Effects of lipid extraction on $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in avian egg yolk

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Background

Avian egg yolk is composed of >50% lipid. Most studies examining nutrient allocation to egg yolk remove lipids from egg yolk by chemical extraction to avoid results being confounded by ^{13}C-depleted lipids.

Currently, it is not known how this chemical lipid extraction affects isotope ratios in egg yolk.

Here we show that chemical lipid extraction changes the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ isotope ratios of egg yolk of two species of sea ducks, king (*Somateria spectabilis*) and spectacled eider (*S. fischeri*). We therefore explore whether arithmetic lipid normalization could be applied to avian egg yolk.

Spectacled Eider (© *R. Fellner*)

King Eider (© *R. Fellner*)

Methods

We collected 20 eggs from wild king eiders nesting on the tundra of northern Alaska. We also obtained 15 eggs from captive spectacled eiders housed at the Alaska Sealife Center (Seward, AK), which were fed a high-quality homogenous diet throughout their life. We manually separated yolk from albumen and then employed the following steps:

- freeze-dried yolk and ground to homogenous powder
- **lipid extraction** with repeated rinses of 2:1 chloroform:methanol solution
- separation of lipid-free yolk and yolk lipids
- stable isotope analysis of whole yolk, lipid-free yolk, and yolk lipids using continuous flow stable isotope ratio mass spectrometry

We then explored whether **arithmetic lipid normalization** would provide plausible results for egg yolk by following the approach of Kiljunen et al. (2006, *J. Anim. Ecol.* 27:345) adjusted for dry egg yolk:

- calculated lipid content (L) of yolk based on molar C:N ratio of whole yolk:

$$L = \frac{96}{1+(0.246\times(C:N)-0.775)^{-1}}$$

- calculated $\delta^{13}C_{normalized}$ with the following formula:

$$\delta^{13}C_{normalized} = \delta^{13}C_{whole\ yolk} + D \times (0.048 + \frac{3.90}{1+287/L})$$

- averaged D over 4 carnivorous duck species from published studies (D = 5‰)

Results

Chemical lipid extraction

Lipid extraction with a 2:1 chloroform:methanol solution led to an increase of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in egg yolk (Fig. 1, Table 1).

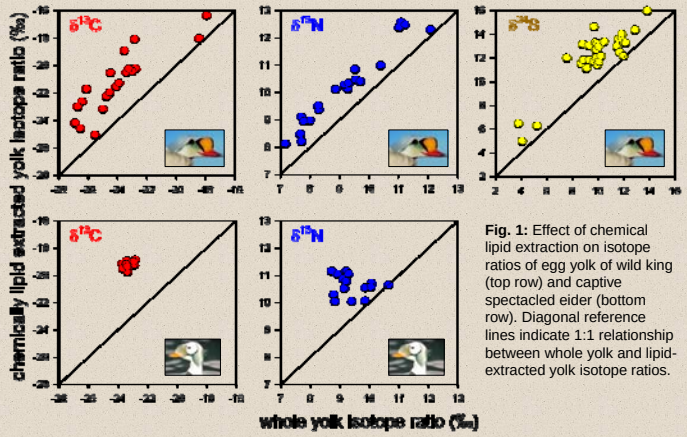

Fig. 1: Effect of chemical lipid extraction on isotope ratios of egg yolk of wild king (top row) and captive spectacled eider (bottom row). Diagonal reference lines indicate 1:1 relationship between whole yolk and lipid-extracted yolk isotope ratios.

Table 1: Average increase in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in egg yolk after chemical lipid extraction.

isotope	species	*n*	Δ (‰)	SD (‰)
$\delta^{13}C$	spectacled eider	15	4.10	0.35
	king eider	20	2.76	1.20
$\delta^{15}N$	spectacled eider	15	1.21	0.74
	king eider	20	1.01	0.33
$\delta^{34}S$	king eider	30	2.33	1.12

Arithmetic lipid normalization

Arithmetic lipid-normalization yielded very accurate prediction of lipid-free yolk $\delta^{13}C$ for captive spectacled eiders, with predicted values very similar to measured values ($\delta^{13}C_{normalized}$ – $\delta^{13}C_{extracted}$ = 0.1‰ ± 0.2‰, paired *t*-test t_{14} = 1.46, p = 0.17).

In wild king eider egg yolk $\delta^{13}C_{normalized}$ and $\delta^{13}C_{extracted}$ were highly correlated (r_s = 0.94, P < 0.001), but $\delta^{13}C_{normalized}$ was on average 1.2‰ higher than $\delta^{13}C_{extracted}$ (t_{19} = -4.43, p < 0.001). The prediction error was greatest in those eggs in which the measured difference between $\delta^{13}C_{extracted}$ and $\delta^{13}C_{lipid}$ deviated most from the constant (D = 5‰) used in the model (R^2 = 0.92).

Discussion

Lipid extraction with a chloroform:methanol solution increased $\delta^{15}N$ and $\delta^{34}S$ isotope ratios in avian egg yolk. The 1-2 ‰ difference could bias the conclusions of nutrient allocation studies. We recommend using whole yolk to analyze $\delta^{15}N$ and $\delta^{34}S$ in egg yolk. Alternatively, lipid-extracted yolk $\delta^{15}N$ could be corrected for the effects of lipid extraction by subtracting 1‰.

Lipid normalization of egg yolk $\delta^{13}C$ provides accurate results for birds feeding on a constant, high-quality diet. Wild migratory birds, however, can obtain macronutrients from different ecosystems, which leads to a non-constant difference between $\delta^{13}C$ of yolk protein and yolk lipids (parameter D). This explains the bias of the lipid normalization model we found in King Eider eggs (Fig. 2).

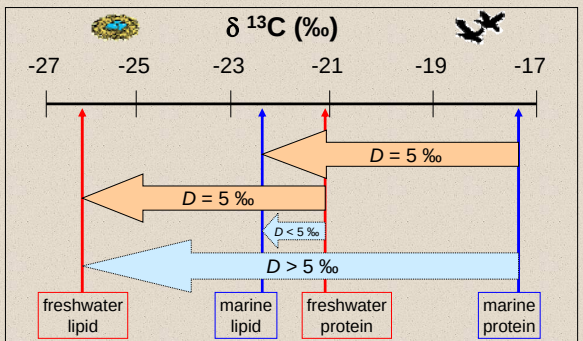

Fig. 2: Schematic presentation of how parameter D may vary in bird species that obtain macronutrients from different ecosystems. The concept is demonstrated for birds that breed in a freshwater environment with food depleted in $\delta^{13}C$, but migrate in a marine environment with food enriched in $\delta^{13}C$. D can assume values greater or smaller (blue arrows) than the hypothetical constant D = 5‰ (orange arrows) depending on which macronutrient is transferred between ecosystems. This violates an assumption of normalization models and can result in biased estimates.

Acknowledgements: We thank the North Slope Borough and Alaska SeaLife Center for funding and logistical support. We would also like to thank numerous field and lab assistants for their help, as well as K. Hobson, D. O'Brien, P. Barboza, and C. Stricker for valuable comments.

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