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GUILLERMO E. PÉREZ[1], KEITH A. HOBSON[2, *], ELENA J. GARDE[1] AND MARTIN GILBERT[3]

[1]Veterinarians Without Borders-Canada, Casilla 609, Valdivia, Chile

[2]Environment Canada, 11 Innovation Boulevard, Saskatoon, SK, S7N 3H5, Canada

[3]Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, New York 10460, USA

*Corresponding author; E-mail: Keith.Hobson@ec.gc.ca

Abstract.—The need for better understanding of migratory movements of wild birds in Asia promoted an evaluation of the usefulness of deuterium in feathers (δD_f) to assign origins. Feathers were sampled from Bar-headed Geese (*Anser indicus*), Whooper Swans (*Cygnus cygnus*), Mongolian Gulls (*Larus vegae mongolicus*), Curlew Sandpipers (*Calidris ferruginea*) and Pacific Golden Plover (*Pluvialis fulva*) in north-central Mongolia, from June to September 2007. Univariate statistical analyses were performed to test for differences between study sites for all species and between growing (blood) and previously grown (non-blood) feathers only for Bar-headed Geese. Values of δD_f in actively growing feathers generally agreed with those expected from integrated isotopic signals in precipitation expected for sampling sites. Values of δD_f from adult migrant birds also indicated varying degrees of movement from north to south expected from the movement of these species in Asia. These results show promise for the isotope approach for establishing origins of molt of migratory waterbirds in Asia in a cost-effective manner without the need for mark and recapture. Projects required to track movements of waterbirds in Asia could benefit by incorporating this approach into study design. However, greater refinement of the δD_f isoscape for Asia is now needed. *Received 18 January 2010, accepted 27 June 2010.*

Key words.—Asia, deuterium, migration, stable isotopes, waterbirds.

Understanding connections between breeding, wintering and stopover sites of wild birds is important for conservation and understanding the evolution of migratory patterns (Webster and Marra 2005). However, tracking migratory movements in birds is not simple, since no one tracking device or technique is without limitations. For example, banding results are typically biased toward regions with high mark-recapture and band return effort (Pollock and Raveling 1982; Hobson *et al.* 2004), and require years of data collection to make strong inferences (Hobson 2003). Geolocators and radio and satellite telemetry are useful for tracking precise migratory movements of individuals (e.g. Combreau *et al.* 1999; Hiroyoshi and Pierre 2005; Stutchbury *et al.* 2009) but may often prevent extrapolations beyond individuals due to typically small sample sizes and may alter the behavior of individuals equipped with these devices (Barron *et al.* 2010). Intrinsic biogeochemical markers such as stable isotopes offer a complementary option to more conventional techniques because they allow ecological and biological inferences from every animal sampled, do not require recapture of a previously marked individual, do not encumber individuals with equipment that may alter their behavior and are not biased spatially by the initial captured sample (West *et al.* 2006; Hobson 2008).

The stable isotope technique is based on the premise that animal tissues have isotopic values in equilibrium with local food webs that in turn vary across continents and landscapes in a predictable manner (Hobson 2008). By matching feather isotope values with those expected based on known isotopic patterns in foodwebs or "isoscapes" the probability of origin of individual birds can be inferred. Feathers are particularly useful isotopic recorders, because they generally grow continuously over several weeks (Grubb 1989) and thus reflect integrated isotopic values of foodwebs at discrete locations. Additionally, they retain this information until the next molt period. The isotopic approach has been used successfully in

North America and Europe (reviewed in Hobson 2008) but similar investigations are lacking in Asia (but see Chang *et al.* 2008) despite considerable interest in emerging conservation and disease surveillance issues involving waterbirds in that region. The focus of this study was to assess the use of feather deuterium values as a retrospective tracking tool for five migratory Asian wild waterbirds as they migrated through and/or molted their feathers in northern Mongolia.

METHODS

Field sampling took place at seven sites (Table 1) in northern Mongolia over 35 sampling dates from 17 June to 24 September, 2007 (also see Wildlife Conservation Society 2007). Molting geese and swans were located at night with spotlights, and then captured from a boat using either nets (geese) or hooks (swans). Shorebirds were captured at night using mist nets. Most other species were incidental captures during the course of swan/goose captures. Following capture, birds were restrained in cloth bags or by using swan jackets (Redfern and Clark 2001) until sampling. All birds were released within one hour of capture and most within 30 min. Feathers were collected opportunistically and were not identified to feather tract for most species. Feathers from adult geese and swans were usually limited to contour feathers (scapular, back or mantle), and occasionally rectrices, inner secondaries or tertials were collected if shed during processing. Scapular feathers were collected from adult Pacific Golden Plover (*Pluvialis fulva*) and hatch-year (HY) Curlew Sandpipers (*Calidris ferruginea*). Rectrices, inner secondaries or tertials were collected from HY Mongolian Gulls (*Larus vegae mongolicus*) at their natal colony. Birds were aged and sexed using either plumage characteristics, or in the case of Anseriformes, through cloacal examination and extrusion of genitalia (Tully *et al.* 2000). Feather samples were stored in paper envelopes and labelled with a unique identifier.

Stable Isotope Analysis

Preparation and stable-hydrogen isotope (δD) analysis of one feather per individual were conducted at the stable isotope facility at the National Hydrology Research Centre, Saskatoon, Saskatchewan, Canada. When a newly-grown feather showed signs of vascularity (e.g. having blood; hereinafter referred to as "blood feather"), we selected the vane portion of this feather for analysis since it would likely provide us with the "local" feather isotopic signal; otherwise, we randomly selected a feather from the collection envelope. Surface oils were removed from feathers by overnight soaking in a 2:1 chloroform:methanol mixture, followed by draining and placing in a fume hood until completely dried. Samples of vane were then cut and 350 ± 10 µg were weighed into 4.0×3.2 mm silver capsules. Isotope analyses of feathers were completed following the comparative equilibration technique described in Wassenaar and Hobson (2003). Using continuous-flow isotope-ratio mass spectrometry (CF-IRMS), samples were pyrolized to H_2 gas. Deuterium isotope ratios were expressed in delta (δ) notation in parts per thousand (‰) relative to the Vienna Standard Mean Ocean Water Standard Light Antarctic Precipitation (VSMOW-SLAP) scale. Measurement precision based on replicate measurements of within-run keratin standards was estimated to be of the order of ±2‰.

Molting Location

Estimates of continental deuterium patterns in mean growing-season precipitation (δD_p) for Asia were derived from Bowen 2009 (www.waterisotopes.org). These data were collected primarily from the International Atomic Energy Agency-Global Network for Isotopes in Precipitation (IAEA-GNIP) for ca. the last 45 y. Continental δD_p data show an expected general decrease in deuterium abundance in precipitation with increasing latitude and altitude in Asia and form the basis for predicting the expected abundance of deuterium in feathers (δD_f) from the average δD_p (i.e. Hobson and Wassenaar 1997; Hobson 2008). Individuals of unknown origin were assigned to a general geographical molt location using expected (mean growing season) δD_p values for Asia and an assumed discrimination factor of -28‰ between δD_p and δD_f based on that calculated for North American waterfowl (Clark *et al.* 2006).

Table 1. Global positioning, expected mean annual and monsoon month δD_p and 95% CI (annual) values in precipitation in seven Global Avian Influenza Network for Surveillance (GAINS) sampling sites in north-central Mongolia. Values of δD_p were obtained using the isotope calculator in www.waterisotopes.org. Approximate elevations were obtained using Google Earth (*http://earth.google.com/*). Latitude and longitude are expressed in decimal degrees (DD).

Site location	Approx. Elevation (m)	Latitude (DD)	Longitude (DD)	δD (‰, V-SMOW)	δD 95% CI (‰)	δD (July -August)
Darkhad Valley	1530	51.20	99.41	-99	6	-87
Erhel Nuur	1540	49.97	99.911	-92	7	-84
Ogii Nuur	1700	47.36	102.81	-83	7	-75
Sangiyn Dalai Nuur	1700	49.26	99.07	-93	7	-84
Sharga Nuur	1330	48.94	101.97	-84	6	-73
Tsegeen Nuur	1520	49.10	101.86	-87	6	-75
Tsengel Nuur	2370	49.77	101.01	-102	8	-88

The application of a fixed discrimination factor is more appropriately replaced with error propagation and probabilistic techniques using actual feather-precipitation calibration algorithms derived for taxa and location of interest (Wunder and Norris 2008; Wunder 2010), but such a relationship has not been established for waterbirds in Asia. So, the use of an approximate precipitation to feather δD discrimination value based on the North American data was deemed appropriate. Three major feather isotopic source areas in Asia (Fig. 1): Region A—northern Asia (<-138‰), B—catchment area (-138 to -104‰) and C—southern Asia (>-104‰) were delineated. The isotopic interval of 34‰ presented for Region B corresponded to latitudes ranging from approximately northern China to mid-Russia, and Regions A and C were north and south of those latitudes, respectively. These arbitrary cut-off values in δD_f were chosen because they bracketed Region B between the lowest and highest 95% CI out of the expected mean annual δD_p values for the latitudes, longitudes and altitudes of the sampling sites (Table 1).

Statistical Analysis

Frequency distributions of δD_f by species were used to examine the overall range of isotopic values and potential multi-modal patterns of locations in which feathers could have been grown. A one-sample Kolmogorov-Smirnov test (Sokal and Rohlf 1995) was used to assess normality in δD_f values and an independent Student's t-test was used to examine potential differences in δD_f between genders, blood vs. non-blood feather, and among study sites. To test for effects of gender, and study site differences in δD_f, a two-way ANOVA was used for Whooper Swan (sampled in multiple locations) and a one-way ANOVA for blood feathers of Bar-headed Goose sampled in Darkhad Valley. In cases where non-blood feathers were not identified to feather tract, they

were not investigated further statistically because their isotopic value could not be interpreted in terms of origin of molt.

RESULTS

Five waterbird species were sampled across seven sampling sites in north-central Mongolia (Table 1). Blood feathers were identified from Bar-headed Goose (*Anser indicus*) and Whooper Swan (*Cygnus cygnus*). All distributions of δD_f values for all datasets within each species were normally distributed (blood feathers from Bar-headed Goose: Z = 0.8, P = 0.5, n = 28; all feathers of Whooper Swan: Z = 1.3, P = 0.08, n = 120; Curlew Sandpiper: Z = 0.5, P = 0.9, n = 7; Pacific Golden Plover: Z = 0.6, P = 0.9, n = 14; Mongolian Gull: Z = 0.7, P = 0.7, n = 42). Two δD_f values from Bar-headed Goose blood-feathers were identified as outliers because they were too enriched (-9‰ and -70‰) from those expected for the study areas (all other values were < -103‰), and were consequently removed from further analyses. In contrast to results for Bar-headed Geese ($T_{112.5}$ = -10.46, P < 0.001), no significant differences in the δD_f values of blood and non-blood feathers in the Whooper Swan sample were found and so they were combined ($T_{50.3}$ = 1.8, P = 0.07). Bar-headed Goose blood feathers were collected primarily in Darkhad Valley (94% of cases) and so no comparisons among sampling sites were possible for this species. Site differences in δD_f values were found between the five sites where Whooper Swan were sampled ($F_{4,119}$ = 15.2, P < 0.001). Tukey's post-hoc analyses revealed two distinct groups of sampling areas that differed in their δD_f values: Sangiyn Dalai Nuur, Sharga Nuur, Tsegeen Nuur and Tsengel Nuur did not differ from each other; however, Darkhad Valley differed from all other sampling areas with lower δD_f values. Gender differences were not found in δD_f values within any study site for the Whooper Swan ($F_{1,119}$ = 0.14, P = 0.71), nor blood feathers of Bar-headed Goose sampled in Darkhad Valley ($F_{1,27}$ = 2.6, P = 0.12). Placements of individuals per species and study site based on δD_f values are presented in Table 2.
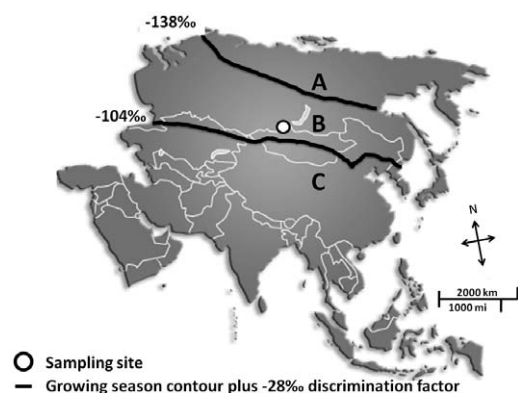

Figure 1. Thick black lines depict the assumed δD_f isoclines for Asia based on an assumed -28‰ precipitation to feather δD discrimination factor (Clark *et al.* 2006) added to mean annual δD_p values provided by Bowen *et al.* (2005). Region A depicts an area of δD_f values expected for feathers grown at northern latitudes (< -138‰). Region B depicts an area of δD_f expected for the study area (-104‰ to -138‰) and Region C for southern molting grounds (> -104‰).

Table 2. Proportion (%) of birds that molted their sampled feather within isotopic Region A, B, or C. Depiction of isotopic regions is found in Fig. 1.

Species	N	Regions A	B	C	mean ± SD (‰)
Bar-headed Geese					
Darkhad Valley					
Blood feather	28	18	82	0	-130 ± 10
Non-blood feather	52	5	33	62	-81 ± 41
Erhel Nuur					
Blood feather	1	0	100	0	-116
Sharga Nuur					
Blood feather	1	0	0	100^	-103
Non-blood feather	24	0	25	75	-85 ± 29
Sangiyn Dalai Nuur*	11	0	36	64	-76 ± 37
Whooper Swan					
Sangiyn Dalai Nuur*	23	9	48	43	-111 ± 17
Darkhad Valley					
Blood feather	3	25	75	0	-135 ± 17
Non-blood feather	31	63	20	17	-140 ± 28
Sharga Nuur					
Blood feather	15	0	80	20	-111 ± 11
Non-blood feather	29	3	59	38	-108 ± 23
Tsegeen Nuur					
Blood feather	3	0	0	100	-99 ± 3
Non-blood feather	1	0	0	100	-83
Tsengel Nuur					
Blood feather	4	0	25	75	-91 ± 35
Non-blood feather	11	8	58	34	-112 ± 20
Curlew Sandpiper*					
Ogii Nuur	7	0	100	0	-124 ± 7
Pacific Golden Plover*					
Ogii Nuur	14	0	0	100	-33 ± 18
Mongolian Gull					
Erhel Nuur					
Blood feather	14	0	0	100	-71 ± 7
Non-blood feather	25	0	0	100	-67 ± 8
Sangiyn Dalai Nuur*	3	0	0	100	-71 ± 7

*non-blood feathers only.
^value of -103‰; right below the cut-off between regions B and C.

DISCUSSION

Our approach to assigning birds to origin in Asia was conservative since we chose three general broad regions of origin based on ranges in expected δD_f values. Once more precise information on the relationship between δD_p and δD_f can be established for Asia, more refined assignment will be possible (e.g. Hobson *et al.* 2009). Adult blood feathers sampled in Region B had δD_f values (mean = -122 ± 15‰) within the range expected from the long-term GNIP dataset and this provided validation for our general approach.

Adult Pacific Golden Plover δD_f values from scapular feathers demonstrated the longest migratory movements of all species sampled, because this species is known to breed in Siberia (Johnson and Connors 1996) and the mean δD_f value of -33‰ clearly indicated that these feathers were molted in southernmost Asian latitudes or Oceania (Johnson and Connors 1996). Similarly, Rocque *et al.* 2006 sampled Pacific Golden Plover (*Pluvialis fulva*) feathers molted on the wintering grounds and found even more positive δD_f values (mean = -24‰) than ours. Our results suggest that scapular feathers in Pacific Golden Plovers were likely grown on or near the wintering grounds prior to spring migration (but see Larson and Hobson 2009).

Locally grown feathers from adult Barheaded Geese and Whooper Swans sampled in the Darkhad Valley had more depleted δD_f values than any other group. Therefore, we reasoned that because the Darkhad Valley consists of a complex of lakes and ponds fed by high elevation runoff, these waters were more depleted in deuterium than adjacent areas outside this valley.

All Curlew Sandpipers captured were juveniles sampled during the southward migration; therefore our expectation was that these birds grew their scapular feathers between their breeding grounds in the tundra of the high Arctic and the study site. All scapular feathers from juvenile Curlew Sandpiper had δD_f values consistent with Region B indicating isotopic equilibrium with diet on their sampling area. In the absence of information on juvenile molt, we reasoned that because none of the birds had δD_f values representative of their breeding area, scapular feathers were grown during the first prebasic molt on their staging area in northern Mongolia.

An unexpected result was that δD_f values from juvenile Mongolian Gulls (mean δD_f = -68‰) did not match those expected for their natal sites, and instead were more consistent with δD_f values south of Mongolia. A review of the monthly patterns of δD_p throughout the sampling areas showed that precipitation is typically more enriched (mean = -84‰) during the monsoonal months (July and August), and so differential contributions of such enriched precipitation to local food webs may have biased inferences of origin to more southern areas. Alternatively, it is possible that juvenile birds show greater enrichment in feather deuterium than adults (Hobson, unpub. data).

Our study has demonstrated that stable isotopes can be used successfully to advance our knowledge of migratory and molting patterns of wild waterbirds in Asia in a rapid and cost effective manner. However, we recognize the need for additional studies. For example, given that the δD_p isoscape for Asia is poorly described and our sampling protocol was not standardized, we encourage future research to better elucidate these patterns for this part of the world (Chang *et al.* 2008). Ground-truthing the relationship between δD_f and δD_p over a large geographic gradient in Asia will allow propagation of error and appropriate isotopic calibrations (Wunder and Norris 2008; Wunder 2010). Similarly, a better understanding of basic molt phenology for most species can be improved, so that intra-specific variability can be accounted for. We recommend that future studies of migratory connectivity using isotopes collect feathers with strong *a priori* expectations of the geographical location of molt and evaluate the potential for molt migrations away from breeding sites (Larson and Hobson 2009). Lastly, we recommend the development of a feather collection protocol to standardize sampling efforts and facilitate isotopic interpretations, the development of an Asian δD_f base map, and the exploration of which rainfall or water source contributes most to regional foodweb δD values in Asia.

LITERATURE CITED

Barron, D. G., J. D. Brawn and P. J. Weatherhead. 2010. Meta-analysis of transmitter effects on avian behaviour and ecology. Methods in Ecology and Evolution 1: 180-197.

Bowen, G. J. 2009. The Online Isotopes in Precipitation Calculator, version 2.2. http://www.waterisotopes.org, accessed 30 January 2009.

Bowen, G. J., L. I. Wassenaar and K. A. Hobson. 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143: 337-348.

Chang, Y., K. A. Hatch, T. Ding, D. L. Eggett, H. Yuan and B. L. Roeder. 2008. Using stable isotopes to unravel and predict the origins of Great Cormorants (*Phalacrocorax carbo sinensis*) overwintering at Kinmen. Rapid Communications in Mass Spectrometry 22: 1235-1244.

Clark, R. G., K. A. Hobson and L. I. Wassenaar. 2006. Geographic variation in the isotopic (δD, δ^{13}C, δ^{15}N, δ^{34}S) composition of feather and claws from Lesser Scaup and Northern Pintail: implications for studies of migratory connectivity. Canadian Journal of Zoology 84: 1395-1401.

Combreau, O., F. Launay, M. A. Bwardi and B. Gubin. 1999. Outward migration of Houbara Bustards from two breeding areas in Kazakhstan. Condor 101: 159-164.

Grubb, Jr. T. 1989. Ptilochronology: feather growth bars as indicators of nutritional status. Auk 106: 314-320.

Hiroyoshi, H. and J. P. Pierre. 2005. Satellite tracking and avian conservation in Asia. Landscape Ecological Engineering 1: 33-42.

Hobson, K. A. 2003. Making migratory connections with stable isotopes. Pages 379-391 *in* Avian Migration (P. Berthold, E. Gwinner and E. Sonnenschein, Eds.). Springer, Berlin, Germany.

Hobson, K. A. 2008. Isotopic methods to track animal movements. Pages 45-78 *in* Tracking Animal Migration with Stable Isotopes (K. A. Hobson and L. I. Wassenar, Eds.). Elsevier, Oxford, UK.

Hobson, K. A., G. J. Bowen, L. I. Wassenaar, Y. Ferrand and H. Lormee. 2004. Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. Oecologia 141: 477-488.

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., M. B. Wunder, S. L. Van Wilgenburg, R. G. Clark and L. I. Wassenaar. 2009. A method for investigating population declines of migratory birds using stable isotopes: Origins of harvested Lesser Scaup in North America. PLoS ONE 4: e7915. doi:10.1371/journal.pone.0007915.

Johnson, O. W. and P. G. Connors. 1996. Pacific Golden-Plover (*Pluvialis fulva*). *In* The Birds of North America. (A. Poole and F. Gill, Eds.). The Birds of North America, Inc., Philadelphia, Pennsylvania.

Larson, K. and K. A. Hobson. 2009. Assignment to breeding and wintering grounds using stable isotopes: a comment on lessons learned by Rocque *et al.* Journal of Ornithology 150: 709-712.

Pollock, K. H. and D. G. Raveling. 1982. Assumptions of modern band-recovery model, with emphasis on heterogeneous survival rates. Journal of Wildlife Management 46: 88-98.

Redfern, C. P. F. and J. A. Clark. 2001. Ringers' Manual. British Trust for Ornithology, Thetford, UK.

Rocque, D. A., M. Ben-David, R. P. Barry and K. Winker. 2006. Assigning birds to wintering and breeding grounds using stable isotopes: lessons from two feather generations among three intercontinental migrants. Journal of Ornithology 147: 395-404.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry, 3rd ed. W. H. Freeman, New York, New York.

Stutchbury, B. J. M., S. A. Tarof, T. Done, E. Gow, P. M. Kramer, J. Tautin, J. W. Fox and V. Afanasyev. 2009. Tracking long-distance songbird migration using geolocators. Science 323: 896.

Tully, T. N., M. P. C. Lawton and G. M. Dorrestein. 2000. Avian Medicine, Elsevier Health Sciences, Oxford, UK.

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.

Webster, M. S. and P. P. Marra. 2005. The importance of understanding migratory connectivity and seasonal interactions: the ecology and evolution of migration. Pages 199-209 *in* Birds of Two Worlds (R. Greenberg and P. P. Marra, Eds.). Smithsonian Institution, Maryland, USA.

West, J. B., G. J. Bowen, T. E. Cerling and J. R. Ehleringer. 2006. Stable isotopes as one of nature's ecological recorders. Trends in Ecology and Evolution 21: 408-414.

Wildlife Conservation Society. 2007. Disease Surveillance: Avian Influenza in Migratory Birds in Mongolia—Results of Extensive Surveys—2006. Ulaanbaatar, Mongolia.

Wunder, M. B. 2010. Using isoscapes to model probability surfaces for determining geographical origins. Pages 251-272 *in* Isoscapes: Understanding Movements, Pattern, and Process on Earth through Isotope Mapping (J. B. West, G. J. Bowen, T. E. Dawson and K. P. Tu, Eds.). Springer, New York, New York.

Wunder, M. B. and D. R. Norris. 2008. Analysis and design of isotope-based studies of migratory animals. Pages 107-128 *in* Tracking Animal Migration with Stable Isotopes. (K. A. Hobson and L. I. Wassenaar, Eds.). Elsevier, Oxford, UK.