Polar Biology (2021) 44:305–314
https://doi.org/10.1007/s00300-020-02793-1

ORIGINAL PAPER

Dynamics of circulating lipoproteins and lipids in Brown Skua (*Stercorarius antarcticus lonnbergi*) during the breeding cycle

A. E. Ibañez[1] · M. Y. Pasquevich[2] · N. Fernandez Machulsky[3] · G. Berg[3] · H. Heras[2] · D. Montalti[1,4] · M. Graña Grilli[5]

Received: 6 September 2019 / Revised: 17 December 2020 / Accepted: 19 December 2020 / Published online: 11 January 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH, DE part of Springer Nature 2021

Abstract

In central-place foragers the breeding cycle is often the period with the highest energy cost, where dietary and stored lipids play a key role. Lipids are mobilized through blood lipoproteins providing fuel to tissues. Thus, the use of food and endogenous resources with high-energy fats is important to sustain individuals' nutritional demands. To evaluate the physiological components associated to energetic demands and their variation regarding life history processes, we analysed the plasma circulating lipoprotein levels, the lipid classes and fatty acid (FA) composition in Brown Skua (*Stercorarius antarcticus lonnbergi*) in breeding stages with different energy requirements: incubation (In), early rearing (Er) and late rearing (Lr). We expected to find differences according to the energy demands of each stage and the sex. The level of Very Low Density lipoproteins was affected by the sex and the breeding stage, whereas Low Density lipoproteins only by the stage. Total plasma lipid content and circulating lipid classes were invariant among the stages studied; however, differences in total plasma FA and monounsaturated FA (oleic and nervonic) abundances were observed among stages. Besides, a decrease in the n-3:n-6 ratio was observed towards the Lr stage. The differences found in lipoproteins may be linked to changes in the energy demands throughout the breeding period. Moreover, the variation observed in FA abundance and n-3:n-6 ratio may be related to a preferential mobilization of FA from stores as fuel or, possibly, to a differential use of the available food resources.

Keywords Antarctic seabirds · Breeding energetic · Fatty acids · Lipid metabolism

Introduction

In migratory seabirds energy demands may fluctuate during the annual cycle affecting their body condition (Hanssen et al. 2005). In particular, the reproduction is often the process with the highest energy investment in bird life (Williams 2005). The nutritional quality of the selected food available has substantial effects on bird fitness and, therefore, on breeding and migratory performance (Blem 1990; Price et al. 2008).

Deposition or utilization of lipids as energy source may be limited by several physiological and environmental factors, such as the rate of lipid transport by plasma lipoproteins and seasonal variations of food resource availability (Pierce and Williams 2005; Williams and Buck 2010). Dietary and endogenous triacylglycerols (TAG) and fatty acids (FA) are transported to different tissues by lipoproteins either for storage or oxidized for energy (Ramenofsky 1990; Klasing 1998; Guglielmo et al. 2002a, b; Cerasale and Guglielmo 2006; Williams and Buck 2010). Thus, the abundance of different lipoprotein classes is related to the metabolic and

✉ A. E. Ibañez
aeibanez@fcnym.unlp.edu.ar

1 Sección Ornitología, División Zoología Vertebrados, Museo de La Plata, Facultad de Ciencias Naturales Y Museo, Universidad Nacional de La Plata, B1900FWA La Plata, Buenos Aires, Argentina

2 Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET, Universidad Nacional de La Plata, La Plata, Argentina

3 Departamento de Bioquímica Clínica, Facultad de Farmacia Y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

4 Instituto Antártico Argentino (IAA), San Martín, Buenos Aires, Argentina

5 Grupo de Investigaciones en Biología de La Conservación, Laboratorio Ecotono, INIBIOMA, CONICET, Universidad Nacional del Comahue, Bariloche, Argentina

nutritional status (Hermier 1997). Regarding the role of fatty acids (FA) as energy source, the FA composition of lipid stores differs across the annual cycle according to the availability of resources (Conway et al. 1994). Moreover, plasma FA composition provides relevant data about the FA with metabolic importance that are being mobilized from stores, as well as those acquired from diet (Iverson et al. 2004; Budge et al. 2006; Price et al. 2008).

Breeding birds have to balance the energy investment between self-maintenance and nestlings (Bourgeon et al. 2009, 2010; Mitchell et al. 2012). Therefore, reproduction restricts the parents' self-foraging ability affecting their body condition. As energy source, stored lipids and proteins are used when there is an insufficient energy income from diet. Thus, most energy requirements are met through the mobilization and oxidation of fat. When fat stores reach a threshold or have been exhausted, the catabolism of body proteins as energy source increases (Carrascal et al. 1998; Groscolas and Robin 2001). Despite the relevance of lipids as energy source, up to our knowledge, lipid dynamics has mostly been studied in laying hens and broiler chicks to evaluate the quality of diets (Hermier et al. 1984; Hermier 1997; Peebles et al. 2004; Navidshad et al. 2010), whereas there are only a few studies conducted in wild migratory birds (Jenni-Eiermann and Jenni 1992).

Brown Skuas (*Stercorarius antarcticus lonnbergi*) are generalist seabirds that breed in subantarctic islands and the Antarctic (Ritz et al. 2008; Graña Grilli and Montalti 2015). Before the Antarctic summer begins, Brown Skuas migrate from winter locations in the Atlantic Ocean to breeding localities in Antarctica (Krietsch et al. 2017). During reproduction a division of labour in adults occurs (Furness 1987), in which males conduct a greater share of foraging to feed females and growing chicks (Burton 1968; Devillers 1978). Previous studies have described a decline of the nutritional and health status of Brown Skuas during breeding as a consequence of parental effort (Graña Grilli et al. 2018; Ibañez et al. 2018). Besides, diet studies during the breeding season have revealed that Brown Skuas often display a flexible foraging behaviour which enables them to supplement terrestrial prey with others from the sea (i.e. fish and mollusc) (Graña Grilli and Montalti 2012, 2015; Graña Grilli et al. 2014; Borghello et al. 2018). Nevertheless, these studies overestimate the proportion and metabolic importance of prey items that mainly contain indigestible material (Votier et al. 2001).

Information about the physiological components linked to the energy demands and its fluctuation is of great interest to understand how life history processes and environmental changes impact on organisms. Regarding the role of Brown Skuas as central-place foragers, the energy expenditure during reproduction may induce variations in body condition, foraging behaviour and in the manner they manage energy reserves. In this fluctuating energy constraint scenario, the use of stored and dietary lipids plays a key role as energy source. Thus, the aim of the present work was to determine the dynamics of circulating lipids and the amount of lipid classes in breeding stages (incubation, early and late care of chicks) which are known to involve different energy demands. For this, the level of lipoproteins, the plasma lipid classes, and FA composition were assessed. Plasma FA composition will provide a comprehensive analysis about the amounts of the circulating plasma FA classes during the different breeding stages. We expect to find differences in the levels of lipoproteins and plasma FA composition as an adaptive physiological mechanism during stages of fluctuating energy demands.

Materials and methods

Sampling site and sample collection

The study was conducted at Potter Peninsula in Isla 25 de Mayo/ King George Island, South Shetland Islands, Antarctica (62°15′0″S, 58°40′0″W) from November to February 2012–2013 and 2013–2014, when Brown Skuas (*S. antarcticus lonnbergi*) are breeding. Three reproductive stages were defined: egg incubation (In), early rearing (Er) and late rearing (Lr) (Fig. 1) (Graña Grilli et al. 2018; Ibañez et al. 2018). For In, sampling was done during incubation of eggs. For Er, the capture was performed about 10–13 days after the chick was found. The Lr stage started when the chicks were completely feathered but still not flying (about 40 days old). At each stage, both adults from 6 nests ($n = 12$ individuals) were captured, weighed and a blood sample was obtained, as previously described (Graña Grilli et al. 2018). Blood samples were collected by venipuncture of the brachial vein. Plasma was obtained using heparin and then by centrifugation during 10 min at $400 \times g$. For serum obtaining blood was incubated for 3 h at 4 °C, and then centrifuged 10 min at $400 \times g$. Plasma and serum were kept at -20 °C until determinations were performed in the laboratory. As plasma volume was not enough in some cases, it was not possible to asses the complete set of parameters for each individual, and samples needed to be pooled. The birds were sexed by molecular techniques (Griffiths et al. 1998).

Lipoprotein analysis

Very Low Density (VLDL), Low Density (LDL) and High Density (HDL) Lipoproteins were determined as previously described (Noble 1968). Briefly, serum lipoproteins (females ($n = 6$) and males ($n = 6$)) were separated by 2% agarose gel electrophoresis using veronal-sodic veronal pH 8.6 solution as running buffer. The electrophoresis was performed at

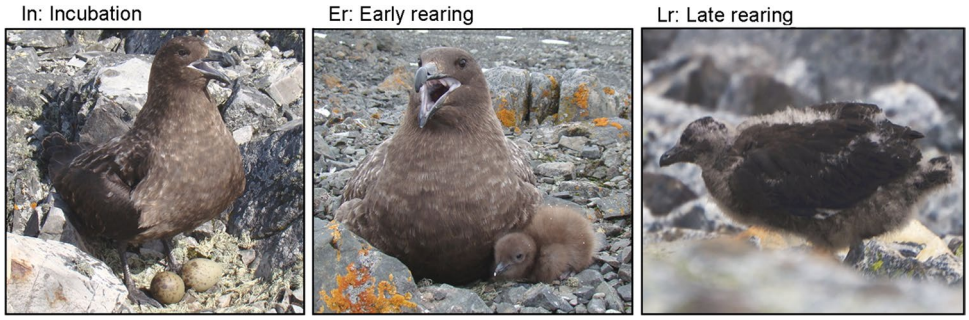

Fig. 1 Breeding stages of the Brown Skua (*Stercorarius antarcticus lonnbergi*). Three moments were defined during the breeding cycle for adult sampling. The In (incubation) stage was considered during egg incubation. Then, the Er (Early rearing) stage was after egg hatching, and, finally, the Lr (Late rearing) when the chicks were completely feathered

3 mA for 20 min. The gel then was fixed for 2 h with fixing solution -ethanol: methanol: isopropanol: water (45:1:1:20)-. After that, gel was dehydrated in a stove at 70 °C for 30 min, and finally gel was stained with Sudan Black Colouring Solution for 2 h. Relative abundance of each lipoprotein was calculated as the area percent of the peak for each protein band with respect to the total area, using the ImageJ software (Schneider et al. 2012).

Circulating lipid characterization

For plasma lipid characterization and FA composition analysis, samples from different individuals ($n_{stage} = 12$) were pooled in four groups each containing three plasma samples. This was repeated for each breeding stage. Plasma samples were pooled because plasma volume was too small to extract a sufficient amount of lipids to finally analyse lipid classes and FA at individual level. Total lipids were extracted from plasma twice with methanol/chloroform (2:1, v/v) following the method of Bligh and Dyer (1959), and total lipid concentrations were determined gravimetrically. Lipid class analysis of plasma was performed by thin layer chromatography (TLC) on silica gel. The separation was conducted with a sequence of two different solvent systems in the same TLC plate (Merck, Darmstadt, Germany). The first development was carried out for 45 min in chloroform: methanol: acetic acid: water (65:25:4:4 v/v/v/v) for polar lipids and then developed in hexane: diethyl ether: acetic acid (80:20:1 v/v/v) for neutral lipids. The standard lipid mix contained: cholesteryl ester (Sigma); triacylglycerol (Triolein, Sigma); free fatty acid (Oleic acid, Sigma); cholesterol (Avanti Polar Lipids); phosphatidyl ethanolamine (Biochemika-Fluka); phosphatidyl choline (Sigma) and sphingomyelin (Sigma) were run in parallel to identify lipid classes. Lipids were revealed with 10% cupric sulphate in 8% orthophosphoric acid (Touchstone et al. 1983) and quantified by densitometry using the ImageJ software (Schneider et al. 2012).

Fatty acids analysis

Fatty acid methyl esters (FAME) from total lipid were prepared with BF3–MeOH according to the method of Morrison and Smith (1992). FAME were analysed by gas–liquid chromatography (GC) in a HP6890 capillary GC (Hewlett Packard, Palo Alto, CA, USA), fitted with an Omegawax 250 fused silica column, 30 m 0.25 mm, with 0.25 lm phase (Supelco, Bellefonte, CA, USA) equipped with a flame ionization detector (FID). The column temperature was programmed for a linear increase of 3 $°C_{min}$ from 175 to 230 °C. Fatty acids were identified by comparing their characteristic retention times with those from a mixture of standard methyl esters run under the same conditions (Heras et al. 2000). Abundance (%) of FA was calculated as the area for each identified peak with respect to the total area.

Statistical analysis

Statistical analysis and plots were performed using R 3.2.1 (R Development Core Team 2015) and GraphPad Software, Inc. (2007). Normality and homogeneity of variance were tested using Kolmogorov- Smirnov and Levene tests. Analysis of lipoprotein abundance in the different breeding stages was conducted using Generalized Linear Mixed models (Zuur et al. 2009) with the package lme4 (function glmer; Bates et al. 2015). Response variables ("VLDL, LDL and HDL abundances") were normalized using log transformation, and the value of the total area of the densitograms for each sample was included in the analysis to account for variation in the total area between samples. The fixed variables were "Sex" and "Breeding stage (In, Er, and Lr)", and the random variable was "Individual". Spearman Correlation test was used to assess possible associations between body mass and lipoproteins level, and as well between lipoprotein classes. One Way ANOVA test (Post Hoc Bonferroni) was used to evaluate possible variations in the lipid classes and in the different FA species during the breeding cycle. For each

result, the statistical test, F_{df} (df: degree of freedom) and p value are shown in brackets, and for correlations Spearman's r and p values are shown. Results with a p value less than 0.05 were considered significantly different.

Results

Serum Lipoproteins

Three lipoprotein classes were determined in serum of Brown Skuas (*S. antarcticus lonnbergi*): Very Low Density (VLDL), Low Density (LDL), and High Density lipoproteins (HDL) (Fig. 2A). The effects of the fixed variables (Sex and Breeding stage) on the response variables are summarized in Table 1, and the effect of the random variable (individual) in Table 2. For VLDL, the sex and breeding stage showed a significant interaction ($F=4.39$, $p=0.033$) (Table 1). On the other hand, only LDL lipoproteins were affected by the breeding stage ($F=4.12$, $p=0.028$) (Table 1). In female skuas, VLDL was the most abundant lipoprotein during incubation (In) stage but represented only 1_3 of the total lipoproteins in later breeding stages (Fig. 2B). LDL abundance peaked during the intensive parental care stage (Er), whereas in the late rearing stage (Lr) HDL was the most abundant lipid carrier in blood (Fig. 2b). On the other hand, lipoproteins in male showed a different pattern where VLDL abundance slightly increased during the Er and Lr stages, whereas LDL was higher in the In stage. Similar to female, there was an increasing trend in HDL abundance through the breeding stages reaching the highest level during Lr stage (Fig. 2c).

Considering the differences addressed in the levels of lipoproteins through the breeding cycle, we then evaluated associations between body mass and the abundance of each lipoprotein class. For both sexes no correlation in VLDL, LDL and HDL levels with body mass was observed. To understand the dynamics of lipid transport by these lipoproteins during the breeding cycle, the correlation between their abundances was analysed (Fig. 3). In males, LDL and HDL were negatively correlated ($r=-0.706$ $p=0.001$) (Fig. 3a), whereas in females this pattern was observed for VLDL and HDL ($r=-0.581$ $p=0.029$) (Fig. 3b). No correlation was observed between LDL and VLDL classes in both sexes.

Plasma lipid content and classes

Total lipid mass in plasma did not show differences between stages. During In, lipid mass was 4.16 ± 0.47 mg; and during Er and Lr stages, 4.15 ± 0.27 mg and 4.28 ± 0.29 mg, respectively. Different lipid classes were determined in the plasma of Brown Skua (*S. antarcticus lonnbergi*): cholesteryl esters, TAG, FA, cholesterol, phosphatidyl ethanolamine, phosphatidyl choline and sphingomyelin (Fig. 4a). Most lipid classes remained invariant during the different breeding stages except the circulating FA that significantly decreased between In and Lr stages (ANOVA, $F_7=5.913$, $p=0.046$) (Fig. 4b).

Plasma fatty acid characterization

The plasma FA composition of Brown Skuas (*S. antarcticus lonnbergi*) contained 27 major FA. Among saturated fatty acids (SFA), palmitic (16:0) and stearic (18:0) acids were the most abundant ones, followed by a long-chain FA, the behenic acid (22:0). No differences in the abundance of these FA were observed during the three breeding stages. MUFA were dominated by oleic (18:1n-9), followed by palmitoleic (16:1n-9) and nervonic (24:1n-9) acids. While no differences were detected in the abundance of palmitoleic acid during breeding, a significant decline in oleic acid (ANOVA, $F_7=7.269$, $p=0.047$) occurred towards the Lr stage. Conversely, a significant increase of nervonic acid abundance was observed during the breeding cycle (ANOVA, $F_7=6.973$, $p=0.048$) (Fig. 5). Among PUFA,

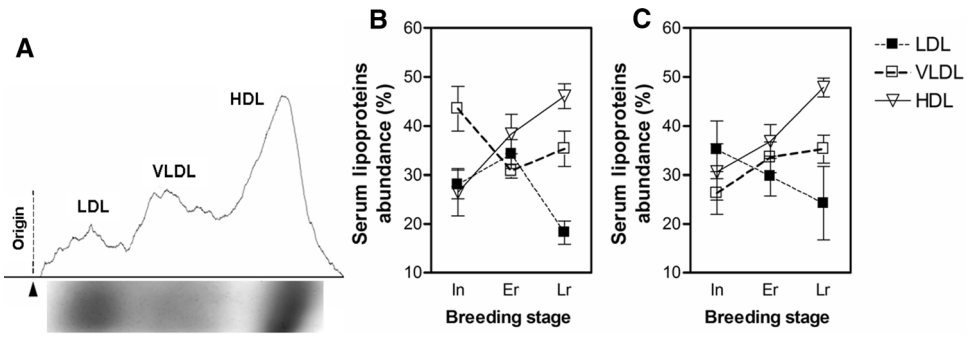

Fig. 2 Serum lipoproteins in breeding Brown Skua (*S. antarcticus lonnbergi*). **a** Agarose gel and densitogram of separated serum lipoprotein classes: LDL, VLDL, and HDL. Abundance of serum lipoproteins in **b** female ($n=6$) and (C) male ($n=6$) Brown Skuas during the different breeding stages (In, Er and Lr). Data were represented as Mean ± SEM of the abundance percentage of each lipoprotein class

Table 1 GLMM modelling to assess variation of serum lipoproteins during Brown Skua (*S. antarcticus lonnbergi*) reproduction

Fixed variables	HDL						LDL						VLDL					
	SumSq	MeanSq	NumDf	DenDF	Fvalue	*p*	SumSq	MeanSq	NumDf	DenDF	Fvalue	*p*	SumSq	MeanSq	NumDf	DenDF	Fvalue	*p*
Sex	0.004	0.004	1	24.999	0.025	0.875	0.038	0.038	1	24.999	0.252	0.619	0.119	0.119	1	6.397	2.061	0.198
Stage	0.727	0.363	2	24.999	2.212	0.130	1.270	0.635	2	24.999	4.116	0.028*	0.042	0.021	2	13.360	0.369	0.698
Stage: Sex	0.307	0.153	2	24.999	0.934	0.406	0.407	0.203	2	24.999	1.319	0.285	0.511	0.255	2	13.916	4.397	0.033*
Area	7.381	7.381	1	24.999	44.879	5.082	11.433	11.433	1	24.999	74.071	$6.044 \times 10–9$	6.977	6.977	1	21.510	120.020	$2.905 \times 10–10$

Breeding stage and sex were considered as fixed variables. The Response variables were the lipoproteins (VLDL, LDL, and HDL) abundances. (*$p < 0.05$)

linoleic (18:2n-6) and linolenic (20:3n-3) acids showed the highest abundance. Only linoleic acid showed an increase, but not significant, during the breeding cycle, reaching higher values during the Lr stage. Abundance analysis of each FA class showed a significant increase in PUFA across the breeding cycle (ANOVA, $F_7 = 6.573$, $p = 0.002$) and Lr stages, whereas no differences were observed in SFA and MUFA (Fig. 6a). Moreover, regarding PUFA abundances through the breeding stages, there was a decrease in the n-3:n-6 ratio from 2.0 in In stage to 0.79 in Lr. Altogether, these results indicate a prevalence of the n-3 family during the initial stages of the reproduction, and an increase in n-6 family nearby the end of the rearing activities (Fig. 6b).

Discussion

Serum lipoproteins during reproduction

Sex-associated differences in lipoproteins abundance across the breeding cycle were observed. These differences may be associated with changes in the body condition and the metabolic status of skuas during each stage. Furthermore, the corresponding division of labour in the parental activities during reproduction may be another factor for these variations (Furness 1987).

Female skuas experienced a loss of body mass with a slight recovery towards the last stage, whereas males experienced a slighter and less significant loss of body mass (Graña Grilli et al. 2018). In this context, the abundance of VLDL in female was higher during the In stage compared to the subsequent stages (Er and Lr) when parental activities increase. The higher values of VLDL may be related in principle to the breeding status (Peebles et al. 2004; Williams and Buck 2010). During the pre-laying stage and egg formation, female seabirds increase the transport of lipids from the liver to oocytes by VLDL (Davis 1997). On the other hand, a better nutritional condition at the beginning of the breeding cycle or an increase in the transport of fats from stores to fuel high-energy expenditure processes as the egg incubation may induce an increase of VLDL abundance (Jenni-Eiermann and Jenni 1992). Afterwards, the decrease of VLDL levels may be associated with the decline of the nutritional status and body condition (Graña Grilli et al. 2018; Ibañez et al. 2018). Stored lipids and proteins may be used as fuel when insufficient energy incomes from diet. Once fat stores have been exhausted, protein utilization increases. Previous works revealed that when lipid stores are low, Brown Skuas shift towards protein catabolism to meet their energy requirements (Graña Grilli et al. 2018) which has been demonstrated in other studies as well (Ibañez et al. 2018). The induction of protein catabolism could indicate that female skuas were in

Table 2 Effects of the random variable (Individual) on serum lipoprotein abundances in breeding Brown Skuas (*S. antarcticus lonnbergi*)

		HDL		LDL		VLDL	
		Variance	StdD	Variance	StdD	Variance	StdD
Random variable	ID	< 0.01	< 0.01	< 0.01	< 0.01	0.0131	0.1146
	Residual	0.1645	0.1645	0.1544	0.3929	0.0581	0.2411

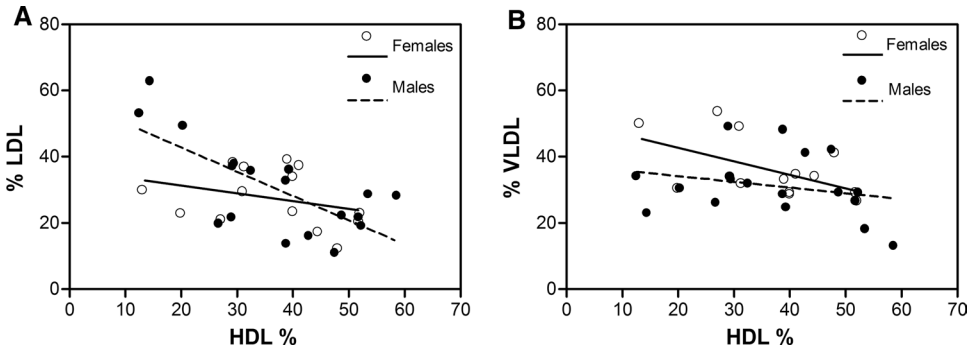

Fig. 3 Dynamics of lipoprotein classes in breeding Brown Skuas (*S. antarcticus lonnbergi*). Correlation between **a** LDL and **b** VLDL *vs.* HDL abundances (%) in female and male skuas

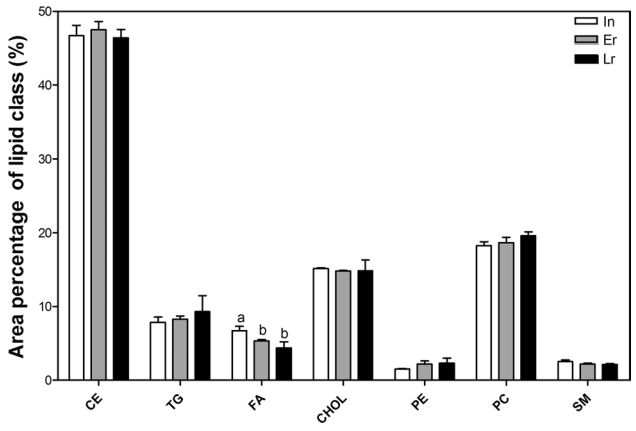

Fig. 4 Plasma lipid classes found in Brown Skua (*S. antarcticus lonnbergi*) during the breeding stages. **a** Lipid classes determination by thin layer chromatography. Lane 1: plasma lipids extracted from skuas during In stage, Lane 2: Er stage, Lane 3: Lr stage, Lanes 4 and 5: standard lipid mix. **b** Area percentage of each lipid class (%) estimated through densitometry analysis of the TLC (*CE* cholesteryl esters, *TAG* triacylglycerols, *FFA* free fatty acids, *CHOL* cholesterol, *PE* phosphatidylethanolamine, *PC* phosphatidylcholine, *SM* sphingomyelin). Data was presented as Mean ± SEM of the area percentage of each lipid class from the total area corresponding to each group of pooled samples (n_{stage} = 4 pools each composed by 3 plasma samples). Statistical results correspond to the *p* values calculated by ANOVA test Post Hoc Bonferroni. Statistically significant differences are indicated as *a* and *b* between stages ($p < 0.05$)

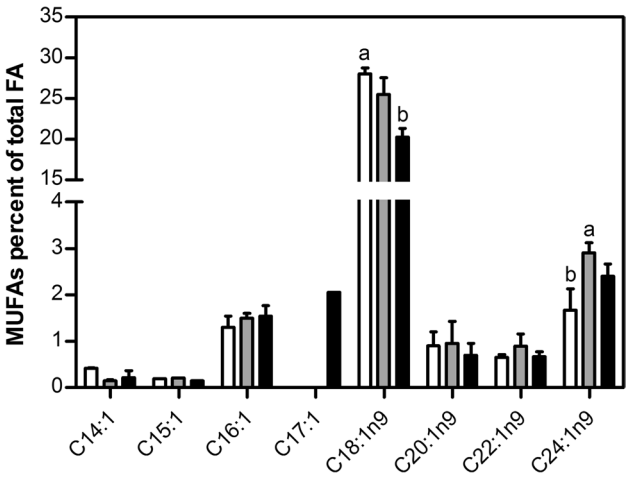

Fig. 5 Plasma fatty acid composition of Brown Skuas (*S. antarcticus lonnbergi*) during the different stages of the breeding cycle (In, Er and Lr) determined by gas chromatography. Results are shown for monounsaturated fatty acids (MUFA) measured in pooled samples (n_{stage} = 4 pools each composed by 3 plasma samples). Data were expressed as the Mean ± SEM percent (%) of each FA species from total FA in plasma. Statistical results correspond to the *p* values calculated by ANOVA test Post Hoc Bonferroni. Statistically significant differences are indicated as *a* and *b* between stages ($p < 0.05$)

poor condition at the onset of the breeding cycle (Graña Grilli et al. 2018; Ibañez et al. 2018) and that, in this context, this is a preferred energy source. In agreement with this, the increased fat catabolism and the reduced feeding opportunities (Graña Grilli et al. 2018) possibly induced a decline in VLDL and an increase in the abundance of LDL during the Er stage.

Conversely, during the In stage male skuas showed higher levels of LDL than VLDL, whereas the abundance of VLDL increased during Er and Lr stages. This suggests that, prior and during the In stage, males showed a deprived body condition; however, in the following stages a slight recovery occured. This is consistent with their feeding behaviour and the division of labour during reproduction (Furness 1987). During the entire breeding period, males undertake a greater share of foraging to ensure females an adequate nutritional condition, whereas females spend more time defending

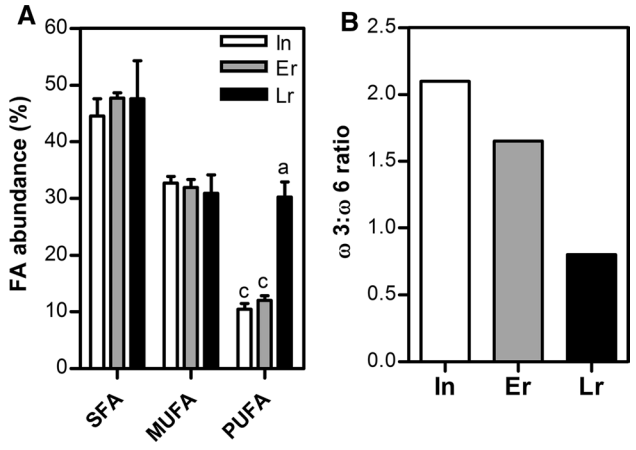

Fig. 6 **a** Plasma FA abundance in the different breeding stages of Brown Skua (*S. antarcticus lonnbergi*). Data represent the Mean ± SEM of the total amount (as the sum of the percent of each FA species) of SFA, MUFA, and PUFA during the different breeding stages (In, Er and Lr). Statistical results correspond to the *p* values calculated by ANOVA test (Post Hoc Bonferroni). Statistically significant differences are indicated as *a* and *c* between stages ($p < 0.01$). **b** PUFA n-3:n-6 ratio during the different breeding stages. Data represent the ratio of the total amount of each FA species for each stage

the territory and nestlings against intruders (Burton 1968; Devillers 1978; Furness 1987). Thus, because of the effort performed, male skuas may reduce their feeding opportunities and consequently their body condition, during the initial breeding stages (In and Er) (Weimerskirch 1990; Tveraa et al. 1998). The slight recovery of VLDL after In stage may be related to an optimization of the foraging strategy allocating resources for not only nest provisioning, but also self-maintenance. Previous reports on other seabird species revealed that the efficiency of the foraging activities probably allows lowering their effort and maintaining stable their body condition (Lormeé et al. 2003). However, such hypotheses need to be tested further using precise metabolic measurements of the male foraging effort and studying the feeding strategy.

Plasma lipids and FA composition

In Brown Skuas plasma circulating total lipids remained invariant during the entire breeding cycle. The levels of TAG were maintained constant indicating that fat reserves were not completely depleted. In addition, FA decreased suggesting that skuas rely on both dietary and/or stored lipids as energy source. However, as it was suggested above, Brown Skuas may already be in an energetic deficit at the initiation of reproduction, and thus, the energy requirements would be compensated by protein catabolism (Graña Grilli et al. 2018; Ibañez et al. 2018). Similar observations were described during the breeding cycle of arctic seabirds where depending on the season they might switch to consume body proteins as a primary energy source after depleting their lipid reserves (Hollmén et al. 2000).

Seasonal variations in FA composition in birds have often been ascribed to preferential mobilization of FA from adipose tissue, seasonal shifts in diet, foraging behaviour and prey preference (Pierce and Williams 2005). In particular, blood FA provide information about dietary lipids (Käkelä et al. 2009) and those mobilized from adipose tissue (Käkelä et al. 2005; Baylin and Campos 2006); thus its composition depends on the metabolic and physiological status (Cooper et al. 2005).

Avian fat stores are dominated by palmitic (16:0), stearic (18:0), palmitoleic (18:1) and linoleic (18:2) acids which comprise about 50–90% of the body fats (Guglielmo et al. 2002a, b; McWilliams et al. 2004; Price et al. 2008). These FA are the principal products of the endogenous FA synthesis in vertebrates. In Brown Skuas it was found that the most abundant circulating FA correspond to these species; however, differences were observed during the breeding cycle. Among SFA palmitic (16:0) and stearic (18:0) acids were the major species. During the Er stage the abundance of palmitic acid slightly increased, while a declining trend in stearic abundance was observed. This may be related to fluctuations and preferential mobilization of FA from stores according to the energy demands. In agreement with this, studies on migratory birds stated that these FA increased in blood as a consequence of the increased mobilization from adipose tissue for fuel (Price et al. 2008). Behenic acid (22:0) showed an increasing trend through the different stages. It was reported that the mobilization of FA from stores increased with the degree of unsaturation and decreased with carbon-chain length, particularly for lengths ≥ 18 (Raclot 2003; Price et al. 2008). Thus, the ultimate mobilization of long-chain SFA may trigger a mechanism to supply fuel during the brooding stages, because of the low availability of short-chain SFA in lipid stores.

Palmitoleic (16:1n-9) and oleic (18:1n-9) acids are the most abundant MUFA in avian adipose tissue and plasma, and are immediate desaturation products of the palmitic and stearic acids, respectively (Pierce and Williams 2005). In Brown Skuas, palmitoleic, oleic and nervonic acids were the major species. Moreover, nervonic acid (24:1n-9) is a product of the oleic acid desaturation. The decline of oleic acid abundance and the increase of nervonic acid may be the result of the endogenous elongation of oleic acid and/or mobilization of nervonic acid from stores to fuel the initial breeding stages (In and Er). Another explanation may be a variation in the food resources selected. Some migratory birds are able to switch their diet to supply the different energy demands through their life cycle, inducing changes in the FA composition of their depots as well as in the energy obtaining rate (Pierce et al. 2005). At this breeding location, skuas nest nearby a breeding colony of pygoscelis penguins

(Aguirre 1995). During the early breeding stages, (In and Er) males supply the nest mainly with penguin eggs (Graña Grilli et al. 2014; Graña Grilli and Montalti 2015) which are rich in oleic acid (Polito et al. 2012). Also, skuas feed on marine resources to supplement terrestrial prey (Graña Grilli et al. 2014; Graña Grilli and Montalti 2015; Borghello et al. 2018). Among marine prey, the fishes *Pleuragramma antarcticum* and *Electrona antarctica* rich in nervonic acid (Reinhardt and Van Vleet 1986) are the most common species consumed (Borghello et al. 2018). Thus, with the advance of the breeding season the decrease of oleic abundance and the increase of nervonic may reflect the use of different food resources in response to a variation in the availability.

Polyunsaturated FA are necessary for the body's metabolism, growth and development (Wathes et al. 2007; Feng et al. 2015). Several reports suggest that the ratio of essential n-3 and n-6 PUFA often depends on the diet and influences the energetic and reproductive performance in birds (Price and Guglielmo 2009; Twining et al. 2016). We observed an increasing trend in the percentage of PUFA across the breeding stages, reaching a maximum by the Lr. Interestingly, linoleic acid (18:2n-6) is a biomarker of marine diet, abundant in pelagic phytoplankton, and is transferred in the food web to fishes (Käkelä et al. 2005). This observation, together with the nervonic acid, possibly may be the result of an increase in the utilization of marine resources in order to optimize the provision of energy during breeding. Moreover, a decrease in n-3:n-6 ratio towards the Lr stage was observed, indicating a greater prevalence of n-3 at the initial stages, whereas n-6 increased towards the Lr stage. Thus, n-3 and n-6 FA played important roles in different moments of the reproduction, which could be associated with the use of particular dietary or endogenous FA to supply the fluctuating energy needs.

In this work we have described the dynamics of blood circulating lipoproteins and the plasma lipids and FA composition in a seabird during the reproduction. The low samples size of this study ($n = 6$ nests) is because of the low number of successful nests since the incubation stage to the fledgling stage. As was reported by Graña Grilli (2014) a decreasing trend of Brown Skua population at this location. In particular, during the seasons studied a high occurrence of nesting failure occurred, therefore, the number of birds that could be sampled at each stage decreased severely. During the season 2012–2013, only three of them got to fledge chicks, whereas during the season 2013–2014 only two nests fledged chicks (Graña Grilli 2014 and Graña Grilli et al. 2018). No association between the parameters measured with body condition was observed; however, the differences addressed were closely related to the endogenous modulation of lipid metabolism to cope with the energy demands of reproduction. Furthermore, the changing seasonal availability of resources across the breeding cycle may also influence the plasma FA composition in skuas. In this context, our results are not representative at individual level; nevertheless, relevant information of trophic ecology at population level was addressed. Altogether, the results presented in our study provide important information about the dynamics of physiological components related to lipid metabolism during a process of fluctuating energy demands. We believe that FA analysis on other tissues, stable isotopes and the use of space by GPS tracking should be conducted in future studies for a broader understanding of the temporal variability and dynamics of the lipid metabolism, as well as, the foraging behaviour of these predators.

Acknowledgements This work was made possible thanks to National Office of the Antarctic (DNA) and Argentine Antarctic Institute (IAA) which provided logistical support to carry out the fieldwork at Potter Peninsula, Isla 25 de Mayo/ King George Island, South Shetland Islands, Antarctica. This work was supported by Proyecto de Investigación Plurianual (PIP- CONICET n° 0158) and Agencia Nacional de Promoción Científica y Tecnológica and Instituto Antártico Argentino (PICTA-2010-0080) (To DM), and partially supported by (PICT-2014-3323) (to AEI). Special thanks to Facundo Xavier Palacio for the help with statistical analysis and to Rosana del Cid for the help with manuscript writing and grammatical edition, as well as to the Editor and reviewers for their interesting and helpful critics for the improvement of the manuscript.

Author contributions AEI was involved in the design of the fieldwork activities, performed experiments and prepared the manuscript, PMY and HH were involved in experimental design of with lipid analysis and provided reactives, FMN and BG were involved in lipoprotein analysis, DM and MGG were involved in the design and founding of project, conducted the fieldwork activities and collaborated with the edition of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The permit to carry out the fieldwork for this work was given by National Office of the Antarctic (DNA). All applicable international, national, and institutional guidelines for sampling, care, and experimental use of animals for the study were followed as established by the Article III, Annex II of the Madrid Protocol, Law 24.216 (Taking, Harmful Intrusion and Introduction of Species) within the framework of the projects evaluated by the Environment Office of the Argentine Antarctic Institute (IAA) and DNA.

References

Aguirre CA (1995) Distribution and abundance of birds at Potter Peninsula, 25 de Mayo (King George) Island, South Shetland Islands, Antarctica. Marine Ornithol 23:23–31

Bates D, Maechler M, Bolker B, Walker S (2015) *lme4*: Linear mixed effects models using Eigen and S4. R package version 1.1.8. http://CRAN.Rproject.org/package=lme4.

Baylin A, Campos H (2006) The use of fatty acid biomarkers to reflect dietary intake. Curr Opin Lipidol 17(1):22–27
Bentzen RL, Powell AN, Williams TD, Kitaysky AS (2008) Characterizing the nutritional strategy of incubating king eiders *Somateria spectabilis* in northern Alaska. J Avian Biol 39:683–690
Blem CR (1990) Avian energy storage Curr Ornithol 7:59–113
Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37(8):911–917
Borghello P, Torres DS, Montalti D, Ibañez AE (2018) Diet of the Brown Skua (*Stercorarius antarcticus*) at Hope Bay, Antarctic Peninsula: differences between breeders and non-breeders. Polar Biol 42:385–394
Bourgeon S, Le Maho Y, Raclot T (2009) Proximate and ultimate mechanisms underlying immunosuppression during the incubation fast in female eiders: Roles of triiodothyronine and corticosterone. Gen Comp Endocrinol 163(1–2):77–82
Bourgeon S, Kauffmann M, Geiger S, Raclot T, Robin JP (2010) Relationships between metabolic status, corticosterone secretion and maintenance of innate and adaptive humoral immunities in fasted re-fed mallards. J Exp Biol 213(Pt 22):3810–3818
Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mamm Sci 22(4):759–801
Burton RW (1968) Breeding Biology of the Brown Skua *Catharacta skua lonnbergi* (Mathews), at Signy Island, Soth Orkney Islands. Br Antarct Surv Bull 15:9–28
Carrascal LM, Senar JC, Mozetich I, Uribe F, Domenech J (1998) Interactions among environmental stress, body condition, nutritional status, and dominance in great tits. Auk 115:727–738
Cerasale DJ, Guglielmo CG (2006) Dietary effects on prediction of body mass changes in birds by plasma metabolites. Auk 123(3):836–846
Conway CJ, Eddleman WR, Simpson KL (1994) Seasonal changes in fatty acid composition of the Wood Thrush. Condor 96(3):791–794
Cooper MH, Iverson SJ, Heras H (2005) Dynamics of blood chylomicron fatty acids in a marine carnivore: implications for lipid metabolism and quantitative estimation of predator diets. J Comp Physiol B 175(2):133–145
Davis RA (1997) Evolution of processes and regulators of lipoprotein synthesis: From Birds to Mammals. J Nutr 127:795S-800S
Devillers P (1978) Distribution and relationships of South American Skuas. Gerfaut 68:374–417
Feng Y, Ding Y, Liu J, Tian Y, Yang Y, Guan S, Zhang C (2015) Effects of dietary omega-3/omega-6 fatty acid ratios on reproduction in the young breeder rooster. BMC Vet Res 11:73
Furness RW (1987) The skuas. A.D. Poyser, Calton
Graña Grilli M, Montalti D (2012) Trophic interactions between Brown and South Polar Skuas at Deception Island. Antarctica Polar Biol 35(2):299–304
Graña Grilli M, Marti LJ, Montalti D (2014) Uniformity of diet composition of Brown Skuas at different ages and between siblings. Rev Bras Ornitol 22:265–269
Graña Grilli M, Montalti D (2015) Variation in diet composition during the breeding cycle of an Antarctic seabird in relation to its breeding chronology and that of its main food resource. Polar Biol 38(5):643–649
Graña Grilli M, Pari M, Ibañez AE (2018) Poor body conditions during the breeding period of a low breeding success seabird population. Marine Biol 165:142
Griffiths R, Double MC, Orr K, Dawson RJ (1998) A DNA test to sex most birds. Mol Ecol 7(8):1071–1075
Groscolas R, Robin JP (2001) Long-term fasting and re-feeding in penguins. Comp Biochem Physiol A Mol Integr Physiol 128:645–655
Guglielmo CG, Haunerland NH, Hochachka PW, Williams TD (2002a) Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a long-distance migrant shorebird. Am J Physiol 282(5):1405-R1413
Guglielmo CG, Williams TD, Zwingelstein G, Brichon G, Weber JM (2002b) Plasma and muscle phospholipids are involved in the metabolic response to long-distance migration in a shorebird. J Comp Physiol B 172(5):409–417
Hanssen SA, Hasselquist D, Folstad I, Erikstad KE (2005) Cost of reproduction in a long-lived bird: incubation effort reduces immune function and future reproduction. Proc R Soc Lond B 272(1657):1039–1046
Heras H, Gonzalez-Baró M, Pollero RJ (2000) Lipid and fatty acid composition and energy partitioning during embryo development in the shrimp *Macrobrachium borellii*. Lipids 35(6):645–651
Hermier D, Chapman MT, Leaclercq B (1984) Plasma lipoprotein profile in fasted and refed chickens of two strains selected for high or low adiposity. J Nutr 114(6):1112–1121
Hermier D (1997) Lipoprotein metabolism and fattening in poultry. J Nutr 127:805S-808S
Hollmén T, Franson JC, Hario M, Sankari S, Kilpi M, Lindstrom K (2000) Use of serum biochemistry to evaluate nutritional status and health of incubating common eiders (*Somateria mollissima*) in Finland. Physiol Biochem Zool 74:333–342
Ibañez A, Graña Grilli M, Figueroa A, Pari M, Montalti D (2018) Declining health status of Brown Skua (*Stercorarius antarcticus lonnbergi*) parents and their offspring during chick development. Polar Biol 41(1):193–200
Iverson SJ, Field C, Bowen WD, Blanchard W (2004) Quantitative fatty acid signature analysis: A new method of estimating predator diets. Ecol Monogr 74(2):211–235
Jenni-Eiermann S, Jenni L (1992) High plasma triglyceride levels in small birds during migratory flight: a new pathway for fuel supply during endurance locomotion at very high mass-specific metabolic rates? Physiol Zool 65(1):112–123
Jenni-Eiermann S, Jenni L (1998) What can plasma metabolites tell us about the metabolism, physiological state and condition of individual birds? An overview. Biol Cons Fauna 102:312–319
Käkelä R, Käkelä A, Kahle S, Becker PH, Kelly A, Furness RW (2005) Fatty acid signatures in plasma of captive herring gulls as indicators of demersal or pelagic fish diet. Mar Ecol Prog Ser 293:191–200
Käkelä A, Crane J, Votier SC, Furness RW, Käkelä R (2006) Fatty acid signatures as indicators of diet in great skuas *Stercorarius skua*, Shetland. Mar Ecol Prog Ser 319:297–310
Käkelä R, Furness RW, Kahle S, Becker PH, Käkelä A (2009) Fatty acid signatures in seabird plasma are a complex function of diet composition: a captive feeding trial with herring gulls. Funct Ecol 23(1):141–149
Klasing KC (1998) Comparative Avian Nutrition. CAB International, New York
Krietsch J, Hahn S, Kopp M, Phillips RA, Peter HU, Lisovski S (2017) Consistent variation in individual migration strategies of Brown Skuas. Mar Ecol Prog Ser 578:213–225
Lormée H, Jouventin P, Trouve C, Chastel O (2003) Sex-specific patterns in baseline corticosterone and body condition changes in breeding Red-footed Boobies *Sula sula*. Ibis 145:212–219
McWilliams SR, Guglielmo C, Pierce B, Klaassen M (2004) Flying, fasting, and feeding in birds during migration: a nutritional and physiological ecology perspective. J Avian Biol 35(5):377–393
Mitchell GW, Wheelwright NT, Guglielmo CG, Norris DR (2012) Short-and long-term costs of reproduction in a migratory songbird. Ibis 154(2):325–337
Morrison WR, Smith LM (1992) Preparation of fatty acid methyl esters and dimethylacetals from lipid with boron fluoride-methanol. J Lipid Res 5:600–608

Navidshad B, Deldar H, Pourrahimi G (2010) Correlation between serum lipoproteins and abdominal fat pad in broiler chickens. Afr J Biotechnol 9(35):5779–5783

Noble R (1968) Electrophoretic separation of plasma lipoproteins in agarose gel. J Lipid Res 9(6):693–700

Peebles ED, Burnham MR, Walzem RL, Branton SL, Gerard PD (2004) Effects of fasting on serum lipids and lipoprotein profiles in the egg-laying hen (*Gallus domesticus*). Comp Biochem Physiol A Mol Integr Physiol 138(3):305–311

Pierce BJ, McWilliams SR (2005) Seasonal changes in composition of lipid stores in migratory birds: causes and consequences. Condor 107(2):269–279

Pierce BJ, McWilliams SR, O´Connor T, Place AR, Guglielmo C, (2005) Effect of dietary fatty acid composition on depot fat and exercise performance in a migrating songbird, the Red-eyed Vireo. J Exp Biol 208(Pt 7):1277–1285

Polito MJ, Koopman HN, Able S, Walsh J, Goebel ME (2012) Physiological constraints and the influence of diet on fatty acids in the yolk of gentoo penguins. Pygoscelis papua J Comp Physiol B 182(5):703–713

Price ER, Krokfors A, Guglielmo GC (2008) Selective mobilization of fatty acids from adipose tissue in migratory birds. J Exp Biol 211(11):29–34

Price ER, Guglielmo CG (2009) The effect of muscle phospholipid fatty acid composition on exercise performance: a direct test in the migratory white-throated sparrow (*Zonotrichia albicollis)*. Am J Physiol 297:R775–R782

R Development Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http:// www.R-project.org/

Raclot T (2003) Selective mobilization of fatty acids from adipose tissue triglycerides. Prog Lipid Res 42(4):257–288

Ramenofsky M (1990) Fat storage and fat metabolism in relation to migration. In: Gwinner E (ed) Bird migration: physiology and ecophysiology. Springer, Berlin Heidelberg, pp 214–231

Reinhardt SB, Van Vleet ES (1986) Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. Marine Biol 91:149–159

Ritz M, Millar C, Miller G, Phillips R, Ryan P, Sternkopf V, Liebers-Helbig D, Peter HU (2008) Phylogeography of the southern Skua complex- rapid colonization of the southern hemisphere during a glacial period and reticulate evolution. Mol Phylogenet Evol 49(1):292–303

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9(7):671–675

Touchstone JC, Levin SS, Dobbins MF, Beers PC (1983) Analysis of saturated and unsaturated phospholipids in biological fluids. J Liq Chrom 6(1):179–192

Twining CW, Brenna JT, Lawrence P, Shipley JR, Tollefson TN, Winkler DW (2016) Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity. PNAS 113:39

Tveraa T, Saether BE, Aanes R, Erikstad KE (1998) Body mass and parental decisions in the Antarctic petrel *Thalassoica antarctica*: how long should the parents guard the chick? Behav Ecol Sociobiol 43(2):73–79

Votier SC, Bearhop S, Ratcliffe N, Furness RW (2001) Pellets as indicators of diet in Great Skuas *Catharacta skua*. Bird Study 48(3):373–376

Wathes DC, Abayasekara DR, Aitken RJ (2007) Polyunsaturated fatty acids in male and female reproduction. Biol Reprod 77(2):190–201

Weimerskirch H (1990) Weight loss of Antarctic Fulmars *Fulmarus glacialoides* during incubation and chick brooding. Ibis 132(1):68–77

Williams TD (2005) Mechanisms underlying the costs of egg production. Bioscience 55(1):39–48

Williams CT, Buck CL (2010) Using fatty acids as dietary tracers in seabird trophic ecology: theory, application and limitations. J Ornithol 151(3):531–543

Zuur A, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York, New York, USA

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.