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# Fatty acid signatures of stomach contents reflect inter- and intra-annual changes in diet of a small pelagic seabird, the Thin-billed prion *Pachyptila belcheri*

Petra Quillfeldt · Juan F. Masello · Paul Brickle · Dominik Martin-Creuzburg

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**Abstract** In diet analyses of seabirds, fatty acid signatures (FAS) can be used to overcome biases due to differential digestion of prey and enable the analysis of very digested diet samples. We applied FAS analysis to stomach contents of a small sub-Antarctic seabird, the Thin-billed Prion *Pachyptila belcheri*, which feeds mainly on squid during incubation and on crustacea during chick rearing. This seasonal dietary switch of Thin-billed prions was reflected in differences in FAS in regurgitates, as were inter-annual differences in diet composition. A discriminant function analysis correctly classified 93.4% of cases with respect to year (2006–2008) and stage of the breeding cycle (incubation versus chick rearing). The dominant types of crustacea in the diet of Thin-billed prions (amphipods *Themisto gaudichaudii*, euphausiids, decapods *Munida gregaria*, and calanoid copepods) were distinguished well by characteristic FAS patterns. However, the FAS of the two main types of prey by volume, amphipods *T. gaudichaudii* and squid

*Gonatus antarcticus*, were similar to each other. Although FAS were successfully applied in the analysis of prey in stomach contents of prions, FAS of some prey species were similar and may not be distinguishable from each other if used in quantitative diet analyses.

## Introduction

Dietary studies of planktivorous seabirds can be particularly useful in order to monitor bottom-up changes in food webs in response to changes in the environmental conditions. However, traditional methods of dietary analysis from stomach contents and regurgitates have several well-known biases (e.g., Barrett et al. 2007), for example due to the fast integration of soft-bodied prey, the selective detainment of squid beaks and the fast disintegration of relatively small otoliths. Traditional methods are increasingly combined with novel methods to assess seabird diets, such as stable isotope and fatty acid analysis. Fatty acid signatures (FAS) can be measured in several samples obtained from seabirds, such as stomach oil (e.g., Wang et al. 2007; Richoux et al. 2010), adipose tissue (e.g., Iverson et al. 2007), and blood plasma (e.g., Käckelä et al. 2010). FAS make use of the fact that fatty acids deliberated from dietary lipids and are the only dietary component that is deposited into tissues with little or known modifications (e.g., Klasing 1998). Therefore, FAS have been found to be sensitive biomarkers in studies of temporal, spatial, and species-specific differences in the diets of seabirds (e.g., Barrett et al. 2007; Iverson et al. 2007; Käckelä et al. 2007; Wang et al. 2009). FAS reflect the composition of the diet during a time window depending on the type of sample. The FAS of stomach contents and oils represent relatively unmodified signatures of recently consumed food (Richoux

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P. Quillfeldt (✉) · J. F. Masello  
Max-Planck-Institut für Ornithologie,  
Vogelwarte Radolfzell, Radolfzell, Germany  
e-mail: petra.quillfeldt@gmx.de

P. Brickle  
Fisheries Department, Falkland Islands Government,  
Directorate of Natural Resources, P.O. Box 598,  
Stanley, Falkland Islands

D. Martin-Creuzburg  
Limnologisches Institut, Universität Konstanz,  
Konstanz, Baden-Württemberg, Germany

et al. 2010). In contrast, the FAS of blood plasma and adipose tissue, turning over in about 5 and 20 days, respectively, need to be calibrated for metabolic modifications in the bird's body by arranging controlled feeding experiments (Foglia et al. 1994; Käkälä et al. 2009, 2010). FAS reflect the composition of the diet, on a time scale depending on the half life for fatty acids, e.g., about 20 days in chicken adipose tissue (Foglia et al. 1994). Thus, while stomach contents and FAS in diet samples generally represent diet consumed during the most recent feeding bout, FAS of adipose tissue represent diets integrated over weeks (e.g., Wang et al. 2010).

Thin-billed prions *Pachyptila belcheri* are small petrels with a potentially very large foraging range that feed on small prey they pick off at the sea surface. From their foraging trips, Thin-billed prions bring back a mix of very digested prey and stomach oil. Thin-billed prions are sensitive to changes in oceanic conditions: sea-surface temperatures have been shown to be negatively correlated with provisioning frequencies to Thin-billed prion chicks (Quillfeldt et al. 2007a, b). A recent study of Thin-billed prions also showed considerable dietary flexibility (Quillfeldt et al. 2010). Based on the analysis of regurgitated food and stomach contents of dead animals, Thin-billed prions preyed mostly on squid during incubation, but switched to mainly amphipods and euphausiids during chick rearing (Table 1, Quillfeldt et al. 2010). The study also suggested that in the year of lowest food availability according to chick provisioning rates, during warm water influence in 2006, Thin-billed prions had difficulties in finding sufficient squid, amphipods, or euphausiids and were forced to switch to lower trophic level prey like very small copepods.

Based on these dietary differences, the aim of this study was to assess the usefulness of FAS for dietary analyses of Thin-billed prions. Specifically, we aimed to see if the dietary differences observed previously in regurgitates (Quillfeldt et al. 2010) could be detected by FAS, as this would enable the analysis of very digested diet samples.

## Materials and methods

### Study site and study species

The Thin-billed prion is a small and abundant sub-Antarctic seabird, known to breed in two main areas: at Crozet and Kerguelen in the Southern Indian Ocean, and at the Falkland Islands/Islas Malvinas (and possibly on some islands off Tierra del Fuego: Clark et al. 1984; Cox 1980) in the Southern Atlantic Ocean. The study was carried out at New Island, Falkland Islands (Fig. 1), in the breeding seasons 2005–2006, 2006–2007, and 2007–2008.

Thin-billed prions show the typical procellariiform pattern of a single-egg clutch and slow chick development, with an average fledging period of 50 days (Strange 1980). Male and female breeding adults feed chicks on return from foraging trips taking one to eight days duration (e.g., Quillfeldt et al. 2007b, 2010).

### Diet sampling

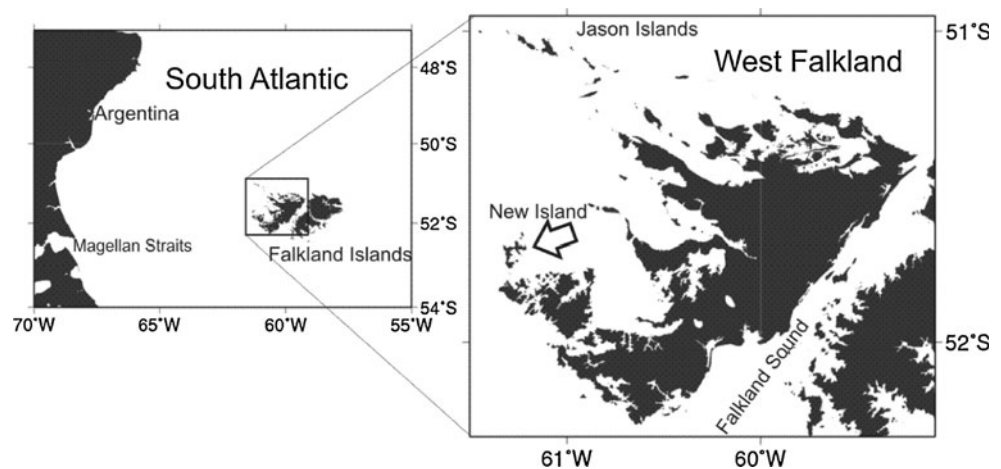
We collected regurgitates and stomach contents in three successive field seasons, as described in Quillfeldt et al. (2010): Most samples were obtained from breeding adults returning to feed chicks, when adults regurgitated during handling or during mist-net captures. Further, we analyzed stomachs of birds found dead. These included adults that had been killed by a feral cat *Felis catus* or by a striated caracara *Phalacrocorax australis*, as well as small chicks that died from hypothermia when water ran into their nest. As adults regurgitate the diet that was brought back to feed the chick, all samples were combined (Quillfeldt et al. 2010) and called “stomach samples” throughout this paper.

The median hatch dates and ranges in the three seasons were January 2 2006 (23 Dec–8 Jan), January 4 2007 (27 Dec–13 Jan), and January 5 2008 (29 Dec–15 Jan). After hatching, chicks were brooded alternately by each parent for 3–8 days (called the “guard period” in petrels). Afterward, chicks remain in the nest on their own during the day over the “chick-feeding period”, while the parents

**Table 1** Average volume per stomach content sample of diet types of Thin-billed prions at New Island, Falkland Islands from regurgitates (data from Quillfeldt et al. 2010)

Season	Crustacea					Squid (%)	Fish (%)
	Amphipods ( <i>Themisto</i> ) (%)	Euphausiids (%)	Decapods ( <i>Munida</i> ) (%)	Copepods (%)	Cirripedia (%)		
1. Chick-feeding 2006 ( <i>N</i> = 17)	32	31	17	17	1	2	–
2. Incubation 2007 ( <i>N</i> = 10)	35	1	–	–	<1	63	–
3. Chick-feeding 2007 ( <i>N</i> = 11)	57	29	–	<1	<1	11	3
4. Incubation 2008 ( <i>N</i> = 20)	9	12	15	3	<1	61	<1

**Fig. 1** Map of the Falkland Islands in relation to southern South America. The arrow indicates the location of the study site, New Island



return to feed their chicks at night, after feeding trips of 1–8 days (Quillfeldt et al. 2007b, 2010). The samples were collected during the incubation and guard period (01 Dec–15 Jan) over two years ( $N = 21$  in 2006–2007 and  $N = 41$  in 2007–2008, subsequently referred to as incubation 2007 and incubation 2008). We collected samples during the chick-feeding period (25 Jan–25 Feb) in the two years 2006 ( $N = 17$ ) and 2007 ( $N = 12$ ). All of the samples were stored at  $-20^{\circ}\text{C}$  in the field and were transported frozen to the laboratory. Here, they were defrosted once to analyze diet contents (Quillfeldt et al. 2010) and were subsequently stored at  $-20^{\circ}\text{C}$  until FAS analysis.

#### Prey species

We analyzed FAS of prey items (i) caught fresh and (ii) separated from selected stomach contents to compare FAS of fresh samples with partially digested samples (for samples sizes see Tables S1–3). Krill from stomach contents was heavily digested, but fragments could be determined as belonging to *Euphausia vallentini*, with possible *E. lucens* and *E. longirostris* additionally found in 2008 (Quillfeldt et al. 2010).

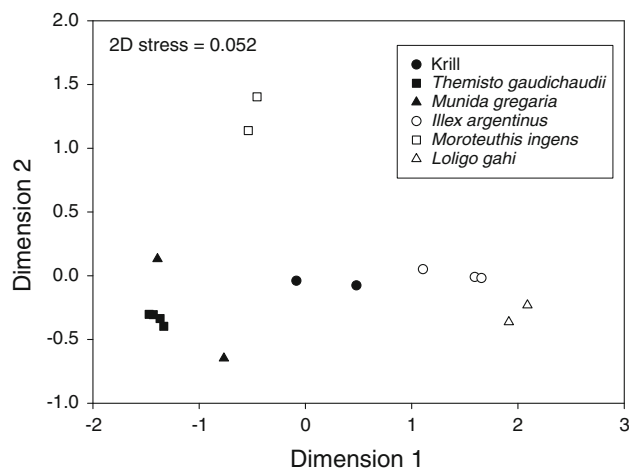
Samples of crustaceans (whole animals of krill *Euphausia lucens* and *Thysanoessa macrura*, lobster krill *Munida gregaria*, and amphipods *Themisto gaudichaudii*) and squid (muscle samples of Argentine shortfin squid *Illex argentinus*, great hooked squid *Onykia ingens*, and Patagonian longfin squid *Loligo gahi*) were obtained during a Falkland Islands Fisheries Department (FIFD) research cruise in February 2006 by bottom and plankton trawling close to the Falkland Islands (for samples sizes see Tables S1, 2). Each of the crustacean samples contained several individuals. Samples were stored frozen at  $-20^{\circ}\text{C}$ .

#### Lipid extraction and fatty acid analysis

Total lipids were extracted from sub-samples of homogenized samples (whole prey samples and stomach contents) using three washes with dichloromethane/methanol (2:1, v/v), and the cell-free extracts were evaporated to dryness under a stream of nitrogen. The lipid extracts were transesterified with  $3\text{ mol l}^{-1}$  methanolic HCl ( $60^{\circ}\text{C}$ , 15 min) according to Mason and Walker (1964). Subsequently, fatty acid methyl esters (FAMES) were extracted three times with 2 ml of iso-hexane and the FAME-containing fraction was evaporated to dryness under nitrogen and resuspended in a volume of 10–20  $\mu\text{l}$  iso-hexane. FAMES were analyzed using gas chromatography on a HP 6890 GC equipped with a flame ionization detector (FID) and a DB-225 (J&W Scientific,  $30\text{ m} \times 0.25\text{ mm}$ , ID  $\times 0.25\text{ }\mu\text{m}$  film) capillary column as described in Martin-Creuzburg et al. (2010). Fatty acids were identified by their retention times and quantified by comparison with internal standards (C17:0 ME and/or C23:0 ME) of known concentrations, using multipoint standard calibration curves determined previously for each FAME with lipid standards purchased from Sigma. The limit for quantification of fatty acids was 20 ng. Fatty acids are reported using shorthand nomenclature as follows:  $a:b_n-x$ , where  $a$  represents the number of carbon atoms,  $b$  the number of double bonds, and  $x$  the position of the first double bond counted from the methyl end. Each fatty acid is reported as weight percent of total fatty acids. It was not possible to clearly distinguish between 18:1n-9 and 18:1n-12. Moreover, we cannot completely exclude that 18:1n-12 is in fact 18:1n-11, a fatty acid potentially synthesised from dietary wax esters (Käkelä et al. 2009).

## Data analysis

Statistical tests were performed in SPSS 11.0. Multidimensional Scaling (MDS) analysis was included to provide a graphical representation of the fatty acid signatures of prey types, reduced to 2–3 dimensions. As a measure of goodness-of-fit, the stress of the solution was given, a low value suggesting a good solution. To compare the similarity of fatty acid signatures between prey types and fresh versus predigested prey, the Renkonen Percent Similarity Index (Renkonen 1938) was used. Differences between years and stages of the breeding cycle were evaluated in a total of 91 diet samples, using a combination of univariate and multivariate techniques. Discriminant function analysis was performed using all 29 determined fatty acids, after we ascertained that this method was more informative than an



**Fig. 2** Multi-dimensional scaling (MDS) plot of whole, undigested crustaceans (black symbols) and squid (white symbols) caught in the vicinity of the Falkland Islands. MDS was run using Ascald procedure with 2-dimensional solution and Young's S-stress formula 1

a priori fatty acid selection (e.g., Iverson et al. 2002) or a stepwise discriminant function analysis in our dataset. Normality was tested using Kolmogorov–Smirnov-Tests. As not all fatty acid data were normally distributed, non-parametric tests were applied in univariate statistics (Mann–Whitney-U-tests), where significance was assumed at  $p < 0.05$ , following Käkälä et al. (2010). In the discriminant analyses, we used untransformed data, but adjusted the significance level to  $p < 0.01$  to account for deviance of some fatty acid data from normality (e.g., Zöfel 2002). Wilk's  $\lambda$  was used to indicate the power of the discriminant analysis to separate groups (smaller values indicating greater success). The percent of cases correctly classified was used to evaluate the performance of the classification function.

## Results

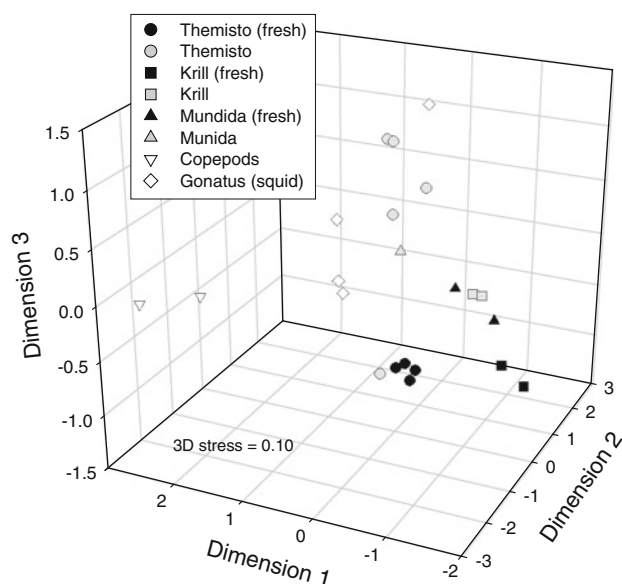
### FAS of crustaceans and squid

Full fatty acid data for crustaceans and squid and fish can be found in Tables S1 and S2 (electronic supplement). With few exceptions, crustaceans and squid species mostly had relatively similar fatty acid patterns (Figs. 2, S1, S2), with consistently high amounts of 20:5n-3 and 22:6n-3.

Among the freshly caught crustaceans, some characteristic differences were observed (see Table 2 for selected fatty acids), such as higher levels of 20:1n-9 in *T. gaudichaudii*, which was present in much lower concentrations in euphausiids and decapods. Copepods were well distinguished (Fig. 3; Table 3) by a characteristic pattern of high levels of 20:4n-6, 22:1n-9 and 22:5n-3, and low levels of 16:0 and 18:1n-9/n-12. Krill species, on the other hand, had the highest values of 22:6n-3 among the crustaceans, although they were exceeded by all squid except *Gonatus*

**Table 2** Eight out of 29 fatty acid values (means  $\pm$  SE) of (a) prey in stomach contents of Thin-billed prions, (b) whole prey items collected at sea. In all crustaceans, each sample contains several individuals

FA	(a) Predigested prey from regurgitates					(b) Whole prey			
	Krill	Copepods	Amphipods	Decapods	Squid	Krill	Amphipods	Decapods	
			<i>Themisto</i>	<i>Munida</i>	<i>Gonatus</i>	<i>Thysanoessa</i>	<i>Euphausia</i>	<i>Themisto</i>	<i>Munida</i>
	(N = 3)	(N = 2)	(N = 5)	(N = 1)	(N = 5)	(N = 1)	(N = 1)	(N = 4)	(N = 2)
16:0	18.31 $\pm$ 1.64	7.61 $\pm$ 1.02	18.36 $\pm$ 2.00	14.97	13.12 $\pm$ 2.54	18.55	16.06	10.18 $\pm$ 0.23	13.92 $\pm$ 1.28
18:1n-9/n-12	8.82 $\pm$ 1.98	3.02 $\pm$ 0.54	13.24 $\pm$ 2.65	10.49	18.61 $\pm$ 7.67	9.36	10.35	9.62 $\pm$ 0.24	9.12 $\pm$ 3.47
20:1n-9	1.20 $\pm$ 0.38	5.94 $\pm$ 0.42	4.14 $\pm$ 1.36	2.23	3.21 $\pm$ 1.10	0.00	0.91	10.71 $\pm$ 0.31	1.10 $\pm$ 0.18
20:4n-6	0.73 $\pm$ 0.11	25.89 $\pm$ 2.64	2.77 $\pm$ 1.77	6.06	3.29 $\pm$ 2.28	1.60	1.78	4.79 $\pm$ 0.32	1.12 $\pm$ 0.13
20:5n-3	15.23 $\pm$ 3.26	3.60 $\pm$ 0.59	15.97 $\pm$ 2.12	16.10	13.08 $\pm$ 2.94	20.13	21.31	18.66 $\pm$ 0.24	23.33 $\pm$ 2.64
22:1n-9	0.23 $\pm$ 0.12	6.40 $\pm$ 0.87	1.68 $\pm$ 0.89	0.95	1.79 $\pm$ 0.59	0.00	0.00	4.24 $\pm$ 0.21	0.30 $\pm$ 0.30
22:5n-3	0.00 $\pm$ 0.00	22.41 $\pm$ 4.23	0.22 $\pm$ 0.22	0.00	0.15 $\pm$ 0.13	0.00	0.00	4.08 $\pm$ 0.42	0.00 $\pm$ 0.00
22:6n-3	22.31 $\pm$ 3.51	5.32 $\pm$ 1.52	11.44 $\pm$ 3.45	16.24	7.41 $\pm$ 1.59	37.47	33.06	22.26 $\pm$ 0.35	24.35 $\pm$ 2.83



**Fig. 3** Multi-dimensional scaling (MDS) plot of whole, undigested crustaceans (*black symbols*), caught in the vicinity of the Falkland Islands, as well as predigested crustacean and squid tissue from Thin-billed prion stomach contents (*gray and white symbols*). MDS was run using Ascral procedure with 3-dimensional solution and Young's S-stress formula 1

*antarcticus* (Table 2). In comparison with freshly caught crustaceans, predigested *Themisto*, *Munida*, and euphausiids from stomach contents contained less long-chained FA (e.g., 20:5n-3 and 22:6n-3), but more long-chained FA (e.g., 14:0, 16:0, 18:1n-9, and 18:4n-3; Table 2, Fig. S1a–c). Squid showed consistent patterns among species (Fig. 2).

## Differences within and between years

Discriminant function analysis revealed a distinct separation between the fatty acid signatures within as well as among years (Wilk's Lambda = 0.032,  $p < 0.001$ ), reflecting different dietary compositions (Fig. 4). Only 6 of 91 samples were not assigned to the right group, and thus, 93.4% of cases were correctly classified. The FAS observed during the incubation period differed between years, as 9 of 29 fatty acids changed in prevalence between the years (Fig. 5). Similarly, annual changes were found in samples from the chick-feeding period in 9 of 29 fatty acids (Fig. 6). In the breeding season 2006–2007, four of 29 fatty acids changed in prevalence from the incubation to the chick-feeding period (Fig. 7).

## Discussion

In the present study, seasonal and annual dietary differences of Thin-billed prions were reflected by FAS in stomach contents, suggesting that FAS analysis might be useful for the analysis of heavily digested diet samples in this species.

## Comparison with conventional dietary analysis

According to regurgitated food, the diet taken during incubation in the two years consisted of over 60% of the squid *Gonatus antarcticus* (Table 1, Quillfeldt et al. 2010). The remainder was almost exclusively the amphipod *T. gaudichaudii* in 2007, but a mix of different crustacea

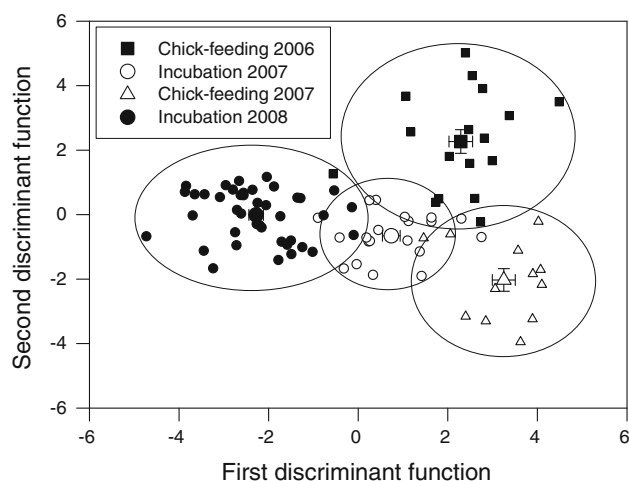
**Table 3** Renkonen percent similarity values among all crustacean and squid samples

	Crustaceans							Squid			
	Eup	Krill*	Cop*	The*	The	Mun*	Mun	Gon*	Ill	Lol	Mor
Thy	91.9	78.0	30.1	68.1	72.9	71.2	81.1	55.7	83.2	78.1	75.1
Eup		79.2	32.5	70.0	75.9	75.1	85.5	60.1	80.5	75.2	77.7
Krill*			37.8	77.2	73.2	80.1	82.6	61.8	71.1	64.4	73.3
Cop*				46.4	50.3	45.5	36.3	42.4	34.5	29.5	36.5
The*					70.7	84.3	75.5	77.9	61.9	58.0	66.1
The						73.9	76.7	62.2	70.8	63.2	72.0
Mun*							83.5	73.2	66.7	60.5	68.1
Mun								65.7	73.2	65.9	73.9
Gon*									56.2	47.0	58.6
Ill										86.3	72.4
Lol											67.1
										<b>Squid</b>	

Samples obtained from stomach content and thus, predigested, are marked with asterisks. All other samples were obtained in a fisheries cruise in February 2006

Abbreviations: *Thy* Thysanoessa, *Eup* Euphausia, *Krill\**, *Cop\** Copepods\*, *The\** Themisto\*, *The* Themisto, *Mun\** Munida\*, *Mun* Munida, *Gon\** Gonatus, *Ill* Illex, *Lol* Loligo





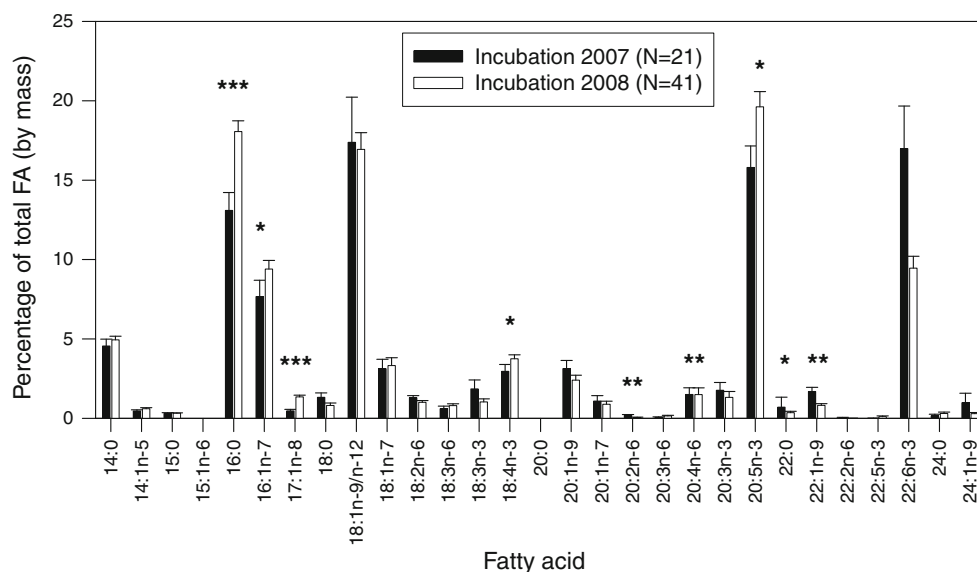
**Fig. 4** Discriminant function analysis, based on data of 29 fatty acids in stomach content samples of Thin-billed prions at New Island. The individual data points and centroids (with *error bars* denoting SEM) of each group are shown. The discriminant function analysis produced three discriminant functions in total, which were all significant. The 1st and 2nd discriminant functions explained 65.9 and 21.8% of the variation, respectively

(9% *T. gaudichaudii*, 12% euphausiids, and 15% decapods *M. gregaria*) in 2008 (Table 1). This was well reflected in the comparison of individual fatty acids. For example, 16:0 is low in *T. gaudichaudii* in comparison to euphausiids and *M. gregaria* (Table S1a), thus causing lower amounts of 16:0 in the incubation period of 2007 (Fig. 5). 17:1n-8 is higher in *M. gregaria* than the other crustacea (Table S1a)

and probably influenced the higher levels of this FA during the incubation period of 2008 (Fig. 5).

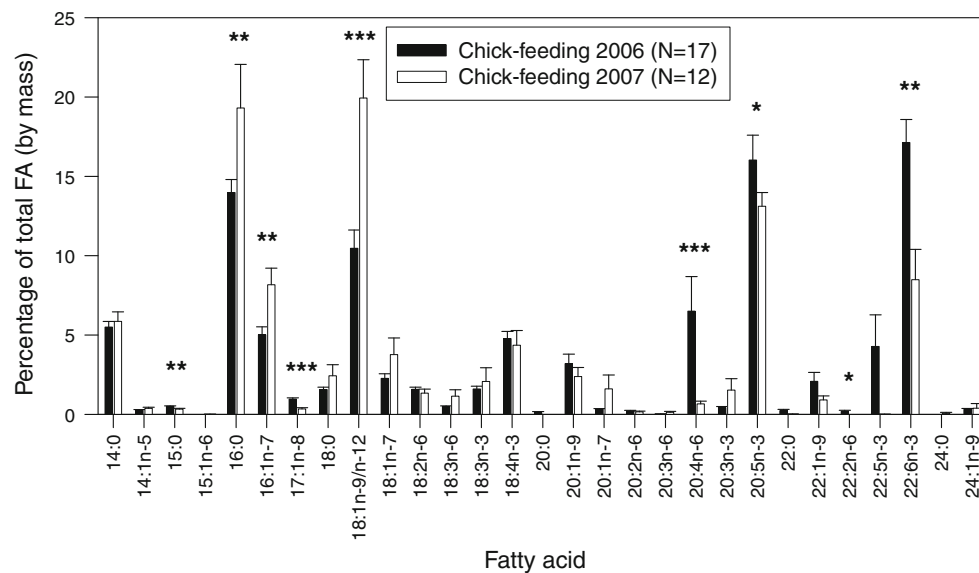
The diet taken during chick feeding consisted mainly of *T. gaudichaudii* in 2007 (57%, with 29% euphausiids and 11% squid *G. antarcticus* being the other important food item (Quillfeldt et al. 2010)). In 2006, squid was almost absent, and the diet fed to chicks consisted of a mixture of crustacea (32% *T. gaudichaudii*, 31% euphausiids, 17% *M. gregaria*, and 17% calanoid copepods). Differences in FAS were especially apparent in the fatty acids characteristic for copepods, with high levels of 20:4n-6 and low levels of 16:0 and 18:1n-9/n-12 in 2006 (Fig. 6).

Statistically, less significant differences in relative amounts of fatty acids were observed when analyzing variations between the phases of the breeding cycle (Fig. 7), even though the magnitude of the dietary change was great. In 2007, Thin-billed prions switched from 63% *G. antarcticus* and 35% *T. gaudichaudii* during incubation to mainly *T. gaudichaudii* during chick feeding in 2007 (57%, with 29% euphausiids and 11% squid *G. antarcticus* being the other important food items; Quillfeldt et al. 2010). The poorer results in this FAS comparison might reflect the relatively similar FAS of the amphipod *T. gaudichaudii* and the juvenile *G. antarcticus* taken by the prions (Table 3: 78% similarity, see also Fig. 3). This might indicate that either both feed on similar prey or *T. gaudichaudii* was consumed by the juvenile *G. antarcticus*. At South Georgia, the diet of adult *G. antarcticus* comprised of crustaceans, 50% of which were *T. gaudichaudii* (Rodhouse et al. 1996). Compared with *G. antarcticus*, the



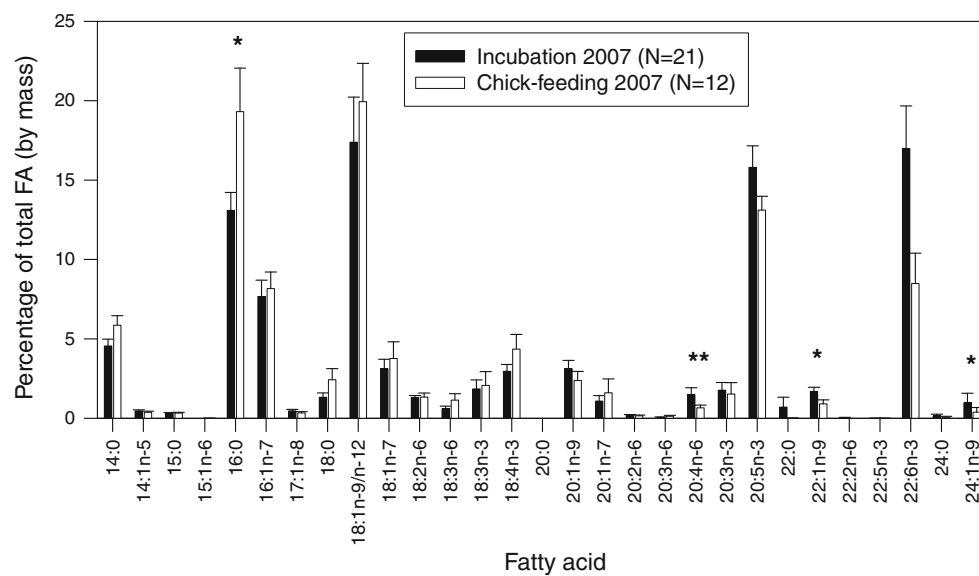
**Fig. 5** Fatty acid values (means  $\pm$  SE) of stomach content samples from adults Thin-billed prions collected during incubation stage in 2007 and 2008. Asterisks denote significant differences between the

2 years in Mann–Whitney–U-tests (\* for  $P < 0.05$ , \*\* for  $P < 0.01$ , \*\*\* for  $P < 0.001$ )



**Fig. 6** Fatty acid values (means  $\pm$  SE) of stomach content samples from Thin-billed prions collected during the chick-rearing period in 2006 and 2007. Asterisks denote significant differences between the

2 years in Mann–Whitney–U-tests (\* for  $P < 0.05$ , \*\* for  $P < 0.01$ , \*\*\* for  $P < 0.001$ )



**Fig. 7** Fatty acid values (means  $\pm$  SE) of stomach content samples of Thin-billed prions collected during the incubation and chick-rearing periods in 2007. Asterisks denote significant differences

between the 2 stages of the breeding cycle in Mann–Whitney–U-tests (\* for  $P < 0.05$ , \*\* for  $P < 0.01$ , \*\*\* for  $P < 0.001$ )

larger specimens of squid of the other species analyzed had very different FAS (Fig. S2). To evaluate inter-study variability, we carried out a comparison with data from Phillips et al. (2003), who also analyzed Falkland Islands specimens. Renkonen similarities between FAS signatures in the present and the former study were high: *Euphausia lucens*: 90%, *Themisto gaudichaudii* 75% and *Munida gregaria*: 90%, *Loligo gahi*: 81%.

Predigested samples versus freshly caught specimens

The FAS of regurgitated crustacea seemed to differ from FAS of freshly caught crustacea (see supplement, Fig S1a–c). In all crustacea we could compare (*Themisto*, *Munida*, euphausiids), stomach contents contained less 20:5n-3 and 22:6n-3, but more 16:0, 18:1n-9, and 18:4n-3.



Regurgitates consisted of predigested dietary items, mixed with stomach oil. Stomach oils are produced uniquely by birds of the order Procellariiformes (e.g., Warham 1977). Stomach oils are a concentrated energy source for chicks, serve to buffer extended periods without a meal, and allow adult Procellariiformes to forage on distant and dispersed food supplies. Stomach oils also serve as defense against predators. Stomach oil is produced from the diet, by combination of specialized gastric anatomy and physiology (e.g., Place et al. 1989; Roby et al. 1993). Aqueous dietary components are rapidly emptied from the proventriculus, while neutral lipids are retained. The chemical composition of stomach oil includes hydrocarbons, monoester waxes, diacylglycerol ethers, triglycerides, diglycerides, monoglycerides, alcohols, cholesterol, and free FAs as well as more polar lipids (e.g., Warham et al. 1976).

Little is known about any selectivity in uptake or release of specific FAs from stomach oil. It has been proposed that the preferential accumulation of neutral lipids, predominantly triacylglycerols, in stomach oil and the rapid gastric emptying of more polar lipids, such as phospholipids may be an important determinant of FA signatures in stomach oil compared to adipose tissue (Wang et al. 2007). This therefore causes a profound difference between stomach content and oil samples and tissue fatty acid profiles. The tissues are metabolically complex, and therefore, stomach contents and oils are likely more straightforward samples to use for FAS analysis in birds which produce them.

However, preferential gastric emptying would, in turn, also influence the composition of the stomach oils, as suggested in the present study. The lipid composition of stomach oils thus depends not only on the composition of recent meals, but also on the relative solubility of the lipids already accumulated in the proventriculus (e.g., Place et al. 1989).

In summary, we identified differences in the FAS of the most important types of crustacea in the diet of Thin-billed prions (amphipods *Themisto gaudichaudii*, euphausiids, decapods *Munida gregaria*, calanoid copepods). However, some caution is required when trying to distinguish the two main types of prey by volume, amphipods *T. gaudichaudii* and squid *G. antarcticus*, as these were less well separated. Therefore, the present data suggest that although FAS can be applied to analyze regurgitates in this species, this method is mainly useful for detecting changes in prey types such as copepods which had markedly different FAS. The results suggest that methods of quantitative dietary analyses using FAS (QFASA, Iverson et al. 2004) would not easily be applicable to diet analyses in Thin-billed prions and should be tested in the future. Further research should be directed to identifying mechanisms leading to FAS changes between freshly caught prey and stomach oils.

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