Chin-spot as an indicator of age in pond bats

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Abstract: In field studies of animal populations, it is often useful to be able to assess the age of an individual. In this paper we investigate the use of chin-spots as an indicator of age in pond bats (*Myotis dasycneme*). During six years of research, from 2002 to 2008, we captured more than 2,500 pond bats. To test whether the chin-spot can be used, we gathered data on chin-spot colour and other indicators of age, such as dental wear, degree of fusion of phalangeal epiphyses and reproductive status. We tested the correlation between chin-spot colour and these indicators of age. We also studied the transformation of these characteristics during bats' life course and therefore between different age classes. We found significant correlations between the colour of the chin-spot and other known age indicators. The results showed a significant relation between colour and the number of days between the first and the last capture. The longer the period between two capture events, the larger the change in colour of the chin-spot. Animals of a known age, captured as juveniles, showed a transformation in coloration from deep purple (class 5) to light-coloured (class 1) over an average time period of two to four years. Most of the juvenile pond bats had a chin-spot coloration in class '4' or '5', most sexual immature animals in class '3' and most mature animals in class '1'. We conclude that the colour of the chin-spot is a reliable predictor of the age class of pond bats.

Keywords: pond bats, determination of age, chin-spot, dental wear, fusion of phalangeal epiphyses, reproductive status, life-history trait, mark-recapture.

Introduction

Researchers capturing bats for scientific purposes often record the individual characteristics of each bat, such as standard biometric measurements, gender, reproductive status and age. Although age is an important property of an animal, year-round suitable methods for estimating age are rare. In a study on Daubenton's bats (*Myotis daubentonii)*, Richardson (1994) found the chin-spot to be a reliable characteristic for estimating age. The author defined chin-spots as: ".... a jet black, often shiny, sharply defined area of skin covering the centre of the lower lip for about 1 mm and tapering, slightly down to the chin. It contrasts markedly with the pink and pale brown skin of the rest of the lower face." Richardson (1994) concluded that animals with a dark chin-spot are juveniles or sub-adults and animals with a light-coloured chin-spot are always adults.

There are several advantages of using the chin-spot as opposed to other age indicators, such as the fusion of the phalangeal epiphyses, dental wear or reproductive status: 1. in autumn, when commonly used characteristics for estimating age become vague, it is possible to use the chin-spot to distinguish between age classes (Rivers et al. 2005). 2. This method does not require any handling of animals during hibernation when seeking to determine their age (Kokurewicz & Speakman 2006). This is especially useful when studying hibernating animals that are sensitive to disturbance.

Until now the chin-spot has only been used as an age indicator for Daubenton's bats. Pond bats (*Myotis dasycneme)* are morphologically very similar to Daubenton's bats. Like Daubenton's bats, some pond bats show a chin-spot and this characteristic could be a practical method to distinguish between age classes. For this reason, we gathered data on chin-spot colour and other indicators of age during an extensive monitoring study on pond bats (Haarsma 2009). We were especially interested in the changes of chin-spot colour during the course of life of a bat and the correlation of these changes with other age indicators.

Materials and methods

Chacteristics used

Protocol

In the Netherlands there is a protocol for assessing the reproductive status, age and health of bats (Haarsma et al. 2009). The purpose of this protocol is to ensure the comparability of datasets between bat researchers. The protocol standard uses five classes to describe each characteristic. In order to simplify comparisons between characteristics, all the characteristics are classed from '1' – extra small (XS)/ absent/ light-coloured to '5'- extra large (XL)/ present/ dark. A manual provides photos and descriptions to identify each class (Haarsma 2008). This allows researchers to make more or less standardised interpretations about age and reproductive status.

During six years of study, between 2002 and 2008, we worked with bat volunteers, capturing pond bats. Each individual was handled and described by the first author using a pilot version of the protocol. (The characteristics discussed in this paper remained unchanged between the pilot and final versions of the protocol). Most of the captured pond bats were individually marked with a ring, a transponder or both. At each recapture of marked individuals, a new description was made which covered the following characteristics: dentition wear, age-class based on interphalangeal fusion and the various aspects of reproductive status. Sometimes, for ethical reasons (e.g. highly pregnant female or a large number of bats) handling time was shortened and some characteristics were skipped.

Dental wear

The dentition of bats is diphyodont. After losing a complete set of deciduous teeth, permanent teeth have fully emerged by the time they are weaned (Fenton 1970). In the course of a lifetime, the tooth surface becomes abraded from repeated mastication (Anthony 1988, Evans 2006). The canines in particular lose their pointed appearance. With age, sometimes dark lines of tartar or dental plaque formed along the edges of the molars. Various investigators have tried to establish a link between different age categories and progressive stages of wear in canines and/or molars (Twente 1955, Sluiter 1961, Phillips et al. 1982). They found a considerable overlap between animals judged to belong to different age groups and the amount of dental wear. Hence they concluded that dental wear may be used as a broad indicator of age in bats, but not a valid characteristic for assigning bats to absolute age groups. Although the accuracy of dental wear in determining age class is still much debated, it is widely used by researchers as it is a relatively visible characteristic. The Dutch bat protocol (Haarsma et al. 2009) uses a combination of dental wear and dental plaque as an indicator, which is scored on a scale from class '1' (no dental wear or plaque) to '5' (heavy dental wear and all molars with plaque).

Interphalangeal fusion

Patterns of closure of the cartilage in finger bones can be used to asses the juvenile status of bats (Felten 1973, Brunet & Austad 2004). Mammals are born with soft cartilage epiphysial plates. At the start of the first autumn after birth these phalangeal epiphyses start to fuse (Elangovan et al. 2002). The fusion of the epiphyses in the bones of the fingers of a bat can be seen by using a torch to illuminate the wing membrane and fingers under the wing. The cartilaginous zones appear lighter than the ossified parts. Some investigators have been able to identify young (of that year) during the winter by the shape of their joints (Davis & Hitchcock 1965). During our research we determined the absence (class '1') or presence (class '5') of fusion in each animal captured. Epiphyseal growth plates which were almost closed were scored as class '3'. We could only use this characteristic during a limited time period (until the end of September), until fusion is complete.

Reproductive status

In most bat species juveniles have different pelage colours than adults (Anthony 1988). Pond bat juveniles are greyish brown and their bellies are greyer than those of adults. There are other distinctive characteristics that also indicate a juvenile status: 1. shorter and softer pelage hairs 2. fluffy grey down on the inside of the thighs and 3. a short dark nose. These characteristics are always accompanied by transparent phalangeal epiphyses and therefore not recorded separately, although we did use them as a reminder to look at the phalangeal epiphyses. During this research we also noticed that juveniles have a shorter, more rounded face, which elongates with aging. The pigmentation of the nose and face of juveniles is dark, older individuals have a lighter face and nose (A.-J. Haarsma, unpublished results). Very old individuals have pink spots, apparently without pigment, especially around their nostrils. Although easily recognisable in age extremes (very young or very old), we found no reliable criteria to describe change in pigmentation of nose and face for each age class; therefore, this characteristic was not used.

Sexual maturity is sometimes used to asses the age classes of bats (Kunz 1973, Encarnação et al. 2006). The onset of sexual development in juveniles is in their first or second autumn. In male bats this can be seen by an increase in the size of the testes and, later the distension of the epidydimus becomes visible through the perianal skin (Entwistle et al. 1998, Encarnação 2004). Before their first spermatogenesis the sheath around both testes and the tip of the epidydimus is dark and heavily pigmented. At the end of the spermatogenesis the epidydimus swells; leading the sheath to become stretched and the pigmentation to become more diffuse. In female bats, the first external signs of sexual development are visible after the first winter in which they mated, as they become pregnant and start to lactate. The distension of the lower abdomen caused by the developing foetus cannot be recognised until late in the pregnancy. Towards the end of pregnancy (in pond bats: week 26; A.-J. Haarsma, unpublished results) and during lactation the nipples become enlarged. The mammary gland can be seen underneath the skin as a yellow disc (Haarsma 2008). During lactation, most females lose most of the hairs in the immediate vicinity of their nipples as a result of repeated suckling by their young. Later the nipples become keratinised, presumably through continued distension and suckling or chewing of the young. After lactation the nipples retain their keratinised, enlarged appearance and this is a sign of parity. During this research we used descriptions of male testes and epidydimes and of female abdomen and nipples, following the protocol for assessing the reproductive status, age and health of bats (Haarsma 2008).

Chin-spot

Classification of the chin-spot was done by visual inspection using a headlamp, with extra-bright LED lights (Black Diamond Zenix IQ and Princton Tec Apex). For a complete inspection of the chin-spot the mouth of the bat had to be slightly opened. The colour of the chin-spot was compared with the colour of the transition zone between the palate and the bottom side of the nose (minimum colour) and the colour of the nose (maximum colour) (see figure 1). For each animal the chin-spot was designated to one of five coloration classes (figure 2):

1. Light-coloured chin-spot that was the same colour as the transition zone between palate and the bottom side of the nose.
2. Barely visible chin-spot, with few pigmented

spots, sometimes only around the edge of the chin.
3. Intermediate chin-spot, which has about 50% or diffuse pigmentation.
4. Visible chin-spot, where more than half of the chin-spot has pigmentation and has an overall purple colour.
5. Completely purple. The chin-spot is densely pigmented with an overall deep purple colour.

We assume the first pond bats are born in the end of week 19 (around the 13th of May) (A.-J. Haarsma, unpublished results). In this week the first female animals without distended bellies and with swollen mammae and bald areas around the mammae, which were taken as a sign of nursing, were observed. The last pregnant female was observed in week 25 (between 18 and 24 June), the week in which the last juveniles will be born. On average pond bats are born in week 22. In the rest of this analysis week 22 will be assigned to as week 0 (or day 0) of the life of a bat. Only recaptured bats that were classified as a juvenile at their first capture, have a known age. This age is based on the number of days between the first and subsequent captures and the estimated day 0 of their lives.

Statistical analyses

Statistical analyses were carried out using SPSS V15. Spearman's rank correlation coefficient was used to test the relation between the ordinal dependent variable 'chin-spot colour' (five categories) and the independent variables 'dental wear', 'fusion phalangeal epiphyses' and 'minimum age' (in years). The relation between change in colour and sexual mature/ sexual immature animals was calculated using binary logistic regression, with sexual maturity as the dependent variable.

Results

Average colour of the chin-spot

We captured and described 787 male (606 individuals) and 1735 female (1321 individuals)

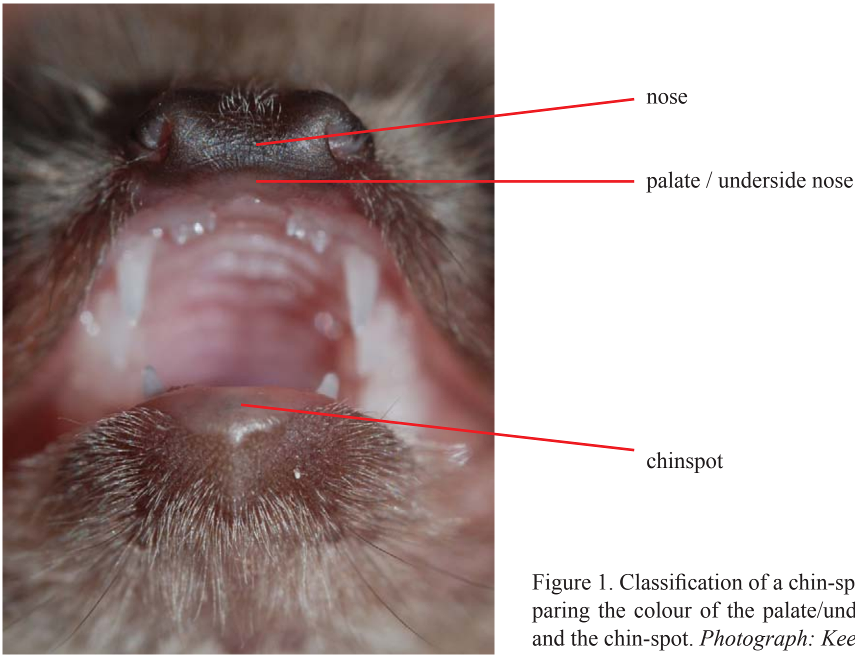

Figure 1. Classification of a chin-spot is done by comparing the colour of the palate/underside of the nose and the chin-spot. *Photograph: Kees van Bochove*.

Figure 2. Photos representing the five coloration classes of the chin-spot. *Photographs: Kees van Bochove (1, 3, 4 and 5) and A.-J. Haarsma (2).*

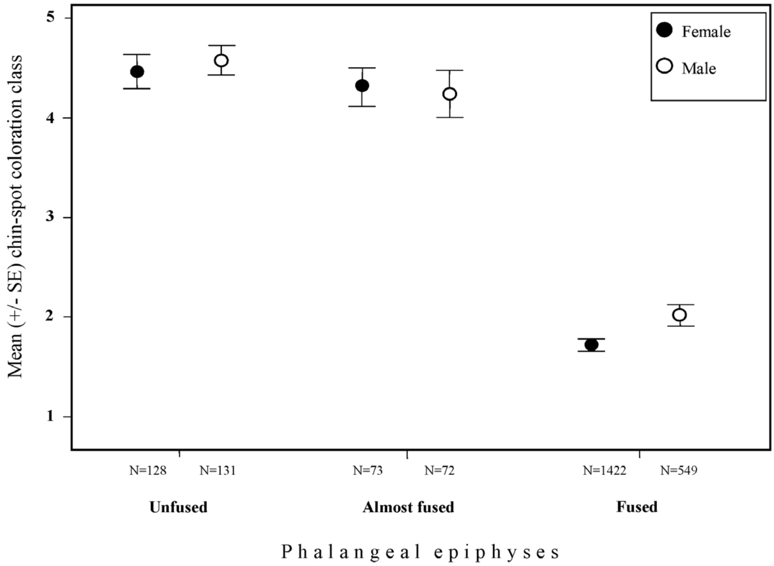

Figure 3. Mean (+/ SE) chin-spot coloration class (class '1'= light-coloured to class '5'= dark purple) plotted against the fusion of the phalangeal epiphyses. For each fusion category sample sizes are given for both females and males.

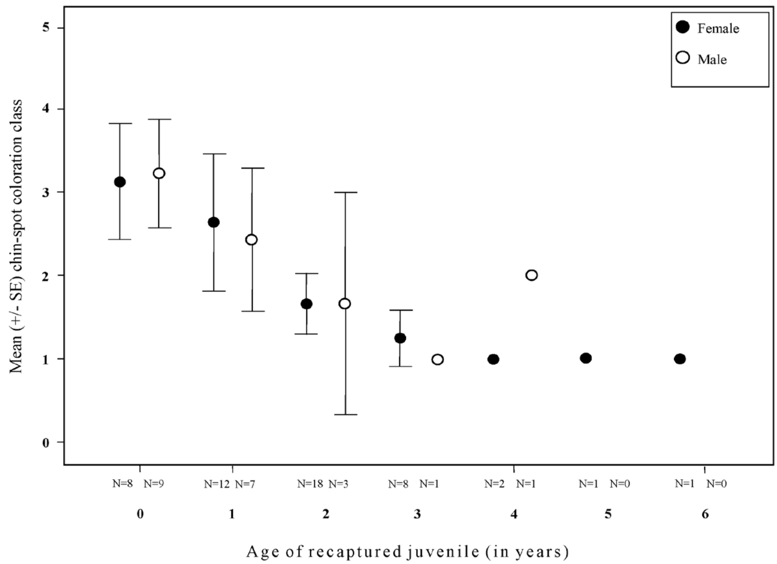

Figure 4. Mean (+/- SE) chin-spot coloration class (class '1'= light to class '5'= dark purple) plotted against calculated age (in years) of recaptured bats, classified as juvenile at first capture. For each year sample sizes are given for both females and males.

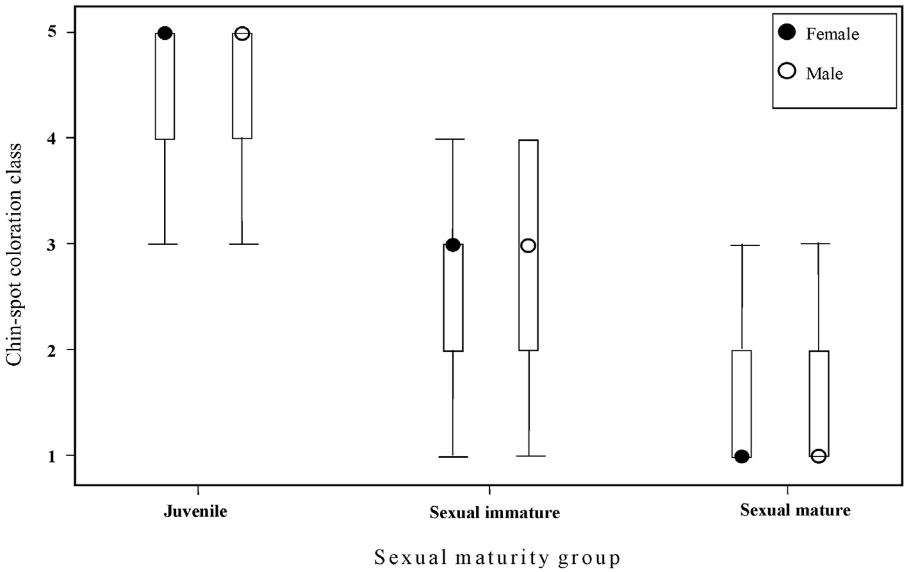

Figure 5. Box plot of the chin-spot coloration class with sexual maturity groups (juvenile, sexual immature and sexual mature). Shown as median, middle 50% and top/bottom 25% of the scores. The results are given for both males and females.

pond bats. We individually marked 501 of the males (82 %) and 1014 of the females (76%). By assessing the fusion of the phalangeal epiphyses we could classify 201 females and 203 males as juveniles. Animals with unfused phalangeal epiphyses generally had a darker chin-spot (class 4 or 5) than animals with fused phalangeal epiphyses (figure 3). We found a significant relation between the fusion of phalangeal epiphyses and the colour of the chin-spot (Spearman's rho = 0.644, n=2110, P=0.000), i.e. a dark chin-spot is associated with unfused phalangeal epiphyses.

Change in the colour of recaptured juveniles

The difference between chin-spot coloration and the fusion of phalangeal epiphyses implies that chin-spot coloration changes during the life of a pond bat. Recaptured bats (those which were classified as juvenile at first capture) hold the key to understanding the speed of transformation of chin-spot colour. This can be done by correlating the age of these bats with changes in chin-spot colour. Although we marked a total of 180 juveniles, we recaptured only 40 of these individuals (27 females and 13 males). We do not know if this was because of a high dispersal rate, low survival of juveniles or fatalities due to marking.

The transformation of the chin-spot of juveniles from dark to light is gradual (figure 4). After two years the first individuals were observed with a class '1' chin-spot. At three years of age the transformation was complete for most individuals. After the age of four no changes in chin-spot coloration class were observed, although a low sample size (n=5) means we can draw no conclusions from this. The relation between a change in pigmentation and the number of years between capture and recapture was significant for both females and males (Spearman's rho for females = 0.600, P=0.000, n=49; Spearman's rho for males = 0.552, P=0.005, n=21).

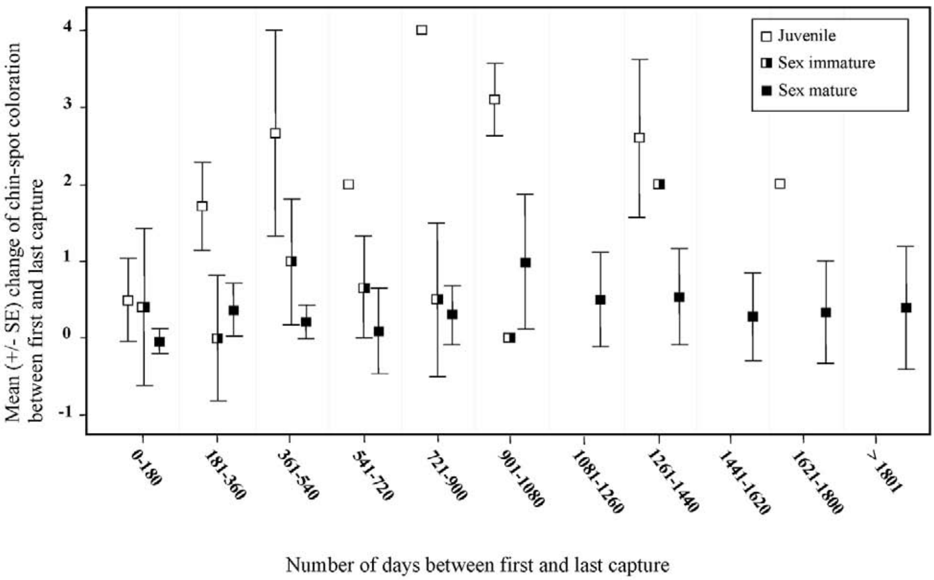

Figure 6. Mean (+/- SE) change between two chin-spot colours plotted against the number of days between the first and the last capture. Animals are grouped according to their sexual maturity during the first catch (juvenile, sexual immature and sexual mature). The largest possible change (four steps) is from a dark chin-spot (class '5') to a light chin-spot (class '1').

Chin-spot in relation to other age indicators

Dental wear

We scored dental wear in 973 female and 428 male pond bats. Dental wear classes '4' and '5' were clustered, because of the low sample size. There was a significant negative correlation between dental wear and chin-spot colour (Spearman's rho = -0.369, *P*=0.000, *n*=1401), i.e. lighter-coloured chin-spots were associated with an increased dental wear. The same result was found when the sexes were analysed separately (Spearman's rho for females = -0.324, *P*=0.000, *n*=973; Spearman's rho for males = -0.398, *P*=0.000, *n*=428,).

Reproductive status

We scored the colour of the chin-spot of 1148 sexually mature females and 278 sexually mature males and for 294 sexually immature females and 366 sexually immature males. A lighter-coloured chin-spot indicated sexual maturity (Wald statistic = 585.54, -2 Log likelihood = 1606.29, df=1, *P*=0.000). The same result was found when the sexes were analysed separately (females: Wald statistic = 327.75, -2 Log likelihood = 888.70, df=1, *P*=0.000; males: Wald statistic= 151.71, -2 Log likelihood = 546.09, df=1, *P*=0.000). Most of the juveniles had a chin-spot with a class '5' coloration, most sexual immature animals had one with class '3'and mature animals had a class '1' chin-spot (figure 5).

Reliability of characteristic

For chin-spot to be a reliable indicator of age the transformation from dark to light must be unidirectional. Furthermore, two independent observations on the same individual must have either the same value or (if the two observations are made over a longer time period) a value that only changes in one direction. To check the reliability of the chin-spot (and the usage of the 5 classes) we compared coloration classes of the chin-spot between the first and last captures (figure 6). In total, the chin-spot was scored twice or more for 208 animals (150 females, 58 males). The maximum change in colour can be four steps, from dark (class '5') to light-coloured (class '1'). The transformation from a dark to a lighter chin-spot was made by 78 animals; the change in colour was the largest for juveniles (between 1 and 4 steps) and sexual immature animals (on average between 0 and 2 steps). 119 individuals, mostly sexual mature animals, obtained the same score at both captures. In 11 individuals (9 females, 2 males) a darker chin-spot was recorded on the second capture.

Conclusions and discussion

The colour of the chin-spot can be used to assess the age class of pond bats. We found significant correlations between the colour of the chin-spot and the other known indicators of age: dental wear, fusion of phalangeal epiphyses and reproductive status. Dark chin-spots corresponded with light dental wear, unfused phalangeal epiphyses and sexual immaturity. By contrast, a light-coloured chin-spot corresponded with intermediate/heavy dental wear, fused phalangeal epiphyses and sexual maturity. The results show a significant relation between the change in colour and the number of days between the first and the last capture. The longer the period between the two capture events, the larger the change in the colour of the chin-spot.

None of the characteristics, dental wear, fusion of phalangeal epiphyses and reproductive status, completely explained the variability of the chin-spot colour. The average coefficients of determination (R^2) for males and females were 14.7%, 41.4% and 36.3% respectively. The chin-spot colour itself is a reliable characteristic, but there is still some overlap between chin-spot coloration classes and the calculated age classes (in years). Therefore we recommend using a combination of characteristics for an accurate age assessment.

For the animals captured as juveniles we observed that the chin-spot coloration transformed from class '5'(deep purple) to class '1' (light-coloured) over between two and four years. After their third winter, almost all the pond bats had a class '1' chin-spot. Most of the pond bats between 0 and 90 days of age (juveniles, with unfused or almost fused phalangeal epiphyses) had a chin-spot with a coloration between class '4'and '5', sexual immature animals class '3' and mature animals class '1'.

The size of pond bats and the combination with other facial characteristics provides sufficient distinction between the five used chin-spot colours to make an accurate age class assessment. Although manual assessment might easily lead to large errors, the results show the opposite. Of the 208 recaptured animals, only 11 individuals were observed with a darker chin-spot on the second capture. Presumably these transformations in colour were based on observer error. To simplify the assessment of chin-spot colour, we advise using only the coloration classes '1', '3' and '5'.

During the production of the manual for the assessment of reproductive status, age and health of bats (Haarsma 2008) experienced bat workers were asked to judge the coloration class of chin-spots shown on a total of 35 pictures of several species of bats. All their judgements were similar, although sometimes there were variations between judgements which did not exceed more than one class. These results confirm that the assessment of chin-spots can be performed accurately by a wide group of users. We advise that bat researchers adopting this technique acquaint themselves with the classification of chin-spot colour by practicing with pictures of chin-spots of several species. Pictures should be displayed with a beamer on a large screen, as a computer screen has too much reflection. We believe that the chin-spot is also applicable as an age indicator for other *Myotis* species. During other studies, in the

Netherlands as well as in France, Germany, Poland and Romania, the first author has seen *Myotis myotis*, *M. nattereri* and *M. bechsteinii* with both dark and light chin-spots.

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Samenvatting

Kinvlek als leeftijd-indicator voor de meervleermuis

Tijdens ecologisch veldonderzoek is het vaak nuttig om de leeftijd van een dier te kunnen bepalen. Bij de watervleermuis (*Myotis daubentonii*) kan de verkleuring van de kinvlek gebruikt worden om de leeftijd van het individu te bepalen. In dit artikel onderzoeken we in hoeverre de verkleuring van de kinvlek ook toepasbaar is voor leeftijdsbepaling bij meervleermuizen (*Myotis dasyneme*). Tijdens zes jaar veldonderzoek, uitgevoerd van 2002 tot 2008, hebben we meer dan 2500 meervleermuizen gevangen. Om de bruikbaarheid van de kinvlek als leeftijd-indicator te bepalen hebben we naast informatie over kinvlekkleur ook informatie over andere leeftijdvariabelen, zoals tandslijtage, vergroeiing van kraakbeenschijven en reproductieve status verzameld. We hebben significante correlaties gevonden tussen de kleur van de kinvlek en andere bekende methoden om leeftijd te bepalen. De resultaten laten een positieve relatie zien tussen de mate van verandering in kleur van de kinvlek en het aantal dagen tussen de eerste en de laatste vangst. De kinvlek van dieren die als juveniel zijn gevangen verbleekte gemiddeld binnen een periode van twee tot vier jaar van kleurklasse ‘5’ (donkerpaars) naar kleurklasse ‘1’ (licht). De kinvlek van juveniele meervleermuizen bevindt zich doorgaans in kleurklasse ‘4’ tot ‘5’, die van de meeste onvolwassen dieren in klasse ‘3’, en die van de meeste sexueel volwassen dieren in klasse ‘1’. We concluderen dat de kleur van de kinvlek een betrouwbaar kenmerk is om de leeftijd van een meervleermuis te bepalen

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