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NOTE

Egg yolk fatty acids as a proxy to quantify diets of female Spectacled Eiders (*Somateria fischeri*)

Shiway W. Wang, Tuula E. Hollmén, and Sara J. Iverson

Abstract: Determining the diets of threatened Spectacled Eiders (*Somateria fischeri* (Brandt, 1847)) in relation to life-history stages will provide information to help identify and characterize their critical habitats. Quantitative fatty acid signature analysis (QFASA) is a novel tool that estimates the proportion of diet items in consumers from their fat depots. We conducted feeding experiments to validate the use of QFASA to estimate the mixed diets of captive female Spectacled Eiders using egg yolk fatty acids (FA) collected in 2008 and 2009. Calibration coefficients (CCs) for individual FA were developed to account for FA modification (due to eider lipid metabolism) from diets of eiders into egg yolk. We also compared the FA profiles between fertile and infertile eggs. Egg yolk FA profiles did not differ significantly between infertile and fertile eggs collected in either year. Using the CCs developed from eggs collected in 2008, QFASA closely estimated the 2009 diet composition of eiders. We conclude that using infertile eggs has the potential to provide a noninvasive method to elucidate diets of breeding female Spectacled Eiders and possibly other avian species, and to provide insight into understanding the sources (i.e., marine wintering or freshwater breeding habitat) and timing (i.e., prebreeding or breeding) of nutrient acquisition during reproduction.

Key words: fatty acid, QFASA, diet estimation, foraging ecology, egg yolk, Spectacled Eider, *Somateria fischeri*.

Résumé : La détermination du régime alimentaire des eiders à lunettes (*Somateria fischeri* (Brandt, 1847)), une espèce menacée, selon l'étape du cycle de vie fournira de l'information qui aidera à cerner et caractériser leurs habitats essentiels. L'analyse quantitative de la signature des acides gras (QFASA) est un nouvel outil pour estimer la proportion des différents composants du régime alimentaire chez les consommateurs à la lumière de leurs dépôts de graisse. Nous avons mené des expériences d'alimentation dans le but de valider la pertinence d'utiliser la QFASA pour estimer les régimes mixtes d'eiders à lunettes femelles en captivité en utilisant des acides gras (AG) de jaunes d'œufs prélevés en 2008 et 2009. Des coefficients d'étalonnage (CE) pour les différents AG ont été établis pour rendre compte de la modification des AG (en raison du métabolisme des lipides des eiders) durant le processus allant de l'alimentation des eiders à la formation des jaunes d'œufs. Nous avons également comparé les profils d'AG d'œufs fécondés et non fécondés. Les profils d'AG de jaunes d'œufs fécondés et non fécondés prélevés durant l'une ou l'autre des années n'étaient pas significativement différents. En intégrant les CE établis pour les œufs prélevés en 2008, la QFASA estimait avec exactitude la composition du régime des eiders en 2009. Nous en concluons que l'utilisation d'œufs non fécondés pourrait constituer une méthode non invasive permettant de faire la lumière sur les régimes alimentaires de femelles reproductrices d'eider à lunettes et possiblement d'autres espèces d'oiseaux, et fournit de l'information pour la compréhension des sources (c.-à-d. habitat d'hivernage en mer ou de reproduction en milieu d'eau douce) et du moment (c.-à-d. avant ou durant l'accouplement) de l'acquisition de nutriments durant la reproduction. [Traduit par la Rédaction]

Mots-clés : acide gras, QFASA, estimation du régime alimentaire, écologie de l'alimentation, jaune d'œuf, eider à lunettes, *Somateria fischeri*.

Introduction

The Spectacled Eider (*Somateria fischeri* (Brandt, 1847)) was listed as threatened under the provisions of the U.S. *Endangered Species Act* in the 1990s (Federal Register 1993). Changes in the dominant clam biomass in the Bering Sea related to the 1976–1977 and 1989 oceanic regime shifts (National Research Council 1996; Hare and Mantua 2000) leading to variation in the availability of food resources may be factors in the recovery of this species (U.S. Fish and Wildlife Service 1996). Spectacled Eiders winter and stage in marine habitats before breeding, and migrate to freshwater tundra habitats to reproduce (Petersen et al. 2000). Their breeding success may be influenced by cross-seasonal effects, as their breeding outcome (i.e., egg laying) likely depends on availability of adequate marine resources prior to arrival on the breeding grounds, but information about timing and sources of critical nutrient acquisition to reproduction is lacking for this species (Petersen et al. 2000). Determining the diet preferences and foraging habitat selection of Spectacled Eiders in relation to seasonal and life-history stages will provide information to help identify and characterize critical habitats of this threatened population.

Fatty acids (FA) have been used to qualitatively infer foraging patterns of marine birds from their adipose tissue (Raclot et al. 1998; Dahl et al. 2003; Connan et al. 2007*a*; Iverson et al. 2007; Williams et al. 2008; Käkelä et al. 2009; Wang et al. 2009; Maranto et al. 2011; Karnovsky et al. 2012; Owen et al. 2013). While using FA profiles of consumers alone only gives a qualitative description of

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consumer diets, using a comprehensive database of potential FA signatures from diet items and accounting for consumer FA metabolism, the relative proportions of different prey consumed in the diet over a period of time (e.g., 2–4 weeks) can be estimated using quantitative fatty acid signature analysis (QFASA) (Iverson et al. 2004). This approach has been validated using adipose tissue from seabirds (Iverson et al. 2007) and sea ducks, including Spectacled Eiders (Wang et al. 2010). QFASA estimates the relative proportion of prey items by calculating the weighted mixture of FA signatures of diet items that most closely resembles that of the consumer's adipose tissue after accounting for metabolism effects, and then uses the relative fat content of each diet item to translate the signature mix into a diet estimate. Rates of biosynthesis, deposition, and metabolism of specific FA within a consumer affect the interpretation of FA data and complicate QFASA estimations of diet. FA signatures of diet items will not exactly match that found in the consumer tissues because of consumer lipid metabolism (i.e., the proportion of some FA observed in the consumer may always be higher, or lower, than that found in diet; Iverson et al. 2004). To account for modification of FA from lipid metabolism, the ratio of an individual FA in the consumer relative to that in the long-term diet is calculated as calibration coefficients (CCs). CCs are calculated for individual FA by feeding consumers a known diet until complete turnover of FA is thought to have occurred, at which point consumer tissue FA composition will most closely represent that of its diet. These CCs are then used in the QFASA model to weight individual FA in diet estimation procedures (Iverson et al. 2004, 2007; Wang et al. 2010). In marine birds, CCs have been developed from adipose tissue of captive Common Murre (*Uria aalge* (Pontoppidan, 1763)) chicks (Iverson et al. 2007), Spectacled Eider and Steller's Eider (*Polysticta stelleri* (Pallas, 1769)) adults (Wang et al. 2010), Tufted Puffin (*Fratercula cirrhata* (Pallas, 1769)) chicks (Williams et al. 2009), Rhinoceros Auklet (*Cerorhinca monocerata* (Pallas, 1811)) chicks (A.S. Kitaysky unpublished data), adipose tissue and blood plasma of Yellow-legged Gull (*Larus michahellis* Naumann, 1840) adults (Käkelä et al. 2009, 2010), and from the egg yolk of Gentoo Penguins (*Pygoscelis papua* (Forster, 1781)) (Polito et al. 2012).

In addition to CCs, appropriate subset(s) of FA must be selected for use in diet estimations, as not all FA provide equal information about diet due to FA origin (i.e., predominantly from diet versus produced de novo by the consumer), consumer metabolism, and levels found in tissue (i.e., trace levels may not be correctly identified) (Iverson et al. 2004). Captive studies are needed to further understand the effects of different CCs and species-specific metabolism on the QFASA model. Furthermore, fat depots other than adipose tissue and blood plasma have not been used to quantitatively estimate the relative contribution of prey in consumer diets.

Few studies have used other lipid depots such as blood plasma (Käkelä et al. 2010) and avian stomach oils to understand the foraging ecology of marine birds (Connan et al. 2005, 2007*a*, 2007*b*; Wang et al. 2007; Owen et al. 2013). In addition to stomach oils, egg yolks are a potential source of lipid from which maternal diets may be inferred (e.g., Polito et al. 2012). Differences in diet have been shown to influence polyunsaturated FA profiles in avian egg yolk (Surai et al. 2001*b*) and some components of egg yolk FA reflect maternal diet (Polito et al. 2012). Egg yolk FA are derived from lipids that are originally assembled in the female liver (Speake et al. 1998). Thus, egg yolk FA may give insight on nutrient acquisition before and during egg formation. Any differences in FA signatures between fertile and infertile eggs may provide insight into their reproductive success. Furthermore, if there were no differences in FA profiles between fertile and infertile eggs, the use of infertile egg yolk FA could provide a noninvasive tool with minimal impacts on productivity during the nesting season (generally May to July). Our goals were to (*i*) examine any differences in FA signatures between fertile and infertile eggs from captive female Spectacled Eiders, (*ii*) develop CCs for individual FA to account for FA modification from diet into egg yolk, and (*iii*) validate QFASA using egg yolks as a proxy for maternal diets.

Materials and methods

Sample collection

The collection of samples for this project was carried out under permits from the Institutional Animal Care and Use Committee (Nos. 05-006 and 08-004) at the Alaska SeaLife Center (ASLC), the Alaska Department of Fish and Game (No. 07-045), and the Federal Fish and Wildlife Permit (No. 065912). Five female Spectacled Eiders housed at the ASLC in Seward, Alaska, USA, were used in this study. Birds were housed in mixed flocks in outdoor pens at ambient conditions and natural saltwater habitats. As pair bonds formed and the nesting season approached, birds were eventually segregated by pairs in individual pens. From February through July 2008, five female Spectacled Eiders were fed a consistent diet of 85% Mazuri sea duck formula (Purina Mills, St. Louis, Missouri, USA) and 15% Atlantic silverside (*Menidia menidia* (L., 1766)). From February through July 2009, the same five birds were fed, on average, a diet of 91% Mazuri, 3% silverside, 1% Atlantic surfclam (*Spisula solidissima* (Dillwyn, 1817)), 2% Antarctic krill (*Euphausia superba* Dana, 1852), and 2% blue mussel (*Mytilus edulis* L., 1758). Diet composition was based on wet mass (g). Daily amounts of each diet item fed and consumed per pen were recorded. The Mazuri sea duck formula consisted of approximately 6.5% lipid, 21.6% protein, 8.4% fiber, 10.9% ash, and 46.6% nitrogen-free extract, and vitamins (available from http://www.mazuri.com/). Mazuri ($n = 15$) and silverside ($n = 15$) were collected throughout the feeding study in 2008, placed in airtight plastic bags, and stored at –20 °C until analysis. Silverside from 2008 consisted of 3.4% lipid. Diet samples were not collected in 2009, therefore FA data for clams, krill, Mazuri, mussel, and silverside from Wang et al. (2010) were used in the QFASA diet estimation. Clams, krill, mussel, and silverside from Wang et al. (2010) consisted of 0.9%, 7.4%, 2.1%, and 5.2% lipid, respectively.

During the breeding season (May to July), birds were monitored daily for egg laying and eggs were collected upon observation. Eggs were artificially incubated at 37.4 °C and 60% relative humidity (Grumbach Incubator, Asslar, Germany) and candled after 3–5 days to determine fertility status, then refrigerated at 4 °C for a maximum of 24 h before yolk was sampled. A total of 49 eggs were collected in 2008 (fertile: $n = 37$; infertile: $n = 11$; fertility status unknown: $n = 1$) and 28 eggs in 2009 (fertile: $n = 9$; infertile: $n = 12$; fertility status unknown: $n = 7$). Sample sizes for each female are given in Table 1. Egg yolks were individually homogenized and then between 0.5 and 1.5 g of yolk was placed in 2 mL of chloroform and 0.01% butylated hydroxytoluene in glass vials with Teflon-lined caps (Budge et al. 2006). Samples were stored at –20 °C until analysis.

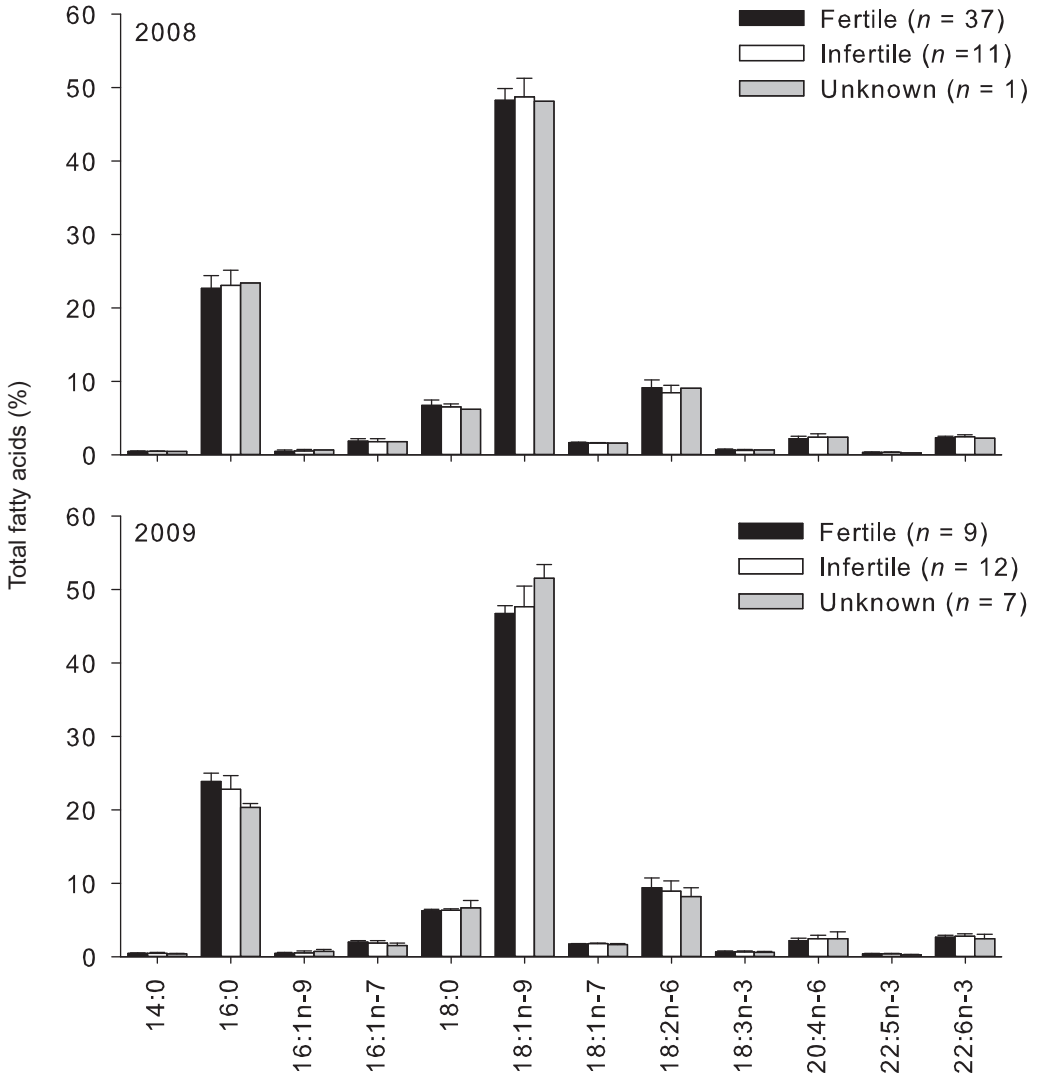
Laboratory analysis

Lipids were quantitatively extracted from egg yolk samples and homogenates of diet items according to (Folch et al. 1957) as modified by Iverson et al. (2001) at ASLC (Seward, Alaska, USA). Fatty acid methyl esters (FAME) were prepared using an acidic transesterification (Budge et al. 2006). FAME were sent to Dalhousie University (Halifax, Nova Scotia, Canada). Cholesterols in egg yolk were removed (as they do not contribute any FA information and lead to the damage of the gas chromatograph column) using thin layer chromatography and a solvent system with a 90:10:1 ratio of petroleum ether to ethyl ether to acetic acid. Additionally, the presence of fatty alcohols resulting from the transesterification of wax esters in diet items was determined using thin layer chromatography. Many seabirds have the ability to assimilate dietary wax esters into their tissues (Roby et al. 1986). To account for wax esters in diets, the alcohols of which are deposited as their corre-

Table 1. Sample sizes for fertile, infertile, and unknown fertility status of egg yolk collected from five female Spectacled Eiders (*Somateria fischeri*) in 2008 and 2009.

	2008				2009			
Female	Fertile	Infertile	Unknown	Total eggs	Fertile	Infertile	Unknown	Total eggs
10012	13	1	0	14	1	0	2	3
10015	6	1	0	7	1	2	1	4
10018	4	2	0	6	4	2	0	6
10019	5	3	0	8	1	5	0	6
10024	9	4	1	14	2	3	4	9
Total	37	11	1	49	9	12	7	28

Fig. 1. Fatty acids (FA) in Spectacled Eider (*Somateria fischeri*) egg yolks in 2008 and 2009 of fertile, infertile eggs, and eggs of unknown fertility status (mean ± 1 SD). FA shown represent 96.9% of the total across all samples.

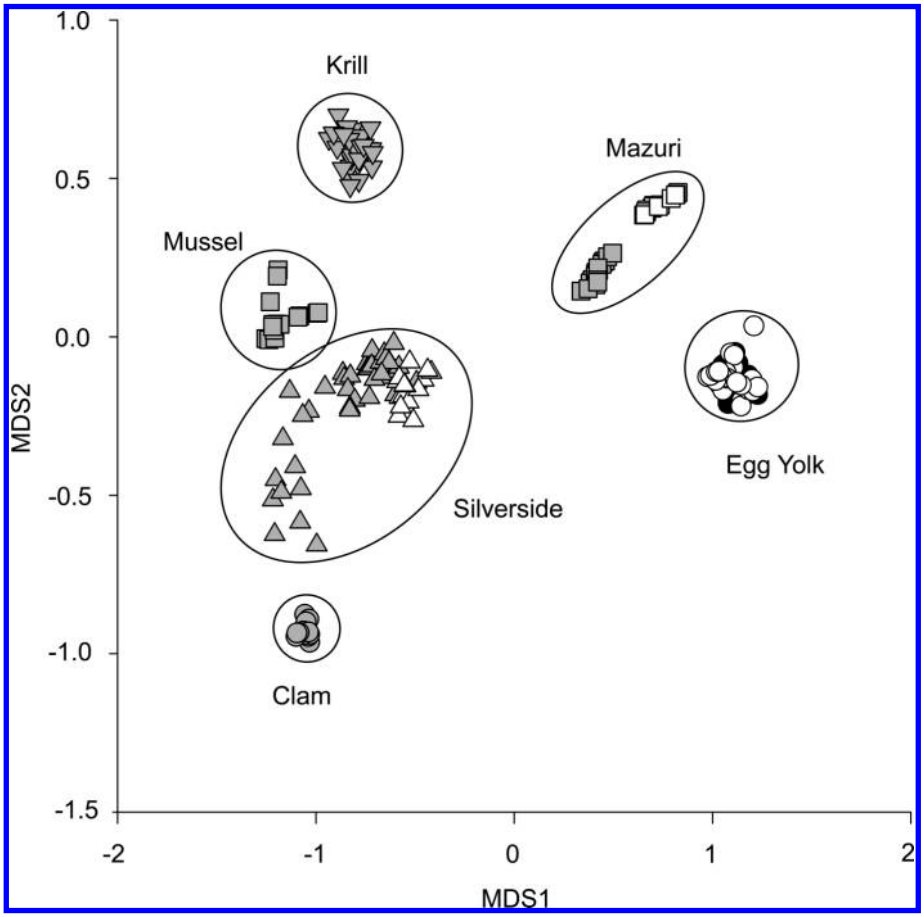


sponding FA in consumer tissues (Budge and Iverson 2003), wax ester alcohols from diet items were converted to their respective FA according to Budge et al. (2006). FAME were quantified using temperature-programmed gas liquid chromatography on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25 mm inner diameter (id) column coated with 50% cyanopropyl-methylpolysiloxane (DB-23) and linked to a computerized integration system (Varian Galaxie software) according to Iverson et al. (2002). Each chromatogram was manually assessed for correct peak identification and reintegrated, where necessary.

Qualitative analysis

FA data often violate the assumptions (normality, independence, and equality of covariance matrices) used in parametric methods, thus we used nonparametric multivariate methods from PRIMER 6 (PRIMER-E) to analyze FA data. Variation in yolk FA composition between fertile eggs, infertile eggs, and eggs with unknown fertility status were examined by calculating Bray–Curtis similarity matrices on all FA present at proportions >0.1% and analysis of similarity (ANOSIM) (Clarke and Green 1988) was performed. Significant differences in FA profiles were identified

Fig. 2. Nonmetric multidimensional scaling plot of Bray–Curtis similarity matrices calculated from fatty acids (FA) present in proportions >0.1% in Spectacled Eider (*Somateria fischeri*) egg yolk and diet items. Gray-shaded symbols indicate diet items collected between September 2007 and January 2008 (Wang et al. 2010). Open symbols indicate Atlantic silverside (*Menidia menidia*) and Mazuri collected between February and July 2008. Open circles indicate egg yolk from 2008, while solid circles indicate egg yolk from 2009. Groupings of 80% similarity (black lines) based on cluster analysis are superimposed to verify adequacy of ordination results. Two-dimensional stress = 0.06.



using the global *R* values and a similarity percentages routine (SIMPER) was used to determine FA contributing to any differences. Global *R* values close to 0 indicate little or no separation between groups and values close to 1 indicate complete separation between groups. Additionally, nonmetric multidimensional scaling (MDS) plots and hierarchical clustering (CLUSTER) with 80% similarity groupings were used to visualize differences in FA profiles of egg yolk and diet items. Select FA were renormalized and data were log(x + 1)-transformed to equalize the weighting of all FA for the ANOSIM, MDS, and SIMPER routines. A repeated-measures analysis of variance (ANOVA) was used to compare differences in percent lipid between fertile and infertile eggs in 2008, and differences in percent lipid between fertile eggs, infertile eggs, and eggs with unknown fertility status in 2009. Repeated-measures ANOVAs were performed in STATISTICA version 12 (Statsoft, Inc., Tulsa, Oklahoma, USA).

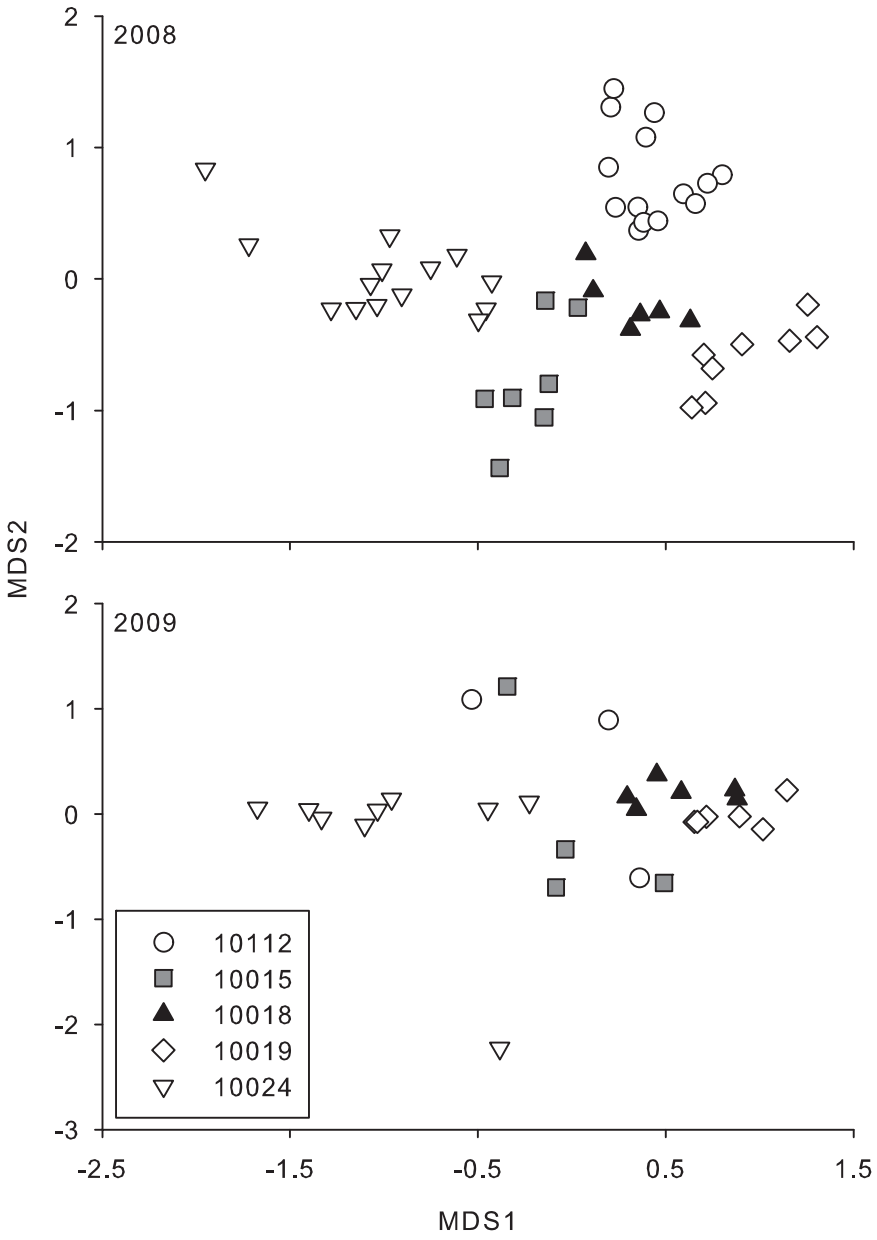
To assess how individual variation in lipid metabolism influenced egg yolk FA profiles, we compared the yolk FA composition between individual females by year using the MDS, ANOSIM, SIMPER, and CLUSTER routines. We also used the ANOSIM routine to examine how variation in lipid metabolism across the laying cycle (egg order) influenced egg yolk FA profiles in 2008 and 2009.

QFASA model

From the known, consistent diet in 2008, we assessed the quantitative characteristics of FA deposition from diet to egg yolk and developed CCs for individual FA for eggs collected in 2008 (Supplementary Table S1)[1]. CCs were calculated by dividing levels of individual FA in egg yolk from each individual egg by mean values of those same FA in the diet for that year then calculated a mean across all eggs. We used four FA subsets in the model to test their relative accuracy. We used the suggested extended and dietary FA subsets from Iverson et al. (2004) and the reduced A and reduced B subsets from Wang et al. (2010) because these subsets performed the best in the QFASA diet estimation of adipose tissue from Spectacled Eider males (Wang et al. 2010). The FA 16:3*n*-1 was removed from all subsets because this FA was not detected in egg yolk or diet items. The FA database of diet items consisted of Mazuri (n = 15), silverside (n = 15), and from Wang et al. (2010): clam (n = 15), krill (n = 39), Mazuri (n = 19), mussel (n = 15), and silverside (n = 39). To use diet items from a different year, we assumed that the FA profiles of diet items in Wang et al. (2010) were similar to those collected between February and July 2008. Diet estimates from eggs were averaged for each female and then the means for all females were averaged for each FA subset used in the QFASA model. Differences between actual and estimated diet were

[1]Supplementary Table S1 are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2013-0293.

Fig. 3. Nonmetric multidimensional scaling plot of Bray–Curtis similarity matrices calculated from fatty acids (FA) present in proportions >0.1% in Spectacled Eider (*Somateria fischeri*) egg yolk for each female (10012, 10015, 10018, 10019, and 10024) in 2008 and 2009. Groupings of 80% similarity based on cluster analysis included all data points for both years (not shown). Two-dimensional stress = 0.12 for both years.



examined using ANOSIM global *R* values and SIMPER was used to determine percent similarity of diets. Fertile eggs, infertile eggs, and eggs of unknown fertility status were pooled because FA profiles were not different among fertility status (see Results).

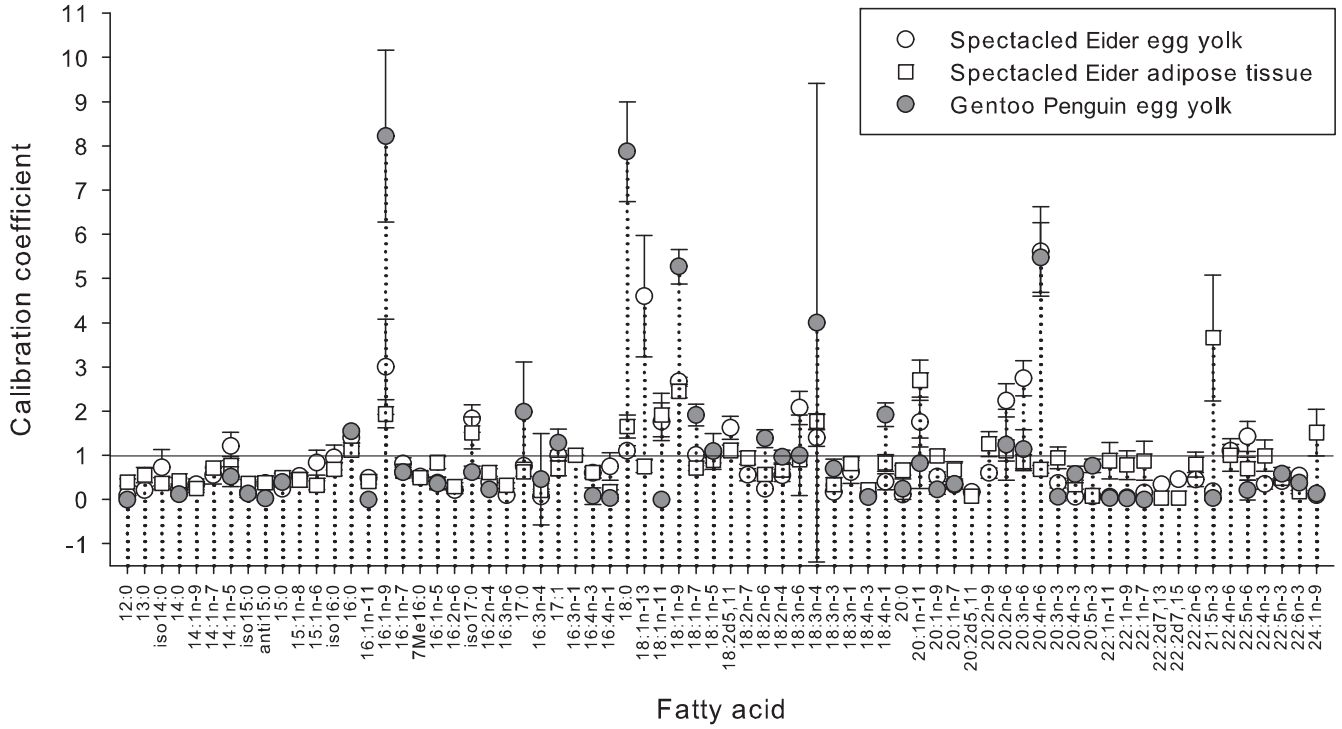
To determine how transferable CCs are between avian species, we estimated the diets of Spectacled Eiders from egg yolk in 2008 using Gentoo Penguin egg yolk CCs from Polito et al. (2012). Four FA subsets similar to the ones listed above were applied in the QFASA model. However, CCs for FA 16:2*n*-6, 18:3*n*-1, 22:2*n*-6, 22:4*n*-6, and 22:4*n*-3 were not reported for Gentoo Penguin egg yolk and thus these FA were removed from the original four FA subsets.

Results

FA 18:1*n*-9, 16:0, and 18:2*n*-6 made up approximately 80% of the egg yolk FA profiles in both years (Fig. 1). Egg yolk FA profiles were not significantly different between fertile and infertile eggs in 2008 (ANOSIM, global $R = 0.06$, $P = 0.17$) and FA profiles were 95% similar (SIMPER). Similarly, egg yolk FA profiles did not differ significantly between fertile eggs, infertile eggs, and eggs with unknown status in 2009 (ANOSIM, global $R = 0.29$, $P = 0.30$) and were 93%–96% similar (SIMPER). The mean (±SD) percent lipid of fertile and infertile eggs in 2008 was 33.1% ± 6.7% and 36.7% ± 6.5%, respectively. In 2009, the mean (±SD) percent lipid of fertile, infertile eggs, and eggs with unknown status was 30.5% ± 6.1%, 32.3% ± 5.2%, and 31.8% ± 9.5%, respectively. There was no significant difference in the percent lipid between fertile and infertile eggs in 2008 (ANOVA, $P = 0.08$), and between fertile eggs, infertile eggs, and eggs with unknown fertility status in 2009 (ANOVA, $P = 0.94$).

Egg yolk FA profiles from both years were most similar to each other relative to FA profiles of diet items (Fig. 2). FA profiles of

Fig. 4. Calibration coefficients (CCs) for diet to egg yolk fatty acids (FA) from Spectacled Eider (*Somateria fischeri*) eggs collected in 2008 (mean ± 1 SD). CCs for diet to adipose tissue for Spectacled Eider males from Wang et al. (2010) and Gentoo Penguins (*Pygoscelis papua*) (Polito et al. 2012) shown for comparison. The horizontal line at 1 represents a 1:1 incorporation of FA from the diet into the egg yolk and adipose tissue.



silverside from Wang et al. (2010) were more variable than those collected in 2008, but most similar to FA profiles of silversides collected from February to July 2008 than to any of the other diet items (Fig. 2). Additionally, FA profiles of Mazuri in 2008 were most similar to that of Mazuri from Wang et al. (2010). The FA profiles of female eiders were most similar to Mazuri than any of the other diet items (Fig. 2).

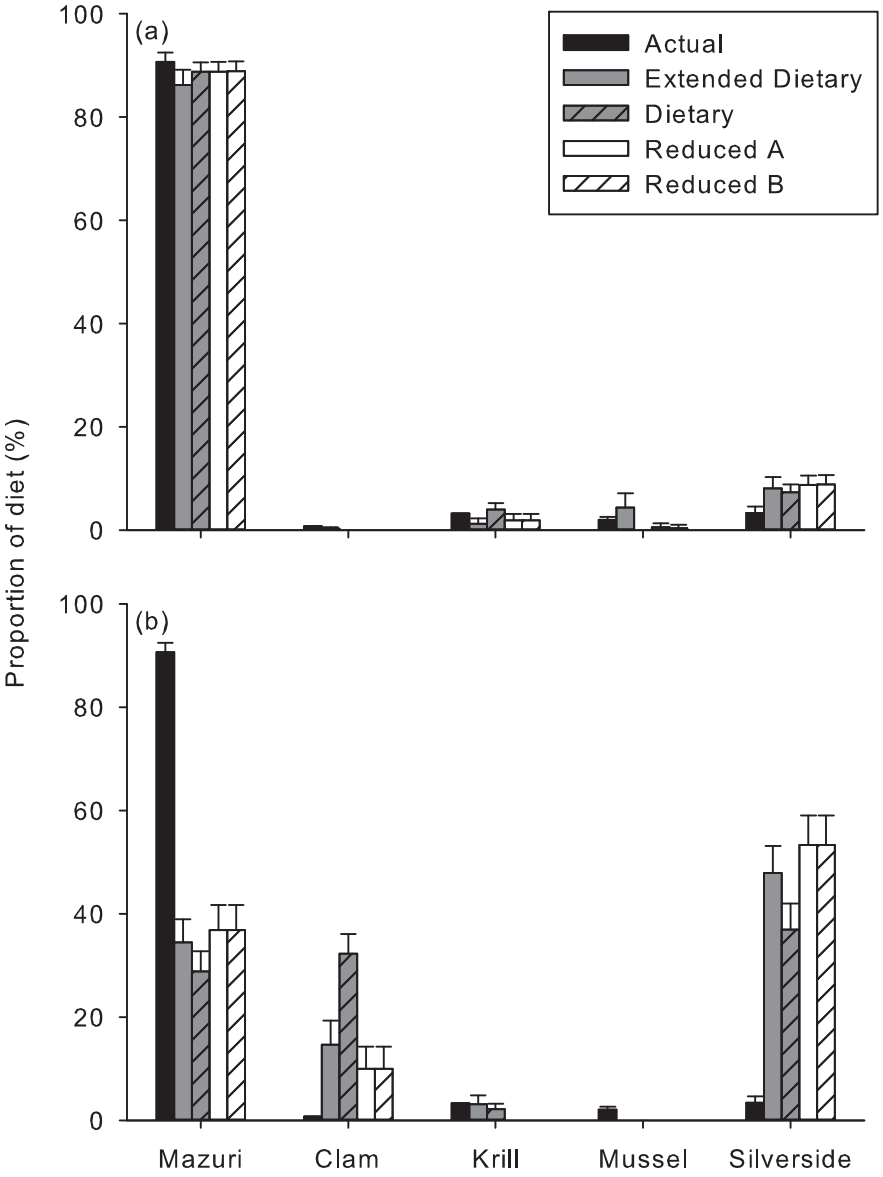
Egg yolk FA profiles were most similar within individual females (Fig. 3) and were significantly different among females in 2008 (ANOSIM pairwise tests, global $R > 0.49$, $P < 0.002$). Similarly in 2009, egg yolk FA profiles differed among all females (ANOSIM pairwise tests, global $R > 0.56$, $P < 0.02$) except between females 10012 and 10015 and females 10018 and 10019 (ANOSIM pairwise tests, $P > 0.05$). Of the 40 FA present in proportions >0.1% in all samples, 24 and 22 FA contributed to 90% of the dissimilarity between females in 2008 and 2009, respectively (SIMPER). Of these FA, nine are considered to arise from both biosynthesis and dietary sources (14:0, 16:0, 16:1*n*-7, 16:1*n*-9, 17:0, 17:1, 18:0, 18:1*n*-9, and 18:1*n*-7), one from mainly biosynthesis (18:1*n*-11), and 12 from mainly dietary sources (16:4*n*-1, 16:4*n*-3, 18:1*n*-13, 18:2*n*-6, 18:3*n*-3, 20:1*n*-11, 20:1*n*-9, 20:2*n*-6, 20:3*n*-6, 20:4*n*-6, 20:5*n*-3, and 22:6*n*-3) (Iverson et al. 2004). In addition to these FA, 18:4*n*-3 and 18:3*n*-6, which are thought to arise from diet, contributed to the dissimilarity between females in 2008. Egg order did not influence FA profiles in 2008 or 2009 (ANOSIM, global $R < -0.03$, $P > 0.60$).

The CCs for egg yolks in 2008 were similar to those for Spectacled Eider adipose tissue for some FA with some notable differences (Fig. 4). For example, CCs for 16:1*n*-9, 18:1*n*-13, 18:3*n*-6, 20:2*n*-6, 20:3*n*-6, and 20:4*n*-6 were much higher in egg yolk than in adipose tissue, while CCs for 20:1*n*-11, 21:5*n*-3, and 24:1*n*-9 were lower in egg yolk than in adipose tissue (Fig. 4). The QFASA estimates using the extended dietary FA subset underestimated the amount of Mazuri by 5%, while diet estimates using all four FA subsets overestimated the amount of silverside in the diet by 4%–5% (Fig. 5*a*). QFASA diet estimates using the reduced A, reduced B, dietary, and extended dietary FA subsets produced similar results and were 93%, 93%, 94%, and 92% similar to the actual diet (SIMPER). QFASA estimates using penguin yolk CCs underestimated the amount of Mazuri in the eider diet (by 54%–62%), overestimated the amount of clam (by 9%–32%), and also overestimated the amount of silverside (by 34%–50%) (Fig. 5*b*). All FA data are available upon request.

Discussion

The results of this study have shown that QFASA accurately estimated maternal diets of captive Spectacled Eiders from egg yolk FA. The dietary FA subset performed the best, but all diet estimates using the other subsets were very similar to the actual diet. All diet estimates overestimated the amount of silverside in the diet but by only 4%–6%. We speculate that this might be because the CCs used were calculated from a diet that consisted of a higher amount of silverside (15%) and CCs may be dependent on diet, along with species and physiological state (Wang et al. 2010; Williams and Buck 2010; Budge et al. 2012; Rosen and Tollit 2012). We also demonstrate that CCs for FA vary somewhat between those derived from egg yolk versus adipose tissue. This is not surprising, as the two tissues undergo different metabolic processing and a greater degree of direct deposition from diet may be expected with adipose tissue. Additionally, egg yolk CCs were derived from females during the breeding season on a diet of 85% Mazuri and 15% silverside, while adipose tissue CCs were derived from males during the nonbreeding season on a diet of 88% Mazuri, 3% krill, 4% silverside, 1% clam, and 4% mussel (Wang et al. 2010). Therefore, factors such as sex, breeding stage, and diet may also contribute to differences between egg yolk and adipose tissue CCs. Another source of CC variation between tissue types could come from the variation within diet items. Diet items were not collected in 2009; therefore our prey database consisted of diet items from Wang et al. (2010), which were collected between

Fig. 5. Quantitative fatty acid signature analysis (QFASA) diet estimates using reduced A, reduced B, dietary, and extended dietary fatty acid (FA) subsets from egg yolks of five Spectacled Eiders (*Somateria fischeri*) collected in 2009 using calibration coefficients (CCs) calculated from (*a*) Spectacled Eider 2008 egg yolks and (*b*) Gentoo Penguins (*Pygoscelis papua*) (Polito et al. 2012). FA subsets used in *b* did not include 16:2*n*-6, 18:3*n*-1, 22:2*n*-6, 22:4*n*-6, and 22:4*n*-3 because values for these FA were not reported for Gentoo Penguin egg yolk. Estimates from eggs were averaged for each female, and then these mean values for all females were averaged for each FA subset used in the QFASA model (mean ± 1 SD).



September 2007 and January 2008, and Mazuri and silverside collected between February and July 2008. The variability of silverside and Mazuri fed in 2009 and similarity to Wang et al. (2010) is unknown, but the MDS plot suggests that the variability of FA profiles between different diet items is greater than the annual variation within the same diet items.

CCs from Spectacled Eider adipose tissue and egg yolk were more similar to each other than to egg yolk CCs from Gentoo Penguins, which showed some striking differences for 16:1*n*-9, 18:0, and 18:1*n*-9. The CCs for these three FA were much higher for penguins than eiders and might be explained by an increase in de novo synthesis of these FA by penguins prior to egg formation (Groscolas 1990). These, and other FA such as 20:4*n*-6 with large CCs, are also likely influenced by differential rates of mobilization from diets and maternal adipose tissue (Polito et al. 2012). Differences in CCs between species may also be explained by differences in diet. Eiders in this study were fed a predominately terrestrial corn-based diet supplemented with marine prey items, while Gentoo Penguins in Polito et al. (2012) consumed a marine diet of Atlantic herring (*Clupea harengus* L., 1758). These differences in CCs between species suggest that using CCs from another species, or likely more important between species fed very different marine-based versus terrestrial-based diets, may not be appropriate for estimating diets of a different species on a different feeding regime. In fact, using penguin yolk CCs to estimate the diets of Spectacled Eiders from egg yolk in 2009 gave inaccurate estimates of eider diets. However, the use of CCs from species with similar physiology and foraging behavior may be transferrable and requires further investigation.

These differences in the diets of captive Spectacled Eiders and Gentoo Penguins are partially reflected in their FA profiles. The FA 16:0, 18:0, and 18:1*n*-9 made up the greatest proportion of egg yolk

FA of captive and wild Gentoo Penguins (Polito et al. 2012), while 16:0, 18:1*n*-9, and 18:2*n*-6 dominated the egg yolk of Spectacled Eiders. Unlike most marine diet items, the terrestrial corn-based Mazuri is dominated by 18:2*n*-6 and hence also reflected in eider adipose tissue (Wang et al. 2010). Variation in FA profiles of egg yolk between species could also be contributed somewhat by phylogenetic differences, as different avian species maintained on the same diet can produce eggs with very different FA profiles (Maldjian et al. 1996; Surai et al. 1999). On the other hand, Speake et al. (2002) found that King Eider (*Somateria spectabilis* (L., 1758)) egg yolk FA showed similar patterns to four other duck species with different diets, habitat, and breeding strategy. Egg yolk FA profiles may be controlled by maternal metabolism, which creates FA profiles characteristic of eggs for similar species (Speake et al. 2002). Egg yolk FA may also be influenced by developmental requirements of the embryo (Polito et al. 2012). Certain FA such as 20:4*n*-6 and 22:6*n*-3 will be conserved in egg yolk because of particular requirements of embryonic tissue for FA (Surai et al. 2001*a*; Speake et al. 2002). Egg yolk FA profiles appear to be influenced by metabolism of individual females but apparently not by the order in which eggs were laid within a female. However, the differences in egg yolk FA profiles between females might also be due to variability in diets consumed by individuals. The quantity of diet items made available to eiders were recorded on a daily basis, but birds were kept in the same pen until pair bonds formed and it was not possible to determine the exact amount each bird consumed during that time. Thus, some birds could have had a preference for and consumed more or less of certain diet items than other birds and this could contribute to the variability seen among females. Further research is needed to determine the magnitude of the effect of maternal metabolism and diet (i.e., marine versus terrestrial) on egg yolk FA profiles and CCs in Spectacled Eiders.

Despite the different factors affecting FA profiles in egg yolk, FA have the potential to provide important insight on the breeding strategies of wild Spectacled Eiders. Large amounts of yolk lipids are deposited into eggs, mostly during the several days prior to ovulation (Klasing 1998), thus egg yolk FA would provide information about female diet during the prebreeding period. Eiders in this study were held on a stable long-term, predominately terrestrial corn-based diet year-round, which makes it difficult to determine whether these captive birds used nutrients directly from diet to build eggs (income breeding strategy) as opposed to using fat and muscle tissue (capital breeding strategy). However, FA analysis could provide a tool to help understand the importance of marine versus freshwater sources in clutch formation in eiders. If marine FA were identified in egg yolk, this would indicate that females were using endogenous sources (thus, capital breeding strategy) for reproduction, while freshwater FA identified in egg yolk would indicate that exogenous sources (thus, income breeding strategy) were used for reproduction. The presence of both marine and freshwater FA in egg yolk would suggest that females incorporate both capital and income breeding strategies. Additionally, FA profiles and the amount of lipid in Spectacled Eider egg yolks did not differ between fertile and infertile eggs. Therefore, applying QFASA techniques using infertile eggs can potentially be used to quantify female diets without impact on potential breeding success of these threatened eiders and other birds. Further work to elucidate CCs obtained from a more typical wild diet for Spectacled Eiders would be important for comparison to both our currently estimated eider CCs and to those of Gentoo Penguins, and would improve confidence in the ability to apply CCs obtained among different species and diets. We conclude that QFASA estimates of Spectacled Eider diets from infertile egg yolks can provide key information to help identify critical habitats, sources, and timing of nutrient acquisition during reproduction, as well as dietary requirements of these threatened populations.

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