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Analysis of fatty acids and fatty alcohols reveals seasonal and sex specific changes in the diets of seabirds

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Abstract

A key challenge in ecology is to find ways to obtain complete and accurate information about the diets of animals. To respond to this challenge in seabirds, traditional methods (usually stomach content analysis or observations of prey at nests) have been supplemented with indirect methods or molecular trophic markers. These techniques have the potential to extend the period of investigation outside the few short months of breeding and avoid biases. Here, we use an analysis of fatty acids (FA) and fatty alcohols (FAL) from blood, adipose tissue and stomach oil to investigate how the diets of male and female common guillemots (*Uria aalge*), black-legged kittiwakes (*Rissa tridactyla*), and northern fulmars (*Fulmarus glacialis*) differed through the sampling period (prelaying and breeding season) and by sex. Diets of both sexes of all three species generally varied across the season, but sex differences were apparent only in fulmars during prelaying. Our study shows that FA analysis can provide significant insights into diets of seabirds, in particular periods of the annual cycle which are not readily studied using traditional methods.

Keywords: Northern fulmar, Black-legged kittiwake, Common guillemot, North Sea, diet, fatty acid, fatty alcohol

Introduction

Reductions in prey quality or availability can negatively impact seabird breeding success, adult survival and recruitment (Lewis et al. 2001; Rindorf et al. 2000; Frederiksen et al. 2004; Cury et al. 2011). Recent declines in many seabird populations (Croxall et al. 2002; Mitchell et al. 2004) are widely believed to have been driven by changes in prey availability that have resulted from broader scale ecosystem change (Edwards and Richardson 2004; Frederiksen et al., 2006; Croxall et al. 2012) and/or commercial fisheries (Arnott et al. 2002; Frederiksen et al. 2008). However, assessments of these interactions are constrained by limited understanding of variation in diet composition. In particular, most information on seabird diets is based on samples of prey brought back to the colony, either by collecting regurgitations or observing prey carried in the bill. Whilst these approaches have greatly improved our understanding of the prey that adults capture to feed chicks, the diet of all other age classes remains poorly documented, particularly outside the breeding season (Wilson et al. 2004; Ronconi et al. 2010). A broader characterization of diet is therefore required to assess how intrinsic and extrinsic factors interact to determine diet, and to develop dietary indicators to monitor change in marine ecosystems (Cairns 1987; Furness and Camphuysen 1997; Einoder 2009).

Studies have shown that the diets of many seabird species change over the course of the breeding season (Annett and Pierotti 1989; Lewis et al. 2001; Suryan et al. 2002; Phillips et al. 2004a). This can be broadly attributed to environmental factors such as weather and the timing of prey availability (Lack 1968; Ainley et al. 1996; Wanless et al. 1998; Lewis et al. 2001; Suryan et al. 2002) or to intrinsic factors associated with breeding stage such as the

need to feed prey of specific size or quality to chicks compared to self-feeding outside these times (e.g. Ito et al. 2010). Disentangling environmental and intrinsic effects is challenging because external conditions and parental duties change simultaneously.

Seasonal changes in diet may also differ between the sexes, since sex is known to influence seabird foraging behaviour as a result of differing reproductive roles, body size or nutritional requirements (Lewis et al. 2002; Phillips et al. 2004a; Forero et al. 2005; Weimerskirch et al. 2006). During the prelaying period, males are typically responsible for nest acquisition and courtship duties (Mawhinney et al. 1999) whereas females have the demands of egg production (Hatch 1990a; Brenninkmeijer et al. 1997). In many species the roles of the two sexes become more similar after laying, with both parents sharing incubation and chick-rearing. Whilst these different constraints on foraging behavior could result in sex specific variation in diet over the season, this has rarely been investigated (Navarro et al. 2009).

One reason for the limited number of studies on seasonal variation in diet is that traditional analysis of prey items provides only a snapshot of diet, often over a narrow time-window during chick-rearing. These techniques are also subject to biases because analysis of regurgitates can overestimate prey items that are slow to pass through the digestive tract, while easily digested prey may be underestimated or missed altogether (Mehlum and Gabrielsen 1993; Votier et al. 2003; Barrett et al., 2007; Polito et al. 2011). Another challenge is that a high proportion of birds can have empty stomachs at the time of capture (Ouwehand et al. 2004; Barrett et al., 2007) and sample composition can be highly variable requiring large sample sizes to determine differences among groups statistically (Polito et al. 2011). Indirect techniques have been developed that aim to bypass these disadvantages and provide a longer term assessment of diet including outside the breeding season. Of these,

stable isotope analysis of carbon and nitrogen in consumer tissues and lipid molecules, such as fatty acids (FAs) or Fatty alcohol (FALs) as trophic markers, have been most widely utilized (reviewed by Barrett 2007; Williams and Buck 2010; Karnovsky et al., 2012). Stable isotopes provide important data on trophic position (e.g. Hobson 1994), but recent work has highlighted that analysis of lipid samples are particularly valuable for describing variation in diet composition for a broad suite of marine predators (Iverson 2009).

Lipids have been used as dietary markers in two main ways. The first is where the composition of FA/FALs is used to show differences in diet between groups; this is sometimes referred to as qualitative FA analysis. The second, generally referred to as quantitative fatty acid signature analysis (QFASA, Iverson et al. 2004) is used to estimate the probable proportions of specific prey types in the diet. QFASA requires a FA prey library of potential prey within the predator's foraging range (e.g. Piche et al. 2010). This means that QFASA is beyond the scope of some studies, particularly for species consuming a wide variety of prey of where foraging ranges are extremely large or poorly defined. However, using FA analysis to identify differences in the diet of groups of animals does not require a prey library. Furthermore, significant steps have been made towards identifying particular lipid markers that can be used to characterize certain prey groups (e.g. Connan et al. 2007; Springer et al. 2007) or identify pelagic or demersal influences (Käkelä et al. 2005). This use of FA/FAL analysis has four advantages. First, it is not biased by differential digestion rates (see Iverson et al. 2004). Second, because lipids are representative of the diet consumed during the formation of a particular tissue (Klasing 1998; Wang et al. 2010), FA/FAL analysis can provide a long-term assessment of diet over periods of days, from analysis of blood (Käkelä et al. 2005), to weeks or months, from analysis of procellarid stomach oil (Wang et al. 2007) or adipose tissue (Wang et al. 2010). This longer term picture is likely to

be more representative of typical diet than the snapshot usually obtained from traditional methods. Third, FA/FAL analysis can be used to investigate diet outside the breeding season and, finally, information can be gained non-lethally from the majority of birds caught. For example, Owen et al. (2010) attempt adipose tissue sampling in 283 birds of two species, of which only two (0.7%) were found to have insufficient fat deposits for sampling. Similarly, in species where it is possible to safely take ~0.5ml blood sample it is possible, with care, in almost all birds (e.g. Owen 2008). However, not all procellariforms will regurgitate stomach oil upon capture.

There are limitations to using FA/FAL to qualitatively compare seabird diets. Currently there is an incomplete understanding of the turnover rates in free-living seabirds leading to imprecision in the estimates of the timescales over which FA/FAL samples indicate diet (Williams and Buck 2010). Some FA/FALs are known to be altered *in vivo* before being laid down and it is not yet known how these processes are affected by nutritional state (Karnovsky et al. 2012). Finally, unlike traditional stomach contents analysis qualitative FA/FAL analysis does not necessarily elucidate the differences in prey species composition that give rise to observed differences in FA/FAL signatures. We seek to better understand the use of FA analysis in its qualitative form as a useful addition to methods for sampling diet.

The objectives of this study were to use FA/FALs to 1) examine seasonal differences in diets, and 2) determine if there were differences in diet between the sexes over the sampling period in black-legged kittiwake (*Rissa tridactyla*), common guillemot (*Uria aalge*), and northern fulmar (*Fulmarus glacialis*) in the North Atlantic. Breeding pairs in these species all share incubation and chick rearing duties, but differ in a number of life history characteristics that

might be expected to influence the extent to which seasonal or sex-related changes in foraging behaviour may constrain prey choice (Table 1).

Methods and materials

Study sites and sample collection.

Tissue samples were collected at the Isle of May, southeast Scotland (56°11'N, 02°33'W) from adult guillemots (blood and adipose tissue) and kittiwakes (blood) during the prelaying and chick-rearing periods of the 2005 and 2006 breeding seasons (Table 2). Fulmar samples (stomach oil and blood) were collected at Eynhallow, Orkney, northern Scotland (59°08'N, 03°07'W), during three time periods: prelaying, incubation and early chick-rearing. Stomach oil is produced in the proventriculus of most procellariiform seabirds and originates from the diet (Roby et al. 1989). Prelaying guillemots were caught using wooden box traps with tip lids while chick-rearing birds were caught with a crook mounted on a 6m pole. Kittiwakes were caught on nests using a nylon noose mounted on an 8m telescopic pole. Fulmars were caught in the air by fleyg net or occasionally from nests using a hand net.

Blood samples were collected using a 25 gauge needle into a 2 ml plain syringe from the brachial vein. Between 0.5 and 2 ml was taken. The blood was immediately transferred to a heparinised cryovial and stored below -70°C in a liquid nitrogen dry shipper within 4 hours of collection to minimise oxidation of lipids. Adipose tissue was sampled from guillemots using the previously described biopsy method which has been shown to be comparable in terms of invasiveness to taking blood samples by syringe (Owen et al. 2010) and involves making a small (0.5cm long and 1-2mm deep) incision just through the skin to sample the adipose tissue that lies just beneath it. Adipose samples were folded into clean sections of

aluminium foil to make a small packet which was itself put inside a cryovial and stored below -70°C. In 2006, paired samples of adipose and blood were collected from individual guillemots to compare two tissue types with different formation times, namely blood (days) and adipose tissue (weeks). Stomach oil was collected from fulmars upon voluntary regurgitation onto clean polythene and transferred to cryovials for storage below -70°C. DNA sexing was carried out on either blood or feathers that were plucked from around the brood patch and stored dry prior to analysis using two CHDll genes (Griffiths et al. 1996).

Lipid Analysis

Lipid extraction was carried out using a variation of the Bligh and Dyer (1959) method as modified by Hanson and Olley (1963). Lipids were extracted from homogenised samples in a methanol, chloroform, water mixture (2:3:1.8 v/v/v; HPLC grade, Rathburn Chemicals Ltd, Scotland, UK). This extraction method has been formally validated by the United Kingdom Accreditation Services (Webster et al. 2006). Following extraction, transesterification was carried out by heating samples at 50ºC overnight (min 12 hours, max 18 hours) in the presence of sulphuric acid and methanol. The resultant fatty acid methyl esters and fatty alcohols were analysed by gas chromatography with flame ionisation detection (GC-FID) in a single run, following the method developed and validated by Webster et al. (2006). An HP6890 GC, incorporating an autosampler, was fitted with a DB23 capillary column (length: 30 m; internal diameter: 0.25 mm; film thickness 0.25 µm, J&W Scientific, Folsom, U.S.A.). Chromatographic peaks were identified manually using standard reference materials to give peak retention times. Peak identity was confirmed in a subset of representative samples using gas chromatography-mass spectroscopy (GC-MS). Peak areas for both FAs and FALs were derived from chromatograms using TotalChrom 6.3.1 (PerkinElmer, Inc.) software. All batches were verified using quality control procedures.

A normalised area percent was calculated for a defined set of 37 peaks which were identified from four standard reference materials which have been used for over 20 years and have been found to include all the major FA/FAL peaks commonly occurring in samples from across different taxonomic groups in the Northeast Atlantic and North Sea region. These were the saturated FAs 14:0, 16:0, 18:0, 20:0, 22:0, the monounsaturated FAs 14:1n-5, 16:1n-7, 18:1n-7, 18:1n-9, 20:1n-11/9, 22:1n-11/9, 24:1n-9, the polyunsaturated FAs 16:2, 18:2n-6, 20:2n-6, 16:3, 18:3n-3, 18:3n-6, 20:3n-3, 16:4, 18:4n-3, 20:4n-3, 20:4n-6, 21:5, 20:5n-3, 22:5n-3, 22:6n-3 and the FALs 14:0, 16:0, 18:0, 22:0, 16:1n-7, 18:1n-9, 20:1n-9, 22:1n-9 and 24:1n-9. Abbreviations used to denote FAs use the format X:Yn-z, where X refers to the chain length and Y the number of carbon-carbon double bonds. The exact position of the double bond is presented using the nomenclature n-z. This gives the position of the first carbon to carbon double bond in the molecule relative to the methyl carbon. Where two components cannot be separated they are referred to with the '/' e.g. 20:1n-11/9. Some very minor and infrequently occurring peaks were not included in the final 37 peaks of interest. One peak was identified on the basis of retention time as corresponding to FA 26:0 and was included in the analysis. Subsequent recent analytical work has indicated that the peak is not 26:0. To date, an exact identity for the peak has not been determined as full interpretation of the mass spectrum fragmentation pattern has not provided an unequivocal outcome. As such, the peak has been labeled as Unidentified peak 1, U1.

Käkelä et al. (2005) used captive herring gulls (*Larus argentatus*) fed on controlled diets to demonstrate that a high value in the ratio 20:4n-6 to the sum of 18:3n-3,18:4n-3 and 20:5n-3 could be used as an index of a diet with a demersal influence, a finding that has since been applied to free-living seabirds in areas across the North Sea (Käkelä et al. 2007). We

224 employed this ratio to provide an indication of the relative proportions of demersal and
225 pelagic prey sources in blood FA profiles of each species. Only blood samples were used as
226 this index has not been validated for other tissue types.
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229 Statistical analysis of FA/FAL data
230
231 Data analysis was performed on those 37 FA/FALs routinely identified across samples. These
232 measurements were rarely normally distributed and so were assessed for log transformation
233 using box plots and tests of skewness and kurtosis before analysis. Canonical variates
234 analysis (CVA) was used to test for differences between groups using the software package
235 GenStat (version 9, VSN International) . CVA forms linear combinations of variables that
236 maximise the ratio of the between-groups and the within-group sum of squares. In effect, a
237 CVA is similar to performing a principal components analysis between the means of groups,
238 after standardising for the covariance structure of observations within the groups.
239 Relationships between groups were plotted using the first and second canonical variates (CV1
240 and CV2) which define the largest and the second largest variances among groups after
241 standardisation. Plots are useful for visualising relationships but show only two dimensions
242 of a multidimensional analysis. Therefore, intergroup distances in multivariate space,
243 measured in Euclidean units, were also calculated. Intergroup distances show the relative
244 similarity or otherwise of lipid compositions between groups after standardisation.
245
246 To assess the significance of differences between groups, as determined by CVA, a
247 subsequent randomization test (Aebischer et al. 1993; Edgington 1995) was developed. Here,
248 original data were redefined by randomising the group allocation of each sample during 1000

simulations. The randomization was performed using all individuals within a species and year with groups defined by breeding stage and sex. This generated a distribution of distances that could be used to assess the probability that a distance as large as the observed one would occur merely by chance, a result which is similar to a p value and considered significant when below 0.05. This test is also a safeguard to increase the certainty with which group separation scores can be assessed when sample sizes were small, as there is no dependence on distributional assumptions for the data. FA/FAL signatures from two tissue types (blood and adipose) collected from guillemots were tested for differences in the mean normalized area percent of each FA component in blood and adipose signatures using *t*-tests.

Results

Seasonal and sex differences in diet

Guillemot

We found seasonal changes (prelay vs chick-rearing) in guillemot blood FA/FAL compositions in both 2005 and 2006 for both males and females (Table 3; Figure 1a,b; Supplementary material available) but there was no evidence of sex differences at any point during our study (Table 4; Figure 1). In 2005, only a small number of birds were sampled. Nevertheless, there was a clear distinction between guillemot blood FA/FAL profiles collected during prelaying and those collected during chick-rearing (Table 3; Figure 1a). The first CV explained 82.8% of the total variation and the second explained 13.3%. FAs 20:4n-6, 20:1n-11/9 and 18:1n-7 had the highest CVA loadings which shows that the groups varied most in their composition of these three FAs. The same seasonal changes were observed in guillemot blood in 2006 (Table 3; Figure 1b), when the first CV explained 92.9% and the

second CV explained 3.0% of the variance between groups. The highest loadings were on FAs 18:0, 18:1n-9 and 22:6n-3.

In 2006, when paired samples were collected from the same individual, the separation between prelaying and chick-rearing birds which was seen in blood samples was also seen in and paired adipose profiles for both males and females (Table 3; Figure 1c). No evidence was found for sex differences in adipose samples collected during the prelaying or chick-rearing stages (Table 4; Figure 1c). First and second CV axes explained 91.6% and 5.8% respectively of the variation in adipose lipid profiles and FAs 18:1n-9, 20:4n-6 and U1 had the highest loading scores.

Comparing tissue types showed that FAs 18:0, 20:5 n-3 and 20:4n-6 were enriched by between 2 and 6 times in blood compared to adipose whereas FAs 14:0, 16:1n-7, 16:2, 20:1n-11/9 and 22:1n-11/9 were enriched by between 2 and 5 times in adipose. 13 FAs/FALs were similar both tissues including FA16:0, 18:1n-9 and 22:6n-3 (Figure 2).

Kittiwake

Seasonal differences were detected in kittiwake FA/FAL profiles of both sexes during 2005 (Table 3; Figure 3a). In 2006, seasonal differences were also evident from the FA/FAL profiles of females but not for males (Table 3; Figure 3b). There was no indication of sex differences in diet at any point in the season in either year of the study (Table 4; Figure 3). In 2005, the first two canonical variates explained 81.3% and 14.6% respectively of the variance between the groups. FAs 18:2n-6, 20:4n-3 and 22:6n-3 had the greatest loadings in the analysis. In 2006, the first two CV's explained 82.5% and 10.2% respectively and FAs 18:1n-9, 20:4n-3 and 22:6n-3 had the highest loadings.

299

300 Fulmar

301 During 2005, the FA/FAL profiles of stomach oil from male fulmars were significantly
302 different between prelaying, incubation and chick-rearing (Table 3; Figure 4a). The greatest
303 difference was between prelaying and chick-rearing birds. FA/FAL profiles of the stomach
304 oil from female fulmars also varied between breeding stages, but only the difference between
305 prelaying and chick-rearing was significant in 2005 (Table 3). Male and female fulmars were
306 found to be consuming different diets during the prelaying period in 2005 (Table 4; Figure
307 4a). This sex difference diminished during incubation and FA/FAL profiles during chick-
308 rearing were closely matched between the sexes. In this analysis CV1 explained 55.2% and
309 CV2, 16.0% of variation. FAs 22:1n-11/9 and FALs 18:0 and 22:1n-9 had the highest
310 loadings on CV1.

311

312 In contrast to 2005, FA/FAL profiles of males from 2006 were not significantly different
313 during any stage of the season (Table 3; Figure 4b) though the largest distance was between
314 prelaying and chick-rearing birds. Also in contrast to 2005, female FA/FAL profiles did vary
315 significantly between all stages of breeding. The sex difference in diet that was seen in
316 prelaying birds during 2005 was repeated in 2006 (Table 4; Figure 4b). As in 2005, male and
317 female FA/FAL profiles were similar in incubating and chick-rearing birds. CV 1 explained
318 52.2% of the variance between groups and CV 2 explained 14.7%. FAs 18:0, 18:1n-9 and
319 20:5n-3 had the highest loadings. The sex difference during the prelaying period was also
320 apparent in the blood samples that were available from this period in both years of the study
321 (Table 4).

322

323 Demersal/pelagic ratio

Based upon the ratio of 20:4n-6 to the sum of 18:3n-3,18:4n-3 and 20:5n-3, the influence of pelagic or demersal prey in the diet did not differ between male and female guillemots (mean ratio male: 1.35±0.21 female: 1.25 ±0.61; Mann-Whitney U: Z=12.0, p=0.142, n=6,8), nor between male and female kittiwakes (mean ratio male: 0.40±0.28 female: 0.37 ±0.12; Mann-Whitney U: Z=61.0, p=0.786, n=12,11). By contrast, the ratio for female fulmars was significantly higher than males (mean ratio male: 0.73±0.34 female: 1.70 ±0.63; Mann-Whitney U: Z=7.0, p=0.004, n=8,9) suggesting that there was a greater influence of demersal prey species in FA/FAL profiles of female fulmars during the prelaying period.

Discussion

The analysis of FA/FALs from various tissues substantially improved our knowledge of the dietary patterns of three common species in the North Atlantic seabird community, provided evidence of seasonal changes in prey taken for all the species and highlighted sex differences that accorded well with our expectations based on life history traits.

Seasonal changes in diet

Previous studies of guillemot diet throughout the breeding range have been dominated by observations of fish brought to the chick (Hatchwell et al. 1992; Barrett et al. 2002). On the Isle of May, these have shown consistent shifts in diet over the 4 – 5 week period chicks are present in the colony, with clupeids, probably sprats (*Sprattus sprattus*) typically replacing 1+ group sandeels (Harris and Wanless 1985; Wilson et al. 2004). The limited data for adult diet obtained by stomach flushing indicate a similar seasonal shift but also highlight that 0 group sandeels contribute substantially to self-feeding (Wilson et al. 2004). Information on diet during incubation and prior to laying is even more fragmentary both on the Isle of May

and to the best of our knowledge, elsewhere. Adults occasionally bring in fish for display (Harris and Wanless 1985) and some of these are eaten. However, it is likely that such items are larger than the typical diet and thus provide a biased sample. We used FA/FALs in guillemot adipose tissue and blood collected from prelaying and chick rearing birds to investigate diet during recent days and also retrospectively to investigate periods when attendance at the colony is sporadic and/or birds are very sensitive to disturbance. The exact periods these samples provide information on are uncertain because rates of lipid turnover in free-living seabirds are poorly understood (Williams et al. 2009; Owen et al. 2010; Wang et al. 2010). However, captive feeding trials in a range of species including guillemots indicate that adipose tissue samples are likely to reflect diet during the month prior to sampling (Foglia et al. 1994; Iverson et al. 2007) and blood samples reflect diet during the previous week to ten days (changes in FA composition detected in 5 days; Käkelä et al. 2005 and within 11 days Käkelä et al., 2009). Assuming this was also the case in our study then adipose samples correspond to guillemot diet about a month before laying and approximately mid-way through incubation. In 2006, both blood and adipose samples collected in the prelaying and chick rearing period showed seasonal differences. Prelaying FA/FAL signatures from both sets of samples were distinct from those during chick rearing suggesting that prelaying diets may not have been dominated by prey types such as sandeel or sprat that guillemots are known to use at this colony whilst raising chicks (Wilson et al. 2004). These findings provide the strongest evidence to date that prelaying diet differs significantly from diet during the breeding season, although what species the birds were taking at that time remains unknown.

Lipid signatures extracted from blood and adipose differed both overall and within individual guillemots. However, despite these differences, the two sets of tissue types provided a similar

ability to determine whether or not there were differences between samples collected at different points in the breeding season. This has also been demonstrated by Käkelä et al. (2010) through captive feeding of yellow legged gulls (*Larus michahellis*). Differences can be due to both metabolic processing and the timescales over which each tissue integrates dietary fatty acids and this study was not designed to separate these effects. Nevertheless, the relative enrichment of individual fatty acids between tissues accorded well with related studies. For example, our finding that mean levels of 18:0, 20:4n-6 and 20:5n-3 were elevated in blood plasma compared to adipose is in line with Käkelä et al. (2010) who found these same components to be enriched in plasma samples compared to diet (18:0 and 20:4n-6) or adipose (20:5n-3). Raclot et al. (1995) also found that 20:5n-3 was used in preference to other fatty acids in penguins whereas 20:1n-9 was preferentially stored in adipose tissue. These findings may explain why 20:5n-3 was enriched in blood compared to adipose tissue in guillemot samples and also why 20:1n-9/11 along with 22:1n-11/9 were found in more than three times the concentration in guillemot adipose tissue than blood.

The standard method for obtaining diet information from kittiwakes has been from regurgitates, providing extensive data on changes in diet during incubation and chick rearing, but only limited data from the prebreeding period (Lewis et al. 2001). On the Isle of May there is typically a sequential change in diet from planktonic crustaceans early in the season to 1+ group sandeels in April and most of May, that are then replaced by 0 group sandeels in late May/early June. Other species such as sprat, rockling or other small gadoids are also recorded, usually towards the end of the season (e.g. Newell et al., 2006). The seasonal changes in diet apparent in the FA/FAL signatures are therefore in line with expectations based on regurgitations. During the years of this study there were unusually high numbers of snake pipefish (*Entelurus aequoreus*) bought to the colony by kittiwakes (Harris et al. 2007).

399 The occurrence of this species was much higher in 2006 when it occurred in 43.4% of 53
400 samples compared to 2005 when it was found in only 1.7% of 116 prey samples (Newell et
401 al. 2006). Only traces were recorded in the diet rather than whole fish, which are boney and
402 difficult to swallow, and therefore it is likely this species made only a small proportion of the
403 biomass of prey consumed, and that which was consumed was of little nutritional value and
404 low lipid content (Harris et al. 2008). Adipose tissue samples were not taken from kittiwakes
405 caught early in the season but such an approach would be feasible. With the proviso of
406 uncertainty about lipid turnover rates, this would extend information about diet further back
407 into the early prelaying period. Such data would be particularly interesting given the recent
408 finding that a high proportion of male kittiwakes on the Isle of May make a major excursion
409 into the mid Atlantic at this time, presumably to exploit a rich feeding area (Bogdanova et al.
410 2011).
411
412 Previous studies of the northern fulmar diet have generally been based upon regurgitates
413 collected from chicks, and have identified a broad range of prey items that include pelagic
414 crustaceans, squid, and fish that may be captured either directly or scavenged from fishery
415 discards (Furness and Todd 1984; Phillips et al. 1999; Ojowski et al. 2001). The relative
416 importance of these different prey types varies spatially (Phillips et al. 1999) and, whilst there
417 is some evidence of seasonal variation (Ojowski et al. 2001), no previous study of
418 regurgitates has extended the sampling period outside chick-rearing. Our results
419 demonstrated that seasonal differences in diet extended beyond this period in both males and
420 females (Table 3). Whilst the strength of this pattern differed slightly between years, this is
421 likely to be at least partly due to low sample sizes for females in 2005 and males in 2006. In
422 both years and sexes, the strongest differences occurred between prelaying and chick-rearing.
423 Prior to the breeding season, both male and female northern fulmars are absent from breeding

colonies for long periods (Hatch 1990b), allowing them to forage over extensive areas and access varied prey resources. Even during incubation, foraging bouts typically last 5-10 days (Mallory et al. 2008). In contrast, foraging trips during early chick rearing last only ~1 – 2 days (Furness and Todd 1984; Hamer et al. 1997; Ojowski et al. 2001; Weimerskirch et al. 2001). Our findings highlight how the demands of chick rearing constrain this species to relatively local foraging areas around breeding colonies, leading to seasonal changes in diet.

Sex differences

We found no evidence of sex differences in the diet of guillemots, a finding that was consistent with the absence of sexual dimorphism and major sex differences in parental duties during the sampling periods (Table 1). Male and female kittiwakes also show relatively little difference in size and parental behaviour (Table 1). None of the diet comparisons between the sexes were statistically significant for kittiwakes, although seasonal variation in diet for males was much less pronounced than females in 2006. Sample sizes were smaller in 2006 than 2005 and thus statistical power was reduced. Diet data from regurgitations could not be analysed by sex as this was not determined for all birds which regurgitated, so there was no way of checking this result independently. Thus further work is needed to check whether males consistently show less seasonal variation.

In contrast to guillemots and kittiwakes, there were marked sex differences in fulmar FA/FAL signatures during the prelaying period, and this effect did not extend into incubation or chick-rearing in either year. Sex differences in diet might be expected in this species given that females are absent from the colony for much longer than males during the prelaying

449 exodus (Macdonald 1977; Hatch 1990b), and males also attend the colony more frequently
450 than females during the winter (Macdonald 1980). Mallory et al. (2009) identified sex-
451 specific changes in the body composition of fulmars following the prelaying exodus,
452 suggesting that females were selecting calcium rich prey to support egg production, whilst
453 males accumulated fat and protein to support incubation. These physiological changes
454 highlight that we cannot rule out the possibility that observed differences in FA signature
455 during the prelaying period could be partly influenced by differences in lipid absorption and
456 allocation as well as dietary intake. At the same time, comparison of the different FA ratios in
457 blood samples collected from prelaying fulmars indicated that females were consuming a
458 higher proportion of demersal prey species than males during this period. The most likely
459 source of demersal prey are discards from demersal fisheries, since fulmars cannot dive
460 beyond the first few metres of the water column (Cramp 1985) and are known to feed on
461 discards (Phillips et al. 1999; Thompson 2006). It is unclear why females would be feeding
462 more upon discards than males. Given that females are smaller and feeding on discards
463 appears to be highly competitive (Hudson and Furness 1989), one might expect females to be
464 excluded by males, as in the giant petrel (Gonzalez-Solis et al. 2000). It is therefore perhaps
465 more likely that males and females are spatially segregated during prelaying. Size related
466 differences in flight energetics could affect the ability of males and females to exploit
467 different foraging areas (Schaffer et al. 2001). Further work using geolocation (Phillips et al.
468 2004b) and GPS devices (Guilford et al. 2008) is now being conducted to test whether these
469 differences in FA/FAL signatures do reflect sex-specific differences in foraging areas.
470
471 Wang et al. (2009) found no sex difference in fulmar adipose tissue samples collected during
472 the pre laying period on Chowiat Island, Alaska. These samples of adipose are representative
473 of diet in the weeks to month previous to sampling whereas the findings of the present study

474 are from stomach oil and therefore representative of the diet over the previous days. By
475 analysing the FA/FAL profiles of adipose samples at an Atlantic colony the longevity of the
476 observed sex difference could be determined. If a sex difference was not found in adipose
477 tissue then our result in stomach oil is likely to be a short term phenomenon linked to
478 specifics of the pre-lay exodus being different for males and females. However, if the
479 opposite result is found then this would suggest that males and females have differences in
480 foraging through a greater part of the year. This would be a result that was indicative of
481 different habits of fulmars in different parts of their global range.
482
483
484 Conclusions
485
486 Analysis of tissue samples are increasingly being used to complement traditional analysis of
487 seabird diet. Stable isotope analyses have successfully compared different groups of seabirds,
488 revealing seasonal, colony and sex-specific variation in the trophic level at which these
489 groups feed (Hedd and Montevecchi 2006; Phillips et al. 2011). Within certain ecosystems,
490 extensive studies of the FA/FAL profiles of both predators and their potential prey have used
491 QFASA to quantify diet composition using these indirect approaches (Iverson et al. 2007;
492 Tucker et al. 2009; Piche et al. 2010). Our results illustrate how FA/FAL analysis can also be
493 used to explore variability in seabird diet in the absence of detailed information on the prey
494 base. Compared with traditional approaches, these indirect methods have the advantage that
495 sampling is not biased by differential digestion rates of prey in the stomach (Votier et al.
496 2003), information is gathered on typical diet rather than a snapshot of the most recent meal
497 and a sample can be collected non-lethally from the majority of birds caught. Thus, FA/FAL
498 analyses provide an important additional tool for elucidating dietary trends over time, both at

a population level and potentially through multiple sampling of tissues from known individuals. The deployment of these techniques alongside novel devices for tracking individual birds now provides the potential to study the foraging movements and diet of breeding and non-breeding birds, thereby providing opportunities to better understand the factors that have driven recent changes in North Sea seabird populations (Mitchell et al. 2004).

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524 **Figure legends**

525

526 **Figure 1** Two-dimensional plot of the first two variates from a canonical variate analysis of
527 FA/FAL profiles in guillemot blood taken from males and females during prelaying and
528 chick-rearing in (a) 2005 and (b) 2006, and (c) from adipose tissue in 2006. Prelaying males
529 (△), prelaying females (▼), chick-rearing males (◇), chick-rearing females (●)

530

531 **Figure 2** Mean area percent ±SD for FA/FAL components in Common guillemot blood and
532 adipose tissue cosampled from 19 birds. Asterisks indicate significant differences between
533 sample types ($p < 0.05$, *t*-test).

534

535 **Figure 3** Two-dimensional plot of the first two variates from a canonical variate analysis of
536 FA/FAL profiles in kittiwake blood taken from males and females during prelaying and
537 chick-rearing in (a) 2005 and (b) 2006. Prelaying males (△), prelaying females (▼), chick-
538 rearing males (◇), chick-rearing females (●)

539

540 **Figure 4** Two-dimensional plot of the first two variates from a canonical variate analysis of
541 FA/FAL profiles in fulmar stomach oil taken from males and females during prelaying,
542 incubation and chick-rearing during (a) 2005 (b) and 2006. Prelaying males (△), prelaying
543 females (▼), incubating males (✳), incubating females (★), chick-rearing males (◇), chick-
544 rearing females (●)

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784 **Table 1** Foraging strategy, body size and prelaying behaviour in the common guillemot,
785 black-legged kittiwake and northern fulmar. Sources: Mitchell et al. 2004; Bogdanova et al.
786 2011; Wanless and Harris 1986.

787

	Common guillemot	Black-legged kittiwake	Northern fulmar
Foraging Strategy	Pursuit diver	Surface feeder	Surface feeder
Dietary breadth during the breeding season	Predominantly Piscivorous	Predominantly Piscivorous	Generalist
Degree of sexual dimorphism	Monomorphic	Monomorphic	Sexually dimorphic (males 11% heavier)
Prelaying behaviour	Females have 1-3 day absence prior to laying	Some males may undertake prelaying exodus	Both sexes make prelaying exodus (Males < 10 days, Females > 14 days)

788

789 **Table 2** Breeding stage, species, sampling dates and type of samples used in this study. BK,
790 Black-legged kittiwake; CG, Common guillemot; NF, Northern fulmar.
791

Breeding stage	Species	Sample Types	Sampling period	
			2005	2006
Prelaying	BK	Blood	9 May -21 May	3 Apr - 7 June
	CG	Blood + Adipose	4 April (blood only)	31March - 3 April
	NF	Blood + Stomach oil	24 April - 26 April	19 April - 20 April
Incubation	NF	Stomach oil	29 May - 31 May	28 May - 31 May
Chick-rearing	BK	Blood	28 July - 2 August	3 July - 1 August
	CG	Blood + Adipose	28 June-3 July	27 June - 5 July
	NF	Blood + Stomach oil	10 July - 22 July	18 July - 20 July

792

Table 3 Seasonal differences in FA/FAL profiles of males and females. Intergroup distances (dist) and significance values (p) are derived from canonical variates analysis and subsequent randomisation test on the FA/FAL profiles extracted from kittiwake, guillemot and fulmar blood, adipose tissue or stomach oil during 2005 and 2006. The number of individuals sampled (n) correspond to the order in the seasonal comparison column.

Species	Year	Sample Type	Seasonal Comparison	Male			Female		
				n	dist	*p*	n	dist	*p*
Guillemot	2005	Blood	Prelay vs chick-rearing	2,4	10.2	0.002 *	2,6	7.8	0.006 *
	2006	Blood	Prelay vs chick-rearing	6,6	15.8	<0.001 *	4,2	16.2	0.002 *
	2006	Adipose	Prelay vs chick-rearing	6,7	13.1	<0.001 *	4,2	11.9	0.043 *
Kittiwake	2005	Blood	Prelay vs chick-rearing	7,3	13.2	0.003 *	8,5	11.1	0.004 *
	2006	Blood	Prelay vs chick-rearing	4,4	5.5	0.117	4,4	10.4	<0.001 *
Fulmar	2005	Oil	Prelay vs chick-rearing	10,5	11.6	<0.001 *	13,14	6.3	<0.001 *
			Prelay vs Incubation	10,13	7.4	<0.001 *	13,3	7.2	0.290
			Incubation vs chick-rearing	11,5	7.5	0.039 *	3,14	8.3	0.157
	2006	Oil	Prelay vs chick-rearing	10,3	9.5	0.069	13,5	12.0	<0.001 *
			Prelay vs Incubation	10,8	5.3	0.315	13,14	5.9	0.022 *
			Incubation vs chick-rearing	8,3	7.8	0.276	14,5	7.6	0.048 *

* Denotes significance at the 5% level

798 **Table 4** Sex differences in FA/FAL profiles during prelaying, incubation and chick-rearing. Intergroup distances (dist) and significance values
799 (*p*) are derived from canonical variates analysis and subsequent randomisation test on the FA profiles extracted from guillemot, kittiwake and
800 fulmar blood, adipose tissue or stomach oil during 2005 and 2006, with n equaling the number of individuals sampled. –, no samples.
801

Species	Year	Sample Type	Comparison	Prelay			Incubation			Chick-rearing		
				n	dist	*p*	n	dist	*p*	n	dist	*p*
Guillemot	2005	Blood	Male vs Female	2,2	6.1	0.130	-	-	-	4,6	2.2	0.751
	2006	Blood	Male vs Female	6,4	4.0	0.656	-	-	-	6,2	3.1	0.963
	2006	Adipose	Male vs Female	6,4	5.1	0.372	-	-	-	7,2	3.9	0.863
Kittiwake	2005	Blood	Male vs Female	7,8	3.0	0.927	-	-	-	3,5	8.0	0.180
	2006	Blood	Male vs Female	4,4	5.9	0.091	-	-	-	4,4	4.0	0.468
Fulmar	2005	Oil	Male vs Female	10,13	7.4	<0.001 *	11,3	7.8	0.169	5,14	1.9	0.868
	2006	Oil	Male vs Female	10,12	6.4	0.022 *	8,14	4.0	<0.566	3,5	9.1	0.126
	2005	Blood	Male vs Female	5,5	15.6	0.033 *	-	-	-	-	-	-
	2006	Blood	Male vs Female	4,3	38.3	0.030 *	-	-	-	-	-	-

802 * Denotes significance at the 5% level

803 **Figure 1**
804

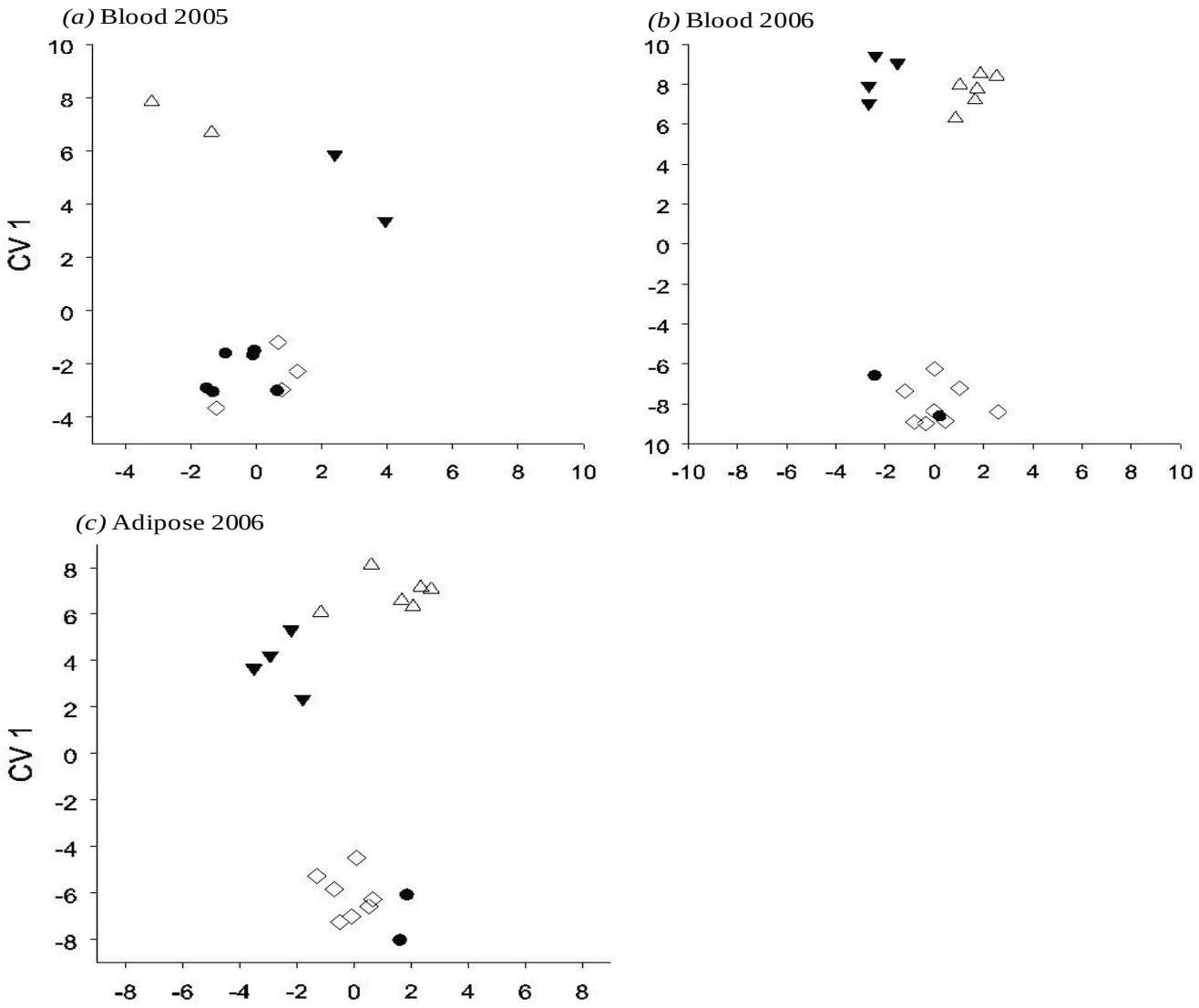
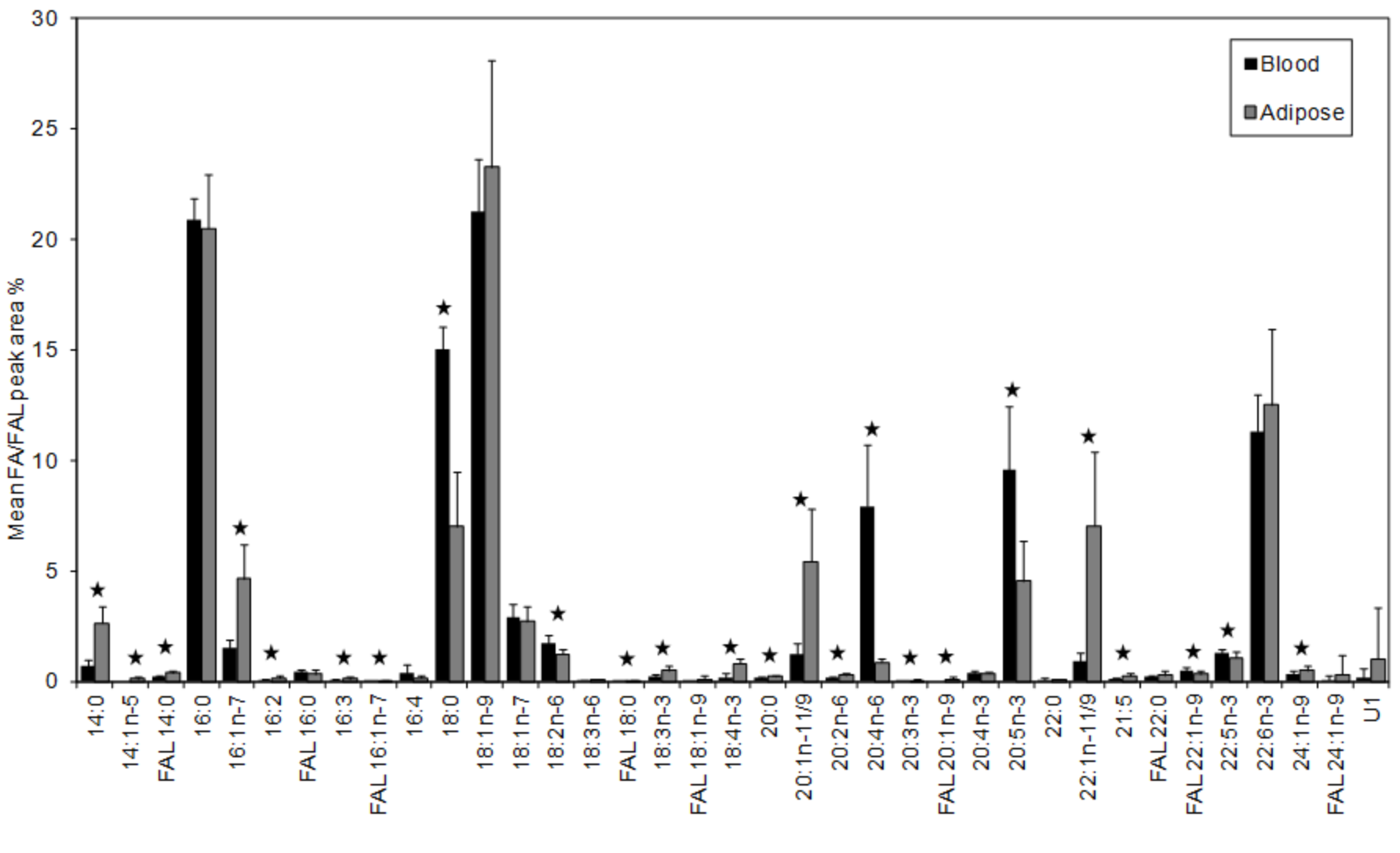
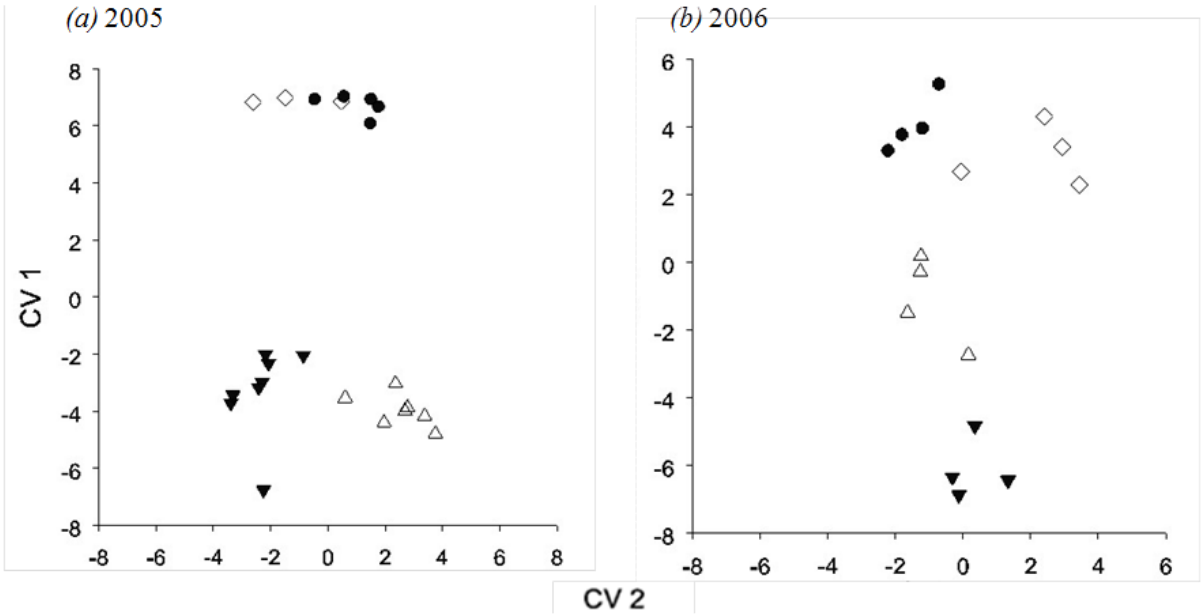


Figure 2

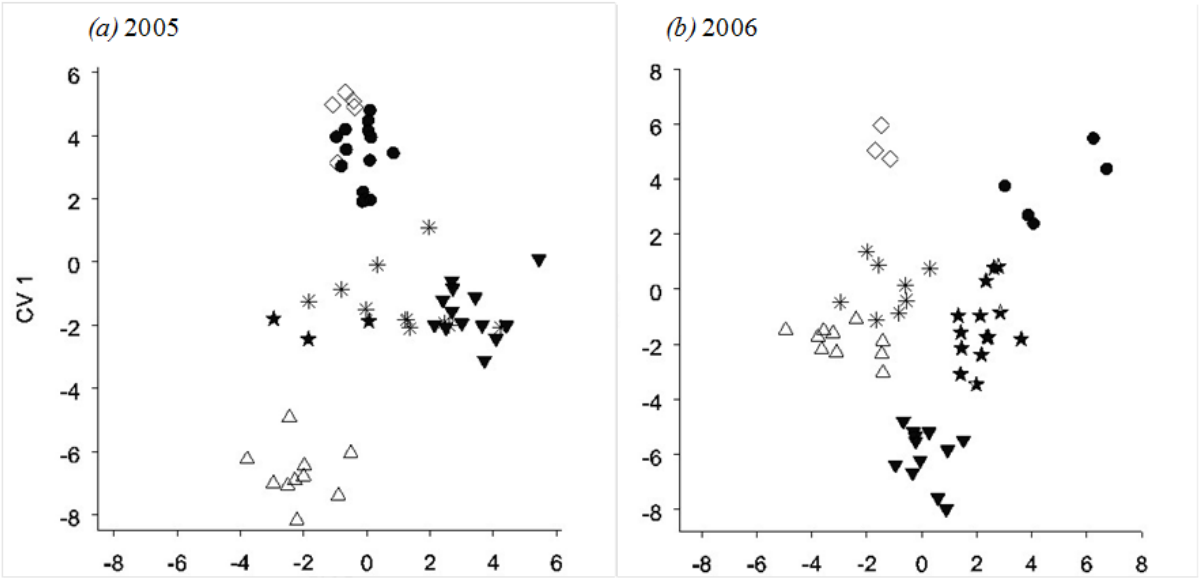

Blood
Adipose
Mean FA/FAL peak area %
0
5
10
15
20
25
30
14:0
14:1n-5
FAL 14:0
16:0
16:1n-7
16:2
FAL 16:0
16:3
FAL 16:1n-7
16:4
18:0
18:1n-9
18:1n-7
18:2n-6
18:3n-6
FAL 18:0
18:3n-3
FAL 18:1n-9
18:4n-3
20:0
20:1n-11/9
20:2n-6
20:4n-6
20:3n-3
FAL 20:1n-9
20:4n-3
20:5n-3
22:0
22:1n-11/9
21:5
FAL 22:0
FAL 22:1n-9
22:5n-3
22:6n-3
24:1n-9
FAL 24:1n-9
U1

807 **Figure 3**
808



809
810

811 **Figure 4**
812



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