MORPHOMETRIC SEX DETERMINATION OF BREEDING ADULT ROYAL *THALASSEUS MAXIMUS* AND SANDWICH TERNS *T. SANDVICENSIS* IN LOUISIANA

MEGAN NEPSHINSKY[1], SABRINA S. TAYLOR[2], JEFF S. LIECHTY[1,3], ASHLEE K. MINOR[1,4], EVA D. WINDHOFFER[1,5] & AARON R. PIERCE[1,6]

[1]*Nicholls State University, 906 E. 1st Street, Thibodaux, Louisiana 70310, USA*
[2]*Louisiana State University and AgCenter, Baton Rouge, Louisiana 70803, USA*
[3]*Current address: Audubon Florida, Florida Coastal Island Sanctuaries, 410 Ware Blvd #702, Tampa, Florida 33619, USA*
[4]*Current address: Southern Illinois University, 1263 Lincoln Drive, Carbondale, Illinois 62901, USA*
[5]*Current address: Louisiana Oil Spill Coordinator's Office, Baton Rouge, Louisiana 70806, USA*
[6]*Current address: Ducks Unlimited, Inc., 125 Southpark Rd., Lafayette, Louisiana 70508, USA (apierce@ducks.org)*

Received 23 September 2020, accepted 07 January 2021

ABSTRACT

NEPSHINSKY, M., TAYLOR, S.S., LIECHTY, J.S., MINOR, A.K., WINDHOFFER, E.D. & PIERCE, A.R. 2021. Morphometric sex determination of breeding adult Royal *Thalasseus maximus* and Sandwich terns *T. sandvicensis* in Louisiana. *Marine Ornithology* 49: 127–132.

Many seabirds have sex-specific traits, including survival, philopatry, behavior, and diet, information that is essential for effective conservation strategies. The difficulty of sexing monomorphic seabirds, however, has resulted in a lack of information on these important traits and an incomplete understanding of the ecology of many species. We used a polymerase chain reaction (PCR)-based sexing approach to determine if Royal *Thalasseus maximus* and Sandwich terns *T. sandvicensis* in Louisiana, USA can be accurately sexed using morphometrics. DNA samples and morphological measurements were obtained from Royal Terns (n = 106) and Sandwich Terns (n = 112) to accurately identify sex and compare gender differences in morphological measurements. In both species, males had greater bill length and head + bill length relative to females. Sexual size dimorphism ranged from 0.0%–4.7% in Royal Terns and 0.4%–3.5% in Sandwich Terns. Using discriminant function analysis, equations correctly assigned sex in 75% of Royal Terns using head + bill and wing chord measurements and in 82% of Sandwich Terns using head + bill and mass measurements. Our methods provide an accurate and economical field-sexing technique for Royal and Sandwich terns, enabling research into sex-based differences in behavior, physiology, and ecology.

Key words: molecular sexing, monomorphic, Polymerase chain reaction (PCR), seabird, sexual size dimorphism

INTRODUCTION

Demographics, behavior, and physiology often differ between male and female seabirds, yet many seabird species are monomorphic. The difficulty of determining sex in monomorphic species has often left sex-specific traits unaddressed by researchers, resulting in an incomplete understanding of species ecology. Recent research has demonstrated sex-biases in survival (Nichols *et al*. 2004), mortality (Ryan & Boix-Hinzen 1999, Gianuca *et al.* 2017), natal philopatry (Becker *et al*. 2007), diet (Nisbet *et al*. 2002), foraging strategies (Lewis *et al*. 2002, García-Tarrasón *et al*. 2015, Gwiazda & Ledwoń 2015), physiology (Ackerman *et al.* 2008), and parental care (Fasola & Saino 1995, Reichert & Becker 2017), highlighting the importance of such information for a comprehensive understanding of seabird natural history. The development of effective sexing techniques in monomorphic species would support research on the extent and importance of currently unexplored sex-specific traits and could help in the design of conservation strategies.

Multiple methods are used to determine the sex of seabirds, including acoustic analysis (Bourgeois *et al*. 2007), cloacal measurements during the breeding season (Copestake *et al.* 1988), and molecular analysis (Morinha *et al*. 2012). Among these methods, molecular analysis is extremely reliable but can be time-consuming and expensive (Dechaume-Moncharmont *et al*. 2011, Morinha *et al*. 2012). A potentially reliable, cost-effective way to determine the sex of sexually-monomorphic birds is to use morphometric measurements (Dechaume-Moncharmont *et al*. 2011). Morphometric measurements can be taken in the field, and they are economical and less invasive. Therefore, recent avian sexing studies have focused on developing discriminant function equations that use morphometric measurements to determine sex within seabird species (Dechaume-Moncharmont *et al*. 2011).

Investigations using morphometric measurements to sex seabirds have focused on identifying those measurements that are most effective in classifying sex. For example, the length of the head plus bill (henceforth head + bill) correctly sexed 73% of Common Terns *Sterna hirundo* and 72% of Arctic Terns *S. paradisaea* (Fletcher & Hamer 2003). In contrast, wing chord and five separate head measurements were used to correctly sex 78% of Sooty Terns *Onychoprion fuscata* (Reynolds *et al*. 2008). Overall, seabird sex classification rates from previous studies have ranged 72.0%–94.0% using morphometric measurements (Fletcher & Hamer 2003, Bluso *et al*. 2006, Palestis *et al*. 2012, Lisnizer *et al*. 2014).

Royal Terns *Thalasseus maximus* and Sandwich Terns *T. sandvicensis* are two monomorphic seabird species that nest abundantly on

Louisiana's coastal islands. Louisiana is experiencing the highest rates of coastal wetland loss in the United States (Couvillion *et al.* 2011), which has important implications to breeding seabirds and emphasizes the importance of understanding their basic biology and ecological needs. Previous research on Royal and Sandwich tern diet composition (Liechty *et al.* 2016) and survivorship (Liechty *et al*. 2017) provided important information, but it did not address the role of sex-bias. Towards enhancing effective conservation strategies for Royal and Sandwich terns, the objective of this study was to determine whether morphometric measurements can be used to reliably determine sex in these species.

METHODS

Study area

The Isles Dernieres Barrier Islands Refuge (IDBIR) is a chain of barrier islands located off the coast of southeast Louisiana (29°03′N, 90°57′W to 29°05′N, 90°36′W; Fig. 1) and is one of the most vulnerable areas to coastal land loss in the United States (Lindstedt 2005). The IDBIR is currently composed of five islands spanning approximately 32.5 km (from east to west): Wine, Trinity, Whiskey, East Raccoon, and West Raccoon. Large breeding colonies (approximately 7 000 breeding pairs) of Royal and Sandwich terns occurred on East Raccoon during the study period (Raynor *et al*. 2012, Windhoffer 2017).

Data collection

Adult Royal and Sandwich terns were captured using a hand net at breeding colonies in May and June 2014–2016. Adults aggressively defend their nests around the time of egg hatching and can be closely approached, facilitating capture. Measurements of tarsus, head + bill length, and bill length were taken to the nearest 0.1 mm using calipers, and un-flattened wing chord was taken to the nearest 1 mm using a wing ruler. Body mass was measured to the nearest gram using a spring scale. Blood samples were taken from the brachial vein following avian bleeding methods outlined in Owen (2011). Blood samples were stored in a 1.5-mL plastic microtube and mixed with 1.0 mL of Queen Lysis Buffer solution (Seutin *et al*. 1991).

Molecular analysis

DNA from each blood sample (blood and buffer mixture) was extracted using the QIAGEN® DNeasy® Blood & Tissue Kit Quick-Start Protocol for nucleated blood (QIAGEN 2011). The protocol was slightly modified to extract DNA from avian blood, such that 20 mL of proteinase K (Product No. 19133) was added to a new 1.5 mL microcentrifuge tube together with 100–150 mL of blood sample. Between 50 mL and 100 mL of phosphate buffered saline (PBS) was added to the mixture to a total of 220 mL. The 1× PBS solution consisted of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4. Otherwise, the extraction followed the manufacturer's protocol. Each set of extractions included a negative control to ensure that there was no cross contamination among samples. The negative control consisted of 20 mL of proteinase K and 200 mL of PBS.

DNA was eluted with 200 mL Buffer AE and the amount of DNA in each sample was measured using a NanoDrop 2000. If the DNA concentration was < 2.0 ng/mL, additional blood was extracted to ensure that there was enough DNA available for amplification. Randomly selected blood samples were processed twice for both Royal Terns (n = 14) and Sandwich Terns (n = 12) to ensure accuracy in sexing methods (Daniel *et al.* 2007).

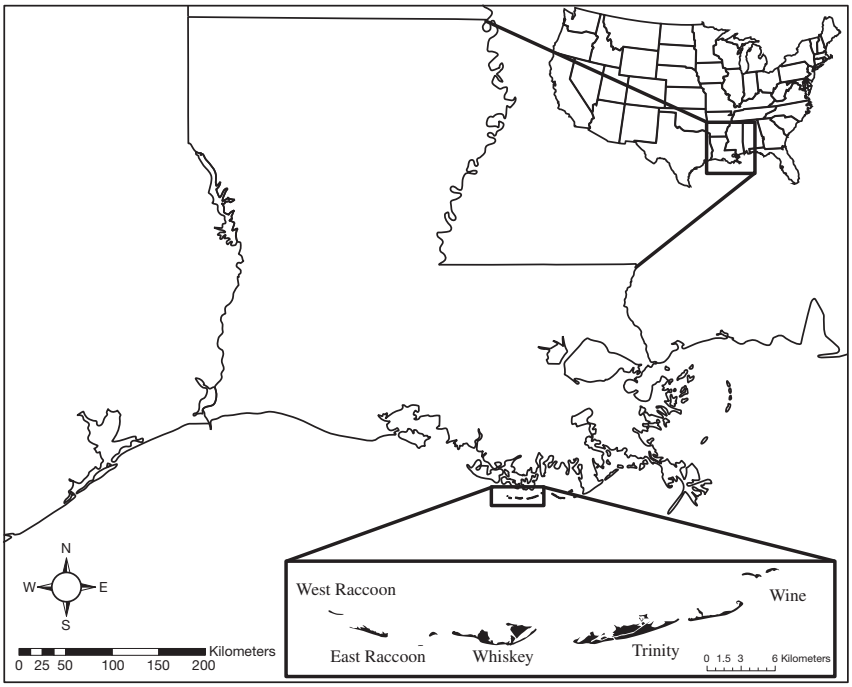

Fig. 1. Location of the Isles Dernieres Barrier Islands Refuge in south Louisiana, USA, including (from east to west): Wine Island, Trinity Island, Whiskey Island, East Raccoon Island, and West Raccoon Island. Inset at top right shows the study location relative to the continental USA.

Following extraction, DNA was amplified with polymerase chain reaction (PCR) and primers CHDaF (5′-TTCTCTCAGATGGTGAGGATG-3′) and CHDaR (5′-TCCTCAATTCCYCCTTTTTATTGA-3′; Peters *et al.* 2014), which amplify the chromo-helicase DNA binding gene on the avian sex chromosomes W and Z. PCR reactions consisted of 10 mM of both the forward and reverse primers, 10× Buffer (New England BioLabs, Product No. B9015S), 8 mM dNTPs (New England BioLabs, Product No. N0447S), 1.5 mM $MgCl_2$ (New England BioLabs, Product No. B9021S), 0.1 mL of *Taq* DNA polymerase (New England BioLabs, Product No. B9014S), 1.0 mL of eluted DNA, and nanopure water for a total reaction volume of 10 mL. The PCR conditions followed Peters *et al.* (2014), which consisted of an initial denaturation step of 94 °C for one minute and then 45 cycles of 94 °C for 20 seconds, 58 °C for 20 seconds, 72 °C for one minute, with a final elongation step of 72 °C for seven minutes.

The PCR products (4 mL) combined with 1.2× EZ-VISION® DNA Dye Loading Buffer (2.0 mL; AMRESCO; Product No. N313) were visualized on 1.0% agarose gels. A molecular standard (New England BioLabs 100 base pair DNA ladder, Product No. N3231S) was loaded in the first well on each row to ensure that the PCR products were in the right size range. For most avian species, including terns, chromosome Z is present in both males and females, and primers amplify a region between 600 and 650 base pairs; in contrast, chromosome W occurs only in females, and primers amplify a region between 400 and 450 base pairs (Lisnizer *et al.* 2014). Thus, the presence of two bands indicated a male bird, whereas a single band indicated a female bird.

Statistical analysis

A univariate two-tailed *t*-test was performed on each morphometric measurement (mass, bill length, head + bill length, wing cord, and tarsus length) to determine if there was a difference between males and females of each species (means ± standard error (SE) reported). Sexual size dimorphism (SSD) was calculated following Fletcher & Hamer (2003). SSD provides an indication of how different males and females are for a given characteristic, with a greater SSD indicating a greater difference between males and females. Discriminant function analysis (DFA) was used to determine if morphometric measurements could be used to reliably sex both species. Each discriminant function equation was created using morphometric measurements (Dechaume-Moncharmont *et al*. 2011). The DISCRIM function in program SAS (SAS Institute, Inc. 2008) was used to classify males and females into groups based on morphometric measurements and to identify which variables were retained by the DFA. In addition, Wilks' Lambda was used to determine if the discriminant function analysis was significant ($P < 0.05$) between males and females for each species. Lastly, a jackknife cross validation was performed under the DISCRIM program to measure the accuracy of the DFA.

RESULTS

Morphometrics and blood samples were collected for 106 Royal Terns and 112 Sandwich Terns over the study period. Molecular analysis identified 74 male and 32 female Royal Terns, and 51 male and 61 female Sandwich Terns. The repeated molecular analysis of a subset of Royal Terns ($n = 14$) and Sandwich Terns ($n = 12$) was 100% consistent with previous results, attesting to the high level of repeatability of the molecular methods used in this study.

Univariate analysis revealed that two of the five morphometric measurements, head + bill and bill, were significantly greater in males than females of both species (Tables 1 and 2). Mean head + bill length for male Royal Terns (range 122.4–139.5 mm) was 4.4 mm greater than in females (range 117.4–129.9 mm), and male mean bill length (range 61.4–72.4 mm) was 3.0 mm greater than in females (range 60.6–69.0 mm). In Sandwich Terns, male mean head + bill length (range 95.9–107.6 mm) was 3.5 mm greater than female head + bill length (range 90.2–107.4 mm), and mean bill length was 1.8 mm greater for males (range 49.6–60.0 mm) than females (range 46.5–60.3 mm). Sexual size dimorphism for the five measurements ranged 0.0%–4.7% in Royal Terns and 0.4%–3.5% in Sandwich Terns.

DFA equations retained the variables head + bill and wing chord for Royal Terns and head + bill and mass for Sandwich Terns. The Wilk's Lambda multivariate analyses revealed that DFAs were significant ($P < 0.01$). The jackknife cross validation revealed that 82.1% of Royal Terns and 75.0% of Sandwich Terns were correctly classified based on the DFA for each species. Lastly, the canonical scores generated by the DFAs were used to illustrate the distribution of males and females based on DFAs for both species (Figs. 2, 3).

DISCUSSION

Molecular analysis was effective in classifying males and females of both Royal and Sandwich terns, such that repeated analyses were

TABLE 1
Mean (± standard error) morphometric measurements and sexual size dimorphism (SSD) of adult male and female Royal Terns *Thalasseus maximus* breeding on the Isles Dernieres Barrier Islands Refuge, Louisiana, USA in May and June 2014–2016

Variable	Male ($n = 74$)	Female ($n = 32$)	*P* value	SSD
Bill (mm)	67.1 ± 0.2	64.1 ± 0.3	< 0.01	4.68%
Head + Bill (mm)	128.0 ± 0.4	123.6 ± 0.5	< 0.01	3.56%
Tarsus (mm)	33.5 ± 0.3	33.5 ± 0.4	0.97	0%
Wing Chord (mm)	376.4 ± 0.7	375.6 ± 2.1	0.68	0.21%
Mass (g)	461.5 ± 3.6	449.7 ± 6.5	0.09	2.62%

TABLE 2
Mean (± standard error) morphometric measurements and sexual size dimorphism (SSD) of adult male and female Sandwich Terns *Thalasseus sandvicensis* breeding on the Isles Dernieres Barrier Islands Refuge, Louisiana, USA in May and June 2014–2016

Variable	Male ($n = 51$)	Female ($n = 61$)	*P* value	SSD
Bill (mm)	54.9 ± 0.3	53.1 ± 0.3	< 0.01	3.39%
Head + Bill (mm)	102.3 ± 0.3	98.8 ± 0.4	< 0.01	3.54%
Tarsus (mm)	26.1 ± 0.3	25.8 ± 0.3	0.29	1.16%
Wing Chord (mm)	297.4 ± 2.1	296.2 ± 1.0	0.57	0.41%
Mass (g)	208.4 ± 1.8	203.9 ± 2.3	0.13	2.21%

100% consistent with each other. The molecular results showed an approximately equal sex ratio among captured Sandwich Terns, but over twice as many Royal Tern males were captured than females. The observed male-dominated sex-bias in sampled Royal Terns could be due to differential allocation of parental duties (Fasola & Saino 1995) or differences in aggression between sexes, whereby tending males were more likely to be captured due to more aggressive defense of young chicks (Yorio & Quintana 1997). The sex-bias of Royal Terns captured in our study highlights the importance of sex-based research and the need for accurate and economical sexing methods.

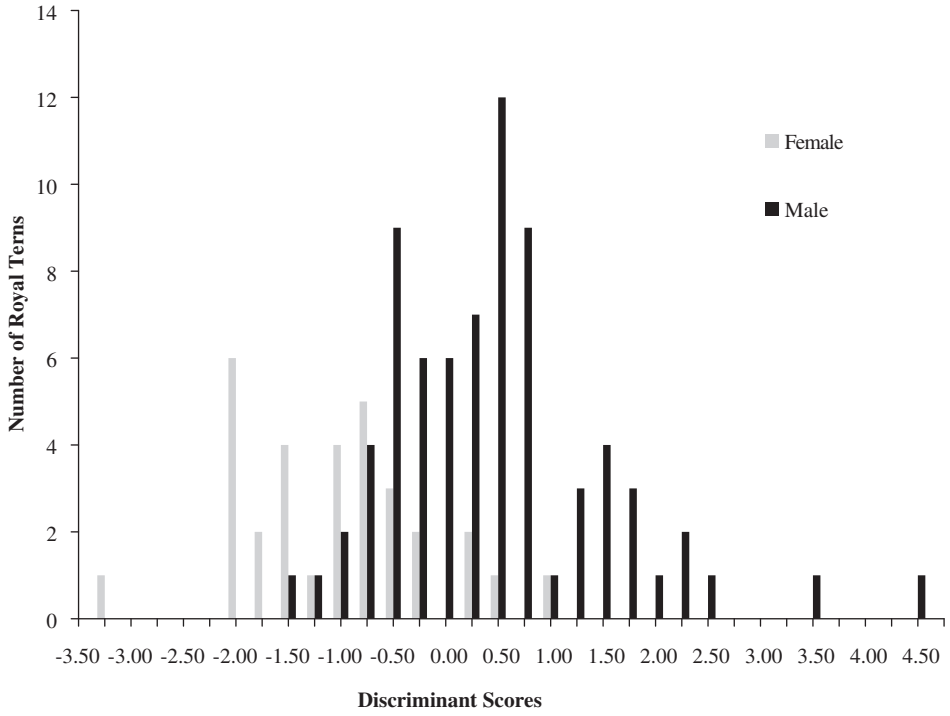

Fig. 2. Frequency distribution of adult breeding male and female Royal Tern *Thalasseus maximus* canonical discriminant function scores based on head + bill length and wing chord length (n = 106) caught in May and June 2014–2016 on the Isles Dernieres Barrier Islands Refuge in Louisiana, USA.

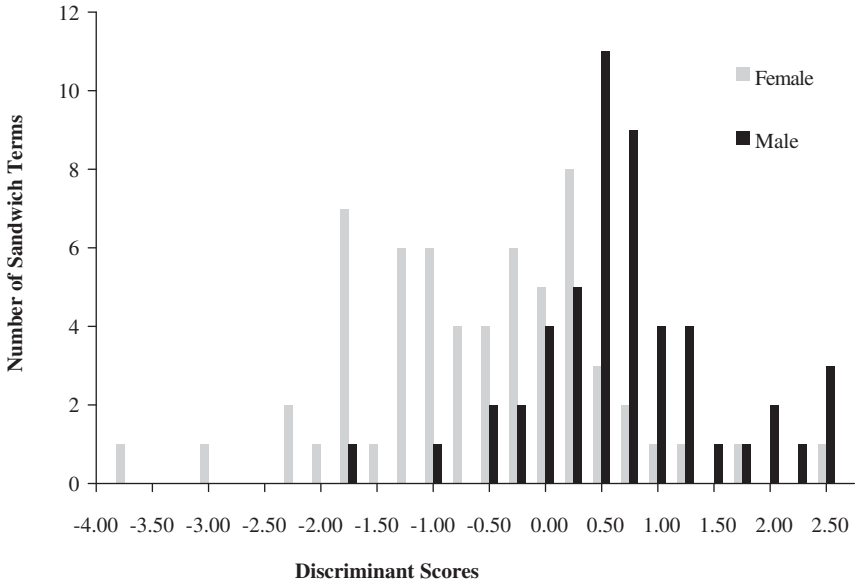

Fig. 3. Frequency distribution of adult breeding male and female Sandwich Tern *Thalasseus sandvicensis* canonical discriminant function scores based on head + bill length and mass (n = 112) caught in May and June 2014–2016 on the Isles Dernieres Barrier Islands Refuge in Louisiana, USA.

Bill and head + bill measurements were found to be significantly greater in males than females for both Royal and Sandwich terns. These measurements also differ between sexes of other tern species, with head + bill frequently used in discriminant functions (Devlin *et al.* 2004, Bluso *et al.* 2006, Nisbet *et al*. 2007, Fletcher & Hamer 2003, Shealer & Cleary 2007, Reynolds *et al*. 2008, Ackerman *et al*. 2008, Ledwoń 2011, Palestis *et al*. 2012, Lisnizer *et al*. 2014). A recent study on Royal Terns and "Cayenne Terns" (*T. s. eurygnathus*) in Patagonia found similar results to ours (Lisnizer *et al.* 2014). In that study, Cayenne Tern males had larger bill depth, bill length, and head length, and male Royal Terns had larger head length than females. Overall, the study correctly sexed 78% and 75% of Cayenne Terns and Royal Terns, respectively. Mean measurements between our study and Lisinzer *et al*. (2014) indicate potential variability between populations, demonstrating the need for establishing morphological determinants of sex for individual populations.

Head + bill and wing chord variables were retained in the DFA equation for Royal Terns and resulted in the 82.1% classification rate. For Sandwich Terns, the DFA equation retained head + bill and mass. The mass variable has been excluded in other studies due to its variability between winter and breeding seasons, in which there are equal foraging rates between males and females in the winter but not in the summer (Croxall 1995). In our study, both Royal and Sandwich terns were banded and measured only during the breeding season, thus eliminating the variability between seasons. However, seabird mass may still fluctuate throughout the breeding season, depending on breeding phase and the availability of food (Paredes *et al*. 2015).

Morphometric measurements may eliminate the need to use the invasive procedures that are required for molecular analysis, such as blood extraction (Owen 2011). The jackknife cross validation in our study confirmed that 82.1% and 75.0% of breeding adult Royal and Sandwich terns, respectively, were correctly classified in our study using morphometric measurements. Both classification rates fall within the range, 72.0%–94.0%, of similar species classifications studies (Fletcher & Hamer 2003, Bluso *et al*. 2006, Palestis *et al.* 2012, Lisnizer *et al.* 2014). The ability to use these basic measurements to determine the sex of breeding adult Royal and Sandwich terns can enhance further investigations into demographics, physiology, diet, and behavior. However, the usefulness of morphometric measurements to determine the sex of Royal and Sandwich terns is clearly limited, and thus, may be inadequate for the enhancement of some investigations that require greater accuracy in sex determination.

ACKNOWLEDGMENTS

Funding and logistical support for this project were provided by Nicholls State Biology Department, Louisiana Universities Marine Consortium, and Louisiana Department of Wildlife and Fisheries. This material is also based upon work that is supported by the National Institute of Food and Agriculture, US Department of Agriculture, McIntire Stennis program (S. Taylor, Louisiana State University AgCenter). Fieldwork was assisted by Ashley Booth and Justin Homer, and William Crenshaw assisted with molecular work in the lab. Handling of Royal and Sandwich terns was conducted under US Geological Survey Bird Banding Permit 23596, Louisiana Department of Wildlife and Fisheries Scientific Collection Permit LNHP-14-005, 15-005, 16-004, 17-009, and Nicholls State University Institutional Animal Care and Use Committee approvals IA038, IA043, IA045, and IA049. We appreciate very much the comments from Darin Lee and anonymous reviewers who helped to improve our paper.

REFERENCES

ACKERMAN, J.T., EAGLES-SMITH, C.A., TAKEKAWA, J.Y., BLUSO, J.D. & ADELSBACH, T.L. 2008. Mercury concentrations in blood and feathers of prebreeding Forster's Terns in relation to space use of San Francisco Bay, California, USA, habitats. *Environmental Toxicology and Chemistry* 27: 897–908.

ACKERMAN, J.T., TAKEKAWA, J.Y., BLUSO, J.D., YEE, J.L. & EAGLES-SMITH, C.A. 2008. Gender identification of Caspian Terns using external morphology and discriminant function analysis. *The Wilson Journal of Ornithology* 120: 378–383.

BECKER, P.H., EZARD, T.H.G., LUDWIGS, J.D., SAUER-GÜRTH, H. & WINK, M. 2008. Population sex ratio shift from fledging to recruitment: consequences for demography in a philopatric seabird. *Oikos* 117: 60–68.

BLUSO, J., ACKERMAN, J., TAKEKAWA, J. & YEE, J. 2006. Sexing Forster's Terns using morphometric measurements. *Waterbirds* 29: 512–517.

BOURGEOIS, K., CURÉ, C., LEGRAND, J. ET AL. 2007. Morphological versus acoustic analysis: what is the most efficient method for sexing yelkouan shearwaters *Puffinus yelkouan*. *Journal of Ornithology* 148: 261–269.

COPESTAKE, P.G., CROXALL, J.P. & PRINCE, P.A. 1988. Use of cloacal sexing techniques in mark-recapture estimates of breeding population size in Wilson's storm-petrel O*ceanites oceanicus* at South Georgia. *Polar Biology* 8: 271–279.

COUVILLION, B.R., BARRAS, J.A., STEYER, G.D. ET AL. 2011. *Land Area Change in Coastal Louisiana from 1932 to 2010*. Denver, USA: US Geological Survey Scientific Investigations Map 3164, 12 p.

CROXALL, J.P. 1995. Sexual size dimorphism in seabirds. *Oikos* 73: 399–403.

DANIEL, C., MILLAR, C.D., ISMAR, S.M.H., STEPHENSON, B.M. & HAUBER, M.E. 2007. Evaluating molecular and behavioral sexing methods for the Australasian gannet (*Morus serrator*). *Australian Journal of Zoology* 55: 377–382.

DECHAUME-MONCHARMONT, F., MONCEAU, K. & CEZILLY. F. 2011. Sexing birds using discriminant function analyses: a critical appraisal. *The Auk* 128: 78–86.

DEVLIN, C.M., DIAMOND, A.W. & SAUNDERS, G.W. 2004. Sexing Arctic Terns in the field and laboratory. *Waterbirds* 27: 314–320.

FASOLA, M. & SAINO, N. 1995. Sex-biased parental-care allocation in three tern species (Laridae, Aves). *Canadian Journal of Zoology* 73: 1461–1467.

FLETCHER, K.L. & HAMER, K.C. 2003. Sexing terns using biometrics: the advantage of within-pair comparisons. *Bird Study* 50: 78–83.

GARCÍA-TARRASÓN, M., BÉCARES, J., BATEMAN, S. ET AL. 2015. Sex-specific foraging behavior in response to fishing activities in a threatened seabird. *Ecology and Evolution* 5: 2348–2358.

GIANUCA, D., PHILLIPS, R.A., TOWNLEY, S. & VOTIER, S.C. 2017. Global patterns of sex- and age-specific variation in seabird bycatch. *Biological Conservation* 205: 60–76.

GONZALEZ-SOLIS, J., CROXALL, J.P. & WOOD, A.G. 2000. Sexual dimorphism and sexual segregation in foraging strategies of northern giant petrels, *Macronectes halli*, during incubation. *Oikos* 90: 390–398.

GWIAZDA, R. & LEDWOŃ, M. 2015. Sex-specific foraging behaviour of the Whiskered Tern (*Chlidonias hybrida*) during the breeding season. *Ornis Fennica* 92: 15–22.

LEDWOŃ, M. 2011. Sexual size dimorphism, assortative mating and sex identification in the Whiskered Tern *Chlidonias hybrida*. *Ardea* 99: 191–198.

LEWIS, S, BENVENUTI, S., DALL–ANTONIA, L. ET AL. 2002. Sex-specific foraging behaviour in a monomorphic seabird. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269: 1687–1693.

LIECHTY, J.S., MINOR, A.K., NEPSHINSKY, M. & PIERCE, A.R. 2017. Apparent survival of Royal Tern *Thalasseus maximus* and Sandwich Tern *T. sandvicensis* at Isles Dernieres Barrier Islands Refuge, Louisiana, USA. *Marine Ornithology* 45: 83–88.

LIECHTY, J.S., FONTENOT, Q.C. & PIERCE, A.R. 2016. Diet composition of Royal Tern (*Thalasseus maximus*) and Sandwich Tern (*Thalasseus sandvicensis*) at Isles Dernieres Barrier Island Refuge, Louisiana. *Waterbirds* 39: 58–68.

LINDSTEDT, D.M. 2005. Renewable resources at stake: Barataria-Terrebonne estuarine system in southeast Louisiana. *Journal of Coastal Research* 44: 162–175.

LISNIZER, N., COTICHELLI, L., YORIO, P., BASSO, N. & GATTO, A. 2014. Using morphometry and molecular markers for sexing South American, Cayenne and Royal terns breeding in Patagonia, Argentina. *Waterbirds* 37: 183–190.

MORINHA, F., CABRAL, J.A. & BASTOS, E. 2012. Molecular sexing of birds: a comparative review of polymerase chain reaction (PCR)-based methods. *Theriogenology* 78: 703–714.

NISBET, I.C.T., BRIDGE, E.S., SZCZYS, P. & HEIDINGER, B.J. 2007. Sexual dimorphism, female-female pairs, and test for assortive mating in Common Terns. *Waterbirds* 30: 169–179.

NISBET, I.C., MONTOYA, J.P., BURGER, J. & HATCH, J.J. 2002. Use of stable isotopes to investigate individual differences in diets and mercury exposures among common terns *Sterna hirundo* in breeding and wintering grounds. *Marine Ecology Progress Series* 242: 267–274.

NICHOLS, J.D., KENDALL, W.L., HINES, J.E. & SPENDELOW, J.A. 2004. Estimation of sex-specific survival from capture–recapture data when sex is not always known. *Ecology* 85: 3192–3201.

OWEN, J.C. 2011. Collecting, processing, and storing avian blood: a review. *Journal of Field Ornithology* 82: 339–354.

PALESTIS, B., NISBET, I., HATCH, J., SZCZYS, P. & SPENDELOW, J. 2012. Morphometric sexing of northwest Atlantic Roseate Terns. *Waterbirds* 35: 479–484.

PAREDES, R., ORBEN, R.A., ROBY, D.D. ET AL. 2015. Foraging ecology during nesting influences body size in a pursuit-diving seabird. *Marine Ecology Progress Series* 533: 261–276.

QIAGEN. 2011. *Quick-Start DNeasy Blood & Tissue Kit Protocol*. Hilden, Germany: Qiagen.

RAYNOR, E.J, PIERCE, A.R., LEUMAS, C.M. & ROHWER, F.C. 2012. Breeding habitat requirements and colony formation by Royal Terns (*Thalasseus maximus*) and Sandwich Terns (*Thalasseus sandvicensis*) on barrier islands in the Gulf of Mexico. *The Auk* 129: 763–772.

REYNOLDS, S.J., MARTIN, G.R., WALLACE, L.L., WEARN, C.P. & HUGHES, B.J. 2008. Sexing Sooty Terns on Ascension Island from morphometric measurements. *Journal of Zoology* 274: 2–8.

RYAN, P. G. & BOIX-HINZEN, C. 1999. Consistent male-biased seabird mortality in the Patagonian toothfish longline fishery. *The Auk* 116: 851–854.

SAS Institute, Inc. 2008. *SAS statistical software v. 9.2*. Cary, North Carolina, USA: SAS Institute, Inc.

SEUTIN, G., WHITE, B.N. & BOAG, P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology* 69: 82–90.

SHEALER, D.A. & CLEARY, C.M. 2007. Sex determination of adult black terns by DNA and morphometrics: tests of sample size, temporal stability and geographic specificity in the classification accuracy of discriminant function models. *Waterbirds* 30: 180–188.

WINDHOFFER, E.D. 2017. *Evaluation of Mammalian Predator Removal and Video Monitoring as Management Tools for Waterbird Conservation*. MSc thesis. Thibodaux, USA: Nicholls State University.

YORIO, P. & QUINTANA, F. 1997. Predation by Kelp Gulls *Larus domincanus* at a mixed-species colony of Royal Terns *Sterna maxima* and Cayenne Terns *Sterna eurygnatha* in Patagonia. *Ibis* 139: 536–541.