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Sex Determination of Least Sandpiper (*Calidris minutilla*) and Western Sandpiper (*Calidris mauri*): Comparing Methodological Robustness of Two Morphometric Methods

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Abstract.—Least (*Calidris minutilla*, $n = 110$) and Western (*Calidris mauri*, $n = 37$) sandpipers collected in Cuban wetlands were sexed by gonadal examination on dissection and used to assess the robustness of two morphometric methods commonly used to assign sex in shorebirds. Discriminant function analyses were performed and then tested by jackknife validation. The best discriminant function for Least Sandpiper included culmen and wing lengths and correctly classified 91% of the birds. Using culmen length, the discriminant function correctly sexed 97% of the Western Sandpiper. Sex ratio and morphometric estimates obtained through sexing Least and Western sandpipers by discriminant functions were not significantly different from the population sexed by gonadal examination on dissection. The range of the bill lengths used for sex determination of Least and Western sandpipers were then assessed by fitting the known-sex data. Classification accuracy of the bill length method was high for Western Sandpiper (95%), but was imprecise (44%) and female biased for Least Sandpiper. Consequently, a female biased sex ratio and under-estimation of male culmen length occurred for Least, but not Western, sandpipers classified by the bill length method. The findings suggest that sex determination based on the single measurement of culmen length is only accurate for Western Sandpiper, a species with high sexual size dimorphism. Linear measurements are also important for sex assignment in Least Sandpiper, but only using a discriminant function analysis approach.
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Key words.— *Calidris mauri*, *Calidris minutilla*, Cuba, discriminant analysis, Least Sandpiper, morphometrics, sexual size dimorphism, Western Sandpiper.

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The ability to sex individuals is essential for avian studies involving population structure, migration patterns, habitat use, foraging behavior, and many other sex-related life history parameters (Wheelwright *et al.* 1994; Mathot and Elner 2004; Markman *et al.* 2006; Remisiewicz and Wennerberg 2006). Diverse criteria and methods have been used to work with birds exhibiting sexually monomorphic plumage. Researchers willing to classify bird sex in such species have relied on behavioral observations at breeding sites (Wheelwright *et al.* 1994), vocalizations (Zavalaga *et al.* 2009), measurements of morphometric variables (Evans *et al.* 1993), laparoscopy (Maron and Myers 1984) and DNA analysis (Shephard *et al.* 2004). Among these methods, sex classification using morphometrics has been used extensively because it is cheaper, is simpler, is less time-consuming, may be used for individuals captured in the past, and provides an immediate assignment for field workers.

Sex classification based on morphometrics is commonly used when species are monomorphic in plumage but show some degree of sexual size dimorphism (SSD) (Dechaume-Moncharmont *et al.* 2011). In several calidridine sandpipers a reverse SSD occurs, with males being smaller than females (Jehl and Murray 1986). Bill length tends to be among the most dimorphic morphometric in shorebirds (Pyle 2008) and has been extensively used as a univariate discriminator factor for sexing Western Sandpiper (*C. mauri*; Page and Fearis 1971), Least Sandpiper (*C. minutilla*; Page 1974a), Dunlin (*C. alpina*; Page 1974b), and Purple Sandpiper (*C. maritima*; Hallgrimsson *et al.* 2008). However, the accuracy of such a method depends on the degree of SSD in the study species (Ackerman *et al.* 2008). In species with low to moderate SSD, where more overlap of morphometrics occurs between sexes, a higher number of misidentified and

unknown sex individuals can be expected relative to those species with high SSD.

Western and Least sandpipers are good examples of species exhibiting marked differences in sex assignment accuracy through bill length. Page and Fearis (1971) and Page (1974a) documented the bill length ranges (BLR) for classifying sexes of Western Sandpiper (males ≤ 24.2 mm and females ≥ 24.8 mm) and Least Sandpiper (males ≤ 17.4 mm and females ≥ 18.7 mm), respectively. The accuracy of sexing Western Sandpiper using BLR was 91% (Page and Fearis 1971). But for Least Sandpiper, which is less sexually dimorphic than Western Sandpiper, the sex assignment accuracy by BLR was 66% (n = 89), leaving an important part of the sampled birds as unsexed (Page 1974a) or potentially incorrectly sexed. Consequently, skewed Least Sandpiper population sex ratios are frequently reported in studies based on the BLR sexing criteria (i.e., female bias population; Butler and Kaiser 1995; Nebel 2006; Lehnen and Krementz 2007). Other considerations challenge the general application of the BLR sexing criteria documented for Western and Least sandpipers. Both BLRs were derived, in large part, from museum specimens of known sex collected on the Pacific Coast (Page and Fearis 1971; Page 1974a). Bill shrinkage in museum materials (Summers 1976) and latitudinal gradient on Western and Least sandpipers bill length (Nebel *et al.* 2002; Nebel 2006) are facts that highlight the need for further testing of the robustness of the application of the BLR sexing criteria.

Discriminant function analysis (DFA) based on morphometric characters has been used to improve sex assignment accuracy in shorebirds (Cartar 1984; Maron and Myers 1984; Ottvall and Gunnarsson 2007). In fact, DFA increases sexing accuracy for Western Sandpiper (i.e., accuracy 100% and 97%; Cartar 1984 and Stein *et al.* 2008, respectively), but no DFA for Least Sandpiper exists yet. Therefore, we explore using DFA as a tool for improving sex assignment accuracy for Least Sandpiper. Also, we used data on Western Sandpiper to contrast species with marked differences in SSD and to test for the robustness of the application of the species' BLR sexing criteria in a different geographical area.

Our goal was to test the robustness of commonly used BLR sexing methods. Specifically, we: 1) assessed the extent of SSD of Least and Western sandpipers with samples from two Cuban estuaries used as stop-over and wintering sites; 2) developed discriminant functions using morphometric data to identify males and females of each species; and 3) assessed the reliability of sex determination by DFA, using two methods for assessing error rate (Dechaume-Moncharmont *et al.* 2011), and contrasted this with the accuracy of BLR (Page and Fearis 1971; Page 1974a). Finally, we considered the effects of the use of each method to describe population sex ratios and species' morphometrics.

METHODS

Study Area

The study was conducted at two Cuban estuaries, Tunas de Zaza and Río Máximo. Tunas de Zaza (21° 38' 34.4" N, 79° 32' 48.1" W) is located on the southern coast of Sancti Spíritus Province, while Río Máximo (21° 44' 03.2" N, 67° 31' 17.9" W) is on the northern coast of Camagüey Province. Both sites are extensive wetlands with important mudflat areas periodically inundated by the effects of tides, winds and flooding regimes from associated rivers. More than 10,000 shorebirds find refuge on these estuaries, but numbers vary according to the migration period and water availability at the sites (Jiménez 2013).

Bird Sampling

Least and Western sandpipers were trapped in mist-nets during 13 visits made to the sites from September 2007 to September 2009. Visits encompassed fall migration (August-October) and winter residency (late November-January) of migratory shorebirds in Cuba.

Linear measurements were taken on all mist-netted birds, including exposed culmen length (from the bill tip to the feather line of the forehead), tarsus length (from middle of midtarsal joint to distal end of tarsometatarsus), wing length (maximum length-flattened and straightened wing; Evans 1986), and body mass. We measured bill and tarsus to the nearest 0.05 mm using carbon fiber vernier calipers. Wing length was recorded to the nearest 1 mm using a stopped ruler. To reduce the potential noise caused by feather condition, we took

special care to exclude from our dataset individuals that were molting or had severely worn primary feathers. Body mass was measured to the nearest 0.5 g with a 60-g Pesola spring scale. All birds caught were aged as being either immature (< 1 year old) or adult based on plumage color and primary feather wear (Pyle 2008). Least and Western sandpipers were sexed by gonadal examination on dissection. All measurements were performed by a single observer.

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to compare all morphometrics measured among sex and age in each studied species. All variables were $\log_{10}(x)$ transformed to meet assumptions of normality and homoscedasticity. The extent of SSD (%) for each species was determined using the Storer's dimorphism index (Storer 1966).

DFA was performed on untransformed data using stepwise selection for "good" predictor variables through the minimization of Wilks' lambda. Given that body mass may not be a reliable measure to include in discriminant functions since it may vary throughout migration and with sites, we excluded it from the analysis to avoid bias in our discriminant functions. Because the sex ratio of Least and Western sandpipers varies with study site latitude (Nebel *et al.* 2002; Nebel 2006), the prior probability of correct classification was based on the relative size of each group. The effectiveness of the DFA was assessed, first in terms of the proportion of birds of known sex that were classified correctly and second by a jackknifed validation (Dechaume-Moncharmont *et al.* 2011).

To test the reliability of sexing these species by a univariate metric such as culmen length, we reclassified the sex of the whole sample based on BLR sex classification criteria found in the literature (Page and Fearis 1971; Page 1974a). To examine the effects of each method on the sex ratio estimation of Least and Western sandpiper populations, we used a contingency table test to compare the proportion of females obtained through gonadal examination, DFA and BLR methods.

We also tested the null hypothesis that morphometrics from DFA- or BLR-sexed birds were not different from the observed values of the population sexed by gonadal examination. In doing this, we used a standardized effect size measurement and its 95% confidence interval (CI). We chose to use the combination of effect size and its 95% CI because they can reveal not only the statistical significance of the effect, but also its direction, magnitude, and uncertainty (Nagakawa and Cuthill 2007). Standardized effect sizes were measured through Cohen's *d* (hereafter *d*; Cohen 1988; Nagakawa and Cuthill 2007), and effect sizes were considered small, medium or large for $d = 0.2$, 0.5 and 0.8, respectively (Cohen 1988). Values for morphometrics are presented as means ± SD. Most of the statistical analyses were performed using Program R (R Development Core Team 2012), and the discriminant analysis was done in SPSS (SPSS, Inc. 2006). All tests were two-tailed and the level of significance was $P < 0.05$.

RESULTS

Sexual Size Dimorphism

We collected Least ($n = 110$) and Western ($n = 37$) sandpipers during the study period. Gonadal examination revealed a male-biased Least Sandpiper population (81 males and 29 females), whereas the population of Western Sandpiper sampled showed the opposite trait (13 males and 24 females).

We did not find any effect of age (Least Sandpiper: $F_{1,106} = 0.70\text{-}2.10$, $P = 0.151\text{-}0.440$; Western Sandpiper: $F_{1,28} = 0.06\text{-}1.60$, $P = 0.215\text{-}0.806$) or age*sex interaction (Least Sandpiper: $F_{1,106} = 0.15\text{-}1.10$, $P = 0.299\text{-}0.700$; Western Sandpiper: $F_{1,28} = 0.10\text{-}2.20$, $P = 0.150\text{-}0.795$) on any of the morphometrics analyzed for the two studied sandpipers. Thus, we pooled immatures and adults together for the rest of the analyses.

Although the range for all morphometrics overlapped (Table 1), interspecific differences existed for most of the variables recorded for the Least and Western sandpipers sexed by gonadal examination. On average, males were significantly smaller than females (Least Sandpiper: $F_{1,106} = 9.00\text{-}68.40$, $P < 0.001\text{-}0.004$; Western Sandpiper: $F_{1,28} = 7.60\text{-}81.11$, $P < 0.001\text{-}0.010$), except for body mass (Least Sandpiper: $F_{1,105} = 0.31$, $P = 0.578$; Western Sandpiper: $F_{1,28} = 0.04$, $P = 0.850$). According to the coefficients of variation, body mass was the most variable measurement for both species. Within species, body mass was more variable in males than females.

Western Sandpiper was considerably more dimorphic than Least Sandpiper for all variables measured (Fig. 1). Culmen length was the most dimorphic character in both species. The overlap range between sexes in this variable was highest for Least Sandpiper and occurred mostly at 18.25-19.90 mm (Fig. 2). Forty-two percent of all individuals ($n = 110$) fell in this interval, representing 69% ($n = 29$) of females and 32% ($n = 81$) of males. Culmen length overlap between Western Sandpiper males and females was minor and occurred at 24.50-25.00 mm with only 5% ($n = 37$) falling within this range (Fig. 2).

Table 1. Morphometrics of Least Sandpiper (*Calidris minutilla*) and Western Sandpiper (*C. mauri*) sexed by gonadal examination on dissection. Individuals were obtained at Tunas de Zaza and Río Máximo estuaries, Cuba, from 2007 to 2009 (F: female; M: male; *n*: sample size; SD: standard deviation; CV: coefficient of variation).

		Least Sandpiper				Western Sandpiper			
Variables	Sex	*n*	Mean ± SD	CV (%)	Range	*n*	Mean ± SD	CV (%)	Range
Culmen length (mm)	F	29	19.4 ± 0.8	4.0	18.2-21.3	24	27.0 ± 1.3	4.8	24.5-29.7
	M	81	17.9 ± 0.8	4.2	16.0-19.9	13	21.8 ± 1.7	7.6	19.0-24.8
Tarsus length (mm)	F	29	21.9 ± 0.5	2.1	21.0-22.7	24	26.4 ± 0.9	3.4	24.3-28.3
	M	81	21.4 ± 0.6	2.7	20.1-23.0	13	24.6 ± 0.7	2.8	23.6-25.5
Wing length (mm)	F	29	88.4 ± 2.3	2.6	84.0-92.0	24	98.2 ± 2.6	2.6	90.0-103.0
	M	81	86.6 ± 1.8	2.1	81.0-92.0	12	94.9 ± 3.1	3.3	89.0-99.0
Body mass (g)	F	29	20.5 ± 1.4	6.8	18.5-23.5	24	26.6 ± 1.9	7.3	22.5-30.5
	M	80	20.4 ± 2.6	12.6	16.0-27.0	12	25.8 ± 2.5	9.8	22.5-30.0

Figure 1. Percentage of sexual size dimorphism (SSD) in morphometrics measured in Least (*Calidris minutilla*) and Western (*C. mauri*) sandpipers inhabiting Tunas de Zaza and Río Máximo estuaries, Cuba, from 2007 to 2009.

Sex Determination by DFA

The DFA revealed that culmen and wing length were the variables that best predicted Least Sandpiper sex (Wilks' lambda = 0.51, $F_{2, 107} = 50.56$, $P < 0.001$). The classification function D_{LESA} = (1.20 x culmen length) + (0.21 x wing length) - 40.20, correctly identified the sex for 91% of the Least Sandpiper sampled. Individuals with $D_{LESA} \leq 0.976$ were classified as males and $D_{LESA} > 0.976$ as females when the posterior probability was set at 0.5. Some overlap was detected for Least Sandpiper males and females, occurring mostly due to the presence of small females (Fig. 3). The function accurately classified 76% ($n = 29$) of females and 96% ($n = 81$) of males. The cross validation procedure (jackknife) yielded the same success rate.

The stepwise DFA indicated culmen length as the most important variable to assign Western Sandpiper sex (Wilks' lambda = 0.23, $F_{1,34} = 113.45$, $P < 0.001$). The resulting discriminant function D_{WESA} = (0.70 x culmen length) - 17.65, with a cut-off point at -0.859, correctly classified the sex of 97% of the collected Western Sandpipers. The function correctly classified 100% ($n = 24$) of females and 92% ($n = 13$) of males. The jackknife procedure yielded the same success rate.

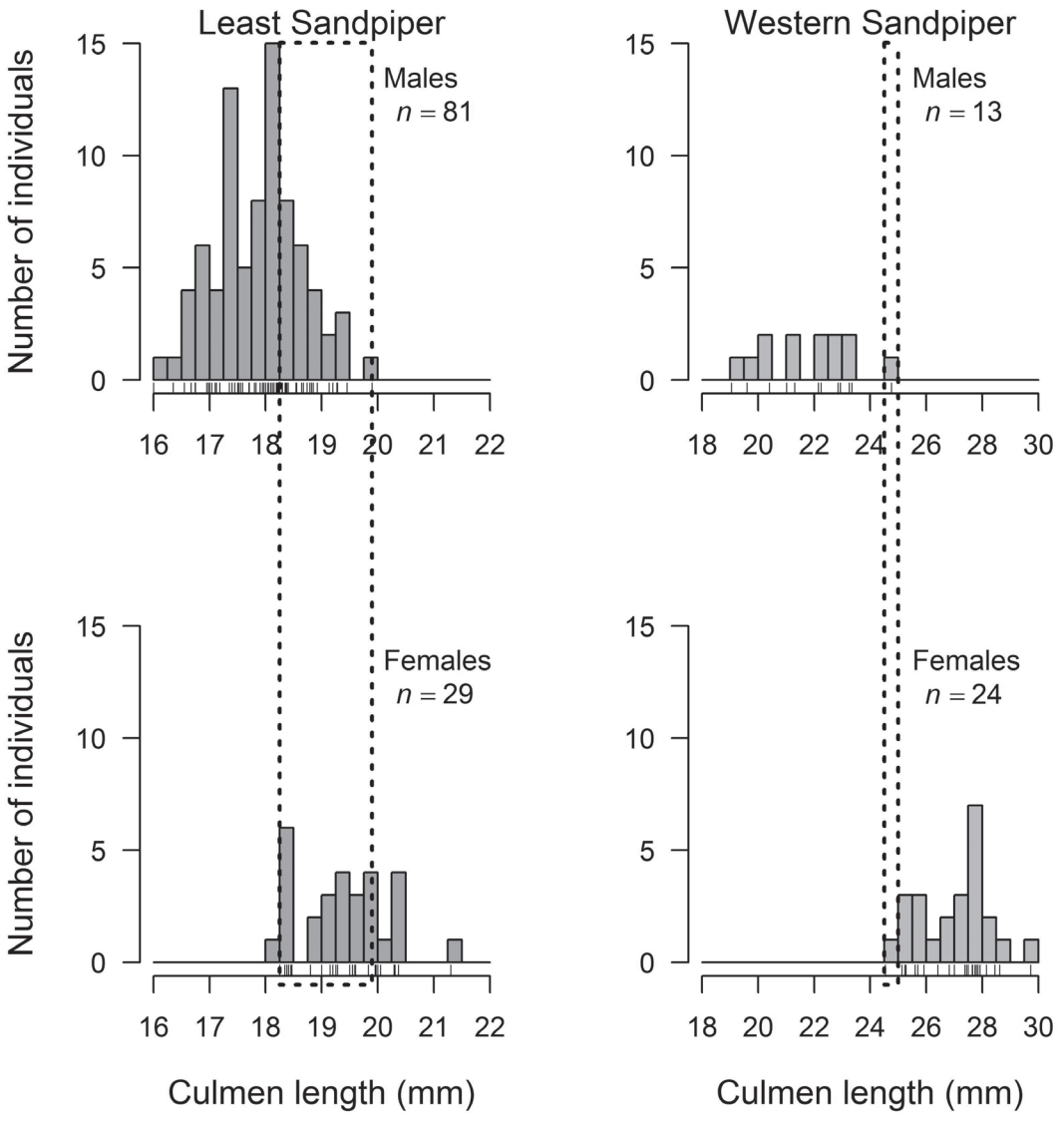

Figure 2. Culmen length (mm) distribution of male and female Least (*Calidris minutilla*) and Western (*C. mauri*) sandpipers. Dashed rectangles show the overlap in culmen length between sampled males and females. The individuals were collected in Tunas de Zaza and Río Máximo estuaries, Cuba, from 2007 to 2009.

Sex Determination by BLR

Sex assignment according to the BLR method was highly inaccurate and unbalanced for Least Sandpiper, yielding 44% correct classification overall. Seventy-six percent (n = 29) of females were accurately classified. However, males were poorly classified with only 32% (n = 81) of males correctly classified. Forty-four percent (42 males and 7 females) of the birds sampled (n = 110) fall into the "unknown" range (17.4-18.7 mm), precluding their identification (Fig. 3). Additionally, the method misidentified 16% (n = 81) of Least Sandpiper males as females because they had culmens longer than 18.7 mm.

Sex classification using the reported BLR for Western Sandpiper correctly classified 95% of the sampled population. Ninety-six percent (n = 24) of females and 92% (n = 13) of males were correctly classified. Only

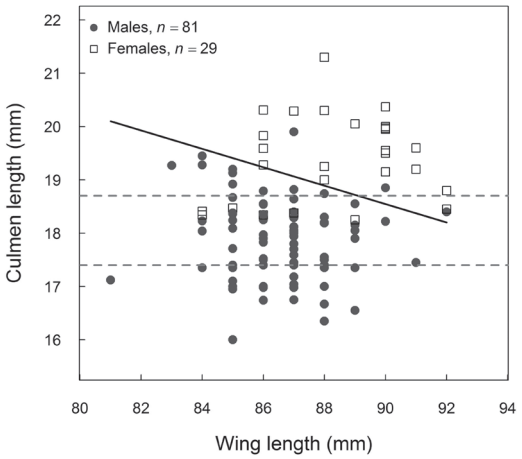

Figure 3. Discriminant function using wing and culmen length measurements to sex female (above solid line) and male (below solid line) Least Sandpipers (*Calidris minutilla*) collected at Tunas de Zaza and Río Máximo estuaries, Cuba, from 2007 to 2009. The lower and upper gray dashed lines (culmen length 17.4 mm and 18.7 mm, respectively) represent the documented bill length range (BLR) criteria for sexing Least Sandpiper (Page 1974a). The area between gray lines encompasses birds that cannot be classified according to the BLR sexing criteria.

two birds (one male and one female) fall into the BLR overlap and none were misidentified.

Robustness of the Sexing Methods

Least Sandpiper female proportion varied among sex classification methods used in this study ($\chi^2_2 = 24.12$, $P < 0.001$). Populations sexed by gonadal examination and populations sexed by DFA resulted in similar proportions of females (0.26 and 0.23, respectively). However, the Least Sandpiper female proportion estimated by the BLR method was approximately 2.2 times higher than the female proportion documented through gonadal examination (0.56). Unlike Least Sandpiper, the female proportion of Western Sandpiper did not differ among sexing methods (gonadal examination: 0.65; DFA method: 0.68; BLR method: 0.66; $\chi^2_2 = 0.06$, $P = 0.969$).

The largest difference in Least Sandpiper morphometrics occurred in the culmen length of males classified by the BLR method (Fig. 4, $d = -1.23$ [-1.65, -0.81]). Ignoring or misclassifying a large proportion of males through BLR caused strong underestimation of male mean culmen length (i.e., 0.84 mm shorter than the population sexed by gonadal examination). The use of the BLR method also caused under-estimation of male tarsus length (Fig. 4). However, the effect size was small ($d = -0.34$) and the estimated 95% CI slightly passed through zero (-0.73, 0.04), suggesting a weak and uncertain effect. The remaining variables measured in BLR-sexed Least Sandpiper showed low and non-significant effect sizes ($d = -0.26$ to 0.10, all 95% CIs widely bounded zero). All morphometric estimates derived from the DFA-sexed population showed low ($d = -0.09$ to 0.41; Fig. 4) and non-significant effects.

In contrast to Least Sandpiper, the resulting high accuracy in Western Sandpiper sex determination by either method precluded any bias in the estimated morphometrics (Fig. 4). Neither the morphometrics obtained from BLR-sexed Western Sandpiper, nor those obtained from DFA-sexed individuals showed differences from the population sexed by gonadal examination. Effect sizes were low in magnitude (-0.15 to 0.09) and their 95% CIs widely bounded zero.

DISCUSSION

Our results clearly showed that the accuracy of the BLR-method for sexing shorebirds largely depends on the magnitude of SSD exhibited by the studied species. The disproportionate dimorphism of Western Sandpiper culmen length allowed the accurate sex determination of males and females by using the sex BLR developed by Page and Fearis (1971), whereas the moderate culmen length dimorphism of Least Sandpiper involved severe bias for sex assignment of this species when using a similar method (Page 1974a).

In contrast to Western Sandpiper, Least Sandpiper showed a low SSD for culmen length as a consequence of both the small between-sex differences in average culmen length and the considerable between-sex

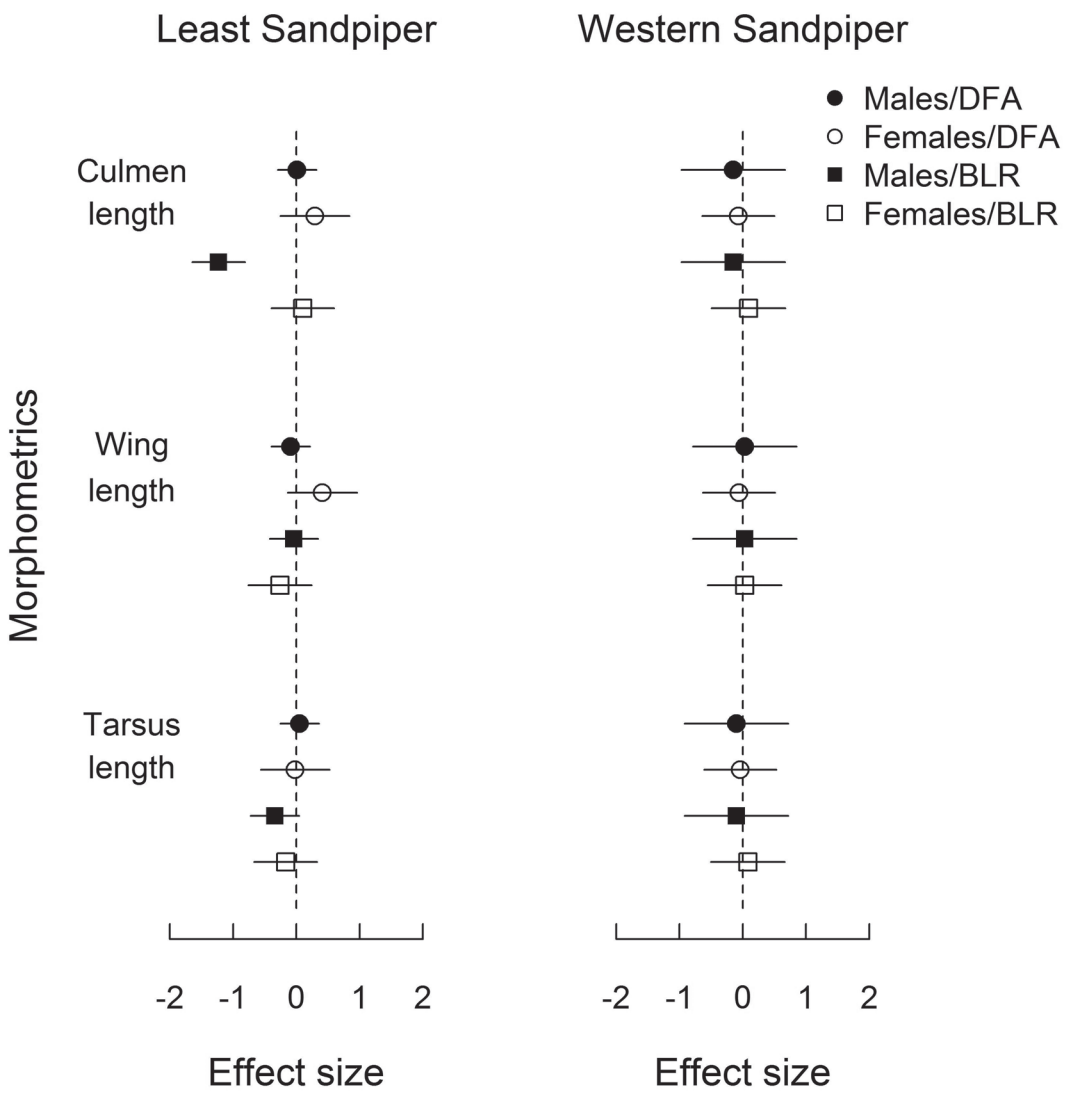

Figure 4. Pair-wise contrast between morphometrics (culmen, wing, and tarsus lengths) from populations of Least and Western sandpipers (*C. minutilla* and *C. mauri*, respectively) sexed by gonadal examination on dissection (zero value) and those obtained by the discriminant function analysis (DFA, circles) and bill length range (BLR, squares) sex classification methods. Closed and open symbols represent males and females, respectively. Effect size (Cohen's *d*) was considered small, medium or large for *d* = 0.2, 0.5 and 0.8, respectively.

overlap exhibited in this character. Consequently, the accuracy of sex assignment based on the single measurement of culmen length was seriously compromised, although differences in mean values between sexes proved significant. The use of the BLR sexing criterion (Page 1974a) will produce a biologically important under-estimation of mean culmen length for Least Sandpiper males. Such under-estimation is caused by restricting the classification only to the smaller males in the population. Results presented here support the cautions raised by several authors about sex ratio and morphometric biases when using the culmen length as the only criterion for sexing Least Sandpiper (Butler and Kaiser 1995; Nebel 2006; Lehnen and Krementz 2007).

Pyle (2008) stated that linear measurements are largely unhelpful in sexing Least

Sandpiper and no other criteria are known. We demonstrated that sex determination for Least Sandpiper in Cuba can be successfully done using linear measurements and a multivariate approach such as the DFA. The discriminant function obtained in this study was a more accurate and reliable predictor of sex than the single measurement of culmen length. We found that 91% of the sampled Least Sandpiper could be accurately sexed when combining culmen and wing length measurements. The DFA accuracy for Least Sandpiper is high and close to the median discriminant rate (i.e., 91.8%) reported for studies seeking to sex cryptic monomorphic bird species through DFA (Dechaume-Moncharmont *et al.* 2011). More specifically, the DFA effectiveness is similar to those reported for other Nearctic calidridine sandpipers (e.g., Sanderling (*C. alba*): Maron and Myers 1984; Wood 1987; Dunlin: Brennan *et al.* 1984; Semipalmated Sandpiper (*C. pusilla*): Cartar 1984). Further, there was a high degree of agreement between the DFA- and the gonadal sexed Least Sandpiper population when determining sex ratio and morphometric estimates. This indicates that Least Sandpiper sex can be accurately determined under field conditions by using our discriminant function.

The best discriminant variables for Least Sandpiper have already been used to discriminate sex in other shorebirds (Brennan *et al.* 1984; Cartar 1984; Maron and Myers 1984; Meissner 2005). Such measurements can be taken easily in the field. Feathered morphometrics, such as wing length, in discriminant analyses may cause potential biases resulting from feather wear and molt (Meissner 2005). The status of the feathers is especially important for the application of the DFA approach developed here. Birds sampled in September and early October need to be carefully examined for feather wear and molt because during this time Least Sandpiper are molting their outermost primary feathers (Galindo-Espinosa *et al.* 2013).

The development of discriminant functions in this study relied on birds collected at two separated estuaries over 2 years. The temporal distribution of samples used, combined with the lack of geographical variation in Least Sandpiper morphometrics within Cuban sites (García-Lau *et al.* 2012), suggests that prediction coefficients were developed from a heterogeneous sample population. Consequently, sex assignments based on the discriminant function derived here should have general applicability across the Cuban range of Least Sandpiper and prediction coefficients robust enough to account for temporal and spatial influences on culmen and wing length size. Further, our discriminant function equation provides an efficient and accurate method for sexing Least Sandpiper in the field, and the approach should be applicable within the species distribution range, even though the specific equations may not be the same (Nebel 2006).

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