

Sex determination of Red Knots *Calidris canutus roselaari* using morphometrics

Zachary M. Pohlen^{1*}, Lucas H. DeCicco^{1,4}, Joseph B. Buchanan², Pavel S. Tomkovich³ & James A. Johnson¹

¹U.S. Fish and Wildlife Service, Migratory Bird Management, 1011 East Tudor Road, Anchorage, AK 99503, USA

²Washington Department of Fish and Wildlife, 1111 Washington Street SE, Olympia, WA 98501, USA

³Zoological Museum, M.V. Lomonosov Moscow State University, 2 Bolshaya Nikitskaya Str., Moscow, 125009, Russia

⁴Current address: Department of Ecology and Evolutionary Biology, Biodiversity Institute, University of Kansas, Lawrence, KS 66045, USA

*Corresponding author: zachary_pohlen@fws.gov

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Researchers often lack tools to classify sex for monomorphic and weakly dimorphic species in the field, an important component of many avian ecology and demography studies. The Red Knot *Calidris canutus roselaari* exhibits minor differences in size and plumage patterns between sexes, although overlap is considerable and sex is not readily apparent in the field. We captured, measured, and molecularly sexed 198 individuals (68.7% males, $n = 136$; 31.3% females, $n = 62$) at two breeding sites and one migratory stopover site and found significant differences between sexes, with females having a longer total head, culmen, and wing than males. We used a jackknifed cross-validated discriminant function analysis (DFA) to correctly identify sex of 85.9% (95% CI 80.2–90.3%) of all *roselaari* knots (94.8% of males and 66.1% of females). When restricting the probability of group membership to >0.7 , we increased our classification accuracy to 92.3% for females ($n = 26$) and 90.3% for males ($n = 121$) while leaving 25.7% unclassified ($n = 51$). We conclude that DFA provides a means for sexing *roselaari* Red Knots when molecular sex determination is not feasible, and we provide a formula for researchers to use in the field.

Keywords

discriminant function
shorebird
sexing
wader

INTRODUCTION

Accurate determination of the sex of individual birds is an important aspect of avian field ecology (Both *et al.* 2003, Durell 2003), demography (Morrison *et al.* 2016), and conservation (Cristol *et al.* 1999). Determining sex in highly dimorphic species can be straightforward, but in monomorphic or weakly dimorphic species, minor differences in plumage or simple morphometric ranges are often unreliable or inadequate to correctly distinguish all individuals (Pyle 1997). Advances in molecular analysis have made sexing more widely available to researchers (Griffiths *et al.* 1998, Baker *et al.* 1999, Fridolfsson & Ellegren 1999), although limitations still exist. In projects where (1) DNA samples are not available, (2) molecular techniques are unaffordable, or (3) immediate determination of males and females is required to meet study objectives (e.g., migratory and foraging behavior), methods to classify sex from plumage or morphometrics may be required.

The presence of small but significant differences in morphometrics between sexes enables statistical techniques that increase accuracy of classification. The use of discriminant function analysis (DFA) to determine the sex of species with weak sexual size dimorphism (SSD) is well established in many avian families with monomorphic species (Phillips & Furness 1997, Weidinger & van Franeker 1998, Bluso *et al.* 2006, Huallacháin & Dunne 2010, Nunes *et al.* 2013). Among Scolopacidae shorebirds, classification accuracy using DFA can be wide-ranging and includes 67% in Common Redshanks *Tringa totanus* (Meissner *et al.* 2018), 77.1% in Common Sandpipers *Actitis hypoleucos* (Meissner & Krupa 2016), 76.0–78.7% in Eurasian Woodcocks *Scolopax rusticola* (Aradis *et al.* 2015), 78.8% in White-rumped Sandpipers *Calidris fuscicollis* (Scherer *et al.* 2014), 82.7–84.7% in Little Stints *C. minuta* (Niemi *et al.* 2018), $\geq 88\%$ in Buff-breasted Sandpipers *C. subruficollis* (Almeida *et al.* 2020), 89–91.5% in Dunlins *C. alpina* (Brennan *et al.* 1984, 1991), and 91%

However, we found females to be significantly larger than males in three measurements: total head, culmen, and wing. The most accurate DFA used total head and wing to correctly classify the sex of 85.9% of adult *roselaari* Red Knots we sampled. This formula performed better on males than females, with only 66.1% of females being correctly sexed.

Combining this DFA with a higher probability of group membership ($P > 0.7$) correctly classified 92.3% of females and 90.3% of males while leaving 25.7% of birds unsexed. The formula we provide, used in combination with restricted discriminant score cut-offs, will allow researchers to classify the sex of most adults with >90% accuracy.

Potential problems exist when incorporating measurement datasets from multiple observers in a DFA. To address this, we used standardized and widely practiced measurements in an effort to reduce between-observer measurement error, but bias may still have reduced the discrimination rate (Gratto-Trevor 2018). Measurement errors, however, have only a moderate impact on discrimination when random bias affects males and females equally (Dechaume-Moncharmont *et al.* 2011).

Our discrimination rates may have also been impacted by pooling all samples from the two breeding sites and stopover site. We are currently unable to assign breeding locality for birds captured during migration, and it is unclear whether there are significant morphometric differences between birds breeding at our two localities. The low sample size of eight males and one female from Wrangel Island made it impossible to compare measurements between the two breeding sites, although differences may exist.

The male-biased sex ratio on the breeding grounds is expected based on breeding biology and timing of captures. Red Knots are socially monogamous and both sexes incubate and with few exceptions, the male cares for the chicks (Tomkovich *et al.* 2018). Nests occur in low densities and are difficult to find, therefore the number of females captured is low, while capturing males during the brood-rearing period is more reliable (Johnson *et al.* 2020).

Differences in linear measurements between sexes were comparable to previous work showing females having longer wings and culmens in *roselaari* Red Knot and culmen having the highest levels of SSD followed by wing and tarsus (Tomkovich 1992). Previous work on *canutus*, *islandica*, and *rufa* showed similar SSD for culmen (range = 2.9–7.1), total head (1.9–4.2), and wing (1.4–2.7) and the same order of SSD among measurements as our *roselaari* birds with culmen the highest followed by total head, wing, and tarsus (Baker *et al.* 1999). However, our DFA performed better than that for other subspecies. For combined Wadden Sea-wintering *canutus* and *islandica*, classification accuracy was 75.9% ($n = 112$), Brazil-wintering *rufa* 80.0% ($n = 90$), and migratory Delaware Bay *rufa* 69.4% ($n = 85$; Baker *et al.* 1999), all of which fell below our 95% CI: 80.2–90.3% in *roselaari*. However, comparisons are difficult without 95% CI from both studies. Differences in discrimination by subspecies

could be explained by differences in morphometrics by subspecies (Tomkovich 1992, Baker *et al.* 2020). Our formula is also only applied to adults on or traveling to the breeding grounds (April–July) and not undergoing primary molt. Including juveniles that could still be growing or individuals on the wintering grounds that may be in some stage of primary feather molt or wear would decrease the formula's accuracy. For this reason, the DFA and subsequent field formula we present here should be used with caution outside of spring migration and the breeding season. Future investigators should evaluate project objectives to determine the consequences of misclassification or acceptance of unidentified individuals when using this equation.

Molecular sexing is a more accurate method for determining sex in Red Knots than using morphometrics (Baker *et al.* 1999). However, when researchers are collecting sex-based samples or require sex-based transmitter deployment in the field, molecular sexing is not possible. Using morphometrics and a DFA is also a less invasive approach when dealing with species of conservation concern or historical datasets without molecular data. Although DFA is not perfect in species with low SSD like Red Knots, our formula and discriminant score cut-offs provide a useful method to address this problem when logistics require it.

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