Skuas (Stercorarius spp.) moult body feathers during both the breeding and inter-breeding periods: implications for stable isotope investigations in seabirds

Journal:	*Ibis*
Manuscript ID	Draft
Manuscript Type:	Original Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Graña Grilli, Maricel; Instituto de Investigaciones en Biodiversidad y Medioambiente, Cherel, Yves; CNRS, UMR7372,
Category:	Antarctic, ecology: trophic (including foraging and diet studies), ecology: migration

Body feathers moult on stable isotope investigations

Skuas (*Stercorarius* spp.) moult body feathers during both the breeding and inter-breeding periods: implications for stable isotope investigations in seabirds

MARICEL GRAÑA GRILLI[1,*] & YVES CHEREL[2]

[1] Laboratorio Ecotono, INIBIOMA (Universidad Nacional del Comahue – CONICET), Quintral 1250 (R8400FRF), San Carlos de Bariloche, Argentina.

[2] Centre d'Etudes Biologiques de Chizé, UMR 7372 du CNRS-Université de La Rochelle, 79360 Villiers-en-Bois, France.

[*] Corresponding author. Email: ggmaricel@comahue-conicet.gob.ar

SUMMARY

Seabirds are mostly believed to moult during the inter-breeding period and the isotopic values of their feathers are related to their diet during such period. We observed Brown Skuas (*Stercorarius antarcticus lonnbergi*) and South Polar Skuas (*S. maccormicki*) moulting on the breeding site at King George Island, Antarctica. This raises a doubt about the reliability of the conclusions drawn up about the feeding localities of birds during the inter-breeding period by means of the determination of the stable isotope values of feathers. We analyzed $\delta^{13}C$ and $\delta^{15}N$ values of growing and fully grown body feathers collected from the same individuals. For both species, $\delta^{13}C$ values of growing feathers indicated feeding areas in the Antarctic zone (breeding grounds), while most fully grown feathers could be assigned to northern latitudes (non-breeding grounds). However, a few fully grown body feathers of Brown Skuas showed isotopic values indicating moulting in the Antarctic zone. Since the time-window (growth period) of those feathers is unknown, they cannot be used with confidence to depict the foraging behaviour of the birds during the non-breeding period. Our results aware about the possibility of drawing up misleading conclusions about the origin of the diet when the moulting pattern of the species is unknown, and show that if the state of development of feathers can be determined the occurrence of moult on the breeding grounds allows the determination of the diet of the same bird during two different periods of its annual cycle through one single feather sampling event.

Keywords: Antarctica, diet, migration, Southern Ocean

INTRODUCTION

Tissues incorporate into their structures the isotopic composition of the food that animals consume, which in turn, reflects its location and trophic level. These features have given researchers the agency to analyze stable isotopes for both the characterization of the diet ($\delta^{15}N$) and identification of the feeding habitats ($\delta^{13}C$) of consumers (Hobson *et al.* 1994, Wolf *et al.* 2009, Jaeger *et al.* 2010a). The characteristic turnover rate of the composition of each tissue results in the isotopic pattern of different tissues allowing the identification of diet and habitats at different temporal scales (Hobson & Clark 1992, Cherel *et al.* 2014). Among the different tissues, feathers set in their inert structure the isotopic signature belonging to the diet while they are synthesized, which remain unchanged after the synthesis process is finished (Hobson & Clark 1992, Bearhop *et al.* 2002). Therefore, the analysis of stable isotopes of feathers allows the identification of the moulting site of birds and, considering an enrichment factor between successive trophic levels, the trophic position during the moulting period (Hobson & Wassenaar 1997, Marra *et al.* 1998, Cherel *et al.* 2014).

It is broadly accepted that due to the costly energetic requirements of moult and breeding, both processes tend to take place out of phase (Hemborg & Lundberg 1998, Dawson 2008). In addition, many long-distance migratory birds moult after breeding or on their wintering sites (Nelson 1979, Löfgren 1984, Schreiber & Burger 2001, Newton 2008). This results in such birds maintaining in their feathers the isotopic signature of the moulting sites, which in many cases are different from the breeding ground (Hobson & Wassenaar 1997, Jaeger *et al.* 2009, Weimerskirch *et al.* 2015, Cherel *et al.* 2016).

This permanence of the isotopic signature of the moulting sites in feathers is particularly valuable in seabirds as generally the breeding period is the only stage when they can be sampled. For this reason, if moulting takes place outside the breeding site, stable isotope analysis of feathers has the potential of allowing the identification of the wintering areas and trophic level of the birds during the non-breeding period (Cherel *et al.* 2000, 2014, 2016, Furness *et al.* 2006, Phillips *et al.* 2007). However, the overlap between breeding and moulting increases towards higher latitudes (Newton 2008) and many species start moulting by the end of their breeding period or even completely overlap both processes (Nelson 1979, Schreiber & Burger 2001, Catry *et al.* 2013, Bourgeois & Dromzée 2014), constituting exceptions to the broadly assumed inter-breeding moult.

Brown Skuas (*Stercorarius antarcticus lonnbergi*) and South Polar Skuas (*S. maccormicki*) breed during the austral summer in the shores of the Antarctic continent and sub-Antarctic Islands and subsequently move northward (Phillips *et al.* 2007, Kopp *et al.* 2011, Weimerskirch *et al.* 2015). The information on the moulting pattern of both or these two species is scarce and is focused mainly on primary feathers (Olsen & Larsson 1997, Newel *et al.* 2003, Votier *et al.*). Both species are supposed to undertake the moult of body feathers after the breeding season (Furness 1987, Olsen & Larsson 1997, Phillips *et al.* 2007) but evidence shows that moulting pattern is highly variable (Votier *et al.* 2015, Weimerskirch *et al.* 2015).

During the austral summer of 2011/12, individuals of Brown and South Polar Skuas were recorded moulting body feathers in Potter Peninsula, King George Island, Antarctica. Here we compare the isotopic composition of two groups of feathers sampled simultaneously from the same individuals: 1) feathers grown on the breeding grounds, and 2) fully grown feathers. We discuss the implications of this situation when drawing up conclusions from the isotopic signature of feathers when the moulting pattern of the species is unknown.

METHODS

A total of nine Brown Skuas and 13 South Polar Skuas were handled during the austral summer of 2011/12 in Potter Peninsula, King George Island, Antarctica. They included three and six moulting Brown and South Polar Skuas, respectively; the birds were adults but only two Brown Skuas were actually breeding. Two growing feathers were taken from the breast and neck of each moulting bird as well as a fully grown feather adjacent to those two. In addition, three developed feathers were taken randomly from the abdomen and back and five fully grown feathers were also sampled randomly from the other six Brown Skuas and seven South Polar Skuas that were not moulting.

In the case of fully developed feathers, five feathers per bird were analyzed to detail the moulting habitat and diet of individuals (Jaeger *et al.* 2009). For growing feathers, two per bird were analyzed. Prior to analyses, feathers where cleaned by immersion in a 2:1 chloroform:methanol solution for 3 min followed by two rinses in methanol and then air dried. Each feather was cut with scissors into small pieces, and a 0.3-0.5 mg subsample was weighed with a microbalance and packed into tin containers. Relative abundance of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined with a continuous flow mass spectrometer (Thermo Scientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112). Results are presented in the usual δ notation (in ‰) relative to Vienna PeeDee Belemnite and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicated measurement errors < 0.15 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

Data was analyzed with R (R Core Team) using GLMM, incorporating the identity of the birds as a random variable in order to avoid the pseudo-replication of feathers taken from the same bird. Values are means ± SD.

RESULTS

Tissue $\delta^{13}C$ and $\delta^{15}N$ values were measured on 127 body feathers (51 from Brown Skuas and 76 from South Polar Skuas) including 17 growing feathers (6 and 11, respectively). Overall feather $\delta^{13}C$ and $\delta^{15}N$ values showed a remarkable range of values, $\delta^{13}C$ ranged from -22.7 to -15.3 ‰ and $\delta^{15}N$ from 8.8 to 19.3 ‰ in Brown Skuas, while in South Polar Skuas the range for $\delta^{13}C$ was from -22.7 to -13.1 ‰ and that for $\delta^{15}N$ from 9.5 to 18.3 ‰. In both species $\delta^{13}C$ values of fully grown feathers differed from that of growing feathers (Brown Skuas: $t = -6.36$, $p < 0.001$; South Polar Skuas: $t = -16.54$, $p < 0.001$). For $\delta^{15}N$, the difference was significant in Brown Skuas ($t = -2.07$, $p = 0.04$) and marginally significant in South Polar Skuas ($t = -1.88$, $p = 0.06$) (Table 1).

Three features were noteworthy. Firstly, growing feathers clustered together and showed little inter-feather isotopic differences when compared to fully grown feathers. Secondly, three fully grown feathers from two Brown Skuas had similar isotopic values compared to growing feathers. Thirdly, there was a ~1‰ gap in $\delta^{13}C$ values with no body feathers between -21.4 and -20.3‰; this gap represents a $\delta^{13}C$ difference between growing and fully grown feathers (Figure 1). Fully grown feathers had similar $\delta^{13}C$ ($t = 1.08$, $p = 0.29$) and $\delta^{15}N$ ($t = -0.30$, $p = 0.76$) values in both species, while growing feathers had similar $\delta^{13}C$ values in both species ($t = 0.75$, $p = 0.47$) but higher $\delta^{15}N$ values were found in South Polar Skuas than in Brown Skuas ($t = 2.57$, $p = 0.04$) (Table 1).

DISCUSSION

Our work adds evidence to the occurrence of moulting during the breeding season of seabirds (Catry *et al.* 2013, Bourgeois & Dromzée 2014, Weimerskirch *et al.* 2015) and therefore, to the simultaneous presence on the birds of feathers developed during both the breeding and inter-breeding periods. Different moulting periods allowed the determination of the isotopic signature of the breeding and wintering sites for the same birds, and using the same tissue. As a consequence, this work also warns about the possibility of drawing misleading conclusions from the analysis of feathers' composition when the moulting pattern is unknown.

As expected, the low $\delta^{13}C$ values of growing body feathers of Brown and South Polar Skuas reflect the $\delta^{13}C$ values of the breeding site in Maritime Antarctica (e.g. Hinke *et al.* 2015). In contrast, two possibilities must be considered when interpreting the isotopic composition of fully grown feathers: (1) if fully grown feathers show a different isotopic signature than the growing ones, it can be assumed that they represent a different site, hence the habitat and diet/trophic position during the inter-breeding period and; (2) if both fully grown and growing feathers show the same isotopic values, it could be that either fully grown feathers could have developed on a wintering site with an isotopic signature similar to that of the breeding grounds, or that feather growth occurred while on the breeding grounds. Therefore, in the case of both groups of feathers having the same isotopic signatures, the origin of the grown feathers cannot be guaranteed, making it impossible to achieve any definite conclusion from them.

In the case of Brown and South Polar Skuas, growing and fully grown feathers have mostly different isotopic signatures. This indicates that each group belongs to the breeding and inter-breeding sites, respectively. However, a few fully grown feathers of Brown Skuas showed isotopic signatures similar to those of growing feathers which, in this case, could suggest that they were grown in the breeding site. The standard deviation of $\delta^{13}C$ and $\delta^{15}N$ values also indicates the different origin of both groups of feathers. Fully grown feathers had a much higher standard deviation than growing feathers, which can be related to birds foraging along a broader geographical range and on a wider range of prey items during the inter-breeding season (Bearhop *et al.* 2004, Weimerskirch *et al.* 2015).

For both species, $\delta^{13}C$ values indicated feeding areas south of the Polar Front in the Antarctic Zone in growing feathers and north of that front in fully grown feathers (Cherel *et al.* 2006, 2016, Quillfeldt *et al.* 2005, Jaeger *et al.* 2010b). The mean $\delta^{13}C$ values of fully grown feathers of Brown Skuas from Potter Peninsula (-17.9 ‰) is identical to that of the population from South Georgia (-17.8 ‰, Phillips *et al.* 2007), suggesting that both populations mainly overwinter in the Subantarctic and Subtropical Zones. However, the high $\delta^{13}C$ (> -16‰) and $\delta^{15}N$ (>16 ‰) values of some feathers from King George Island indicate that some birds wintered over neritic waters that are marked by elevated $\delta^{13}C$ and $\delta^{15}N$ baseline levels, most likely closely-located to the Patagonian shelf (Granadeiro *et al.* 2014). South Polar Skuas from King George Island migrate far north, overwintering in the northern hemisphere between 30 and 55°N (Kopp *et al.* 2011). The isotopic values of fully grown body feathers showed large $\delta^{13}C$ and $\delta^{15}N$ variations that indicate various moulting habitats that cannot be determined due to the lack of appropriate isoscapes. Notably, however, the mean South Polar skua $\delta^{13}C$ value (-17.3 ‰) was close to that (-17.7 ‰) of Atlantic puffins (*Fratercula arctica*) from around 47°N (Hedd *et al.* 2010).

Beginning moult as soon as breeding activity stops (Dawson 2008) could be an explanation for finding moulting skuas during the summer time, since most of the sampled birds were not breeding. However, two birds were breeding (one of them was moulting primary feathers), which shows that skuas, as some other seabirds, may moult during their breeding period (Catry *et al.* 2013, Bourgeois & Dromzée 2014). They therefore presented simultaneously feathers whose isotopic signature belong to different moulting localities (Fox *et al.* 2007), thus raising the possibility of drawing up wrong conclusions if the growing feathers are not identified as newly developed. Therefore, this work directs the attention towards the condition of development of feathers when sampling species for which the moulting pattern is unknown or can show unexpected changes, in order to avoid assigning wrong origins to the analyzed feathers.

Analysis of stable isotopes of feathers from adults and chicks has allowed the study of the trophic ecology of birds in two different periods of their annual cycle, namely the inter-breeding and breeding periods, respectively (Cherel *et al.* 2000, 2014, Jaeger *et al.* 2010a). The moult of some body feathers in the breeding grounds opens the possibility for studying the trophic ecology of adults in two different periods of the annual cycle for the same individuals, and through the analysis of the same tissue type (feather). Such kind of study can also be induced by measuring stable isotopes on plucked feathers early in the breeding season and collecting the replacement feathers later on (Nisbet *et al.* 2002, Quillfeldt *et al.* 2005). This is, however, a more invasive method that requires more than one capture of the birds.

ACKNOWLEDGMENTS

The authors thank G. Guillou for stable isotope analysis and to R. McCabe for language correction. The fieldwork was made possible by the Instituto Antártico Argentino. MGG thanks the Company of Biologists for the travelling fellowship that allowed her doing this work.

REFERENCES

Bearhop S., S. Waldron, S.C. Votier and R.W. Furness. 2002. Factors that influence assimilation rates and fractionation of Nitrogen and Carbon stable isotopes in avian blood and feathers. Physiological and Biochemical Zoology 75: 451–458.

Bearhop S., C.E. Adams, S. Waldron, R.A. Fuller and H. MacLeod. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology 73: 1007–1012.

Bourgeois K. and S. Dromzeé. 2014. Moulting strategies of the Yelkouan Shearwater *Puffinus yelkouan* during the breeding season. Journal of Ornithology 155: 265–271.

Catry P., M. Poisbleau, M. Lecoq and R.A. Phillips. 2013. Differences in the timing and extent of annual moult of black-browed albatrosses *Thalassarche melanophris* living in contrasting environments. Polar Biology 36: 837–842.

Cherel Y., K.A. Hobson and H. Weimerskirch. 2000. Using stable-isotope analysis of feathers to distinguish moulting and breeding origins of seabirds. Oecologia 122: 155–162.

Cherel Y., R.A. Phillips, K.A. Hobson and R. McGill. 2006. Stable isotope evidence of diverse species-specific and individual wintering strategies in seabirds. Biology Letters 2: 301–303.

Cherel Y., M. Connan, A. Jaeger and P. Richard. 2014. Seabird year-round and historical feeding ecology: blood and feather $\delta^{13}C$ and $\delta^{15}N$ values document foraging plasticity of small sympatric petrels. Marine Ecology Progress Series 505: 267–280.

Cherel Y, Quillfeldt P, Delord K, Weimerskirch H (2016) Combination of at-sea activity, geolocation and feather stable isotopes documents where and when seabirds molt. Front Ecol Evol 4:3.

Dawson A. 2008. Control of the annual cycle in birds: endocrine constraints and plasticity in response to ecological variability. Philosophical Transactions of the Royal Society B. 363: 1621–1633.

Fox T.A.D., T.K. Christensen, S. Bearhop and J. Newton. 2007. Using stable isotope analysis of multiple feather tracts to identify moulting provenance of vagrant birds: a case study of Baikal Teal *Anas formosa* in Denmark. Ibis 149: 622–625.

Furness R.W. 1987. The Skuas. T & AD Poyser, Calton.

Furness R.W., J.E. Crane, S. Bearhop, S. Garthe, A. Käkelä, R. Käkelä, A. Kelly, U. Kubetzki, S.C. Votier and S. Waldron. 2006. Techniques to link migration patterns of seabirds with diet specialization, condition and breeding performance. Ardea 94: 631–638.

Granadeiro J.P., P. Brickle and P. Catry. 2014. Do individual seabirds specialize in fisheries' waste? The case of black-browed albatrosses foraging over the Patagonian Shelf. Animal Conservation 17: 19-26.

Hedd A., D.A. Fifield, C.M. Burke, W.A. Montevecchi, L. McFarlane Tranquilla, P.M. Regular, A.D. Buren and G.J. Robertson. 2010. Seasonal shift in the foraging niche of Atlantic puffins *Fratercula arctica* revealed by stable isotope (δ^{15}N and δ^{13}C) analyses. Aquatic Biology 9: 13–22.

Hemborg C. and A. Lundberg. 1998. Costs of overlapping reproduction and moult in passerine birds: an experiment with the pied Fycatcher. Behavioural Ecology and Sociobiology 43: 19–23.

Hinke J.T., M.J. Polito, M.E. Goebel, S. Jarvis, C.S. Reiss, S.R. Thorrold, W.Z. Trivelpiece and G.M. Watters. 2015. Spatial and isotopic niche partitioning during winter in chinstrap and Adélie penguins from the South Shetland Islands. Ecosphere 6: 125.

Hobson K.A. and R.G. Clark. 1992. Assessing avian diets using stable isotopes I: Turnover of 13C in tissues. Condor 94: 181–188.

Hobson K.A., J.F. Piatt and J. Pitocchelli. 1994. Using stable isotopes to determine seabird trophic relationships. Journal of Animal Ecology 63: 786–798.

Hobson K.A. and L.I. Wassenaar. 1997. Linking breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. Oecologia 109: 142–148.

Jaeger A., P. Blanchard, P. Richard and Y. Cherel. 2009. Using carbon and nitrogen isotopic values of body feathers to infer inter- and intra-individual variations of seabird feeding ecology during moult. Marine Biology 156: 1233–1240.

Jaeger A., M. Connan, P. Richard and Y. Cherel. 2010a. Use of stable isotopes to quantify seasonal changes of trophic niche and levels of population and individual specialization in seabirds. Marine Ecology Progress Series 401: 269–277.

Jaeger A., V. Lecomte, H. Weimerskirch, P. Richard and Y. Cherel. 2010b. Seabird satellite tracking validates the use of latitudinal isoscapes to depict predators' foraging areas in the Southern Ocean. Rapid Communications in Mass Spectrometry 24: 3456–3460.

Kopp M., H.-U. Peter, O. Mustafa, S. Lisovski, M.S. Ritz, R.A. Phillips and S. Hahn. 2011. South polar skuas from a single breeding population overwinter in different oceans though show similar migration patterns. Marine Ecology Progress Series 435: 263–267.

Löfgren L. 1984. Ocean birds. Alfred A. Knopf, Inc., New York.

Marra P.P., K.A. Hobson and R.T. Holmes. 1998. Linking winter and summer events in a migratory bird by using stable-carbon isotopes. Science 282: 1884–1886.

Nelson B. 1979. Seabirds. Their biology and ecology. A & W Publishers, Inc., New York.

Newell D., S.N.G. Howell and D. López-Velasco 2013. South Polar and Great Skuas: the timing of primary moult as an aid to identification. British Birds 106: 325–346.

Newton I. 2008. The migration ecology of birds. Academic Press, London.

Nisbet I.C.T., J.P. Montoya, J. Burger and J.J. Hatch. 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.

Olsen K.M. and H. Larsson. 1997. Skuas and Jaegers. Pica Press, Sussex.

Phillips R.A., P. Catry, J.R.D. Silk, S. Bearhop, R. McGill, V. Afanasyev and I.J. Strange. 2007. Movements, winter distribution and activity pattern of Falkland and brown skuas: insights from loggers and isotopes. Marine Ecology Progress Series 345: 281–291.

Quillfeldt P., R.A.R. McGill and R. Furness. 2005. Diet and foraging areas of Southern Ocean seabirds and their prey inferred from stable isotopes: review and case study of Wilson's storm petrel. Marine Ecology Progress Series 295: 295–304.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Schreiber E.A. and J. Burger. 2001. Biology of marine birds. CRC Press, Boca Raton.

Votier S., S. Bearhop, R.W. Furness, D. Newell and P. Harvey. Ageing and moult in Catharacta skuas - some comments. At: http://www.surfbirds.com/mb/Features/skua-identification.html (Acceded on 20 May 2015).

Weimerskirch H., A. Tarroux, O. Chastel, K. Delord, Y. Cherel and S. Descamps. 2015. Population-specific wintering distributions of south polar skuas over the three oceans. Marine Ecology Progress Series, in press.

Wolf N., S.A. Carleton and C. Martínez del Rio. 2009. Ten years of experimental animal isotopic ecology. Functional Ecology 23: 17–26.

Table 1. Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of fully grown and growing body feathers of adult Brown and South Polar Skuas. Values are means ± SD with ranges in parentheses.

	N feathers (n birds)	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**C:N mass ratio**
Brown Skuas				
Fully grown	45 (9)	- 17.9 ± 1.7	12.9 ± 3.3	3.12 ± 0.03
		(-22.4--15.3)	(8.8-19.3)	(3.05-3.19)
Growing	6 (3)	- 22.6 ± 0.1	11.1 ± 0.9	3.13 ± 0.02
		(-22.7--22.5)	(9.9-11.8)	(3.09-3.16)
South Polar Skuas				
Fully grown	65 (13)	- 17.3 ± 1.5	12.6 ± 2.0	3.13 ± 0.03
		(-20.1--13.1)	(9.5-18.3)	(3.07-3.24)
Growing	11 (6)	- 22.4 ± 0.3	11.7 ± 0.3	3.13 ± 0.02
		(-22.7--21.8)	(11.3-12.2)	(3.10-3.16)

Figure 1. Isotopic values (δ^{13}C and δ^{15}N) of fully grown (black symbols) and growing (white symbols) body feathers of adult Brown and South Polar Skuas.