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Research Papers

Establishing Winter Origins of Migrating Lesser Snow Geese Using Stable Isotopes

Détermination des sites d'hivernage de Petites Oies des neiges en migration au moyen d'isotopes stables

*Viviane Hénaux*¹, *Larkin A. Powell*¹, *Mark P. Vrtiska*², and *Keith A. Hobson*³

ABSTRACT. Increases in Snow Goose (*Chen caerulescens*) populations and large-scale habitat changes in North America have contributed to the concentration of migratory waterfowl on fewer wetlands, reducing resource availability, and enhancing risks of disease transmission. Predicting wintering locations of migratory individuals is critical to guide wildlife population management and habitat restoration. We used stable carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), and hydrogen ($\delta^2\text{H}$) isotope ratios in muscle tissue of wintering Snow Geese to discriminate four major wintering areas, the Playa Lake Region, Texas Gulf Coast, Louisiana Gulf Coast, and Arkansas, and infer the wintering locations of individuals collected later during the 2007 and 2008 spring migrations in the Rainwater Basin (RWB) of Nebraska. We predicted the wintering ground derivation of migrating Snow Geese using a likelihood-based approach. Our three-isotope analysis provided an efficient discrimination of the four wintering areas. The assignment model predicted that 53% [95% CI: 37-69] of our sample of Snow Geese from the RWB in 2007 had most likely originated in Louisiana, 38% [23-54] had wintered on Texas Gulf Coast, and 9% [0-20] in Arkansas; the assessment suggested that 89% [73-100] of our 2008 sample had most likely come from Texas Gulf Coast, 9% [0-27] from Louisiana Gulf Coast, and 2% [0-9] from Arkansas. Further segregation of wintering grounds and additional sampling of spring migrating Snow Geese would refine overall assignment and help explain interannual variations in migratory connectivity. The ability to distinguish origins of northbound geese can support the development of spatially-adaptive management strategies for the midcontinent Snow Goose population. Establishing migratory connectivity using isotope assignment techniques can be extended to other waterfowl species to determine critical habitat, evaluate population energy requirements, and inform waterfowl conservation and management strategies.

RÉSUMÉ. L'accroissement des populations d'Oies des neiges (*Chen caerulescens*) et les changements d'habitat à grande échelle en Amérique du Nord ont contribué à la concentration de la sauvagine migratrice sur des milieux humides de moins en moins nombreux, à la diminution de la disponibilité des ressources alimentaires et à l'augmentation du risque de transmission de maladies. La capacité à prévoir les sites d'hivernage d'individus migrants est essentielle pour orienter la gestion des populations fauniques et la restauration d'habitat. Nous avons utilisé des ratios d'isotopes stables de carbone ($\delta^{13}\text{C}$), d'azote ($\delta^{15}\text{N}$) et d'hydrogène ($\delta^2\text{H}$) dans les tissus musculaires d'Oies des neiges en hiver afin de différencier quatre aires d'hivernage importantes – la région de Playa Lake au Texas, la côte du golfe au Texas, la côte du golfe en Louisiane et l'Arkansas – et de prévoir les sites d'hivernage d'individus récoltés durant la migration printanière de 2007 et celle de 2008 dans la région de Rainwater Basin (RWB) au Nebraska. Nous avons calculé la prévision des sites d'hivernage des Oies des neiges migratrices à l'aide d'une approche fondée sur la vraisemblance. Notre analyse des trois isotopes a permis de différencier efficacement les quatre aires d'hivernage. Le modèle d'attribution a prévu que 53 % [95 % IC : 37-69] de notre échantillon d'oies de RWB en 2007 provenait fort probablement de la Louisiane, 38 % [23-54] avait hiverné sur la côte du golfe au Texas et 9 % [0-20] en Arkansas; l'attribution a montré que 89 % [73-100] de notre échantillon en 2008 provenait fort probablement de la côte du golfe au Texas, 9 % [0-27] de la côte du golfe en Louisiane et 2 % [0-9] de l'Arkansas. Une division plus fine des aires d'hivernage et davantage d'échantillons d'oies en migration printanière permettraient d'améliorer l'attribution dans l'ensemble et de contribuer à expliquer les variations interannuelles de la connectivité migratoire. La capacité à distinguer la provenance des oies se dirigeant vers le nord peut contribuer à l'élaboration de stratégies de gestion évolutive fondées sur la répartition géographique.

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de la population d'Oie des neiges du milieu du continent. L'établissement de la connectivité migratoire au moyen de techniques d'attribution isotopique peut être appliqué à d'autres espèces de sauvagine afin de déterminer l'habitat essentiel, d'évaluer les besoins énergétiques des populations, et d'appuyer les efforts de conservation et les stratégies de gestion de la sauvagine.

Key Words: *carbon-13*, *Chen caerulescens*, *hydrogen-2*, *migratory connectivity*, *nitrogen-15*, *Rainwater Basin*, *Snow Goose*, *spring migration*, *stable isotope*

INTRODUCTION

Establishing wintering areas of northbound or breeding and natal areas of southbound individuals allows insight into processes affecting various stages of the life cycle of populations of migratory birds. For example, the extent to which environmental conditions on wintering grounds drive spring migration timing and affect individual life cycles is essential in understanding major variations in population dynamics related to overwinter survival (Saino et al. 2004). The strength of the connections between breeding, winter, and stopover habitats can influence the ability of migratory populations to evolve and adapt to selective pressures induced by global changes (review in Webster et al. 2002, Webster and Marra 2005). In addition, continental management of target species requires connections be made between wintering habitats and critical stopover locations used as part of their annual life cycle. Indeed, a good knowledge of migratory connectivity is critical for designing conservation strategies (Martin et al. 2007), implementing adaptive harvest of game populations (Hobson et al. 2009), and predicting potential infectious disease spread in wildlife (Prosser et al. 2009, Gunnarsson et al. 2012).

Snow Goose (*Chen caerulescens*) management requires spatial derivation of migratory populations. North American Snow Goose populations have dramatically increased over the last decades, causing serious ecological and economic problems (Ankney 1996, Jefferies et al. 2004a, Abraham et al. 2005). The rapid growth in Snow Goose populations has also resulted in an extension of the species' wintering range. Originally dependent on the salt and brackish marshes of the Gulf of Mexico, Snow Geese of the midcontinent population first extended their range to irrigated rice prairies of Texas and Louisiana and now also feed on agricultural crops farther north (Alisauskas and Hobson 1993, Abraham et al. 2005). Studying migratory origins of Snow Geese would also provide information needed for habitat management. Landscape changes in North America have affected the number of wetlands available to spring migrants (Dahl 1990). In Nebraska's Rainwater Basin (RWB) region, more than 85% of historical wetlands have been converted to agriculture (Smith 1998). Wetland habitat is particularly critical during spring because the RWB is a key stopover site for millions of migratory ducks, geese, shorebirds, and cranes (Bishop and Vrtiska 2008). Migrant waterbirds stop in the RWB for short periods to acquire the nutrient reserves necessary for migration further north and reproduction. Loss of wetlands has resulted

in higher concentrations of wintering and migrant waterbird populations, which reduces the availability of food resources and enhances the risk of transmission and spread of infectious diseases (Friend 1992).

Stable isotope analyses of specific animal tissues are now widely used to infer diet, geographical origin, and migratory connectivity (Hobson 1999, Webster et al. 2002, Hobson and Wassenaar 2008, Inger and Bearhop 2008). In contrast to genetic or extrinsic markers, e.g., individual tags or remote-sensing devices, this method offers the advantage of not requiring recapture of individuals and may provide reliable information on migratory pathways in species with regional to continental distribution (Rubenstein and Hobson 2004, Hebert and Wassenaar 2005, Hobson and Norris 2008). This approach relies on the fact that food web isotopic signatures are reflected in the tissues of individuals and that such signatures can vary spatially based on a variety of biogeochemical processes (Hobson 2008). Consequently, organisms moving between isotopically distinct food webs can carry with them information on the location of previous feeding. Importantly, this technique is not biased to the distribution of any initially marked populations, in contrast to exogenous marker methods that require marking birds in several wintering regions and then recapturing those that happened to go to the RWB.

Assessments of movements have largely been aimed at southward migration, because most migratory birds in North America conveniently molt flight feathers after breeding and before migration (Hobson and Wassenaar 1997). Gathering information on northward migration, however, demands the use of different tissues. Determining winter origins of spring migratory birds involves three steps (Rubenstein and Hobson 2004, Hobson 2011): (1) choosing an appropriate tissue representing the period of integration appropriate to the time spent on the wintering grounds, (2) isotopically characterizing and differentiating among wintering populations of interest, and (3) inferring geographical wintering locations based on isotopic similarity in tissues of wintering and migrating individuals.

Previous studies using hydrogen ($\delta^2\text{H}$) measurements of animal keratin-based tissues ($\delta^2\text{H}_k$) to characterize terrestrial source areas have used continent-wide, amount-weighted, growing-season $\delta^2\text{H}$ values in precipitation ($\delta^2\text{H}_p$) and a knowledge of the calibration relationship between $\delta^2\text{H}_p$ and $\delta^2\text{H}_k$ (reviewed by Hobson 2008, Hobson et al. 2012).

Table 1. Sampling dates, number of adult Snow Geese (*Chen caerulescens*) collected, and mean (\pm SE) stable hydrogen ($\delta^2\text{H}$), carbon ($\delta^{13}\text{C}$), and nitrogen ($\delta^{15}\text{N}$) isotope signatures in four major wintering areas of the central flyway and in Nebraska's Rainwater Basin (RWB) during spring migrations 2007 and 2008.

Sampling places	Sampling dates (n = number of individuals)	$\delta^2\text{H}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Playa Lake Region	13 Dec 2006 ($n = 5$)	-170.3 \pm 3.9	-20.3 \pm 0.2	7.0 \pm 0.2
Texas Coast	28 Jan - 2 Mar 2007 ($n = 10$); 18 Jan - 11 Feb 2008 ($n = 17$)	-118.1 \pm 1.7	-19.9 \pm 0.5	8.0 \pm 0.1
Louisiana Coast	19 Feb 2007 ($n = 6$); 5 Feb 2008 ($n = 8$)	-130.7 \pm 1.2	-22.8 \pm 0.3	7.6 \pm 0.2
Arkansas	12 Feb 2007 ($n = 9$)	-144.7 \pm 2.1	-23.1 \pm 0.6	7.0 \pm 0.1
Nebraska RWB	27 Feb - 20 Mar 2007 ($n = 35$); 7-12 Mar 2008 ($n=11$)	n/a	n/a	n/a

n/a = not applicable

However, isoscapes for other elements such as carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) can be more complex because they result from differences in plant photosynthetic pathway (C_3 vs. C_4 , CAM plants), water-use efficiency mechanisms, nitrogen fixation process, soil moisture conditions, and human activities, i.e., irrigation, pollution, and land use practices (see papers in Michener and Lajtha 2007). Constructing local, fine-scale isoscapes from measurements of multiple stable isotope values in prey tissues has been shown to be valuable to determine migration corridors of large carnivores (Hénaux et al. 2011). However, for generalist species, like Snow Geese, the ideal approach involves measuring stable isotope ratios in the tissues of interest (e.g., Yerkes et al. 2008). Accordingly, we documented isotopic differences among four major wintering areas of Snow Geese by measuring $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^2\text{H}$ in muscle tissues of wintering geese and subsequently predicted the wintering origin of spring migrants using a likelihood-based inference approach.

METHODS

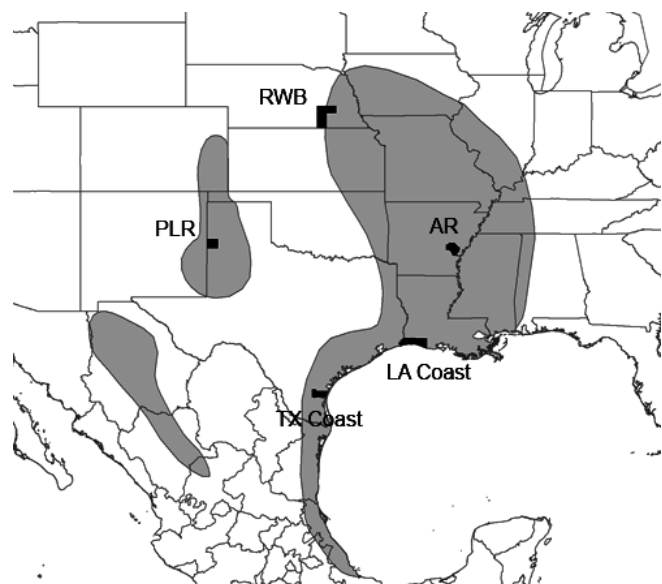
Tissue selection and collection

In contrast to studies that inferred breeding origin of migratory individuals from isotopic values in feather tissue grown after breeding, we relied on metabolically active tissue to determine the wintering grounds of lesser Snow Geese that stop in the RWB of Nebraska during spring migration. With a turnover half-life of the order of two weeks for large-bodied geese (see Discussion), we assumed that the muscle tissue of a goose recently arrived at the RWB should reflect the isotopic composition of the diet consumed at its wintering place, i.e., a dietary integration of two to three half-lives.

Snow geese migrating through the Mississippi and Central flyways winter in several distinct areas in the Playa Lake Region, central Mexico, the Gulf coast from Mexico to Louisiana, and along the Mississippi Alluvial Valley (Drewien et al. 2003, Jefferies et al. 2004b, Ridgely et al. 2007). We obtained wings from apparently healthy Snow Geese from hunter-shot birds or collected by shotgun during the winters 2006/2007 and 2007/2008 at four major wintering locations: Playa Lake Region ([PLR] Parmer County, Texas), Texas Gulf

Coast ([TX Coast] Kleberg County, Texas), Louisiana Gulf Coast ([LA Coast] Cameron and Vermilion Parishes, Louisiana), and eastern Arkansas ([AR] Arkansas County, Arkansas; Fig. 1). We also collected wings from hunter-shot or collected Snow Geese at multiple sites in the RWB in February-March 2007 and 2008 (Table 1). One wing from each individual was frozen separately for storage.

Fig. 1. Location of the collection sites of midcontinent Snow Geese (*Chen caerulescens*) in the Playa Lake Region (PLR), Texas Coast (TX Coast), Louisiana (LA Coast), and Arkansas (AR), and in the Rainwater Basin of Nebraska (RWB) in 2007 and 2008. The Snow Goose wintering range in Central and Mississippi flyways (grey area) was provided by Ridgely et al. (2007).



Stable isotope analyses

Following thawing and dissection of the wing, 1-cm³ samples of muscle (bicep) tissue were dried at 60°C during ≥ 24 hours, and ground to powder. Because of the potential influence of lipids on $\delta^{13}\text{C}$ analyses (Post et al. 2007), we removed lipids

from powdered muscle using a 2:1 chloroform:methanol overnight soaking and rinsed. Stable-hydrogen isotope measurements were performed on H₂ derived from high-temperature flash pyrolysis of samples and continuous-flow isotope-ratio mass spectrometry (CF-IRMS). Pure H₂ was used as the sample analysis gas and the isotopic reference gas. A Eurovector 3000TM (Eurovector, Milan, Italy) high temperature elemental analyzer (EA) with autosampler was used to automatically pyrolyse muscle samples to a single pulse of H₂ gas (and N₂ and CO gas). The resolved H₂ sample pulse was then introduced to the isotope ratio mass spectrometer (Micromass IsoprimeTM [Manchester, England] with electrostatic analyzer) via an open split capillary. Repeated analysis of hydrogen isotope intercomparison material IAEA-CH-7, routinely included as a check, yielded an external repeatability of better than ±1.5‰. Estimates of the δ²H value of the nonexchangeable H in unknown samples were derived using the comparative equilibration technique of Wassenaar and Hobson (2003, 2006).

For δ¹³C and δ¹⁵N measurements, approximately 1 mg of each sample was loaded into tin cups. Our mass spectrometer consisted of a Europa Robo Prep combustion system interfaced with a Europa Tracermat continuous-flow isotope-ratio mass spectrometer. Samples were analyzed as five unknowns separated by two in-house albumin standards. Based on within-run standard measurements, we estimate our analytical error to be ±0.1‰ for δ¹³C measurements and ±0.3‰ for δ¹⁵N measurements. All stable isotope values are expressed in the δ notation (in units of per mil, ‰). The standards are Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW–SLAP) for hydrogen, Pee Dee Belemnite (PDB) standard for carbon, and AIR standard for nitrogen. Stable hydrogen isotope analyses were conducted at the Environment Canada Stable Isotope facility in Saskatoon, Canada. Stable carbon and nitrogen analyses were conducted at the Department of Soil Science Laboratory, University of Saskatchewan, Saskatoon.

Isotopic segregation of wintering areas

We included only adult-plumaged geese and did not account for sex because we assumed males and females to have identical diet in the same habitat (e.g., Alisauskas et al. 1988). We used mixed-effect models to evaluate difference in isotopic ratio between years in both LA Coast and TX Coast and also between dates (within year) in TX Coast. These analyses indicated no significant random effects (results not shown), so we combined samples collected in 2007 and 2008 for each wintering area.

We conducted a linear discriminant analysis to determine the proportion of individuals from each wintering area whose isotopic signature correctly represented their wintering habitat. We calculated mean stable isotope values in lipid-extracted muscle tissues within each wintering region and ran a multiple analysis of variances (MANOVA) to determine

whether these areas were segregated isotopically. We conducted these analyses on ranked data (nonparametric test) because data for δ²H (Shapiro test: $P = 0.002$) and δ¹³C values ($P = 0.006$) were not normally distributed. All data were homoscedastic (Levene's test: all $P > 0.06$). Variations of each isotope among areas were further evaluated using ANOVA (on ranks). We used R 2.11.0 (R Development Core Team 2010) for all statistical analyses.

Stable isotope assignment approach

We used the likelihood-based assignment method developed by Royle and Rubenstein (2004) to evaluate the likely origin, among the four discrete wintering populations, of each Snow Goose collected in the RWB. The basis of this approach was that we considered all significant wintering areas used by our migrant population sampled and that migrating Snow Geese may be associated with the location most likely to produce the observed tissue isotope values. We defined the expected stable isotope value (for any given isotope X) in Snow Goose muscle tissue at each wintering place, $w = 1, 2, \dots, W$, as

$$y_{w,\delta X} = \mu_{w,\delta X} \pm \sigma_{w,\delta X} \quad (1)$$

where μ and σ represent the mean δX value and standard deviation, respectively. We used

$$y^*_{\delta X,i} \quad (2)$$

to denote the isotopic value measured in the muscle tissue of the goose i collected at RWB. To determine the most likely wintering origin of this individual, we evaluated the likelihood

$$f(y^*_{\delta X,i} | \mu_{w,\delta X}, \sigma_{w,\delta X}) \quad (3)$$

defined as the conditional probability of

$$y^*_{\delta X,i} \quad (4)$$

given that the individual came from the wintering area w . In our study, we considered three elements (hydrogen, carbon, and nitrogen), therefore, the likelihood,

$$f(y^*_{\delta^2H, \delta^{13}C, \delta^{15}N} | \mu_{w,\delta^2H}, \sigma_{w,\delta^2H}, \mu_{w,\delta^{13}C}, \sigma_{w,\delta^{13}C}, \mu_{w,\delta^{15}N}, \sigma_{w,\delta^{15}N}) \quad (5)$$

evaluated at each wintering place w , was a multivariate normal likelihood with location parameter μ and variance–covariance matrix Σ as follows:

$$\mu = \begin{pmatrix} \mu_{\delta^2\text{H}} \\ \mu_{\delta^{13}\text{C}} \\ \mu_{\delta^{15}\text{N}} \end{pmatrix} \quad (6)$$

$$\Sigma = \begin{pmatrix} \sigma_{\delta^2\text{H}}^2 & \sigma_{\delta^2\text{H},\delta^{13}\text{C}} & \sigma_{\delta^2\text{H},\delta^{15}\text{N}} \\ \sigma_{\delta^2\text{H},\delta^{13}\text{C}} & \sigma_{\delta^{13}\text{C}}^2 & \sigma_{\delta^{13}\text{C},\delta^{15}\text{N}} \\ \sigma_{\delta^2\text{H},\delta^{15}\text{N}} & \sigma_{\delta^{13}\text{C},\delta^{15}\text{N}} & \sigma_{\delta^{15}\text{N}}^2 \end{pmatrix} \quad (7)$$

The covariance between two isotopes at each wintering place w was calculated, for example between $\delta^2\text{H}$ and $\delta^{13}\text{C}$, as:

$$\sigma_{\delta^2\text{H},\delta^{13}\text{C}} = \rho_{\delta^2\text{H},\delta^{13}\text{C}} \sigma_{\delta^2\text{H}} \sigma_{\delta^{13}\text{C}} \quad (8)$$

where

$$\rho_{\delta^2\text{H},\delta^{13}\text{C}} \quad (9)$$

is the correlation coefficient between $\delta^2\text{H}$ and $\delta^{13}\text{C}$.

Following Royle and Rubenstein (2004), we determined the probability that Snow Goose i originated from w as:

$$\pi_{w,i} = \frac{f(\mathbf{y}_i^* | \mu_w, \sigma_w)}{\sum_{w=1} f(\mathbf{y}_i^* | \mu_w, \sigma_w)} \quad (10)$$

where

$$f(\mathbf{y}_i^* | \mu_w, \sigma_w) \quad (11)$$

denotes the three-isotope likelihood. Lastly, we calculated the most likely wintering areas of Snow Geese migrating via the RWB as:

$$\bar{\pi}_w = \frac{\sum_i \pi_{w,i}}{n} \quad (12)$$

where n is the number of individual geese collected at the RWB. We used bootstrapping with 1000 resamplings to evaluate the 95% confidence interval. The upper bound for wintering areas that provided any of the geese sampled at the RWB was calculated as:

$$\bar{\pi}_w(\text{upper}) = \frac{-\ln(\alpha)/W}{n} \quad (13)$$

where $\alpha = 0.05$ is the level of significance (adapted from Jovanovic and Levy 1997). We also conducted the likelihood assignment analysis using only one or two isotopes to contrast results with the tri-isotope analysis.

RESULTS

Isotopic segregation of wintering grounds

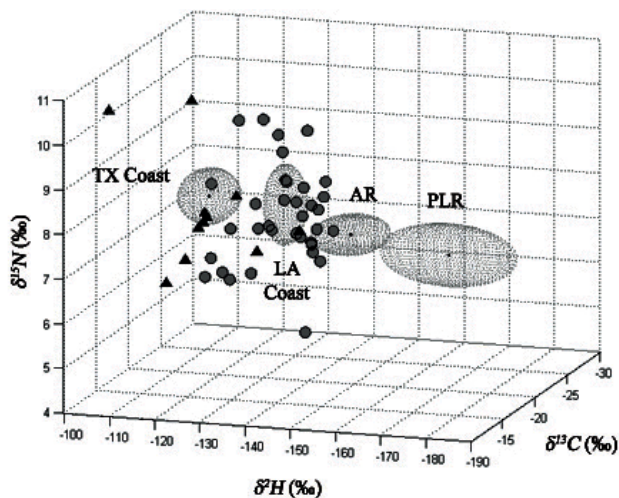
The discriminant analysis misclassified one AR (11%) and three TX geese (10%) in LA, and one LA goose (7%) in AR. Isotopic values in Snow Goose muscle tissues showed significant variations among the four wintering areas (MANOVA: $F_{3,54} = 14.50$, $P < 0.001$). As expected from the continental isoscape and expected deuterium (^2H) enrichment at marine-influenced sites, i.e., brackish marshes, $\delta^2\text{H}$ values followed a decreasing southeast-northwest pattern, ranging from -118.1 ± 1.7 ‰ (SE) and -130.7 ± 1.2 ‰ in TX and LA coastal areas, respectively, to -144.7 ± 2.1 ‰ in AR and -170.3 ± 3.9 ‰ the PLR (Fig. 2). All differences in $\delta^2\text{H}$ values between wintering areas ($F_{3,54} = 46.37$, $P < 0.001$) were significant (Tukey: $P < 0.006$), except between the PLR and AR ($P = 0.43$).

There was a latitudinal gradient in stable carbon isotope distribution with more ^{13}C -depleted diet in AR (-23.1 ± 0.6 ‰) and LA Coast (-22.8 ± 0.3 ‰) than in TX Coast (-19.9 ± 0.5 ‰) and PLR (-20.3 ± 0.2 ‰; Fig. 2). Our analysis showed divergent $\delta^{13}\text{C}$ values among all sites ($F_{3,54} = 10.86$, $P < 0.001$), except between LA Coast and AR ($P = 1.00$) and TX Coast and PLR ($P = 0.92$; other combinations: $P < 0.012$).

We found more ^{15}N -enriched muscle tissues on the TX Coast (8.0 ± 0.1 ‰) and LA Coast (7.6 ± 0.2 ‰) than in AR (7.0 ± 0.1 ‰) and PLR (7.0 ± 0.2 ‰; Fig. 2). Variations in $\delta^{15}\text{N}$ values among sites were significant ($F_{3,54} = 9.01$, $P < 0.001$) between AR and both LA ($P = 0.008$) and TX Coast ($P < 0.001$) as well as between the PLR and both TX Coast ($P = 0.008$) and LA Coast ($P = 0.057$); isotopic signatures were similar in AR and PLR ($P = 1.00$) and TX Coast and LA Coast ($P = 0.85$). This pattern reflected more ^{15}N -enriched muscle

tissues on the TX Coast ($8.0 \pm 0.1\text{‰}$) and LA Coast ($7.6 \pm 0.2\text{‰}$) than in AR ($7.0 \pm 0.1\text{‰}$) and PLR ($7.0 \pm 0.2\text{‰}$; Fig. 2).

Fig. 2. Stable isotopic signatures in muscle tissues of spring migrating Snow Geese (*Chen caerulescens*) collected in the Rainwater Basin (RWB) of Nebraska and at four potential wintering areas: Playa Lake Region (PLR), Texas Gulf Coast (TX Coast), Louisiana Coast (LA Coast), and Arkansas (AR). Ellipsoids centered on small black dots indicate the mean (and 95% confidence interval) muscle isotopic pattern in the four wintering areas. Grey dots and black triangles represent the isotopic signatures of RWB Snow Geese collected in 2007 and 2008, respectively.



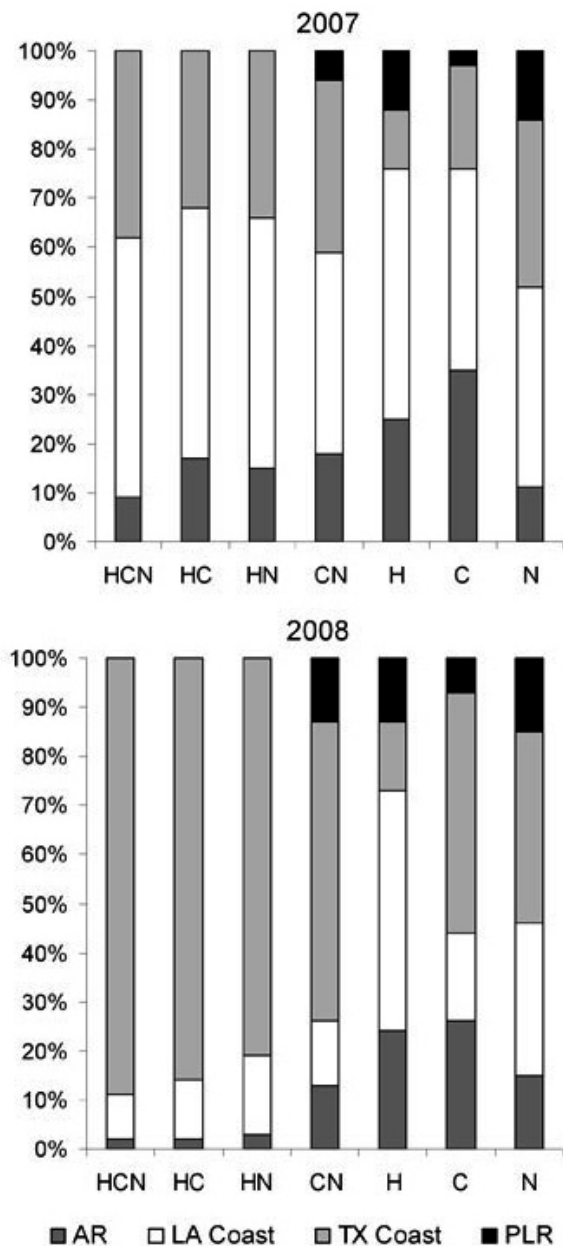
Origin of Snow Geese migrating through the RWB

The maximum likelihood assignment approach from our tri-isotope assessment predicted that among the wintering regions considered in our study the LA Coast was the most likely origin for 53% [95% CI: 37-69] of the geese collected in the RWB during the 2007 spring migration; the TX Coast was predicted as the origin for 38% [23-54] and AR was the most likely origin for 9% [0-20] of the geese in our sample (Fig. 2). The predicted winter distribution of birds differed in 2008 (TX Coast: 89% [73-100], LA Coast: 9% [0-27], and AR: 2% [0-9]). Our tri-isotope assessment predicted that none of the geese collected in 2007 (0% [0-13]) and 2008 (0% [0-40]) came from the PLR (Fig. 2). Snow Geese shot in the same Nebraska county came from two or three wintering places.

The assignment analyses based on $\delta^2\text{H}$ and $\delta^{13}\text{C}$ or $\delta^2\text{H}$ and $\delta^{15}\text{N}$ provided similar results to the tri-isotope predictions (Fig. 3). The two-isotope analysis with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ predicted

increased probabilities of origin from AR and PLR, and reduced probabilities from LA and TX Coast, but the most likely origin remained the same compared to the tri-isotope approach. The single-isotope analyses provided more contrasting inferences.

Fig. 3. Predicted winter origin of spring migrating Snow Geese (*Chen caerulescens*) collected in Nebraska's Rainwater Basin in springs of 2007 and 2008. Predictions (%) derived from tri-, dual-, or single-isotope analyses with stable isotopes of hydrogen (H), carbon (C), and nitrogen (N).



DISCUSSION

Our study emphasizes the potential of using stable isotope measurements of multiple elements in Snow Geese muscle tissues to establish migratory connectivity between wintering and spring stopover sites. The combination of stable hydrogen, carbon, and nitrogen isotopic ratios from ground-truthed samples provided a marked segregation of four major wintering areas within the Central and Mississippi flyways. Our likelihood-based inference approach predicted that most migrating Snow Geese that we sampled in the RWB wintered along both the LA and TX Coasts in 2007, but primarily on the TX Coast in 2008. We also found that few Snow Geese came from eastern AR or the Mississippi Alluvial Valley (located between LA and AR; Fig. 2) but none from the PLR.

Although no direct experimental information on elemental turnover rates in Snow Goose muscle is available, turnover rate or mean isotopic residence time scales allometrically with body mass (Carleton and Martínez del Río 2005). Based on body mass and the assumption that muscle turnover rate will be similar to whole blood turnover (see also Haramis et al. 2001), we predicted that isotopic measurements in muscle tissue of Snow Geese easily represented a dietary integration over the previous month. Given that a large number of Snow Geese is present in November in southern Texas (Hobaugh 1984), late October/early November in Arkansas (James and Neal 1986), and early November for the Playa Lake Region (Baar et al. 2008) and southwest Louisiana (Jónsson and Afton 2006), we expect all our wintering birds were in equilibrium with the local diets. After a few-day spring migratory flight, average residency time for waterfowl species in the RWB was estimated, from observation and modeling, to be less than 10 days (Frederikson and Reid 1988, Pearse et al. 2011). Consequently, we are confident that the muscle tissue of the RWB Snow Geese represented their winter habitat and was not affected by the changes in diet and habitat during the early phase of spring migration.

Alisauskas and Hobson (1993) indicated the potential of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses of muscle tissues to characterize winter diets and habitats used by spring staging Snow Geese. In our study, muscle isotopic signatures from $\geq 89\%$ of the winter-shot geese represented their wintering habitat. Depending on abundance and distribution of crops, Snow Geese in the midcontinent population may consume a large variety of food. The observed spatial variation in Snow Goose $\delta^{13}\text{C}$ values is related to the proportion of C_3 , e.g., rice, wheat, and C_4 plants, e.g., corn, sorghum, in diet (Tieszen et al. 1983), whereas higher $\delta^{15}\text{N}$ values in muscle tissues of Snow Geese collected in TX coast and LA generally characterized the estuarine/marsh habitat vs. upland environment in AR and TX Playa (Alisauskas and Hobson 1993). We found that $\delta^2\text{H}$ values provided an additional discrimination of wintering location, with a typical decreasing southeast-northwest pattern expected from the precipitation isoscape for North America (Hobson 1999,

Bowen et al. 2005). The large variation in muscle $\delta^2\text{H}$ values (min: -183; max: -160‰) and the low number of individuals collected in the PLR may explain that the $\delta^2\text{H}$ signature at this site was not significantly different from that in AR. Our tri-isotope analysis enhanced the characterization and segregation of wintering habitats over the use of only one or two isotopes (Fig. 2), and has been shown to improve the assignment of migrating individuals to a geographic wintering region (Royle and Rubenstein 2004). We note that the higher $\delta^2\text{H}$ ($> -115\text{‰}$) and $\delta^{15}\text{N}$ ($\geq 10\text{‰}$) values in two RWB Snow Geese suggest that these individuals may have wintered in marine-influenced marsh areas located south of the TX coast sampling site, i.e., Mexican Gulf coast. We recommend wildlife managers to collect samples from additional areas, including northern and coastal Mexico, Kansas, and Missouri to get a full coverage of the wintering grounds in the Central and Mississippi flyways (Fig. 1). Snow geese may change diet over winter and also rely on several foraging habitats during short periods depending on resource availability, hunting pressure, and climate conditions (Hobaugh 1984, 1985, Hill and Frederick 1997). Thus, sampling should cover both the natural wetlands and agricultural environments within a specific region, e.g., coastal marshes and rice fields in coastal LA and TX (Bellrose 1980). In addition, given the predicted preferential use of the coastal area by migrating Snow Geese, the use of stable sulfur isotopes ($\delta^{34}\text{S}$) may be valuable in further discriminating estuarine and marsh habitats from less ^{34}S enriched terrestrial habitats (Hobson 1999). Monitoring the presence of adventitious color on the head plumage of the geese, which results from feeding in wetlands with deposits of iron oxides, may also provide a good tool to identify Snow Geese that recently used coastal marshes (Alisauskas et al. 1998).

The breeding and wintering range of Snow Geese in North America and links between summer and winter locations had been well established from resighting and recoveries of marked Snow Geese (Cooke et al. 1995, Johnson 1996, Williams et al. 2008). On the other hand, recoveries of spring migrants were too limited to document northward migration with precision, reporting only few recoveries in the RWB (Cooke et al. 1995, Johnson 1996). Ours is the first study to demonstrate the spring migratory connectivity between both LA and TX Coast and the RWB. Although sampling dates and locations in the RWB were similar in both years, our results showed large variations in the potential origins of migrating Snow Geese between springs (Fig. 2). The reasons for this pattern are unclear. We believe our small sample sizes in the RWB may influence overall assignment. Following Thompson (1987), we calculated that a sample size of 510 Snow Geese at the RWB is needed to get predicted assignment probabilities that lie within $\pm 5\%$ of the true probabilities, and 127 individuals with an error margin of $\pm 10\%$ (at a significance level $\alpha = 0.05$). Although snow geese usually exhibit high wintering fidelity (Johnson 1996, Williams et al. 2008), it has

been suggested that rigorous winter conditions in the Mississippi flyway may alter this pattern. In addition, high spring hunting pressure (e.g., Light Goose Conservation Order) may also influence regional movements (Béchet et al. 2003). Besides, interannual deviations in precipitation $\delta^2\text{H}$ patterns (Brewster 2009) can alter $\delta^2\text{H}$ isotopic spatial patterns in vegetation and may thus limit the precision of the assignment approach (Wunder and Norris 2008, but see Hobson 2011); the spatial distributions of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in food webs are likely less prone to unpredictable between-year variations (Bukata and Kyser 2007). We did not detect marked variations in $\delta^2\text{H}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ patterns in LA and TX Coast samples between years; yet, interannual deviance can vary spatially and may require year-specific correction algorithms for assignment (Van Wilgenburg et al. 2012). Thus, we also recommend calibration of the isoscapes of wintering areas using known-origin tissues that were the same tissue type and from the same year as the samples from the RWB geese (Hobson et al. 1999, Wunder and Norris 2008). However, if only spring migrating Snow Geese are collected, our findings indicate that applying the dual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ approach with the current isotopic segregation of wintering grounds would not affect overall assignment predictions.

The distribution and abundance of Snow Geese in the RWB is influenced by natural and anthropogenically caused variations in water conditions, hunting pressure (Vrtiska and Sullivan 2009, Webb et al. 2010), and migration chronology, which in many species is related to spring environmental variables, e.g., climatic conditions, food availability (Jonzén et al. 2006, Bridge et al. 2010, Studds and Marra 2011). Additional sampling of birds in the western RWB wetlands is needed to assess spatial distribution of geese in the RWB according to their wintering areas; heterogeneous spatial distribution in the RWB of waterfowl from diverse wintering areas was previously reported (Pearse et al. 2011). Snow Geese populations in the western RWB may be comprised of more birds from the PLR and we fully expect this technique to be able to detect such differences. Expanding our study to additional wintering areas would allow RWB managers to obtain better information of the origin of migrating Snow Geese and adjust the timing of spring harvests to target subpopulations from different wintering areas. Combining origin information with winter census would also provide information on the number of Snow Geese that are expected to use the RWB wetlands each spring, so that adequate food resources and space may be provided. Snow Geese pose a significant threat of an avian cholera outbreak because of its potential role as carrier of the disease bacteria *Pasteurella multocida* (Samuel et al. 2005). This infectious disease is one of the most important affecting North American waterfowl and has caused tens of thousands of bird deaths in some years in wintering places in the Mississippi and Central flyways (Samuel et al. 2007) and in the RWB (Blanchong et al. 2006).

Therefore, a better understanding of patterns of migratory connectivity may inform decisions made in future years to predict the most likely route of disease spread and prevent disease occurrence and transmission; for example, with preventive habitat management in the RWB or winter culls if infectious pathogens circulate in wintering Snow Goose populations predicted to migrate through the RWB.

Our isotopic approach to delineating population origins of spring migrant Snow Geese at RWB can be easily applied to management and conservation programs in other flyways if unique regional isotopic signatures corresponding to wintering locations can be established. The study of movements between wintering grounds and other spring locations could outline the overall connectivity network of the Snow Goose population. Isotopic analyses of both muscle and feather tissues could delineate spatial connectivity at large scales, with the ability to infer both wintering and breeding origins (e.g., Yerkes et al. 2008). For studies establishing migratory connectivity of endangered species, the use of cellular blood, which exhibits a temporal integration of elements similar to muscle (Hobson and Clark 1993), would be a nondestructive approach to identify previous origins of migratory individuals. Combining isotopic analyses with body condition or diet studies facilitates the detection of key habitats, as in the American Redstart *Setophaga ruticilla* (Marra et al. 1998), improve our knowledge of waterfowl population requirements, and favor the restoration of critical environments.

Better understanding of the connectivity between breeding, wintering, and migration areas is critical to inform the management strategies of the continental Snow Goose population in North America. Our findings suggest it is possible to discriminate wintering locations of migratory birds at a useful spatial scale within a species' winter range. Our study highlighted the connectivity between LA and TX Gulf Coasts and the eastern part of the RWB. Such assessments show the potential of our methods to inform habitat, harvest, and disease management of Snow Geese and other migratory birds.

Responses to this article can be read online at:
<http://www.ace-eco.org/vol7/iss1/art5/responses/>

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