parents may produce more offspring of the more dispersing sex because of the higher probability to find a better habitat (Faust & Thompson, 2000).

Food availability represents one major characteristic of habitat quality and typically shows considerable spatial and temporal variation resulting in varying nestling mortality (e.g. Gordon et al., 2000). Since in sexually-dimorphic species nestlings of the two sexes might differ in mortality rates (e.g. Teather & Weatherhead, 1989; Anderson et al., 1993), food-related sex-specific nestling survival can be an important mechanism linking habitat quality and secondary brood sex ratio. In this case, brood sex ratio should change during the nestling period and, at fledging, patterns in secondary brood sex ratio should reflect patterns of food availability. For example, seasonal changes or spatial gradients in food availability should become visible in a varying secondary brood sex ratio. However, though a link between food availability and secondary brood sex ratio is often proposed, empirical evidence is rare.

In species with sexually size dimorphic nestlings there are two possible scenarios of nestling mortality under limited food conditions. First, the individuals of the larger sex show an increased mortality during periods of food limitation (Teather & Weatherhead, 1989; Weatherhead & Teather, 1991) and when breeding success is low (Hörnfeldt et al., 2001). In this case, the larger sex grows faster during the nestling period (Teather & Weatherhead, 1994) and consequently needs more energy (Fiala & Congdon, 1983; Slagsvold et al., 1986; Teather & Weatherhead, 1988). Second, the mortality of the smaller sex can be higher because it is at disadvantage when competing for food (Mock, 1985; Anderson et al., 1993; Oddie, 2000) or because the individuals of the bigger sex kill their smaller siblings (Bortolotti, 1986; Sheldon et al., 1998). Though it is recognised that food limitation often results in a sex-specific nestling mortality in sex-dimorphic species, results on the susceptible sex are inconsistent. Nocturnal raptors (i.e. owl species) represent an excellent example of a species group with varying extent of sex-dimorphism and inconsistent results in terms of the more susceptible sex during the nestling period. In owls with sex-dimorphism females are the larger sex. Kekkonen et al. (2008) showed that in tawny owls (*Strix aluco*) the bigger sex (i.e. females) required more energy to grow and was therefore more sensible to poor food conditions. Similarly, Brommer et al. (2003) showed that in Ural owls (*Strix uralensis*) the fledging weights of females were lower under poor food conditions whereas the weights of the males remained unchanged. In contrast, in tengmalm's owls (*Aegolius funereus*) the female nestlings had a lower mortality than their male siblings (Hipkiss et al., 2002). Hipkiss et al. (2002) suggested that female nestlings are superior in competing for food because adult female tengmalm's owls are slightly larger than males and female nestlings are also heavier than male nestlings of the same age.

In southern Germany, mortality of little owl nestlings (*Athene noctua*) results from limited food input to the nest as shown by a food supplementation experiment (Perrig et al., 2014, submitted). In addition, little owls show a small but consistent size dimorphism in adult birds (van Nieuwenhuyse et al., 2008). Therefore, the little owl represents an ideal study organism to investigate the relationships between nestling mortality and secondary brood sex ratio.

This master thesis aims (1) to investigate the occurrence of a biased secondary brood sex ratio in little owls of Central Europe, and (2) to investigate the role of nestling mortality in the underlying mechanisms resulting in a biased secondary brood sex ratio. This study was not designed to disentangle different sex allocation hypotheses. Rather, we investigate whether nestling mortality is sex-specific and identify whether sex-specific nestling mortality is associated with sex-specific nestling rank or sexual nestling dimorphism. We hypothesise that a possible sexual dimorphism in nestlings results in a biased sex ratio at fledging of the brood. In this case, we expect that the brood sex ratio is related to the age of the brood. Since nestling mortality is strongly associated with food availability, we further expect that the seasonal change in brood sex ratio is related to the change in food availability and that habitats with low food availability show a stronger bias in the brood sex ratio.

Methods

Study species

The little owl (*Athene noctua*) is a Eurasian nocturnal generalist raptor who feeds on small mammals, birds, insects, and earthworms (van Nieuwenhuyse et al., 2008; Müller, 2012). The little owl is a monogamous species and both parents have to be in good condition to bring up the juveniles (van Nieuwenhuyse et al., 2008). In Western Europe the female starts laying of one to seven eggs at the end of April (van Nieuwenhuyse et al., 2008). After an incubation period of 28 days the eggs hatch at about one-day intervals (van Nieuwenhuyse et al., 2008). Little owl nestlings fledge at day 28 to 32 post-hatching (van Nieuwenhuyse et al., 2008). During the nestling period the survival rate of the hatched little owls ranges from 27% to 86% (van Nieuwenhuyse et al., 2008; Thorup et al., 2010; Perrig et al., 2014, submitted). Possible causes for the death of owlets are predation, chilling, cainism, and starvation (van Nieuwenhuyse et al., 2008).

Adult female little owls are on average heavier than males. Body mass of females is 170 – 250 g and that of males 160 – 240 g. The greatest difference in body mass between the sexes occurs during the breeding season and the smallest at the end of summer and in fall (van Nieuwenhuyse et al., 2008). Adult female little owls show also longer tarsi than males (van Nieuwenhuyse et al., 2008).

Study populations

The main sample of secondary brood sex ratios came from a little owl study area in the German county of Ludwigsburg, Baden-Württemberg (Table A1, see Appendix). In this study area we investigated brood sex ratio in the years 2009 to 2013. For 2013, we added also samples from volunteer little owl ringers of other study areas: data of four further study areas in Baden-Württemberg, of four study areas in Rheinland-Pfalz, Germany, of a study area in the Netherlands, and of a study area in Denmark were available (Table A1, see Appendix).

For the statistical analyses we classified the study areas into 7 study regions by pooling data of study areas that were close to each other. Because we had only limited data for some very small study areas, we added these data to a study region with similar landscape even though they were far away from each other. This resulted in seven study regions with more than 20 broods except Denmark for which only 6 broods were available and the landscape differed considerably (Table A1, Fig. A1, see Appendix).

Habitat

Using Google Earth we classified the breeding sites into two habitat classes based on the fact that grassland provides higher food availability than arable farmland (Apolloni, 2013). In an area with a radius of 100 meters around the breeding site (ca. 3.14 ha) we estimated the proportion of grassland available (habitat class 0 = 0 – 50% grassland; habitat class 1 = 50 – 100% grassland). Vineyards were categorized as half grassland, half arable land.

Sex determination

The sex determinations of the feather samples from the year 2013 were done by LABOklin Labor für Klinische Diagnistik GmbH & Co. KG in Bad Kissingen, Germany (729 feather samples). For 40 nestlings two feather samples were analyzed to investigate the repeatability of sex determination.

In the years 2009 to 2012 the sex determinations of feather samples were done by IDEXX GmbH in Ludwigsburg, Germany (344 feather samples).

Measurements of nestlings

Nestlings in all study areas were ringed between day 8 and 45 from hatching (1051 individuals from 369 broods). Simultaneously, we weighted the nestlings using an electronic balance and measured the tarsus length and the length of the 8th and 9th primary feather with a calliper to the nearest 0.1 mm. Finally, we sampled 3 to 5 growing breast feathers for sex determination (1033 individuals, 491 males and 542 females, from 354 broods).

To investigate sex-specific nestling growth and mortality, we asked the involved little owl ringers to conduct a second nest visit in broods with early measurements (second control at day 18 to 61 from hatching; 250 broods; brood sex ratio of 247 broods). The second nest visit allowed recording of nestling mortality.

We calculated the age of the nestlings using the length of the primary 9 and the published relationship between age and primary length (Juillard, 1979). Since not all volunteer little owl ringers agreed to take all the measurements, we did not have all data for all individuals. When measuring length of primary 8 instead of primary 9 (149 nestlings from 63 broods), we calculated the age of the nestlings based on the correlation between length of primary 8 and age from birds with both measurements:

age [days] = (0.221 * primary 8 [mm]) + 12.721

For extreme values of the length of primary 8, we adjusted the age of the nestlings according to age values of birds with data of both primary 8 and primary 9. For those few nestlings without any measurement of feather lengths (49 nestlings from 18 broods) we determined the nestling age based on the correlation between body weight and age from birds with both measurements:

age [days] = (0.1203 * weight [g]) + 5.5478

Feather length was excluded from analyses because we determined the age with the help of the feather length. We determined the ranks of nestlings in the brood on the basis of the feather length of the primary 9. Because only one brood showed a brood size of six and 20 broods a brood size of fife, we pooled ranks 4 to 6 for the statistical analysis. Because the little owl nestlings were ringed at different age, we calculated the residuals for the weight and for the tarsus length from a mean growth curve (Perrig et al., 2014, submitted) to be able to compare these values among all nestlings.

Statistical analyses

The statistical analyses were done using R (version 3.0.2, R Development Core Team 2013) with the package arm (Gelman & Su, 2013). In a binomial model, we evaluated the secondary brood sex ratio at the last nest visit using a generalized linear mixed model (GLMM) with only intercept and binomial error distribution. Breeding site was included as random factor since broods at the same site in different years were included in the Ludwigsburg study area.

For 236 broods the secondary brood sex ratios at ringing and at a second nest visit were available. To compare the secondary brood sex ratios of the same broods at ringing and at a second nest visit we used generalized linear mixed models (GLMMs) with only intercept and binomial error distribution. Breeding site, study area, and year were included as random factors.

For the statistical analysis we added date as numeric variable (date number 1 = April 25). To investigate factors influencing the secondary brood sex ratio we included data from all nest visits. The factors influencing the secondary brood sex ratio were evaluated with a generalized linear mixed model (GLMM) with binomial error distribution. The included fixed factors were hatching date, age of the brood at measurement, habitat class, and brood size at measurement. Brood identity, breeding site, study area, and year were used as random factors.

To investigate seasonal effects on brood size we used a generalized linear mixed model (GLMM) with brood size at ringing as dependent variable and with poisson error distribution. We included hatching date, age of the brood, and habitat class as fixed factors. As random factors we used brood identity and breeding site. To investigate seasonal effects on the original brood size we used a generalized linear mixed model (GLMM) with original brood size as dependent variable and with poisson error distribution. We included hatching date and habitat class as fixed factors. As random factors we used brood identity and breeding site. To investigate seasonal effects on the number of dying individuals per brood between hatching and ringing we used a generalized linear mixed model (GLMM) with the number of dying individuals per brood between hatching and ringing as dependent variable and with poisson error distribution. We included original brood size, hatching date, age of the brood, and habitat class as fixed factors. As random factors we used brood identity and breeding site. To investigate seasonal effects on body weight we used a linear mixed model (LMM) with weight residuals of individual nestling as dependent variable.

We included sex, nestling rank at ringing, and hatching date as fixed factors. Additionally, interactions between sex and nestling rank and between sex and hatching date were included. As random factors we used brood identity, breeding site, study area, and year. To investigate seasonal effects on nestling rank we used a linear mixed model (LMM) with nestling rank at ringing as dependent variable. We included sex, and hatching date as fixed factors. Additionally, an interaction between sex and hatching date was included. As random factors we used study area and year.

For the investigation of the nestling survival two nest visits were required. We just included data of individuals that were younger than 39 days at the second nest visit. We could assume that up to this age the young little owls were in the nest boxes in the daytime and consequently we could be sure that the missing individuals were dead. To investigate nestling survival we used a generalized linear mixed model (GLMM) with survival of individual nestling as dependent variable and binomial error distribution. We included hatching date, age of the nestling at ringing, time between controls, sex, habitat class, brood size at ringing, nestling rank at ringing, and weight residuals at ringing as fixed factors. As random factors we used brood identity, breeding site, and study area. We excluded year as random factor because the time between controls and year were highly correlated.

Measurements of body mass and tarsus length were used to investigate sex dimorphism. To investigate sex differences of nestlings we used a generalized linear mixed model (GLMM) with sex of individual nestling as dependent variable and binomial error distribution. We included nestling rank at ringing, weight residuals at ringing, and tarsus residuals at ringing as fixed factors. Additionally, interactions between nestling rank and weight residuals and between nestling rank and tarsus residuals were included. Non-significant interactions were removed. As random factors we used brood identity, breeding site, study area, and year. To correct for overdispersion, we included an observation level random factor.

For all analyses, the 95% credibility intervals (CrIs) were obtained from a simulated distribution (5000 simulations) and they were used to determine the significance of the fixed factors.

Results

Repeatability of sex determinations

Repeated sex determinations of the 40 individuals revealed 100% agreement in sex determination of the same individual (Fig. A2, see Appendix).

Secondary brood sex ratio

The overall secondary brood sex ratio from the total of 365 broods (considering the last nest visit of each brood) showed that the brood sex ratio was biased and deviated significantly from a ratio of 1:1. The mean secondary brood sex ratio (proportion males) in little owl broods was 0.462 (GLMM with only intercept; 95% CrI: 0.427 to 0.497; N = 365 broods; random factor (SD): breeding site 0.431; Fig. 1).

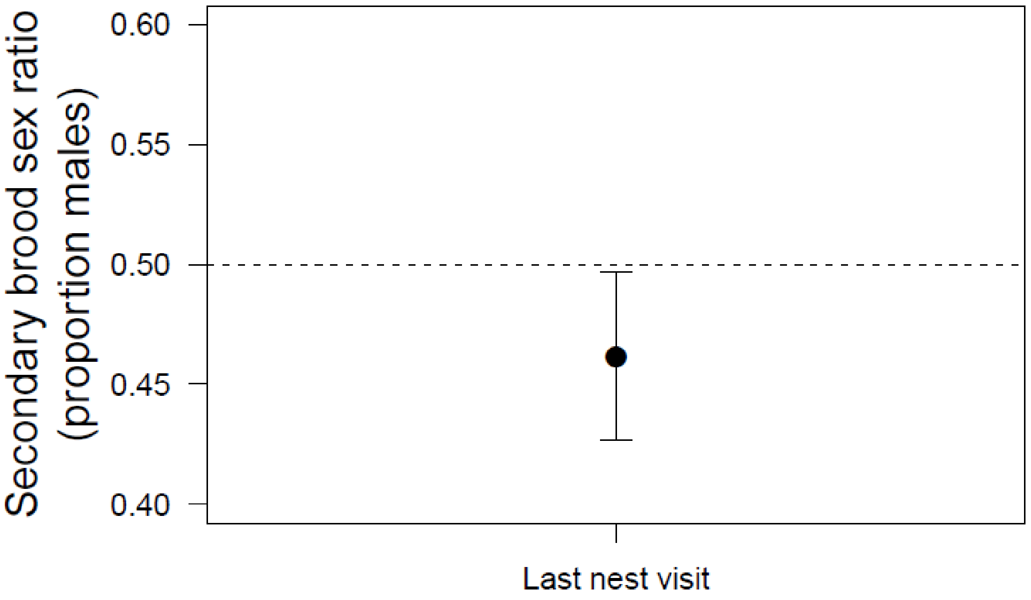

Figure 1: The secondary brood sex ratio at the last nest visit, with the 95% credibility interval.

Factors affecting the secondary brood sex ratio

The secondary brood sex ratio was not influenced by the study area ($\chi^2 < 0.001$, df = 1, p = 0.990). In all seven areas the secondary brood sex ratio at the last nest visit was not significantly different from a balanced sex ratio (Fig. A3, see Appendix).

Also the year did not influence the secondary brood sex ratio ($\chi^2 < 0.001$, df = 1, p = 0.992). In all five years (2009 – 2013) the secondary brood sex ratio at the last nest visit was not significantly different from a balanced sex ratio (Fig. A4, see Appendix).

The secondary brood sex ratio of the same broods was neither significantly different from 0.5 at ringing nor at a second nest visit. At ringing the secondary brood sex ratio (proportion males) was 0.479 (GLMM with only intercept; 95% CrI: 0.436 to 0.523; N = 236 broods; random factors (SD): breeding site 0.567, study area 0.000, year 0.000). From the ringing to the second nest visit the secondary brood sex ratio decreased to 0.460 (GLMM with only intercept; 95% CrI: 0.412 to 0.508; N = 236 broods; random factors (SD): breeding site 0.542, study area 0.000, year 0.000). The reduction in the proportion of males between two nest visits of the same broods revealed a higher male than female mortality in the period between the nest visits.

In the analysis considering all available secondary brood sex ratio data the proportion of males in the broods tended to decrease with increasing age of the brood. Although this decline was not significant, broods reached a significantly female-biased sex ratio at ca. day 26 from fledging (Table 1, Fig. 2).

Table 1: Factors affecting the secondary brood sex ratio. N = 592 observations, 356 broods, 304 breeding sites, 7 study areas, 5 years

Fixed factors	Estimate	95% CrI	
Intercept	0.581	-0.438	1.638
Hatching date	-0.016	-0.032	0.001
Age of the brood	-0.010	-0.029	0.009
Habitat class (grassland)	-0.124	-0.479	0.223
Brood size	0.016	-0.135	0.169

Random factors (SD)	
Brood identity	0.738
Breeding site	0.815
Study area	0.000
Year	0.000

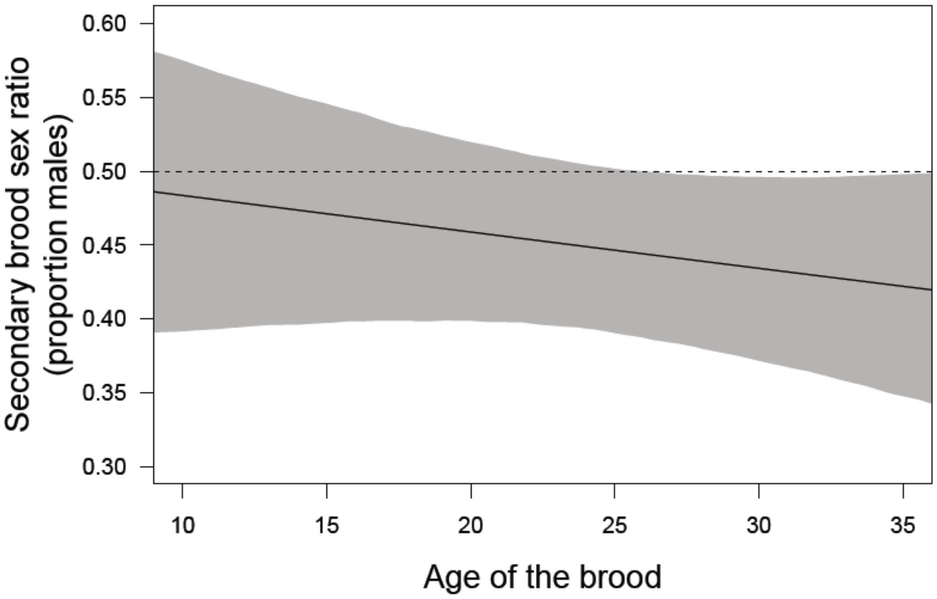

Figure 2: The secondary brood sex ratio in relation to the age of the brood. The 95% credibility interval is shown in grey.

The proportion of males in the broods tended to decrease with increasing hatching date (Table 1, Fig. 3). This decline resulted in a significantly female biased sex ratio after May 24 (date number 30).

Neither habitat class nor brood size at the nest visit showed a significant effect on secondary brood sex ratio (Table 1).

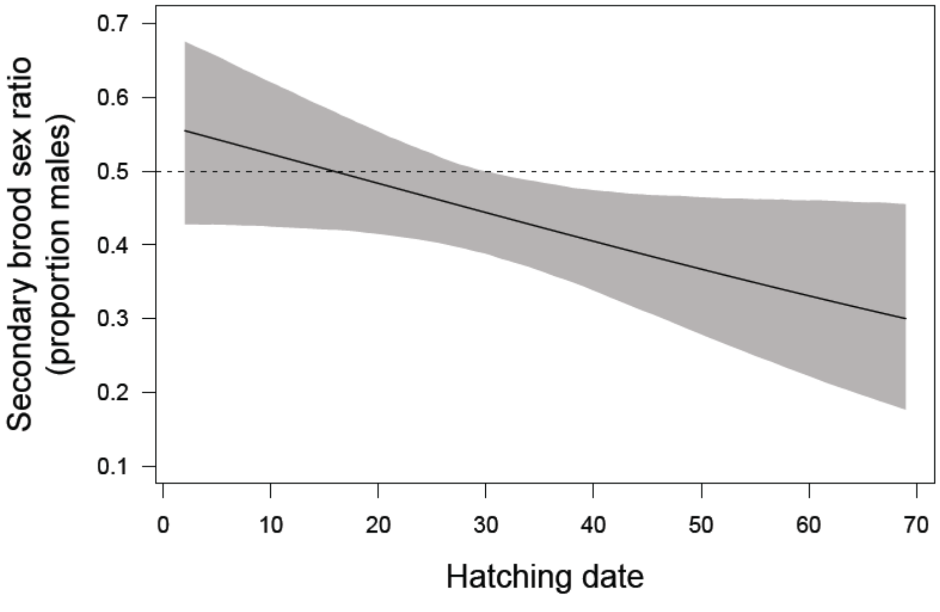

Figure 3: The secondary brood sex ratio in relation to the hatching date (date number 0 = April 24). The 95% credibility interval is shown in grey.

Nestling survival

The brood size significantly decreased with increasing hatching date and with increasing age of the brood (fixed factors: hatching date: estimate = -0.010, 95 % CrI: -0.015 to -0.005, age of brood: estimate = -0.019, 95 % CrI: -0.027 to -0.012; N = 610 observations, 360 broods; random factors (SD): brood identity 0.000, breeding site 0.000). In contrast, the original brood size was not influenced by hatching date (fixed factor: hatching date: estimate = -0.004, 95 % CrI: -0.009 to 0.001; N = 419 observations, 224 broods; random factors (SD): brood identity 0.000, breeding site 0.000), suggesting that nestling mortality between hatching and ringing increased seasonally. This was corroborated by the analysis of the factors affecting the number of dying individuals per brood between hatching and ringing, showing an increase of nestling mortality with increasing hatching date and with increasing age of the brood (fixed factors: hatching date: estimate = 0.017, 95 % CrI: 0.005 to 0.030, age of brood: estimate = 0.040, 95 % CrI: 0.024 to 0.055; N = 419 observations, 224 broods; random factors (SD): brood identity 0.322, breeding site 0.415).

Survival of little owl nestlings between ringing and the second nest visit depended on the time between these two nest visits (Table 2a). As expected, longer periods were associated with lower survival rates. The survival rate decreased with higher rank (i.e. ranks of later hatched nestlings within a brood) and with lower weight residuals (Table 2a, Fig. 4). Nestling survival tended to be negatively related to brood size at ringing (Table 2a). Neither hatching date nor habitat class showed an effect on nestling survival (Table 2a).

Table 2: Factors affecting nestling survival: a) including body weight, b) excluding body weight. Significant fixed factors according to the 95% CrI are marked with asterisks. N = 552 individuals, 197 broods, 146 breeding sites, 5 study areas

	a) Model including body weight			**b) Model excluding body weight**		
Fixed factors	**Estimate**	**95% CrI**		**Estimate**	**95% CrI**	
Intercept	10.512*	2.491	18.499	10.055*	2.651	17.573
Hatching date	0.020	-0.050	0.089	0.009	-0.056	0.073
Age at ringing	-0.064	-0.262	0.138	-0.044	-0.242	0.150
Time between nest visits	-0.297*	-0.514	-0.080	-0.249*	-0.455	-0.043
Sex (male)	-0.685	-1.473	0.105	-0.858*	-1.620	-0.084
Habitat class (grassland)	0.052	-1.425	1.314	0.026	-1.136	1.208
Brood size at ringing	-0.679	-1.413	0.046	-0.680	-1.389	0.030
Rank at ringing	-0.394*	-0.761	-0.024	-0.461*	-0.826	-0.099
Weight residuals at ringing	0.041*	0.011	0.071	-	-	-
Random factors (SD)						
Brood identity		1.586			1.934	
Breeding site		1.833			1.066	
Study area		0.022			0.001	

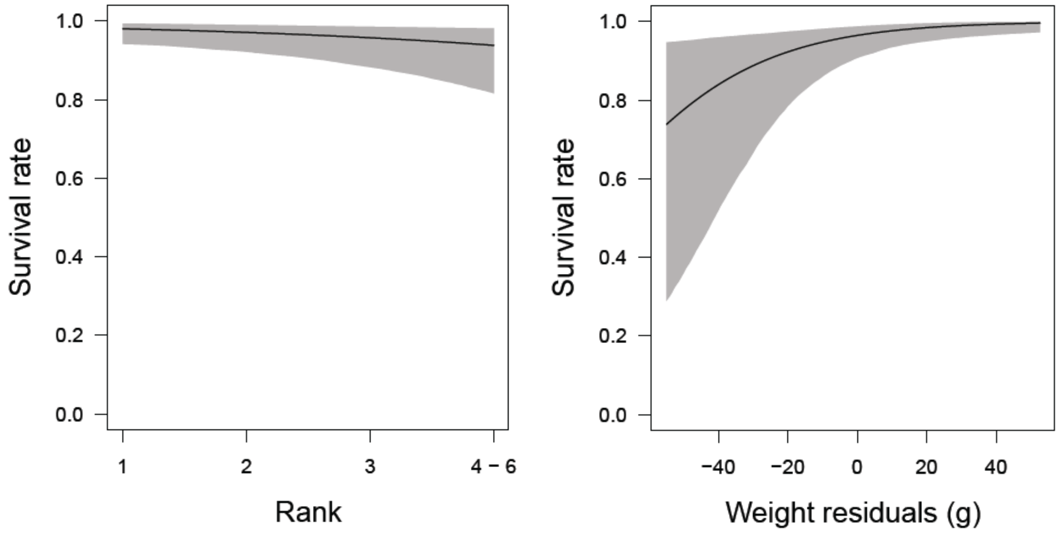

Figure 4: The survival rate in relation to rank and weight residuals. The 95% credibility intervals are shown in grey.

If we excluded body weight from the model, sex had a significant effect on the survival rate (Table 2b). Male nestlings showed significant lower survival than female nestlings in the same rank.

Sex dimorphism

Female little owl nestlings were significantly heavier but not significantly larger (in terms of tarsus length) than male nestlings at ringing (Table 3, Fig. 5). Sexes did not differ in rank within the brood. However, the interaction between rank and weight residuals had a significant effect on sex (Table 3), showing that in our data set at ringing this sexual dimorphism was only present in the first hatched nestlings of the brood but not in the last hatched nestlings (Fig. 5).

Table 3: Sex dimorphism in little owl nestlings at ringing. Significant fixed factors according to the 95% CrI are marked with asterisks. N = 569 individuals, 216 broods, 162 breeding sites, 6 study areas, 5 years

Fixed factors	**Estimate**	**95% CrI**	
Intercept	-0.019	-0.427	0.391
Rank	-0.022	-0.186	0.146
Weight residuals	-0.037*	-0.061	-0.013
Tarsus residuals	-0.015	-0.091	0.061
Rank x weight residuals	0.011*	0.001	0.021

Random factors (SD)	
Brood identity	0.000
Breeding site	0.503
Study area	0.000
Year	0.000
Observation level	0.000

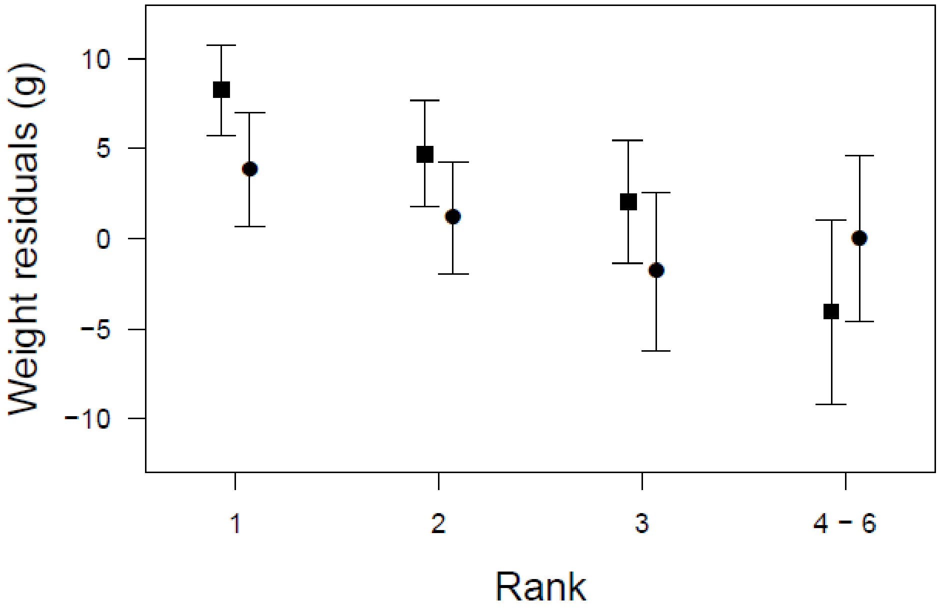

Figure 5: Weight residuals for females (squares) and males (circles) with the 95 % confidence intervals (CIs) in relation to rank.

As already shown, the weight residuals depended on sex and rank. Moreover, weight residuals of males but not that of females tended to decrease with increasing hatching date (fixed factor: sex x hatching date: estimate = -0.126, 95 % CrI: -0.276 to 0.022; N = 771 Individuals; random factors (SD): brood identity 11.642, breeding site 8.699, study area 1.915, year 5.358, residual 8.688).

Nestling rank was not influenced by hatching date (fixed factor: hatching date: estimate = -0.005, 95 % CrI: -0.014 to 0.004; N = 778 Individuals; random factors (SD): study area 0.156, year 0.262, residual 0.999).

Discussion

This thesis provides clear evidence for a female-biased brood sex ratio in little owls at the time of fledging. Our results show that the proportion of males within a brood declined with the age of the brood. Nestling survival was reduced in the latest-hatched nestlings of a brood and in nestlings with low body mass. This survival pattern resulted in a female-biased brood sex ratio at fledging because male little owl nestlings showed reduced body weight compared to female nestlings leading to increased male nestling mortality.

In our study we could not identify a size dimorphism in the tarsus length of male and female little owl nestlings, although adult female little owls have longer tarsi than males (van Nieuwenhuyse et al., 2008). On the other hand the age-corrected body weight of nestlings differed between the sexes, i.e. females were heavier than males, corresponding with the fact that adult female little owls are slightly heavier than adult males (van Nieuwenhuyse et al., 2008). However, higher-ranked (i.e. later-hatched) nestlings showed no difference in the age-corrected body weight between female and male nestlings. One reason for this could be that some higher-ranked nestlings already died before ringing and that these nestlings were more males than females. This would explain why the absolute value for the weight residuals of females in ranks 4 – 6 was lower than that of males. The difference in the age-corrected body weight at ringing could be the result of faster development and weight gain of female nestlings (e.g. Teather & Weatherhead, 1994). Alternatively, the difference might develop due to a higher parental investment into female nestlings during the nestling period (Droge et al., 1991; Radford & Blakey, 2000; McDonald et al., 2005). In this case the parents should have a higher benefit of investing into female nestlings than into males. This would be the case if females have a higher mating probability than males. Aviles and Parejo (2003) found that heavier little owl nestlings had a yellower beak than lighter nestlings and that parents from big broods fed the nestlings with yellower beaks more. We do not know whether the colour of the beak differs between sexes. However, if yes, the female nestlings would get more food from their parents and consequently show higher body weight and survive better. To test this hypothesis further investigation is needed. It could also be that female nestlings are more aggressive than their male siblings and therefore compete more for their food and consequently get more food. To verify this hypothesis experimentation is needed because Bortolotti (1986) observed the opposite pattern: individuals of the smaller sex (i.e. males) of bald eagle nestlings were more active and agile, and fledged earlier than individuals of the larger sex.

Mortality of higher-ranked (i.e. later-hatched) nestlings was higher. Nestlings in higher ranks were smaller than their siblings because they were younger. Therefore in conditions of limited food provision, it is likely that they were in disadvantage when competing for food with their siblings (Oddie, 2000). Lighter nestlings had a lower survival rate than heavier nestlings. Thus, our results corroborate the importance of nestling weight for nestling survival shown in multiple bird species (e.g. Magrath, 1991; Perrig et al., 2014, submitted). In our study the nestling survival tended to decrease with increasing brood size. A reason why this was not more pronounced could be that female little owls could regulate the number of eggs they lay according to food supply. So the brood size at hatching is lower in situations with bad food supply than in situations with good food supply. Another reason could be that many nestlings already died before ringing. So the brood size at ringing was already adjusted to the food situation. When excluding body weight from the survival model, the survival rate of female nestlings was higher than that of male nestlings because of the lower weight of male nestlings compared to females.

Our study showed that the main effect on secondary brood sex ratio was sex-specific mortality of nestlings during the nestling period. Four results provide strong evidence for the occurrence of regular nestling mortality and existence of a seasonal pattern. First, brood size at ringing decreased with the age of the brood. Second, brood size at ringing decreased during the breeding season whereas the original brood size did not change during the breeding season. Third, the number of individuals dying in the period between hatching and ringing increased with the age of the brood and during the breeding season. At last, survival analysis between ringing and a second nest visit revealed that last-hatched and lightest nestlings showed the highest mortality rates. These results coupled with the fact that male nestlings showed lower body weight than female nestlings and therefore experienced lower survival probability indicate that nestling mortality in little owls should result in a biased brood sex ratio at fledging and at the end of the breeding season. Accordingly, the bias in the secondary brood sex ratio increased with the age of the brood and in the course of the breeding season. Since nestling mortality is shown to depend on the amount of food provided to the nest (Perrig et al., 2014, submitted), our results suggest that factors affecting the provisioning rate also affect the secondary sex ratio. In addition to characteristics of the parents, food availability and food accessibility in the breeding habitat are major factors affecting the amount of energy brought to the nest (Müller, 2012; Apolloni, 2013). We therefore believe that spatial and annual variation in food supply should result in spatially and temporally varying secondary brood sex ratios. However, our sample size of brood sex ratios in different areas and years was too small to identify such variation.

The seasonal pattern in secondary brood sex ratios might arise due to a seasonal decrease in food supply. This is supported by the fact that the vole density decreased during the breeding season from April to July in the study area of Ludwigsburg (Apolloni, 2013). However, we do not know whether an increase in nestling mortality over the breeding season is due to declining food supply or due to the possibility that breeding parents who start with egg-laying later are of lower quality than early breeding parents (Sydeman & Eddy, 1995; González-Solís et al., 2005). To disentangle these two possibilities experimentation is needed. Moreover, we could not exclude the possibility that additionally, mothers produced more male eggs early than late in the breeding season because male owlets (and thereby their parents) benefit more from an early hatching date (Daan et al., 1996).

In this study the habitat classes neither had an effect on nestling survival nor on secondary brood sex ratio. This was unexpected because Apolloni (2013) showed that food availability in grassland was higher than in arable land. A reason why we might not find any effect of habitat could be that our main study year generally showed extremely low vole densities (own unpublished data). So possibly the differences in vole occurrence between grassland and arable land were very small.

In conclusion, at the end of the breeding season of our study years more female than male little owls have fledged. The proximate mechanism underlying this pattern is a sex dimorphism in nestling body mass resulting in higher nestling mortality rates of males than of females at conditions of low food input to the nest. These results suggest a link between habitat quality in terms of food availability and the sex ratio of juvenile birds entering the population after fledging. This could have multiple consequences on the demography of the population. First, tertiary sex ratio in regions of low habitat quality might still be female-biased affecting the effective population size and thus population growth rate. Second, emigration rates in populations of high nestling mortality with higher number of female fledglings might be increased because dispersal propensity is higher in female little owls than in males (e.g. Zens, 2005), again negatively affecting population growth rate. Reduced nestling survival not only reduces population growth rate by low productivity but also by a bias in the secondary brood sex ratio. Thus, temporal or spatial variation in nestling survival results in disproportional variation in population growth rate. Especially prone to such negative demographic effects might be small and fragmented populations or populations at the edge of the species distribution.

Acknowledgements

This master thesis could just be done by the support of many dedicated people. First of all, I want to thank my main supervisor, Dr. Martin Grüebler from the Swiss Ornithological Institute for all his various support during the whole work. He was always approachable for questions and took his time for discussions. My gratitude goes also to Dr. Beat Naef-Daenzer from the Swiss Ornithological Institute and Prof. Dr. Lukas Keller from the Institute of Evolutionary Biology and Environmental Studies at the University of Zurich for their support. I am very grateful to Herbert Keil for letting me attend him while ringing the owlets and for letting me investigate broods in his area. I also want to thank Vanja Michel for his help and advice as my supervisor in the field. Additionally, I am deeply grateful for the support of Marco Perrig and Dr. Fränzi Korner in statistics. Just with the help of many little owl ringers in Germany, the Netherlands, and Denmark it was possible to get such a big dataset. Therefore I want to express my gratitude to Joscha Erbes, Jochen Fischer, Gerhard Fritz, Barbara Geiger, Wolfgang Graef, Thomas Henschel, Rudi Holleitner, Bernd Hoos, Johanna Hurst, Lars Bo Jacobsen, Werner Kneule, Gerhard Lörcher, Heinz Michels, Leander Möbius, Alexander Neu, Philip Rössler, Volker Schlie, Dieter Schneider, Pascal Stroeken, Bruno Vollmar, Ronald von Harxen, and all their cooperators.

References

Alonso-Alvarez, C. 2006. Manipulation of primary sex-ratio: an updated review. *Avian and Poultry Biology Reviews* 17: 1 – 20.

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

Ankney, C. D. 1982. Sex ratio varies with egg sequence in lesser snow geese. *Auk* 99: 662 – 666.

Apolloni, N. 2013. Habitat selection and home range use of adult little owls: Recognizing the importance of scale. *Master thesis, University of Bern.*

Avilés, J. M. & Parejo, D. 2013. Colour also matters for nocturnal birds: owlet bill coloration advertises quality and influences parental feeding behaviour in little owls. *Oecologia* 173: 399 – 408.

Badyaev, A. V., Hill, G. E., Beck, M. L., Dervan, A. A., Duckworth, R. A., McGraw, K. J., Nolan, P. M. & Whittingham, L. A. 2002. Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science* 295: 316 – 318.

Badyaev, A. V., Oh, K. P. & Mui, R. 2006. Evolution of sex-biased maternal effects in birds: II. Contrasting sex-specific oocyte clustering in native and recently established populations. *Journal of Evolutionary Biology* 19: 909 – 921.

Blount, J. D., Surai, P. F., Nager, R. G., Houston, D. C., Møller, A. P., Trewby, M. L. & Kennedy, M. W. 2002. Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. *Proceedings of the Royale Society of London, Series B: Biological Sciences* 269: 29 – 36.

Bortolotti, G. R. 1986. Influence of sibling competition on nestling sex ratios of sexually dimorphic birds. *The American Naturalist* 127: 495 – 507.

Bradbury, R. B. & Blakey, J. K. 1998. Diet, maternal condition, and offspring sex ratio in the zebra finch, *Poephila guttata*. *Proceedings of the Royal Society of London, Series B: Biological Sciences* 265: 895 – 899.

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.

Cichon, M., Sendecka, J. & Gustafsson, L. 2005. Male-biased sex ratio among unhatched eggs in great tit *Parus major*, blue tit *P. caeruleus* and collared flycatcher *Ficedula albicollis*. *Journal of Avian Biology* 36: 386 – 390.

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

Dijkstra, C., Daan, S. & Buker, J. B. 1990. Adaptive seasonal variation in the sex ratio of kestrel broods. *Functional Ecology* 4: 143 – 147.

Droge, D. L., Gowaty, P. A. & Weathers, W. W. 1991. Sex-biased provisioning: A test for differences in field metabolic rates of nestling eastern bluebirds. *The Condor* 93: 793 – 798.

Ellegren, H. 2000. Evolution of the avian sex chromosomes and their role in sex determination. *Trends in Ecology and Evolution* 15: 188 – 192.

Faust, L. J. & Thompson, S. D. 2000. Birth sex ratio in captive mammals: Patterns, biases, and the implications for management and conservation. *Zoo Biology* 19: 11 – 25.

Ferrer, M., Newton, I. & Pandolfi, M. 2009. Small populations and offspring sex-ratio deviations in eagles. *Conservation Biology* 23: 1017 – 1025.

Fiala, K. L. & Congdon, J. D. 1983. Energetic consequences of sexual size dimorphism in nestling Red-winged Blackbirds. *Ecology* 64: 642 – 647.

Fisher, R. A. 1930. The genetical theory of natural selection. *Clarendon Press, Oxford*.

Gelman, A. & Su, Y.-S. 2013. arm: Data Analysis Using Regression and Multilevel/Hirarchical Models. R package version 1.6-10. URL http://CRAN.R-project.org/package=arm.

González-Solís, J., Becker, P. H., Wendeln, H. & Wink, M. 2005. Hatching sex ratio and sex specific chick mortality in common terns *Sterna hirundo*. *Journal of Ornithology* 146: 235 – 243.

Gordon, C. A., Cristol, D. A. & Beck, R. A. 2000. Low reproductive success of black skimmers associated with low food availability. *Waterbirds: The International Journal of Waterbird Biology* 23: 468 – 474.

Hardy, I. C. W. 2002. Sex ratios: concepts and research methods. *Cambridge University Press, Cambridge*.

Hasselquist, D. & Kempenaers, B. 2002. Parental care and adaptive brood sex ratio manipulation in birds. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 357: 363 – 372.

Hipkiss, T. 2002. Brood sex ratio and sex differences in Tengmalm's owl. *Doctoral dissertation, Umea University*.

Hipkiss, T., Hörnfeldt, B., Eklund, U. & Berlin, S. 2002. Year-dependent sex-biased mortality in supplementary-fed Tengmalm's owl nestlings. *Journal of Animal Ecology* 71: 693 – 699.

Hörnfeldt, B., Hipkiss, T. & Eklund, U. 2001. Juvenile sex ratio in relation to breeding success in Capercaillie (*Tetrao urogallus*) and Black Grouse (*T. tetrix*). *Ibis* 143: 627 – 631.

Juillard, M. 1979. La croissance des jeunes Chouettes chevêches, *Athene noctua,* pendant leur séjour au nid. *Nos Oiseaux* 35: 113 – 124.

Kekkonen, J., Kolunen, H., Pietiäinen, H., Karell, P. & Brommer, J. E. 2008. Tawny owl reproduction and offspring sex ratios under variable food conditions. *Journal of Ornithology* 149: 59 – 66.

Kilner, R. 1998. Primary and secondary sex ratio manipulation by zebra finches. *Animal Behavior* 56: 155 – 164.

Komdeur, J., Magrath, M. J. L. & Krackow, S. 2002. Pre-ovulation control of hatchling sex ratio in the Seychelles warbler. *Proceedings of the Royal Society of London (B)* 269: 1067 – 1072.

Komdeur, J. & Pen, I. 2002. Adaptive sex allocation in birds: the complexities of linking theory and practice. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 357: 373 – 380.

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

Magrath, R. D. 1991. Nestling weight and juvenile survival in the blackbird, *Turdus merula*. *Journal of Animal Ecology* 60: 335 – 351.

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

Mock, D. W. 1985. Siblicidal brood reduction: The prey-size hypothesis. *The American Naturalist* 125: 327 – 343.

Müller, M. 2012. Das Nahrungsspektrum von Steinkäuzen (*Athene noctua*) im Landkreis Ludwigsburg (D). *Bachelor thesis, Zurich University of Applied Sciences ZHAW.*

Oddie, K. R. 2000. Size matters: competition between male and female great tit offspring. *Journal of Animal Ecology* 69: 903 – 912.

Oigarden, T. & Lifjeld, J. T. 2013. Primary sex ratios vary with clutch size in the size-dimorphic White-throated Dipper (*Cinclus cinclus*). *Journal of Ornithology* 154: 91 – 97.

Perrig, M., Grüebler, M. U., Keil, H. & Naef-Daenzer, B. 2014. Brood provisioning affects physical development, behaviour and survival in Little Owl (*Athene noctua*) nestlings. *Ibis submitted*.

R Development Core Team 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Radford, A. N. & Blakey, J. K. 2000. Intensity of nest defence is related to offspring sex ratio in the great tit *Parus major*. *Proceedings of the Royal Society of London, Series B: Biological Sciences* 267: 535 – 538.

Romano, A., Ambrosini, R., Caprioli, M., Bonisoli-Alquati, A. & Saino, N. 2012. Secondary sex ratio covaries with demographic trends and ecological conditions in the barn swallow. *Evolutionary Ecology* 26: 1041 – 1053.

Royle, N. J., Surai, P. F. & Hartley I. R. 2003. The effect of variation in dietary intake on maternal deposition of antioxidants in zebra finch eggs. *Functional Ecology* 17: 472 – 481.

Sheldon, B. C., Merilä, J., Lindgren, G. & Ellegren, H. 1998. Gender and environmental sensitivity in nestling collared flycatchers. *Ecology* 79: 1939 – 1948.

Slagsvold, T., Roskaft, E. & Engen, S. 1986. Sex ratio, differential cost of rearing young, and differential mortality between the sexes during the period of parental care: Fisher's theory applied to birds. *Ornis Scandinavica* 17: 117 – 125.

Sydeman, W. J. & Eddy, J. O. 1995. Repeatability in laying date and its relationship to individual quality for common murres. *The Condor* 97: 1048 – 1052.

Teather, K. L. & Weatherhead, P. J. 1988. Sex-specific energy requirements of great-tailed grackle (*Quiscalus mexicanus*) nestlings. *Journal of Animal Ecology* 57: 659 – 668.

Teather, K. L. & Weatherhead, P. J. 1989. Sex-specific mortality in nestling Great-tailed Grackles. *Ecology* 70: 1485 – 1493.

Teather, K. L. & Weatherhead, P.J. 1994. Allometry, adaptation, and the growth and development of sexually dimorphic birds. *OIKOS* 71: 515 – 525.

Thorup, K., Sunde, P., Jacobsen, L. B. & Rahbek, C. 2010. Breeding season food limitation drives population decline of the little owl *Athene noctua* in Denmark. *IBIS* 152: 803 – 814.

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

Van Nieuwenhuyse, D., Génot, J.-C. & Johnson, D. H. 2008. The little owl: conservation, ecology and behavior of *Athene noctua*. *Cambridge University Press, Cambridge*.

Weatherhead, P. J. & Teather, K. L. 1991. Are skewed fledgling sex ratios in sexually dimorphic birds adaptive? *The American Naturalist* 138: 1159 – 1172.

Zens, K.-W. 2005. Langzeitstudie (1987 – 1997) Zur Biologie, Ökologie und Dynamik einer Steinkauzpopulation (*Athene noctua*) im Lebensraum der Mechernicher Voreifel. *Ph.D. thesis, Rheinische Fridrich-Wilhelm-Universität Bonn.*

Appendix

Table A1: Area classification for the little owl populations that were included in our study.

Study region, Year	**Study area**	**Federal state, Country**	**Observers**	**Number of broods**
Ludwigsburg, 2009	Ludwigsburg	Baden-Württemberg, Germany	Herbert Keil	17
Ludwigsburg, 2010	Ludwigsburg	Baden-Württemberg, Germany	Herbert Keil	30
Ludwigsburg, 2011	Ludwigsburg	Baden-Württemberg, Germany	Herbert Keil	43
Ludwigsburg, 2012	Ludwigsburg	Baden-Württemberg, Germany	Herbert Keil	31
Ludwigsburg, 2013	Ludwigsburg	Baden-Württemberg, Germany	Herbert Keil Jolanda Humbel	52
Heilbronn, 2013	Heilbronn	Baden-Württemberg, Germany	Jochen Fischer Wolfgang Graef	18
	Ortenaukreis	Baden-Württemberg, Germany	Gerhard Lörcher	3
	Karlsruhe	Baden-Württemberg, Germany	Gerhard Fritz	2
Köngen, 2013	Esslingen	Baden-Württemberg, Germany	Werner Kneule Heinz Michels Dieter Schneider Philip Rössler	29
Bad Dürkheim, 2013	Bad Dürkheim	Rheinland-Pfalz, Germany	Leander Möbius Rudi Holleitner Volker Schlie	41
	Neustadt	Rheinland-Pfalz, Germany	Bernd Hoos	6
Mainz, 2013	Mainz-Bingen	Rheinland-Pfalz, Germany	Barbara Geiger Thomas Henschel Alexander Neu Bruno Vollmar	29
	Bad Kreuznach	Rheinland-Pfalz, Germany	Joscha Erbes	5
Netherlands, 2013	-	Netherlands	Ronald von Harxen Pascal Stroeken	57
Denmark, 2013	-	Denmark	Lars Bo Jacobsen	6
Total number of broods				**369**

Figure A1: Study regions (shown with red circles).

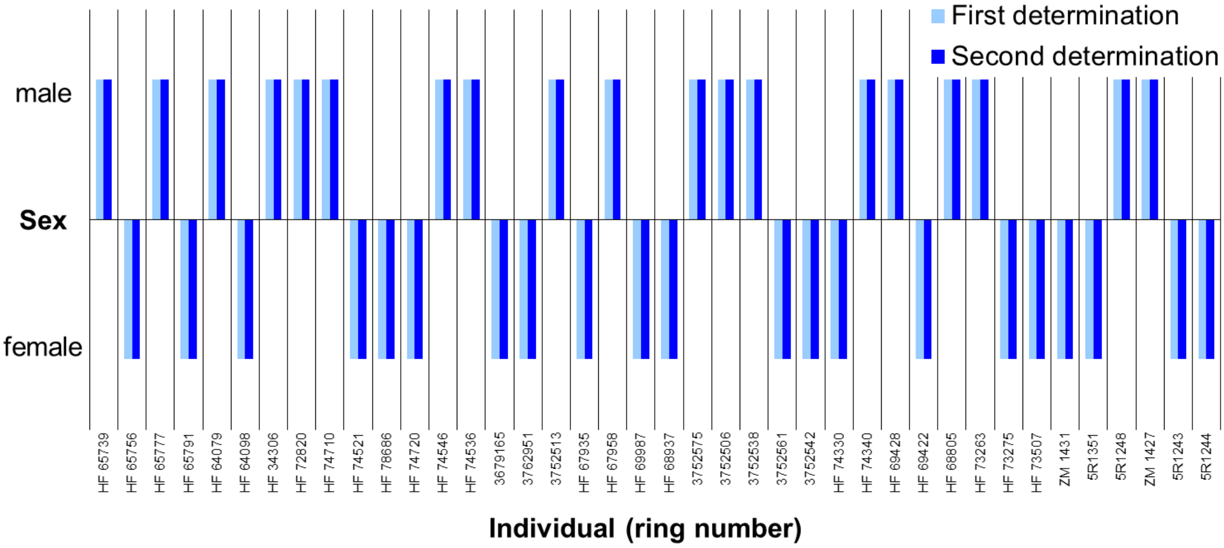

Figure A2: Repeatability of sex determinations from 40 nestlings.

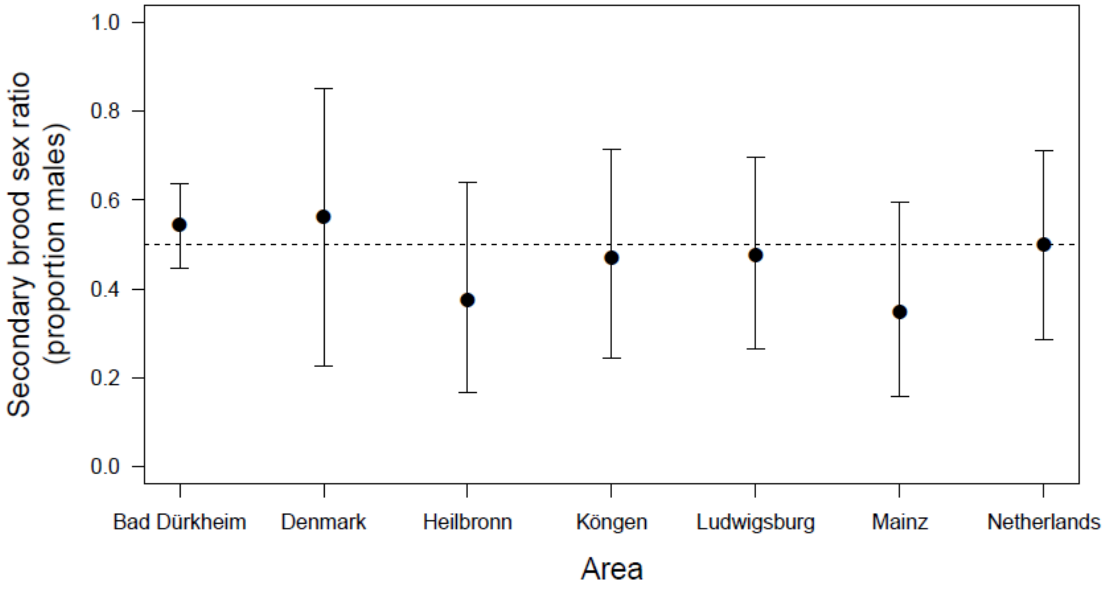

Figure A3: The secondary brood sex ratio in the different study areas in 2013 with the 95% credibility intervals.

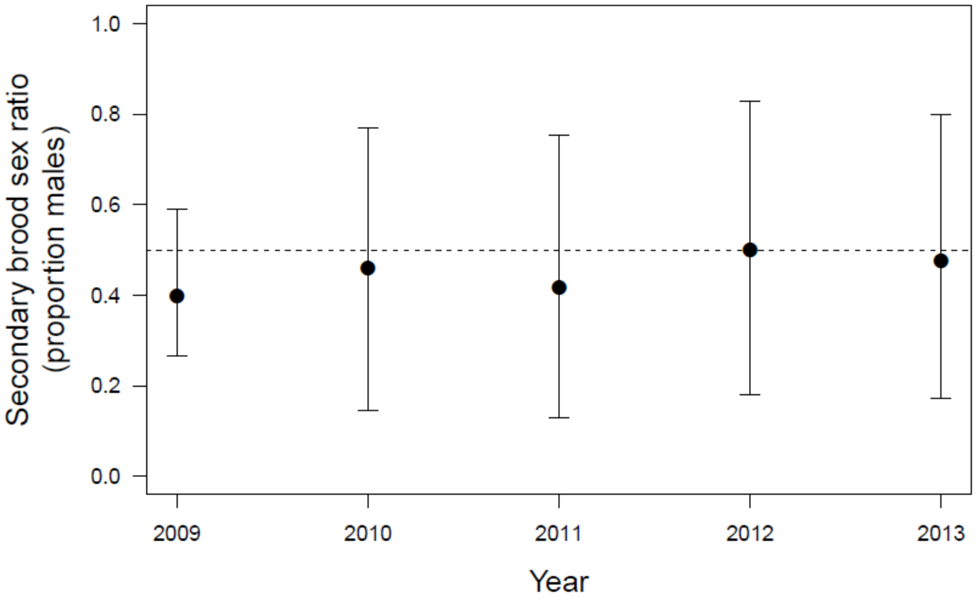

Figure A4: The secondary brood sex ratio in the different years in the county of Ludwigsburg, Germany, with the 95% credibility intervals.

Statement of Authorship:

I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

place, date signature