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LACK OF STABLE-ISOTOPE DIFFERENCES BETWEEN CANADA GOOSE POPULATIONS NESTING IN THE SUBARCTIC AND TEMPERATE ZONES

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Abstract. Ratios of stable isotopes in feathers have great potential for identifying the connectivity of bird migrations and the origin of harvested individuals of game species. In particular, the relationship between the hydrogen ratio due to latitudinal variation in precipitation (δD_p) and that in feathers (δD_f) is often used to determine unknown individuals' latitude of origin. We assessed this relationship between Canada Geese nesting in the temperate zone (*Branta canadensis maxima*) and the subarctic (*B. c. interior*). For this game species, the origin of harvested birds is important for developing management that maintains a desirable level of harvest while ensuring continued viability of all subspecies and breeding populations. We collected freshly grown primaries from three populations of *interior* and five of *maxima* and analyzed them for δD , $\delta^{13}C$, and $\delta^{15}N$. Multivariate analysis suggested no overall differences in isotopic composition between subspecies. A univariate assessment indicated a significant difference in $\delta^{15}N_p$, despite substantial overlap between subspecies, and no difference in δD_f or $\delta^{13}C_f$. Of particular interest is the lack of difference in δD_f , despite the large latitudinal differences in δD_p and between the subspecies' breeding ranges. Values of δD_f averaged $-131.85\% \pm 1.36$ for *interior*, $-131.63\% \pm 0.71$ for *maxima*, and we found no overall relationship between δD_f and δD_p . Overall, our results suggest that δD , $\delta^{13}C$, and $\delta^{15}N$ alone have limited ability to discriminate between subspecies *interior* and *maxima* and hence have limited applicability for estimating the origin of harvested birds and/or identifying molt migrants of *maxima*.

Key words: deuterium, harvest derivation, migration, stable isotopes, waterfowl.

Falta de Diferencias en los Isótopos Estables entre Poblaciones de *Branta canadensis* que Anidan en las Zonas Subártica y Templada

Resumen. Los cocientes de isótopos estables en las plumas tienen un gran potencial para identificar la conectividad de las aves migratorias y el origen de individuos recolectados de especies cinegéticas. En particular, el cociente entre la tasa de hidrógeno dado por la variación latitudinal en la precipitación (δD_p) y aquel en las plumas (δD_f) se emplea usualmente para determinar la latitud de origen desconocida de los individuos. Evaluamos la relación entre individuos de *Branta canadensis* que anidan en la zona templada (*B. c. maxima*) y subártica (*B. c. interior*). Para esta especie cinegética, el origen de las aves recolectadas es importante para desarrollar estrategias de manejo que mantengan un nivel deseable de colecta mientras que se asegura la viabilidad continuada de todas las subspecies y poblaciones reproductivas. Colectamos primaries desarrolladas frescas de tres poblaciones de *interior* y cinco de *maxima* y las analizamos para δD , $\delta^{13}C$ y $\delta^{15}N$. Los análisis multivariados sugirieron que no existe una diferencia global en la composición isotópica entre las subspecies. Una evaluación univariada indicó una diferencia significativa en $\delta^{15}N_p$, a pesar de una superposición sustancial entre subspecies y falta de diferencias en δD_f o $\delta^{13}C_f$. De interés particular es la falta de diferencias en δD_f , a pesar de las grandes diferencias latitudinales en δD_p y entre los rangos de cría de las subspecies. Los valores de δD_f promediaron $-131.85\% \pm 1.36$ para *interior*, $-131.63\% \pm 0.71$ para *maxima* y no encontramos una relación global entre δD_f y δD_p . En general, nuestros resultados sugieren que δD , $\delta^{13}C$ y $\delta^{15}N$ individualmente tienen una habilidad limitada para discriminar entre las subspecies *interior* y *maxima* y por ende tienen una aplicabilidad limitada para estimar el origen de las aves recolectadas y/o para identificar migrantes mudando de *maxima*.

INTRODUCTION

Understanding connections between breeding, migration stopover, and wintering sites used by migratory birds is essential to their conservation (Webster and Marra 2005), as factors affecting individuals' fitness and survival during one season may have lasting effects into other parts of the annual cycle

(e.g. Norris et al. 2004). Determining population identity is particularly important for migratory waterfowl and other game species in which multiple breeding populations, each with different conservation and management objectives, mix during fall and winter hunting seasons. Information on the population composition of harvested samples (harvest derivation) is important for developing management strategies that

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maintain a desirable and sustainable level of harvest while ensuring the continued viability of all breeding populations.

Ratios of naturally occurring stable isotopes vary geographically because of regional patterns in geology, precipitation, evapotranspiration, anthropogenic inputs, and other factors. The isotopes are acquired through diet and their ratios remain fixed in feathers and other metabolically inert structures. Therefore, stable-isotope ratios have great potential for determining the location of molt of migratory birds of unknown geographic origin (reviewed in Rubenstein and Hobson 2004, Hobson 2005). In particular, ratios of stable hydrogen in feathers (δD_f) are linked via the local food web to latitudinal variation in precipitation (δD_p). This link has been well established for many populations of passerines (e.g. Hobson and Wassenaar 1997, Chamberlain et al. 1997), raptors (Lott and Smith 2006), and waterfowl (e.g. Hebert and Wassenaar 2005, Clark et al. 2006, Coulton et al. 2010). This relationship has been used to identify the geographic origin of unknown samples by means of either a generic discrimination factor (e.g., Hobson et al. 2006, Kelly 2006, Norris et al. 2006a, Boulet et al. 2006) based on the general relationship between δD_f and a model of continent-wide variation in δD_p , or by establishing a baseline of δD_f values from samples of known origin and then applying this baseline to determine the origin of unknown birds (e.g. Hobson and Wassenaar 1997, Chamberlain et al. 1997, Lott and Smith 2006). There are a variety of factors that may cause deviations from the general relationship between δD_f and δD_p (Hobson 2005, Norris et al. 2006b). For example, δD_f values may deviate from predicted values because of age (Meehan et al. 2003, Langin et al. 2007), habitat (Lott et al. 2003), variation in molt strategies (Larson and Hobson 2009, Rocque et al. 2009), inter- and intra-individual differences (Smith and Dufty 2005, Smith et al. 2008), lack of analytical reproducibility (Smith et al. 2009), and differences in feather-cleaning methods (Paritte and Kelly 2009). In addition to δD , ratios of carbon and nitrogen isotopes in birds also vary in large-scale geographic patterns due to natural and anthropogenic factors (Hobson 1999, Wassenaar and Hobson 2000), and a multi-isotope approach often improves geographic resolution over one based on δD alone (e.g., Wunder et al. 2005, Hebert and Wassenaar 2005). For the Canada Goose (*Branta canadensis*) and other game species, stable isotopes may provide an alternative to traditional mark-recapture or genetic methods (e.g., Scribner et al. 2003) used to delineate the origins of harvested birds (Hobson et al. 2006, 2009, Ashley et al. 2010); however, the factors stated above stress the importance of investigating the nature of isotopic variation in the species of interest before using these techniques to make inferences about geographic source.

The Canada Goose is an important game species throughout North America and is divided into a number of subspecies and discrete breeding populations that can be grouped into those migrating long distances to breed in arctic and subarctic regions of North America (subarctic-nesting) or those breeding in southern Canada and the United States

(temperate-nesting). Two subspecies are harvested regularly in Ohio, the subarctic-nesting interior subspecies (*B. c. interior*) and the temperate-breeding giant subspecies (*B. c. maxima*). There is a desire to control the overabundant giant subspecies while limiting harvest of the subarctic-nesting interior subspecies, but harvest management is complicated by the two subspecies' co-occurrence during fall and winter hunting seasons.

Several methods have been used to determine the geographic origin of harvested Canada Geese, including band returns, morphology, molecular markers, and stable-isotope analysis of feathers (e.g., Moser and Rowley 1990, Caccamise et al. 2000, Scribner et al. 2003, Mylecraine 2008). The species' patterns of molt are well known; flight feathers are molted simultaneously every year on the breeding grounds (Mowbray et al. 2002), so isotope ratios in flight feathers have the potential to trace the origins of migrating or wintering birds. Caccamise et al. (2000) found significant variation in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ among Canada Goose populations in the Atlantic Flyway, but δD_f may prove to be more effective at determining the birds' source because of the large differences in latitude and δD_p between locations of breeding (and therefore of molt). Using δD_p , Marra et al. (2009) found that migratory Canada Geese were responsible for a collision leading to a crash of a commercial airplane; however, their analysis did not include Mississippi Flyway populations and was intended to determine whether the birds were migratory but not their specific geographic origin.

The use and interpretation of stable isotopes for discriminating among Canada Goose populations is complicated by molt migrations (Swift et al. 2009). For example, many non-breeders or failed breeders of the giant subspecies travel long distances north to molt within the breeding grounds of the interior subspecies (Abraham et al. 1999, Nichols et al. 2004). For these individuals, population identity, defined by breeding or natal location, differs from that implied by where the feathers were molted: molt migrants of *B. c. maxima* should have the same isotopic signature as *B. c. interior*. Previous studies of isotope ratios in Canada Goose feathers have not addressed this issue; however, if there are identifiable isotopic differences between breeding areas, genetic techniques (Scribner et al. 2003, Mylecraine et al. 2008) can be used to distinguish the subspecies, and the two datasets could be combined to determine both the subspecies identity and location of molt of individual birds. This method could have potential for estimating the derivations of harvested as well as identifying individual molt migrants and estimating the prevalence of molt migration in *B. c. maxima*.

In the current study, we explored developing baseline isotope values for Canada Geese from subarctic-nesting populations of *B. c. interior* and temperate-breeding populations of *B. c. maxima*. Of each subspecies, we sampled only first-year juveniles and/or recaptured adults that had been banded as juveniles. We collected samples during the annual molt to

ensure subspecies identity. The large latitudinal difference between these two groups ($>15^\circ$) exceeds the minimum separation in latitude generally required for individual samples to be assigned to location on the basis of δD_f (Farmer et al. 2008), so we expected to find significant differences between the two subspecies. We also included $\delta^{13}C$ and $\delta^{15}N$ because the combination of these three isotopes has been used successfully for other bird species (e.g., Wunder et al. 2005, Szymanski et al. 2007). Although Caccamise et al. (2000) used $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ for Canada Geese in the Atlantic Flyway, we chose not to include $\delta^{34}S$ in our study because it can be affected by marine influences that may have affected our samples (Hobson et al. 1997, Lott et al. 2003). Caccamise et al. (2000) found that in New Jersey $\delta^{34}S$ in coastal and inland populations differed, while birds resident in coastal New Jersey could not be distinguished from migrants. Once developed, this baseline for δD_p , $\delta^{13}C$, and $\delta^{15}N$ could then be used to discriminate between *B. c. interior* and *B. c. maxima* in harvested samples and to identify molt migrants. The specific objectives of our study are to (1) identify isotopic differences (δD , $\delta^{13}C$, and $\delta^{15}N$) between the interior and giant subspecies of the Canada Goose, (2) assess the relationship between δD_f and continent-wide patterns of δD_p in these populations, and (3) determine whether this technique is sufficient for determining the location of molt of harvested birds.

METHODS

SAMPLE COLLECTION

In 2005, we collected primary feathers from Canada Geese while banding during the period when the birds are flightless at the end of the breeding season (14–29 June for *B. c. maxima*, 9–31 July for *B. c. interior*). To ensure sampled geese originated from the sampled subspecies/population, we sampled only pre-fledgling juveniles and/or recaptured adults that had been banded as juveniles, and we collected only newly emerged primaries grown at that location. The sample of *B. c. interior* included three subpopulations from two populations defined for management: on the mainland coast of southern James Bay ($n = 25$) and on Akimiski Island ($n = 31$) of the southern James Bay population and the Mississippi Valley population along the coast of Hudson Bay ($n = 25$). The sample of *B. c. maxima* included five Ohio subpopulations: at and near Killdeer Plains Wildlife Area ($n = 20$), at and near Magee Marsh Wildlife Area ($n = 20$), at and near Mercer Wildlife Area ($n = 20$), Mosquito Creek Wildlife Area ($n = 20$), and Salt Fork Wildlife Area ($n = 20$) (Fig. 1).

ISOTOPIC DETERMINATION

We obtained a single subsample from the distal end of each primary, cleaned samples of surface oils and debris with a 2:1 chloroform:methanol solution, allowed them to dry overnight in a fume hood, and submitted them to the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University) for

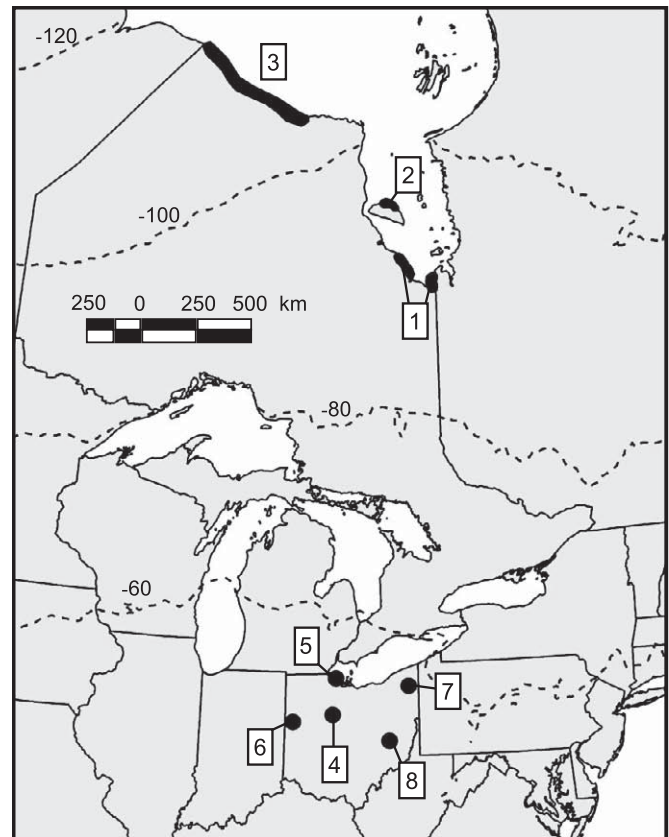


FIGURE 1. Locations of eight Canada Goose populations sampled for stable-isotope analysis, including three subpopulations of the Interior subspecies (*B. c. interior*) in Ontario and Nunavut, two of the southern James Bay population (1, mainland; 2, Akimiski Island) and one of the Mississippi Valley population (3, Hudson Bay), and five subpopulations of the Mississippi Flyway population of the Giant subspecies (*B. c. maxima*) in Ohio (4, Killdeer Plains Wildlife Area; 5, Magee Marsh Wildlife Area; 6, Mercer Wildlife Area; 7, Mosquito Creek Wildlife Area; 8, Salt Fork Wildlife Area). All feathers were collected during banding in 2005. Contours are of annual δD_p (‰) (Bowen and Revenaugh 2003).

analysis of δD , $\delta^{13}C$, and $\delta^{15}N$. For δD , 350 $\mu g \pm 20 \mu g$ of each feather sample was placed into a 3.5 \times 5.0 mm silver capsule. Samples and calibrated keratin standards were analyzed with a Delta Plus XL mass spectrometer by the pyrolysis and continuous-flow isotope-ratio mass spectrometry (CF-IRMS) techniques described by Wassenaar and Hobson (2003). We used comparative equilibration with three keratin standards (chicken feathers, whale baleen, and cow hoof; Wassenaar and Hobson 2003). Standard deviations for repeated samples were 1.6, 1.4, and 2.0 for chicken feathers ($n = 26$), whale baleen ($n = 9$), and cow hoof ($n = 8$), respectively. We report values of δD for the nonexchangeable component of feathers, expressed in δ notation, as parts per thousand (‰) relative to the standard scale of Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation. Duplicate analyses, performed on approximately 10% of the feather samples, yielded a mean standard deviation

of 1.4‰. For $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis, feather samples were weighed, placed into tin capsules, and analyzed by CF-IRMS techniques with a Delta Plus mass spectrometer interfaced with a Carlo Erba elemental analyzer. We report measurements in δ notation relative to the Pee Dee Belemnite standard for $\delta^{13}\text{C}$ and atmospheric air for $\delta^{15}\text{N}$. Data were normalized by four International Atomic Energy Association reference standards (CH6, CH7, N1, and N2). Repeated analysis ($n = 47$) of an internal laboratory standard (peach leaves; National Institute of Standards and Technology 1547), yielded standard deviations of ± 0.04 for $\delta^{13}\text{C}$ and ± 0.10 for $\delta^{15}\text{N}$. Duplicate analyses, performed on approximately 10% of the feather samples, yielded mean standard deviations of 0.09‰ for $\delta^{13}\text{C}$ and 0.11 for $\delta^{15}\text{N}$.

STATISTICAL ANALYSES

We used multivariate analysis of variance (MANOVA, PROC GLM) to examine overall differences in stable-isotope ratios in feathers of *B. c. interior* and *B. c. maxima*. We also ran separate univariate analyses of variance (ANOVA, PROC GLM) for each of the three stable isotopes individually. Within each of the two subspecies, we used MANOVA and univariate ANOVA to examine differences among individual populations. We report mean values \pm SE.

To examine the relationship between δD_f and latitudinal variation in δD_p , we used GIS-based grids (Bowen 2009) to estimate values of δD_p for the growing season (Bowen et al. 2005) and entire year (Bowen and Revenaugh 2003) for each source population, and we used correlation analysis to assess the relationship between mean δD_f for each subpopulation and

either growing-season or annual δD_p . We used PROC CORR to calculate Pearson correlation coefficients and assess their significance with Student's t -test. We used SAS (SAS Institute 2001) for all statistical analyses.

RESULTS

Mean values of δD , $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ in feathers are presented in Table 1, by subspecies and population. We found no significant difference between the interior and giant Canada Goose subspecies in overall δD_f , $\delta^{13}\text{C}_f$, or $\delta^{15}\text{N}_f$ isotopic composition (MANOVA, Wilks' λ , $F_{3,177} = 0.97$, $P = 0.1296$). Looking at each isotope individually, we found no difference between subspecies in δD_f ($F_{1,179} = 0.02$, $P = 0.8790$); δD values averaged $-131.85 \text{‰} \pm 1.36$ for *B. c. interior* and $-131.63 \text{‰} \pm 0.71$ for *B. c. maxima*, and there was substantial overlap between the two groups (Fig. 2a). Despite large latitudinal differences between populations, we found no overall correlation between mean δD_f and either mean growing-season δD_p ($r = 0.31$, $n = 8$, $P = 0.4569$; Fig. 3a) or mean annual δD_p ($r = 0.25$, $n = 8$, $P = 0.5498$; Fig. 3b). We also found no significant differences between the subspecies in $\delta^{13}\text{C}_f$ ($F_{1,179} = 1.47$, $P = 0.2273$); $\delta^{13}\text{C}_f$ averaged $-25.24 \text{‰} \pm 0.06$ for *B. c. interior* and $-25.07 \text{‰} \pm 0.12$ for *B. c. maxima*, with substantial overlap (Fig. 2b). We did find significant differences between the two subspecies in $\delta^{15}\text{N}_f$ ($F_{1,179} = 4.86$, $P = 0.0287$). Values for *B. c. interior* averaged $5.38 \text{‰} \pm 0.12$, while those for *B. c. maxima* averaged $6.00 \text{‰} \pm 0.23$; however, there was substantial overlap in values between the two groups (Fig. 2c).

TABLE 1. Collection location (latitude and longitude) and isotopic composition of feathers from three populations of *Branta canadensis interior* and five of *B. c. maxima*, including mean (SE) δD_f , $\delta^{13}\text{C}_f$, and $\delta^{15}\text{N}_f$. All values of δ are in parts per thousand.

Subspecies and population	Latitude (N)	Longitude (W)	n	δD_f (SE)	$\delta^{13}\text{C}_f$ (SE)	$\delta^{15}\text{N}_f$ (SE)
<i>B. c. interior</i>			81	$-131.85 (1.36)$	$-25.24 (0.06)$	$5.38 (0.12)$
Mississippi Valley (Hudson Bay coast)	55.74	86.08	25	$-145.12 (1.71)$	$-25.25 (0.09)$	$4.65 (0.14)$
Southern James Bay (Akimiski I.)	53.18	81.17	31	$-131.25 (1.02)$	$-25.31 (0.11)$	$5.68 (0.22)$
Southern James Bay (mainland)	51.48	80.16	25	$-119.32 (1.22)$	$-25.14 (0.09)$	$5.72 (0.20)$
<i>B. c. maxima</i>			100	$-131.63 (0.71)$	$-25.07 (0.12)$	$6.00 (0.23)$
Mosquito Creek Wildlife Area	41.43	80.83	20	$-134.05 (1.90)$	$-25.36 (0.17)$	$6.41 (0.41)$
Magee Marsh Wildlife Area	41.62	83.18	20	$-136.52 (1.55)$	$-24.33 (0.31)$	$5.81 (0.57)$
Killdeer Plains Wildlife Area	40.72	83.28	20	$-129.65 (0.92)$	$-25.13 (0.16)$	$6.12 (0.48)$
Mercer Wildlife Area	40.53	84.55	20	$-126.16 (1.26)$	$-25.05 (0.26)$	$6.09 (0.41)$
Salt Fork Wildlife Area	40.08	81.47	20	$-131.77 (1.10)$	$-25.47 (0.30)$	$5.55 (0.66)$

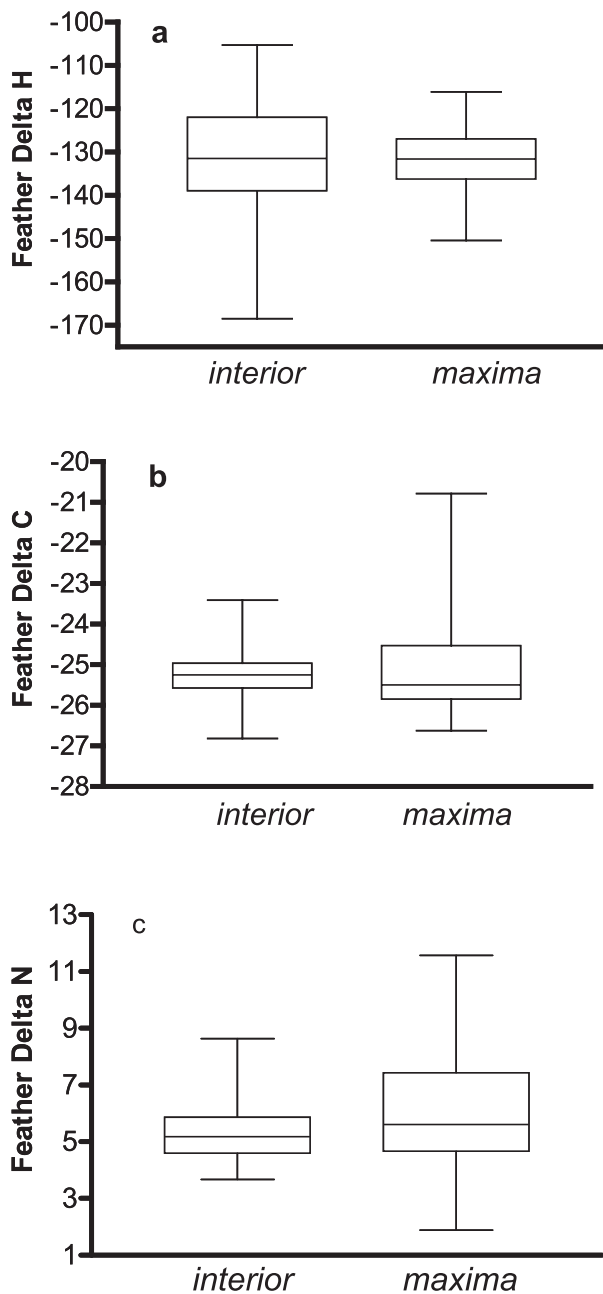


FIGURE 2. Box and whisker plots, showing stable-isotope ratios in feathers of the Interior (*Branta canadensis interior*) and Giant (*B. c. maxima*) subspecies of the Canada Goose. (a) δD , (b) $\delta^{13}C$, and (c) $\delta^{15}N$. Samples of *B. c. interior* ($n = 81$) comprise feathers from three subpopulations; those of *B. c. maxima* ($n = 100$) comprise feathers from five subpopulations (see Fig. 1). The box extends from the 25th percentile to the 75th percentile, with a line at the median. The whiskers show the highest and lowest values.

Within each subspecies, we found significant differences in isotopic composition among the various populations. Among the three populations of *B. c. interior*, results indicated significant overall differences (MANOVA, Wilks' λ , $F_{6,152} = 0.28$, $P < 0.0001$) in isotopic composition. Univariate

analyses revealed significant differences in δD_f ($F_{2,78} = 89.66$, $P < 0.0001$), and we found a fairly strong but not statistically significant correlation between δD_f and both growing-season δD_p ($r = 0.98$, $n = 3$, $P = 0.1127$; Fig. 3a) and annual δD_p ($r = 0.98$, $n = 3$, $P = 0.1175$; Fig. 3b) for *B. c. interior*. Results also indicated significant differences in $\delta^{15}N_f$ ($F_{2,78} = 9.31$, $P = 0.0002$) but no difference in $\delta^{13}C_f$ among these populations ($F_{2,78} = 0.82$, $P = 0.4441$). Among the five populations of *B. c. maxima*, results indicated overall differences (MANOVA, Wilks' λ , $F_{4,95} = 0.56$, $P < 0.0001$) in isotopic composition. Univariate analyses specified significant differences in δD_f ($F_{4,95} = 8.24$, $P < 0.0001$) among populations of *B. c. maxima*, and, using either growing-season δD_p ($r = 0.57$, $n = 5$, $P = 0.3162$) or annual δD_p ($r = 0.64$, $n = 5$, $P = 0.2386$), we found a trend toward increasing δD_f with increasing δD_p in this subspecies. We found significant differences among populations of *B. c. maxima* in $\delta^{13}C_f$ ($F_{4,95} = 3.18$, $P = 0.0170$) but not in $\delta^{15}N_f$ ($F_{4,95} = 0.40$, $P = 0.8117$).

DISCUSSION

Ratios of stable isotopes in feathers have great potential for revealing the connectivity of avian migrations (reviewed in Hobson 2005) and identifying the source of harvested samples of game species (Hobson et al. 2006, 2009, Ashley et al. 2010). In particular, the relationship of latitude with δD_p and δD_f is often used to determine birds' latitude of origin (e.g., Chamberlain et al. 1997, Hobson and Wassenaar 1997, Clark et al. 2006). In our study, using a combination of δD_f , $\delta^{13}C_f$ and $\delta^{15}N_f$, we found no overall difference between *B. c. interior*, nesting in the subarctic, and *B. c. maxima*, nesting in the temperate zone. Of particular interest was the lack of difference between subspecies in δD_f , despite large differences in latitude and δD_p between locations of breeding and molt. Farmer et al. (2008) estimated that in eastern North America the minimum distance required for distributions of δD_p to be distinguished at the 80% confidence level is 6.8° of latitude. The two subspecies sampled for our study were separated by 10–15°, well above this threshold, but we found their distributions of δD_p to overlap. These results suggest that δD_f alone cannot be used to determine the location of molt of *B. c. interior* and *B. c. maxima* and that the potential of δD_f for estimating the origin of Canada Geese harvested in Ohio or for distinguishing molt migrants of *B. c. maxima* from birds molting locally is very limited.

Despite the lack of difference in δD_f between *B. c. interior* and *B. c. maxima*, we found significant variation among populations within each of the two subspecies. In particular, we found a significant correlation between δD_f and δD_p within the three populations of *B. c. interior*. All three were sampled from coastal areas with marine influence and were separated by a maximum distance of 4.2° of latitude. We also found a nonsignificant trend toward δD_f increasing with δD_p among the five populations of *B. c. maxima*. These populations were sampled

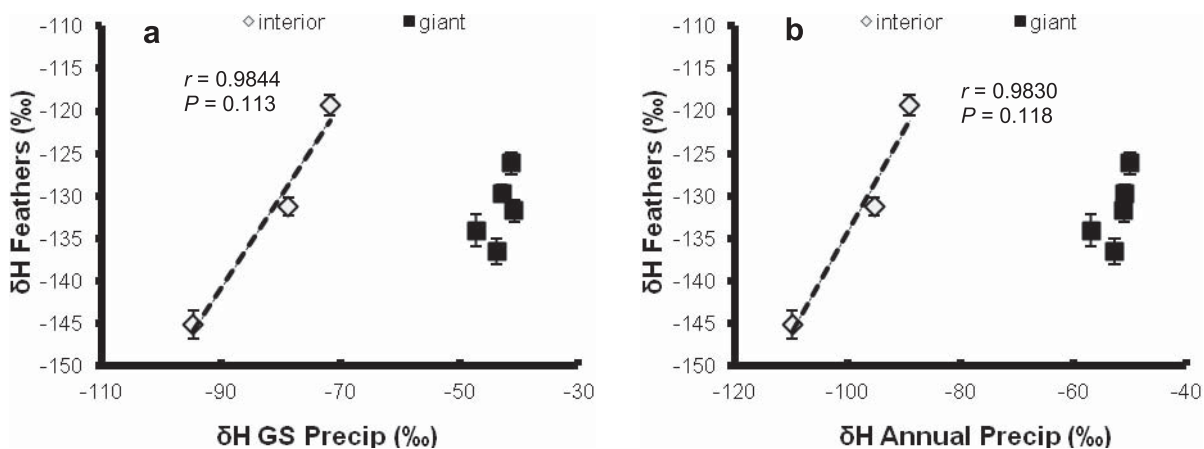


FIGURE 3. Correlation between δD in feathers (δD_f) and precipitation (δD_p) for three subpopulations of the Interior subspecies (*Branta canadensis interior*) and five of the Giant subspecies (*B. c. maxima*) of the Canada Goose. (a) δD_f vs. growing-season δD_p ; (b) δD_f vs. annual δD_p . δD_f values (\pm SE) were obtained from analysis of feathers collected from birds of known origin in 2005; δD_p values were obtained from GIS maps of growing-season (Bowen et al. 2005) and annual (Bowen and Revenaugh 2003) δD_p .

from inland freshwater habitats and were separated by a maximum of only 1.3° of latitude. The three populations of *B. c. interior* fell closer to the values expected from relationships between δD_f and growing-season δD_p found for the Mallard (*Anas platyrhynchos*) and Northern Pintail (*A. acuta*) ($\delta D_f = -57 + 0.83\delta D_p$; Hebert and Wassenaar 2005) and for the Lesser Scaup (*Aythya affinis*) ($\delta D_f = -27.8 + 0.95\delta D_p$; Clark et al. 2006). In Ohio, *B. c. maxima* appears to much more depleted in δD_f than expected from these relationships. This may suggest that its foods are disconnected from local growing-season precipitation; however, both of these previous studies were conducted in the western United States and may not be applicable to eastern Canada Goose populations. Overall, these results suggest that δD_f varies with latitudinal variation in δD_p at a more local scale (within subspecies), but that these differences are eliminated at a larger scale, with no overall relationship between δD_f and δD_p when the comparison is between the two subspecies.

For some bird species, a multiple-isotope approach, using $\delta^{13}C_f$ and $\delta^{15}N_f$ in addition to δD_p , has improved estimates of geographic origin (e.g., Wunder et al. 2005, Szymanski et al. 2007) over those based on δD_f alone. Other recent studies of waterfowl have employed $\delta^{34}S$ in combination with $\delta^{13}C_p$, $\delta^{15}N_p$, and δD_f (Clark et al. 2006, Coulton et al. 2010). In the Atlantic Flyway, Caccamise et al. (2000) was able to distinguish migratory populations of the Canada Goose from northern Quebec and resident populations from New Jersey by using $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. In our study, we found no significant difference between *B. c. interior* and *B. c. maxima* in $\delta^{13}C$. Although we identified significant differences between the two in $\delta^{15}N$, there was considerable overlap, and the addition of $\delta^{13}C$ and $\delta^{15}N$ did not improve our ability to discriminate between them; multivariate analyses failed to distinguish the two groups, even when all three isotopes were considered.

The samples of *B. c. interior* obtained for this study cover the coastal portions of the breeding ranges of the Mississippi Valley and southern James Bay populations; however, the portions of these populations breeding inland may have an isotopic signature very different from that of coastal birds. Although a large portion of the population inhabits Akimiski Island and the mainland coasts of James Bay, it extends south to 50° N. Inland portions of the population are less accessible and not as well studied. Future studies should consider including samples from inland locations. Furthermore, our sample of *B. c. maxima* represents a small portion of the subspecies range, so our results may not be applicable in other areas where the two subspecies mix and/or to other subspecies in North America. In a previous study, designed to determine the migratory status of geese in the Atlantic Flyway, Marra et al. (2009) found that individuals from New York City averaged isotopically heavier, but not significantly so, than individuals breeding in Newfoundland. These results from the Atlantic Flyway, coupled with our results in the Mississippi Flyway, suggest that δD_f may have limited applicability for specifying the source and subspecies of Canada Geese over a wider portion of the species' range, and that indistinguishability of populations may not be limited to the two subspecies we sampled. We recommend caution in applying isotope analyses to identify the source of Canada Geese. At a minimum, further baseline sampling of other subspecies and populations is required.

Our results suggest that δD_f alone, or in combination with $\delta^{13}C_f$ and $\delta^{15}N_p$, does not distinguish *B. c. interior* and *B. c. maxima*, despite the large latitudinal difference between the two subspecies' breeding ranges. Previous studies have identified several factors that may limit the applicability of δD_f and other stable isotopes for discriminating among populations. Fox et al. (2009) cautioned that in the Greylag Goose (*Anser*

anser) the use of both exogenous (from local sources) and endogenous (acquired at previous locations) protein stores to produce feathers can result in isotope ratios that do not reflect the local isoscape. Actual δD_f values may also deviate from predicted values because of age (Meehan et al. 2003, Langin et al. 2007), variation in molt strategies (Larson and Hobson 2009, Rocque et al. 2009), inter- and intra-individual variation (Smith and Dufty 2005, Smith et al. 2008), inter-annual differences in δD_p (Farmer et al. 2008, Coulton et al. 2009), and diet and habitat differences (Lott et al. 2003). In particular, because the values of δD in sea water is higher than in local precipitation, the relationship between δD_p and δD_f may not be valid for species that forage in marine environments (Lott et al. 2003).

The lack of difference in δD_f we found between *B. c. interior* and *B. c. maxima* may indicate a difference between them in biology or physiological ecology. For example, differences in habitat and/or diet may contribute to the lack of difference in δD_f between the two subspecies, despite the wide differences in latitude and δD_p . In Ohio and most of the Mississippi Flyway *B. c. maxima* is restricted to freshwater wetlands, but coastal populations of *B. c. interior* have a strong marine influence (O et al. 2006). Recent studies have used $\delta^{13}C$ to determine whether the origin of waterfowl is freshwater or marine, with values less than -20‰ indicating freshwater and those greater than -20‰ indicating marine habitats (Yerkes et al. 2008, Ashley et al. 2010). Although *B. c. interior* feeds extensively in coastal intertidal and supratidal areas (O et al. 2006), none of the samples of either subspecies had the $\delta^{13}C$ greater than -20‰ that would indicate a marine influence, according to the threshold used in these studies (Yerkes et al. 2008, Ashley et al. 2010). Analysis of $\delta^{34}S$ has also been used to identify birds exposed to marine habitats, with values of $\delta^{34}S$ higher for birds feeding in marine environments (Hobson et al. 1997, Lott et al. 2003). Among Canada Goose populations in the Atlantic Flyway, Caccamise et al. (2000) found that in New Jersey $\delta^{34}S$ in coastal and inland populations differed, while coastal New Jersey populations could not be distinguished from migrants from northern Quebec. Feathers from birds with elevated $\delta^{34}S$ also have been shown to deviate strongly from the relationship expected between δD_p and δD_f , and this should be taken into account for species that may forage in marine habitats (Lott et al. 2003). The influence of a marine-derived diet on isotopic composition of *B. c. interior* should be explored further. Differences between coastal and inland populations of this subspecies should also be explored, as isotope ratios of inland populations with little marine influence may differ from those of the coastal populations we sampled. In addition, the inclusion of $\delta^{34}S$ may benefit future attempts to use stable isotopes to distinguish *B. c. interior* and *B. c. maxima*, though *B. c. interior* populations from inland areas, with little marine influence, may be expected to have an $\delta^{34}S$ signature similar to that of the inland *B. c. maxima*.

In addition to habitat (marine vs. freshwater) differences, dietary differences may influence isotope ratios. Canada Geese

readily adapt to feed on agricultural crops, which tend to dominate the diets of most populations when they are available (Mowbray et al. 2002). Agricultural land lies within and around the wildlife areas in Ohio where we sampled Giant Canada Geese, so the potential influence of agricultural crops in the diet is greater than for *B. c. interior*. Recent studies (Yerkes et al. 2008, Ashley et al. 2010) have used a threshold value for determining agricultural influence, with $\delta^{15}N$ greater than 9‰ indicating an agricultural source. None of the samples of *B. c. interior* we analyzed was above this threshold, but 14% of those of *B. c. maxima* were. Although $\delta^{15}N_f$ values of the two subspecies overlapped substantially, average values were higher for *B. c. maxima* ($6.00\text{‰} \pm 0.23$) and lower for *B. c. interior* ($5.38\text{‰} \pm 0.12$). This result is consistent with Alisauskas and Hobson (1993), who found higher average $\delta^{15}N$ in muscle tissue of Snow Geese in areas of corn agriculture (95% CI: $7.93\text{--}9.03\text{‰}$), than in marshes (95% CI: $7.06\text{--}8.02\text{‰}$). These results suggest a possible agricultural influence on the diet of Giant Canada Geese. We suggest that further study of the influence of these differences in diet (agricultural vs. natural) as well as habitat (marine vs. inland) on isotope ratios in the Canada Goose is warranted prior to any future attempts to identify individuals' origins with stable isotopes.

For our study in particular, the interpretation of δD results is also limited by the resolution of the base maps of δD_p . Growing-season and annual δD_p maps were interpolated from a network of locations sampled by the International Atomic Energy Association (Bowen and Revenaugh 2003, Bowen et al. 2005). In North America, these stations are clustered in the Great Lakes region, the Atlantic Flyway, and the western boreal region, sites of many studies of stable isotopes in waterfowl (Hebert and Wassenaar 2005, Clark et al. 2006, Coulton et al. 2010). Sampling in the Hudson Bay region (Bowen and Revenaugh 2003, Bowen et al. 2005) is lacking, increasing the possibility of interpolation error in this area. Future studies would benefit from the addition of precipitation sampling in this region to confirm and/or refine estimates of δD_p .

Overall, our results suggest that δD , $\delta^{13}C$, and $\delta^{15}N$ alone have limited ability to discriminate between *B. c. interior* and *B. c. maxima* and so have limited applicability for estimating the derivations of harvested birds and/or identifying molt migrant individuals within *B. c. maxima*. Of particular interest is the lack of variation in δD_p despite the wide differences in latitude and δD_p between the breeding ranges of these two subspecies. Our results also exemplify, for any species of interest, the importance of calibrating local δD_f values with a baseline of locally grown feathers (as suggested by Hobson 2005, Lott and Smith 2006, Farmer et al. 2008) before attempting to assign individuals of unknown origin to a geographic source.

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