

4-21-2014

Barn Owl Sex Determination

Lillian McKinley

College of Arts and Sciences, Boise State University

Sex Determination of Barn Owls via Morphometric Measurements and Plumage Variation

Lillian A. McKinley

Mentor: Dr. Marc Bechard

Abstract

The Barn Owl (*Tyto alba*) follows the normal birds of prey trend that females are larger in size than males and it has also been reported that female Barn Owls have more brown plumage than male Barn Owls, which tend to be mostly white. However, no studies have attempted to quantify this. In this study, the mass, wing chord, tail length, and plumage of 40 Barn Owls (24 females and 16 males) from Southwestern Idaho were recorded and quantified. I found that the most accurate method of sex determination in Barn Owls is plumage variation. Males generally have less than 20% brown plumage on the ventral side of their bodies, and females generally have over 20% brown plumage on their ventral sides. Mass is the next most accurate measurement for sex determination; the use of plumage and mass together resulted in accurate sex determination of 82.5% of the Barn Owls in this study. The sex of all of the Barn Owls in this study was accurately determined using the model criteria outlined.

Introduction

The sex of birds of prey is usually determined using one of two methods: plumage or size. Plumage is often the most obvious way to tell the difference between male and female birds, because male birds often have bright and colorful plumage and female birds generally have drab and dull colored plumage (Hill 1993). In species that do not have plumage variation, size difference is used for sex determination. The most common measurement of size is body mass, but other measurements can also be used including wing chord, tail length, and footpad.

The Barn Owl (*Tyto alba*) follows this general trend in that females are larger than males, but there is overlap, which often makes sex determination difficult (Amadon 1975). It has also been noted that female Barn Owls seem to have darker (more brown) plumage than the mostly white males, but this variation is usually not quantified (Marti 1990 and Roulin 1999). Some males are nearly pure white and others have abundant small black spots (Marti 1992). Females are usually a darker color than males, and they have larger and more abundant black spots than males. However, the plumage variation completely overlaps between the sexes of Barn Owls (Marti 1992). The present study proposes that if both of these sex determination methods (size and plumage) are quantified and used together, then a model could be developed that would allow for sex determination of all Barn Owls in the field.

Methodology

Forty Barn Owls from Southwestern Idaho were sampled, 26 were suspected to be females and 14 were suspected to be males. The Barn Owls were hand captured from nest boxes in the Treasure Valley area, specifically from Boise, Kuna, Middleton, and Caldwell. After capture, the owls were processed for approximately 15 min. to take measurements and a blood sample. The owls were wrapped in an aba and had their eyes and head covered with a breathable cloth material. To ensure that no Barn Owls were sampled more than once, each owl was banded with a USGS aluminum alloy band.

The measurements taken were mass, wing chord, tail length, and footpad. The mass was taken with a digital scale while the owl was in the aba and had the head covering on. The mass of the aba and head covering was 70 gm, which was subtracted from the total mass, and the net mass was recorded. The wing chord was measured by folding the wing, but leaving the natural curve. Then a ruler was used to measure the distance from the highest point of the wrist joint to

the longest primary feather. The right wing was always measured for consistency. The tail length was measured using a tape measure. A deck feather of the tail was measured from the point at which it emerged from the body to the tip of the vane. The footpad measurement was taken using calipers. The right foot was opened and stretched out, and the total length of the pad was measured between the talons of the hallux and third toe.

For plumage determination the Barn Owls were held in front of a black background and four pictures were taken: front (ventral) wings in, front (ventral) one wing outstretched, back (dorsal) wings in, and back (dorsal) one wing outstretched. The pictures were saved on a SD memory card and labeled appropriately. The pictures were analyzed by printing a copy using photo paper and photo quality ink. Then a piece of acetone paper with a 1cm² grid was laid over the picture. The picture that was used in the calculations for this study was the ventral with wings in. The body/chest of the Barn Owl was outlined in red and all areas of brown plumage were marked/shaded with blue (Fig. 1). The percent of brown plumage was then calculated by taking the total number of grid squares that were shaded blue and dividing that by the total number of grid squares that the Barn Owl's body took up.



Figure 1: Picture of the ventral side of a Barn Owl with wings in. A piece of acetone paper has been laid over the picture and the chest/body of the Barn Owl is outlined and the brown plumage has been shaded in blue.

To verify the sexes of the captured Barn Owls, blood samples were taken from each individual and DNA analysis was done to determine the sex. The blood samples were taken from the right medial metatarsal vein. Approximately 0.5 mL of blood was taken using a one milliliter syringe with a 25 gauge needle. This amount of blood did not exceed 1% body mass of the Barn Owls. Then the blood was spread on a Whatman filter paper card and placed in an appropriately labeled envelope.

The blood samples were analyzed at the Idaho Fish and Games Wildlife Health Lab located in Caldwell, Idaho. First the DNA was extracted from the whole blood using a Qiagen Blood Extraction Kit. The samples that had a low amount of DNA extracted were then concentrated to increase the amount of DNA. Next the DNA was amplified via PCR. The primer pair P2 and P8 was used because it had previously been tested of a variety of bird species (Cerit and Avanus 2007). Twenty-five microliters of each primer and 60 μ L water were added together. This primer mix (1 μ L per sample) and Qiagen Taq mastermix (5 μ L per sample) were combined to form the overall PCR mastermix for this analysis. Then 7 μ L of this PCR mastermix was added to 3 μ L of DNA for each sample in 40 wells of a PCR plate. This plate was then run through a PCR machine to amplify the DNA.

Finally, the PCR products were loaded and run on a thick 5% agarose gel. The agarose was made by adding 100 mL of 1xTAE buffer to 5 gm of agarose, then the solution was mixed and brought to a boil on a spin/hot plate. After boiling 18 μ L of Sybr Green stain was added and the entire solution was poured into a tray with either a 15 or 20 space comb. One to 2 μ L of loading dye was mixed with 3 to 7 μ L of the PCR products for each sample. Controls were made by mixing 1 to 2 μ L of loading dye with 3 to 9 μ L of 100 base pair DNA ladder. The PCR product/loading dye mixtures were pipetted into the wells of the gel and the gels were run at

100V for 1.5 to 2 hours. The gels were then analyzed for bands to indicate ZW (female) or ZZ (male). The W chromosome is slightly larger than the Z chromosome, so the female DNA separated into two bands on the gels and the male DNA stayed as one band. The banding patterns were visualized on a gel box and photographs were taken of the completed gels.

Statistical analysis was performed on plumage variation and all 4 measurements to determine the degree of difference between the sexes. The statistical test used was a two-tailed t-test with unequal variance (Samuels and Witner 1999). An alpha value of 0.05 was used; with $p < 0.05$ indicating a statistically significant difference and $p > 0.05$ indicating no difference between the sexes.

Results

Using a two-tailed t-test all of the morphometric measurements and plumage showed significant difference ($p < 0.05$) between the sexes, with females always being larger than males. However, every variable had some degree of overlap. Plumage had the largest statistical difference ($t = 2.06$, $p = 1.66 \times 10^{-9}$, $n = 40$) and least amount of overlap (Fig. 2).

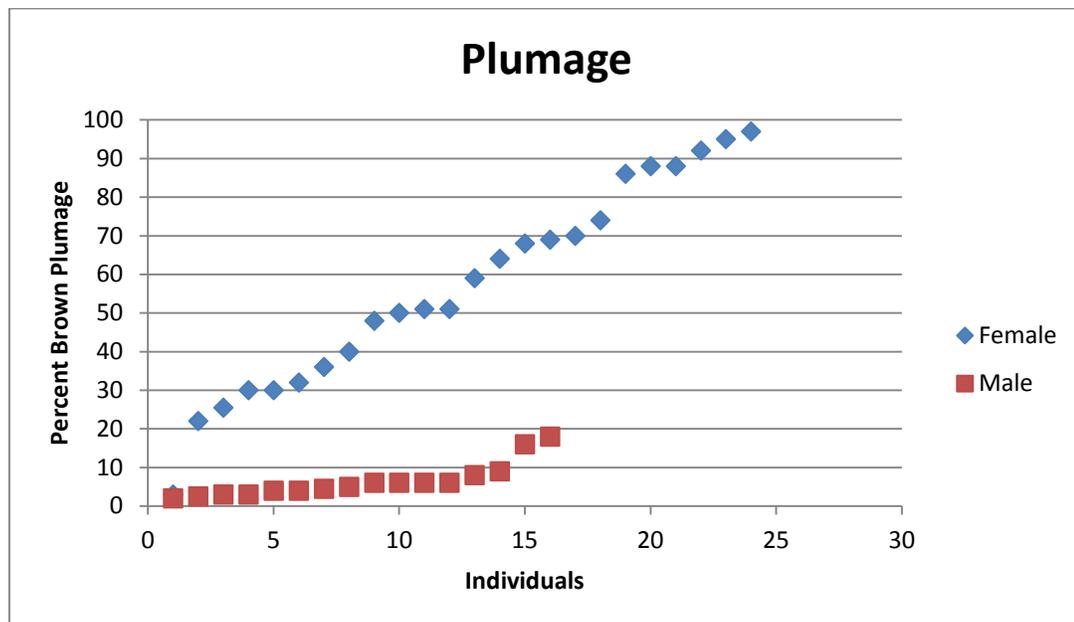


Figure 2: Percent of brown plumage of each individual Barn Owl categorized by sex.

The mean percent brown plumage for male Barn Owls was 6% and all 16 male Barn Owls had less than 20% brown plumage. The mean percent brown plumage for females was 57% and 23 of 24 female Barn Owls had more than 20% brown plumage (Table 1).

Females	97%	95%	92%	88%	88%	86%	74%	70%	69%	68%
	64%	59%	51%	51%	50%	48%	40%	32%	30%	30%
	30%	25.5%	22%	3%						
Males	18%	16%	9%	8%	6%	6%	6%	6%	5%	4.5%
	4%	4%	3%	3%	2.5%	2%				

Table 1: Percent of brown plumage on the chest of each of the forty Barn Owls in this study (using the picture of the ventral side with wings in).

The values for mass showed some overlap, but the two-tailed t-test showed statistically significant difference between males and females ($t=2.03$, $p=9.68 \times 10^{-7}$, $n=40$) (Fig. 3). All but 2 of the male Barn Owls in this study had a mass that was under 500 grams; and of the 26 females only 3 had a mass under 500 grams. Seven of the 26 female Barn Owls were even over 600 grams.

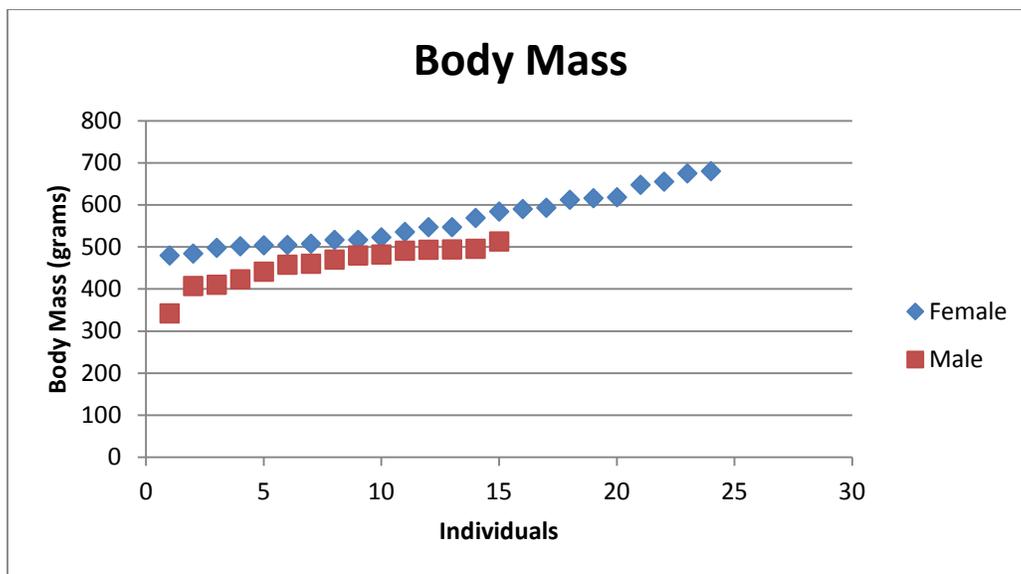


Figure 3: Body Mass of male versus female Barn Owls ($n=40$).

Of the remaining morphometric measurements, footpad was also statistically significant difference ($t= 2.05$, $p=0.0013$, $n=40$), but there was more overlap between the sexes (Fig. 4).

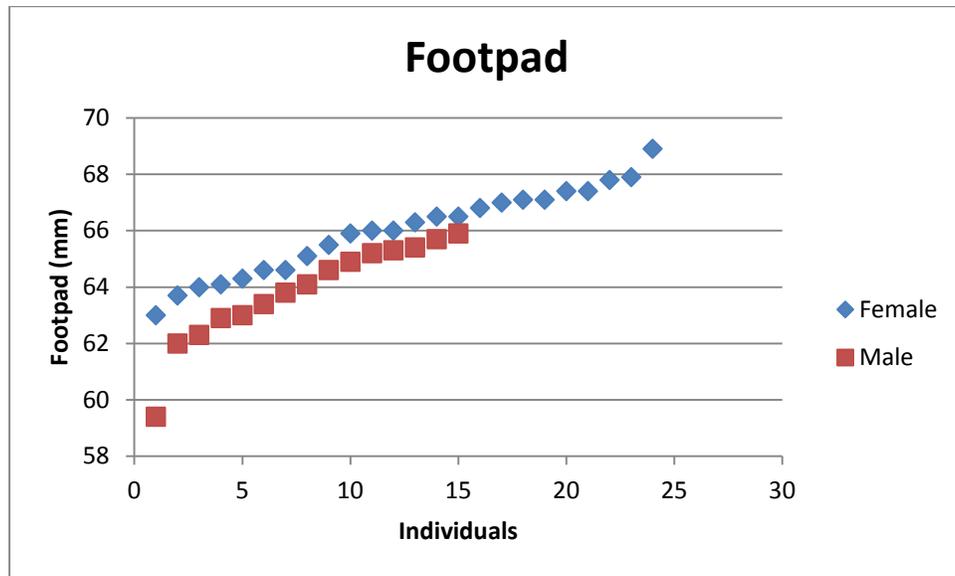


Figure 4: Graph of the difference in footpad, between the 40 male and female Barn Owls in this study.

Tail length was also significantly different ($t=2.03$, $p=0.0028$, $n=40$), but there was again considerable overlap (Fig. 5).

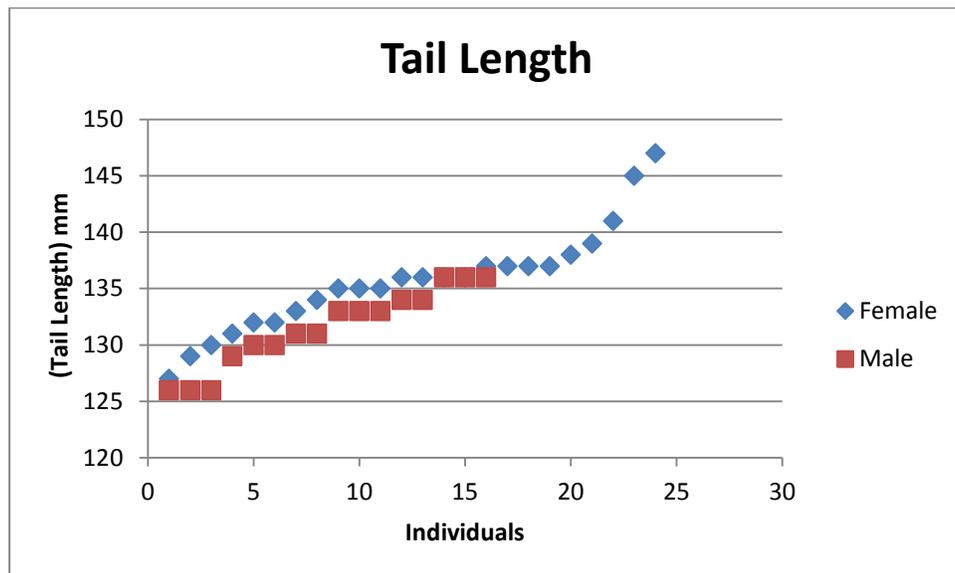


Figure 5: Graph of the tail length difference between male and female Barn Owls.

The final morphometric measurement, wing chord, was still significantly different ($t=2.06$, $p=0.0073$, $n=40$). This measurement had the least significance of all of the morphometric measurements taken in this study. This resulted in the highest degree of overlap between the sexes, but still females were generally larger than males (Fig. 6).

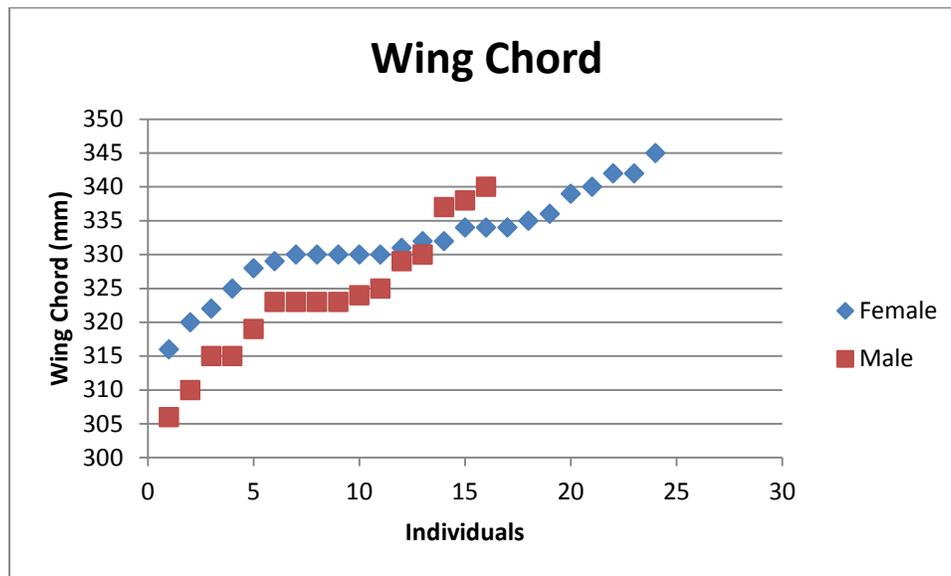


Figure 6: Wing Chord of the forty Barn Owls in this study, categorized by sex.

From the five variables, I made a model of sex determination using the criteria outlined in Table 2.

Table 2: Sex Determination Model Criteria					
	Plumage (%)	Mass (gm)	Footpad (mm)	Wing Chord (mm)	Tail Length (mm)
Female	>20%	>500	>65	>327	>134
Male	≤20%	≤500	≤65	≤327	≤134

Table 2: The criteria used to determine the suspected sex of all 40 Barn Owls sampled.

The model in Table 2 allowed for an accurate determination of sex for all Barn Owls in this study that I could confirm through DNA analysis. This ended up being 33 samples, because, 7 samples produced blanks in the gels due to low DNA concentrations (Fig. 7).

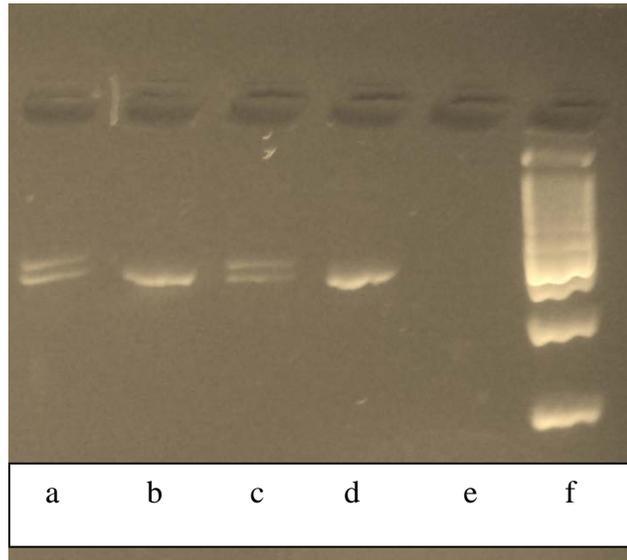


Figure 7: Banding patterns of the gels. Wells from left to right: (a) two bands (female), (b) one band (male), (c) two bands (female), (d) one band (male), (e) no banding (too low concentration), (f) DNA ladder.

Discussion

My study reinforces the general trend that female Barn Owls have significantly larger mass than male Barn Owls. It also shows that females have significantly larger wing chords, tail lengths, and footpads than males. This contradicts the results of other studies that showed there is too much overlap for there to be any significant difference in morphometric measurements other than mass (Taylor 2011, Marti 1990). However, my study did find significant difference between all the morphometric measurements and was able to use them to accurately determine the sex of the Barn Owls.

My study also reinforces previous research that female Barn Owls have darker plumage (more brown) and male Barn Owls have more white plumage (Marti 1990, Roulin 1999, Roulin et al. 2008, Van den Brink et al. 2011). The trend that females have larger and more abundant spotting was observed in this study, which reinforces previous studies done by Roulin et al. (2008) and Taylor (2011). However, the focus of the present study is on the overall plumage coloration not the spottiness.

The main finding of my study was the quantification of the plumage coloration in Barn Owls. It shows that all (16 of 16) male Barn Owls had less than 20% brown plumage on their chests, and that most female Barn Owls (23 of 24) had at least 20% brown plumage on their chest. The majority of female Barn Owls have over 57% brown plumage on their chests. This plumage variation is very obvious when observing and capturing Barn Owls. Other researchers had previously published the trend that female Barn Owls had darker plumage than male Barn Owls, but the plumage coloration was rarely quantified.

My study shows that plumage is a very good method of sex determination for Barn Owls. This method has the most significance and highest accuracy of all variables used to determine sex. Mass is the next best measurement to use for sex determination in Barn Owls with a high accuracy. The majority of females weighed over 500 grams and the majority of males weighed less than 500 grams. By just using plumage and mass, the sex of 82.5% of the Barn Owls could be easily and accurately determined. This is less accurate than Carl Marti, who reported 97% accuracy using just mass and plumage (1992). With a larger sample size the percentage in the present study would likely be closer to the latter value.

The remaining morphometric measurements (wing chord, tail length, and footpad) have substantially less accuracy, but are still significantly different between the sexes. These measurements should not be used alone for sex determination of Barn Owls, but can be used in conjunction with plumage or mass. These remaining morphometric measurements were analyzed for the 7 Barn Owls in which sex was not definitely determined with my mass and plumage model. The sex was assumed to be whatever category the majority (3 out of 5) of the measurements fell into. This allowed for accurate sex determination of 100% of the Barn Owls in the present study.

Acknowledgements

I would like to thank James McKinley for providing assistance with field work and sampling. Also, I would like to thank the staff, especially Tricia Hosch-Hebdon, at the Idaho Fish and Game Wildlife Health and Forensics Laboratory for providing the use of their lab equipment and support and assistance with the DNA analysis portion of this study.

Literature Cited

- Amadon, D. (1975). Why are female birds of prey larger than males? *Journal of Raptor Research*. 9:1-11.
- Cerit, Harun and Kozet Avanus. Sex Determination CHDW and CHDZ Genes of Avian Sex Chromosomes in *Nymphicus hollandicus*. *Turkey Journal of Veterinary Animal Science*. 31(6):371-374.
- Hill, Geoffrey E. (1993). Male Mate Choice and the Evolution of Female Plumage Coloration in the House Finch. *Evolution*. 47(5):1515-1525.
- Marti, Carl D. (1990). Sex and Age Dimorphism in the Barn Owl and a Test of Mate Choice. *The Auk*. 107(2):246-254.
- Marti, Carl D. (1992). Barn Owl. *The Birds of North America*. (1):1-16.
- Roulin, Alexandre. (1999). Nonrandom pairing by male barn owls (*Tyto alba*) with respect to a female plumage trait. *Behavioral Ecology*. 10(6):688-695.
- Roulin, A., Gasparini, J., Bize, P., Ritschard, M., and Richner, H. (2008). Melanin-Based Colorations Signal Strategies to Cope with Poor and Rich Environments. *Behavioral Ecology and Sociobiology*. 62(4):507-519.
- Samuels, Myra and Jeffery Witmer. (1999). *Statistics for the Life Sciences*. New Jersey: Prentice Hall Publishing.

Taylor, Iain R. Age and sex determination of barn owls *tyto alba alba*. *Ringing and Migration*.
14(2):94-102.

Van den Brink, V., Dolivo, V., Falourd, X., Dreiss, A., and Roulin, A. (2011). Melanic color-dependent antipredator behavior strategies in barn owl nestlings. *Behavioral Ecology*.
23:473-480.