# Nest-box Monitoring Guide Forstenrieder Park

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# 1. Introduction to nest-box monitoring

This document serves as a guide to explain the procedures for the nest monitoring of the great tits (*Parus major*) and the blue tits (*Cyanistes caeruleus*) at the Forstenrieder Park. The sections below are intended to inform observerson how to proceed during fieldwork and on how to fill in the nest data sheets during the nest box monitoring.

## 1.1 Breeding season overview

While males begin to establish their territories already in January, it will take up to March until you can see males and females exclusively flying around together. At the end of March, early April, the nest is built by the female only. At the beginning of the breeding season, females can take between one and two weeks to build a nest. Later in the season (e.g. second broods), a nest will usually be built within 1-2 days. Most of the time, the nest material is gathered in the near surroundings of the nest nest-box. After nest completion, the female will lay one egg per day. Eggs are usually laid in the early mornings. For that reason, nest-monitoring activities should be carried out in the late morning or in the afternoon to avoid disturbing females when laying eggs. If there are periods of cold weather, females can pause laying for one to two days. The clutches of a great tit usually consist of 6-12, while the clutches of the blue tits consist of 8-15 eggs. Both species lay whitish eggs with red spots, which only differ in size. Great tit females usually start incubating after the last egg is laid while blue tits can sometimes start incubating before the last egg is laid. In both species, only the female incubates. The incubation period of great tits lasts between 12-15 days and 10-14 days for blue tits, depending on air temperatures. Upon hatching, the female will brood the chicks by herself. Males will help to provide the chicks with food. Chicks remain in the nest for up to 19-22 days until fledging. After fledging, the chicks will remain in their parents’ territories and will be fed for another 2-3 weeks.

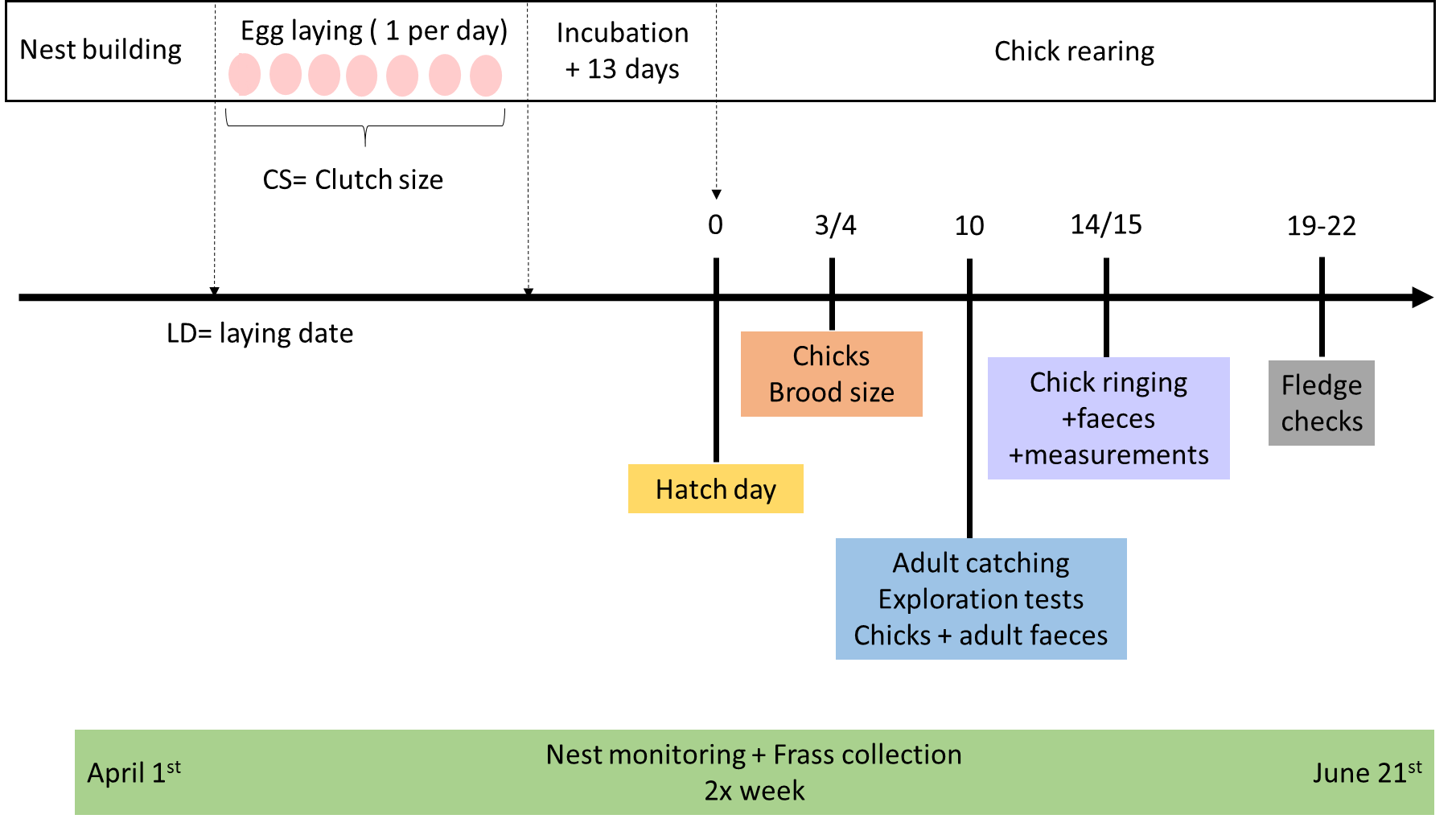


Figure 1. Overview breeding season

## 1.2 Nest monitoring

Nest-boxes will be checked twice a week from the end of March onwards. Two checks per week will be carried out throughout the stages of nest building, egg laying, and start of incubation (Figure 1). When the nest is in advanced stage (e.g. IN/CN see section 2) check carefully if there are already any eggs. During laying, the female often covers them with nest materials. The checks continue until the eggs are found to be warm, indicating that the female is incubating. Write down if any of the eggs are broken.

### *1.2.1 Incubation check*

If the female is not present, note if the eggs are:

* **no** = indicates that no eggs are in the nest
* **cc** = eggs are cold and covered with nest material
* **co** = eggs are cold and not covered
* **wc** = eggs are warm and covered with nest material
* **wo** = eggs are warm not covered

Count the eggs. To do this, either push the female gently on the side with a pencil or let her fly out. **Make sure you keep holding the lid so that it cannot fall onto** **the female.** When it is done, immediately leave the nest-box, to allow thefemale to return quickly. **Always count number of eggs** when incubated as the clutch size allows to determine the expected hatch day. Importantly, **never take the female in your hands** as she might abandon the nest when caught. **If the eggs are still cold after 10 days the last** **one was laid** remove the nest from the nest-box.

### *1.2.2 Hatch check*

Two days before the expected hatch date (i.e. 14 days after the start of incubation) check if any chick has hatched. If no chick has hatched, continue to visit the nest-box **daily** until at least one chick has hatched (i.e. hatch date). If the female is on the nest during the checks, **read her color rings**. **To do this, cover the nest-box opening with one hand and gently lift the female up on one side using a pencil.** Afterwards, let the female fly away or push her gently to the side using a pencil and check if there are any hatchlings. You do not need to count the hatchlings, just note that there is at least one. **On the hatch day, the chicks have an age of day 0**. If chicks seem to be older than day 0, try to estimate their age (See picture Age). Write down their age. If the female is not on the nest and you find any dead chicks, remove them for the nest and write it down on the nest form.

In the case the female is not on the nest and no chicks have hatched, feel if the eggs are warm and count their number. **It might be that the female laid additional eggs after she started** **incubating. If eggs remain cold for more than 5 days after the expected hatch day, remove the nest**.

### *1.2.3. Brood size check (day 3 or 4)*

At day three for great tits and four for blue tits after hatching, the nest will be visited to determine the brood size. The brood size is the total number of eggs minus the number of unhatched eggs. Therefore, count the number of chicks alive, chicks dead and remaining eggs. It is important to count the latter because chicks that have died might have been already removed from the nest by the parents.

### *1.2.4 Adult catching, faeces collection and exploration test (day 10)*

1. Catching and handling aggression score

When the chicks are 10 days old, the parents will be caught and subjected to the exploration test. Before you start, set up a testing/ringing station outside the focal birds view (ca. 50-100m from the focal nest box). Set up the testing cage by putting it on a flat stable surface. Ad a disinfected sheet of plastic at the bottom of the cage, for the feces collection. Face the front away from any roads/paths or potential disturbance and keep your gear behind the cage. **Check that all perches are in position (lowered to the bottom position)** and that all doors (main and side) are closed! Place the camera in front of the cage, so that the entire cage, including the side compartment can be seen. Get the banding equipment ready.

When everything is set up, go to the nest-box, cover the entrance hole and arrange the net over the top of the nest box\slide a bird bag from the top to catch the adult underneath. If no adult is in the box, remove the net and quickly set up the spring trap. If the female or male is present, grab it, perform the handling aggression test.



Figure2: Position of the handler during the handling aggression (HA) test. The bird is facing outward. Both legs are held still (between the thumb, middle and forefinger) in one hand and the observer is moving their finger (other hand) horizontally towards the bird: 4 times stopping at 2-3cm from the break and 4 times touching the bird’s beak. Figure from Caizergues et al. 2022.

|  |  |  |  |
| --- | --- | --- | --- |
| Score | Wings spread | Tail feathers spread | Bird strikes fingers |
| **0** | No | No | No |
| **1** | No | No | Yes, but only if provoked |
| **2** | No | Yes | Yes, spontaneously |
| **3** | Yes | Yes | Yes, spontaneously |

When the bird displayed one reaction specific to one score and another reaction specific to another score, it received an average score between the two. For example, a bird that struck without any provocation (score 2) but did not have its wings and tail feathers spread (score 1) would be scored as 1.5.

Then put it in a bird bag. **Use a fresh bag for every bird!** **Properly close the bag and start the stopwatch!** Remove the net and set up the spring trap to catch the other parent. This procedure should be done in a thorough but quick manner to avoid that you will be seen by the other parent. **Write down the trap set up time** and move away quickly. **Check the trap about every 10 minutes.** When the trap is closed, go to the nest-box and slide your hand under the lid and grab the bird. A bird bag might come handy to prevent the bird from escaping. Once you grabbed the bird, put it in a bird bag and start the stopwatch. In case the trap has triggered, but no adult is caught, reset the trap. You might have to change the stick and/or its position in a way that it is unreachable for the chicks. If you have several unsuccessful trials or the trap is not closed after 30 min, watch the nest-box from the distance to see if the parents are around. If an adult enters the nest-box without triggering the trap, run to the nest-box, cover the entrance hole (stick or bird bag), and grab the bird. Follow the steps mentioned above.

1. Exploration, breath rate, morphometrics, blood and faeces samples
2. Take the bird out of the bag and ring it (one aluminum ring). Write down the ring number, colour combination and sex. Double or triple check that the ring number is correct. There should be 7 Letter/Digits ordered as LDLDDDD (e.g. C3F6823). Determine the bird age and sex.
3. Put the bird into the side compartment of the exploration cage. Cover the plastic door with a bird bag and **stop the stopwatch** to get the **handling time**.
4. Fill in the **camera sheet** and the first fields of the **exploration form** (nest-box, trap set up time, catching time, etc.).
5. After the bird has been in the side compartment for ~ 30 seconds, start the camera and film the camera sheet. This is needed to assign the taken videos to the right bird later.
6. Open the door between side compartment and main cage and shake the bird bag to make the bird move into the main cage. **Start the stopwatch** and video record the bird’s behavior for **2 minutes**.
7. After 2 minutes, take the bird bag from the transparent plastic door and open the door of the side compartment. Use the bird bag to make the bird go into the side compartment. Once the bird is in the compartment, close the slide door and take it out to measure its breathing rate.
8. To measure the breathing rate, count the number of breaths (in or out) for 30 seconds. Do not restart the stopwatch. During that time, note the number of struggles during the 30 seconds to assess the docility score of the bird.
9. Next, re-read the bird ring and take the standard measurements that are usually taken when a bird is caught (**fat score, tarsus 3x, wing length 3x, P3, weight, muscle score, bill**).
10. Then, we will assess the adult for ticks in the head. First, we will scan for ticks in the right ear, you can use a Q-tip to help you move (and hold) the feathers around it. Then, continue with the top of the head. After this, grab the bill firmly with your fingers and turn the head of the adult to scan the left ear. Then, and while grabbing the bill, pull the head back and blow so you can scan the throat. Finally, check for ticks around the bill.
11. Finally, 10 μl (0.01 ml) of blood will be collected for genetic analysis. Use a Q-tip and some sanitizer to disinfect and expose the brachial vein of the left wing. Blood sampling involves puncturing the brachial vein with a sterile needle and then drawing off a blood drop from the skin with a capillary tube. The blood will be immediately stored in an Eppendorf tube containing 0.75 ml of Queen’s buffer. Put the capillary tube into the Eppendorf tube, close and shake it to make the blood blend with the solution. Press a cotton swab gently on the puncture site to ensure complete coagulation of the wound. Close the wing (leave the cotton swap) and put the bird into another bird bag.
12. Search the first bird bag and plastic sheet for bird faeces. If found, collect with disinfected spoon and place in an Eppendorfer tube with alcohol. Mark each tube with the date\_plot & nest box number\_ ring number (eg. 22.04.23­\_E28\_B5K0221). Clean the plastic sheet with disinfectant for the next use. Make sure that no contaminations are left!
13. Go to the nest box, take the complete brood, count them and measure total weight by placing them in the round plastic cup. Then, if possible collect faeces from the chicks pooled in one tube. When in the tube, destroy the faecal sacs by using a toothpick (**use a new one each time!**). Mark each tube with the date\_plot & nest box number (eg. 22.04.23\_E28). Clean the plastic cup with disinfectant for the next use. Make sure that no contaminations are left! Put the chicks pack into the nest while counting them again. For each nest it is important to collect "negative control samples". Open and close a sample tube in the field, while pretending to fill it with the tools you used, but without adding a sample. (label with date\_plot & nest box number\_C (eg. 22.04.23­\_E28\_C).
14. Release the adult into its nest-box or nearby if not possible.

### *1.2.5 Chick ringing and measurements (day 14 or 15)*

At **day 14 for great tits and 15 for blue tits** after hatching, the chicks will be temporarily removed from their nest to take measurements and collect faeces. Go to the nest-box and count the number of alive and dead chicks, as well as the number of eggs, if there are any. If all chicks are dead, remove the nest and check if there are any eggs buried in the nest material. If so, you might have to adjust the brood size estimated on day 3.

If the nest is still active, t**ake all the** **chicks** and put them in a bird bag. **Use a new bird bag for each nest box!** Go to your ringing station (near the box so the parents do not try to get in) to ring and measure the chicks. Process one chick after the other in the following way: first, write down the ring number and ring the chick. Next, take the chick’s fat score (See picture fat) and morphometrics, including tarsus, wing length, P3 and weight. Processed chicks go together in a new bird bag to make things easier. After the procedure, all nestlings will be returned to their nest (estimated time of this procedure is ~ 20 min). Take faeces from the first bird bag/whenever possible and put them all together in an Eppendorf. When in the tube, destroy the faecal sacs by using a toothpick (use a new toothpick each time!). Mark each tube with the date\_plot & nest box number (eg. 22.04.23­\_E28). For each nest it is important to collect "negative control samples". Open and close a sample tube in the field, while pretending to fill it with the tools you used, but without adding a sample. (label with date\_plot & nest box number\_C (eg. 22.04.23­\_E28\_C).

### *1.2.6 Fledge check (day 19)*

Go to the nest-box, cover the entrance hole and carefully open the lid. **Approaching the nest** **at this period may cause the chicks to jump out!** Check if there are any chicks in the nest-box. The fledge date is the day when more than half of the young left the nest. Continue checking the nest-box every other day until all chicks fledged. When you find only 1-2 chicks in the nest, check again the following day (except if brood size on day 14 or 15 was 1-2). Once all chicks fledged, remove the nest and check if there are any eggs buried in the nest material. If so, you might have to adjust the brood size estimated on day 3. In the case that the remaining chicks are dead, read their rings and indicate on the nest sheet under chick status that they died after day 14/15.

# 2. How to fill in the nest form

## 2.1 Brood overview

***Brood ID:*** *The brood ID will be generated once you entered the nest into the database. Only**fill in the brood ID after the nest was entered.*

**Plot:** The plot the nest-box is located in (A, B, C, D, E or F).

**Nest-box:** Unique nest-box ID, which is written outside on the nest-box.

**Species:** The species breeding in the nest-box. Write GT = great tit, BT = blue tit, CT = coaltit and NH = nuthatch.

**Density treatment:** Density treatment of the area in which the nest box is located. Write 1= GL/BL, 2=GH/BL, 3= GH/BH, and 4=GL/BH.

**Nest box type:** Write GT= nest box for a GT, BT= nest box for a BT

**Female ringed:** Is the female ringed yes/no?

**Female colour code:** Ring combination of the female.

**Male ringed:** Is the male ringed yes/no?

**Male colour code:** Ring combination of the male.

## 2.2 Nest visits

**Visits:** Number of the visit to the nest.

**Date:** Enter day/month/year each time the nest is monitored.

**Time:** Enter time the nest was monitored, use 24h format.

**Eggs:** The number eggs counted for each nest visit.

**Chicks:** The number of live counted for each nest visit.

**Dead:** The number of dead chicks counted for each nest visit.

**Nest stage:** Choose a code to describe the condition of the nest every time you visit.

* **HN** = Half a nest,evidence of nesting material; nest building appears to be inprocess. No cup shape yet.
* **IN** = Incomplete nest with cup shape,but without final lining.
* **CN** = Complete nest, nest appears structurally complete with cup and final lining withhair/soft materials.
* **DN** = Nest appears to be damaged and/or torn apart.

**Adult activity:** Choose a code to describe the activity of adults when monitoring the nest.

* **NO** = No adults seen.
* **BA** = Female seen building the nest or carrying nest material.
* **AA** = Adult hissing or giving alarm calls when you approach the nest.
* **FA** = Adults seen carrying food to the nest.
* **VA** = Adult(s) seen in vicinity of the nest
* **OA** = During the check, an adult is in or flushes out of the nest-box while opening thelid.

**Chick stage:** Choose a code to describe the development stage of the young.

* **HY** = Hatching young, a chick is emerging or has just hatched from an egg.
* **NY** = Naked young, nestlings with no or little down feathers (usually day 1- 4, seeFigure 2)
* **PY** = Partially feathered, outermost flight feathers on the wing are emerging, encasedwithin a pointed sheath, OR feathers have broken through the sheath and have a stubby brush-like appearance, OR the flight feathers appear fully grown but tail feathers and body feathers are not yet complete. Some skin visible.
* **FY** = The tail feathers are short and most body feathers are visible, little or no skinvisible. Nestlings are well feathered and look ready to leave the nest (whether or not they can fly).
* **YY** = Young presumed or known to have fledged.
* **NY** = No young, use only when presumed to be dead or predated

**Mgmt. activity:** Management activity – Choose a code to describe action taken at the nest.

* **NB** = Regular nest box check
* **HC** = Hatch check
* **BSC** = Brood size check on day 3 or 4
* **ET** = Exploration test is performed
* **CM** = Chick measurements on day 14 or 15
* **FC** = Fledge check on day 19 or after

**Observer:** ID of theperson who conducted the monitoring

## 2.3. Chick summary (day 10)

Nr. Chicks: Write the number of alive chicks

Total weight: Write in g

Faeces taken: Y/N

## 2.4 Chick measurements (day 14/15)

**Date:** Date (DD/MM/YY) the chick was measured.

**Ring number:** Number of the chick’s band.

**Time:** Enter the time chick was measured, use 24h format.

**Chick stage**: Enter the same code as in chick stage (see above).

**Fat:** Fat score of the chick according to the following scores (see also picture):

* 0 = No fat at the furcular
* 1 = Yellow fat can only be found in small patches on both the sides of the oesophagus. The furcular depression is fat free and has a dark red color.
* 2 = Yellow fat can be found in patches around the oesophagus. The furcular depression has small traces of fat and is light-red.
* 3 = The fat is evenly distributed along both sides of the oesophagus. Half of the furcular depression is covered with fat and it has a yellow-reddish color. The tip of the furcular depression remains more or less fat free.
* 4 = The space on both sides of the oesophagus and the furcular depression are completely filled with fat. The color is light yellow.

**Tarsus:** Length of the right tarsus in mm. Make the measurement from the notch on themetatarsus to the top of the bone above the folded toes.

**Wing:** Length of the right wing in mm. Wing-length is determined as maximum chord whichis the length of the flattened and straightened wing, and it is the distance between the bend of the wing and the longest primary

**P3:** Measure stretched P3 using the pin scale (see cheat sheet)

**Weight:** Weight of the chick in grams.

**Observer:** ID of the person who conducted the measurements

**Faeces taken:** Y/N

**Bill:** feathers to tip

## 2.5 Nest summary

Fill in after the chicks fledged or the nest is done.

**First egg:** The April date the female laid the first egg.

**Incubation start:** The April date the female started to incubate the eggs.

**Estimated hatch:** The April date the first chick hatched.

**Estimated fledge:** The April date half of the chicks or more left the nest-box.

**Clutch size:** Maximum number of eggs counted in the nest.

**Unhatched eggs:** Maximum number of eggs that failed to hatch.

**Live chicks:** Maximum number of chicks that where alive at day 3.

**Fledged:** Total number of chicks that fledged.

## 2.6 General comments

You can provide additional information about the nest visits or the outcome of a nest.

# 3. FRASS collection

Twice a week during the plot checks FRASS samples will be collected. Go to the two FRASS collector in your plot and carefully brush the sample from the fabric towards the center. Remove bigger branches and leaves after brushing them down onto the collector. Place your sample tube underneath and open to clip to collect the sample. Make sure to collect the full sample! Label the sample with date\_time\_plot\_FRASS collector number (eg. 05.06.2023\_07:23\_E\_1). For each collector it is important to collect "negative control samples". Open and close a sample tube in the field, while pretending to fill it with the tools you used, but without adding a sample. date\_time\_plot\_FRASS collector number\_C (eg. 05.06.2023\_07:23\_E\_1\_C).