# Sexing Adult and Nestling Red-Shouldered Hawks Using Morphometrics and Molecular Techniques

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# SEXING ADULT AND NESTLING RED-SHOULDERED HAWKS USING MORPHOMETRICS AND MOLECULAR TECHNIQUES[1]

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ABSTRACT.—Sexing of raptors is important for understanding their ecology and demography. Males and females of monomorphic species such as Red-shouldered Hawks (*Buteo lineatus*) may be distinguished using molecular and morphometric techniques. We collected blood samples and morphometric measurements from adult and nestling Red-shouldered Hawks in southern Ohio. We determined sex via amplification of the sex-linked chromo-helicase-DNA-binding gene and polymerase chain reaction. We used a suite of morphometric measurements to generate a recursive partitioning classification tree and in a linear discriminant analysis to determine the sex of adults and nestlings. For adults, the recursive partitioning tree utilized only mass to distinguish sexes, with an overall successful classification rate of 94%. For nestling hawks aged approximately 3 wk and older, mass and toepad (footpad) length were used to distinguish the sexes, with an overall successful classification rate of 91%. The ability to sex adults and nestlings in the field is valuable for studies of dispersal, survival, and behavior.

KEY WORDS: *Red-shouldered Hawk*; Buteo lineatus; *morphometrics*; *nestling*; *raptor*; *sexing*.

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## DETERMINACIÓN DEL SEXO DE INDIVIDUOS DE *BUTEO LINEATUS* ADULTOS Y PICHONES USANDO TÉCNICAS MORFOMÉTRICAS Y MOLECULARES

RESUMEN.—La determinación del sexo en rapaces es importante para entender su ecología y demografía. Los machos y las hembras de especies monomórficas tales como *Buteo lineatus* pueden ser diferenciados utilizando técnicas moleculares y morfométricas. Colectamos muestras de sangre y tomamos medidas morfométricas de adultos y pichones de *B. lineatus* en el sur de Ohio. Determinamos el sexo por medio de la amplificación del gen de unión de ADN cromo-helicasa ligado al sexo y de la reacción en cadena de la polimerasa. Usamos un grupo de medidas morfométricas para generar un árbol de clasificación de partición recursiva y un análisis de discriminación lineal para determinar el sexo de adultos y polluelos. Para los adultos, el árbol de partición recursiva sólo utilizó la masa para distinguir los sexos, con una tasa total de clasificación exitosa del 94%. Para los pichones de halcón de aproximadamente 3 semanas de edad y mayores, la masa y el largo de la almohadilla de los dedos (almohadilla plantar) fueron utilizados para diferenciar los sexos, con una tasa total de clasificación exitosa del 91%. La capacidad para determinar el sexo de adultos y polluelos en el campo es valiosa para estudios de dispersión, supervivencia y comportamiento.

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Many raptors have monomorphic plumage, with reversed sexual size dimorphism to varying degrees. Accipiters are among the most dimorphic of the families, with males less than two-thirds the mass of females in some species (Ferguson-Lees and Christie 2001). Many *Buteo* species are moderately dimorphic; Red-tailed Hawk (*Buteo jamaicensis*) males average 82% of the size of females, and Red-shouldered Hawks (*B. lineatus*) 85% (Ferguson-Lees and Christie 2001).

The ability to sex raptors is important to our understanding of their ecology. The two sexes of a raptor species may have different prey bases, dispersal distances, migration patterns, survival rates, and parental behaviors. For example, female Eurasian Sparrowhawks (*Accipiter nisus*) have greater natal dispersal distances than males (Newton and Marquiss 1983), as well as greater breeding dispersal distances (Newton 2001). Being able to distinguish the sexes at the initial handling of a bird allows a more detailed and precise description of these and other ecological measures when the bird is subsequently observed.

Modern molecular techniques (Dubiec and Zagalska-Neubauer 2006) allow accurate sexing using DNA. However, morphometric sexing can be efficiently done in the field, and is less expensive, less time-consuming, and less invasive than molecular sexing. Morphometric sexing can be done easily by researchers with limited funding and limited access to technology, in field situations where molecular sexing may be impractical. In addition, if large numbers of birds must be sexed in order to gain statistically significant sample sizes, as in many banding studies where there is a low recovery/encounter rate (e.g., 4–5%, Dykstra et al. 2004), then the costs of DNA sexing may be prohibitive, whereas the cost of morphometric sexing is negligible.

The suburban population of Red-shouldered Hawks in southwestern Ohio has been studied for more than 15 years, and much is known about its ecology (Dykstra et al. 2000, 2003, 2009). Red-shouldered Hawks in this study area are year-round residents, with nest-site selection and reproductive rates similar to those in nearby rural, forested habitat (Dykstra et al. 2000). Natal dispersal for a sample of these birds averaged 18 km (Dykstra et al. 2004), but it could not be determined whether male and female dispersal distances and mortality rates differed, because the sexes could not be distinguished at banding and fewer than half the birds were sexed when encountered or recovered. The ability to reliably sex these hawks, including the nestlings, would provide greater insight into the ecology of these suburban birds.

Our objective in this study was to determine whether adult and nestling Red-shouldered Hawks could be effectively sexed using morphometric measurements, and if so, to derive techniques for sexing them in the field.

METHODS

**Study Areas.** We studied Red-shouldered Hawks in two regions of southern Ohio. The southwestern Ohio study area (SWOH), in Hamilton, Clermont, and Warren counties in the suburbs of Cincinnati, is composed of residences surrounded by lawns and nonnative plantings, interspersed with small areas of natural forest, which are dominated by second-growth mixed mesophytic, oak-hickory (*Quercus* spp., *Carya* spp.) and American beech-sugar maple (*Fagus grandifolia, Acer saccharum*) associations.

The Hocking Hills study area (HH) in southeastern Ohio comprises portions of Wayne National Forest, Hocking State Forest, Zaleski State Forest, and associated private lands in Athens, Hocking, Vinton, and Perry counties. The predominant forest type is oak-hickory. Plantations of white pine (*Pinus strobus*) and red pine (*P. resinosa*) also are common.

**Measurements of Adult Hawks.** We trapped adult hawks in the SWOH study area using a bal-chatri trap baited with a mouse (*Mus musculus*), between 23 February 2008–12 July 2009, with most of the birds trapped during the winter months. We banded adults with USGS aluminum bands (banding permit #23352) and weighed them using a 1000-g Pesola scale to the nearest 5 g. We measured the following physical characteristics: bill length (culmen length) to the nearest 0.1 mm, toepad length (termed ''footpad length'' by some researchers) from the base of central talon to the base of the hallux to the nearest mm, length of the first and second secondary to the nearest mm, wing chord (unflattened) to the nearest mm, and the tarsus length from the intertarsal joint to the distal end of the final leg scale before the toes (Pyle 1997, 2008) to the nearest 0.1 mm. Bill length and tarsus were measured with a digital caliper (Swiss Precision Instruments, Garden Grove, California, U.S.A.), toepad and secondaries with a standard 33-cm ruler, and wing chord with a metal meterstick. For toepad, talons were extended as in Bortolotti (1984a; measurement termed ''footpad'' therein). We then collected

a blood sample, 0.1–0.3 ml, by puncturing the brachial vein with a sterile needle, and collecting the blood in 1–2 micropipettes. Blood samples were immediately transferred to 1.5-ml vials containing Queen's Lysis Buffer solution (Seutin et al. 1991), kept in a cooler until the end of the day ($<$10 hr), and then frozen until DNA extraction.

We also made the same measurements on, and collected blood from, birds that were submitted alive to a local raptor rehabilitation organization. For such birds, mass was measured immediately after the bird was submitted, before any treatment or feeding. We did not measure mass if the bird had been admitted with a condition that may have affected body mass (e.g., dehydration, starvation), based on the assessment of qualified raptor rehabilitators.

Finally, we also sampled birds found dead within the study area, which were then frozen until data collection. For these birds we snipped samples of breast muscle tissue for molecular sexing. As above, measurements were omitted if there was evidence of conditions that might influence them.

**Measurements of Nestlings.** Red-shouldered Hawk nest locations were previously known to us from an ongoing long-term study (Dykstra et al. 2000, 2004). We climbed to 28 nests in southwestern Ohio and 21 nests in Hocking Hills between 19–28 May 2008 and 17 May–22 June 2009 to band young. Nestlings were banded at approximately 2–5 wk old, and fledge when approximately 6–7 wk old (Dykstra et al. 2008). We measured the same physical characteristics for nestlings as described for adults (above), and collected and handled blood samples as for adults.

**Molecular Sexing.** Genomic DNA was isolated from blood samples by standard phenol-chloroform-isoamyl alcohol isolation, precipitated in 7.5 M ammonium acetate and 70% ethanol, washed with 70% ethanol, and resuspended in Tris-EDTA (pH 8.0). Sex was determined via amplification of the sex-linked chromo-helicase-DNA-binding (CHD) gene following a modified protocol from Han et al. (2009). P2 (TCTGCATCGCTAAATCCTTT), P8 (CTCCCAAGGATGAGRAAYTG) and P0 (ATTGAGTTGGAACCAGAICA) primers were used to amplify the CHD-W and CHD-Z alleles. Polymerase chain reaction (PCR) was performed in 10 µl reactions with Fidelitaq™ Master Mix (Affymetrix/ USB, Santa Clara, California, U.S.A.), 1.25 mM of additional MgCl$_2$, 0.5 µM of each of three primers (P0, P2, P8) and 2.5 µl of a 1:20 dilution of genomic DNA in Tris-EDTA (pH 8.0). PCR cycles were conducted on a Veriti Thermal Cycler (Applied Biosystems, Carlsbad,

California, U.S.A.) under cycling conditions of 94°C for 4 min followed by 30 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C, and then a final extension cycle for 10 min at 72°C. PCR products were separated on 2.0% agarose TBE gels stained with ethidium bromide and run for approximately 100 volt-hours. Gels were visualized on a 320-nm light box and photographed with a digital camera (UVP PhotoDoc-IT™ Imaging System, UVP, Upland, California, U.S.A.). Males express the homogametic condition with a single ≈400 bp band and females the heterogametic with two bands, one ≈400 bp band shared with males corresponding to the Z-linked allele and a second ≈500 bp band corresponding to the W-linked allele. Sex determination was made for each individual sample by scoring digital photographs by eye.

**Statistical Analysis.** For adults, we used Kruskal-Wallis tests to compare mass and morphometric measurements of birds submitted to the rehabilitation center or found dead to those of birds caught in the wild on a bal-chatri trap, to determine whether the conditions that caused the birds to be brought for rehabilitation (e.g., injury, etc.) affected their masses and/or other measurements. Males and females were tested separately.

For nestlings, mean secondary length was assumed to be an index to nestling age, based on Penak (1982), who showed that growth of secondaries in Red-shouldered Hawk nestlings was independent of brood size. We divided the nestlings into two groups, those with mean of first and second secondary of 78–118 mm (''younger nestlings'') and those $>$118 mm (''older nestlings''). According to the findings of Penak (1982), mean secondaries of 78–118 mm corresponded to nestlings age 21–29 d, and mean secondaries $>$118 mm corresponded to nestlings ≥30 d in Quebec. Nestlings with mean secondary length $<$78 mm were excluded from further analysis. We ignored any year-to-year variation in nestling growth and mass and combined data from both years, because in this case such variation is useful for the development of a sexing method that is robust enough to distinguish males and females despite variation in local prey availability, weather conditions, and/or the number of young in the nest. In addition, incorporating year effects would have reduced our statistical power unacceptably.

Statistics were performed in R (R Development Core Team 2008). We tested all variables for normality by the Lilliefors (Kolmogorov-Smirnov) test. Variables with non-normal distribution were transformed using a log-transformation before inclusion

Table 1. Mean morphological measurements for adult Red-shouldered Hawks in southwestern Ohio, 2008–09. Sex determined by molecular sexing techniques. Bonferroni-corrected $P$-value = 0.008.

| MEASUREMENT | MALES MEAN $\pm$ SD ($n = 21$) | FEMALES MEAN $\pm$ SD ($n = 15$) | $F$ | $P$ |
|---|---|---|---|---|
| Mass (g) | 573 $\pm$ 55 | 768 $\pm$ 104 | 53.40 | <0.001 |
| Mean of 1st and 2nd secondary (mm) | 181 $\pm$ 5 | 197 $\pm$ 5 | 87.23 | <0.001 |
| Toepad (mm) | 65 $\pm$ 2 | 71 $\pm$ 2 | 64.74 | <0.001 |
| Wing chord (mm) | 327 $\pm$ 8 | 348 $\pm$ 8 | 58.20 | <0.001 |
| Tarsus length (mm) | 71 $\pm$ 3 | 74 $\pm$ 3 | 5.99 | 0.02 |
| Bill length (mm) | 22 $\pm$ 1 | 24 $\pm$ 1 | 55.24 | <0.001 |

in statistical tests. MANOVA was performed for all variables, using log-transformed variables where needed. Mean secondary length was not used for sexing because it was used as an index to nestling age. We generated a recursive partitioning tree from the scaled data for toepad length, tarsus length, mass, bill length, and wing chord using the rpart package in R.

Linear discriminant analysis was performed on the scaled data using the MASS package lda function in R. Toepad length, tarsus length, mass, bill length, and wing chord were included in the discriminant function. Two resampling methods, a jackknifed classification and a randomized sample validation, were used to assess the validity of the linear discriminant analysis (McGarigal et al. 2000). Data were scaled to have an equal variance of 1 using the R scale function for recursive partitioning and linear discriminant analyses. We conducted separate analyses for adult birds, all nestlings, and the younger nestlings (mean secondaries 78–118 mm).

RESULTS

We collected blood samples and made measurements for 45 adult or subadult Red-shouldered Hawks, and 86 nestlings. For nestlings, 14 birds were young (mean of first and second secondary that was <78 mm), so these were excluded from our analysis. DNA extraction and/or PCR failed for six adults and three of the remaining nestlings, so these were also excluded. At least one measurement was missing for two nestlings, which were excluded, leaving a final nestling dataset of 67 nestlings. Adults lacking one or more measurement were also excluded, leaving a final dataset of 36 adults/subadults (35 in adult plumage, one hatch-year in December), 30 of which were trapped with a bal-chatri, five submitted alive to the rehabilitation organization, and one found dead.

Masses and all other measurements of the birds submitted to the rehabilitation center or found dead did not differ significantly from those of birds trapped on a bal-chatri in the wild (Kruskal-Wallis test; all $P > 0.05$) and fell within 1 SD of the sample mean for all birds, so these birds were included in the dataset.

**Adult Red-shouldered Hawks.** Only bill length deviated significantly from a normal distribution (D = 0.1917, $P = 0.002$); wing chord was also transformed because of non-normality when all nestlings were combined (D = 0.13, $P = 0.01$ for all nestlings; D = 0.13, $P = 0.16$ for adults). Morphometrics of adult males and females differed significantly (MANOVA, Wilks = 0.18, $F_{1,34} = 21.98$, $P < 0.001$). Statistically significant differences between males and females were found for toepad length, mean secondary length, bill length, mass, and wing chord ($n = 36$, Bonferroni corrected $P$-value = 0.008, Table 1).

For adults, the recursive partitioning tree utilized only mass and resulted in a single split at 623.5 g (Fig. 1a). Two males (of 21; 10%) were misclassified as females while no females (of 15) were misclassified, resulting in an overall successful classification rate of 94%. Linear discriminant analysis for sex in adults returned an overall 97% correct (93% correct for females and 100% correct for males). The jackknifed classification matrix returned an overall 92% correct classification (80% correct for females and 100% for males). Randomized sample validation found the adult discriminant function resulted in an overall increase in predictive power of 31% (47% increase in predictive power for females and 19% increase for males).

**Nestling Red-shouldered Hawks.** For the younger nestlings alone ($n = 47$), no variables differed significantly from a normal distribution (Lilliefors test); however, bill length and wing chord were transformed for symmetry with the adults' data.
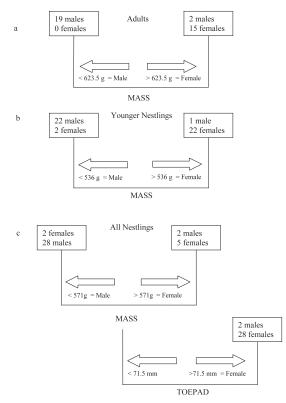
Figure 1. Recursive partitioning decision tree for (a) adult, (b) younger nestling, and (c) all nestling Red-shouldered Hawks in southern Ohio.

Morphometrics of these males and females differed significantly (MANOVA, Wilks = 0.23, $F_{1,45}$ = 21.77, $P < 0.001$). Male and female younger nestlings differed significantly in toepad length, bill length, and mass (Bonferroni corrected $P$-value = 0.008, Table 2). For older nestlings alone ($n = 20$), no variables differed significantly from a normal distribution (Lilliefors test); bill length and wing chord were log-transformed for symmetry with other data. Morphometrics for older nestlings differed significantly by sex (MANOVA, Wilks = 0.21, $F_{6,13}$ = 8.01, $P < 0.001$), with females having greater mass and toepad length (Table 2).

For the younger nestlings, a recursive partitioning tree was generated from scaled nestling data for toepad length, tarsus length, mass, bill length, and wing chord using the rpart package in R. The recursive partitioning tree generated from the younger nestling data also utilized only mass and resulted in a single split at 536 g (Fig. 1). Two females (of 24; 8%) were misclassified as males

while a single male (of 23; 4%) was misclassified as a female.

A separate linear discriminant analysis was performed on the scaled nestling data. As in the adult analysis, toepad length, tarsus length, mass, bill length, and wing chord were included in the discriminant function. Linear discriminant analysis for younger nestlings returned an overall 98% correct (96% correct for females and 100% correct for males). A jackknifed classification matrix for sex returned an overall 96% correct classification with 96% correct for both sexes. Compared to a linear discriminant function derived from a randomized sample, this discriminant function resulted in an overall increase in predictive power of 35% (30% increase in predictive power for females and 40% increase for males).

For the older nestlings, we had too few samples ($n = 20$) to generate a recursive partitioning tree or conduct linear discriminant analysis. Older nestlings were included in the combined nestling dataset.

In the combined dataset for all nestlings ($n = 67$), wing chord deviated significantly from normal distribution (Lilliefors test, D = 0.13, $P = 0.01$); thus, wing chord was log-transformed before inclusion in the linear discriminant analysis and the recursive partitioning tree. For symmetry with adult analyses, bill length was also log-transformed. Linear discriminant analysis and recursive partitioning included mass, toepad, wing chord, bill length, and tarsus length for the combined nestling dataset. The linear discriminant analysis for all nestlings returned an overall 97% correct (97% correct for both sexes). A jackknifed classification matrix for sex returned overall 97% correct classification and 97% correct for both sexes. Compared to a linear discriminant function derived from a randomized sample, this discriminant function resulted in an overall increase in predictive power of 36% above chance (29% increase in predictive power for females and 43% increase for males).

For all nestlings combined, the recursive partitioning tree utilized only mass and toepad in discriminating among the sexes. An overall accuracy of 91% was achieved with these variables, with two females misclassified as males (of 35; 6%), and four males misclassified as females (of 32; 13%; Fig. 1).

The analyses for all nestlings together and for younger nestlings alone had similar statistical significance. Thus, for younger nestlings, either classification tree

Table 2.   Mean morphological measurements for nestling Red-shouldered Hawks in southwestern Ohio, 2008–09. Sex determined by molecular sexing techniques. Younger nestlings were defined as those with mean of first and second secondaries between 78–118 mm, older nestlings as those with mean of >118.5 mm. Bonferroni-corrected $P$-value = 0.008.

| MEASUREMENT | YOUNGER MALES MEAN ± SD ($n = 23$) | YOUNGER FEMALES MEAN ± SD ($n = 24$) | $F$ | $P$ | OLDER MALES MEAN ± SD ($n = 9$) | OLDER FEMALES MEAN ± SD ($n = 11$) | $F$ | $P$ |
|---|---|---|---|---|---|---|---|---|
| Mass (g) | 494 ± 28 | 578 ± 41 | 66.48 | <0.001 | 575 ± 43 | 640 ± 33 | 14.81 | 0.001 |
| Mean of 1st and 2nd secondary (mm) | 96 ± 12 | 96 ± 11 | 0.002 | 0.97 | 137 ± 10 | 135 ± 14 | 0.19 | 0.67 |
| Toepad (mm) | 69 ± 2 | 73 ± 2 | 39.28 | <0.001 | 69 ± 2 | 74 ± 1 | 45.22 | <0.001 |
| Wing chord (mm) | 175 ± 15 | 175 ± 13 | <0.001 | 0.99 | 227 ± 15 | 225 ± 19 | 0.073 | 0.79 |
| Tarsus length (mm) | 71 ± 3 | 72 ± 3 | 1.65 | 0.21 | 73 ± 2 | 75 ± 2 | 6.94 | 0.02 |
| Bill length (mm) | 17 ± 1 | 18 ± 1 | 9.89 | 0.003 | 18 ± 1 | 19 ± 1 | 4.95 | 0.04 |

may be used for sexing. For older nestlings, the ''all nestlings'' classification tree (Fig. 1c) must be used. Because mass and toepad were the variables utilized for sexing all nestlings in the recursive partitioning tree, we plotted the relationship of these two variables for all nestlings (Fig. 2).

### DISCUSSION

We determined that adult male and female Red-shouldered Hawks in southern Ohio could be distinguished using mass alone. For adults, mass was also the most dimorphic of the traits we measured, with average male mass only 75% of average female mass. Mass is easy to measure and is commonly measured by most banders, even when other measurements are not taken.

We also determined that nestlings older than approximately 3 wk could be sexed using a combination of mass and toepad length. For nestlings, toepad is also relatively easy to measure and has good repeatability (Bortolotti 1984a) and reproducibility (C. Dykstra and J. Hays unpubl. data). The analysis for younger nestlings alone and that for all nestlings combined were similarly significant, despite the fact that a larger range of masses and ages was included in the ''all nestlings'' analysis. We believe this occurred because the toepad of nestlings was apparently fully grown or nearly so by 3 wk of age, as evidenced by the similarity of mean toepad length in older and younger nestlings (Table 2). With toepad nearly full grown, many nestlings were separable by that feature alone (Fig. 1; 28 females identified by toepad size alone, with only 2 males misclassified as females).

Comparison of adult and nestling means suggested that even the older nestlings (aged approximately 30–40 d) had not reached adult size in terms of secondary length, wing chord, and bill length, as means for these measures were greater in adults than in older nestlings. Nestling females were lighter in mass than adult females, but older nestling males' masses equaled those of adult males. Conversely, toepad lengths were significantly greater in older nestlings than in adults ($t$-test; $t = 5.528$, $P < 0.001$; $n = 36$ adults and $n = 20$ older nestlings). Repeated measurements on individual birds that were measured as nestlings and later as adults confirmed that toepad decreased as the bird aged (average of $3.5 \pm 0.7$ mm, $n = 10$, considering only nestlings measured at approximately 3 wk or older; C. Dykstra and J. Hays unpubl. data). Bortolotti (1984a) also found that mean adult eagles' toepads were smaller than those of older nestlings; however, he attributed the difference to the difficulty of extending the toes of mature eagles, as adult birds clenched their toes tightly. We do not believe this is a significant problem for Red-shouldered Hawks, which also clench their toes tightly, but are much smaller and weaker than Bald Eagles. Published information on this topic is scarce, and further data are needed to elucidate the causes of an apparent decrease in toepad length with age.

Juvenile Red-shouldered Hawks in California were successfully sexed using wing chord and mass (Pitzer et al. 2008) to differentiate males and females. However, the birds in California represent a different subspecies, *Buteo lineatus elegans*, which is generally smaller than eastern Red-shouldered Hawks, *B. l. lineatus* (Wheeler 2003). For example,
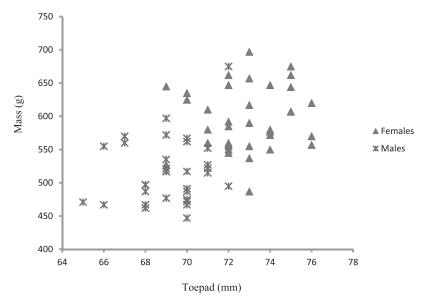
Figure 2. Relationship between mass and toepad for all Red-shouldered Hawk nestlings age approximately 3 wk or older (mean of first and second secondaries >78 mm; see text for details).

mean mass of the juvenile California birds measured by Pitzer and colleagues (2008) was $467 \pm 8$ (SE) g and $542 \pm 25$ g for males in the Marin headlands north of San Francisco and California's Central Valley, respectively, and $582 \pm 14$ g and $656 \pm 18$ g for females in those locations, compared to mean masses of 573 g and 768 g for adult males and females in our study (Table 1). Due to the size difference, we would not expect criteria from the California birds to be directly applicable to birds of the eastern subspecies. It is probable that each subspecies will require an independent confirmation of sexing criteria for its region. The fact that we and Pitzer et al. (2008) were able to distinguish the sexes based on a suite of measurable characteristics suggests that there is sufficient size differentiation between sexes of Red-shouldered Hawks that they should be distinguishable at other locations as well. A meta-analysis of morphometric sexing for all the subspecies would be interesting and informative.

For other raptor species, various combinations of morphometrics have been used to sex adult birds. For Swainson's Hawks (*Buteo swainsoni*) wintering in Argentina, measurements of the forearm, tail, and wing chord discriminated males and females (Sarasola and Negro 2004). Red-tailed Hawk juveniles in California were separated using tarsus depth, hallux claw, and wing chord, whereas adults were

distinguished using tarsus depth, culmen, and wing chord (Pitzer et al. 2008). In the intermountain west, Donohue and Dufty (2006) found that wing chord, mass, hallux, and culmen could be used to sex juvenile Red-tailed Hawks, and wing chord and mass to sex adults. For Golden Eagles (*Aquila chrysaetos*), hallux claw and culmen length differed between males and females (Bortolotti 1984b).

Nestling *Buteo* hawks have rarely been sexed morphometrically. However, morphometric equations were developed for Bald Eagle (*Haliaeetus leucocephalus*) nestlings (Bortolotti 1984a) and have been widely applied in nestling banding for this species. In Bald Eagles, the combination of toepad and bill depth was used to distinguish the sexes. In addition, nestling Booted Eagles (*Hieraaetus pennatus*) nearing fledging age were successfully sexed using the combination of tail length, forearm length, bill, and tarsus length (Balbontín et al. 2001).

An important contribution of our study is the ability to sex nestling Red-shouldered Hawks. We could only sex nestlings older than approximately 3 wk old and some mid-size nestlings could not be sexed with 100% accuracy. However, the ability to sex most nestlings will greatly improve our capacity to describe dispersal and survival patterns for this species, in addition to offering greater insight into behavior in studies of nestling aggression and development.

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