Toe-pad Length and Body Mass are Reliable Indicators of Sex in Barred Owls

Hermary M. Gonzales [1*],
Daniel F. Hofstadter [1],
Whitney A. Watson [1,2],
M. Zachariah Peery [1]

[1] Department of Forest and Wildlife Ecology
University of Wisconsin–Madison, Madison, WI
[2] Department of Fish, Wildlife, and Conservation Ecology
New Mexico State University, Las Cruces, NM
* Corresponding author: HMG, 1630 Linden Drive, Russell Labs, Madison, WI 53706; hgonzales@wisc.edu

ABSTRACT

*The expansion of Barred Owls (*Strix varia*) into the Pacific Northwest and California threatens the Northern Spotted Owl (*S. occidentalis caurina*) and western forest ecosystems. In response, many studies have been conducted to better understand Barred Owl behavior, demography, and ecological effects. However, Barred Owls are difficult to sex in the field using existing techniques, which prompts the need for a reliable method to distinguish males and females. Here, we describe an accurate method of sexing Barred Owls in hand using toe-pad length in combination with body mass. We measured and analyzed the toe-pad lengths and body mass of 136 subadult and adult Barred Owls (64 females and 72 males) that were lethally collected and had their sex confirmed based on examination of gonads. Females had statistically larger toe-pads and body mass standardized by time of year than males. We then developed a predictive equation based on toe-pad length and standardized body mass that accurately determined the sex of 99% of males and 98% of females. Thus, our study provides a rigorous means for sexing Barred Owls in hand and facilitates ecological studies of this species in its invasive range, although additional assessments in other parts of the range are merited.*

INTRODUCTION

Barred Owls (*Strix varia*) are native to the forests of eastern North America but have expanded their range into the Pacific Northwest and California over the past century (Mazur and James 2021). In their novel range in western North America, Barred Owls have become invasive and now occur at high densities, threatening federally threatened Northern Spotted Owls (*S. occidentalis caurina*) with extinction (Livezey 2009; Franklin et al. 2021; Wiens et al. 2021). However, owing to their generalist diets, Barred Owls pose a threat not only to Northern Spotted Owls, but also to various prey species (Wiens et al. 2014; Kryshak et al. 2022; Holm et al. 2016). Accordingly, many studies have been conducted to better understand Barred Owl behavior and demographics to mitigate effects on Spotted Owls and western forest ecosystems (Hofstadter et al. 2022; Wiens et al. 2014). In these studies, it is often important to know the sex of individual Barred Owls, however, there is not a standard and reliable method to sex Barred Owls in the field.

Barred Owls, as with many other avian species, are a sexually monomorphic species without distinguishable external characteristics such as plumage. Discerning sexes from Barred Owl vocalizations can be difficult for inexperienced observers (Odom and Mennill 2010). While females tend to be larger than males, sex cannot be distinguished visually (Mazur and James 2021). Even when in hand, body mass alone does not provide an easy and accurate sex determination because their body mass can fluctuate throughout the year (Acker 2020). Sex can be determined either by examining the gonads during necropsies or molecularly through techniques such as polymerase chain reaction (Steiner et al. 2016), but both methods are prohibitively expensive and neither technique is suitable for fieldwork when sex needs to be determined and individuals cannot be collected.

Toe-pad lengths have been used to accurately determine the sex of other bird species when in hand, such as the Barred Owl's congener, the Northern Spotted Owl (Fleming et al. 1991). Similarly, toe-pad length and body mass were used to determine the sex of Swainson's Hawks (*Buteo swainsoni*; Kochert and McKinley 2008) and toe-pad length, body mass, and wing chord length were used for Golden Eagles (*Aquila chrysaetos*; Edwards and Kochert 1986). In this paper, we describe a new method to accurately sex Barred Owls using toe-pad lengths and body mass for reliable sex determination of Barred Owls.

METHODS

Study area and sample selection.– We measured the toe-pads of 175 Barred Owls that were collected through experimental lethal removal studies for the benefit of Spotted Owls in Mendocino, Sonoma, and Marin counties in coastal California following field protocols established by Diller et al. (2014, 2016) under federal and state permits (United States Fish and Wildlife Service permits MB24592D and California Department of Fish and Wildlife permits S-193180001-19337). From this sample, we selected 136 Barred Owls (64 females and 72 males) including both sub-adult (first or second-year birds) or adults (three years or older), determined from the molt pattern of their flight feathers (Pyle 1997). Specifically, we selected birds that had their sex confirmed from observing gonads during the tissue archiving and study skin preparation process at the California Academy of Sciences in San Francisco, California, where their tissues were collected for genetic, diet, and other studies. A majority of these individuals were collected during the fall, winter, and spring (n = 128) with the remaining few in summer (n = 8). We used all 136 individuals to analyze toe-pad lengths, but for our analysis on body mass, we excluded one female who did not have its body mass taken due to poor health conditions, giving us a sample size of 135 Barred Owls (63 females and 72 males). We omitted three juveniles (fledglings that had not yet dispersed their natal territories) because their toe-pads may not have been fully developed, and omitted a Barred x Spotted Owl hybrid because of the uncertainty about whether the hybrid measurements would reflect an intermediate measurement between Barred and Spotted Owls.

Measuring toe-pad length and body mass.– We defined our toe-pad length as the distance between the base of the talon of the first digit (the hallux or hind toe) to the base of the talon of the third (middle) digit, similar to the measurement made by Edwards and Kochert (1986). With one hand, we fully spread the talons of each foot and used vernier calipers in the other hand to record both left and right toe-pad lengths to the nearest 0.01 mm (Figure 1). We averaged both left and right toe-pad lengths of each individual and rounded the value to the nearest 0.1 mm to account for differences that could have resulted from imperfect measurements. Additionally, we weighed each individual in the field using a spring scale with 10g increments to assess whether a combination of morphometric measurements resulted in reliable sex determination of individuals. Both measurements were taken immediately after birds were lethally collected in the field to most closely approximate measurements taken on live birds.

Statistical analysis – We used two-sample independent t-tests with an alpha level of 0.05 to test differences between male and female toe-pad lengths and body mass. We tested for differences in both measured body mass and body mass standardized to account for fluctuations in body mass throughout the year. To standardize body mass, we calculated Z-scores for two-month bins using the following equation: $\mathbf{Z = (x - \mu) / \sigma}$, where $\mathbf{x}$ was the individual's raw body mass and $\boldsymbol{\mu}$ and $\boldsymbol{\sigma}$ are mean and standard deviation for the two-month bin in which the individual was collected (Table 2).

Additionally, we used generalized linear models (McCullagh and Nelder 1989) within an information-theoretic framework (Burnham and Anderson 2002) to develop a predictive equation between sex and toe-pad length and standardized body mass, with toe-pad length and standardized body mass as independent variables and sex as a binomial response. The most general form of the equation could be expressed as: $\mathbf{Pr(S_i) = [exp\ (\beta_0 + \beta_1 x_1 + \beta_2 x_2)] / [1 + exp\ (\beta_0 + \beta_1 x_1 + \beta_2 x_2)]}$, where $\mathbf{Pr(S_i)}$ represents the probability that the individual i is a male (and $\mathbf{1 - Pr(S_i)}$ is the probability that the individual is a female), $\boldsymbol{\beta_0}$ is the intercept, $\boldsymbol{\beta_1}$ is the coefficient for toe-pad length, $\mathbf{x_1}$ is the individual's toe-pad length, $\boldsymbol{\beta_2}$ is the coefficient for body mass, and $\mathbf{x_2}$ is the individual's standardized body mass. If $\mathbf{Pr(S_i)}$ was greater than 0.5 we assumed individual **i** was male; if $\mathbf{Pr(S_i)}$ was less than 0.5 we assumed individual i was female.

We evaluated support for four competing models: a null model, body mass alone, toe-pad length alone, and toe-pad length and body mass together. We considered models as those within 5 ΔAIC_c (second-order Akaike Information Criterion corrected for small sample sizes; Arnold 2010) of the top model to be supported. All analyses were conducted in R version 4.2.3 (R Core Team 2021).

RESULTS

Females had toe-pad lengths that were statistically greater (mean = 67.8 mm, SD = 1.6) than males (mean = 62.8 mm, SD =1.3, p <0.001, t = 20.4; Table 1). There was overlap of toe-pad lengths between 64.5 mm and 65.1 mm, with 10 males and 3 females in this range (Figure 2A), but 86% of the males sampled had a toe-pad length less than 64.5 mm and 95% of the females sampled had a toe-pad length greater than 65.1 mm.

Females also had, on average, larger body mass (mean = 926 g, SD = 120) than males (mean = 731 g, SD = 68.2, p < 0.001, t = 11.8; Table 1). There was overlap of body mass between 720 g and 890 g, with 41 males and 30 females in this range, where 43% of the males sampled had body mass below 720 g and 52% of females sampled had body mass greater than 890 g. When standardized, there was still a significant difference between sexes using body mass (p < 0.001, t = 14.8), but with less overlap between the sexes. There was overlap between standardized body mass of -0.43 and 0.22, with 16 males and 20 females in this range. About 78% of males had standardized body mass less than -0.43 and 68% of females had standardized body mass greater than 0.22. The overlap was minimized further when the Barred Owls were separated into their month of collection, with a total of 11 Barred Owls, 7 males and 4 females, overlapping (Figure 2B).

We found strongest support for the generalized model that included both toe-pad length and standardized body mass (Table 3). Toe-pad length alone was also competitive in our model-selection and within 4.40 ΔAIC_c of our top model. However, even with the penalty of including another parameter, there was stronger support for the model including both toe-pad length and body mass, suggesting the addition of body mass can facilitate the determination of sex. There was little support for the model containing body mass alone, and for the null model, which had ΔAIC_c of 31.31, and 176.43 respectively (Table 3).

The model containing both toe-pad length and standardized body mass could be expressed as: $\mathbf{Pr(S_i) = \{exp\ (363.028 - 5.613\ x_1 - 8.985\ x_2\ )\} / \{1 + exp\ (363.028 - 5.613\ x_1 - 8.985\ x_2\ )\}}$. When we used this equation to predict the sex of 135 Barred Owls included in statistical analyses, the equation accurately determined the sex of 99% of males and 98% of females, leaving 1 male and 1 female incorrectly sexed. The one female that was not properly sexed was an anomalously small female collected in January that had a $\mathbf{Pr(S_i)}$ of 0.86, a body mass of 860 g, standardized body mass of -0.10, and toe-pad length of 64.5 mm. The male, however, had a $\mathbf{Pr(S_i)}$ of 0.49, a body mass of 790 g, standardized body mass of -0.01, and toe-pad length of 64.7 mm. It was the only individual that had a $\mathbf{Pr(S_i)}$ close to 0.5, as the next closest individual had a $\mathbf{Pr(S_i)}$ of 0.67.

DISCUSSION

Our results indicate that in combination, toe-pad length and body mass constitute a reliable way to identify the sex of Barred Owls in the hand. We found strong evidence that female Barred Owls have longer toe-pads than males. There was some overlap between the toe-pad lengths of males and females in the 64.5 mm and 65.1 mm range, with males falling within this range more than females. Reasonable separation has also been found in congeneric Northern Spotted Owls where toe-pad lengths less than 69 mm corresponded to males while toe-pad lengths greater than or equal to 70 mm corresponded to females (Fleming et al. 1991). Although Barred Owls are larger than Northern Spotted Owls, their smaller toe-pads can likely be attributed to their generalist dietary habits compared to Northern Spotted Owls predating more exclusively on rodents (Wiens et al. 2014; Kryshak et al. 2022). These patterns are common in other raptor species where females have longer toe-pads than males (Edwards and Kochert 1986; Kochert and McKinley 2008).

Measured body mass alone may be a somewhat less reliable method for sexing Barred Owls, likely in part because of within-year variation, as birds are heavier during the winter than they are in the summer (Table 2). Additionally, due to these seasonal fluctuations, there is more room for overlapping body mass. When body mass is standardized according to the date of collection, the number of Barred Owls with overlapping body mass decreased by 49%. Thus, standardizing body mass with the date of collection allows us to take seasonal fluctuations into account and decrease the number of individuals with overlapping body mass.

Here, we provide a predictive logistic equation that will allow researchers to sex Barred Owls accurately with toe-pad length and date-standardized body mass. To apply this model, researchers will need to calculate the date-standardized body mass (Z) for each sampled individual using means and standard deviations in body mass for the appropriate two-month bin (Table 2). This model is expected to facilitate the sexing of Barred Owls with negligible error rates. We do, however, caution the classification of sex when $\mathbf{Pr(S_i)}$ is close to 0.5.

We omitted the three juvenile male Barred Owls from our study owing to the uncertainty regarding whether juvenile toe-pad lengths follow the same pattern as the adult toe-pad lengths. Unfortunately, we were not able to test the equation with the juvenile Barred Owls because their body mass were not measured. However, after analyzing the toe-pad lengths of adults, we determined that toe-pad lengths of the juvenile Barred Owls fell within the range of toe, pad lengths for adult males – and these individuals were confirmed to be males by examination of their gonads by personnel at the California Academy of Sciences. Thus, while we can infer that male juvenile toe-pad lengths fall in the same range as adult male measurements, we expect this is similarly true for juvenile and adult female Barred Owls, but future research is necessary to confirm this hypothesis.

Determining the sex of Barred Owls in the field is crucial to understanding the effects of their range expansion into western North America. Here, we describe a methodology of accurately sexing Barred Owls in the field using toe-pad length and body mass in conjunction. However, we caution that further research may be necessary to determine whether these thresholds are upheld in other populations of Barred Owls that may differ morphologically, such as in eastern North America or other parts of their expanded range in western North America. This methodology could be especially useful in managing Barred Owl populations through lethal removals to benefit Spotted Owl populations, as managers can determine the sex of the individuals immediately after collection and learn more about Barred Owl demographics through observing sex ratios of both colonizing and recolonizing Barred Owls (at sites where removals have occurred previously). Moreover, Barred Owl studies that do not involve the lethal collections but still require sex determination, such as capture-resight or juvenile dispersal studies "(Watson et al. (2023)," will benefit from field-based sex determination methods. Using toe-pad lengths and body mass may be more convenient than using vocalizations, especially for inexperienced observers (Odom and Mennill 2010). It may be more difficult to measure toe-pad lengths on live Barred Owls, but it is feasible with two people: one person to hold the Barred Owl and its toes in place and another person to take the measurement. Accordingly, toe-pad lengths and body mass are reliable measures for determining sex in the field, creating more potential for understanding the life history of Barred Owls and predicting the trajectory of their population expansion.

ACKNOWLEDGEMENTS

We thank Mark Higley for giving us the idea of sexing Barred Owls using toe-pad measurements. We thank Robert Douglas and Mike Stephens for their assistance during Barred Owl removals. We also thank Jack Dumbacher, Moe Flannery, Martha Velez, Daniela Sanchez, Adan Deeb, and Augie Kramer for their assistance with sex determination of Barred Owls at the California Academy of Sciences. Additionally, thank you to R. J. Gutiérrez, Anu Kramer, Emma Fehlker Campbell, William Vince Berigan, Sheila Whitmore, and other members of the Peery Lab for providing helpful comments and suggestions on earlier drafts of this manuscript.

Fig. 1. Measuring toe-pad lengths of Barred Owls lethally collected from Mendocino, Sonoma, and Marin counties in coastal northern California, from 2021 and 2022. The digits are numbered, and the measurements were taken from the hallux, digit 1, to the middle digit, digit 3. (Photo: Emma Fehlker Campbell)

TABLES

Table 1. Summary of the toe-pad lengths and body masses measured from male and female Barred Owls collected from Mendocino, Sonoma, and Marin Counties in California, USA.

Sex	Toe-pad Length (mm)			Body Mass (g)		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Male	62.8	59.6	65.1	731	600	890
Female	67.8	64.5	71.7	926	720	1300

Table 2. The means and standard deviations for body mass per two-month bin, which correspond to the date Barred Owls were collected and measurements taken. This table is meant to aid in body mass standardization for Barred Owls collected or captured in the field.

Two-month Bin	Mean (g)	Standard Deviation (g)
January-February	874	150
March-April	791	133
May-June	733	99
July-August	795	58
September-October	855	98
November-December	886	91

Table 3. Generalized linear modeling results of the four models used to examine the relationships between Barred Owl sex and morphometrics. The covariates were toe-pad length and standardized body mass. k is the number of parameters included in the model and w_i is Akaike's weight of the model.

Model	*k*	ΔAIC_c[a]	w_i
Toe-pad lengths + standardized body mass	3	0.00	0.90
Toe-pad lengths	2	4.43	0.10
Standardized body mass	2	31.31	0.00
Intercept only	1	176.43	0.00

[a]Akaike's information criterion corrected for sample size (AIC_c) of top model was 13.66.

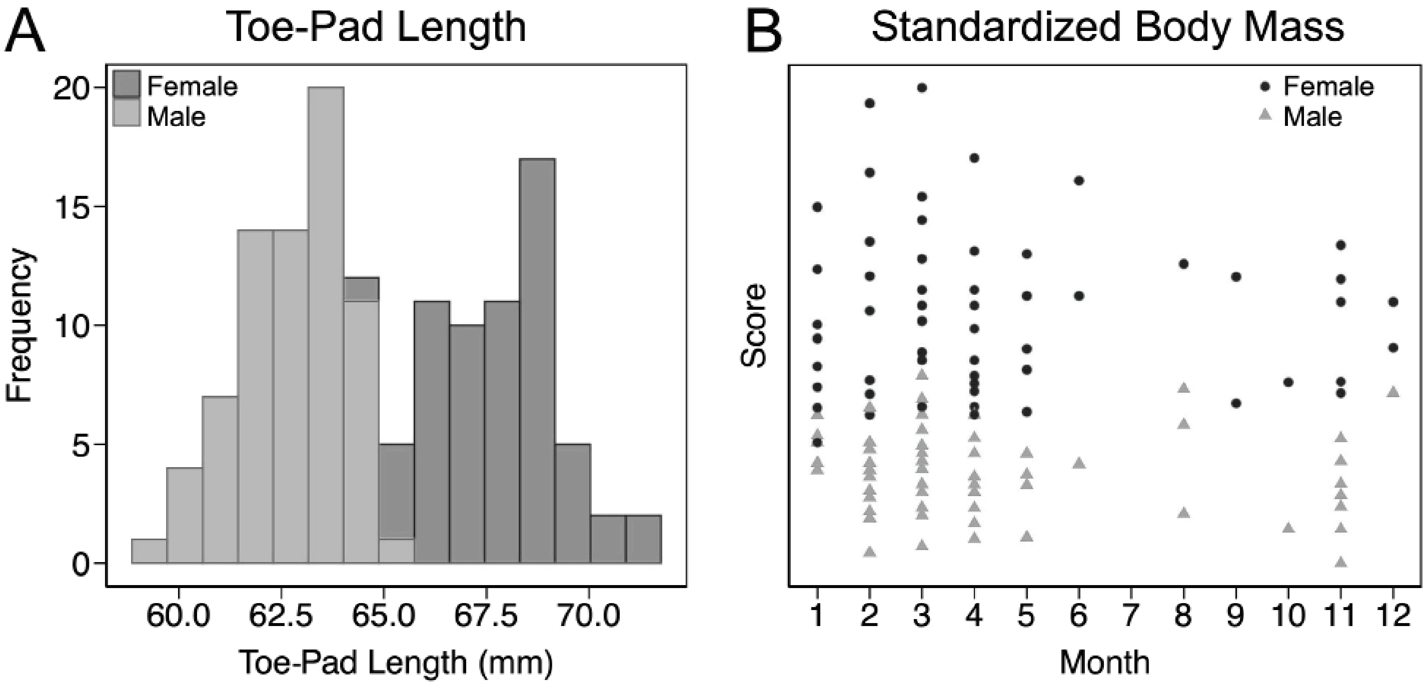

Fig. 2. (A) Toe-pad lengths of 136 Barred Owls and (B) standardized body mass of 135 Barred Owls that were lethally collected through experimental removal studies in Mendocino, Marin, and Sonoma counties in coastal California from 2021 and 2022. All individuals were sexed by analyzing their gonads in a laboratory setting.

LITERATURE CITED

Acker, J.M. 2020. Seasonal weight changes in Barred Owls by sex. *North American Bird Bander* 45: 106 - 111.

Arnold, T.W. 2010. Uninformative parameters and model selection using Akaike's Information Criterion. *Journal of Wildlife Management* 74: 1175 - 1178.

Burnham, K.P. and D.R. Anderson. 2002. Model selection and multimodel inference: A practical information-theoretic approach, 2nd edition. Springer-Verlag, New York, NY.

Diller, L.V., K.A. Hamm, D.A. Early, D.W. Lamphear, K.M. Dugger, C.B. Yackulic, C.J. Schwarz, P.C. Carlson, and T.L. McDonald. 2016. Demographic response of Northern Spotted Owls to Barred Owl removal. *Journal of Wildlife Management* 80: 691 - 707.

Diller, L.V., J.P. Dumbacher, R.P. Bosch, R.R. Bown, and R.J. Gutiérrez. 2014. Removing Barred Owls from local areas: Techniques and feasibility. *Wildlife Society Bulletin* 38: 211 - 216.

Edwards, T.C., and M.N. Kochert. 1986. Use of body weight and length of footpad as predictors of sex in Golden Eagles. *Journal of Field Ornithology* 54: 317 - 319.

Fleming, T.L., J.B. Buchanan, and L.L. Irwin. 1991. Footpad dimorphism as a possible means to determine sex of adult and juvenile Northern Spotted Owls (*Strix occidentalis caurina*). *North American Bird Bander* 16: 66 - 68.

Franklin, A.B., K.M. Dugger, D.B. Lesmeister, R. J. Davis, J.D. Wiens, G.C. White, J.D. Nichols, J.E. Hines, C.B. Yackulic, C.J. Schwarz, S.H. Ackers, L.S. Andrews, L.L. Bailey, R. Bown, J. Burgher, K.P. Burnham, P.C. Carlson, T. Chestnut, M.M. Conner, K. E. Dilione, E. D. Forsman, E. M. Glenn, S. A. Gremel, K. A. Hamm, D.R. Herter, J. M. Higley, R.B. Horn, J. M. Jenkins, W.L. Kendall, D.W. Lamphear, C. McCafferty, T.L. McDonald, J.A. Reid, J. T. Rockweit, D. C. Simon, S. G. Sovern, J. K. Swingle and H. Wise. 2021. Range-wide declines of Northern Spotted Owl populations in the Pacific Northwest: A meta-analysis. *Biological Conservation* 259: 109168.

Hofstadter, D.F., N.F. Kryshak, C.M. Wood, B.P. Dotters, K.N. Roberts, K.G. Kelly, J.J. Keane, S.C. Sawyer, P.A. Shaklee, H.A. Kramer, R. Gutiérrez, and M.Z. Peery. 2022. Arresting the spread of invasive species in continental systems. *Frontiers in Ecology and the Environment* 20: 278 - 284.

Holm, S.R., B.R. Noon, J.D. Wiens, and W.J. Ripple. 2016. Potential trophic cascades triggered by the Barred Owl range expansion. *Wildlife Society Bulletin* 40:615 - 624.

Kochert, M.N. and J.O. McKinley. 2008. Use of body mass, footpad length, and wing chord to determine sex in Swainson's Hawks. *Journal of Raptor Research* 42: 138 - 141.

Kryshak, N.F., E.D. Fountain, D.F. Hofstadter, B.P. Dotters, K.N. Roberts, C.M. Wood, K.G. Kelly, I.F. Schwarcz, P.J. Kulzer, A.K. Wray, H.A. Kramer, J.P. Dumbacher, J.J. Keane, P. A. Shaklee, R.J. Gutiérrez, and M.Z. Peery. 2022. DNA metabarcoding reveals the threat of rapidly expanding Barred Owl populations to native wildlife in western North America. *Biological Conservation* 273: 109678. https://doi.org/10.1016/j.biocon.2022.109678

Livezey, K.B. 2009. Range expansion of Barred Owls, Part I: Chronology and distribution. *American Midland Naturalist* 161: 49 - 56.

Mazur, K.M. and P.C. James. 2021. Barred Owl (*Strix varia*), version 1.1. In *Birds of the World* (A.F. Poole and F.B. Gill, eds.). Cornell Lab of Ornithology, Ithaca, NY.

McCullagh, P. and J.A. Nelder. 1989. Generalized linear models, 2nd edition. Chapman and Hall/CRC, Boca Raton, FL.

Odom, K.J. and D.J. Mennill. 2010. A quantitative description of the vocalizations and vocal activity of the Barred Owl. *Condor* 112: 549 - 560.

Pyle, P. 1997. Flight-feather molt patterns and age in North American owls. *Monographs in Field Ornithology* no. 2. American Birding Association, Colorado Springs, CO.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Steiner, G.,G. Preusse, C. Zimmerer, M.-E. Krautwald-Junghanns, V. Sablinskas, H. Fuhrmann, E.Koch, and T.Bartels. 2016 . Label free molecular sexing of monomorphic birds using infrared spectroscopic imaging. *Talanta* 150: 155 - 161.

Watson, W.A., C.M. Wood, K.G. Kelly, D.F. Hofstadter, N.F. Kryshak, C.J. Zulla, S.A. Whitmore, V.O'Rourke, J.J. Keane, and M. Z. Peery. 2023. Passive acoustic monitoring indicates Barred Owls are established in northern coastal California and management intervention is warranted. *Ornithological Applications* duad017, https://doi.org/10.1093/ornithapp/duad017

Wiens, J.D., K.M. Dugger, J.M. Higley, D.B. Lesmeister, A.B. Franklin, K.A. Hamm, G. C. White, K.E. Dilione, D.C. Simon, R. R. Bown, P.C. Carlson, C.B. Yackulic, J. D. Nichols, J.E. Hines, R.J. Davis, D.W. Lamphear, C. McCafferty, T.L. McDonald, and S.G. Sovern. 2021. Invader removal triggers competitive release in a threatened avian predator. *Proceedings of the National Academy of Sciences* 118: e2102859118, https://doi.org/10.1073/pnas.2102859118

Wiens, J.D., R.G. Anthony, and E.D. Forsman. 2014. Competitive interactions and resource partitioning between Northern Spotted Owls and Barred Owls in western Oregon. *Wildlife Monographs* 185:11 - 50.

Barred Owl
Can Stock Studios