Online Resource 1 – Detailed methods

At-sea distribution and foraging tactics of a monomorphic tropical seabird.

Authors

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Molecular sexing

Approximately 3 mm of the root tip of 3 – 4 dry breast feathers per individual were used to isolate DNA using a Chelex® extraction method Rishworth et al. (2014); adapted from Ellegren (1992). The feathers were sectioned into 200 µl 5% Chelex 100 resin (BioRad) with 1 µg proteinase K. The mixture was vortexed for 20 s and then incubated at 56°C for 2 h. After 2 h, another 1 µg proteinase K was added to the mixture and vortexed for 20 s and incubation continued for a further 2 h. After 4 h of incubation at 56°C, the mixture was vortexed for 10 s and incubated at 95°C for 10 min, re-vortexed and centrifuged at 36000 RPM. The supernatant was extracted from the mixture and 1 µl of the supernatant was used to measure the DNA yield, using a NanoDrop® 2000c Spectrophotometer (Thermo Scientific). The remaining supernatant was stored at - 40°C. The 2550F (5'-GTTACTGATTC GTCTACGAGA-3') and 2718R (5'-TTGAAATGATCCAGTGCTTG-3') primers were used to amplify fragments of the sex-linked CHD-1 gene (Fridolfsson and Ellegren 1999). After amplification, these primers allow for the detection of females as two fragments (ZW) and males as a single fragment (ZZ).

Polymerase chain reactions (PCR) in a 15 µl solution containing: 4.3 µl of molecular grade H_2O, 7.5 µl of GoTaq® G2 Hot Start Green Master Mix (Promega), 0.6 µl; 50 nmol of each primer and 40 – 410 ng (2 µl; 50 nmol) DNA, were performed using a C1000 Touch Thermal Cycler (BioRad). Initial denaturation of the DNA was at 94°C for 2 min, followed by 43 additional cycles of denaturation at 94°C for 30 s, a constant annealing temperature of 50°C for 30 sec and extension at 72°C for 45 s. A final extension step of 5 min at 72°C was added after the last cycle. PCR products (5 µl) were separated on a 1.8% agarose gel with 1 x TAE buffer, stained with 2.5 µl GelRed™ Nucleic Acid Gel stain (Biotium). After electrophoresis at 100 V for 45 min, the bands were visualised under ultraviolet light and the sexes of the WTS identified. In some cases, no feathers were sampled in which case sex could not be established. Molecular sexing was conducted at the Nelson Mandela University, South Africa.

Data analysis

Due to low sample sizes for 2017/18 (n = 2) and 2018/19 (n = 4), the effect of year on trip metrics were not investigated and foraging trips for the three breeding seasons were pooled for data analysis. During chick rearing, all foraging trips were classified as either short (≤ 4d) or long (> 4d) foraging trips. This classification was based on a Ward Hierarchical clustering approach which was used to identify groups based on foraging trip duration and maximum distance travelled (Online Resource 2 Fig. S3) and the frequency distribution for trip durations (see Results; Congdon et al. 2005; Weimerskirch et al. 2020). Differences in foraging trip parameters between sexes and breeding stages, as well as between long and short trips (Congdon et al. 2005) during early chick rearing, were tested using Linear Mixed Models (LMM) with Gaussian distribution of error terms, using an Identity link function (package lme4; Bates et al. 2015). Since several trips were made per individual, unique individual 'ID' was added as a random effect for all models. The LMM residuals normality and homogeneity were visually assessed, and trip parameters log transformed where needed. Outliers in maximum foraging range and trip duration resulted in a deviation of model assumptions; however, their removal had no influence on the study results.

To identify important areas utilized by WTS, the spatial distribution of WTS was estimated by producing core areas and home range kernel utilization distributions (50% UD and 90% UD respectively; Worton 1989) for each individual, using kernel analysis with a grid of 0.1 x 0.1° cells. An averaged smoothing parameter for each breeding stage was estimated using 'href' method (adehabitatHR package; Callenge et al. 2006). To determine whether our sample size was representative of the population breeding on Réunion Island, we used saturation curves based on the 50 % UD and 90 % UD. As per Lascelles et al. (2016), foraging trips were treated as individual samples and an increasing number of foraging trips were randomly selected. For each step, the mean and confidence interval were calculated for 100 iterations and a nonlinear asymptotic regression was modelled from the mean. The representative value was calculated by using the kernel UD at the population level and dividing it by the area of the kernel UD at the asymptote.

Overlaps of core areas and home ranges utilized by different sexes over different breeding stages were calculated using Bhattacharyya's affinity (BA; Fieberg and Kochanny 2005) using kernel analysis (adehabitatHR package; Calenge 2006). The extent of overlap between sexes during different breeding stages ranged from 0 (no overlap) and 1 (complete overlap). To test if there was a difference in the spatial distribution utilized by the male and female WTS within different breeding stages, a randomization technique was used, using BA as a measure of spatial overlap. The randomization procedure was used to test the null hypothesis that there were no differences between sexes within different stages (Cleasby et al. 2015). Following Cleasby et al (2015), for both breeding stages, the bird's sex was assigned randomly using the observed sex ratio for the selected breeding stage. To test whether the overlap of the core areas and home ranges were significantly different between sexes, a null distribution for BA was generated based on 1000 randomizations of the data used. P-values were estimated by the proportion of random overlaps that were less than the observed overlap.

Multiple trips were recorded from several individual birds during chick rearing (Online Resource 2 Table S2) which may exhibit pseudo-replication that could bias results (Augé et al. 2013). We tested for pseudo-

replication using methods described following (Lascelles et al. 2016). The 50 % kernel UD was identified for each of the trips performed by an individual and to estimate the proximity between these areas, the Hausdorff distance was calculated (Munkres 2018). For every combination of trips for each individual bird, the distance between core use areas were calculated and compared to a data group reference distribution. The reference distribution was calculated by randomly selecting the same number of trips for each individual bird and then between each individual, the Hausdorff distance was calculated for core use ranges. A Mann-Whitney U-test was used to compare individual distances between the population-level distances. To avoid biases in the random sample, this process was repeated 100 times and a p-value was calculated. The significance threshold was set at $\alpha = 0.1$. One incubating bird completed multiple trips and site fidelity was assessed by visually inspecting the trips.

References

Augé A, Chilvers B, Moore A, Davis L (2013) Importance of studying foraging site fidelity for spatial conservation measures in a mobile predator. Animal Conservation 17: 61-71

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67: 1-48

Calenge C (2006) The package "adehabitat" for the R software: a tool for the analysis of space and habitat use by animals. Ecological modelling 197: 516-519

Cleasby IR, Wakefield ED, Bodey TW, Davies RD, Patrick SC, Newton J, Votier SC, Bearhop S, Hamer KC (2015) Sexual segregation in a wide-ranging marine predator is a consequence of habitat selection. Marine Ecology Progress Series 518: 1-12

Congdon BC, Krockenberger AK, Smithers BV (2005) Dual-foraging and coordinated provisioning in a tropical Procellariiform, the Wedge-tailed Shearwater. Marine Ecology Progress Series 301: 293-301

Ellegren H (1992) Polymerase-chain-reaction (PCR) analysis of microsatellites: a new approach to studies of genetic relationships in birds. Auk 109: 886-895

Fieberg J, Kochanny CO (2005) Quantifying home-range overlap: the importance of the utilization distribution. Journal of Wildlife Management 69: 1346-1359

Lascelles BG, Taylor P, Miller M, Dias M, Oppel S, Torres L, Hedd A, Le Corre M, Phillips R, Shaffer SA (2016) Applying global criteria to tracking data to define important areas for marine conservation. Diversity and Distributions 22: 422-431

Munkres JR (2018) Elements of algebraic topology. CRC Press

Rishworth GM, Connan M, Green DB, Pistorius PA (2014) Sex differentiation based on the gular stripe in the apparently monomorphic Cape Gannet. African Zoology 49: 107-112

Weimerskirch H, de Grissac S, Ravache A, Prudor A, Corbeau A, Congdon BC, Mcduie F, Bourgeois K, Dromzée S, Butscher J (2020) At-sea movements of wedge-tailed shearwaters during and outside the breeding season from four colonies in New Caledonia. Marine Ecology Progress Series 633: 225-238

Worton BJ (1989) Kernel methods for estimating the utilization distribution in home-range studies. Ecology 70: 164-168