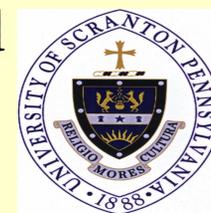


Assessing variability in feather coloration by gender and indices of immune function in Common Yellowthroats captured in northeastern Pennsylvania



Natalia M. Maass, University of Scranton, Robert J. Smith, University of Scranton,
Margret I. Hatch, Penn State Worthington Scranton

Introduction: Birds gain their feather coloration from two sources of pigment, carotenoids and melanins (McGraw 2006). Melanins are synthesized from amino acids, and create black, grey, and brown pigments. Carotenoids on the other hand are acquired through diet, and are responsible for the reds, yellow, and orange pigments (Browner III and Hill, 1998). Carotenoids serve two primary functions in birds - feather ornamentation (mate choice) or acting to enhance immune function (McGraw 2006). Consequently, birds must balance carotenoid allocation between immune function and the need to attract a mate.

Carotenoid allocation may be affected by a number of factors, including age, gender and state of an individual's immune system. For example, because older birds precede younger birds in arrival at the migratory destination, territories of older individuals are likely to be higher quality (Smith and Moore 2005), providing more food resources, including carotenoid-rich resources. Further, due to gender-related differences in roles crucial to mate choice, carotenoid pigmentation may be more important to one sex (typically males) than the other (Hill 1990). Finally, because carotenoid coloration is often positively associated with health or immune capacity (McGraw 2006) there should be relationships between measures of immune function such as differential leukocyte (white blood cell) counts and carotenoid pigmentation in feathers.

Past studies have examined relationships between brightness, hue, and saturation and carotenoid content of a feather. For example, Saks et al. (2003) found that the concentrations of carotenoids extracted from the tail feathers of male green finches were positively correlated with the increasing feather saturation and hue, although there was no relationship with brightness. Faris (2011) found that canthaxanthin and canary xanthophyll A concentrations in tail feathers of male American Redstarts were positively correlated with hue and saturation though he, as well, did not find relationships with brightness. The carotenoid lutein plays a significant role in the yellow coloring of Common Yellowthroat feathers (Guzy and Ritchison, 1999), which should be reflected by quantifiable variation in feather coloration. Hence our objective was to test the hypothesis that carotenoid pigmentation in feathers is influenced by age, gender and immune function in Common Yellowthroats.

Methods: We collected feathers from Common Yellowthroats captured during the 2006 spring migratory and breeding seasons, Lackawanna State Park (Dalton, PA) and privately owned lands adjacent to the park (Figures 1 and 2). Birds were captured using mist nets and standard netting protocol. We opened nets at sunrise, closing them in early afternoon unless weather conditions were unfavorable. All birds were identified to species, gender and age according to Pyle (1997).

Upon capture we recorded date, time and collected an assortment of morphological measures [see Smith and Hatch (2008) for a more complete description of the study]. We collected blood samples, preparing a single-cell layer blood slide, fixed the slide with 100% methanol, then stained it using a Hema3 (Sigma Aldrich) staining kit. We conducted differential white blood cell counts using oil immersion microscopy at x1000 (Hatch et al. 2009).

The feather coloration variables we were interested in included brightness (the total amount of light reflected from the area of the surface), saturation (the degree of color purity), and hue (the measure of which wavelengths of the visible spectrum contribute most to the reflected light, Montgomerie, 2006). To measure feather variables we used an Ocean Optics spectrometer (Model USB 2000), a fiber optic probe, and a forty-five degree probe holder. We collected 3 readings each from 3 randomly selected locations on each feather, for a total of 9 readings per feather. We used the average reading for each feather for all subsequent analyses.

We processed readings using Spectra Suite (Ocean Optics 2007) and CLR: Colour Analysis Program v1.02 software (Montgomerie 2008) then used general linear models to compare the color variables by age and sex using SPSS 16.0 statistical software (SPSS 2008). We also used Spearman's correlational analyses to look for relationships between feather coloration variables and immune function, as measured by differential white blood cell count.



Figure 1: Photograph of male Common Yellowthroat. Females lack the black mask over the eyes.

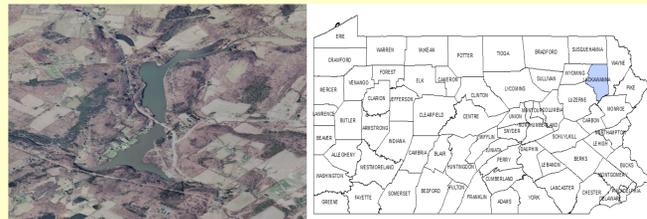


Figure 2: Location of field site on state map and over head photograph of site.

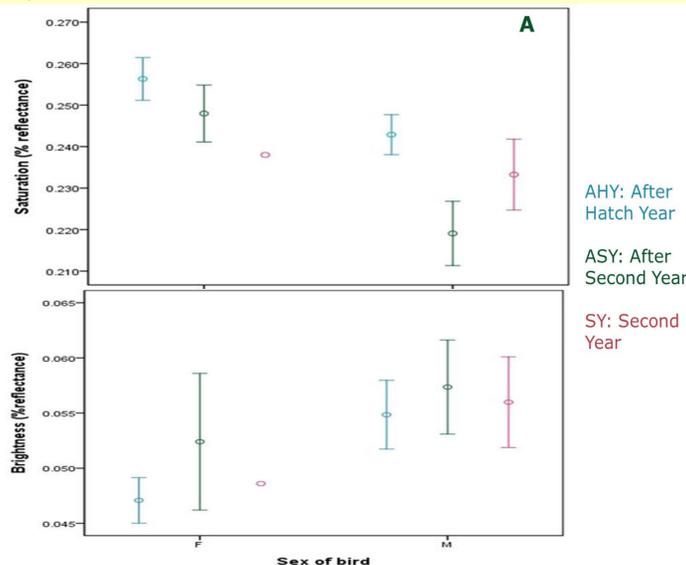


Figure 3a and 3b: Average brightness and saturation of male and female Common Yellowthroats grouped by age. Bars represent ± one standard deviation.

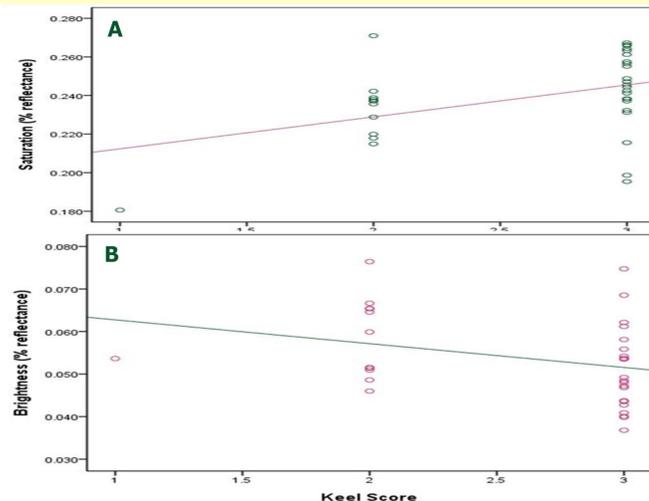


Figure 4a and 4b: Scatterplot of keel score in relation to feather saturation and brightness. Keel score is the amount of breast muscle a bird has, on a scale of 0-3. Keel scores and feathers were collected in 2006.

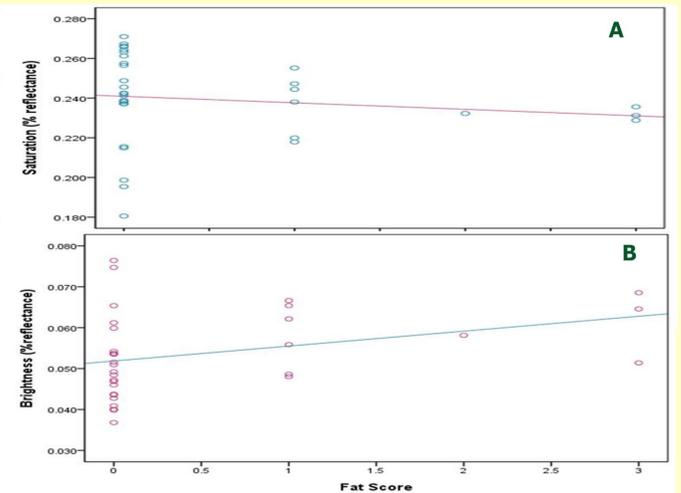


Figure 5a and 5b: Scatterplot of fat score in relation to feather saturation and brightness. Fat score is the visual indicator of how much fat a bird has, on scale of 0-5. Fat scores and feathers were collected in 2006.

Results: We found no relationship between total white blood cell count and brightness ($r=-0.13$, $n=35$, $P=0.94$), hue ($r=-0.03$, $P=0.87$) nor saturation ($r=0.05$, $n=35$, $P=0.77$). We did find that the relationships between gender ($F=3.41$, $df=1$, $P=0.08$, Figure 3a), age ($F=2.29$, $df=2$, $P=0.12$, Tuke Figure 3a) and saturation approached significance. AHY and ASY females were more saturated than males (Tukey, $P=0.04$). We also found an effect of gender on brightness ($F=6.52$, $df=1$ $P=0.02$, Figure 3b).

We found a positive association between fat score and brightness ($r=0.40$, $n=35$, $P=0.029$, Figure 4b) and a negative association between fat score and saturation that approached significance ($r=-0.29$, $n=35$, $P=0.09$, Figure 4a). We also found that keel score was positively associated with saturation ($r=0.41$, $n=35$, $P=0.02$, Figure 5a) and negatively associated with brightness ($r=-0.33$, $n=35$, $P=0.05$, Figure 5b).

Discussion:

- Contrary to our original hypothesis, our measures of immune function did not appear to affect any of the feather coloration variables. More work, notably using additional measures of immune function is necessary to further evaluate the hypothesis that immune function affects carotenoid pigmentation in Common Yellowthroats.
- In agreement with our hypothesis about feather coloration attributes and gender, we found that male rectrix feathers were brighter than female rectrices and that female rectrix feathers were more saturated than males. Given this species particular mating system [(males establish and defend territories, females appear to choose a particular male least at partly based on brightness of yellow bib feathers (Guzy and Ritchison 1999, Tarof et al. 2005)] our data support the hypothesis that brightness of tail feathers may also play a role in female choice.
- More work is necessary to better evaluate the gender differences in brightness and saturation. One hypothesis is that females have darker tail feathers to minimize predator attraction while incubating and males utilize brighter tail feathers as a secondary ornament (in addition to mask and bib attributes) to display health and/or individual quality (Tarof et al. 2005).
- Our results relating fat score to brightness (positive) are in line with the hypothesis that brightness may signal individual quality. Birds in better energetic condition grew brighter feathers. Further, the fact that males were brighter than females suggests that brightness in rectrix feathers may also be involved in female choice.
- The negative association between keel score and brightness was unexpected. There is some evidence that accumulation of carotenoid pigments at levels needed for pigmentation of thousands of contour feathers in goldfinches exerts long-term stress and contributes to skeletal muscle breakdown (Huggin et al. 2010), which we may be seeing evidence of in Common Yellowthroats.

Literature Cited:

- Faris, M. H. Determination and Quantitation of Carotenoids in *Setophaga ruticilla* Deathers. MA thesis. University of Scranton 2011. Print.
- Guzy, M.J. and G. Ritchison. 1999. Common Yellowthroat (*Geothlypis trichas*). The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology. Retrieved from the Birds of North America Online.
- Hatch M.I., R.J. Smith, J.C. Owens (2009). "Arrival timing and hematological parameters in Gray Catbirds (*Dumetella carolinensis*).". *Journal of Ornithology* 151.3: 545-52. Print.
- Hill, G. E. (1990). "Female house finches prefer colourful males: sexual selection for a condition-dependent trait." *Animal Behaviour* 40(3): 567-572.
- Hill, G. E. and W. R. Browner. (1998). "Melanin-based plumage coloration in the house finch is unaffected by coccidial infection." *Proceedings of the Royal Society of London, Series B: Biological Sciences* 265: 1401: 1105-1109.
- Huggins, K.A., K.J. Navarra, M.T. Mendonça, and G.E. Hill. (2010). "Determinants of Carotenoid Pigments: The Dark Side of Bright Coloration." *Naturwissenschaften* 97.7: 637-44. Print.
- McGraw, K. J. (2006). "Mechanics of carotenoid-based coloration." *Bird Coloration: Mechanisms and Measurements*. G. E. Hill and K. J. McGraw, eds. Cambridge, Harvard University Press: 177-242.
- Montgomerie, R. (2006). "Analyzing colors." *Bird Coloration: Mechanisms and Measurements*. G. E. Hill and K. J. McGraw, eds. Cambridge, Harvard University Press: 177-242.
- Montgomerie R. (2008). CLR: Colour Analysis Programs v1.02 ed. Queen's University, Kingston, ON, Canada.
- Ocean Optics. (2007) Spectrasuite Spectrometer Operating software.
- Pyle, P. Identification Guide to North American Birds. Bolinas (Calif.): Slate Creek, 1997. Print.
- Saks, L., K. McGraw, and P. Horak (2003). "How feather colour reflects its carotenoid content." *Functional Ecology* 17.4: 555-561.
- Smith, R. J., and F. R. Moore (2005). "Arrival timing and seasonal reproductive performance in a long-distance migratory landbird." *Behavioral Ecology and Sociobiology* 57: 231-239.
- Smith, R.J., and M.I. Hatch (2008). "A comparison of shrub dominated and forested habitat use by spring migrating landbirds in northeastern Pennsylvania." *The Condor* 110.4: 682-93. Print.
- Tarof, S.A., P. O'Dunn, and L.A. Whittingham (2005). "Dual functions of a melanin-based ornament in the common yellowthroat." *Proceedings of the Royal Society B: Biological Sciences* 272.1568: 1121-127. Print.