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EFFECTS OF LIPID EXTRACTION ON STABLE ISOTOPE RATIOS IN AVIAN EGG YOLK: IS ARITHMETIC CORRECTION A RELIABLE ALTERNATIVE?

Steffen Oppel,[1,6] Rebekka N. Federer,[2,3] Diane M. O'Brien,[1,4] Abby N. Powell,[1,4,5] and Tuula E. Hollmén[2,3]

[1]*Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775, USA;*
[2]*School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks, Alaska 99775, USA;*
[3]*Alaska SeaLife Center, Seward, Alaska 99664, USA;*
[4]*Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska 99775, USA; and*
[5]*U.S. Geological Survey, Alaska Cooperative Fish and Wildlife Research Unit, University of Alaska Fairbanks, Fairbanks, Alaska 99775, USA*

Abstract.—Many studies of nutrient allocation to egg production in birds use stable isotope ratios of egg yolk to identify the origin of nutrients. Dry egg yolk contains >50% lipids, which are known to be depleted in ^{13}C. Currently, researchers remove lipids from egg yolk using a chemical lipid-extraction procedure before analyzing the isotopic composition of protein in egg yolk. We examined the effects of chemical lipid extraction on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of avian egg yolk and explored the utility of an arithmetic lipid correction model to adjust whole yolk $\delta^{13}C$ for lipid content. We analyzed the dried yolk of 15 captive Spectacled Eider (*Somateria fischeri*) and 20 wild King Eider (*S. spectabilis*) eggs, both as whole yolk and after lipid extraction with a 2:1 chloroform:methanol solution. We found that chemical lipid extraction leads to an increase of (mean ± SD) 3.3 ± 1.1‰ in $\delta^{13}C$, 1.1 ± 0.5‰ in $\delta^{15}N$, and 2.3 ± 1.1‰ in $\delta^{34}S$. Arithmetic lipid correction provided accurate values for lipid-extracted $\delta^{13}C$ in captive Spectacled Eiders fed on a homogeneous high-quality diet. However, arithmetic lipid correction was unreliable for wild King Eiders, likely because of their differential incorporation of macronutrients from isotopically distinct environments during migration. For that reason, we caution against applying arithmetic lipid correction to the whole yolk $\delta^{13}C$ of migratory birds, because these methods assume that all egg macronutrients are derived from the same dietary sources. *Received 12 June 2008, accepted 27 May 2009.*

Key words: ^{13}C, egg yolk, eiders, lipid correction, ^{15}N, ^{34}S, *Somateria*, stable isotopes.

Efectos de la Extracción de Lípidos sobre los Cocientes de Isótopos Estables en la Yema de Huevos de Aves: ¿es la Corrección Aritmética una Alternativa Confiable?

Resumen.—Muchos estudios sobre la asignación de nutrientes en la producción de huevos en las aves usan cocientes de isótopos estables presentes en la yema para identificar el origen de los nutrientes. La yema seca del huevo contiene >50% de lípidos, los cuales se sabe que son pobres en ^{13}C. Los métodos utilizados actualmente remueven los lípidos de la yema utilizando un procedimiento químico de extracción antes de analizar las composiciones isotópicas de las proteínas de la yema. En este estudio, examinamos los efectos de la extracción química de lípidos sobre los isótopos $\delta^{13}C$, $\delta^{15}N$ y $\delta^{34}S$ de yemas de huevos de aves y exploramos la utilidad de un modelo de corrección aritmética de los lípidos para ajustar el $\delta^{13}C$ de toda la yema por el contenido total de lípidos. Analizamos la yema seca de huevos de 15 individuos cautivos de *Somateria fischeri* y de 20 individuos silvestres de *S. spectabilis*, considerando tanto los análisis de la yema completa como los de la extracción de lípidos con una solución 2:1 de cloroformo:metanol. Encontramos que la extracción química de lípidos lleva a un aumento de (media ± DE) 3.3 ± 1.1‰ en $\delta^{13}C$, 1.1 ± 0.5‰ en $\delta^{15}N$ y de 2.3 ± 1.1‰ en $\delta^{34}S$. La corrección aritmética de lípidos entregó valores precisos para el $\delta^{13}C$ de lípidos extraídos en los individuos cautivos que fueron alimentados con una dieta homogénea de alta calidad. Sin embargo, la corrección aritmética de lípidos no fue confiable para los individuos silvestres de *S. spectabilis,* probablemente debido a que durante la migración éstos incorporan diferentes macro-nutrientes provenientes de ambientes isotópicamente diferentes. Por esta razón, sugerimos tener precaución en la aplicación de la corrección aritmética de lípidos del $\delta^{13}C$ de toda la yema de aves migratorias, debido a que estos métodos suponen que todos los macro-nutrientes del huevo derivaron de la misma fuente de alimentos.

[6]Present address: Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, UK. E-mail: steffen.oppel@gmail.com

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Tracing the origin of nutrients used for reproduction is one of the key goals of many ecological studies, and stable isotopes are increasingly used to determine the sources of nutrients acquired and utilized by animals (Hobson 1995, Gannes et al. 1998, Fry 2006). In many avian studies, stable isotope ratios of egg components have revealed valuable information about the proportional contribution of exogenous and endogenous nutrient sources to egg synthesis (Hobson et al. 1997, 2004, 2005; Gauthier et al. 2003; Morrison and Hobson 2004).

Most of the energy in an egg is stored in the yolk (Sotherland and Rahn 1987); hence, studies that examine nutrient allocation to reproduction in birds often focus on egg yolk. Dry avian egg yolk is composed of ≥50% lipids in most bird species (Carey et al. 1980, Sotherland and Rahn 1987, Burley and Vadehra 1989), and lipids are depleted in ^{13}C, compared with proteins and carbohydrates (DeNiro and Epstein 1977). Thus, differences in lipid content of samples can confound the interpretation of carbon stable isotope ratios (Post et al. 2007), and many ecological studies either chemically remove lipids from target tissues or apply an arithmetic correction to account for the higher abundance of lighter carbon isotopes in lipids (Kiljunen et al. 2006, Sweeting et al. 2006, Post et al. 2007).

Arithmetic lipid correction has been developed for fish and invertebrate tissues (Kiljunen et al. 2006, Sweeting et al. 2006, Smyntek et al. 2007); however, the reliability of these corrections for avian yolk, with its higher lipid content, has not been established (Post et al. 2007, Logan et al. 2008, Mintenbeck et al. 2008). The current lack of a reliable arithmetic correction has made chemical lipid extraction necessary for analysis of carbon stable isotope ratios in avian egg yolk (Gauthier et al. 2003, Hobson et al. 2005, Bond et al. 2007). Lipid extraction is assumed to normalize carbon stable isotope ratios but has also been shown to affect nitrogen stable isotope ratios in fish and invertebrate tissues (Søreide et al. 2006, Sweeting et al. 2006, Logan and Lutcavage 2008). We are aware of only one study on the isotopic effects of lipid extraction in avian eggs, but that study (Ricca et al. 2007) did not separate yolk and albumen components of eggs. Thus, it is unknown how chemical lipid extraction affects the carbon, nitrogen, and sulfur stable isotope ratios in avian egg yolk.

We used data from two sea duck species, captive Spectacled Eiders (*Somateria fischeri*) and wild King Eiders (*S. spectabilis*), to examine differences in carbon, nitrogen, and sulfur stable isotope ratios between whole egg yolk and chemically lipid-extracted yolk. We then used two different arithmetic lipid correction approaches, based on empirically derived constants and published parameters, respectively, to evaluate whether they could reliably estimate carbon stable isotope ratios for lipid-free yolk. On the basis of our results, we formulated a set of recommendations for ornithologists to account for lipids in avian egg yolk.

Methods

Study species.—King and Spectacled eiders are sea ducks that nest during a short season in the Arctic and spend the rest of the annual cycle in coastal marine areas at high northern latitudes. During the breeding season, these species lay 4–7 eggs in every clutch. Eggs average ~67 g in King Eiders (Suydam 2000) and ~71 g in Spectacled Eiders (Petersen et al. 2000).

Egg collection.—We collected 40 King Eider eggs on the Arctic coastal plain of Alaska in June 2006 and 2007. The study area was located 30 km south of the Beaufort Sea, near Teshekpuk Lake (70°26′N, 153°08′W). We searched the study area on foot for nests and collected 1 fresh egg from 20 nests each year. Eggs were boiled in the field and subsequently kept frozen until separation and analysis (Gloutney and Hobson 1998).

Fifteen eggs laid by captive Spectacled Eiders housed at the Alaska SeaLife Center (Seward, Alaska) were collected in May and June 2006. These birds were raised and maintained in captivity and fed a homogeneous diet consisting mostly of ground corn, wheat, and fish meal (Mazuri Sea Duck Diet, Purina Mills, St. Louis, Missouri) throughout their life. Fresh yolk was separated from albumen and kept frozen until analysis.

Laboratory techniques.—Following common practice used in most isotopic studies of avian eggs, we lyophilized and then homogenized yolk samples from each egg ($n = 40$ King Eider, $n = 15$ Spectacled Eider) by grinding with a mortar and pestle. We then removed lipids by rinsing samples with a 2:1 chloroform:methanol solution (Bligh and Dyer 1959). We used ~5 mg of dry yolk sample and soaked samples multiple times for 24 h each until the solvent wash was completely clear. We extracted dissolved lipids manually with a pipette and kept them uncovered under a fume hood at room temperature until all solvent had evaporated. Lipid samples were then kept frozen until analysis. For each component (whole yolk, lipid-free yolk, yolk lipids), we placed 0.2–0.4 mg in small tin capsules for $\delta^{13}C$ and $\delta^{15}N$ analysis. We used 0.9–1.2 mg of whole and lipid-free yolk for $\delta^{34}S$ analysis. We analyzed $\delta^{13}C$ and $\delta^{15}N$ for both King and Spectacled eider eggs but analyzed $\delta^{34}S$ only for King Eider eggs.

We measured stable isotope compositions by continuous-flow isotope ratio mass spectrometry (CF-IRMS) at the Alaska Stable Isotope Facility (University of Alaska Fairbanks, $\delta^{13}C$ and $\delta^{15}N$) using a Finnigan Delta^{plus}XP CF-IRMS and at the Stable Isotope Ratio Facility for Environmental Research (University of Utah, $\delta^{34}S$) using a Finnigan MAT Delta S CF-IRMS. We report results in delta (δ) notation in relation to the internationally recognized standards (Vienna-PeeDee Belemnite for C, atmospheric air for N, Vienna-Canyon Diabolo Troilite for S) according to the following equation: $\delta X\ (‰) = ([R\text{sample}/R\text{standard}] - 1) \times 1{,}000$, where X denotes either ^{13}C, ^{15}N, or ^{34}S and R represents the ratio of $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$, in relation to the respective standards. Standard deviation of known laboratory standards (peptone $\delta^{13}C = -15.8‰$ and $\delta^{15}N = 7.0‰$; bovine liver $\delta^{34}S = 8.0‰$) run concurrently with samples was estimated to be 0.1‰, 0.2‰, and 0.3‰ for carbon, nitrogen, and sulfur isotope measurements, respectively.

Arithmetic correction for lipids in yolk.—We used two approaches to assess the validity of arithmetic correction for $\delta^{13}C$ in egg yolk of captive Spectacled Eiders and wild King Eiders. Approach 1 was based on an empirically derived constant, and approach 2 was based on a model incorporating lipid content and a correction constant derived from published values for the isotopic difference between yolk lipid and protein.

Approach 1: Correction using an empirically derived constant.—Because the difference in $\delta^{13}C$ between whole and lipid-free tissues (defined here as $\Delta\delta^{13}C$) approaches a constant at high C:N ratios (Kiljunen et al. 2006, Logan et al. 2008), a simple, empirically derived correction factor may be the most appropriate

approach for correcting lipid-rich tissues such as avian egg yolk (Ricca et al. 2007). Thus, we calculated the isotopic difference between whole yolk and lipid-extracted yolk ($\Delta\delta^{13}C = \delta^{13}C_{\text{whole yolk}} - \delta^{13}C_{\text{lipid-free yolk}}$) for each yolk sample and tested for a relationship with the C:N ratio of whole yolk using a simple linear regression. We then compared the actual $\Delta\delta^{13}C_{\text{sample}}$ of each sample with the species-specific correction constant $\Delta\delta^{13}C_{\text{mean}}$ by calculating absolute residuals ($|\Delta\delta^{13}C_{\text{sample}} - \Delta\delta^{13}C_{\text{mean}}|$) for each egg. These residuals indicate the error associated with using a constant species-specific correction factor instead of manually extracting lipids from each yolk sample to obtain $\delta^{13}C$ of lipid-free yolk. We report means (± SD) of those absolute residuals separately for King and Spectacled eiders.

Approach 2: Correction using published parameters.—We used a common arithmetic correction model that is based on lipid content (calculated from the C:N ratio) and the isotopic difference between yolk lipid and yolk protein, defined as parameter *D* (McConnaughey and McRoy 1979, Kiljunen et al. 2006, Logan et al. 2008). Published values of *D* are available for several bird species (Appendix). Thus, we examined whether these published estimates of *D* could be used to accurately estimate $\delta^{13}C_{\text{corrected}}$ of King and Spectacled eider egg yolk.

We calculated the percentage lipid content of egg yolk from the C:N ratio of whole yolk using a standard equation (McConnaughey and McRoy 1979) adjusted for the total percentage of protein and lipid in dried avian egg yolk (96%; Burley and Vadehra 1989). The C:N ratio was very similar in yolk of both species in our study and resulted in an average lipid content of 71 ± 1.5% (Table 1). We therefore simplified the correction factor of the original arithmetic correction model (Kiljunen et al. 2006, Logan et al. 2008) and calculated lipid-corrected yolk $\delta^{13}C_{\text{corrected}}$ using the following equation:

$$\delta^{13}C_{\text{corrected}} = \delta^{13}C + 0.82 \times D \quad (1)$$

where $\delta^{13}C$ is the measured value of the whole yolk sample and *D* is the isotopic difference in $\delta^{13}C$ between yolk lipids and lipid-free yolk (Kiljunen et al. 2006). We used an average *D* for carnivorous ducks for our model (*D* = 5‰; Appendix) and compared the $\delta^{13}C_{\text{corrected}}$ estimates to actual values of chemically extracted yolk samples ($\delta^{13}C_{\text{extracted}}$) using a paired-samples two-tailed *t*-test with $\alpha = 0.05$. We report this model prediction error (‰; $\delta^{13}C_{\text{corrected}} - \delta^{13}C_{\text{extracted}}$) separately for Spectacled and King eiders. We then calculated *D* for our yolk samples on the basis of $\delta^{13}C$ of yolk lipids and lipid-free yolk and estimated whether measurements of *D* for individual King Eider eggs were related to the magnitude of the prediction error.

TABLE 1. Lipid content, difference in $\delta^{13}C$ between whole and chemically lipid-extracted yolk ($\Delta\delta^{13}C$), difference in $\delta^{13}C$ between yolk lipids and chemically lipid-extracted yolk (parameter *D*), and C:N ratio of whole yolk from 20 wild King Eider and 15 captive Spectacled Eider eggs.

	King Eider		Spectacled Eider	
	Mean ± SD	Range	Mean ± SD	Range
Lipid content (%)	70.5 ± 1.2	68.3–72.0	71.8 ± 1.6	68.6–74.4
$\Delta\delta^{13}C$ (‰)	2.8 ± 1.2	0.4–4.7	4.1 ± 0.3	3.6–4.5
D (‰)	4.3 ± 1.7	0.5–6.3	5.5 ± 0.3	4.7–5.9
$C:N_{\text{whole yolk}}$	14.4 ± 0.7	13.2–15.3	15.3 ± 1.1	13.3–17.1

TABLE 2. Comparison of whole yolk and chemically lipid-extracted yolk isotope values and the difference between whole and chemically lipid-extracted yolk of captive Spectacled Eider and wild King Eider eggs (mean ± SD). All differences are statistically significant with $P < 0.001$.

Species	Isotope (δ)	*n*	Whole yolk (‰)	Lipid-free yolk (‰)	Difference (‰)
Spectacled Eider	^{13}C	15	–23.3 ± 0.3	–19.2 ± 0.2	4.1 ± 0.4
	^{15}N	15	9.4 ± 0.6	10.6 ± 0.4	1.2 ± 0.7
King Eider	^{13}C	20	–24.0 ± 2.4	–21.2 ± 2.3	2.8 ± 1.2
	^{15}N	20	9.2 ± 1.4	10.2 ± 1.4	1.0 ± 0.3
	^{34}S	30	9.8 ± 2.3	12.1 ± 2.4	2.3 ± 1.1

RESULTS

Extraction of lipids from eider egg yolk led to a significant increase in sulfur, nitrogen, and carbon isotope ratios of yolk samples (Table 2). The C:N ratio of whole yolk samples ranged from 13.2 to 17.1 (Table 1). There was no relationship between C:N ratio of whole yolk and the difference ($\Delta\delta^{13}C$) between whole yolk $\delta^{13}C$ and lipid-extracted yolk $\delta^{13}C$ in either King or Spectacled eider eggs (linear regression, $P = 0.60$ and 0.24, respectively; Fig. 1). Thus, $\Delta\delta^{13}C$ (Table 1) could be used as a constant to correct whole yolk $\delta^{13}C$ for lipid content by adding it to $\delta^{13}C_{\text{whole yolk}}$. If we used the species-specific $\Delta\delta^{13}C$ to lipid-correct whole yolk samples, the mean absolute residuals were within the range of measurement error for captive Spectacled Eiders (0.2 ± 0.1‰). However, $\Delta\delta^{13}C$ was highly variable in King Eider eggs (Table 1), and mean absolute residuals for lipid-corrected yolk were >4× larger than measurement error (0.9 ± 0.8‰).

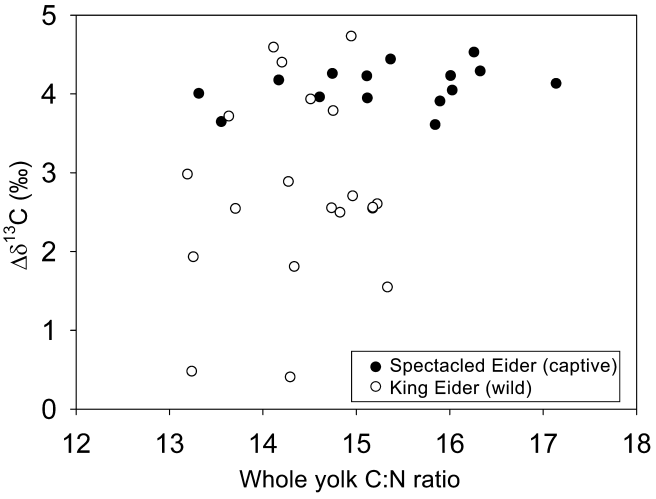

FIG. 1. Relationship between the C:N ratio of whole yolk and the difference ($\Delta\delta^{13}C$) between whole yolk $\delta^{13}C$ and chemically lipid-extracted yolk $\delta^{13}C$ of wild King Eider (white circles) and captive Spectacled Eider (black circles) eggs.

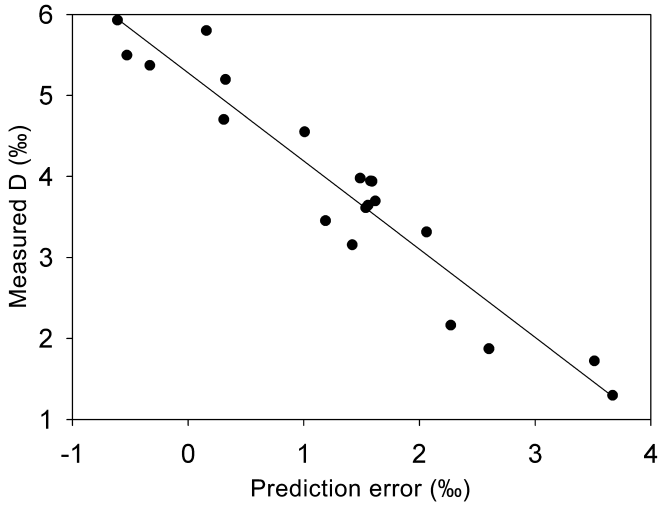

FIG. 2. Relationship describing the isotopic difference between lipid and protein fraction in egg yolk measured in our samples (measured D) as a function of the prediction error (‰; $\delta^{13}C_{corrected} - \delta^{13}C_{extracted}$) of lipid-corrected yolk samples from 20 wild King Eider eggs (linear regression, $b = -1.1$, $r^2 = 0.92$). We calculated $\delta^{13}C_{corrected}$ using a constant of D = 5‰ based on published values.

The arithmetic correction model based on lipid content and published values of D (Equation 1) provided results similar to those of the correction using an empirically derived $\Delta\delta^{13}C$: it predicted $\delta^{13}C$ extremely well in Spectacled Eider yolk (prediction error = 0.0‰ ± 0.2‰, paired t-test, $t = -0.73$, df = 14, $P = 0.47$) but poorly in wild King Eider yolk (prediction error = 1.3 ± 1.2‰, $t = -4.94$, df = 19, $P < 0.001$). Using this model allowed us to evaluate a key assumption of lipid correction models, namely that the mean difference in $\delta^{13}C$ between yolk lipids and lipid-free yolk (D) is constant. Both $\Delta\delta^{13}C$ and D were much more variable in King than in Spectacled eider yolk (Table 1). The prediction error of the lipid correction model in King Eider eggs increased significantly with increasing deviation of actually measured D from the constant used in the model (Fig. 2). Thus, nonconstant values of D in a wild migratory bird resulted in poor performance of the lipid correction model.

DISCUSSION

We found that the current practice of using chemical lipid extraction to prepare avian yolk samples for isotope analysis affects the $\delta^{15}N$ and $\delta^{34}S$ values of treated samples. The magnitude of the increase in both $\delta^{15}N$ (1.1‰) and $\delta^{34}S$ (2.3‰) is sufficient to alter the conclusions of nutrient allocation studies. Therefore, we recommend using whole yolk samples instead of lipid-extracted yolk samples for analysis of $\delta^{15}N$ and $\delta^{34}S$.

The effect of lipid extraction on $\delta^{15}N$ in the present study is similar to the increase of 0.3–2.8‰ found in invertebrate and fish tissues (Sotiropoulos et al. 2004, Søreide et al. 2006, Sweeting et al. 2006). This is most likely attributable to accidental leaching of proteins from yolk because the polar solvent used (methanol) is not lipid specific. Some polar structural lipids are associated with proteins, and when polar lipids are removed, the loss of associated proteins could lead to an increase in $\delta^{15}N$ (Sotiropoulos et al. 2004, Sweeting et al. 2006). A different solvent (e.g., diethyl ether) that does not remove polar lipids did not alter $\delta^{15}N$ in whole eggs (Ricca et al. 2007) but may be less effective in removing all lipids (Manirakiza et al. 2001, Schlechtriem et al. 2003).

We also found a large and previously undocumented increase in $\delta^{34}S$ in lipid-extracted King Eider yolk samples. Sulfur is mainly associated with sulfur-bearing amino acids in proteins, and incidental loss of proteins associated with polar structural lipids could also cause a loss of isotopically depleted sulfur. A further potential cause of the increase in $\delta^{34}S$ is the loss of sulfolipids through the extraction process (C. Stricker pers. comm.). Sulfolipids are vital components of all photosynthetic membranes and are the most abundant biological sulfur compound after amino acids (Benson 1963, Harwood and Nicholls 1979). They are soluble in chloroform, and removal by lipid extraction may therefore alter the $\delta^{34}S$ signature of yolk samples (Joyard et al. 1988).

Given that chemical lipid extraction affects both nitrogen and sulfur isotope ratios, an arithmetic lipid correction for $\delta^{13}C$ of whole yolk samples would be preferable. Because of the lack of a relationship between C:N ratio and $\Delta\delta^{13}C$ in our yolk samples, lipid correction could be accomplished by adding a constant ($\Delta\delta^{13}C$) to whole yolk $\delta^{13}C$ (Ricca et al. 2007). This constant would need to be determined experimentally by lipid-extracting a subset of yolk samples and analyzing $\delta^{13}C$ in both whole and lipid-extracted yolk, because published values are sparse and $\Delta\delta^{13}C$ may range from 1.1‰ to 4.1‰ among different species (Hobson 1995, present study). We also found that an arithmetic correction based on C:N ratios and published values of the isotopic difference between yolk lipid and yolk protein in carnivorous ducks provided highly accurate results for captive Spectacled Eiders fed a homogeneous high-quality diet. By contrast, both arithmetic lipid-correction approaches performed poorly for wild King Eider eggs, in that predicted $\delta^{13}C_{corrected}$ deviated by as much as 3.7‰ from measured $\delta^{13}C_{extracted}$ (Fig. 2). This difference can significantly affect the outcome of mixing models and thus alter the conclusions of isotopic studies (Phillips 2001).

Why is lipid correction accurate for captive but not for wild eiders? We suggest that prediction error in wild migratory birds results from the variable origin of macronutrients in egg yolk. If protein and lipid in egg yolk are derived from isotopically different sources, the isotopic differences between yolk lipid and protein (parameter D), or between whole yolk and lipid-free yolk ($\Delta\delta^{13}C$), are confounded by the isotopic difference between the sources. Migratory birds can obtain macronutrients for egg production at different stages of the annual life cycle (Hobson et al. 2000) and from isotopically distinct environments (Fig. 3). This process can result in variation in D and $\Delta\delta^{13}C$ (Table 1), thereby violating the assumption that these parameters are constant. Indeed, variability in measured D in wild King Eider eggs explained most of the prediction error (Fig. 2). We recommend careful consideration of the potential origins of lipids and protein in yolk before any arithmetic correction is applied.

Recommendations for future studies.—In summary, we caution against the interpretation of $\delta^{34}S$ and $\delta^{15}N$ values from chemically treated yolk samples. We do not recommend arithmetic lipid correction of egg yolk $\delta^{13}C$ for wild birds that are likely to

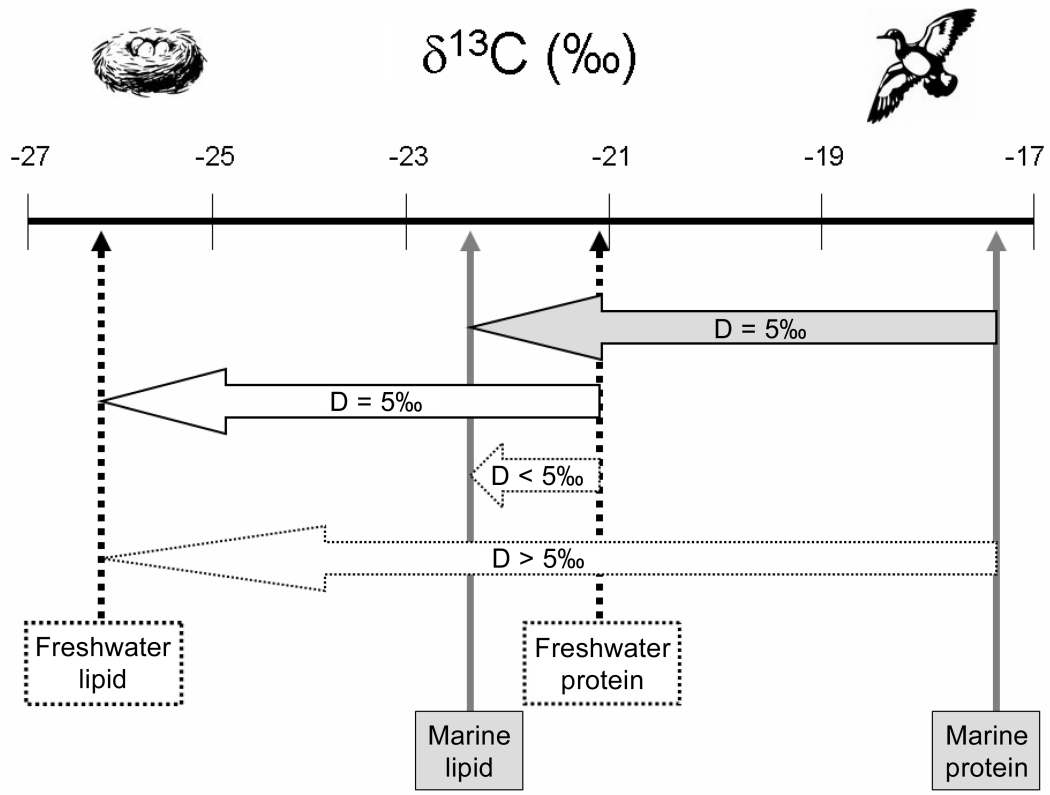

Fig. 3. Schematic presentation of how parameter *D* (the isotopic difference between lipid and protein fractions in egg yolk) can vary in bird species that obtain macronutrients from different ecosystems. The concept is demonstrated for birds that breed in a freshwater environment with food depleted in $\delta^{13}C$ but migrate or winter (or both) in a marine environment with food enriched in $\delta^{13}C$. *D* can assume values greater or smaller than the hypothetical constant *D* = 5‰, depending on which macronutrient is transferred between ecosystems.

differentially allocate lipids and protein from isotopically distinct diet sources or environments. Yolk samples from those species should be analyzed in both the lipid-extracted (for $\delta^{13}C$) and bulk form (for $\delta^{34}S$ and $\delta^{15}N$) if inference from multiple isotopes is desired.

For species that likely derive lipids and proteins from the same source and environment, we recommend the following steps to minimize analytical costs. (1) Analyze whole yolk for $\delta^{13}C$ and $\delta^{15}N$, and calculate C:N ratios from standard output of isotopic analysis. (2) Choose at least 10 yolk samples representing the entire range of C:N ratios for that species, chemically lipid extract yolk, analyze $\delta^{13}C$ of lipid extracted yolk, and calculate $\Delta\delta^{13}C$ for these 10 samples. (3) Analyze the relationship between C:N ratio and $\Delta\delta^{13}C$ for these 10 samples; if variation in $\Delta\delta^{13}C$ around the regression line is within the range of measurement uncertainty (SD < 0.3‰), then arithmetic lipid correction is possible for the remaining eggs of that species. (4) If there is no relationship between $\Delta\delta^{13}C$ and the C:N ratio, use the simple correction of adding the average of measured $\Delta\delta^{13}C$ to whole yolk (Ricca et al. 2007). (5) Otherwise, use the regression relationship between C:N ratio and $\Delta\delta^{13}C$ to arithmetically correct yolk samples (Post et al. 2007). Although the model suggested by Kiljunen et al. (2006) is robust for lipid-correcting yolk, it is likely that over the range of lipid content in avian egg yolk, the relationship between $\Delta\delta^{13}C$ and C:N ratio will be linear (Sotherland and Rahn 1987, Ricca et al. 2007) and a simple correction based on $\Delta\delta^{13}C$ will be sufficient. We encourage researchers to report all isotopic measurements of egg yolk in future studies and validate the model that provided accurate predictions in our study of captive Spectacled Eiders for species in the wild.

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protocol #05-29 and complies with current U.S. laws. Mention of trade or product names is for descriptive purposes only and does not imply endorsement by the U.S. government.

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Appendix. Published data on the mean difference in $\delta^{13}C$ between chemically lipid-extracted yolk and yolk lipids (parameter *D*) from a variety of bird species. *D* can be used as a constant in arithmetic lipid-correction models. Multiple rows for a single species represent different study years or study sites. Data for *D* from species in bold (carnivorous ducks) were used in our model for sea ducks.

Species	*n*	$\delta^{13}C$ lipid-free yolk	$\delta^{13}C$ yolk lipids	*D*	Source
Double-crested Cormorant (*Phalacrocorax auritus*)	10	–22.8	–25.3	2.5	Hobson et al. 1997
	6	–23.9	–25.7	1.8	Hobson et al. 1997
Greater Snow Goose (*Chen caerulescens atlantica*)	20	–25.3	–29.2	3.9	Gauthier et al. 2003
	20	–25.0	–28.6	3.6	Gauthier et al. 2003
	25	–24.5	–27.3	2.9	Gauthier et al. 2003
	18	–25.0	–27.1	2.1	Hobson et al. 2000
Ross's Goose (*C. rossii*)	18	–25.8	–28.0	2.2	Hobson et al. 2000
Mallard (*Anas platyrhynchos*)	16			2.7	Hobson 1995
Redhead (*Aythya americana*)	32	–29.2	–32.9	3.7	Hobson et al. 2004
King Eider (*Somateria spectabilis*)	44	–23.8	(–26.4)[a]		Lawson 2006
Long-tailed Duck (*Clangula hyemalis*)	29	–23.6	(–25.9)[a]		Lawson 2006
Barrow's Goldeneye (*Bucephala islandica*)	24	–24.7	–31.0	6.3	Hobson et al. 2005
Ruddy Turnstone (*Arenaria interpres*)	24	–25.3	–30.4	5.1	Morrison and Hobson 2004
Parasitic Jaeger (*Stercorarius parasiticus*)	10	–24.4	–27.5	3.1	Hobson et al. 2000
Bonaparte's Gull (*Chroicocephalus philadelphia*)	8	–24.8	–29.0	4.2	Hobson et al. 2000
Ring-billed Gull (*Larus delawarensis*)	10	–23.6	–26.8	3.2	Hobson et al. 2000
Mew Gull (*L. canus*)	23	–25.0	–27.9	2.9	Hobson et al. 2000
Herring Gull (*L. argentatus*)	12	–24.0	–26.2	2.2	Hobson et al. 1997
	5	–21.8	–23.2	1.4	Hobson et al. 1997
	12	–21.7	–25.6	3.9	Hobson et al. 1997
	24	–24.9	–30.7	5.8	Hobson et al. 2000
California Gull (*L. californicus*)	10	–26.2	–30.4	4.2	Hobson et al. 2000
Common Tern (*Sterna hirundo*)	10	–22.3	–22.8	0.5	Hobson et al. 2000
Arctic Tern (*S. paradisaea*)	25	–24.8	–26.7	1.9	Hobson et al. 2000
Black Tern (*Chlidonias niger*)	10	–26.9	–27.4	0.5	Hobson et al. 2000
Caspian Tern (*Hydroprogne caspia*)	16	–25.0	–27.0	2.0	Hobson et al. 1997
	10	–18.6	–21.2	2.6	Hobson et al. 1997
	9	–22.8	–27.7	4.9	Hobson et al. 2000
Prairie Falcon (*Falco mexicanus*)	2			3.7	Hobson 1995
Peregrine Falcon (*F. peregrinus*)	6			3.5	Hobson 1995
Gyrfalcon (*F. rusticolus*)	4			3.3	Hobson 1995
Japanese Quail (*Coturnix japonica*)	9			2.7	Hobson 1995

[a] Yolk lipid value calculated from mass balance equations based on egg yolk lipid content of pelican, gull, and gannet eggs.