

Drivers of intrapopulation variation in resource use in a generalist predator, the macaroni penguin

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Supplement 1. Concurrently collected guano samples

There were 50 guano samples collected opportunistically during the 2012 breeding season (Table S1.1; Table S3.1). Samples were immediately sealed in a microcentrifuge tube (Eppendorf, Hamburg) to minimise nitrogen volatilization (Jianjun *et al.*, 2009), and stored at 20°C until prepared for analysis. Guano was taken to represent diet within ca. 3 days (Bird *et al.*, 2008). Guano samples were freeze dried for 12-24 hours and were homogenised using a ball mill (TissueLyser II, Qiagen, Manchester, UK).

Guano samples were analysed at the Godwin Laboratory, University of Cambridge (UK). Single subsamples of 0.7 mg aliquots were analysed in tin capsules. To correct for instrumental drift, each analytical sequence included 3 internal standards (Caffeine [IAEA, Austria]; Alanine and Nylon [Sigma–Aldrich, Germany]) for every 16 unknown samples of guano.

Two dietary classes were identified in guano (Fig. S1.1 A-E); the difference between the two classes within guano ($\sim 3\text{ ‰}$) was slightly larger than observed in blood plasma or feathers. The proportion of individuals in each class was similar to the blood plasma results, albeit with smaller sample sizes for guano. Consequently, it appears that ammonia volatilization from the guano was negligible. Sex-specific isotope ratios are shown in Table S1.1.

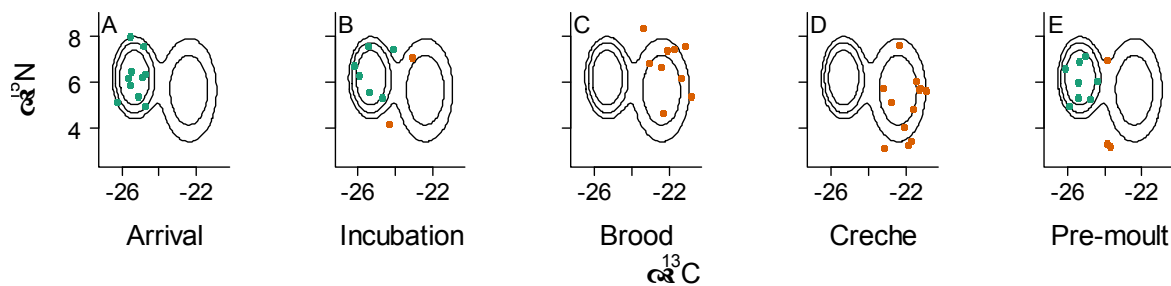


Fig. S1.1. Stable isotope ratios of guano samples collected from macaroni penguins during different phases of the breeding season. Two distinct dietary classes: class 1 (light blue) and class 2 (orange). Data points shown as de-standardised. Density contours shown for total data set at 0.25, 0.5 and 0.75.

Table S1.1. Stable isotope signatures of guano collected from macaroni penguins at Bird Island, South Georgia, by sex and breeding phase.

Sample	Year	Phase	n	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		C:N	
				M	F	M	F	M	F
Guano	2012	PB	10	-25.4±0.5	-24.9±0.1	6.4±1.0	5.6±0.9	2.0±0.7	1.9±0.3
		Inc	8	-25.4±0.8	-24.5±1.2	6.0±1.5	6.6±0.9	2.8±1.7	1.3±0.0
		BG	9	-	-22.1±0.9	-	6.7±1.2	-	2.5±1.1
		Cr	12	-22.0±1.0	-21.9±0.7	6.0±1.2	4.6±1.2	2.7±1.4	3.0±1.7
		PM	11	-24.8±0.8	-25.2±1.0	5.7±1.3	5.5±1.7	2.3±1.0	2.2±1.4

References

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Supplement 2. Effect of feather colour and feather cleaning

Black and white feathers collected concurrently from the same individual during the 2012 breeding season were used to test for pigment enrichment of ^{13}C and ^{15}N ($n=20$; Michalik *et al.*, 2010). Feathers were cleaned of surface contaminants following the cleaning procedure detailed in the main text. Cleaned and un-cleaned black feathers from the same individual (all males) were also prepared for stable isotope analysis in order to test ^{15}N fractionation from the cleaning process ($n=16$; Sweeting *et al.*, 2006).

The effects of feather colour and cleaning on stable isotope ratios were examined using mixed models (Zuur *et al.*, 2009). The inclusion of individual as a random effect to account for non-independence of observations was tested using a likelihood ratio test. Treatment (colour or cleaning), and in the analysis of colour; sex and an interaction between sex and treatment were included as fixed effects. Models with different fixed effect structures were compared via stepwise model selection with the Akaike Information Criterion (AIC). Here, more than 2 ΔAIC units was taken to indicate strong support for the model with the lower AIC (Burnham and Anderson, 2002). Parameter estimates from selected models were derived using restricted maximum likelihood (REML) estimation (Zuur *et al.*, 2009). When investigating the effect of feather cleaning, clusters were identified within the residuals that could not be attributed to the available covariate information (e.g. cleaning, supp. info. S3, Fig. 3.1). This violates the assumption that the spread of possible isotopic values is the same within each cleaning treatment (the homogeneity assumption; Zuur *et al.*, 2007). Because this analysis was concerned with examining the effect of cleaning only, individuals were manually assigned to clusters in order to control for the unexplained variance ($\delta^{15}\text{N} > 11.25$; $\delta^{13}\text{C} > -20$; supp. info. S3). This variable was included as a fixed effect alongside cleaning and sex. All analyses were performed in the program R using the statistical packages *nlme* and *RLRsim* (Pinheiro *et al.*, 2014; Scheipl and Bolker, 2014).

This study showed that white feathers were enriched significantly in both ^{13}C and ^{15}N , confirming the importance of considering differences in levels of pigmentation when interpreting stable isotope ratios in feathers (Michalik *et al.*, 2010). White feathers were significantly enriched in ^{13}C (linear mixed model: $df=19$, $t = 18.19$, $p<0.01$) and ^{15}N (linear mixed model: $df=19$, $t = 9.88$, $p<0.01$) compared to black feathers. There was no evidence for ^{15}N fractionation as a result of the cleaning process (Table S2.1).

References

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Table S2.1. Influence of feather colour and chloroform:methanol cleaning on the stable isotope signature for samples collected from macaroni penguins at Bird Island, South Georgia. Notation; (.) intercept only model, (S) sex, (C) colour, (R) chloroform:methanol rinse, (G) cluster grouping.

Treatment	Isotope	Model	<i>df</i>	AIC	Δ AIC
Colour	$\delta^{13}\text{C}$	C	4	55.3	0.00
		C + S	5	57.2	1.92
		C * S	6	59.5	4.25
		.	3	111.1	55.78
		S	4	112.8	57.55
	$\delta^{15}\text{N}$	C	4	8.6	0.00
		C + S	6	9.8	1.28
		C * S	5	11.0	2.40
		.	3	42.4	33.83
		S	4	44.6	36.08
Cleaning	$\delta^{15}\text{N}$	G	4	-6.5	0.00
		R + G	5	-3.7	2.80
		.	3	20.9	27.41
		R	4	23.5	30.01

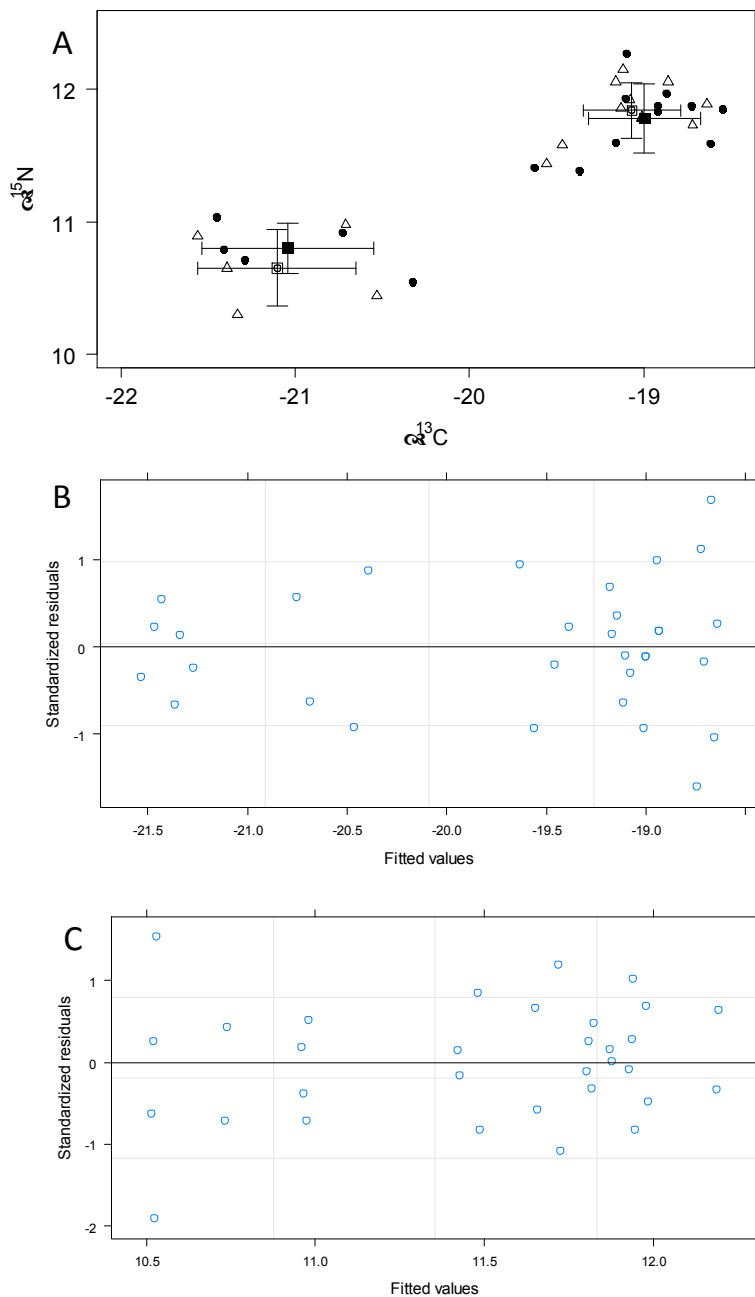
Supplement 3. Example of clustering within a sample type

Fig. S3.1. Example of clustering within a sample type illustrated using black feathers collected from males at the individual level. A) Difference between feathers that have been cleaned of surface contaminants (black circles) and not cleaned (triangles); feathers reflect the pre-moult foraging trip of the 2002 breeding season. Standard deviations shown for clusters assigned by $\delta^{15}\text{N} > 11.25$. B) Standardised residuals for random effects model $\delta^{13}\text{C} \sim \text{cleaning}$. C) Standardised residuals for random effects model $\delta^{15}\text{N} \sim \text{cleaning}$.

Table 3.1. Number of individuals within each isotopic class by sex and time. Class numbers reflect the order of classes within a sample type (e.g. for plasma, 1st = Class 3, 2nd = Class 4).

	Year	Period of synthesis	<i>Total</i>		Number of individuals (<i>n</i>)							
			M	F	1 st class		2 nd class		3 rd class		4 th class	
					M	F	M	F	M	F	M	F
Feather	2001	Pre-moult	20	20	11	13	9	7				
	2002	Pre-moult	20	20	14	10	6	10				
	2003	Pre-moult	14	19	9	11	5	8				
	2011	Pre-moult	20	22	10	8	10	14				
	2012	Pre-moult	21	20	13	9	8	11				
Blood	2012	Winter	12	-	12	-	0	-				
Plasma		Incubation	7	15	7	9	0	6				
		Brood	-	9	-	0	-	9				
		Crèche	15	14	0	0	15	14				
		Pre-moult	16	14	8	6	8	8				
Blood cells	2012	Winter	12	8	12	8	0	0	0	0	0	0
		Incubation	7	15	0	0	7	5	0	10	0	0
		Brood	-	9	-	0	-	1	-	8	-	0
		Crèche	15	12	0	0	0	0	15	3	0	9
		Pre-moult	14	13	0	0	0	0	5	4	9	9
Guano	2012	Winter	8	2	8	2	0	0				
		Incubation	4	4	3	3	1	1				
		Brood	-	9	-	0	-	9				
		Crèche	4	8	0	0	4	8				
		Pre-moult	7	4	5	3	2	1				

N.B. Dietary types in blood cells are not comparable with other tissues.

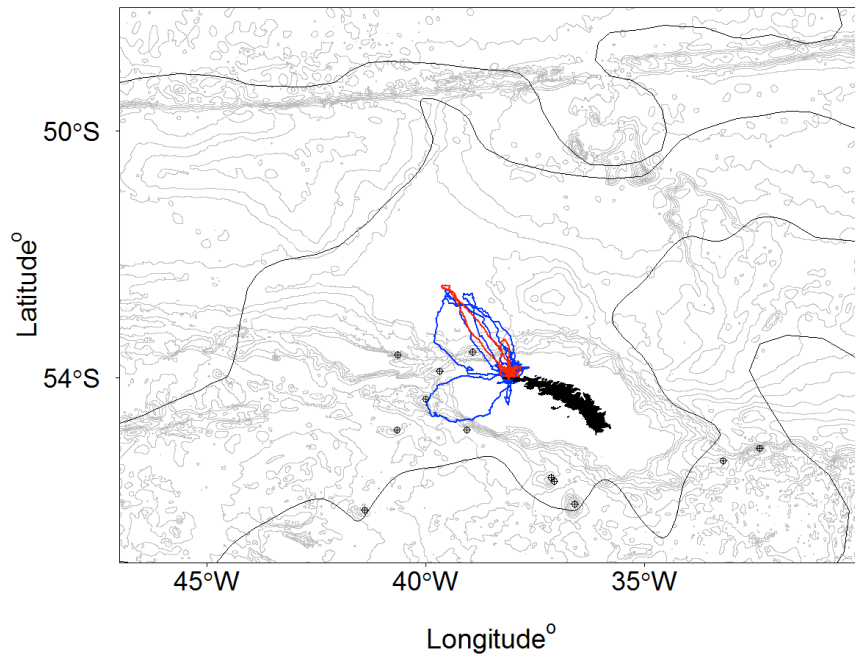


Fig. S3.2. Comparison of the foraging distribution during the crèche period of 2002 (blue, $n=15$) and 2012 (red, $n=11$). Preliminary analysis to examine whether feather samples should be standardised. Mean maximum distance travelled in 2002 = 65 km, $SD=65$; Mean maximum distance travelled in 2012 = 28 km, $SD=32$. Average position of ocean fronts are shown as black lines; top down: Sub-Antarctic Front; Antarctic Polar Front; Southern Antarctic Circumpolar Current Front, and Southern Antarctic Circumpolar Current Boundary. The general pattern of the surface current flow in the Scotia Sea is from west to east in line with the major ocean fronts. The study area, Bird Island, is at the western end of South Georgia. Bathymetry contours (grey lines) every 500m, and seamounts shown with black circles.