Scharf, W.C., J. Kren, P.A. Johnsgard, and L.R. Brown. 2008. Body weights and species distributions of birds in Nebraska's central and western Platte Valley. Papers in Ornithology (MS #1042), Digital Commons University of Nebraska, http://digitalcommons.unl.edu/biosciornithology/43

Wilson, R.E. and K.G. McCracken. 2008. Specimen shrinkage in Cinnamon Teal. *Wilson Journal of Ornithology* 120:390-392.

Winker, K. 1993. Specimen shrinkage in Tennessee Warblers and "Traill's" flycatchers. *Journal of Field Ornithology* 64:331-336.

Zar, J.H. 1999. Biostatistical analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.

Sexing California Clapper Rails using Morphological Measurements

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ABSTRACT

California Clapper Rails (Rallus longirostris obsoletus) *have monomorphic plumage, a trait that makes identification of sex difficult without extensive behavioral observation or genetic testing. Using 31 Clapper Rails (22 females, 9 males), caught in south San Francisco Bay, CA, and using easily measurable morphological characteristics, we developed a discriminant function to distinguish sex. We then validated this function on 33 additional rails. Seven morphological measurements were considered, resulting in three which were selected in the discriminate function: culmen length, tarsometatarsus length, and flat wing length. We had no classification errors for the development or testing datasets either with resubstitution or cross-validation procedures. Male California Clapper Rails were 6-22% larger than females for individual morphological traits, and the largest difference was in body mass. Variables in our discriminant function closely match variables developed for sexing Clapper Rails of Gulf Coast populations. However, a universal discriminant function to sex all Clapper Rail subspecies is not likely because of large and inconsistent differences in morphological traits among subspecies.*

INTRODUCTION

Identifying the sex of wild birds on the basis of morphological measurements can be difficult or impossible to accomplish for birds with monomorphic plumage and little size dimorphism. Although many alternative techniques can be used to determine the sex of individuals, these techniques are often either too time-consuming (behavioral sampling), expensive (genetic sampling), or disruptive (laparotomy) to be useful in the field. As an alternative to these often problematic techniques, individuals with relatively little size dimorphism can often be sexed using the mathematical approach of discriminant function analysis (Amat et al. 1993, Bluso et al. 2006, Perkins 2007, Ackerman et al. 2008). Discriminant function analysis, when applied to unknown individuals, describes the best combination of variables to distinguish between two known groups (e.g., male and female) (Khattree and Naik 2000). Morphological characteristics collected at the time of capture, such as mass, culmen, tarsus and wing length, are convenient candidate variables for use in discriminant functions. Discriminant functions that have been developed to distinguish the sex of Clapper Rails (*Rallus longirostris*) used wing chord, exposed culmen, and tarsus as the best factors to distinguish sex in rails along the Gulf Coast (Perkins 2007).

When applying discriminate functions to unknown birds, it is important that the population of unknown individuals is drawn from the same population from which the discriminant function was calculated. All three west coast subspecies of Clapper Rail are larger than east coast subspecies, suggesting discriminant functions developed for other populations may not apply throughout the species range (Orthmeyer et al. 1995, Perkins 2007). The endangered California Clapper Rail (*R. l. obsoletus*) is the largest of Clapper Rail subspecies. Resident only to tidal marshes of San Francisco Bay and Suisun Bay, the California Clapper Rail is sexually dimorphic (males are ~20% heavier than females) (Albertson 1995, Eddleman and Conway 1998). Albertson (1995) developed a discriminant function for sexing California Clapper Rails. One limitation of Albertson's (1995) study was that many of the morphological characteristics considered in previous discriminant functions were not evaluated. Therefore, our objectives were to examine sexual size dimorphism in California Clapper Rails using external measurements, and to develop a discriminant function that can be used for sexing individuals of this subspecies.

METHODS

Study Area - The largest estuary on the west coast of the United States, San Francisco Bay (37.8°N, 122.4°W) includes the last remaining range of the California Clapper Rail (Gill 1979, Eddleman and Conway 1998). Formerly located throughout San Francisco Bay and along coastal estuaries from Monterey Bay to Humboldt Bay (Silliman 1915, Gill 1979), California Clapper Rails now inhabit isolated intertidal margins in both the north and south arms of San Francisco Bay and Suisun Bay (Albertson and Evans 2000).

Collection and Measurements - During the winters of 2006 – 2008, we captured rails at three sites in south San Francisco Bay: Colma Creek Marsh in South San Francisco, Cogswell Marsh in Hayward, and Laumeister Marsh in East Palo Alto. Rails were captured in modified double-drop-door mammal traps set in tidal channels during low tide cycles, and by hand or dip net on foot or in boats during high tide.

From each rail, we collected a series of morphological measurements: exposed culmen, tarsus length (tarsometatarsus bone), mid-toe length, wing chord, wing flat, tail length (longest rectrix), and body mass (Dzubin and Cooch 1992). We used digital calipers to measure culmen, tarsus, and mid-toe to the nearest 0.01 mm. Wing chord, flat wing, and tail length were measured to the nearest millimeter using a stopped wing rule (Sutherland et al. 2004). We measured mass to the nearest 5 g using a 500-g Pesola spring scale (Pesola AG, Barr, Switzerland). Two researchers measured the majority of captured rails, but eight observers obtained at least some measurements (measurement differences between observers was less than 5%: range 0-10%). While this may increase error associated with the measurement, it also provides more general application to other studies (Devlin et al. 2004). The sex of all rails was field-identified using previous research on museum specimens as a guide (Albertson 1995). To verify sex of rails, a drop of blood was collected from half of captured birds for sex chromosome visualization of PCR enhanced DNA at Zoogen Services, Inc®, Davis, CA.

Statistical Analyses - We tested for univariate differences in morphological measurements between male and female rails using an analysis of variance. We calculated sexual size dimorphism (SSD) as the difference between male and female measurements relative to male measurements (Bluso et al. 2006, Ackerman et al. 2008). We used a stepwise discriminant function analysis (PROC STEPDISC in SAS, SAS Institute 2004) to identify the best measurements to classify the sex of the rails. Variable entrance and removal in the model were assessed using an F-test on Wilks' lambda values with a significance level of 0.15. Variables selected by the model were analyzed using PROC DISCRIM to calculate discriminant scores, posterior probabilities, and resubstitution and cross-validation error measures (Lachenbruch and Mickey 1968, Chardine and Morris 1989, Lance et al. 2000). The resulting discriminant functions were applied to a testing dataset comprised of morphological measurements collected from birds for which genetic sex identity was not available. For all analyses prior probabilities assumed an equal sex ratio (i.e., 50:50). Discriminant scores (D_{Male} and D_{Female}) were defined as $D = -0.5\,(x - \mu)'\Sigma^{-1}(x - \mu)$, where $(x - \mu)'\Sigma^{-1}(x - \mu)$ represents the squared distance of a rail with measurements x from a subpopulation (sex) with mean μ and variance matrix Σ (Khattree and Naik 2000, SAS Institute 2004). Rails were classified into the sex for which the largest D score was measured using the combined form $D_{Male} - D_{Female}$, where males had $D_{Male} - D_{Female} > 0$ and females had $D_{Male} - D_{Female} < 0$. Under the assumption that the variance matrix Σ was constant, we simplified discriminant scores into linear expressions without changing the effect of scoring between sexes (SAS Institute 2004).

RESULTS

Between 3 Jan 2007 and 5 Mar 2008, we captured and measured 64 California Clapper Rails. Thirty-six were field classified as female and 28 as male. We confirmed sex for 36 rails using genetic information (26 females, 10 males) with no contradictory field classification. A complete series of morphological measurements was not available for five individuals which were removed from the discriminant function analysis. Sexual size dimorphism was observed for all variables, but average mass for male rails was 22% larger than females (Table 1).

Our discriminant factor analysis indicated that tarsus, culmen length, and flat wing lengths were

Table 1. Morphological Measurements (mean ± SD [range]) and Sexual Size Dimorphism (SSD) of 25 Female and 10 Male California Clapper Rails in South San Francisco Bay, 2007 and 2008.

Measurement	Female	Male	t_{33}	p	SSD (%)
Mass (g)	326.4 ± 23.93 [275-385]	417.8 ± 33.55 [370-482]	9.08	<0.001	21.88
Culmen (mm)	56.51 ± 1.66 [52.39-59.53]	62.25 ± 1.68 [59.23-65.11]	9.22	<0.001	9.22
Tarsus (mm)	53.35 ± 2.33	59.05 ± 1.60	10.28$^{\#}$	<0.001	9.65
	[50.32-56.6]	[56.93-60.46]			
Wing Chord (mm)	151.76 ± 3.7	162.89 ± 3.82]	7.67$^{\&}$	<0.001	6.83
	[145-158]	[158-168]			
Wing Flat (mm)	154.84 ± 3.14	166.56 ± 3.71	9.14&	<0.001	7.03
	[149-160]	[161-172]			
Mid-Toe (mm)	41.85 ± 2.23	46.72 ± 2.29	5.78	<0.001	10.42
	[37.24-47.48]	[42.6-50.69]			
Tail Length (mm)	64.52 ± 3.94	68.8 ± 3.99	2.89	0.006	6.22
	[55-70]	[64-77]			

$^{\#}$ = 22 Females used in calculation (t_{30})
$^{\&}$ = 9 Males used in calculation (t_{32})

the best morphological measurements separating male and female California Clapper Rails (Wilks' $\lambda = 0.1278$: $F_{3,\,27} = 61.43$, $p < 0.001$). A test for homogeneity between covariance matrices of male and female rails was negative ($\chi^2 = 1.62$, df = 6, $p = 0.95$), thus a pooled covariance matrix was used in analysis (Morrison 1990).

Discriminant scores obtained from our model were:

$$D_{Female} = (Tarsus * 11.79173 + Culmen * 20.31156 + Flat\ Wing * 12.633) - 1866$$

$$D_{Male} = (Tarsus * 13.34666 + Culmen * 22.23522 + Flat\ Wing * 13.55629) - 2216$$

Thus, the combined form was:

$$D_{Male} - D_{Female} = (Tarsus * 1.55493 + Culmen * 1.92366 + Flat\ Wing * 0.92329) - 350$$

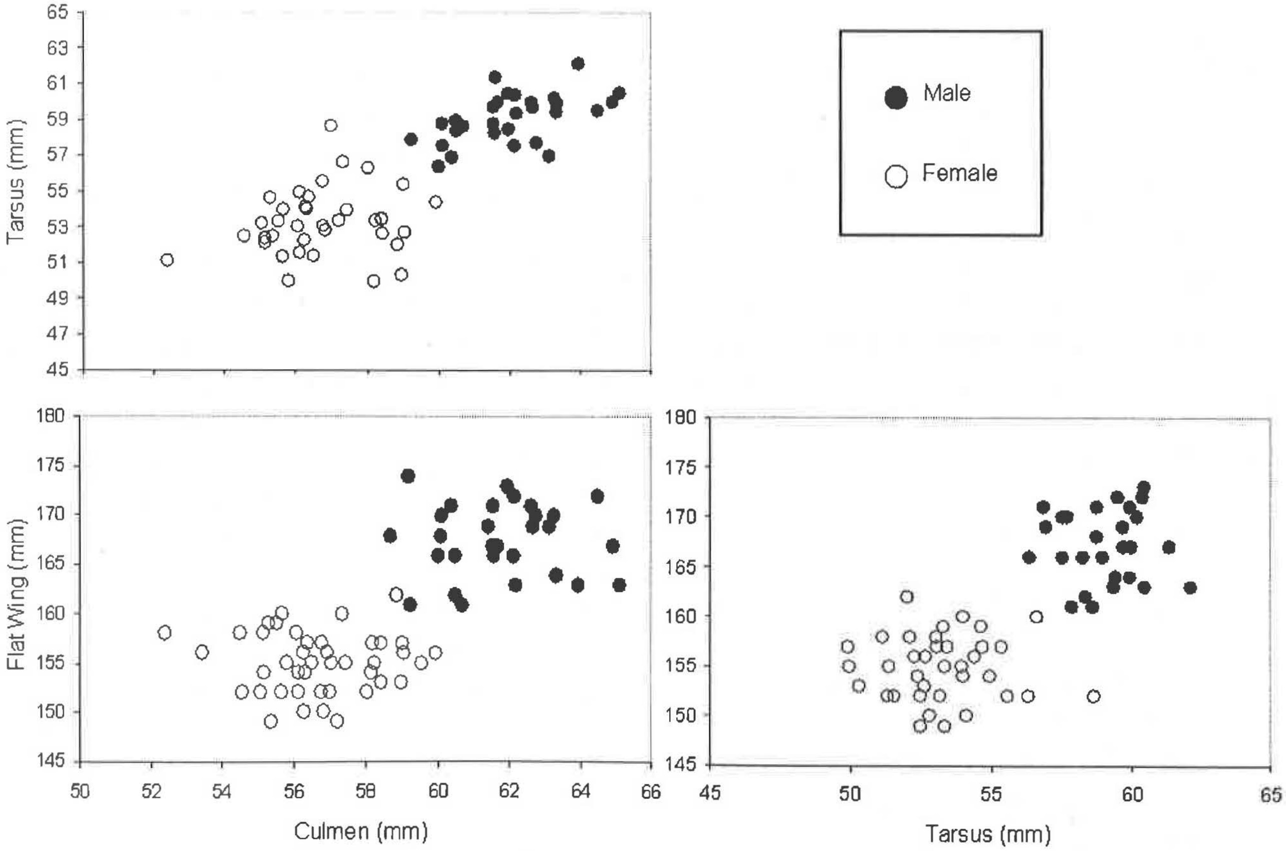
We classified rails as male when $D_{Male} - D_{Female}$ was > 0 and as females when $D_{Male} - D_{Female}$ was < 0.

Correct classification under resubstitution and cross-validation tests were 100% for both sexes. Results from the "tested" dataset containing measurements of rails without genetic verification of sex also resulted in 100% correct classification of field identification (Fig. 1). Posterior probability of classification into the field identified sex ranged from 0.9233 for males and 0.9823 for females.

DISCUSSION

Male California Clapper Rails in San Francisco Bay were significantly larger than females in all seven morphological measures taken. Mass showed the greatest difference between the sexes among the variables we measured (Table 1). However, mass was not selected in the best combination of measures to discriminate between sexes. Mass had larger variability within each sex, which increased uncertainty of classification. This larger variability could be due to numerous factors including timing of feeding relative to capture or body condition changes relative to breeding season.

Fig. 1. Relationships between male and female morphological measurementsused in discriminant function to distinguish sex of California Clapper Rails.



Furthermore, mass is more likely to change throughout the year, potentially making a discriminant function developed from birds captured in one season unuseable for birds captured in other seasons. Rather, we selected three other measurements as the best factors to include in a discriminant function. These measurements were culmen length, tarsometatarsus length, and flat wing length.

Two measurements that were included in our discriminant function (culmen and tarsometatarsus lengths) are identical to discriminant function measures developed for Gulf Coast Clapper Rails (Perkins 2007). The third measure used for the Gulf Coast conspecifics was wing chord (Perkins 2007). Wing chord and flat wing length were highly correlated measures in our data ($r = 0.9837$), but we feel wing chord is slightly more difficult to measure consistently across observers, indicated by slightly higher variance in our data (Table 1). Both our study and Perkin's (2007), correctly classified the sex of 100% of rails. For many discriminant functions, intermediate morphological traits result in high uncertainty in classifications. In these situations, cut-off values are often applied to the posterior probability class assignments to reduce the instance of misclassification (Ackerman et al. 2008). For birds classified in our study, high posterior probabilities suggest that there is no need for a cut-off value to increase the success in classifying birds. Therefore, we believe that when our technique is applied correctly, genetic sexing of adult California Clapper Rails is not necessary and birds may be sexed through field measurements (Ackerman et al. 2008).

Our discriminant function provides researchers with a greater ability to identify sex of California Clapper Rails using field measurements. Metrics used in the discriminant function are not interchangeable and observer bias can compromise the applicability of the discriminant function (Devlin et al. 2004, Bluso et al. 2006). Tarsus, for example, can be measured as either tarsus bone length (length from the back of the tarsus to the front of the metatarsus joint) or total (diagonal) tarsus (from the lower part of the tarsus joint to the metatarsus joint; Baldwin et al. 1931, Miller et al. 1998, Dzurbin and Cooch 1992).

Flat wing measurements may be more precise than wing chord. We recommend use of flat wing over wing chord measurements in most field studies because maintaining a natural curvature of the primary feathers is often difficult in field situations. Flat wing measurement obtained from museum specimens are less likely to be influenced by prolonged storage or preparation of the specimens (Winkler 1998).

Use of a single discriminant function for all Clapper Rails is problematic because multiple subspecies may occur within a small geographic area, and relatively large variation in size may be found among many of these subspecies (Eddleman and Conway 1998). California Clapper Rails show greater sexual size dimorphism in body mass than Gulf Coast Clapper Rails (24% vs. 18%; calculations from data presented in Perkins 2007), but sexual dimorphism for characteristics selected for use in discriminant functions appear similar between subspecies (males 7-11% larger than females). However, Clapper Rail morphological traits vary between subspecies, and the use of a discriminant function developed for one subspecies would be inappropriate when applied to a different subspecies. For example, all Gulf Coast Clapper Rails key out as female when using our discriminant function because California Clapper Rails are larger in most measurements than other subspecies. Body mass, wing chord, tarsus and tail length are all greater for California Clapper Rails than Gulf Coast Clapper Rails, but culmen was smaller for birds of the same sex. This suggests that a single discriminant function is not likely to differentiate the sexes across subspecies. Future research could investigate the potential to develop a discriminant function for Clapper Rail subspecies of known sex (Orthmeyer et al. 1995, Perkins 2007).

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LITERATURE CITED

Ackerman, J.T., J.Y. Takekawa, J.D. Bluso, J.L. Yee, and C.A. Eagles-Smith. 2008. Gender identification of Caspian Terns using external morphology and discriminant function analysis. *Wilson Journal of Ornithology* 120:378-383.

Albertson, J.D. 1995. Ecology of the California Clapper Rail in south San Francisco Bay. M.A. Thesis. San Francisco State University, San Francisco, CA.

Albertson, J.D. and J. Evans. 2000. California Clapper Rail. In Goals project, 2000. Bayland ecosystem species and community profiles: life histories and environmental requirements of key plants, fish and wildlife. (P.R. Olofson, ed.), pp. 332-340. San Francisco Bay Regional Water Quality Control Board, Oakland, CA.

Amat, J.A., J. Vinuela, and M. Ferrer. 1993. Sexing Chinstrap Penguins (*Pygoscelis antarctica*) by morphological measurements. *Colonial Waterbird* 16:213-215.

Baldwin, S.P., H.C. Oberholser, and L.G. Worley. 1931. Measurements of birds. Scientific Publication of the Cleveland Museum of Natural History 2:1-121.

Bluso, J.D., J.T. Ackerman, J.Y. Takekawa, and J.L. Yee. 2006. Using morphological measurements to sex Forster's Terns. *Waterbirds* 29:511-516.

Chardine, J.W. and R.D. Morris. 1989. Sexual size dimorphism and assortive mating in the Brown Noddy. *Condor* 91:868-874.

Devlin, C.M., A.W. Diamond, and G.W. Saunders. 2004. Sexing Arctic Terns in the field and laboratory. *Waterbirds* 27:314-320.

Dzubin, A. and E.G. Cooch. 1992. Measurements of geese: general field methods. California Waterfowl Association. Sacramento, CA.

Eddleman, W.R. and Conway, C.J. 1998. Clapper Rail (*Rallus longirostris*). *In* Birds of North America, no. 340 (A. Poole and F. Gill, eds.). The Birds of North America, Inc. Philadelphia, PA.

Gill, R. 1979. Status and distribution of the California Clapper Rail (*Rallus longirostris obsoletus*). *California Fish and Game* 65:36-49.

Khattree, R. and D.N. Naik. 2000. Multivariate data reduction and discrimination with SAS software. SAS Institute, Inc., Cary, NC.

Lachenbruch, P.A. and M.R. Mickey. 1968. Estimation of error rates in discriminant analysis. Technometrics 10:1-11.

Lance, R.F., M.L. Kennedy, and P.L. Leberg. 2000. Classification bias in discriminant function analyses used to evaluate putatively different taxa. *Journal of Mammalogy* 81:245-249.

Miller, S.L., M.A. Gregg, A.R. Kuritsubo, S.M. Combs, M.K. Murdock, J.A. Nilsson, B.R. Noon, and R.G. Botzler. 1998. Morphometric variation in Tundra Swans: relationships among sex and age classes. *Condor* 90:802-815.

Morrison D.F. 1990. Multivariate statistical methods, Third Edition. McGraw Hill Inc., New York, NY.

Orthmeyer, D.L., J.Y. Takekawa, C.R. Ely, M.L. Wege, and W.E. Newton. 1995. Morphological differences in Pacific Coast populations of Greater White-fronted Geese. *Condor* 97:123-132.

Perkins, M. 2007. The use of stable isotopes to determine the ratio of resident to migrant King Rails in southern Louisiana and Texas. M.Sc. thesis. Louisiana State University, Baton Rouge, LA.

SAS Institute, Inc. 2004. SAS OnlineDoc®. Version 9.1.2. SAS Software, SAS Institute Inc., Cary, NC. <http://v8doc.sas.com/sashtml/> (27 May 2008).

Silliman, O.P. 1915. Range of the California Clapper Rail. *Condor* 17:201.

Sutherland, W.J., I. Newton, and R.E. Green. 2004. Bird ecology and conservation: a handbook of techniques. Oxford University Press, New York, NY.

Winkler, K. 1998. Suggestions for measuring external characters of birds. *Ornitologia Neotropica* 9:23-30.

Remembering Kathy Klimkiewicz 1943 - 2008

Almost every bird bander in North America is aware that much of Kathy's adult life was devoted to birds and particularly to the banding of birds. For 35 years Kathy was one of the USGS Bird Banding Laboratories' biologists and through her correspondence and telephone calls banders were encouraged and, in perhaps her greater role, called onto the carpet for forms not submitted, improper age and sex codes, questionable identifications, poor handwriting, and any general lack of integrity in banding record keeping. As a teenager I was one of several of Kathy's young banding protégées and can attest to her demand for neat handwriting, careful data collection, organization, and the penalties for failure to perform.

Few, however, know that Kathy came to be a government biologist more through passion than a traditional science career. While obtaining a Masters at Radford College in 1965 she took an ornithology course by Dr. Donald Messersmith who propelled her into the serious study of birds, and by 1966 she had obtained her Master Banding Permit. By the end of her active banding career she and her subpermitees (at least 13) had banded over 100,000 individuals of 171 species.

After graduating, Kathy moved to the Washington, DC, area and began teaching in the public schools. During the summers she would volunteer at the Bird Banding Lab and in 1973 she began working full time for the Lab.

During her time at the Bird Banding Laboratory she was also active in the local birding community, taking young birders (like me) bird watching and actively participating in the Maryland Ornithological Society. She was active in all of the early bird atlas projects, Christmas Bird Counts, and regional bird projects. While her activities became more circumscribed as her health deteriorated, she ran Breeding Bird Survey routes, despite her lack of mobility, until the year of her death; running 103 different surveys across 11 different routes.

Particularly early in her career Kathy was very active in Eastern Bird Banding and Inland Bird Banding Associations, attending meetings and working on various collaborative projects. While not a requirement of her position, she managed to produce or coauthor 28 scientific publications. North American bird banders all knew Kathy. She and a few of the other biologists, rather than the four Lab directors she worked under, were the persons who banders corresponded with and became the face of the program. Her impacts reached every bander and her passing will affect banders and the Bird Banding Laboratory for years to come. Because of Kathy's generosity, several of us have gone on to become biologists (with very neat handwriting) in our own right and are now passing on similar lessons.

- Sam Droege