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An examination of iris color variation in Northern Saw-whet Owls (*Aegolius acadicus*) relative to sex and age

Un examen de la variación del color del iris en el Tecolote Oyamelero Norteño (*Aegolius acadicus*) en relación con el sexo y la edad

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ABSTRACT. The factors that influence avian iris color variation encompass a range of variables, including demographic characteristics such as sex, age, and body condition, as well as environmental factors including diet and habitat. Some pigments found in irises, such as carotenoids, are energetically costly to produce, especially for predators who consume little to no plant-based food resources. In this study, we investigated the influence of sex, age, and location on iris color variation in a sample of 566 Northern Saw-whet Owls (*Aegolius acadicus*) captured during banding operations stationed along the Mississippi flyway in Minnesota, Wisconsin, and Indiana between 2016 to 2022. We predicted that: (1) female owls would display lighter iris colors on average than males; and (2) hatch-year birds would display lighter iris colors than older individuals. We tested these predictions using generalized linear models and interpreted our results using predicted probabilities. We found no significant difference in iris colors between males and females when using field-assigned sex or physiological proxies, e.g., body mass. In contrast, we found that after-second-year individuals displayed darker-colored irises than hatch-year, after-hatch-year, and second-year birds. Finally, owls at the Wisconsin banding station were significantly more likely to display darker-colored irises than at other banding stations. These findings highlight the complexity in the mechanisms driving carotenoid variation displayed in avian irises. Iris color may serve as a valuable standardized index of body condition that can be utilized in addition to conventional metrics, such as fat and keel scores.

RESUMEN. La variación del color del iris en las aves se ve influenciada por una serie de variables, incluyendo características demográficas como el sexo, la edad y la condición corporal, así como factores ambientales como la dieta y el hábitat. Algunos pigmentos que se encuentran en los iris, como los carotenoides, son energéticamente costosos de producir, particularmente en depredadores con una ingesta limitada o nula de recursos vegetales. En este estudio, investigamos la influencia del sexo, la edad y la ubicación en la variación del color del iris. Para ello, analizamos una muestra de 566 individuos de Northern Saw-whet Owl (*Aegolius acadicus*), capturados durante operaciones de anillamiento estacionadas a lo largo de la ruta migratoria del Mississippi, en Minnesota, Wisconsin e Indiana entre 2016 y 2022. Nuestras predicciones fueron: (1) las hembras mostrarían, en promedio, colores de iris más claros que los machos; y (2) las aves nacidas durante el año en curso (Hatch-Year) presentarían colores de iris más claros que los individuos de mayor edad. Probamos estas predicciones utilizando modelos lineales generalizados e interpretamos nuestros resultados utilizando probabilidades predichas. El análisis reveló que no hubo diferencias significativas en el color del iris entre machos y hembras, ya sea utilizando el sexo asignado en el campo o indicadores fisiológicos como la masa corporal. En contraste, encontramos que los individuos de segundo año mostraron iris de color más oscuro en comparación que los individuos de primer año, las nacidas el año anterior y las de segundo año. Finalmente, los tecolotes capturados en la estación de anillamiento de Wisconsin mostraron una probabilidad significativamente mayor de tener iris de color más oscuro en comparación con los de otras estaciones. Estos hallazgos subrayan la complejidad de los mecanismos subyacentes a la variación de carotenoides observada en los iris de las aves. El color del iris podría constituir un índice estandarizado valioso de la condición corporal, complementando métricas convencionales como las puntuaciones de grasa y quilla.

Key Words: *body condition; carotenoids; Indiana; Minnesota; Mississippi flyway; mist netting; owl populations; Wisconsin*

INTRODUCTION

Carotenoids, molecules used in the production of red, orange, and yellow coloration in birds (Brush 1990), are a highly energetically expensive pigment because they are exclusively derived through diet and cannot be independently synthesized by animals (McGraw 2006). Carotenoid-based pigmentation within avian plumage, skin color, or irises may be indicative of an individual's overall health or migratory condition (Passarotto et al. 2020) because pigment intensity acts as an intraspecific signal of age and mate quality for some species (Wolfenbarger 1999, Pryke et al. 2001, Pérez-Rodríguez and Viñuela 2008, Simons et al. 2014). Carotenoids are an especially limiting micronutrient for birds that are predatory or have carrion-based diets (Olson and Owens 1998, Blanco et al. 2014, Delhey et al. 2022) because carotenoid content in vertebrate food items is generally lower than that of plant-based items (Olson 2006). Carotenoid pigmentation

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of raptors may signal successful resource allocation toward self-maintenance (Sumasgutner et al. 2018), because internal carotenoid availability is essential for controlling oxidative stress from free radicals (Camplani et al. 1999, Alonso-Alvarez et al. 2008).

The Northern Saw-whet Owl (*Aegolius acadicus*) is a migratory owl native to North America (Rasmussen et al. 2020). The species' breeding range is extensive, ranging from southern Canada to the northern United States, spanning from the Pacific Northwest to the northeast (De Ruyck et al. 2012, Rasmussen et al. 2020). Some populations are non-migratory (Contreras 2000), but the majority migrate southward during the fall to wintering sites throughout the United States (Rasmussen et al. 2020). Northern Saw-whet Owl banding stations are prominent in the United States and Canada, and typically operate during their fall and spring migratory periods (Beckett and Proudfoot 2011). This extensive coverage allows for the examination of birds migrating to and from different breeding populations (Confer et al. 2014, Rasmussen et al. 2020), especially because there is some evidence for route fidelity in this species (Beckett and Proudfoot 2011).

The iris color of Northern Saw-whet Owls is noted to vary from yellow to a deep golden hue (Rasmussen et al. 2020), when the birds are caught during banding operations. This pigmentation consists of a mixture of carotenoids, pteridines, and purines (Oliphant 1987); however, little is known about the potential factors responsible for iris-color variation. Additionally, it is uncertain whether these pigments change prior to energetically expensive periods, such as migration, or if they differ within different population demographics. Higher fat scores and low keel scores are associated with lighter colored irises for this species (Wails et al. 2018), suggesting tradeoffs between pigment function and fat-storage requirements. Whereas iris color in isolation may not be a robust predictor of long-term body condition for Northern Saw-whet Owls (Wails et al. 2018), it may provide inferences to the short-term, pre-migratory condition that individuals are in when captured at banding stations (Passarotto et al. 2020).

This study's aim was to explore variation in iris color relative to sex, age class, and banding station among a sample of Northern Saw-whet Owls caught in Wisconsin, Minnesota, and Indiana. Because of predicted higher energy expenditure required during the previous nesting period, we predicted that female Northern Saw-whet Owls may display lighter iris colors than males in the fall. Additionally, we predicted that hatch-year birds may display lighter iris colors with less visible carotenoid concentration than older individuals because younger birds likely need to utilize more antioxidants for immunomodulatory functions (Chew 1996, Blount et al. 2003) during immunosuppressive periods of high stress, e.g., migration (O'Neal and Ketterson 2012), because they have less time to endogenously develop their immune system compared to adults (O'Neal and Ketterson 2012).

STUDY AREA
Banding stations that collaborated on this project and whose data were utilized were located within Purdue University in Tippecanoe County, Indiana, and the Carpenter St. Croix Valley Nature Centers in St. Croix County, Wisconsin, and Washington County, Minnesota.

The Purdue University Northern Saw-whet Owl Banding Station is split between two banding sites: Martell Forest (40°26′07.6″N, 87°02′13.8″W) and Purdue Wildlife Area (PWA; 40°27′34.3″N, 87°03′31.2″W) in West Lafayette, Indiana. The sites are fairly close together at 2.57 km apart. Martell encompasses 193 ha, 70% of which is mixed oak-hickory and maple-beech forest. PWA is approximately 117 ha and is composed of a variety of ecosystems including wetlands, prairie, oak savannas, red and white pine stands (*P. strobus*), and mixed hardwood forests. The average temperature in West Lafayette during the fall, i.e., September to November, ranges from 25.2 °C to 0.2 °C, and the average precipitation is 7.7 cm (https://usclimatedata.com). During the spring banding season, i.e., February to March, average temperatures range from 9.4 °C to -6.8 °C with an average precipitation of 5.7 cm (https://usclimatedata.com).

The Carpenter St. Croix Valley Nature Center NSWO Station is split between five banding sites near Hastings, Minnesota (*n* = 3; 44°46′0.5″N, -92°48′53.34″W), and Hudson, Wisconsin (*n* = 2; 44°54′27.3″N, -92°44′44.9″W), along the St. Croix River. The banding sites at Carpenter St. Croix Valley Nature Center in Hastings, Minnesota, encompass approximately 172 ha that alternate between pine, oak savanna, restored prairie, and forested ravine habitats near the St. Croix River. The average temperature in Hastings during the fall, i.e., September to November, ranges from 21.6 °C to -3.3 °C, and the average precipitation is 6.6 cm (https://usclimatedata.com). The Carpenter St. Croix Valley Nature Center in Hudson, WI banding sites cover 121 ha and consists of a mosaic of restored prairie, oak savanna, and wooded bluffs along the St. Croix River. The average temperature in Hudson during the fall (September to November) ranges from 23.6 °C to -1.9 °C, and the average precipitation is 7.3 cm (https://usclimatedata.com).

METHODS

Field methods
The Purdue banding station targeted Saw-whet Owls during their fall and spring migratory period in 2016 to 2022 following the Project Owlnet protocol (http://projectowlnet.org). Exact season start/end timing was weather dependent, but the fall banding season typically occurred from late September to late November and spring banding occurred from early January to mid-March. Not all collaborating banding stations participated in the spring banding season, so spring data were not utilized in statistical analyses. Sampling frequency occurred a minimum of two nights per week during spring and fall, averaging four nights during peak season at the Purdue Banding Station and three nights at the Carpenter St. Croix Valley Nature Center stations. Banding sites at Purdue consisted of either four or five, 2.6 m x 12 m, 60 mm nylon mist nets set in a cross-design formation, or similar formation. Carpenter stations had several formations across in Minnesota and Wisconsin, including nets arranged in F-shaped, long-line, and perpendicular pairs. In the center of the Purdue nets, we set a FoxPro NX4 (2016 to 2020), or a FoxPro Hammerjack 2 (2021 to 2022) loaded with a 5.5-minute audio file of Northern Saw-whet Owl vocalizations (http://projectowlnet.org), including their advertising call, *ksew* call, whine, and bill snap. The Carpenter stations utilized Edge by Expedite MP3 Caller & Speakers loaded with the same recording. Nets were opened 30 minutes after civil sunset and checked a maximum of

Fig. 1. Northern Saw-whet Owls (*Aegolius acadicus*) from Tippecanoe, Indiana, between 2016 and 2022, being evaluated for iris color, using a color chart consisting of Benjamin Moore paint chips glued vertically on a Popsicle stick for in-field assessment. The colors, as shown, are: A: Bold Yellow (336), B: Golden Orchards (329), C: Abstracta (322), and D: Oxford Gold (315). All external lights are off and the only light source is a handheld flashlight (Timlon 7W, 350 lm Tactical LED Flashlight).

every 60 minutes and a minimum of every 15 minutes. Banding ceased between 11:00 p.m. and 12:00 a.m. unless an owl was captured, upon which an additional hour was added for every owl captured up until 4:00 a.m. The audio lure was played on repeat once nets opened and silenced when an owl was being removed from the net.

When a Northern Saw-whet Owl was successfully captured, banders placed a United States Geological Survey (USGS) 4-short aluminum band on the tarsometatarsus of each new capture. Banders recorded, at minimum, the wing chord in millimeters, rectrices in millimeters, and weight in grams of both new and recaptured owls. Additional data collected across stations included culmen in millimeters, tarsus in millimeters, presence or absence of parasites, and keel scores. We aged individuals using an ultraviolet flashlight (NVTED Ultraviolet Handheld Blacklight Flashlight, 51 LED, 395 nm) to determine molt limits (Weidensaul et al. 2011; http://projectowlnet.org). Sex was determined by mass and wing-chord measurements (Pyle 1997). We compared iris color of each banded owl to a discrete color scale of paint chips provided by the commercial paint supplier Benjamin Moore, glued vertically on a popsicle stick by holding an individual vertically next to the color chart (Fig. 1), then turning off all external lights and shining a Timlon 7W, 350 lm Tactical LED Flashlight at the individual and color chart until at least two observers agreed on an iris color. The colors utilized were 336 Bold Yellow, 329 Golden Orchards, 322 Abstracta, and 315 Oxford Gold (Fig. 1). To minimize bias between stations, all stations were provided with a color chart, flashlight, and protocol for standardization.

All applicable ethical guidelines for the use of birds in research have been followed, including those presented in the Ornithological Council's Guidelines to the Use of Wild Birds in Research (Fair et al. 2010). The Purdue Banding Station captured and handled birds in compliance with the Purdue Animal Care and Use Committee guidelines, protocols #1110000078E003 and #995013.

Statistical analysis methods

Our analyses included only individuals captured in the fall across the three sites, because the Minnesota and Wisconsin banding sites only operated during the fall. Our dataset included a total of 566 independent data points, i.e., tagged individuals with only one observation each. Of those, we had data on sex for 439 individuals, and data on body mass for 559 individuals. We tested the association between body mass and sex with a general linear model with body mass as dependent variable and sex as independent variable. We found that sex significantly explained the variation in body mass (F1, 430 = 256, P < 0.001, Adjusted R^2 = 0.37), whereby females (96 g ± 0.32) were larger than males (79.60 g ± 0.98). We ran two models, one where we included body mass as a covariate, because of the higher availability of data, as well as a model with sex as a covariate. For all statistical analyses, we binned age into three classes: age class 1 for hatch-year, age class 2 for after-hatch-year and second-year, and age class 3 for after-second-year birds. We binned hatch-year birds as age class 1 because they were in the process of completing their first fall migration. We binned after-hatch-year and second-year birds together for age class 2, because both age groups have already experienced at least one breeding season prior to capture and have already made one prior fall migration. Furthermore, because after-hatch-year and second-year birds are experiencing their second fall migration, they may have increased fitness advantages compared to hatch-year owls taking on their first migration. Finally, after-second-year birds are in at least their third fall migration, so we binned them into age class 3. Our final sample sizes considering age class and station were as follows: for Minnesota, age class 1 was 166, age class 2 was 54, and age class 3 was 28; for Wisconsin, age class 1 was 138, age class 2 was 71, and age class 3 was 59; and for Indiana, age class 1 was 42, age class 2 was 8, and age class 3 was 0.

For our main analysis, the dependent variable, eye color, consisted of four discrete values along an ordinal scale of increasingly darker irises: 336 Bold Yellow, 329 Golden Orchards, 322 Abstracta, and 315 Oxford Gold. We therefore ran a generalized

linear model with ordinal-dependent variable following Melamed and Doan (2023). One of the key assumptions of this model is the parallel regression assumption, also known as the proportional odds assumption, by which the coefficients characterizing the association between the dependent and the independent variables do not change for the different discrete categories of the dependent variable (Ari and Yildiz 2014). We checked for this assumption using the Brant test (Brant 1990) and found that we violated this assumption ($X^2_{10} = 37.17$, P < 0.001). We followed the suggestions outlined by Melamed and Doan (2023) and pooled the ordinal categories into two groups and conducted a generalized linear model with a binary dependent variable instead.

We dichotomized our dependent variable into zeros (eye color codes = 329 and 336) and ones (eye color codes = 315 and 322), such that higher values of our model would represent the probability of darker irises. Hence, our generalized linear model followed a binomial distribution with a logit link function. Our independent variables were age class at three levels, i.e., age class 1, age class 2, and age class 3; station at three levels, i.e., Minnesota, Wisconsin, and Indiana; and body mass continuous. In principle, we could not add to this model the interaction between age class and station, because the Indiana station did not catch any individuals of age class 3, failing to meet the orthogonality assumption for interaction effects. Nevertheless, to aid in the interpretation of our results, we ran a second model with the same independent factors, but without including the data from the Indiana station, allowing us to include the interaction effects between age class and station. To interpret our results, we used predicted probabilities for the sake of simplicity, following Winter (2020). We used the R-package emmeans (Lenth 2023) to estimate the predicted probabilities of our models. All analyses were conducted in, and figures developed with, R version 4.3.1 version 2023-06-16 ucrt (R Core Team 2023) and R-Studio version 2023.6.2.561 (Posit team 2023).

RESULTS
Our modeling approach found that the probability of darker eye color was significantly affected by age class and station. Our first generalized linear model, including body mass, age class, and station, yielded an overall significant outcome ($\chi^2 = 43.33$, d.f. = 5, P < 0.001, log-likelihood ratio test = -365.15); however, the overall proportion of the variability explained in eye color was low (Pseudo $R^2 = 5.60\%$). Although body mass was not significant ($\chi2 = 1.84$, d.f. = 1, P = 0.175), age class ($\chi^2 = 27.11$, d.f. = 2, P < 0.001) and station ($\chi^2 = 14.37$, d.f. = 2, P < 0.001) were. The probability of having darker eyes increased as age class increased, whereby age class 3 had the highest probability (Fig. 2). More specifically, age class 3 had a significantly higher probability of a darker eye than age class 2 (z-ratio = -3.57, P = 0.001) and age class 1 (z-ratio = -4.42, P < 0.001). The difference between age class 1 and age class 2 (z-ratio = -0.66, P = 0.787) was not significant. In terms of relative differences between age classes, age class 3 had a 56.21% higher probability of darker eyes relative to age class 1, and a 46.77% higher probability relative to age class 2 (Fig. 2). We note that these results may have been influenced by the unbalanced sample sizes across age classes, with lower sample sizes relative to older individuals (age class 1 = 346, age class 2 = 133, age class 3 = 87). Regarding the sampling stations, the probability of birds displaying darker eyes was significantly

Fig. 2. The predictive mean (± SE) probability of Northern Saw-whet Owls (*Aegolius acadicus*) in our sample having darker eyes relative to age class, at three banding stations along the Mississippi Flyway in Indiana, Minnesota, and Wisconsin, USA. Age class 3 has a significantly higher probability of a darker eye than age class 2 (z-ratio = -3.57, P = 0.001) and age class 1 (z-ratio = -4.42, P < 0.001). There is no significant difference between age class 1 and age class 2 (z-ratio = -0.66, P = 0.787).
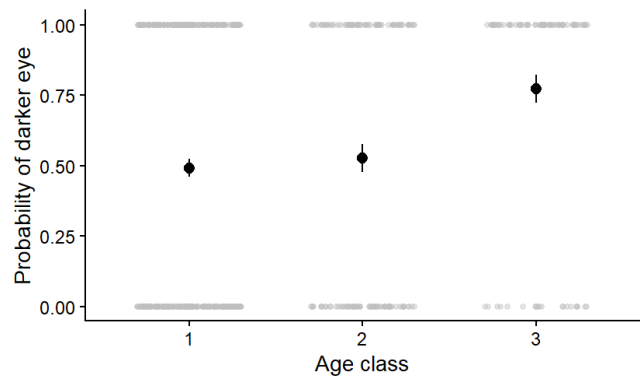

higher in the Wisconsin station (P = 0.658, SE = 0.032) compared to the Minnesota station (P = 0.496, SE = 0.038; z-ratio = -3.59, P = 0.001), but without significant differences between the Minnesota and Indiana stations (P = 0.657, SE = 0.071; z-ratio = -2.06, P = 0.098) and the Wisconsin and Indiana stations (z-ratio = 0.003, P = 1.000). Again, these results may have been affected by the unbalanced sample sizes across stations (Wisconsin = 268, Minnesota = 248, Indiana = 50).

Our next generalized linear model, including sex, age class, and station, yielded qualitatively the same results as our first generalized linear model that utilized body mass as a proxy for sex, with an overall significant outcome ($\chi^2 = 44.72$, d.f. = 5, P < 0.001, log-likelihood ratio test = -278.95). Similarly, the overall proportion of the variability explained in eye color was low (Pseudo $R^2 = 7.42\%$). Sex was not significant ($\chi^2 = 1.20$, d.f. = 1, P = 0.273), whereas age class ($\chi^2 = 29.33$, d.f. = 2, P < 0.001) and station ($\chi^2 = 14.19$, d.f. = 2, P < 0.001) were.

We ran an extra generalized linear model to establish whether the observed direct relationship between increasing age class and greater probability of darker eye remained the same across stations with high enough sample size by removing the Indiana station and including the interaction effect between age class and station. The overall model was significant ($\chi^2 = 42.22$, d.f. = 6, P < 0.001, log-likelihood ratio test = -332.66), with a low proportion of the variation explained (Pseudo $R^2 = 6\%$). Age class ($\chi^2 = 28.37$, d.f. = 2, P < 0.001) and station ($\chi^2 = 12.71$, d.f. = 1, P < 0.001) were both significant, but body mass ($\chi^2 = 1.09$, d.f. = 1, P = 0.297) and the interaction between age class and station ($\chi2 = 0.06$, d. f. = 2, P = 0.970) were not. When plotting the probability of darker eyes across age classes and station, we found that a similar pattern for age class within each station to that found in the previous analysis with the three stations (Fig. 3). More specifically, for the Minnesota station, the probability of darker eyes in age class 3

Fig. 3. The predictive mean (± SE) probability of Northern Saw-whet Owls (*Aegolius acadicus*) having darker eyes relative to age and banding station in Minnesota and Wisconsin, USA. In Minnesota, the probability in age class 3 is significantly higher than age class 1 (z-ratio = -2.85, P = 0.012), with the other two age class comparisons not being significant (age class 2 versus age class 1, z-ratio = -0.58, P = 0.832; age class 2 versus age class 3, z ratio = -2.16, P = 0.078). In Wisconsin, the probability in age class 3 is significantly higher than age class 1 (z-ratio = -3.34, P = 0.002) and age class 2 (z-ratio = -2.83, P = 0.013) with no significant differences between age class 1 and age class 2 (z-ratio = -0.28, P = 0.958).
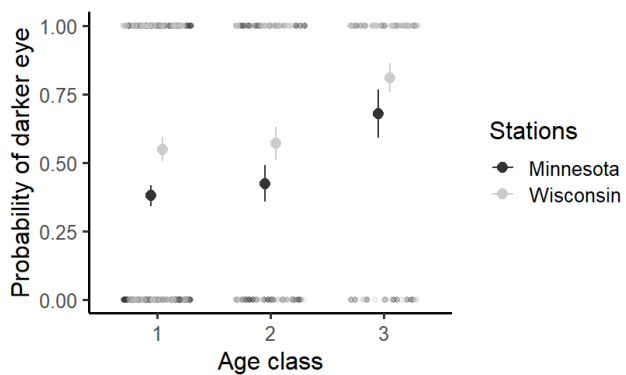
was significantly higher than age class 1 (z-ratio = -2.85, P = 0.012), with the other two age-class comparisons not being significant (age class 2 versus age class 1, z-ratio = -0.58, P = 0.832; age class 2 versus age class 3, z ratio = -2.16, P = 0.078); and for the Wisconsin station, the probability of darker eyes in age class 3 was also significantly higher than age class 1 (z-ratio = -3.34, P = 0.002) and age class 2 (z-ratio = -2.83, P = 0.013) with no significant differences between age class 1 and age class 2 (z-ratio = -0.28, P = 0.958).

DISCUSSION
Our investigation yielded insights into the physiological drivers shaping avian iris color in Northern Saw-whet Owls across the Mississippi flyway. We a priori predicted that sex and age class would impact iris coloration. Whereas we found an age class effect, iris color did not vary significantly with sex or body mass, which was a proxy of sex in our study. Overall, our data show that younger owls of age classes 1 and 2 displayed lighter irises compared to older owls of age class 3. Additionally, we found a significant difference in the probability of darker eyes between the Wisconsin and Minnesota stations, despite their proximity.

Because not all stations evaluated sex in the field, and female Northern Saw-whet Owls are significantly heavier than males, we had more data with body mass, so we used body mass as a proxy for sex in one of the models. Although we anticipated females to display lighter colored irises because of energy expenditures associated with nesting, we found that neither body mass nor sex were statistically significant variables in explaining iris color. Our results are consistent with similar passerine studies that found few to no significant relationships between sex and iris color (Craig

and Hulley 2004, Negro et al. 2017, Polakowski et al. 2020, Corbett et al. 2023). Whereas incubation solely occurs by female Northern Saw-whet Owls, males do feed females on the nest (Rasmussen et al. 2020), which suggests that there may similar energetic costs between sexes. Our results may also be a result of disproportionate migration between sexes, where female Northern Saw-whet Owls migrate earlier and are captured more frequently than males (Stock et al. 2006, Brittain et al. 2009).

We found that age class 3 individuals were more likely to display darker colored irises than both age class 1 and 2 birds. This followed our prediction and matches similar assessments by Wails et al. (2018), who found that HY female Northern Saw-whet Owls were more likely to exhibit lighter colored irises than older individuals. Similarly, a study with Brazilian Tanager (*Ramphocelus bresilius*) iris coloration served as a reliable morphological feature to distinguish young birds, where adult birds exhibited more vibrantly red-colored irises than dull-brown-eyed juveniles (Nogueira and Alves 2008). Associations between iris color and individual health are also documented in American Kestrels (*Falco sparverius*) with older birds displaying darker, more pigmented irises that are likely the result of greater individual health, experience, and migratory performance compared to younger individuals (Mueller et al. 2013). This presence of greater carotenoid pigmentation in older Northern Saw-whet Owls may indicate effective allocation of resources toward self-preservation (Sumasgutner et al. 2018), as well as a greater balance between pigment function and fat-storage requirements (Wails et al. 2018).

The lack of significance between age class 2 iris color and age class 1 may be due to our smaller sample size of age class 2 captures. Data only consisted of a total of 30 age class 2 captures across all stations and sampling years, compared to 449 captures for age class 1 and 87 captures for age class 3. The small sample size of age class 2 relative to the other age classes may mask relevant relationships when comparing morphological differences (Bissonette 1999). Additional data on these older individuals are necessary to better interpret if there are differences in iris color as individuals reach older age classes.

Northern Saw-whet Owls captured in Wisconsin were significantly more likely to have dark-colored irises, regardless of age class, compared to individuals captured in Minnesota, but not Indiana. These results may be due to observer differences between stations. There are multiple challenges to describing iris color in the field, such as using descriptors that can lead to misinterpretation, e.g., Bold Yellow, and a lack of general data standardization across stations (Joseph et al. 2024). Although all the participating stations in the study utilized the same color chart, using quantitative methods to record iris color, such as calibrated digital photographs, may further reduce observer bias (Joseph et al. 2024). Additional measures to further standardize this process would increase the value of the data collected and could allow for more meaningful connections regarding the variation of eye color to be explored.

If iris color is potentially attributed to body condition and food availability for owls (Wails et al. 2018), then it is possible that significantly darker coloration could reflect conditions within the Wisconsin banding site. Northern Saw-whet Owls reside within Wisconsin during the breeding season, with known populations

present year-round (Mueller and Berger 1967, Bielefeldt and Rosenfield 1993). Although migratory individuals from outside Wisconsin pass through on their way further south in the fall, these same migrant populations also pass through adjacent Minnesota (Erdman et al. 1997). Northern Saw-whet Owls in Wisconsin and Minnesota primarily reflect Great Lakes migrants and some western Ontario migrants (Confer et al. 2014), whereas those in Indiana primarily reflect the central Ontario migrants passing through the Ohio River Valley (Brittain et al. 2009, Neumann et al. 2020). Whereas individuals do not necessarily adhere to strict migratory routes (Cannings 1993), the likelihood of capturing birds with darker colored irises may reflect conditions within particular breeding populations during fall migration. However, further study is needed to reduce inter-observer variation between banding stations in order to extrapolate these relationships further.

Understanding variation in iris color holds broader implications for field ornithology, especially when used as an additional aging criterion (Snyder and Snyder 1974, Bortolotti et al. 2003, Polakowski et al. 2020). Additionally, iris color may serve as a vital marker of speciation (Joseph et al. 2024), or as an indicator of environmental stressors, such as the presence of pollutants (Bortolotti et al. 2003). Whereas our findings contribute to our understanding of iris color variation in a sample of Northern Saw-whet Owls, they also underscore the need for comprehensive and interdisciplinary research to identify similar relationships across various avian families.

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