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A novel non-invasive method for measuring the body mass of hole nesting birds during breeding

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ABSTRACT

Capturing birds during egg-laying or incubation to determine their condition often results in brood abandonment – up to 40% of broods for the great tit *Parus major*. At the same time, the weight of birds during the feeding of young is most often completely different (lower) than during incubation. Hole nesting birds are frequently the object of research, as they easily accept artificial nesting sites, i.e., nest boxes. We tested the possibility of determining the weight of an incubating female great tit without its capture by modifying the nest box to make it easily removable, allowing the female to be weighed along with the box. By taking measurements in such a way, we were able to determine the weight of the incubating female without breeding losses and brood abandonment. We also present changes in the weight of the same individuals (N = 15) during successive broods in the same season. These females were weighed twice during the incubation and feeding of nestlings during two broods (6 measurements in total per season). The presumed pattern was found, i.e., reduction in weight during the feeding of young. Females at the beginning of incubation of the first and second broods had similar weights, indicating that they rebuild their condition very quickly after the first brood's young have fledged. The proposed method is particularly recommended for determining the weight of birds during the initial stages of reproduction (nest building, egg laying and incubation) during which birds are most vulnerable to disturbances after having been captured.

INTRODUCTION

The body weight of an individual is a basic component of the measurements/estimators of its condition (e.g., Peig and Green 2010). To determine the investment of birds at different stages of reproduction, condition should be determined at a given nesting stage. This is particularly important because the female body weight of birds varies throughout reproduction due to different expenditures for nest building, egg laying, incubation and feeding young (see e.g., Moreno 1989, Blem and Blem 2006, Neto and Gosler 2010).

However, capturing females to weigh them during some stages of breeding, especially when they are on or near the nest, may result in brood abandonment (see Kania 1992). The data gathered to date indicate that it is the safest to capture adult birds when nestlings are large, as the rate of brood abandonment is very low at this stage (reviewed in Kania 1992, see also Götmark 1992). Capturing birds, especially in the early stages of reproduction, can lead to abandonment of the existing brood, followed by the construction of a new nest and the production of new eggs. This generates additional costs for the birds, which raises problems in interpreting the results collected in this way regarding, for example, the cost of reproduction. During the relatively safe capture of adult birds with young just before fledging, various biometric measurements (such as wing and tarsus length) can be collected. These can then be used to determine the bird's condition (regardless of method, e.g., residuals from the regression of body mass vs. body size parameters, scaled index, etc. – see Peig and Green 2010), as they do not change during a particular breeding season. However, body mass, the second parameter used for these analyses usually differs in females during the feeding of nestlings and, for example, incubation, especially in small passerines (e.g., Cichoń 2001, Suarez *et al.* 2005, Blem and Blem 2006, Neto and Gosler 2010). Considering the high risk of capturing females at early stages of breeding (e.g., more than 40% of brood abandonments by females caught on the nest during incubation for the great tit *Parus major* – Kilgas *et al.* 2007, Dubiec 2011), and taking into account the welfare of the birds, there is a need for another, relatively easy way to determine a female bird's weight at a given stage of reproduction.

Many studies on bird breeding ecology and reproduction costs at particular stages of nesting are conducted on birds using nest boxes. Nest boxes are a very useful tool, because they can be modified in various ways to ensure the collection of a variety of data (cf. e.g., Zarybnicka *et al.* 2016, Surmacki and Podkowa 2022b), as well as to facilitate bird captures (e.g., Steward 1971, Stutchbury and Robertson 1986, te Mervelde *et al.* 2011, Zhang *et al.* 2019 and many others). However, no proposals have emerged to date on how to determine the mass of a female during nest building or incubation without causing the clutch/brood to be abandoned. One proposed method is to use a box-net attached to the tree with a nest box, but this capture method has also been shown to cause brood abandonment (20%), as well as failing to catch many birds (te Marvelde *et al.* 2011). Newly proposed methods should be quite easy to apply in the field and allow the female to be weighed while taking into account the specificity of the early stages of reproduction, when the female is present at the nest only for a certain, usually short period of time (nest building, egg laying) or for longer periods when the female is less willing to leave the nest box (egg incubation and brooding of young). It is particularly important for such a method to allow the same individual to be weighed several times during the same season, which is necessary for analyzing the condition of birds with two (or even more) broods per season.

When planning to weigh birds during the early stages of breeding, we decided that such a method must take into account two aspects. First, the weighed bird must be assigned to a specific nesting site and brood; and at the same time, we did not want to physically capture the bird at the nesting site, due to both the high probability of nest abandonment and the possible behavioral changes that capturing the bird causes (e.g. Schlicht & Kempenaers 2015, Seress *et al.* 2017). Therefore, it is best to determine the weight of the bird while it is in the nest box – whether during nest building and arriving with nesting material, or during egg laying or incubation. Second, we did not want to modify the nest box itself for such weighing, especially the dimensions of its interior, because at that time the nest has already been built or is just being built, and the dimensions of a nest box affect the size of the nests and clutch (see e.g., Karlsson and Nilsson 1977, Moller *et al.* 2014). Thus it is not possible to modify nest box dimensions during breeding. Also, since various data have been collected for many populations for many years using specific nest boxes (e.g., Lambrechts *et al.* 2010), using the same nest boxes in the planned research as were used in previous years does not disrupt long-term data collection, allowing for comparable results, etc.

Thus, the aim of the study was to develop a method for weighing hole nesting bird – mostly females breeding in nest boxes – several times without capturing them, which may cause brood abandonment. The study also sought to determine changes in female body mass between incubation and nestling feeding. These changes may potentially differ in the case of birds breeding twice a year, as the investment in the first brood may affect a bird's condition during the next brood, which has so far been overlooked in previous studies. We were particularly interested in determining whether the weight of females starting the first and second broods is similar, or whether females are not able to rebuild their condition so quickly after the first brood. The study was conducted on the great tit *Parus major*, a population that breeds in nest boxes, where about 40% of pairs raise two broods per year (Harnist 2017).

STUDY AREA

The study was conducted in an approximately 60 year-old pine forest located in Sękocin, about 10 km SW of Warsaw (52⁰05 N, 20⁰52 E). Around 260 nest boxes were hung in this area in parallel lines every 50 meters forming a grid. The boxes are wooden with a hinged front wall for opening, having internal dimensions of 11 x 11 cm at the bottom and a 21 cm height from the bottom to the entrance hole. The boxes are hung about 2.5 m above the ground. The nests themselves are built in perforated plastic liners (see Fig. 1F), which allows them to be removed, accurately weighed and measured without destroying the nest structure. The most common hole nesters breeding in these nest boxes are great tits and pied flycatchers *Ficedula hypoleuca*. Various aspects of their ecology have been studied at this site for many years (e.g., Mazgajski & Rykowska 2008, Dubiec *et al.* 2018, Harnist *et al.* 2020).

METHODS

Taking into account the two conditions presented in the introduction, it was decided to weigh the birds when they are inside the nest boxes. Therefore, as a first step, new nest boxes, identical to those used to date, were modified so that they could be quickly and repeatedly removed from the tree. To this end, roller slides (25 cm long standard slides used for drawers, for example) were attached to the rear wall and the top of wooden mounting strip of each box (Fig. 1A,B). The strip was permanently attached to the tree, while the body of the nest box was placed in the slides, making the box stable, wobble-free, and fully removable (Fig. 1C). Such boxes were hung in 2019, replacing some of the existing boxes.

As we have been working in this study area since 2005, many boxes are available to the birds, so we were not able to replace all the nest boxes with such modified ones before the breeding season. If the birds chose existing, standard nest boxes, we replaced them with the modified ones at the latest when 3 eggs were laid, and hung them as much as possible in exactly the same place, with the same exposure, height, etc. Since the nest boxes were identical in all these seasons, it was possible to transfer the plastic liner with the nest and sometimes the eggs to such a new modified nest box.

A base for the scale with a bracket was also designed and specially made for weighing the nest box together with the bird and its brood. This was mounted to the nest box mounting strip each time weighing was conducted (Fig. 1D,E). An appropriate height pad was also needed, as the roller slides were longer and protruded outside the nest box rear wall (cf. Fig. 1B). In this way, the arriving bird would find the nest box in the same location, only that it was not attached to the tree during weighing, but rested stably on the scale (Fig. 1E). The scale base was properly leveled each time during installation so that the weight and its measurements would not be affected. To accustom the bird to the potential presence of the scale, a fixed plastic dummy, shaped and colored to resemble the scale, was attached earlier under the occupied box.

There were at least two reasons why we decided to weigh the nest box on a tree-mounted base, rather than removing it and weighing it on the ground. First, weighing it on the ground would have been possible only during incubation, when the bird was sitting inside the nest box, but it would have been impossible to take measurements this way at other stages of reproduction – nest building or egg laying. Second, removing the nest box and weighing it off of the tree would not have been safe, because the nest box with the brood and the bird inside would have to travel in the hands of the researcher down the tree on a ladder a long way from its location to the ground. This could be risky both for the brood (e.g., possible breakage of the eggs in the nest) and the bird itself – during this maneuver, the female could become very frightened, fly away and not return to the nest, abandoning

Fig. 1. Modified nest box. A – roller slides attached to the top of wooden mounting strip, B – roller slides attached to the rear wall of the nest box, C – nest box placed stable in the slides, D – nest box mounting strip is long enough to mount a base for the scale, E – a set ready for measurements: nest box with a height pad on the scale, note the base for the scale with a bracket mounted to the strip, F – great tit nest built in a plastic liner.

the brood (or in the best case we would have to wait for it, or try again to weight it later). Therefore, this would be too risky and time consuming. On the other hand, mounting a weighing base to the mounting strip each time was considered to be potentially relatively unobtrusive to the birds.

The key in this method is to record the weight of the bird when it visits the nest box with nest material or enters the nest box for incubation. We chose a scale that records data at small intervals and, using a Bluetooth module, sends the data to a mobile device via an application (we used the OHAUS Scout SJX6201 scale with a weight capacity of up to 6 kg, and an accuracy/resolution of 0.1 g).

Measuring the bird's weight was done in two ways. Initially, the bird was flushed out of the nest box by opening it, and then the scale base and entire measurement set up was assembled and we waited until the bird returned. Then the measurements began to be taken with the bird already incubating. However, we found during the surveys that incubating birds sit tight and had to be flushed out of the nest when the nest box was opened. Therefore, during the next survey period, we first set up the measurement equipment, i.e., the base and scale, slowly removed the nest box and weighed the whole set – the height pad, the nest box with the bird, nest and clutch without opening the nest box, and only then was the nest box opened so that the bird would fly out. Then the whole set was weighed again, but without the bird. Only about 10% of the females reacted to scale mounting by leaving the nest box. The measurements finished when results presented by the scale were stabilizing, especially in short windless condition.

Birds were weighed at the beginning of incubation – usually on the third day (3–4) and at the end of incubation before the chicks hatched – usually on the tenth (9–10) day of incubation (day 1 of incubation = the first day after the end of egg laying when the clutch was complete). The birds were later captured, ringed, weighed and measured (tarsus and wing length) on the 13th day of the chicks' life (day of hatching = 1). The same procedures were performed for the birds from the first and second broods. Importantly, we included the same individuals raising a second brood in the study, which allowed us to compare changes in females' weight over such a long period of time – that is, at 6 points throughout the entire breeding season. We will not analyze the factors affecting changes in female weight/condition here, as this will be the subject of a detailed analysis in another paper. At the moment, it is important to show the potential of the results collected by the proposed method, to determine how large such changes in mass are within the same individual, how they vary between individuals as well as between individual broods, and, most importantly, whether it is possible to measure body mass without capturing the birds, and thus without causing brood losses and brood abandonment. In the case of the great tit, abandonment associated with capturing an incubating bird to take biometric measurements, sometimes also taking blood samples, etc., can be more than 40% (Kilgas *et al.* 2007, Dubiec 2011). Also, subsequent captures of female great tits on replacement or second clutches during the season increases the frequency of brood abandonment (Kania 1989).

Data analysis

The main problem investigated in the study was to determine the weight of the bird (female) from the measurements and to assess the reliability of the results obtained. The results of the measurements of the nest box weight with nest and eggs with or without incubating females provided by the scale were subsequently recorded on a computer. A number of values were obtained, indicated by the scale during weighing.

We found that it was difficult to obtain only a single, constant measurement in field conditions – the scale did not indicate only one value, but values were characterized by a certain variability. Therefore, for the entire set of recorded values, histograms of the distribution of the obtained data were prepared, and the mean, median and modal values (modes, sometimes more than one) were determined for two sets of data – the nest box containing a nest with a clutch, and the same nest box additionally with the incubating female. In the case of several modal values in the dataset, the one that was closest to the mean and median values was selected. It was assumed that the modal value should best indicate the real measured value and reflects the mass of the system under study. However, we also calculated how large the differences were between the so-defined modal value and the average or median obtained from all recorded measurements. Additionally, for each method of calculating the mass of a given system, the differences between the calculated mass of the female from the results of the mean, median and modal values of the measurements were calculated. The range of variability of the system's mass during weighing was also counted, i.e., how large the range of results was within the weighing period.

As the results of female weight changes were presented for the same individuals, the Wilcoxon signed-rank test to matched samples was used in the analyses (due to the lack of the normal distribution of all these variables). Analyses were performed using Statistica 13. Unless otherwise stated, results are presented as mean ± SD.

RESULTS

For the evaluation of the proposed weighing method a set of data collected in 2020 for 51 pairs of measurements (nest boxes without a bird and with a bird) was carefully analyzed. On average, during a measurement, a nest box with a nest and clutch and height pad weighed 4255.88 ± 316.32 g (range 3369.5–4942.2 g) while the mass of this set together with a bird weighed 4276.02 ± 316.36 g (range 3388.3–4961.9 g). The average number of measurements for one weighing of a nest box with a brood but without a bird was 82.1 ± 77.0 (range 4–403) while for a nest box with an incubating female – 93.3 ± 88.6 (range 13–511). On the other hand, the range of variation of the obtained values for weighing a nest box without a bird was on average 3.64 ± 3.04 g (range 0.1–15.5 g) and on average 4.06 ± 3.93 g (range 0.3–19.5 g) for a nest box with a bird inside. The number of measurements recorded influenced the range of variability – a positive correlation was found between the number of measurements and the variability of measurements for both types of measurements – both an 'empty' nest box and one with a bird inside (r_s – 0.744 and r_s = 0.735, respectively, $P < 0.05$ in both cases). The more measurements, the greater the range of variability. The average difference between the modal value and mean was 0.24 ± 0.23 g (range 0.0–0.9 g), between the mode and median: average – 0.16 ± 0.2 g (range 0.0–0.8 g), and between the median and mean: average -0.09 ± 0.1 g (range 0.0–0.5 g).

The weight of the incubating female was determined based on these measurements. The mean female weight calculated from the mean or median values was similar to the female weight calculated from the modal values (0.01 ± 0.35 g, range -0.9–1.0 g. and 0.0 ± 0.26 g range -0.9–0.8 g respectively).

Female mass changes during the breeding season

In the 2020 and 2021 breeding seasons, the proposed weighing method allowed us to weigh 13 females raising two broods (two individuals were measured in two consecutive seasons, N = 15): twice during incubation and then after capture during the feeding of nestlings (6 measurements in total). The same pattern was observed in both broods – a higher female weight at incubation, which decreased during nestling feeding (Wilcoxon T test > 3.0, $P < 0.002$ in all cases). During the first brood, the weight of the female at the beginning of incubation was lower than at the end (Wilcoxon test T = 9.50, $P < 0.007$). A similar relationship was not found during the second brood (Wilcoxon test T = 46.5, $P < 0.7$). The weight of the female at the beginning of incubation of the first and second broods was similar (T = 41.0, $P > 0.47$), while it differed at the end of incubation (T = 12.5, $P < 0.02$). The weight of the female when feeding the chicks was lower during the second brood (T = 3.00, $P < 0.01$, Fig. 2).

DISCUSSION

Application of the method

The proposed method for determining the weight of a female (or both birds, in the case when both partners of a given species incubate) during breeding is easy to use, but it requires both preparation of the nest boxes before the season and certain procedures in the field while preparing the nest boxes for weighing.

Despite some disturbance in the immediate surroundings of the nest box (the need to use an electric screwdriver to attach the scale base to the mounting strip), 100% of the great tit females in our study population returned to the nest after our measurements, with only about 10% of the incubating females reacting to scale mounting itself by flying out of the nest box. This observation greatly improved the measurements, resulting in our ability to weigh 90% of the females twice during incubation in the 2020–2021 seasons. There was not a single case of brood abandonment as a result of this procedure. In comparison – when using a box trap, nest abandonments were observed and not all birds were captured (te Marvelde *et al.* 2011). Thus, this method can be successfully used to determine the weight of a female, even during such a sensitive stage of breeding as egg incubation. Potentially, it is possible to also use this method to determine the weight of a female during nest building or egg laying.

We also found that in the case of the great tit, it is possible to replace a nest box holding an already-built nest (and sometimes even the first eggs laid) with one used for our research without having the brood abandoned. Thus, it is not necessary to immediately replace all the nest boxes in a study area before starting a study, which is important when there is a large number of nest boxes available for the research. It is, of course, difficult to say how other species of hole nesting birds will behave in a similar situation, this remains to be determined.

The possibility of removing the nest boxes means that they can be removed for the winter, so that they and especially their metal elements of roller slides do not deteriorate as quickly, enabling them to be used for a longer period of time for research. It should be kept in mind that many sedentary hole nesting birds also use nest boxes for roosting during the winter (e.g., Busse and Olech 1968, Tyller *et al.* 2012, Typiak *et al.* 2019), so some nest boxes should be left on the trees.

Such modifications to existing nest boxes (enabling them to be weighed along with the

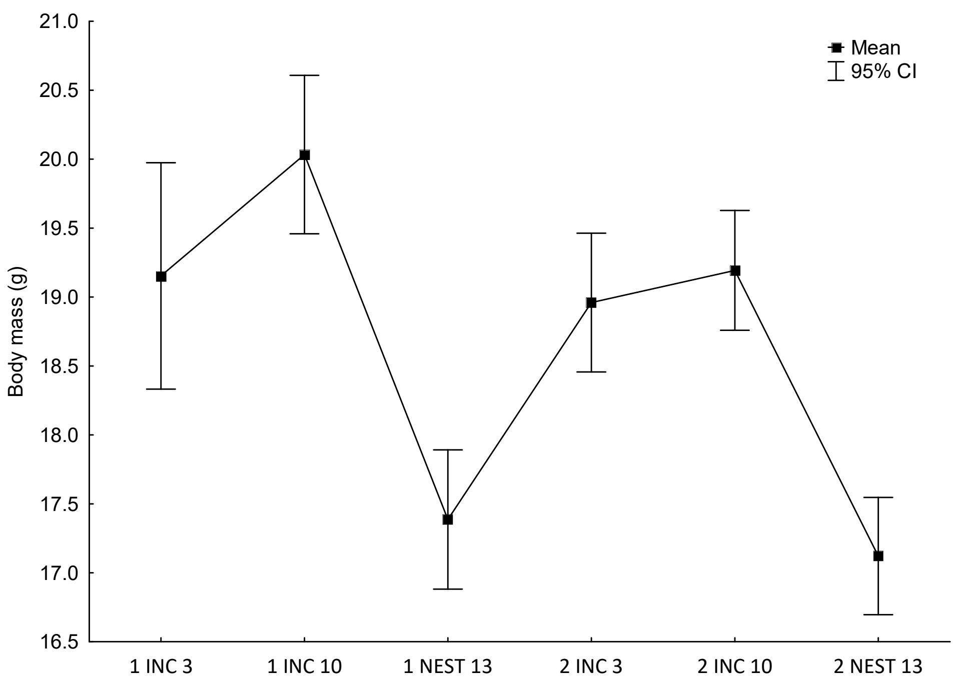

Fig. 2. Changes of great tit females' mass during first and second broods (data from the same individuals, N = 15). 1 – first brood, 2 – second brood. INC – incubation (days 3 and 10), NEST 13 – feeding of 13-day-old nestlings.

female) allow us to work with nest boxes of identical dimensions in subsequent seasons, which means that any work on determining the condition of females does not interfere, for example, with collecting other long-term data on a particular population at the same time. With the scale transmitting measurement data to an external device, one does not need to observe the nest box to see whether a bird has arrived/flushed, etc., because this can be deduced from the measurements. However, it is better to observe the nest box and synchronize the measurements with what is happening to/in the nest box. This, for example, allowed us to weigh a male that came and sat on the nest box roof.

The problem with this method is that the longer it takes for the measurements to be taken, the greater is the variation of the obtained results (mainly due to the wind and some movement of the trees). This makes it more difficult to determine the actual weight of the bird itself. Hence, the observation that the female of the great tit usually sits tight on the nest during incubation long enough to be weighed together with the box, and only after that leaving to allow us to weigh the nest box itself, has improved the work and reduced the number of measurements. Nevertheless, short measurements are best, especially when the bird is absent from the nest box, which can even be weighed unattached to the tree, thus avoiding variability in the measurements. Keep in mind, however, that the data from many measurements are similar, and whether the mean, median or modal value is then calculated does not affect the final determination of the weight of the female. The average differences between these values were up to 0.24 g, which is about 1.2% of a female's weight (the average weight of the female during incubation – 20.16 g), so it seems that any of these ways of determining the female's weight is acceptable.

The proposed method works best with wooden boxes attached to the tree with a mounting strip, because the base of the scale can also be attached this way, but the method can be applied, for example, to wood-concrete Schwegler-type nest boxes, which are also used in studies of hole nesting birds. These types of nest boxes are easily removed, but the difficulty here may be in placing the nest box on the scale exactly in the same place where it was hanging, in the case of weighing a bird that has already left the nest box, or during another period in the breeding cycle, such as nest building.

Changes in female mass during the breeding season

We found the expected pattern of change in female weight, i.e., higher mass during incubation and a marked decrease during nestling feeding (e.g., Suarez *et al.* 2005, Blem and Blem 2006, Neto and Gosler 2010). Both a similar decline in weight between incubation and nestling feeding as well as similar females mass during these two periods that we observed were found in another great tit population, but capturing these birds led to frequent brood abandonment (Kilgas *et al.* 2007). In our study, however, by using the proposed method, we were able to determine the weight of the same individuals during their first and second broods, mostly without capturing them, as repeated captures of the same individuals could potentially increase the abandonment of the next brood (Kania 1989).

This method made it possible to determine how quickly females rebuild their condition after the young from the first brood fledge. It is known that a female great tit starts a second brood very quickly, even before the young of the first brood become independent, and in rare cases, when the chicks of the first brood are still in the nest (e.g., Gosler 1993, Surmacki and Podkowa 2022a, own data). In the studied population, nestlings of the first brood leave the nest most often at the age of 20 days (range 17–22, Dubiec and Mazgajski 2023), so females start laying the eggs of the next brood at an average of 9 ± 4 days after the first-brood young fledge (range: 4–19 days, N = 15). Body weight rebuilds very quickly and at the beginning of incubation of the first and second broods, females had a similar weight (Fig. 2).

Unexpectedly, a higher weight of females was found at the end of incubation than at the beginning with the first brood, but this should be confirmed with a larger number of females. Importantly, the proposed method allows for weighing a specific individual multiple times, e.g., on a specific day in the planned experiment, or before and after the experiment, or at a specific moment of the breeding cycle, for example, during or just after egg laying. This can enable further research to be performed relating to the costs of reproduction, incubation, changes in bird mass during incubation and testing related hypotheses (such as adaptive mass adjustment, programmed anorexia or physiological stress hypotheses – see e.g., Suarez *et al.* 2005, Neto and Gosler 2010 and many others).

We recommend this method for determining the weight of females and their condition during the initial stages of reproduction, when the probability of abandoning the brood is high.

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