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On eye lens weights and other age criteria of the Brown hare (*Lepus europaeus* Pallas, 1778)

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Abstract

Described the eye lens growth of 42 age-known brown hares (*Lepus europaeus*) by regression models including confidence limits. In addition, lens weights of 369 free-living specimens from the wild were compared with three other indicators of age: "Stroh-sign" i.e. epiphyseal protrusion of the ulna, flexibility of processus lacrimalis and degree of ossification of skull sutures. Due to the slow growth rate and the high individual variability of lens weights in adults estimation of years of age is not possible, but segregating juveniles and adults can be achieved by determination of eye lens weights. Furthermore, within juvenile hares eye lens weights serve for estimation of the date of birth. The "Stroh-sign" appears to be only a crude indicator for separating juveniles from adults. Both the processus lacrimalis and the state of ossification of skull sutures are of no value for a reliable discrimination of juvenile and adult hares.

Introduction

Among the various methods proposed for ageing brown hares (STROH 1931; KLEYMANN and SCHNEIDER 1974; FRYLESTAM and V. SCHANTZ 1977; HABERMEHL 1985) the determination of dry eye lens weights has been reported to be sensible enough for the discrimination of juveniles (i.e. young of the year or individuals not older than 9–10 months of age) and adult hares (CABOŃ-RACZYŃSKA and RACZYŃSKI 1972; PEPIN 1974). In adults years of age can be assessed with sufficient accuracy by counting the annual periosteal adhesion lines of the mandible (FRYLESTAM and V. SCHANTZ 1977; PASCAL and KOVACS 1983). In juveniles periods of births have been estimated roughly by PEGEL (1986) using the eye lens growth curve presented by PEPIN (1974), which was constructed according to an equation based on a non-linear regression of eye lens weight on real age of hares. However, the appropriate solution for ageing by using eye lens weights requires a regression of real age on eye lens weights, thereby minimizing variation of age (comp. e.g. MYERS and GILBERT 1968 for wild rabbits, *Oryctolagus cuniculus*; DAPSON 1980). Moreover, PEPIN's growth curve does not include confidence intervals as recommended by DAPSON (1980). The reference curve for ageing brown hares by means of eye lens weights published by BROEKHUIZEN and MAASKAMP (1979) has been fitted by eye.

The main objectives of this paper are 1. to give a new non-linear regression model for the growth of eye lenses based on age-known brown hare individuals, 2. to present both the curves and the confidence limits of the regression of age on eye lens weight and 3. to evaluate the reliability of various other methods for ageing brown hares by comparison with ages as indicated by dry eye lens weights, respectively.

Material and methods

From a total of 411 hares presently used 359 individuals were shot by hunters in the course of regular hunts in autumn 1988 in different parts of Austria covering a wide range of environmental conditions

(comp. HARTL et al. 1989). Further 10 specimens were obtained during an extra hunt in summer 1989 in the region of "Marchfeld" (approx. 30 km east of Vienna). In addition, a sample of 42 individuals was taken from the brown hare breed at the Forschungsinstitut für Wildtierkunde und Ökologie (Vet. Med. University) in Vienna. The latter specimens were ear-tagged animals maintained in cages and fed commercial pellets *ad libitum*; their exact days of birth were known.

All animals were sexed by inspection of internal reproductive organs. Palpation of the lateral ulnar knob close to the carpal joint (epiphyseal protrusion – "Stroh's sign" of juvenile individuals; comp. STROH 1931) has been carried out on one forelimb and repeated on the other one whenever the first check gave a negative result.

In 167 heads of hares obtained from hunters the praeorbital lateral processus lacrimalis had been proved for flexibility by palpation through the pelt before eyeballs were removed. The processus lacrimalis is supposed to remain flexible in juveniles until sufficient ossification has occurred; thus it may serve as a criterion to discriminate juveniles and adults (HABERMEHL 1985). In 172 hares stemming from the various hunting areas both eye balls were removed within 30 minutes to 6 hours after death. The eyeballs of the 42 cage-reared individuals were removed immediately after death. There was no predetermined schedule for sacrificing cage-reared hares for the purpose of the present study. Other individuals ($n = 197$) were either stored cool at 4 °C for variable time (up to 10 days) or frozen at -20 °C for 1 to 2 months before eye balls were removed. Fixation of total eye balls was performed in 10% formalin for 2 months. Desiccation of the lenses of 344 individuals was carried out in a normal air pressure oven ("Heraeus type TU HO") at 100 °C for 24 hours solely. The lenses of 55 hares were weighed after drying for 24 hours and 48 hours, respectively. Weighing of a further sample of 58 lenses from 35 hares was done after 24, 48, 72, 96, 120 und 161 hours of desiccation. Weighing was performed on an analytical balance to the nearest 0.1 mg. The weights of 396 lenses were determined immediately after drying for 24 hours and once again after having been left for cooling off at normal room temperature for 15–30 minutes. In 269 specimens age category was determined according to the ossification of skull sutures following the scheme provided by CABOŃ-RACZYŃSKA (1964) and additionally after a slightly modified scale. For all statistical tests significance was considered at the 0.05% level of probability if not mentioned otherwise.

Results

The drying process and intraindividual variation

The course of reduction of lens weights during 161 hours of desiccation is shown in Figure 1. The average increase of dry weights of lenses permitted to cool off for 15–30 minutes

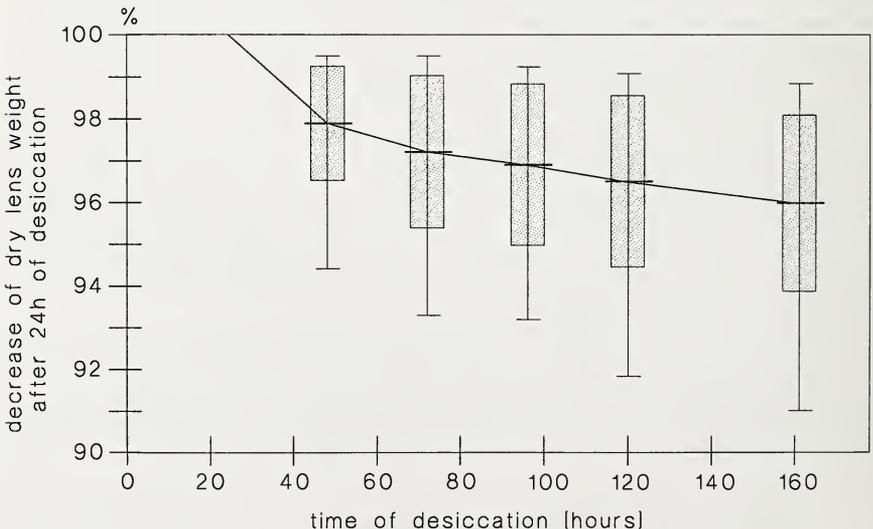


Fig. 1. Reduction of eye lens weights of Brown hares ($n = 58$ lenses from 35 specimens). Lens weights are given in % of the respective weights after 24 h of drying. Mean, standard deviation, minimum and maximum is given for weights gained after 48, 72, 96, 120 and 161 h, respectively

after removing from the oven was $\bar{x} = 0.3$ mg (s.d. = 0.31, min. = -0.6, max. = 1.6, n = 396 lenses after drying for 24 hours). The intraindividual differences (right vs. left) of dry lens weights were found to be $\bar{x} = 7.45$ mg (s.d. = 6.6, min. = 0.0, max. = 23.0, n = 42 lenses dried for 24 hours), $\bar{x} = 5.1$ mg (s.d. = 4.3, min. = 0.0, max. = 16.2, n = 42 lenses dried for 48 hours) and $\bar{x} = 3.4$ mg (s.d. = 2.9, min. = 0.0, max. = 12.7, n = 42 lenses dried for 161 hours). Weights of lighter lenses of pairs averaged $\bar{x} = 1.22\%$ (s.d. = 1.0, max. = 3.9, min. = 0.0) less than the heavier ones, respectively, after drying for 161 hours. In 82.6% of lens pairs that lens which had been the heavier one of both after 24 hours of drying remained to be the heavier one of both after 48 hours of drying remained to be the heavier one throughout the total period of desiccation (161 hours). Between the dry lens weights (DLW) gained after 24 hours (DLW₂₄) and 48 hours (DLW₄₈) of desiccation a very strong correlation ($R^2 = 0.9999$, n = 26) was found; the linear regression of DLW₄₈ on DLW₂₄ (DLW₄₈ = 0.975 × DLW₂₄) including the 95% confidence intervals is shown in Figure 2.

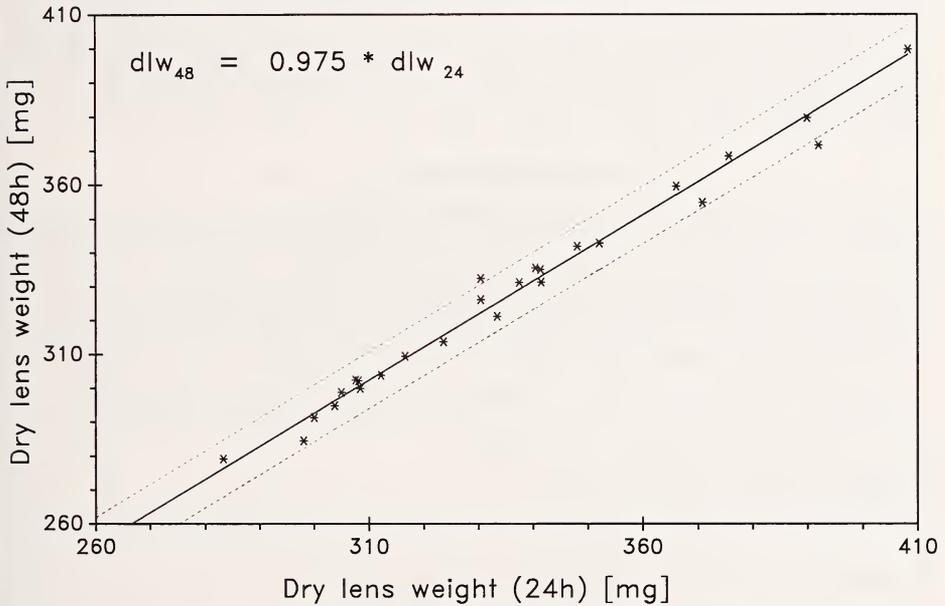


Fig. 2. Relationship of lens weights gained after 24 and 48 h of drying, respectively. Dotted lines indicate 95% confidence range

Accordingly further analyses concerning dry lens weights were performed on the basis of DLW₂₄. Furthermore, any individual dry lens weight was calculated as the arithmetic mean of both lenses, with few exceptions in cases of damages of one eye.

Eye lens growth and the ageing curve

In the literature (e.g. MYERS and GILBERT 1968; CONNOLLY et al. 1969) normally transformations of $x = \frac{a}{\text{Age} + b}$ and $y = \log(\text{DLW})$ are found to be applied to make their relations linear. The parameters a and b are evaluated by testing several values and using the best. Trying a non-linear approach by optimizing these parameters using a gradient method (comp. HARTLEY 1961) no convergence to an optimal solution was found

presently. All results proved inferior to using logarithmic transformations for age and DLW_{24} . Retransforming the results of the linear regression yields the representations:

$$DLW_{24} \text{ (mg)} = 29.76 \times \text{age (days)}^{0.3721}, R^2 = 0.9582 \text{ for all age-known specimens (n = 42),}$$

$$DLW_{24} \text{ (mg)} = 23.11 \times \text{age}^{0.4326}, R^2 = 0.9932 \text{ and}$$

$$\text{age (days)} = 0.0007646 \times DLW_{24} \text{ (mg)}^{2.2958}, R^2 = 0.9932 \text{ for individuals younger than 454 days of age (n = 20).}$$

Growth of eye lenses of 42 pen-raised brown hare specimens is characterized by means of the regression curve in Figure 3. There was no indication of different growth of lenses in respect of the sex (comp. PEPIN 1974). According to the confidence limits in Figure 3 interindividual variation in eye lens weights increased especially from the second year of life onward, after main growth has been already accomplished. In Figure 4 the relationship of real age and DLW_{24} calculated separately for the first 453 days of life (20 individuals), to reduce the 95 % confidence interval during the main growth period, is presented. To enable direct estimation of age by using DLW_{24} , the equation and the curve including the 95 % confidence limits of the regression calculation of age on DLW_{24} based on the same 20 individuals is presented in Figure 5. In addition, estimated ages and respective lower and upper confidence limits are given in Table 1 for selected DLW_{24} (as recommended by DAPSON 1980).

Eye lens weights and other age indicators

In Figure 6 the frequency distribution of DLW_{24} of hares collected during the autumnal hunting season and the respective percentage of individuals exhibiting a "Stroh-sign" within each DLW_{24} -class are presented. In both sexes hares without a "Stroh-sign" occurred first in the DLW_{24} -class 185–195 mg (see Fig. 6), indicating an age of 123 (97–155 conf. range) days (comp. Fig. 5). Within the DLW_{24} -class 275–285 mg (317 days of age, 237–425 conf. range; comp. Fig. 5) no hare showed a "Stroh-sign"; however, few animals

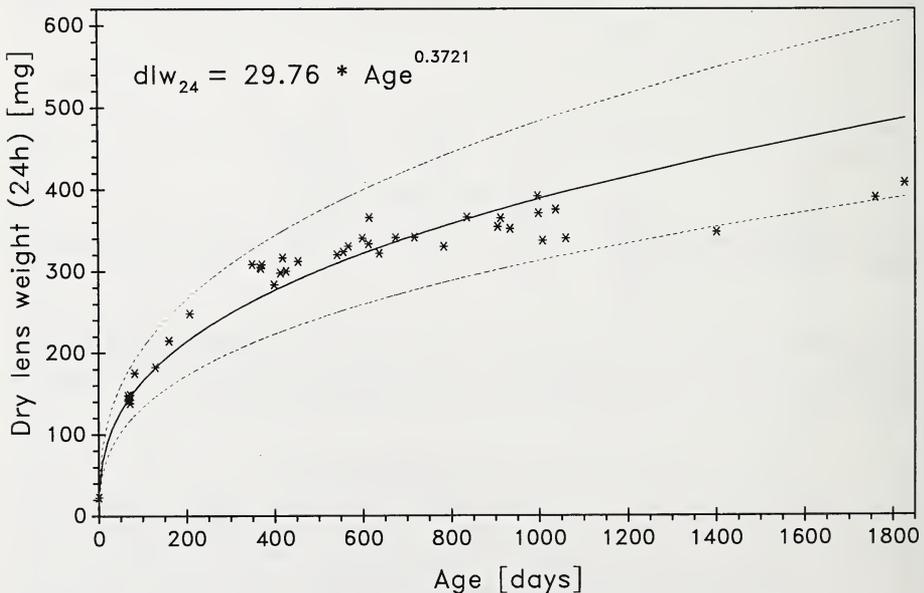


Fig. 3. Growth curve of age-known pen-reared Brown hares (n = 42 specimens). Dotted lines indicate 95 % confidence range

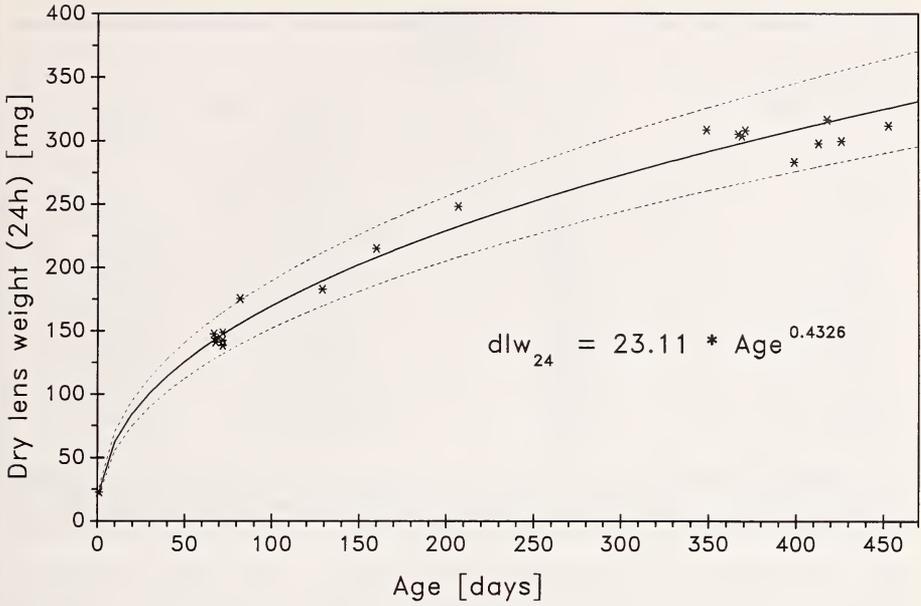


Fig. 4. Growth curve of age-known pen-reared Brown hares ($n = 20$) younger than 454 days of age. Dotted lines indicate 95 % confidence range

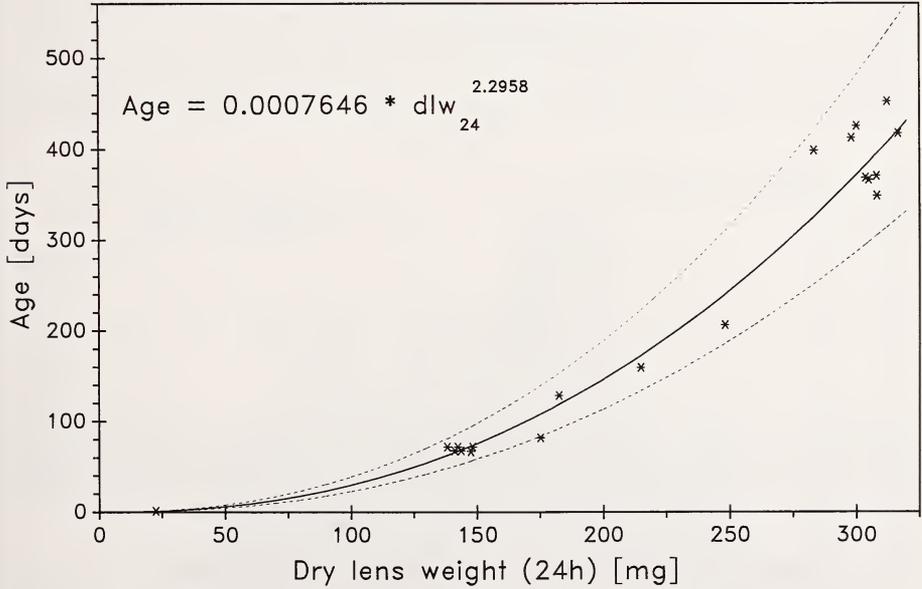


Fig. 5. Reference curve for age-estimation of Brown hares younger than 454 days. The regression calculation is based on the same individuals ($n = 20$) as in fig. 4. Dotted lines indicate 95 % confidence range

Table 1. Estimated ages and 95 % confidence intervals of Brown hares based on the regression equation

Lens weight (mg)	Estim. age (days)	95 % confidence interval (days)	95 % confidence interval (months)
25	1.5	1.1–1.4	0
50	6.1	5.1–7.2	<0.3
75	15.4	12.7–18.7	0.4–0.6
100	29.9	24.3–36.7	0.8–1.2
125	49.8	40.2–61.8	1.3–2.1
150	75.7	60.6–94.7	2.0–3.2
175	107.9	85.7–135.8	2.9–4.5
200	146.6	115.8–185.7	3.9–6.2
225	192.1	150.9–244.6	5.0–8.5
250	244.7	191.3–313.0	6.4–10.4
275	304.6	237.1–391.2	7.9–13.0
300	371.9	288.4–479.6	9.6–16.0
325	446.9	345.3–578.4	11.5–19.3

$y = 0.0007646 \times x^{2.2958}$ for selected values of dry lens weights (24 h of desiccation). Regression equation and variance were based on dry lens weights of 20 hares 453 days old or less (comp. Fig. 5).

with a “Stroh-sign” were detected in some higher DLW₂₄-classes. This reveals, that the disappearance of the “Stroh-sign” may already occur within the fourth month of life and it might be still present at least in some specimens older than one year. There was no tendency of the “Stroh-sign” to disappear earlier in one of the sexes (Kruskal-Wallis-test on DLW₂₄ in male and female “Stroh-positive” hares). Few individuals occurred in DLW₂₄-class 275–285 mg (see Fig. 6) indicating an age of 10.6 (7.9–14.2) months (comp. Fig. 5); this corresponds to the cessation of reproduction during the period October–December in central European hare populations, consequently leading to a low number of individuals 8–14 months of age in the autumnal sample (comp. CABOŃ-RACZYŃSKA and RACZYŃSKI 1972; BROEKHUIZEN and MAASKAMP 1979; PEGEL 1986). Thus, within the

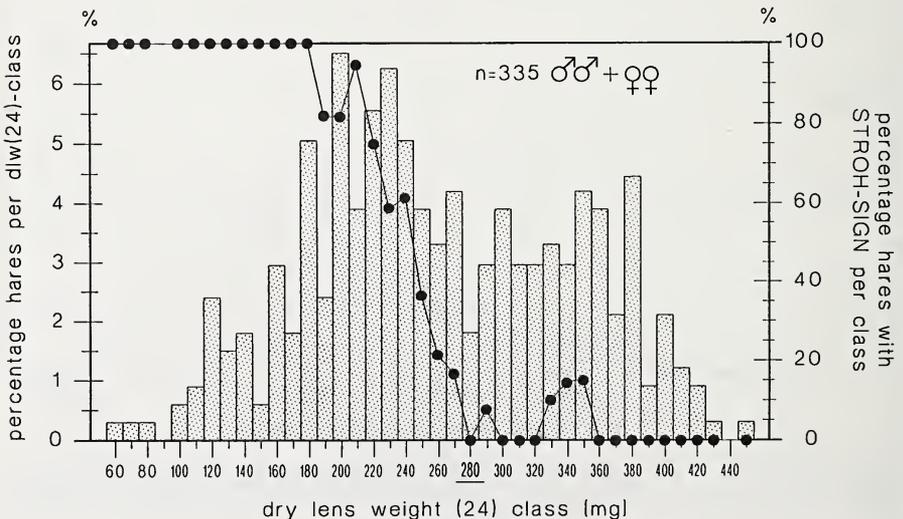


Fig. 6. Frequency distribution of eye lens weights of 335 wild living Brown hares sampled during the autumnal hunting season 1988 (histogram, left ordinate). Percentage occurrence of specimens with a “Stroh-sign” in each lens weight class (full circles, right ordinate)

present autumnal sample all specimens with $DLW_{24} < 275$ mg are considered as juveniles (young of the year). When discriminating juveniles and adults using the "Stroh-sign" and comparing the results with respective DLW_{24} values 100% agreement was achieved in hares sampled in October; however, accordance was lower in specimens from November (84.1%) and the first half of December (79.2%).

Palpable flexibility of the processus lacrimalis in juvenile hares ($DLW_{24} < 275$ mg, $n = 90$) was given in only 55.6%. Moreover, DLW_{24} did not differ significantly between juveniles (palpable "Stroh-sign") with a flexible processus lacrimalis on the one hand and with an ossified one on the other. In adults 10% of the processus were flexible.

Ages of skulls were categorized into four consecutive classes according to the progress of the ossification of sutures (sutura sagittalis, sutura frontalis, sutura parietotemporalis and sutura coronaria) as described by CABOŇ-RACZYŃSKA (1964). However, it appeared, that ossification was generally somewhat reduced in the present material as compared to the hares investigated by CABOŇ-RACZYŃSKA (1964): especially sut. parietotemporalis and sut. coronaria hardly showed an ossification. Therefore, presently the oldest age class (number 4 in CABOŇ-RACZYŃSKA 1964) was characterized by (almost) complete ossification of sut. sagittalis and sut. frontalis but not necessarily by ossification of sut. parietotemporalis and sut. coronaria. The relationship of skull age classes and the respective DLW_{24} values is presented in Figure 7. Despite of extended zones of overlap significant differences in

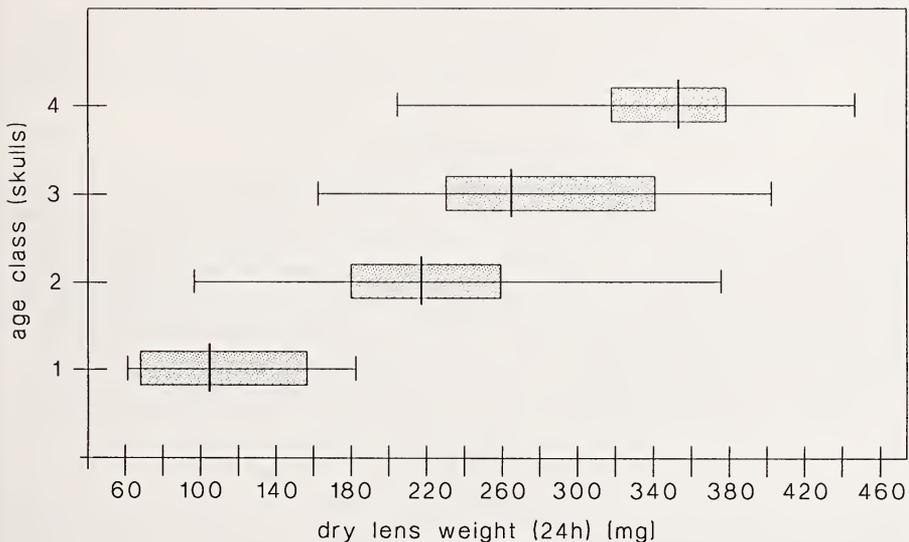


Fig. 7. Box plots of lens weights in four age classes of brown hare skulls (n of class 1 = 6, n of class 2 = 153, n of class 3 = 50, n of class 4 = 60). Age classes of skulls were determined according to the slightly modified scheme of ossification of sutures provided by CABOŇ-RACZYŃSKA (1964); comp. also section "results". Medians, range of second and third quarters, minima and maxima are given

DLW_{24} were found between the four age categories of the skulls (Kruskal-Wallis-test of DLW_{24} in the four groups, $p < 0.0001$, $d.f. = 3$) confirming the progress of ossification with increasing age. No tendency of sex-specific differences concerning ossification of skulls was detected (Kruskal-Wallis-tests on DLW_{24} values between the sexes for each of the four skull age groups). Within a sample of age-known brown hares from the breed ($n = 27$) skull age class four (indicating the highest step of ossification) was reached first at an age of 418 days. However, among specimens at least 418 days old ($n = 22$), only 31.8%

could be attributed to age class 4, 31.8 % to age class 3 and 36.4 % to age class 2. The maximal age of an individual in age class 2 (start of ossification of sut. sagittalis and sut. frontalis) was 962 days!

Discussion

Eye lens weights of hares are reported to depend to a certain degree on the specific procedures of desiccation as temperature, duration, use of hygroscopic substances and application of reduced air pressure (ANDERSEN and JENSEN 1972; CABOŃ-RACZYŃSKA and RACZYŃSKI 1972; PEPIN 1974; BROEKHUIZEN and MAASKAMP 1979). Freezing and decomposition prior to fixation reduced lens weights in cottontails (*Sylvilagus floridanus*) (PELTON 1970) and in raccoons (*Procyon lotor*) (MONTGOMERY 1963) but not in domestic sheep (LONGHURST 1964). According to MONTGOMERY (1963) lens weights of raccoons were not affected by decomposition when lenses were left within the eye balls for up the three days at room temperature. Apart from such influences, present findings concerning intraindividual variability suggest occurrence of fluctuating asymmetry (comp. e.g. SOULÉ 1967) in lens masses, possibly determined by varying potentials for maintaining developmental homeostasis (ZAKHAROV 1981).

Most of water evaporation of lenses was already accomplished after 24 hours of desiccation at 100 °C (Fig. 1). Thus, mean values of DLW₂₄ were used for the construction of a lens growth curve extending from one to 1800 days of age (Fig. 3). The regression curve in Figure 3 does not appear to represent the best fitting line and the rate of lens growth in adult specimens is most likely to be slower than indicated by the respective regression formula; however, there was no simple relationship feasible to convey the saturation of DLW₂₄ at a level of approx. 350 mg (comp. Fig. 3). Using more parameters to explain the relationship of DLW₂₄ and age would raise the degree of uncertainty about these parameters and consequently increase the confidence region. Because of the reduced growth rate and the considerable individual variation of DLW₂₄ in adult specimens determination of DLW₂₄ is an inappropriate technic for assessing years of age in adult brown hares. Nevertheless, the rapid growth of eye lenses and the comparatively narrow confidence interval of DLW₂₄ within the first year of life (Fig. 4) enable estimating the months of age in juveniles (Fig. 5 and Tab. 1). Thereby, the approximate month of birth of a juvenile hare can be determined by dating back from the date of hunt.

However, it is emphasized, that the present growth curves have been constructed by using captive animals which may have somewhat heavier lens weights than free-living hares (comp. e.g. RONGSTAD 1966 for cottontail rabbits, *Sylvilagus floridanus*). Furthermore, differences in the rate of lens growth between various wild populations may also occur (comp. CONNOLLY 1969 for the black-tailed jack rabbit, *Lepus californicus*). The low frequency of DLW₂₄ ranging from 275 to 285 mg (Fig. 6), indicating an age of 10.6 (7.9–14.2) months (comp. Fig. 5), is in good correspondence with the reproductive pause of central European hare populations (October–December), consequently leading to few individuals 9–14 months of age in the autumnal sample. Accordingly hares with DLW₂₄ < 275 mg were presently determined as young of the year. Similar DLW limits for juveniles were found by CABOŃ-RACZYŃSKA and RACZYŃSKI (1972), PEPIN (1974), BROEKHUIZEN and MAASKAMP (1979) and PEGEL (1986).

When using the "Stroh-sign", which occasionally may already be absent in animals 4 or 5 months of age (Fig. 6), to segregate juveniles and adults in the present autumnal hare sample, the chance of incorrect ageing as compared with the respective DLW₂₄ increased from 0 % in October to approx. 20 % in November and the first half of December. This is a consequence of higher portions of older juveniles in the samples from November/December (comp. e.g. CABOŃ-RACZYŃSKA and RACZYŃSKI 1972). Since one or the other juvenile individual may have a DLW₂₄ > 275 mg (comp. Tab. 1) and consequently would

not have been grouped as a juvenile for the purpose of this comparison, the percentages of wrong ageing in November and December as given above should even be somewhat higher. In this study, occasionally adult hares (DLW₂₄ > 275 mg) were found to have a "Stroh-sign" (Fig. 6); they are considered, however, as having been categorized as „Stroh-positive“ by mistake. When segregating juveniles and adults within a given sample by using the "Stroh-method" the percentage of wrong determinations depends both on the time of the year the animals have been sampled and the particular age structure of the juveniles within the sample (comp. also CABOŃ-RACZYŃSKA and RACZYŃSKI 1972). In conclusion, the "Stroh-method" is merely a crude way to discriminate juvenile and adult brown hares. If applied in analyses for hunting management it must be taken into account, that the percentage of juveniles per sample estimated by the "Stroh-method" represents only a minimal value and that no general constant for correction can be given.

Ossification of the skull sutures starts already within the first year of life and increases generally with age, but the high individual variability (Fig. 7) does not allow age estimations by using the scheme of CABOŃ-RACZYŃSKA (1964). A reliable discrimination of juveniles and adults is impossible by this method. Also, the flexibility of the processus lacrimalis did not prove useful for segregation of juvenile and adult hares.

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Zusammenfassung

Über Augenlinsengewichte und andere Altersmerkmale beim Feldhasen (Lepus europaeus Pallas, 1778)

Bei insgesamt 411 Feldhasen aus Österreich wurden die Trockengewichte der Augenlinsen, die Verknöcherung der Ellen-Epiphyse („Stroh'sches Zeichen“), des Processus lacrimalis und der Verknöcherungsgrad der Schädelnähte ermittelt. Anhand der Linsengewichte von 42 Tieren mit bekanntem Alter aus einer Feldhasenzucht wurden Regressionsmodelle des Linsenwachstums sowie eine Regression zur Altersschätzung von Hasen erstellt. Während die Linsengewichte eine Trennung von Jung- und Althasen und bei Junghasen außerdem eine Einteilung in einzelne Geburtsperioden ermöglichen, ist bei adulten Tieren wegen des verlangsamten und individuell stark variierenden Linsenwachstums keine Altersgliederung nach Jahren möglich. Das „Stroh'sche Zeichen“ ist nur als grober Indikator, der Processus lacrimalis und die Schädelverknöcherung sind überhaupt nicht zur Trennung von Jung- und Althasen geeignet.

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