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DETERMINATION OF SEX USING MORPHOMETRICS IN THE NORTHERN WATERTHRUSH (*PARKESIA NOVEBORACENSIS*) AND SWAINSON'S THRUSH (*CATHARUS USTULATUS*)

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ABSTRACT.—Many species of passerines are sexually monochromatic and thus sex cannot be determined based on plumage characteristics. Northern Waterthrushes (*Parkesia noveboracensis*) and Swainson's Thrushes (*Catharus ustulatus*) are two such species. The objective of this study is to examine morphological differences between males and females of both species and determine whether this information may be used to determine sex. With genetically sexed birds, I indicate wing chord values can be used to determine sex with 95% confidence. This information would allow field researchers to determine sex of 58% of Northern Waterthrushes and 33% of Swainson's Thrushes with 95% confidence of correct assignment. If age is taken into account, the proportion of individuals for which sex can be determined increases to 62% and 38%, respectively. This information may be used by avian ecologists in future studies of behavioral ecology, conservation biology, or evolutionary biology these species. *Received 1 December 2014. Accepted 24 March 2015.*

Key words: genetic sex determination, sexual dimorphism.

Sex may be determined in many passerine species based on vocalization, plumage, size, or external breeding characteristics (e.g., brood patch and cloacal protuberance; Pyle 1997). However, it is often impossible to determine the sex of sexually monochromatic species outside of the breeding season. Alternative methods such as laparotomy or genetic techniques are available to determine the sex of these individuals. In the past, researchers who needed to know the sex of a monochromatic species used laparotomy and subsequent visual inspection of the gonads which has been done in both laboratory-based and field-based studies (e.g., Bailey 1953, Wingfield and Farner 1976, Ketterson and Nolan 1986, Romero et al. 1997). If done correctly, laparotomies seem to have little or no effect on the physical heath and behavior of birds (Piper and Wiley 1991). However, standards for the methods used when conducting these procedures have become more stringent and require specialized equipment making it difficult to conduct in the field (see Fair et al. 2010).

Many avian species can be sexed based on differences between the intron size of the chromobox-helicase-DNA (CHD) binding genes on the W chromosome and the Z chromosome (Griffiths et al. 1998, Jensen et al. 2003). Therefore, when amplified via Polymerase Chain Reaction (PCR) and viewed using gel electrophoresis, males being the homogametic sex show just one band because both introns are the same size. Females, on the other hand, are the heterogametic sex and thus show two bands. This simplified method works only in those species with different intron sizes; however, alternative methods are available that may be used in other cases (e.g., Han et al. 2009).

The Northern Waterthrush (*Parkesia noveboracensis*) and the Swainson's Thrush (*Catharus ustulatus*) are sexually monochromatic species and additional efforts are required to sex individuals. Eaton (1957) described intraspecific variation in various body size measurements of breeding Northern Waterthrushes. Females had on average smaller wing, tail, culmen, and tarsus lengths than males. Most notably, Eaton (1957) showed that, compared to other measurements, wing chord overlapped the least between the sexes. Using tower kills during fall migration in Florida, Hicks (1967) quantified wing length of male and female Swainson's Thrushes that were sexed by visual inspection of the gonads. Males were generally larger, but there was substantial overlap between sexes likely as a result of limited sample size ($n = 31$). Although both studies provided valuable information, they failed to indicate how this information might be applied by other researchers for determining sex in the field. Collectively, these studies indicate that size dimorphism may be useful to determine sex in both these species but that additional research is necessary for this information to be useful for avian ecologists.

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The objective of this study was to analyze the morphometric differences between male and female Northern Waterthrushes and Swainson's Thrushes that were genetically sexed as part of a larger study on circulating testosterone levels in migrants (Covino et al. 2015). My goal was to utilize observed patterns in morphological measurements and determine whether these may be used to predict the sex of these two species. Ultimately this would allow avian ecologists to determine sex in future studies of these species without having to conduct additional sampling.

METHODS

Migrating Northern Waterthrushes and Swainson's Thrushes were captured during their spring migration from 2011–2014 at Johnson's Bayou, Louisiana (29° 45′ N, 93° 37′ W), and Appledore Island, Maine (42° 58′ N, 70° 36′ W). After capture via mist-net, birds were banded with a USGS aluminum leg band, and measurements of wing chord (unflattened wing length; nearest 0.5 mm), tail length (nearest 0.5 mm), and tarsus length (nearest 0.1 mm) were taken by one of eight experienced bird banders. Age was determined as either second-year (SY) or after-second-year (ASY) based on flight feather wear and shape, amount of edging on the primary coverts, and/or the presence of a molt limit within covert sets (see Pyle 1997). Occasionally a bird's age was recorded as the less specific age class of after-hatching-year (AHY) if we were unable to determine age resulting from conflicting amounts of wear on the rectrices and remiges or to an intermediate amount of edging present on the primary coverts. From these individuals, a small blood sample (~100 μl) was collected into heparinized capillary tubes (#22-362-566, Fisher Scientific, Pittsburgh, PA, USA) from the brachial vein and samples were kept on ice until centrifuged. The plasma and cellular portions were separated, and the cellular portion was suspend in approximately 500 μl of blood lysis buffer (50 mM TRIS, 10 mM EDTA, 1% SDS, 0.1 M NaCL), and stored at −20 °C until DNA extraction.

I extracted the DNA using a DNeasy Tissue Kit (#69506, QIAGEN Inc., Valencia, CA, USA) following standard protocol for nucleated erythrocytes. PCR was used to amplify the CHD binding gene, different versions of which are found on the W and Z chromosomes of birds (Griffiths et al. 1996, 1998). I used the P2 (5′- TCTGCATCGCTAAATCCTTT-3′) and P8 (5′ - CTCCCAAGGATGAGRAAYTG - 3′) primers for amplification. Samples were processed using a Pure TAQ Ready-To-Go PCR Bead (#27-9557-01, Amersham Biosciences Corp., Piscataway, NJ, USA), 5 μl DNA, 2 μl of each primer, and 16 μl distilled water. PCR cycle was as follows: 60 sec at 95 °C, then 30 cycles of 60 sec at 95 °C, 60 sec at 50 °C, and 60 sec at 72 °C followed by a 3 min final extension at 72 °C, and holding at 4°. PCR products were run on a 1.5% or 2% agarose gel, stained with ethidium bromide, and visualized under UV light. Samples with one band at 325 bp were determined to be male and those with two bands, one each at 325 and 375 bp, were determined to be female.

To validate combining data from my two study locations, I used Cohen's D to investigate the effect size difference for each body size measurement by sex. With the combined dataset I tested for morphometric differences between males and females with general linear models (GLM) for each of the three body size measurements. Age was also incorporated into these models as an independent variable since feather wear may differ in songbirds depending on their age, however those individuals that could only be aged as AHY were excluded from these analyses. I performed linear discriminant function analyses to determine the accuracy of classifying birds to the appropriate sex by their morphometric measurements. Following the Bird Banding Laboratory's 95% reliability rule for sexing, I indicate the range of wing chord measurements for which sex can be determined in each of these two species by wing chord alone as this is the most common and most reliable measurement taken by field ornithologists. Analyses were performed using the statistical software package R version 3.1.0 (R Core Team 2014).

RESULTS

A total of 223 Northern Waterthrushes and 339 Swainson's Thrushes were sexed using genetic analyses (Table 1). There was a higher proportion of male Northern Waterthrushes sampled in Louisiana (63%) while the majority of individuals sampled in Maine were female (66%). The numbers of male and female Swainson's Thrushes sampled at each location were similar, 56% male in Louisiana and 51% male in Maine. There was only a small amount of non-overlap in the morphometrics of each sex between our two sampling locations ($D < 0.3$) thus data from the two sites were pooled in all subsequent analyses.

TABLE 1. Age and sex of the Northern Waterthushes and Swainson's Thrushes captured at two sampling locations during spring migration.

Species	Location	Sex (*n*)	% AHY	% SY[a]
Northern Waterthrush	Louisiana	Male (72)	49%	51%
		Female (40)	40%	59%
	Maine	Male (38)	16%	41%
		Female (73)	12%	75%
Swainson's Thrush	Louisiana	Male (136)	9%	42%
		Female (106)	5%	57%
	Maine	Male (49)	4%	51%
		Female (48)	2%	47%

[a] Of those who could be aged more precisely than AHY

Sex Differences in the Northern Waterthrush.— Overall, ASY Northern Waterthrushes were larger than SY birds and males were larger than females (Table 2; Fig. 1A). Wing and tail lengths of males were significantly longer than in females (wing: $t = 8.7$, df = 156, $P < 0.0001$; tail: $t = 4.2$, df = 135, $P < 0.001$), but tarsus length was not different between the sexes ($t = 0.69$, df = 167, $P = 0.49$). Wing chord was the only body size measure that was significantly different between age groups, with ASY birds being the larger of the two (wing: $t = 2.3$, df = 156, $P = 0.025$; tail: $t = 2.0$, df = 135, $P = 0.05$; tarsus: $t = 1.2$, df = 137, $P = 0.24$).

Using wing chord classified 86% of all Northern Waterthrushes to the correct sex. This classification was not improved by the addition of tarsus length and tail length into the model (83% correctly classified). After excluding all birds with wing chords between 72 mm and 75.5 mm, the proportion correctly classified increased to 96% (Table 3). While the exclusion of individuals with intermediate wing chords allows a greater than 95% correct classification rate for sex, it left only 58.3% of individuals for which this assignment could be made. When age was also taken into consideration, the number of individuals not excluded increased slightly to 61.8% of those birds for which age could be determined (see Table 3 for percent assignment by age).

Sex Differences in the Swainson's Thrush.— ASY Swainson's Thrushes were larger than SY individuals and males larger than females (Table 2; Fig. 1B). Similar to the Northern Waterthrush, wing and tail length of male Swainson's Thrushes were significantly longer than in females (wing: $t = 11.5$, df = 319, $P < 0.0001$; tail: $t = 7.8$, df = 269, $P < 0.0001$; tarsus: $t = 1.4$, df = 272, $P = 0.15$) while wing chord was the only measurement that was significantly different between ASY and SY individuals (wing: $t = 2.8$, df = 319, $P = 0.01$; tail: $t = 1.6$, df = 269, $P = 0.11$; tarsus: $t = 0.40$, df = 272, $P = 0.69$).

There was a greater amount overlap in the wing chord between male and female Swainson's Thrushes compared to Northern Waterthrushes (Fig. 1). Only 79% of Swainson's Thrushes would be correctly classified to the appropriate sex using wing length measurements. The ability to correctly classify Swainson's Thrushes by sex was not improved by including tarsus and tail length into the analysis (77% correctly classified). By excluding individuals with wing chords between 93 mm and 100 mm, a 97.3% correct classification rate was achieved (Table 3). Based on this rule, 67% of the population would fall into the intermediate wing chord range and thus sex could only be assigned for 33% of all Swainson's Thrushes.

TABLE 2. Summary statistics of body size measurements in Northern Waterthrushes and Swainson's Thrushes.

Species		Wing chord (mm)		Tarsus length (mm)		Tail length (mm)	
		n	Mean (± SE)	*n*	Mean (± SE)	*n*	Mean (± SE)
Northern Waterthrush	Sex						
	Male	110	75.9 (0.19)	88	20.8 (0.08)	85	51.9 (0.22)
	Female	113	72.1 (0.16)	105	20.7 (0.06)	103	49.4 (0.16)
	Age						
	SY	97	72.9 (0.23)	89	20.8 (0.07)	87	49.7 (0.20)
	ASY	60	75.2 (0.33)	49	21.1 (0.09)	49	51.1 (0.30)
Swainson's Thrush	Sex						
	Male	185	98.9 (0.18)	153	27.6 (0.13)	151	69.3 (0.25)
	Female	154	94.9 (0.19)	129	27.0 (0.30)	128	65.8 (0.26)
	Age						
	SY	155	96.2 (0.24)	128	27.3 (0.31)	126	66.9 (0.30)
	ASY	165	97.9 (0.24)	145	27.3 (0.13)	144	68.4 (0.29)

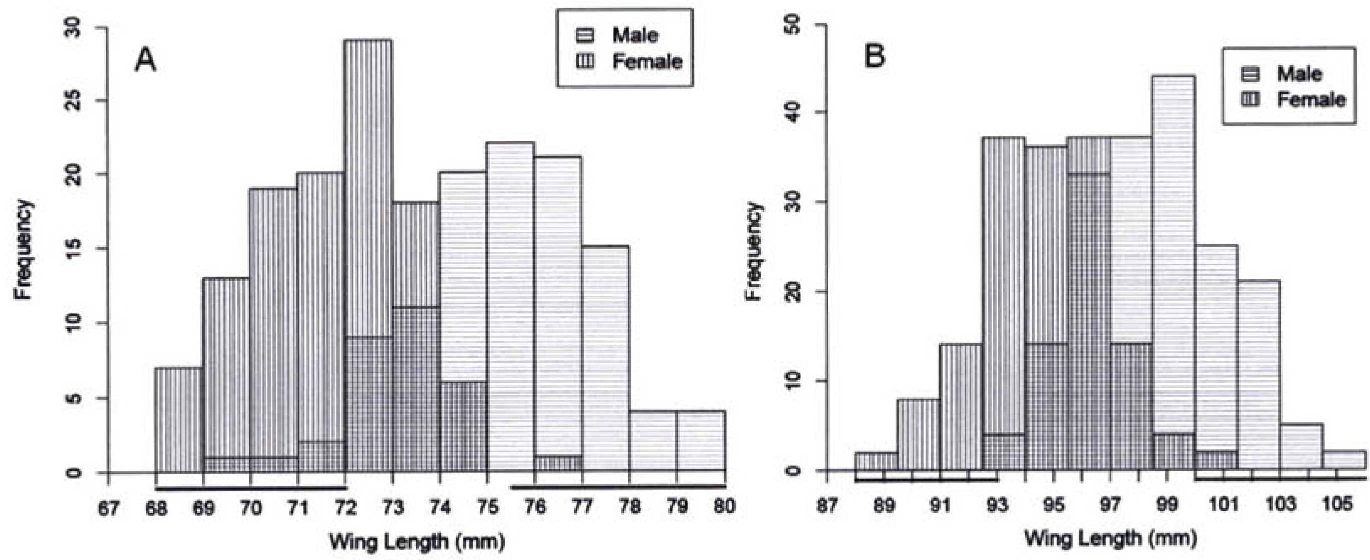

FIG. 1. Histograms of wing chord for male (horizontal stripes) and female (vertical stripes) Northern Watherthrushes (A) and Swainson's Thrushes (B). Birds were sampled during spring migration from April through June of 2011–2014 at two sites: Johnson's Bayou, Louisiana and Appledore Island, Maine. Crossed areas indicate overlap between the sexes. The presence of the horizontal bars below the x-axis indicates when wing chord may be used to determine sex with 95% accuracy.

This proportion could be increased to 37.5% of individuals for which age could be determined when age is also taken into account (See Table 3 for assignment percentage by age).

DISCUSSION

In many songbird species, measures of body size overlap between males and females which prevents researchers from determining sex of individuals based on size dimorphism. The majority of male Northern Waterthrushes, however, are substantially larger than females, thus allowing for accurate sex determination for well over half of all individuals. Although there is more overlap in wing chord between male and female Swainson's Thrushes, my results demonstrate that sex may be determined based on wing chord for at least one-third of all individuals. Generally, these results indicate that researchers can accurately (with greater than 95% confidence) determine sex in most Northern Waterthrushes and many Swainson's Thrushes using wing chord. Even though genetic sexing is not prohibitively expensive (estimated laboratory costs are US$6–$7/individual), this information is beneficial for avian ecologists since measuring wing chord has no additional cost and requires less training.

These results are similar to previous studies indicating that male Northern Waterthrushes and Swainson's Thrushes are larger than females. Similar to the North American Bird Banding Manual (USFWS and CWS 1977) which indicates that Swainson's Thrushes can be determined male if their wing chord is 99 mm or greater, my data suggest that birds whose wing chord is 100 mm or greater can be determined male with greater than 95% confidence. My results for female Swainson's Thrushes differ from the Bird Banding Manual such that my study puts the cutoff at 93 mm and less while the manual uses 90 mm and below (USFWS and CWS 1977). This difference may result from the likelihood that my study only

TABLE 3. Wing chord ranges for which sex can be correctly determined with 95% confidence.

	Northern Waterthrush				Swainson's Thrush			
	Male (mm)	Female (mm)	% correctly classified	% assignable[a]	Male (mm)	Female (mm)	% correctly classified	% assignable[a]
All Age Groups	>75.0	<72.5	96.2%	58.3%	>99.5	<93.5	97.3%	33.0%
ASY	>75.0	<73.0	97.6%	60.0%	>99.5	<95.0	96.1%	46.7%
SY	>74.0	<72.5	96.4%	62.8%	>100.0	<93.5	95.3%	27.7%

[a] Calculated based on the total number of individuals in each group.

included the Olive-backed subspecies (*C. u. swainsoni*) while the Bird Banding Manual reports combined data for both "eastern" and "western" birds, which likely includes at least two subspecies. Additionally, the sample size for Swainson's Thrushes in this study was very large ($n = 339$) but the Bird Banding Manual fails to indicate what sample sizes were used to draw conclusions (USFWS and CWS 1977). My wing chord data for males and females of both Northern Waterthrushes and Swainson's Thrushes are similar to those reported in Pyle (1997).

This study on Northern Waterthrushes and Swainson's Thrushes indicates a higher confidence for determining sex using wing chord alone rather than utilizing additional measures of body size. Measuring wing chord is likely more reliable than tarsus of tail measurements when quantifying body size in songbirds (Gosler et al. 1998). If not done properly, tarsus measurements may produce high inter-measurer variability due to incorrect placement of the calipers being used and learning proper tool placement seems to be more difficult for tarsus measurements compared to measurements of wing chord (KMC, pers. obs.). While I cannot rule that out in this study, the mean tarsus lengths were very similar between males and females of both species which likely also contributed to the lack of use for this measurement in differentiating between the sexes. Similarly for tail length, inconsistencies may result from improper ruler placement or slight differences in how the bird is held during measurement (KMC, pers. obs.). Measurements used in this study were taken by eight different bird banders and this may have contributed to these results.

Determining sex in wild birds may be important for studies of breeding ecology as well as studies outside the breeding season. The timing or distance of migratory routes may differ for males and females (*sensu* Ketterson and Nolan 1983) and thus important insights into these aspects of a species' biology may only be possible if researchers know the sex of the birds they are studying. However, conservative estimates reveal that field researchers are unable to determine sex on the majority of passerine species based on physical characteristics: 51% of North American passerines (Pyle 1997) and 57% of 334 passerines in Reeve and Pfennig (2003). Future studies of monochromatic species in which sex is determined genetically or by other means should publish morphometric data to allow for their use in determining sex without the need for additional efforts.

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