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Short communication

Is reproductive strategy of Alpine mountain hares adapted to different elevations?

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ABSTRACT

In *Lepus*, litter size is inversely related to the duration of the reproductive season resulting in a consistent yearly production of around ten leverets per female. In high latitudes, animals have few litters with large litter sizes, whereas in low latitudes, several litters with small litter sizes are common per year. Knowledge on reproductive performance of Alpine mountain hares (*Lepus timidus varronis*) is scarce. In this study, we analysed 89 hares from Grisons, Switzerland, by examining placental scars and eye lens weight. The general aim of the survey was to examine the reproductive performance of female Alpine mountain hares. In particular, we focused on the question whether this subspecies adjusts the reproductive strategy in relation to elevation such as other *Lepus* species do in relation to latitude. All adults of our sample reproduced. 39% of the females littered twice and 61% three times a year with a median litter size of 3.00. We identified a significant effect of elevation on litter size, whereas the elevation did not influence the number of litters. We found no significant difference of yearly reproductive output across elevation range. Hence, some reproductive parameters seem to indicate that the Alpine mountain hare changes the reproductive strategy in relation to elevation similar to the mountain hares living further north do in relation to latitude.

Due to their wide distribution across the globe (Schai-Braun and Hackländer, 2016), hares (genus *Lepus*) are a suitable model taxon to study effects of latitude, elevation, or climatic conditions on reproductive performance. Litter size in the genus *Lepus* is inversely related to the length of the breeding season resulting in a persistent annual production of about ten leverets per female in almost all species (Flux, 1981). Only one litter per year is feasible in the extreme north and the litter size ranges between six and seven (e.g. *Lepus othus* Anderson and Lent, 1977; *Lepus arcticus* Parker, 1977). The breeding season encompasses around six months with 3–4 litters and an average litter size of 2–5 leverets at mid latitudes (e.g. *Lepus europaeus* Flux, 1965; *Lepus americanus* Keith et al., 1966). Breeding lasts throughout the year, and eight litters are produced with 1–2 young each near the equator (*Lepus capensis* Flux, 1969). The breeding season is shorter and litter size is larger at high elevation in snowshoe hare (*Lepus americanus*) populations inhabiting the Central Rocky Mountains (Dolbeer and Clark, 1975). Hence, the snowshoe hare seems to apply a similar reproductive strategy in areas at high elevation and high latitude. Within the reproductive season, litter size in the genus *Lepus* increases from the first litter and declines towards the last litter (Flux, 1981).

In this study, we use Alpine mountain hares (*Lepus timidus varronis*) as a model to evaluate the effects of elevation and climatic conditions (the combination of both mimicking latitudinal effects) on the reproductive performance within one species.

The reproductive biology of mountain hares has been addressed by many studies. Mountain hare females have predominantly 2–3 litters annually (Angerbjörn, 1986; Höglund, 1957; Kauhala et al., 2005; Pehrson and Lindlöf, 1984) resulting in 5–8 leverets per female during one reproductive period (Flux, 1970; Kauhala et al., 2005). The start of breeding varies with temperature (Angerbjörn, 1986; Myrberget, 1983) and differences in reproduction exist between subspecies. The general goal of this study was to investigate the reproductive performance (measured by litter size and number of litters during one reproductive season) of female Alpine mountain hares under different conditions. Our hypotheses were: (1) litter size increases and number of litters decreases at higher elevations resulting in a constant yearly reproductive output independent of elevation; (2) altitudinal effects on the reproductive performance are affected by other environmental factors such as climatic conditions; and (3) litter size varies seasonally with the first litter being smaller than the second litter, and the third litter

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being smaller than the second litter. We tested these hypotheses by examining placental scars and eye lens weight of female Alpine mountain hares collected in the canton Grisons.

We collected 89 female Alpine mountain hares during the hunting periods in October and November 2003–2005 in the canton Grisons, Switzerland. This canton is located entirely in the Alpine region, and is biogeographically particularly rich encompassing three of the six existing biogeographical regions in Switzerland (Gonseth et al., 2001).

Eyeballs were stored in 10% formalin solution for further age determination (Suchentrunk et al., 1991) and uteri were frozen at −18 °C until further analysis of placental scars (Bray et al., 2003). Hunters recorded the elevation at the spot the hare was shot. Moreover, the body weight of every hare was measured to the nearest 5 g. The biogeographical region was determined according to the classification of each municipality by the Swiss Federal Office for the Environment (Gonseth et al., 2001).

Eye lenses were removed from the eyeballs and dried at 100 °C. Lenses were weighed to the nearest 0.1 mg after 24 h (Suchentrunk et al., 1991). We plotted the frequencies of dried eye lens weights to distinguish between young of the year and older females (Kauhala and Soveri, 2001). We assumed that dried eye lens weight increases with female age although no age groups can be classified within adult females (Broekhuizen and Maaskamp, 1979; Suchentrunk et al., 1991). In case of damaged eyeballs, hares were classified as adults when Stroh's sign (Stroh, 1931) was not detectable (n = 9).

We determined the number and age of placental scars according to Bray et al. (2003) to quantify the number of litters and litter sizes of mountain hares. The uteri were immersed for eight minutes in a 10% solution of ammonium sulphide for staining and then rinsed out under tap water. In a second step, the uteri were soaked for eight minutes in a solution consisting of a 20% potassium hexacyanoferrate solution and a 1% chloric acid solution in equal ratio. After that, the uteri were rinsed out again under tap water. The duration of the staining process was reduced from ten to eight minutes in contrast to Bray et al. (2003) because of the darker tint of the uteri (Hackländer et al., 2001). The uteri were put under a 6–16 x magnification zoom binocular microscope after the staining and examined for scars. Scars and their age were grouped into litters according the intensity of staining colour, visibility of the crater, of the antimesometrial depression and the two black bands (Bray et al., 2003). The analysis of the scars was made independently by JG and KH.

All analyses were computed with the software R 3.3.0 (R Core Team, 2016). We analysed each response variable by linear mixed-effects modelling using the package lme4 (Bates et al., 2015). Because no subadult (young of the year) female had delivered leverets, the response variables number of litters, litter size and yearly reproductive output were investigated in adult animals only with a full model including the covariates elevation and biogeographical region (3 levels). The response variable litter size was further analysed by a model including the covariate season (1st vs. 2nd and 1st vs. 2nd vs. 3rd litter) for individuals having delivered twice and three times separately. All models included year as random factor in order to account for the different years of the study. Moreover, the model comprising the covariate season included hare identity as random factor in order to allow paired testing for the different litter sizes of a female during the reproductive season. The full models were used to create a set of models with all combinations of the independent variables using the package MuMln (Bartoń, 2016). *P*-values and estimates (β) were extracted by model averaging (including all models delta AIC < 10). Residuals of the full models were checked for normal distribution by QQ-plots and histograms. Post-hoc tests were computed for the covariate season using the Tukey's all-pair comparisons method of the package multcomp (Hothorn et al., 2008). We visually checked normality of the

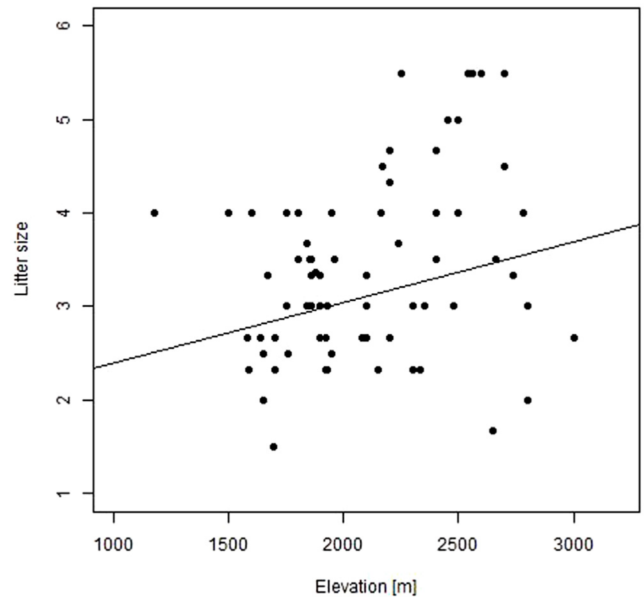

Fig. 1. Relationship between Alpine mountain hares' litter size and elevation with the regression line drawn from the model summary (n = 74; statistically significant). See text for details of statistics.

model residuals by a normal probability plot. The homogeneity of variances and goodness of fit were examined by plotting residuals versus fitted values (Faraway, 2006).

We initially included interaction effects in all models tested. However, since there were never any significant interaction effects in our linear mixed-effects models ($p > 0.10$), these factors were omitted from the models before re-calculation.

Mountain hares were collected within an altitudinal range of 1180–3000 m a.s.l. (median = 2100 m, SD = 383.67 m; Fig. A.1). The distribution of the eye lens weights showed two pinnacles (max = 408 mg, min = 91 mg, median = 267 mg, SD = 71.81 mg; Fig. A.2). We made the division between subadult and adults at the gap between the two pinnacles, i.e. at 210 mg. From the 89 Alpine mountain hares, 75 were adults and 14 subadults. Every adult doe had leverets during the previous reproductive season, whereas no subadult had been reproducing, i.e. had no placental scars. Hence, the number of litters was dependent on whether the female was subadult or adult.

The number of offspring born over the period of a year ranged from 3 to 14 young with an average of 8.59 (SD 2.22) young per adult female (Fig. A.3). We found no significant effect of elevation on number of litters or yearly reproductive output, resp. (each $p > 0.10$; Table A.1). Nevertheless, we detected a significant influence of elevation on litter size indicated by the regression line drawn with the values form the model summary ($p = 0.07$; $\beta = 0.001$, Fig. 1). Moreover, we found no significant effect of biogeographical region on the number of litters, litter size, or yearly reproductive output, resp. (each $p > 0.10$).

The litter size ranged from 1 to 7 leverets with a median of 3.00 (SD 1.47) leverets per litter and year (Fig. A.5a). Females had two (39%) or three (61%) litters per year. Mean litter size per female and year varied from 1.5 to 5.5 leverets (median = 3.33, SD = 0.95; Fig. A.5b). For females with two litters per year (n = 28), the litter size was not dependent on the season with a median of 4.0 (SD 1.6) leverets in the first, and 4.0 (SD 1.4) in the second litter ($p > 0.10$; Fig. 2). However for females having delivered three times per year (n = 46), the litter size depended significantly on the season with a median of 2.0 (SD 1.3) leverets in the first, 4.0 (SD 1.4) in the second, and 3.0 (SD 1.1) in the third litter (2nd Litter − 1st Litter: