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FIELD PRESERVATION TECHNIQUES FOR THE ANALYSIS OF STABLE-CARBON AND NITROGEN ISOTOPE RATIOS IN EGGS

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Abstract.—Tissue preservation is a challenge faced by researchers collecting eggs in the field. Use of stable isotope analyses of eggs will undoubtedly increase as a means to trace directly avian trophic relationships and nutrient allocation to reproduction, and so field preservation techniques that do not alter stable-isotope ratios are needed. We evaluated effects of several preservation techniques on stable-carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios in lipid-free yolk and albumen, and stable-carbon isotope ratios in yolk lipid. Drying, freezing, boiling then freezing eggs, and storage of egg components in 70% ethanol did not alter stable-carbon and nitrogen isotope abundance in egg components, and we recommend these preservation techniques prior to isotopic analysis. Boiling eggs prior to freezing facilitates future separation of albumen and yolk. Maintaining boiled eggs at 6 C for 7 d reduced ^{13}C abundance in lipid-free yolk, while maintenance at 6 C for 50 d increased ^{15}N abundance in albumen. Preservation in Formalin should be avoided as it reduced ^{13}C abundance in lipid-free yolk and albumen but did not affect yolk lipids.

TÉCNICAS DE PRESERVACIÓN DE CAMPOS PARA EL ANÁLISIS DE RAZONES DE ISÓTOPOS ESTABLES DE CARBONO Y DE NITRÓGENO EN HUEVOS

Sinopsis.—La preservación de tejidos es un reto enfrentado por investigadores colectando huevos en el campo. El análisis de huevos mediante isótopos estables sin duda aumentará como manera de trazar directamente las relaciones tróficas de aves y la alocación de nutrientes a la reproducción. Por tanto, se necesitan ténicas de campo que no alteren las razones entre isótopos estables. Nosotros evaluamos los efectos de varias técnicas de preservación en las razones de isótopos estables de carbono ($^{13}C/^{12}C$) y nitrógeno ($^{15}N/^{14}N$) en yemas libres de lipidos y albúmina y razones de isótopos estables de carbono en lipido de yema. Secar, congelar, herviry entonces congelar los huevos, y almacenar los componentes de huevos en etanol 70% no alteró la abundancia de isótopos estables de carbono y de nitrógeno en los componentes de huevos, y recomendamos estas técnicas de preservación previo a un análisis isotópico. Hervir huevos antes de congelarlos facilita separaciones futuras de albúmina y yema. Mantener huevos hervidos a 6 C por 7 d redujo la abundancia de ^{13}C en yemas sin lipidos, mientras que mantenerlos a 6 C por 50 d aumentó la abundancia de ^{15}N en la albúmina. Se debe evitar la preservación en formalina, ya que reduce la abundancia de ^{13}C en yemas y albúminas libres de lipidos pero no afectaria lipidos de las yemas.

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The measurement of stable-carbon and nitrogen isotope abundance in avian tissues can provide valuable ecological information on diets of individuals as well as populations (reviewed by Hobson 1995). Until recently, stable-isotope analysis of birds has focused almost exclusively on tissues from young and adults (Hobson 1987, 1990; Hobson and Sealy 1991; Hobson et al. 1994; Mizutani et al. 1990). However, application of this technique has recently expanded to include eggs. In particular, Hobson (1995) demonstrated that stable-carbon and nitrogen isotope analyses of avian eggs can provide both dietary information and insights into metabolic pathways linking endogenous and exogenous reserves to reproduction.

The stable-isotope technique measures the relative abundance of naturally occurring stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$). Egg yolk lipids, lipid-free yolk, albumen, shell carbonates, and shell membranes are all materials amenable to isotopic analysis. While eggshells are easily preserved without treatment, a problem frequently faced by researchers collecting whole eggs is preservation of samples under field conditions and transportation of samples back to the laboratory. Much avian research is conducted in remote locations where preservation of tissues must conform to available resources. Proper storage techniques must conserve the original stable-carbon and nitrogen isotope signature without alteration due to elemental exchange or chemical and/or biological degradation (Gearing 1991). However, we know little about effects of preservation techniques on stable-carbon and nitrogen isotope abundance in eggs and other tissues. Therefore, our objective was to evaluate effects of several preservation techniques on stable-carbon and nitrogen isotopic abundance of yolk lipids, lipid-free yolk, and albumen.

METHODS

A random sample of 50 Japanese Quail (*Coturnix japonica*) eggs, all laid the same day, was obtained from the University of Saskatchewan Animal Care Quail colony comprising 200 laying females. Females were of similar age and were raised from hatch on isotopically homogenous diets (Hobson 1995). Thus, we are confident that all eggs initially had similar stable-carbon and nitrogen isotope ratios.

To evaluate effects of field preservation techniques on stable-isotope ratios, eight treatments were established each containing five randomly selected eggs. We examined effects of freezing, boiling, and preservation in 70% ethanol and 10% formaldehyde (hereafter Formalin) on stable-isotope ratios. With the possible exception of freezing, these preservation techniques are easily accomplished in the field. As a control, fresh yolks were separated from the albumen, and egg tissues were dried at 60 C to constant mass and then stored in a 2:1 chloroform:methanol solution for 50 d. The chloroform:methanol solution extracts lipids from tissues (Bligh and Dyer 1959). Following treatments, yolks were separated from the albumen and egg tissues were dried at 60 C to constant mass and then placed in a 2:1 chloroform:methanol solution for 48 h to extract lipids. Yolk lipids, lipid-free yolk, and

TABLE 1. Egg preservation techniques.

Control (C1)	Separated[a], dried[b], preserved in 2:1 chloroform : methanol solution for 50 d, then dried.
T1	Whole egg frozen[c] for 50 d, then separated, dried, and extracted[d].
T2	Whole egg boiled[e], then frozen for 50 d, then separated, dried, and extracted.
T3	Whole egg boiled, then 7 d at room temperature[f], and then frozen until day 50, then separated, dried, and extracted.
T4	Whole egg boiled, then 7 d at 6 C[g], then frozen until day 50, then separated, dried, and extracted.
T5	Whole egg boiled, then 50 days at (6 C), then separated, dried, and extracted.
T6	Separated, preserved in 70% Ethanol[h] for 50 d, dried, then extracted.
T7	Separated, preserved in 10% Formalin[h] for 50 d, dried, then extracted.

[a] Separated egg into yolk and albumen components.
[b] Each individual egg component oven-dried at 60 C to constant mass.
[c] Frozen at −20 C.
[d] Lipids extracted using a 2:1 ratio of chloroform : methanol.
[e] Boiled for 10 min.
[f] In an open plastic bag at 20 C.
[g] In a closed plastic bag in a refrigerator.
[h] Yolk and albumen preserved separately at room temperature.

albumen were then prepared for isotopic analyses following standard protocols as outlined in Hobson (1995). Mass spectrometric analyses were performed on all egg components at the stable isotope facility of the Department of Soil Science, University of Saskatchewan, using a continuous-flow Europa 20:20 isotope-ratio mass spectrometer interfaced with a Robo Prep elemental analyzer. Based on several hundred replicate measurements of laboratory standards, we estimate measurement error to be ±0.1‰ and ±0.3‰ for stable-carbon and nitrogen isotope ratios, respectively. All isotope values are expressed in delta (δ) notation relative to the PeeDee Belemnite (PDB) and atmospheric (AIR) standards for carbon and nitrogen, respectively (Hobson 1995).

We used an analysis of variance (ANOVA) to test for effects of preservation techniques on the stable-carbon and nitrogen isotopic abundance for each egg component separately. Dunnett's tests (SAS 1991) were used to determine if stable-isotope ratios of tissues differed according to preservation technique compared with the control.

RESULTS

Preservation techniques had a significant effect on $\delta^{13}C$ and $\delta^{15}N$ values in albumen and lipid-free yolk (ANOVA: Lipid-free yolk: Carbon $F_{7,32} = 43.8$, $P = 0.0001$, Nitrogen: $F_{7,32} = 4.8$, $P = 0.0009$; Albumen: Carbon: $F_{7,32} = 60.3$, $P = 0.0001$, Nitrogen: $F_{7,32} = 4.4$, $P = 0.0016$), but did not effect $\delta^{13}C$ values in yolk lipids (ANOVA: Yolk lipid: Carbon: $F_{7,32} = 1.4$, $P = 0.23$; Table 2). Dunnett's tests revealed that preservation in Formalin (T7) significantly reduced $\delta^{13}C$ values in lipid-free yolk and albumen,

TABLE 2. Mean $\delta^{13}C$ and $\delta^{15}N$ values (‰) for each egg component preserved using various techniques.

	Yolk lipid	Lipid-free yolk		Albumen	
Technique	$\delta^{13}C$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
C1	−26.6 ± 0.2	−23.3 ± 0.3	8.5 ± 0.1	−23.1 ± 0.2	7.3 ± 0.3
T1	−26.6 ± 0.2	−23.2 ± 0.3	8.5 ± 0.3	−23.1 ± 0.2	7.3 ± 0.2
T2	−26.4 ± 0.3	−23.3 ± 0.4	8.7 ± 0.1	−23.2 ± 0.3	7.5 ± 0.4
T3	−26.2 ± 0.3	−23.3 ± 0.3	8.8 ± 0.3	−23.3 ± 0.2	7.1 ± 0.2
T4	−26.4 ± 0.4	−23.8 ± 0.4	8.1 ± 0.3	−23.3 ± 0.1	7.5 ± 0.1
T5	−26.5 ± 0.4	−23.4 ± 0.2	8.6 ± 0.2	−23.1 ± 0.1	7.7 ± 0.1
T6	−26.3 ± 0.5	−23.2 ± 0.3	8.4 ± 0.2	−23.1 ± 0.3	7.2 ± 0.2
T7	−26.7 ± 0.4	−25.7 ± 0.2	8.5 ± 0.2	−25.5 ± 0.3	7.6 ± 0.2

while maintaining boiled eggs at 6 C for 7 d (T4) significantly reduced $\delta^{13}C$ values in lipid-free yolk (Table 3). Furthermore, $\delta^{15}N$ values in albumen were significantly enriched when boiled eggs were maintained at 6 C for 50 d (T5, Table 3).

DISCUSSION

Our study indicates that freezing (T1) and boiling then freezing (T2) eggs does not significantly alter $\delta^{13}C$ and $\delta^{15}N$ values in the egg components examined compared with the control. Under remote field conditions, boiling or drying of egg components may be the only preservation techniques available. A compromise must be made when drying between quickly arresting bacterial action and loss of volatile organics; Gearing (1991) recommended drying temperatures of 40–80 C. In our study, fresh yolk and albumen dried to constant mass at 60 C in 4 and 3 d, respectively, but drying time will vary with sample size. Preservation of egg yolk and albumen in 70% ethanol (T6) had no effect on stable-isotope ratios. Storage of samples in 70% ethanol or, in cases where researchers intend to remove lipids prior to analysis, in a 2:1 chloroform : methanol solution, is

TABLE 3. Mean differences (‰) in $\delta^{13}C$ and $\delta^{15}N$ values between control and treatment preservation techniques for each egg component and results of Dunnett's test for significance.

Control:	Yolk lipid	Lipid-free yolk		Albumen	
treatment	$\delta^{13}C$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
C1:T1	−0.02	0.08	0.05	0.03	0.02
C1:T2	0.14	−0.03	0.22	−0.11	0.14
C1:T3	0.41	0.03	0.3	−0.21	−0.20
C1:T4	0.13	−0.55[a]	−0.36	−0.2	0.21
C1:T5	0.03	−0.13	0.13	0.0	0.41[a]
C1:T6	0.27	0.08	−0.1	0.0	−0.07
C1:T7	−0.16	−2.44[a]	0.03	−2.39[a]	0.24

[a] $P < 0.05$.

a suitable option, especially in hot or humid conditions where freezing or drying are difficult. Storage of boiled eggs at 6 C for various periods (T4, T5) caused significant decreases in $\delta^{13}C$ values in lipid-free yolk and significant increases in $\delta^{15}N$ values in albumen. This may have occurred because eggs were stored in sealed plastic bags and the subsequent restriction of air circulation may have accelerated biological degradation.

Storage of egg components in Formalin is not appropriate. Previously, Formalin was shown to deplete $\delta^{13}C$ and $\delta^{15}N$ values in zooplankton by 2–3 and <1‰, respectively (Mullin et al. 1984). Changes in $\delta^{13}C$ but not $\delta^{15}N$ values was confirmed for all egg components. Formalin likely alters $\delta^{13}C$ values through direct incorporation of isotopically lighter Formalin-based carbon into the sample or through leaching out of the more soluble organics into solution (Gearing 1991).

In conclusion, where it is necessary to store eggs for considerable periods in the field, we recommend that fresh egg components be dried, frozen, or boiled then frozen. If this is not possible, then storage in 70% ethanol is preferred or, in cases where lipids are to be ultimately separated from samples, in a 2:1 chloroform : methanol solution.

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