

## Capercaillie (*Tetrao urogallus*) eggshell pigmentation, maculation and thickness

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Eggshells properties (mainly thickness, pigmentation and shape) vary within avian taxa across species, and intraspecifically with age, environmental conditions or individual features of the animals. The properties and appearance of eggshells are important for successful breeding both for birds kept in captivity and in breeding centres and those living in their natural environment. The presented study focuses on the association between the pigmentation, maculation and eggshell thickness of Western Capercaillie (*Tetrao urogallus*) kept in captivity. The eggs were collected during the entire egg-laying season in order to determine the factors which influence eggshell pigmentation and thickness across the laying period. We found that at the equator surface the lightest eggs had a tendency to be thinner than the darkest ones. In addition, eggshells with a smooth pattern were thinner at the equator, especially when compared to the mottled ones. There was no statistically significant association between the eggshell overall lightness (eggshell pigmentation except the concentrated pigment spots), maculation and eggshell thickness at the blunt and sharp ends of the eggshell. Eggshells were on average 5.5% thicker at spots than in other areas. The obtained results suggest that the spot pigment forms an additional layer on the Capercaillie eggshell and does not compensate for the loss of thickness, as observed in some bird species (Passeriformes, Falconiformes). A negative association between the eggshell thickness and the laying date (comparing eggs laid in April and June) was also observed, despite the availability of a variety of food and *ad libitum* calcium supplementation during the entire reproductive season. The eggshell coloration was not related to the period of egg-laying. We have shown the effect of pigmentation on one of the most important parameters of eggshell quality, shell thickness. In the case of Capercaillie, the places of eggshell thinning are not related to concentrated pigment spots. This suggests that the pigment deposition and its role may be different from other bird orders (Charadriiformes, Falconiformes, Passeriformes).



## 1. Introduction

The egg coloration of early avian species was white, similarly to reptile eggs (Kilner 2006). Modern bird eggs vary in shape, background pigmentation (base-colour) and maculation (spottiness). Across all avian species, open-nesting species such as Capercaillie (*Tetrao urogallus*) tend to lay more pigmented eggs than hole-nesting species (Lack 1968). These eggshell pigments belong to two blood-derived groups: protoporphyrin that provides brown, red, yellow and black colours, and biliverdin, responsible for the blue-green coloration (Kilner 2006). Capercaillie eggs are of different shades of brown which indicates protoporphyrin pigmentation (Kennedy & Vevers 1976).

All eggshell pigments are deposited in the shell gland during the latest stages of shell formation (Poole 1965, Roberts 2004), and their functions are complex. While egg pigmentation seems to be genetically predetermined or fixed, the pigment intensity and pigment spread may change in birds with age (Odabaşı *et al.* 2007) and the egg laying order (Gosler *et al.* 2005). Eggshell pigmentation may even be affected by weather conditions (Avilés *et al.* 2007). There are many hypotheses regarding the function of egg pigmentation. One of the first and most common hypotheses suggests that it prevents egg depredation (Blanco & Bertelotti 2002, Sanchez *et al.* 2004), especially in ground nesters (Weidinger 2001) and brood parasites (Davies & Brooke 1989a,b, Avilés *et al.* 2007).

It has also been observed that less pigmented eggs were characterized by higher embryo mortality related to an excessive water-loss (Higham & Gosler 2006). According to more recent theories focusing on eggshell pigmentation on thermal properties during embryogenesis (Moreno & Osorno 2003), embryos developing in the lighter eggs are more resistant to high temperature as they are exposed to less heat gain from solar radiation (Westmoreland *et al.* 2007, Magige *et al.* 2008, Maurer *et al.* 2011b). Rogers & Krebs (1996) stated that chicks that hatched from eggs which were exposed to light during incubation, showed structural asymmetries in the brain, and consequently performed better in a number of behavioural tasks (in cavity nesters, especially parrots,

where lateralization is strongly developed). This may explain secondary development of white eggshells. Pigmentation must allow light to penetrate the eggshell in order for the embryo to establish the circadian rhythm (Zeman *et al.* 1999).

Eggshell pigmentation and maculation are affected by many factors, with variation between and within species, and research implies that they impact the breeding success in birds. Embryonic light stimulation plays a significant role in the regulation of chromatin repair by photo-reactivation (Thoma 1999). This is important during numerous cell divisions in the developing embryo, where an erroneous division may have critical consequences on the organism. In addition, pigments may prevent pathogen infections. Ishikawa *et al.* (2010) showed light-dependent antimicrobial pigmental activity against *Staphylococcus aureus* and *Bacillus cereus*.

Some authors (Higham 2006, Kilner 2006, Gosler *et al.* 2011) stated that pigments also play a structural function affecting eggshell thickness and strength. Those factors are linked with dietary calcium availability (Graveland & Drent 1997, Dhondt & Hochachka 2001, Tilgar *et al.* 2005), pesticide levels (Ratcliffe 1970) or environmental acidification (Nybø *et al.* 1997). However, the relationship of those factors with pigmentation and maculation has not been examined in many bird species, including Western Capercaillie. For example, several studies (Gosler *et al.* 2005, Higham & Gosler 2006, Jagannath *et al.* 2008, Sanz & García-Navas 2009) have reported an association between the maculation pattern and the eggshell thickness. They suggested that pigments play a role in compensating for the eggshell thinning caused by structural variations in the shell and calcium deficiency. Similarly, Solomon (1987, 1997) suggested that protoporphyrin may strengthen a crystalline matrix by acting as a shock absorber. Not only maculation, but also lightness influence eggshell thickness (Orłowski *et al.* 2017). The pale eggs collected from Common Quail (*Coturnix coturnix*) had thinner eggshells compared to the dark ones. However, the eggshell lightness itself has been investigated to a much lesser extent than maculation.

Many studies have shown direct and indirect pigmentation impacts on eggshell properties (Darnell-Middleton *et al.* 1998, Gosler *et al.* 2005,

Bain *et al.* 2006, Higham & Gosler 2006, Jagannath *et al.* 2008, Sanz & García-Navas 2009), but also on other egg parameters, like egg shape, that cannot be overlooked. Even within one species it is easy to observe the diversity related to egg length and width. Thus, in our analyses we also studied associations between egg shape, eggshell lightness, strength and laying period.

The main aim of this study was to examine whether the overall pigmentation (eggshell pigmentation except the concentrated pigment spots) and maculation of the Western Capercaillie eggshell compensates the loss of eggshell thickness, as in the other bird orders (Passeriformes, Falconiformes) (Gosler *et al.* 2005, Higham & Gosler 2006, Jagannath *et al.* 2008, Sanz & García-Navas 2009). The majority of authors have not clearly distinguished these two traits, probably because they are related to each other.

We decided to separate them to see which of them can have a more important impact on eggshells. Based on our previous observations (Rosenberger *et al.* 2016) we predicted that pigments may influence eggshell properties. One of the prerequisites for such conclusions was the observations of the ease of abrading the pigment from the surface of the eggshell, suggesting that the pigment is deposited on the surface of the shell rather than penetrating its structure. In this work we focused on this issue carefully while selecting eggs for measures.

## 2. Material and methods

### 2.1. Study animals

The eggs were collected from 39 females maintained in the Capercaillie Breeding Centre in Wisła Forestry situated in the Silesian Beskid (49°32'05.4"N, 18°55'58.1"E). The birds were kept in wooden aviaries and during spring and summer they were allowed to use a big fenced yard that was part of their natural habitat (Łukaszewicz *et al.* 2011, Rosenberger *et al.* 2016). All females were kept in similar conditions and had permanent access to a variety of natural vegetation and invertebrates. Before and during the laying period, the Capercaillie diet was supplemented *ad libitum* with pigeon grit rich in crushed shellfish shells.

### 2.2. Eggshell evaluation

After incubation, 150 unhatched eggs were collected for further examination. Because we were not sure which eggs were laid by which birds (some eggs were laid outside the nest or in the other one) we were not able to exclude pseudo-replication. The strength (crush test) of unhatched eggs with undamaged shells was measured using the EGG Force Reader (ORKA Food Technology LTD). The eggs were placed in the egg cradle vertically, with the blunt end directed upwards. Then, a force gauge was applied to the upper surface with a gradual increase in the applied pressure. The moment of eggshell cracking was recorded to the nearest 0.001 kg. An electronic calliper was used to measure the maximum egg length and width, to the nearest 0.01 mm. After the evaluation of the eggshell, each egg was opened to define its content (unfertilized egg or containing a dead embryo).

Due to the fact that there are no available tables describing the embryo development of the Capercaillie, we adopted tables created for the Helmeted Guineafowl (*Numida meleagris*) embryo (Niedziółka *et al.* 2010), which have a similar developmental period (Capercaillie hatch at 26<sup>th</sup> and Helmeted Guineafowl at 27<sup>th</sup> day of incubation). Out of 150 eggs, 84 were unfertilised, 66 contained dead embryos at different developmental stages (very early death – up to 4<sup>th</sup> day of incubation – 5 eggs, early death – up to 7<sup>th</sup> day – 5 eggs, moderate death- up to 19<sup>th</sup> day – 12 eggs, late death – up to 26<sup>th</sup> day 44 – eggs). Eggs with embryos older than four days were excluded from later analyses because of their possible influence on the eggshell thickness (Vanderstoep & Richards 1970), which has been also revealed in our previous studies on Capercaillie (Rosenberger *et al.* 2017).

The relationships between the coloration of the eggshell, its thickness and strength were analysed. The eggshell overall coloration (without concentrated pigment spots) was determined using the Konica Minolta Chroma Meter CR-400 (Konica Minolta Sensing, Osaka, Japan), that use pulsed xenon lamp as a light source during measurements. For each eggshell, three measurements on the eggshell surface from 8-mm diameter area were recorded, and then the mean lightness (L\*) was calculated. The measurement surface was chosen in terms of two criteria: the pigmentation



Fig. 1. Types of maculation of Capercaillie eggshells: 1 – mottled, 2 – smooth, 3 – spotted.

was well preserved (not erased or dirty) and avoiding measurements at a point of concentrated pigment spots. Twenty darkest ( $L^*$  between 51.58 and 69.53), 20 medium ( $L^*$  between 72.42 and 76.18) and 20 palest eggshells ( $L^*$  between 77.87 and 84.8) were chosen from all the unfertilised eggs and eggs with dead embryos up to the 4<sup>th</sup> day of development.

To test the correlation between the type of maculation, eggshell thickness and strength, three categories of maculation were distinguished from the gathered eggshells, and all eggs were assigned to these categories. Of the unfertilised eggs (84 eggs) and the eggs with early stage mortality (5 eggs), the following eggs were distinguished (see Fig. 1): 14 mottled eggs containing many small, indistinct, merged spots that covered the entire eggshell surface (hereafter: mottled); 16 eggs

with a smooth background pattern without pigment spots (hereafter: smooth); 10 eggshells with a smooth background pattern and clearly visible spots (hereafter: spotted) (Fig. 1). Mottled eggs were the most numerous and smooth eggshells constituted the least numerous group. There were more spotted eggshells than those included in the study, but most contained dead embryos and were excluded from analyses. It was difficult to unambiguously classify maculation of part of the eggshell resulting in small numbers inside groups.

As it was assumed that pigments determining maculation may influence the eggshell thickness, we selected 27 eggs with clearly visible spots for further analysis. From those eggs, 96 pairs (at spot point and near it) of measurements were obtained from different sites at the spot points and near them. The eggshell thickness was measured at



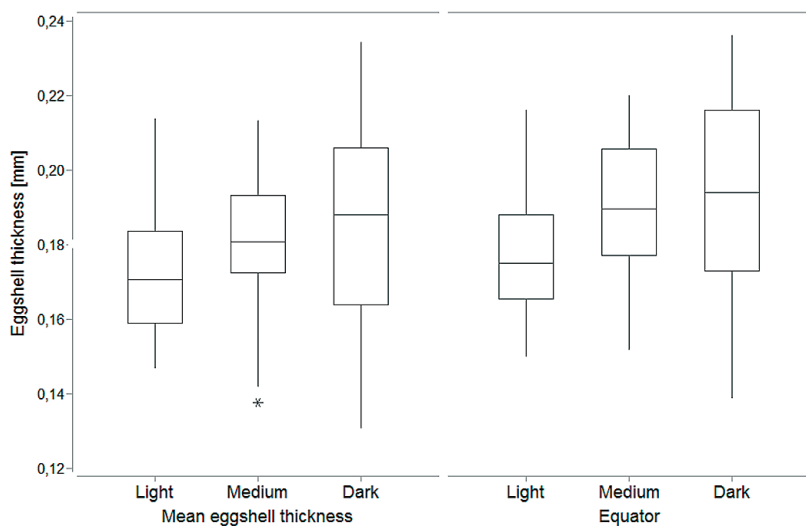


Fig. 2. Eggshell thickness in light ( $n = 20$ ), medium ( $n = 20$ ) and dark ( $n = 20$ ) eggs at the equator surface in relation to the lightness of the Capercaillie eggshell. Horizontal lines in the square indicate the 25<sup>th</sup> percentile, median, 75<sup>th</sup> percentile, vertical lines indicate data range.

each spot point and near it, up to 2 mm from the border of the spot. We did not classify the measurement site, because the Capercaillie eggs had limited numbers of spots that made dividing them based on the measurement site impossible.

The possible impact of the laying period on the eggshell thickness, lightness, strength, width and length was analysed by comparing unfertilised eggs from the first clutches (April) with those from late clutches (June) (20 eggs in each group). Thanks to video monitoring of every nest and manual checking when female made recess during laying and incubation period, we were able to know approximately when the particular egg was laid.

All the selected eggshell samples were washed in running water, and eggshell membranes were mechanically removed with tweezers. Three eggshell samples were then obtained from each egg: one was at the equator surface, one 1 cm from the sharp end and one from the blunt end. Eggshell thickness was measured to the nearest 0.001 mm using a micrometer (Insize 3580-25A) with a 0.2-mm spline diameter.

### 2.3. Statistical analyses

All statistical analyses were carried out using Minitab statistical software (ver. 17). Most data, apart from the thickness measured at and near the pigment spot, and  $L^*$ , had a normal distribution. A

two-sample t-test was used to analyse the influence of the clutch laying time on egg length, width and eggshell thickness. A one-way analysis of variance with a post-hoc Tukey was carried out to test the association between maculation, eggshell thickness and strength. Association between  $L^*$  and eggshell thickness and strength was tested using Kruskal-Wallis H test. T-test for paired comparisons was used to compare the eggshell thickness at the pigmented spot point and near it. Analyses were conducted using 95% confidence interval (significant results) and 99% confidence interval (highly significant results).

### 3. Results

Kruskal-Wallis H-test showed no significant differences in the mean eggshell thickness ( $p = 0.220$ ;  $H_2 = 3.03$ ), thickness at the blunt end ( $p = 0.276$ ;  $H_2 = 2.58$ ) and the sharp end ( $p = 0.718$ ;  $H_2 = 0.66$ ) between light, medium and dark eggs ( $L^*$ ). At the equator surface, the lightest eggs were not statistically significantly thinner compared to the darkest eggs, but there was a tendency ( $p = 0.065$ ;  $H_2 = 5.46$ ) (Fig. 2).

The lightest eggs had slightly thinner shells at all the measured regions than medium and dark eggs. Eggshell  $L^*$  was not associated with eggshell strength ( $p = 0.621$ ;  $H_2 = 0.95$ ).

The type of maculation was not associated with mean eggshell thickness calculated based on

Table 1. Eggshell thickness in Capercaillie eggs with different types of maculation (smooth  $n = 16$ ; spotted  $n = 10$ ; mottled  $n = 14$ ) tested using the one-way Anova (confidence level 95% and 99%) and subsequent post-hoc tests.

Eggshell type/ measurement	Mean eggshell thickness [mm]	95% CI	99% CI	SD
<i>Mean thickness</i>				
Smooth eggshell	0.186 <sup>aA</sup>	0.175; 0.196	0.157; 0.183	0.019
Spotted eggshell	0.173 <sup>A</sup>	0.161; 0.186	0.157; 0.190	0.017
Mottled eggshell	0.170 <sup>aA</sup>	0.160; 0.180	0.172; 0.200	0.021
<i>Thickness at blunt end</i>				
Smooth eggshell	0.176 <sup>aA</sup>	0.166; 0.187	0.151; 0.177	0.018
Spotted eggshell	0.163 <sup>aA</sup>	0.151; 0.175	0.146; 0.180	0.017
Mottled eggshell	0.164 <sup>aA</sup>	0.154; 0.173	0.162; 0.190	0.021
<i>Thickness at the equator</i>				
Smooth eggshell	<b>0.198</b> <sup>aA</sup>	0.187; 0.209	0.164; 0.191	0.022
Spotted eggshell	<b>0.180</b> <sup>abA</sup>	0.168; 0.193	0.163; 0.197	0.014
Mottled eggshell	<b>0.178</b> <sup>bA</sup>	0.168; 0.188	0.183; 0.212	0.020
<i>Thickness at sharp end</i>				
Smooth eggshell	0.183 <sup>aA</sup>	0.170; 0.196	0.153; 0.186	0.024
Spotted eggshell	0.176 <sup>aA</sup>	0.161; 0.192	0.1559; 0.197	0.026
Mottled eggshell	0.169 <sup>aA</sup>	0.157; 0.182	0.165; 0.201	0.024

the measurements of the equator, sharp and blunt end (t-test for independent samples:  $F_{2,37} = 2.56$ ;  $p = 0.091$ ), thickness at the blunt end ( $F_{2,37} = 2.04$ ;  $p = 0.145$ ) and sharp end ( $F_{2,37} = 1.14$ ;  $p = 0.332$ ). However, maculation was significantly related to the thickness at the equator surface ( $F_{2,37} = 4.26$ ;  $p = 0.022$ ). Eggshells with a smooth pattern were about 10% thinner in this area, when compared to mottled ones (Table 1). Even though maculation had a significant association with the eggshell thickness at the equator, we observed a tendency for the smooth eggshells to be thinner than the spotted ones, and the spotted eggshells to be thinner than the mottled ones in all the measured regions. No association between eggshell strength and type of maculation was found ( $F_{2,29} = 0.26$ ;  $p = 0.771$ ).

To investigate eggshell thickness at spot point and near it we ran a t-test for paired comparisons using two confidence levels. In both analyses the differences were statistically significant. The eggshells were notably thicker at the spot point ( $p < 0.001$ ,  $t\text{-value} = 7.55$ ) in the all eggs. The mean eggshell thickness at the spot point was 0.301 mm, and 0.285 mm near spot point. The difference between those two measurements was on average 5.5%. Unsurprisingly, a two-sample t-test showed

that early clutches had highly significantly thicker eggshells than late clutches ( $t\text{-value} = 3.52$ ;  $p = 0.001$ ;  $df = 31$ ) (Fig. 3). In all the measured eggshell regions, including the blunt end ( $t\text{-value} = 3.24$ ;  $p = 0.003$ ;  $df = 29$ ), the equator surface ( $t\text{-value} = 2.65$ ;  $p = 0.007$ ;  $df = 31$ ) and the sharp end ( $t\text{-value} = 3.65$ ;  $p = 0.001$ ;  $df = 34$ ), the relationship between the laying time and eggshell thickness was highly significant. L\* was not associated with the time of laying ( $t\text{-value} = -1.41$ ;  $p = 0.167$ ;  $df = 36$ ), even though there was a slight tendency for eggs to be brighter in late clutches. The relationship between early and late clutches in terms of the type of maculation was not assessed due to the limited number of eggs with different maculation patterns.

#### 4. Discussion

While eggshell thickness was related to the type of maculation and to a lesser extent pigmentation lightness, there was no evident impact of those factors on the eggshell strength. However, our results showed that the pigment in Capercaillie eggs has a positive effect on the total eggshell thickness, which is most marked at the equator and the con-

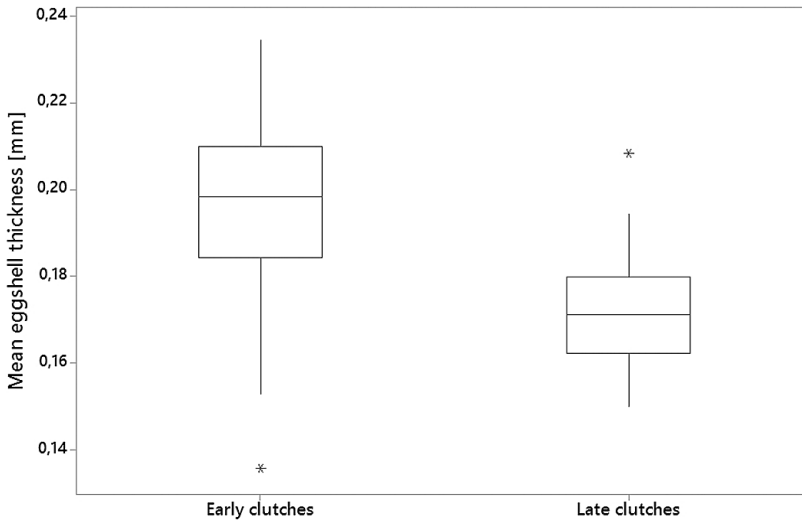


Fig. 3. Eggshell thickness at the beginning ( $n = 20$ ) and at the end ( $n = 20$ ) of the laying period in Capercaillie. The horizontal lines in the square indicate the 25<sup>th</sup> percentile, median, 75<sup>th</sup> percentile, the vertical lines indicate the data range, \* represents an outlier.

centrated pigment spot. Type of maculation seems to be more important than pigmentation lightness itself, even when these two factors are connected to each other. Therefore, maculation may be one of the indicators of eggshell thickness, which may be useful in breeding centres, where the eggs are often incubated artificially or by domestic fowl.

Recent studies provide evidence that pigmentation may compensate the loss of eggshell thickness. In the Great Tit (*Parus major*), subsequent eggs in clutches varied in maculation and pigmentation: first-eggs were slightly lighter than later ones (Gosler *et al.* 2005, Higham & Gosler 2006). Considering that pigment seems to compensate the loss in eggshell thickness, it may be assumed that later laid eggs have thinner and more pigmented eggshells.

In our study  $L^*$  value was not significantly associated with early (April) and late (June) clutches however, and eggs laid in June were little lighter, what was contrary to the findings in the Great Tit. A further study of the changes in eggshell pigmentation intensity in relation to the laying sequence in Capercaillie is thus recommended.

Studies carried out on Helmeted Guineafowl (*Numida meleagris*) revealed that unpigmented eggs had a thinner eggshell compared to typical eggs (Darnell-Middleton *et al.* 1998), which is very similar to our observations in Capercaillie eggs with a smooth pattern. We assume that pigmentation in those two species has a similar role (contrary to the mechanism of deposition in

Passeriformes or Falconiformes) (Gosler *et al.* 2005, Higham & Gosler 2006, Jagannath *et al.* 2008, Sanz & García-Navas 2009). Lack of eggshell pigmentation may result from disordered, unfinished or incorrect calcification process. In the study carried out in the Common Quail (*Coturnix coturnix*), eggs laid too early, i.e. after 21.5 hours after oviposition (3.5h before laying), were unpigmented (Woodard & Mather 1964). In the case of Capercaillie, it is impossible to determine the exact moment of pigmentation, but it may be presumed that some eggs with a smooth pattern were laid too early, leading to lack of pigmentation. Therefore, calcification and pigmentation processes may have been disturbed resulting in loss of thickness.

Eggshell colour comprises of not only pigmentation but also spot pattern, usually referred to as maculation. Sanz & García-Navas (2009) found that the eggshells in Blue Tits (*Cyanistes caeruleus*) were thicker when the spots were widely distributed on the eggshell surface. In the later studies, García-Navas *et al.* (2011) did not observe a decrease in the size and intensity of the pigment spots in birds that received calcium supplementation, but found that provisioning calcium-rich material during the egg-laying period led to a wider distribution of pigment spots. In Eurasian Sparrowhawks (*Accipiter nisus*), eggshells with more spots had higher levels of DDE (Dichlorodiphenyldichloroethylene), which caused eggshell thinning.

As in the Great Tit, the spots in the Eurasian Sparrowhawk eggshells most likely compensated for the loss of eggshell thickness as the eggshells were noticeably thinner at the protoporphyrin spot point (Jagannath *et al.* 2008). If this is indeed the role of pigmentation in these species, the pigment deposited at thinner points, would play small, but still a compensatory role in the eggshell thickness loss, because the pigmentless spots would be even thinner. All the birds in the Capercaillie Breeding Centre that laid the eggs, lived in the same environmental conditions and were fed the same diet. Therefore, it seems likely that in Capercaillies, the type of maculation depends mostly on genetic factors, considering the variety of the observed eggshell spot pattern. We presume that contrary to Blue Tits, in Capercaillies the additional calcium supplementation did not affect the maculation probably due to large differences in the eggshell maculation patterns and other functions of the pigment.

Although we distinguished three maculation categories, most of the collected eggs were pigmented in such a way that their clear assignation to a particular group was difficult (due to intermediate forms). Capercaillie female may build their nests in different places, hence maculation may act as camouflage depending on nest location, increasing the chance of clutch survival. Maybe the ecological aspect would explain the diversity of maculation patterns, which seems to be an interesting subject for further investigation.

Another hypothesis is that the pigment in Capercaillie, and maybe other Galliformes, form an additional layer increasing the total eggshell thickness. We speculate that there are two different explanations why poor pigmented eggs have thinner shells: in case of some smooth pattern eggs with slightly rough eggshell, small amount of pigment might be a consequence of disturbed calcification process. On the other hand, in case of eggs where process of eggshell formation was not disturbed, pigments made additional layer increasing the total eggshell thickness. The compensation of shell thinning in spot point was not well marked in all bird species. In the case of Black-headed Gull (*Larus ridibundus*), the eggshell in the maculated areas was significantly thinner after removing the eggshell membrane and cuticula (Maurer *et al.* 2011a,b). Those authors speculated that the thin-

ning of the calcite layer may be a consequence of, rather than the cause for the observed maculation, if protoporphyrin and calcium competed for the deposition pathway.

Studies carried out in other species revealed that the eggshell was thinner at the spot point than the area next to it, by approximately 7.5% in the Great Tit (Sanz & García-Navas 2009), by 3.3% in the Sparrowhawk (Jagannath *et al.* 2008), and by 1.2% in the Black-headed Gull (Maurer *et al.* 2011a,b). In the Capercaillie, there was on average 5.5% difference in the eggshell thickness between spotted and smooth eggshell areas, but the spot points were thicker, not thinner as in other presented studies. Similar processes of eggshell thickening at the spot point were reported in the Common Quail (Orłowski *et al.* 2017) and Black Grouse (*Lyrurus tetrix*) eggshell (unpublished data). Hence, protoporphyrin in Galliformes likely has different functions than in other studied bird orders. It may be interesting to study this phenomenon in other bird species.

Strength is one of the most important eggshell properties. The crush test is commonly used to evaluate hatching in commercial chicken eggs. Smooth patterned eggs may be a little more fragile, so putting them under domestic hens can be risky. This was confirmed by our observations in Capercaillie Breeding Centre in Wisła Forestry (Rzońca, pers. information). We used the crush test in our experiment, but as a matter of fact the result of the test should not prejudice the eggshell quality. The instrument used to measure chicken eggshell strength is designed to measure it vertically because commercial eggs are transported and artificially incubated in this position. Our study showed lack of association between L\* value, maculation type and Capercaillie eggshell strength, but eggshells were thinner at the equator surface.

This remark may be important because birds incubate their eggs horizontally in nature: although when we did not found a connection between eggshell lightness and maculation with its strength, more surveys should be conducted. In our previous studies (Rosenberger *et al.* 2017), we stated that eggshell strength is determined by the eggshell thickness and shape. It was documented that shorter and wider eggs are more resistant to cracking. This may also explain the lack of significant correlation between the eggshell strength,



colour of pigmentation and type of maculation. In our opinion, more tests are required to determine the eggshell strength in a horizontal position. Bain *et al.* (2006) indicated that in eggs with light shells, microcracks occurred when a smaller force is applied than required to fracture the eggshell completely. Hence, the lighter eggs with undetectable microcracks may be more prone to dehydration and pathogen invasion.

Furthermore, we found previously that eggs were wider (rounder) at the end of the laying season (Rosenberger *et al.* 2017). We assume this may result from the Capercaillie anatomy of the ovary, which, as a result of their laying performance, is well developed and wider. Knowing that the egg shape has a significant impact on the eggshell strength, we can assume that altered shape made them more resistant to cracking and could have partly compensated their loss of thickness, observed in this study. Moreover, it may be an adaptation mechanism to the loss of eggshell thickness during the laying season. Despite this, late clutches are still more susceptible to breakage, and the altered egg shape did not compensate shell thinning.

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### **Metson munankuoren väriyty, kuviointi ja paksuus**

Munankuoren ominaisuudet (paksuus, väriyty ja muoto) vaihtelevat lintulajien välillä mutta myös lajinsisäisesti, riippuen iästä, ympäristöolosuhteista ja yksilön ominaisuuksista. Munankuoren ominaisuudet ja ulkonäkö ovat tärkeitä lisääntymismenestyksen kannalta sekä luonnonvaraisilla

lajeilla että vankeudessa. Tässä tutkimuksessa selvitettiin munankuoren väriytyksen, kuvioinnin ja kuoren paksuuden yhteyttä vankeudessa kasvateilla metsoilla. Munia kerättiin koko munintakauden ajan jotta voitaisiin myös selvittää, miten kuoren kuviointi ja paksuus muuttuvat munintakauden aikana. Havaittiin, että vaaleimmat munat olivat ohuempia munan keskikohdalta kuin tummemmat munat. Myös tasaväriset munat olivat ohuempia keskikohdastaan, erityisesti verrattuna pilkullisiin muniin.

Munankuoren väriytyksellä ei ollut yhteyttä paksuuteen munankuoren pyöreämmässä tai terävämmässä päässä. Kuori oli 5.5 % paksumpi tummien pilkkujen kohdalta kuin muualta. Tulokset viittaavat siihen, että metson munissa pilkkujen pigmentti ei kompensoi munankuoren ohenemista, kuten muilla lintulajikoilla Charadriiformes, Falconiformes, Passeriformes). Munankuoren väriyty ei muuttunut munintakauden aikana. Munankuoret olivat ohuempia myöhemmin munintakaudella (huhtikuu vs kesäkuu), vaikka ravintoa ja kalsiumlisäravinnetta oli saatavilla rajoittamattomasti.

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