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Tools and Technology

A Morphometric Model to Predict the Sex of Virginia Rails (*Rallus limicola*)

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ABSTRACT Virginia rails (*Rallus limicola*) are secretive marsh birds found in freshwater wetlands across much of North American. There is currently no known way to differentiate between the sexes in the field. We suggest the use of morphometric discriminant analysis as an effective method to separate males and females. We compared the length of the culmen, tarsus, wing chord, and middle toe of live birds captured at Ottawa National Wildlife Refuge (Ottawa Co., OH) during the springs of 2002–2011 and museum specimens measured the summer of 2011. We genetically determined the sex of a subset of samples using an intronic region of the chromo-helicase-DNA-binding gene on the Z and W chromosomes. For live birds, 81% of males and 70% of females were classified correctly; and for museum specimens, 71% of males and 80% of females were classified correctly. This technique provides an accurate and simple method of determining Virginia rail sex that can contribute to efforts to better understand population demographics. © 2013 The Wildlife Society.

KEY WORDS dimorphism, morphometrics, museum specimens, non-invasive, Ottawa National Wildlife Refuge, *Rallus limicola*, sex differences.

The ability to monitor population demographics is a critical step in understanding species and developing targeted conservation priorities. Without demographic information, managers cannot track changes over time or adapt policy regulations in a sustainable way (Mills 2007). This monitoring can be achieved through a variety of methods (e.g., mark–recapture, point sampling, radio telemetry), but regardless of what approach is used, understanding demographic parameters of a population is critical. Determining the sex ratio in a population is important to understanding breeding and survivorship dynamics as well as whether or not habitat management actions differentially affect sexes, and whether sexes exhibit different susceptibility to environmental stressors or harvesting. For many bird species, the sex ratio is easy to observe because males and females look different based on plumage characteristics, but not all species are sexually dimorphic in this way.

The Virginia rail (*Rallus limicola*) is a secretive marsh bird species that occurs in freshwater mudflats and vegetated areas with low water levels and dense vegetation across most of North America; the species is part of approximately 50% of bird species in the world that are not sexually dimorphic through plumage (Griffiths et al. 1998). Currently, there is no mechanism available to determine an individual Virginia rail's sex other than to sample tissue and analyze DNA. This process is invasive, time-consuming, and costly, making it undesirable and prohibitive to many managers. Hence, there is a need to develop alternative means to determine Virginia rail sex ratios to support resource management and conservation efforts.

The Virginia rail's role as a game species across much of its range underscores this need. Although Virginia rails are currently listed as a species of least concern at a national and international level, declines since the 1980s have been observed across their range and the cause of their decline is unclear (Conway and Eddleman 1994, IUCN 2013). Declines could be attributed to the loss of palustrine wetlands, but there are likely other contributing factors (Tiner 1984, Conway 1995, Poole et al. 2005). Hunting pressure is thought to have decreased since the early 1900s, but there is currently no accurate monitoring of birds harvested or population demographics (Raftovich et al. 2012). There is thought to be considerable migratory overlap between the sexes, which makes collecting demographic data on hunted birds additionally challenging because hunting seasons occur during the autumn when there are no visible breeding characteristics (Conway and Eddleman 1994, Conway 1995). The development of an effective, non-invasive, low-cost method to determine sex will help managers better understand and monitor potential demographic differences in these declines and guide conservation planning (Sayre and Rundle 1984).

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Many species of birds have been shown to be sexually dimorphic in ways other than visible plumage characteristics. Size-based dimorphism is common, with males typically being larger than females (Weimerskirch et al. 2009). When gross size and obvious plumage are not sufficient to differentiate sex, morphometric modeling has proven useful in determining the sex of many bird species, including king, clapper, and Iberian water rails (*R. elegans*, *R. longirostris*, *R. aquaticus*; Perkins et al. 2009, Fuertes et al. 2010). For example, exposed culmen has been important for sex determination in a wide range of bird species, including several waterbirds (Herring et al. 2008, 2010; Fuertes et al. 2010). Wing chord (the length of the closed, uncompressed wing) is also commonly used to differentiate between the sexes of a variety of bird species (Ryan et al. 1989, Pyle 1997, Brady et al. 2009, Fuertes et al. 2010). Tarsus length is often used in waterbirds, along with the combination of the middle toe (Ryan et al. 1989, Gucking et al. 2004, Fuertes et al. 2010). These morphometric measurements are easy to record and well-suited for inclusion in citizen-science and hunter collection programs. The objective of this study was to develop a predictive model to determine a Virginia rail's sex based on morphometric analyses of field and museum data, and then test the model with DNA-derived sex data.

STUDY AREA

Live birds were captured at Ottawa National Wildlife Refuge. Ottawa National Wildlife Refuge was a 3,600-ha wetland complex managed by the U.S. Fish and Wildlife Service in Ottawa and Lucas counties, Ohio, USA. Virginia rails used this complex as stop-over habitat during spring and autumn migration and also for breeding throughout the summer. Black Swamp Bird Observatory has monitored Virginia rail populations at Ottawa National Wildlife Refuge since 2001 to determine migratory timing in the region.

MATERIALS AND METHODS

Sample Collection

Live birds.—We captured adult birds at Ottawa National Wildlife Refuge using live traps based on Kearns et al. (1998) during the springs of 2002–2011. We opened the traps each year from 15 March and through late May. We checked the traps once daily shortly after dawn and all birds captured were banded and all measurements taken by the same person. We collected one primary feather from each bird for genetic analysis. Of the birds captured, 52 samples were randomly selected and the sex of each was determined using genetic analysis of a size difference in an intronic region in (chromo-helicase-DNA-binding; CHD) genes occurring on the W and Z sex chromosomes (Griffiths et al. 1998).

Museum specimens.—One individual, who was trained by the person who did the live bird measurements, measured all the museum specimens, from the following U.S. collections: University of Michigan Museum of Zoology, Ann Arbor, Michigan; Michigan State University Museum of Natural History, East Lansing, Michigan; The Field Museum, Chicago, Illinois; Cleveland Museum of Natural History, Cleveland, Ohio; and the Smithsonian Museum of Natural History, Washington, D.C.

We measured museum specimens of both adult and juvenile birds. Juvenile and adult birds were separated by breast color. Adults exhibit a largely unmarked rusty breast, while juveniles possess a white and dark brown or gray mottled chest (Conway 1995; Fig. 1). Specimens exhibiting intermediate plumage were not included in this study. Birds that had no known sex, or birds with tags that indicated that sex was not determined through dissection were omitted as well. Individuals with incomplete sets of measurements because of broken or missing specimen parts were also not included in the analysis.

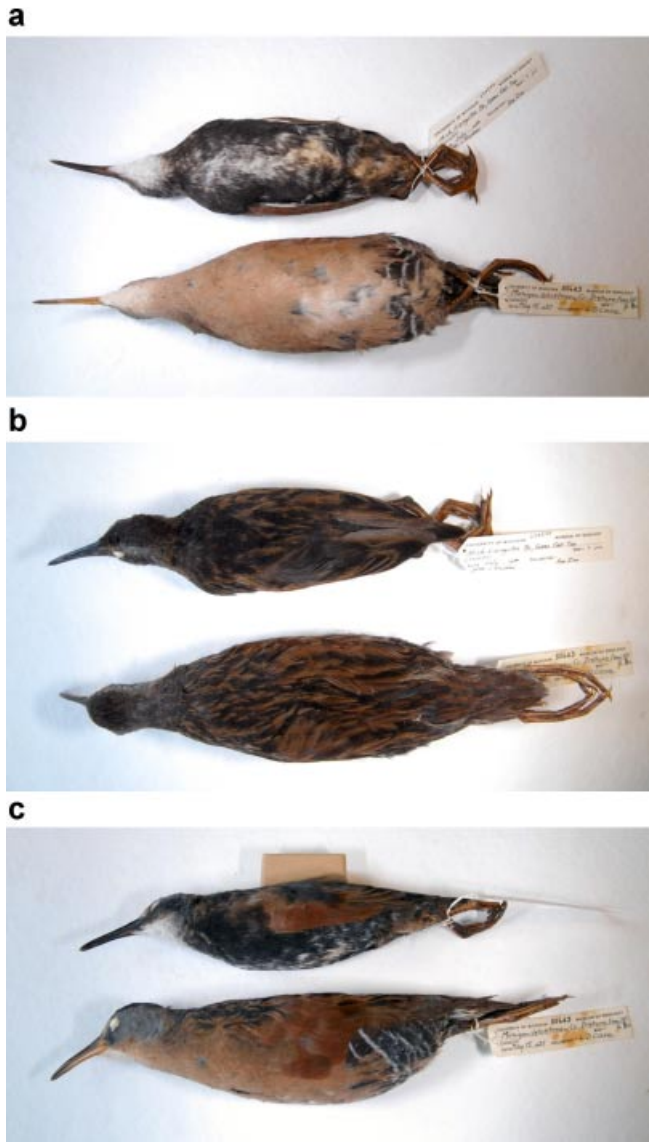

Figure 1. Age-related differences in Virginia rail (*Rallus limicola*) chest plumage color. Juvenile above adult in each panel: (a) ventral view; (b) dorsal view; and (c) side view.

Morphometric Measurements

Exposed culmen, tarsus, middle toe, and wing chord were measured to the nearest mm on each bird. Wing chord was measured on the naturally folded wing, starting at the curve of the wrist and extending to the longest primary feather. The joints at either end of the tarsus were bent to 90° and the length measured from the outside of the joint to the outside of the joint. We carefully bent a pipe cleaner to the same dimensions of the leg for birds where this was not possible. The middle toe was extended straight and measured from the inside of the right side of the toe to the beginning of the toenail. Exposed culmen was measured from the tip of the bill to the farthest bit of exposed upper culmen. These measurements were chosen for ease of repeatability and their use in determining the sex of other species of birds (Helms and Drury 1960, Phillips 1975, Perkins et al. 2009).

Analysis

Genetic.—We randomly selected a subset of the feathers of live-birds for genetic sex determination. We extracted DNA from primary flight feathers using Qiagen DNeasy tissue extraction kits (QIAGEN, Inc., Valencia, CA). An intron in the CHD gene on both the Z and W sex chromosomes from each individual was amplified following Griffiths et al. (1998). Size differentiation by agarose gel was not possible because of only a 3-base-pair difference between Z and W amplified fragments, similar to king and clapper rails (Perkins et al. 2009). Therefore, we fluorescently labeled the P8 primer from Griffiths et al. (1998) and determined fragment size using an ABI PRISM 3100 Genetic Analyzer scored with Genemapper v.4.1 based on the ROX 350 internal size standard (Life Technologies Corp., Carlsbad, CA).

Statistical.—Analysis of variance (ANOVA; Tukey–Kramer honestly significant difference test) was used to compare morphometric measurements between positively identified female and male Virginia rails. Unless otherwise noted, sample groups did not exhibit heteroscedasticity. Step-wise discriminant analysis with equal prior probabilities was used to determine whether sex could be differentiated accurately using the morphometric measurements (Afifi and Clark 1996, Perkins et al. 2009). We conducted all analyses using JMP 10® software (SAS Institute, Inc., Cary, NC). These analyses were done separately for field and museum samples. Museum samples were further grouped by age as juvenile or adult. All live-captured birds were adults as they were captured in the spring before the breeding season. We developed discriminant functions for both live-captured and museum specimens, with a randomly selected group of half of the samples, and we used the other half for cross-validation to produce an unbiased assessment of the discriminant function. Percentages of correct classification were used as measures-of-goodness for the classification procedure.

RESULTS

In the live sample group ($n = 52$) genetic sexing identified 20 adult females and 32 adult males. Museum specimens totaled 286 samples, with 125 adult male; 128 adult female, 20 juvenile male, and 13 juvenile female. Results of the ANOVA indicated that for live samples males are slightly larger than females, on average, for all 4 morphometric measurements, with overlap in the range of measurements (Table 1). For the museum samples, adult males were also larger than adult females for each of the 4 measurements, but a larger tarsus for males versus females was the only measurement difference found between juveniles of the sexes (Table 1).

Stepwise discriminant analysis indicated that culmen and tarsus were the most important morphometric measurements for differentiating live female and male Virginia rails (Fig. 2). Cross-validation of live samples resulted in 70% correct classification for females and 81% correct classification for

Table 1. Mean ± 1 standard deviation and range (in parenthesis) of Virginia rail (*Rallus limicola*) morphometric measurements recorded (mm) for live and museum samples. Live samples were captured at Ottawa National Wildlife Refuge, Ohio, USA during the springs of 2002–2011. Museum specimens were measured from several US museums during the summer of 2011.

Sample[a]	Live		Museum			
Sex	Male	Female	Male		Female	
Age	Ad ($n = 32$)	Ad ($n = 20$)	Ad ($n = 125$)	Juv ($n = 20$)	Ad ($n = 128$)	Juv ($n = 13$)
Wing chord	103.50 ± 3.24 (96–112) A	99.35 ± 4.70 (92–106) BCD	101.004 ± 5.09 (88–114) AB	100.05 ± 4.29 (92–109) ABC	96.20 ± 4.89 (82–110) D	95.78 ± 3.32 (91–101) CD
Tarsus	42.34 ± 1.31 (40–45) A	39.95 ± 1.85 (37–43) B	32.30 ± 2.55 (26–39) C	30.25 ± 3.24 (29–36) C	32.35 ± 1.95 (25–36) D	28.92 ± 1.50 (27–31) D
Culmen	39.72 ± 1.37 (37–43) A	36.70 ± 2.68 (32–42) BC	37.36 ± 2.97 (30–44) B	35.45 ± 2.63 (31–39) CD	34.54 ± 2.47 (28–41) D	32.92 ± 2.33 (29–37) D
Middle toe	35.56 ± 1.29 (33–39) A	33.60 ± 2.50 (30–37) B	33.29 ± 2.47 (27–39) B	33.45 ± 2.26 (29–38) BC	31.57 ± 2.14 (25–39) D	31.23 ± 2.13 (28–35) CD

[a] Sample sex was determined genetically for live samples and via dissection for museum samples. Age was determined by chest plumage color. For each measurement, groups sharing a letter do not differ statistically ($P > 0.05$).

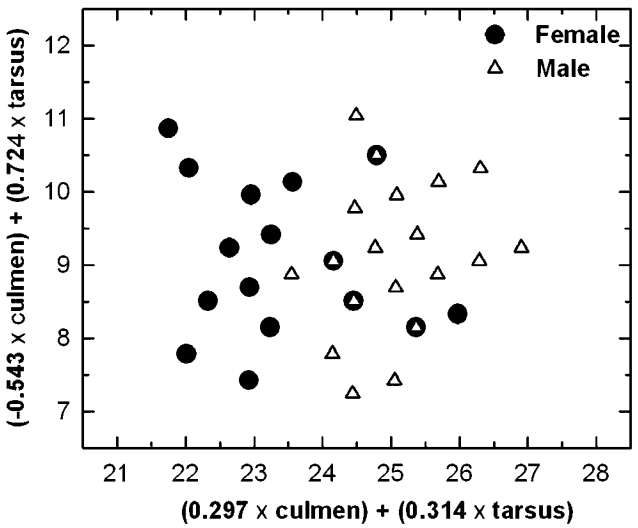

Figure 2. Discriminant functions used to distinguish sexes of live Virginia rail (*Rallus limicola*) samples collected during the springs of 2002–2011 at Ottawa National Wildlife Refuge, Ohio, USA. Closed circles are female; open triangles are male. Note some overlapping symbols.

males. The discriminant analysis produced the following canonical classification functions:

$$v1 = \alpha + (0.297 \times \text{culmen}) + (0.314 \times \text{tarsus})$$

and

$$v2 = \alpha + (-0.543 \times \text{culmen}) + (0.724 \times \text{tarsus})$$

These 2 discriminant functions explained 73.7% of the total variance.

For museum samples, tarsus, culmen, and wing chord were the most important measurements for differentiating adult female and male Virginia rail specimens (Fig. 3). Cross-validation indicated 80% correct classification for females and 71% correct classification for males. Classification functions for adult museum specimens were:

$$v1 = \alpha + (0.236 \times \text{culmen}) + (0.201 \times \text{tarsus}) + (0.074 \times \text{wing chord}), v2 = \alpha + (0.306 \times \text{culmen}) + (-0.044 \times \text{tarsus}) + (-0.185 \times \text{wing chord})$$

and

$$v3 = \alpha + (0.177 \times \text{culmen}) + (-0.344 \times \text{tarsus}) + (0.054 \times \text{wing chord})$$

These 3 discriminant functions explained 61.2% of the total variance. For juvenile museum samples, tarsus and culmen were the most important morphometric measurements for differentiating female and male Virginia rails specimens (Fig. 4). Cross-validation indicated 100% correct classification for females and 100% correct classification for males. Classification functions for juvenile museum specimens were:

$$v1 = \alpha + (0.47 \times \text{culmen}) + (0.347 \times \text{tarsus}), \text{ and}$$
$$v2 = \alpha + (-0.33 \times \text{culmen}) + (0.402 \times \text{tarsus})$$

These 2 discriminant functions explained 87.5% of the total variance.

DISCUSSION

We found that both Virginia rails migrating through Ottawa National Wildlife Refuge, and those found in museum collections can successfully have their sex predicted based on morphometrics. A discriminant function model using all 4 measurements is able to correctly classify individuals 80% of the time based on tarsus, culmen, wing chord, and middle toe length. This provides an accurate means of sex determination for this species, which can potential contribute to management and conservation efforts.

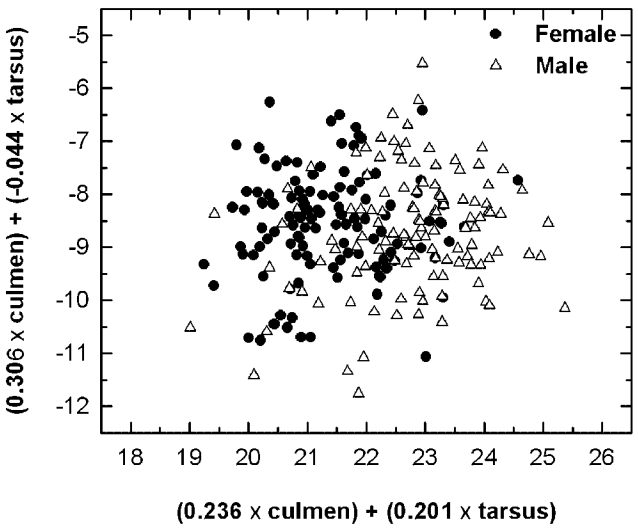

Figure 3. First 2 discriminant functions used to distinguish sexes of adult Virginia rail (*Rallus limicola*) museum specimens measured during the summer of 2011. Closed circles are female; open triangles are male. Note some overlapping symbols.

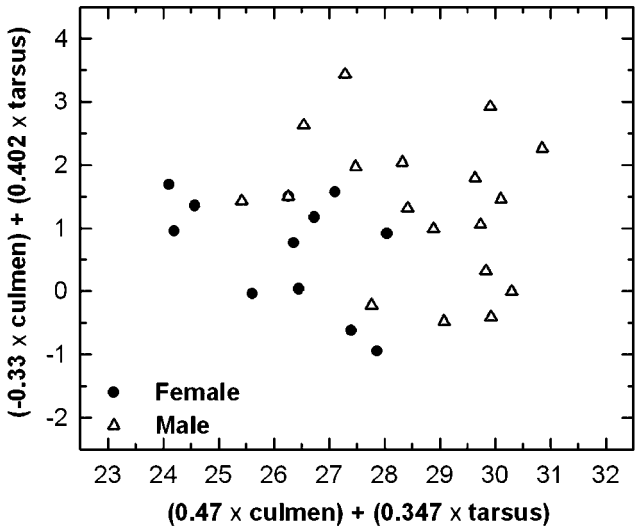

Figure 4. Discriminant functions used to distinguish sexes of juvenile Virginia rail (*Rallus limicola*) museum specimens measured during the summer of 2011. Closed circles are female; open triangles are male. Note some overlapping symbols.

Differences were found between museum specimens and live birds, but model predictions were consistent in describing the differences between males and females in both groups. Although there was overlap across all categories, males are larger in general. Similar trends have been found in king, clapper, Iberian water, and Inaccessible Island rails (*Atlantisia rogersi*; Ryan et al. 1989, Perkins et al. 2009, Fuertes et al. 2010).

Museum specimens have been well-documented to have variable amounts of shrinkage between species and between sexes across species (Winker 1993, Wilson and McCracken 2008). This shrinkage has been found to be less significant in measurements such as culmen and tarsus that do not involve joints or ligaments (Winker 1993). We recognize that there may be instances of shrinkage within our museum specimens and as a result we do not suggest that our classification functions for museum specimens be applied to live birds. These functions do show that the relationship between male and female Virginia rails extends beyond the birds that migrate through Ottawa National Wildlife Refuge remains the same, suggesting that our live bird model could be applied in other parts of the Virginia rail's range.

Our 80% correct classification rate falls within the range of published discriminate functions for sex determination in other bird species. Other rails have been correctly classified with >75% accuracy (Perkins et al. 2009, Fuertes et al. 2010). Classification efforts using morphometrics for many other bird species, including gulls (Laridae), hawks (Accipitridae), ravens (Corvidae), and cormorants (Phalacrocoracidae) have been able to achieve >90% accuracy (Mawhinney and Diamond 1999, Donohue and Dufty 2005, Bedrosian et al. 2008, Liordos and Goutner 2008, Herring et al. 2010). Still, models with >75% accuracy have been shown to be useful to understanding population demographics in terns (Sternidae), shearwaters (Procellariidae), puffins (Alcidae), shrikes (Laniidae), and finches (Fringillidae; Gucking et al. 2004, Bluso et al. 2006, Brady et al. 2009, Van Rooij and Griffith 2010, Friars and Diamond 2011). We contend that our results are likely of interest and will prove useful to natural resource professionals that study secretive marsh-birds, but additional work should be completed to increase our accuracy and the model's application. For example, power analysis indicated that to be 90% confident of detecting a difference of 0.1 mm at an alpha equal to 0.05 would require sampling approximately 1,053 samples. Such a sample size is not unfeasible given harvesting, and this work suggests that with a larger sample of birds, a classification function could easily be developed with a greater level of accuracy for field implementation. Our study also presents a way of determining the sex of Virginia rails with a limited number of morphometric measurements. Although minimizing the number of measurements is desirable from an efficiency perspective in field work and citizen-science involvement, incorporating additional measurements, such as tail length, could also help better discriminate between the sexes and is unlikely to be burdensome. To help reduce potential variability across observers that record measurements, we recommend that citizen-science involvement efforts include instruction and/or materials (e.g., standard protocols) to train volunteers on proper measurement techniques (Booney et al. 2009).

Overall, this analysis identifies a novel, noninvasive, and low-cost technique for determining sex of this species through multiple morphological measurements with a degree of confidence that would not otherwise be possible in the field. The ability to accurately determine the sex of Virginia rails is an important, significant step toward a better understanding of their population dynamics, food habits, migrational timing, winter distribution, molt timing, vocalizations, habitat use, and many other parts of their natural history. This model can be easily applied to captured birds and allows for both adult and juvenile birds to be sexed with confidence through these measurements.

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