Is the eye lens method of age estimation reliable in voles?

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A b s t r a c t . We examined the reliability of laboratory-derived calibration curves for age determination of field individuals of the common vole, *Microtus arvalis*. The sex-specific calibration curves for age determination based on the relationship between eye lens mass and age derived in the laboratory were applied to a live-trapped field population of common vole. When comparing the individual's age to the length of its trapping history, we found a slight tendency for underestimation of real age. These errors were observed slightly more in females than in males and in individuals captured over a longer time. This could mean that growth rates in captive animals, especially older ones, and in females are greater than those from the field. The month of first trapping has no effect on the presence of the error. We suggest that, in population studies with a special concern for ageing individuals over the whole life span, other methods should be examined, such as those measuring insoluble eye lens proteins or calibration curves based on more than one predictor.

Introduction

Mammalian populations are often structured into age classes because age is among the most important predictors of survival rates. Several methods have been used for age determination of small mammals. The simplest but less reliable techniques were derived from measurements of body size and growth of certain body parts (G e b c z y n s k a 1964, A d a m c z e w s k a - A n d r z e j e w s k a 1973a, F u l l e r 1988, G a r d e & E s c a l a 1996) or skull (S t e i n e r & R a c z y n s k i 1976, G a r d e & E s c a l a 1997). Other possibilities are observations of growing annuli in teeth enamel and bones (A n s o r g e 1995), ossification of bones epiphyses (B r o e k h u i z e n & M a a s k a m p 1979), concretion of cranial sutures (M e a d 1967) or teeth growth and development (A d a m c z e w s k a - A n d r z e j e w s k a 1973a, Z e j d a et al. 1992).

In many mammals, including voles, age is often estimated using eye lens mass because it tends to vary much less than other body measurements with environmental conditions (L o r d 1959). However, the regression relationships between age and eye lens mass are usually established under laboratory conditions (T h o m a s & B e l l i s 1980, A n d o & S h i r a s h i 1997) and are only rarely validated in field populations (L e L o u a r n 1971, H l a v á č 1978, H a n s s o n 1983a, M o r a v e c 1985). Eye lens growth in field populations is affected by seasonal variation in many environmental factors, such as photoperiod, weather or food resources, and may also respond to population density and phase of the population cycle (H a n s s o n 1983a,b). Detailed capture-recapture studies

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provide an excellent opportunity to assess the reliability of a laboratory-derived eye lens mass method in natural populations. Here, we compare the eye lens estimates of age in the common vole (*Microtus arvalis* (Pallas, 1778)) to those obtained from capture-recapture data. This is important because there are hypotheses suggesting that density-dependent changes in age are causally related to vole population dynamics (B o o n s t r a 1994, T k a d l e c & Z e j d a 1998) and the eye lens method has been applied to test their predictions (E r g o n et al. 2001, J á n o v á et al. 2003).

Material and Methods

In 2001–2003, a common vole population was studied by the capture-recapture method in an alfalfa field near the village of Drnholec, Czech Republic (48°53'30''N, 16°27'30''E). Trapping was undertaken once or twice a month for 4–5 days and live traps were checked twice a day. In a total of 254 recaptured voles that died in live traps, eyes were removed and fixed in 10% formalin for at least 3 weeks. The preserved lenses were weighed to the nearest 0.1 mg ("formalin mass"), then dried for 24 hours at 55°C and weighed again ("dry mass").

A sample of 57 wild-caught voles from the same population was brought to the laboratory and bred in captivity. Young from F1 and F2 generations born in the laboratory were used to develop the regression model describing the relationship between eye lens mass of individuals and their age. We used several general linear models to fit the formalin and dry masses of eye lenses separately for 121 males and 129 females whose ages ranged from 15 to 262 days. Age and lens mass was log transformed. We selected the model with the lowest values of AIC (B u r n h a m & A n d e r s o n 1998:48) as the most parsimonious one. As the final estimate of age we used a mean value from ages predicted by formalin estimation and dry lens mass estimation to reduce the uncertainty associated with both methods. As recommended by D a p s o n (1980), we used $s_{yx}/\overline{y}$, the standard error of estimate divided by the mean of log (eye lens mass) to compare the precision of regression curves for males and females.

We assessed the reliability of eye lens estimates (ELM) of age, calculated using calibration curves derived under laboratory conditions, by comparing them with estimates from capture-recapture data (CR). To obtain more accurate CR estimates of age, we first sorted out a subset of 22 field animals that were captured for the first time as young animals, still coated with juvenile fur, with body mass below 13.5 g in a period from April to August. Based on laboratory-derived data for growth rates in young common voles, we calculated their approximate ages at capture using the equation: age (days) = (body mass – 1.384)/0.609 (unpublished data). By this method, we estimated most ages to be about 10 to 20 days. These animals were then considered as individuals of a known age. In these animals, we could relate the ELM and CR estimates of age to each other by linear regression. The closer to 1, the slope of regression line, the greater accuracy of age estimation by ELM. The influence of sex, date of first trapping and length of trapping history on value of ELM error of estimation (CR minus ELM estimation) was analysed by multiple regression.

The dead animals from the live traps (n = 254) were examined for age by ELM estimation and the estimates then compared to the length of their trapping history. The length of the trapping history was increased by 10 days in these animals, i.e., the lowest trappable age as detected in a previous group, and further considered as the minimum age (MA) estimate. We assessed the relationship between ELM and MA estimates by linear regression.

Clearly, the MA estimates should fit the line or be underestimates because some individuals were apparently older than 10 days at their first capture. If the ELM estimation is reliable, the scatter of points should not be constrained from above but linearly bounded from below by a line with a slope of 1. Allowing for some sampling error, only a few points should fall below the lower equality line because the age of field animals cannot obviously be lower than the MA. To analyse influences of sex, length of trapping history and date of first trapping on the accuracy of estimation, we distinguished correct estimates which were equal or higher than MA (i.e., including overestimation), and false estimates which were lower than MA (i.e. underestimation). The effect of sex (χ^2 test for the number of correctly and false estimated males and females) and the time span (length of trapping history of correctly against wrongly estimated individuals) of individual recaptures were assessed by the nonparametric Mann-Whitney test (the time span of correctly against wrongly estimated individuals). The effect of the first trapping date (all years together for each month) on the presence and the size of the underestimation were tested by χ^2 test. The general linear modelling, nonparametric tests and correlations were calculated using the statistical program Statistica (StatSoft 2001).
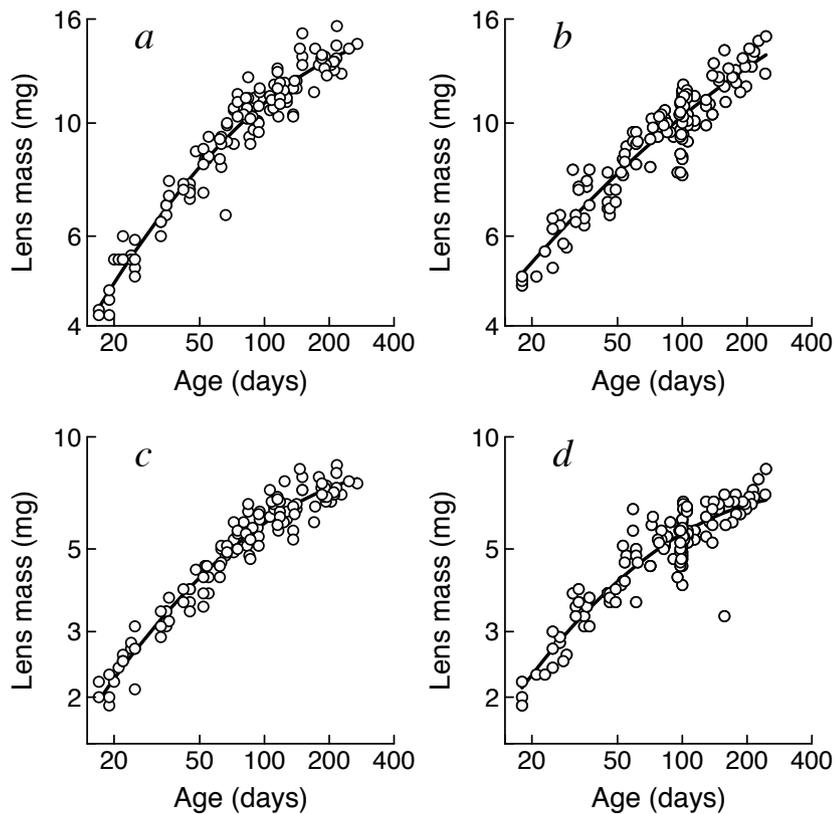

Fig. 1. Relationships between age and formalin (*a*, *b*) and dry (*c*, *d*) eye lens mass in captive male (*a*, *c*) and female (*b*, *d*) common voles. The following equations were used to fit the data: males – log (formalin mass) = –1.2753 + 1.2432×log (age) – 0.0969×(log (age))², log (dry mass) = –2.6857 + 1.5349×log (age) – 0.1233×(log (age))²; females – log (formalin mass) = –0.1100 + 0.7153×log (age) – 0.0401×(log (age))², log (dry mass) = –2.5228 + 1.4940×log (age) – 0.1251×log (age)².

Results

The effect of age on eye lens mass in laboratory-bred animals was conditioned by sex (interaction sex × ln (age) for formalin mass of lenses: $F_{1,247} = 8.56$; $p = 0.004$; for dry mass: $F_{1,247} = 6.509$; $p = 0.011$). Consequently, we constructed calibration curves separately for each sex. Based on the lowest values of AIC, we selected the quadratic log-log model giving the best fit (Fig. 1): log (lens mass) = $b_0 + b_1$ log (age) + b_2 log^2 (age). Judging by the $s_{yx}/\bar{y}$, the fit of calibration curves was better in males than females (formalin mass: males = 0.035, females = 0.040; dry mass: males = 0.054, females = 0.077). We fitted a linear regression passing through the origin to compare the ELM estimates of age for 22 individuals (first captured as juveniles) with the CR ones (Fig. 2a). The slope differed from 1 (b = 0.842, 95% c.i. 0.752–0.932) suggesting that the ELM method slightly underestimated the true age of individuals. Neither sex nor the date of first capture influenced the error (sex: $t_{20} = 0.51$, $p = 0.62$; date: $t = 0.64$, $p = 0.53$).

The ELM age of dead animals ranged from 21 to 251 days. When compared to their MAs by fitting a linear regression passing through the origin, the slope was higher than 1 (b = 1.169, 95% c.i. 1.090–1.247), suggesting that the MAs were mostly underestimates as expected (Fig. 2b). Underestimation was more common in females than males ($\chi^2 = 4.388$; $p < 0.05$). The month of first capture had no effect on the accuracy of estimation ($\chi^2 = 2.498$; $p > 0.05$). Individuals with recaptures over longer periods (therefore older ones) were found to be underestimated more often (Mann-Whitney: $z = -6.789$; $p < 0.01$; the incorrectly estimated individuals were on average recaptured over a period of 83.4 days while correctly aged animals were recaptured over 33.4 days).
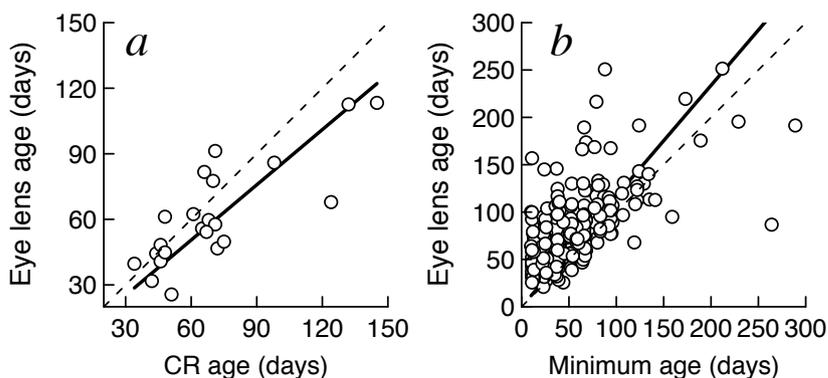

Fig. 2. The comparison between ages of common voles from the field, estimated from capture-recapture (CR) data, and eye lens mass in individuals captured first as juveniles (*a*) and for the whole sample (*b*) in which the minimum age (MA) was used. A linear regression passing through the origin was used, with the expected slope of 1 depicted as the dashed line.

Discussion

The eye lens method to determine age of small rodents in natural populations is often based on the calibration curve derived from laboratory data (H a g e n et al. 1980, M a l l o r y et al. 1981, J á n o v á et al. 2003). Here we evaluated the reliability of this method of aging in the common vole by comparing the ELM estimates with those obtained directly from capture-

recapture data. Although performing well at younger ages, the ELM method was frequently found to underestimate systematically the age of older individuals, especially that of females. As a consequence, the amount of between-year variation in age structure can be reduced in vole population analyses and result in a distorted view of density-dependent changes in the oldest component throughout the population cycle.

The curves describing the relationship between age and eye lens mass show the very steep growth over the first three months of life (T h o m a s & B e l l i s 1980, A n d o & S h i r a s h i 1997, T a k a h a s h i & S a t o h 1997) and a much slower increase at older age. This fact itself makes estimates of age less precise in older animals in which small differences in lens mass translate into large differences in age. In addition to the amplified sampling error, there is a systematic error further squeezing the variation in age. The greater growth rates in captive animals compared with those in the field could be one reason for this observation (A d a m c z e w s k a - A n d r z e j e w s k a 1973b). In captivity, voles are capable of retaining growth in lens mass even over the winter months whereas those in natural winter populations clearly undergo a period of deceleration in most somatic growth rates. Consequently, the estimated differences in age between different phases of the population cycle may have been severely underestimated, putting less emphasis on the importance of changes in age structure to changes in numbers than they really deserve. For instance, J á n o v á et al. (2003, note that owing to a typographic error the ages for males and females are confounded in Table 1) observed in the common vole that the pattern of ageing with density was less pronounced compared to other *Microtus* species, such as *M. pennsylvanicus* and *M. californicus* (B o o n s t r a 1994). In fact, the density-dependent differences in age observed between the population phases were likely to be underestimates, producing a biased pattern of variation in age throughout the population cycle. As growth rates for other body measurements may show the same pattern of seasonal variation, they are likely to underestimate ages of old animals as well. On the other hand, the lens growth rates observed over a breeding season do not have to be always lower than those in caged individuals (H a n s s o n 1983a).

The achieved precision of estimation as measured by $s_{yx}/\overline{y}$ is in good agreement with similar studies in other small rodents, falling in a range of 0.04 to 0.066 (A n d o & S h i r a s h i 1997). The systematic differences in eye lens growth rates between males and females were found in some voles, with the male rates being higher (M a r t i n e t 1966, L e L o u a r n 1971) but not in others (T h o m a s & B e l l i s 1980, M a l l o r y 1981, T a k a h a s h i & S a t o h 1997). However, it is the female rodents that seem to exhibit more variation in eye lens mass reducing further the accuracy of estimation (M a r t i n e t 1966, H a r d y et al. 1983, R o w e et al. 1985, S t o c k r a h m et al. 1996, T a k a h a s h i & S a t o h 1997). This is unfortunate as it is the females which are more critical to demographic processes in mammalian populations and whose age variation is needed to be known more accurately.

Although the eye lens method of age estimation is the prime methods in wild animals, the estimates for old individuals should be treated with caution, especially in females. We suggest that, in population studies with a special concern for ageing individuals over the whole life span, other methods should be examined. Perhaps, better results would be obtained by analyzing insoluble eye lens proteins, a method producing excellent results in oldfield mice (D a p s o n & I r l a n d 1972) and meadow voles (S t u m p & A n t h o n y 1983). Another possibility to increase precision of EL estimation could be the inclusion of an

additional variable in a multiple regression model for calibration curves. A useful candidate could be a body mass, which varies significantly with that of eye lens in the common vole (J á n o v á et al. 2007). Therefore, calibration curves based on more predictors could also be a route to the improved determination of age in field animals.

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