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# Differentiating Sex and Species of Western Grebes (*Aechmophorus occidentalis*) and Clark's Grebes (*Aechmophorus clarkii*) and Their Eggs Using External Morphometrics and Discriminant Function Analysis

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**Abstract.**—In birds where males and females are similar in size and plumage, sex determination by alternative means is necessary. Discriminant function analysis based on external morphometrics was used to distinguish males from females in two closely related species: Western Grebe (*Aechmophorus occidentalis*) and Clark's Grebe (*A. clarkii*). Additionally, discriminant function analysis was used to evaluate morphometric divergence between Western and Clark's grebe adults and eggs. *Aechmophorus* grebe adults ( $n = 576$ ) and eggs ( $n = 130$ ) were sampled across 29 lakes and reservoirs throughout California, USA, and adult sex was determined using molecular analysis. Both Western and Clark's grebes exhibited considerable sexual size dimorphism. Males averaged 6-26% larger than females among seven morphological measurements, with the greatest sexual size dimorphism occurring for bill morphometrics. Discriminant functions based on bill length, bill depth, and short tarsus length correctly assigned sex to 98% of Western Grebes, and a function based on bill length and bill depth correctly assigned sex to 99% of Clark's Grebes. Further, a simplified discriminant function based only on bill depth correctly assigned sex to 96% of Western Grebes and 98% of Clark's Grebes. In contrast, external morphometrics were not suitable for differentiating between Western and Clark's grebe adults or their eggs, with correct classification rates of discriminant functions of only 60%, 63%, and 61% for adult males, adult females, and eggs, respectively. Our results indicate little divergence in external morphology between species of *Aechmophorus* grebes, and instead separation is much greater between males and females. Received 22 June 2015, accepted 11 September 2015.

**Key words.**—*Aechmophorus clarkii*, *Aechmophorus occidentalis*, California, Clark's Grebe, discriminant function analysis, morphometrics, niche partitioning, sexual size dimorphism, Western Grebe.

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Determining the sex of individuals is essential to many avian ecological studies. Researchers use various techniques to assign sex to birds in the field, including plumage and size (Pyle 2008), vocalizations (Carlson and Trost 1992; Eda-Fujiwara *et al.* 2004; Bourgeois *et al.* 2007), and behavior (Jodice *et al.* 2000). For species with sexually monochromatic plumage, overlapping morphological measurements between the sexes, and sex-specific vocalizations and behaviors that either are not apparent or cannot be readily observed, an alternative means for sex determination is necessary. Molecular sexing (Griffiths *et al.* 1998; Dubiec and Zagalska-Neubauer 2006) is one alternative, but it can be costly and invasive, and the collection of blood or tissue samples requires additional training and permitting. Moreover, molecular sexing does not allow for sex

determination at the time of capture, which is necessary in some ecological studies. Conversely, discriminant function analysis (DFA) of morphological measurements is an inexpensive and minimally invasive means for assigning sex to individuals in real-time, and has been used widely among bird taxa (Dechaume-Moncharmont *et al.* 2011). Using individuals of known sex (either through behavior, molecular sexing, or laparotomy), DFA generates a linear discriminant function, where values of key morphological measurements can be entered to predict the sex of an individual. The accuracy of the discriminant function is reported in the proportion of individuals of known sex that are assigned to the correct sex based on the discriminant function.

Discriminant functions for sex determination often are developed from individuals

captured from a single population, or over a relatively small geographic area. One potential limitation of sex determination by DFA may exist if there is a high degree of variation in morphological measurements among geographically disparate populations. In such instances, a discriminant function developed from individuals from a population in one location may be less accurate or even unusable for assigning sex to individuals from populations at other locations (Evans *et al.* 1993; Martínez-Abraín *et al.* 2006; Herring *et al.* 2010). Thus, conducting a DFA using measurements from individuals sampled over a large geographic area may improve the utility of the resulting discriminant function at sex determination across large portions of the species' range.

Similar in size and appearance, Western Grebes (*Aechmophorus occidentalis*) can be distinguished from Clark's Grebes (*A. clarkii*) by differences in plumage and bill coloration, yet within each species, plumage is sexually monochromatic (LaPorte *et al.* 2013). Males are typically larger than females, and differences in bill morphometrics have been suggested as a means for determining sex of individuals (Pyle 2008; LaPorte *et al.* 2013). However, overlap in morphometrics exists between males and females, making it difficult to distinguish smaller males from larger females.

In addition to their similar size and plumage, Western and Clark's grebes lay eggs that are similarly sized and colored (LaPorte *et al.* 2013). Because nesting colonies often include both Western and Clark's grebes, similarities in egg morphology make it difficult to identify eggs to a particular species. Identifying eggs to species often is necessary for studies on breeding success, demography, behavior, and environmental contaminants. Observing the incubating bird is one direct way to identify eggs to species (assuming interspecific brood parasitism does not occur). However, in cases where nests are abandoned, through disturbances such as recreational boating, or if the attending adult flushes before it can be observed, identifying eggs to a particular species by observing the attending adult often is not possible.

We used DFA to study divergence in external morphology between sexes and species of *Aechmophorus* grebe adults and eggs. Our objectives were to: 1) develop discriminant functions to accurately identify the sex of adult Western and Clark's grebes using external morphological measurements; 2) identify cut-off points for key morphometrics that can be used in the field to accurately assign sex to Western and Clark's grebes; 3) use DFA to evaluate divergence in external morphology between Western and Clark's grebes and the potential for interspecific niche partitioning; and 4) evaluate the utility of using egg measurements and DFA for differentiating between Western and Clark's grebe eggs.

## METHODS

### Study Area

We captured Western and Clark's grebes (hereafter, grebes) at 29 lakes and reservoirs (hereafter lakes) in California between April and October of 2012 and 2013. Capture lakes were distributed throughout the State of California, USA, from the Oregon border (Tule Lake) to the Mexican border (Lower Otay Reservoir), and included lakes in coastal areas, in California's Central Valley, and at high elevations along the Sierra Nevada Mountains (Fig. 1). We sampled grebes at 16 lakes in 2012 and 17 lakes in 2013 (four lakes were sampled in both years). At each lake, grebes were sampled once over a few consecutive days, except at three lakes (Clear Lake, Lake Berryessa, and Lake San Antonio), where grebes were sampled on three to five separate occasions each year, and at Topaz Lake where one grebe was captured in 2012 and eight were captured in 2013.

Grebes were captured at night from boats using a night-lighting technique, where spotlights were directed at grebes to disorient them until they were captured using a long-handled landing net (King *et al.* 1994; Whitworth *et al.* 1997). Grebes were held in individual animal crates lined with towels until processing and released near the site of capture. We distinguished Western Grebes from Clark's Grebes using plumage (black head feathers extend below the eyes in Western Grebe) and bill color (yellow-green in Western Grebe, orange-yellow in Clark's Grebe; LaPorte *et al.* 2013). We weighed each grebe with a digital bench scale ( $\pm 2$  g; Ohaus ES6R) or spring scale ( $\pm 20$  g; Pesola spring scales). Measurements included head-to-bill length (distance from the back of the head to the tip of the bill), nares-to-bill length (distance from the distal end of the nares to the tip of the bill), exposed culmen length, bill depth (measured at the proximal end of the nares), short tarsus length (distance from the middle of



**Figure 1.** Locations of the lakes and reservoirs in California, USA, where Western and Clark's grebes were captured (both filled and unfilled circles) and eggs were sampled (unfilled circles) during 2012-2013.

the midtarsal joint to the end of the tarso-metatarsus, measured on the right leg), and flattened wing length (measured on the right wing). All structural measurements were measured to the nearest 0.01 mm with digital calipers, except for wing length, which was measured to the nearest 1 mm with a wing ruler. Each bird was

banded with a metal U.S. Geological Survey leg band, and a drop of blood was collected on a ZooMark card for DNA sex determination (Zoogen Services, Inc.).

We sampled grebe eggs at seven lakes in California (Fig. 1). One egg was randomly selected from each active nest and collected for contaminant analyses. We

sampled eggs only from those nests where we were able to observe the incubating parent and thus were certain of species identification. We measured length and width of each egg to the nearest 0.01 mm using digital calipers. We calculated egg volume as  $V = K_v LW$ , where  $V$  is egg volume ( $\text{mm}^3$ ),  $L$  is egg length (mm),  $W$  is egg width (mm), and  $K_v$  is an egg volume coefficient of 0.507 (Hoyt 1979). Lastly, we calculated egg elongation as the ratio of egg length to egg width.

#### Statistical Analyses

We used two-way analyses of variance (ANOVAs) with main effects of species and sex and a species by sex interaction term to test for differences in each of the seven morphometrics (PROC GLM; SAS Institute, Inc. 2013). We used Tukey's test for multiple comparisons to investigate differences between: 1) male and female Western Grebes; 2) male and female Clark's Grebes; 3) Western Grebe males and Clark's Grebe males; and 4) Western Grebe females and Clark's Grebe females. Differences were considered significant at  $P \leq 0.05$ . Prior to conducting ANOVAs, we evaluated whether the data met the assumption of equal variance using Levene's test of homogeneity of variance and that the residuals were normally distributed. Sexual size dimorphism of each morphometric was assessed for each species by subtracting the mean value of females from the mean value of males and then dividing the absolute value of that number by the mean value of females. Morphometric differences between the two species for each sex was assessed by subtracting the mean value of Clark's Grebes from the mean value of Western Grebes and then dividing the absolute value of that number by the mean value of Clark's Grebes. We tested for grebe morphometric differences among lakes using separate ANOVAs (one for each measurement of each species) with the main effects of lake and sex, and a sex by lake interaction term.

We performed separate DFAs on Western and Clark's grebes to identify sex using the morphometrics head-to-bill length, nares-to-bill length, exposed culmen length, bill depth, and short tarsus length. We excluded mass as a potential variable in the DFAs because grebes can exhibit large body mass fluctuations seasonally (Jehl 1997), making mass a less useful trait to identify a bird's sex throughout the year. We also excluded flattened wing length because wing molt can occur in late summer, fall, or winter (Humble *et al.* 2013), making wing measurements less useful for identifying a bird's sex year-round. In the first step of our DFA, we used Pearson's correlation analysis (PROC CORR; SAS Institute, Inc. 2013) to determine if any of the seven morphometrics were highly correlated (multicollinearity). We found that head-to-bill length, nares-to-bill length, and exposed culmen length were all highly correlated (Western Grebes:  $r^2 \geq 0.92$ ; Clark's Grebes:  $r^2 \geq 0.94$ ). Thus, to simplify discriminant functions, and to accommodate different bill measurement preferences among biologists, we conducted separate DFAs using the morphometrics of bill depth, short tarsus length, and either head-to-bill length, nares-to-bill length, or exposed culmen length.

We used a forward selection stepwise discriminant analysis (PROC STEPDISC; SAS Institute, Inc. 2013) to select the best measurements for differentiating males and females. At each step, we used a Wilks' lambda  $F$ -statistic to enter the measurement that provided the model with the most discriminatory power, and removed the measurement that provided the least discriminatory power. The significance level for measurement entry or removal from the model was 0.15 (Hosmer and Lemeshow 2000). Once no measurements could be entered or removed, the stepwise selection process was concluded. We then used PROC DISCRIM (SAS Institute, Inc. 2013) to model the discriminant function, calculate discriminant scores (D), and obtain posterior probabilities that each grebe was classified as a female based on morphometrics. We used an *a priori* probability of a randomly captured grebe being a female of 0.50. Discriminant function accuracy (proportion of grebes assigned to the correct sex) was assessed by resubstitution, and validated using the leave-one-out (jackknife) cross-validation procedure, where each grebe was assigned a sex classification using a function derived from the entire sample minus the focal grebe (Manly 1994). We identified potential multivariate outliers by measuring Mahalanobis distances and examined the leverage of these outliers by removing them from the data set and modeling a new discriminant function and re-estimating discriminant function accuracy (Dechaume-Moncharmont *et al.* 2011). We calculated the cut-off values where discriminant scores yielded 75%, 50%, and 25% probability of being classified as a female by plotting the posterior probability that each grebe was classified as a female (PP), along with the corresponding D score, against a logistic curve using PROC NLIN (SAS Institute, Inc. 2013) where  $PP = 1/(1+\exp(k(D-D_{mid})))$ , and the constants  $k$  (the steepness of the curve) and  $D_{mid}$  (the midpoint of D) were estimated using an iterative curve-fitting process.

Lastly, we ran separate DFAs using only one of each of the five morphometrics used in the main DFAs, and used the classification error rates to identify which single measurement most accurately predicted grebe sex. For all the above DFA steps, separate analyses were conducted for Western Grebes and Clark's Grebes.

We performed between-species DFAs for males and females separately using the morphometrics head-to-bill length, nares-to-bill length, exposed culmen length, bill depth, and short tarsus length. Because Western and Clark's grebes can be distinguished using plumage and bill color, our intent was not to develop a discriminant function to identify male or female grebes to species. Rather, we conducted DFAs to test if Western and Clark's grebe morphometrics differed to the point that species differentiation, and potentially niche separation could occur in these two highly-sympatric species. As with the species-specific DFAs for identifying grebe sex, we used stepwise DFA (PROC STEPDISC, PROC DISCRIM; SAS Institute, Inc. 2013) to identify morphometrics that best separated species of grebes (Western Grebe male vs. Clark's Grebe male; Western Grebe female vs. Clark's Grebe female). We used an *a priori*

probability of 0.50 for a captured grebe being a Western Grebe or Clark's Grebe. Discriminant function accuracy (proportion of adult grebes assigned to the correct species) was assessed by resubstitution, and validated using the leave-one-out cross-validation procedure.

We used a one-way ANOVA to test for differences in grebe egg morphometrics between the two grebe species. The percent difference in each egg morphometric between the two species was assessed by subtracting the mean value of Clark's Grebe eggs from the mean value of Western Grebe eggs and then dividing the absolute value of that number by the mean value of Clark's Grebe eggs. We then used stepwise DFA (PROC STEPDISC, PROC DISCRIM; SAS Institute, Inc. 2013) using the morphometrics of egg length, egg width, egg volume, and egg elongation. We used an *a priori* probability of 0.50 for a randomly collected egg being that of a Western or a Clark's grebe. Discriminant function accuracy (proportion of eggs assigned to the correct species) was assessed by resubstitution and validated using the leave-one-out cross-validation procedure.

## RESULTS

We captured and obtained DNA sex determination from 373 Western Grebes (189 males and 184 females) and 203 Clark's Grebes (91 males and 112 females). An average of 14 Western Grebes (Range = 1-97) and 8 Clark's Grebes (Range = 1-61) were captured at each lake. We obtained mass and morphological measurements from all captured birds, with the exception that we could not sample the flattened wing length of 75 molting grebes since many grebes captured in late July through October were in the process of molting their primary feathers.

Between sexes, males were significantly larger than females in all seven morphological measurements, with the greatest sexual size dimorphism observed in bill depth (25.0-25.7%) and mass (23.7-24.3%; Table 1). For both species, morphometrics were similar among lakes, and there were no significant sex by lake differences (all *P*-values  $\geq 0.09$ ), with the exception that mass of male Western Grebes varied among lakes ( $F_{22, 324} = 1.7, P = 0.03$ ). The significant sex by lake interaction for mass of Western Grebes may have been due to differences in lake conditions, but also seasonal changes in mass, as some lakes were sampled as early as April, whereas others were sampled as late as October.

**Table 1. Mean ( $\pm$  SD) morphological measurements, *P*-values of Tukey's test for multiple comparisons, percent sexual size dimorphism (SSD), and percent species differences from a two-way ANOVA with a species by sex interaction term for differences between male and female Western and Clark's grebes sampled in California, USA, 2012-2013.**

	Western Grebes ( $\pm$ SD)			Clark's Grebes ( $\pm$ SD)			Species Differences (Males)			Species Differences (Females)		
	Males	Females	<i>P</i>	% SSD	Males	Females	<i>P</i>	% SSD	<i>P</i>	%	<i>P</i>	%
Mass (g)	1,309 (162)	1,058 (129)	< 0.001	23.7	1,272 (122)	1,023 (114)	< 0.001	24.3	0.14	2.9	0.14	3.4
Head-to-bill length (mm)	133.36 (5.01)	116.73 (4.92)	< 0.001	14.2	131.06 (4.77)	113.70 (4.10)	< 0.001	15.3	0.001	1.8	< 0.001	2.7
Nares-to-bill length (mm)	60.38 (2.95)	52.46 (2.81)	< 0.001	15.1	59.14 (3.15)	50.73 (2.43)	< 0.001	16.6	0.004	2.1	< 0.001	3.4
Exposed culmen length (mm)	72.81 (3.45)	63.20 (2.90)	< 0.001	15.2	71.25 (3.54)	61.01 (2.70)	< 0.001	16.8	0.001	2.2	< 0.001	3.6
Bill depth (mm)	13.27 (0.81)	10.62 (0.70)	< 0.001	25.0	13.05 (0.72)	10.38 (0.66)	< 0.001	25.7	0.10	1.7	0.03	2.3
Short tarsus length (mm)	79.58 (2.84)	73.50 (2.45)	< 0.001	8.3	79.30 (2.65)	72.85 (2.91)	< 0.001	8.9	0.86	0.4	0.18	0.9
Flattened wing length (mm)	204 (6)	192 (5)	< 0.001	6.3	200 (5)	187 (6)	< 0.001	7.0	< 0.001	2.0	< 0.001	2.7

Between species, Western Grebe males were, on average, approximately 0.4-2.9% larger than Clark's Grebe males in all morphological measurements, although significant differences occurred only in head-to-bill length, nares-to-bill length, exposed culmen length, and flattened wing length (Table 1). Similarly, female Western Grebes were, on average, 0.9-3.6% larger than female Clark's Grebes in all morphological measurements, with significant differences occurring in head-to-bill length, nares-to-bill length, exposed culmen length, bill depth, and flattened wing length (Table 1). The greatest morphometric differences between species occurred in the two measurements associated only with bill length (nares-to-bill length and exposed culmen length; Table 1).

Western Grebe Discriminant Function Analyses for Sex Identification

The combination of head-to-bill length, bill depth, and short tarsus length performed best at discriminating male and female Western Grebes (*Wilks*  $\lambda = 0.17$ :  $F_{3, 369} = 618.74$ ,  $P < 0.001$ ). This function correctly classified 98% of male (three misclassifications) and 98% of female (four misclassifications) Western Grebes. Leave-one-out cross-validation also correctly classified 98% of male (four misclassifications) and 98% of female (four misclassifications) Western Grebes. DFAs performed by substituting either nares-to-bill length or exposed culmen length for head-to-bill length resulted in similar functions and correct classification rates. Western Grebe discriminant functions using either of the three correlated bill length measurements are presented in equations 1-3 (Table 2). For the function with head-to-bill length, bill depth, and short tarsus length, we identified a total of 18 potential multivariate outliers using Mahalanobis distances (nine males, nine females). Removing these individuals only modestly increased the proportion of correctly sexed males (resubstitution: 99%; leave-one-out: 98%) and females (resubstitution: 99%; leave-one-out: 99%), indicating they had little effect on the DFA.

**Table 2. Discriminant function equations for sex determination of Western ( $n = 203$ ) and Clark's ( $n = 373$ ) grebes using external morphological measurements, where  $D > 0$  indicated an individual was male and  $D < 0$  indicated an individual was female. Also shown are the percentages of males and females correctly classified using each equation.**

Species	Equation Number	Discriminant Function Equation	Males Correctly Classified	Females Correctly Classified
Western Grebes	1	$D = 0.488 \times (\text{Head-to-bill length}) + 0.304 \times (\text{Short tarsus length}) + 3.796 \times (\text{Bill depth}) - 129.621$	98%	98%
	2	$D = 0.665 \times (\text{Nares-to-bill length}) + 0.386 \times (\text{Short tarsus length}) + 4.405 \times (\text{Bill depth}) - 115.350$	98%	98%
	3	$D = 0.691 \times (\text{Exposed culmen length}) + 0.372 \times (\text{Short tarsus length}) + 3.979 \times (\text{Bill depth}) - 122.943$	97%	99%
	4	$D = 4.668 \times (\text{Bill depth}) - 55.732$	96%	96%
Clark's Grebes	5	$D = 0.690 \times (\text{Head-to-bill length}) + 4.447 \times (\text{Bill depth}) - 136.525$	99%	100%
	6	$D = 0.761 \times (\text{Nares-to-bill length}) + 4.906 \times (\text{Bill depth}) - 99.246$	99%	98%
	7	$D = 0.719 \times (\text{Exposed culmen length}) + 0.269 \times (\text{Short tarsus length}) + 4.608 \times (\text{Bill depth}) - 122.010$	99%	99%
	8	$D = 0.894 \times (\text{Head-to-bill length}) - 109.352$	98%	98%
	9	$D = 5.768 \times (\text{Bill depth}) - 67.543$	98%	98%

When head-to-bill length, bill depth, and short tarsus length were used together, there was little overlap between Western Grebe males and females where the probability of correct sex assignment was reduced (Fig. 2A). Western Grebes with discriminant scores between -1.10 and 1.10 had a less than 75% chance of being assigned to the correct sex (Fig. 3A). Only 2% ( $n = 373$ ) of Western Grebes exhibited discriminant scores within this range. Outside of this range, the discriminant function correctly assigned 99% ( $n = 367$ ) of Western Grebes to the correct sex.

Evaluation of discriminant functions comprised of a single measurement revealed that bill depth at the proximal end of the nares was the single most accurate morphometric for predicting Western Grebe sex (*Wilks*  $\lambda = 0.24$ :  $F_{1,371} = 1,152.29$ ,  $P < 0.001$ ). Bill depth alone correctly identified 96% of males (eight misclassifications) and 96% of females (seven misclassifications). Leave-one-out cross-validation also correctly classified 96% of male (eight misclassifications) and 96% of female (seven misclassifications) Western Grebes using bill depth alone. A simplified discriminant function for sex determination of Western Grebes based on bill depth alone is presented in equation 4 (Table 2).

#### Clark's Grebe Discriminant Function Analyses for Sex Identification

Similar to the DFA for Western Grebes but without tarsus length, the combination of head-to-bill length and bill depth performed best at discriminating male and female Clark's Grebes (*Wilks*  $\lambda = 0.14$ :  $F_{2,200} = 596.81$ ,  $P < 0.001$ ). This function correctly classified 99% of male (one misclassification) and 100% of female Clark's Grebes. Leave-one-out cross-validation correctly classified 99% of male (one misclassification) and 99% of female (one misclassification) Clark's Grebes. DFAs conducted by substituting either nares-to-bill length or exposed culmen length for head-to-bill length resulted in similar functions (although short tarsus length was retained in the function with exposed culmen length) and similar correct classification rates. Clark's Grebe

discriminant functions using either of the three correlated bill length measurements are presented in equations 5-7 (Table 2). For the function with head-to-bill length and bill depth, we identified a total of 16 potential multivariate outliers using Mahalanobis distances (12 males, 4 females). Removing these individuals had no effect on the proportion of correctly sexed males (resubstitution: 99%; leave-one-out: 99%) and only modestly increased the proportion of correctly sexed females (resubstitution: 100%; leave-one-out: 100%), indicating they had little effect on the DFA.

When head-to-bill length and bill depth were used together, there was little overlap between Clark's Grebe males and females where the probability of correct sex assignment was reduced (Fig. 2B). Clark's Grebes with discriminant scores between -1.10 and 1.10 had a less than 75% chance of being assigned to the correct sex (Fig. 3B). Only 1% ( $n = 202$ ) of Clark's Grebes exhibited discriminant scores within this range. Outside of this range, the discriminant function correctly assigned 99% ( $n = 200$ ) of Clark's Grebes to the correct sex.

Evaluation of discriminant functions using only a single measurement revealed that head-to-bill length (*Wilks*  $\lambda = 0.21$ :  $F_{1,201} = 778.87$ ,  $P < 0.001$ ) or bill depth (*Wilks*  $\lambda = 0.21$ :  $F_{1,201} = 775.73$ ,  $P < 0.001$ ) alone were equally accurate at predicting Clark's Grebe sex. Functions using only one of these bill measurements correctly identified 98% of males (two misclassifications) and 98% of females (two misclassifications). Leave-one-out cross-validation also correctly classified 98% of male (two misclassifications) and 98% of female (two misclassifications) Clark's Grebes. Simplified discriminant functions for sex determination of Clark's Grebes based on either head-to-bill length or bill depth alone are presented in equations 8 and 9 (Table 2).

#### Western Grebe vs. Clark's Grebe Discriminant Function Analyses for Adults

Head-to-bill length alone performed best at discriminating male Western Grebes and

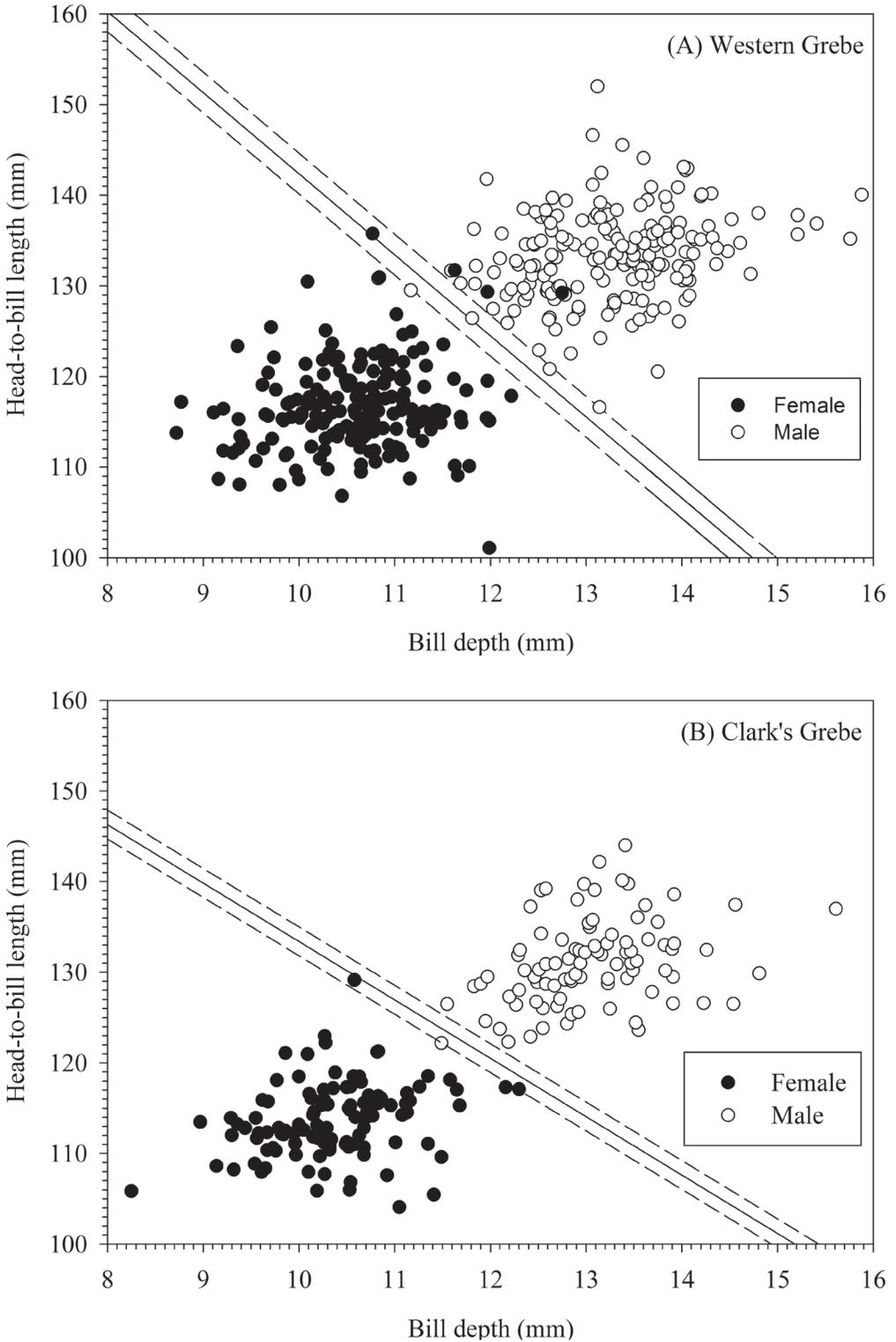


Figure 2. Head-to-bill length, bill depth, and the individuals classified as male (above solid line) and female (below solid line) according to discriminant functions for (A) Western Grebes and (B) Clark's Grebes captured in California, USA, during 2012-2013. The area between the dashed lines denotes the morphometric space where the discriminant function had a < 75% probability of assigning the correct sex.

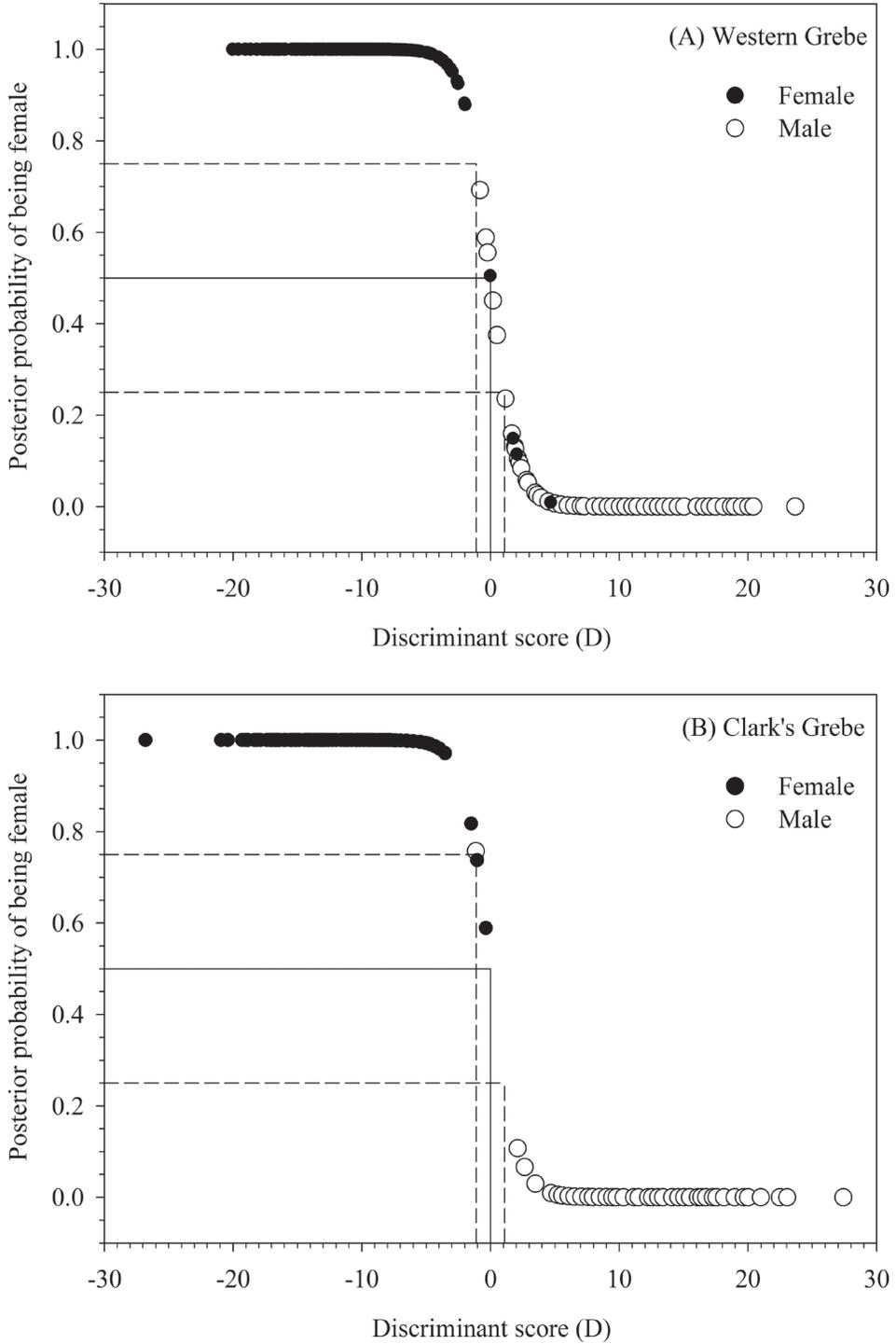


Figure 3. Probability of being a female (A) Western Grebe or (B) Clark's Grebe according to discriminant functions developed for each species from individuals captured in California, USA, during 2012-2013. The Western Grebe discriminant function included the measurements head-to-bill length, bill depth, and short tarsus length, whereas the Clark's Grebe discriminant function included the measurements head-to-bill length and bill depth. The solid line denotes the cutoff point for discriminant scores where the probability of being female was 50%. The dashed lines denote the cutoff points for discriminant scores where the probability of being female was 25% and 75%.

male Clark's Grebes (*Wilks*  $\lambda = 0.95$ :  $F_{1, 278} = 13.40$ ,  $P < 0.001$ ). However, this function correctly classified only 59% of male Western Grebes (77 misclassified as male Clark's Grebes) and 62% of male Clark's Grebes (35 misclassified as male Western Grebes). Leave-one-out cross-validation also correctly classified only 59% of male Western Grebes (77 misclassified as male Clark's Grebes) and 62% of male Clark's Grebes (35 misclassified as male Western Grebes). There was considerable overlap in head-to-bill length measurements between males of the two grebe species, so much so that most individuals had less than a 75% probability of being assigned to the correct species (Fig. 4A). Thus, DFA could not be used to successfully differentiate between males of these two grebe species.

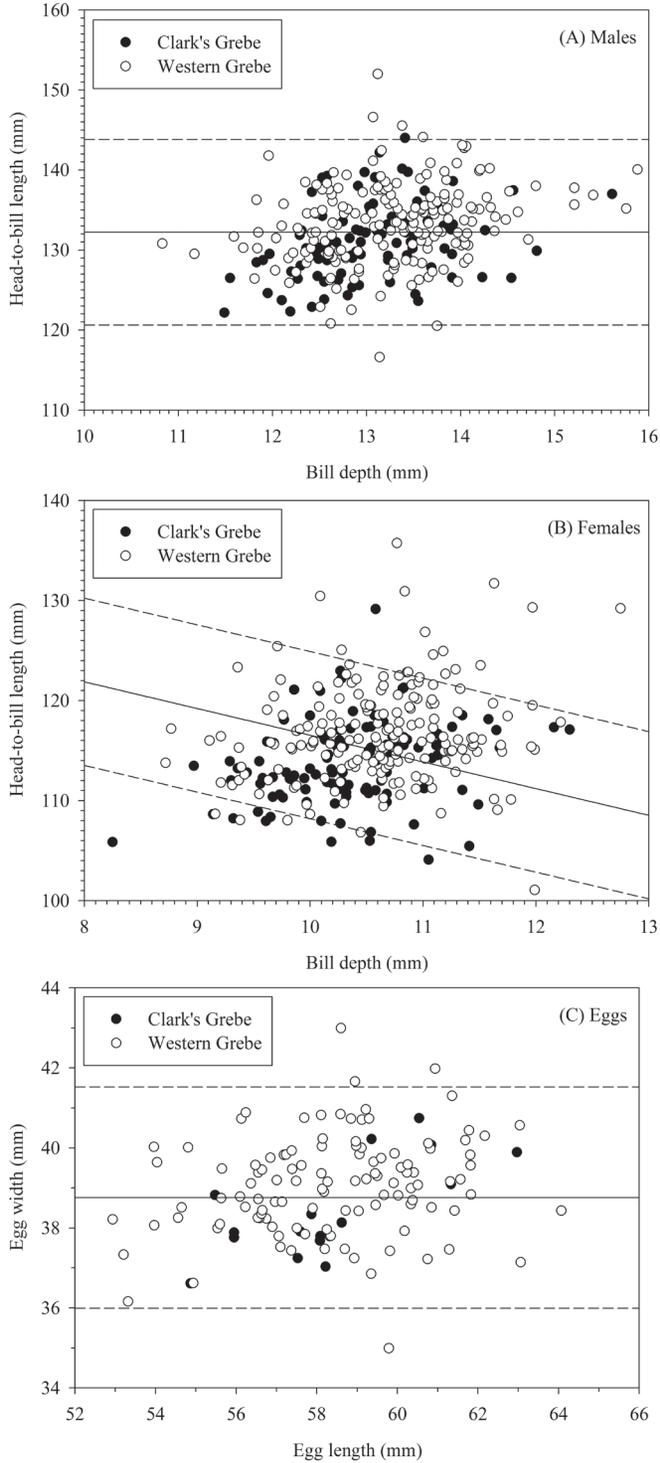
The combination of head-to-bill length and bill depth performed best at discriminating female Western Grebes and female Clark's Grebes (*Wilks*  $\lambda = 0.90$ :  $F_{2, 293} = 16.79$ ,  $P < 0.001$ ). However, similar to the poor function for assigning species to male grebes, this function correctly classified only 63% of female Western Grebes (69 misclassified as female Clark's Grebes) and 63% of female Clark's Grebes (41 misclassified as female Western Grebes). Leave-one-out cross-validation also correctly classified only 63% of female Western Grebes (69 misclassified as female Clark's Grebes) and 63% of female Clark's Grebes (41 misclassified as female Western Grebes). We identified a total of 20 potential multivariate outliers using Mahalanobis distances (15 Western Grebe females, 5 Clark's Grebe females). Removing these individuals reduced the proportion of correctly identified Western Grebe females (resubstitution: 62%; leave-one-out: 62%), and Clark's Grebe females (resubstitution: 61%; leave-one-out: 61%). There was considerable overlap in head-to-bill length measurements between females of the two species, such that most individuals had less than a 75% probability of being assigned to the correct species (Fig. 4B). Thus, as with males, DFA could not be used to successfully differentiate between females of these two grebe species.

#### Western Grebe vs. Clark's Grebe Discriminant Function Analyses for Eggs

We sampled 114 Western Grebe eggs and 16 Clark's Grebe eggs. An average of 16 Western Grebe eggs (Range = 1-30) and four Clark's Grebe eggs (Range = 2-7) were sampled at each lake. There were no significant species differences in any of the four egg measurements evaluated (Table 3). Egg length was essentially identical between the two species, and egg width was, on average, 1.6% wider in Western Grebe eggs, which resulted in an egg volume 3.2% greater in Western Grebes than in Clark's Grebes. A discriminant function with egg width alone was selected for discriminating Western Grebe eggs from Clark's Grebe eggs (*Wilks*  $\lambda = 0.97$ :  $F_{1, 128} = 3.41$ ,  $P = 0.07$ ). However, this function only correctly classified 59% of Western Grebe eggs (47 misclassifications) and 63% of Clark's Grebe eggs (six misclassifications). Leave-one-out cross-validation also only correctly classified 59% of Western Grebe eggs (47 misclassifications) and 63% of Clark's Grebe eggs (six misclassifications). Thus, DFA could not be used to successfully differentiate eggs between these two grebe species.

#### DISCUSSION

Western and Clark's grebes exhibited substantial sexual size dimorphism with males being significantly larger than females in each of the seven morphological measurements examined. The greatest sexual size dimorphism was observed in mass (24%) and those measurements associated with bill morphology (14-26%; bill depth, exposed culmen length, nares-to-bill length, and head-to-bill length). In contrast, sexual size dimorphism in short tarsus length and flattened wing length was relatively smaller (6-9%). Livezey and Storer (1992) suggested that sexual size dimorphism in *Aechmophorus* grebes may result from a combination of factors including sexual selection and intersexual partitioning of feeding niche. Disproportionately large sexual dimorphism in bill morphology relative to other morphological



**Figure 4.** Head-to-bill length and bill depth of (A) male and (B) female Western and Clark's grebes, and (C) length and width of Western and Clark's grebe eggs collected in California, USA, during 2012-2013. In each plot, the solid line denotes where the respective discriminant functions classified individuals as Western Grebes (above line) and Clark's Grebes (below line). Due to the poor performance of these discriminant functions, most individuals lie in the area between the dashed lines, which represents the morphometric space where the discriminant function had a  $< 75\%$  probability of assigning the correct species.

**Table 3.** Mean ( $\pm$  SD) egg measurements, *F*-statistics, and *P*-values from a one-way ANOVA of species differences, and percent species differences for 130 eggs of Western and Clark's grebes sampled in California, USA, 2012-2013.

	Western Grebes ( $\pm$ SD)	Clark's Grebes ( $\pm$ SD)	$F_{1, 129}$	<i>P</i>	% Species Differences
Egg length (mm)	58.41 (2.27)	58.33 (2.25)	0.02	0.90	0.1
Egg width (mm)	39.06 (1.24)	38.45 (1.23)	3.41	0.07	1.6
Egg volume (mm <sup>3</sup> )	45.25 (3.64)	43.84 (4.24)	2.02	0.16	3.2
Egg elongation <sup>a</sup>	1.50 (0.07)	1.52 (0.04)	1.41	0.24	1.4

<sup>a</sup>Egg length divided by egg width.

traits (Selander 1966; this study), coupled with observations of potential sex differences in prey fish size during brood rearing (Forbes and Sealy 1990), suggest that intersexual niche partitioning may be a driver of sexual size dimorphism in these species.

Large sexual size dimorphism in morphometrics associated with bill size is common among grebes (Pyle 2008), and has been used in sex identification of various grebe species (Nuechterlein and Storer 1982; Piersma 1988; Kloskowski *et al.* 2006; Amat *et al.* 2014). However, formal discriminant functions for sex assignment of live birds have only been published for Great Crested Grebe (*Podiceps cristatus*; Piersma 1988), Eared Grebe (*P. nigricollis*; Jehl *et al.* 1998), and Red-necked Grebe (*P. grisegena*; Kloskowski *et al.* 2006). Livezey and Storer (1992) developed a discriminant function for species and sex assignment of Western and Clark's grebes, but they used skeletal measurements obtained from museum specimens, which are not measurable in the field on live birds. Ratti *et al.* (1983) developed a discriminant function for differentiating species of *Aechmophorus* grebes in the field, but not for sex identification.

The discriminant functions we developed in this study provide a highly accurate method for sex determination of *Aechmophorus* grebes in the field. From our discriminant functions, we provide morphometric cut-off values to accurately determine sex of *Aechmophorus* grebes in the hand. A Western Grebe with a bill depth  $\geq 12$  mm and either head-to-bill length  $\geq 127$  mm, nares-to-bill length  $\geq 57$  mm, or exposed culmen length  $\geq 69$  mm is likely a male, whereas a Western Grebe with a bill depth  $\leq 11.5$  mm and either head-to-bill length  $\leq 126$  mm,

nares-to-bill length  $\leq 56$  mm, or exposed culmen length  $\leq 68$  mm is likely a female (Fig. 2A). Similarly, a Clark's Grebe with a bill depth  $\geq 11.5$  mm and either head-to-bill length  $\geq 126$  mm, nares-to-bill length  $\geq 56$  mm, or exposed culmen length  $\geq 66$  mm is likely a male, whereas a Clark's Grebe with a bill depth  $\leq 11$  mm and either head-to-bill length  $\leq 125$  mm, nares-to-bill length  $\leq 55$  mm, or exposed culmen length  $\leq 65$  mm is likely a female (Fig. 2B). These rules correctly assigned sex to  $> 96\%$  of Western and Clark's grebes in our sample.

Two factors suggest that the discriminant functions we developed are robust for sexing Western and Clark's grebes throughout much of their range. First, morphometrics common to this study and those reported for grebes sampled from the United States and Canada (LaPorte *et al.* 2013) had similar values and degrees of sexual size dimorphism. Second, we measured a large number of grebes ( $n = 576$ ) from 29 separate lakes and reservoirs throughout California, ranging from Tule Lake on the border with Oregon to Lower Otay Reservoir on the border with Mexico. Because our sample was collected from a broad geographic area, and included numerous local breeding populations, the resulting discriminant functions are likely less susceptible to reductions in accuracy due to geographic variation in morphometrics (Evans *et al.* 1993; Herring *et al.* 2010). However, the discriminant functions presented here are unlikely to perform well with *Aechmophorus* grebes sampled from the Mexican Plateau, which are notably smaller than grebes from the United States and Canada (LaPorte *et al.* 2013).

Compared to sex differences within species, morphological measurements were

small (< 4%) between species. Morphological measurements that were significantly different between species, and which showed the greatest species differences, were the two associated only with bill length (2-4%; nares-to-bill length, exposed culmen length, Table 1). Ratti *et al.* (1983) and Storer and Nuechterlein (1985) also found significant interspecific differences in bill measurements, more so than other morphometrics. Disproportionate interspecific differences in bill morphology compared to other morphometrics, coupled with potential differences in foraging behavior (Ratti 1985; Nuechterlein and Buitron 1989) between Western Grebes and Clark's Grebes, may indicate that interspecific niche partitioning exists between these two species. Yet, although statistically significant, the small (< 4%) differences in bill measurements between Western Grebes and Clark's Grebes could not be used to differentiate them (only 60% of males and 63% of females were correctly classified), indicating little divergence in external morphology between the two species. In contrast, Ratti *et al.* (1983) developed a discriminant function based on nares-to-bill length only, which correctly classified 75% of males and 95% of females to the correct species at the Bear River Migratory Bird Refuge in Utah. This was despite the fact that the observed nares-to-bill length means by species and sex were similar to those observed in our study. Overall, our results indicate that there is little difference in external morphometrics between Western and Clark's grebes, and sex differences are far greater within species than between species.

Western Grebe and Clark's grebe eggs cannot be differentiated using egg morphometrics. The egg discriminant function performed poorly at assigning eggs to species, with an overall error rate of 39%. We conclude that the egg measurements used in this study are not a suitable method for differentiating eggs of Western and Clark's grebes. These results suggest that using egg morphometrics to assign eggs to species may be similarly difficult to detecting conspecific brood parasitism (McRae 1997; Ådahl *et al.* 2004).

Substantial sexual size dimorphism, especially in bill measurements, allowed for the development of highly accurate discriminant functions for distinguishing male from female Western Grebes and male from female Clark's Grebes. Yet, morphometric differences of adults and eggs between species were small compared to large sex differences within species. Further, the development of accurate discriminant functions to differentiate between Western and Clark's grebe adults or eggs was not possible, indicating little divergence in external morphology between the two species. Indeed, Western and Clark's grebes are very closely related, and only in 1985 were they separated into two distinct species (American Ornithologists' Union 1985). Our results suggest that Western and Clark's grebes occupy a similar ecological niche and that the potential for ecological separation is much greater between males and females of both species, than between species.

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