

# Fatty acid signatures of stomach oil and adipose tissue of northern fulmars (*Fulmarus glacialis*) in Alaska: implications for diet analysis of Procellariiform birds

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**Abstract** Procellariiforms are unique among seabirds in storing dietary lipids in both adipose tissue and stomach oil. Thus, both lipid sources are potentially useful for trophic studies using fatty acid (FA) signatures. However, little is known about the relationship between FA signatures in stomach oil and adipose tissue of individuals or whether these signatures provide similar information about diet and physiology. We compared the FA composition of stomach oil and adipose tissue biopsies of individual northern fulmars ( $N = 101$ ) breeding at three major colonies in Alaska. Fatty acid signatures differed significantly between the two lipid sources, reflecting differences in dietary time scales, metabolic processing, or both. However, these signatures exhibited a relatively consistent relationship between individuals, such that the two lipid sources provided a similar ability to distinguish foraging differences among individuals and colonies. Our results, including the exclusive presence of dietary wax esters in stomach oil but not adipose tissue,

are consistent with the notion that stomach oil FA signatures represent lipids retained from prey consumed during recent foraging and reflect little metabolic processing, whereas adipose tissue FA signatures represent a longer-term integration of dietary intake. Our study illustrates the potential for elucidating short- versus longer-term diet information in Procellariiform birds using different lipid sources.

**Keywords** Fatty acid signature analysis · *Fulmarus glacialis* · Stomach oil · Alaska · Diet analysis

## Introduction

The composition of fatty acids (FA) in predator fat stores (e.g., adipose tissue, blubber), the predator's FA signature, can be used to qualitatively infer trophic levels, as well as spatial and temporal patterns in foraging behavior of free ranging seabirds and marine mammals (Iverson 1993; Iverson et al. 1997a, b; Kirsch et al. 2000; Brown et al. 1999; Raclot et al. 1998; Dahl et al. 2003). Given a comprehensive database of prey FA signatures and an accounting for predator FA metabolism, it is possible also to estimate the proportions of different prey types in the diet using quantitative fatty acid signature analysis (QFASA, Iverson et al. 2004, 2006, 2007). Both FA signature analysis and QFASA are based on the observations that FAs in the marine environment are complex and diverse and that prey species can often be characterized by their FA signatures (e.g., Budge et al. 2002; Iverson et al. 2002), that predators have a limited ability to biosynthesize FAs (Cook 1991), and that FAs of carbon chain length  $\geq 14$  in diet are incorporated into the body fat of predators with little change or in a predictable manner—thus FAs can be used as qualitative and quantitative tracers of prey consumption (Iverson 1993; Iverson et al. 2004; Budge

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et al. 2006). FAs consumed in amounts above immediate energy requirements are re-esterified primarily to triacylglycerols and deposited in adipose tissue (or blubber). Previous studies using FAs have focused primarily on sampling these reservoirs to gain qualitative and quantitative insight into trophic relations (e.g., Raclot et al. 1998; Dahl et al. 2003).

However, in some animals other depots of FAs exist, which may be of interest and use in diet studies. In particular, members of the order Procellariiformes are unique among seabirds because they produce FA-rich stomach oils in addition to storing FAs in adipose tissue (Lewis 1966; Warham 1977; Roby et al. 1993, 1997; Taylor et al. 1997). Procellariiform stomach oils are formed in the proventriculus of both adults and chicks by a combination of specialized gastric anatomy and physiology (Roby et al. 1989, 1992, 1993; Place et al. 1989), which rapidly empties aqueous dietary components from the proventriculus while retaining the neutral lipids (Roby et al. 1989). The chemical composition of stomach oil includes hydrocarbons, monoester waxes (wax esters), diacylglycerol ethers, triacylglycerols, diacylglycerols, monoacylglycerols, alcohols, cholesterol, and free FAs, as well as more polar lipids (Lewis 1966, 1969; Cheah and Hansen 1970a, b; Clarke and Prince 1976; Warham et al. 1976). Although quantitative work has not been done, close resemblances have been noted between the lipid composition of Procellariiform stomach oil and other oils found in marine systems (Lewis 1966; Cheah and Hansen 1970a, b), and further studies on the composition of stomach oil and variability between individuals confirmed that stomach oil originates from the diet (Lewis 1969; Cheah and Hansen 1970a; Clarke and Prince 1976; Imber 1976; Watts and Warham 1976; Warham 1977; Jacob 1982; Place et al. 1989). More recently, Connan et al. (2005, 2007) have used lipid classes in stomach oils of Procellariiformes to infer their diets.

The color of stomach oil has also been found to be correlated with diet (Warham et al. 1976), ranging from colorless, to shades of yellow, orange, red, amber, deep reddish-brown, and green. Colorless oils may represent lipids of meso- and bathypelagic fishes, red samples contain carotenoids and esterified astaxanthin pigments found in planktonic crustaceans or in squids that have consumed crustaceans, and green coloration may arise from the contribution of bile (Fisher 1952; Lewis 1969; Warham 1977).

Despite the influence of diet on Procellariiform stomach oil, and the relative ease and non-invasiveness of collecting it, little work has been done in comparing the composition of stomach oil and adipose tissue within individuals. Previous comparative studies relied on small sample sizes and limited quantitative analysis to distinguish FA signatures (Rosenheim and Webster 1927; Lovern 1938; Cheah and Hansen 1970a; Bishop et al. 1983; Horgan and Barrett 1985; Clarke 1989). Thus, it is unclear whether the two sources of lipids provide similar information about diets.

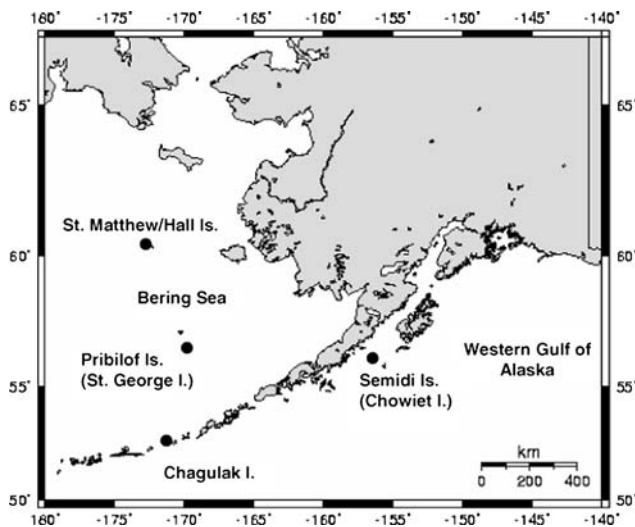
There remains limited understanding of whether stomach oil and adipose tissue represent similar time frames in the integration of dietary FA intake, whether stomach oil represents non-selective direct uptake of all dietary FA (i.e., the lipid composition of stomach oils may reflect not only the composition of recent meals but also the relative solubility of each class of lipids in the stomach oils already accumulated, Place et al. 1989), or whether greater modification and biosynthesis of FAs occur in adipose tissue compared to the proventriculus.

Northern fulmars (*Fulmarus glacialis*) are an abundant Procellariiform and generalist predator distributed throughout the North Pacific and North Atlantic. The population of fulmars in North America is estimated at 2.1 million individuals with 70% occurring in four distinct and accessible breeding colonies in Alaska (Hatch and Nettleship 1998). Thus, Alaskan fulmars are tractable subjects for investigating the relationship between stomach oil and adipose tissue. The objectives of this study were to (1) characterize the relationship between FAs in stomach oil and adipose tissue of individual northern fulmars, and (2) evaluate how well FAs discriminate between the two lipid sources. We expected that FA signatures from stomach oil and adipose tissue would differ because adipose tissue FAs are comprised of dietary FAs that have been influenced by metabolic processing and also include additional FAs biosynthesized by the birds, whereas stomach oils contain only dietary FAs that have not been processed metabolically, although they may have experienced selective uptake or release (Place et al. 1989). Furthermore, we did not expect to find a consistent pattern for all FAs in stomach oil versus adipose tissue within individual birds, i.e., the relative levels of different FAs in stomach oil and adipose tissue would vary independently due to variability in short- and long-term diets and integration times. Roby et al. (1989) suggested that the length of time for stomach oil to form in 4-week-old Antarctic giant petrel chicks (*Macroneptes giganteus*) is less than 12 h, whereas adipose tissue likely reflects longer-term diet integration (Iverson et al. 2007).

## Materials and methods

### Study sites and sample collection

Samples from adult fulmars and chicks were collected from three of the four major fulmar colonies in Alaska during the breeding season of 2004: Chowiet Island in the Semidi Islands group in the western Gulf of Alaska (56°05'N, 156°45'W), St George Island in the Pribilof Islands group in the eastern Bering Sea (56°35'N, 170°35'W), and Chagulak Island in the central Aleutian Archipelago (52°35'N, 171°10'W) (Fig. 1). Samples were not collected on the same dates at the three islands due to logistical constraints.



**Fig. 1** Locations of the four major northern fulmar colonies in Alaska. (1) Semidi Islands ( $56^{\circ}05'N$ ,  $156^{\circ}45'W$ ) in the western Gulf of Alaska; (2) Chagulak Island ( $52^{\circ}35'N$ ,  $171^{\circ}10'W$ ) in the eastern Aleutian Islands; (3) Pribilof Islands ( $56^{\circ}35'N$ ,  $170^{\circ}35'W$ ) in the eastern Bering Sea; and (4) St. Matthew and Hall Islands ( $60^{\circ}40'N$ ,  $173^{\circ}10'W$ ) in the central Bering Sea. Studies were not undertaken at the latter site

Adult birds were captured using a modified dip net, a noose-pole, or by hand from their nests. Adipose tissue and stomach oil samples were collected from adults on Chagulak I. in July ( $N=26$ ) and St George I. in June ( $N=25$ ) and August ( $N=20$ ). Adipose tissue and stomach oil samples were collected from chicks captured by hand at their nests on Chowiet I. during August ( $N=30$ ). To collect stomach oil, the bird's head was positioned in a Whirl-Pak<sup>®</sup> immediately upon capture to ensure minimal loss of sample when the bird willingly regurgitated during handling. Oil samples averaged 80 ml. Within several hours of being collected, the stomach oil was transferred to glass vials with teflon caps containing chloroform with 0.01% butylated hydroxytoluene (BHT) as antioxidant for storage. Upon returning from the field, all stomach oil samples were stored frozen until analyzed.

Synsacral adipose tissue samples were obtained from adults and chicks using a live biopsy method (Enderson and Berger 1968). The sampled area was disinfected with chlorhexidine and an incision approximately 0.5 cm in length was made just through the skin. A tissue sample of approximately 0.1 g was excised from the subcutaneous fat reservoir and placed in a vial containing chloroform with 0.01% BHT. The incision sites were closed with Vet-bond<sup>®</sup> to insure rapid healing, which minimized the chances of infection. Upon returning from the field, all adipose tissue samples were stored frozen until analyzed.

Morphometric measurements were taken from all individuals. Wing length and mass were used to estimate the ages of chicks using a logarithmic curve fitted to changes in length with age from a sample of known-age chicks (Hatch 1979). Chicks sampled on Chowiet I. in August were

estimated to be  $22 \pm 5.7$  (SD) days old on average. The age of chicks at fledging is unknown in Alaska, however, the mean age at fledging in Scotland is 53 days (Hatch and Nettleship 1998). On Chowiet I. in 2002, the first observed fulmar hatching occurred on July 16 and the first chick was observed to fledge on September 10 (Wang 2002).

#### Lipid extraction and analysis

Lipids were extracted using a modified Folch extraction (Folch et al. 1957; Iverson et al. 2001). FA methyl esters were prepared directly from 100 mg of the pure extracted lipid (filtered and dried over anhydrous sodium sulfate) using 3.0 ml Hilditch reagent (0.5 N H<sub>2</sub>SO<sub>4</sub> in methanol) and 1.5 ml methylene chloride with 0.01% BHT, capped under nitrogen, and heated at 100°C for 1 h. FA methyl esters were then extracted into hexane, concentrated, and brought up to volume (50 mg/ml) with high purity hexane. Identification and quantification of FA methyl esters were performed on samples using temperature-programmed gas liquid chromatography as described previously (Iverson et al. 1997b, 2004; Budge et al. 2002) on a Perkin-Elmer Autosystem II Capillary FID gas chromatograph fitted with a polar flexible fused silica column (30 m × 0.25 mm i.d.) containing 50% cyanopropyl polysiloxane (0.25 μ film thickness; J&W DB-23; Folsom, CA, USA) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson, San Jose, CA, USA). All sample chromatograms and FA identifications were individually checked, corrected, and reintegrated as necessary. FAs are expressed as mass percent of total FAs.

The dietary lipids of most animals, and especially carnivores, consist of triacylglycerols (three FAs esterified to a glycerol backbone) that are the primary storage form of lipids in animals. However, in the marine environment a number of fish and invertebrates store their lipids primarily as wax esters, which are comprised of a single FA esterified to a long-chain fatty alcohol (e.g., Benson et al. 1972). Many seabirds have the ability to digest and assimilate dietary wax esters (Roby et al. 1986). The presence of fatty alcohols resulting from the transesterification of wax esters in stomach oil and adipose tissue was determined using thin layer chromatography. In order to account for wax esters in diets, the alcohols of which are deposited as their corresponding FA in the adipose tissue (Budge and Iverson 2003), wax ester alcohols were converted to their respective FAs. The transesterification process converts the FAs in acyl lipids to FA methyl esters, but fatty alcohols and dimethylacetals are also generated if wax esters are present. Thus, we used thin layer chromatography and a modified Jones' reagent (13.5 g CrO<sub>3</sub>, 6.4 ml H<sub>2</sub>SO<sub>4</sub>, 43.6 ml distilled water) to oxidize the alcohols and dimethylacetals to free FAs, which were methylated and quantitatively recombined with FA methyl esters from the same sample according to Budge and Iverson (2003).

## Data analysis

Sixty-nine FAs were routinely identified and quantified in northern fulmar lipid samples. Because the number of parameters (FAs) must not exceed the group sample size (<20 in this study), FAs that were used in statistical analyses were chosen by the following criteria: (1) those that were primarily dietary in origin (e.g., Iverson et al. 2004), (2) those that exhibited the highest overall variances, and (3) those that had an overall mean of  $\geq 0.35\%$  of the total FAs. Seventeen FAs met these criteria. Despite the large variance of one of those FAs (22:5n-3), it was excluded from the analyses because it may be an intermediate between 20:5n-3 and 22:6n-3 (e.g., Ackman et al. 1988). All analyses were thus performed using 16 FAs, of which ten are considered to be exclusively dietary and six could arise from both diet and biosynthesis (Iverson et al. 2004). The 16 FAs accounted for 90.8% by mass of the total FAs (Table 1). Percentages of these FAs were first renormalized over 100% and their value was then divided by the value for one of the 16 FAs, 18:0, which was used as a reference (Aitchison 1986). The resulting 15 ratios were then log transformed and used in the statistical analyses.

Differences among and between adipose tissue and stomach oil samples were evaluated using a combination of univariate and multivariate techniques. A MANOVA was performed on the subset of 16 FAs to evaluate differences in FA signatures between the two lipid sources and among locations—adults sampled at Chagulak I. in July and at St George I. in June and August and chicks sampled on Chowiet I. in August. Following the MANOVA, a Tukey's multiple comparison with a Bonferroni adjustment was used to evaluate differences between individual FAs from stomach oil and adipose tissue in adults on Chagulak I. in July, St George I. in June and August, and chicks on Chowiet I. in August. Differences from the results of the Tukey's multiple comparison were considered significant at  $P < 0.0008$ , Bonferroni adjustment. Discriminant analyses were performed to reveal patterns of variation between stomach oil and adipose tissue FAs among the four sampling groups according to Budge et al. 2002 and Iverson et al. 2002. Wilk's  $\lambda$  was used as the test of significance of the discriminant analyses to separate groups. The number of observations correctly classified and the squared Mahalanobis distances to group centroids were used to evaluate the performance of the discriminant analyses. Classifications were cross-validated using a jack-knife procedure (SAS 2000). The predicted group membership of individuals based on the jack-knife procedure was examined to determine into which group individuals were misclassified. Differences were considered significant at  $P < 0.05$ .

**Table 1** Fatty acid composition of adipose tissue and stomach oil of adult northern fulmars on Chagulak I. in July 2004, St George I. in June and August 2004, and of chicks on Chowiet I. in August 2004

Fatty acid	Adipose tissue	Stomach Oil	Mean ratio
<b>Chagulak I. July (N = 26)</b>			
Saturated	14.43 ± 0.53	18.48 ± 1.68	
14:0*	3.19 ± 0.19 <sup>1</sup>	4.97 ± 0.24 <sup>2</sup>	0.66 ± 0.04 <sup>a</sup>
16:0*	8.55 ± 0.39 <sup>1</sup>	11.11 ± 0.31 <sup>2</sup>	0.78 ± 0.04 <sup>a</sup>
17:0	0.10 ± 0.01	0.07 ± 0.003	
18:0*	2.84 ± 0.11	1.61 ± 0.10	1.90 ± 0.11 <sup>a,b</sup>
20:0	0.30 ± 0.01	0.15 ± 0.01	
Monounsaturated	70.77 ± 1.08	58.91 ± 1.09	
16:1n-11	0.29 ± 0.02	0.34 ± 0.01	
16:1n-7*	3.35 ± 0.26 <sup>1</sup>	6.30 ± 0.21 <sup>2</sup>	0.53 ± 0.04 <sup>a</sup>
18:1n-11	0.53 ± 0.04	0.33 ± 0.03	
18:1n-9*	13.05 ± 0.71 <sup>1</sup>	13.08 ± 0.77 <sup>2</sup>	1.08 ± 0.08 <sup>a</sup>
18:1n-7*	2.95 ± 0.21 <sup>1</sup>	3.62 ± 0.21 <sup>2</sup>	0.83 ± 0.05 <sup>a</sup>
18:1n-5	0.43 ± 0.02	0.62 ± 0.03	
20:1n-11*	19.57 ± 0.74 <sup>1</sup>	12.02 ± 0.37 <sup>1</sup>	1.64 ± 0.06 <sup>a</sup>
20:1n-9*	4.11 ± 0.16 <sup>1</sup>	3.92 ± 0.22 <sup>2</sup>	1.10 ± 0.05 <sup>a</sup>
20:1n-7	0.47 ± 0.02	0.29 ± 0.01	
22:1n-11*	22.97 ± 1.40 <sup>1</sup>	17.09 ± 0.93 <sup>1</sup>	1.37 ± 0.05 <sup>a</sup>
22:1n-9*	1.52 ± 0.09 <sup>1</sup>	1.25 ± 0.09 <sup>1</sup>	1.31 ± 0.08 <sup>a,b</sup>
22:1n-7	0.30 ± 0.01	0.25 ± 0.02	
Polyunsaturated	12.60 ± 0.52	18.73 ± 0.88	
16:2n-4	0.24 ± 0.02	0.47 ± 0.03	
16:3n-6	0.21 ± 0.02	0.27 ± 0.03	
16:4n-1	0.18 ± 0.03	0.64 ± 0.14	
18:2n-6*	0.99 ± 0.01 <sup>1</sup>	0.92 ± 0.04 <sup>2</sup>	1.12 ± 0.05 <sup>a</sup>
18:3n-3*	0.43 ± 0.03 <sup>1</sup>	0.51 ± 0.02 <sup>2</sup>	0.85 ± 0.05 <sup>a</sup>
18:4n-3*	1.25 ± 0.16 <sup>1</sup>	2.03 ± 0.26 <sup>2</sup>	0.79 ± 0.15 <sup>a</sup>
20:2n-6	0.26 ± 0.01	0.25 ± 0.01	
20:4n-6*	0.25 ± 0.01 <sup>1</sup>	0.29 ± 0.01 <sup>2</sup>	0.89 ± 0.04 <sup>a</sup>
20:4n-3	0.31 ± 0.03	0.47 ± 0.02	
20:5n-3*	2.54 ± 0.31 <sup>1</sup>	6.83 ± 0.73 <sup>2</sup>	0.45 ± 0.09 <sup>a</sup>
21:5n-3	0.22 ± 0.02	0.33 ± 0.03	
22:5n-3	1.21 ± 0.07	0.51 ± 0.04	
22:6n-3*	4.12 ± 0.30 <sup>1</sup>	4.31 ± 0.31 <sup>2</sup>	1.03 ± 0.11 <sup>a</sup>
24:1n-9	0.96 ± 0.09	1.20 ± 0.11	
<b>St. George I. June (N = 25)</b>			
Saturated	19.23 ± 0.48	31.87 ± 1.97	
14:0*	7.62 ± 0.41 <sup>1</sup>	20.20 ± 1.71 <sup>2</sup>	0.46 ± 0.05 <sup>b</sup>
16:0*	8.61 ± 0.30 <sup>1</sup>	11.40 ± 0.21 <sup>2</sup>	0.76 ± 0.03 <sup>a</sup>
17:0	0.11 ± 0.02	0.06 ± 0.01	
18:0*	2.47 ± 0.14	1.33 ± 0.25	2.36 ± 0.16 <sup>a</sup>
20:0	0.37 ± 0.01	0.34 ± 0.02	
Monounsaturated	68.72 ± 1.20	53.10 ± 2.07	
16:1n-11	0.30 ± 0.02	0.23 ± 0.01	
16:1n-7*	2.93 ± 0.12 <sup>1</sup>	3.54 ± 0.20 <sup>2</sup>	0.85 ± 0.04 <sup>b</sup>
18:1n-11	0.68 ± 0.04	0.25 ± 0.07	
18:1n-9*	6.93 ± 0.61 <sup>1</sup>	3.23 ± 0.60 <sup>1</sup>	3.24 ± 0.32 <sup>b</sup>
18:1n-7*	1.65 ± 0.19 <sup>1</sup>	1.20 ± 0.24 <sup>1</sup>	2.15 ± 0.22 <sup>b</sup>
18:1n-5	0.46 ± 0.01	0.52 ± 0.01	
20:1n-11*	28.31 ± 1.39 <sup>1</sup>	20.84 ± 1.01 <sup>1</sup>	1.45 ± 0.07 <sup>a</sup>

**Table 1** continued

Fatty acid	Adipose tissue	Stomach Oil	Mean ratio
20:1n-9*	3.79 ± 0.12 <sup>1</sup>	2.83 ± 0.10 <sup>2</sup>	1.36 ± 0.04 <sup>b</sup>
20:1n-7	0.503 ± 0.03	0.19 ± 0.04	
22:1n-11*	20.91 ± 0.79 <sup>1</sup>	16.65 ± 0.55 <sup>1</sup>	1.29 ± 0.07 <sup>a,b</sup>
22:1n-9*	1.18 ± 0.06 <sup>1</sup>	0.67 ± 0.06 <sup>1</sup>	2.07 ± 0.19 <sup>c</sup>
22:1n-7	0.24 ± 0.01	0.15 ± 0.01	
Polyunsaturated	10.08 ± 0.88	12.16 ± 1.54	
16:2n-4	0.29 ± 0.02	0.44 ± 0.04	
16:3n-6	0.11 ± 0.01	0.07 ± 0.01	
16:4n-1	0.23 ± 0.03	1.08 ± 0.13	
18:2n-6*	0.66 ± 0.03 <sup>1</sup>	0.36 ± 0.04 <sup>1</sup>	2.37 ± 0.24 <sup>b</sup>
18:3n-3*	0.24 ± 0.02 <sup>1</sup>	0.20 ± 0.03 <sup>1</sup>	1.81 ± 0.22 <sup>b</sup>
18:4n-3*	0.92 ± 0.11 <sup>1</sup>	1.83 ± 0.23 <sup>2</sup>	0.59 ± 0.07 <sup>a</sup>
20:2n-6	0.14 ± 0.01	0.09 ± 0.01	
20:4n-6*	0.22 ± 0.02 <sup>1</sup>	0.24 ± 0.06 <sup>2</sup>	1.21 ± 0.10 <sup>a</sup>
20:4n-3	0.18 ± 0.02	0.26 ± 0.05	
20:5n-3*	1.99 ± 0.24 <sup>1</sup>	4.97 ± 0.63 <sup>2</sup>	0.52 ± 0.08 <sup>a</sup>
21:5n-3	0.18 ± 0.02	0.17 ± 0.04	
22:5n-3	1.24 ± 0.08	0.49 ± 0.11	
22:6n-3*	2.90 ± 0.43 <sup>1</sup>	2.33 ± 0.46 <sup>1</sup>	2.10 ± 0.32 <sup>b</sup>
24:1n-9	0.62 ± 0.05	0.42 ± 0.03	
<b>St. George I. August (N = 20)</b>			
Saturated	16.26 ± 0.54	19.50 ± 1.21	
14:0*	3.44 ± 0.23 <sup>1</sup>	5.01 ± 0.33 <sup>2</sup>	0.71 ± 0.04 <sup>a</sup>
16:0*	9.09 ± 0.38 <sup>1</sup>	11.33 ± 0.32 <sup>2</sup>	0.81 ± 0.03 <sup>b</sup>
17:0	0.15 ± 0.01	0.55 ± 0.06	
18:0*	3.32 ± 0.14	2.51 ± 0.20	1.42 ± 0.08 <sup>b</sup>
20:0	0.27 ± 0.06	0.11 ± 0.01	
Monounsaturated	61.83 ± 1.52	51.01 ± 0.61	
16:1n-11	0.30 ± 0.02	0.33 ± 0.02	
16:1n-7*	3.86 ± 0.24 <sup>1</sup>	5.77 ± 0.16 <sup>2</sup>	0.67 ± 0.04 <sup>a,b</sup>
18:1n-11	0.94 ± 0.10	0.91 ± 0.08	
18:1n-9*	12.80 ± 0.76 <sup>1</sup>	12.15 ± 1.04 <sup>1</sup>	1.12 ± 0.06 <sup>a</sup>
18:1n-7*	3.61 ± 0.23 <sup>1</sup>	4.23 ± 0.30 <sup>2</sup>	0.88 ± 0.04 <sup>a</sup>
18:1n-5	0.49 ± 0.02	0.56 ± 0.03	
20:1n-11*	17.44 ± 0.96 <sup>1</sup>	12.06 ± 0.60 <sup>1</sup>	1.50 ± 0.11 <sup>a</sup>
20:1n-9*	3.54 ± 0.16 <sup>1</sup>	2.98 ± 0.12 <sup>1</sup>	1.20 ± 0.05 <sup>a,b</sup>
20:1n-7	0.47 ± 0.05	0.32 ± 0.07	
22:1n-11*	17.03 ± 1.19 <sup>1</sup>	10.83 ± 0.73 <sup>1</sup>	1.72 ± 0.18 <sup>a</sup>
22:1n-9*	1.13 ± 0.09 <sup>1</sup>	0.72 ± 0.05 <sup>1</sup>	1.61 ± 0.11 <sup>a,c</sup>
22:1n-7	0.22 ± 0.02	0.16 ± 0.02	
Polyunsaturated	19.33 ± 0.97	25.89 ± 0.63	
16:2n-4	0.19 ± 0.01	0.19 ± 0.01	
16:3n-6	0.29 ± 0.02	0.44 ± 0.03	
16:4n-1	0.25 ± 0.03	0.59 ± 0.06	
18:2n-6*	0.98 ± 0.05 <sup>1</sup>	0.82 ± 0.03 <sup>1</sup>	1.19 ± 0.04 <sup>a</sup>
18:3n-3*	0.55 ± 0.05 <sup>1</sup>	0.63 ± 0.05 <sup>1</sup>	0.89 ± 0.05 <sup>a</sup>
18:4n-3*	1.96 ± 0.21 <sup>1</sup>	3.15 ± 0.28 <sup>1</sup>	0.65 ± 0.05 <sup>a</sup>
20:2n-6	0.29 ± 0.01	0.3 ± 0.02	
20:4n-6*	0.34 ± 0.03 <sup>1</sup>	0.43 ± 0.13 <sup>1</sup>	1.08 ± 0.07 <sup>a</sup>
20:4n-3	0.47 ± 0.03	0.64 ± 0.06	
20:5n-3*	4.79 ± 0.49 <sup>1</sup>	9.24 ± 0.23 <sup>2</sup>	0.51 ± 0.05 <sup>a</sup>
21:5n-3	0.34 ± 0.02	0.49 ± 0.01	
22:5n-3	1.40 ± 0.11	0.94 ± 0.11	

**Table 1** continued

Fatty acid	Adipose tissue	Stomach Oil	Mean ratio
22:6n-3*	6.88 ± 0.37 <sup>1</sup>	7.43 ± 0.35 <sup>2</sup>	0.94 ± 0.05 <sup>a</sup>
24:1n-9	0.61 ± 0.05	0.58 ± 0.04	
<b>Chowiet I. August (chicks N = 30)</b>			
Saturated	15.54 ± 0.50	16.15 ± 0.57	
14:0*	2.46 ± 0.11 <sup>1</sup>	3.87 ± 0.11 <sup>2</sup>	0.64 ± 0.16 <sup>a</sup>
16:0*	9.55 ± 0.35 <sup>1</sup>	9.81 ± 0.37 <sup>2</sup>	0.99 ± 0.20 <sup>b</sup>
17:0	0.28 ± 0.01	0.65 ± 0.07	
18:0*	3.00 ± 0.10	1.69 ± 0.16	2.07 ± 0.75 <sup>a,b</sup>
20:0	0.25 ± 0.01	0.13 ± 0.01	
Monounsaturated	68.87 ± 1.08	64.56 ± 1.28	
16:1n-11	0.37 ± 0.02	0.32 ± 0.01	
16:1n-7*	3.61 ± 0.18 <sup>1</sup>	6.04 ± 0.10 <sup>2</sup>	0.60 ± 0.18 <sup>a</sup>
18:1n-11	0.58 ± 0.04	0.35 ± 0.03	
18:1n-9*	18.11 ± 0.59 <sup>1</sup>	15.46 ± 0.61 <sup>2</sup>	1.21 ± 0.26 <sup>a</sup>
18:1n-7*	3.07 ± 0.18 <sup>1</sup>	3.12 ± 0.19 <sup>2</sup>	1.02 ± 10.31 <sup>a</sup>
18:1n-5	0.57 ± 0.02	0.72 ± 0.03	
20:1n-11*	17.20 ± 0.76 <sup>1</sup>	12.67 ± 0.51 <sup>1</sup>	1.39 ± 0.32 <sup>a</sup>
20:1n-9*	5.01 ± 0.13 <sup>1</sup>	4.57 ± 0.15 <sup>2</sup>	1.12 ± 0.20 <sup>a</sup>
20:1n-7	0.51 ± 0.02	0.37 ± 0.02	
22:1n-11*	18.26 ± 0.82 <sup>1</sup>	19.20 ± 0.92 <sup>2</sup>	1.01 ± 0.38 <sup>b</sup>
22:1n-9*	1.36 ± 0.05 <sup>1</sup>	1.49 ± 0.06 <sup>2</sup>	0.95 ± 0.23 <sup>b</sup>
22:1n-7	0.22 ± 0.01	0.25 ± 0.01	
Polyunsaturated	12.73 ± 0.60	15.12 ± 0.90	
16:2n-4	0.35 ± 0.02	0.52 ± 0.03	
16:3n-6	0.16 ± 0.01	0.26 ± 0.02	
16:4n-1	0.06 ± 0.01	0.16 ± 0.02	
18:2n-6*	1.28 ± 0.02 <sup>1</sup>	1.08 ± 0.03 <sup>2</sup>	1.21 ± 0.19 <sup>a</sup>
18:3n-3*	0.53 ± 0.02 <sup>1</sup>	0.55 ± 0.02 <sup>2</sup>	0.99 ± 0.26 <sup>a</sup>
18:4n-3*	0.64 ± 0.07 <sup>1</sup>	1.09 ± 0.10 <sup>2</sup>	0.61 ± 0.29 <sup>a</sup>
20:2n-6	0.3 ± 0.01	0.25 ± 0.01	
20:4n-6*	0.33 ± 0.02 <sup>1</sup>	0.36 ± 0.02 <sup>2</sup>	0.97 ± 0.43 <sup>a</sup>
20:4n-3	0.32 ± 0.02	0.41 ± 0.03	
20:5n-3*	1.95 ± 0.21 <sup>1</sup>	4.10 ± 0.45 <sup>2</sup>	0.54 ± 0.33 <sup>a</sup>
21:5n-3	0.12 ± 0.01	0.21 ± 0.02	
22:5n-3	1.26 ± 0.09	0.69 ± 0.09	
22:6n-3*	4.77 ± 0.24 <sup>1</sup>	3.90 ± 0.33 <sup>2</sup>	1.39 ± 0.55 <sup>a,b</sup>
24:1n-9	0.67 ± 0.03	1.52 ± 0.08	

Values are percentage of mean mass ± SE of FAs (31 out of 69, including reference FA 18:0) which averaged ≥0.2% among samples analyzed  
*Mean ratio* mean ratios of adipose tissue and stomach oil ± SE

\* Designates the 16 FAs used in analyses

Adipose tissue and oil FA values for each group that do not share a common superscript number were significantly different using the Tukey's multiple comparison test and the Bonferroni adjustment ( $P < 0.0008$ )

Ratio values for the same FAs that do not share a common superscript letter were significantly different between groups ( $P < 0.003$ , Tukey's multiple comparison test)

Statistical tests were performed on renormalized log-transformed data not shown here

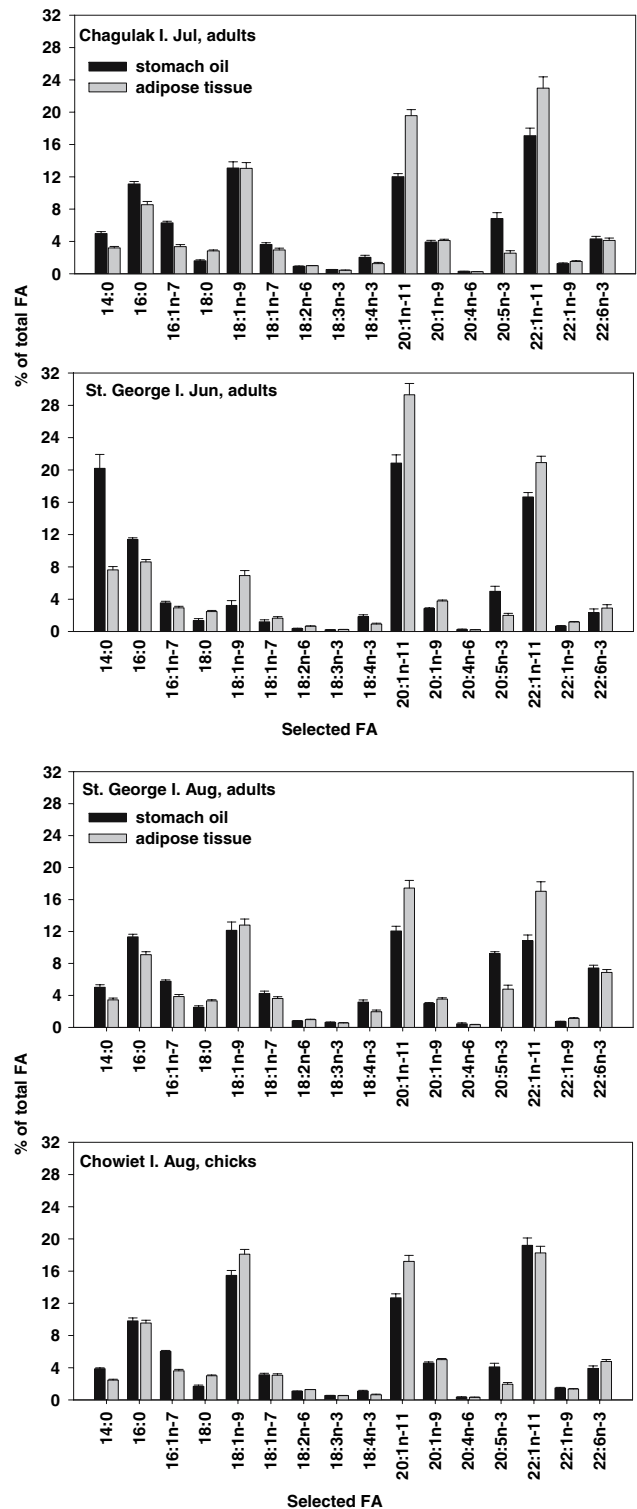
Ratios of adipose tissue and stomach oil FAs were also calculated to evaluate patterns between the two lipid sources. A MANOVA was performed on the subset of 16

FAs to evaluate differences in FA ratios among locations—adults sampled at Chagulak I. in July and at St George I. in June and August and chicks sampled on Chowiet I. in August. Following the MANOVA, a Tukey's multiple comparison was used to evaluate differences between individual FA ratios between adults on Chagulak I. in July, St George I. in June and August, and chicks on Chowiet I. in August. Differences from the results of the Tukey's multiple comparison were considered significant at  $P < 0.003$ . Discriminant analyses were performed to reveal patterns of FA ratios among the four sampling groups according to Budge et al. 2002 and Iverson et al. 2002. Wilk's  $\lambda$  was used as the test of significance of the discriminant analyses to separate groups. The number of observations correctly classified was used to evaluate the performance of the discriminant analyses. Classifications were cross-validated using a jack-knife procedure (SAS 2000). The predicted group membership of individuals based on the jack-knife procedure was examined to determine into which group individuals were misclassified. Differences were considered significant at  $P < 0.05$ . Ratios of adipose tissue and stomach oil FAs were combined into single means across all fulmars sampled and compared with ratios ("calibration coefficients") obtained between adipose tissue FAs of common murre (*Uria aalge*) chicks and that of their long-term dietary FAs (Iverson et al. 2007). All statistical analyses were performed using the SAS statistical software (SAS 2000). Data are presented as means  $\pm$  standard errors.

## Results

Thin layer chromatography confirmed the presence of wax ester alcohols in most of the stomach oil samples from northern fulmars. However, no wax esters were detected in any of the adipose tissue samples, including the adipose tissue of individuals with wax esters in their stomach oil. All wax ester alcohols were converted to their corresponding FA to gain the complete FA signature for comparative purposes.

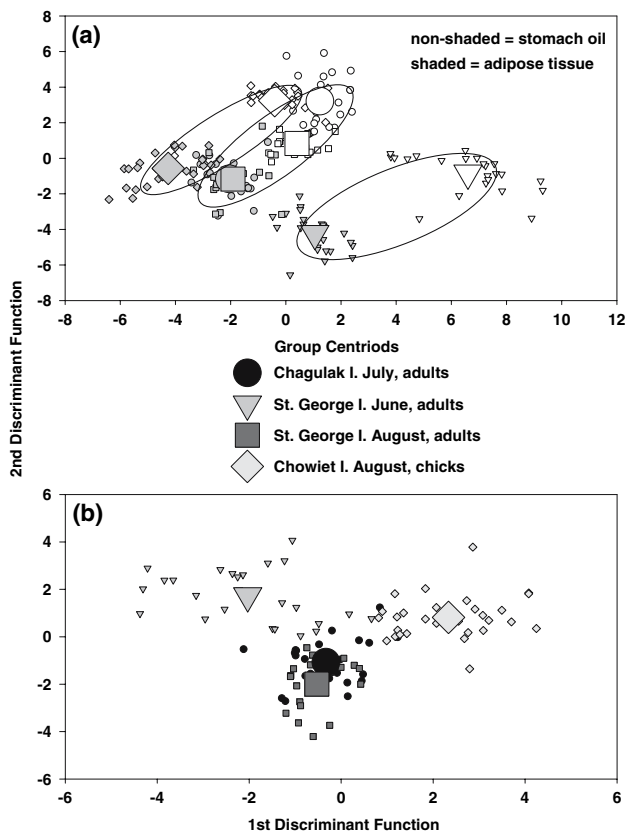
There were significant differences in FA signatures between stomach oil and adipose tissue of adults on Chagulak I. in July and St George I. in June and August and chicks on Chowiet I. in August (MANOVA, Wilk's  $\lambda$ ,  $P < 0.001$ ; Table 1). Levels of 12 FAs differed significantly ( $P < 0.0008$ ) between adipose tissue and stomach oil of adults at Chagulak I. Seven FA's differed for adults on St George I. in June, six differed for adults on St George I. in August and 14 differed between the two lipid sources for chicks on Chowiet I. (Fig. 2; Table 1). Between groups, individual FAs varied in how they differed between the two lipid sources but 14:0, 16:0, 16:1n-7, 18:4n-3, 20:4n-6 and



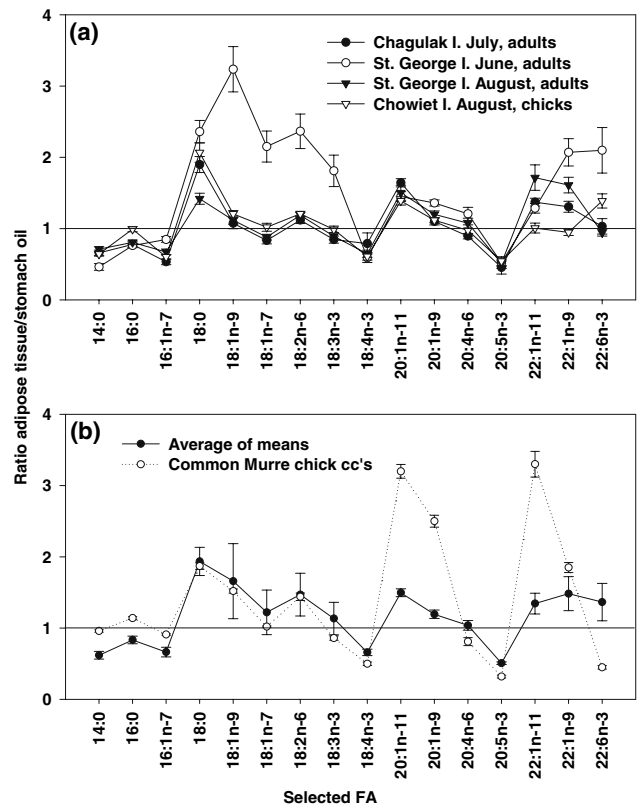
**Fig. 2** The 16 most abundant FAs in northern fulmar lipids, including reference FA 18:0, illustrating characteristic differences in patterns between lipid sources for adults on Chagulak I. in July ( $N = 26$ ), St. George I. in June ( $N = 25$ ) and August ( $N = 20$ ), and chicks on Chowiet I. in August ( $N = 30$ ) 2004. Stomach oil and adipose tissue samples were collected from the same individuals. Bars are means and vertical lines are 1 SE. See Table 1 for statistical comparisons

20:5n-3 were always higher ( $P < 0.0008$ ; Table 1; Fig. 2). There were no clear patterns in the other FAs tested (18:1n-9, 18:1n-7, 20:1n-11, 20:1n-9, 22:1n-11, 22:1n-9, 18:2n-6, 18:3n-3 and 22:6n-3), which were variably higher, lower, or nearly equal between the two lipid sources.

Results of discriminant analysis confirmed the findings from the MANOVA and revealed a significant ability to distinguish between stomach oil and adipose tissue in the four sampling groups (Fig. 3a). Mahalanobis distance is measured in terms of standard deviations from the centroid. Centroids more than 1.96 Mahalanobis distance units apart have less than 0.05 chance of belonging to the same group; 3 units would correspond to a chance of less than 0.01. The Mahalanobis distances between lipid sources for each location



**Fig. 3** Plots of the discriminant scores and group centroids for the first and second discriminant functions comparing FA signatures of stomach oil and adipose tissue of adult northern fulmars on Chagulak I. in July ( $N = 26$ ), St. George I. in June ( $N = 25$ ) and August ( $N = 20$ ), and in chicks on Chowiet I. in August ( $N = 30$ ) 2004. (a) Discriminant analysis of stomach oil and adipose tissue FAs across locations. The first and second discriminant functions explained 96.6% of the variation between stomach oil and adipose tissue FA signatures across locations; 76.9% of samples were correctly classified by lipid source (jack-knife procedure,  $P < 0.001$ ). Ellipses illustrate the similar ability of the two lipid sources to distinguish foraging differences among individuals and colonies. (b) Discriminant analysis of the ratios between adipose tissue and stomach oil FAs within individuals across locations. The first and second discriminant functions explained 89.7% of the variation, 73.1% of the individuals were correctly classified to location ( $P < 0.001$ )



**Fig. 4** (a) The mean ratios between stomach oil and adipose tissue for the 16 most abundant FAs in northern fulmar lipids for adults on Chagulak I. in July ( $N = 26$ ), St. George I. in June ( $N = 25$ ) and August ( $N = 20$ ), and chicks on Chowiet I. in August ( $N = 30$ ) 2004 (vertical lines are 1 SE). Adipose tissue and stomach oil samples were collected from the same individuals. See Table 1 for statistical comparisons. (b) Average of the ratios for all individuals in comparison to calibration coefficients (CC's, ratios between diet and adipose tissue FAs) from common murre chicks, vertical lines are 1 SE ( $N = 13$ , data from Iverson et al. 2007)

ranged from 14 to 49 standard deviation units ( $P < 0.002$ ) indicating clear discrimination between them.

The MANOVA and discriminant analysis on the quantitative ratio of FAs between stomach oil and adipose tissue within individuals across locations showed greatest similarity between adults on Chagulak I. in July and St. George I. in August, with the other two groups more distant (Wilk's  $\lambda$ ,  $P < 0.001$ , MANOVA; Fig. 3b). Examination of the individual mean ratios between stomach oil and adipose tissue for the 16 most abundant FAs in fulmar lipids revealed similarities and differences among sampling groups (Fig. 4a). For all groups except adults sampled on St. George in June, ratios were well-clustered and followed similar patterns among groups. In contrast, ratios for St. George adults in June differed considerably in at least 6 of the 16 FAs (Fig. 4a). Finally, we combined ratios into single means across all fulmars sampled and compared these with ratios (“calibration coefficients”) obtained between adipose tissue

FAs of common murre chicks and that of their long-term dietary FAs (Iverson et al. 2007; Fig. 4b). In large part, these two sets of ratios were similar, but several FA's differed notably, including the long-chain monosaturated isomers of 20:1, 22:1 and the polyunsaturated 22:6n-3.

## Discussion

The results of our study show that the FA composition of stomach oil and adipose tissue differed within individual northern fulmars (Fig. 2), but in a relatively consistent way across individuals (Fig. 4a), such that the two lipid sources provided a similar ability to distinguish foraging differences among individuals and colonies (Fig. 3a). Consistent with previous studies (e.g., Connan et al. 2005, 2007), our data demonstrate that both stomach oil and adipose tissue FA composition in northern fulmars clearly reflect their marine-based diets, but nevertheless differed in their FA signatures.

Differences in FA signatures of stomach oil and adipose tissue could arise from several causes, the two most important of which would likely be (1) differences in metabolic processing that FAs in the two depots experience, including potentially different retention and deposition of FAs, and (2) differences in the dietary time scales reflected in the two sources. The production of stomach oil has been hypothesized to be an adaptation which allows breeding adult Procellariiformes to enhance provisioning rates to chicks while foraging on distant and dispersed food supplies (Ashmole 1971; Warham 1977; Laugksch and Duffy 1986; Roby et al. 1993, 1997; Obst and Nagy 1993; Taylor et al. 1997). This hypothesis was supported in an experiment conducted by Roby et al. (1997), who showed that storing oil in the proventriculus has energetic advantages for seabirds which frequently experience periods of fasting, because it reduces the metabolic costs of storing fat reserves from assimilated FAs and later re-mobilizing them when fasting. Thus, it should be advantageous for seabirds that form stomach oil to directly metabolize FAs in the oil rather than depositing them first in adipose tissue and later mobilizing them during periods of fasting, as the latter bears a cost of 25–30% of the assimilated energy (Ricklefs 1974; Spady et al. 1976; Roby et al. 1989, 1997). However, although this might imply direct and unmodified reflection of dietary FAs in stomach oil, preferential accumulation of neutral lipids, predominantly triacylglycerols, in stomach oil and rapid gastric emptying of more polar lipids, such as phospholipids, may be an important contributor to differences between FA signatures in stomach oil and diet. Currently, little is known about any selectivity in uptake or release of specific FAs from stomach oil.

In contrast to stomach oil FAs, the FAs found in adipose tissue have been digested and assimilated, i.e.,

released from the glycerol or alcohol backbone, re-esterified and passed through the circulation, then released, taken up and re-esterified into adipose tissue. Although it has been shown that many FAs from diet are deposited in adipose tissue at an almost 1:1 ratio (Iverson et al. 2004, 2006, 2007), it is also known that the effects of metabolism within the predator can affect levels of specific FAs in adipose stores causing selective release of FA from adipose tissue (e.g., Groscolas 1990; Raclot and Groscolas 1993, 1994, 1995; Klasing 1998; Iverson et al. 2004). Cooper et al. (2005) showed that in grey seals (*Halichoerus grypus*), individual dietary FAs likely undergo predictable but differential metabolism before their assimilation into chylomicrons, the triacylglycerol-rich lipoproteins synthesized in the small intestine of mammals that act as the primary transport lipoproteins for dietary FAs in blood. This results in differences between FA composition of seal blubber and their prey. In birds, dietary triacylglycerols are absorbed in the small intestine and incorporated into portomicrons, which are the primary transport lipoproteins (Klasing 1998). Portomicrons are routed through the liver, where elongation and desaturation of FA can occur, along with synthesis of monounsaturated FAs. The liver repackages the dietary and synthesized lipids and the resulting triacylglycerols are carried to peripheral tissues for energy or stored in the adipose tissue for later use (Klasing 1998). Because dietary FAs must pass through the liver, the possibility of modification is potentially greater in birds than in most mammals. Additional effects of predator metabolism include possible selective mobilization of FAs from adipose tissue during fasting as has been shown in rats and penguins (Groscolas 1990; Raclot and Groscolas 1993). However, selective mobilization was not shown to occur during fasting in juveniles of three different species of pinnipeds (S. J. Iverson, unpublished data). While we cannot address this in the current study, especially as we do not know the dietary FA composition which influenced the adipose tissue stores we sampled, it will be important to understand how individual dietary FAs are processed in birds through controlled feeding studies during different metabolic states and compare FA signatures between diet, stomach oil and adipose tissue to quantify the amount of modification or mobilization that might occur between each lipid source.

A second possible reason underlying differences between stomach oil and adipose tissue FAs in fulmars pertains to the different dietary time spans reflected in the two lipid sources—e.g., stomach oil reflects the most recent meal(s), whereas adipose tissue should represent a longer term integration of FA intake. The most recent meals would likely have a different FA pattern than the average diet if heterogeneous meals are consumed over time. Again,



controlled feeding studies would be extremely useful in addressing such questions.

The finding that wax esters were present in stomach oil but not in adipose tissue of the same birds supports the notion that lipids in stomach oils had not undergone metabolic processing and that, as proposed by Budge and Iverson (2003), wax ester fatty alcohols are converted to FAs after digestion and deposition in adipose tissue. This observation is consistent with our understanding of the metabolic fate of wax esters in predators that store FAs as triacylglycerols (Budge and Iverson 2003). Many seabirds have the ability to digest and assimilate dietary wax esters efficiently (Roby et al. 1986), and wax ester alcohols contribute significantly to the FA composition of adipose tissue in predators (Budge and Iverson 2003). In comparing FA signatures between stomach oil and adipose tissue, we accounted for the wax ester alcohols that can be deposited into the adipose tissue. The technique described in Budge and Iverson (2003) generates a FA signature of prey containing wax esters that is equivalent to that which the predator has available for deposition as FA upon digestion of that prey, and thus we are confident that our treatment of the samples was not a source of the significant differences found between stomach oil and adipose tissue FAs in fulmars.

Discriminant analysis revealed a distinct separation between stomach oil and adipose tissue using the subset of indicator FAs (Fig. 3a). However, discriminant analysis using the individual ratios of FAs in oil to adipose tissue (Fig. 3b) indicated otherwise. It suggested that either (1) a consistent relationship between adipose tissue and stomach oil FAs does not exist, or (2) a consistent relationship does exist but there is significant spatial variation in the diet that accounts for the differences in signatures. Not all FAs occurred at lower or higher levels in stomach oil or adipose tissue consistently in all four sampling groups (Fig. 2; Table 1), suggesting that physiological mechanisms alone do not explain the differences in lipid source signatures, but that a combination of diet and physiology influences the differences. We suggest that differences in FA signatures of adipose tissue and stomach oil are due not only to the presence of biosynthesized FAs in adipose tissue, but may also be explained by a combination of two effects: (1) different deposition or mobilization characteristics of individual FAs from oil into adipose tissue, and/or (2) temporal differences, with stomach oil signatures representing a short-term diet and adipose tissue signatures reflecting diet integrated over a longer interval. In the latter case, large variation in FA signatures of stomach oil and adipose tissue would be indicative of a highly variable diet over time, whereas close similarities between the signatures of the two lipid sources would indicate a temporally homogenous diet.

This study is the first to compare the FA composition of stomach oil and adipose tissue of individual northern fulmars in detail. Fatty acid signature analysis of both stomach oil and adipose tissue has the potential of being extremely informative, with stomach oil potentially providing information on the most recent meals and adipose tissue revealing a diet integrated over a longer period of time. Our investigation has shown that there are differences between stomach oil and adipose tissue signatures; however, the biological importance of these differences remains to be evaluated. By eventually using QFASA (Iverson et al. 2004) to model the diets of fulmars using stomach oil and adipose tissue and a library of known prey FAs, it may be possible to determine what these differences in signatures represent. To accurately estimate the diet of predators using QFASA, calibration coefficients must be calculated through captive feeding studies to account for lipid metabolism (Iverson et al. 2004, 2007, Cooper et al. 2005). However, if stomach oil FAs indeed represent most dietary FAs without metabolic processing (e.g., Fig. 4b), then stomach oil compositions without calibration could be used to model the diet of fulmars. Most fulmars in this study readily regurgitated stomach oil upon capture. If the results of the models are consistent with results reported here then stomach oil and adipose tissue FAs reflect essentially the same diet, and the less invasive method of collecting stomach oil would give the same information as the relatively more invasive method of sampling adipose tissue. But, stomach oil analysis could only be substituted for adipose tissue if the overall diet of fulmars varied little over time—otherwise, investigation of both lipid sources would be necessary to understand the mean longer-term (adipose tissue) and more recent (stomach oil) diets.

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