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Camille Aleixo Hoffmaster

**DIMORFISMO SEXUAL CRÍPTICO EM PLUMAGEM DE MOCHO-AMOLADOR,
*AEGOLIUS ACADICUS***

Belo Horizonte

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Camille Aleixo Hoffmaster

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*AEGOLIUS ACADICUS***

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Coorientadora: Dra. Lia Nahomi Kajiki

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ATA DE DEFESA DE DISSERTAÇÃO

CAMILLE ALEIXO HOFFMASTER

Ao trigésimo primeiro dia do mês de outubro do ano de dois mil e vinte e quatro, às quatorze horas e trinta minutos, ocorreu a defesa de Mestrado da Pós-Graduação em Zoologia, de autoria da Mestranda Camille Aleixo Hoffmaster intitulada: **“Dimorfismo Sexual Críptico em Plumagem de Mocho-amolador, *Aegolius acadicus*.”**. Abrindo a sessão, o Presidente da Comissão, Prof. Dr. Marcos Rodrigues, após dar a conhecer aos presentes o teor das Normas Regulamentares do Trabalho Final, passou a palavra para a discente para apresentação de seu trabalho.

Esteve presente a Banca Examinadora composta pelos membros: Guilherme Henrique Silva de Freitas, Priscilla Esclarski, e demais convidados. Seguiu-se a arguição pelos examinadores, com a respectiva defesa da discente.

Após a arguição, apenas os examinadores permaneceram na sala para avaliação e deliberação acerca do resultado final, a saber: o trabalho foi **APROVADO COM ALTERAÇÕES**.

Nada mais havendo a tratar, o Presidente da Comissão encerrou a reunião e lavrou a presente ata, que será assinada por todos os membros participantes da Comissão Examinadora.



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RESUMO

Este estudo investiga o dimorfismo sexual críptico no mocho-amolador (*Aegolius acadicus*), analisando traços de plumagem e morfologia, com ênfase nas características da cabeça e das sobrancelhas. Utilizando dados de imagem do Projeto OwlNet e fotografias em UV de espécimes de museu, identificamos diferenças na forma da cabeça e das sobrancelhas entre os sexos. Os machos geralmente apresentaram cabeças arredondadas e sobrancelhas em formato de V, enquanto as fêmeas tinham cabeças achatadas e sobrancelhas em formato de C. A fotografia UV revelou uma coloração violeta-magenta na plumagem branca do disco facial, sugerindo um possível papel na sinalização noturna. Os resultados ampliam nosso entendimento sobre o dimorfismo sexual nesta espécie e demonstram a utilidade da fotografia UV para pesquisas ornitológicas. Este estudo contribui com uma abordagem não invasiva para a identificação de sexo, oferecendo percepções sobre o comportamento e as adaptações de aves noturnas.

Palavras-chave: Mocho-amolador; *Aegolius acadicus*; ultravioleta; determinação de sexo; disco facial; padrões de plumagem branca.

ABSTRACT

This study explores cryptic sexual dimorphism in the Northern Saw-whet Owl (*Aegolius acadicus*) by analyzing plumage and morphological traits, with a focus on head and eyebrow features. Utilizing image data from Project OwlNet and UV photography of museum specimens, we identified differences in head shape and eyebrow shape between sexes. Males typically exhibited round heads and V-shaped eyebrows, while females had flat heads and C-shaped eyebrows. UV photography revealed a violet-magenta coloration and fluorescence on the facial disk plumage, suggesting a potential role in nocturnal signaling. These findings enhance our understanding of sexual dimorphism in this species and demonstrate the utility of UV photography for avian research. This study also contributes a non-invasive approach to sex identification, offering insights into nocturnal avian behavior and adaptations.

Keywords: Saw-whet Owl; *Aegolius acadicus*; ultra-violet; sex prediction; facial disk; white plumage patterns.

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LISTA DE ABREVIATURAS E SIGLAS

CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
MD	Maryland
PA	Pennsylvania
UV	Ultravioleta

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1 INTRODUCTION

The ability to accurately determine the sex of individual animals is crucial for various research applications, including evolutionary ecology (e.g., Clutton-Brock 1986), conservation genetics (e.g., Griffith and Tiwari 1995), and demographic and behavioral studies (e.g., Anciães and Del Lama 2002; Holt et al. 2016). Understanding sex ratios in wildlife is essential for setting conservation priorities and managing threatening processes, as these ratios play a significant role in population persistence (Gowans et al. 2000; Rowe and Dawson 2009; Székely 2014; Cooke et al. 2020). However, studying sex ratios in Strigiformes can be particularly challenging due to their cryptic and often invariant morphology (Clutton-Brock 1986; Wink et al. 2009).

Raptors in general are not known for their plumage sexual dimorphism; for most owls, body mass and wing chord are used to differentiate sexes since females are usually bigger than males (Owens and Hartley 1998; Sunde et al. 2003). Northern Saw-whet Owls are not any different; individuals must be closely analyzed in hand for sexing through size dimorphism (except when seen exhibiting gender-specific behaviors) (Dunn et al. 2001; Clay et al. 2020). Other methods like genetic analysis (Hogan et al. 2008) and morphometric modelling of captured animals (Hurley et al. 2007), can be used to determine the sex of non-sexually dimorphic, cryptically dimorphic or dichromatic species. However, these methods often face limitations due to financial, ethical, or labor constraints (Hogan et al., 2008).

The Northern Saw-whet Owl *Aegolius acadicus* (Gmelin, JF 1788) is a very small, round-headed owl without ear-tuffs (Appendix 1). The brownish facial disk has a blackish spot between the base of the bill and eyes, and a whitish area around the eyes in the form of radial white streaks extending towards the disk edge (Sibley 2003; Weick 2006a). Although the sexes appear visually similar, females are, on average, more than 50g heavier than males during the breeding season, though this difference reduces to just 16g in autumn (Johnsgard 1988; Mikkola 2013b). Despite this reversed sexual dimorphism (i.e., females being larger than males), gender determination in the field is quite challenging due to the apparent sexual monomorphism (Sunde et al. 2003; Delgado and Penteriani 2004).

Recent studies indicate that owls, like many diurnal raptors, can perceive ultraviolet (UV) light, enhancing their night vision (Mikkola 2013a). Despite lacking UV/V cones, owls detect UV light through heightened rod sensitivity, making UV-reflective feathers appear brighter (Höglund et al. 2019). Although most birds of prey lack conspicuous markings, they still require visual cues to identify breeding pairs or territorial males from a distance. Surprisingly, even nocturnal species may use visual signals more than previously assumed

(Olmos and Rodrigues 1990; Penteriani et al. 2007, 2010; Penteriani and Delgado 2017; Camacho et al. 2019). Research also suggests that species appearing monomorphic in visible light may show sexual dimorphism under UV light (Burkhardt 1988; Andersson et al. 1998, 1999; Hunt et al. 2001; Eaton and Lanyon 2003).

The necessity for nocturnal species to convey information visually, in low-light conditions, may have driven the convergent evolution of white visual signaling during twilight among unrelated species (Penteriani et al. 2007). This adaptation optimizes the limited light available at dawn and dusk (Penteriani et al. 2006). Many Strigiformes exhibit white plumage patches, which sometimes play a role in sexual communication. Eagle Owls (*Bubo bubo*), for instance, use a white badge on their neck as a courtship display to attract females (Penteriani et al. 2007; Camacho et al. 2019). Other white markings present in owls' facial disks, may or may not be related to sexual communication, but possibly indicate sexual dichromatism (Penteriani et al. 2006).

Recent studies indicate that UV wavelengths influence avian mate choice, as many bird species can perceive UV reflectance in the 320-400 nm range (Cuthill et al. 2000; Hunt et al. 2001). While the Tawny Owl (*Strix aluco*) cannot see in this range (Bowmaker & Martin 1978), no data exists on the vision of the Northern Saw-whet Owl, though owls may detect UV light using photopigments in the visible range (Koivula et al. 1997; Jacobs 1992; Penteriani and Delgado 2017; Di Marzio et al. 2023). Weidensaul et al. (2011), for instance, found that UV light can be used as an aid in age classification of owls. While most birds' tetrachromatic vision enables them to perceive feathers that appear white to humans as colored due to UV reflectance, owls have a much narrower spectrum, but higher sensitivity of cone vision (Jane and Bowmaker 1988; Wu et al. 2016; Höglund et al. 2019).

Sexual dichromatism often evolves because of sexual selection pressures (Owens and Hartley 1998). Increasing evidence suggests that the development of secondary sexual traits, such as plumage, results from mutual sexual selection within and between sexes, encompassing mate choice and competition (Berglund et al. 1996; Jones and Hunter 1999; Amundsen 2000; Daunt et al. 2003). For instance, Holt et al. (2016) observed sexual color dimorphism between males and females of Long-eared Owls (*Asio otus*) while scoring underwing coverts, tarsometatarsus, and facial disks. Their results also suggest that by autumn of their first year, and perhaps throughout their lives, Long-eared Owls can be reliably sexed by plumage color.

Different methods, such as those developed by Cooke et al. (2020), enable to sex Powerful Owls (*Ninox strenua*) accurately through measurements of facial disk features using highly varied images taken by amateur photographers. By using a sex-specific plumage

character, the authors were able to find that females and males differ in how their facemask feathers extend beyond the head's contour. This technique is particularly valuable for cryptic or hard-to-capture species and has the potential to be a cost-efficient approach, providing essential information on species dynamics such as sex ratios, social organization, and behavior (Cooke et al. 2020).

The Northern Saw-whet Owl is one of the smallest and most widespread owls in North America, with a range extending from southern Canada to the northern United States. Many individuals migrate south during the fall, peaking in October (Mueller and Berger 1967; Holroyd and Woods 1975; Weir et al. 1980; Duffy and Kerlinger 1992; Contreras 2000; Whalen and Watts 2002; Hamilton 2002; Beckett and Proudfoot 2011). Despite its wide range and abundance, much remains to be learned about its distribution and movements, behavior, and populations (Johnsgard 1988; Rasmussen et al. 2020). For example, the sex of individuals has been evaluated through size dimorphism in the field, but it can be challenging because it requires a lot of experience from banders.

This nocturnal and cryptic species is mostly silent outside of courtship and early breeding seasons, making it difficult to assess their true abundance through vocalization records (Swengel and Swengel 1995; Duncan et al. 2009). However, banding studies, particularly in eastern North America during fall migration, show that Northern Saw-whet Owls, especially hatch-year individuals, are more common than sight and sound observations suggest (Cannings 1993). While banding efforts have provided valuable insights, many aspects of the species' ecology remain unknown (Marks et al. 2015).

These owls exhibit prominent white V shapes on their faces, broad brown streaks on their underparts, and white spots on their scapulars, traits shared by both sexes (Smithe 1975; Weick 2006b; Sibley 2003; Appendix 1). Recent studies suggest that facial disk features may serve as sex-specific plumage characters, offering new possibilities for sexing owl individuals (Holt et al. 2016; Cooke et al. 2020). Consequently, the distinctive white features on the Northern Saw-whet Owl's facial disk may be an excellent candidate for identifying cryptic sexual dimorphism. This study aims to test whether males and females can be differentiated based on plumage phenotypes and anatomical features (such as head shape, eyebrow shape, and eyebrow thickness) and investigate the occurrence of cryptic sexual dimorphism. Specifically, we hypothesize that males and females differ in head shape, with males displaying a rounder head and females a flatter head. Additionally, we hypothesize that UV reflectance on the white facial disk plumage differs between the sexes, with males and females exhibiting distinct reflectance patterns. Lastly, we aim to determine whether these differences can be detected and

quantified using photographic analysis, providing an objective method for identifying cryptic sexual dimorphism in this species.

2 MATERIALS AND METHODS

2.1 Study area

The study area is in Eastern North America, at various Northern Saw-whet Owl (*Aegolius acadicus*) banding stations operated by Project OwlNet. Project OwlNet is a collaborative network of more than 100 owl migration banding sites, which annually band 8,000 to 15,000 Northern Saw-whet Owls (<http://www.projectowl.net.org/>). The data used for the present study was collected at banding stations in the states of Pennsylvania and Maryland.

Data from four banding stations on the east side were collected and ceded for this project. Scott Weidensaul, head of three banding stations located in Pennsylvania, made data available collected between 2018–2022 containing photos taken of the facial disks of all Northern Saw-whet Owls banded during migration (Table 1; Appendix 2). Between 4–10 November 2022, C.A.H. collected data in the Northern Saw-whet Owl banding station located at the Smithsonian Environmental Research Center in Edge Water, Maryland (Fig. 1).

Table 1. Locations and coordinates of Northern Saw-whet Owl banding stations in Pennsylvania, where data collection was conducted and ceded.

Banding Station	Coordinates
King's Gap State Park, Cumberland Co., PA	40°05'32"N 77°16'06"W
Small Valley Girl Scout Camp, Dauphin Co., PA	40°29'38"N 76°47'01"W
Hidden Valley Golf Course, Schuylkill Co., PA	40°37'14"N 76°16'07"W
Smithsonian Environmental Research Center, Edge Water, MD	38°54'04"N 76°33'16"W

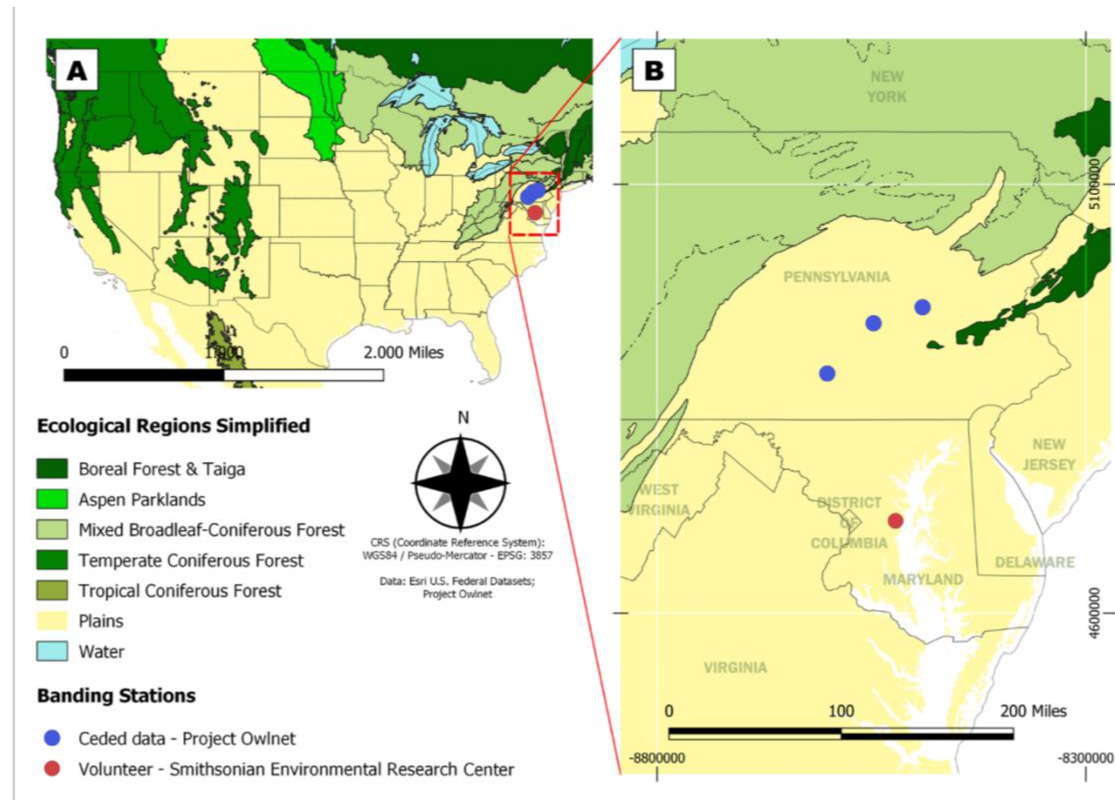


Figure 1. Locations of Northern Saw-whet Owl banding stations in Pennsylvania (blue) and Maryland (red) where data were collected for this study. These sites are part of the Project OwlNet network, which supports research on Northern Saw-whet Owl migration and population dynamics.

2.2 Data collection

2.2.1 Defining facial features on Northern Saw-whet Owl's facial disk

Three categories were established to assess cryptic sexual dimorphism: eyebrow shape, head shape, and eyebrow thickness. For this analysis, clear and measurable criteria were defined to categorize these features based on standardized observations. Eyebrow shape was categorized into two distinct forms: a C shape, characterized by a rounded curve, and a V shape, defined by a pointed angle. Head shape was classified based on visual assessment as either round or flat, using the curvature of the head profile as a distinguishing criterion. Eyebrow thickness was measured at its widest point; eyebrows thicker than 1 cm were classified as thick, while those thinner than 1 cm were classified as thin. These categorizations were applied systematically to photographic and field data, ensuring consistent application of criteria across all samples. It is important to note that these categories are based on preliminary observations.

2.2.2 Image selection and dimorphism description

A comprehensive review of images provided by S. Weindensaul ($n = 1,612$ sexed individuals), collected from three banding stations during the fall migration seasons of 2018–2022, resulted in 70 Northern Saw-whet Owls (35 males and 35 females) with known sex and adequate images for analysis. The sex of these birds was determined using size dimorphism criteria, a procedure conducted by the head bander of each station, as outlined in the table in Appendix 2.

We focused our analysis on hatch-year individuals, as they provided a balanced sample size with reliable sex confirmation based on size-based dimorphism. Throughout five years across three banding stations, only five males were aged and classified as after hatch year, second year, or after second year. Images excluded from this analysis were either unsuitable for selection and analysis or unavailable in the image bank. Despite the substantial number of Northern Saw-whet Owls captured and photographed by Project OwlNet, only approximately 2.5% ($n = 40$) were identified as males through size dimorphism, which limited the overall sex ratio available for this study.

From a total of 1,612 Northern Saw-whet Owls captured, banded, and photographed, only 35 were hatch-year males with known sex and suitable images for scoring analysis. The limited number of after-hatch year and second-year males ($n = 5$) precluded their inclusion, as they were insufficient for a robust statistical assessment. Consequently, our analysis was restricted to hatch-year birds to ensure data consistency and adequate representation of both sexes.

2.2.3 Catching, banding and ultraviolet light

Netting procedures along with banding, processing owls and ageing have been described in detail to obtain data that can be accurately compared from year to year and can be accessed on the Northern Saw-whet Owl Project website (<http://www.projectowl.net.org/>). Trapping is expected to occur on all suitable nights between 1 October to 25 November, some extending until early December. Four 12 m, 60 mm mist nets ("thrush nets") strung between aluminum conduit poles are placed in the same location each year. The banding stations are operated from half an hour after sunset until at least 23:00 EST each night, weather permitting, even if no owls are being caught. An audio lure with a male Northern Saw-whet Owl toot call is used continuously, broadcasting at the center of the net line to attract individuals. Mist nets

should be checked with no more than an hour between each inspection. All owls that were captured and banded, had photographs taken with their band number (Fig. 2).



Figure 2 – Fieldwork at the Smithsonian Environmental Research Center, Edgewater, MD. Top row: 1) Extracting a Northern Saw-whet Owl from mist nets; 2) Melissa banding the owl. Bottom row: 3) Photographing methods used in data collection; 4) Measuring and documenting the owl's morphometrics.

2.2.4 Northern Saw-whet Owl Project

Individuals were sexed by measuring the wing chord and weight, and they determined the gender according to the mass/wing chord chart (Appendix 2). With a 13-watt compact fluorescent blacklight (Feit Electric BPESL15T/BLB), the ventral flight feathers fluoresce an intense magenta color with the ultraviolet (UV) light positioned 15 cm away in a dark room. Using a UV blacklight, owls can be aged according to their molting pattern classified as hatch year, after hatch year, second year/third year and after second year/after third year (Weidensaul et al. 2011). There appears to be little information suggesting that brief exposure to UVA light experienced during normal banding operations would be harmful to owls, but caution is warranted. They make an effort to keep exposure as brief as possible.

Furthermore, for the present study, the same UV light from Project OwlNet was used on the owls captured ($n = 2$ males, 2 females) at the Smithsonian Environmental Research Center banding station for a better understanding of cryptic plumage traits and to describe feather patterns that could indicate cryptic sexual dimorphism. The photos were taken of their facial disk with a Canon EOS Digital Rebel T3i and Canon Zoom Lens EF-S 18-55mm 1:4-5.6 (adapted for astrophotography with UV filter removed) camera at a 90° angle 60 cm away from the owl, under a UV flashlight (also 60 cm away from the owl), in a completely dark room, while keeping exposure as brief as possible. Northern Saw-whet Owl's facial disks photographed were analyzed after to detect any possible UV signals.

2.2.5 Museum specimen samples

We analyzed the specimens at the Ornithology Collection within the Museum of Comparative Zoology, Harvard University, Cambridge, MA, on 14 October and 18 November 2022. At Harvard's Ornithology Collection, photographs of the facial disk of *A. acadicus* specimens were taken under controlled conditions. A total of 20 specimens were selected for UV photography, using the same camera in a light-controlled environment illuminated solely by a black light positioned 60 cm away from the subject (uvBeast 365nm Black Light UV Flashlight) (Fig. 3). This subset of 20 specimens was chosen based on feather integrity and correct anatomical positioning, and any specimens in poor conservation state were excluded from UV photography and analysis. Additionally, conventional photographs were taken for all *A. acadicus* specimens in the collection ($n = 53$) using an iPhone 13 Max Pro. The sex of all specimens used in the study was indicated on their tags.

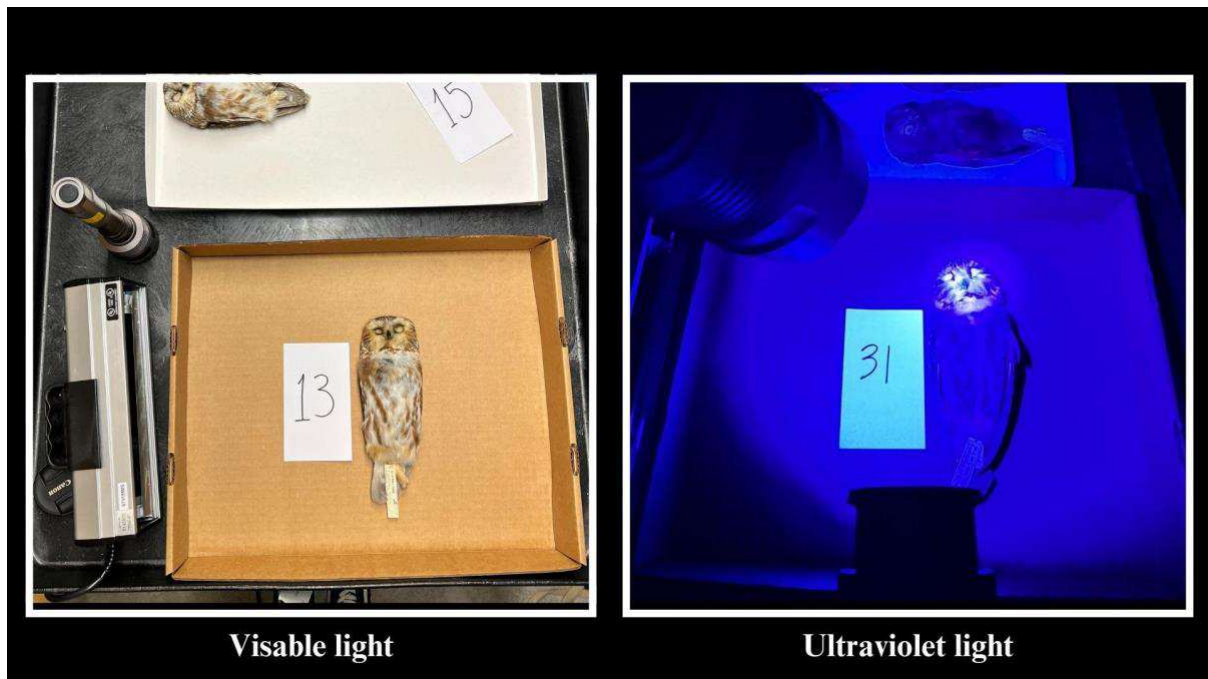


Figure 3. *Aegolius acadicus* specimen imaging and handling at the Museum of Comparative Zoology, Harvard University. Specimens were photographed under visible and UV light conditions to assess potential cryptic sexual dimorphism.

2.3 Data analysis

2.3.1 Image selection and dimorphism description

A total of 354 photographs of museum specimens from the Harvard Ornithology Collection were taken, from which 20 specimens were selected for UV photography based on their labeled sex, head orientation, and preservation quality. Of the 53 museum specimens available, 20 were selected for UV photography based on their labeled sex, head orientation, and preservation quality. From these, only 4 images (2 males and 2 females) met all criteria for the final analysis (Fig. 6). Differences in photograph quality arose due to pandemic constraints, the absence of a tripod, and the need to operate equipment independently with limited resources.

2.3.2 UV light and analysis of photographs

UV were images captured in a light-controlled environment using a Canon EOS Digital Rebel T3i (Astro). These images were used to examine whether white plumage markings on the facial disk appeared more distinct under UV light, potentially highlighting morphological traits associated with cryptic sexual dimorphism. The analysis focused on determining whether these markings highlighted morphological traits associated with cryptic sexual dimorphism.

2.3.3 Morphological traits and quantification

Data on 70 hatch-year Northern Saw-whet Owls were analyzed (Appendix 3). Morphological traits were classified and quantified based on field observations and prior studies. Three traits were analyzed: eyebrow shape (C-shaped for females, V-shaped for males), head shape (flat for females, round for males), and eyebrow width (thick for females, thin for males). Each trait was recorded as a binary variable (1 = trait present, 0 = trait absent; Appendix 4). The goal of this analysis was to determine whether these traits reliably differentiated males and females.

2.3.4 Statistical analysis

To investigate the relationship between sex and morphological traits in *A. acadicus*, logistic regression analysis was employed. Logistic regression is appropriate for this analysis because it models the probability of a binary outcome (presence or absence of a trait) as a function of one or more predictor variables (in this case, sex) (Sperandei 2014). The sex was categorized as a factor with two levels (male and female) and each trait was considered a binary variable. Separate logistic regression models were fitted for each trait to evaluate the effect of sex on the likelihood of the trait being present. The general form of the logistic regression model used is:

$$\text{logit}(P) = \beta_0 + \beta_1 \times \text{Sex}$$

In the logistic regression models, P represents the probability of the trait being present. The intercept (β_0) corresponds to the log-odds of the trait being present when the sex is female, while the coefficient (β_1) for the sex variable represents the difference in log-odds of the trait being present for males relative to females. The models were implemented in RStudio using the `glm()` function from the `stats` package, with a binomial family and a logit link function. The `stats` package is included by default in R and is widely used for fitting generalized linear models, including logistic regression. Data preprocessing was performed using RStudio, and additional packages such as `dplyr` and `ggplot2` were utilized for data manipulation and visualization, respectively.

3 RESULTS

3.1 Statistical analysis

Overall, the results highlight the presence of sexual dimorphism in hatch-year Northern Saw-whet Owls, with head shape and eyebrow shape serving as significant predictors of sex. (Fig. 4). Differences were observed in head shape and eyebrow shape between the sexes, but not in eyebrow thickness. Head shape is a meaningful predictor of sex in this species ($p = 0.046$) with a predicted probability of approximately 80% for males round head shape and 75% for females flat head shape (Fig. 5). Eyebrow shape also showed sexual dimorphism, with males more frequently associated with a V-shaped eyebrows (predicted probability of approximately 80%) and females with a C-shaped eyebrow (predicted probability of approximately 75%) (Fig. 5). This distinction was supported by the logistic regression model ($p = 0.001$). In contrast, eyebrow thickness did not differ significantly between sexes, with males slightly more likely to have thin eyebrows (predicted probability of around 55%) and females more likely to have thick eyebrows (predicted probability of around 55%) (Fig. 5). However, this difference was not statistically significant ($p = 0.883$).

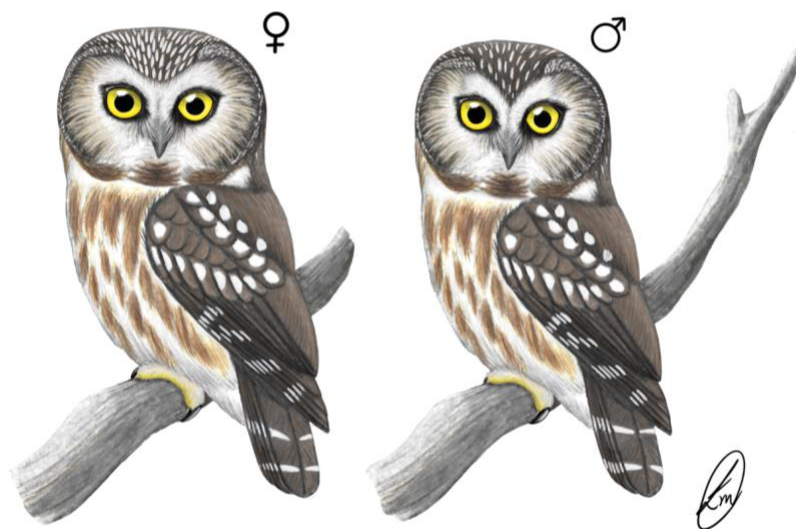


Figure 4. Illustration comparing female (left) and male (right) Northern Saw-whet Owls (*A. acadicus*). Illustration by Leonardo Marujo.

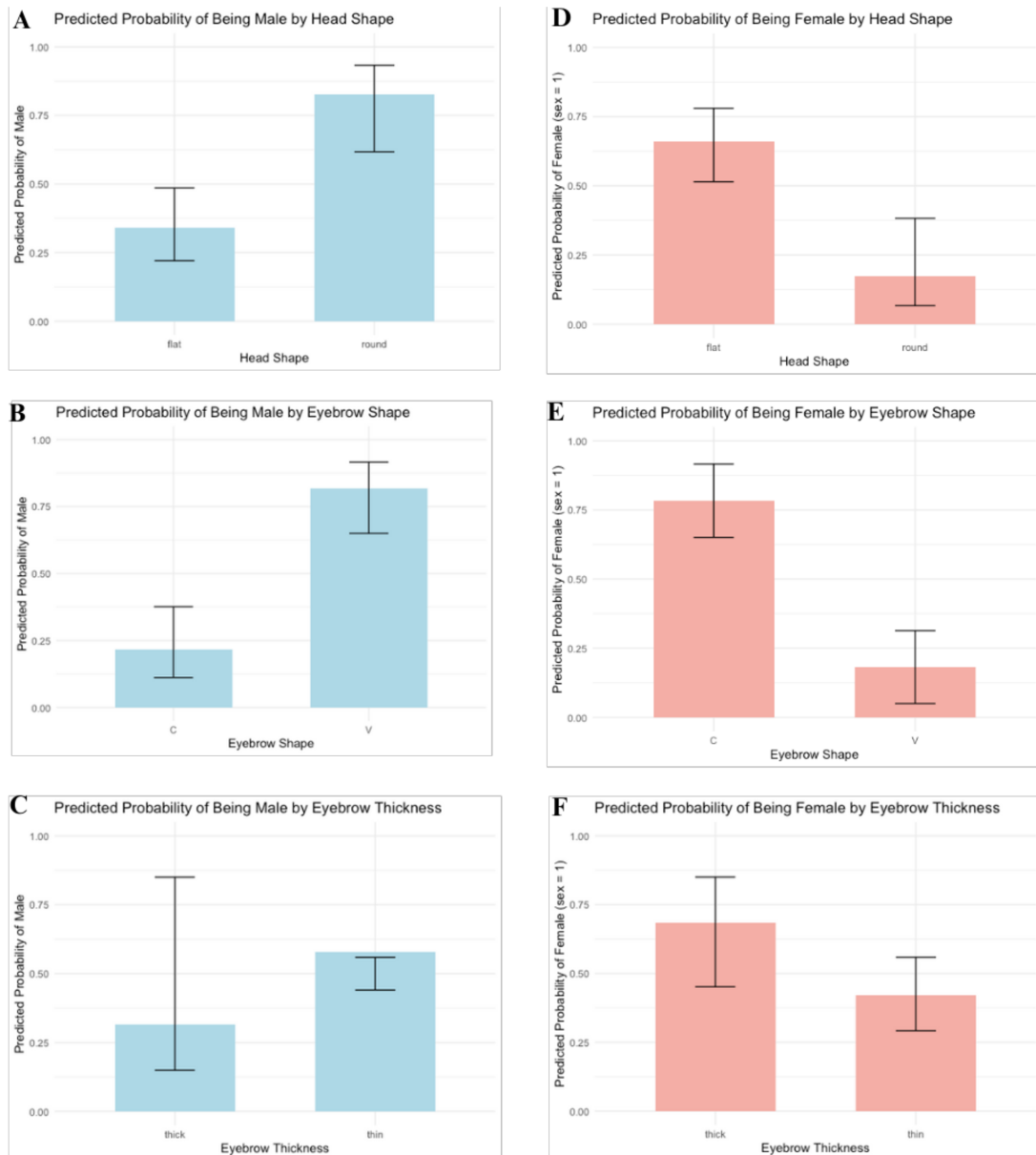


Figure 5. Predicted probabilities of being male or female based on head shape (A and D), eyebrow shape (B and E), and eyebrow thickness (C and F) in Northern Saw-whet Owls, estimated using logistic regression models. Error bars represent 95% confidence intervals.

3.2 Ultraviolet light and museum specimen samples

3.2.1 Museum specimen

The white plumage patches in the owl's facial disks displayed a violet-magenta coloration when exposed to ultraviolet (UV) light in a controlled environment. This effect was observed under a black light and captured using a camera without a UV filter. The specific morphological traits selected for analysis, which appeared consistent and non-reactive under

visible light, were visually enhanced under UV light, reflecting a distinct violet-magenta hue (Fig. 6). While qualitative differences in the intensity of coloration between male and female specimens were observed, no quantitative measurements were taken to confirm these differences. It is important to note that the appearance of fluorescence or reflectance may be influenced by the nature of light incidence and the equipment used for image capture. Further standardized testing and color calibration would be required to assess the significance of these observations.

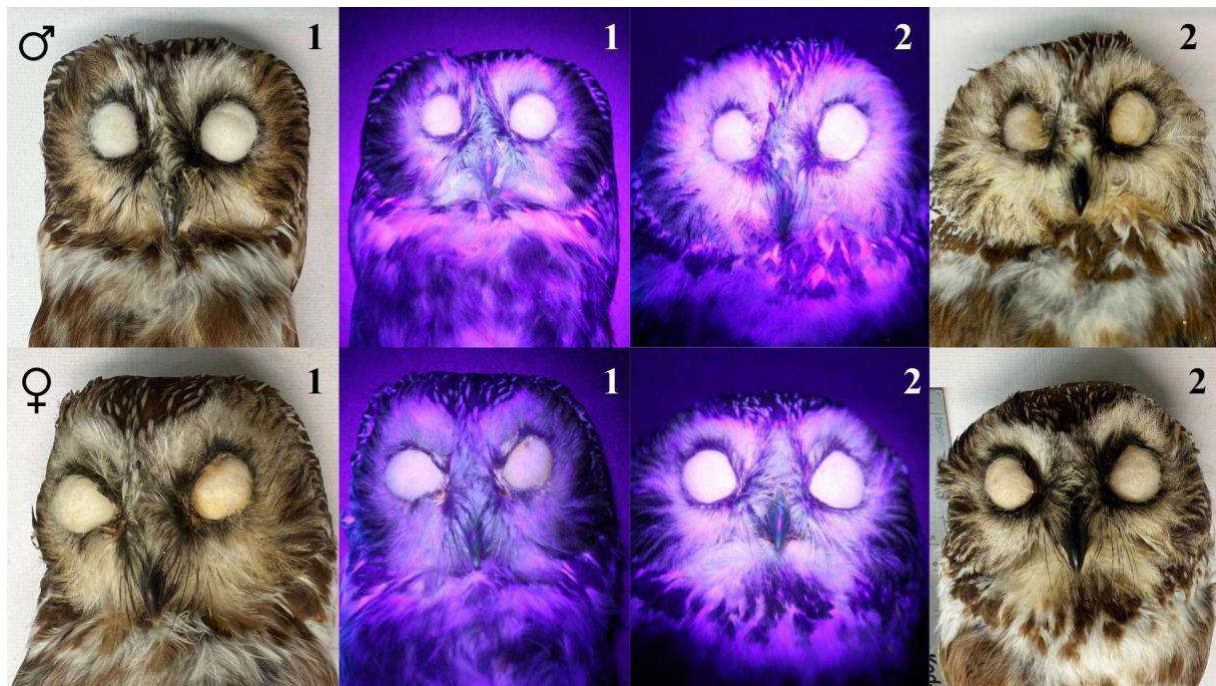


Figure 6. Facial disks of *Aegolius acadicus* specimens under visible and UV light conditions. The first row depicts male specimens, and the second row depicts female specimens. UV images were captured with an astro-modified camera at Harvard University's Museum of Comparative Zoology, and conventional images were taken using an iPhone 13 Pro Max. Specimens are labeled as follows: Male 1 (top left, specimen N° 323743 collected in 1916, Vancouver), Male 2 (top right, specimen N° 10147 collected in 1885, Maine), Female 1 (bottom left, specimen N° 322049 collected in 1914, Washington), Female 2 (bottom right, specimen N° 29549 collected in 1891, Massachusetts).

3.2.2 Live specimen

Live female Northern Saw-whet Owls exhibited a more intense violet-magenta coloration in their facial disks compared to museum specimens, likely due to pigment degradation in preserved specimens over time (Fig. 7). While the coloration observed in museum specimens under UV light appeared subtle, live females displayed a vibrancy that was distinctly more pronounced. The sample size of live specimens was limited, and while subtle differences in UV reflectivity between males and females were noted, these observations cannot

be confirmed with the current data. Further studies with larger sample sizes are needed to validate these potential patterns.

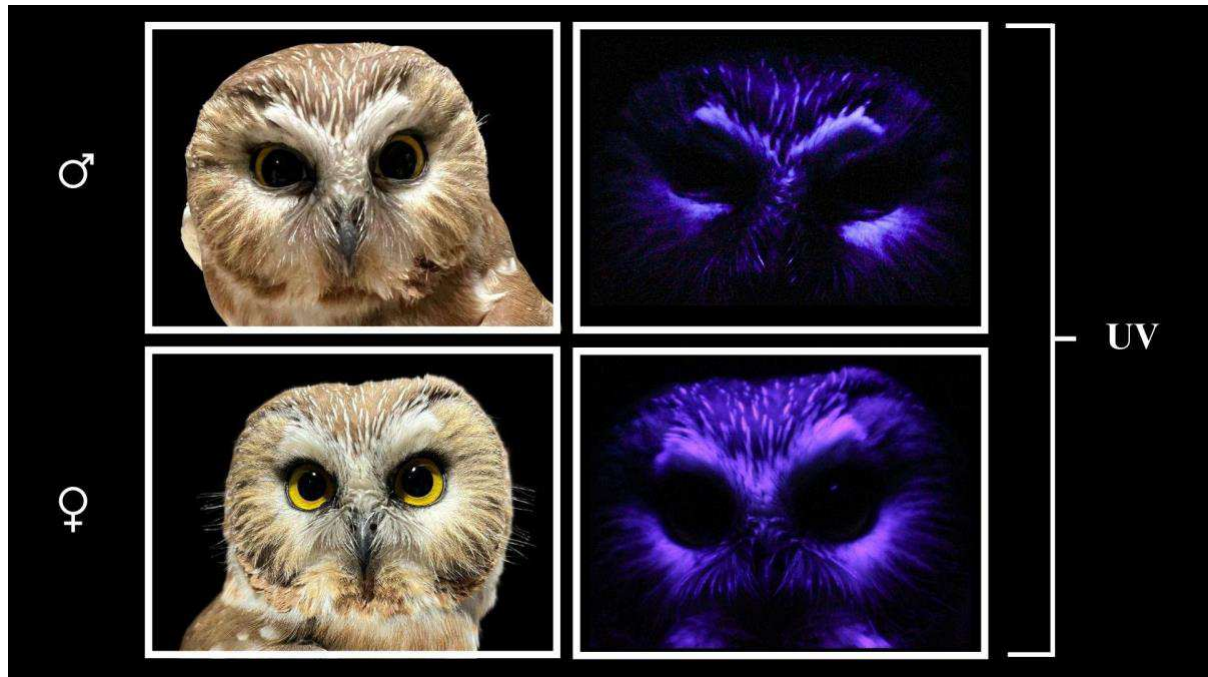


Figure 7. Visible and UV light images of Northern Saw-whet Owls captured at the Smithsonian Environmental Research Center. Top row: Male owl, bottom row: Female owl.

4 DISCUSSION

Our study presents evidence of cryptic sexual dimorphism in hatch-year Northern Saw-whet Owls, primarily observed through differences in head shape and eyebrow shape. While males were more likely to exhibit a round head and V-shaped eyebrows, females typically showed a flat head and C-shaped eyebrows. Eyebrow thickness was not significantly different between the sexes, as no meaningful differences were found. Differences in plumage coloration found in the UV light photographs were observed, but they were subtle and not statistically significant. Employing UV photography allowed us to visualize previously undetected details in this species, such as the violet-magenta appearance of white plumage patches on the facial disks. However, it is important to note that this coloration may not represent true fluorescence, as no controlled spectral analysis was conducted to confirm this property. While subtle variations were noted between the sexes, they could not be quantified with the current methods or sample size.

The resulting images of the museum specimen photographed under a UV light highlighted the spectral response of the sensor in the UV, reflecting a violet-magenta color

detected in the Northern Saw-whet Owls' facial disks (Weidensaul et al. 2011; Crowther 2022). The limited sample size restricted our ability to generalize these findings. In addition, variations in image selection and quality, influenced by head orientation and other factors, posed challenges to consistently measuring traits. UV images of museum specimens highlighted the sensor's response to UV wavelengths, but it is not possible to definitively confirm whether this represents a true UV signal or artifacts of camera sensitivity. In contrast to Weidensaul et al. (2011), who found visible fluorescence in ventral wing surfaces, the violet-magenta coloration in the facial disks observed here was only visible in photographs taken with a modified camera. Since this coloration is not visible to the naked eye, it may not represent fluorescence as commonly defined. Further controlled studies are needed to explore this phenomenon. Moreover, expanding the use of UV light photography could uncover further dimorphic features, as a growing number of bird taxa have been shown to see in UV wavelengths, with UV reflectivity playing a role in mate selection and hunting behaviors (Bennett and Cuthill 1994; Viitala et al. 1995; Bowmaker et al. 1997; Wilkie et al. 1998; Cuthill et al. 2000; Hunt et al. 2001; Arnold et al. 2002; Hausmann et al. 2003; Weidensaul et al. 2011; Di Marzio et al. 2023). Understanding these nuances contributes to a broader comprehension of sexual dimorphism in this species.

The UV-reflecting coloration was not conclusively more pronounced in either sex, as no statistically significant differences were demonstrated. While some observations suggested possible differences in live specimens, this could not be reliably tested, and sample sizes were too small to confirm trends. Although the sample size of live specimens was limited, this finding raises intriguing questions about the potential role of UV reflectivity in female fitness and health conditions. Just as larger female size has been linked to reproductive success, the intensity of UV fluorescence in female plumage could also correlate with health or genetic quality, potentially influencing mate choice (Galván et al. 2018; San-Jose et al. 2019). However, the observation raises interesting questions about potential links between UV reflectivity and fitness indicators in females. The role of dietary carotenoids and nutrition in pigment production could be explored in future studies, as these factors are often linked to plumage coloration and may provide signals of health and reproductive quality (Weaver et al. 2018).

Owls, despite lacking UV/V cones, can detect UV light. This increases the sensitivity of their cone vision, allowing them to see UV-reflecting feathers as brighter signals at night (Höglund et al. 2019). This sensitivity to UV light might explain the widespread ultraviolet reflectivity documented in avian plumage, as observed by Mullen and Pohland (2008). Di

Marzio et al. (2023) extended this understanding by demonstrating that fluorescence is common across all European owl species, including the Snowy Owl (*Bubo scandiacus*), which has also been proven to integrate coloration, behavior and environment to maximize the efficacy of their visual displays (Bortolotti et al. 2011). They suggest that fluorescence may be linked to eumelanin and could persist longer in owls living in northern regions, indicating that fluorescence might also be present in other owl species, such as neotropical owls. Further research could clarify whether the fluorescence signal complements, replaces, or interacts with other visual signals, which may reveal critical insights into owl behavior and contribute to conservation efforts by elucidating their ethological adaptations (Galván et al. 2018; Di Marzio et al. 2023). This emerging understanding of visual communication in low-light environments suggests that the white plumage markings of nocturnal owls may serve adaptive purposes beyond camouflage, opening new avenues for research on how nocturnal light conditions shape the evolution of visual traits (Warrant 2004).

Ultra-violet reflecting plumage found in Northern Saw-whet Owl facial disks resembled the violet-magenta coloration described in Weidensaul et al. (2001) study by photographing ventral surfaces of *A. acadicus* wings under ultraviolet light. The discovery of UV-reflecting plumage in Northern Saw-whet Owls suggests that UV vision could play an important role in their nocturnal communication. Visual signaling under moonlight appears more significant for nocturnal birds than previously assumed (Penteriani and Delgado et al. 2017). Although moonlight reflects less UV than sunlight (Henry et al., 1995), it still provides enough illumination to enhance the visibility of white plumage patches, which many species use for social and reproductive signaling (Penteriani and Delgado, 2017). This suggests that Northern Saw-whet Owls may leverage these traits under moonlight for mating or territorial displays, using the contrast against darker surroundings to improve communication (Johnstone and Norris, 1993; Iida, 1995). As these owls are mostly silent outside of courtship, further research on UV reflectivity and visual cues under moonlight could offer valuable insights into their behavior and communication strategies (Hunt et al., 2001; Penteriani et al., 2010). Thus, owl activity, particularly during times of increased moonlight, may be partially driven by enhanced visual cues, facilitating communication or influencing mate selection during the breeding season (Roulin et al. 2001; Penteriani et al. 2006; Penteriani et al. 2007; Penteriani et al. 2010; Penteriani and Delgado 2017; San-Jose et al. 2019).

Our study also highlights the potential for developing a non-invasive sexing technique for Northern Saw-whet Owls, similar to the field methods proposed by Holt et al. (2016) and

Cooke et al. (2020). Here, we identified head and eyebrow shape as significant predictors of sex. As Holt et al. (2016) noted, age-related variation in plumage does not significantly affect the accuracy of sexing techniques, which suggests that this may also be a factor in Northern Saw-whet Owls. By focusing on hatch-year individuals, we maintained consistency, yet future studies should assess whether these dimorphic traits persist beyond the first year. Understanding accurate sex ratios and the role of visual signaling in Northern Saw-whet Owls can provide crucial insights into their population health and reproductive success. Improved non-invasive sex determination methods would allow for more effective monitoring of population dynamics, aiding in the identification of at-risk groups and informing conservation strategies.

Photographic analysis offers a valuable complementary tool for sexing cryptic species like the Northern Saw-whet Owl. Although this approach enhances traditional methods, our sample revealed a skewed sex ratio, with significantly fewer males captured, and many individuals could not be sexed using the Project OwlNet protocol's size-based criteria (i.e., > 95% probability of correct sex for any individual). This difficulty underscores the challenges of accurately determining sex in cryptic species, even with close examination. The observed sex ratio may reflect sex-biased survival or migration behaviors, as seen in other owl species. In some species, younger males are known to migrate alongside females, while older males, recognizing the importance of maintaining their territories, migrate in distinct directions to arrive earlier for breeding (Pérez et al. 2014; Lehikoinen et al. 2017). This behavior, although energetically costly, provides a reproductive advantage (Kokko et al. 2006). These potential patterns highlight areas for further research. Integrating photographic analysis into existing sex determination protocols could enhance the accuracy of studies on these elusive species. This is particularly important as it could help prioritize conservation efforts in areas where Northern Saw-whet Owl populations may be declining.

Photographic analysis, while valuable, also presents challenges due to the subtlety of the cryptic traits in Northern Saw-whet Owls. The facial disk characteristics used in our logistic regression analysis—such as head and eyebrow shape—require careful examination and a trained eye to identify consistently. The potential for bias in scoring these traits highlights the importance of standardizing training for observers to ensure reliability. This complexity underscores that while non-invasive methods offer promise, their accuracy can depend on the observer's familiarity with these cryptic characteristics. Advances in technology, such as high-resolution imaging and AI-driven pattern recognition, could further enhance the accuracy of sex determination by helping identify subtle morphological traits automatically. Implementing

these technologies could reduce observer bias and improve the scalability of photographic methods for sexing cryptic species. Future studies could explore the integration of such tools to facilitate more consistent and widespread data collection.

Our findings contribute to the understanding of cryptic sexual dimorphism in owls and provide insights into the evolutionary pressures that may affect these traits and highlight the need for further research to validate these results across larger sample sizes and age groups. The exclusion of older males and the focus on hatch-year individuals were necessary for consistency but may have limited broader applicability. The differences in UV-reflecting plumage and morphological characteristics observed in Northern Saw-whet Owls highlight the importance of continued research across diverse geographic regions and age groups. Refining these methodologies could also support citizen science initiatives, enabling amateur birdwatchers and volunteers to contribute valuable data on Northern Saw-whet Owls. Expanding the reach of this research through public involvement could lead to a more comprehensive and participatory approach to owl conservation. By expanding our understanding of these unique visual traits, we can better understand how environmental factors influence the evolution and behavior of nocturnal species.

5 CONCLUSION

In conclusion, our study reveals cryptic sexual dimorphism in Northern Saw-whet Owls and highlights the potential significance of UV light in nocturnal visual communication. The observed differences in head shape, eyebrow shape, and UV-reflecting plumage between sexes suggest that visual cues may play a role in social signaling and mate selection in this species. The disproportionate number of captured females and the difficulty in identifying sex using traditional methods underscore the complexities of studying this species.

Expanding this research to other owl species will be crucial to determine if similar cryptic dimorphism exists across the Strigidae family. Additionally, increasing the sample size of live individuals and examining owl photoreceptors, particularly oil droplets, are essential next steps. Analyzing these photoreceptors will help confirm whether Northern Saw-whet Owls can perceive UV signals, thus providing more insight into their use of visual cues.

By integrating UV photography and non-invasive sexing techniques, we can improve our understanding of the behavioral and ecological adaptations of Northern Saw-whet Owls. This approach not only enhances data collection but also supports conservation efforts by offering a minimally disruptive method for monitoring owl populations. Our findings lay the groundwork for future research on the interplay between morphology, behavior, and

environmental light, emphasizing the need for further study across geographic regions and age groups to fully grasp the adaptive significance of these traits.

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8 APPENDIXES

8.1 Northern Saw-whet Owl illustrations

Illustrations of the Northern Saw-whet Owl's facial disk and plumage patterns extracted from the field guides: Field Guide to the Birds of North America National Geographic Society (Fig. 1) and The Sibley Field Guide to Birds of Western North America (Fig. 2). The third illustration is from Cornell Lab of Ornithology's website: Birds of the World (<https://birdsoftheworld.org/bow/species/nswowl/cur/introduction>) by Hilary Burn (Fig. 3). Fourth illustration is from the annotated and illustrated checklist (Weick 2006b), part three, owls in flight, the upward wing stroke showing wing form and underwing pattern (Fig. 4).



Figure 1 – The upperparts are reddish-brown, while the underparts are white with reddish streaking. The bill is dark, and the facial disks are reddish without a distinct dark border. Juveniles exhibit a stronger reddish coloration above and a tawny-rust hue below (National Geographic Society 1987).

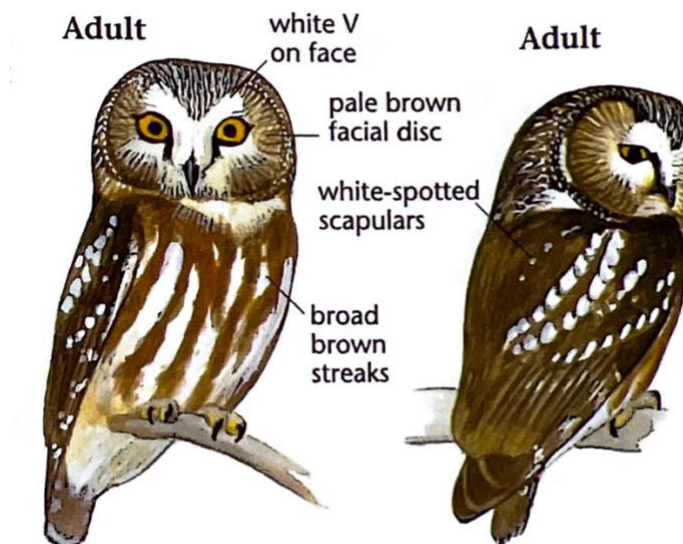


Figure 2 – The smallest owl in the northern region, characterized by a fluffy appearance and a large head. Distinguishing features include a prominent white V-shaped marking on the face, broad brown streaks on the underparts, and white spots on the scapulars (Sibley 2003).



Figure 3 – The head is large and round with a circular facial disk and no ear tufts. Irides range from yellow to golden. Adults have brown upperparts with white streaks and spots, a white facial disk with pale brown sides, and a tuft of black feathers between the eyes. Underparts are white with broad brown stripes, and wings are rounded with a short tail (Rasmussen et al. 2020).



Figure 4. *Aegolius acadicus* upward wing stroke from Weick 2006b.

8.2 Sex determination for Northern Saw-whet Owls using weight and wing chord

Boundaries for assignment of sex in Northern Saw-whet Owls* > 95% probability of correct sex for any individual (table extracted from Project OwlNet Protocol).

Wing Chord	Mass (grams)			
	Male	Unknown		Female
120	≤88	≥89	≤92	≥93
121	≤87	≥88	≤92	≥93
122	≤87	≥88	≤92	≥93
123	≤86	≥87	≤91	≥92
124	≤85	≥86	≤91	≥92
125	≤85	≥86	≤90	≥91
126	≤84	≥85	≤90	≥91
127	≤84	≥85	≤90	≥91
128	≤83	≥84	≤89	≥90
129	≤82	≥83	≤89	≥90
130	≤82	≥83	≤89	≥90
131	≤81	≥82	≤88	≥89
132	≤80	≥81	≤88	≥89
133	≤80	≥81	≤88	≥89
134	≤79	≥80	≤87	≥88
135	≤78	≥79	≤87	≥88
136			≤87	≥88
137			≤87	≥88
138			≤86	≥87
139			≤86	≥87
140			≤86	≥87
141			≤85	≥86

*When using these criteria to sex owls as a part of banding schedule preparation you must include the following remark for each owl. **Sex determined using the wing-mass DF available from Project OwlNet** (<http://www.projectowl.net/>).

8.3 Morphometric data sheet for selected Northern Saw-whet Owls

Morphometric data sheet detailing measurements for 70 Northern Saw-whet Owls captured by Project OwlNet from 2018–2022 (35 males and 35 females). Sex determination was based on wing length measurements, following the >95% probability threshold. Data were collected from three banding stations: Hidden Valley Golf Course, Schuylkill Co., PA (HIDDENVY); Small Valley Girl Scout Camp, Dauphin Co., PA (SMALLVY); and King’s Gap State Park, Cumberland Co., PA (KINGSGAP). These measurements were used in the logistic regression analysis for sex differentiation based on cryptic dimorphic traits.

<i>n</i>	Band Number	Location	Date	Age	Weight	Sex	Tail	Wing
1	1014-85369	HIDDENVY	11/3/18	HY	79.9	M	67	129
2	1583-00172	HIDDENVY	11/7/18	HY	82.6	M	67	128
3	1014-53501	HIDDENVY	11/10/18	HY	81.9	M	66	130
4	1583-00174	HIDDENVY	11/18/18	HY	69.2	M	69	131
5	1583-00176	HIDDENVY	11/18/19	HY	79.4	M	68	130
6	1104-31424	HIDDENVY	10/14/20	HY	74.6	M	69	125
7	1533-06863	HIDDENVY	10/21/20	HY	76.4	M	67	132
8	1104-31440	HIDDENVY	10/22/20	HY	80.5	M	64	133
9	1104-31462	HIDDENVY	11/3/20	HY	77.1	M	65	135
10	1533-06865	HIDDENVY	11/4/20	HY	72.2	M	66	133
11	1533-06867	HIDDENVY	11/8/20	HY	73.6	M	66	126
12	1104-31493	HIDDENVY	11/12/20	HY	81.8	M	69	129
13	1014-53670	HIDDENVY	11/2/21	HY	80.1	M	65	130
14	1014-53682	HIDDENVY	11/3/21	HY	70.2	M	64	130
15	1533-06872	HIDDENVY	11/4/21	HY	75.5	M	60	126
16	1533-06873	HIDDENVY	10/20/22	HY	78.4	M	67	130
17	1104-31181	SMALLVY	10/30/18	HY	79.4	M	61	131
18	1104-31208	SMALLVY	11/4/18	HY	75.1	M	71	135
19	1104-31247	SMALLVY	11/10/18	HY	77.9	M	64	130
20	1583-00437	SMALLVY	11/8/18	HY	78.5	M	65	129
21	1104-31299	SMALLVY	11/14/20	HY	75.8	M	65	134
22	1583-00446	SMALLVY	10/20/20	HY	78.8	M	70	133
23	1583-00450	SMALLVY	11/7/20	HY	75.6	M	66	129
24	1583-00454	SMALLVY	11/7/20	HY	76.1	M	66	129
25	1583-00460	SMALLVY	11/2/21	HY	76.8	M	67	127
26	1583-00462	SMALLVY	11/7/21	HY	74.7	M	61	126
27	1583-00480	SMALLVY	11/9/22	HY	75.1	M	67	129
28	1583-00479	SMALLVY	11/9/22	HY	79.9	M	65	127
29	1124-25251	SMALLVY	11/16/22	HY	78.5	M	61	127
30	1353-64754	KINGSGAP	11/11/18	HY	74.5	M	69	134

31	1353-64755	KINGSGAP	11/14/18	HY	77	M	70	134
32	1353-64760	KINGSGAP	10/27/20	HY	84.3	M	66	127
33	1353-64769	KINGSGAP	11/13/20	HY	79	M	67	132
34	1104-35616	KINGSGAP	10/20/21	HY	81.5	M	69	130
35	1353-64772	KINGSGAP	10/28/21	HY	79.1	M	66	130
36	1014-85311	HIDDENVY	10/7/18	HY	93.9	F	70	141
37	1014-85312	HIDDENVY	10/9/18	HY	87.8	F	71	140
38	1014-85313	HIDDENVY	10/12/18	HY	95.7	F	70	137
39	1014-85314	HIDDENVY	10/12/18	HY	90.5	F	65	138
40	1014-85315	HIDDENVY	10/14/18	HY	90.4	F	72	140
41	1014-85318	HIDDENVY	10/16/18	HY	88.5	F	68	135
42	1014-85319	HIDDENVY	10/16/18	HY	96.3	F	73	144
43	1014-85328	HIDDENVY	10/18/18	HY	96.4	F	74	141
44	1014-53563	HIDDENVY	10/23/19	HY	95.6	F	72	139
45	1014-53564	HIDDENVY	10/25/19	HY	102.7	F	72	134
46	1014-53580	HIDDENVY	11/8/19	HY	98.3	F	72	137
47	1014-53581	HIDDENVY	10/8/19	HY	94.6	F	74	146
48	1014-53587	HIDDENVY	11/12/19	HY	94.6	F	76	146
49	1014-85325	SMALLVY	10/25/18	HY	98.3	F	69	139
50	1104-31149	SMALLVY	10/15/18	HY	95.7	F	71	138
51	1104-31151	SMALLVY	10/16/18	HY	99.5	F	69	141
52	1104-31160	SMALLVY	10/21/18	HY	98.6	F	70	138
53	1104-31161	SMALLVY	10/21/18	HY	100.1	F	69	140
54	1104-31271	SMALLVY	10/28/19	HY	94	F	72	140
55	1104-31272	SMALLVY	10/29/19	HY	102.8	F	69	135
56	1104-31289	SMALLVY	11/18/19	HY	104.1	F	69	140
57	1104-31292	SMALLVY	10/13/20	HY	104	F	75	140
58	1104-31293	SMALLVY	10/13/20	HY	94.6	F	71	141
59	1104-31294	SMALLVY	10/13/20	HY	96.3	F	72	138
60	1104-31296	SMALLVY	10/14/20	HY	89	F	69	140
61	1014-85523	KINGSGAP	10/13/18	HY	86.8	F	72	139
62	1014-85527	KINGSGAP	10/16/18	HY	91.4	F	70	139
63	1014-85528	KINGSGAP	10/16/18	HY	94	F	72	138
64	1014-85535	KINGSGAP	10/25/18	HY	94	F	67	138
65	1104-31510	KINGSGAP	11/13/19	HY	93.6	F	73	147
66	1104-31522	KINGSGAP	10/16/20	HY	100.7	F	72	137
67	1104-31525	KINGSGAP	10/16/20	HY	99.9	F	76	135
68	1104-31531	KINGSGAP	10/21/20	HY	98.9	F	71	135
69	1104-31536	KINGSGAP	10/24/20	HY	92.4	F	70	135
70	1104-3159	KINGSGAP	10/24/20	HY	91	F	70	141

8.4 Data analysis with facial disk quantification for Northern Saw-whet Owls

Scoring sheet for quantifying facial disk differences between sexes in 70 hatch-year Northern Saw-whet Owls. Morphological traits—Eyebrow Shape (C or V), Head Shape (Flat or Round), and Eyebrow Width (Thick or Thin)—were analyzed and recorded as binary variables (1 = presence, 0 = absence). Data were organized into Excel spreadsheets for assessment of sexual dimorphism traits in logistic regression analysis.

<i>n</i>	Individual ID	Sex	Eyebrow Shape		Head Shape		Eyebrow Width	
			C (Female)	V (Male)	Flat (Female)	Round (Male)	Thick (Female)	Thin (Male)
1	1014-85369	Male	0	1	0	1	0	1
2	1583-00172	Male	1	0	0	1	0	1
3	1014-53501	Male	0	1	0	1	0	1
4	1583-00174	Male	0	1	0	1	0	1
5	1014-53576	Male	0	1	1	0	0	1
6	1104-31424	Male	0	1	0	1	0	1
7	1533-06863	Male	0	1	0	1	0	1
8	1104-31440	Male	1	0	0	1	0	1
9	1104-31462	Male	0	1	0	1	0	1
10	1533-06865	Male	0	1	0	1	0	1
11	1533-06867	Male	0	1	0	1	0	1
12	1104-31493	Male	0	1	1	0	1	0
13	1014-53670	Male	0	1	0	1	0	1
14	1014-53682	Male	0	1	0	1	1	0
15	1533-06872	Male	0	1	1	0	1	0
16	1533-06873	Male	0	1	0	1	0	1
17	1104-31181	Male	0	1	0	1	0	1
18	1104-31208	Male	0	1	1	0	1	0
19	1104-31247	Male	1	0	1	0	0	1
20	1583-00437	Male	0	1	0	1	0	1
21	1104-31299	Male	1	0	1	0	1	0
22	1583-00446	Male	0	1	1	0	0	1
23	1583-00450	Male	1	0	1	0	0	1
24	1583-00454	Male	0	1	1	0	0	1

25	1583-00460	Male	0	1	1	0	0	1
26	1583-00462	Male	0	1	1	0	0	1
27	1583-00480	Male	0	1	1	0	0	1
28	1583-00479	Male	0	1	0	1	0	1
29	1124-25251	Male	0	1	1	0	0	1
30	1353-64754	Male	0	1	1	0	0	1
31	1353-64755	Male	1	0	1	0	0	1
32	1353-64760	Male	0	1	0	1	0	1
33	1353-64769	Male	1	0	1	0	1	0
34	1104-35616	Male	1	0	0	1	0	1
35	1353-64772	Male	0	1	0	1	0	1
36	1014-85311	Female	1	0	1	0	0	1
37	1014-85312	Female	0	1	0	1	0	1
38	1014-85313	Female	0	1	1	0	0	1
39	1014-85314	Female	1	0	1	0	1	0
40	1014-85315	Female	1	0	1	0	0	1
41	1014-85318	Female	1	0	1	0	1	0
42	1014-85319	Female	1	0	1	0	1	0
43	1014-85328	Female	1	0	1	0	0	1
44	1014-53563	Female	0	1	1	0	0	1
45	1014-53564	Female	1	0	1	0	0	1
46	1014-53580	Female	1	0	1	0	1	0
47	1014-53581	Female	1	0	1	0	1	0
48	1014-53587	Female	1	0	1	0	1	0
49	1014-85325	Female	1	0	1	0	0	1
50	1104-31149	Female	1	0	1	0	1	0
51	1104-31151	Female	1	0	1	0	0	1
52	1104-31160	Female	1	0	1	0	1	0
53	1104-31161	Female	1	0	1	0	1	0
54	1104-31271	Female	1	0	1	0	0	1
55	1104-31272	Female	1	0	1	0	0	1
56	1104-31289	Female	1	0	1	0	0	1
57	1104-31292	Female	1	0	1	0	0	1

58	1104-31293	Female	1	0	1	0	1	0
59	1104-31294	Female	0	1	0	1	0	1
60	1104-31296	Female	1	0	1	0	1	0
61	1014-85523	Female	1	0	1	0	0	1
62	1014-85527	Female	0	1	1	0	0	1
63	1014-85528	Female	1	0	0	1	0	1
64	1014-85535	Female	1	0	1	0	1	0
65	1104-31510	Female	1	0	1	0	0	1
66	1104-31522	Female	1	0	1	0	1	0
67	1104-31525	Female	0	1	1	0	1	0
68	1104-31531	Female	1	0	0	1	0	1
69	1104-31536	Female	1	0	1	0	0	1
70	1104-31539	Female	1	0	1	0	0	1
