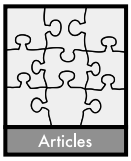


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# Fatty acid signatures of female Brünnich's guillemots *Uria lomvia* suggests reliance on local prey for replacement egg production

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The laying of smaller replacement eggs has been described as a time-saving adaptation because chicks generally grow faster once hatched than inside the egg. However, chicks hatched from smaller eggs have, potentially, lower survival. Consequently, the smaller replacement egg represents a benefit to the female in terms of preserving its own condition at a cost to their offspring. We test these ideas by measuring adult mass changes and plasma lipid concentration changes in male and female Brünnich's guillemots *Uria lomvia* breeding on Coats Island, Nunavut. Though males lost more mass than females, these differences were not significant. Between laying the first and replacement egg, plasma fatty acid concentrations declined in females and increased in males, suggesting that females mobilise less lipid to preserve their condition after laying the replacement egg. In females, plasma lipid concentrations of the dominant fatty acids found in the eggs (16:0 and 18:1) declined between the laying of first and replacement eggs while plasma concentrations of 20:1 increased in both males and females. We compared the fatty acid signatures of first and replacement egg to look for evidence of differences between the lipid sources for their production. Principal component and discriminant function analyses showed that the fatty acid signatures of replacement eggs were closer to the signatures of the local prey than those of first eggs. We suggest that females rely on local sources of energy to a greater degree for the production of the replacement egg than the first egg, but that endogenous reserves of certain nutrients are important for the production of both eggs.

During egg production, birds can either use reserves accumulated within the female (endogenous) or resources obtained directly from the environment (exogenous), with many species using a combination of both strategies (Gauthier et al. 2003, Morrison and Hobson 2004, Williams 2005, Houston et al. 2006). The choice of strategy has important implications for evolutionary fitness because loss of accumulated reserves in birds relying on endogenous stores (''capital breeders'') may reduce adult survival if foraging conditions deteriorate when these supplies need to be replenished. In contrast, for breeders using exogenous stores (''income breeders'') deterioration of foraging conditions at the time of laying can result in delayed laying, smaller clutch size or inability to replace eggs (Hipfner et al. 2003, 2004, Williams 2005, Houston et al. 2006). The loss of the first clutch can compound these issues, depending on whether the replacement clutch is produced from additional endogenous reserves or from exogenous sources, if foraging conditions change between first and replacement clutches (Hipfner et al. 2003, 2004). Arctic breeders are under particular stress because food abundance and the reproductive period are constrained by a narrow seasonal window (Gauthier et al. 2003, Morrison and Hobson 2004). For example, in some Arctic species later-laid eggs, including replacement eggs, are usually smaller with lower yolk to albumin ratios, resulting in lighter but not structurally smaller chicks (Birkhead and Nettleship 1982, 1984, Hipfner et al. 2003). For birds relying on endogenous reserves, we might expect that these symptoms would be reduced, compared to those relying primarily on exogenous nutrients.

Lipids are an important energy source for embryo development (Arnold et al. 1991, Williams 2005). Nonetheless, few studies have examined whether lipids supplied to the egg originate from endogenous or exogenous sources. Although lipid concentrations may not change between first and replacement eggs (Hipfner et al. 2003), the origin of these lipids may change. Because there is conservative transfer of fatty acids in neutral lipids (Lee et al. 1971, but see Budge et al. 2002, Christie 2006), their use as biomarkers in predator-prey relationship studies has been increasing (i.e. Hooker et al. 2001, Wang et al. 2007, Käkelä et al. 2007). For example, whole body fatty acid composition in fish can be used to identify diet of predators at upper trophic levels (Kirsch et al. 1998, Iverson et al. 2002, Beck et al. 2007).

Brünnich's guillemots *Uria lomvia* are highly colonial, cliff-breeding seabirds of Arctic waters. They employ a

life-history strategy that is typical for a marine bird, with several years deferred breeding, a high annual adult survival rate, and a single-egg clutch. Egg-size is comparatively large, the Arctic summer is brief, placing a premium on early breeding, and foraging may be constrained by ice cover at the start of the season (Hipfner et al. 2003, Gaston et al. 2005). Consequently, guillemot eggs are usually considered to be created from endogenous stores, although recent work on other Arctic breeders has shown that exogenous sources may be more important than previously envisioned (Morrison and Hobson 2004, Gauthier et al. 2003). The situation is further complicated for replacement eggs. Previous studies on replacement eggs in guillemots showed that replacement eggs are lighter than first eggs with lower yolk-to-albumen ratio, but with similar proportion (once corrected for egg mass) of eggshell, total protein and total lipids and similar amino acid makeup (Hipfner et al. 2003). Eggs laid by inexperienced females during first breeding attempts are similar in size (Hipfner et al. 2003). Egg size increases and laying date advances with female age and only about 20–30% of females relay after they lose their egg, mainly because those females that lose eggs tend to be young and inexperienced. Consequently, it is unlikely that seasonal reductions in egg size reflect time-saving, evolutionary strategies for murres because: (1) experienced females that lay late, and concurrently with young females, lay full-sized first eggs (Hipfner et al. 1997), (2) a large egg confers a time-saving advantage during posthatching chick development (Hipfner and Gaston 1999, Hipfner et al. 2001a,b), and (3) feeding conditions do not deteriorate late in the season to an extent that the ability of capable parents to raise chicks is affected (de Forest and Gaston 1996, Hipfner 1997, 2001). Thus, physiological constraints, rather than strategic adjustments, are likely more important in limiting egg production in guillemots and potentially other long-lived seabirds. As amino acid and calcium (eggshell proportion) content do not change between first and replacement eggs, it is possible that lipid composition plays an important role in the physiological constraints of guillemots feeding on calcium- and protein-rich marine life (Hipfner et al. 2003).

Here, we use fatty acid analysis on Brünnich's guillemots, to examine whether lipids in replacement eggs are mobilized from endogenous or exogenous reserves. If fatty acids in the first egg derive largely from stored fat, but those in the second egg include a greater proportion of fats derived from the local prey base (because endogenous stores had been depleted by the first egg), we expected to see a change in the fatty acid signature between first and replacement eggs, to more closely approach that characteristic of the local prey base. Based upon theories of life-history strategy, and the observations that egg size is reduced in replacement eggs, we predicted that females would preserve their own body condition at an expense to the offspring, by laying a smaller replacement egg. If male and female diets are similar during the pre-laying period we predicted that their plasma fatty acids should be similar prior to laying, but might differ post-laying, with those fatty acids most important in egg synthesis being depleted in females, relative to their mates.

# Methods

## Experimental design and sample collection

The study was conducted in 2004 at the Brünnich's guillemot colony on Coats Island, Nunavut (62° 57′N, 82° 00′W) in the eastern Canadian low Arctic. We arrived at the colony before the date of mean egg lay and mapped the position of breeding sites on an accessible part of the breeding area (plot S, Hipfner et al. 1997). Breeding sites were observed daily and on the day of egg laying, and replacement egg laying, the incubating adult was caught and approximately 3 ml f blood was withdrawn from the brachial vein using a syringe and 27 gauge butterfly needle. The blood was transferred immediately to a 3 ml vaccutainer lined with heparin. Immediately after capture, adults were weighed with a pesola spring balance ($\pm$1 g). The birds were released within 5 min of capture.

Before the bird was released, the egg was removed and replaced with a painted hard boiled chicken egg. The adult was then released and its behaviour observed to ensure that it began to incubate the chicken egg. All adults accepted the chicken egg as their own, though some took a few extra minutes to begin incubating. We continued to observe the site and after the first incubation change-over, we also caught and blood-sampled the mate. Once both members of the pair had been sampled we removed the chicken egg. Observations of the site recommenced after ten days to observe the laying of a replacement egg, which occurred approximately 14 d later. The same sampling procedure was used after the laying of the replacement egg. Eleven first and replacement eggs were collected from the same breeding sites and eight complete sets of adult plasma (plasma samples from both adults of the pair at first and replacement egg laying) were collected and analysed. A few adults were too wary to be caught twice and in one case, insufficient plasma was recovered after centrifugation.

Upon arriving at the camp (within 1 h), blood samples were centrifuged until sufficient plasma was obtained. The plasma was transferred to cryovials and immediately frozen in a propane freezer ($-20^\circ$ C) and stored there until being shipped to Ottawa in a dry nitrogen shipper. The sex of adult Brünnich's guillemots was identified using DNA markers.

Each egg was weighed and measured (length and breadth $\pm$0.5 mm). The eggs were then cracked and the contents of each egg were scrambled thoroughly with an egg beater until 2 min after the egg appeared homogenous. Approximately 6 ml of each egg were then transferred to cryovials. Egg samples were frozen in a propane freezer ($-20^\circ$ C) and stored until being shipped to Ottawa in a dry nitrogen shipper.

Prey samples were collected opportunistically during the 2003 field season. On many occasions, fresh prey items were found on the cliff ledges. Details of prey species, sample sizes and fatty acid composition are available in Jacobs (2008) and appendix 1. Each item was collected, weighed, measured (fork length) and then frozen in a plastic bag at $-20^\circ$ C until shipping on wet ice in a cooler to Ottawa. Mean masses of different species were similar to

those estimated from larger samples collected at Coats Island 1981–2007 (Elliott and Gaston 2008).

The protocols described here were approved by the Animal Care Committee of the Univ. of Ottawa under the permit BL-172.

### Fatty acid analysis

We transferred 0.05 g of each egg sample into 6 ml of Folch reagent (chloroform: methanol 2:1 v/v). The resulting mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was filtered and KCl (0.25% in distilled water) was added to eliminate water-soluble compounds. This mixture was centrifuged at 3,000 rpm for 10 min to facilitate the separation of an aqueous phase. This phase was eliminated with a water-powered vacuum pump and 2 ml methanol was added to each tube. Samples were frozen at $-20^{\circ}$ C and tubes topped with $N_2$ to prevent oxidation.

Lipid extraction for prey items followed the protocol for egg fatty acid analysis except that the lipids from the whole prey item were extracted and then a subsample of the fat was removed for analysis. This was done because the whole prey items are not homogenous and direct subsampling of a single tissue would have introduced insurmountable error given that some species were not fish. Consequently, only percent composition was analysed.

Plasma was then added to Folch reagent (2:1 v/v). An internal standard (Heptadecanoic acid, 17:0, 30 mg/100 ml in hexane) was added for gas chromatography (GC) analysis of the non-esterified fatty acids (NEFA). The resulting mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was filtered and KCl (0.25% in distilled water) was added to eliminate water-soluble compounds. This mixture was centrifuged at 3,000 rpm for 10 min to facilitate the separation of an aqueous phase. This phase was eliminated with a water-powered vacuum pump and the remaining solvent was evaporated under $N_2$ at $70^{\circ}$ C.

Total lipids were separated into neutral lipids (NL), non-esterified fatty acids (NEFA) and phospholipids (PL) to analyse their fatty acid composition. Supelclean solid-phase extraction tubes (LC $NH_2$, 100 mg: Sigma) were conditioned with hexane. The fats were resuspended in choloroform and transferred into the columns. The NL fraction was eluted by flowing chloroform:isopropanol (2:1 v/v) through the columns. The NEFA fraction was eluted by flowing isopropyl ether:acetic acid (98:2 v/v) through the columns. The PL fraction was eluted by flowing methanol through the columns. An internal standard (17:0, 30 mg/100 mL hexane) was added to each lipid fraction. The solvent from all samples was evaporated under $N_2$ at $70^{\circ}$ C and the lipids were resuspended in Folch reagent and frozen at $-20^{\circ}$ C with $N_2$ until methylation.

Methylation is required to increase the volatility of the lipid samples for GC analysis. NEFA were resuspended in methanol (100 ul), dimethoxypropane (1 ml) and 6N HCl (50 ul). The mixture was vortexed and incubated at room temperature for 20 min and evaporated under $N_2$ at $70^{\circ}$ C. The methylated fats were resuspended in isooctane and transferred into GC autosampler tubes.

Fats were resuspended in an acetyl chloride solution (7.2 ml acetyl chloride in 100 mL methanol). The samples were incubated at $90^{\circ}$ C for 2 h after which the acetyl chloride in methanol was evaporated under $N_2$ at $70^{\circ}$ C. The fats were resuspended in methanol and evaporated under $N_2$ at $70^{\circ}$ C. The fats were resuspended in isooctane and transferred to GC autosampler tubes.

All samples were then analysed using Gas Chromatography (HP 5890 series II with HP 7673 autosampler and flame-ionization detector). The retention times of the fatty acids were compared with those from known standards and the concentration of each fatty acid was determined by comparing the size of the peak of the internal standard with that of all the other fatty acids. The analyses of two plasma samples were incomplete as no NL (male after first lay), or PL (male after replacement lay) were detected. These samples were excluded from the statistical analysis.

### Statistical analyses

Fatty acid concentrations in plasma, eggs, and prey were analysed using MANOVA with all fatty acid concentrations as dependents within the same analysis. Individual analyses of fatty acids were done in a univariate posthoc test of MANOVA and Bonferroni adjustments were made for multiple comparisons. To compare the fatty acid signatures of first and replacement eggs, univariate analyses do not account for interactions in variability and therefore, principal component analysis (for variable reduction) and discriminant function analysis are required (Grahl-Nielsen 1999). Fatty acid signatures of eggs and female plasma were compared using either only a discriminant function analysis, or a principal component analysis (PCA) with eigenvalues of greater than 1.0 followed by discriminant function analysis on the first three factors to identify if the there were significant differences in the fatty acid signatures. The results from both of these approaches are presented because a discriminant function analysis generally should not be conducted when there are fewer sample replicates than variables. Consequently, data reduction techniques, such as a PCA may be favourable in this case. For comparison of fatty acid signatures of eggs and plasma, and of eggs and prey, arcsine transformed percent contributions of each of the fatty acids were used. All statistical analyses were conducted using SYSTAT version 8 software and the level of significance was set at 0.05 when not indicated otherwise.

## Results

### Adult mass changes

In 2004, all but two males ($n=8$) lost mass while five females ($n=8$) gained mass between first and replacement egg laying (average mass change males: $-18\,g \pm 34$; females: $-13\,g \pm 38$). Males did not lose significantly more mass than females ($F_{1,14}=0.1$, $P=0.79$), nor were there differences in total mass between the sexes ($F_{1,14}=1.2$, $P=0.29$; Table 1).

Table 1. Body mass of breeding Brünnich's guillemots (n = 16), and measurements of first and replacement eggs (n = 22) at Coats Island in 2004. Values presented are means (±SE). Results of ANOVAs for differences between means are also presented.

| | Male mass (g) | Female mass (g) | Mass (g) | Length (mm) | Width (mm) | Volume[a] ($mm^3$) | Lipid concentration (mg/ml) |
|---|---|---|---|---|---|---|---|
| First eggs | 989 (31) | 955 (55) | 109.4 (2.3) | 80.5 (0.9) | 50.6 (0.5) | 206211 (4395) | 42.16 (3.24) |
| Replacement eggs | 971 (41) | 942 (62) | 103.5 (2.6) | 79.6 (0.7) | 49.6 (0.6) | 196084 (5260) | 39.44 (2.46) |
| % Difference | 1.02 | 1.01 | 5.3 | 1.1 | 2.0 | 4.9 | 7.1 |
| F | 0.99 | 0.2 | 5.36 | 1.42 | 2.99 | 4.2 | Table 2 |
| P[b] | 0.34 | 0.66 | 0.03 | 0.25 | 0.10 | 0.05 | Table 2 |

[a]Volume = (length × width$^2$).
[b]With the Bonferroni correction for multiple comparisons, the level of significance is set at P = 0.013.

## Egg measurements

First laid eggs were heavier, wider, and longer and greater in volume index (mean difference 5%) than replacement eggs, though the differences were not significant (Table 1).

## Egg fatty acids

Phospholipids accounted for 50% of the total fatty acid concentration, while NL and NEFA accounted for approximately 49 and 1% respectively. Fatty acids 16:0 and 18:1 were highest in concentration in both the first and replacement eggs. The only fatty acid to show a significant change in concentration between first and replacement eggs was 20:1 (Table 2), which made up <3% of total lipids. This suggests that the fatty acid composition of eggs is highly conserved (Fig. 1).

Results from the principal component analysis of fatty acid concentrations (mg/ml) are presented in Fig. 2. The first three factors accounted for 68% of the variation in the data. Fatty acid signatures of first and replacement eggs were separated along the component 2 axis. The two fatty acids with the highest loading in this component were 20:1 and 22:1 and these were the only ones which increased in concentration in the eggs (Table 2). Consequently, the fatty acid signatures of first and replacement eggs were differentiated by changes in the concentration of 20:1 and 22:1. Conducting a discriminant function analysis on data with a large number of variables can decrease the power of the analysis and is inadvisable in this case, but there was no significant difference in the fatty acid signatures between first and replacement eggs ($F_{1,15} = 1.54$, $P = 0.31$). However, a discriminant function analysis of the first, second, and third factors of the principal component analysis showed that there was a significant difference in the loadings of these factors between first and replacement eggs ($F_{1,3} = 4.36$, $P = 0.03$) and that they are significantly spread.

## Plasma fatty acids

There was a significant interaction between sex and treatment (after first or replacement egg laying) on total plasma fatty acid concentrations ($F_{1,26} = 6.25$, $P = 0.02$). The total plasma lipid concentration was significantly higher in females than males after laying the first egg ($F_{1,13} = 10.3$, $P = 0.007$) and suggests that females were mobilising more lipids than males at this time. In females, the concentration of plasma lipids decreased between first and replacement egg laying. In males, plasma lipid concentrations increased. After laying the replacement egg, there was no significant difference in the plasma concentrations of lipids between males and females ($F_{1,13} = 0.04$, $P = 0.84$; Fig. 3).

In males, the fatty acid composition was similar after the laying of the first and replacement eggs (Fig. 4). However,

Table 2. Fatty acid concentrations (mg/ml ± SE) of first and replacement eggs of Brünnich's guillemots breeding at Coats Island in 2004 (n = 22).

| Fatty acid | Mean concentration (mg/g) | | $F_{1,20}$ | P |
|---|---|---|---|---|
| | Egg 1 (SE) | Egg 2 (SE) | | |
| 14:0 | 0.19 (0.03) | 0.17 (0.02) | 0.10 | 0.76[a] |
| 16:0 | 10.09 (0.83) | 9.08 (0.71) | 0.61 | 0.44 |
| 16:1 | 0.20 (0.05) | 0.37 (0.12) | 2.00 | 0.17 |
| 18:0 | 2.34 (0.12) | 2.17 (0.06) | 1.13 | 0.30 |
| 18:1 | 21.34 (1.72) | 19.92 (1.49) | 0.24 | 0.63 |
| 18:2 | 0.22 (0.04) | 0.21 (0.03) | 0.03 | 0.86 |
| 20:1 | 0.49 (0.06) | 1.02 (0.08) | 26.24 | <0.001 |
| 20:4 | 1.81 (0.80) | 1.02 (0.09) | 0.96 | 0.34 |
| 22:1 | 0.07 (0.02) | 0.12 (0.01) | 5.74 | 0.03 |
| 20:5 | 2.29 (0.11) | 2.22 (0.09) | 0.45 | 0.51 |
| 22:5 | 0.51 (0.04) | 0.42 (0.04) | 2.50 | 0.13 |
| 22:6 | 2.54 (0.15) | 2.64 (0.09) | 0.23 | 0.64 |
| Total egg fat | 42.16 (3.24) | 39.44 (2.46) | 0.46 | 0.51 |

[a]With the Bonferroni correction for multiple comparisons, the level of significance is set at P = 0.004.

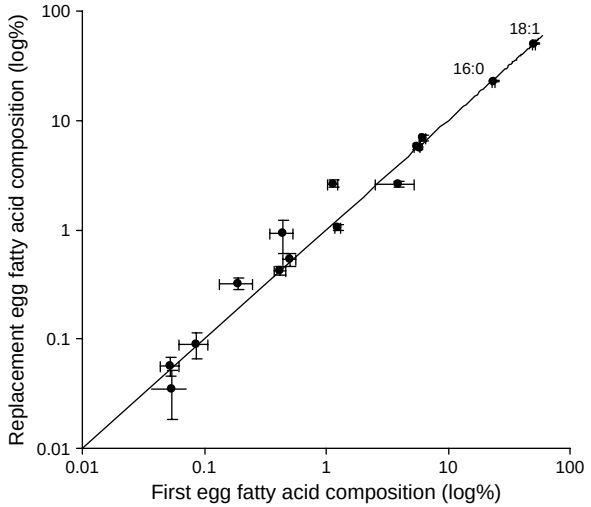


Figure 1. Fatty acid composition (% ± SE) for individual fatty acids for first versus replacement eggs for Brünnich's guillemots at Coats Island in 2004. Fatty acids 16:0 and 18:1 are identified. A trendline representing the equation x = y is presented for comparison of values.

the concentrations of 16:0 and 18:1 increased significantly (16:0: $F_{1,12} = 8.29$, $P = 0.01$, 18:1: $F_{1,12} = 15.05$, $P = 0.002$). In females, those fatty acids found in the highest concentration in the eggs decreased in concentration significantly between first and replacement egg laying (16:0: $F_{1,14} = 10.94$, $P = 0.01$, and 18:1: $F_{1,14} = 4.89$, $P = 0.04$, Fig. 4). These comparisons show that 16:0 and 18:1, which make up a high proportion of egg lipids, are depleted in females after laying the replacement egg (Fig. 5).

The percent contribution of each fatty acid to the total lipid composition show some differences in males versus females after laying the first and replacement eggs. Females showed higher percent contributions of both 16:0 and 18:1 after laying the first egg, which were then depleted in females after laying the replacement egg. Both the contributions of 16:0 and 18:1 declined to below that of males. In addition, males had higher contributions of the fatty acid 20:5 than females (after first egg: $F_{1,13} = 6.65$, $P = 0.02$; after replacement egg: $F_{1,13} = 7.60$, $P = 0.02$).

Based upon the changes in fatty acid composition of first and replacement eggs, and the principal component analysis of those data, we compared the changes in the concentration of fatty acids 20:1 and 22:1 in the plasma of the adults. Changes in the concentration of fatty acids in the first and replacement eggs are reflected in one of the two fatty acids in the adult plasma. Plasma concentrations of 20:1 increased significantly in males ($F_{1,12} = 6.83$, $P = 0.02$), but not in females ($F_{1,14} = 1.26$, $P = 0.28$).

A discriminant function analysis using the raw plasma fatty acid concentrations of females showed that there is no significant difference in the fatty acid signatures between laying the first and replacement eggs ($F_{14} = 0.35$, $P = 0.9$). A discriminant function analysis using the first three PCA factors shows that there is a significant difference in the fatty acid signatures of females after laying the first egg and after laying the replacement egg ($F_3 = 4.09$, $P = 0.03$).

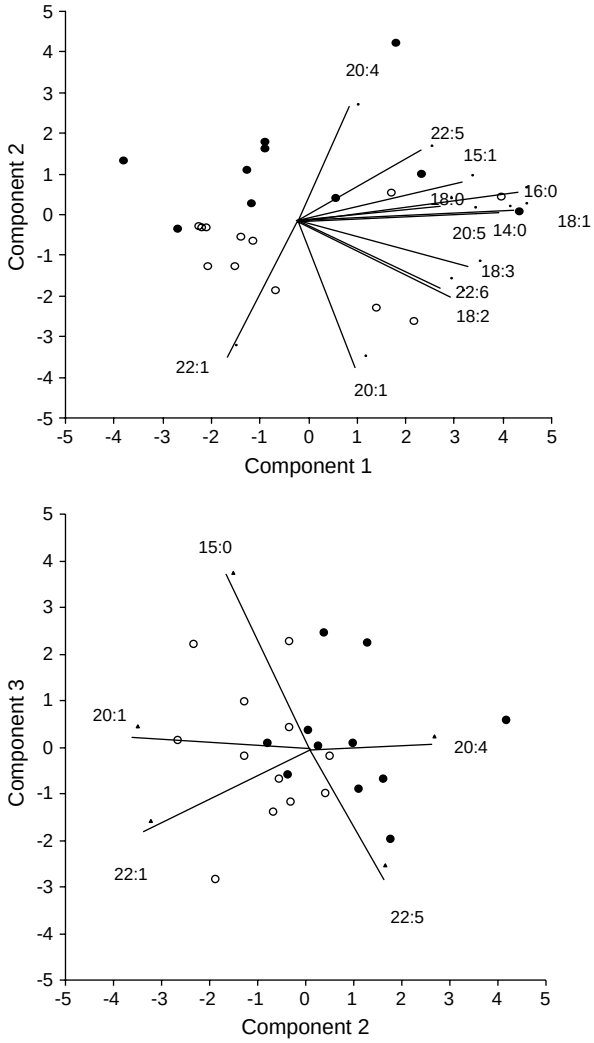


Figure 2. Components (a) 1 vs. 2, and (b) 2 vs. 3 for first (dark circles) and replacement eggs (open circles) from a principal component analysis for Brünnich's guillemots at Coats Island in 2004. All fatty acids with component loadings of less than 0.5 for both components are excluded from the figure.

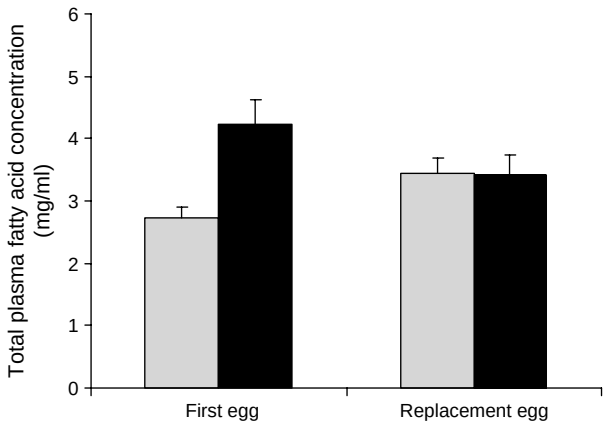


Figure 3. Total plasma lipid concentrations (mg/ml ± SE) of male (light bars), and female (dark bars) Brünnich's guillemots just after laying the first and replacement eggs at Coats Island in 2004.

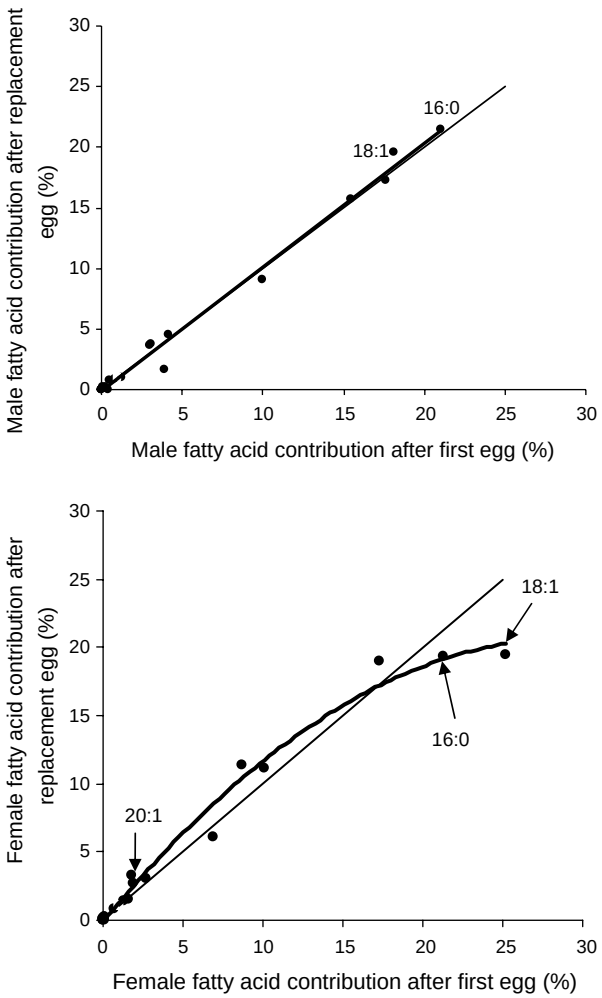


Figure 4. Plasma fatty acid composition (% of each fatty acid) for (a) male (trendline in bold, $r^2 = 0.98$, $y = 0.98x - 0.11$), and (b) female (trendline in bold, $r^2 = 0.99$, $y = -0.02x^2 + 1.42x - 0.13$) Brünnich's guillemots after laying of the first and replacement egg at Coats Island in 2004. Equation $x = y$ is also presented (fine line).

### Prey fatty acids

Species varied significantly in percent total 20:1 ($F_{8,15} = 3.21$, $P = 0.036$, Appendix 1). Of the species analysed, capelin, sandlance, and squid had the highest percent 20:1 (Appendix 1). Other fatty acids showed no significant differences among species ($F_{8,15} < 2.44$, $P > 0.05$). All prey species had high concentrations of 16:0, 18:1, 20:5 and 22:6 (Appendix 1).

Principal component analysis showed that the fatty acid signatures of replacement eggs were closer to the fatty acid signatures of prey than those of first laid eggs. The first and second components accounted for 54 and 10% of the variation respectively. There was still quite a separation between eggs and prey as they are very different tissues (Fig. 6).

## Discussion

Although our data did not show significant differences in the size of first and replacement eggs, studies with larger sample sizes have shown that first and replacement egg dimensions differ significant and that the magnitude of the difference is similar to that observed in our study (e.g. 6%, Hipfner et al. 2003).

Plasma lipid concentrations in females just after laying the first egg were higher than just after laying the replacement egg (Fig. 3). This suggests that females were mobilising fewer lipids after laying the replacement egg than after laying the first. Production of the first and second eggs both take 14–16 d despite the smaller size of second eggs (Hipfner et al. 1997) showing that energy mobilization rate must be slower for the second egg. The relative contribution of yolk is the same or smaller in the replacement eggs (Hipfner et al. 2003), and lipid content did not differ significantly between first (4.2%) and replacement eggs (3.9%). These differences, along with no change in adult mass, suggest that females preserve their own condition at a cost to the chick when producing the replacement egg. Chicks hatched from larger eggs show faster wing feather growth and depart from the colony at a younger age than those from smaller eggs, presumably leading to better survival after departure (Hipfner and Gaston 1999).

The local availability of prey may influence the decision on whether to lay smaller eggs more quickly or delay laying in order to produce a larger egg. For example, in one year, the egg size of Brünnich's guillemots increased with date throughout the laying period (Gaston and Nettleship 1981), and was linked with evidence of unusually abundant prey. The effects of prey availability (lipids and nutrients) during egg production may be particularly challenging because most high latitude birds time reproduction so that chick-rearing coincides with maximum prey availability (Williams 2005). The apparent depletion of 16:0 and 18:1 in females forced to lay a replacement egg (Fig. 4) suggests that certain fatty acids may be limiting in egg production. This would place a premium on selecting prey with a high 16:0 and 18:1 content.

The apparent depletion of 16:0 and 18:1 in females concurrent with their high concentration in first and replacement eggs provides some support for a contribution of endogenous stores in replacement egg production; 16:0 showed little variability among prey items, suggesting that a dietary switch could not be responsible for the change (Appendix 1). Only those fatty acids found in high concentration within the eggs decreased in female plasma between the two layings, with a change in the opposite direction in males. There is a 4–5 d lag between the end of the deposition of lipids in the yolk and the time of laying in alcids (Astheimer 1986), therefore concentration of plasma fatty acids of the females after lipid deposition to the yolk reflects energy mobilisation for activities other than egg production. Unlike females, in males all fatty acids which changed in concentration increased, resulting in increased plasma fatty acid concentration after the laying of the replacement egg, possibly reflecting increased exercise (see review: McWilliams et al. 2004). It is possible that males have to increase their exercise between layings in order to ensure that the site is occupied full-time during the period when the female is recharging her nutritional condition; a shorter at-sea period may require proportionately greater exercise while at-sea. The inter-gender differences we observed in mass and lipid allocation in response to egg

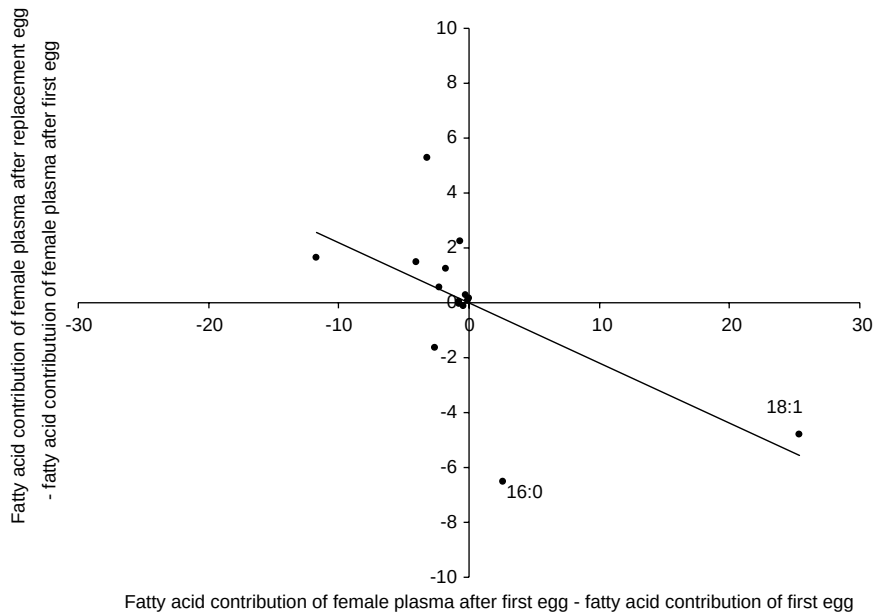


Figure 5. Comparison of changes in percent lipid composition between female Brünnich's guillemot plasma (after laying the first egg) and the first egg, and differences in percent lipid composition of female plasma after laying the first egg and the replacement egg at Coats Island in 2004 ($y = -0.219x - 0.015$, $r^2 = 0.37$).

production mirror the inter-gender differences in response to chick-rearing by penguins, where males rely on endogenous stores and females on exogenous stores (Green et al. 2007).

The fatty acid signatures of first and replacement eggs differed in the concentrations of 20:1 and 22:1, with the signatures of replacement eggs being closer to those of the prey than first eggs (Fig. 6). When dominant fatty acids were removed from the calculation of percent contribution, 20:1 increased from 4.6% to 9.8%, and 22:1 increases from 0.7% to 1.2% in eggs (Table 2), and 20:1 increased significantly in the plasma, from 13% to 20%. All local prey items had higher representation of 20:1 than was found in the eggs, suggesting that replacement eggs were produced from nutrients originating in the local prey base; capelin, squid and sand lance have particularly high 20:1 percent composition (Henderson et al. 1984, Lea et al. 2002, Jacobs 2008) and may have played an important role in the nutrition of females during replacement egg production. Further evidence that the increase in 20:1 represents a change in the prey base is shown by its increase in male plasma. Exogenous sources of energy for egg production have been shown to be more important than thought previously in other avian species (Bromley and Jarvis 1993, Perrins 1996, Hobson et al. 1997, Gauthier et al. 2003, Morrison and Hobson 2004), and it is likely that this applies also to Brünnich's guillemots.

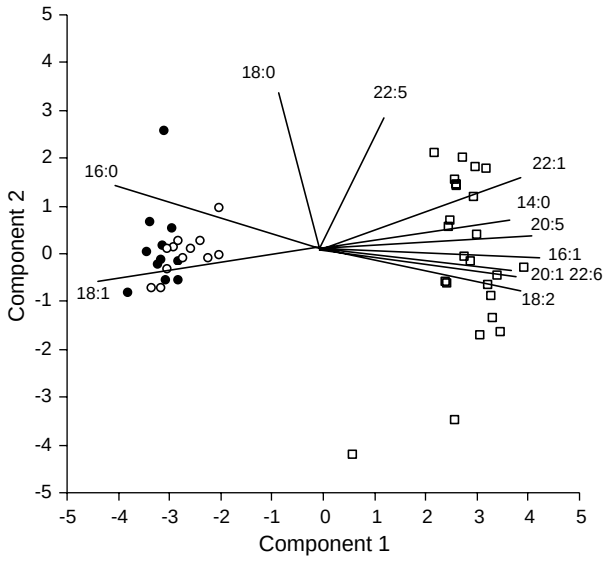


Figure 6. Components 1 vs. 2 for first (dark circles) and replacement (open circles) eggs and prey (open squares) from a principal component analysis of Brünnich's guillemots breeding at Coats Island in 2004.

The information available on adult diet in Brünnich's guillemots in the eastern Canadian Arctic comes from stomach content analysis (Gaston and Bradstreet 1993, Gaston and Hipfner 2000), and stable isotopes (Woo et al. 2008). Stomach content analysis is limited in temporal resolution and by differential digestibility of prey items (Gaston and Bradstreet 1993, Iverson et al. 2002, Beck et al. 2007) while stable isotopes are limited in their ability to resolve multiple, similar prey inputs (Beck et al. 2007, Käkelä et al. 2007, Woo et al. 2008). Fatty acid analysis provides an opportunity to complement these techniques, as many fatty acids are conserved and the multiple fatty acids available allow for the resolution of many prey inputs (Hooker et al. 2001, Iverson et al. 2002, Wang et al. 2007, Käkelä et al. 2007). For example, the diets of Brünnich's guillemots differ between high and low Arctic populations (Gaston and Bradstreet 1993, Gaston and Hipfner 2000), which is reflected in differences in fatty acid signatures (Jacobs 2008). As males had higher percent contribution of 20:5, which was highest in amphipods, our fatty acid results

suggest that males may consume a higher percentage of amphipods, a result that was not apparent from previous analyses of stomach contents and stable isotopes. This observation is consistent with the males' nocturnal feeding schedule (Kober and Gaston 2003), as amphipods are more likely to be encountered at night due to diel vertical migration. Also, males provision their chicks with amphipods more often than females do (KHE unpubl. data).

Nonetheless, interpretation of diet through fatty acid signatures is complex, due to differential conservation of different fatty acids (Iverson et al. 2002, Pierce and McWilliams 2005, Wang et al. 2007). For example, individual variation in fatty acid signatures can be due to geographic (Budge et al. 2002), or dietary (Kirsch 1998, Iverson et al. 2002, Pierce and McWilliams 2005) effects, and there is an interaction between these two (Budge et al. 2002). The importance of differential fatty acid conservation is emphasized in our study. In adult plasma, fatty acids 16:0, 18:0, 18:1, 20:5, and 22:6 account for 83% of all lipids, and in eggs, fatty acids 16:0 and 18:1 account for 75% of all lipids. These fatty acids are highly conserved because their concentration and percent contribution do not change. In the prey, percent contributions of 16:0, 18:0, and 18:1 (except for sculpin), were lower than in the plasma and percent contributions of 16:0 and 18:1 were also lower than in eggs (Appendix 1). Brünnich's guillemots are therefore either: 1) preferentially selecting these fatty acids from their energy stores, or 2) creating them *de novo* to generate the appropriate concentrations. Consequently, we suggest that these fatty acids are essential for either survival (in adult plasma), or chick development (in eggs) and that those fatty acids found in lower concentrations can be used as biomarkers to identify changes in diet (see Kirsch et al. 1998, Iverson et al. 2002).

We show that the fatty acid compositions are different in both first and replacement eggs and adult plasma, likely reflecting an exogenous source for replacement egg production. Two possibilities exist to explain the differences in fatty acid composition between first and replacement eggs: 1) females rely upon lipid sources close to the breeding colony for the production of both the first and replacement eggs but the composition of prey species changes during the time of the deposition of lipids, 2) females make substantial use of lipid sources obtained at the wintering grounds by storing that lipid during migration for the production of the first egg and use a greater proportion of lipid sources near the breeding colony for the production of the second egg. The time for the development of the first egg in guillemots is about 14 d (Hatchwell and Pellatt 1990), that they generally arrive on the breeding colony several weeks before median egg laying (Gaston and Hipfner 2000), that migration is an energetically costly activity (McWilliams et al. 2004), and that storing lipid for use after migration may present little adaptive advantage. Consequently, it seems likely that females rely upon local food sources for the development of both eggs, that the reliance is greater for the second egg and that for both eggs endogenous stores are important for certain elements.

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Appendix 1. Percent fatty acid composition (±SE) of prey items collected off the ledges at the Coats Island Brünnich's guillemot colony in 2003. Sample sizes in parentheses (pooled value from several individuals for amphipods).

| Species | Percent fatty acids composition | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Amphipod (1.3 g) | Capelin (5) | Cod (3) | Fish Dr. (1) | Sandlance (4) | Sculpin (5) | Shanny (3) | Shrimp (1) | Squid (1) |
| 14:0 | 2.3 | 1.6 (0.4) | 1.3 (0.4) | 0.9 | 1.7 (0.2) | 1.2 (0.3) | 1.1 (0.2) | 1.3 | 1.4 |
| 15:0 | 0.3 | 0.1 (0.1) | 0.1 (0.1) | 0 | 0.1 (0.1) | 0.1 (0.0) | 0.2 (0.1) | 0.4 | 0.4 |
| 15:1 | 1.2 | 1.4 (1.4) | 0 | 0 | 0 | 0.5 (0.5) | 0 | 0 | 0 |
| 16:0 | 15.0 | 12.5 (1.2) | 15.0 (1.0) | 14.0 | 14.1 (0.5) | 12.1 (2.2) | 12.9 (0.4) | 13.0 | 13.8 |
| 16:1 | 5.7 | 6.7 (1.3) | 10.1 (1.7) | 7.4 | 7.3 (0.7) | 9.0 (1.4) | 10.6 (1.1) | 7.3 | 7.1 |
| 18:0 | 6.3 | 4.7 (0.3) | 5.2 (0.8) | 6.6 | 6.1 (0.8) | 5.1 (0.8) | 4.4 (0.3) | 6.8 | 2.5 |
| 18:1 | 16.3 | 11.9 (0.5) | 17.6 (1.4) | 18.0 | 10.8 (1.2) | 26.5 (8.8) | 18.2 (1.4) | 18.9 | 19.4 |
| 18:2 | 2.8 | 1.6 (0.2) | 1.4 (0.4) | 1.9 | 1.4 (0.3) | 1.8 (0.1) | 1.7 (0.2) | 1.1 | 1.2 |
| 20:1 | 3.5 | 10.7 (2.1) | 7.4 (0.7) | 2.8 | 9.6 (0.7) | 4.8 (1.2) | 6.1 (1.4) | 3.4 | 12.9 |
| 22:0 | 0 | 0.1 (0.0) | 0.5 (0.1) | 0.2 | 0.1 (0.0) | 0.1 (0.1) | 0.2 (0.1) | 0.2 | 0.5 |
| 20:4 | 2.2 | 1.8 (1.4) | 0.5 (0.2) | 1.4 | 0.3 (0.2) | 0.6 (0.3) | 2.1 (0.6) | 2.5 | 0.9 |
| 22:1 | 2.3 | 9.7 (2.5) | 8.2 (3.0) | 7.1 | 12.3 (3.5) | 4.1 (2.1) | 8.2 (3.1) | 14.7 | 3.0 |
| 20:5 | 22.7 | 14.2 (1.4) | 15.2 (1.5) | 20.7 | 14.2 (1.7) | 14.8 (2.5) | 17.2 (0.4) | 19.6 | 15.1 |
| 22:5 | 5.2 | 1.5 (0.8) | 3.2 (2.1) | 3.1 | 3.8 (1.8) | 2.1 (1.1) | 4.2 1.1) | 3.2 | 0 |
| 22:6 | 13.8 | 21.3 (2.6) | 14.1 (2.2) | 15.6 | 17.7 (2.1) | 17.0 (3.6) | 11.0 (3.5) | 7.5 | 21.5 |