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# Stable isotopes reveal regional movement patterns in an endangered bustard

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**Abstract** Stable isotope analysis is a valuable technique to infer animal movement between isotopically distinct landscapes. For birds in terrestrial systems, it is usually only applied at continental scales, often relying on global isotopic patterns. In contrast, we used this technique to investigate movement patterns of Ludwig's bustard (*Neotis ludwigii*) at a regional scale, where such information is needed to improve the conservation status of this species. We analysed carbon and nitrogen isotopic compositions of feathers from bustards across two biomes of the semi-arid rangelands of the Karoo, South Africa, to investigate movement and explore sex and age movement strategy differences. We used a linear discriminant function analysis based on growing feathers to classify fully grown feathers to a Succulent or Nama Karoo biome origin. Six of 12 birds for which all primary feathers were analysed had at least one feather classified as having grown in the Succulent Karoo, supporting the theory that these birds are partial migrants. Feathers from two satellite-tracked bustards broadly supported the conclusions of the analysis, although food base differences resulting from local rainfall variation probably obscured geographic signals at finer scales. There was no apparent difference in movement strategies between the sexes, but juvenile feathers were almost exclusively assigned to the Nama Karoo, suggesting that most breeding occurs in this biome. Adult and juvenile feathers also had significantly different isotope ratios, which could relate to diet or to differing metabolic processes. This study demonstrates that with a good understanding of the system, carbon and nitrogen stable isotopes can be useful to infer general movement patterns of birds at a regional level.

**Key words:** carbon, feathers, Karoo, movement, *Neotis ludwigii*, nitrogen, stable isotopes.

## INTRODUCTION

In order to conserve migratory or nomadic species, it is important to know how they use their environment in space and time (Rubenstein & Hobson 2004; Hobson 2008). The analysis of stable isotope ratios has long been used as a relatively cheap and simple way to study movement ecology, because animals migrating between isotopically distinct landscapes ('isoscapescapes') incorporate isotopic signatures of diet in their body tissues (Hobson 2008; Wassenaar 2008; Hobson *et al.* 2012). Studies to date have focussed on patterns at broad, usually continental, geographical scales (e.g. Yohannes *et al.* 2007; Szép *et al.* 2009; Symes & Woodborne 2010) where distinct isotopic gradients are known to exist. However, an understanding of movement at much smaller scales is often needed, where this technique remains largely untested.

Ludwig's bustard (*Neotis ludwigii*) provides a good case study to investigate the applicability of isotopic movement analysis across a smaller area. This species is endemic to the dry and sparsely populated land-

scapes of western South Africa and Namibia, where it frequently collides with overhead power lines (Jenkins *et al.* 2011). Collision mortality rates are so high that Ludwig's bustard was classified as globally Endangered on the IUCN Red List in 2010 (BirdLife International 2012). The power grid in southern Africa is extensive and growing, and line marking mitigation measures are expensive to apply retrospectively (Jenkins *et al.* 2010; Eskom 2012), so they will not be expanded without a more detailed knowledge of where birds are likely to be and therefore collide. Limited movement information is available through census counts of Ludwig's bustards, which suggest that they are nomadic and partially migratory with at least some of the population moving west to the winter rainfall parts of their range at this time of year (Allan 1994; Shaw 2013). This observation has also been broadly confirmed using satellite tracking, with eight tagged birds generally moving west in winter (Shaw 2013). However, stable isotope analysis provides a much simpler method to evaluate movements of a much larger number of individuals, and to potentially assess different age and sex strategies.

Stable isotope ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) are commonly used to study trophic and movement ecology, and have been used successfully to infer diet and geographic origin of historic and modern

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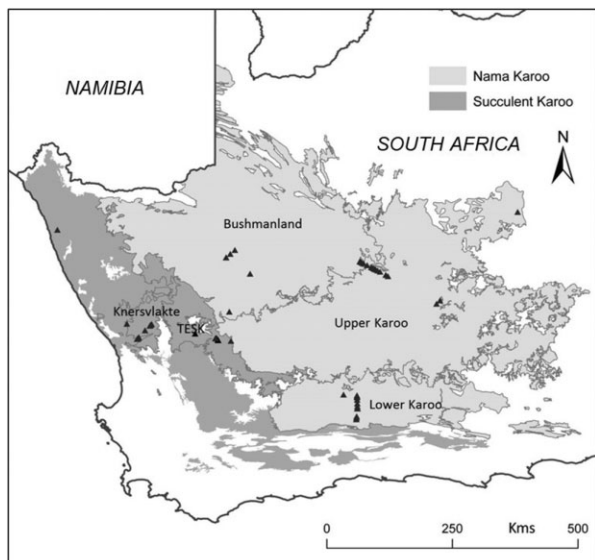
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African animals (e.g. Johnson *et al.* 1998; Symes & Woodborne 2010; Codron *et al.* 2011). This is possible because these isotope ratios vary in plant tissues depending on which photosynthetic pathway is used and how it fixes nitrogen (Kelly 2000; Rubenstein & Hobson 2004). Different pathways of carbon fixation result in different plant  $^{13}\text{C}/^{12}\text{C}$  ratios;  $\text{C}_3$  plants have depleted  $^{13}\text{C}$  relative to  $\text{C}_4$  plants. CAM (crassulacean acid metabolism) plants can exhibit a range of  $\delta^{13}\text{C}$  values depending on whether this pathway is used consistently (Ehleringer *et al.* 1986; O'Leary 1988; Kelly 2000; Marshall *et al.* 2007). Carbon isotope signatures in plants are directly reflected in the tissue of consumers (with some fractionation), but the pattern in nitrogen isotopes is more complicated because the heavy isotope  $^{15}\text{N}$  is preferentially incorporated in consumer tissues, resulting in  $\delta^{15}\text{N}$  enrichment with increasing trophic level. In addition,  $\delta^{15}\text{N}$  in consumers is generally more enriched in dry than wet habitats, relating to metabolic processes under nutritional and water stress (Johnson *et al.* 1998; Kelly 2000; Rubenstein & Hobson 2004). Because both carbon and nitrogen isotopic compositions vary with environmental conditions, analysing them together can increase the power to discriminate between different locations (Kelly 2000; Rocque *et al.* 2006).

In South Africa, Ludwig's bustard is largely restricted to the Karoo (Allan 2005). This region covers approximately one third of South Africa (Fig. 1), and the land is predominantly used for extensive free-range stock farming (Hoffman *et al.* 1999). The western Succulent Karoo biome is a semi-desert shrubland characterised

by fairly predictable winter rainfall (mean annual precipitation 100–200 mm) and a mild, even climate (mean annual temperature of 16.8°C). Further inland, the arid Nama Karoo biome has a continental climate, which results in more extreme temperatures (–5 to 43°C). It also has less reliable summer rainfall (mean annual precipitation 70–500 mm, generally increasing from west to east), and is subject to periodic droughts (Mucina & Rutherford 2006). These environmental conditions have resulted in strong divergence of plant photosynthetic types between the two Karoo biomes.  $\text{C}_3$  grasses and CAM types (mainly succulents) dominate the Succulent Karoo, and  $\text{C}_4$  grasses dominate the Nama Karoo, with an increase in  $\text{C}_4$  plants towards the north-east linked to increasing summer rainfall (Vogel *et al.* 1978; Bond *et al.* 1994; Midgley & van der Heyden 1999).

In this paper we investigate the suitability of stable isotopes to assess bird movements at a regional geographical scale. Feathers are commonly used in bird isotope studies as they are metabolically inert, reflecting the diet of the bird at the time of feather growth (Rubenstein & Hobson 2004). We therefore infer movements of Ludwig's bustards by analysing carbon and nitrogen isotope ratios of feathers from birds killed by power lines across the Karoo, and validate patterns detected from growing feathers by comparing our results with feathers sampled from two satellite-tracked birds. We then use isotope data to infer the origins of juvenile birds (and hence the location of breeding areas) and to assess whether migratory movements between the two biomes can be inferred for non-tracked bustards. Based on the vegetation differences between the two biomes, we expect feathers grown while birds are in the Succulent Karoo to be more depleted in  $^{13}\text{C}$  relative to feathers grown in the Nama Karoo. However, the pattern for nitrogen is difficult to predict, given that both the Nama and Succulent Karoo can be water stressed. In addition, Ludwig's bustards are opportunist foragers across their range, with the bulk of their diet comprising arthropods and vegetable matter (Earlé *et al.* 1988; Allan 2005), so if birds consume these components in equal proportions in each biome there is unlikely to be a trophic nitrogen isotopic composition difference. However, there may be a dietary difference between adult and juvenile birds, so we also compare adult and juvenile feathers to investigate this possibility.



**Fig. 1.** Collection sites for Ludwig's bustard feathers (black triangle for each bird) in the Nama and Succulent Karoo biomes, South Africa, with the five bioregions in which growing feathers were collected.

## METHODS

### Feather samples

Ludwig's bustard wing feathers were collected from 56 individuals across the Karoo from 2009 to 2012, with most

samples from five main bioregions (Knervlakte, Transescarpment Succulent Karoo (TESK), Bushmanland, Upper Karoo and Lower Karoo; Fig. 1). Most feathers were taken from carcasses found under power lines where an entire wing was collected from each bird where possible, with additional feathers obtained from two satellite-tracked bustards that were recovered dead after being tracked for nine and 12 months respectively (all details of satellite tracking methodology presented in Shaw 2013).

The vast majority of our samples were from primary feathers, which are the main outer flight feathers that attach to the manus of the wing. Ludwig's bustards have 10 primaries on each wing, which are traditionally labelled from P1 (innermost) to P10 (outermost). Ludwig's bustards have protracted sequential primary moults, resulting in several generations of feathers in the primaries of older birds, and age gradients sometimes visible even within a group of primaries belonging to the same moult generation (Cramp & Simmons 1980; Allan 2005; J. Shaw, unpubl. data, 2012). Ludwig's bustards are strongly sexually dimorphic with no overlap in wing length (Allan 2005), so this was the main measurement used to assign sex to individuals. Juveniles were identified by the presence of narrow, pointed primaries that are substantially shorter than those grown in subsequent moults; these juvenile primaries are replaced by the second year in most bustard species (Cramp & Simmons 1980; Jarrett & Warren 1999; J. Shaw, unpubl. data, 2012). Long bones are fully grown within a few months of attaining independence, so the discrimination of sexes was clear even among individuals retaining some juvenile feathers. In many cases we also used tarsus length to aid identification; there is very little overlap in this measurement between the sexes (Allan 2005).

Samples were firstly taken from all growing wing feathers. These were mostly primaries but also included eight secondary feathers (inner flight feathers) and two coverts (contour feathers that cover the bases of the flight feathers). This gave a total of 43 growing feathers from 39 individuals; four individuals had two growing feathers, so only the newest was used to relate isotope values in growing feathers to collection location. All 10 primary feathers were then sampled on wings that had at least one growing primary ( $n = 11$ ) or for which protracted periods of satellite tracking data were obtained ( $n = 2$ , one of which was in moult, giving  $n = 12$  whole wings). In addition, 30 carcasses retained at least some juvenile primaries. Samples were taken from the juvenile P9 ( $n = 29$  individuals) for consistency or P10 ( $n = 1$ , where P9 had been dropped already). One of the wings with juvenile feathers also had a growing primary (P1) so it was included as a whole wing sample, and had juvenile feathers from P7 to P10. To see if there was an isotopic difference between juvenile and adult feathers grown in post-juvenile moults, the newest and oldest adult feathers on 28 of the 30 wings with juvenile primaries were sampled (the other two had no adult primaries). Altogether, we analysed 222 samples from 56 individuals (34 males and 22 females).

### Laboratory methods

Feather samples were cut from the tips (1 cm) of old feathers and the base of growing feathers (the base represents the

most recently grown material and should thus be most representative of the site where the bird died). Feather samples were washed with distilled water to remove contaminants, dried overnight in paper envelopes in a drying oven at 30°C, and cut into small pieces with stainless steel scissors (Wassenaar 2008). Many studies wash feathers in a chloroform : methanol mix to remove surface oils, but we did not consider this to be necessary. Knoff *et al.* (2001) compared these two washing methods and found that it made no difference to carbon isotopic values. In addition, Post *et al.* (2007) have suggested for terrestrial animals that if the carbon : nitrogen ratio is less than 4 in all samples, then lipid concentrations are already uniformly low and lipid extraction will have little influence on results (the maximum ratio from our samples was 3.42).

Analysis of stable carbon and nitrogen isotopes was then carried out by the Archaeology Department, University of Cape Town. Between 0.4 and 0.5 mg of each sample was weighed into a tin cup using a Sartorius M2P micro balance (which measures to the nearest µg), and then enclosed by squashing the cup. The samples were combusted in a Flash 2000 organic elemental analyser and the gases passed to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a ConFlo IV gas control unit (all equipment made by Thermo Scientific, Bremen, Germany). Isotope ratios are expressed as  $\delta$  values in parts per thousand (per mil, ‰) relative to Vienna Pee-Dee Belemnite (VPDB) for carbon and atmospheric nitrogen for nitrogen. Internal laboratory standards used were seal bone (UCT), DL Valine (Sigma) and Merck Gel (Merck). All in-house standards had been calibrated against IAEA (International Atomic Energy Agency) standards. Accuracy ranged from 0.009–0.064 standard deviations for  $\delta^{15}\text{N}$  and 0.006–0.128 for  $\delta^{13}\text{C}$ .

### Statistical analyses

Statistical analyses were run in R (R Core Team 2012). The data were tested for normality (Shapiro–Wilk test, generalized Shapiro–Wilk test for multivariate normality (library *mvShapiroTest*; Gonzalez Estrada & Villasenor Alva 2009)) and homogeneity of variance ( $F$  test, Bartlett test). Parametric tests were used where these assumptions were met and non-parametric tests where they were not. We first compared carbon and nitrogen isotope ratios by sex in the growing feathers and then all feathers using two sample Welch's  $T$ -tests and Wilcoxon rank sum tests.

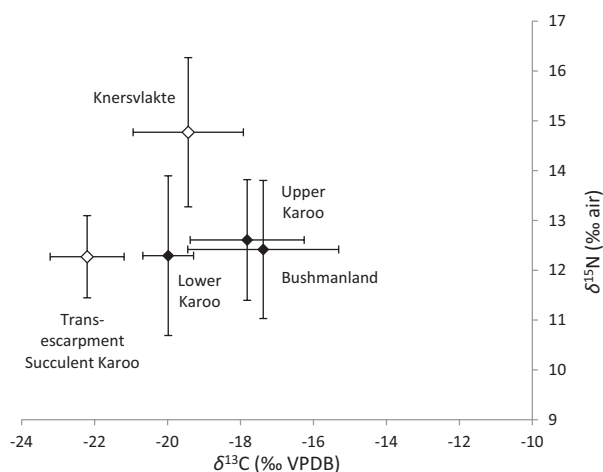
The regional effect for growing feathers was first examined by bioregion (ANOVAs and MANOVA) and then by biome (Fig. 1) with Welch's  $T$ -tests and a MANOVA to assess the variation in both carbon and nitrogen isotope compositions by region simultaneously (Quinn & Keough 2002). A linear discriminant function analysis (DFA) (library *MASS*; Venables & Ripley 2002) was performed to find the relationship between the isotope compositions of the two elements (dependent variables) and biome (grouping variable) and to classify non-growing feathers to a biome origin. The predictive accuracy of the discriminant function was tested in two ways: the model was used to classify the origin of the growing feathers used in the analysis, then a more robust jackknife classification procedure was run which took out one observation at a time, and classified it based on the model

determined by the remaining observations (Quinn & Keough 2002). The effect of age was investigated with a paired T-test comparing the average isotope ratios of the oldest and newest adult feathers with the juvenile feather (or the average ratio for the wing with four juvenile feathers) for the 28 wings that had both adult and juvenile feathers for each element.

## RESULTS

There was no significant difference between males ( $n = 29$ ) and females ( $n = 10$ ) in the isotopic composition of either element in growing feathers ( $T$ -tests:  $\delta^{13}\text{C}$ :  $t = -1.190$ , d.f. = 16.46,  $P = 0.251$ ;  $\delta^{15}\text{N}$ :  $t = 0.75$ , d.f. = 15.08,  $P = 0.465$ ), or between males ( $n = 135$ ) and females ( $n = 87$ ) across all feathers (Wilcoxon tests:  $\delta^{13}\text{C}$ :  $W = 6000.5$ ,  $P = 0.785$ ;  $\delta^{15}\text{N}$ :  $W = 5938.5$ ,  $P = 0.889$ ), so thereafter sexes were pooled.

For the growing feather samples,  $\delta^{13}\text{C}$  varied significantly between bioregion locations (Fig. 2, Table 1) (ANOVA:  $F = 6.881$ , d.f. = 4,  $P = 0.0004$ ), with a Tukey's honest significant difference test showing significant differences between TESK and Bushmanland ( $P = 0.002$ ) and between TESK and Upper Karoo ( $P = 0.0004$ ). Regional differences in  $\delta^{15}\text{N}$  were bordering on significance at the 5% level (ANOVA:  $F = 2.585$ , d.f. = 4,  $P = 0.054$ ), with a tendency for higher  $\delta^{15}\text{N}$  values in the Knersvlakte (Fig. 2, Table 1). A MANOVA that tested both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  simultaneously showed a strong difference between bioregions ( $F = 4.399$ , d.f. = 4,  $P = 0.0003$ ). These analyses indicated some isotopic composition differences between bioregions in the Succulent and Nama Karoo, so



**Fig. 2.** Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $\pm 2$  SE) isotope values of growing wing feathers from Ludwig's bustards recovered from the Succulent Karoo (white diamonds) and Nama Karoo (black diamonds) by bioregion: Knersvlakte ( $n = 8$ ), Trans-escarpment Succulent Karoo ( $n = 8$ ), Bushmanland ( $n = 5$ ), Lower Karoo ( $n = 7$ ) and Upper Karoo ( $n = 11$ ).

based on these results and the small sample size we classified the growing feathers more broadly by biome (Table 1). We first ran these analyses with the full moulting feather dataset ( $n = 39$ ), and then without feathers from the Hantam Karoo, which falls in the TESK bioregion ( $n = 33$ , hereafter termed the reduced dataset) because these samples were from the boundary between the Succulent and Nama Karoo so may have obscured the biome effect.

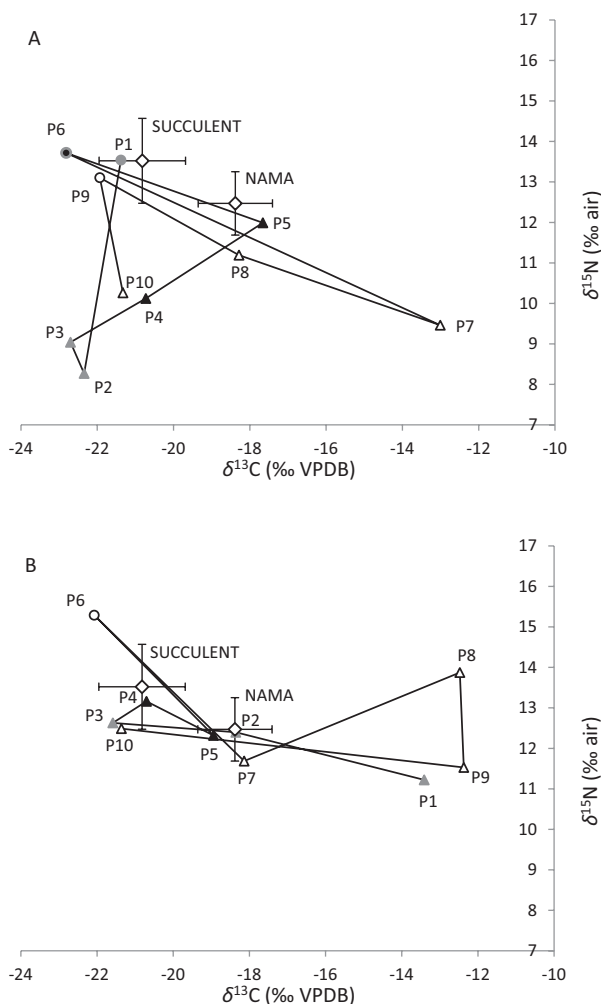
$T$ -tests for the full dataset showed a significant difference between  $\delta^{13}\text{C}$  in the two biomes ( $t = 3.263$ , d.f. = 33.05,  $P = 0.003$ ), but not between  $\delta^{15}\text{N}$  ( $t = -1.61$ , d.f. = 30.06,  $P = 0.118$ ). A MANOVA taking the isotopic compositions of both elements into account found a strongly significant difference between the two biomes ( $F = 7.175$ , d.f. = 1,  $P = 0.002$ ), with  $\delta^{13}\text{C}$  accounting for most of this effect ( $\delta^{13}\text{C}$ :  $F = 10.532$ , d.f. = 1,  $P = 0.0025$ ;  $\delta^{15}\text{N}$ :  $F = 2.698$ , d.f. = 1,  $P = 0.109$ ). Tests on the reduced dataset also highlighted differences between the biomes, with  $\delta^{15}\text{N}$  now the stronger influence;  $T$ -tests for  $\delta^{13}\text{C}$  ( $t = 1.959$ , d.f. = 16.44,  $P = 0.067$ ) and  $\delta^{15}\text{N}$  ( $t = -2.655$ , d.f. = 16.22,  $P = 0.017$ ), and MANOVA for the isotopic compositions of both elements ( $F = 5.719$ , d.f. = 1,  $P = 0.008$ , with  $\delta^{13}\text{C}$ :  $F = 3.995$ , d.f. = 1,  $P = 0.0544$ ; and  $\delta^{15}\text{N}$ :  $F = 7.429$ , d.f. = 1,  $P = 0.0105$ ). As the isotopic compositions of both elements contributed to the relationship between the two areas, we performed DFAs with the discriminant function classifying 82% of the observations to the correct regions using all the data, and 72% using the jackknife procedure with the full dataset (and 88% and 82% with the reduced set, respectively). As the reduced set classification performed better, this model was then applied to the fully grown feathers to infer where they were grown.

## Satellite-tracked birds

Bird A was tagged in the Succulent Karoo near Vanrhynsdorp ( $31^{\circ}29'S$ ,  $18^{\circ}40'E$ ) on 16 October 2010, staying in this biome near Nieuwoudtville ( $31^{\circ}21'S$ ,  $19^{\circ}04'E$ ) over the next few months (Appendix S1). On 18 December he moved east into the Nama Karoo between Calvinia and Brandvlei ( $31^{\circ}05'S$ ,  $20^{\circ}11'E$ ) and over the next six months ranged up to 75 km east of the biome boundary ( $31^{\circ}04'S$ ,  $20^{\circ}46'E$ ) before heading west to the Succulent Karoo around Loeriesfontein ( $30^{\circ}46'S$ ,  $19^{\circ}26'E$ ) a few weeks before his death in July 2011. When he died, bird A's primary moult score was  $8^3 5^2 4^0 4^0$  (following Ginn & Melville 1983) with only P6 (the actively growing feather) classified as originating in the Succulent Karoo by the DFA (Fig. 3A). The rate of moult in Ludwig's bustard is not known, but it is likely that some if not all of P1–6 were replaced during the 265 days over which the bird was tracked.

**Table 1.** Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $\pm$  SE) values of growing Ludwig's bustard feathers by Karoo biome and bioregion (sample sizes given in parentheses)

Biome	Bioregion	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Succulent Karoo	Knersvlakte (8)	$-19.43 \pm 0.76$	$+14.77 \pm 0.75$
	Trans-escarpment Succulent Karoo (8)	$-22.20 \pm 0.51$	$+12.27 \pm 0.41$
	<i>Pooled Succulent Karoo</i> (16)	$-20.82 \pm 0.57$	$+13.52 \pm 0.52$
Nama Karoo	Bushmanland (5)	$-17.38 \pm 1.03$	$+12.42 \pm 0.69$
	Lower Karoo (7)	$-19.98 \pm 0.35$	$+12.29 \pm 0.80$
	Upper Karoo (11)	$-17.82 \pm 0.78$	$+12.61 \pm 0.61$
	<i>Pooled Nama Karoo</i> (23)	$-18.38 \pm 0.49$	$+12.47 \pm 0.39$

**Fig. 3.** Primary feather  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (P1–P10) from satellite-tagged Ludwig's bustards A and B. Symbol shape indicates predicted feather origin (triangles = Nama Karoo, circles = Succulent Karoo) and symbol colour indicates feather age (black = fresh, new (moult score 5), grey = moderately worn (moult score 8), white = old, worn (moult score 0) and black with grey border = growing (moult score 4)). Also shown are the mean ( $\pm 2$  SE) isotope composition values of known growing feathers from the Succulent and Nama Karoo (diamonds).

Bird B was tagged in the Nama Karoo between Calvinia and Brandvlei ( $31^{\circ}05'S$ ,  $20^{\circ}11'E$ ) on 27 June 2011, and his satellite tag transmitted for 346 days (Appendix S1). In July, he moved west and stayed around the boundary of the two biomes ( $30^{\circ}58'S$ ,  $19^{\circ}47'E$ ) until October when he headed east to the Beaufort West area ( $32^{\circ}25'S$ ,  $22^{\circ}33'E$ ) of the Nama Karoo, making no further visits to the Succulent Karoo. His moult score when recovered was  $8^3 5^2 0^3$  with only P6, presumably the oldest primary, classified as having been grown in the Succulent Karoo (Fig. 3B).

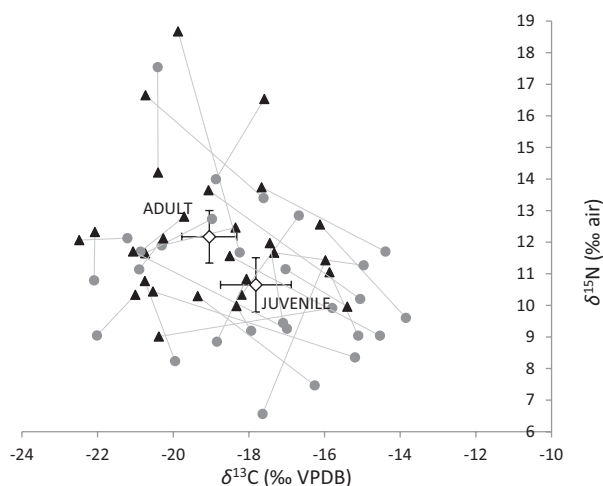
The DFA feather origin classification suggests that of the other complete sets of primaries analysed (six males and four females), four had at least one feather of Succulent Karoo origin (two males and two females), so together with the satellite-tracked birds, six of 12 sampled birds visited the Succulent Karoo. The mean number of primaries ascribed to the Succulent Karoo was only  $1.3 (\pm 0.59 \text{ SE}, \text{ range } 0\text{--}6)$ , suggesting that relatively little moulting occurs in the west of the range.

### Juvenile versus adult feathers

We compared the probable origin of adult and juvenile feathers for all feather samples ( $n = 222$ ), using collection location for growing feathers and predicted location (assigned by DFA) for fully grown feathers. Most adult feathers (83%) were identified as having a Nama Karoo origin, and nearly all juvenile feathers (97%) were ascribed to this region. Juvenile and adult feathers from the same wings (Fig. 4) were significantly different for the isotopic compositions of both elements ( $\delta^{13}\text{C}$  adult: mean  $\pm$  SE =  $-19.05 \pm 0.36$ , juvenile =  $-17.82 \pm 0.47$ ;  $\delta^{15}\text{N}$  adult =  $+12.17 \pm 0.42$ , juvenile =  $+10.70 \pm 0.43$ ; paired  $T$ -tests:  $\delta^{13}\text{C}$ :  $t = -3.065$ , d.f. = 27,  $P = 0.005$ ;  $\delta^{15}\text{N}$ :  $t = 3.770$ , d.f. = 27,  $P = 0.0008$ ).

### DISCUSSION

As well as revealing previously unknown aspects of Ludwig's bustard ecology valuable to the conservation



**Fig. 4.** Average  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of adult (black triangles) and juvenile (grey circles) Ludwig's bustard feathers from the same wings ( $n = 28$ ), with pairs joined by light grey lines and the mean ( $\pm 2$  SE) adult and juvenile values across all 28 samples (white diamonds).

of this species, the results of our study indicate that stable isotopes can be used to successfully infer coarse movements for this bird over a relatively small area. This highlights the potential of this technique to infer regional movements of other species, particularly in areas where neighbouring biomes have distinct food bases.

The growing Ludwig's bustard feathers matched the expected  $\delta^{13}\text{C}$  pattern, with feathers grown in the Succulent Karoo depleted in  $^{13}\text{C}$  compared with the Nama Karoo. The relatively enriched  $\delta^{13}\text{C}$  of feathers from the Knersvlakte was surprising, but this may be because succulents that use the CAM photosynthetic pathway dominate the vegetation of this bioregion (Mucina & Rutherford 2006). The isotopic distinction between the two biomes is supported by recent work which uses  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  vegetation isoscapes to map feather isotopic clusters in Africa (Hobson *et al.* 2012). These isoscapes suggest a clear delineation between the biomes for  $\delta^{13}\text{C}$  values (depleted in the Succulent Karoo compared with the Nama Karoo), with a more gradual change in  $\delta^{15}\text{N}$  values, which are increasingly depleted from northwest to southeast. The most enriched  $\delta^{15}\text{N}$  values are mapped in the northwestern parts of both biomes towards the Kalahari Desert (Hobson *et al.* 2012). The enriched Knersvlakte  $\delta^{15}\text{N}$  values are compatible with this isoscape, but we would have expected a higher  $\delta^{15}\text{N}$  composition for the Bushmanland samples. However, nitrogen isotope ratios can vary greatly under different environmental influences, and this outcome may have resulted from wetter conditions at the time these feathers were grown (Kelly 2000; Codron *et al.* 2005).

Half of the birds for which all primary feathers were analysed appear to have grown at least one feather in the

Succulent Karoo, supporting the partial migration theory (Allan 1994). The results suggest that both male and female bustards move between biomes, and that there are no marked differences in migration strategies between the sexes. This conclusion is supported by observational data, with similar sex ratios observed in the two biomes (Succulent Karoo male: female ratio 1:1.4, Nama Karoo 1:1.8, D. Allan unpubl. data 1989). However, there was a difference between the origin of adult and juvenile feathers, with all but one of 30 juveniles apparently originating in the Nama Karoo. Although Ludwig's bustards have been reported to breed in both biomes (Allan 2005), our results suggest that most breeding occurs in the Nama Karoo. This seems more likely than an alternative explanation of lower chick survival in the Succulent Karoo, given that satellite-tracked Ludwig's bustards have moved purposefully and repeatedly to specific areas in the Nama Karoo in early summer, apparently to breed (Shaw 2013). There is currently very little evidence for movement of birds between South Africa and Namibia, but it would be interesting to analyse feather material from Namibia to see whether such a movement, if it is isotopically discernible, is evident. The significant difference between the isotope ratios of juvenile and adult feathers is likely confounded by the geographic pattern, but the nitrogen signal in particular is much stronger for age than biome. Adults have higher  $\delta^{15}\text{N}$  values which may relate to a number of factors including diet (if they feed on different food items to juveniles, e.g. more or different invertebrates), and the different metabolic processes involved in tissue growth of juveniles.

While the DFA was moderately effective at classifying the region of feather growth correctly, our results should be interpreted with some caution. First, the analysis was based on a relatively small sample (although this is also the case in similar studies, e.g. Rocque *et al.* 2006; Wakelin *et al.* 2011), and there were too few samples to confidently use DFA with the bioregion data. We also assumed that growing feathers were formed using income (i.e. recently ingested) resources at the location where the bird was recovered. These assumptions may not always be valid, for example satellite tracking has revealed that while Ludwig's bustards often stay in one area for many months, they can also move considerable distances in a matter of days (Shaw 2013). In addition, there are unknown aspects of Ludwig's bustard ecology that may have influenced results, including suspended moult and potential use of capital resources (i.e. those from body stores) in feather growth (e.g. Klaassen *et al.* 2001). Given the unpredictable nature of the Karoo environment, that birds may not breed in drought years and that they are likely long-lived (Allan 2005; Shaw 2013), the former at least seems likely, and further investigation of this subject may help to clarify the interpretations of this study.

Although we know little about the rate of moult in Ludwig's bustards, the predicted feather origins do provide a plausible match to the satellite tracking data available for two birds. However, the scale of the analysis is probably too crude to discriminate movements close to the border between the two biomes (especially considering the DFA including the border Hantam Karoo samples was less accurate at classification). Bird A's feathers were classified as from the Nama Karoo with only the newest from the Succulent Karoo; this bird spent much of the tracking period in the area bordering the two biomes but died in the Succulent Karoo. Bird B moved far into the Nama Karoo, and all of his newest feathers were correctly classified with Nama Karoo origins. His only primary feather allocated to the Succulent Karoo was old and was thus probably grown before the satellite tag was deployed.

Comparing the satellite and isotope data also highlights the wide variability in measured feather isotope ratios (Fig. 3A,B). This variation could in part relate to a shift in food base at local scales. Ludwig's bustards are generalist foragers, but their isotope composition values may change if for example the bird concentrates feeding for a time on a locust outbreak, which occur in response to erratic localised rainfall (Earlé *et al.* 1988; Allan 2005). Therefore the usefulness of this technique is limited at a finer scale, and the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for birds with all primary feathers sampled cannot be reliably used to estimate how far birds move. A recent study of southern African ducks similarly found that high variance in isotopic patterns made inferring scales of mobility difficult (Mutumi 2010). An additional possibility is that seasonal dietary changes may have influenced the general patterns we detected, but Ludwig's bustard is a poorly known species and there are no seasonal dietary data currently available to investigate this. However, given their broad feeding habits and that our samples were collected across all seasons in several years, this seems more likely to have added further variation rather than influenced the general patterns shown.

Feather stable isotope analysis in terrestrial systems is usually applied at continental scales for birds with predictable moult patterns (e.g. Rocque *et al.* 2006; Szép *et al.* 2009; Hobson *et al.* 2012). We have found that this technique has been a valuable complement to satellite tracking for Ludwig's bustards, and our study shows that stable isotope analysis has value in smaller regions for birds that are little known and difficult to track in other ways. However, while broad conclusions can be drawn, at finer scales the variation in rainfall and patchy food resources are probably too great to maintain clear geographic signals. While testing other elements was outside the scope of this study, there is great potential for further work to improve our ability to infer origins of feathers and clarify some of the patterns observed for this species. Widely used in avian

movement studies (e.g. Yohannes *et al.* 2007; Symes & Woodborne 2010; Wakelin *et al.* 2011; Hobson *et al.* 2012), analysis of hydrogen isotope ratios in particular would be valuable. Hydrogen varies with rainfall patterns (which would help to differentiate winter from summer rainfall regions), altitude and elevation, and can also be used to clarify dominance of different photosynthetic pathways in the food base (Hobson *et al.* 2003; Rubenstein & Hobson 2004; Marshall *et al.* 2007). Other elements, such as strontium and sulphur, have also been used successfully in avian movement studies and could improve resolution of our predictions in this environment (e.g. Szép *et al.* 2009). In addition, further insight into Ludwig's bustard moult patterns, diets across seasons and the potential use of capital resources in feather growth would be valuable.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Appendix S1.** Satellite tracked positions (2010–2012) of two Ludwig's bustards in the Karoo, South Africa which also had feathers analysed for stable isotopes.