

Sexing Young Snowy Owls

Author(s) :Mathew T. Seidensticker, Denver W. Holt, Jennifer Detienne, Sandra Talbot, and Kathy Gray

Source: Journal of Raptor Research, 45(4):281-289. 2011.

Published By: The Raptor Research Foundation

DOI:

URL: <http://www.bioone.org/doi/full/10.3356/JRR-11-02.1>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

THE JOURNAL OF RAPTOR RESEARCH

A QUARTERLY PUBLICATION OF THE RAPTOR RESEARCH FOUNDATION, INC.

VOL. 45

DECEMBER 2011

No. 4

J. Raptor Res. 45(4):281–289

© 2011 The Raptor Research Foundation, Inc.

SEXING YOUNG SNOWY OWLS

MATHEW T. SEIDENSTICKER,¹ DENVER W. HOLT AND JENNIFER DETIENNE
Owl Research Institute, P.O. Box 39, Charlo, MT 59824 U.S.A.

SANDRA TALBOT

USGS Molecular Ecology Laboratory, Alaska Science Center, 1011 East Tudor Road, Anchorage, AK 99503 U.S.A.

KATHY GRAY

Math and Statistics Department, California State University, Chico, 400 West First Street, Chico, CA 95929 U.S.A.

ABSTRACT.—We predicted sex of 140 Snowy Owl (*Bubo scandiacus*) nestlings out of 34 nests at our Barrow, Alaska, study area to develop a technique for sexing these owls in the field. We primarily sexed young, flightless owls (38–44 d old) by quantifying plumage markings on the remiges and tail, predicting sex, and collecting blood samples to test our field predictions using molecular sexing techniques. We categorized and quantified three different plumage markings: two types of bars (defined as markings that touch the rachis) and spots (defined as markings that do not touch the rachis). We predicted sex in the field assuming that males had more spots than bars and females more bars than spots on the remiges and rectrices. Molecular data indicated that we correctly sexed 100% of the nestlings. We modeled the data using random forests and classification trees. Both models indicated that the number and type of markings on the secondary feathers were the most important in classifying nestling sex. The statistical models verified our initial qualitative prediction that males have more spots than bars and females more bars than spots on flight feathers P6–P10 for both wings and tail feathers T1 and T2. This study provides researchers with an easily replicable and highly accurate method for sexing young Snowy Owls in the field, which should aid further studies of sex-ratios and sex-related variation in behavior and growth of this circumpolar owl species.

KEY WORDS: *Snowy Owl; Bubo scandiacus; molecular sexing; plumage characteristics; sex; sex-ratios.*

DETERMINACIÓN DEL SEXO DE INDIVIDUOS JÓVENES DE *BUBO SCANDIACUS*

RESUMEN.—Predijimos el sexo de 140 polluelos de *Bubo scandiacus* pertenecientes a 34 nidos en nuestra área de estudio Barrow, Alaska, con el objetivo de desarrollar una técnica para la determinación del sexo de estas lechuzas en el campo. Determinamos el sexo principalmente de lechuzas jóvenes, individuos no voladores (38–44 d de edad), mediante la cuantificación de las marcas en el plumaje de las remeras y la cola, prediciendo el sexo y colectando muestras de sangre para comprobar nuestras predicciones de campo mediante técnicas moleculares de determinación del sexo. Clasificamos y cuantificamos tres marcas diferentes del plumaje: dos tipos de barras (definidas como las marcas que llegan hasta el raquis) y puntos (definidos como las marcas que no llegan hasta el raquis). Predijimos el sexo en el campo con base en el supuesto de que los machos tenían más puntos que barras y que las hembras tenían más barras que puntos en las remeras y timoneras. Los datos moleculares indicaron que identificamos correctamente el sexo del 100% de los pichones. Modelamos los datos utilizando los métodos de clasificación “bosques aleatorios” y

¹ Email address: mat_seidensticker@yahoo.com

dendrogramas de clasificación. Ambos modelos indicaron que el número y tipo de marcas en las plumas secundarias son las más importantes en la clasificación del sexo de los polluelos. Los modelos estadísticos verificaron nuestra predicción cualitativa inicial de que los machos tienen más puntos que barras y las hembras más barras que puntos en las plumas de vuelo P6–P10 tanto para las plumas de las alas como de la cola T1 y T2. Este estudio proporciona a los investigadores un método fácilmente reproducible y de alta precisión para la determinación del sexo de individuos jóvenes de *B. scandiacus* en el campo, que debe ayudar a realizar más estudios sobre la razón de sexos y variaciones en el comportamiento relacionadas con el sexo y el crecimiento de esta especie de lechuza circumpolar.

[Traducción del equipo editorial]

Like most raptors, owls tend to exhibit reversed sexual size dimorphism in external morphology (Earhart and Johnson 1970, Snyder and Wiley 1976, McGillivray 1987, Duncan 1996). However, as a group, owls typically exhibit little to no sexual color dimorphism in plumage characteristics (D. Holt unpubl. data). In most cases, using plumage characteristics to differentiate sex is challenging and for some owl species thought to be impossible. Exceptions include Short-eared (*Asio flammeus*) and Long-eared owls (*Asio otus*). In these species, width of band markings on the flight feathers and color variation in plumage, respectively, can be used to reliably determine sex (Holt and Leasure 1993, Arroyo et al. 2000, D. Holt unpubl. data).

One notable example of sexual color dimorphism in owls is the Snowy Owl (*Bubo scandiacus*). Snowy Owls are a large, circumpolar species that breed almost exclusively on the arctic tundra and are considered nomadic (Mikkola 1983, Cramp 1985, Parmelee 1992) or irruptive migrants (Holt and Zetterberg 2008). Snowy Owls have delayed plumage maturation (Parmelee 1992) and exhibit noticeable sexual dimorphism in plumage characteristics (Josephson 1980, Cramp 1985, Pyle 1997a, 1997b). Adult females are darker than males and birds that are <1 yr old tend to be darker than adults. At least four plumage classes have been recognized (Josephson 1980).

Snowy Owls presumably lose plumage markings over time (Josephson 1980, Pyle 1997a, 1997b). Adult male basic plumage is almost pure white (few to no markings) and adult female basic plumage is white with extensive black/dark brown barring. Males may reach definitive basic plumage after their third, fourth, or fifth year of life (Pyle 1997a). However, the rate at which plumage markings are lost and how that rate varies among individuals and between the sexes is essentially unknown. As a result, it is unclear exactly how many years it takes Snowy Owls to reach adult plumage.

Sex is known to influence many demographic aspects in birds, making its identification important

when marking individuals for behavioral or ecological studies (Greenwood 1980, Short and Balaban 1994). Thus, being able to identify sex of individuals is important for knowledge of sex-ratios, among many other life-history traits (Ellegren and Sheldon 1997).

From 1992 to present, DWH has conducted a long-term ecological study of Snowy Owls at Barrow, Alaska. During this study, observed differences in the plumage characteristics of flightless nestlings after 4 wk of age were noted. We presumed that these differences reflected sex, but we could not find detailed information about using plumage characteristics to determine sex of nestlings. Because we were interested in incorporating brood sex-ratio data into this long-term project, we conducted this study to quantify and investigate sex-related differences in plumage markings of young, flightless Snowy Owls.

STUDY AREA AND METHODS

We conducted research (June–September) on a 127-km² area of coastal tundra at Barrow, Alaska (71°18'N, 156°46'W). Barrow is bordered by the Chukchi Sea on the west and the Beaufort Sea to the east. The coastal tundra at Barrow has low relief and is dominated by a pattern of ice-wedge polygons, shallow directionally oriented lakes, drained lake basins, and small ponds (Brown et al. 1980). During the summer months, the area experiences a cool and humid climate with mean temperatures ranging from 2–4°C and average relative humidity consistently above 80% (Brown et al. 1980). Barrow is well-known as a traditional nesting ground for Snowy Owls and infrastructure there allows researchers to routinely access the tundra and monitor nests.

We primarily collected data from nestlings 38–44 d old. We were able to closely determine the age of each nestling because we monitored nests every 3 d from incubation until fledging. For all individuals 38–44 d of age, we: (1) quantified plumage markings, (2)

measured bill length, width, and depth, (3) predicted sex based on plumage, (4) collected blood samples, and (5) compared our sex predictions in the field with laboratory molecular sexing techniques.

To investigate whether our technique could be applied to owls younger or older than 38–44 d, we collected data from nestlings 28–35 d and several owls >44 d old. We also captured and applied this technique to several other owls during an irruptive migration in the Mission Valley of west-central Montana in 2006 (Holt and Zetterberg 2008). We examined molt, and referenced Pyle (1997b) and our own experience to determine the age of each owl captured in winter 2006.

Quantifying Plumage Markings. We classified three types of plumage markings: transverse bars (TBs), irregular bars (IBs), and spots (SPOTs). We defined bars as markings that touched the rachis of the feather, and we considered TBs as markings that had complementary sides on both feather vanes, analogous to “mirror images” and formed a contiguous band across the rachis that touched both feather vanes, and IBs as markings that touched the rachis but did not form a contiguous band across the rachis and touch both feather vanes. We defined SPOTs as markings that did not touch the rachis (Fig. 1).

We counted the number of each type of marking on primary remiges P6–P10, secondary remiges S1–S5 for both wings and on the central two rectrices T1–T2. We did not sample all the remiges and rectrices because we wanted to minimize the amount of time that each owlet was handled.

Sometimes markings that touched the rachis appeared, to our eyes, oddly shaped or pigmented. In general, plumage markings that were longer than wide we considered bar-like, and those that were approximately equal in length and width we considered spot-like. We did not quantify shape (i.e., bar-like or spot-like) of these atypical markings. We used photographs taken in the field to make qualitative observations of unusual differences in marking shape between the sexes.

Measuring Bill Morphology. We used calipers accurate to 0.5 mm to measure bill length, width, and depth. We measured bill length as the distance between the distal edge of the cere to the tip of the bill. We measured bill width as the distance, measured from the widest point at the proximal base of the bill, between the paired maxillary bones that help support the tomlia (cutting edges) of the upper bill. We measured bill depth as the maximum dis-

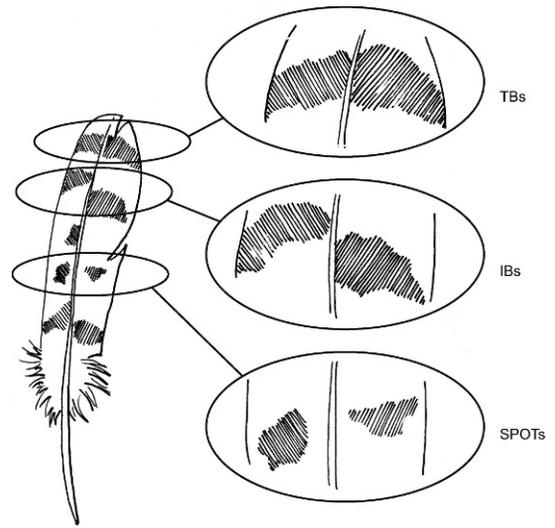


Figure 1. Depiction of plumage markings: transverse bars (TBs)—markings that have complementary sides on both feather vanes, analogous to “mirror images” and forming a contiguous band across the rachis that touched both feather vanes; irregular bars (IBs)—markings that touch the rachis but did not form a contiguous band across the rachis and touch both feather vanes; spots (SPOTs)—markings that did not touch the rachis.

tance, measured at the proximal base of the bill, from the top edge of the bill (culmen) to the bottom edge of the bill (gonys).

Predicting Sex. Based on prior qualitative field observations, we predicted that males would have few to no bars and more spots while females would have more bars and few to no spots on the primary, secondary, and tail feathers. Accordingly, we predicted the sex of each owlet we handled and recorded our prediction in the field. We predicted sex after counting plumage markings. We used the count data to support our qualitative assumption that males have few to no bars and more spots and females the opposite.

Collecting Blood Samples. We collected blood samples by puncturing the brachial vein using a sterilized 25-gauge needle and then collecting the emergent blood in a capillary tube. We immediately transferred the blood from the capillary tubes to Eppendorf tubes containing Longmire Buffer solution. We sterilized puncture sites with alcohol and ensured bleeding had stopped before releasing the owls. We labeled each blood sample with our prediction of sex, along with the individual’s corresponding band number. We froze samples upon

returning from the field and kept them frozen until transport to the lab. We sent blood samples to the USGS Molecular Ecology Laboratory at the Alaska Science Center, Anchorage, Alaska, for processing.

Molecular Sexing. Z and W chromosomes are the sex chromosomes in birds. Each sex has two sex chromosomes with the female being the heterogametic sex (ZW). We determined the sex of each owl using DNA-based sex identification techniques described in Griffiths et al. (1998), modified to allow electrophoresis on a LI-COR 4200L automated sequencer (LI-COR, Lincoln, Nebraska, U.S.A.). This method of molecular sexing relies on size differences between the chromo-helicase dehydrogenase gene on the W-chromosome (CHD-W) relative to a homologous but nonactive locus on the Z-chromosome (CHD-Z). Due to rapid mutation of an intron associated with the non-transcribing CHD-Z, this locus is generally larger or smaller than the CHD-W gene and can be distinguished based on fragment size when the two genes are co-amplified in a single polymerase chain reaction (PCR).

The CHD-Z and CHD-W genes of Snowy Owls were amplified via PCR, using primers P2 (TCTGCATCGCTAAATCCTTT) and P8 (CTCCCAAGGATGAGRAAYTG). The forward primer was directly labeled with the infrared fluorophore IRD700 or IRD800 (LI-COR Inc., Lincoln, Nebraska, U.S.A.) and used in a PCR cocktail containing 1 μ L of DNA extract, 10.0 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50.0 mM KCl, 0.01% (w/v) gelatin, 0.2 mM dNTPs, 3.7 pmoles unlabeled P8 primer, 4.0 pmoles unlabeled P2 primer, 0.3 pmoles labeled P8 primer, 0.1 μ g bovine serum albumin, and 0.75 units *Taq* polymerase (United States Biochemical, Cleveland, Ohio, U.S.A.). The PCR reaction profile was identical to that reported in Handel et al. (2006). Fluorescently-labeled PCR products were electrophoresed on an 18-cm or 25-cm, 48-well, 6% polyacrylamide gel (Acryl/Bis; Amersco, Solon, Ohio, U.S.A.), on a LI-COR 4200L (LICOR, Inc., Lincoln, Nebraska, U.S.A.) automated sequencer. Initially, PCR products from known females and known males nesting at Barrow, Alaska, were electrophoresed against an M13 DNA ladder of known size. Females produced two fragments that differed in size: the 373-base-pair (bp) CHD-W fragment, and the 368-bp CHD-Z fragment. Males produced two bands of the same size: the 368-bp CHD-Z gene. These samples, or samples sized against them, were included in subsequent gels as standards typically occupying 6–9 lanes. Products with a single band of 368 bp (ZZ) were classified as

males; individuals demonstrating two bands, one at 368 bp and one at 373 bp (ZW) were classified as females.

Statistical Analyses. We calculated summary statistics and applied multivariate analysis to the data. We calculated confidence intervals to compare the mean number of markings on the remiges and rectrices between males and females. Due to growth and development of young owls, and possible variation in measurement error, we did not use bill-size measurements in the statistical modeling; bill-size data were simply reported as means \pm SE.

Data modeling. We used classification trees and random forests to model the count data. Although principle component analysis (PCA) or discriminant function analysis (DFA) are often used in predicting sex in birds (see Bortolotti 1984, Balbontín et al. 2001, Counsilman et al. 1994) we chose to apply random forests and classification trees to our data because we felt that these two methods were the most appropriate techniques to meet our objectives. Classification trees, introduced by Breiman et al. (1984), model a categorical variable by binary partitioning of predictor variables (Cutler et al. 2007). The binary partitioning takes the researcher down the branches of a tree until a prediction of class membership is made at the final node. Cross validation is commonly used to find a parsimonious model that will perform well on new data (Tan et al. 2006).

Breiman (2001) proposed random forests as an extension to classification trees. A random forest model consists of many (500–2000) classification trees produced by incorporating resampling methods to both the variables and the observations (Liaw and Wiener 2002). For each classification tree, some of the observations are left out due to the bootstrap sampling method. Additionally, the predictor variables that can be used at an individual node is a randomly selected subset of all the variables (Prasad et al. 2006). The observations that are left out due to the bootstrap sampling are called out-of-bag observations and are used to compute the misclassification rate of the model. Each individual tree results in a prediction for an observation and a majority vote among all the trees decides the class prediction for an observation. This procedure has been shown to perform very well compared to other models and is robust to overfitting (Breiman 2001). One advantage of random forests over PCA or DFA is that random forests can handle a large number of predictor variables (Breiman 2001). We used ran-

dom forests as an exploratory tool in order to determine which plumage markings were the best predictors of sex. Random forests produce a measurement of the importance of the predictor variables. We felt that this was important to the analysis since it is likely the case that a number of different plumage markings could be used to accurately predict sex. The random forest algorithm estimates the importance of a variable by randomly permuting the values of the out-of-bag observations. The measure of importance is calculated by taking the difference between the misclassification rate for the permuted observations and the original out-of-bag observations and dividing this by the standard error (Cutler et al. 2007).

As a field tool, the random forests model is not practical since it produces a complicated model to predict sex and requires a computer to apply the prediction algorithm. The main objective of this analysis was to create a simple sexing algorithm that can be easily applied in the field. To this end, we created a classification tree for the ten most important variables to accurately and easily determine sex in the field. All statistical analysis was performed using R (R Development Core Team 2005).

RESULTS

During the 2002–06 breeding seasons, we quantified plumage markings, measured morphology, predicted sex, and collected blood from 140 Snowy Owl nestlings 38–44 d old from 34 nests. Molecular data validated that we had correctly sexed 100% of the nestlings in the field. Molecular data also verified that we correctly sexed all 28–35-d-old nestlings ($n = 15$) and owls captured during irruptive migration ($n = 4$, each in its first year of life, based on molt patterns representing one generation of remiges and rectrices). Because the flight feathers of nestlings 28–35 d old had just begun to grow and were rather short, it was difficult to differentiate marking types. For the greatest reliability, we recommend predicting sex after young Snowy Owls are >5 wk old.

Because we sampled multiple nestlings from each nest, there was some lack of independence. Although this probably had little effect on the predictive models, we acknowledge that lack of independence undoubtedly affected confidence interval size. However, we treated the observations as independent, as there was not a significant nest effect in our sample. Exploratory analysis of confidence intervals indicated marked differences in mean num-

ber of plumage markings between males and females. Confidence intervals showed significant differences in mean number of plumage markings between males and females for all feathers that we sampled except: left P8, right P8, and right P9 (Table 1). Morphometric data indicated that females may have a larger bill size than males, which seemed consistent with reversed sexual size dimorphism in owls (Table 2).

We used only the ten most important variables as indicated by analysis of confidence intervals to model the data (Table 3). The random forests model determined the number of IB markings on the left S4 feather to be most important. When used as a single variable in the classification tree model, the number of IB markings on the left S4 feather was the best predictor of sex. The cross-validated classification tree error rate using the number of IBs on the left S4 feather was 1.4%. This yielded a general classification rule: if an individual had zero or one IB on left S4, it was classified as male; if it had >1 IB, it was classified as female. Although the variables were highly correlated, the most important variable does not negate the importance of other variables (K. Gray pers. comm.). Indeed, nine other variables also predicted sex with 95% or greater accuracy when used in the classification tree model (Table 3).

In general, on the secondary feathers, males most often had markings that did not touch the rachis (spots). Conversely, females most often had markings that touched the rachis (bars). Both statistical models indicated that the total number and type of markings on the secondary feathers were the most important variables for determining sex of young Snowy Owls, verifying our initial prediction that males have few bars and more spots and females have more bars and few spots on the remiges (Fig. 2).

In general, the models misclassified males as females. These misclassifications arose from these individuals having >1 IB marking on secondary feathers.

Indeed, some of the individuals that we correctly predicted as male in the field had, by our definition, >1 IB marking on one or more of the secondary feathers. Based on our field observations, male markings on the secondary feathers (whether bars or spots) were most often spot-like in shape and/or barely touched the rachis. In females, most often markings on the secondary feathers (whether bars or spots) were bar-like in shape. However, because

Table 1. Mean number of plumage markings per feather for males and females. Confidence intervals are for the difference between mean number of markings for males and females. If males have more markings, both numbers are negative. If the difference was not significant, then the confidence interval ranges from a negative value to a positive one.

| FEATHER | MALE | FEMALE | 95% CONFIDENCE INTERVAL FOR DIFFERENCE (FEMALE-MALE) |
|-----------|------------------|------------------|---|
| | MEAN \pm SE | MEAN \pm SE | |
| Left P10 | 3.87 \pm 0.187 | 4.63 \pm 0.149 | (0.291,1.237) |
| Left P9 | 4.97 \pm 0.197 | 5.54 \pm 0.166 | (0.054,1.07) |
| Left P8 | 6.09 \pm 0.156 | 5.76 \pm 0.148 | (-0.751,0.098) |
| Left P7 | 5.93 \pm 0.217 | 6.99 \pm 0.155 | (0.531,1.586) |
| Left P6 | 5.03 \pm 0.169 | 7.03 \pm 0.131 | (1.576,2.4223) |
| Left S1 | 2.52 \pm 0.179 | 4.96 \pm 0.107 | (2.022,2.850) |
| Left S2 | 2.55 \pm 0.176 | 5.14 \pm 0.124 | (2.164,3.016) |
| Left S3 | 2.78 \pm 0.158 | 5.07 \pm 0.112 | (1.903,2.672) |
| Left S4 | 2.78 \pm 0.141 | 5.07 \pm 0.107 | (1.937,2.638) |
| Left S5 | 3.29 \pm 0.131 | 5.31 \pm 0.128 | (1.658,2.382) |
| Right P10 | 3.67 \pm 0.166 | 4.61 \pm 0.156 | (0.488,1.389) |
| Right P9 | 5.14 \pm 0.169 | 5.37 \pm 0.156 | (-0.234,0.676) |
| Right P8 | 5.83 \pm 0.175 | 5.87 \pm 0.150 | (-0.409,0.503) |
| Right P7 | 5.93 \pm 0.204 | 6.75 \pm 0.136 | (0.334,1.303) |
| Right P6 | 5.00 \pm 0.215 | 7.06 \pm 0.125 | (1.564,2.549) |
| Right S1 | 2.55 \pm 0.204 | 4.86 \pm 0.099 | (1.859,2.758) |
| Right S2 | 2.41 \pm 0.187 | 5.13 \pm 0.127 | (2.273,3.169) |
| Right S3 | 2.72 \pm 0.175 | 4.96 \pm 0.105 | (1.829,2.638) |
| Right S4 | 2.74 \pm 0.154 | 4.92 \pm 0.102 | (1.811,2.542) |
| Right S5 | 3.14 \pm 0.133 | 5.24 \pm 0.101 | (1.764,2.425) |
| T1 | 3.90 \pm 0.146 | 2.76 \pm 0.109 | (-1.50,-0.777) |
| T2 | 4.20 \pm 0.159 | 2.80 \pm 0.116 | (-1.79,-1.01) |

we did not define and collect categorical data of shape (i.e., bar-like or spot-like) for each marking that we counted, it was not included as a variable in the statistical models. Although our field observations of marking shape were qualitative in nature, the data suggested that marking shape may be important in determining sex of young Snowy Owls and may help avoid misclassification if it is included in the predictive models.

DISCUSSION

Sexual color dimorphism in plumage characteristics has been documented in Snowy Owls. However,

these studies have focused primarily on rank variables such as: the extent or degree of mottling/barring and overall color of the body plumage (Josephson 1980, Cramp 1985). Although Pyle (1997a, 1997b) does specify that counting the number of bars on the central rectrices can be used as a means for differentiating sex, to our knowledge this study is the first to define and quantify different types of plumage markings on the flight feathers and central rectrices of wild Snowy Owls.

Measures such as scoring the degree of mottling/barring or overall color of the body plumage often carry potential of observer bias and other confound-

Table 2. Bill morphometrics and mass (mean \pm SE (range)) for Snowy Owl nestlings 38–44 d of age. Bill length, width, depth ($n = 63$) both sexes. Mass ($n = 62$) both sexes.

| SEX | BILL LENGTH (mm) | BILL WIDTH (mm) | BILL DEPTH (mm) | MASS (g) |
|---------|----------------------------------|----------------------------------|----------------------------------|------------------------------------|
| Males | 23.7 \pm 0.11 (21.3 – 26.0) | 31.5 \pm 0.32 (19.5 – 36.4) | 20.6 \pm 0.26 (18.3 – 31.2) | 1373.5 \pm 12.2 (1074 – 1549) |
| Females | 25.1 \pm 0.17 (19.4 – 27.1) | 33.5 \pm 0.25 (29.3 – 38.5) | 21.8 \pm 0.19 (18.7 – 26.0) | 1684.7 \pm 39.6 (999 – 1949) |

Table 3. Ten most important feather variables generated from the random forest model for predicting sex of Snowy Owls 38–44 d of age and classification rules for the number of IB markings (most important plumage marking for determining sex) when used as a single variable in the classification tree model.

| FEATHER VARIABLE: NUMBER OF IB MARKINGS | RANDOM FOREST IMPORTANCE | CLASSIFICATION TREE CROSS-VALIDATION ERROR RATE | CLASSIFICATION RULE |
|--|-----------------------------|--|------------------------------------|
| Left S4 | 0.098 | 2/140 (1.43%) | 0 or 1 IB = male, otherwise female |
| Left S2 | 0.082 | 3/140 (2.14%) | 0 or 1 IB = male, otherwise female |
| Right S3 | 0.062 | 4/140 (2.86%) | 0 IB = male, otherwise female |
| Left S5 | 0.049 | 3/140 (2.14%) | 0 or 1 IB = male, otherwise female |
| Right S2 | 0.045 | 4/140 (2.86%) | 0 or 1 IB = male, otherwise female |
| Left S3 | 0.039 | 3/140 (2.14%) | 0 or 1 IB = male, otherwise female |
| Right S4 | 0.033 | 6/140 (4.29%) | 0 IB = male, otherwise female |
| Left S1 | 0.032 | 5/140 (3.57%) | 0 or 1 IB = male, otherwise female |
| Right S5 | 0.018 | 5/140 (3.57%) | 0 or 1 IB = male, otherwise female |
| Right S1 | 0.002 | 7/140 (5.00%) | 0 IB = male, otherwise female |

ing factors such as individual variation in marking color and plumage wear. Often, the rank variables and sampling protocol are subjective and, if not well-defined, can lead to confusion. We believe that count data of types of plumage markings is a more objective measure than rank data for predicting sex of Snowy Owls in the field because: (1) counting is efficient, (2) counting is easily replicated and, (3) counting reduces observer bias. The results of this study supported that notion and indicated that counting types of plumage markings to determine sex can be applied to Snowy Owls >4 wk of age up to hatch-year (HY), and possibly older.

It is known that plumage in some wide-ranging bird species varies geographically. However, Marthinsen et al. (2009) reported no phylogeographic structure and high levels of gene flow in Snowy Owls. Further, annual movements of Snowy Owls can link disparate regions and individuals may breed in Alaska one year and Russia or Canada in the subsequent year (Fuller et al. 2003). Environmental factors may affect plumage, but we are not aware of any data indicating this for Snowy Owls. Given evidence of high genetic diversity and nomadic tendencies, we feel that geographic variation in plumage is likely not significant in Snowy Owls.

Assuming that males lose all or most of their plumage markings over time (i.e., pure white, or nearly so, adult basic plumage) and females retain much of them, this method is likely applicable to all Snowy Owls older than 4 wk of age. However, incomplete adult prebasic molts in second year (SY) and older birds (Cramp 1985) and the rate that individuals lose plumage markings could be confounding factors

that may lead to incorrect sex classification using this technique. For example, if you were to apply this technique to an after-hatch-year (AHY) or older Snowy Owl, it is possible that the owl may have molted the left S4 feather, in which case the classification rule would be ineffective. However, several other variables on the flight feathers and tail were accurate (>95%) predictors of sex and could be used to reliably classify sex if left S4 was missing (Table 3).

Currently, we are not aware of any quantitative data about the rate at which Snowy Owls lose plumage markings. Although this could be a confounding factor, we feel that the unknown length of time it takes Snowy Owls to reach definitive plumage is probably not much of a problem in misclassifying sex because sex is independent of age and age is based on molt pattern, not plumage markings. Because males presumably lose plumage markings with each successive molt and females retain them, identifying AHY males should become easier because they will have lost IB markings, especially on the secondary feathers. For example, a HY male with one IB marking on left S4 will most likely have one or zero IB markings on left S4 as an AHY. Even if he retains that one IB marking he will, according to our classification rule, be predicted male 99% of the time because he has one or zero IB markings on left S4. Conversely, a HY female with four IB markings on left S4 should have at least four IBs on left S4 as an AHY. According to our classification rule, an AHY owl that has two IBs on left S4 would be correctly predicted female 99% of the time because it had more than one IB on left S4. Our method is 99% effective for IB markings on left S4 in HY birds. If males lose markings over time and females retain them (or lose markings more slowly

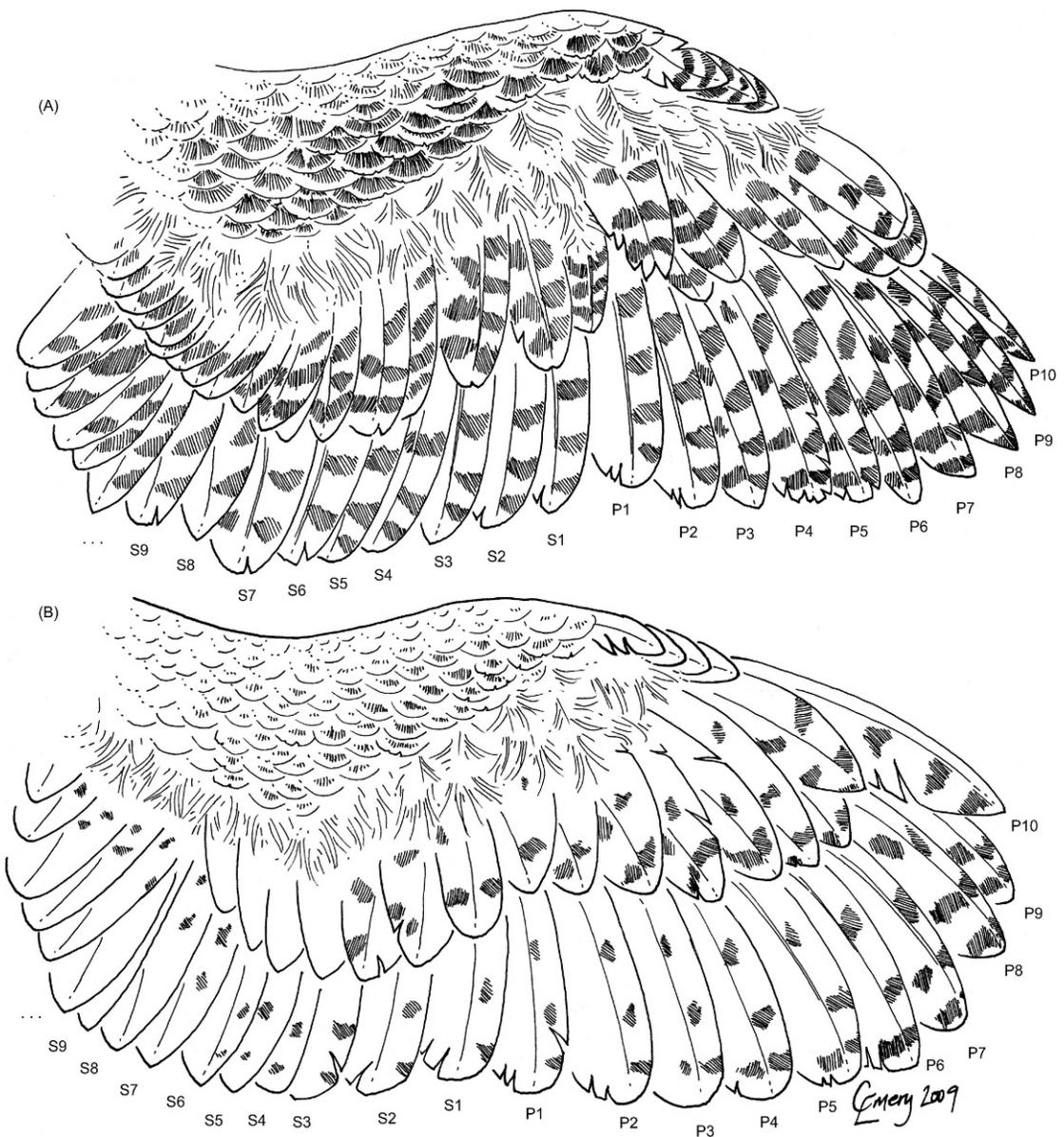


Figure 2. Typical (A) female wing, (B) male wing. We predicted sex in the field assuming that males had more spots than bars on the flight feathers and females vice versa.

than males) our method should remain just as effective in Snowy Owls age AHY.

This study provides researchers with a simple and easily replicable field technique that allows them to gather highly reliable data about sex-ratios and sex-related variation in growth and behavior of young, flightless Snowy Owls. Although more data are needed to determine the efficacy of this technique

on older birds, our initial results indicate this technique may be applied to HY and older Snowy Owls with great reliability. This will aid further studies on various facets in the life history of adult Snowy Owls.

ACKNOWLEDGMENTS

We thank Ukpeagvik Inupiat Corporation (UIC) for granting research permits and access to our study area,

Barrow Arctic Science Consortium (BASC) for logistical support, and the North Slope Borough Department of Wildlife Management. We also thank two anonymous reviewers for their helpful comments. We especially thank artist Claire Emery for providing the line drawings.

LITERATURE CITED

- ARROYO, B.E., T. DECORNULIER, AND V. BRETAGNOLLE. 2000. Sex and age determination of Short-eared Owl nestlings. *Condor* 102:216–219.
- BALBONTÍN, J., M. FERRER, AND E. CASADO. 2001. Sex determination in Booted Eagles (*Hieraetus pennatus*) using molecular procedures and discriminant function analysis. *Journal of Raptor Research* 35:20–23.
- BORTOLOTTI, G.R. 1984. Sexual size dimorphism and age-related size variation in Bald Eagles. *Journal of Wildlife Management* 48:72–81.
- BREIMAN, L. 2001. Random forests. *Machine Learning* 45:5–32.
- , J.H. FREIDMAN, R.A. OLSHEN, AND C.J. STONE. 1984. Classification and regression trees. Wadsworth and Brooks/Cole, Monterey, CA U.S.A.
- BROWN, J., K.R. EVERETT, P.J. WEBBER, S.F. MACLEAN, JR., AND D.F. MURRAY. 1980. The coastal tundra at Barrow. Pages 1–29 in J. Brown, P.C. Miller, L.L. Jeisen, and F.L. Bunnell [Eds.], *An arctic ecosystem*. US/IBP No. 12. Dowden, Hutchinson, and Ross, Stroudsburg, PA U.S.A.
- COUNSILMAN, J.J., K. NEE, A.K. JALIL, AND W.L. KENG. 1994. Discriminant analysis of morphometric characters as a means of sexing mynas. *Journal of Field Ornithology* 65:1–7.
- CRAMP, S. 1985. Handbook of the birds of Europe, the Middle East and North Africa: the birds of the western palearctic, Vol. IV. Oxford University Press, Oxford, U.K.
- CUTLER, D.R., T.C. EDWARDS, K.H. BEARD, A. CUTLER, K.T. HESS, J. GIBSON, AND J.J. LAWLER. 2007. Random forests for classification in ecology. *Ecology* 88:2783–2792.
- DUNCAN, J. 1996. Techniques to age and sex Great Gray Owls. *Birders Journal* 5:240–246.
- EARHART, C.M. AND N.K. JOHNSON. 1970. Size dimorphism and food habits of North American owls. *Condor* 72:251–264.
- ELLEGREN, H. AND B.C. SHELDON. 1997. New tools for sex identification and the study of sex allocation in birds. *Trends in Ecology and Evolution* 12:255–259.
- FULLER, M., D. HOLT, AND L. SCHUECK. 2003. Snowy Owl movements: variation on the migration theme. Pages 359–366 in P. Bertold, E. Gwinner, and E. Sonnenschein [Eds.], *Avian migration*. Springer-Verlag, Heidelberg, Germany, and New York, NY U.S.A.
- GREENWOOD, P.J. 1980. Mating systems, philopatry, and dispersal in birds and mammals. *Animal Behavior* 28:1140–1162.
- GRIFFITHS, R., M.C. DOUBLE, K. ORR, AND R.J.G. DAWSON. 1998. A DNA test to sex most birds. *Molecular Ecology* 7:1071–1075.
- HANDEL, C.M., S.M. PAJOT, S.L. TALBOT, AND G.K. SAGE. 2006. Use of buccal swabs for sampling DNA from nestling and adult birds. *Wildlife Society Bulletin* 34:1094–1100.
- HOLT, D.W. AND S.M. LEASURE. 1993. Short-eared Owl (*Asio flammeus*). In A. Poole and F. Gill [Eds.], *The birds of North America*, No. 62. The Academy of Natural Sciences, Philadelphia, PA and The American Ornithologists' Union, Washington, DC U.S.A.
- AND S.A. ZETTERBERG. 2008. The 2005 to 2006 Snowy Owls irruption migration to western Montana. *Northwestern Naturalist* 89:145–151.
- JOSEPHSON, B. 1980. Aging and sexing Snowy Owls. *Journal of Field Ornithology* 51:149–160.
- LIAW, A. AND M. WIENER. 2002. Classification and regression by random forest. *R News* 2:18–22.
- MARTHINSEN, G., L. WENNERBERG, R. SOLHEIM, AND J.T. LIFJELD. 2009. No phylogeographic structure in the circumpolar Snowy Owl. *Conservation Genetics* 10:923–933.
- MCGILLIVRAY, W.B. 1987. Reversed sexual dimorphism in ten species of northern owls. Pages 59–65 in R.W. Nero, R.J. Clark, R.J. Knapton, and R.H. Hamre [Eds.], *Biology and conservation of northern forest owls*. USDA Forest Service, Gen. Tech. Rep. GTR-RM 142, Ft. Collins, CO U.S.A.
- MIKKOLA, H. 1983. Owls of Europe. Buteo Books, Vermillion, SD U.S.A.
- PARMELEE, D.F. 1992. Snowy Owl (*Nyctea scandiaca*). In A. Poole, P. Stettenheim, and F. Gill [Eds.], *The birds of North America*, No. 10. The Academy of Natural Sciences, Philadelphia, PA and The American Ornithologists' Union, Washington, DC U.S.A.
- PRASAD, A.M., L.R. IVERSON, AND A. LIAW. 2006. Newer classification and regression tree techniques: bagging and random forests for ecological prediction. *Ecosystems* 9:181–191.
- PYLE, P. 1997a. Identification of North American birds, Part 1: Columbidae to Ploceidae. Slate Creek Press, Bolinas, CA U.S.A.
- . 1997b. Flight-feather molt patterns and age in North American owls. Monographs in Field Ornithology No. 2. American Birding Association, Colorado Springs, CO U.S.A.
- R DEVELOPMENT CORE TEAM. 2005. R: A language and environment for statistical computing, reference index version 2.x.x. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org> (last accessed 5 July 2011).
- SHORT, R.V. AND E. BALABAN. 1994. The differences between the sexes. Cambridge University Press, Cambridge, U.K.
- SNYDER, N.F.R. AND J.W. WILEY. 1976. Sexual dimorphism in hawks and owls of North America. Ornithological Monograph No. 20. American Ornithologists Union, U.S.A.
- TAN, P., M. STEINBACH, AND V. KUMAR. 2006. Introduction to data mining. Pearson Education, Inc., Boston, MA U.S.A.

Received 11 January 2011; accepted 9 July 2011