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Pros and cons of different methods of measuring egg coloration – photocolorimetry vs. spectrometry

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Color measurement plays an important role in numerous studies on evolution and function of egg coloration. Recent availability of compact, portable spectrometers and digital cameras resulted in considerable number of studies testing different hypotheses with the use of more objective color measurements. Each of the methods, however, has its advantages and limitations. In the present study we evaluate color variation in UV-rich and blue-green European Starling eggs using two different methods – color measurement from digital photography and spectrometric color measurement. Next, based on the values obtained from both devices, we classify the eggs to color categories and we compare the results of the classifications. We found that two measures of egg color intensity – saturation and BGC – were significantly correlated, however classification of blue-green egg color intensity based on saturation values obtained from a digital photography taken in a darkroom reflected egg colors more accurately, at least for human observer, than classification based on BGC values measured by spectrophotometer. We suggest that differences in classification of some eggs using spectrometric measurements may have resulted from the differences in egg glossiness. However, spectrometric measurement allowed analyses of UV component of egg color. The possibility of collecting and analyzing data in this range of the spectrum is the most pronounced advantage of spectrometric measurements of eggs coloration.

Key words: egg coloration, photocolorimetry, spectrometry, color measurement, blue-green and UV component of egg color

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1. Introduction

Color measurement plays an important role in numerous studies on evolution and function of egg coloration. The assessment of color differences between eggs and clutches as well as objective classification of eggs to color classes is the first and unavoidable step of such studies. Color measuring instruments can be divided into two categories – filter-based and spectrometer-based devices. Filter-based devices use three special filters, whose transmission characteristics are matched to the maximum sensitivity of three color-specific types of cones in the eye (at the red, green and blue wavelengths). Analyzed images from digital cameras are recorded in the same three color space: Red-Green-Blue (RGB). The method of measurement of color from digital images is called photocolorimetry. Spectrometers, on the other hand, have much more detectors with their sensitivity distributed across the UV-VIS spectrum, so they measure the energy present over a range of wavelengths as the spectral power distribution. Recent availability of compact, portable spectrometers and digital cameras resulted in considerable number of studies testing different hypotheses with the use of both photocolorimetry (Nguyen et al. 2007, Sezer & Tekelioglu 2009, Berg et al. 2009, McCormack & Berg 2010) and spectrometry (Moreno et al. 2006, Siefferman et al. 2006, Soler et al. 2008, Hanley et al. 2008). Each of the methods, however, has its advantages and limitations. Spectrometer measurements are precise, but time-consuming procedures. This refers both to color measurement and further analyses. Taking the photo of an egg or a clutch in a portable dark room takes much less time than spectrometric measurement, which reduces the risk of nest desertion. Spectrometers are also more expensive and require some experience in color measurements but some of them cover UV spectrum, which was proved to play an important role in birds color vision (Smith et al. 2002). Eggs of some species are UV-rich (Avilés et al. 2006), thus color analyses constrained to visible spectrum may not reveal important aspects of egg coloration.

As mentioned above, a main task of color measuring is color comparison. Thus in the present study we evaluate color variation in UV-rich and blue-green eggs of the European Starling *Sturnus vulgaris* using two different methods – photocolorimetry and spectrometry. Next, based on the values obtained from both devices, we classify the eggs to color categories and compare the results of the classifications. We also classify egg coloration according to human perception and test which of the two objective methods, photocolorimetry or spectrometry, produced more accurate results, at least from human perspective of color vision.

2. Material and methods

2.1. Study area and species

The study was conducted in a population of European Starlings in Czeszewo forest, north-west Poland (52°06′43′′ N 17°29′52′′ E) during the breeding season of 2009 in nest boxes installed earlier in the same year. The European Starling lays

nonspotted blue-greenish eggs (Cramp 1998) with a small peak at the ultraviolet wavelength (Avilés et al. 2006). Nest-boxes were inspected in every three days to minimize the risk of nest desertion by females. As the female lays one egg a day, the date of laying could be precisely determined without disturbing birds every day. The color of each egg was measured five days after clutch completion. This ensured that all analyzed eggs were at the same stage of development, which is important, because eggs may differ in color with respect to the number of days elapsed between laying and color measurement (Moreno et al. 2006).

Clutches contained 4–6 eggs. The range of laying dates of the study nests was of five days. We studied egg coloration of 21 eggs randomly chosen from 21 different clutches – one egg was collected from each clutch.

2.2. Color measurements

We analyzed color variability in European Starling eggs using three approaches. First, we assessed egg colors from digital photography of the eggs taken in the portable dark chamber. Using Adobe Photoshop CS we obtained values of saturation, hue and brightness. These parameters make up the three distinct attributes of color (Williamson and Cummins 1983). Saturation is a chromatic purity: freedom from dilution with white and hence vividness of hue. Hue is the quality of a color as determined by its dominant wavelength. Brightness of a colored surface depends upon the illumination and its reflectivity. Under the same light conditions perceived brightness is the logarithmic function of the light reflected from the surface.

Second, we assessed egg colors using measurements of reflectance spectra (300–700 nm) obtained from portable spectrophotometer Photon Control SPM-002 connected with deuterium-halogen lamp SPL-1DH, reflectance probe SPA-200U and Spectrosoft Pro v. 2.3.1. Software. Color of each egg was measured on three randomly selected areas of the surface of the egg along the long egg axis (Avilés et al. 2006). Each area was measured five times and the mean values were used in further analyses. The spectrophotometer covers the reflectance spectrum from 300 to 800 nm in intervals of 1 nm. Reflectance was measured with the probe placed at a constant distance from the egg surface (2mm) and reaching the egg at 45°, which is the recommended angle for glossy objects (Soler et al. 2008). Measurements were relative and referred to a standard white reference and to the dark, both calibrated before the measurement of each clutch. Reflectance, the term frequently used in our paper, is the proportion of light reflected at given wavelength. Mean reflectance (of entire spectrum or its range) was calculated as the sum of reflectances for each nanometer in the given spectral region divided by the number of nanometers. Blue-green chroma (BGC) was calculated as the proportion of total reflectance in the blue-green region of the spectrum ($BGC = Ref_{480–550\ nm}/Ref_{300–700\ nm}$, where $Ref_{480–550\ nm}$ is the sum of reflectances of wavelengths between 480 and 550 nm and $Ref_{300–700\ nm}$ is the sum of reflectances of wavelengths between 300 and 700 nm; Siefferman et al. 2006), which corresponds to the region of the lowest absorbance of biliverdin (Falchuk et al. 2002) and because starling eggs reflect light maximally in it (Fig. 1). UV chroma (UVC) was calculated analogously to BGC as the proportion of total reflectance in UV re-

gion of the spectrum (UVC = $Ref_{300–340\ nm}/Ref_{300–700\ nm}$) because starling eggs showed the highest UV reflectance between 300 and 340 nm (Fig. 1).

Third, five human observers (two authors of the study and three students involved in a field study) ordered the eggs from the palest to the darkest. Two of the observers were males and three were females. Observers' age varied from 24 to 38. The palest egg was given score 1, the second in the order score 2 and so on. If the colors of two or more eggs were judged by the observer as the same, the eggs were placed in the same position in the order and given the same score. The mean values of scores produced by five observers for each egg were used in further analyses.

Classification of eggs to color categories (pale, medium and dark) was based on K-means cluster analysis with 3 cluster centers. Differences between egg categories were tested with ANOVA. We used Spearman correlation to test to what degree BGC, saturation and human scores of egg color correspond to each other. All analyses were conducted in SPSS 16.

3. Results

3.1. Classification of egg color intensity

We perceived majority of collected starling eggs as intermediate and a few eggs as intensively blue or almost white. Thus, using K-means cluster analysis with 3 cluster centers, we divided 21 collected eggs into three categories: pale, medium and dark, using three different methods: (i) color measurement from digital photography based on saturation, (ii) spectrophotometric color measurement based on blue-green chroma (BGC) and (iii) color scores given by human observers.

Saturation of the studied eggs varied from 2% to 51% and showed the highest coefficient of variation (57%) in comparison to hue (10%) and brightness (7%). We entered saturation values in K-means cluster analysis with 3 cluster centers and divided eggs into color categories (pale, medium and dark) according to the graph presented in Fig. 2A. Eggs with saturation below 15% (n = 6) were classified as pale, medium eggs (n = 11) matched saturation between 16–30% and dark eggs (n = 4) were characterized by BGC above 55%. Differences between the three categories were highly significant ($F = 18.157$, $p < 0.001$).

Next, we entered BGC values of the same eggs in K-means cluster analysis with 3 cluster centers which resulted in classification of eggs presented in Fig. 2B BGC of the studied eggs varied from 49% to 57% and showed relatively low coefficient of variation (4%). Eggs with BGC below 52% (n = 5) were classified as pale, medium eggs (n = 13) matched BGC between 52–55% and dark eggs (n = 3) were characterized by BGC above 55%. Differences between the three categories were highly significant ($F = 48.647$, $p < 0.001$). Six eggs (marked in Fig. 2B with red asterisks) were classified into different categories than using saturation values.

Finally, we entered egg color scores given by human observers in K-means cluster analysis with 3 cluster centers which resulted in classification of eggs presented in Fig. 2C. Color scores of studied eggs varied from 1 to 10.4 and showed high coef-

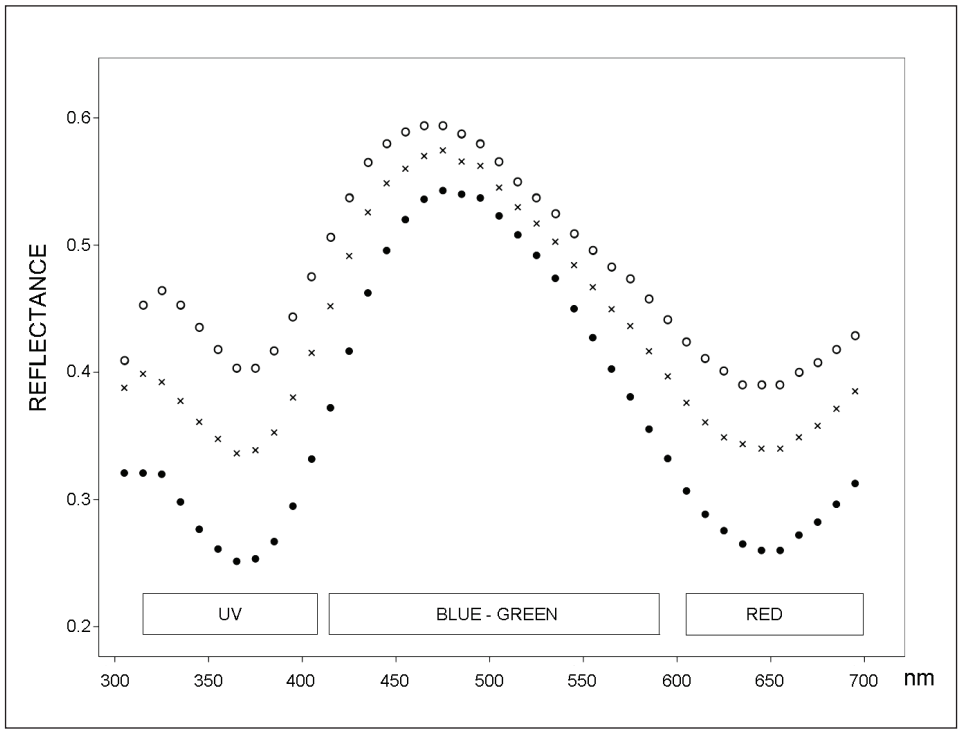

Figure 1. Reflectance values of pale (○), medium (x) and dark (●) eggs in the range 300–700 nm. Each plot is the mean of reflectances of three eggs from given color category.

ficient of variation (44%). Eggs with color scores below 4 (n = 5) were classified as pale, medium eggs (n = 12) matched scores between 4 – 7 and dark eggs (n = 4) were characterized by color scores above 7. Differences between the three categories were highly significant ($F = 73.611$, $p < 0.001$).

It can be seen that BGC based classification do not fully correspond with the classification based on saturation values. There were 6 eggs classified differently by the two methods (marked in Fig. 2B with red asterisks). The results obtained from spectrometry also do not match classification of egg colors based on human perception in 5 cases (marked in Fig. 2C with small asterisks). In contrast, the classification based on saturation values corresponded well with the classification based on human scores of egg colours – only one egg (no. 13) was classified differently by the two methods (marked in Fig. 2C with green asterisk). There was higher correlation between saturation values of eggs and human scores (Spearman's correlation: $r = 0.91$, $p < 0.001$) than between saturation and BGC (Spearman's correlation: $r = 0.49$, $p = 0.025$) or BGC and human scores (Spearman's correlation: $r = 0.54$, $p = 0.012$). Thus saturation seems to reflect color differences of eggs more accurately than BGC, at least from human perspective. The most pronounced examples of differences in classification based on BGC and saturation/human scores are eggs number 19 (the darkest in our collection) and 20 (one of the palest). The BGCs of these two eggs were

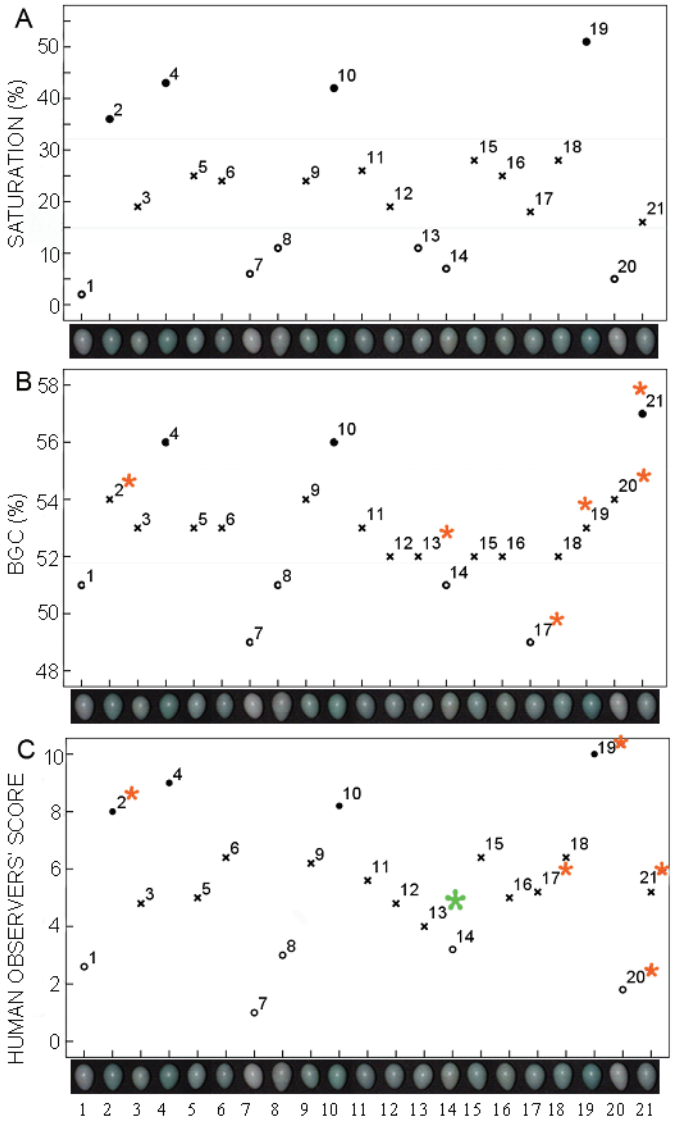

Figure 2. Classification of starling eggs colors based on three different methods: A – values of saturation obtained from digital photography taken in a darkroom, B – values of BGC measured with a spectrophotometer, C – human observers' color scores. In both analyses K-means cluster analysis with 3 cluster centers was used in order to distinguish three color categories: ○ – pale eggs, x – medium eggs and ● – dark eggs. Eggs marked with asterisk in 2 B were classified differently using BGC values than using saturation values. In 2 C red asterisks mark differences between classification based on BGC and human scores and green asterisk mark difference between classification based on saturation and human scores.

very similar so they were classified as medium eggs, despite the fact their colors were quite different as perceived by humans and presented on the photographs. Similarly, the egg 21 was classified as very dark according to its BGC, however it did not look so (classifications based both on saturation values from digital photography and human observers' scores placed this egg among the eggs of medium color).

3.2. Additional information of egg color revealed by spectrometric measurement

Detailed analysis of mean reflectance in spectrum 300–400 nm (UV), 400–600 nm (blue-green) and 600–700 nm (red) revealed that the eggs differed almost twice as more in UV (cv = 15%) and red spectrum (cv = 15%) than in blue-green region (cv = 8%).

Intensity of blue-green (BGC) and UV (UVC) coloration of starling eggs was strongly and negatively correlated (Spearman's correlation: r = –0.67, p = 0.001; Fig. 3). Mean UV reflectance of dark eggs ($\bar{x}$ = 23%, SD = 0.008, n = 3) was lower than this of medium eggs ($\bar{x}$ = 30%, SD = 0.023, n = 13) and pale eggs ($\bar{x}$ = 36%, SD = 0.037, n = 5), and the difference between the three categories of eggs was significant (F = 22.730, $p < 0.001$).

4. Discussion

We found that two measures of egg color intensity, saturation and BGC, were significantly correlated, however classification of blue-green egg color intensity based on saturation values obtained from a digital photography taken in a darkroom reflected egg colors better, at least for human observer, than classification based on BGC values measured by spectrometer. This is a surprising result as both saturation and blue-green chroma are related to colorimetric purity, or degree of paleness of a color, which is calculated using the formula: P = L/(Lw+L), where L is the luminance of the spectral color and Lw is the luminance of the white that is mixed with the spectral color. Note that the formula used to calculate BGC (BGC = $R_{400–580\ nm}/R_{300–700\ nm}$, where $R_{400–580\ nm}$ is the reflectance of the spectral color and $R_{300–700\ nm}$ is a total reflectance of an egg) by Siefferman et al. (2006) is in fact the same as formula of colorimetric purity. In case of spectrometric color measurement the reflectance, which is the measure of the amount of light reflected from a surface, is the analogue of the luminance, which is the measure of the amount of light being emitted or reflected off a particular surface. Thus L = $R_{400–580\ nm}$ and Lw+L = $R_{300–700\ nm}$. If so, both methods should produce the same color classifications, however they did not.

We suggest that differences in classification of some eggs using spectrometric measurements may have resulted from the differences in egg glossiness. The precision of spectrometer can be disrupted by the differences in surface gloss, as spectrometry consists in the measurement of the scattering of the illuminating light on the surface. Thus differences in the surface gloss may produce dissimilar results of measurements of equally pigmented objects. This should be especially taken into consideration

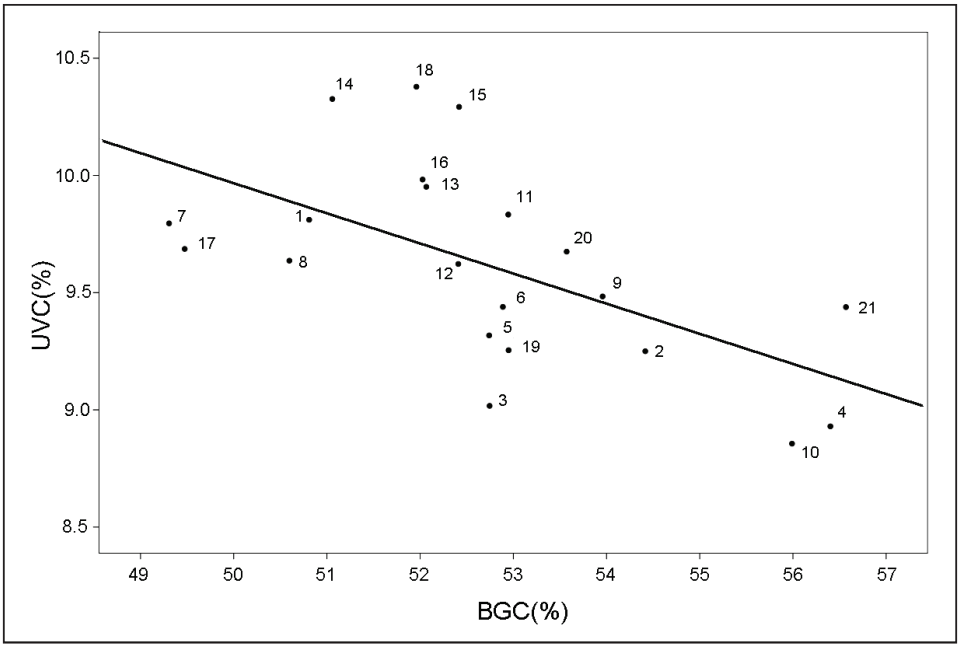

Figure 3. Relation between intensity of blue-green (BGC) and UV (UVC) coloration of starling eggs (n=21). Eggs are numbered according to Fig. 2.

when measuring egg coloration as eggs are often more or less glossy. Digital photography is less sensitive to the differences in surface gloss than spectrophotometry, especially in that saturation of the photographed eggs was measured outside the central area where the eggs reflected the flash. Conversely, spectrometric measurements must be taken from the area where the light is reflected from the surface. Although we conducted measurements with a probe placed at 45° as recommended for glossy objects, the probability that the differences in egg glossiness induced some distortions in color measurement seems high. We suggest that this is the most probable explanation of misclassifications of some eggs using BGC. The differences in color measurement of the same trait using various methods were also found by Evans et al. (2010). Similarly to our results, despite these discrepancies, general results obtained from different methods were comparable.

Although egg color classification based on BGC seemed less accurate, spectrometric measurement allowed analyses of aspects of coloration which were not possible based on saturation values obtained from digital photography. First, it revealed UV component of egg color. Second, it allowed detailed analyses of the relation between intensity of UV and blue-green coloration of eggs. As birds photoreceptors are UV sensitive (Chen et al. 1984, Hart et al. 1998) and UV component plays an important role in color perception in birds (Derim-Oglu & Maximov 1994, Bize et al. 2006, Ayala et al. 2007), the possibility of collecting data in this range of the spectrum is the most pronounced advantage of spectrometric measurements of eggs coloration.

Based on our analyses we recommend using portable spectrometers in studies of color perception of UV sensitive species while digital camera would be more effective device in studies covering only VIS spectrum. Moreover, further analyses of data collected from digital photographs are much faster (raw data from a spectrometer need time consuming transformation before getting, for example, BGC values). This fact enables using much more measurements in analyses based on saturation. Beside color parameters, photographs also provide additional data such as size or shape of analyzed objects, which may be important in some studies. Finally, a digital camera is much more affordable than a portable spectrometer, thus the cost of the device does not constitute a constraint in project budget. Taking into consideration all pros and cons of using the two devices, we suggest that using digital camera is sufficient in all studies on animal coloration, except from these conducted on UV sensitive species.

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