Stable-Hydrogen Isotope Analysis (δD) of Asian Wild Aquatic Bird Feathers Reveal Migratory Movements

Project Report for the Wildlife Conservation Society

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EXECUTIVE SUMMARY

Understanding migratory movements and connectivity in wild birds is of great theoretical and practical importance, especially in context of their role in the spread and establishment of diseases such as avian influenza. Conventional means of tracking birds with exogenous markers is not appropriate to this investigation due to the chance encounter of unmarked individuals. To identify the approximate origins of 27 wild bird species sampled for avian influenza in northern Mongolia in 2007, we used stable-hydrogen isotope analysis of feathers and claws. This approach has met with success in North America. Based on isoscapes of mean growing season patterns of precipitation δD in Asia, we inferred approximate latitudes for each individual based on an algorithm developed for avifauna in North America.

The objectives of this study were the following:

1.-To assess the application of measurements of deuterium in feathers (δD_f) and claws (δD_c) as a tracking tool for migratory wild aquatic birds throughout Asia.

2.- To uncover migratory movement patterns and general origins of wild aquatic birds sampled in Mongolia, in order to identify potential flyways that may play a role in routes for disease dissemination.

Field sampling took place at seven Global Avian Influenza Network for Surveillance (GAINS) sites in north-central Mongolia, from June to September 2007. Capture techniques included spotlighting, mist netting and opportunistic finding of dead, injured or sick birds. Feathers and claws were selected for isotopic analyses. Univariate statistical analyses were performed to test for differences between study sites, age and genders.

In general, δD_f values ranged widely in our sample encompassing the entire range of isotopic values expected for Asia, from northernmost to southernmost latitudes. The wide distribution in δD_f values

showed that, without exception, all species analyzed statistically had isotopic signatures outside of those expected for the catchment areas, indicating varying degrees of movement. We identified expected δD_f values for regions of Asia and compared these to their actual δD_f of those feathers collected. Through this approach we found that all Pacific Golden Plovers, Mongolian Gulls and 82% of Swan Geese individuals had δD_f values indicative of latitudes south of southern Mongolia, while 76% of Whooper Swans sampled in Darkhad Valley, 77% of Bar-headed Geese (blood feathers) and 86% of Curlew Sandpipers had isotopic signatures indicative of north of northern Mongolia. Most results were compatible with our current understanding of the species range, and for those that were not, the results warrant further investigation because they could be suggestive of unreported geographic ranges of a species, or be further explained by species-specific physiology and moult patterns, regional weather patterns or environmental influences.

Based on the results found in this study, our recommendations include: 1) development of a feather collection protocol to standardize sampling efforts and facilitate isotopic interpretations, 2) development of an Asian δD base map for feathers to establish a ground tested isotopic template for Asia, and 3) explore δD values of other water sources such as ground water and run-off, particularly around sampling sites, to investigate potential variability between δD abundance in precipitation and surface waters.

The stable isotope technology used in this study proved useful as a resource to unravel migratory movements of wild aquatic birds of Asia. The results presented herein may also be beneficial for other researchers interested in expanding the application of this technology to include studies on bird migration, disease dissemination and illegal wildlife trade in new regions and species in Asia.

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INTRODUCTION

Wild aquatic birds are widely thought to play a role as global disseminators of avian influenza viruses because they are natural reservoirs of non lethal (low pathogenic) forms and many species fly long-distances from breeding to wintering grounds (reviewed in Olsen *et al.* 2006). Asia is presently a global hotspot for cases of avian influenza virus in humans and poultry, and although new laboratory evidence suggests that particular bird species can act as asymptomatic carriers (Keawcharoen *et al.* 2008), the precise role of wild birds in the ecology of avian influenza outbreaks is unknown mainly due to a paucity of information regarding migratory bird movements and a poor understanding of virus transfer from wild birds to domestic poultry and vice versa (Feare and Yasué 2006). Most of the migratory tracking work done on birds in Asia is biased towards satellite telemetry done on a few larger species (e.g., Combreau *et al.* 1999; Hiroyoshi and Pierre 2005), therefore population-level inferences are lacking. Examining the movements of and viral prevalence in wild aquatic birds in Asia would form an essential building block for a clearer understanding of disease outbreaks.

For decades, extrinsic markers such as leg bands and neck collars have been used to monitor movements of various bird species (Dunn and Hussell 1995), but their application requires considerable effort and cost relative to the volume of data retrieved (Hobson 2003) and results are geographically biased towards the site of marking and recapture (Hobson et al. 2004). Radio and satellite telemetry are useful for tracking precise migratory movements but are only available for large-sized birds and incur high costs of tracking equipment and time (Hobson 2003). Given the limitations of these conventional tracking techniques and devices, scientists are now turning to intrinsic biogeochemical markers such as stable isotopes, which is a relatively new non lethal approach that allows one to draw ecological and biological inferences from every animal sampled (West *et al.* 2006). This approach is based on the fact that animal tissues have isotopic signatures, which reflect those in the food webs they equilibrate with and that isotopic patterns across continents have structure that can be used to infer origins of animals that move among such isotopically distinct food webs. Feathers are particularly useful recorders of moult origins of birds because they provide a metabolically inert isotopic record of food webs experienced during the feather growth period (Hobson 2008). A milestone in the application of this technology to tracking movements of migratory animals, however, came with the realization that continent-wide patterns in the abundance of stable-hydrogen isotopes (deuterium) in rainfall in North America were transferred through the food webs to consumers, making it possible to track movements across latitudinal gradients (Hobson and Wassenaar 1997). Since then, the wildlife scientific community has increasingly employed the technology, especially in migratory connectivity studies in North America (Webster *et al.* 2002; Hobson 2005), Europe (Hobson *et al.* 2004), Africa (Yohannes *et al.* 2007) and potentially South America (Pérez *et al.* 2008). Furthermore, stable isotopes are now beginning to be applied in disease surveillance (Chang *et al.* 2008), microbial and wildlife forensics (Bowen *et al.* 2005; Kreuzer-Martin and Jarman 2007), and illegal wildlife trade (Cerling *et al.* 2007).

This study forms part of a larger avian influenza program in Mongolia led by the Wildlife Conservation Society with the greater goal of providing insight into the movement patterns and general origins of wild aquatic birds sampled for avian influenza viruses. Specifically, the focus of this part of the study was to assess the application of feather and claw deuterium measurements as a tracking tool for migratory wild aquatic birds throughout Asia.

Caveat

Tracking long-distance movements of birds using stable-hydrogen isotope measurements relies fundamentally on the presence of continental stable-hydrogen isotope patterns, and a known relationship between the abundance of deuterium in feathers (δD_f) of known origin, and the long term average precipitation (δD_p) at a given location (Hobson and Wassenaar 1997; Kelly and Finch 1998;

Hobson *et al.* 2004). Fortunately, estimates of continental deuterium patterns in precipitation are available, because the International Atomic Energy Agency-Global Network for Isotopes in Precipitation (IAEA-GNIP) has been collecting isotope in precipitation data for ~40 years (Bowen *et al.* 2005) and these data show a general decrease in deuterium abundance with increasing latitude and altitude in Asia. Although some isotopic patterns have been investigated in areas in Asia (Araguás-Araguás *et al.* 1998; Vuille *et al.* 2005; Yamanaka *et al*. 2007), they have not yet been applied to wildlife tracking or forensics, and so a tissue-precipitation relationship has not been established. This relationship is important because the natural process of feather formation from the elemental form of hydrogen available as water in food webs changes (discriminates) as it is ultimately assimilated into feathers (Hobson *et al.* 2004). This forms the basis for predicting the expected deuterium abundance in feathers from the average abundance of deuterium in precipitation. In North America for example, various discrimination factors have been reported; including, -25‰ for songbirds (Hobson and Wassenaar 1997); -34‰ for Cooper's Hawks (Meehan *et al.* 2001), -27‰ for Red-winged Blackbirds (Wassenaar and Hobson 2000), and -28‰ for Lesser Scaups (Clark *et al.* 2006). The application of a fixed discrimination factor is more appropriately replaced with error propagation and probabilistic techniques based on actual algorithms derived for taxa of interest (Wunder and Norris 2008), but such a relationship has not been established for waterbirds in Asia necessitating the use of the application of an approximate value based on the North American data.

METHODOLOGY

Field Sampling

Collection site and date

Field sampling took place at 7 Global Avian Influenza Network for Surveillance (GAINS) sites (Figure 1 and Table 1) during 35 sampling dates from June 17TH to September 24TH, 2007 in northern Mongolia during routine avian influenza disease surveys. For more information on avian influenza surveys in Mongolia, please refer to (Gilbert 2007) or visit www.gains.org.

Capture techniques

Capture techniques included spotlighting, mist netting and opportunistic finding of dead, injured or sick birds. Following capture, birds were restrained in bags or using swan jackets (as appropriate for species) until sampling. All birds were released within one hour of capture and most appreciably less. Moulting Shelduck geese and swans were located at night with spotlights, and then captured from a boat using either nets (shelducks and 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; or in the case of cormorants, by approaching on foot with a net.

Sample collection

In general, collected feathers were unidentified for most species. Feathers collected from ducks, geese and swans were usually limited to contour feathers (scapular, back or mantle), and occasionally rectrices or inner secondaries (tertials) were collected if shed during processing. The claw with the least wear, usually the middle toe, was clipped and collected. Mass (g), wing chord length (mm), and short tarsus length (mm) (Redfern and Clark 2001) were recorded from most birds. Birds were aged and sexed using either plumage characteristics, or in the case of Anseriformes through cloacal examination and extrusion of genitalia. Feather and claw samples were stored in paper envelopes and plastic vials, respectively, and labelled with a unique identifier. Samples were then shipped from Mongolia to Canada for stable isotope analysis.

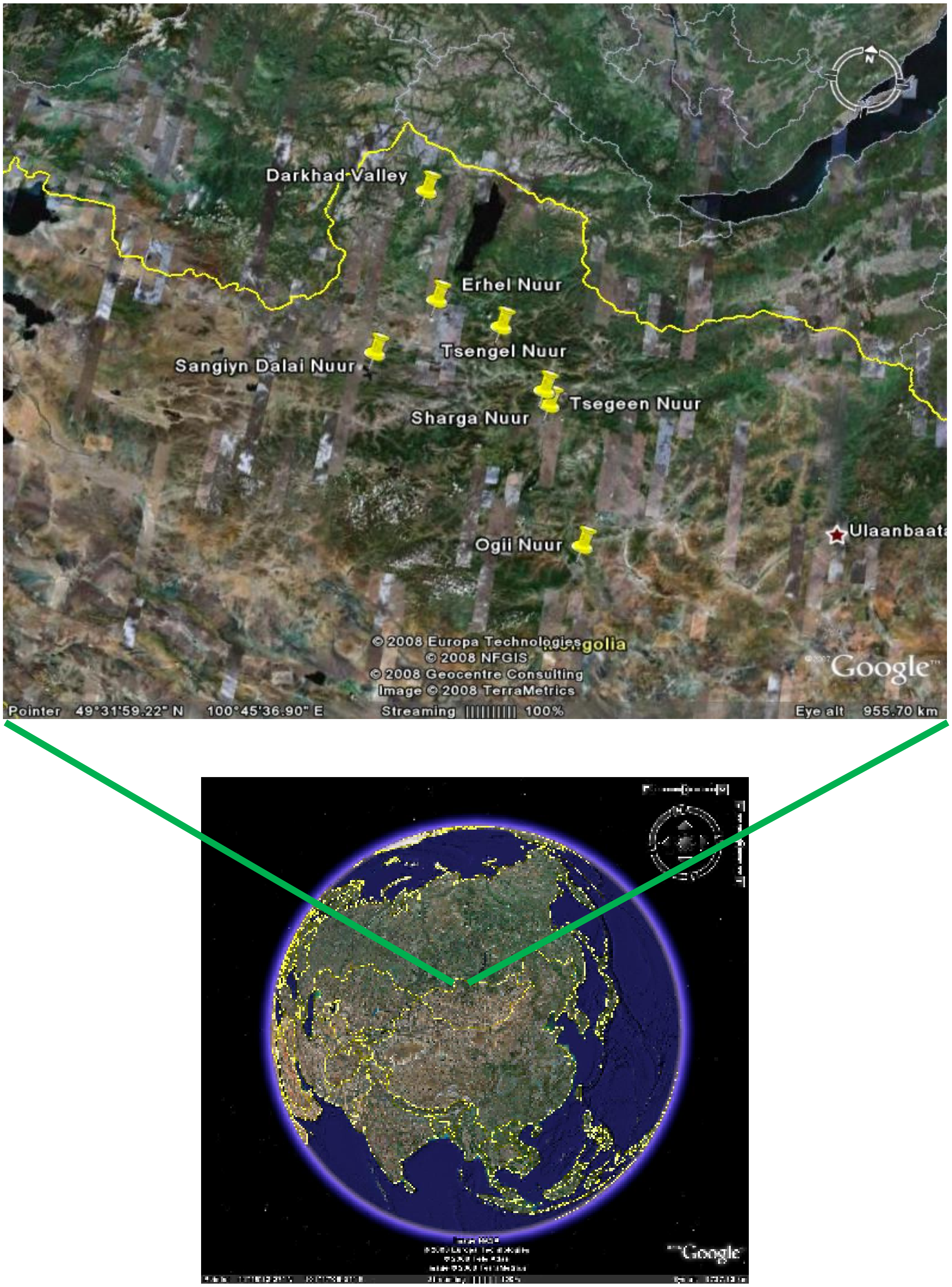

Figure 1. Location of the 7 sampling sites in north-central Mongolia.

Table 1. Global positioning of the 7 sampling sites, in north-central Mongolia.

Site location	Extended name of location	Latitude	Longitude
Darkhad Valley	Darkhad Valley Renchinlhumbe soum, Khovsgol aimag	51.19736	99.41078
Erhel Nuur	Erhel Nuur, Alag Erdene soum, Khovsgol aimag	49.96677	99.90592
Ogii Nuur	Ogii Nuur, Ogiinuur soum, Arkhangai aimag	47.36325	102.8113
Sangiyn Dalai Nuur	Sangiyn Dalai Nuur, Burentogtoh soum, Khovsgol aimag	49.26023	99.0688
Sharga Nuur	Sharga Nuur, Bayan Agt soum, Bulgan aimag	48.94365	101.9703
Tsegeen Nuur	Tsegeen Nuur, Bayan Agt soum, Bulgan aimag	49.10107	101.8607
Tsengel Nuur	Tsengel Nuur, Tunel soum, Khovsgol aimag	49.76619	101.0126

Plastic-handled Dacron swabs were used to collect samples for avian influenza screening. Two swabs were collected from the cloaca and the trachea (or with smaller species the oropharynx), with one stored in viral transport media and the other in a lysis buffer containing guanidine isothiocyanate. Swabs in viral transport media were stored at 4 $^{\circ}$C until they could be frozen in liquid nitrogen (not more than six hours post-collection) and cold chain maintained until analysis at the laboratory. Swabs stored in lysis buffer were screened for influenza A using reverse transcriptase polymerase chain reaction (RT-PCR) at the University of California Davis. The duplicates of any samples found to be positive for influenza A were submitted for viral isolation using embryonated chickens eggs at the United States Department of Agriculture, Southeast Poultry Research Laboratory.

Tissue selection

Feathers and claws were selected for isotopic analyses because they are the most widely used tissues in ornithological isotopic studies to-date. They are both metabolically inert following formation; locking in isotopic signatures typical of the geographical area of growth. Feathers retain this information until replaced or moulted – typically once a year. Conversely, claws are continuously growing tissues and theoretically provide time-integrated information depending on claw growth rates (Bearhop *et al.* 2003; Clark *et al.* 2006).

Stable Isotope Analysis

Stable-hydrogen isotope preparation and analysis of feathers and claws were conducted at the Stable Isotope Hydrology and Ecology Laboratory in the National Hydrology Research Centre, Environment Canada, Saskatoon, Saskatchewan, Canada by Dr. Len Wassenaar. The majority of the collection envelopes contained numerous unidentified feathers (described previously). One feather was selected per individual for stable isotope analysis. If a newly grown or growing feather was showing signs of vascularity (e.g., having blood; hereinafter referred to as "blood feather") we selected it for analysis based on the expectation that those feathers would provide us with the "local" isotopic signal; otherwise, we selected any feather from the collection envelope. A small distal section of each feather vane was cut and the distal 5 mm portion of the claw was trimmed and placed in individual glass vials for cleaning. Surface materials were removed from feathers and claws by overnight soaking in a 2:1 chloroform:methanol mixture, followed by draining and placing in a fume hood until completely dried. Samples were then cut and 350±10µg were weighed into 4.0 x 3.2 mm silver capsules. Isotope analyses of feathers and claws were completed following the comparative equilibration technique using keratin standards as references, described in Wassenaar and Hobson (2003). Using continuous flow – isotope ratio mass spectrometry (CF-IRMS), samples were pyrolized into H_2 gas. Deuterium ratios were expressed in delta notation in parts per thousand (‰) relative to the Vienna Standard Mean Ocean Water Standard Light Antarctic Precipitation (VSMOW-SLAP) scale.

Establishing moulting location

We assigned individuals of unknown origin to a general geographical moult location along a latitudinal gradient, using expected isotopic values from the mean annual growing-season precipitation (δD_p) abundance for Asia, from a deuterium base map created using ArcGIS and the grid files available in http://www.waterisotopes.org/ [accessed March 5th 2008], developed by Bowen et al. (2005) (Figure 2). Given that a discrimination factor for Asia has not yet been established, we hypothesized that a discrimination between the abundance of deuterium in precipitation vs. feathers is likely to occur and behave similarly to those calculated for North America waterfowl. Therefore, we created a theoretical base map for deuterium abundance in feathers for Asia by adding a discrimination factor of -28‰ presented by Clark et al. (2006). Application of this discrimination value delineated 3 major isotopic source areas in Asia (Figure 3): Region A - northern Asia (>-118‰), B - catchment area (-118 to -88‰) and 8C – southern Asia (<-88‰). We reasoned that the isotopic range of -30‰ presented for Region B corresponded to latitudes ranging from approximately mid-Russia to southern Mongolia, and Regions A and C would be north and south of those latitudes respectively. When both feather and claw samples from the same individual were available, we examined the concordance of isotopic information between them (e.g., Clark et al. 2006; Hobson *et al.* 2006).

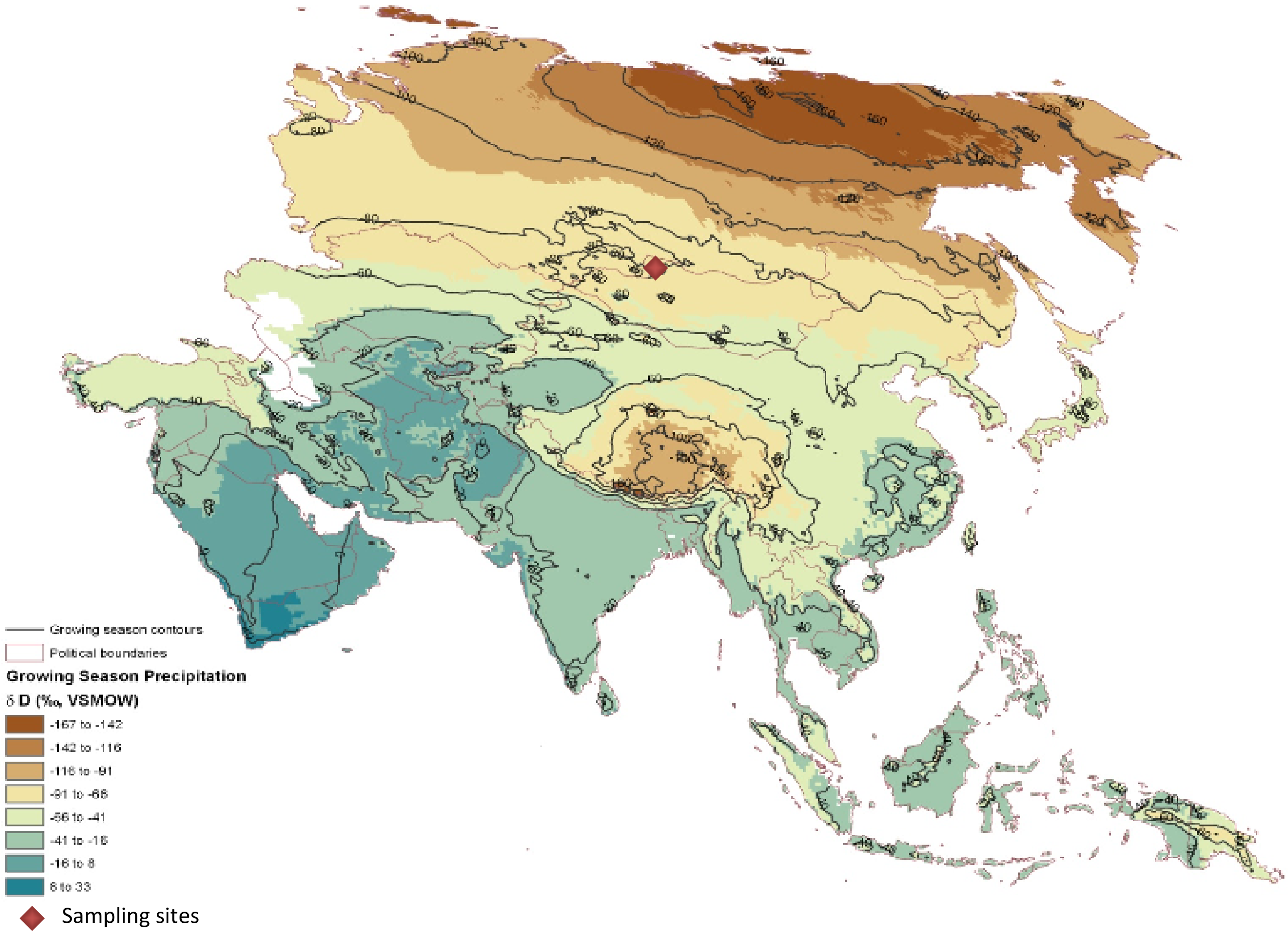

Figure 2. Average growing-season δD pattern in precipitation in Asia. Isotopic values are expressed in parts per thousand (‰) in relationship to the Vienna Standard Mean Ocean Water. Red diamond depicts the general location of the collection sites in north-central Mongolia.

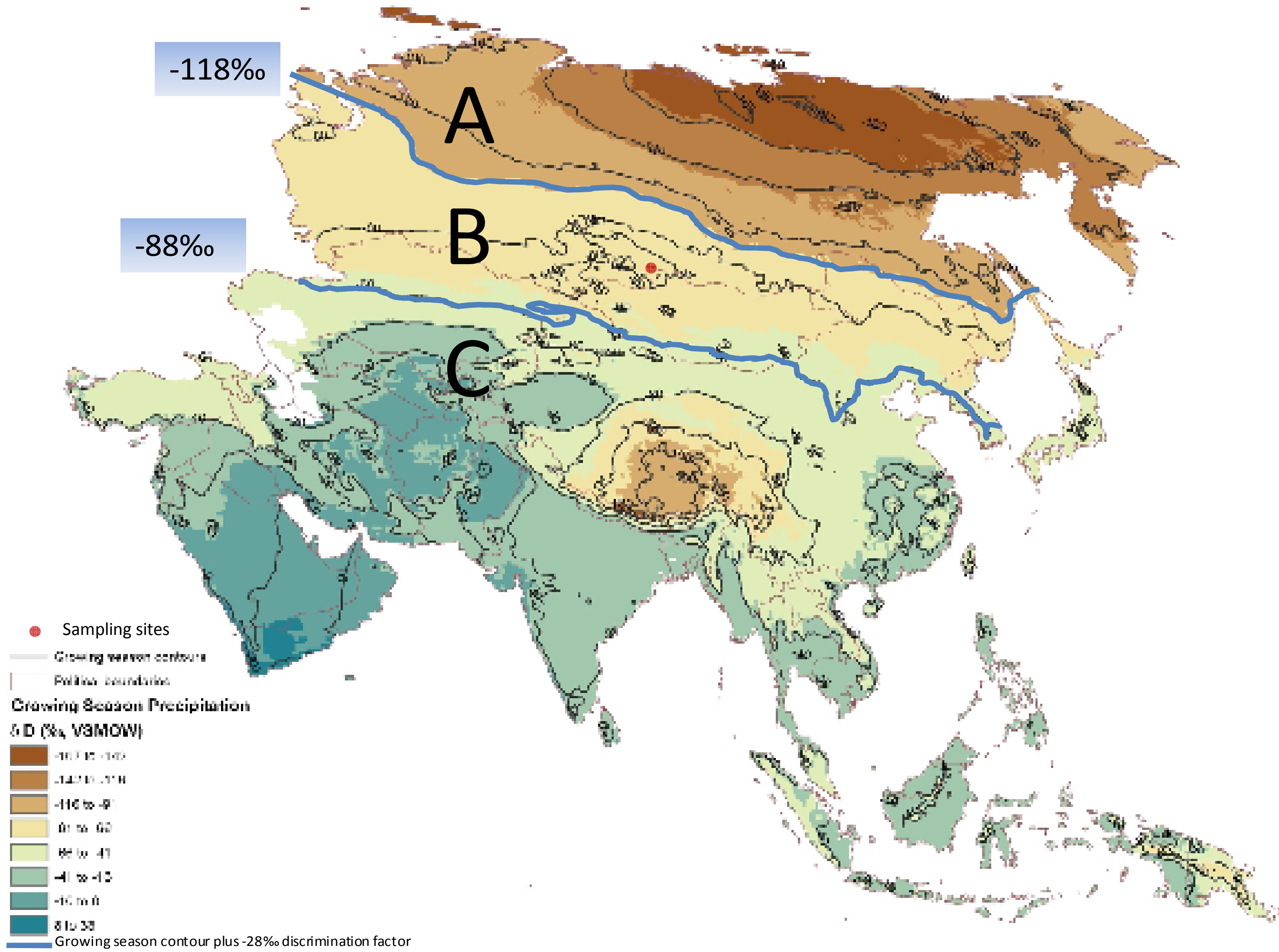

Figure 3. Thick blue lines depicts the assumed deuterium abundance in feather isoclines for Asia, from adding a -28‰ discrimination factor (Clark et al. 2006). Thin black contour lines are the deuterium in precipitation isoclines without adding a discrimination factor. Regions A, depicts an area of δDf values expected for feather grown at northern latitudes (> -118‰). Region B depicts an area of δDf expected for within the catchment area (-118‰ to -88‰). Region C depicts southern moulting grounds (< -88‰).

Statistical Analysis

We depicted deuterium abundance in feather (δD_f) data using frequency distributions in order to examine the overall range of isotopic values and potential multi-modal patterns of locations in which feathers could have been grown. To assess for normality in δD_f and deuterium abundance in claws (δD_c) we used a One-Sample Kolmogorov-Smirnov test (Sokal and Rohlf 1995) and used a Pearson's correlation test to examine the potential linear relationship between them. We used an Independent Sample *T*-test to examine potential differences in δD_f and δD_c, between age, sex, blood feather vs. non-blood feather, and among- and within study sites. To test for effects of gender, age and study site differences in δD_f and δD_c, we used a One-way ANOVA. Mongolian Gulls were sampled at four sites, so we used a univariate analysis to investigate differences in δD_f among them.

RESULTS

A total of 461 individuals comprising 27 waterbird species (Table 2) caught at 7 sites in Mongolia (Figure 1) were analyzed for their deuterium composition in feathers (δD_f) and claws (δD_c). Expected mean annual abundance of deuterium in precipitation (δD_p) were fairly consistent across all sampling sites (Table 3). Combining all species across all sampling sites, δD_f ranged widely from -185‰ (Eurasian Wigeon, *Anas penelope*) to 36‰ (Black-throated Loon, *Gavia arctica*). Of the 27 species examined, only 10 had sample sizes large enough (> 5 samples) to analyze statistically. See Table 4 for species' names and isotope values for those with small sample sizes.

Bar-headed Geese

Out of 119 Bar-headed Geese (*Anser indicus*), 118 healthy moulting flightless adults were captured between July 14th and July 30th 2007, and 1 sick adult was caught on June 25th, 2007. Eightytwo Bar-headed Geese were sampled in Darkhad Valley (39 males, 41 females, 2 gender unknown), 25 in Sharga Nuur (11 males, 14 females), 11 in Sangiyn Dalai Nuur (7 males, 4 females), and 1 sick female in Erhel Nuur. Two blood-feather δD values in the dataset were identified as outliers (too enriched) and consequently removed from further analyses. Blood feathers were collected primarily in Darkhad Valley (94% of cases); therefore, no comparisons of deuterium values among sampling sites were made. δD values of blood feathers, non-blood feathers and claws were normally distributed (Kolmogorov Smirnov Z = 0.887, P = 0.41; Z = 1.078, P = 0.196; Z = 0.0815, P = 0.520, respectively)(Figure 4). The δD values of blood and non-blood feathers were different (T-test = -10.46, df = 112.5, P < 0.001), as well as those between blood feathers and claws (T-test = -5.93, df = 84.1, P < 0.001). We did not find a correlation between δD values in blood feathers and δD_c (r = 0.27, P = 0.15, n = 30). On average, blood feathers were more depleted in deuterium than claws (Table 5). No gender differences were found in δD_f and δD_c (T-test = -1.38, df = 28, P = 0.18; $F_{1,114}$ = 0.002, P = 0.97, respectively). In general, the wide δD_f values indicate that Bar-headed Geese moult their feathers in each of the three isotopic Regions presented here (Table 6). As expected, δD of blood feathers displayed a narrow distribution indicating that all were grown at a similar location; however they represented more northern latitudes than those expected for their breeding signature in northern Mongolia (see Figure 5).

Table 2. List and sample size (n) of 27 wild aquatic bird species, sampled in north-central Mongolia during the summer and fall of 2007; examined for their isotopic composition in either feathers and/or claws.

Scientific name	Common English name	Common Mongolian name	*n*
Anas crecca	Common Teal	Ногоохон Нугас	2
Anas penelope	Eurasian Wigeon	Хадуур элсэг	2
Anser anser	Greylag Goose	Бор Галуу	1
Anser cygnoides	Swan Goose	Хошуу Галуу	17
Anser fabalis	Bean Goose	Буурал Галуу	21
Anser indicus	Bar-headed Goose	Хээрийн Галуу	119
Arenaria interpres	Ruddy Turnstone	Алаг хайргач	2
Caladris ferruginea	Curlew Sandpiper	Хадуур элсэг	7
Calidris minuta	Little Stint	Одой элсэг	1
Calidris subminuta	Long-toed Stint	Шавар Элсэг	1
Charadrius alexandrinus	Kentish (snowy) Plover	Тэнгисийн Хиазат	2
Charadrius dubius	Little-ringed Plover	Нарийн Хиазат	2
Chlidonias leucopterus	White-winged Tern	Буурал хараалзай	4
Cygnus cygnus	Whooper Swan	Гангар Хун	120
Gallinago stenura	Pintail Snipe	Замбын Хараалж	1
Gavia arctica	Black-throated Loon	Хилэн Омруут Ахууна	1
Larus vegae mongolicus	Mongolian Gull	Монгол цахлай	42
Melanitta fusca	White-winged Scoter	Дөрт Нугас	2
Phalocrococax carbo	Great Cormorant	Тураг гогой	53
Philomachus pugnax	Ruff	Ноололдой	3
Pluvialis fulva	Pacific golden Plover	Азийн Сүвээ Цагаан	14
Podiceps cristatus	Great crested Grebe	Отгот Шунгуур	1
Sterna hirundo	Common Tern	Эгэл хараалай	2
Tadorna ferruginea	Ruddy Shelduck	Ангир	34
Tringa erythropus	Spotted Redshank	Хар Хөгчүү	1
Tringa gareola	Wood Sandpiper	Шугуйн Хөгчүү	3
Tringa totanus	Common Redshank	Улаанхөлт хөгчүү	3

Table 3. Expected mean annual deuterium (δD) and 95% CI values in precipitation for seven study sites in north-central Mongolia. Values were obtained using isotope calculator in www.waterisotopes.org. Approximate elevations were obtained using the free software Google Earth (http://earth .google.com/).

Sampling Site	Approx. Elevation (m)	δD (‰, V-SMOW)	δD 95% CI (‰)
Darkhad Valley	1530	-99	6
Erhel Nuur	1540	-92	7
Sharga Nuur	1330	-84	6
Shagiyn Dalai Nuur	1700	-93	7
Ogii Nuur	1700	-83	7
Tsegeen Nuur	1520	-87	6
Tsengel Nuur	2370	-102	8

Table 4. Deuterium feather (δD_f) and claw (δDc) values of those species with small sample size (n < 5). "Region" refers to the expected continental area (i.e., Region A, B, or C) that the observed δD_f value falls within.

Species	δD_f (‰)	Feather type	Region	δD_c (‰)	Gender	Age	Sampling site	Capture date	Capture method
Eurasian Wigeon	-147	NS	**A**	-132	U	A	Ogii Nurr	2007-08-20	Mist net
Eurasian Wigeon	-185	NS	**A**	-160	U	A	Sangiyn D.N	2007-09-14	Spotlight
Common Teal	-155	B	**A**	-130	M	A	Ogii Nuur	2007-08-21	Mist net
Common Teal	-153	S	**A**	-150	U	J	Sharga Nuur	2007-09-24	Dead
Little Stint	-138	S	**A**	-	U	J	Ogii Nuur	2007-08-26	Mist net
Little-ringed Plover	-130	NS	**A**	-	U	A	Ogii Nuur	2007-08-18	Mist net
Little-ringed Plover	-107	NS	**B**	-	U	J	Ogii Nuur	2007-08-24	Mist net
Wood Sandpiper	-132	NS	**A**	-	U	J	Ogii Nuur	2007-08-18	Mist net
Wood Sandpiper	-127	NS	**A**	-88	U	J	Ogii Nuur	2007-08-18	Mist net

Wood Sandpiper	-107	NS	**B**	-	U	J	Ogii Nuur	2007-08-18	Mist net
Common Redshank	-103	S	**B**	-	U	J	Ogii Nuur	2007-07-30	Mist net
Common Redshank	-113	S	**B**	-	U	J	Ogii Nuur	2007-07-30	Mist net
Common Redshank	-87	S	**C**	-	U	J	Ogii Nuur	2007-07-30	Mist net
Greylag Goose	-104	NS	**B**	-90	M	A	Sharga Nuur	2007-07-29	NS
Long-toed Stint	-98	NS	**B**	-	U	J	Ogii Nuur	2007-08-18	Mist net
Ruddy Turnstone	-111	NS	**B**	-93	U	J	Ogii Nuur	2007-08-24	Mist net
Ruddy Turnstone	-111	NS	**B**	-99	U	J	Ogii Nuur	2007-08-24	Mist net
Black-throated Loon	36	NS	**C**	-	U	A	Sharga Nuur	2007-07-30	Spotlight
Common Tern	-29	NS	**C**	-40	U	A	Ogii Nuur	2007-08-20	Mist net
Common Tern	-1	NS	**C**	-32	U	A	Ogii Nuur	2007-08-20	Mist net
Great-crested Grebe	-67	NS	**C**	**-**	U	A	Sangiyn D.N	2007-07-13	Spotlight
Kentish Plover	-46	NS	**C**	-	U	A	Ogii Nuur	2007-08-21	Mist net
Kentish Plover	-60	NS	**C**	-	U	A	Ogii Nuur	2007-08-24	Mist net

Pintail Snipe	-59	NS	**C**	-16	U	A	Ogii Nuur	2007-08-18	Mist net
Spotted Redshank	-42	NS	**C**	-11	U	A	Ogii Nuur	2007-08-18	Mist net
White-winged Scoter	-60	NS	**C**	-	F	A	Ehrel Nuur	2007-06-17	Sick
White-winged Scoter	-79	NS	**C**	-57	F	A	Sharga Nuur	2007-07-29	Spotlight
White-winged Terns	-84	NS	C	-54	U	J	Ogii Nuur	2007-08-18	Mist net
White-winged Terns	-82	NS	C	-49	U	J	Ogii Nuur	2007-08-20	Mist net
White-winged Terns	-87	NS	C	-80	U	J	Ogii Nuur	2007-08-24	Mist net
White-winged Terns	-70	NS	C	-54	U	J	Ogii Nuur	2007-08-25	Mist net
Ruff	-139	S	A	-112	U	J	Ogii Nuur	2007-08-20	Mist net
Ruff	-139	S	A	-109	U	J	Ogii Nuur	2007-08-20	Mist net
Ruff	-142	S	A	-127	U	J	Ogii Nuur	2007-08-20	Mist net

NS : not specified; U: unknown; M: male; F: female; A: adult; J: juvenile; S: scapular feather; B: blood feather

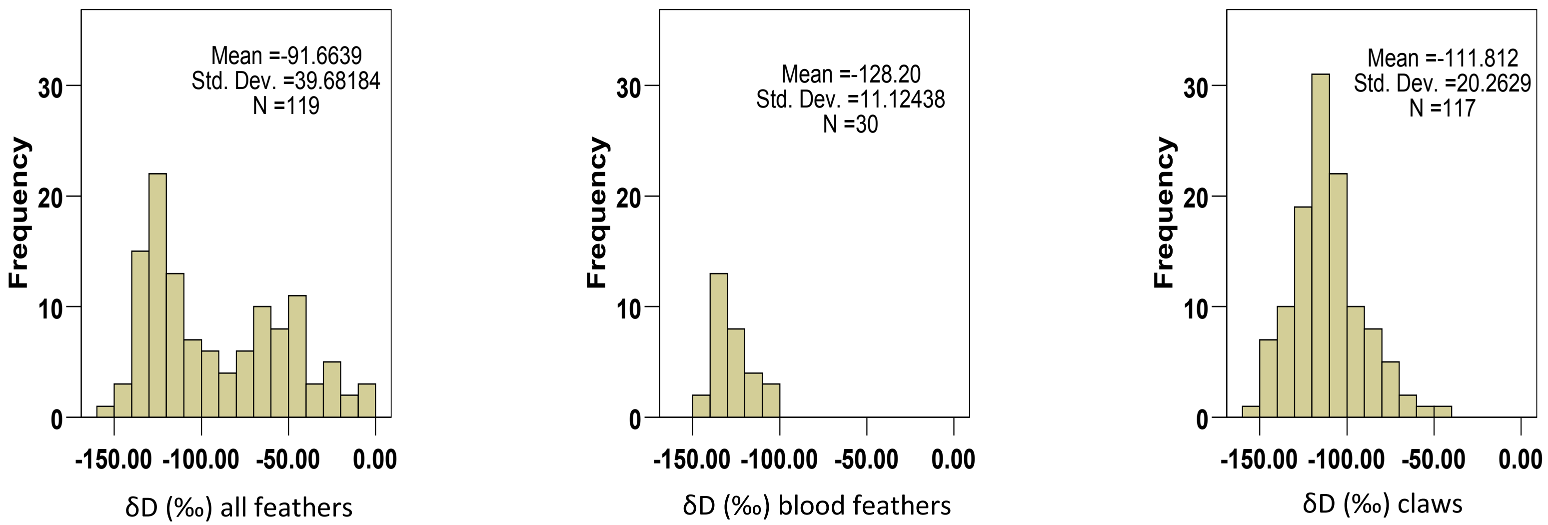

Figure 4. Frequency distribution of δD values of all feathers, blood feathers and claws from Bar-headed Geese sampled in north-central Mongolia in 2007.

Table 5. Mean, standard deviation (SD), confidence interval (CI), and number of samples (n) of deuterium values of blood feather and claw samples of Bar-headed Goose sampled in north-central Mongolia during the summer of 2007.

Tissue	*n*	Mean	SD	95% CI	
				Lower	Upper
Blood Feather	30	-128.2	11.1	-132.4	-124.0
Claw	115	-111.9	20.4	-115.7	-108.2

Table 6. Proportion (%) of birds that moulted their sampled feather within isotopic Region A, B, or C. Depiction of isotopic regions is found in Figure 3.

Species	*n*	Regions		
		A	**B**	**C**
Bar-headed geese				
All feathers	*117*	39	19	42
Blood feathers	*30*	77	23	-
Swan geese	*17*	6	12	82
Bean geese	*21*	33	43	24
Whooper swan				
Sangiyn Dalai Nuur	*23*	26	65	9
Darkhad Valley	*34*	76	18	6
Sharga Nuur	*44*	25	66	9
Tsegeen Nuur	*4*	-	75	25
Tsengel Nuur	*15*	27	53	20
Curlew sandpiper	*7*	86	14	-
Pacific golden plover	*14*	-	-	100
Great cormorant	*53*	2	34	64
Mongolian gull	*42*	-	-	100
Ruddy shelduck				
Males	*13*	-	23	77
Females	*15*	33	40	27

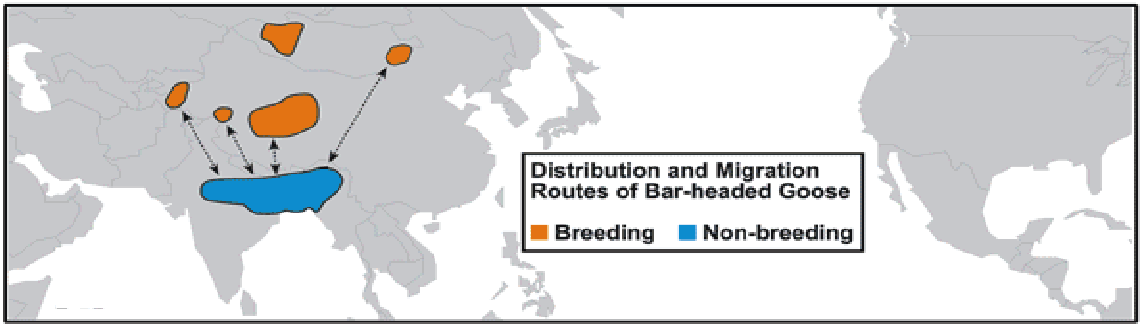

Figure 5. Breeding (orange) and wintering (blue) distribution and migration route of Bar-headed Geese. The migratory direction of northernmost Bar-headed Geese population is not indicated (courtesy of Webster et al. 2006).

Swan Geese

A total of 17 healthy moulting adult Swan Geese (*Anser cygnoides*) were sampled in northern Mongolia in 2007; 15 in Sharga Nuur (5 males, 10 females) and 2 in Tsegeen Nuur (1 male, 1 female), between July 28th and July 31st, 2007 (Figure 1). Feathers and claws were collected for all individuals except two (feathers only). Distributions of δD_f and δD_c were normal (both, $P > 0.07$) (Figure 6). The δD values of feathers and claws were significantly different (T-test = 6.5, df = 30, $P < 0.001$). On average δD_c values were more depleted than δD_f values (mean ± SD, -113‰±12.1 and -88‰±10.1, respectively). No gender differences were found in δD_f and δD_c (both $P > 0.4$). In general, most individuals had δD feather signatures similar to those found in latitudes similar to southern Mongolia and interestingly, 12 out of 17 individuals had feather δD values between -83‰ to -88‰, which are within the known range of the species. Generally, migratory populations of Swan Geese breed in Mongolia and eastern Russia and overwinter in southern and eastern China (http://www/werc/usgs.gov).

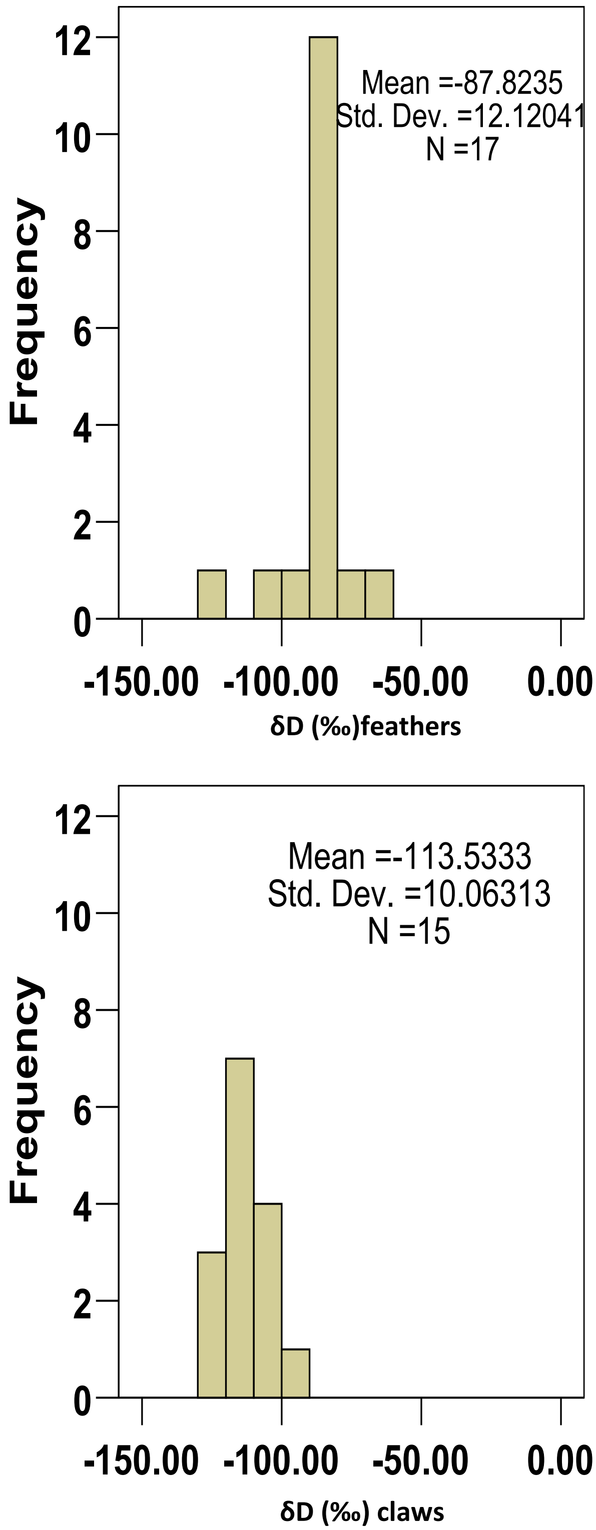

Figure 6. Frequency distribution of Swan Geese sampled in Sharga Nuur and Tsegeen Nuur, in north-central Mongolia in 2007.

Bean Geese

Twenty one healthy moulting adult Bean Geese (*Anser fabalis*) were sampled in Sangyin Dalai Nuur (11 males, 9 females, 1 unknown) between July 11th and July 14th, 2007 (Figure 1). Feathers and claws were collected for all individuals except for one, for whom there was only a feather. Distribution of δD_f and δD_c were normal (both $P > 0.8$) (Figure 7). No gender differences were found in δD_f and δD_c for Bean Geese ($F_{1,18} = 0.12$, $P = 0.92$ and $F_{1,17} = 0.007$, $P = 0.94$, respectively). We found no mean significant difference (*T*-test = 1.7, df = 32.7, $P = 0.1$), nor a correlation ($r = -0.28$, $P = 0.24$, $n = 20$) between δD_f and δD_c. On average, δD_f were more enriched than δD_c (Figure 7; Table 7). Bean Goose δD feather values represented δD values spanning all three regions (Table 6). Five migratory subspecies have been described for this species, most of which breed widely across northern Eurasia and winter in China, Korea, Japan and southern Europe (Kear 2005). These data fit well within the described range since the lowest δD_f values observed for this species was -76‰, and all other values correspond to areas north of their southernmost wintering area.

Whooper Swans

A total of 120 healthy moulting flightless adult Whooper Swans (*Cynus cygnus*) were sampled in Darkhad Valley ($n = 34$; 13 males, 21 females), Sangiyn Dalai Nuur ($n = 23$; 7 males, 15 females, 1 gender unknown), Sharga Nuur ($n = 44$; 26 males, 18 females), Tsegeen Nuur ($n = 4$; 2 males, 2 females) and Tsengel Nuur ($n = 15$; 8 males, 7 females) between July 8th and August 2nd, 2007. In 92% of the cases we had both the feather and claw from the same individual. Deuterium feather and claw values had a normal distribution (all $P > 0.09$). We found no differences in δD_f values between blood and non-blood feathers (*T*-test = 1.8, df = 50.3, $P = 0.07$); although we found that the δD_f values among the 5 sampling sites were different ($F_{4,119} = 15.2$, $P < 0.001$). Frequency distributions in δD_f values in each of the sampling areas were examined graphically (Figure 8). Tukeys post-hoc analyses revealed 2 distinct groups of sampling areas that differed in their δD_f values: Sangiyn Nuur, Sharga Nuur, Tsegenn Nuur, and Tsengel Nuur did not differ from each other. However, Darkhad Valley differed from all other sampling areas (more depleted in δD_f) (Table 6). No gender differences in δD_f and δD_c were found within study sites (all $P > 0.2$). We found a positive relationship between feathers and claw δD values in birds sampled in Sharga Nuur ($r = 0.243$, $P = 0.004$, $n = 43$, Y-intercept $P < 0.0001$).

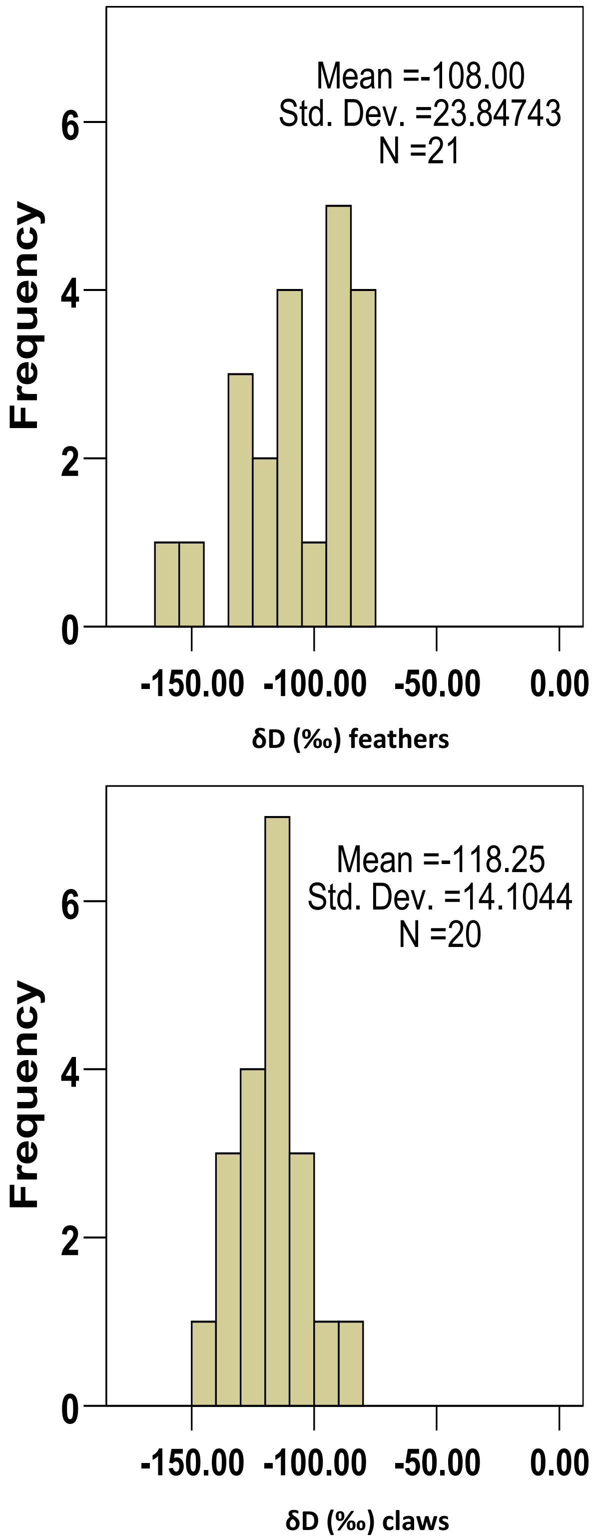

Figure 7. Frequency distribution of δD values in feathers and claws of Bean Geese sampled in Sangiyn Dalai Nuur, north-central Mongolia in July 2007.

Table 7. Mean, standard deviation (SD) and confidence interval (CI) in deuterium values of feathers and claws collected from Bean Goose in north-central Mongolia during the summer of 2007.

Tissue	*n*	Mean	SD	95% CI	
				Lower	Upper
Feather		-108.0	23.8	-120.4	-98.3
Claw		-118.3	14.1	-125.3	-111.3

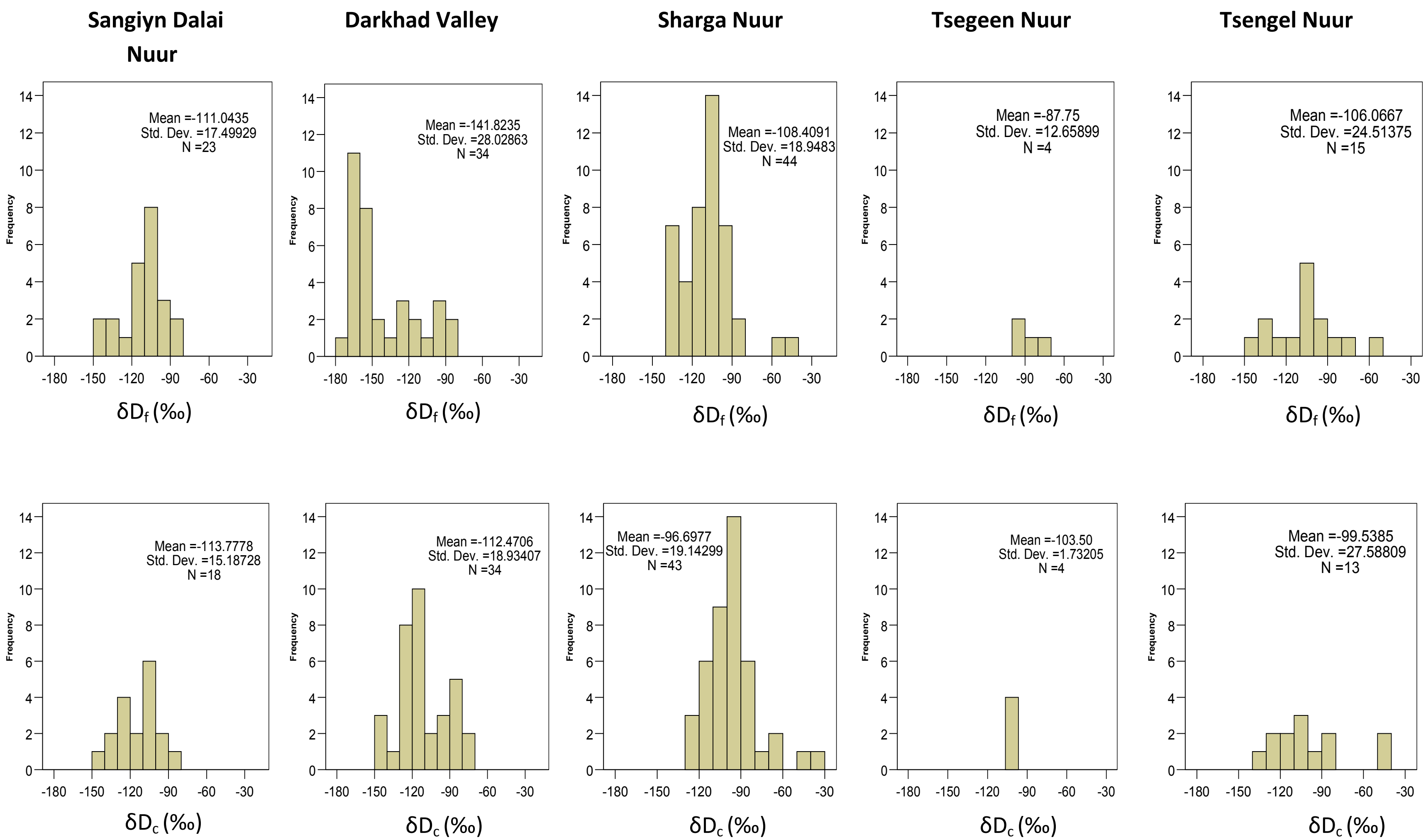

Figure 8. Frequency distribution of feather and claw δD values of Whooper Swans sampled in 5 locations in north-central Mongolia in 2007.

Curlew Sandpiper

Seven healthy gender unknown juvenile Curlew Sandpipers (*Calidris ferruginea*) were sampled in Ogii Nuur between August 24th and 26th 2007. Graphical representation of Curlew Sandpiper isotopic distribution of scapular feathers is given in Figure 9. Even though claw material was obtained from all 7 individuals, we only had enough material from 3 to analyze isotopically. Distribution of both δD_f and δD_c were normal (both $P > 0.9$). On average, δD_f values were more depleted than δD_c (mean δD_f = 123‰, mean δD_c = -104‰), and there was no relationship between δD_f and δD_c ($r = 0.5$, $P = 0.67$, $n = 3$). Most Curlew Sandpipers had deuterium values representative of those found in Region A (Table 6).

Pacific Golden Plover

A total of 14 healthy gender unknown adult Pacific Golden Plovers (*Pluvialis fulva*) were sampled in Ogii Nuur from August 18th to 26th, 2007. Isotopic distribution of Pacific Golden Plover scapular feathers and claws are shown in Figure 9. Both feather and claw δD values had a normal distribution (both $P > 0.9$). For all individuals, except one, we had both feather and claw deuterium values. Although the mean between δD_f and δD_c were similar (*T*-test = -0.6, df = 25, $P = 0.6$), we found no correlation between δD values of feathers and claws ($r = 0.51$, $P = 0.07$, $n = 13$). Without exception, all Pacific golden plover feather deuterium values were consistent with values originating from Region C (Table 6).

Great Cormorant

Out of 53 healthy gender unknown juvenile Great Cormorants (*Phalacrocorax carbo*), 52 birds were caught by boat and spotlight method in Sangiyn Dalai Nuur, on September 14th and 15th, 2007 and 1 was opportunistically mist netted in Ogii Nuur on August 23rd, 2007. For all individuals both feather and claw samples were collected. Feather and claw δD values had a normal distribution (both $P > 0.4$). We found a positive relationship between δD_f and δD_c ($r = 0.42$, $P = 0.002$, $n = 53$) (Figure 10). On average, δD_f were more depleted than δD_c (Figure 11). The Great Cormorant is widely distributed around the world occurring in North America, Europe, Africa, Asia and Oceania (http://www.bsc-eoc.org/avibase/species [Accessed April 3rd, 2008]) and their moult pattern is poorly understood (Hatch *et al.* 2000). Our data suggest that 98% of individuals moulted their feathers south of latitudes corresponding to southern Russia.

Mongolian Gulls

A total of 42 Mongolian Gull (*Larus vegae mongolicus*) samples were provided. Thirty-nine dead juvenile Mongolian Gulls were sampled on nesting islands in Erhel Nuur (38 gender unknown and 1 male) and 3 live, gender unknown juveniles in Sangiyn Dalai Nuur between August 6th and September 14th, 2007. Feather samples were analyzed from all 42, but only 6 claw samples were provided for analysis. Distribution of δD_f and δD_c had a normal distribution (both $P > 0.7$) (Figure 12). No concordance between δD_f and δD_c was found ($r = 0.48$, $P = 0.33$, $n = 6$). δD_f between study sites and between blood and non-blood feathers were similar ($F_{1,41} = 1.26$, $P = 0.27$, $F_{1,41} = 3.19$, $P = 0.08$, respectively). All Mongolian Gull feather deuterium values were consistent with values originating from Region C (Table 6).

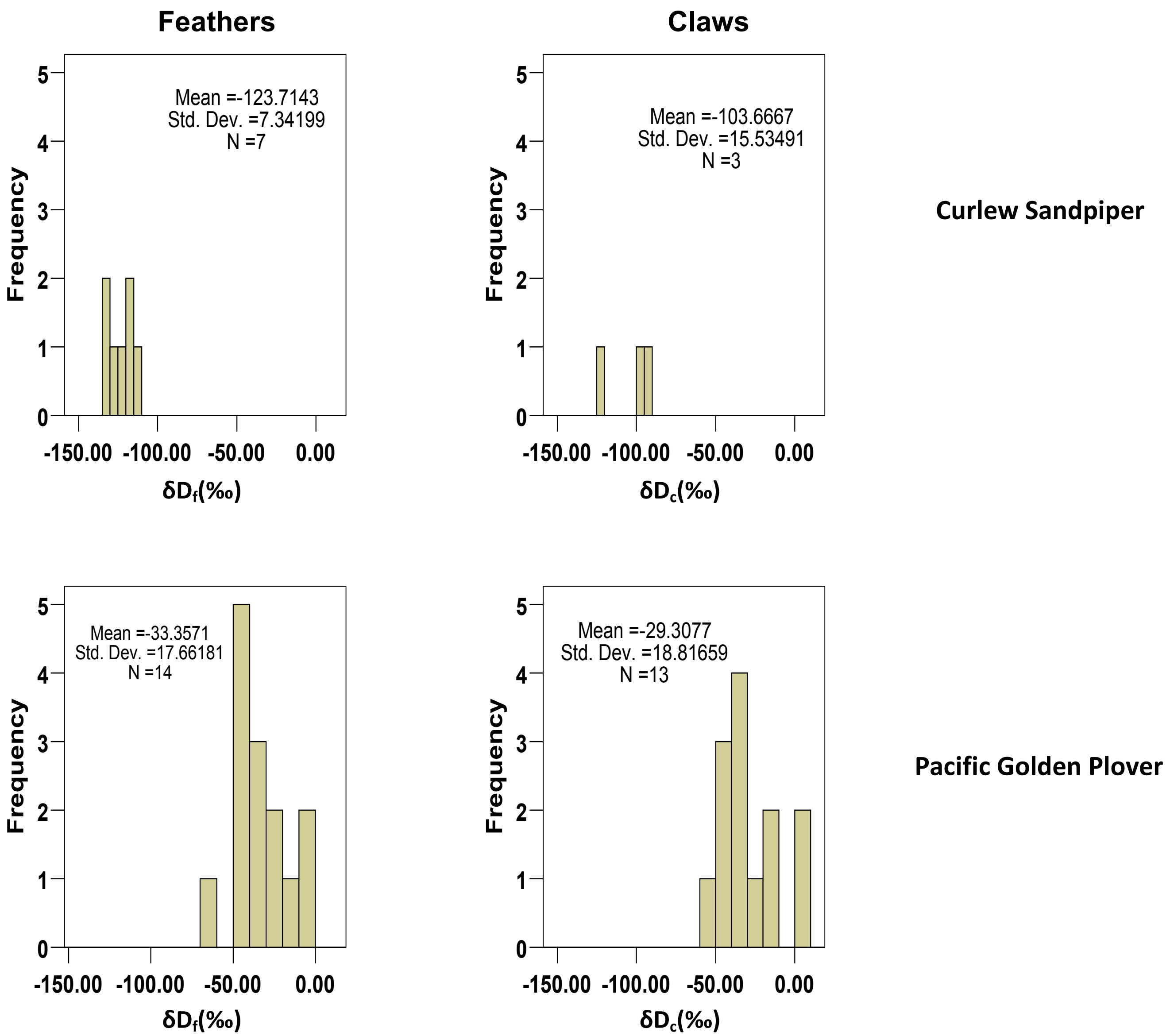

Figure 9. Frequency distribution of deuterium values in feathers and claws of Curlew Sandpipers and Pacific Golden Plovers sampled in Ogii Nuur in north-central Mongolia in 2007.

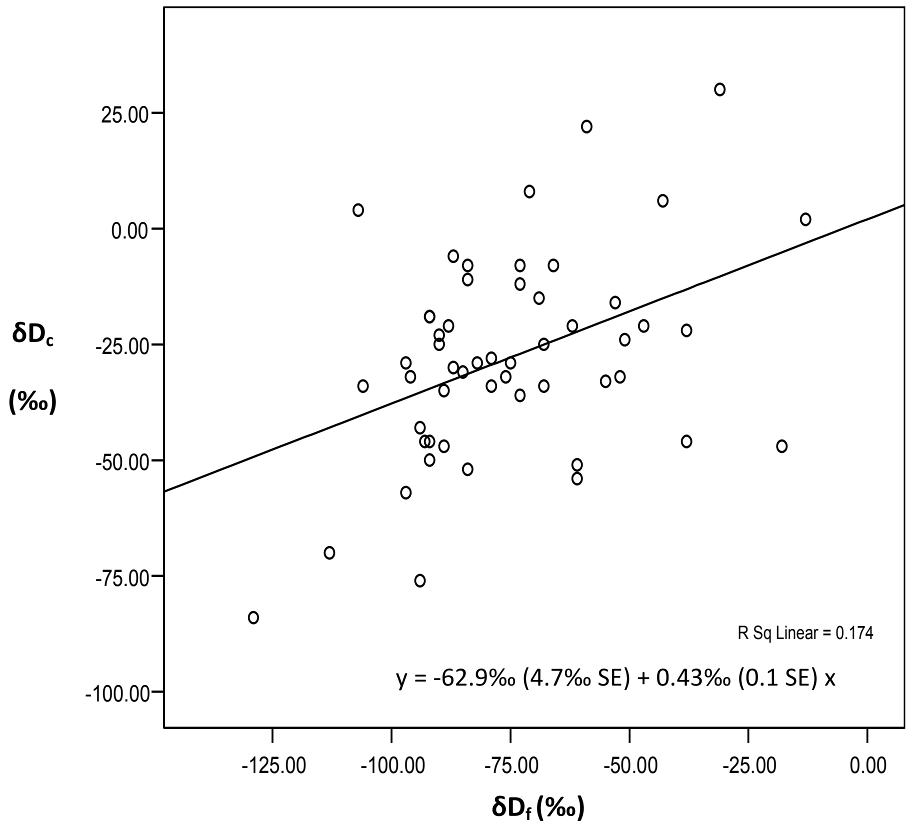

Figure 10. Relationship between δD values in feathers and claws of 53 juvenile Great Cormorants sampled in August and September-2007 in north-central Mongolia (52 sampled in Sangiyn Dalai Nuur and 1 in Ogii Nuur). Y-intercept significantly different from zero (P < 0.002)

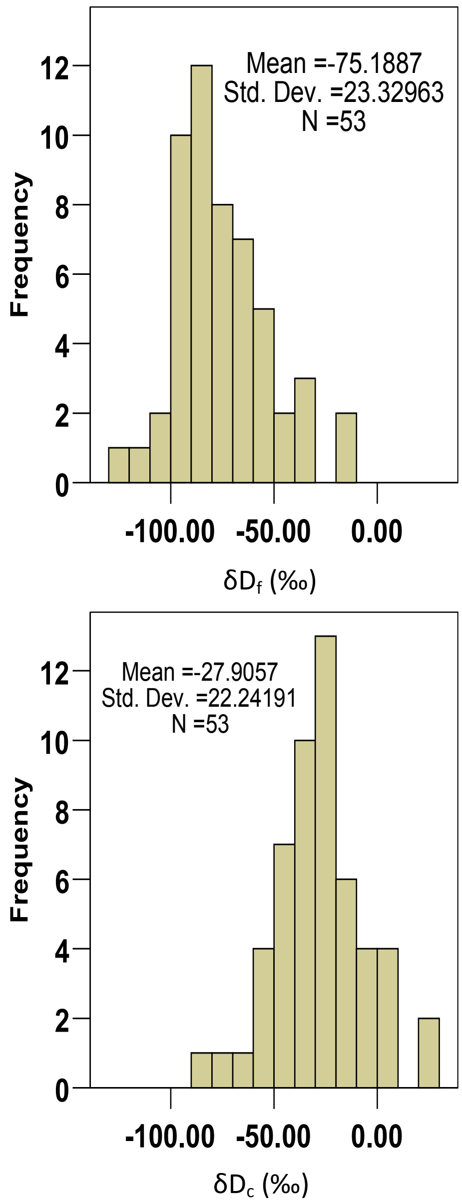

Figure 11. Frequency distribution of δD values in feather and claws of juvenile Great Cormorants sampled in Mongolia during August and September 2007.

Ruddy Shelduck

Thirty four Ruddy Shelducks (*Arenaria interpres*) were sampled. One dead adult (1 female) was sampled in Sharga Nuur. Four live adult and 1 juvenile in Tsegeen Nuur (3 males, 2 females), and 18 live adults and 10 live juveniles were sampled in Tsengel Nuur (10 males, 12 females, 6 gender unknown); all healthy. All sampling occurred between July 29th and August 6th, 2007. In all cases, except for one, we obtained both δD_f and δD_c values. Both δD_f and δD_c values were normally distributed (all $P > 0.7$) and were similar across sampling sites (both $P > 0.09$). There was no difference in δD_f between blood- and non-blood feathers (*T*-test = 1.19, df = 32, $P = 0.2$). We found gender differences in δD_f ($F_{1,26} = 5.8$, $P = 0.02$) and genders were subsequently separated in further analyses. Females were significantly more depleted in δD_f than males (Figure 13). We tested for potential differences among study sites and between age classes within males and females and found no difference (All $P > 0.2$). No correlations were found between δD of feathers and claws in males or females (both $P > 0.08$). No clear moulting patterns are observed in female Ruddy Shelducks but most males appear to moult feathers in Region C (Table 6). These findings concur with overall distribution of the species, which has a wide breeding distribution extending from Eastern Europe and northern Africa through the Middle East, central Asia, southern Siberia, Mongolia, China and northern India (Kear 2005) and wintering in China (Quan *et al.* 2001), India (Mishra and Humbert-Droz 1998), Korea, Myanmar, Thailand and Vietnam and central Africa (Kear 2005). Populations of eastern Eurasia are migratory and winter in southern India and China (Kear 2005), all well within the isotopic values observed here.

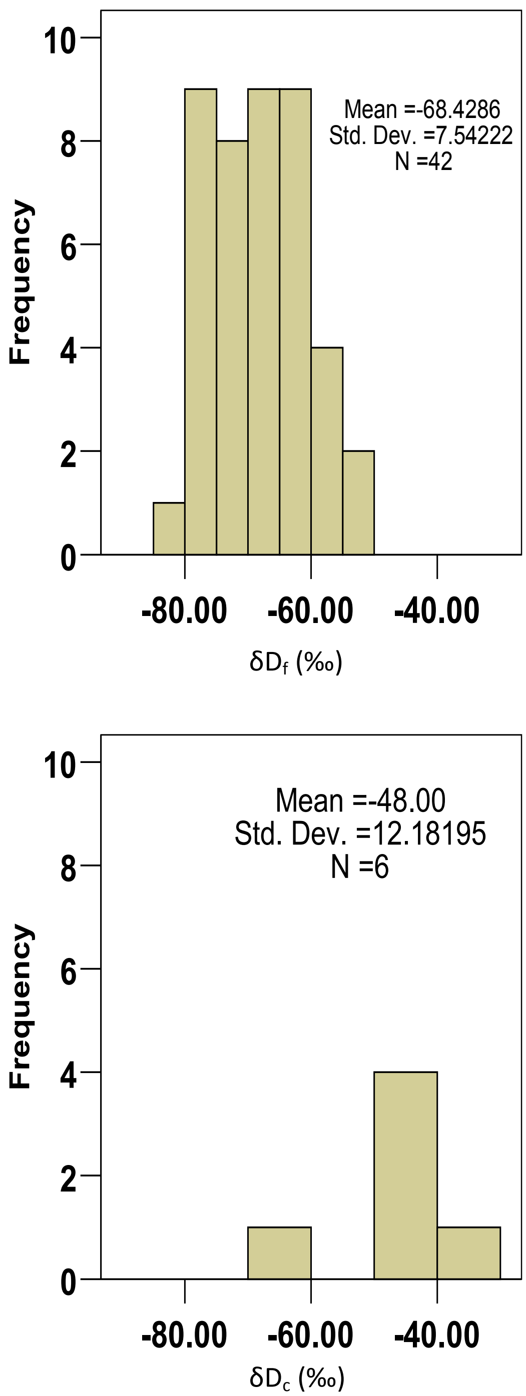

Figure 12. Frequency distribution of isotopic feather and claw values of Mongolian Gulls sampled in Mongolia in 2007.

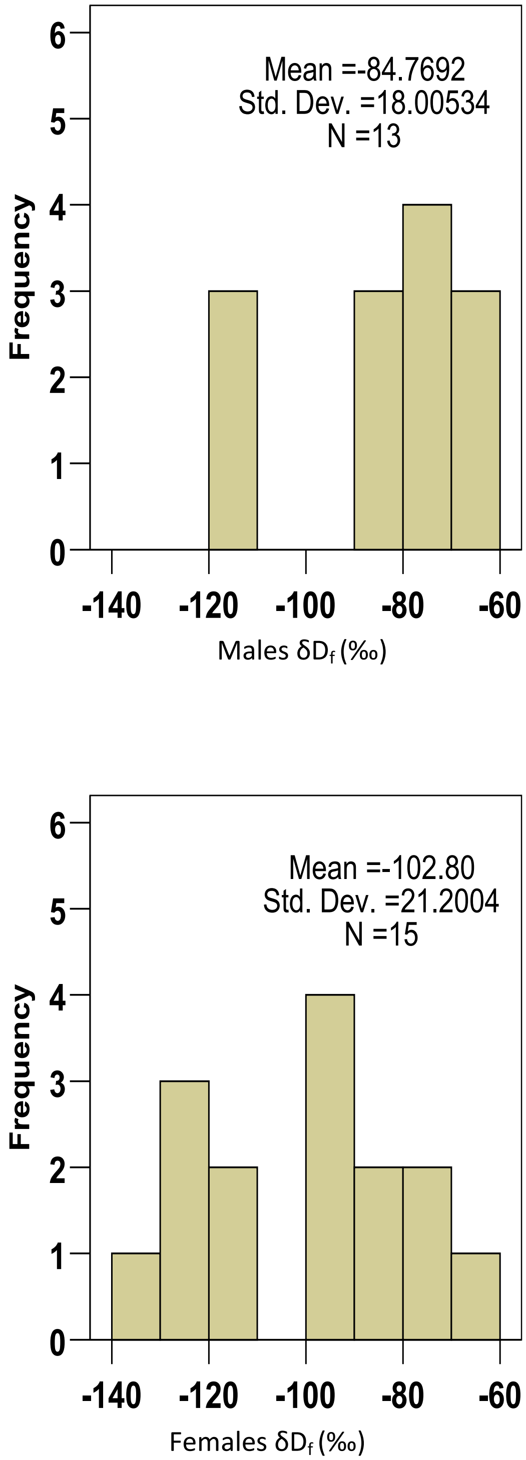

Figure 13. Frequency distribution of feather deuterium values of male and female Ruddy Shelduck sampled in north-central Mongolia in 2007.

DISCUSSION

Our investigation revealed a range of latitudinal origins of feather moult from northern to southern Asia. We explored the average deuterium values from blood feathers in all species (-104‰) and the approximate average expected growing season δD_p value across all sampling sites in north-central Mongolia (-80‰), and suggested an approximate discrimination factor of ~-24‰. We felt that using a discrimination factor presented by Clark et al. (2006) of -28‰, was a more reasonable choice given that it was calculated from an aquatic species of known origin, and worked reasonably well with the known distribution of these species examined here. The wide distribution in δD_f values showed that, without exception, all species analyzed statistically showed isotopic signatures from outside of those expected for Region B, indicating varying degrees of movement from the location in which the collected feather was grown, to the capture site.

All Pacific Golden Plovers and Mongolian Gulls and 82% of Swan Geese individuals had δD_f values representative of latitudes south of southern Mongolia, in Region C (below the -60‰ isoline, Figure 3). Our findings agreed with reports that Pacific Golden Plovers complete a prebasic moult soon after arriving on the wintering grounds (Johnson and Connors 1996), which extends from East and Southeast Asia to Australia, Oceania and Africa (Johnston and McFarlane 1967; Johnson and Connors 1996) (Figure 14). Pacific Golden Plover individuals begin their prebasic moult on the breeding grounds of north-central Siberia and western Alaska (Johnson and Connors 1996). However, based on the scapular feathers collected, we found that no individuals appeared to have started their moult on the breeding grounds.

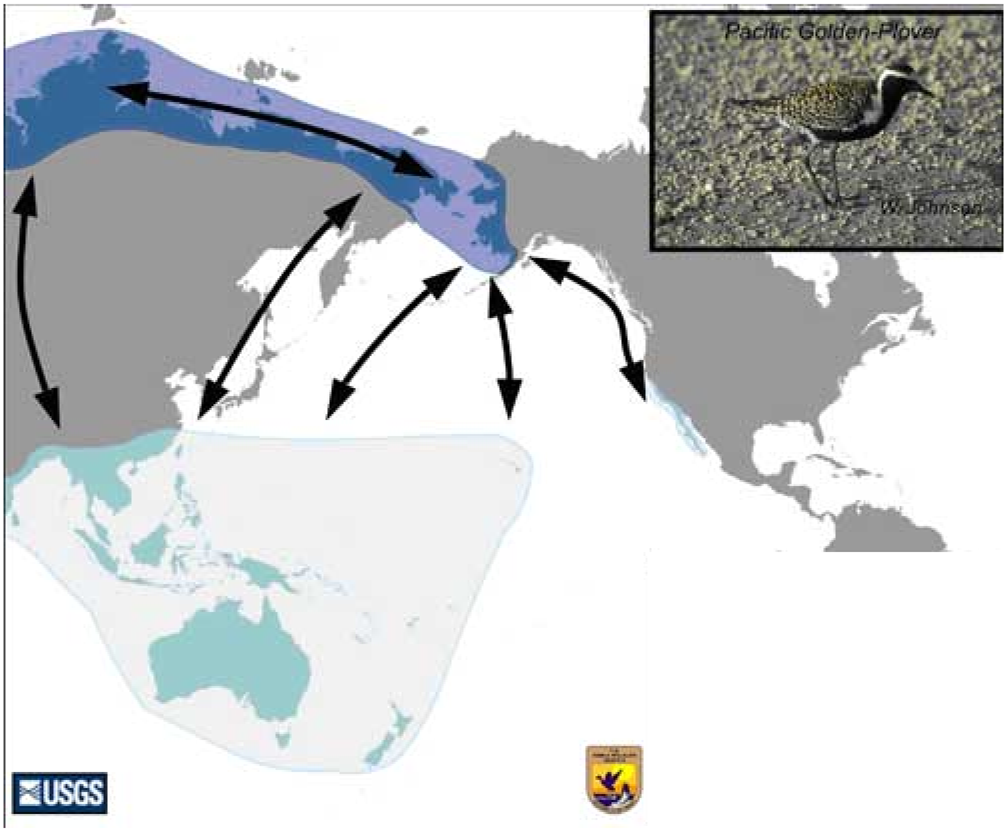

Figure 14. Breeding and wintering distribution and possible migration pathways of Pacific Golden Plover in Asia and North America. (Map courtesy of USGS website: http://alaska.usgs.gov/science/biology/avian_influenza/species/species.php?code=PAGP)

Similarly, although Mongolian Gulls can occur in Russia, Mongolia, and China (http://www.bsc-eoc.org/avibase/species [Accessed April 3rd, 2008]) moulting in our sample appears to have occurred exclusively at latitudes found south of Mongolia (mean δD_f = -68‰). This does not concur with our expectations because all Mongolian Gulls sampled were juveniles and their δD_f values were more enriched than expected for birds that have not yet left their natal sites. There are a few possible explanations for the high δD_f values for this species. First, physiological processes related to heat stress during feather moult have been suggested as a cause of enriched body and δD_f values in some species (Mckechnie et al. 2004, Smith and Dufty 2005). Second, discrimination factors vary among taxonomic groups (Langin et al. 2007), and perhaps the discrimination factor between precipitation and Mongolian Gull feathers is closer to 0‰ vs. -28‰. Identifying such variables would require monitoring captive birds throughout their moult period while controlling certain ambient variables. Third, the temporal variability in the patterns of δD_p does not always match the long-term average (Powell and Hobson 2006). Reviewing the monthly patterns of δD_p throughout Mongolia in http://www.waterisotopes.org/ [accessed June 30th, 2008], δD_p is more enriched in δD during the summer months (June to September) compared to all other seasons. When feathers are grown during periods of high precipitation, the δD_f values may more closely represent this period, resulting in dramatic deviations from the annual average (Powell and Hobson 2006). This could be tested by examining δD values of known diets of Mongolian Gull throughout the season and especially during the expected moulting period.

Very little is known about the migratory movements of the Swan Goose. Migratory populations breed primarily in Mongolia and eastern Russia and overwinter in southern and eastern China, while non-migratory populations occur throughout Asia (http://www.werc.usgs.gov). Information from global positioning system (GPS) transmitters mounted on three birds in north-eastern Mongolia has shown movement through northern Korea and eastern China (http://www.werc.usgs.gov/sattrack/swangoose/overall.html). Given that the feathers collected were not identified, it is impossible to know whether they were indeed moulted at more southern latitudes or whether the discrimination factor for this species, similar to the possibilities discussed for the Mongolian Gull and other species, should be more depleted than the one used in this study. Alternatively, seasonal variation in δD_p values or influences of ground water can also cause more enriched δD_f values. Future studies identifying specific feather tracts collected, and a finer resolution base map and δD values of other regional water sources, such as ground water and run-off, would assist in resolving uncertainties.

The species with results most consistently demonstrating moult at northern latitudes of Region A, were the Whooper Swans (sampled in Darkhad Valley), Bar-headed Geese (blood feathers) and Curlew Sandpipers (> 75% of individuals in each species). Whooper Swans breed in northern latitudes from Iceland to north-eastern Siberia down to northern China and winter in coastal areas in Europe, the Black and Caspian Seas, or eastern Asia (Kear 2005). Our data suggest that Whooper Swans moulted their feathers at latitudes north of central Russia. Interestingly, all Whooper Swan were sampled during a flightless moulting period, which lasts about 30 days, and in Mongolia occurs mainly in July (MG, pers. observ.) but can extend into September (Kear 2005) in other regions.

The migratory movements and ecology of Bar-headed Geese are not well understood (Javed et al. 2000). In general, the Bar-headed Goose breeds in central Asia and winters in southern Asia (Uttangi 1987, Scott and Milsom 2007) (Fig 5). Out of five marked individuals, two birds banded in eastern Krygyzstan were spotted in Pakistan (Roberts 1991) and three birds banded at Qinghai Lake, China were recovered in India and Bangladesh (Uttangi 1987, Kear 2005). Satellite telemetry data from birds caught in India and Nepal have shown birds flying over the Himalayas to summer in the Tibetan Plateau (http://www.werc.usgs.gov). However, most recent re-sighting data from four neck-collared individuals showed them wintering in Karnataka and Maharashtra, India; considerably south of the wintering range given in Figure 5 (WCS unpublished data). Adults moult their primary and secondary feathers about a month after young hatch, and sub adults, unsuccessful breeders, and non breeders moult slightly later than successful breeders (Kear 2005). Bar-headed Geese blood feather values were representative of more northern latitudes than expected for the catchment area. Therefore, whether these swans and geese began growing blood feathers prior to moving into Mongolia or whether the discrimination factors might be different for these species because of other dietary inputs (i.e., feeding in wetlands sourced by high altitude runoff with more depleted deuterium values) cannot be answered at this point, without knowledge of the specific feather tracts collected and sampling of potential water sources.

Curlew Sandpipers begin their prenuptial and flight feather moult on the wintering grounds before migrating back to the breeding grounds in the tundra of Arctic Siberia (Thomas and Dartnall 1971; Frodin *et al.* 1993), because this is the only period of time where energy is freely available for moulting (Thomas and Dartnall 1971). Our data do not concur with previous observations and suggest that most curlew sandpipers (86%) moult, at least their scapular feathers, on or near their breeding grounds, probably before stopping over in Mongolia on their way to southern Eurasia (Frodin *et al.* 1993), Africa (Wymenga *et al.* 1990), and Australia (Weishu and Purchase 1987). Other authors have also noted variations in timing of moulting between geographical areas (Elliot *et al.* 1976; Barter 1986).

An important point to consider when interpreting isotopic data to track migratory movements of animals is that the relationship between δD_p and δD_f is stable for food webs where H_2 is derived from precipitation. This relationship loses its strength when significant ground water is incorporated into a food system. In situations where birds feed in aquatic food webs, such as lakes and wetlands, weaker δD_p and δD_f relationships are expected (Hobson 2005). Moreover, bodies of standing water are prone to change seasonally in δD values as a result of interactions between evaporation and precipitation (Gibson 2002). In much of Asia, monsoon rains drive the hydrological systems (Hirose and Nakamura 2002) and in the collection areas most precipitation falls during the month of July and August, overlapping with the moulting period of many species sampled here (MG, pers. observ.). At this time it is unclear how much of the food web system in Asia is driven by H_2 from precipitation alone, versus influences from precipitation combined with other water sources such as ground water and/or high elevation run-off. Therefore, in addition to exploring the δD_p and δD_f correlation, the relationship between δD_f of wetland-associated species and seasonally weighted average δD for surface water sources should also be explored.

Claws are a continuously growing tissue at their base, although once grown the tissue becomes metabolically inert retaining the isotopic information of where a specific segment of claw grew. This represents a continuous time of integration from tip to base. However, factors such as wear will influence the expected time period that a claw length represents. Since the growth rate of claws and the discrimination factor between precipitation and claws are unknown for waterfowl in general, the interpretation of δD_c values depends on finding a correlation between δD_f and δD_c, so that δD_c can be converted into δD_f, using the regression equation derived from the correlation. In this manner, δD_c values can be compared to the δD_f base map (i.e., Figure 3). A common error in δD_c interpretation is to place δD_c values directly into a δD_f base map. Although we found a positive relationship between δD values of claws and feathers of Whooper Swans sampled at Sharga Nuur and all Great Cormorants, these relationships were not consistent across all other species and Whooper Swans sampled at all other sites. Correlations were found in other studies in North American species (Clark et al. 2006, Hobson et al. 2006) between δD of feathers and claws, however Clark et al. (2006) state that it is essential to evaluate the species specific patterns and rates of growth in claws in captive and wild species, before reliable inferences can be made.

Although our ability to assign birds in Asia to their breeding, stopover, or wintering sites is still limited, δD_f values have provided valuable information on movements of bird populations in Asia in one season, far exceeding those obtained from neck-collaring and satellite telemetry, over several seasons. However with this great potential comes a need to better understand biological, ecological and biogeochemical factors driving isotopic abundances in Asia. Currently, there are several assumptions inherent to the application of stable-hydrogen isotopes for establishing migratory movements. First, that the feather selected would provide at least a minimum estimate of the numbers of individuals that moulted away from the catchment area. The occurrence of eccentric or interrupted moult experienced by some species (i.e., Pacific Golden Plover; Johnson and Connors 1996) certainly complicates feather selection, but stable isotope analysis offers a feasible solution to investigating moulting patterns (Pérez and Hobson 2006). Sampling individuals from different latitudinal "populations" may help tease out assignment variations in isotopic signatures associated with moulting trade-off in relationship to longer vs. shorter distance migration and breeding seasons (Howell *et al.* 1999; Pérez and Hobson 2006). Second, the precipitation base map and discrimination used here would have to be ground-truthed with feathers of known residents collected at known locations. The precipitation base map was interpolated from long-term IAEA data set and errors are likely to be encountered when used in assignment (Hobson 2005; Lott and Smith 2006). Perhaps some of these species grew their feathers during the monsoon rain periods and their isotopic signature may not match those depicted by annual growing season average. These are questions requiring further investigation.

This study suffered from two major limitations, which jeopardized the meaningfulness of results presented here. One major limitation was the lack of feather-sampling standardization (e.g., collecting the same feather tract across the same species) in consideration of the known moulting schedule of the species examined. The second limitation was the absence of a known relationship between the abundance of δD_f of known origin, and δD_p at a given location. Isotopic studies are designed so that feather analysis will provide information on known moulting origins, and although it is sometimes expected that these areas coincide with breeding origins, at least for flight feathers; it is not always consistent within and among species, and moulting can also occur at stopover site(s) or wintering grounds (Pyle 1997; Leu and Thompson 2002). Having an understanding of migration and moulting patterns by species is essential for establishing expected isotopic values for the general geographic location of the growth of specific feather tracts, regardless of where the birds were sampled (e.g., Mazerolle *et al.* 2005; Pérez and Hobson 2006); especially considering that waterfowl are notorious for moult migrations. Despite the above-mentioned limitations, we derived biologically plausible stories for most of the species sampled, emphasizing the potential for the application of stable-hydrogen isotopes as a geo-referencing tool for birds in Asia and as an aid for investigating the role of wild birds in the spread of avian influenza.

In summary, we identified expected δD_f values for regions of Asia and compared these to the actual δD_f to test this technology in a new region and on novel species. To our knowledge, this is the first document of its kind to compile what is currently known about the range and migration of some important Asian waterbirds together with some actual isotopic values. Most results were compatible with what is currently understood about the species range, and for those that were not, the results warrant further investigation because they could be suggestive of either unreported geographic ranges of a known species, or be further explained by species-specific physiology, moult patterns, high altitude moults or regional weather patterns and environmental influences. Based on this study and the promising results found, our recommendations include: 1) development of a feather collection protocol to standardize sampling efforts and facilitate isotopic interpretations, 2) development of an Asian δD base map for feathers to establish a ground-tested isotopic template and 3) explore δD values of surface water sources particularly around sampling sites to investigate potential variability between δD abundance in precipitation and surface waters.

At a time when avian influenza is expanding its westward range threatening human and poultry populations as well as biodiversity (Daszak *et al.* 2000), our lack of information of avian disease ecology continues to stand in the way of understanding the role of migratory birds in the cross-continental spread of the H5N1 avian influenza virus. Stable isotopes have been demonstrated here as a valid and state-of-the-art technology that can be used to advance our knowledge of important diseases in a rapid and cost effective manner. The results presented in this report may be a useful resource for others in expanding the application of this technology to include more studies on bird migration, disease dissemination and illegal wildlife trade in new regions and species.

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