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Age determination in leverets of European hare *Lepus europaeus* based on body measurements

Yves Bray, Stephane Champely & Dominique Soyez


The objective of our work was to provide allometric relationships between different body measurements and age of young leverets of European hare *Lepus europaeus* (less than two months old) for use in live-trapping studies. Five morphometric variables were measured to predict the age of leverets: body mass, skull length, skull width, hind foot length and ear length. The hares came from two rearing centres near Paris (France) and were handled in 1995 and 1996. Measurement repeatability of each variable was compared in a pilot study (N = 51 leverets) and the effect of rearing centre on the growth curve was tested for the first 30 days after birth. We used a complementary sample (N = 168 leverets) to establish a model of age determination using I-splines regression. The skull length was the best candidate variable based on measurement repeatability, comparability between the two rearing centres and goodness of fit. I-spline modelling has minimal underlying statistical assumptions. In addition, it provides easy-to-use and powerful plots of the relation between age and measurements, including confidence limits estimated by a bootstrap procedure.

Key words: age criteria, European hare, *Lepus europaeus*, morphometrics, non-parametric modelling

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related patterns in demographic parameters to identify key stages in the life cycles of animals. For European hare *L. europaeus*, the population growth rate seems to be highly dependent on breeding success (Marboutin & Péroux 1995), but the relative influence of changes in fecundity, juvenile survival and dispersal has never been documented. As part of an analysis of dispersal patterns in European hares from central France (Bray 1998), we investigated early movements and survival of wild new-born leverets using radio-tagged animals. It was therefore of great importance that leverets be aged accurately when first captured.

As in other mammal species (Morris 1972), the methods available for age determination in hares largely depend on whether animals are dead or alive. Some criteria have already been proposed for age determination of dead hares, and the most reliable criteria is eye-lens weight (Walhovd 1966, Pépin 1973, Broekhuizen & Maaskamp 1979, Suchentrunk, Willing & Hartl 1991). As far as live animals are concerned, it is possible to roughly distinguish between young (≤ 7 months of age) and adults based on the disappearance of the epiphyseal cartilage on the distal part of the ulna (Stroh 1931). Of other methods based on the ossification process (Lloyd 1968, Frylestam & von Schantz 1977, Pascal & Kovacs 1983) only Stroh’s can be used under field conditions without laboratory equipment. Determining the age of very young specimens, such as leverets, has often been based on growth curves obtained under various circumstances. Sometimes the age has been estimated from repeated weighing of hand-reared hares (Hedinger 1948, Pépin 1973, Biadi & Nouhaut 1975). Other estimations have been based on recapture of young leverets found in the field and maintained in close captivity (Pilarska 1969, Broekhuizen 1971) or on leverets born and maintained in large enclosures where natural conditions were imitated (Pielowski 1971). The resulting growth curves were highly variable (Fig. 1), suggesting that different local conditions and genetic backgrounds may induce changes in growth rates and, as a by-product, uncertainty in the prediction of the age. Last but not least, these curves only deal with body mass, and they do not provide any estimate of confidence limits. Other body measurements have already been used (e.g. length of hind foot, ear or skull), but the people who used these measurements were interested either in describing the species (Flux 1967), or in distinguishing between young and adults (Broekhuizen 1979).

Therefore, we investigated growth curves of five body measurements in hand-reared leverets, because recapture and repeated handling of wild animals would have been more invasive and would likely have induced more stress-related bias in growth rates. Furthermore, sample sizes could be increased when using hand-reared hares. The actual birth dates of the hares used were known, and the stability of results obtained for different rearing centres and observers would help in assessing the external validity of the method.

**Material and methods**

We used leverets up to two months of age from two rearing centres; measurements were done in a first step by two observers to assess repeatability where some error measurement of variables could occur. Autocorrelation in data was limited by subsampling when repeated measurements of the same individuals occurred. Changes in body measurements as a function of age were modelled by means of semi-parametric non-linear functions (I-splines; Ramsay 1988). These functions assume limited underlying statistical assumptions, and provide easy-to-use and powerful plots of the relation between age and measurements, including confidence limits estimated by a bootstrap procedure.

**Sample collection**

Data on age and body measurements were collected during 1995-1996, in two rearing centres located close to Paris (France). For one rearing centre, data were only available for the first month of age. As measuring accu-
Figure 2. Skull length (SL; condylobasal length) corresponded to the distance between the back of the skull and the tip of nose (A); maximum skull width (SW; larger value of bizigomatic breadth) (B); hind foot length (FL; including nail) was measured as the distance between the foremost nail point and the end of the heel bone (C); ear length (EL; notch to tip) was measured excluding hairs (D).

Accuracy may partly depend on an observer-effect, a pilot study of measurement repeatability was conducted, based on the comparison of paired data from two observers. A total of 51 leverets were used, from which both observers recorded independently the same body measurements.

Then, a complementary sample was obtained, to achieve a total sample size of $N_1 = 219$. Measurements ($N_2 = 522$) were performed from birth to the age of 65 days, every third day during the first month, and every week during the second month.

Variables and measurements
We considered five variables that could be related to body growth and age: body mass (BM), skull length (SL) and width (SW), hind foot length (FL; including nail), and ear length (EL; notch-to-tip excluding hairs). Mass was measured to the nearest gram using an electronic weighing machine, and further transformed using cube root. Skull length and width were measured to the nearest 0.1 millimetre using a calliper rule. Hind foot and ear lengths were measured to the nearest millimetre using a steel scale. Details on measurements of SL, SW, FL, and EL are presented in Figure 2.

Statistical analyses
We performed systematic subsampling within data from every leveret, to limit time-related autocorrelation in measurements. Serial autocorrelation was no longer significant when consecutive data were spaced using a 6-day period. For example, when computed from long series, the first-order coefficient of serial autocorrelation (time-step = three days) ranged within 0.70-0.75 in SL (upper 95% confidence limits: 0.57), whereas the second-order coefficient of correlation (time-step = six days) ranged within 0.42-0.48 (upper 95% confi-
dence limits ≥ 0.8). As a result, the statistical analysis was based on N3 = 388 measurements in any of the five variables analysed.

**Covariance analysis of growth curves**

In order to compare the growth curves and to test the equality of the growth curves from the two rearing centres, we used an extension of the classical covariance analysis suitable for growth curves.

Let xij be the age of leveret i from centre j (j = 1, 2) and yj(xij) be the response variable. In order to gain some flexibility in the modelling, we used a semi-parametric approach based on B-splines (de Boor 1978). This can be done using two nested linear models of the form:

\[
y_j(x_{ij}) = B(x_{ij}) + \varepsilon_{ij}
\]

against

\[
y_j(x_{ij}) = B_j(x_{ij}) + \varepsilon_{ij}
\]

where B() and Bj() were (cubic) B-splines with v degrees of freedom, and \( \varepsilon_{ij} \) was the conventional error term assumed independently normal with zero mean and constant variance.

The model in Equation 1 consists of one growth curve common to the two centres, whereas the model in Equation 2 represents two different growth curves.

To compare the two models, the corresponding test of the centre effect is an F-test. An example of such computations using the S-Plus® software was described in Chambers & Hastie (1991: 280).

**Modelling with I-splines**

Let \((x_i, y_i)\) be the data at hand where x is the age of the leveret and y a growth variable. A monotone spline regression (we used the notations from Ramsay 1988) was used to model the growth curves:

\[
y(x_i) = \alpha_0 + \sum \alpha_j I_j(x_i) + \varepsilon_{ii}
\]

where \( \varepsilon_i \) was the error term and \( I_j() \) a set of monotonous
transformation functions called I-splines. They corresponded here to a piecewise polynomial of degree \( k \) defined on an interval \([L, U]\) with \( m \) interior knots positioned at appropriate \( x \)-quantiles.

The parameters \( \alpha_j \) were estimated in a least square sense with respect to positive constraints on the parameters for \( j > 0 \). These positivity constraints were sufficient to ensure monotonicity.

The resulting fit was summarised by the classical coefficient of determination (percentage of variance explained by the model). We developed S-Plus® routines based on the gradient projection algorithm to fit this model (see the Appendix in Ramsay 1988).

Calibration by bootstrapping residuals

First, our aim was to obtain prediction intervals for the response variable \( y \) computed on a grid of \( x \) values. Secondly, the resulting plot was inverted to provide calibration intervals for \( x \).

The I-spline model (Equation 3) was expressed as

\[
y(x) = \mu(x) + \varepsilon.
\]

The errors \( \varepsilon \) were assumed to be a random sample from an unknown distribution having null expectation and constant variance.

In order to define a prediction interval at \( x = x_{\text{new}} \), we used a bootstrap approach. As a matter of fact, a direct mathematical solution seemed difficult for two reasons. First, the residuals were clearly non-normal (Fig. 3B) and second, the model was fitted by a complex optimisation routine.

To calculate a bootstrap data set \( y^*(x_{\text{new}}) \) for \( y(x_{\text{new}}) \) and thus a prediction interval by using appropriate quantiles, one needed to sample independently bootstrap data sets \( \mu^*(x_{\text{new}}) \) for \( \mu(x_{\text{new}}) \) and \( \varepsilon^* \) for \( \varepsilon \), and then to compute:

\[
y^*(x_{\text{new}}) = \mu^*(x_{\text{new}}) + \varepsilon^* \tag{4}.
\]

For bootstrapping \( \varepsilon^* \) in Equation 4, let \( r_i \) be the residuals of the I-spline fitting (Fig. 3A):

\[
r_i = y_i - \hat{\mu}_i.
\]

The 'adjusted errors'

\[
e_i = r_i \sqrt{\frac{n}{n-p}} \tag{5}
\]

were closer to errors (Efron & Tibshirani 1993: 122). The bootstrap dataset \( \varepsilon^* \) was obtained by sampling with replacement of the adjusted errors \( e_i \) (see Fig. 3B).

For bootstrapping \( \mu^*(x_{\text{new}}) \) in Equation 4, we used the approach described as 'bootstrapping residuals' in Efron & Tibshirani (1993: 111).

First, bootstrap responses \( y_i^* \) were generated using

\[
y_i^* = \hat{\mu}_i + \varepsilon_i^*.
\]

Second, the I-spline model was fitted to the new data \((x_i, y_i^*)\) giving bootstrap parameters \( \alpha_j^* \) (see Fig. 3C). Third, one could predict

\[
\mu^*(x_{\text{new}}) = \alpha_0^* + \sum j\alpha_j^*I_j(x_{\text{new}}) \tag{6}.
\]

Results

Collinearity between body measurements

The five predictors were strongly correlated (all cross-correlation coefficients within the range of 0.83-0.99), and this collinearity prevented consideration of the potential of multivariate calibration (Brown 1982). Therefore, the problem was to select the best univariate predictor from the set of body measurements, based on measurement repeatability, comparability between rearing centres and quality of the modelling.

Reliability of body measurements

Measurement repeatability

In the pilot study which was done on 51 leverets, a paired t-test was performed on the data measured by each of the two observers. Skull length (SL) and skull width (SW) were the most promising variables (Table 1). Despite the fact that the two observers were experienced and had discussed the protocol for a long time, they failed to reproduce comparable measurements for hind foot (FL) and ear lengths (EL).

Rearing centre variability

The two rearing centres were compared using hares less than 30 days old. The differences in the growth curves obtained from the two rearing centres depended on each body measurement (Fig. 4). The covariance analysis (using cubic B-splines with df = 4) statistically evidenced this centre effect (Table 2). Nevertheless, the most comparable variable was skull length.

Table 1. Repeatability of the body measurements obtained by the two observers (\( N = 51 \) leverets) assessed using paired t-test.

<table>
<thead>
<tr>
<th>Variables</th>
<th>( \overline{y_i} - \overline{y_j} ) (mm)</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skull length</td>
<td>0.31</td>
<td>0.19</td>
<td>0.11</td>
</tr>
<tr>
<td>Skull width</td>
<td>0.13</td>
<td>0.07</td>
<td>0.06</td>
</tr>
<tr>
<td>Foot length</td>
<td>2.37</td>
<td>0.43</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ear length</td>
<td>3.70</td>
<td>0.45</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Growth curves modelling
The I-spline model was fitted to the five measurements using polynomial with $k = 3$ degrees and $m = 2$ interior knots. Every variable was well fitted by the versatile I-spline model, but the coefficient of determination was largest using skull length ($R^2_{SL} = 0.93$; $R^2_{\sqrt[3]{BM}} = 0.93$; $R^2_{FL} = 0.91$; $R^2_{EL} = 0.88$; $R^2_{SW} = 0.79$). Moreover, its residuals were more homoscedastic.

Considering the three criteria (measurement repeatability, comparability between the two rearing centres and goodness of fit by the I-spline model), the skull length was the variable of choice. So, the calibration intervals were defined only for this measurement.

Age calibration based on skull length
To compute the prediction intervals for skull length, we used a grid of 65 age values regularly spaced from day 1 to day 65. Each 95% prediction interval was defined using 1,000 bootstrap predicted values (Equation 4).

These intervals were inverted to provide calibration intervals (Fig. 5). It was clear from the plot that a good

Table 2. F-test of rearing centre effect in covariance analysis of B-splines curves. This test compares the sum of squared residuals between the common growth curve model (Equation 1) and the two growth curves model (Equation 2) expressing the differences between the two rearing centres.

<table>
<thead>
<tr>
<th>Variables</th>
<th>F</th>
<th>P ($^*10^{-5}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skull length</td>
<td>4.7</td>
<td>35</td>
</tr>
<tr>
<td>Foot length</td>
<td>5.3</td>
<td>11</td>
</tr>
<tr>
<td>Ear length</td>
<td>6.0</td>
<td>2</td>
</tr>
<tr>
<td>Cube root of body mass</td>
<td>10.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Skull width</td>
<td>26.3</td>
<td>0</td>
</tr>
</tbody>
</table>
estimation of the age may be given for values of skull length within the range of 40–75 mm. For skull lengths larger than 80 mm, the calibration intervals were not superiorly bounded and the median curve became almost vertical. So estimating the age of leverets older than 40 days could be somewhat hazardous.

Discussion

Until now, age determination in young European hare leverets in the field has been based on the relationship between body mass and age. However, growth curves described in the literature differ greatly (see Bray 1998 for a review). According to the chosen growth curve the weight of a weanling (aged 1 month) ranged (see Fig. 1) from 250 g (Broekhuizen 1971) to 1,000 g (Hediger 1948). Moreover, individual variations were not considered as the growth curve only provided a mean value. Considering this variability, it seems difficult to select the best-fitting curve. Indeed, body mass was influenced by the animal’s condition, and may vary with geographical situation (Cabon-Raczynska & Raczynski 1974, Broekhuizen & Maaskamp 1979, Morris 1972, Pepin 1979), as shown for other species (see Myers 1958, Dudzinski & Mykytowycz 1960 for wild rabbits Oryctolagus cuniculus). The birth weight and growth rates during the first month of life both varied with litter size (Biadi & Nouhaud 1975), and individual variations among leverets of the same litter were significant (Piełowski 1971).

Our results provide different relationships between body measurement and age together with confidence limits. Skull length was unambiguously the best variable as it lowered the effect of both observer and centre and resulted in a better fit.

Morris (1972) suggested that variables using rigid linear dimensions vary less according to season or nutritional status than other volumetric variables such as body mass. In the European hare, the relationships between linear measurement and age have been poorly documented. Broekhuizen & Maaskamp (1979) have provided a relationship between hind foot length and age for leverets less than five months old. The growth of the hind foot decreased rapidly before the age of two months, so the authors argued that it was only possible to distinguish adults from leverets less than two months old. Previously, Cabon-Raczynska & Raczynski (1964) showed allometric growth of hind feet and ears and emphasised that the growth of these dimensions decreased rapidly before the age of six months. Keith (1968) provided more detailed information on hind-foot/age relationship in captive-reared snowshoe hares, and Flux (1967) outlined collinearity between body mass and these variables in young hares. These studies have shown a strong growth of hind feet and ears during the first month of life, but a greater variability of curves using soft organs (Flux 1970). Our results also showed a lower measurement repeatability of the ear length.

In most of the five variables studied, we found significant differences between the two rearing centres: growth curves of body mass, skull length and hind foot length had steeper slopes in the second centre, contrary to the growth curve of the ear. Skull width varied according to rearing centre more than other variables, and the individual variation for a given age was greater. Although the growth of skull length was less influenced by the rearing centre effect than other variables, some variability due to the animals’ origin, health status and rearing conditions still remained, and should be documented more completely. Some complementary sets of body measurements from other rearing centres would help us to assess more accurately the actual level of residual variability and, as a result, to define meaningful and robust age classes to account for such residual variance. Further experiments would confirm the applicability of our findings to wild leverets.

Field data on showshoe hares (Keith 1968) showed that wild hares grew more slowly during their first three months of life than individuals in captivity. Conversely, data collected by Pepin (1974) in France, showed that after the age of 40 days wild European hares were heavier than captive hares. This result is in accordance with Petrusewicz’s (1970) findings in Poland. Because growth rates in wild young hares seem to be higher, models established in rearing centres could likely overestimate the actual age when used in the wild. Nevertheless, the growth curves using hand-
reared leverets (symbolised with ●, ○ and ♦ in Figure 1) were higher and, despite the possible sampling variations, seemed more stable in particular before the age of 30 days than curves based on wild leverets kept in captivity (Pilarska 1969 (□), Broekhuizen 1971(■)) or living under field conditions in large enclosures in Poland (Pielowski 1971(A)). In this way, the first curves (circles) could be more accurate than the last (squares and triangular symbols) in age determination of wild leverets. Considering the variability in growth rates, our non-parametric model provides at least a robust relative ranking of leverets (according to meaningful age classes). Relative age determination is, indeed, biologically meaningful for a great number of studies, e.g. changes in survival or spacing behaviour of growing juveniles.

Considering linear variables, only cranial measurements did not differ significantly between observers in our study. Skull length was the best candidate variable when considering goodness-of-fit and external validity (centre effect and repeatability).

From a practical point of view, Figure 5 is very easy to use as it allows a quick estimation of age in leverets from skull measurement. For example, a skull length of 70 mm corresponds to an age of 23 days (95% confidence limits ranged within 17-32 days). Calibration limits widen with age. They become unbounded when the skull length is above 80 mm. Therefore, the method should be limited to leverets less than 40 days old; for older leverets no confidence limits can be computed.

Using only skull length to estimate the age of leverets may be risky as measurement errors always occur. In order to detect such errors, one could also use the strong relationship between skull length and body mass (r = 0.99) to check compatibility between these two measurements (Fig. 6).

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