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Age estimation of black stork (Ciconia nigra) nestlings from wing, bill, head, and tarsus lengths at the time of ringing

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Abstract: Black stork nestlings were measured (wing, bill, head, and tarsus lengths) at weekly intervals in central Poland in 2012, 2014, and 2015. The aim of the study was to provide growth equations based on nestling measurements to allow age estimation of black stork nestlings. The hatching hour and date of nestlings in five nests were determined using trail cameras. The age of the measured nestlings ranged from 18 to 53 days. Wing, bill, and head lengths showed linear growth, while tarsus growth was only linear for nestlings not exceeding 35 days old. Within the age range studied, wing length grew 9.6 mm per day, head length grew 2.3 mm per day, and bill length grew 1.8 mm per day. The study provides the first growth parameters for the black stork.

Key words: Breeding ecology, growth formulas, ringing, biometrics, storks, trail cameras

Compared to the white stork (*Ciconia ciconia*), the black stork (*Ciconia nigra*) is a species whose breeding biology has been much less intensively studied. One obvious reason for this discrepancy is that the black stork is a timid species, sparsely distributed in damp forest areas. In addition, nests of this species are situated high in the trees. As a result, the growth of black stork nestlings has not yet been described. Most studies of this species are concerned with country status (Lohmus et al., 2005; Czuchnowski and Profus, 2008), habitat preferences (Rosenvald and Lohmus, 2003), or survival and migration (Bobek et al., 2008; Tamas, 2011; Cano and Telleria, 2013). Of these topics, survival and migration studies are particularly dependent on ringing nestlings. However, although black stork nestlings are ringed intensively in many countries (e.g., Bobek et al., 2008; Tamas, 2011), age estimation formulas are not available (Janssen et al., 2004). Our aim was to provide ringers with simple growth equations based on nestling biometrics.

The black stork is a large (c. 3000 g), long-lived migratory bird inhabiting Palearctic forests of Europe and Asia, with an isolated population in South Africa (Snow and Perrins, 1998). Black storks breed as single pairs, preferably in old, undisturbed forests, interspersed with streams or swamps, where they feed mainly on small fish and amphibians (Snow and Perrins, 1998; Hampl et al., 2005). Usually they lay clutches of 3-5 (range: 2-6) eggs and raise one brood in the breeding season. Incubation lasts 35-36 days and the fledging period is 63-71 days. Black storks are semialtricial and nidicolous. Nestlings hatch asynchronously (Snow and Perrins, 1998). In Poland, the black stork population is estimated to hold approximately 1400-1600 pairs, with a density of 0.5 pairs 100 km^{-2} of total area (Zieliński et al., 2011). In central Poland the mean distance between neighboring nests occupied by different pairs is 8.9 km (Zieliński et al., 2017).

Trail cameras (type Ltl-6210M and Ltl-6310WMG) were used to monitor the progress of breeding of black stork pairs. The main role of the trail cameras was to determine the time and date of hatching of each nestling. Cameras were placed 1.5–3 m from nests prior to the commencement of egg-laying. One photograph and a short video recording were taken every hour during day and night. Trail cameras were placed in 18 different nests during the years 2012-2016. However, breeding was successful in only five nests, mainly due to pine marten predation on black stork nestlings and inclement weather. Of these five nests of different pairs included in the analysis, three nests were situated in oak (*Quercus* sp.) trees and two in Scots pines (*Pinus sylvestris*), 12-17 m above the ground. The nests were located in central Poland, in the Lodz province. The nests studied were separated by a mean distance of 34

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km. Each nest with nestlings was visited three to five times at about weekly intervals during the nestling period.

Measurement error might be a consequence of a lack of well-defined measurement landmarks, flexibility of structures, and even differential experience of the researchers (Perktaş and Gosler, 2010). Thus, to obtain comparable data among ringers, measurements should be taken in exactly the same way. Wing length (right wing, maximum chord measurement) was measured to the nearest 1 mm with a ruler cut off at the zero line (Busse, 2000). In older nestlings, primary wing feathers were straightened to their maximum length. Head (total length), bill, and tarsus lengths were measured with a sliding caliper to the nearest 0.1 mm. The head length was measured from the tip of the bill to the most exposed part of the occipital bone. However, caution must be paid while measuring head length when nestlings are about 25-40 days old. Nestlings at that age have a thick layer of down and are growing contour feathers on their heads. Thus, to obtain reliable readings, one arm of the caliper should be pressed to the occipital bone. The bill length was measured from the tip of the bill to the line between the naked bill sheath and the feathered part of the bill. In young nestlings, this line is clearly visible. In older nestlings, the line is covered with feathers and the measurement was taken after drawing the feathers aside to make the line visible.

During visits to nests, older nestlings show aggressive behavior towards the intruder. They also make defensive sounds, sometimes regurgitate food, and actively wave their wings. To calm down the nestlings, a cotton cloth was placed over them as quickly as possible. Thus, the nestlings immediately stopped fighting and the risk that they could hurt each other or fall was eliminated.

Nestling age was calculated in hours. For each nestling, we calculated hatching time (mean time of the last picture with an egg and the first picture with a nestling). For each nest, we calculated the mean hatching time for nestlings who hatched on the first day of hatching, and the hatching time for nestlings who hatched on the last day of hatching. Knowing the hatching time and the time of every visit to the nest to measure the nestlings, it was possible to calculate nestling age in hours. On the first visit to the nest, nestlings were individually marked with small plastic rings, later replaced with bigger ones.

A total of 268 measurements were taken for wing, head, bill, and tarsus lengths from 17 nestlings from five nests. The age of the nestlings included in the analysis ranged from 18 to 53 days.

Because measurements of nestlings from the same brood and multiple measurements of the same nestling in subsequent inspections are not independent, the individual nestling values were treated as unit records and analyzed using linear mixed models, with brood ID and nestling ID being included as random factors controlling for clustering (Heck et al., 2010). Parameters of the models were estimated by applying restricted maximum likelihood. Because the denominator degrees of freedom cannot be counted directly in hierarchical models, they were approximated using the Satterthwaite method that is implemented in the IBM SPSS procedure MIXED, used in this study (Heck et al., 2010).

In further analysis, we used two categories to reflect hatching order of nestlings: FIRST, for nestlings hatched on the first day of hatching, and LAST, for nestlings hatched on the last day of hatching. To check whether there were significant differences in the measurements of nestlings belonging to these two categories, nestling hatching category (FIRST/LAST) was included as a fixed factor in the linear mixed model. In cases where hatching order caused a difference in the pattern of growth of nestlings, the interaction between the hatching order factor and the nestling age covariate should significantly affect a given morphometric trait.

In nest Kolumna1, two nestlings hatched on 9 May 2012. The mean hatching time for the nestlings hatched on 9 May was 0814 hours. The third nestling hatched on 10 May 2012 at 0323 hours. In nest Kolumna2, three nestlings hatched on 12 May 2012. The mean hatching time for the nestlings hatched on 12 May was 1351 hours. The fourth nestling hatched on 14 May 2012 at 1457 hours. In nest Spala, two nestlings hatched on 20 May 2014 (mean hatching time: 0814 hours). The third nestling hatched two days later, on 22 May 2014 (0917 hours). In nest Kutno, two nestlings hatched on 23 May 2015 (mean hatching time: 0643 hours) and one on 24 May 2015 (1208 hours). In nest Grotniki, three nestlings hatched on 19 May 2015 (mean hatching time: 1549 hours) and the fourth nestling hatched on 21 May (0412 hours).

In the studied nests, the difference in size between nestlings hatched on the first day and those that hatched a day or two later was visible in the photographs taken every hour by the trail camera following hatching, so it was possible to discriminate the nestlings hatched on the first day (FIRST) from the nestlings hatched on the last day (LAST) by their size.

The interaction of hatching order (FIRST/LAST) × age (in hours) was nonsignificant for wing length ($F_{1;54.65} = 0.02$; $P = 0.887$), head length ($F_{1;49.68} = 0.1$; $P = 0.75$), bill length ($F_{1;50.32} = 0.14$; $P = 0.71$), and tarsus length ($F_{1;16.45} = 0.27$; $P = 0.61$), meaning that the hatching order of nestlings did not affect the pattern of growth. Therefore, measurements of nestlings from all broods were pooled.

The growths of wing (WL), head (HL), and bill length (BL) appeared to be linear (Figure). Because the growth of tarsus length for nestlings older than 35 days was nonlinear (Figure), the linear growth parameters of the tarsus were

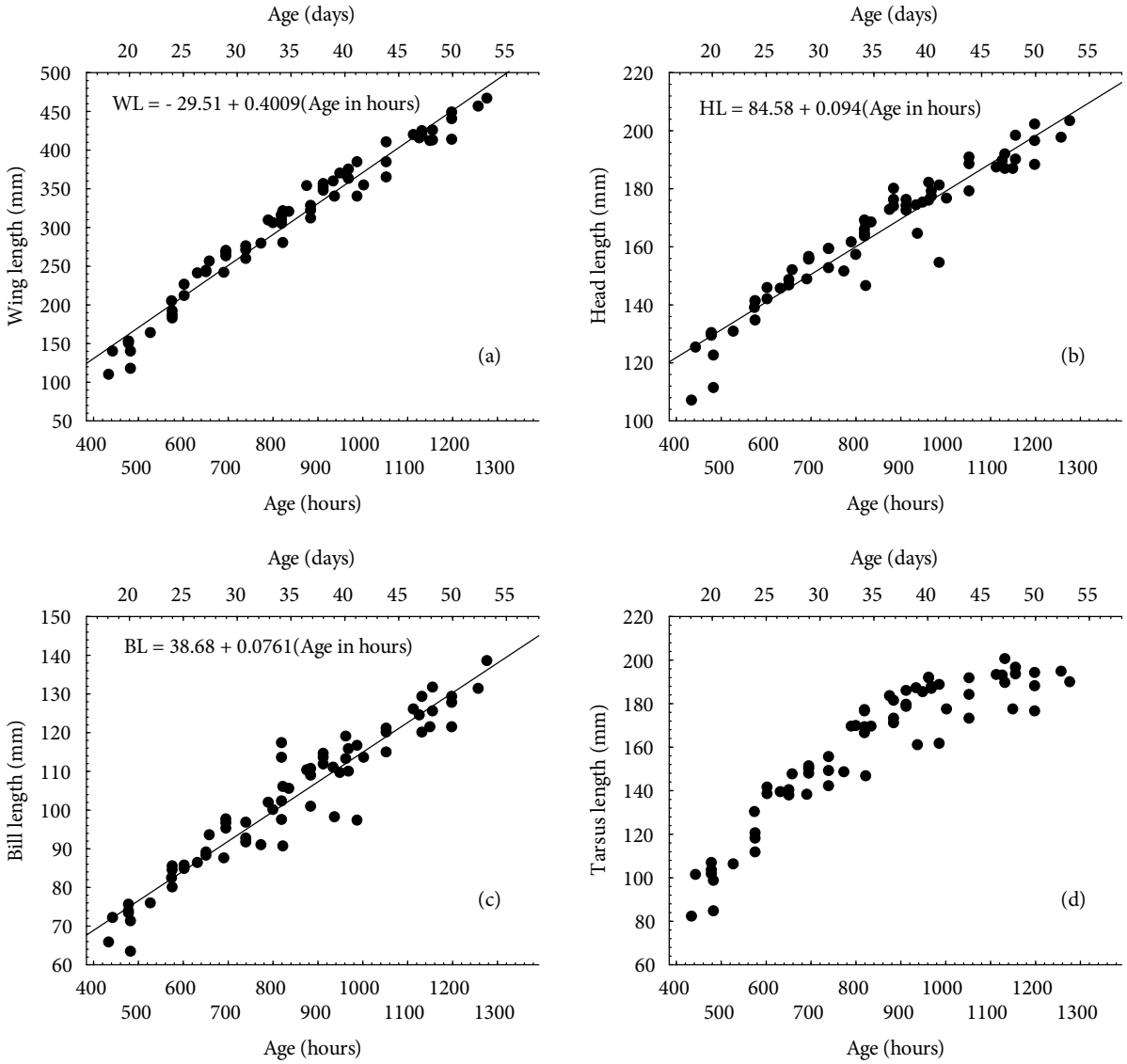

Figure. Measurement data plotted for all nestlings as a function of age for the black stork: a) wing length (WL), b) head length (HL), c) bill length (BL), and d) tarsus length.

estimated for the age range of 18-35 days in this case. The linear growth parameters (age in hours, measurements in mm) for wing, head, bill, and tarsus lengths are given in the Table and the growth equations for wing, head, and bill lengths are shown in the Figure. Wing length grew 0.4009 mm per hour (9.6 mm per day), head length grew 0.0941 mm per hour (2.3 mm per day), bill length grew 0.0761 mm per hour (1.8 mm per day), and tarsus length grew 0.195 mm per hour (4.7 mm per day).

Nestling age can be calculated from wing length (WL), head length (HL), bill length (BL), and tarsus length (TL) (all measurements in mm) by applying the following equations (derived from the Table):

Age in hours = 73.61 + WL/0.4009;
Age in hours = –898.83 + HL/0.0941;
Age in hours = –508.28 + BL/0.0761;
Age in hours = –49.25 + TL/0.1945.

Due to recent interest in phenology, data on the timing of avian reproduction have been analyzed for an increasing number of bird species (Crick, 2004). In studies on white stork breeding ecology, laying and hatching dates were either directly recorded or calculated based on nestling measurements (Jovani and Tella, 2004; Fulin et al., 2009). These studies found a predominant impact of the timing of breeding on the reproductive performance of white stork pairs. However, without a precise age estimation

Table. Growth parameters of wing (WL), head (HL), bill (BL), and tarsus (TL) lengths (Y variables) of black stork nestlings in central Poland, estimated by linear mixed modeling. Repeated measures on the same brood (brood ID) were included as a random effect and the Satterthwaite method was used to approximate degrees of freedom.

Measurement	Intercept	SE	df	t	slope	SE	df	t
WL[a] (mm)	–29.51	7.397	1; 64.27	–3.99***	0.4009	0.008	1; 55.53	48.72***
HL[a] (mm)	84.58	2.392	1; 57.13	35.37***	0.0941	0.002	1; 50.58	41.46***
BL[a] (mm)	38.68	2.029	1; 61.52	19.06***	0.0761	0.002	1; 51.183	37.74***
TL[b] (mm)	9.58	3.459	1; 30.95	2.77**	0.1945	0.004	1; 17.451	45.73***

[a] Age range of nestlings for wing, head, and bill lengths: 18-53 days.
[b] Age range of nestlings for tarsus length: 18-35 days.
***P < 0.001; **P < 0.01.

of nestlings based on their measurements, calculation of laying and hatching dates would not be possible.

The present study gave an opportunity to assess some methodological aspects of black stork ringing, especially with regard to the best age and method to ring nestlings. According to our experience, the safest time window to ring nestlings is between 30 and 40 days after hatching. Nestlings at that age are not guarded by the parents anymore (Snow and Perrins, 1998) and are strong enough to defend themselves against most predators. Slightly younger nestlings could also be ringed, but this should only be attempted during warm and dry weather. Ringing should be finished at least 2 h before darkness to let parents return to their nests during daytime. Disturbing parents that guard smaller nestlings might result in a prolonged period when nestlings are left unprotected from rain and predators. In addition, after returning to the nest, parents carefully clean small nestlings. This may be problematic for nestlings: in one nest we observed that when nestlings were 27 days old, the parent stork tried to remove the ring with its beak, which could hurt the nestling's leg. On the other hand, the ringing of nestlings older than 40 days might cause some nestlings to fall from the nest, because they at first try to frighten an intruder with aggressive behavior and later move to the nest rim. This is particularly dangerous in large broods when four or five large nestlings wave wings at the nest edge, potentially knocking each other from the nest.

In addition, in the white stork, a closely related species, it was shown that the release of corticosterone into the blood in response to stress was strongly age-dependent, with a low response in young nestlings and a rapid increase in older ones, reaching adult-like responses near fledging (Blas et al., 2006; Corbel and Groscolas, 2008). The increased level of corticosterone affects such aspects of bird behavior as foraging activity or aggression, allowing nestlings to overcome the source of stress, but which may also have detrimental consequences to growth, body condition, and survival (Blas et al., 2006; Corbel and Groscolas, 2008). Thus, to diminish the stress suffered by nestlings during ringing and to increase nestling safety, it is highly recommended to use a small blanket to cover all the nestlings immediately after the ringer arrives at the nest.

In conclusion, within the age range studied, the wing, head, and bill lengths clearly show linear growth and, therefore, all these measurements might be used to estimate the age of nestlings. Although the proposed method of age determination of nestling black storks is sufficient for practical purposes in most ringing schemes, it will certainly require further elaboration in the case of more detailed studies of the species biology. In particular, the reliability of age estimation would be improved by providing the respective confidence limits (cf. Perktaş and Gosler, 2010), which are not available at this stage.

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