



## EFFECT OF CLEANING REGIME ON STABLE-ISOTOPE RATIOS OF FEATHERS IN JAPANESE QUAIL (*COTURNIX JAPONICA*)

JACQUELINE M. PARITTE<sup>1</sup> AND JEFFREY F. KELLY

Department of Zoology and Oklahoma Biological Survey, University of Oklahoma, 111 East Chesapeake Street, Norman, Oklahoma 73019, USA

**ABSTRACT.**—Stable-isotope analysis of feathers is an increasingly important source of information on diet and movement of birds. Feathers are typically cleaned with a solvent before analysis, but the effects of this cleaning on the resulting data have not been examined critically. We conducted an experiment to determine whether a cleaning regime affected hydrogen ( $\delta D$ ), carbon ( $\delta^{13}C$ ), and nitrogen ( $\delta^{15}N$ ) stable-isotope ratios in feathers of Japanese Quail (*Coturnix japonica*). A paired design was used to clean feathers with 2:1 chloroform:methanol or detergent. Results after initial cleaning indicated enrichment of hydrogen by ~40‰ in feathers treated with 2:1 chloroform:methanol-treated compared with other treatments but no correlation between treatments. We found a similar pattern among treatments for carbon, but the effect was on the order of 0.2‰. Nitrogen values showed no discernible correlation, but both uncleaned and 2:1 chloroform:methanol treatments had enriched values, on average, compared with detergent treatments. Further, variance among samples was high for hydrogen and nitrogen measurements. After recleaning with alternate treatments, differences in mean hydrogen-isotope ratios were no longer evident, data for hydrogen and carbon became less variable, and carbon maintained its initial pattern. We suggest a standard method of cleaning feathers, first with a dilute detergent solution, then with 2:1 chloroform:methanol solvent. This study has implications for increasing repeatability of hydrogen, carbon, and nitrogen stable-isotope measurements, which would increase the validity of inter-laboratory comparisons and the utility of large-scale projects using compiled data sets. Received 9 November 2007, accepted 29 September 2008.

Key words: 2:1 chloroform:methanol, carbon-13, deuterium, nitrogen-15, stable-isotope ratios, standard cleaning method.

### Efecto del Régimen de Limpieza sobre Los Cocientes de Isótopos Estables de Plumas de *Coturnix japonica*

**RESUMEN.**—El análisis de isótopos estables de plumas es una fuente de información creciente sobre la dieta y el movimiento de las aves. Las plumas son típicamente limpiadas con un solvente antes del análisis, pero el efecto de esta limpieza sobre los datos resultantes no se ha evaluado de forma crítica. Realizamos un experimento para determinar si un régimen de limpieza afectaba los cocientes de isótopos estables de hidrógeno ( $\delta D$ ), carbono ( $\delta^{13}C$ ) y nitrógeno ( $\delta^{15}N$ ) de *Coturnix japonica*. Se utilizó un diseño pareado en que se limpiaron las plumas con cloroformo:metanol 2:1 o detergente. Después de la limpieza inicial los resultados indicaron un enriquecimiento de hidrógeno de un ~40‰ en las plumas tratadas con cloroformo:metanol 2:1 en comparación con los otros tratamientos, pero no se observó correlación entre los tratamientos. Encontramos un patrón similar entre los tratamientos para el carbono, pero el efecto fue del orden del 0.2‰. Los valores de nitrógeno no mostraron una correlación discernible, pero tanto los tratamientos sin limpieza como el de cloroformo:metanol 2:1 presentaron, en promedio, valores enriquecidos en comparación con los tratamientos de detergente. Además, la varianza entre muestras fue alta para las medidas de hidrógeno y nitrógeno. Después de limpiar nuevamente con tratamientos alternativos, las diferencias en los cocientes medios de isótopos de hidrógeno ya no fueron evidentes, los datos para hidrógeno y carbono se tornaron menos variables y el carbono mantuvo su patrón inicial. Sugerimos un método estándar para limpiar las plumas, primero con una solución diluida de detergente, luego con solvente cloroformo:metanol 2:1. Este estudio tiene implicancias para aumentar la repetibilidad de las medidas de isótopos estables de hidrógeno, carbono y nitrógeno, lo que aumentaría la validez de las comparaciones entre laboratorios y la utilidad de proyectos a grandes escalas utilizando bases de datos compilados.

STABLE-ISOTOPE ANALYSIS has become increasingly important in avian ecology for tracing dietary inputs (DeNiro and Epstein 1978, 1981; Hobson et al. 1999; Wolf et al. 2002), food webs (Hobson et al. 1993, 1994; Kelly 2000; Birchall et al. 2005), and movement patterns (Cherel et al. 2000, Kelly et al. 2005, Mazerolle et al. 2005, Dunn et al. 2006) of birds. The technique analyzes within-sample

relative quantities of hydrogen, oxygen, carbon, nitrogen, or sulfur stable isotopes, which vary naturally as a result of physical, chemical, and biological phenomena.

As stable-isotope approaches have been rapidly incorporated into ecology, a multitude of sample-preparation methods has been employed. However, discovery of novel applications for isotopes

<sup>1</sup>E-mail: [jparitte@ou.edu](mailto:jparitte@ou.edu)

has tended to overshadow advances in methodology. More attention has been given recently to detailing the accuracy and precision of published results (Jardine and Cunjak 2005); furthering laboratory experiments to test assumptions (Gannes et al. 1997); developing correction techniques, such as those for exchangeable hydrogen (Wassenaar and Hobson 2000a, 2003); and identifying sources of intrasample variation in stable-isotope analysis to minimize the effect on data (Wassenaar and Hobson 2006, Wunder and Norris 2008). Wunder and Norris (2008), when comparing  $\delta D$  error from data interpolation to analytical error, concluded that analytical error was highly influential in the incorrect assignment of birds to geographic origin; however, their definition of analytical error considered only mass-spectrometric measurement error, not error from sample preparation. Understanding the effect of various sample-preparation methods on the mean and variance of stable-isotope measurements is an important component of analytical error and requires controlled comparisons for each sampled tissue type.

In avian isotope studies, primarily those that estimate geographic origin, feathers are commonly sampled because they are metabolically inert once grown (Hobson and Wassenaar 1997) and are relatively unintrusive to sample (Jaspers et al. 2007). Feathers are typically cleaned with a solvent before analysis to remove residual dirt and oil, yet there is no standard cleaning method and effects of differences in cleaning methods on resulting data have not been examined critically. We found only one attempt at a comparison of cleaning methods in the literature. Bensch et al. (2006), before analyzing feathers for an African Willow Warbler (*Phylloscopus trochilus*) study, compared isotope ratios in detergent-cleaned and uncleaned feathers and found no difference in measurements. This comparison is significant; however, more comprehensive studies are needed to determine the most effective feather-cleaning method and to move toward standard sample-preparation protocol. Creating detailed cleaning protocols is a complex process, but it may improve the consistency of data, with the goal of obtaining the best possible estimate of the true isotope value in the tissue. If cleaning regimes influence stable-isotope ratios, developing a consistent routine with appropriate cleaning agents can increase repeatability of analyses and reduce the risk of isotopic fractionation due to cleaning, thereby improving the efficacy of hydrogen, carbon, and nitrogen values in their applications and increasing the reliability of comparison among data sets.

We evaluated three common feather-washing techniques to determine how they influence hydrogen, carbon, and nitrogen stable-isotope ratios. From these experiments, we elucidate the presence and magnitude of bias that is introduced into isotope data through choice of cleaning agent and suggest which method produces the most consistent data.

#### METHODS

We reviewed 69 publications that used feathers for stable-isotope analysis (Appendix) and found reports of 11 cleaning agents used, the two most common being a solvent of 2:1 chloroform:methanol (43%; 30 publications) and detergent (15%; 10 publications). Another eight publications (12%) either did not clean feathers before processing or did not document the cleaning method. On the basis of this survey, we chose to use 2:1 chloroform:methanol, detergent, and no cleaning as the cleaning treatments for this study. Further, few publications

mentioned comprehensive specifics of cleaning procedures (e.g., duration of washing–drying, agitation method), so our experimental protocol here is based on how we clean feathers in our lab.

We obtained 17 frozen Japanese Quail (*Coturnix japonica*) of similar age, mass, and diet from the Sutton Avian Research Center in Bartlesville, Oklahoma (36°N, 96°W; elevation 230 m). To reduce the isotopic variation seen among feathers in individual birds, we used only the two first primaries from each bird, both of which should reflect isotope ratios in symmetrical patterns. Because Japanese Quail have only two of any corresponding feather, comparing three cleaning treatments requires that both the left and right feathers receive two of the three treatments, such that one treatment is assigned to both the left and right feathers. This duplicate treatment allows for transitive comparison of the other two treatments. If replicated treatments yield the same values from both left and right primaries, which should yield similar isotope ratios, the other two treatment values should then be comparable.

To do this, we removed left and right first primary feathers (Fig. 1) and randomly assigned each feather to one of two cleaning treatments—2:1 chloroform:methanol and detergent or detergent and no cleaning—such that each Japanese Quail received both treatments. We halved feathers along the rachis.

We randomly cleaned one feather, half with one of the two cleaning types in its assigned treatment and the other half with the other cleaning type. We placed feather halves cleaned in 2:1 chloroform:methanol in a 120-mL sealed jar with the solvent and shook the jar for 30 s under a fume hood. We then removed the feather and allowed it to air dry for 24 h under a fume hood. Similarly, we cleaned the feather halves in detergent once with 1 L of a 1:30 solution of Fisher Versa-Clean (Fisher Scientific, Pittsburgh, Pennsylvania; catalogue number 04-342) detergent:deionized water, then rinsed three times in three 1-L jars of deionized water and allowed to air dry for 24 h under a fume hood. We changed the deionized water after every five feathers to prevent detergent accumulation. We left the uncleaned feather halves in their original condition. We then sampled feathers for  $\delta D$ ,  $\delta^{13}C$ , and  $\delta^{15}N$  analyses. After this first sample was taken, to further test for effects of solvent residue on isotope values and data variation, we recleaned feather halves treated with the 2:1 chloroform:methanol or detergent treatment with the other treatment in its respective pair and resampled for  $\delta D$ ,  $\delta^{13}C$ , and  $\delta^{15}N$  analyses.

To ensure that any variation in feather stable-isotope ratios was not attributable to variation along the feather, we took  $\delta D$  samples from only the last centimeter of the feather and  $\delta^{13}C$ / $\delta^{15}N$  samples from the next-to-last centimeter. One centimeter of feather provides enough tissue for two samples, allowing us to resample the same section of feather a second time after additional treatment. We used entire feathers, including rachis and barbs, for analysis. To reduce further sample variation due to isotopic variation within feather parts, we cut each sample to contain proportional quantities of the rachis and barbs.

*Stable-isotope analyses.*—Hydrogen stable-isotope samples were run at the Environment Canada Stable Isotope Hydrology and Ecology Research Laboratory in Saskatoon, Saskatchewan, as described in Wassenaar and Hobson (2003). The  $\delta^{13}C$  and  $\delta^{15}N$  samples were run at the Stable Isotope Laboratory of the Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque.

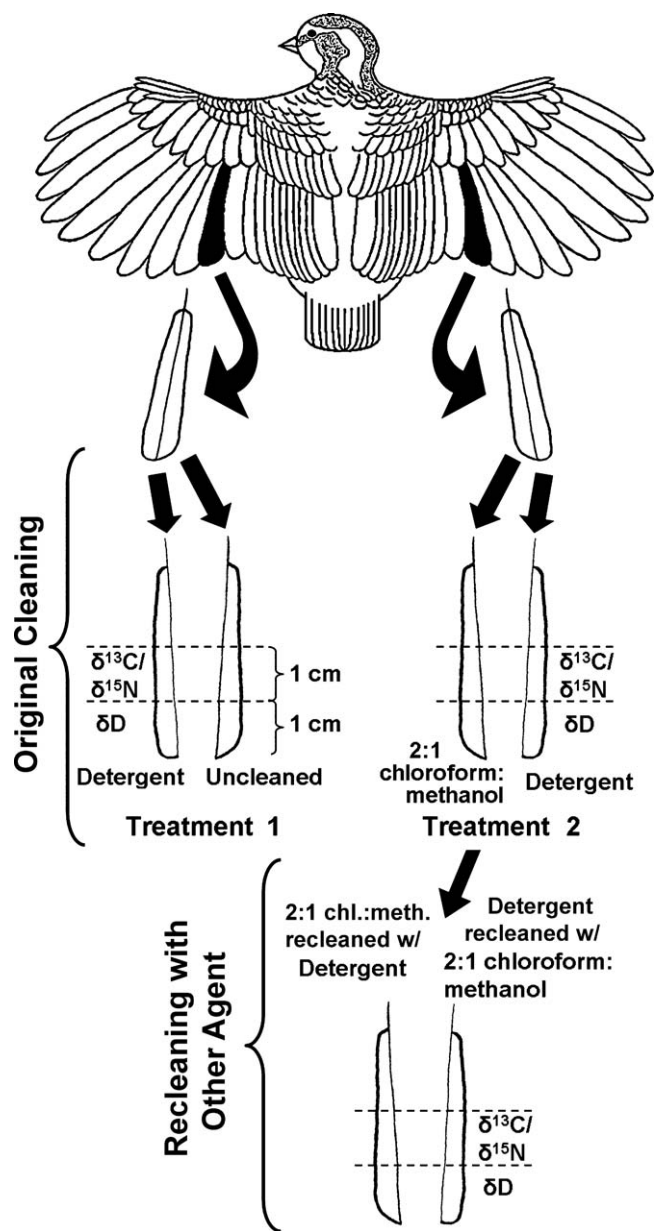


FIG. 1. Schematic of treatment pairs, with treatments assigned randomly to left and right wings and feather halves.

*Statistical analyses.*—To determine whether cleaning treatments yield similar results, we performed paired *t*-tests to compare sample means. We also provide least-squares best-fit regression lines as a descriptor of data colinearity. If treatments yield the same results, data points will fall along a 1:1 line (slope = 1, intercept = 0). We also used these tests to compare data from the detergent treatments from left and right wings of the same individual. These data should also fall along a 1:1 line if left and right wings produce equivalent values. For the recleaning experiment, we used the aforementioned *t*-tests along with Levene's test for equal variance to determine whether within-sample variation decreased with recleaning.

RESULTS

In the uncleaned–detergent treatment, uncleaned values were lighter than detergent values for  $\delta D$  (means,  $t = -2.86$ ,  $df = 14$ ,  $P = 0.013$ ; regression, Fig. 2A) but heavier than detergent means for  $\delta^{15}N$  (means,  $t = 3.37$ ,  $df = 16$ ,  $P = 0.004$ ; Fig. 2E). Uncleaned and detergent means for  $\delta^{13}C$  were not different (means,  $t = 0.64$ ,  $df = 16$ ,  $P = 0.529$ ; regression, Fig. 2C). For detergent–2:1 chloroform:methanol pairs, 2:1 chloroform:methanol values were heavier for all three elements (means:  $\delta D$ ,  $t = 11.91$ ,  $df = 16$ ,  $P < 0.001$ ;  $\delta^{13}C$ ,  $t = 4.018$ ,  $df = 16$ ,  $P = 0.001$ ;  $\delta^{15}N$ ,  $t = 6.66$ ,  $df = 16$ ,  $P < 0.001$ ; regressions:  $\delta D$ , Fig. 2B;  $\delta^{13}C$ , Fig. 2D;  $\delta^{15}N$ , Fig. 2F).

When we compared detergent treatments from the left and right wings of each individual, we found no differences between means ( $\delta D$ ,  $t = -0.48$ ,  $df = 16$ ,  $P = 0.64$ ;  $\delta^{13}C$ ,  $t = 0.03$ ,  $df = 16$ ,  $P = 0.97$ ;  $\delta^{15}N$ ,  $t = -0.30$ ,  $df = 16$ ,  $P = 0.77$ ). There were 1:1 trends in  $\delta D$  and  $\delta^{13}C$ , though there was not a trend for  $\delta^{15}N$  (Fig. 3).

The recleaned feathers showed less isotope-ratio variation than those cleaned only once (Table 1 and Fig. 4). There were no differences between means for  $\delta D$  and  $\delta^{13}C$  ( $\delta D$ ,  $t = -0.55$ ,  $df = 16$ ,  $P = 0.59$ ;  $\delta^{13}C$ ,  $t = 0.78$ ,  $df = 16$ ,  $P = 0.44$ ) and only a marginal difference for  $\delta^{15}N$  ( $t = 2.14$ ,  $df = 16$ ,  $P = 0.05$ ). There were 1:1 trends for  $\delta D$  and  $\delta^{13}C$  (Fig. 4). The  $\delta^{13}C$  trend is strengthened if an outlier point is excluded ( $y = 0.60[0.19]x - 7.32[3.43]$ , where the numbers in brackets are SE;  $r^2 = 0.42$ ). For  $\delta^{15}N$ , the linearity deviates slightly from 1:1.

DISCUSSION

These experiments show that cleaning method affects hydrogen, carbon, and nitrogen stable-isotope ratios of bird feathers. Cleaning methods are not interchangeable, and cleaning only once with a given agent may not be sufficient for both precise and accurate isotope analysis. For feather cleaning before analysis, we propose first using detergent, then recleaning with 2:1 chloroform:methanol. Although some studies we reviewed (Hobson 1999, Wassenaar and Hobson 2000b) cleaned feathers multiple times with the same solvent, we did not test this and make no judgment about its effectiveness.

*Challenges.*—In stable-isotope analysis, absolute values of the samples measured are never known, but repeated measures of homogenized samples can improve confidence in the measured isotope ratio. Cleaning agents may remove contaminants but may also change feather isotope values, either by leaving a residue with a different enough stable-isotope ratio to change the measured value or by causing atom exchange, a phenomenon that involves removing atoms from the feather and replacing them with atoms from the cleaning agent. Atom exchange is most commonly seen with hydrogen (Wassenaar and Hobson 2003) and varies with pH, temperature, and solvent (Schimmelmann 1991, Campbell et al. 1995).

To infer how different cleaning treatments affected the feather isotope ratios in relation to the actual isotope values of the feathers, we examined the direction of isotopic shift after recleaning (Fig. 5). The case in which recleaning with a second solvent shifts isotope ratios toward those of feathers cleaned only in this solvent may suggest that this solvent is driving the isotope ratio of the sample. If values shift either in the opposite direction or

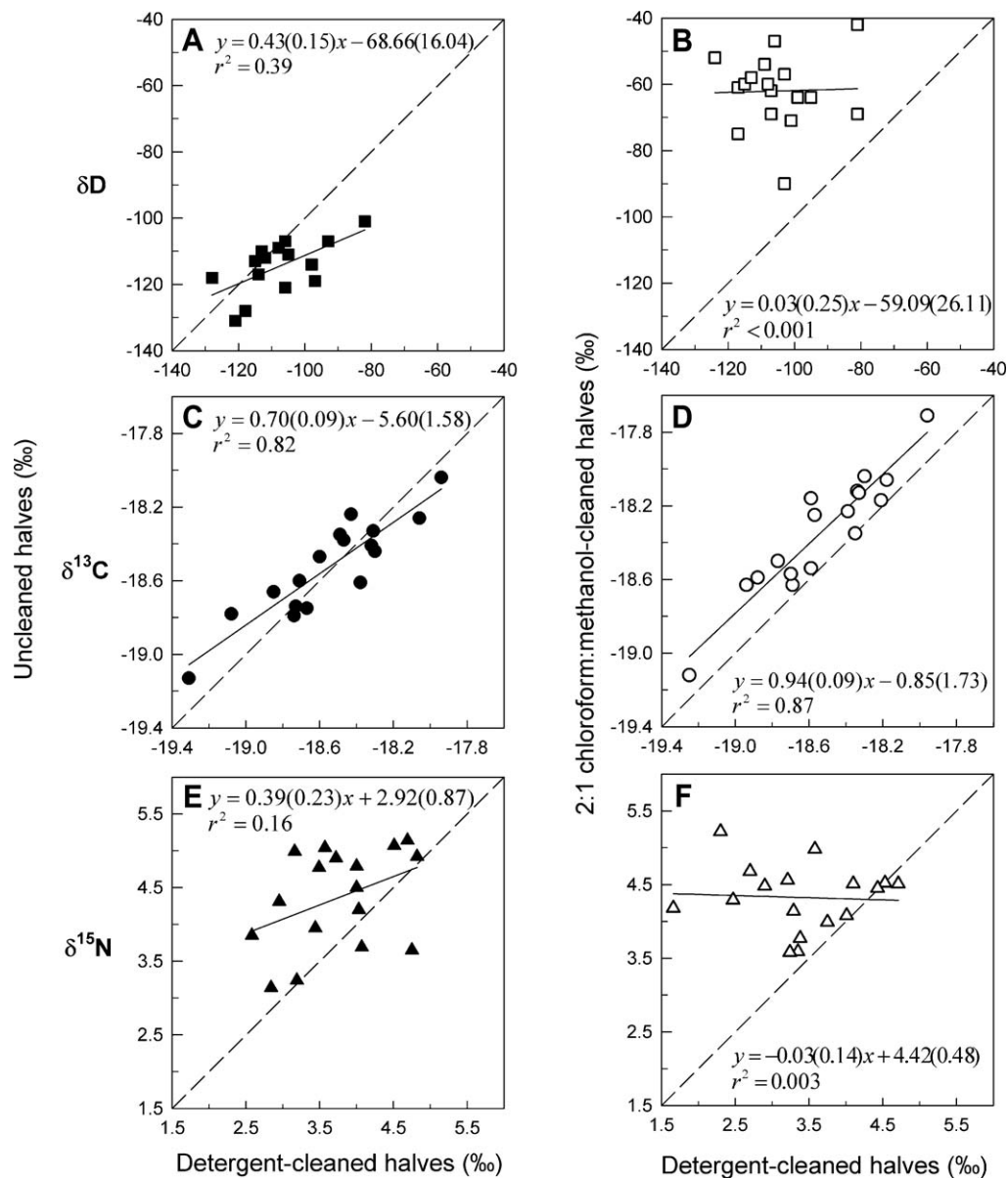


FIG. 2. Stable-isotope ratios of hydrogen (squares; A and B), carbon (circles; C and D), and nitrogen (triangles; E and F) for paired treatments after initial cleaning of Japanese Quail feathers. Solid shapes represent uncleaned versus detergent-cleaned feathers, and open shapes represent 2:1 chloroform:methanol-cleaned feathers. Best-fit lines and equations are based on least squares. Equations include SE in parentheses for slope and intercept. In D, the slope is not significantly different from 1 and the y-intercept is not significantly different from zero.

beyond those of the feathers cleaned only in this solvent, it is unlikely that the solvent is primarily responsible for shifts in isotope ratios.

For hydrogen, both recleaned treatments have stable-isotope ratios closer to those of the original 2:1 chloroform:methanol treatment, which is consistent with the idea that 2:1 chloroform:methanol may be driving  $\delta D$  values. Given that both chloroform and methanol are highly volatile, the potential for residue from either solvent is low. However, if we consider the known relationship between precipitation and feather  $\delta D$  (Hobson et al. 1999) and also that the predicted value for feather  $\delta D$  at Bartlesville,

where the Japanese Quail were raised, is  $-71 \pm 11\text{‰}$  (based on water data from Bowen and Revenaugh [2003] and a standard  $-25\text{‰}$  fractionation from water from Hobson et al. [1999]), our treatments of detergent recleaned with 2:1 chloroform:methanol ( $-74.12 \pm 3.77\text{‰}$ ; Table 1) and 2:1 chloroform:methanol recleaned with detergent ( $-73.35 \pm 6.11\text{‰}$ ) accurately reflect the predicted value, thus supporting the notion that these treatments reflect the true isotope values of the feathers. The mean for the 2:1 chloroform:methanol-only treatment ( $-62.06 \pm 11.12\text{‰}$ ) is also statistically similar to the predicted value (though heavier than the recleaned treatments), but, with a variance two to three times that



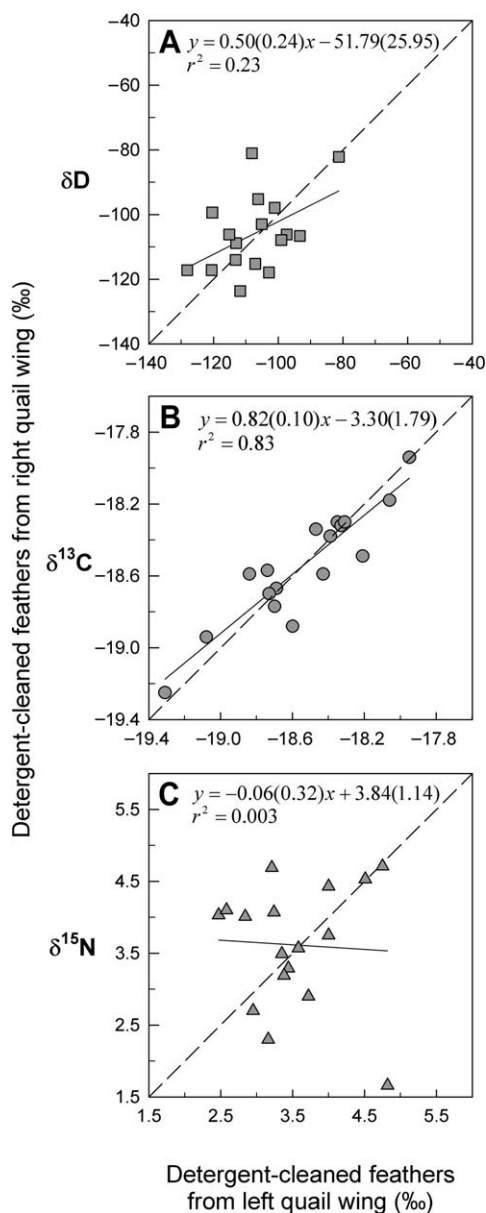


FIG. 3. Comparison of detergent treatments from left and right wings of Japanese Quail, for hydrogen (squares; A), carbon (circles; B), and nitrogen (triangles; C). The slope in B is not significantly different from 1 and the y-intercepts in A and B are not significantly different from zero.

of the recleaned treatments, these data may be less useful for geographic assignment. Moreover, because all individuals were given the same water with the same  $\delta D$ , regardless of the actual value, we did not expect a large  $\delta D$  range for feathers, as is seen in the recleaned-treatment data. This result further suggests that some cleaning procedures can yield results with low variance.

Carbon means did not shift when feathers were recleaned. This stability supports the notion that carbon stable-isotope ratios are robust to contamination and that carbon exchange is insignificant. However, there were differences in the variance resulting

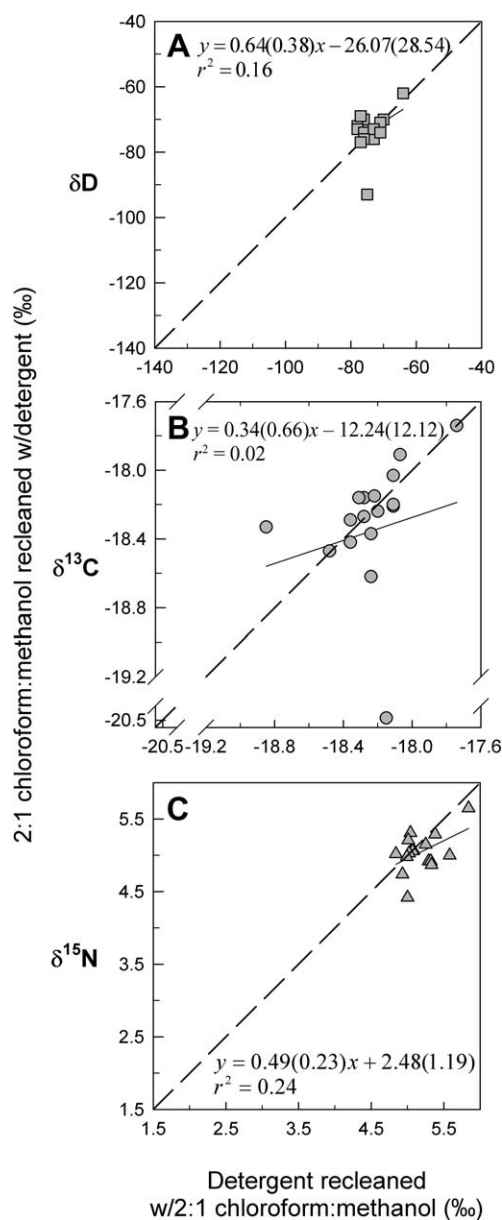


FIG. 4. Comparison of stable-isotope ratios for hydrogen (squares; A), carbon (circles; B), and nitrogen (triangles; C) of recleaned feather halves. In A and B, slopes are not significantly different from 1 and y-intercepts are not significantly different from zero. It is clear that the y value of one of the feather pairs is an outlier among all data in the experiment. We were unable to identify the source of this discrepancy.

from recleaning. The increased variation in the values from the feathers cleaned in 2:1 chloroform:methanol and recleaned in detergent makes us reluctant to suggest using these cleaning agents in this order. The other treatment, cleaning with detergent and recleaning with 2:1 chloroform:methanol, maintains variation equivalent to that seen in the two original treatments, which may argue for use of these cleaning agents in this order.

TABLE 1. Comparison of variance between feather halves subjected to both original cleaning and recleaning with detergent and 2:1 chloroform:methanol.

Element	Treatment	Mean $\pm$ SD (‰)	Levene's test for equality of variances	
			<i>F</i>	Significance
H	Detergent	$-105.06 \pm 11.62$	8.20	0.007
	Detergent, recleaned with 2:1 chloroform:methanol	$-74.12 \pm 3.77$	4.14	0.050
	2:1 chloroform:methanol	$-62.06 \pm 11.12$		
	2:1 chloroform:methanol, recleaned with detergent	$-73.35 \pm 6.11$		
C	Detergent	$-18.53 \pm 0.32$	3.59	0.067
	Detergent, recleaned with 2:1 chloroform:methanol	$-18.24 \pm 0.22$	0.12	0.733
	2:1 chloroform:methanol	$-18.34 \pm 0.33$		
	2:1 chloroform:methanol, recleaned with detergent	$-18.35 \pm 0.58$		
N	Detergent	$3.34 \pm 0.83$	11.11	0.002
	Detergent, recleaned with 2:1 chloroform:methanol	$5.18 \pm 0.26$	5.09	0.031
	2:1 chloroform:methanol	$4.33 \pm 0.45$		
	2:1 chloroform:methanol, recleaned with detergent	$5.04 \pm 0.26$		

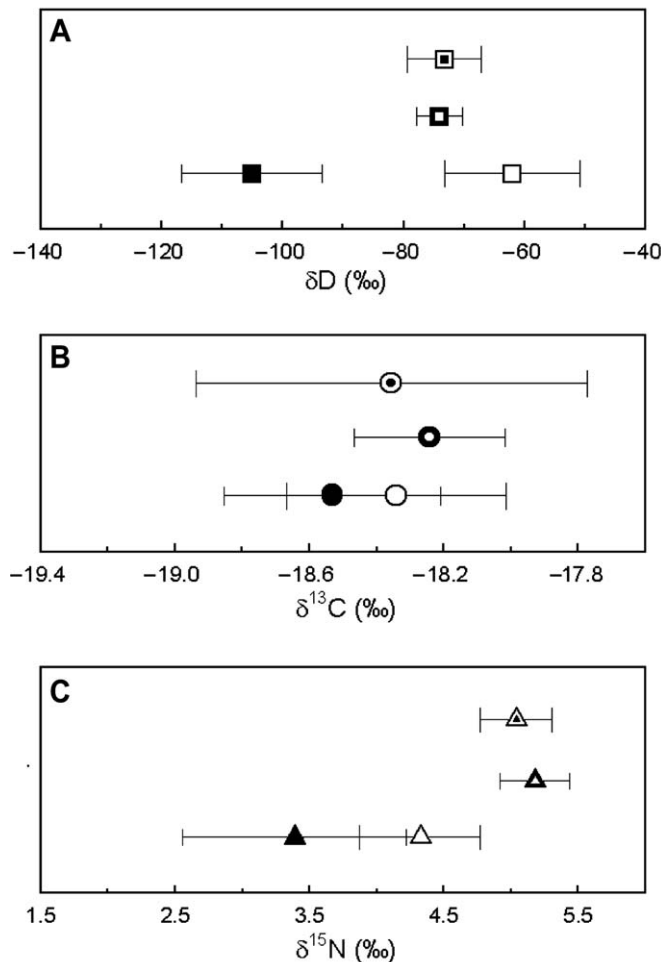


FIG. 5. Means  $\pm$  SD for hydrogen (squares; A), carbon (circles; B), and nitrogen (triangles; C) stable-isotope ratios of original detergent (dark shapes) and 2:1 chloroform:methanol (open shapes) treatments compared with the movement of the recleaned treatments, detergent-cleaned feathers recleaned in 2:1 chloroform:methanol (dark shapes with open centers) and 2:1 chloroform:methanol-cleaned feathers recleaned in detergent (open shapes with dark centers).

Nitrogen values of recleaned feathers are higher than those of feathers that received either of the original treatments. Because neither chloroform nor methanol contains nitrogen, the possibility of residue from these solvents is limited. Further, nitrogen exchange has not been documented, other than with terminal metal nitrides (Woo 1993). Thus, we suggest that treatment influence related to residue or exchange from either chloroform or methanol is negligible. Detergent residue may have an effect, but because both recleaned treatments have similar means with low variance and both data sets fall outside the range of the two original treatments, we believe that contamination is not a factor and that the range of recleaned feather isotope ratios contains the actual feather isotope values.

*Implications.*—In avian ecology, hydrogen stable isotopes are primarily used to assign birds to a particular geographic location. An isotopic precipitation gradient in relation to latitude, altitude, and distance from the coast is well documented and transfers reliably to animal tissues (Hobson et al. 1999, Bowen and Revenaugh 2003). However, error in calculating tissue isotope ratios can alter geographic placement of birds. For the two initial treatments in the present study,  $SD \approx 22\text{‰}$ , and the difference between their means is  $\sim 43\text{‰}$ . Within a treatment, this can place a bird that belongs in Oklahoma as far north as South Dakota and southern Minnesota, or as far south as southern Texas. Comparing treatments, this range extends north into southern Canada and south to coastal Mexico. Recleaned values, which show no difference between means and  $SD \approx 8\text{--}12\text{‰}$ , result in a condensed geographic area within which a breeding bird may be assigned. Our Oklahoma bird would most likely still be placed in Oklahoma after these treatments, with a possible range from Kansas to north Texas.

Carbon stable isotopes are used for diet analysis but also indicate trophic level, with 1–2‰ enrichment per trophic step. The observed  $\geq 1\text{‰}$  within-treatment variation may lead to misclassification of trophic level; for example, a herbivore may be classified as a carnivore, or vice versa. Observed variation is not expected to significantly affect diet analysis unless isotopic distinction between dietary elements is small.

Nitrogen is used mainly to identify trophic level with 3–5‰ enrichment per trophic step. With an almost 2‰ difference in

means between cleaning treatments, cleaning-method differences may misclassify an organism by half a trophic level. If a cleaning method with higher variance is chosen, additional error may occur.

In conclusion, choice of cleaning solvents affects stable-isotope ratios of bird feathers, a determination that should be considered in future experiments. This seemingly small detail in the overall scheme of a project may have serious implications for reliability and interpretation of data both within and among data sets. We propose a standard two-step cleaning method using both detergent and 2:1 chloroform:methanol. Because our data suggest that the order of cleaner use may influence variation in data, we specifically suggest cleaning with detergent first, then recleaning with 2:1 chloroform:methanol. This approach, however, should be considered a starting point in moving toward standardized preparation techniques for isotope samples.

#### ACKNOWLEDGMENTS

The authors thank the G. M. Sutton Avian Research Center for providing quail; V. Atudorei and Z. Sharp for carbon and nitrogen isotope analysis; K. A. Hobson and L. I. Wassenaar for hydrogen isotope analysis and insightful review; and B. Bestelmeyer, M. Engel, M. Kaspari, L. Wallace, A. Fudickar, L. Niemann, and R. Soto for review and advice. This research was supported by a National Science Foundation Graduate Research Fellowship, the George M. Sutton Scholarship in Ornithology, and the University of Oklahoma. This work was submitted in partial fulfillment of J.M.P.'s Ph.D. at the University of Oklahoma. All experiments complied with the appropriate U.S. regulations.

#### LITERATURE CITED

- BEARHOP, S., R. A. PHILLIPS, R. MCGILL, Y. CHEREL, D. A. DAWSON, AND J. P. CROXALL. 2006. Stable isotopes indicate sex-specific and long-term individual foraging specialisation in diving seabirds. *Marine Ecology Progress Series* 311:157–164.
- BEARHOP, S., R. A. PHILLIPS, D. R. THOMPSON, S. WALDRON, AND R. W. FURNESS. 2000. Variability in mercury concentrations of Great Skuas *Catharacta skua*: The influence of colony, diet and trophic status inferred from stable isotope signatures. *Marine Ecology Progress Series* 195:261–268.
- BEARHOP, S., D. R. THOMPSON, R. A. PHILLIPS, S. WALDRON, K. C. HAMER, C. M. GRAY, S. C. VOTIER, B. P. ROSS, AND R. W. FURNESS. 2001. Annual variation in Great Skua diets: The importance of commercial fisheries and predation on seabirds revealed by combining dietary analyses. *Condor* 103:802–809.
- BEARHOP, S., D. R. THOMPSON, S. WALDRON, I. C. RUSSELL, G. ALEXANDER, AND R. W. FURNESS. 1999. Stable isotopes indicate the extent of freshwater feeding by cormorants *Phalacrocorax carbo* shot at inland fisheries in England. *Journal of Applied Ecology* 36:75–84.
- BEARHOP, S., S. WALDRON, S. C. VOTIER, AND R. W. FURNESS. 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiological and Biochemical Zoology* 75:451–458.
- BENSCH, S., G. BENGTSOON, AND S. ÅKESSON. 2006. Patterns of stable isotope signatures in Willow Warbler *Phylloscopus trochilus* feathers collected in Africa. *Journal of Avian Biology* 37:323–330.
- BIRCHALL, J., T. C. O'CONNELL, T. H. E. HEATON, AND R. E. M. HEDGES. 2005. Hydrogen isotope ratios in animal body protein reflect trophic level. *Journal of Animal Ecology* 74:877–881.
- BOWEN, G. J., AND J. REVENAUGH. 2003. Interpolating the isotopic composition of modern meteoric precipitation. *Water Resources Research* 39:1299–1312.
- CACCAMISE, D. F., L. M. REED, P. M. CASTELLI, S. WAINWRIGHT, AND T. C. NICHOLS. 2000. Distinguishing migratory and resident Canada Geese using stable isotope analysis. *Journal of Wildlife Management* 64:1084–1091.
- CAMPBELL, S., M. T. RODGERS, E. M. MARZLUFF, AND J. L. BEAUCHAMP. 1995. Deuterium exchange reactions as a probe of biomolecule structure. Fundamental studies of gas phase H/D exchange reactions of protonated glycine oligomers with D<sub>2</sub>O, CD<sub>3</sub>OD, CD<sub>3</sub>CO<sub>2</sub>D, and ND<sub>3</sub>. *Journal of the American Chemical Society* 117:12840–12854.
- CHAMBERLAIN, C. P., S. BENSCH, X. FENG, S. ÅKESSON, AND T. ANDERSSON. 2000. Stable isotopes examined across a migratory divide in Scandinavian Willow Warblers (*Phylloscopus trochilus trochilus* and *Phylloscopus trochilus acredula*) reflect their African winter quarters. *Proceedings of the Royal Society of London, Series B* 267:43–48.
- CHAMBERLAIN, C. P., J. D. BLUM, R. T. HOLMES, X. FENG, T. W. SHERRY, AND G. R. GRAVES. 1997. The use of isotope tracers for identifying populations of migratory birds. *Oecologia* 109:132–141.
- CHEREL, Y., K. A. HOBSON, F. BAILLEUL, AND R. GROSCOLAS. 2005a. Nutrition, physiology, and stable isotopes: New information from fasting and molting penguins. *Ecology* 86:2881–2888.
- CHEREL, Y., K. A. HOBSON, AND S. HASSANI. 2005b. Isotopic discrimination between food and blood and feathers of captive penguins: Implications for dietary studies in the wild. *Physiological and Biochemical Zoology* 78:106–115.
- CHEREL, Y., K. A. HOBSON, AND H. WEIMERSKIRCH. 2000. Using stable-isotope analysis of feathers to distinguish moulting and breeding origins of seabirds. *Oecologia* 122:155–162.
- CLEGG, S. M., J. F. KELLY, M. KIMURA, AND T. B. SMITH. 2003. Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's Warbler (*Wilsonia pusilla*). *Molecular Ecology* 12:819–830.
- DENIRO, M. J., AND S. EPSTEIN. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42:495–506.
- DENIRO, M. J., AND S. EPSTEIN. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45:341–351.
- DUNN, E. H., K. A. HOBSON, L. I. WASSENAAR, D. J. T. HUSSELL, AND M. L. ALLEN. 2006. Identification of summer origins of songbirds migrating through southern Canada in autumn. *Avian Conservation and Ecology* 1:4.
- EVANS OGDEN, L. J., K. A. HOBSON, AND D. B. LANK. 2004. Blood isotopic ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) turnover and diet-tissue fractionation factors in captive Dunlin (*Calidris alpina pacifica*). *Auk* 121:170–177.
- GANNES, L. Z., D. M. O'BRIEN, AND C. MARTÍNEZ DEL RIO. 1997. Stable isotopes in animal ecology: Assumptions, caveats, and a call for more laboratory experiments. *Ecology* 78:1271–1276.
- GRAVES, G. R., C. S. ROMANEK, AND A. R. NAVARRO. 2002. Stable isotope signature of philopatry and dispersal in a migratory

- songbird. *Proceedings of the National Academy of Sciences USA* 99:8096–8100.
- HEBERT, C. E., AND L. I. WASSENAAR. 2005. Feather stable isotopes in western North American waterfowl: Spatial patterns, underlying factors, and management applications. *Wildlife Society Bulletin* 33:92–102.
- HOBSON, K. A. 1999. Stable-carbon and nitrogen isotope ratios of songbird feathers grown in two terrestrial biomes: Implications for evaluating trophic relationships and breeding origins. *Condor* 101:799–805.
- HOBSON, K. A., R. T. ALISAUSKAS, AND R. G. CLARK. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analyses of diet. *Condor* 95:388–394.
- HOBSON, K. A., L. ATWELL, AND L. I. WASSENAAR. 1999. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proceedings of the National Academy of Sciences USA* 96:8003–8006.
- HOBSON, K. A., Y. AUBRY, AND L. I. WASSENAAR. 2004a. Migratory connectivity in Bicknell's Thrush: Locating missing populations with hydrogen isotopes. *Condor* 106:905–909.
- HOBSON, K. A., G. J. BOWEN, L. I. WASSENAAR, Y. FERRAND, AND H. LORMEE. 2004b. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. *Oecologia* 141:477–488.
- HOBSON, K. A., R. B. BRUA, W. L. HOHMAN, AND L. I. WASSENAAR. 2000. Low frequency of “double molt” of remiges in Ruddy Ducks revealed by stable isotopes: Implications for tracking migratory waterfowl. *Auk* 117:129–135.
- HOBSON, K. A., AND R. G. CLARK. 1992. Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. *Condor* 94:189–197.
- HOBSON, K. A., K. P. MCFARLAND, L. I. WASSENAAR, C. C. RIMMER, AND J. E. GOETZ. 2001. Linking breeding and wintering grounds of Bicknell's Thrushes using stable isotope analyses of feathers. *Auk* 118:16–23.
- HOBSON, K. A., J. F. PIATT, AND J. PITOCHELLI. 1994. Using stable isotopes to determine seabird trophic relationships. *Journal of Animal Ecology* 63:786–798.
- HOBSON, K. A., S. VAN WILGENBURG, L. I. WASSENAAR, H. HANDS, W. P. JOHNSON, M. O'MEILIA, AND P. TAYLOR. 2006. Using stable hydrogen isotope analysis of feathers to delineate origins of harvested Sandhill Cranes in the central flyway of North America. *Waterbirds* 29:137–147.
- HOBSON, K. A., AND L. I. WASSENAAR. 1997. Linking breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia* 109:142–148.
- HOBSON, K. A., AND L. I. WASSENAAR. 2001. Isotopic delineation of North American migratory wildlife populations: Loggerhead Shrikes. *Ecological Applications* 11:1545–1553.
- HOBSON, K. A., L. I. WASSENAAR, AND E. BAYNE. 2004c. Using isotopic variance to detect long-distance dispersal and philopatry in birds: An example with Ovenbirds and American Redstarts. *Condor* 106:732–743.
- HOBSON, K. A., L. I. WASSENAAR, B. MILÁ, I. LOVETTE, C. DINGLE, AND T. B. SMITH. 2003. Stable isotopes as indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community. *Oecologia* 136:302–308.
- JARDINE, T. D., AND R. A. CUNJAK. 2005. Analytical error in stable isotope ecology. *Oecologia* 144:528–533.
- JASPERS, V. L. B., S. VOORSPOELS, A. COVACI, G. LEPOINT, AND M. EENS. 2007. Evaluation of the usefulness of bird feathers as a non-destructive biomonitoring tool for organic pollutants: A comparative and meta-analytical approach. *Environment International* 33:328–337.
- KAUSHAL, S. S., AND J. WALSH. 2002. Comparison of nitrogen isotope ratios in feathers from seven species of Colorado breeding birds. *Western North American Naturalist* 62:246–248.
- KELLY, J. F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Canadian Journal of Zoology* 78:1–27.
- KELLY, J. F. 2006. Stable isotope evidence links breeding geography and migration timing in wood warblers (Parulidae). *Auk* 123:431–437.
- KELLY, J. F., V. ATUDOREI, Z. D. SHARP, AND D. M. FINCH. 2002. Insights into Wilson's Warbler migration from analyses of hydrogen stable-isotope ratios. *Oecologia* 130:216–221.
- KELLY, J. F., K. C. RUEGG, AND T. B. SMITH. 2005. Combining isotopic and genetic markers to identify breeding origins of migrant birds. *Ecological Applications* 15:1487–1494.
- KLAASSEN, M., T. BAARSPUL, T. DEKKERS, AND P. VAN TIENEN. 2004. The relationship between carbon stable isotope ratios of hatchling down and egg yolk in Black-headed Gulls. *Journal of Field Ornithology* 75:196–199.
- LOTT, C. A., T. D. MEEHAN, AND J. A. HEATH. 2003. Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: Influence of marine prey base. *Oecologia* 134:505–510.
- LOTT, C. A., AND J. P. SMITH. 2006. A geographic-information-system approach to estimating the origin of migratory raptors in North America using stable hydrogen isotope ratios in feathers. *Auk* 123:822–835.
- MAZEROLLE, D. F., AND K. A. HOBSON. 2005. Estimating origins of short-distance migrant songbirds in North America: Contrasting inferences from hydrogen isotope measurements of feathers, claws, and blood. *Condor* 107:280–288.
- MAZEROLLE, D. F., K. A. HOBSON, AND L. I. WASSENAAR. 2005. Stable isotope and band-encounter analyses delineate migratory patterns and catchment areas of White-throated Sparrows at a migration monitoring station. *Oecologia* 144:541–549.
- MEEHAN, T. D., C. A. LOTT, Z. D. SHARP, R. B. SMITH, R. N. ROSENFELD, A. C. STEWART, AND R. K. MURPHY. 2001. Using hydrogen isotope geochemistry to estimate the natal latitudes of immature Cooper's Hawks migrating through the Florida Keys. *Condor* 103:11–20.
- MEEHAN, T. D., R. N. ROSENFELD, V. N. ATUDOREI, J. BIELEFELDT, L. J. ROSENFELD, A. C. STEWART, W. E. STOUT, AND M. A. BOZEK. 2003. Variation in hydrogen stable-isotope ratios between adult and nestling Cooper's Hawks. *Condor* 105:567–572.
- MEHL, K. R., R. T. ALISAUSKAS, K. A. HOBSON, AND D. K. KELLETT. 2004. To winter east or west? Heterogeneity in winter philopatry in a central-Arctic population of King Eiders. *Condor* 106:241–251.
- MIZUTANI, H., M. FUKUDA, AND Y. KABAYA. 1992.  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichment factors of feathers of 11 species of adult birds. *Ecology* 73:1391–1395.



- MIZUTANI, H., M. FUKUDA, Y. KABAYA, AND E. WADA. 1990. Carbon isotope ratio of feathers reveals feeding behavior of cormorants. *Auk* 107:400–403.
- MIZUTANI, H., Y. KABAYA, AND E. WADA. 1991. Nitrogen and carbon isotope compositions relate linearly in cormorant tissues and its diet. *Isotopenpraxis* 27:166–168.
- MIZUTANI, H., AND E. WADA. 1988. Nitrogen and carbon isotope ratios in seabird rookeries and their ecological implications. *Ecology* 69:340–349.
- MØLLER, A. P., AND K. A. HOBSON. 2004. Heterogeneity in stable isotope profiles predicts coexistence of populations of Barn Swallows *Hirundo rustica* differing in morphology and reproductive performance. *Proceedings of the Royal Society of London, Series B* 271:1355–1362.
- MØLLER, A. P., K. A. HOBSON, T. A. MOUSSEAU, AND A. M. PEKLO. 2006. Chernobyl as a population sink for Barn Swallows: Tracking dispersal using stable-isotope profiles. *Ecological Applications* 16:1696–1705.
- NETO, J. M., J. NEWTON, A. G. GOSLER, AND C. M. PERRINS. 2006. Using stable isotope analysis to determine the winter moult extent in migratory birds: The complex moult of Savi's Warblers *Locustella luscinioides*. *Journal of Avian Biology* 37:117–124.
- NEWTON, I., K. A. HOBSON, A. D. FOX, AND M. MARQUISS. 2006. An investigation into the provenance of Northern Bullfinches *Pyrrhula p. pyrrhula* found in winter in Scotland and Denmark. *Journal of Avian Biology* 37:431–435.
- NORRIS, D. R., P. P. MARRA, R. MONTGOMERIE, T. K. KYSER, AND L. M. RATCLIFFE. 2004. Reproductive effort, molting latitude, and feather color in a migratory songbird. *Science* 306:2249–2250.
- PAIN, D. J., R. E. GREEN, B. GIEBING, A. KOZULIN, A. POLUDA, U. OTTOSSON, M. FLADE, AND G. M. HILTON. 2004. Using stable isotopes to investigate migratory connectivity of the globally threatened aquatic warbler *Acrocephalus paludicola*. *Oecologia* 138:168–174.
- PASZKOWSKI, C. A., B. A. GINGRAS, K. WILCOX, P. H. KLATT, AND W. M. TONN. 2004. Trophic relations of the Red-necked Grebe on lakes in the western boreal forest: A stable-isotope analysis. *Condor* 106:638–651.
- PEARSON, S. F., D. J. LEVEY, C. H. GREENBERG, AND C. MARTÍNEZ DEL RIO. 2003. Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. *Oecologia* 135:516–523.
- PODLESAK, D. W., S. R. McWILLIAMS, AND K. A. HATCH. 2005. Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. *Oecologia* 142:501–510.
- ROMANEK, C. S., K. F. GAINES, A. L. BRYAN, JR., AND I. L. BRISBIN, JR. 2000. Foraging ecology of the endangered Wood Stork recorded in the stable isotope signature of feathers. *Oecologia* 125:584–594.
- RUBENSTEIN, D. R., C. P. CHAMBERLAIN, R. T. HOLMES, M. P. AYRES, J. R. WALDBAUER, G. R. GRAVES, AND N. C. TUROSS. 2002. Linking breeding and wintering ranges of a migratory songbird using stable isotopes. *Science* 295:1062–1065.
- SCHIMMELMANN, A. 1991. Determination of the concentration and stable isotopic composition of nonexchangeable hydrogen in organic matter. *Analytical Chemistry* 63:2456–2459.
- SMITH, A. D., AND A. M. DUFTY, JR. 2005. Variation in the stable-hydrogen isotope composition of Northern Goshawk feathers: Relevance to the study of migratory origins. *Condor* 107:547–558.
- SMITH, R. B., E. C. GREINER, AND B. O. WOLF. 2004. Migratory movements of Sharp-shinned Hawks (*Accipiter striatus*) captured in New Mexico in relation to prevalence, intensity, and biogeography of avian hematozoa. *Auk* 121:837–846.
- SMITH, R. B., T. D. MEEHAN, AND B. O. WOLF. 2003. Assessing migration patterns of Sharp-shinned Hawks *Accipiter striatus* using stable-isotope and band encounter analysis. *Journal of Avian Biology* 34:387–392.
- THOMPSON, D. R., AND R. W. FURNESS. 1995. Stable-isotope ratios of carbon and nitrogen in feathers indicate seasonal dietary shifts in Northern Fulmars. *Auk* 112:493–498.
- THOMPSON, D. R., R. W. FURNESS, AND S. A. LEWIS. 1995. Diets and long-term changes in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in Northern Fulmars *Fulmaris glacialis* from two northeast Atlantic colonies. *Marine Ecology Progress Series* 125:3–11.
- WASSENAAR, L. I., AND K. A. HOBSON. 2000a. Improved method for determining the stable-hydrogen isotopic composition ( $\delta\text{D}$ ) of complex organic materials of environmental interest. *Environmental Science and Technology* 34:2354–2360.
- WASSENAAR, L. I., AND K. A. HOBSON. 2000b. Stable-carbon and hydrogen isotope ratios reveal breeding origins of Red-winged Blackbirds. *Ecological Applications* 10:911–916.
- WASSENAAR, L. I., AND K. A. HOBSON. 2001. A stable-isotope approach to delineate geographical catchment areas of avian migration monitoring stations in North America. *Environmental Science and Technology* 35:1845–1850.
- WASSENAAR, L. I., AND K. A. HOBSON. 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes in Environmental and Health Studies* 39:211–217.
- WASSENAAR, L. I., AND K. A. HOBSON. 2006. Stable-hydrogen isotope heterogeneity in keratinous materials: Mass spectrometry and migratory wildlife tissue subsampling strategies. *Rapid Communications in Mass Spectrometry* 20:2505–2510.
- WENNERBERG, L., M. KLAASSEN, AND Å. LINDSTRÖM. 2002. Geographical variation and population structure in the White-rumped Sandpiper *Calidris fuscicollis* as shown by morphology, mitochondrial DNA and carbon isotope ratios. *Oecologia* 131:380–390.
- WOLF, B. O., C. MARTÍNEZ DEL RIO, AND J. Y. BABSON. 2002. Stable isotopes reveal that saguaro fruit provides different resources to two desert dove species. *Ecology* 83:1286–1293.
- WOO, L. K. 1993. Intermetal oxygen, sulfur, selenium, and nitrogen atom transfer reactions. *Chemical Reviews* 93:1125–1136.
- WUNDER, M. B., C. L. KESTER, F. L. KNOPE, AND R. O. RYE. 2005. A test of geographic assignment using isotope tracers in feathers of known origin. *Oecologia* 144:607–617.
- WUNDER, M. B., AND D. R. NORRIS. 2008. Improved estimates of certainty in stable-isotope-based methods for tracking migratory animals. *Ecological Applications* 18:549–559.
- YOHANNES, E., K. A. HOBSON, D. J. PEARSON, AND L. I. WASSENAAR. 2005. Stable isotope analyses of feathers help identify autumn stopover sites of three long-distance migrants in northeastern Africa. *Journal of Avian Biology* 36:235–241.

Associate Editor: A. M. Dufty, Jr.

APPENDIX. References reviewed to determine the prevalence of cleaning agents and the elements analyzed in stable-isotope studies using bird feathers.

Cleaning agent	References (elements analyzed are in parentheses)
2:1 chloroform:methanol	Cherel et al. 2005a; Cherel et al. 2005b (C, N); Dunn et al. 2006 (D); Graves et al. 2002 (D); Hobson 1999 (C, N); Hobson and Wassenaar 1997 (D); Hobson and Wassenaar 2001; (D, C); Hobson et al. 1999; (D); Hobson et al. 2000 (D, C); Hobson et al 2003 (D, C, N); Hobson et al. 2004a (D); Hobson et al. 2004b (D, O); Hobson et al 2004c (D); Hobson et al. 2006 (D); Lott and Smith 2006 (D); Mazerolle and Hobson 2005 (D); Mazerolle et al 2005 (D); Mehl et al 2004 (C, N); Møller and Hobson 2004 (D, C, N); Møller et al. 2006 (C, N); Newton et al. 2006; Smith and Dufty 2005 (D); Smith et al. 2003 (D); Smith et al. 2004 (D); Wassenaar and Hobson 2000a (D); Wassenaar and Hobson 2000b (D, C); Wassenaar and Hobson 2001 (D, C); Wassenaar and Hobson 2003 (D); Wunder et al. 2005 (D); Yohannes et al. 2005 (D, C, N)
Detergent	Chamberlain et al. 1997 (D, C); Clegg et al. 2003 (D); Kelly 2006 (D); Kelly et al. 2002 (D); Kelly et al. 2005 (D); Lott et al. 2003 (D, S); Meehan et al 2001 (D); Meehan et al. 2003 (D); Romanek et al. 2000 (C, N)
Some uncleaned, some cleaned with detergent	Bensch et al. 2006
Uncleaned	Bearhop et al. 2006 (C, N); Neto et al. 2006 (D, C, N)
Not documented	Chamberlain et al. 2000 (C, N); Hobson et al. 2001 (D); Norris et al. 2004 (D); Evans Ogden et al. 2004 (C, N); Rubenstein et al. 2002 (D, C);
0.25M sodium hydroxide and water	Bearhop et al. 1999 (C, N); Bearhope et al. 2001 (D, C, N); Bearhop et al. 2001 (C, N); Bearhop et al. 2002 (C, N); Pain et al. 2004 (D, C, N)
Water—distilled, deionized or not documented	Caccamise et al. 2000 (C, N, S); Mizutani and Wada 1988 (C, N); Mizutani et al. 1990 (C); Mizutani et al. 1992 (C, N); Paszkowski et al. 2004 (C, N)
Ether	Hobson and Clark 1992 (C); Nobson et al. 1993 (N); Pearson et al. 2003 (C, N); Podlesak et al. 2005 (C)
Chloroform	Klaassen et al. 2004 (C); Wennerberg et al. 2002 (C)
Chloroform–acetone	Thompson and Furness 1995 (C, N); Thompson et al. 1995 (C, N)
2:1 chloroform:ether	Cherel et al. 2000. (C, N)
Freeze-dried and stored in a dessicator	Kaushal and Walsh 2002
Methanol	Hebert and Wassenaar 2005
Physiological salt solution	Mizutani et al. 1991