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# Use of Morphometric Measurements to Differentiate Between Species and Sex of King and Clapper Rails

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**Abstract.**—King Rails (*Rallus elegans*) and Clapper Rails (*Rallus longirostris*) are large, secretive waterbirds whose ranges overlap in brackish marshes along the Atlantic and Gulf Coasts. King and Clapper Rails are difficult to separate by physical appearance and there is currently no reliable method to distinguish between the two species. Here, the relative effectiveness of using discriminant analysis of morphometric measurements to identify and sex King and Clapper Rails was examined. Mean measurements of wing, tarsus, and weight were different between male King and Clapper Rails and between female King and Clapper Rails. However, for all measurements except culmen, male Clapper Rails and female King Rails were not different. Discriminate analysis of morphometric measurements revealed that wing, tarsus, and culmen measurements differentiated between King and Clapper Rails, but cross-validation results for male Clapper Rails were only 73%. Male King Rails were larger than female King Rails for all morphometric measurements and male Clapper Rails were larger than female Clapper Rails for all morphometric measurements except for the tail. Wing and tarsus measurements differentiated between male and female King Rails and wing, tarsus, and culmen measurements differentiated between male and female Clapper Rails. Received 6 February 2008, accepted 12 August 2009.

**Key words.**—Clapper Rail, discriminant analysis, King Rail, Louisiana, morphometric measurements, Texas.

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King Rails (*Rallus elegans*) and Clapper Rails (*Rallus longirostris*) are large secretive waterbirds whose ranges overlap along the Atlantic and Gulf Coasts; both species breed and winter in coastal Louisiana and Texas (Meanley 1992; Eddleman and Conway 1998). King Rails inhabit fresh to brackish wetlands, while Clapper Rails inhabit saline to brackish wetlands (Meanley 1992; Eddleman and Conway 1994). Both King and Clapper Rails inhabit brackish marsh habitat; where their ranges often overlap and they are thought to hybridize (Meanley and Wetherbee 1962).

King and Clapper Rails are difficult to distinguish by physical appearance, although the King Rail is larger than the Clapper Rail. Clapper Rails are more drab in color, gray or dull brown, in comparison to the brighter, reddish coloration of King Rails (Eddleman and Conway 1998), but the subspecies of Clapper Rail that occurs along the

Gulf Coast has coloration similar to the King Rail (Meanley 1992). King Rails and Clapper Rails are sexually monomorphic in plumage, although males are larger than females for both species (Meanley 1992; Eddleman and Conway 1998).

Graves (2001) found that King Rails and Clapper Rails could be distinguished through discriminant analysis of sonograms of their calls; however, acquiring these data was difficult. Discriminant analysis using morphometric measurements has been used to determine the sex of bird species that are sexually size dimorphic but do not differ in appearance (Azure *et al.* 2000; Cuthbert *et al.* 2003; Shephard *et al.* 2004). It has also been used to separate bird species or subspecies that differ in size but not in appearance (Cuthbert *et al.* 2003; Pearce and Bollinger 2003). Size differences for multiple morphometric measurements have been observed between King and Clapper Rails, however,

this difference has never been quantified (Meanley 1992; Eddleman and Conway 1998). This size difference may be a useful method for separating King and Clapper Rails. The difference in size between the sexes of both species may also be a useful technique for sexing these rails in the field.

The objectives of this study were to use discriminant analysis of morphometric measurements to (1) differentiate between King and Clapper Rails and (2) differentiate between the sex of King and Clapper Rails.

#### METHODS

Study sites in Texas included McFaddin National Wildlife Refuge (NWR) and Anahuac NWR. In Louisiana, the study sites included Rockefeller Wildlife Refuge, Marsh Island Wildlife Refuge, Cameron Prairie NWR, Sweet Lake Land and Oil, Inc., and privately owned rice farms in Jefferson Davis and Rapides Parishes.

Rails were captured from 24 September 2004 until 8 April 2005, and from 17 October 2005 until 23 March 2006; we also captured resident King and Clapper Rails from 14-24 May 2005 and from 11-12 July 2006 (IACUC AE04-07) (Perkins 2007). We assumed rails captured in freshwater marsh were King Rails and rails captured in tidal salt marsh were Clapper Rails (Eddleman and Conway 1998). The King and Clapper Rails captured in brackish marsh were tentatively identified in the field based on coloration, size, and habitat type at the capture location. Rails weighing greater than 400 g were tentatively identified as King Rails (Eddleman and Conway 1998).

All rails captured were banded with an individually-numbered aluminum leg band, and the outer feather from each side of the tail was pulled from each bird for genetic analysis. Each rail was weighed to the nearest 1 g; the exact weight of any rail weighing over 500 g was visually estimated by the distance the scale was pulled beyond the 500 g maximum ( $N = 2$ ). Measurements of the wing chord and tail were taken to the nearest 1 mm using a 30 cm wing rule; tarsus and exposed culmen were measured with calipers to the nearest 0.01 mm (Pyle 1997).

Genetic analysis was used to ascertain the sex of each King and Clapper Rail. The collected retrices were placed in a labeled bag and frozen in a -20 C freezer within 24 hours after collection. They were stored there for up to a month, and then transferred to an ultra-cold freezer (-80°C) where they were stored for up to one year. The DNA was extracted from the feathers using a Qiagen mini-kit (QIAGEN Inc., Chatsworth, CA). The DNA was then refrigerated for up to eight months. Rails were genetically sexed using the method described in Griffiths *et al.* (1998). However, because only a two base pair difference differentiated the CHD-W and CHD-Z (chromo-helicase-DNA-binding) genes used for distinguishing the sexes in the two species under investigation, amplification products (with a fluorescent 6-FAM tag attached via the upstream primer) were resolved by electrophoresis on an ABI Prism 310 Genetic Analyzer in the presence of an internal size standard (GeneScan-400HD [ROX]; Applied Biosystems, Inc, Foster City, CA).

SAS 9.1.2. was used for all statistical analyses (SAS Version 9.1.2., 2002-2003). All morphometric measurements of the positively identified male King Rails ( $N = 16$ ), female King Rails ( $N = 10$ ), male Clapper Rails ( $N = 11$ ), and female Clapper Rails ( $N = 12$ ) were compared using ANOVA.

Discriminant analysis was used to determine if morphometric measurements could be used to differentiate between King and Clapper Rails, as well as to differentiate between the sexes of both species. For these analyses, the sample of positively identified, genetically sexed King and Clapper Rails was used. Standard step-wise procedures were carried out in order to determine the best discriminant function for each model, and cross-validation was used to calculate probabilities. In order to determine if discriminant analysis of morphometric measurements could be used to differentiate between King and Clapper Rails, the rails were separated into four species and sex categories. Once determined, the best discriminant function to separate these four rail categories was then used to predict the correct category of the unknown rails ( $N = 184$ ).

Discriminant analysis was used to determine if morphometric measurements could be used to sex King and Clapper Rails. These analyses were done separately for King and Clapper Rails; for each analysis, the rails were grouped by males and females. Once determined, the best discriminant function for each model was then used to predict the sex of the rails that were identified as King Rails or Clapper Rails using both field identification and discriminant analysis of the morphometric measurements.

#### RESULTS

The method for genetically sexing birds was successful for 249 of 254 King and Clapper Rails samples. Ninety-one females and 158 males were identified. The sex of five rails could not be determined using this analysis.

Morphometric measurements of 233 genetically sexed adult King and Clapper Rails were used in this study. Juvenile rails, rails with incomplete measurement, sets and rails that could not be genetically sexed, were excluded from the study. Of the rails used, 26 were positively identified as King Rails because they were captured in freshwater areas, and 23 were positively identified as Clapper Rails because they were captured in tidal salt marsh.

Results of the ANOVA indicated that differences existed in the morphometric measurements for the positively identified, genetically sexed King and Clapper Rails (wing  $F_{3,45} = 47.59$ ,  $P \leq 0.001$ ; tail  $F_{3,45} = 10.72$ ,  $P \leq 0.001$ ; tarsus  $F_{3,45} = 44.02$ ,  $P \leq 0.001$ ; culmen  $F_{3,45} = 12.62$ ,  $P \leq 0.001$ ; weight  $F_{3,45} = 23.64$ ,  $P \leq 0.001$ ). Male King Rails were larger than female King Rails for all morphometric mea-

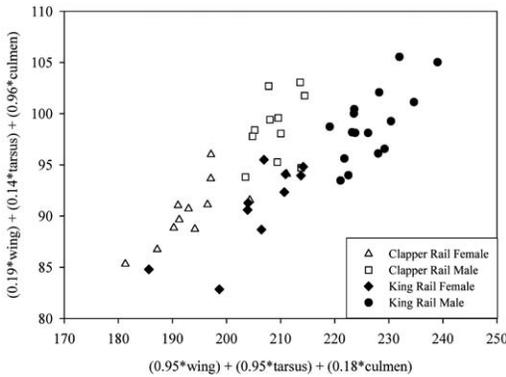
surements, and male Clapper Rails were larger than female Clapper Rails for all morphometric measurements except the tail (Table 1). There was overlap in the culmen measurement between species; and for all other measurements, male Clapper Rails and female King Rails were not different. Mean measurements of wing, tarsus, and weight were different between male King and Clapper Rails and between female King and Clapper Rails.

Step-wise discriminant analysis of morphometric measurements indicated that the

most important variables for separating male King Rails, female King Rails, male Clapper Rails and female Clapper Rails were wing, tarsus, and culmen (Fig. 1). Cross-validation results for the positively identified rails were 100% for male King Rails, 80% for the female King Rails, 73% for the male Clapper Rails, and 75% for the female Clapper Rails. The classification formulas produced by this discriminant analysis were:  $v1 = \alpha + 0.95$  (Wing) + 0.95 (Tarsus) + 0.18 (Culmen) and  $v2 = \alpha + 0.19$  (Wing) + 0.14 (Tarsus) + 0.96 (Culmen).

**Table 1. Mean, standard deviation, and range (in parenthesis) of positively identified male and female King Rails (*Rallus elegans*) and male and female Clapper Rails (*Rallus longirostris*) and those identified by both field techniques and genetic sexing, and by discriminant analysis. Rows sharing a letter do not differ ( $P > 0.05$ ).**

	Positively Identified King and Clapper Rails			
	King Rail Male N = 16	King Rail Female N = 10	Clapper Rail Male N = 11	Clapper Rail Female N = 12
Wing (mm)	165.9 ± 3.7 (158-173) C	151.4 ± 5.9 (138-158) B	153.3 ± 3.3 (140-162) B	143.3 ± 7.1 (132-160) A
Tail (mm)	68.7 ± 4.4 (59-77) B	60.5 ± 6.0 (48-69) A	63.7 ± 3.3 (60-67) A	61.6 ± 2.8 (56-65) A
Exposed Culmen (mm)	61.3 ± 3.2 (56-67) AB	56.8 ± 3.4 (50-62) C	64.4 ± 3.2 (59-69) A	58.7 ± 2.3 (56-64) BC
Tarsus (mm)	61.0 ± 2.9 (56-66) C	54.2 ± 2.8 (47-57) B	54.6 ± 2.3 (52-60) B	50.4 ± 1.6 (47-53) A
Weight (g)	369.6 ± 34.9 (320-425) C	309.0 ± 37.5 (262-353) B	329.4 ± 26.7 (289-377) B	272.6 ± 20.7 (243-315) A
	King and Clapper Rails Identified by Field Techniques and Discriminant Analysis			
	N = 81	N = 35	N = 11	N = 13
Wing (mm)	166.3 ± 5.0 (155-178)	153.5 ± 3.9 (144-161)	152.5 ± 5.6 (144-166)	141.3 ± 4.4 (134-150)
Tail (mm)	68.6 ± 3.5 (60-77)	63.3 ± 3.6 (57-73)	63.0 ± 4.5 (56-71)	58.7 ± 4.6 (52-66)
Exposed Culmen (mm)	63.0 ± 3.0 (57-70)	56.6 ± 2.1 (53-60)	65.2 ± 2.8 (61-71)	59.6 ± 2.3 (55-64)
Tarsus (mm)	61.4 ± 2.6 (57-71)	54.4 ± 1.8 (51-59)	55.4 ± 2.0 (53-59)	49.1 ± 2.2 (46-53)
Weight (g)	395.0 ± 40.1 (292-490)	317.4 ± 41.6 (242-384)	337.3 ± 25.5 (291-371)	278.2 ± 20.6 (250-311)



**Figure 1.** Discriminant functions used to separate female Clapper Rails (*Rallus longirostris*), male Clapper Rails, female King Rails (*Rallus elegans*), and male King Rails.

Our discriminant function was used to predict the classification of the remaining 184 rails. These predicted classifications were then compared with the original classifications based upon field identifications and genetic sexing. The predicted classification and the original classification were the same for 140 of the 184 rails (76%). Discriminant function predicted a different species, but the same sex as the original classification for 27 of the rails (Table 2). The method predicted the same species, but a different sex from the original classification for 13 of the rails, and predicted different species and sex for four of the rails (Table 2). The mean, standard deviation, and range for the wing, tail, exposed culmen, tarsus, and weight measurements of the 140 rails that were identified by both original classifications and discriminant analysis are shown in Table 1.

Wing and tarsus measurements were indicated by stepwise discriminant analysis to be the most important variables for separating King Rails by sex. Cross-validation results were 100% for both male and female King Rails. The classification formula produced by this discriminant analysis was:  $v1 = \alpha + 0.99 (\text{Wing}) + 0.9 (\text{Tarsus})$ . The discriminant function correctly classified the sex of 96% of the rails that were identified as King Rail using both field identification and discriminant analysis of the morphometric measurements (Fig. 2). For Clapper Rails, wing, tarsus, and culmen measurements were determined by stepwise discriminant analysis to be the most important variables for separating the sexes. Cross-validation results were 91% for male Clapper Rails and 92% for female Clapper Rails. The classification formula produced by this discriminant analysis was:  $v1 = \alpha + 0.82 (\text{Wing}) + 0.90 (\text{Tarsus}) + 0.87 (\text{Culmen})$ . The discriminant function correctly classified the sex of 100% of the rails that were identified as Clapper Rail using both field identification and discriminant analysis of the morphometric measurements (Fig. 3).

DISCUSSION

Previous morphometric measurements taken from King and Clapper Rails suggest that the two differ in size, with the Clapper Rail being smaller than the King Rail (Meanley 1969, 1992; Eddleman and Conway 1998). Little morphometric data for King Rails has been published; the most widely used set of measurements was taken from 18

**Table 2.** Differences seen for field identified, genetically sexed King Rails (*Rallus elegans*) and Clapper Rails (*Rallus longirostris*), and the predicted classification of these rails by discriminant analysis of their morphometric measurements.

Field and Genetic Identification	Discriminant Analysis Identification	Number of rails
Female Clapper Rail	Female King Rail	4
Female King Rail	Female Clapper Rail	5
Male King Rail	Male Clapper Rail	18
Male Clapper Rail	Female Clapper Rail	6
Female King Rail	Male King Rail	3
Male King Rail	Female King Rail	4
Female King Rail	Male Clapper Rail	3
Male King Rail	Female Clapper Rail	1

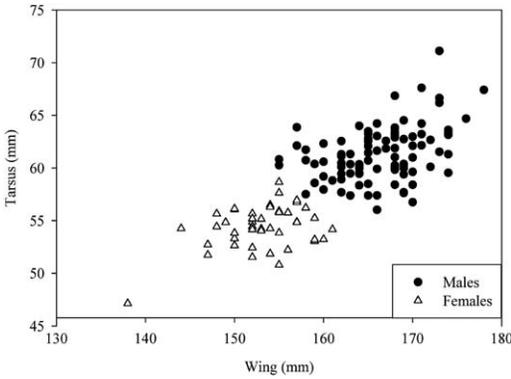


Figure 2. Wing and tarsus measurements of King Rails (*Rallus elegans*); shown by discriminant analysis to separate males and females.

male and 14 female museum specimens (Meanley 1969, 1992). Although more detailed morphometric data has been published for the Clapper Rail (Eddleman and Conway 1998), only one publication has compared the measurements of these two species. Meanley (1969) compared measurements of weight, wing, tail, exposed culmen, tarsus, and middle toe without claw. For all measurements except culmen, the male Clapper Rail averaged smaller than the male King Rail; the female Clapper Rail averaged smaller than the female King Rail for all measurements. The measurements for the male Clapper Rail and female King Rail were

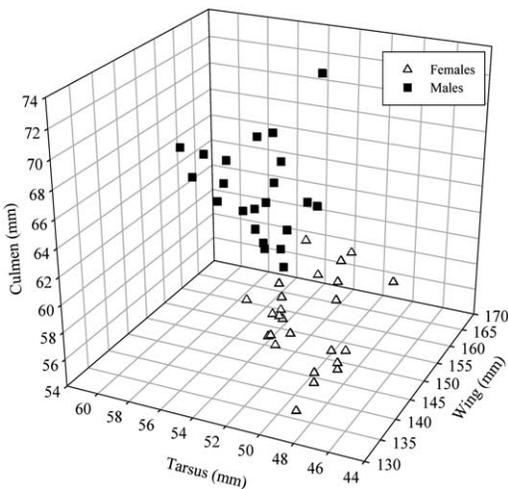


Figure 3. Wing, culmen, and tarsus measurements of Clapper Rails (*Rallus longirostris*); shown by discriminant analysis to separate males and females.

similar, for half of the measurements the male Clapper Rail was smaller and for half the female King Rail was smaller. The morphometric measurements for the two species have never been compared using multivariate statistics.

The morphometric measurements taken showed that male Clapper Rails were smaller than male King Rails and female Clapper Rails were smaller than female King Rails. They showed no difference between male Clapper Rails and female King Rails (Table 1). This suggests that morphometric measurements may be helpful in distinguishing between these two species as long as the rail's sex is known. However, the use of discriminant analysis of morphometric measurements was only moderately successful for distinguishing between King and Clapper Rails collected in southern Louisiana and Texas. Therefore, the use of discriminant analysis of morphometric measurements, combined with educated field identification and genetic sexing, may be the best method for distinguishing between King and Clapper Rails in areas where their populations overlap.

The variability of this discriminant analysis may have been due to King and Clapper Rails having overlapping morphometric measurements. However, it also may have been due to inaccurate field identification of the rails used in the discriminant function. We assumed that all rails captured in freshwater marshes were King Rails and all rails captured in tidal salt marshes were Clapper Rails. This tidal salt marsh is in close proximity to brackish marsh where King Rails are thought to reside. Since movements of the rails in this region have not been well studied, it is unknown whether King Rails could have ventured into this salt marsh. Genetic analyses were not conducted for this study; however, Avise and Zink (1988) found no conclusive proof of genetic differences between King Rails and Clapper Rails using mtDNA and allozyme assays. This is the only genetic study on these species that we are aware of and rails were collected near the same areas as our study.

Another possible explanation for the variability of the discriminant model is the

possible inclusions of hybrids. King and Clapper Rails are thought to hybridize in brackish marsh, and hybridization has been witnessed in southwestern Louisiana (Meanley and Wetherbee 1962). The variability of this model should not have been due to differences in size between migratory and resident King Rails, because all the King Rails used in this study were most likely residents to Louisiana and Texas (Perkins 2007).

King and Clapper Rails have sexually monomorphic plumage and both sexes incubate the eggs; therefore, it has not been possible to correctly sex them in the field. Both King and Clapper Rails are sexually size dimorphic, with males averaging larger than females for most measurements. The use of discriminant analysis of morphometric measurements appears to be a good method for determining sex in both King and Clapper Rails, particularly in habitats where their populations do not overlap or where the species is known.

The use of discriminant analysis of morphometric measurements to differentiate King or Clapper Rail species or sex should also be used with caution in regions outside the Gulf Coast. This is because there are many recognized subspecies of the Clapper Rail, which vary in size (Eddleman and Conway 1998). This study only included the Gulf Coast population of Clapper Rails. This subspecies ranges from the Florida Panhandle across the Gulf Coast to northern Mexico. Therefore, it is unknown whether discriminant analysis of morphometric measurements of Atlantic and Pacific Coast Clapper Rails would be useful in differentiating species or sex. Also, it is currently unknown if there are size differences among different populations of King Rails, such as migratory and resident populations. Further research is needed to determine if this analysis would be successful for other populations of King and Clapper Rails.

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#### LITERATURE CITED

- Awise, J. C. and R. M. Zink. 1988. Molecular genetic divergence between avian sibling species: King and Clapper Rails, Long-billed and Short-billed Dowitchers, Boattailed and Great-tailed Grackles, and Tufted and Black-crested Titmice. *Auk* 105: 516-528.
- Azure, D. A., D. E. Naugle, J. E. Toepfer, G. Huschle and R. D. Crawford. 2000. Sexing American Bitterns, *Botaurus lentiginosus*, using morphometric characteristics. *Canadian Field-Naturalist* 114: 307-310.
- Cuthbert, R. J., R. A. Phillips and P. G. Ryan. 2003. Separating the Tristan Albatross and the Wandering Albatross using morphometric measurements. *Waterbirds* 26: 338-344.
- Eddleman, W. R. and C. J. Conway. 1994. Clapper Rail. Pages 167-179 in *Migratory shore and upland game bird management in North America* (T. C. Tacha and C. E. Braun, Eds.). The International Association of Fish and Wildlife Agencies, Washington, D.C.
- Eddleman, W. R. and C. J. Conway. 1998. Clapper Rail (*Rallus longirostris*). In *The Birds of North America*, No. 340 (A. Poole and F. Gill, Eds.). The Academy of Natural Sciences, Philadelphia, Pennsylvania.
- Graves, C. A. 2001. Avian use of tidal marshes across a salinity gradient at Savannah National Wildlife Refuge, Georgia-South Carolina. Unpublished M.S. thesis, University of Tennessee, Knoxville, Tennessee.
- Griffiths, R., M. C. Double, K. Orr and R. J. G. Dawson. 1998. A DNA test to sex most birds. *Molecular Ecology* 7: 1071-1075.
- Meanley, B. 1969. Natural history of the King Rail. United States Department of Interior, North American Fauna, number 67, Washington, D.C.
- Meanley, B. 1992. King Rail (*Rallus elegans*). In *The Birds of North America*, No. 3 (A. Poole and F. Gill, Eds.). The Academy of Natural Sciences, Philadelphia, Pennsylvania.
- Meanley, B. and D. K. Wetherbee. 1962. Ecological notes on mixed populations of King Rails and Clapper Rails in Delaware Bay marshes. *Auk* 79: 453-457.
- Pearce, J. M. and K. S. Bollinger. 2003. Morphological traits of Pacific Flyway Canada Geese as an aid to subspecies identification and management. *Journal of Field Ornithology* 74: 357-369.
- Perkins, M. 2007. The use of stable isotopes to determine the ratio of resident to migrant King Rails in southern Louisiana and Texas. Unpublished M.S. thesis, Louisiana State University, Baton Rouge, Louisiana.
- Pyle, P. 1997. Identification Guide to North American Birds, Part 1. Slate Creek Press, Bolinas, California.
- SAS Institute 2002-2003. Version 9.1.2. SAS Institute, Cary, North Carolina.
- Shephard, J. M., C. P. Catterall and J. M. Hughes. 2004. Discrimination of sex in the White-bellied Sea-eagle, *Haliaeetus leucogaster*, using genetic and morphometric techniques. *Emu* 104: 83-87.