Manual for assessment of reproductive status, age and health in European Vespertilionid bats

Anne-Jifke Haarsma

Electronic publication
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Cover photo: *Myotis mystacinus*, Bart Noort.

With photographs by (in alphabetical order)

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PREFACE

Since the start of mistnet research in the Netherlands in 2001, bats could be studied in the hand. Characteristics such as age, sexual status and health could be described and used to answer fundamental questions. All researchers adapted their own methods to describe these characteristics. Notations and used identification keys for characteristics sometimes conflicted. In most cases only rough descriptions were made, which later could not be checked. For example, four researchers noted two categories to describe the age of a caught bat: juvenile and adult. However, one researcher did this by checking the patterns of closure of the cartilaginous epiphyseal growth plates, another by looking at the chinspot, a third used sexual maturity as a sign of adulthood and the last used fur colour. Instead of describing the characteristic used, they only noted the outcome of their analysis: juvenile or adult. Therby making these four datasets incomparable.

Bats are caught for various reasons, such as identification of species, collecting rabies samples and answering specific research questions. The moment of capture is a stressful event for the bat; after disentanglement from the mistnet most bats become more relaxed. Subsequently each bat has to endure a waiting period and a relative short handling period. Due to this relatively large disturbance the number of capture events should be limited, both to the benefit of the individual bat and to the group of bats, especially on catch events near roost sites or on commuting routes. Therefore it is desirable to minimize disturbance and to get the highest benefit from each captured bat by making the data usable for several research questions. The collection of a common data set by different researchers is essential to achieve a better understanding of bat ecology and hence a higher level of bat protection.

This manual is meant to achieve comparable data sets by the description of analytical characteristics such as age, sexual status and health. By collecting as much rough data as possible about characteristics, interpretations about age, reproductive status and health of a bat can be made afterwards by an impartial computer. If knowledge about certain characteristics varies, re-interpretations could still be possible. This manual can be a helpful tool for both students, newcomers to the study of bats and experienced field workers and aims to ensure that everyone can describe bats in a similar manner.

For the manual to be successful, the variability of each character must be known for each species, especially for complicated characteristics, such as those concerning sexual activity. However, the variability of those characteristics has not been adequately described and/or photographed for all species. Hence I have chosen for an electronic publication which is easier to update with new photos and descriptions. Bat workers are encouraged to send their comments, photos and their experiences using this manual.

Peter Eekelder

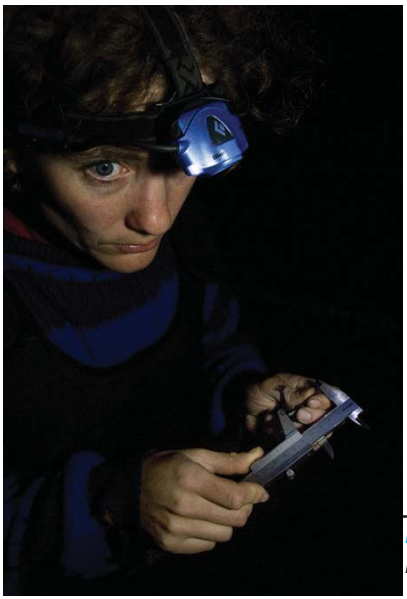


Figure 1: *Author during bat reserach.*

ACKNOWLEDGEMENTS

Many people helped me by sharing their experience in the field identification of bats. Especially Johannes Regelink and Thijs Bosch who helped me make and promote the first complete dataset for the description of bats.
I am also grateful for all (approximately 200) volunteers who helped capture several bat species and used the first forms to describe the bats and made photographs of characteristics. Especially René Janssen, Jaap van Schaik and Bart Kranstauber who used the form to capture and process over 750 bats during a research on the swarming behaviour of bats in front of limestone caves. Furthermore, I also thank Joy de Wit and Jaap van Schaik for reading and correcting the manuscript.

A-J Haarsma

Figure 2: *Volunteer during bat reserach.*

HOW TO CAPTURE AND PROCESS A BAT

Bats can be caught by a variety of techniques both at roost sites and in free flight. Most common is the use of mistnets. The use of mist nets to catch bats can be traced back to as early as 1932. Prior to that time, and for many years thereafter, the primary method of documenting bats during the summer was by shooting them as they flew. Numerous accounts of using mist nets to capture bats appeared in Barbour and Davis (1969). These mistnet were adopted from ornithologists and were not very bat friendly. Today, most researchers use a portable netting system with interlocking poles, tension ropes and mistnets adapted for catching bats. Mistnets suitable for catching European bats have a smaller 'bag' and a finer mesh size (approximately 30-38 mm), so bats will get less entangled. In the Netherlands an adapted version of the white German mistnets is often used. These mistnets are bat friendly and, especially for catching swarming bats in front of hibernaculasare, very successful.

General advice how to disentagle bats from a mistnet are given in '' The bat workers manual'' available for free in electronic format (http://www.jncc.gov.uk/page-2861). In the Netherlands batworkers are encouraged to use the standard procedure (see attachment) for handling and capuring bats. In this procedure both ethical and practical recomondations are made.

To make the most benefit of caught bats, process bats according to a fixed method (form A, see attachment). On this form standard information can be noted, such as number and size of used mistnets, as well as the time at which the nets were opened and closed. Also a sketch of the placement of the mistnets (including net lenghts and key geographic/environmental features) can be made. This information is essential for later analysis of the effectiveness of placement of mistnets and for a comparason between capture nights.

For the study of physical characteristics described in this manual, hang bats in bags in sequence in accordance with catch sequence (figure 3). Put every caught bat in a seperate bag, attach the bag to a line or otherwise make sure the right order of catch can later be followed. Note time of catch, species and flying direction (e.g. in or out of roost, from north to south).

The next step is (after a short relaxing period for the bat) to describe each individual bat with form B (see attachment). This form includes all characteristics given in this manual.

Peter Eekelder

Figure 3: *Hang bags in sequence accordance with catch. Bags should always be hung up and never laid down on the ground, preferably in a warm location without draft, like the inside of a car.*

ETHICAL REMARKS

Once a bats is captured, care is needed to ensure that it is classified and measured quickly and without causing harm. In the Netherlands batworkers are encouraged to use the standard procedures enclosed (see attachment), which advises to release a bat within one hour after capture. Pregnant females within half an hour. Lactating females with young attached should be released immediately, without further disturbance.

After capture, bats must be kept in a soft cloth bag. Bags should always be hung up and never placed on the ground as to avoid accidentally stepping on a bag. Also, bats can start walking in their bag and either fall into the water and drown, or walk off and be forgotten. If possible, captured bats should be placed in a warm location without draft, like the inside of a car (figure 3). For both ethical and research purposes it is necessary to place each bat in a separate bag. Bags should be large enough for a bat to move freely and hang upside down.

Being handled can be very stressful for a bat if not done correctly. Take the bat in palm of your left hand (for right-handed people), face up, and put your thumb under its chin. If this is done correctly, the bat can flex its head without being able to bite the researcher. Always allow the feet to grip the fingers of the hand. This, together with the freedom of movement in a correct grip, makes that most bats will give up their struggle and relax. This is possible even with larger and stronger species such as Eptesicus serotinus (figure 4).

A-J Haarsma

Figure 4: *In a correct grip even larger species such as Eptesicus serotinus and Nyctalus noctula will give up their struggle and relax.*

Avoid heat stress by holding a bat in your palm for too long. Never hold bats solely by their forearms, elbows or wing tips. This will cause flight muscles to be strained, especially in the case of bats like Eptesicus who will not stop making pumping motions.

Before releasing the bat, make sure it's not in semi-torpor. It should be fully aware of its surroundings. A bat should fly away by itself, so never throw it up into the air! Some species of bat need a small gravity drop in order to gather sufficient flight speed (figure 5). Always ensure there is enough free flying space.

Leonie de Kluys

Figure 5: *A bat should fly away by itself. Some species of bat need a small gravity drop in order to gather sufficient flight speed.*

ANALYSIS

The manual can be used to study the different characteristics of age, sexual status and health. Later questions can be studied such as:

- Timing of reproductive stadia
- Correspondence between changes of two characteristics
- Difference in behaviour of groups of bats (e.g. males versus females, sexually active bats vs. sexually inactive bats)
- Health of different groups
- Reproductive success (ratio between adults and juveniles)

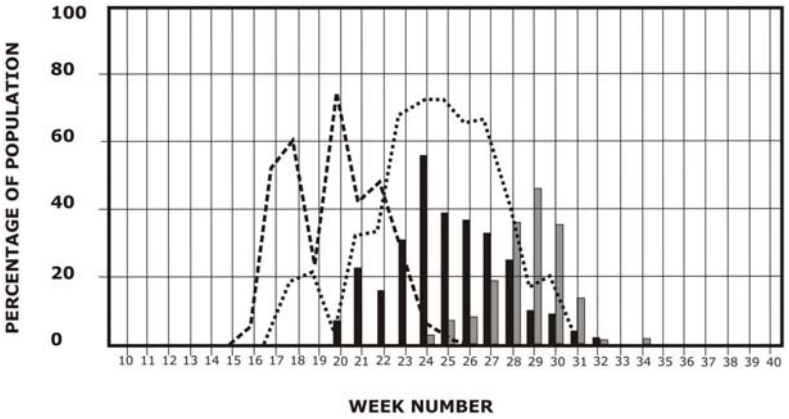


Figure 6: *Example output of an analysis. Percentage of population showing bald spots (black bars) or the typical dark hairs which indicate regrow of a former bald spot (grey bars) per week. Also in this figure, the timing of reproductive stadia of females: pregnant (dotted line) and nursing (dotted line) are indicated in percentage of the population. (Haarsma unpublished data)*

DESCRIBING CHARACTERISTICS

In this manual almost all characteristic is subdivided into 5 categories:

1 = XS/ Minimum/ Absent
2 = S/ near minimum
3 = M/ intermediate
4 = L/ near maximum
5 = XL/ maximum/ Present

By always using these 5 categories, comparing between characteristics is easier. Also filling in data on form B (see attachment) becomes easier and quicker. Unfortunately not every characteristic could be discribed in 5 categories. In that case codes are used, for example nipple status: A- active, R- regrowth of nipple hair, P - active in previous season.

Beginners are advised to fill in the forms, using only the categories 1, 3 and 5. More experienced bat workers are adviced to use all possible codes.

Peter Eekelder

Figure 7: *Researcher filling in the forms.*

MATERIALS

Four materials are needed: 1:Calliper, 2:Spring Balance, 3:Camera, 4: x10 hand lens

1. Calliper

There are several types of callipers: vernier calliper, dial calliper, or electronic/digital. For work in moist conditions (during night fog or rain) a vernier calliper is recommended. A calliper can take several readings: outside, inside, depth, and step. The most common measurement taken for bats is the forearm length; this is done with the outside technique. Measurements of finger length is most often done with the inside technique. Although this measurement is not applied in this manual, it is mentioned for completeness' sake. How to handle a bat during measurement is described in the next paragraph.

Outside Measurement
Outside measurements are the most basic you can do with a calliper. Open the calliper jaws, place the calliper over the object to be measured, and slide the jaws until they contact the object. Read the measurement. Although seemingly easy, several errors can be made resulting in inaccurate measurements. For example, if the callipers are held not straight (i.e. perpendicular to the object) the measurement will not be accurate. Likewise, if there is dirt on the jaws or the object the measurement will not be accurate.

Inside Measurement
The smaller jaws on the top of the calliper are used for inside measurements. Slide the calliper close, place the inside-measuring jaws into the space to be measured, and slide the jaws apart as far as they will go. Read the measurement. It's a little harder to keep things lined up correctly when you are taking an inside measurement. Be sure that the callipers are not cocked, or you will not get an accurate measurement.

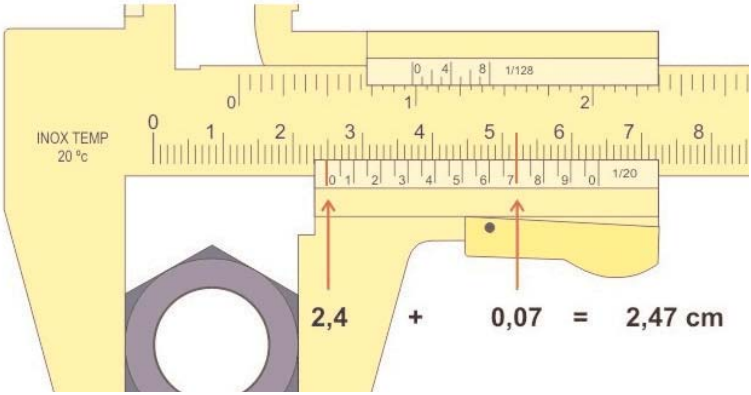


Figure 8: *Using a vernier calliper to take measurements on a nut. The 1st two digits are decided by the location of the 0 on the lower scale. The last digit is determined by the 1st line on the lower scale that exactly matches the upper scale.*

2. Spring balance

There are several types of scales, most common used are digital weight scales and spring balances. For work in moist conditions (during night fog or rain) spring balances are recommended; they reliable, durable and require no batteries. A spring balance should have an tare (zero adjustment) screw and a crocodile clamp. The net weight of the balance should correspond to the maximum weight of the studied bats (if plastic weighing bats are used). A 30 gram spring balance is recommended

Figure 9: *A pesola spring balance with a tare screw.*

3. Camera

A camera is recommended to take pictures of abnormalities found in bats, for later comparison of characteristics described in this manual and a as a way to describe catch location. A camera should have a flashlight and a close-up function (minimal distance between camera and object approximately 30 cm)

4. 10 X hand lens

A hand lens is recommended to inspect small details, such as dental plaque. A 10 X lens is sufficient.

Bertrik Sikken

Figure 10: *Researcher making photo's.*

FOREARM LENGTH

Used for species identification and as a correction factor for weight (body condition index)

How to measure a bat's forearm

To take forearm length (for right-handed people), keep the bat in the palm of your left hand, with your thumb under its chin to prevent it from biting. Fixate the folded forearm of the bat with your thumb and the tip of your middle finger. The top end of the calliper is placed on the bat's wrist, the inner end of the calliper can be balanced against the remaining fingers of the left hand. The maximum forearm length is taken between the elbow and the wrist. It's important to ensure that the moveable jaws of the calliper are well attached to the elbow and wrist and that the elbow is held parallel to the calliper. The forearm of a bat is slightly bendable, which could result in an underestimation of the actual forearm length. Before taking readings, make sure the parts of the calliper are allowed to make slight sideward movements.

Bart Noort

Figure 11: *Incorrect measurement with calliper.*

Peter Eekelder

Figure 12: *Correct measurement with calliper.*

How to measure finger length

For right-handed people, it's best to hold the bat in your left palm, curling your fingers round the bat's body, with your thumb under its chin. Then place the bat on a flat surface, with your hand covering the bat. While opening the wing, turn your hand clockwise and place your thumb on the bat's finger and your index finger on the bat's forearm. Straighten the bat's finger with your thumb. The outer end of the calliper is best attached to the inside of the wrist and the lower part of the calliper is opened until it matches the length of the finger.

WEIGHT

There are considerable changes in body mass during the course of a day and a year. Measuring is valuable only in combination with time of capture.In a single night a bat can increase its weight considerably.

Weighing method
Put the bat in a light, but strong plastic bag. With the tare screw the spring balance can be adjusted to zero. This way you don't have to subtract the weight of the bag. With stronger species use stronger (i.e. thicker) bags. Do not allow the bat too much room in the bag, otherwise it will tear the bag with its thumb claws.

When weighing a bat, hold balance spring by the tip, otherwise the measurement can be incorrect (figure 13).

Peter Eekelder

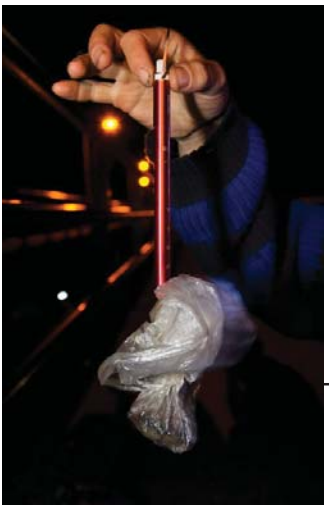


Figure 13: When weighing a bat, hold balance spring by the tip.

DENTITION

The dentition of bats is diphyodont. After losing a complete set of deciduous teeth, permanent teeth are formed (Fenton 1970). In the course of a lifetime, tooth surface becomes abraded from repeated chewing (Anthony 1988). Especially the canine lose their pointy appearance. With age, sometimes dark lines of tartar /dental plaque are formed alongside 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). They found a considerable overlap between animals judged to belong to different age groups and the amount of tooth wear. Hence they concluded that dental wear may be a broad indictor of age in bats, but not a valid characteristic for assigning bats to absolute age groups. Although it is still debated whether dentition can function as an accurate age determinant (Philips et al. 1982), dental wear is still widely used by researchers because of its relative ease.

Looking at a bat's teeth
Not all bats are co-operative. To check the dental wear and plaque a bat has to open its mouth and keep it open. This can best this is done by gently pressing a fingernail against side of the mouth of the bat. Or by gently pulling at the hairs of the neck of the bat and thus bending the head backward (figure 14). During this motion the bat will also automatically open its mouth.

Dental wear can be easily seen without a hand loupe. Inspect wear from both front and side. Teeth of bats with a tough diet, like Eptesicus serotinus and Myotis myotis, abrade first along the inside of the mouth of the bat. This is most obvious from the front, although the teeth appear relatively sharp in a sideways look.

What to look for?
Dental plaque, best seen using a 10 X hand loupe and headlight. It takes practice to shine enough light on bat to see through hand loupe. The dental plaque can be seen as a line along the base of molars (near the gums). This line may have an inconspicuous yellow colour and sometimes have a darker colour (figure 15).

5 categories
In this manual we score dental wear and tartar /dental plaque in two separate categories.

Wear = dental wear:

1 = No wear
2 = Little wear
3 = Intermediate wear
4 = Obvious wear
5 = Complete wear

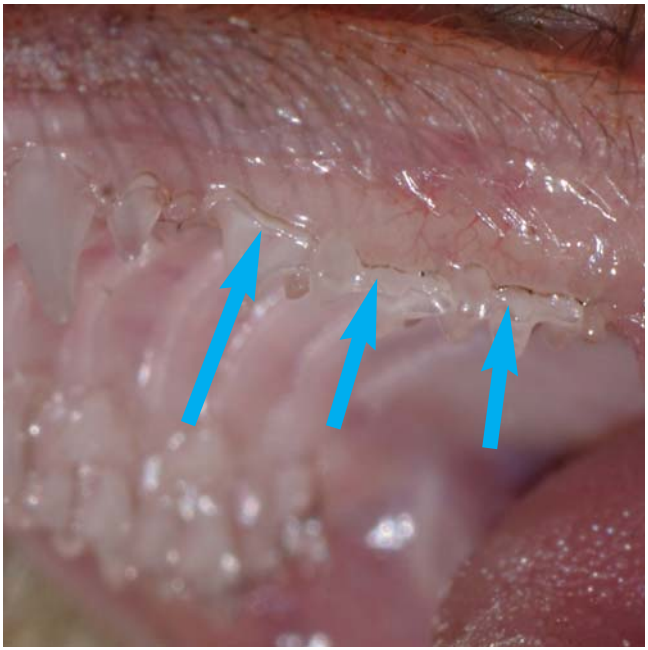
Please note whether you see wear in the upper or the lower jaw.

DP = dental plaque:

1 = No dental plaque
2 = Little dental plaque, yellow coloration
3 = Intermediate dental plaque
4 = Obvious dental plaque, several teeth with black lines
5 = Abundant dental plaque. All tteeth have black lines

JOhannes Regelink

Figure 14: A bat will automatically open its mounh when the hairs of the neck are genty pulled back.



Domini Dalessi

Figure 15: With a handloupe dark lines of tartar/ dental plaque are visable alongside the molars

----------DENTAL PLAQUE----------

Figure XX: *Myotis myotis. Bart Noort*

Figure XX: *Myotis mystacinus. Bart Noort*

Figure XX: *Myotis dasycneme. Kees Bochove*

Figure XX: *Myotis mystacinus. Bart Noort*

Figure XX: *Myotis dasycneme. Kees Bochove*

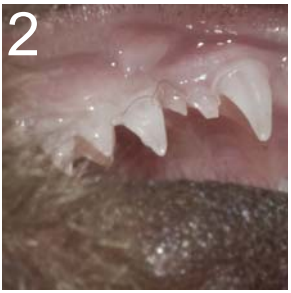


Figure XX: *Myotis mystacinus. Bart Noort*

Figure XX: *Myotis brandtii. Rene Janssen*

Figure XX: *Myotis emarginatus. Domin Dalessi.*

Figure XX: *Myotis emarginatus. Jonas Mortelmans.*

----------DENTAL WEAR----------

Figure XX: *Myotis daubentonii. Peter Eekelder.*

Figure XX: *Myotis myotis. Bart Noort.*

Figure XX: *Vespertilio murinus. Jan Buys.*

Figure XX: *Myotis mystacinus. Bart Noort.*

Figure XX: *Myotis dasycneme. Kees Bochove.*

Figure XX: *Myotis dasycneme. Kees Bochove.*

Figure XX: *Myotis mystacinus. Bart Noort.*

Figure XX: *Myotis emarginatus. Rene Janssen.*

Figure XX: *Myotis bechsteinii. Raymond Haselager.*

----------DENTAL WEAR----------

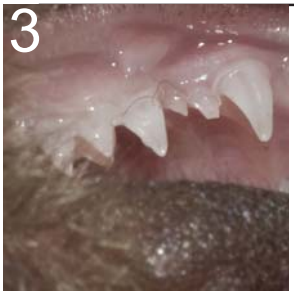


Figure XX: *Myotis mystacinus. Bart Noort.*

Figure XX: *Myotis brandtii. Rene Janssen.*

Figure XX: *Myotis myotis. Bart Noort.*

Figure XX: *Myotis myotis. Raymond Haselager.*

Figure XX: *Myotis emarginatus. Jonas Mortelmans.*

AGE

Patterns of closure of the cartilaginous epiphyseal growth plates in long bones can be used to assess 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 (average 75 days, Elangovan et al. 2002) these phalangeal epiphyses start to fuse. The fusion of the epiphyses in the bones of their fingers can be made visible using a torch, which illuminates the wing membrane and fingers from under the wing. The cartilaginous zones appear lighter than ossified parts of the bones. Additionally, some investigators are able to identify young of the year during winter by the shape of their joints (Davis and Hitchcock, 1965). In this manual we use the absence or presence of fusion. This characteristic is only usable during a limited time (60-75 days after birth), until fusion is complete. In the same period juveniles can also be recognized due to the fact that in most bat species juveniles have different pelage colours than adults (Anthony 1988). Most myotis juveniles are greyish brown and their belly is greyer than the adult form (figure 19-23). Most juveniles have a thicker, denser fur, especially on the inside of their thighs. Juvenile Schreiber's bats can be recognized by the difference between the colour of the fur on their head and that on their lower body.

In most bat species juveniles can be recognized by the shape of their face, which elongates during aging. The pigmentation of nose and face of juveniles is dark; older individuals have a lighter face and nose. Very old individuals have pink spots, apparently without pigment, especially around their nostrils. Although easily recognisable in age extremes (very young or very old), these characteristics are not used in this manual.

Sexual maturity can also be used to assess the age of bats (Kunz 1973). In their first autumn young of the year begin their sexual development. The assessment of characteristics related to reproductive status is described in the next paragraph.

In a study on Daubenton's bats (*Myotis daubentonii*), the chinspot was found and proven to be a reliable mark for estimating age (Richardson 1994). This author defined chinspots as follows: "The chinspot consists of 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." Animals with a dark chinspot are juveniles or sub-adults and animals with a light chinspot are always adults. This might be also true for other myotis species. The chinspot has also proved to be a valid characteristic in age estimation of pond bats (Haarsma,in press).

Visualizing epiphyseal growth plates

Patterns of closure of growth plates can be seen against a light background. This is commonly done by illuminating the wing from below with a torch (see figure 16 and 17). The growth plates near the joints appear as light (translucent) cartilaginous gap (or actually 3 light gaps) and can best be seen in the first and second finger. In very young animals the joints are straight, later they develop into a more rounded, knuckle-like shape.

5 categories

For each animal the closure can be recorded in one of five stages.

1 = Not joined (three large gaps. Joints are straight)
2 = Some joining visible
3 = Intermediate joining visible
4 = Almost joined
5 = Joined (joints rounded, knuckle-like. One gap visible)

Bertrik Sikken

Figure 16: By illuminating the wing from below with a torcheath, patterns of closure of growth plates can be seen against a light background.

A-J Haarsma

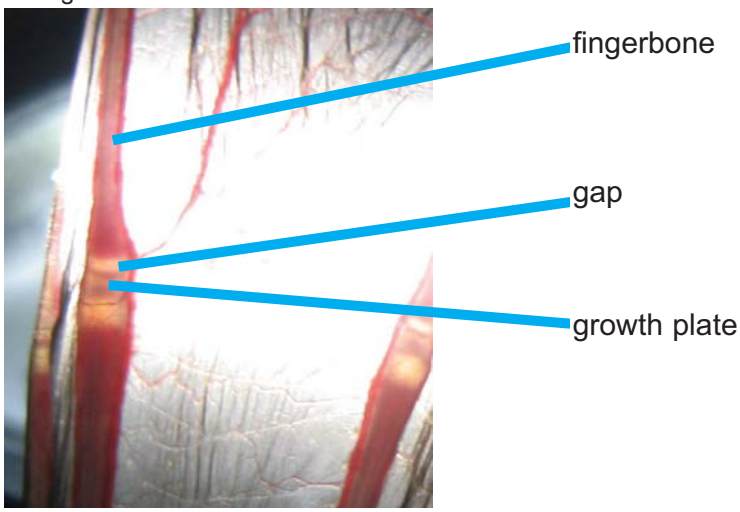


Figure 17: The growth plates near the joints appear as a light (translucent) cartilaginous gap (or actually 3 light gaps). In very young animals the joints are straight.

Visualizing a chinspot

Classification of a chinspot is done by visual inspection using a headlamp. Make sure your headlamp is strong enough, because colour can depend on available light. The mouth has to be slightly opened for a complete inspection of the chin. Colour of the chinspot is compared with colour of palate/ underside of nose (figure 18).

5 categories

For each animal the chinspot can be recorded in five coloration shades:

1 = Chinspot absent. Chinspot the same colour as palate or underside nose

2 = Barely visible. Chinspot with few pigmented spots, sometimes only around the edge of the chin

3 = Intermediate chinspot. About 50% of the chinspot has pigmentation/ chinspot has diffuse pigmentation

4 = Visable. More then half of chinspot has pigmentation. Overall colour purple

5 = Completely purple. Chinspot is densely pigmented. Overall colour deep purple.

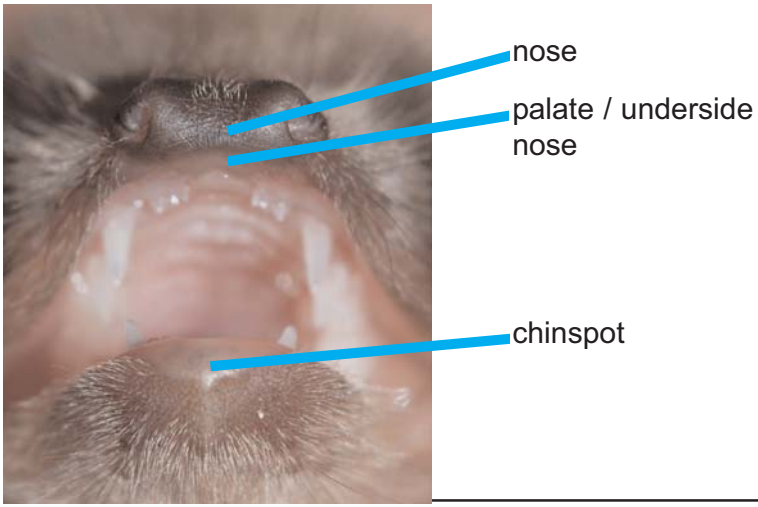
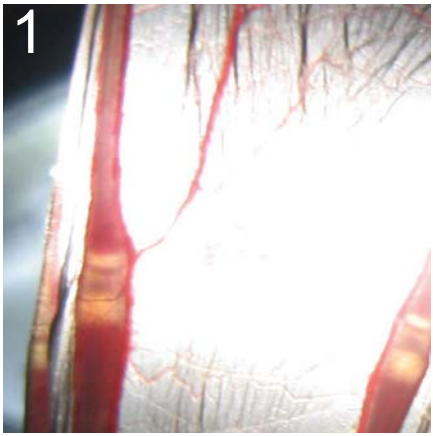


Figure 18: Classification of a chinspot is done by comparing colour of palate/ underside of nose with chinspot.

----------EPIPHYSEAL GROWTH PLATES----------

Bertrik Sikken



A-J Haarsma

Peter Eekelder

Rene Janssen

Kees Bochove

Kees Bochove

----------PELAGE COLOUR----------

Domini Dalessi

Figure 19: *An adult and juvenile Myotis emarginatus.*

Jan Buys

Figure 20: *A juvenile Miniopterus schreibersi.*

John Mulder

Figure 21: *A juvenile Myotis emarginatus.*

----------PELAGE COLOUR----------

Rene Janssen

Figure 22: *A group of Myotis brantii's bats. The juvenile (with circel) has a more dark pelage colour then the adults.*

Yves Adams

Figure 23: *A group of Myotis myotis bats. The juvenile (with circel) has a more dark pelage colour then the adults.*

----------CHIN SPOT----------

----------BECHSTEINS BAT----------

Raymond Haselager

Floris Brekelmans

Gerard Mäscher

----------MOUSE BAT----------

Raymond Haselager

Rene Janssen

----------OTHER BAT----------


Nyctalus noctula. Rene Janssen


Barbastellus barbastellus. Rene Janssen


Plecotus auritus. Rene Janssen

----------POND BAT----------

Kees Bochove

----------POND BAT----------

A-J Haarsma

Kees Bochove

Kees Bochove

Kees Bochove

----------MYSTACINUS BAT----------

Rene Janssen

----------EMARGINATUS BAT----------

Bart Noort

Rene Janssen

----------DAUBENTON BAT----------

Rene Janssen

Jan Buys

Rene Janssen

----------Brandt BAT----------

Rene Janssen

----------Nattereri BAT----------

Rene Janssen

Jonas Mortelmans

REPRODUCTIVE STATUS

Reliable assessment of the reproductive status of bats is important to field and laboratory studies. However, it is often difficult to assess reproductive status accurately (Kunz, 1988; Crighton & Krutzsch, 2000). To prevent incorrect judgement of reproductive status, this manual uses a combination of all identifiable facets. In later analyses steps can be retraced and judgements revised.

Males

The position of the testes varies among bat families. In many vespertilionids they are descended at birth and lie close to and on either side of the base of the penis, where they form bulges beneath the skin. In Rhinolophus the testes and penis are located much higher on the abdomen.
Peak spermatogenic activity of most European bats occurs in autumn, declines over winter and ceases in spring.Some species, like Plecotus auritus and Pipistrellus nathuisii, have a second often much smaller peak in their spermatogenic activity in early spring. Accessory glands are enlarged in periods when mating occurs, but regressed at other times of the year. Corresponding to seasonal spermatogenesis the testes of some species of bats undergo a seasonal migration between the abdomen and the scrotal pouches. This phenonomen of seasonal testicular descent has not been described for European bats.
Puberty is reached in most bats in the year following birth. However, in some vespertilionids some individuals achieve sexual maturity in their first autumn, while some horseshoe bats take several years to become sexually mature.

In males sexual reproductive status can by assessed by visual inspection of the testes and epidydimus through the perianal skin (Entwhistle , Racy, Speakman, The reproductive cycle and determination of sexual maturity in male brown long-eared bats). During spermatogenesis the size of the testis increases. After their release from the testis, spermatozoa

descend to the lowest part of the epididymidis. This causes a shrinkage of the testes and, correspondingly, a swelling to the epididymides. Before their first spermatogenesis the sheath around the tip of the epidydimus (tunica caudalis) is dark and heavily pigmented. At the end of the spermatogenesis the epidydimus swells; as a result the sheath becomes stretched and pigmentation becomes more diffuse. The epididymal tubules appear white through the skin. In most species the pigmented sheath does not return to its former dark colour, and the colour of the tunica caudalis can be used as a distinguishing mark between sexually mature and immature males. This distinction can be obscured by the deposition of adipose tissue around the reproductive organs, especially in hibernation (Racey, 1974).

Females

The nipples of bats are located near the armpits. Females have a single pair of mammary glands and nipples. Rhinolophus have a pair of fake nipples in their pubic region, used by young bats to hang on to their mothers. Mating takes place in autumn and in some species continues in spring. In most species of bats, the sperm is stored and after oestrus in spring the females become pregnant. Towards the end of pregnancy nipples and mammary glands start to enlarge. After lactation the nipples retain their enlarged appearance.

Puberty is reached in most bats in the year following birth. However, in some vespertilionids some individuals achieve sexual maturity in their first autumn, while some horseshoe bats take several years to become sexually mature.

In females sexual reproductive status is difficult to diagnose from the outside. The first external signs of sexual development are visible after their first winter, as they become pregnant or start to lactate. Distension of the lower abdomen caused by the developing foetus cannot be recognized until late in the pregnancy. By gently palpating the abdomen the foetus can be felt as a hard lump. Towards the end of pregnancy and during lactation the nipples

become enlarged. The mammary gland can be seen underneath the skin as a yellow disc. Near the armpits the demarcation line between yellow and pink is visible as the fur is less dense in this region.

During lactation, most females lose most of the hairs in the immediate vicinity of their nipples as a result off fanatic suckling by their young. In most species the hairs on top of the nipples remain and tend to stick together like a paintbrush (a tuft of hairs). At the end of the lactation period the nipples have become dark and keratinized. This is presumably the result of continued distension and suckling/chewing by the young. After lactation nipples retain their keratinized enlarged appearance; such nipples can be used as a criteria of the bat's parity (i.e. having given birth). Most of the hairs in the vicinity or the nipples regrow and during regrowth they are identifiable as wavy grey hair.

Checking a male bat's reproductive status
In order to check the reproductive organs of a male bat, the researcher has to hold the bat by its neck between thumb and index finger. With the other hand the feet and tail membrane are held (figure 26).

The penis colour can be seen by gently flipping the penis into an erect position and checking the underside (figure 24). In species like Pipistrellus nathusii the penis darkens during the mating season.
The testes are located on either sides of the penis and can be made visible by gently pressing on both sides of the abdomen. The epidydimes have descended along the root of the tail. They can only be made visible by pushing the tail root up and stretching the tail membrane, sometimes a torch from underneath is needed (figure 27).

A-J Haarsma

Figure 24: The penis colour can be seen by gently flipping the penis in erect position and checking the underside

Johannes Regelink

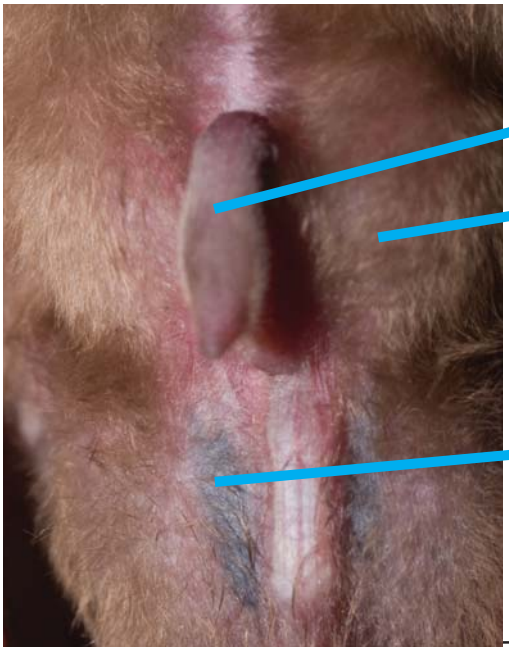


Figure 25: Location of the different reproductive organs of a male Nyctalus bat.

A-J Haarsma

Figure 26: *In order to check the reproductive organs of a male bat, the researcher has to hold the bat by its neck between thumb and index finger. With the other hand the feet and tail membrane are held*

A-J Haarsma

Figure 27: Sometimes a torch from underneath is needed to visualize the epidydimes. They are located along the root of the tail.

5 categories
For each animal the reproductive status can be recorded by the following characteristics:

P colour = penis colour (especially visible on underside of penis. Give in colour shades of white.
B=brown, R=red, O=orange,
followed by, 1 very light; 2 light; 3 intermediate; 4 dark; 5 very dark.

Epd colour = epidydimes colour:
1 very light; 2 light; 3 intermediate; 4 dark; 5 very dark.

BG = buccal glands:

1 = not present
2 = slightly visible
3 = visible (light yellow)
4 = obvious (yellow)
5 = very obvious (extruding and yellow)

Epd size = size of epidydimes

1 = absent
2 = little
3 = intermediate
4 = large (almost descended)
5 = very large (completely descended)

Ts = testes swollen:

1 = not swollen
2 = slightly swollen
3 = intermediate
4 = obviously swollen
5 = very obviously swollen

Epd fill = filling of epidydimus

1 = empty
2 = little filling
3 = intermediate
4 = round and almost filled (only tip empty)
5 = very rounded and filled

Checking female bats' reproductive status

The nipples can be found next to the armpits. By gently blowing through the fur, the nipple can be revealed (figure 28). Most often the tuft of hairs on the nipple itself will advertise the nipple. At the base, these hairs tend to be a different colour than the other hairs. If the female is lactating a yellowish gland (mammary gland) underneath skin can be seen. Near the armpits the demarcation line between yellow and pink is visible as the fur is less dense in this region (figure 29 and 30). A nipple can be classified to 4 different stadia: active (figure 33), active untill two weeks ago (figure 32) and active during previous season (figure 31). A nipple of a immature animal is classified as not active.

5 categories

For each animal the reproductive status can be recorded by the following characteristics:

Np bald = nipples bald

- 1 = not bald.
- 2 = some baldness.
- 3 = intermediate baldness.
- 4 = bald.
- 5 = surrounding of nipple completely bald.
- R= regrowth of hair around nipple.

MG= mammary gland

- 1 = not present.
- 3 = maybe present.
- 5 = present.

Np colour = nipple colour

- 1 = light (same colour as skin)
- 3 = light brown (a little bit darker then skin)
- 5 = obviously darker than skin (visible if animal has lactated in the previous season)

Np size = nipple size:

- 1 = very small.
- 2 = small.
- 3 = intermediate.
- 4 = large.
- 5 = very large and stretched.

Bertrik Sikken

Figure 28: *The nipples can be found next to the armpits. By gently blowing through the fur, the nipple can be revealed.*

Kees Bochove

Figure 29: *No mammary gland present.*

Kees Bochove

Figure 30: *Mammary gland present.*

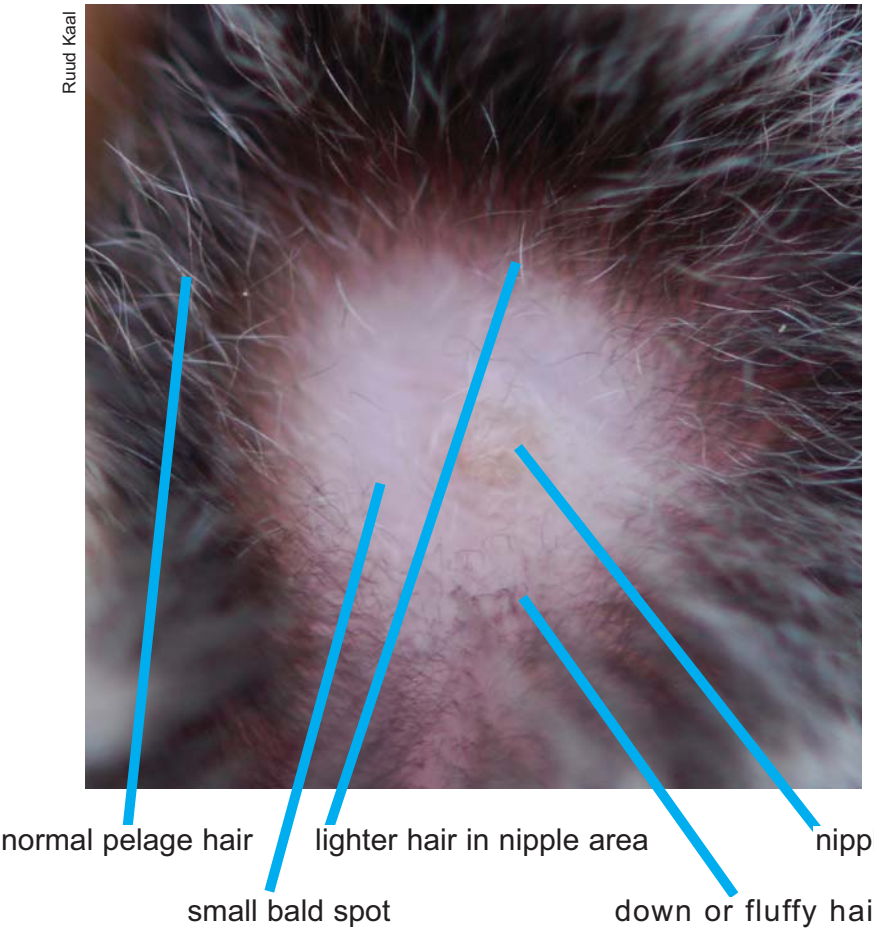


Figure 31: A nipple classified as active during previous season, can be identified by looking at several characteristics.

Kees Bochove

Figure 32: A nipple classified as regrowth, can be identified by looking at several characteristics.

Raymond Haselager

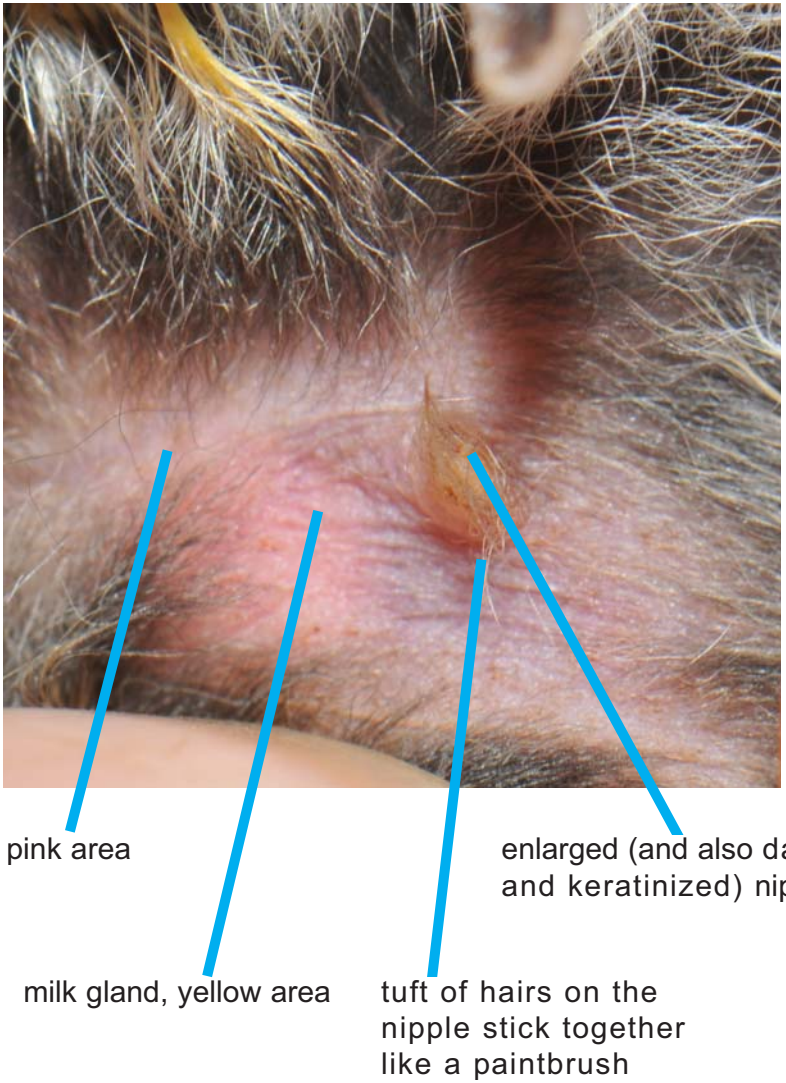
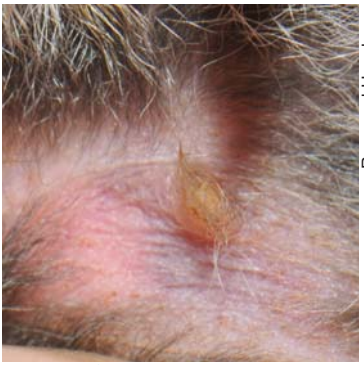


Figure 33: A nipple classified as active (female is lactating), can be identified by looking at several characteristics.

----------NIPPLES----------

Peter Eijkelder

Figure 34: *Myotis daubentonii. MG = 5, NP bald = 5*



Raymond Haseleger

Figure 35: *Myotis myotis. MG=5, NP =.4.*

Kees Bochove

Figure 36: *Myotis dasycneme. MG = 1, NP bald = T*

Peter Eejkelder

Figure 37: *Myotis daubentonii. MG = 1, NP bald = T*

Kees Bochove

Figure 38: *Myotis dasycneme. MG = 1, Nipple colour = 5, nipple size = 5, Previous season active*

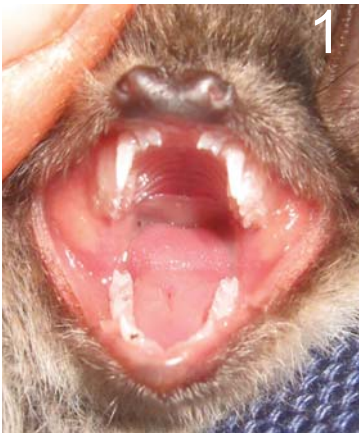
Kees Bochove

Figure 39: *Myotis dasycneme. MG = 1, Nipple colour = 1, nipple size =1, Never active.*

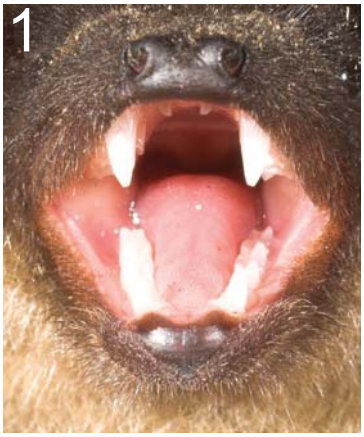
----------BUCCAL GLAND----------

Jan Buys

A-J Haarsma



Yves Adams



----------TESTES----------

A-J haarsma

Figure 40: *Myotis dasycneme. Epd size = 3, Epd fill = 2.*

Kees Bochove

Figure 41: *Myotis dasycneme. Epd size = 5, Epd fill = 3.*

Johannes Regelink

Figure 42: *Nyctalus noctula. TS = 5*

Johannes Regelink

Figure 43: *Pipistrellus pipistrellus. TS = 5*

----------TESTES----------

Yves Adams

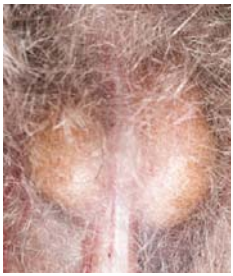


Figure 44: *Myotis dasycneme. Epd size = 5, Epd fill = 4, colour = 1.*

Jan Buys

Figure 45: *Vespertilio murinus. Epd size = 5, Epd fill = 5, colour = 4.*

A-J haarsma

Figure 46: *Myotis dasycneme. Epd size = 5, Epd fill = 5, colour = 1.*

Jan Buys

Figure 47: *Myotis daubentonii. TS = 5, Epd size = 1, Epd fill = 1*

Domin Dalessi

Figure 48: *Myotis emarginatus. Epd size = 5, Epd fill = 5, colour = 4.*

----------PENIS----------

Rene Janssen

Figure 49: *Myotis brandtii, R4*

Bart Noort

Figure 50: *Myotis emarginatus, B3*

Rene Janssen

Figure 51: *Pipistrellus pipistrellus, R5*

Jan Buys

Figure 52: *Myotis daubentonii, R3*

ABNORMALITIES

It is worth noting any unusual coloration, injuries or deformity to re-identify individual animals. Not only can they be used to analysed the health of a population, they can sometimes be used to monitor changes or healing processes in individual bats. Besides identifying individuals by their abnormalities, with some effort individual bats can also be identified by the pattern of veins and scars in their wings.
A number of bats have serious tears to the wings, which nonetheless have healed. Bite marks are sometimes apparent, especially in male bats' ears. The pond bat (*Myotis dasycneme*) seems to be afflicted by a strange phenomenon, most lactating and pregnant bats show bald spots between their shoulder blades (Haarsma, in press).
Leucism (a partial or total lack of eumelanin and phaeomelanin), frequently misinterpretated as partial albinism, is a frequently observed in all bat species. Furthermore, all sorts of warts and fungi can be found. The fusarium or white nose syndrome is the most well-known infliction of bats in America; a non-lethal version of the syndrome has also been seen in Europe.

5 categories
For each animal the baldness can be recorded in one of five stages:

1 = No baldness
2 = Little baldness
3 = Intermediate baldness
4 = Obvious baldness
5 = Very obvious baldness (large bald spot)
R = regrowth (regrowth of hair; grey or shorter hairs visible).

Please note location of bald spot (for example: between shoulder blades, around navel.

Kees bochove

Kees bochove

Figure 53: *The beginnings of regrowth of hair. These hairs grow in a tipicular circel, basis of each hair darker and more grey then normal hairs.*

Figure 54: *Advanced stadia of regrowth of hair. The hairs can still be indentified as freh, by there shorter and darker apparance.*

Kees bochove

Rene Janssen

Figure 55: *A frequent observed injuries or deformity on the joints of pond bats.*

Figure 56: *Leucism on wings.*

Kees bochove

Figure 57: *by the pattern of veins and scars in their wings. Pond bat*

Gerard Mäscher

Figure 58: *Leucism. Natters bat*

Kees bochove

Figure 59: *Wart on nose. Pond bat*

Gerard Mäscher

Figure 60: *Many warts. Pond bat*

Gerard Mäscher

Figure 61: *White nose syndrome. Pond bat*

Bart Noort

Figure 62: *Warts on ears. Myotis*

Kees bochove

Figure 63: *An intermediate bald spot. Pond bat*

Figure 64: *Animals in a maternity roost with baldspots.Myotis dasycneme*

----------PARASITES----------

Raymond Haseleger

Jonas Mortelmans

Rollin Verlinde

Rollin Verlinde

Jan Buys

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GUIDELINES FOR CATCHING BATS

General

- The leading researcher is always responsible for the entire catching attempt. When necessary he/she must be able to quickly take over from assistants, or if necessary choose to close the net.
- Catching is prohibited within a 40 meter radius of known summer roosts of the following species: Myotis dascycneme, Myotis emarginatus, Eptesicus serotinus, Myotis myotis and Vespertilio murinus. This also includes catching the bats at the exit of the roost!
- A mistnet must be put up so that it can be closed quickly, in the event that a larger amount of bats is caught than can be properly handled.
- A mistnet must be put up so that a bat can be reached quickly, regardless of where it lands in the net, and without endangering the animal.
- Open mistnets are to be checked at least once every 10 minutes (when a large amount of bats is caught, a higher frequency of checking is necessary!). When placing nets, attention must be paid to whether this checking frequency is achievable.
- Bats must be extracted from the net within 2 minutes. When placing nets, the total length of net per person must be held low enough to achieve this.
- Bats that are still entangled in the net after 2 minutes of attempted extraction must receive priority treatment. This entails either that the extraction be handed over to a more experienced catcher, or that the bat be cut out of the net using a small pair of scissors.
- All captured bats must be released within an hour of capture. Under exceptional circumstances (ex: telemetry) an animal may be kept for a longer period. In this case, feeding it is required, it is therefore advisable to take some sort of food such as mealworms to each capture attempt.
- Pregnant or lactating females must receive priority in

- Nearly full term females and females with attached newborns must be released immediately.
- When releasing bats allow them to fly away under their own power. If necessary they can be placed on a tree (ensure that the bat is not undercooled!)
- Try to prevent undercooling as much as possible. This includes placing the captured bats in a draft-free location. Bats which do become undercooled must be warmed up prior to release.

Catching near roosts or flight paths

- A capture attempt in the vicinity of a roost must be composed of at least 2 people
- To minimize disturbance of flight paths, a route may only be caught during either the evening or the morning migration.

Catching above water

- The leading researcher is responsible! He/she must also be prepared to enter the water and help if and when necessary.
- A net above water may never be unmanned, so catching both above land and water is only possible if multiple researchers are present.
- Nets above water must be visible at all times. When placing the nets it must be ensured that this is the case.
- Special care must be taken to ensure that animals do not touch the water during capture.

FORM A

Location	
Coordinates	
Date	
Observer	
Names of people helping	
Remark	

	air	1 cm	10 cm	30 cm
Temp begin				
Temp end				
Water temp				
Rain				
Mist				
Wind (Bft)				
Wind direction				
Cloud (%)				

	Observation Method	**Length**	**Time open**	**Time close**	**Location/ remarks**
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Behaviour (see *)
1: N turned
2: N escaped
3: N passed
4: N caught

Observation method (see *)
1: Batdetector
2: Visual detection
3: Mistnet

Make drawing on the back op paper!

						Behaviour (see *)			
Nr	**Species**	**Time**	**Flies from … to ..(direction)**	**Observation method (see *)**	**Remarks**	**1**	**2**	**3**	**4**

Date: **FORM B**

Location:

Also see FORM A								Age		Dent				Fur		♂						♀					Parasites/ diarea/ markings/ feaces/ abnormal fur colour/ broken teeth/ other remarks
Nr	**Time**	**Bag**	**Direction**	**Net**	**RC**	**Species**	**♀♂**	**CS**	**Eph**	**Wear**	**DP**	**FA**	**Weight**	**Bold**	**Where**	**P colour**	**BC**	**Ts**	**Epd size**	**Epd filling**	**Epd colour**	**Np size**	**Np bold**	**Np colour**	**MG**	**Abd**	

Also see FORM A	Use corresponding numbers on both form A and B. Write on form B **Time**, **Bag** (bag number), **Direction** (from X to Y), **net** (net number)
Other	**RC = recapture:** if visible with number of first capture occasion **CS = chinspot:** 1 not visible, 2 barely visible, 3 intermediate visible, 4 visible, 5 completely purple **Eph = epiphyseal growth plates:** 1 not joined (large gap); 2 some joining visible; 3 intermediate joining visible; 4 almost joined; 5 joined (joins rounded, knuckle like) **Wear = dental wear:** 1 XS; 2 S, 3 M; 4 L; 5 XL. Note: please note whether in upper or lower jaw). **DP = dental plaque:** 1 XS, 2 S, 3 M, 4 L (several teeth have black lines), 5 XL (all teeth have black lines) **FA = forearm length in millimetres** **Bald =** 1 no baldness; 2 little baldness; 3 intermediate baldness; 4 obvious baldness; 5 large bald spot, R regrowth (regrowth of hair; grey or shorter hairs visible) **Where =** if bald spots are visible, where are they located on the body of the bat? (for example: between shoulder blades, around navel)
♂	**P colour = penis colour (especially visible on underside of penis):** Give basis colour in shades of white, B=brown, R=red, O=orange, followed by, 1 (light) to 5 (dark). **BG = buccal glands:** 1 not present; 2 slightly visible; 3 visible; (light yellow); 4 obvious; 5 very obvious (extruding and yellow) **Ts = testes swollen:** 1 XS; 2 S; 3 M; 4 L; 5 XL **Epd size = size of epidydimus:** 1 absent; 2 little; 3 intermediate; 4 large; 5 very large (completely descended) **Epd fill = filling of epidydimus:** 1 empty; 2 little filling; 3 intermediate; 4 round and almost filled; 5 very rounded and filled **Epd colour = colour of epidydimus:** 1 (light) to 5 (dark), or B (completely black), W (completely white), B/W (white with a black tip)
♀	**Np size = nipple size:** - 1 XS; 2 S; 3 M; 4 L; 5 XL (also stretched) **Np bald = nipples bald:** 1 not; 2 little baldness; 3 intermediate; 4 bald; 5 surrounding of nipple completely bald, R regrowth of hair around nipple **Np colour = nipple colour:** 1 light (same colour as skin); 3 light brown (a little bit darker then skin); 5 obviously darker than skin (visible if animal has lactated in previous season) **MG= mammary gland (yellowish gland underneath skin):** 1 not present, 3 maybe present, 5 present. **Abd = size abdomen:** P? maybe pregnant, P pregnant, N normal