ORIGINAL ARTICLE

Effects of population structure and density on calf sex ratio in red deer (*Cervus elaphus*)—implications for management

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Abstract

There is ongoing interest to assess what factors affect offspring sex ratio, especially in ungulates. Wildlife managers might be interested in influencing this sex ratio for two reasons: either in order to limit population growth more effectively by reducing the proportion of females born or to increase revenues by a higher proportion of trophy bearing males in the population. While previous studies mostly focused on how maternal traits affect offspring sex ratio, we included here also male traits in our analysis. We achieved this by investigating data from 30 areas covering entire Lower Austria, collected over the past 12 years from both hunted red deer and those killed in road accidents. We focused our analyses on parameters that can be easily assessed by managers on the population and individual level, i.e. the numbers of animals culled in different age/sex classes and their body mass. We found that the proportion of females among calves increased with population density. Furthermore, we found that calf sex ratio (i.e. the proportion males among calves aged between 2 and 7 months) increased with increasing proportions of adult females and males older than 10 years, independent of the density effect. We conclude that wildlife managers interested in the effective reduction of red deer abundance and/or increasing the proportion of males among offspring should select a culling regime leading to a low population density dominated by adult, prime-aged females and males. This can be achieved by over-proportional removal of young females and warranting that a high number of strong males reach an age of at least 10 years.

Keywords Age · Body mass · Hunting bags · Juvenile sex ratio · Paternal effects · Trivers-Willard effect

Introduction

Despite a long history of studying offspring sex ratio variation, there is ongoing interest in this field (Cameron 2004; Gomendio et al. 2006; Hamel et al. 2016; Trivers and Willard 1973). This is especially true for ungulates in temperate zones where offspring sex ratio is an important issue in the management of game species (Clutton-Brock and Lonergan 1994). On one hand, managers are interested in maximising the number of high-quality mature males for trophy hunting (Clutton-Brock and Lonergan 1994). However, on the other

hand, there might be an interest in limiting population growth rate and thus population size effectively in order to reduce economically costly damages to forests and agriculture (Reimoser and Putman 2011), to limit the spreading of wild-life diseases (Gortázar et al. 2006), or to reduce the risk of traffic collisions with wildlife. These conflicts of interest exist in all overabundant wildlife species subject to trophy hunting.

Red deer hunting bags have increased considerably during the last decades across Europe (Fig. 1), presumably indicating increases in population size such as it happens in wild boar (*Sus scrofa*) (Apollonio et al. 2010; Vetter et al. 2015), and causing exactly the afore mentioned problems. A male-biased offspring sex ratio could support controlling population growth via reduced recruitment of females into the population and simultaneously increase the relative abundance of the trophy-bearing sex.

In some species, and also red deer, offspring sex ratios have been shown to be affected by the reproducing female as an evolutionary strategy to maximise its inclusive fitness (e.g. Landete-Castillejos et al. 2001, 2004). On the one hand, local resource competition (LRC) predicts that females which are in

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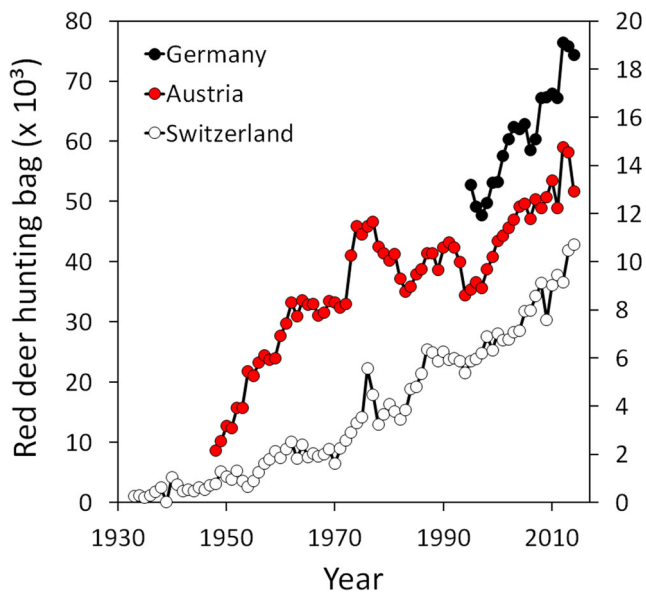
Fig. 1 Development of red deer hunting bags (in thousands) from Germany (black, left axis), Austria (red, left axis), and Switzerland (white, right axis) over most of the last century

bad shape, e.g. due to high competition or low resource availability, should produce mainly dispersing male rather than stationary female offspring in order to reduce direct competition with own offspring (Clark 1978). On the other hand, the Trivers and Willard model (TWM) predicts that in highly dimorphic and polygynous species, only females in good shape should produce male offspring. Weak mothers will produce weak male offspring with poor chances to produce grandchildren, whereas condition-dependent fitness differences between female offspring are much less pronounced (Trivers and Willard 1973). Nevertheless, these two models are not necessarily mutually exclusive (compare Landete-Castillejos et al. 2001), and both, LRC and TWM, make predictions about how female condition influences the primary sex ratio of the offspring, i.e. sex ratio at conception (Bodmer and Edwards 1960). In fact, most studies investigating offspring sex ratio in ungulates focused on potential influences of maternal traits like age, condition, or social rank (e.g. Bonenfant et al. 2004; Clutton-Brock et al. 1984; Clutton-Brock and Iason 1986; Côté and Festa-Bianchet 2001; Flint et al. 1997; Gomendio et al. 1990; Kruuk et al. 1999; Landete-Castillejos et al. 2004, 2005; Post et al. 1999; Wauters et al. 1995).

The sex of offspring, however, not only might be affected by the mother but also can be affected by the father. Only a few studies so far investigated whether male traits were associated with offspring sex ratio (white-tailed deer (*Odocoileus virginianus*): Ozoga and Verme (1985); moose (*Alces alces*): Sæther et al. (2004); reindeer: Holand et al. (2006); bighorn sheep (*Ovis canadensis*): Douhard et al. (2016)). Concerning red deer, only one study investigated paternal effects on

offspring sex ratio (Gomendio et al. 2006) and showed that male red deer bearing large antlers sire a higher proportion of sons (Gomendio et al. 2006). Whether this highly interesting effect, found in an artificial insemination experiment under very controlled conditions, also exists in free ranging and naturally reproducing red deer, however, has never been tested so far.

Finally, biased offspring sex ratios may result from differential mortality of male and female offspring, in utero and after birth, which are unrelated to maternal or paternal evolutionary strategies, but caused by environmental factors like food availability (Clutton-Brock et al. 1985; McMillen 1979). Especially in dimorphic species, the sex with the higher energetic requirements (males in red deer) might show increased mortality rates (Clutton-Brock et al. 1985). Given that primary sex ratios are impossible to measure or to infer in a natural setting (Fiala 1979), it is difficult to differentiate such sex-specific mortality from potential parental evolutionary strategies affecting offspring sex mentioned above.

In red deer, previous studies investigating effects on offspring sex ratio yielded inconsistent results. For instance, in some studies, higher population densities, which are a good indicator for per capita food availability in ungulates (Messier 1991), caused decreasing sex ratios, i.e. lower proportions of males (e.g. Kruuk et al. 1999; Mysterud et al. 2000) which would support TWM. Other studies, in contrast, found no effect (Bonenfant et al. 2004) or even a higher proportion of male offspring (Post et al. 1999), which would support LRC. This inconsistency might reflect that relative differences between mothers within a population affect offspring sex ratio more than absolute body mass or condition, as to be expected from TWM (Charnov 1982).

Another explanation for the observed inconsistent effect of the condition of mothers on offspring sex ratio is provided by Saltz and Blaustein (2001). They argue that conception, birth, and lactation occur in most ungulates in different seasons due to long gestation time and that the condition of a mother at conception, therefore, is a poor predictor for her condition during lactation, the period of highest maternal investment (e.g. Oftedal 1984, 1985; Speakman and McQueenie 1996), in contrast to age (Carrión et al. 2010; Saltz and Blaustein 2001). Thus, if mother's age is not accounted for, spurious correlations between offspring sex ratio and age-dependent factors like social rank (Clutton-Brock et al. 1982) may be found (Saltz and Blaustein 2001). In fact, several studies on ungulates identified an effect of maternal age on offspring sex ratio with older mothers producing a higher proportion of sons compared to younger females (e.g. roe deer (*Capreolus capreolus*): Wauters et al. (1995); mountain goat (*Oreamnos americanus*): Côté and Festa-Bianchet (2001); red deer: Landete-Castillejos et al. (2004)). However, other studies failed to identify such an effect (e.g. reindeer (*Rangifer tarandus*): Reimers and Lenvik (1997); fallow deer

(*Dama dama*): Birgersson (1998); red deer: Landete-Castillejos et al. (2005); mountain goat (*Oreamnos americanus*): Hamel et al. (2016)).

In our analyses of potential parameters affecting offspring sex ratio in wild red deer, we focused on measures that are easy to assess for managers, i.e. the number of animals culled in different age/sex classes and the average body mass in these classes. We thus included not only effects of females on juvenile sex ratio in our analysis but, for the first time, also tested whether males affect juvenile sex ratios in free ranging and naturally reproducing red deer. We choose Lower Austria as study area, because total juvenile sex ratio during the study period was strongly female biased, which is rather unexpected for red deer (Clutton-Brock and Iason 1986). At the same time, however, there was considerable variation in the sex ratio of calves (i.e. calves aged between 2 and 7 months) and population structure among different sub-regions and years.

Methods

Study area and data availability

Red deer hunting bag data were collected between 2004 and 2015 along with data on traffic accidents involving red deer from all hunting grounds in Lower Austria (geographical centre 48.27° N, 15.77° E) with information about sex and age class (females: juvenile, yearling, adult; males: juvenile, yearling, 2–4 years old (class III), 5–10 years old (class II), and > 10 years old (class I)). Harvested animals and road kills were classified/aged by the respective hunter retrieving the animal based on tooth replacement, eruption, and wear (Lowe 1967). For the analyses, only hunting grounds with at least 5 years of bagged red deer were considered. Due to the small size of the hunting grounds and the resulting low number of individuals shot or killed in traffic accidents annually per class and ground, the remaining hunting grounds were summarised in 37 larger, continuous areas (Fig. 2). Seven of these areas were excluded from further analyses due to small overall hunting bags (less than a total of 50 animals have been shot there per year, i.e. approximately less than 0.5 animals per 100 ha and year). Including these seven areas would have made the analysis unreliable due to low total numbers, especially when considering single classes (e.g. adult females, class I males, etc.). The resulting data set thus covered 30 areas (mean ± SD = 153.29 ± 61.60 km², Fig. 2) and 12 years. Data from one area, however, was only available for 11 years leading to a final N of 359 area-years.

Hunting in (Lower) Austria is organised via hunting grounds that are leased by one or more hunters. The number of deer to shoot in a given year and game class is applied for by the tenant(s) of the respective hunting ground every 3 years with respect to previous hunting bags (with yearling red deer being included in adult females and class III males depending on their sex). Importantly, especially for abundant age classes (i.e. calves, adult females and class III males), these numbers represent minimum numbers rather than an allowed maximum and therefore do not impose a limit on red deer hunting bags. Accordingly, numbers applied for are regularly exceeded by actual hunting bags, in 62% of all years and areas for adult females and class III males (adult females 0.82 ± 0.13 (mean ± SEM) surplus animals shot/100 ha, $t = 6.16$, $P < 0.001$; class III males 0.51 ± 0.09 surplus animals shot/100 ha, $t = 5,67$, $P < 0.001$) as well as in 59% of all years and areas for class II males (0.31 ± 0.02 surplus animals shot/100 ha, $t = 5,67$, $P < 0.001$). Only class one males were not shot more than applied for (0.01 ± 0.02 surplus animals shot/100 ha, $t = 0.96$, $P = 0.491$), likely due to the low abundance of this class. Thus, with exception of class one males, hunting bags are not limited by any legal regulations. An overview about the average number of animals hunted or killed in traffic accidents per year and area in each age class is shown in Fig. 3.

Testing the reliability of hunting bag data

The goal of the present study was to determine effects on juvenile sex ratio in Lower Austrian red deer populations based on hunting bag and roadkill data. Use of this data is only justified if it reflects changes in the abundance of single game classes within populations largely unbiased. Thus, we performed two pre-analyses before testing our main question concerning effect on juvenile sex ratio. First, in order to test whether hunting bag data reliably reflect the number of red deer as well as the calf sex ratio (i.e. proportion of male calves) present in a given area and year, we calculated separate linear mixed effects models (R-package nlme; Pinheiro et al. 2015) correlating the log-transformed hunting bag data of each game class (see below) with the respective roadkill data including area as random effect, assuming that road kills represent a random sample from the population (Mysterud 2004; compare Vetter et al. 2015 for a similar approach). This was done for the number of adult females, yearling females, class I males, class II males, and class III males, yearling males, and total number of animals (all $N = 359$), as well as for calf sex ratio ($N = 51$, Table 1). The lower sample size in the model on calf sex ratio was due to the fact that in this analysis, we considered only data points with at least three calves killed in traffic accidents in a given area and year as otherwise sex ratios calculated from roadkill data might have been highly unreliable due to chance effects. We chose this number as a compromise for obtaining reliable sex ratio estimates and a reasonable sample size, because the number of animals killed in traffic accidents was low.

Second, in order to assess the effect of hunting on red deer populations, we compared numbers from 1 year with those of

Fig. 2 Map showing the federal state of Lower Austria and in colour the 30 areas used for the analyses. The seven areas that were excluded from the analyses due to low numbers of bagged red deer (see above) are shown in dark grey, black borders indicate political districts. Areas mostly free of red deer (i.e. red deer bagged in less than five of the 12 years) are shown in light grey. We included a small map of Austria at the bottom for size comparison where Lower Austria is indicated in grey

Fig. 3 Number of **a** red deer shot per 100 ha and **b** red deer roadkills per 100 ha in Lower Austria between 2004 and 2015 averaged over years and areas for all game classes. Error bars show the standard error of the means
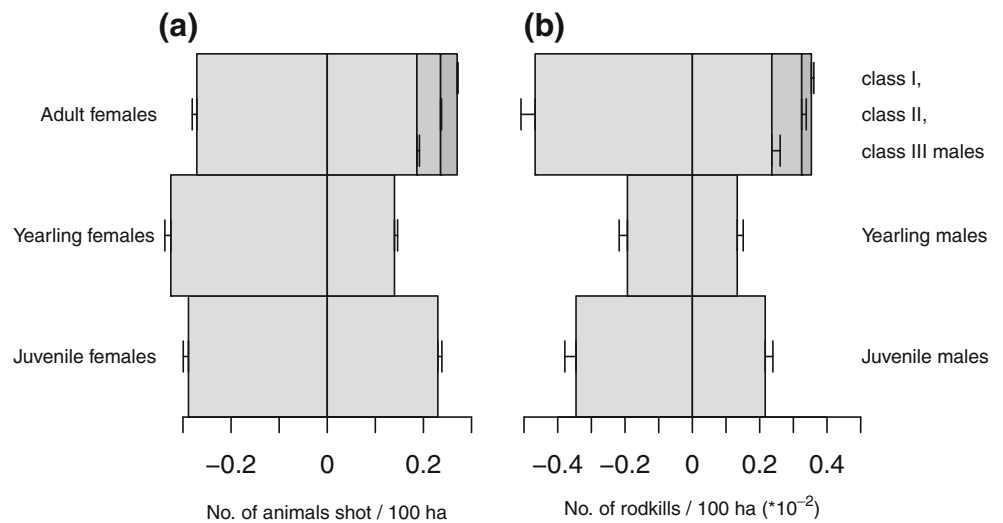
Table 1 Regressions between hunting bag and road kills for different age classes to test for testing the reliability of red deer hunting bag data from Lower Austria (2004–2015)

	Slope ± SE	t	P	N
Juvenile sex ratio	*0.113 ± 0.037*	*3.06*	*0.004*	*51*
Total no. animals	*0.011 ± 0.003*	*3.25*	*0.001*	*359*
No. adult females	*0.032 ± 0.015*	*2.14*	*0.033*	*359*
No. yearling females	*0.060 ± 0.019*	*3.19*	*0.002*	*359*
No. class I males	0.132 ± 0.072	1.83	0.068	359
No. class II males	0.023 ± 0.042	0.54	0.590	359
No. class III males	*0.064 ± 0.019*	*3.36*	*< 0.001*	*359*
No. yearling males	− 0.058 ± 0.056	− 1.04	0.299	359

Significant correlations between number of roadkills and number of shot deer are italicized

the previous year. We calculated seven linear mixed effects models with the previous year's hunting bags as predictors. For four models, we used hunting bags as the dependent variable because they reflected abundance well (total numbers, numbers of yearling and adult females, numbers of class III males, see 'Results'). For three models, we used numbers of road kills as dependent variable because hunting bags did not reflect abundance (numbers of class I, class II and yearling males, see 'Results'). For the yearling class, the hunting bag of the previous year was the number of shot calves. For female adults and class III males, previous year's hunting bags were calculated as the sum of female yearlings and female adults and the sum of male yearlings and class III males shot in the previous year, respectively. Given that in males class I, class II and class III encompass several years of age and the actual age of the shot individuals was not known; we used only the numbers of shot class I and class II males in the previous year as the previous year's hunting bags for these classes. All seven models additionally contained area as a random effect.

Standardising body mass data

In addition to sex, age class and harvest date location, hunters also delivered data on dressed body mass (i.e. body mass after removing all internal organs) from 4965 animals shot between 2013 and 2015. They noted whether the carcasses have been weighed with or without the head. Body mass of red deer is subject to seasonal variation and differs between sexes (Mitchell et al. 1976). In order to make body mass data comparable within age/sex classes despite seasonal changes and different weighing methods (i.e. with/without head), we computed separate generalised additive models (R-package mgcv (v. 1.8–7), Wood 2011) for adult females, 2 to 4-year-old males (class III; minor reproductive effort Yoccoz et al. 2002), and older males (class I and II; major reproductive effort Yoccoz et al. 2002) including a smooth spline of day

of year as fixed effect, area, year of harvest and weighing method (with vs. without head) as random effects. For modelling body mass of juveniles and yearlings, both sexes were pooled and sex was included as an additional fixed effect. Based on these models (see Supporting Information, Fig. S1), we predicted the body mass of each individual for 1st of August of the respective harvest year. This was done by adding the residual of the each data point to the value predicted by the model of the respective sex and age class at that date. In order to make body mass data comparable across different weighing methods, in the model prediction, the weighing method was set to 'with head' for all animals. Standardised body mass data were subsequently averaged for each age/sex class, area and year of harvest.

Testing effects on body mass

Although body mass was mainly intended to serve as an independent variable in the analysis of effects on offspring sex ratio (see below), we were also interested in whether body mass itself was affected by density (total number of animals shot/100 ha). Therefore, we computed a linear mixed effects model on body mass using the R-package nlme (Pinheiro et al. 2015), where we allowed different intercepts and slopes for all age/sex classes and also included area as a random effect.

Testing effects on sex ratio

Our prior analyses showed that sex ratios calculated from hunting bags reflect those calculated from a random sample (i.e. roadkill data). As much more calves are shot than killed in traffic accidents, sex ratios calculated from hunting bags are likely more accurate than those calculated from roadkill data and thus were used for further analyses. Due to gestation time and regulations concerning hunting season, all harvested calves were between 1 and 7 months of age (see also Supplementary Material, Fig. S1a). In order to identify factors affecting the sex ratio in calves, we computed a linear mixed effects model (R-package nlme; Pinheiro et al. 2015) on the percentage of male calves as a function of the previous year's (i.e. the year of mating and early foetal development) numbers of individuals of the three major reproductive classes (i.e. female adults, class I males and class II males (Clutton-Brock et al. 1982)), as well as the total number of animals shot in the previous year. Including the total number of animals harvested not only allowed testing for effects of population density (see 'Results', Table 1) but also adjusted the number of individuals from the three classes for the total number of individuals so that, for the single game classes, we in fact tested the effect of their relative abundance (i.e. their proportion) within the population. All variables were normalised to 100 ha in order to make them comparable between the areas of different sizes. Hereby, sex ratio, number of female adults and

density (i.e. variables for which the hunting bag correlated significantly with the roadkill data; Table 1) were taken from the hunting bag due to the larger sample size and thus higher accuracy of the estimate (compare Vetter et al. 2015). Because hunting bags of class I and class II males did not reliably reflect changes in abundance, as indicated by comparison with the roadkill data (Table 1), roadkill data were used as indicators of abundance of these age classes. Similar to all other analyses, area was included as a random effect in this model ($N = 329$; $n_{areas} = 30$, $n_{years} = 11(10)$). We did not include the number of class III males and yearling females in this model, as this would have decreased the quality of the model drastically due to co-linearity issues (i.e. variance inflation factors > 5). Preliminary analyses further showed that including class III males showed no significant effects on juvenile sex ratio if density was considered ($P > 0.7$). In contrast, the number of female yearlings showed a negative effect on juvenile sex ratios, reflecting the same age effect as we found with adult females (see 'Results' and 'Discussion'). R^2 statistics for the model was calculated utilising the R package piecewiseSEM (Lefcheck 2016). To test how influential very high-density populations were, we repeated this analysis excluding areas and years with a total density above three red deer shot per 100 ha, which also excluded those where more than 0.65 adult female were shot.

Body mass variables have not been included in this analysis, as body masses from animals of different classes were only available for the last 3 years. Including these body mass data would have decreased the available sample sizes for the model too much. Therefore, potential effects of previous year's female adult, class I and class II male body mass on the juvenile sex ratio, were tested in a separate linear mixed effects model (R-package nlme; Pinheiro et al. 2015), also including area as a random effect ($N = 23$).

All statistical analyses were performed in R.3.2.2 (R Core Team 2015). Inspection of the distribution of residuals of generalised linear mixed effects models by means of histograms, quantile-quantile and residual versus fitted values plots gave no evidence for serious deviations from normality (after logarithmic transformation of the response variable in the case of the models on the reliability of hunting bag data; see above). Further, we tested for temporal and spatial autocorrelation for all models calculated using the autocorrelation function (acf) and spatial and temporal variograms (R-packages gstat (Gräler et al. 2016; Pebesma 2004) and nlme (Pinheiro et al. 2015), respectively). Some models showed autocorrelation and few showed evidence for heteroscedasticity. For these models, the best correlation and/or variance structure was determined via the Akaike's Information Criterion (AICc; Akaike 1973) corrected for small sample size (Hurvich and Tsai 1989). A table with all models and their respective correlation and/or variance structure is given in the Supporting Information (Table S1).

Results

Evaluating demographic data

The calf sex ratio calculated from the hunting bag and the numbers of adult females, yearling females, class III males and the total number of animals shot were significantly correlated to the respective numbers in the roadkill data (Table 1). In contrast, this was not true for the number of class I males, class II males, and yearling males (Table 1).

Hunting bag of the previous year was significantly and positively correlated with the current hunting bag for the total bag, and bags of adult females and class III males. No significant correlation was found in yearlings of either sex as well as in class I and II males (Table 2).

Effect of population density on body mass

Density was negatively associated with body mass of all age/sex classes in Lower Austrian red deer (Fig. 4).

Effects on calf sex ratio

The calf sex ratio in Lower Austria was during the study period female biased (hunting bags mean ± SD = 44 ± 8% male juveniles, $X^2 = 388.5$, $P < 0.001$; roadkill 40 ± 22% male juveniles, $X^2 = 9.7$, $P = 0.002$) and ranged from 21 to 64% male calves in the different areas.

The proportion of male calves correlated negatively with the total number of bagged individuals per 100 ha as a measure for population density (Fig. 5a, Table 3). In contrast, the number of shot adult females per 100 ha, as a measure of the proportion of this age class in an area in the population, correlated positively with the proportion of males among calves (Fig. 5b, Table 3). Finally, we also could identify, independent from other so far found effects, a trend for an positive effect of the number of class I males involved in traffic accidents per 100 ha, the best measure

Table 2 Correlations of deer shot in each age and sex class between numbers in previous and current year for evaluating the effect of hunting on red deer in Lower Austria between 2004 and 2014

	Slope ± standard error	t	P	N
Total no. of red deer	*1.03 ± 0.01*	*121.71*	*< 0.001*	*329*
No. of yearling females	0.12 ± 0.07	1.82	0.070	329
No. of adult females	*1.03 ± 0.02*	*52.91*	*< 0.001*	*329*
No. of yearling males	0.004 ± 0.002	1.85	0.065	329
No. of class III males	*0.99 ± 0.02*	*59.32*	*< 0.001*	*329*
No. of class II males	− 0.001 ± 0.005	− 0.18	0.856	329
No. of class I males	0.003 ± 0.003	1.03	0.305	329

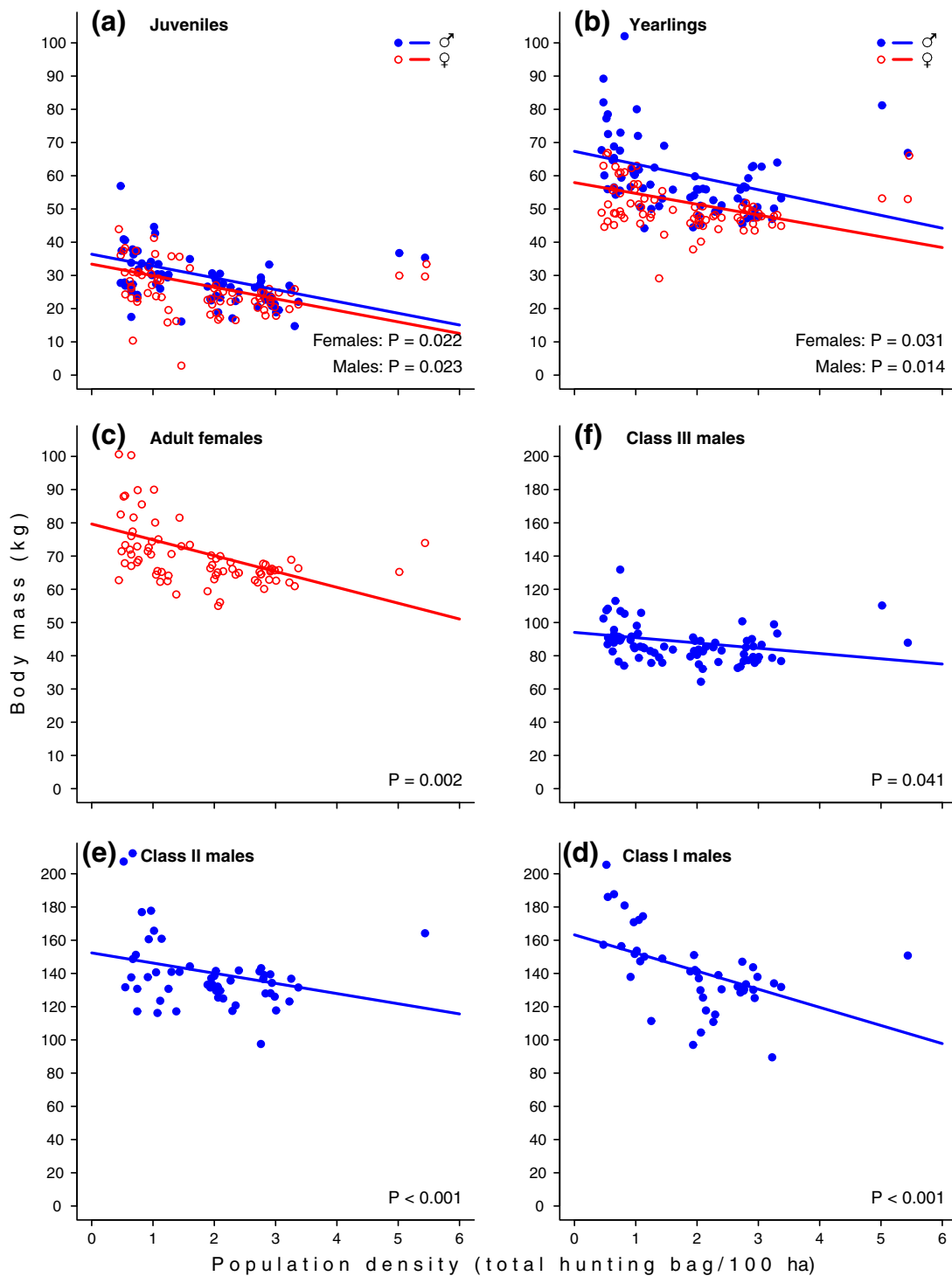Significant correlations are italicized

Fig. 4 Associations of population density (i.e. total number of red deer shot per 100 ha) with body mass of **a** juveniles, **b** yearlings, **c** adult females, **d** class I males, **e** class II males, and **f** class III males in Lower Austrian red deer populations between 2013 and 2015

for the proportion of this age class in an area, on calf sex ratio (Fig. 5c, Table 3). The amount of class II males within an area, as indicated by the number of class II males involved in traffic accidents per 100 ha, was not significantly related to calf sex ratio (Table 3). Conditional R^2 of the model was $R^2 = 0.27$.

When we excluded areas and years with total densities above three deer shot per 100 ha and adult female densities above 0.65 animals shot per 100 ha from the model, these results changed only marginally and we still found the negative effect of density (-0.05 ± 0.02; $p = 0.02$), the positive effect of the number of adult females (0.30 ± 0.10;
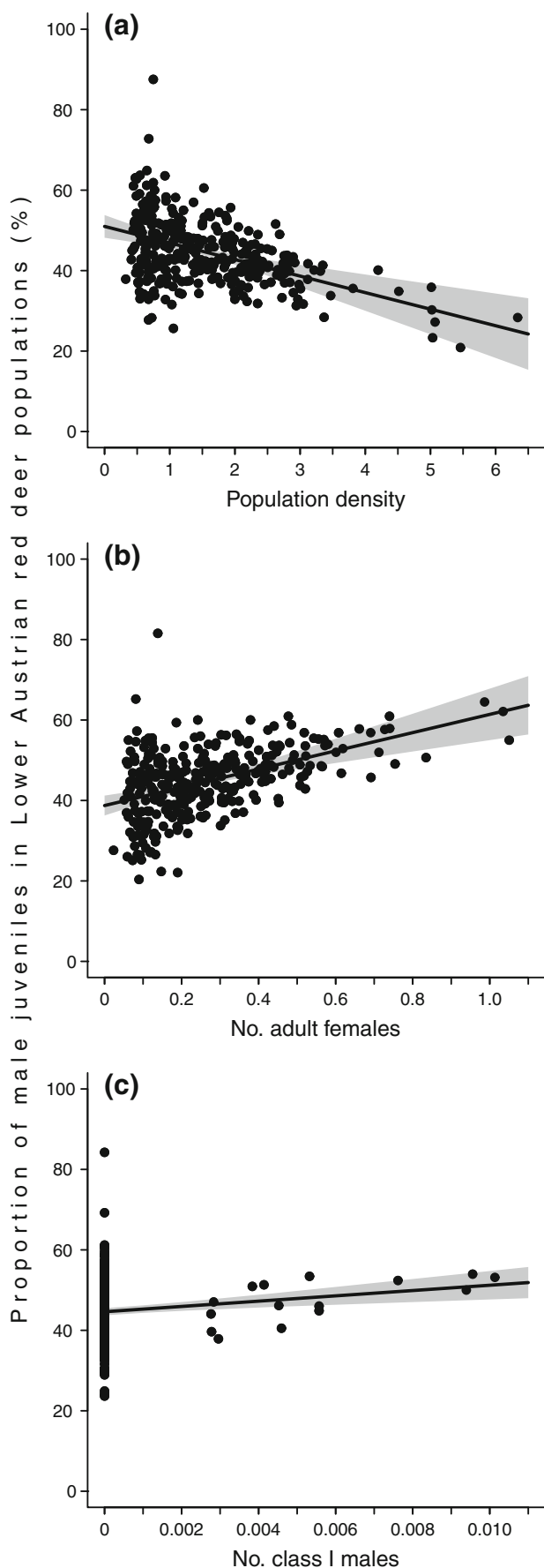
Fig. 5 Partial effects on juvenile sex ratio in Lower Austrian red deer between 2004 and 2015 by **a** population density (i.e. total number of red deer shot per 100 ha), **b** the number of adult females (i.e. number of adult females shot per 100 ha), and **c** the number of class I males (i.e. the number of class I males killed in traffic accidents per 100 ha)

$p = 0.005$) and no effect of class II males (0.77 ± 1.86, $p = 0.68$). Only the effect of class I males changed slightly and turned out to be significant now (7.30 ± 3.60, $p = 0.044$). Conditional R^2 of the reduced model was $R^2 = 0.28$.

Neither the average body mass of adult females (slope $\pm$ SE $= 1.2*10^{-3} \pm 2.6*10^{-3}$, $t = 0.45$. $P = 0.658$) nor that of class I (slope $\pm$ SE $= 6.9*10^{-4} \pm 9.3*10^{-4}$, $t = 0.32$. $P = 0.466$) or class II (slope $\pm$ SE $= -7.3*10^{-4} \pm 10.0*10^{-4}$, $t = -0.73$. $P = 0.474$) males was significantly related to calf sex ratio.

Discussion

Reliability of hunting bag data and selective hunting

Our analyses show that total hunting bags were significantly correlated to numbers of roadkills in our study areas and therefore can be considered a good proxy for the actual number of red deer present, like for the wild boar (Vetter et al. 2015). This holds true for the total hunting bag, female age classes and class III males. In contrast, not only class I and class II males, which are subject to trophy hunting and thus are likely hunted selectively, but also male yearlings, which are usually spared by hunters to judge their individual potential later in life when they became class III males, showed no such correlations between hunting bags and numbers of roadkills (Table 1).

Table 3   Results of the linear mixed effects model on calf sex ratios (i.e. proportion of male calves) in Lower Austrian red deer

	Slope ± standard error	t	P
Population density	*− 0.04 ± 0.02*	*− 2.32*	*0.020*
No. of adult females	*0.23 ± 0.09*	*2.62*	*0.010*
No. of class I males	6.59 ± 3.51	1.88	0.061
No. of class II males	0.55 ± 1.80	0.31	0.760

Explanatory variables in the model were population density (i.e. total number of bagged individuals per 100 ha), the number of adult females (i.e. number of bagged adult females per 100 ha), and class I and II males (i.e. the respective numbers of individuals killed in traffic accidents per 100 ha in these two reproductively most active classes). Given the long gestation, time-independent variables were taken from the respective previous year, i.e. the year when mating and insemination happened. Due to collinearity issues, class III males and yearling females have not been considered in this model but proved non-significant in preliminary analyses. The model had a conditional R^2 of 0.27. Significant correlations are italicized

The effects of hunting (i.e. previous year's hunting bag) on abundance of red deer (Table 2) confirmed these results. In class I, class II and yearling males as well as in yearling females, abundance was not correlated to the previous year's harvest. However, in adult females, class III males and also for the total number of individuals across all game classes, a significant link could be identified between abundance and previous year's harvest. If hunting had a major influence on red deer populations, one would expect a negative correlation between the previous year's harvest and the current abundance or harvest (i.e. the more deer were shot in a given year the fewer animals should be present the following year). Interestingly, however, the identified correlations were positive indicating that hunting in Lower Austria had no or only very little regulatory effect on the red deer population. Thus, for adult females, class II males and total abundance of red deer hunting bags can be considered as a random sample, and changes represent a good proxy for the actual population trends.

In addition to absolute numbers in different classes, also the calf sex ratio found in traffic accident data was well reflected in the hunting bag, indicating the lack of sex-specific selective hunting of calves. This is important from an analytical point of view, as hunting bags vastly exceed the numbers of road kills and thus provide better estimates of true sex ratios.

Effects of body mass and density

We found that density negatively affected body mass of all age classes in both sexes (Fig. 4). Thus, our results here match findings of previous studies showing similar effects of density on red deer body mass (e.g. Mysterud et al. 2001; Post et al. 1999) and are therefore in line with the general assumption that population density in ungulates is a good indicator for LRC (Messier 1991), which itself might affect offspring sex ratio via maternal condition (Cameron 2004).

LRC predicts that females should produce predominantly the dispersing sex (typically males) under low food availability (i.e. high densities). Our results, however, showed that population density negatively affected the proportion of males among calves (Fig. 5a). Raising a male offspring is energetically more demanding for a female red deer than raising a female offspring (Clutton-Brock et al. 1981) and, due to the higher competition between males and the resulting much higher variation in male reproductive success compared to females, a less than optimal investment in a son has more severe fitness consequences for the mother than a similarly reduced investment in a daughter (Trivers and Willard 1973). Thus, it seems reasonable to deduct that females are selected for producing a higher proportion of daughters at higher densities which indicates a reduced per capita food availability (Messier 1991). This deduction is corroborated by previous studies of red deer (e.g. Kruuk et al. 1999;

Mysterud et al. 2000), and the results presented here rather suggest an effect according to the Trivers-Willard model. Both, LRC and TWM, however, make predictions about adaptive manipulation of offspring sex ratio by the mother (Clark 1978; Trivers and Willard 1973). Clutton-Brock et al. (1985) propose a different mechanism and argue that in dimorphic species like red deer, the faster growing sex, due to higher energy requirements, is more susceptible towards food shortage. Such a mechanism affecting foetal and/or juvenile mortality differently in male and female offspring could also explain our result of increasingly female-biased offspring sex ratios with increasing population density (i.e. reduced per capita food-availability). The data presented here, however, does not allow for differentiating between effects of parental evolutionary strategies like TWM and sex-specific foetal/juvenile mortality independent of such strategies, which are also not mutually exclusive, as neither primary sex ratios nor maternal investment were assessed. Nevertheless, our analysis confirms also for Lower Austria a negative link between population density and the proportion of male calves which can be used by wildlife managers to affect red deer sex ratio.

In contrast, the average body masses of adult females, class I and class II males were in Lower Austrian red deer not associated with the sex ratio of calves. It seems likely that this lack was due to the rather small sample size, as body mass data were only available for the last three sampling years and not for all hunting areas. However, a meta-analysis on this issue indicated, first, that maternal condition should be used instead of body mass and, second, that maternal condition reliably shows an effect on the sex of the offspring in ungulates only when measured around conception but not at other times during the reproductive cycle (Cameron 2004). Body mass data in the present study, however, were not corrected for body size and collected throughout the hunting season and thus are likely too heterogeneous to allow for the identification of potential effects. Saltz and Blaustein (2001), in contrast, argue that, due to the long gestation time, maternal condition at the time of implantation of the embryo is only a poor indicator for the maternal condition during lactation, when maternal condition becomes most critical due to the high energetic demands of lactation for females. Other parameters like social rank, population density or age better predict future maternal condition and therefore should have a higher influence on offspring sex (Saltz and Blaustein 2001). Accordingly, it has been found that milk production was not affected by hind weight at mating but only by post-calving hind weight (Carrión et al. 2008) which in turn also was not affected by hind weight during mating but by age (Carrión et al. 2010). It is therefore not surprising that we did not find an effect of previous years' body mass on offspring sex ratio, although, however, potential effects on offspring sex could have been also masked by sex-specific foetal/juvenile mortality as discussed above.

Effect of female and male age

Our results show that calf sex ratios in Lower Austrian red deer were independent of the density effect described above, additionally related to the age structure of females present in an area (Fig. 5b). In populations with a higher proportion of adults among females, the calf sex ratio tended to be more male-biased. As to be expected from this result, the proportion of juveniles among females in a population correlated negatively with the proportion of males among calves (results not shown). Unfortunately, the strong negative correlation between proportions of adult and juvenile females in populations prevented testing for the independent influence of both female age classes on calf sex ratio in a single model. Studies on different ungulate species yielded contrasting result on this issue (e.g. positive effects of female age on offspring sex ratio are reported for roe deer (Wauters et al. 1995) and mountain goats (Côté and Festa-Bianchet 2001), but no such effects were found in reindeer (Reimers and Lenvik 1997) and fallow deer (Birgersson 1998)). However, the majority of studies targeting this issue specifically in red deer are in line with our results (e.g. Landete-Castillejos et al. 2004; Lowe 1969). In fact, social rank and post-calving body mass of female red deer, which is a good predictor for energy availability during lactation, are positively associated with age (Carrión et al. 2008, 2010; Clutton-Brock et al. 1986; Thouless and Guinness 1986), and female body condition is known to peak in prime age females (Clutton-Brock et al. 1982). Social rank and age both have been demonstrated to be positively associated with the probability of producing a male calf by red deer mothers multiple times (Clutton-Brock et al. 1984, 1986; Clutton-Brock and Iason 1986; Flint et al. 1997; Gomendio et al. 1990; Kruuk et al. 1999; Luna-Estrada et al. 2006). No matter how this age effect is mediated (i.e. via maternal modification of the primary sex ratio, via sex-specific maternal investment, or via an increased mortality of male calves from younger mothers with less energy available for lactation), it shows that managers could increase the proportion of male calves by fulfilling the majority of the required harvest in female classes preferentially with younger females.

Interestingly, not only maternal but also paternal age seems to be an important factor affecting juvenile sex ratio in red deer, as indicated by our finding that the proportion of males among calves increased with the proportion of class I males in an area (Fig. 5c). Although this result is in line with those from other ungulate species (reviewed in Mysterud et al. 2002), it needs to be interpreted with care. Class I males were only killed in traffic accidents in few areas and years, whereas in the majority of area-years, no class I roadkills occurred (see Fig. 5c). Due to the low number of roadkills, such data are a very rough proxy for actual abundance. There were likely some areas where no class I males have been killed in traffic accidents although there were some present in the population.

This could also explain the large variation in calf sex ration that we found in populations where no class I males were involved in traffic accidents (Fig. 5c). Nevertheless, it was also shown in moose (Sæther et al. 2004), reindeer (Holand et al. 2003, 2006), and by trend in white-tailed deer (Ozoga and Verme 1985), that older males sire a higher proportion of male offspring. And finally, also the only previous study investigating paternal effects on offspring sex ratio in red deer, Gomendio et al. (2006), showed that males with larger antlers are not only more fertile but also produce more sons, independent of the date of conception. Further, this study clearly demonstrated that the male effect on sex determination of offspring was independent of maternal influences as females had been inseminated artificially, thus excluding any potential influence of female choice. The authors attribute the identified differences in offspring sex ratios mainly to genetically inherited individual differences in male quality (Gomendio et al. 2006). Although antler size is known to be affected by age, Gomendio et al. (2006) unfortunately neither incorporated male age into their statistical models nor did they report the age of the sires. This renders it difficult to interpret their results with respect to our study. Our results suggest two potential explanations for a mother-independent effect of paternal age on offspring sex: It might have been a real age effect in the sense that class I males produced a higher proportion of sons. Alternatively, if physical strength and the tendency to produce sons are genetically linked, it might also be that this was rather an effect of competitiveness than of age, i.e. strong males always sired a higher percentage of sons, but these males were also more competitive, survived better and were more likely to become class I males. Such a genetic link could also explain the observed increase in the proportion of male calves with the increase in the proportion of class I males in an area.

Implications for managers

The unusual low proportion of male red deer calves in Lower Austria leads to an increased proportion of females in the population enhancing the growth potential of the population. This effect creates a positive feedback loop as higher population densities further reduce the proportion of male offspring born or surviving to about 6 months of age, or both. In order to break up this vicious circle, it is mandatory to reduce population density. This would not only result in an increase in the proportion of male calves but might additionally even reduce mortality also in older male classes which are known to be less efficient in extracting energy from overgrazed pastures than females and thus suffer even more from food shortage and high densities compared to female red deer (Clutton-Brock et al. 1982). Our results implicate further that the reduced population should contain a high proportion of prime-aged females and males in order to achieve a more balanced, or

even male-biased calf sex ratio. Altogether, such a culling regime would not only effectively reduce overabundant populations and the associated problems but also increase the number of trophy bearers and hence revenues from hunting.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Data accessibility The datasets analysed during the current study are available from the corresponding author on reasonable request.

References

Akaike H (1973) Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Csádki F (eds) Proceedings of the 2nd International Symposium on Information Theory. Akademiai Kiado, Budapest, Hungary, Tsahkadsor, Armenia, USSR, pp 267–281. doi:https://doi.org/10.1007/978-1-4612-1694-0_15

Apollonio M, Andersen R, Putman RJ (2010) European ungulates and their management in the 21st century. Cambridge University Press, Cambridge

Birgersson B (1998) Adaptive adjustment of the sex ratio: more data and considerations from a fallow deer population. Behav Ecol 9:404–408. https://doi.org/10.1093/beheco/9.4.404

Bodmer WF, Edwards AWF (1960) Natural selection and the sex ratio. Ann Hum Genet 24:239–244. https://doi.org/10.1111/j.1469-1809.1960.tb01735.x

Bonenfant C, Gaillard JM, Loison A, Klein F (2004) Sex-ratio variation and reproductive costs in relation to density in a forest-dwelling population of red deer (*Cervus elaphus*). Behav Ecol 14:862–869. https://doi.org/10.1093/beheco/arg077

Cameron EZ (2004) Facultative adjustment of mammalian sex ratios in support of the Trivers-Willard hypothesis: evidence for a mechanism. Proc R Soc Lond B 271:1723–1728. https://doi.org/10.1098/rspb.2004.2773

Carrión D, García AJ, Gaspar-López E, Landete-Castillejos T, Gallego L (2008) Development of body condition in hinds of Iberian red deer during gestation and its effects on calf birth weight and milk production. J Exp Zool A 309A:1–10. https://doi.org/10.1002/jez.425

Carrión D, Gaspar-López E, Landete-Castillejos T, Gallego L, García AJ (2010) Effect of reproductive rest on the subsequent breeding in Iberian red deer hinds (*Cervus elaphus hispanicus*). Mamm Biol 75:211–218. https://doi.org/10.1016/j.mambio.2010.01.007

Charnov EL (1982) The theory of sex allocation vol 18. Monographs in population biology. Princeton University Press, Princeton

Clark AB (1978) Sex ratio and local resource competition in a prosimian primate. Science 201:163–165. https://doi.org/10.1126/science.201.4351.163

Clutton-Brock TH, Iason GR (1986) Sex ratio variation in mammals. Q Rev Biol 61:339–374. https://doi.org/10.2307/2826773

Clutton-Brock TH, Lonergan ME (1994) Culling regimes and sex ratio biases in highland red deer. J Appl Ecol 31:521–527. https://doi.org/10.2307/2404447

Clutton-Brock TH, Albon SD, Guinness FE (1981) Parental investment in male and female offspring in polygynous mammals. Nature 289:487–489. https://doi.org/10.1038/289487a0

Clutton-Brock TH, Guinness FE, Albon SD (1982) Red deer. Behavior and ecology of two sexes. Wildlife behavior and ecology. Edinburgh University Press, Edinburgh

Clutton-Brock TH, Albon SD, Guinness F (1984) Maternal dominance, breeding success and birth sex ratios in red deer. Nature 308:358–360. https://doi.org/10.1038/308358a0

Clutton-Brock TH, Albon SD, Guinness FE (1985) Parental investment and sex differences in juvenile mortality in birds and mammals. Nature 313:131–133. https://doi.org/10.1038/313131a0

Clutton-Brock TH, Albon D, Guiness FE (1986) Great expectations: dominance, breeding success and offspring sex ratios in red deer. Anim Behav 34:460–471. https://doi.org/10.1016/S0003-3472(86)80115-4

Côté SD, Festa-Bianchet M (2001) Offspring sex ratio in relation to maternal age and social rank in mountain goat (*Oreamnos americanus*). Behav Ecol Sociobiol 49:260–265. https://doi.org/10.1007/s002650000301

Douhard M, Festa-Bianchet M, Coltman DW, Pelletier F (2016) Paternal reproductive success drives sex allocation in a wild mammal. Evolution 70:358–368. https://doi.org/10.1111/evo.12860

Fiala KL (1979) On estimating the primary sex ratio from incomplete data. Am Nat 115:442–444 http://www.jstor.org/stable/2460728

Flint APF, Albon SD, Jafar SI (1997) Blastocyst development and conceptus sex selection in Red Deer, *Cervus elaphus*: studies of a free-living population on the isle of rum. Gen Comp Endocrinol 106:374–383. https://doi.org/10.1006/gcen.1997.6879

Gomendio M, Clutton-Brock TH, Albon SD, Guinness FE, Simpson MJ (1990) Mammalian sex-ratios and variation in costs of rearing sons and daughters. Nature 343:261–263. https://doi.org/10.1038/343261a0

Gomendio M, Malo AF, Soler AJ, Fernandez-Santos MR, Esteso MC, Garcia AJ, Roldan ERS, Garde J (2006) Male fertility and sex ratio at birth in Red Deer. Science 314:1445–1447. https://doi.org/10.1126/science.1133064

Gortázar C, Acevedo P, Ruiz-Fons F, Vicente J (2006) Disease risks and overabundance of game species. Eur J Wildl Res 52:81–87. https://doi.org/10.1007/s10344-005-0022-2

Gräler B, Pebesma E, Heuvelink G (2016) Spatio-temporal interpolation using gstat. R J 8:204–218

Hamel S, Festa-Bianchet M, Côté SD (2016) Offspring sex in mountain goat varies with adult sex ratio but only for mothers in good condition. Behav Ecol Sociobiol 70:123–132. https://doi.org/10.1007/s00265-015-2031-9

Holand Ø, Røed KH, Mysterud A, Kumpula J, Nieminen M, Smith ME
(2003) The effect of sex ratio and male age structure on reindeer
calving. J Wildl Manag 67:25–33. https://doi.org/10.2307/3803058

Holand Ø, Mysterud A, Røed KH, Coulson T, Gjøstein H, Weladji RB,
Nieminen M (2006) Adaptive adjustment of offspring sex ratio and
maternal reproductive effort in an iteroparous mammal. Proc R Soc
B 273:293–299. https://doi.org/10.1098/rspb.2005.3330

Hurvich CM, Tsai C-L (1989) Regression and time series model selection
in small samples. Biometrika 76:297–307. https://doi.org/10.1093/
biomet/76.2.297

Kruuk LEB, Clutton-Brock TH, Albon SD, Pemberton JM, Guinness FE
(1999) Population density affects sex ratio variation in red deer.
Nature 399:459–461. https://doi.org/10.1038/20917

Landete-Castillejos T, Garcia A, Langton S, Inglis I, Gallego L, Garde J
(2001) Opposing offspring sex ratio variations with increasing age
and weight in mouflon mothers (Ovis musimon). Acta Vet Hung 49:
257–268. https://doi.org/10.1556/004.49.2001.3.1

Landete-Castillejos T, Gortázar C, Vicente J, Fierro Y, Garcia A, Gallego
L (2004) Age-related foetal sex ratio bias in Iberian red deer (Cervus
elaphus hispanicus): are male calves too expensive for growing
mothers? Behav Ecol Sociobiol 56:1–8. https://doi.org/10.1007/
s00265-004-0764-y

Landete-Castillejos T, García A, López-Serrano FR, Gallego L (2005)
Maternal quality and differences in milk production and composi-
tion for male and female Iberian red deer calves (Cervus elaphus
hispanicus). Behav Ecol Sociobiol 57:267–274. https://doi.org/10.
1007/s00265-004-0848-8

Lefcheck JS (2016) piecewiseSEM: piecewise structural equation model-
ling in r for ecology, evolution, and systematics. Methods Ecol Evol
7:573–579. https://doi.org/10.1111/2041-210X.12512

Lowe VPW (1967) Teeth as indicators of age with special reference to
Red deer (Cervus elaphus) of known age from Rhum. J Zool (Lond)
152:137–153. https://doi.org/10.1111/j.1469-7998.1967.tb01881.x

Lowe VPW (1969) Population dynamics of the Red Deer (Cervus
elaphus L.) on Rhum. J Anim Ecol 38:425–457. https://doi.org/10.
2307/2782

Luna-Estrada AA, Vera-Avila HR, Mora O, Anguiano-Serrano B,
Vasquez-Pelaez CG, Shimada A (2006) Effect of pre-mating nutri-
tional status in red deer (Cervus elaphus scoticus) hinds on the sex
ratio of their offspring. Small Rum Res 65:154–160. https://doi.org/
10.1016/j.smallrumres.2005.05.039

McMillen MM (1979) Differential mortality by sex in fetal and neonatal
deaths. Science 204:89–91. https://doi.org/10.1126/science.571144

Messier F (1991) The significance of limiting and regulating factors on
the demography of moose and white-tailed deer. J Anim Ecol 60:
377–393. https://doi.org/10.2307/5285

Mitchell B, McCowan D, Nicholson IA (1976) Annual cycles of body
weight and condition in Scottish Red deer, Cervus elaphus. J Zool
(Lond) 180:107–127. https://doi.org/10.1111/j.1469-7998.1976.
tb04667.x

Mysterud A (2004) Temporal variation in the number of car-killed red
deer Cervus elaphus in Norway. Wildl Biol 10:203–211

Mysterud A, Yoccoz NG, Stenseth NC, Langvatn R (2000) Relationships
between sex ratio, climate and density in red deer: the importance of
spatial scale. J Anim Ecol 69:959–974. https://doi.org/10.1111/j.
1365-2656.2000.00454.x

Mysterud A, Yoccoz NG, Stenseth NC, Langvatn R (2001) Effects of age,
sex and density on body weight of Norwegian red deer: evidence of
density–dependent senescence. Proc R Soc B 268:911–919. https://
doi.org/10.1098/rspb.2001.1585

Mysterud A, Coulson T, Stenseth NC (2002) The role of males in the
dynamics of ungulate populations. J Anim Ecol 71:907–915. https://
doi.org/10.1046/j.1365-2656.2002.00655.x

Oftedal OT (1984) Milk composition, milk yield and energy output at
peak lactation: a comparative review. In: Peaker M, Vernon RG,
Knight CH (eds) Physiological strategies in lactation, vol 51.
Symposia of the Zoological Society of London. Academic Press,
London, pp 33–85

Oftedal OT (1985) Pregnancy and lactation. In: Hudson RJ, White RG
(eds) Bioenergetics of wild herbivores. CRC, Boca Raton, pp 215–
238

Ozoga JJ, Verme LJ (1985) Comparative breeding behavior and perfor-
mance of yearling vs prime-age white-tailed bucks. J Wildl Manag
49:364–372. https://doi.org/10.2307/3801533

Pebesma EJ (2004) Multivariable geostatistics in S: the gstat package.
Comput Geosci 30:683–691. https://doi.org/10.1016/j.cargo.2004.
03.012

Pinheiro J, Bates D, DebRoy S, Sarkar D, and the R Development Core
team (2015) nlme: Linear and Nonlinear Mixed Effects Models (R
Package Version 3.1–122). http://CRAN.R-project.org/
package=nlme

Post E, Forchhammer MC, Stenseth NC, Langvatn R (1999) Extrinsic
modification of vertebrate sex ratios by climatic variation. Am Nat
154:194–204. https://doi.org/10.1086/303224

R Core Team (2015) R: a language and environment for statistical com-
puting. R Foundation for Statistical Computing, Vienna. http://
www.R-project.org/

Reimers E, Lenvik D (1997) Fetal sex ratio in relation to maternal mass
and age in reindeer. Can J Zool 75:648–650. https://doi.org/10.1139/
z97-082

Reimoser F, Putman R (2011) Impacts of wild ungulates on vegetation:
costs and benefits. In: Putman R, Apollonio M, Andersen R (eds)
Ungulate management in Europe: problems and practices.
Cambridge University Press, pp 144–191. doi:https://doi.org/10.
1017/CBO9780511974137.007

Sæther BE, Solberg EJ, Heim M, Stacy JE, Jakobsen KS, Olstad R (2004)
Offspring sex ratio in moose Alces alces in relation to paternal age:
an experiment. Wildl Biol 10:51–57

Saltz D, Blaustein J (2001) Progeny sex ratio variation in ungulates:
maternal age meets environmental perturbation of demography.
Oikos 94:377–384. https://doi.org/10.1034/j.1600-0706.2001.
940220.x

Speakman JR, McQueenie J (1996) Limits to sustained metabolic rate:
the link between food intake, basal metabolic rate, and morphology
in reproducing mice, Mus musculus. Physiol Zool 69:746–769
http://www.jstor.org/stable/30164228

Thouless CR, Guinness FE (1986) Conflict between red deer hinds: the
winner always wins. Anim Behav 34:1166–1171. https://doi.org/10.
1016/S0003-3472(86)80176-2

Trivers RL, Willard DE (1973) Natural selection of parental ability to
vary the sex ratio of offspring. Science 179:90–92. https://doi.org/
10.1126/science.179.4068.90

Vetter SG, Ruf T, Bieber C, Arnold W (2015) What is a mild winter?
Regional differences in within-species responses to climate change.
PLoS One 10:e0132178. https://doi.org/10.1371/journal.pone.
0132178

Wauters LA, de Crombrugghe SA, Nour N, Matthysen E (1995) Do
female roe deer in good condition produce more sons than daugh-
ters. Behav Ecol Sociobiol 37:189–193. https://doi.org/10.1007/
BF00176716

Wood SN (2011) Fast stable restricted maximum likelihood and marginal
likelihood estimation of semiparametric generalized linear models. J
R Statist Soc B 73:3–36. https://doi.org/10.1111/j.1467-9868.2010.
00749.x

Yoccoz NG, Mysterud A, Langvatn R, Stenseth NC (2002) Age- and
density-dependent reproductive effort in male red deer. Proc R Soc
B 269:1523–1528. https://doi.org/10.1098/rspb.2002.2047