Spatial patterns of the isotopic composition of bird feathers and their implications

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by

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Certificate

This is to certify that this dissertation entitled ‘Spatial pattern of the isotopic composition of bird feathers and their implications’ towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/ work carried out by Yuvraj Date at Indian Institute of Science Education and Research under the supervision of Dr. Shreyas Managave,Department of ECS, during the academic year 2019-2020.

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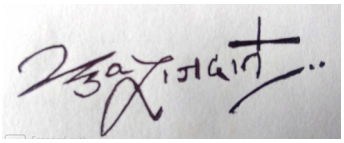
This thesis is dedicated to

Aai, Anna, Aniruddha

for their constant love and support

Declaration

I hereby declare that the matter embodied in the report entitled ‘Spatial pattern of the isotopic composition of bird feathers and their implications’ are the results of the work carried out by me at the Department of Earth and Climate Science, Indian Institute of Science Education and Research, Pune, under the supervision of Dr. Shreyas Managave and the same has not been submitted elsewhere for any other degree



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Abstract

Studying isotopic composition of bird feathers is an emerging field. It can yield important information regarding diet and provenance of the birds. In this study we have carried stable isotopic analysis for the feather museum samples. These samples are obtained from all over the India by BNHS. In this study $\delta^{15}N$ feather values were dependent on tropic level. $\delta^{13}C$ feather values were determined by C3 & C4 proportion in diet of the birds. $\delta^{34}S$ values were dependent on underlying geology of the place but also showed some dependence on diet. Seabird species used showed enriched $\delta^{34}S$ and $\delta^{15}N$ values and this can be used to distinguish between marine diet and terrestrial diet. $\delta^{18}O$ and $\delta^{34}S$ of bird feathers appears to have a spatial control and hence could be useful in studying migration of birds in the Indian context. We have demonstrated here that the birds from different geographical regions/biomes in India have distinct isotopic signatures. Baseline data created in the present study will form basis to study migration of birds in India.

Chapter 1 Introduction

Studying isotopic composition of bird feathers is an emerging field. It can yield important information regarding diet and provenance of the birds. If used in conjunction with traditional observations, it can be a great tool in avian research.

Use of stable isotopes in bird migration studies has recently been initiated. Hydrogen and oxygen isotopic compositions of precipitation are influenced by factors such as the source of water vapor, temperature and the amount of precipitation. Relative influence of these factors varies spatially and often leads to geographically varying isotopic signature of precipitation. Studies have shown that the plants do preserve such distinct isotopic signature of precipitation. The same isotopic signature is further carried and preserved in the food chain, although with some modifications. Similarly, the carbon and nitrogen isotopic compositions of plants (and subsequently the food chain) often show distinct geographical patterns. The sulfur isotopic composition also varies geographically as the different rocks often have different sulfur isotopic composition. As C, H, N, O and S constitutes the major composition of animal tissues, its isotopic composition give clues to identify its geographical location. Migration of birds or animals between such isotopically distinct locations can be deciphered by the isotopic studies of appropriate tissues (e.g. bird feathers) wherein, once incorporated, the isotopic signature gets ‘locked’ and do not change subsequently. In this way, the comparison of isotope ratios in feathers of a bird could reveal its migration route because the feather isotopic ratios would mirror the food sources of the sites visited.

Isotope approach to study the migration of birds has certain advantages over traditional studies such as bird ringing. Success of the latter greatly depends upon finding the ringed bird while in the case of the former any individual bird would suffice, provided it carries the distinct isotopic signature of the region where it stayed/ grown. Isotopic studies are also likely to complement regular ringing studies by

narrowing on identifying the probable regions from where the birds are migrating (Hobson et al. 2018). One of the implications of this is in identifying breeding places of migratory (either local or regional or intercontinental) birds, migratory routes and thereby helping in prioritizing areas for habitat conservation (Robinson et al. 1999).

Migration of the birds within the Indian region is not well understood (Balachandran 2002). The potential of using the isotopic composition feathers to find out migratory routes of birds from Indian region is not explored. The first step towards successfully applying this approach is to demonstrate that the birds from different geographical regions/biomes in India have distinct isotopic signatures. In order to understand the regional isotopic signatures a baseline data needs to be created. The present study aims at this by carrying out a preliminary study of the isotopic analysis of feathers of resident birds from different parts of India. This data will be generated using feathers from BNHS's bird collection. The outcome of this pilot study will form the basis for interpretation of further studies.

1.1 The theory behind C, H, N, O, S isotopic studies

Isotopes are two or more forms of the same element having the same number of protons but different numbers of neutrons. Few of them are stable, easily measurable and can be used in ecological and migration studies. Isotopes of carbon, hydrogen, nitrogen, oxygen, and sulfur are most useful in migratory studies and called ''light isotopes'' These elements are the building blocks of life. The isotopic ratio of these elements varies widely in nature. Other element's isotopes are not used because either they are radioactive and not stable or they are present in a very trace amount and are hard to detect using IRMS. Heavier elements stable isotopes are difficult to measure, costly and susceptible to contamination very easily. Some heavy stable isotope exhibits very little natural variation thus cannot be used for migration studies however in some cases strontium (Sr) has been used in migration studies.

Element	Isotope	Abundance
Carbon	^{12}C	98.9‰
	^{13}C	1.1‰
Nitrogen	^{14}N	99.6‰
	^{15}N	0.4‰
Sulfur	^{32}S	94.99‰
	^{33}S	0.75‰
	^{34}S	4.25‰
	^{36}S	0.01
Hydrogen	^{1}H	99.98‰
	^{2}H	0.02‰
Oxygen	^{16}O	99.76‰
	^{17}O	0.04‰
	^{18}O	0.20‰

Table 1.1: Stable isotopes and their composition

All elements (CHNOS) have one highly abundant isotope and other rare heavier isotope. Directly measuring isotopic ratio in tissue is difficult, so rare to an abundant ratio of the isotope is measured using Isotopic ratio mass spectrometer (IRMS). Details about IRMS are given in chapter 2.

1.1.1 Stable isotope method

For migration studies light isotopes of the element carbon, hydrogen, nitrogen, oxygen, and sulfur are used. These five elements comprise the primary building block of the biosphere, hydrosphere and the atmosphere. Relative differences are measured between the combusted gaseous sample and reference using IRMS(Isotope ratio mass spectrometry)

$$\delta_X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

Where *X* is a stable isotope of interest

Isotopic fractionation occurs when the chemical, biological or physical process results in a changing of stable isotope ratio of source and reactant. A stable isotopic method exists due to fractionation. Because of fractionation, there are differences in

isotopic signatures. Isotopic fractionations of light isotopes in nature are widespread and diverse.

The key advantage of using feathers is they are metabolically inert and grown over a very short period of time at a specific location. But, it's not always the case, some birds develop their feathers in enroute and the isotopic variations are captured over the length of the feather. For maternally fed animals their feathers will reflect the signature of the place from where they are getting their food.

1.2 Nitrogen

Atmospheric nitrogen is inert as compared to its other forms. It is turned into fix nitrogen by microorganisms and can be used by plants (Hoefs 2015).

N_2 fixation	$N_2 \rightarrow N_{org}$	−2 to +2 ‰
NH_4^+ assimilation	$NH_4^+ \rightarrow N_{org}$	+14 to +27 ‰
NH_4^+ oxidation (nitrification)	$NH_4^+ \rightarrow NO_2^-$	+14 to 38 ‰
Nitrite oxidation (nitrification)	$NO_2^- \rightarrow NO_3^-$	−12.8 ‰
Nitrate reduction (denitrification)	$NO_3^- \rightarrow NO_2^-$	+13 to +30 ‰
Nitrite reduction(denitrification)	$NO_2^- \rightarrow NO$	+5 to +10 ‰
Nitrous oxide reduction(denitrification)	$N_2O \rightarrow N_2$	+4 to +13 ‰
Nitrate reduction (nitrate assimilation)	$NO_3^- \rightarrow NO_2^-$	+5 to +10 ‰

Table 1.2: Nitrogen isotope fractionations for microbial cultures (Casciotti 2009).

Stable nitrogen isotopes are used to determine animal diets and their position and interaction with other trophic levels. Nitrogen isotopes are not used for tracing animal's origin but can be used to fine-tune the origin. $\delta^{15}N$ values of plant and animal vary largely at a small spatial scale due to numerous influences. This includes natural as well as anthropogenic factors like agriculture, land use, use of fertilizers, sewage disposal and industries. $\delta^{15}N$ values of plant and soil vary tremendously due to different processes and pools involved thus hard to model (Pardo and Nadelhoffer 2010; Amundson et al. 2003) compiled data and created a

global isoscape for $\delta^{15}N$. Their finding shows that soil and plant $\delta^{15}N$ value decrease with increasing mean annual precipitation and decreasing mean annual temperature.

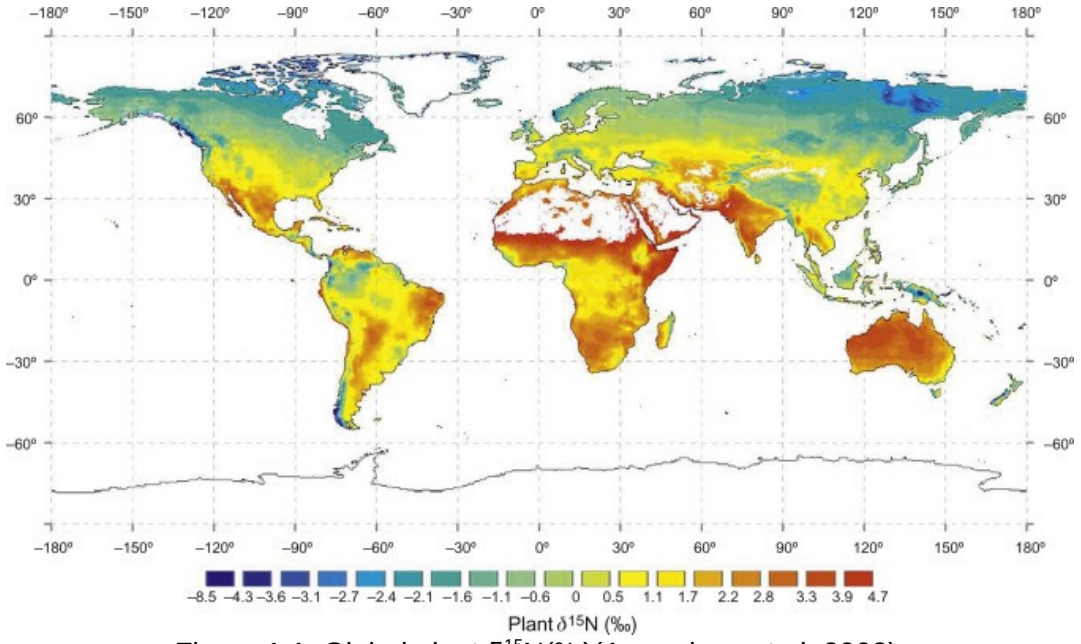


Figure 1.1: Global plant $\delta^{15}N$(‰)(Amundson et al. 2003).

$\delta^{15}N$ values in feathers are used to identify biome supporting animals during their tissue growth. The terrestrial source of nitrogen is more depleted than the marine source. In the terrestrial sources, agricultural $\delta^{15}N$ is more enriched than non-agricultural $\delta^{15}N$. Places with high temperatures have food webs with high $\delta^{15}N$ values than cooler or wetter areas(Hobson 1999).

$\delta^{15}N$ measurements are used to determine trophic positions. With the increase in trophic level, $\delta^{15}N$ values are increased with 2.5‰ to 5‰ (Layman et al. 2012). It's challenging to use this information in omnivores birds as their diet keeps changing. But if the information on the bird's diet is known then we can distinguish between carnivore vs frugivore birds.

Source:

Nitrogen is not present in carbohydrates and fats. So, the main source of nitrogen in bulk tissue is through the dietary proteins. Nitrogen is easily incorporated from essential amino acids with little isotopic discrimination, as they are easily incorporated into body. Nitrogen from non-essential amino acids shows more

discrimination as it goes through physiological processes. Diet with poorer quality shows high discrimination than a diet with a higher quality.

Physiological & biosynthetic factors also affect $\delta^{15}N$. Animals adapted to arid conditions recycle urea to conserve water this changes their $\delta^{15}N$ values (Ambrose and DeNiro 1986).Geese breeding at high altitude fast and undergo protein catabolism which increases body $\delta^{15}N$ values (Hobson, Alisauskas, and Clark 1993).

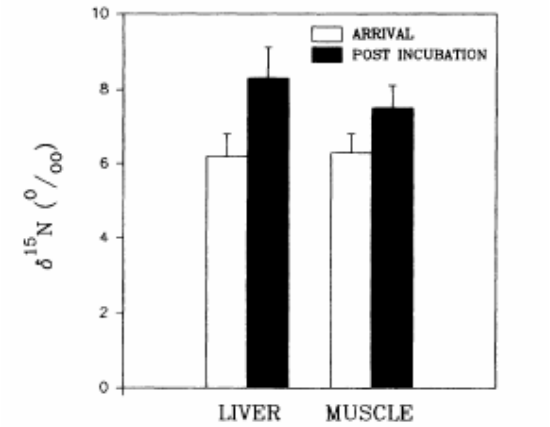


Figure 1.2: Stable nitrogen isotope ratios of the tissues of female Ross' Geese taken upon arrival and after incubation at Karrak Lake, Northwest Territories.

1.3 Carbon

Like nitrogen, carbon isotopic composition is also used to determine animal diets and their trophic interaction. Carbon isotopes are used to get information about biomes which are supporting animals. In terrestrial plants, $\delta^{13}C$ values differ with photosynthetic pathways used to fix the atmospheric carbon dioxide. In marine systems, $\delta^{13}C$ values are affected by algal growth. C3 plants show more depleted $\delta^{13}C$ values as compared to the C4 plants. This is the result of specific structure of C4 plants which got evolved to conserve water. C4-pathway involves PEP carboxylase at the beginning whereas such pathway is absent in C3-pathway. Due to different ways of fixing the carbon atoms, overall, C4 plants get $\delta^{13}C$ values from -12 to -16 ‰. Stomatal opening, plant water use and climate also play a key role in determining $\delta^{13}C$ values of plant tissue. With a scarcity of water stomata close which increases $\delta^{13}C$ of a plant. Closing of stomata decreases CO2 diffusion out of the leaf this results in the enrichment of $\delta^{13}C$ (Hemming et al. 2005).

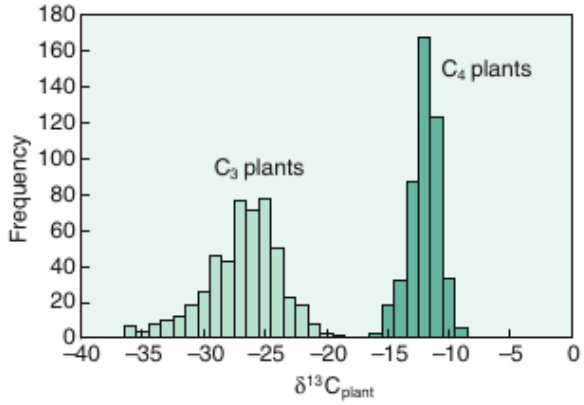


Figure 1.3: Histogram of $\delta^{13}C$ values of C3 Vs C4 plant (Ehleringer and Cerling 2002).

Pathway	$\delta^{13}C$ (‰)
C3	-20 to -37
C4	-12 to -16
CAM	-10 to -20
Phytoplankton	-18 to -25

Table 1.3: Different photosynthetic pathways and $\delta^{13}C$ range

Sources:

Carbon is present in dietary protein fat and carbohydrates. Lipids in the diet are transferred directly with very little isotopic discrimination. Carbohydrates are used for energy and they are burned to release CO_2 as their byproduct So, it is not stored in bulk tissue. The $\delta^{13}C$ value of bulk tissue is more likely to be associated with the dietary proteins (Hobson 2018). Like nitrogen, carbon is also used to infer diet and biome information. $\delta^{13}C$ values are heavily influenced by anthropogenic factors like agriculture, land use, irrigation, etc.

Carbon isoscape is made by modeling the expected area covered by C3 Vs C4 plants. This modeling is done by using available satellite data, crop records temperature and rainfall of the place. C3 & C4 plants differ physiologically. C4 plants are found in an area having a temperature of more than 22^0C and precipitation 25 mm or more per month (West et al. 2005; Suits et al. 2005; Still and Powell 2010 ;Still et al. 2003).

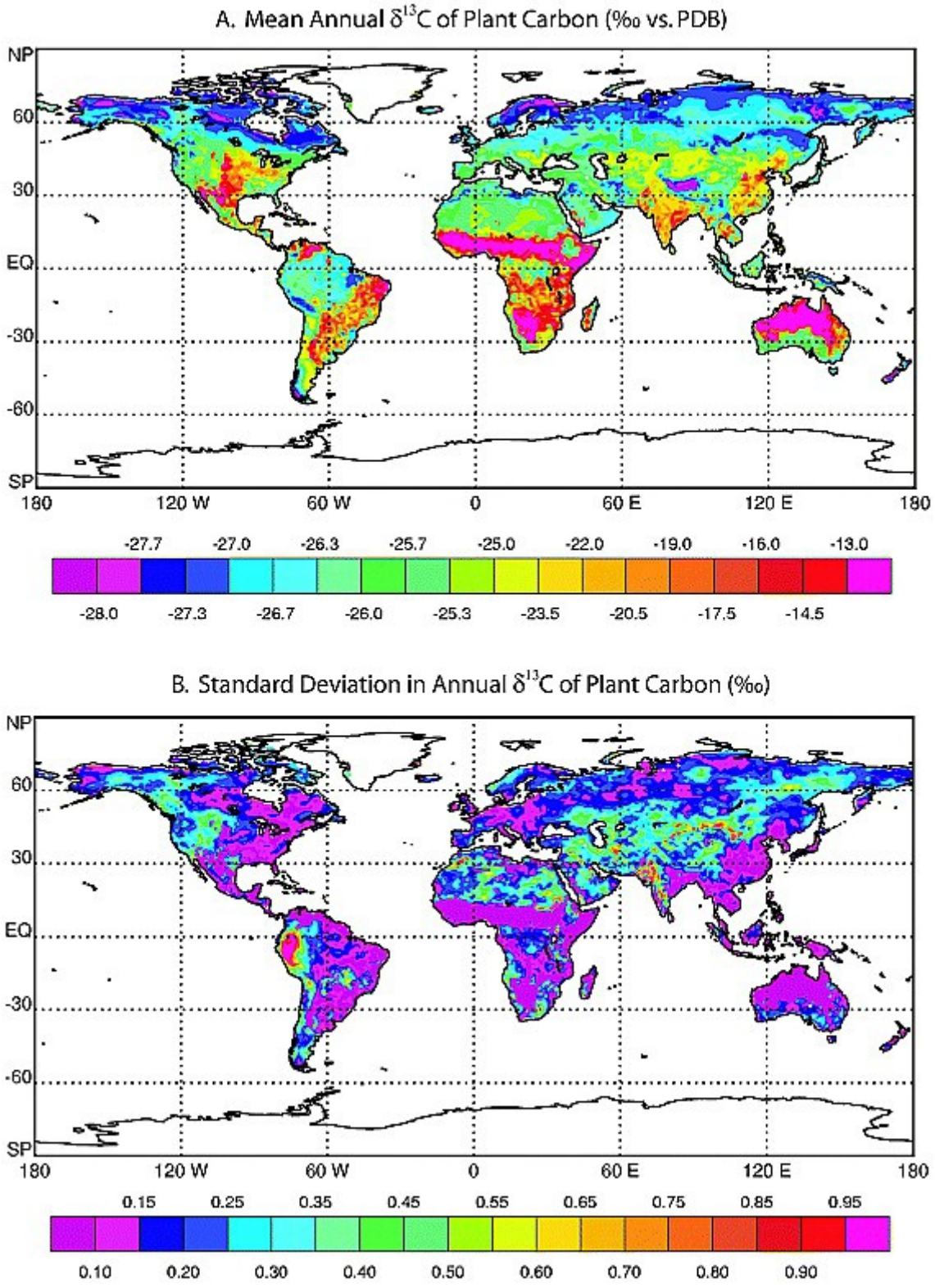


Figure 1.4: Mean annual $\delta^{13}C$ and standard deviation in $\delta^{13}C$ (Suits et al. 2005)

1.4 Sulfur:

Sulfur shows little isotopic discrimination. Source of sulfur in animal tissue is dietary proteins containing Sulfur-bearing amino acids. Sulfur shows very little isotopic discrimination as essential amino acids are readily absorbed by the body giving very little time in isotopic discrimination. So, sulfur isotopes can be used as direct tracers in the food web and migration studies.

Naturally occurring sulfur isotope variation is due to different microbial processes. Many microorganisms use sulfur for their metabolism. Chemical reactions involving different sulfur compounds also adds to the variation (Hoefs 2015).

Marine $\delta^{34}S$ values are influenced by the sea while terrestrial $\delta^{34}S$ values depend upon the geology of the place. Marine-derived sediments and volcanic rocks have enriched values compared to other sources. Animals related to anaerobic sediments in marshes for their diet also show enrichment in $\delta^{34}S$ values. Sulfur isotopes are used to distinguish between animals belonging to marine Vs terrestrial habitat.

1.5 Hydrogen and Oxygen

In migration and animal movement tracking using isotopic studies Hydrogen is the most important isotope used. Hydrogen isotope is the most robust among all stable light isotopes used for migratory studies. $\delta^{2}H$ values vary significantly globally. $\delta^{2}H$ has a long-range of variation around ~500‰ and very less analytical error ~2‰. This provides a very high signal to noise ratio. Sources of Hydrogen in the animal body are through water and food. The only problem with Hydrogen is it's exchangeable with atmospheric vapor. A comparative equilibrium approach is taken to remove this effect (Wassenaar and Hobson 2003).

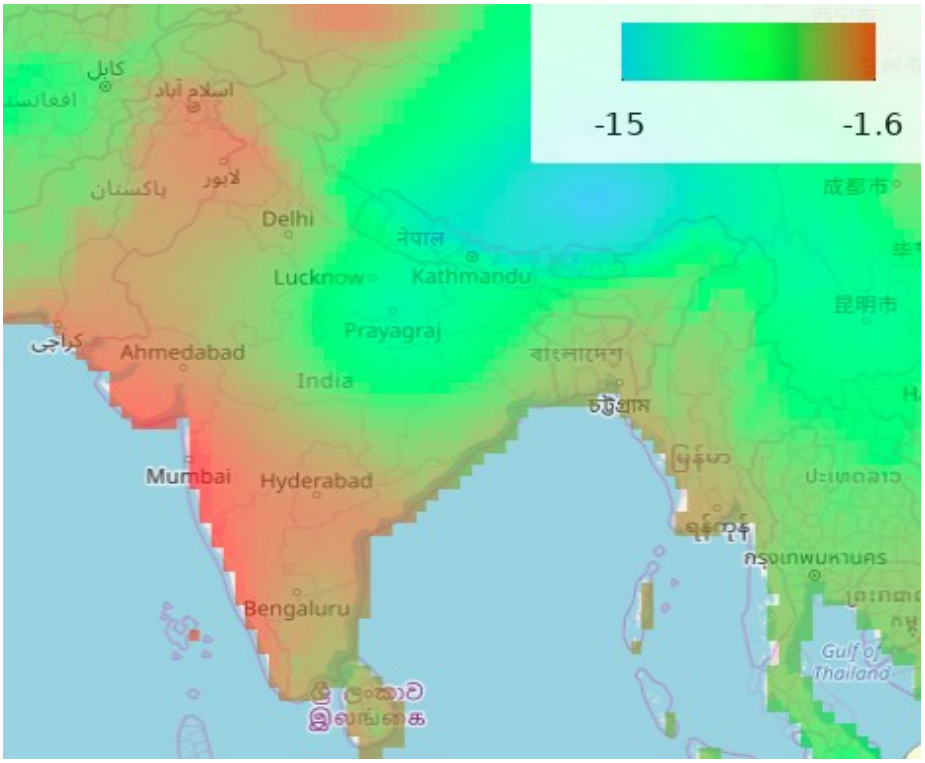
Unlike hydrogen, oxygen present in the bulk tissue is nonexchangeable. The global range of oxygen isotope is ~15‰. $\delta^{18}O$ has a high analytical error of around ~0.5‰ making it less robust than Hydrogen isotope.

δ^2H and $\delta^{18}O$ values change with temperature, elevation, moisture, and rainfall. Heavier isotopes are the first to be removed from the cloud system. So as the cloud system proceeds it gets depleted in δ^2H and $\delta^{18}O$ isotope this creates gradient. Hydrological mixing, evaporation also affect δ^2H values. This spatial pattern created by rainfall is connected to terrestrial food webs through plants. Water in plants is unfractionated as well as fractionated (in case of leaves water). Leaf water gets enriched in δ^2H and $\delta^{18}O$ with evaporation. Leaf water isotopic composition is important as it is the potential source of the animal diet.

Hydrogen is acquired through diet and water. Oxygen sources in animal tissue are mainly oxygen inhaled from the air, dietary oxygen, oxygen from water and sinks in various biosynthetic pathways which produce H_2O and CO_2. Since there are many sources and sinks for oxygen in the body, oxygen isotope can be complicated sometimes in providing information about migratory animals.

Processes are similar by which $\delta^{18}O$ global distribution appears. So, $\delta^{18}O$ are tightly coupled with δ^2H values. So most times $\delta^{18}O$ studies do not provide any additional information in the migratory studies.

Isoscape for hydrogen and oxygen can be created using online tools http://isomap.org This contains globally collected data for precipitation hydrogen and oxygen isotopic values. This tool can be used to predict the isotopic value of δ^2H and $\delta^{18}O$ at a given place



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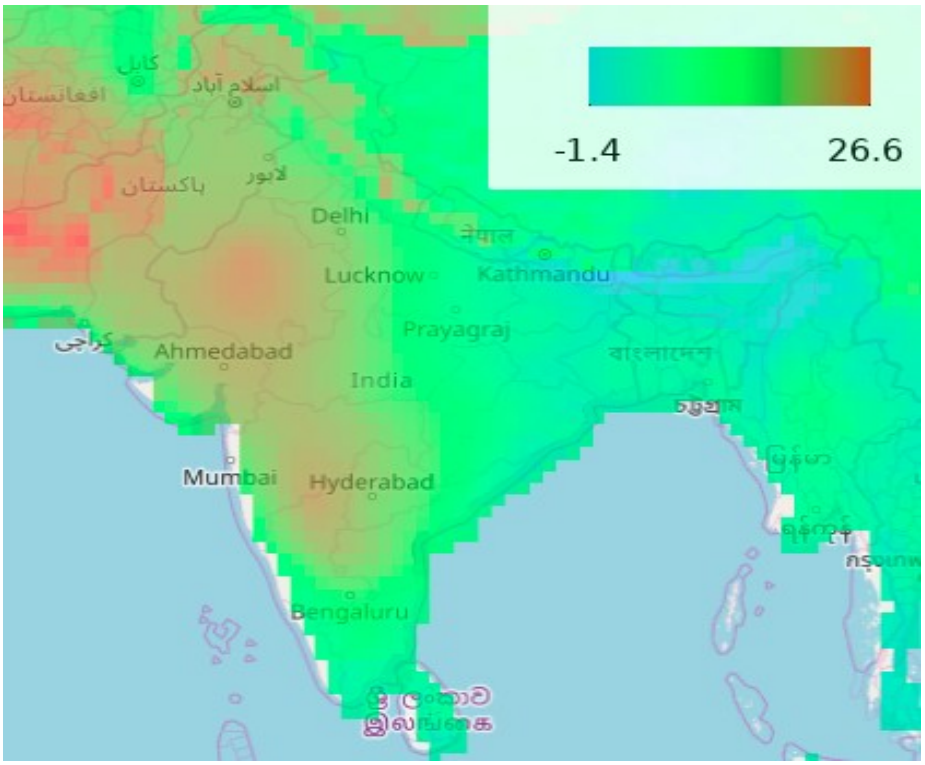


[b]

Figure 1.5: Precipitation $\delta^{18}O$ isoscape for India [a]. Leaf water $\delta^{18}O$ isoscape for India [b]. Created using www.isomap.org (Bowen, Wassenaar, and Hobson 2005)

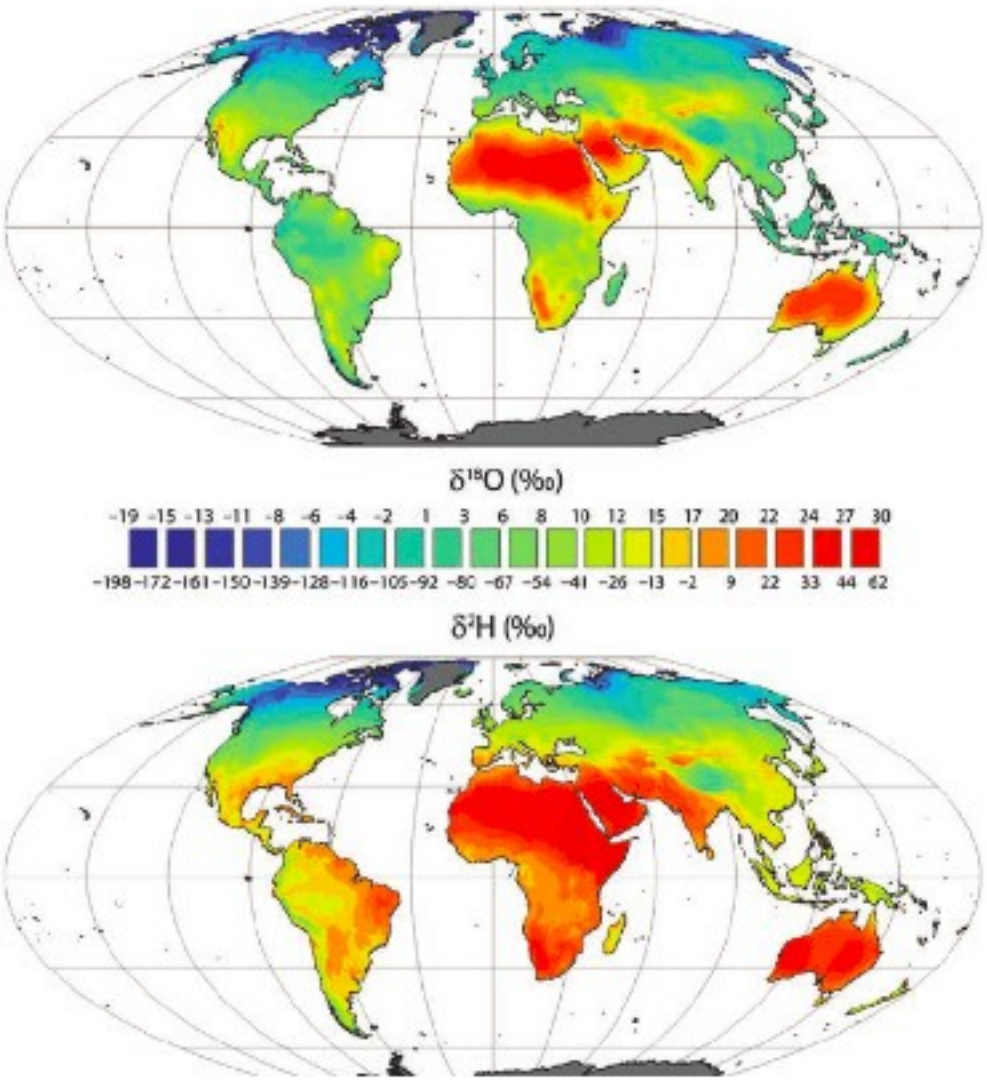


Figure 1.6: Global mean annual average $\delta^{2}H$ and $\delta^{18}O$ leaf water isoscape (West, Kreuzer, and Ehleringer 2010)

Chapter 2 Materials & Methods

2.1 Samples and sample locations

All the feather samples, except that of the Bar-headed Goose, analysed in the present study are from museum specimens obtained from the Bombay Natural History Society (BNHS). The sample of Bar-headed Goose was collected during a field visit conducted by BNHS. The samples analysed (Table 2.1) are from ~62 locations spanning all over India (Figure 2.1). A sample from Pakistan was analysed as well. The museum samples of BNHS have been collected over an extended period of time. Out of the specimens chosen for the isotopic analysis, the oldest specimen was collected during 1876 and the newest, during 2016. The information about location, gender and the date/month of collection is available for almost every specimen. No chemicals were applied on the external part of the museum specimens. Therefore, the feathers do not contain traces of preservatives. BNHS regularly cleans the specimens with a brush. The feathers coming out during cleaning were used for the analysis (Figure 2.2).

Species	**Scientific Name**
House Sparrow	*Passer domesticus*
Red-vented Bulbul	*Pycnonotus cafer*
Common Myna	*Acridotheres tristis*
Indian Robin	*Copsychus saularis*
House Crow	*Corvus splendens*
Spotted Owlet	*Athene brama*
Red-billed Tropicbird	*Phaethon aethereus*
Masked Booby	*Sula dactylatra*
Brown Booby	*Sula leucogaster*
Red-footed Booby	*Sula sula*
Lesser Frigatebird	*Fregata ariel*
Great Frigatebird	*Fregata minor*
Bar-headed Goose	*Anser indicus*

Table 2.1: Species analysed in this study

Most of the species analysed in the present study are local resident species. Local resident species were chosen to get an idea about geographical variation in the stable isotopic compositions of feathers of different species. Further, species belonging to different trophic level were considered. Coastal seabird species are also analysed to compare the isotopic variations between coastal and inland species. To check the evolution of isotopic composition of feathers during migration, a feather of Bar-headed Goose was analysed.

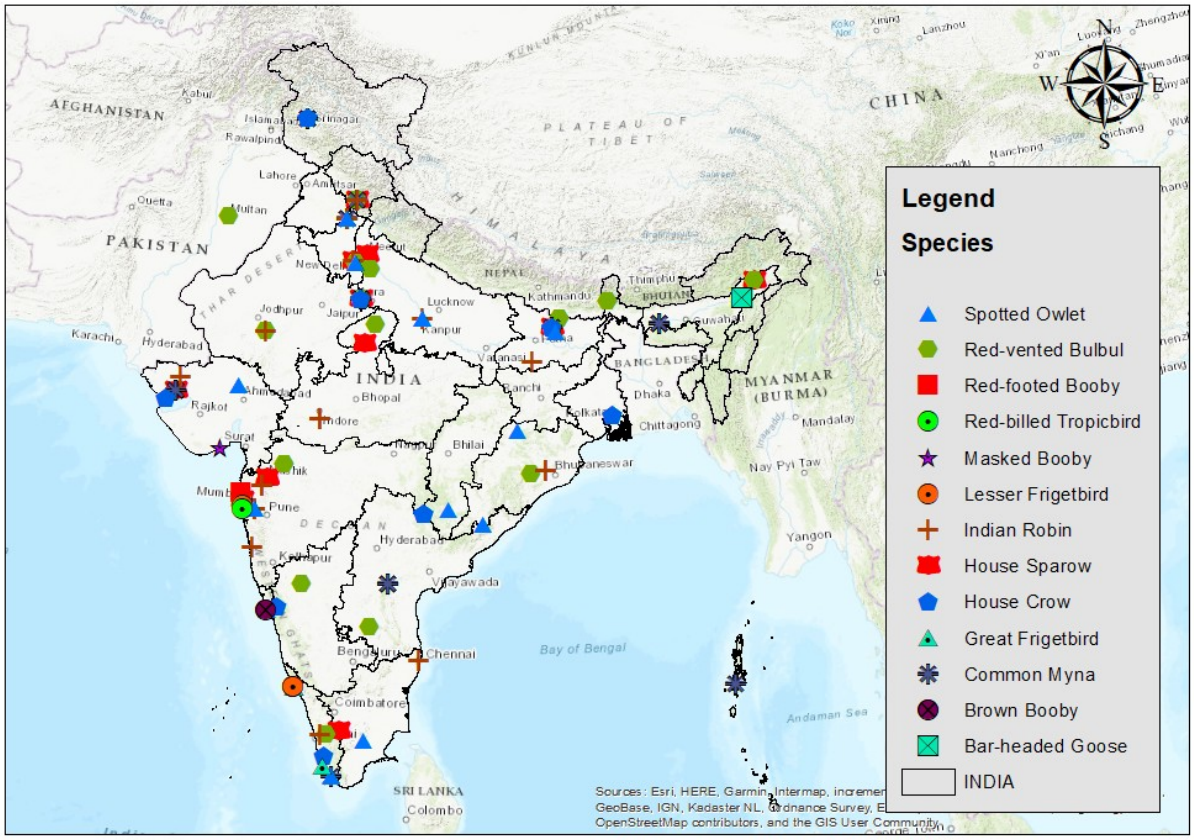


Figure 2.1. Locations of the specimens selected in the present study

2.2 Sampling strategy

The analysed feathers are typically chest feathers (Figure 2.2). Weight of the individual feathers ranged from less than 1mg to 4-5 mg. Typically, 3 to 6 feathers from individual specimens were chosen for isotopic analysis.

To check for the heterogeneity in the isotopic compositions, we have analysed multiple feathers from the same specimen, multiple specimens of the same species from a give location and multiple species from the same location. Because of limited availability of feathers from a given specimen and limited number of species/specimen from a given location, the heterogeneity study could not be conducted for every specimen and every location.

Feathers were cut into half sections not taking rachis as rachis gives variability in isotopic values (Bontempo et al. 2014). Half of its part was used for carbon, nitrogen and sulfur isotope analysis and the other half was used for Oxygen isotope analysis. For some species a single half of the feather was not enough for isotopic analysis. In such cases two feathers were used.

We had two long Bar-headed Goose feathers as shown in the Figure 2.2b. One of them was used for analysis. We cut the feather into 6 sections equidistant from each other to get the idea about evolution of isotopic composition of feather during its migratory route.

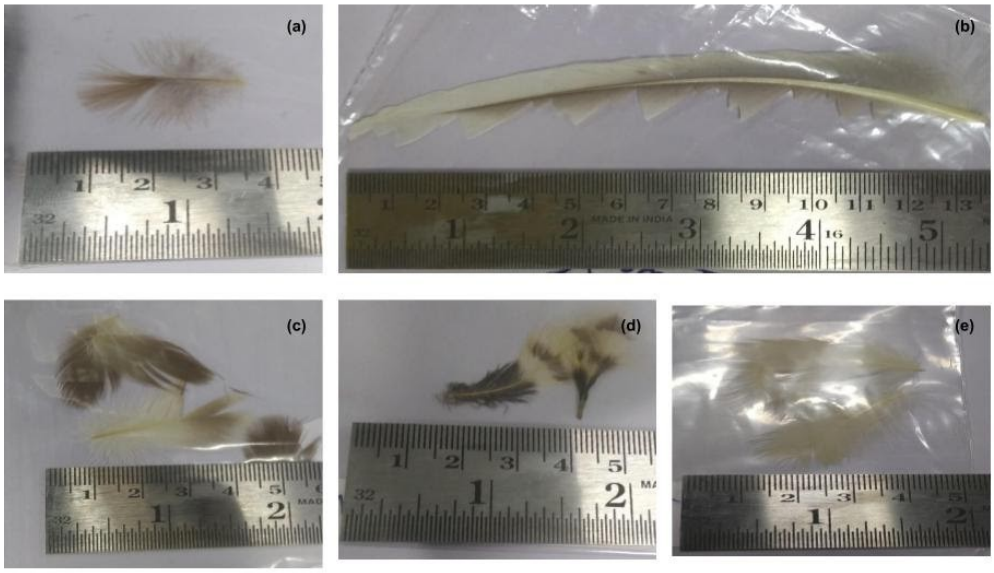


Figure 2.2. Photographs showing typical feathers analysed in the present study. The feathers in the photo belong to Common myna (a), Bar-headed Goose (b), *Sula leucogaster* (c), Spotted owlet (d) and *Sula dactylatra* (e).

2.3 Sample cleaning

Following procedure was used to prepare the samples for isotopic analysis.

- Feathers were first cleaned using Mili Q water 3 times
- All dust and sticky substances were removed manually using hand gloves
- Feathers were then cleaned in an ultrasonicator. Feathers were dipped in Milli Q water inside the test tubes. Ultrasonication was done for 10 minutes. Ultrasonication ensures that feathers are cleaned without any damage.
- After ultrasonication, feathers were dried in a hot air oven at the temperature 45 degrees for 24 hours to remove any water or moisture content.
- Natural oils and lipids are present on the feather. Since they are made of hydrocarbons they affect C and H isotopes. Surface oils have different isotopic values than the bulk tissue. So, it is essential to remove surface oil before isotopic analysis. To remove surface oils and lipids solvent of Chloroform: Methanol in 2:1 v/v ratio was used. Samples were dipped inside the solvent and were kept under a fume hood. This solvent treatment was done for 24 hours during which after 8 Hours solvent was changed with a fresh one (Paritte and Kelly 2009).
- After these samples were air-dried inside a fume hood for 24 hours.
- Cleaned and completely dried feather samples were kept in sterile 20 ml Borosil glass vials with a screw cap and were labeled with sample identification.

2.4 Sample weighing and packing

2.4.1 For CNS stable isotopic analysis

- Half part of feathers was used for CNS analysis and the other half was kept in a glass vial for hydrogen and oxygen analysis.
- A calibrated microbalance (Mettler Toledo) was used for sample weighing.

- Feathers were cut using cleaned scissors. Rachis was not taken for analysis as it shows variable isotopic value than the rest of the feather (Bontempo et al. 2014).
- Feathers were transferred into a tin boat using tweezers. Tin boats used were made from 35 x 35 mm tin foil.
- Around 1 mg of feather sample was packed. The tin capsule was carefully closed without any stray edge, loose side or sample material coming outside the capsule.
- Packed samples were put into the 96 well sterile Tarsons tissue culture plate
- The sample's name and its weight with its position in 96 well culture plate were recorded.
- After packing of every sample, all the weighing and packing tools were cleaned with methanol and Kimwipes.
- Standards were weighed in 4 x 4 x 11mm tin boats
- These packed samples with standards were added to the autosampler.

2.4.2 For OH analysis

- Other half of the feather remaining after CNS isotopic analysis was used for OH analysis
- Feathers and standards were kept in laboratory conditions for more than 96 hours. The laboratory temperature was set at 22^0C. This is also known as a comparative equilibrium approach as Hydrogen is exchanged with the ambient moisture. This process reaches its equilibrium at more than 96 hours.
- Around 1 mg of the feather sample was weighed and packed according to the procedure mentioned above.
- For OH analysis samples are packed in silver foil. For this 40 x 40mm silver foils for elemental analysis were cut into 3 equal rectangles.
- Using a clean metal rod it was pressed to have a shape of a boat.
- Around 1 mg of feather sample was packed. The boat was carefully closed without any stray edge, loose side or sample material coming outside the capsule.

- After packing of every sample, all the weighing and packing tools were cleaned with methanol and Kimwipes.
- Standards used for OH analysis were weighed and packed in silver foil.
- These packed samples with standards were then loaded in the autosampler.

2.5 Isotopic measurements

2.5.1 CNS analysis using combustion mode

- For combusting the samples Elementar Vario Pyro cube was used
- Samples were combusted in the oxygen-rich environment. Helium was used as a carrier gas.
- Combustion tube was operated at 1150^0C
- Combustion can create the following compounds CO_2, H_2O, N_2, NO_x, SO_2 and SO_3 and volatile halogens.
- After combustion the oxidized species were made to move through the reduction tube. Reduction tube was filled with copper fillings and was kept at 850^0C. The oxidised species got reduced in the reduction tube.
- Silver wool is used in the reduction tube to remove halogens. After reduction the species of interest coming out were N_2, CO_2 and SO_2.
- Absorption tube filled with phosphorus pentoxide was used to remove water vapour content of the gas.
- The separation of the gases were carried out through an Advanced Purge and Trap (APT) method. The pyro cube contains two columns, one to trap SO_2 and other to trap CO_2. The column dedicated to SO_2 was set at 55^0C wherein N_2 and CO_2 are not retained but SO_2 is trapped. In another APT column temperature is maintained at 200^0C. Here CO_2 is retained and N_2 is passed directly to IRMS. After that APT column for CO_2 is heated and analysis for CO_2 is done in a similar way. In the last step, the APT column for SO_2 is heated to release SO_2 into IRMS
- Isoprime precisiON was used to give the relative isotopic ratio.

- Before every batch linearity and stability runs were given.

2.5.2 OH analysis by pyrolysis mode

- For analysis Elementar Vario Pyro cube was used in pyrolysis mode
- In the pyrolysis mode, the sample gasses went through a tube filled with glassy carbon chips. This tube's temperature is set at 1450^0C and its packing is optimized for a higher carbon surface area.
- From the pyrolysis tube gas went to an absorption tube consisting of sodium hydroxide and phosphorus pentoxide to remove acidic and basic pyrolysis products and water.
- In O-H mode gas first traveled through the CO APT column where CO is retained and H_2 went in IRMS. After analysis of H_2, CO column heats up and CO is released into IRMS.
- Isoprime precisiON was used to give the relative isotopic ratio.
- Before every batch linearity and stability runs were given.

2.5.3 Standards

- Following reference materials were used to assign isotopic composition to the analyzed gas species.

Name	δ^{15}N (Air)	δ^{13}C (VPDB)	δ^{34}S (VCDT)	δ^{18}O (VSMOW)
Sulfanilamide 2	-6.27	-27.85	-5.66	
Sulfanilamide 1	-3.21	-27.75	-8.91	
Merk Sucrose		-12.11		
IAEA-CH3		-24.72		
IAEA S1			-0.3	
IAEA S2			22.7	
CBS				2.5
KHS				21.46

Table 2.2. Reference materials used during the isotopic analysis and their isotopic ratios (given in ‰)

The measured isotopic ratios are reported with respect to standards (given in Table 2.3) and are given in "δ" notation in permil (‰).

It is calculated as

$$\delta_X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R_{sample} and $R_{standard}$ respectively are the isotopic ratio of the species x (i.e. $^{2}H/^{1}H$ for Hydrogen; $^{13}C/^{12}C$ for carbon; $^{15}N/^{14}N$ for nitrogen; $^{18}O/^{16}O$ for oxygen; and $^{34}S/^{32}S$ for sulfur) in the sample and standard.

All samples are measured and reported with respect to international scales which are defined by the original primary reference standards as given in Table 2.3

Primary reference standard	Isotope ratio	Absolute abundance ratio
Vienna Standard Mean Ocean Water (VSMOW)	$^{2}H/^{1}H$	0.00015576
Vienna Pee Dee Belemnite (VPDB)	$^{13}C/^{12}C$	0.0112372
Vienna Standard Mean Ocean Water (VSMOW)	$^{18}O/^{16}O$	0.0020052
Atmospheric Nitrogen (air)	$^{15}N/^{14}N$	0.0036765
Vienna Canon Diablo Troilite (VCDT)	$^{34}S/^{32}S$	0.0450045

Table 2.3. Normalizing isotopic ratios for various isotopes

Repeated measurements of the laboratory reference materials gave the reproducibility better than 0.15, 0.45, 0.35 and 0.3 ‰ for carbon, nitrogen, sulfur and oxygen, respectively.

Chapter 3 Results & Discussion

3.1 Variation in the isotopic composition among different species

The variation in the isotopic composition of different species of birds reflect mainly their diet and geographical distribution. Nitrogen isotopic composition reflect trophic level of the species. Carbon isotopic composition on the other hand reflects relative contribution of C3 and C4 plants in the diet of the birds. Sulfur isotopic composition likely reflects bulk Sulfur isotopic composition of the environment (i.e. water and rocks/soils). Oxygen isotopic composition is inherited from the oxygen isotopic composition of the environmental water which in turn depends on isotopic composition of precipitation and subsequent evaporative enrichment. This section deals with description of isotopic variability among different species.

3.1.1. $\delta^{15}N$ variations

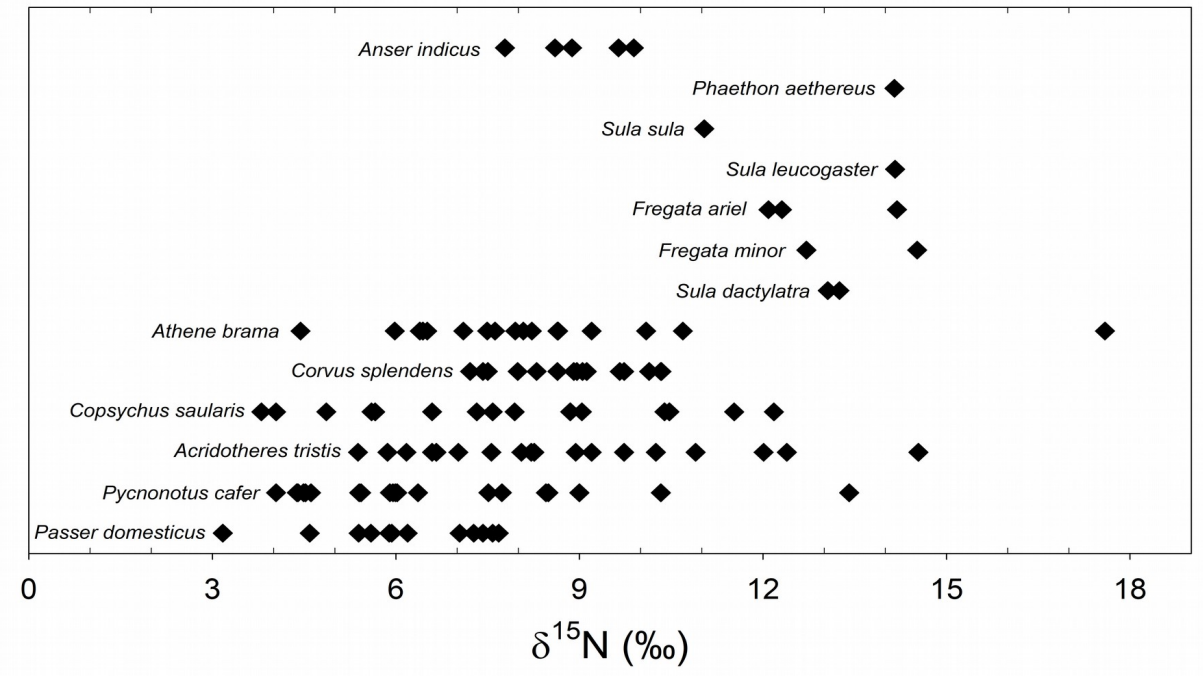


Fig 3.1. Nitrogen isotopic variation

Minimum value obtained during nitrogen analysis was 3.2‰ in case of House Sparrow obtained from Shimla, Himachal Pradesh. The highest value for nitrogen analysis was 17.6‰ in case of Spotted Owlet for a sample obtained from Madurai, Tamil Nadu. House Sparrows $\delta^{15}N$ values are close to each other except the lowest value of 3.2‰ for the bird obtained from Shimla. House Sparrow shows the lowest mean and low standard deviation implying their diet consist of less insect and among all other species used in the analysis they are at bottom of food chain. House Sparrows are absent from undisturbed forest and grasslands. They are found nearby human settlements. They are dependent on grains, weed, grass seeds this is reflected in their lower $\delta^{15}N$ value.

After Sparrow, Red-vented Bulbul's nitrogen values are also on lower side. They are found in dry scrubs, open forests, plains and cultivated lands. They are frugivore in nature but their diet also includes plant buds, nectar and insects. Though their $\delta^{15}N$ values are on lower side few higher $\delta^{15}N$ values can be explained by shifting of their diet on insects. Indian Robin are found in open woodland and cultivated areas close to human habitation. Indian Robin are insectivore in nature and their mean nitrogen isotopic value is higher than that of House Sparrow and Red-vented Bulbul. Common Myna are mostly found near human habitat. For food they depend mostly on insects in absence of insects it can become agricultural pest as it shifts its diet to grains, fruits and nectar. Common Myna are also a known scavenger. This variety in their diet depending on the availability of food is indicated in its high standard deviation. House Crow is opportunistic omnivorous bird which lives near human settlements. It is good scavenger, consumes human waste and leftover. It feeds on insect small mammals and corps of dead animals. The lower standard deviation in case of house crow implies that its tropic level is similar in all the sampling areas. Spotted Owlet lives in open habitat. Spotted Owlets feeds on rodents, frogs, small birds and insects. It is higher on the food chain and it's $\delta^{15}N$ isotopic values are relatively higher as well. Though its mean $\delta^{15}N$ values are lower than Myna it can be explained as owlets diet does not contain any agricultural material which may be enriched in $\delta^{15}N$ due to fertilizers. Marine food sources are more enriched isotopically in $\delta^{15}N$ than that of the terrestrial sources (Hobson 1999),

also seabird species diet mostly constitute of the small fish, flying fish, squids placing them higher on the food chain. This is reflected in their highest $\delta^{15}N$ values which is totally distinct from all the other inland species.

Species(Nitrogen)	No.	Minimum	Maximum	Mean ± Std
House Sparrow	12	3.2	7.7	6.1 ± 1.4
Red-vented Bulbul	15	4.0	13.4	6.8 ± 2.5
Common Myna	11	5.4	14.5	9.2 ± 2.8
Indian Robin	14	3.8	12.2	7.7 ± 2.7
Spotted Owlet	12	4.4	17.6	8.5 ± 3.2
House Crow	9	7.2	10.3	8.9 ± 0.9
Seabird species	9	11.0	14.5	13.3 ± 1.1

Table 3.1. Nitrogen isotopic variation among analyzed species

3.1.2. $\delta^{13}C$ variations

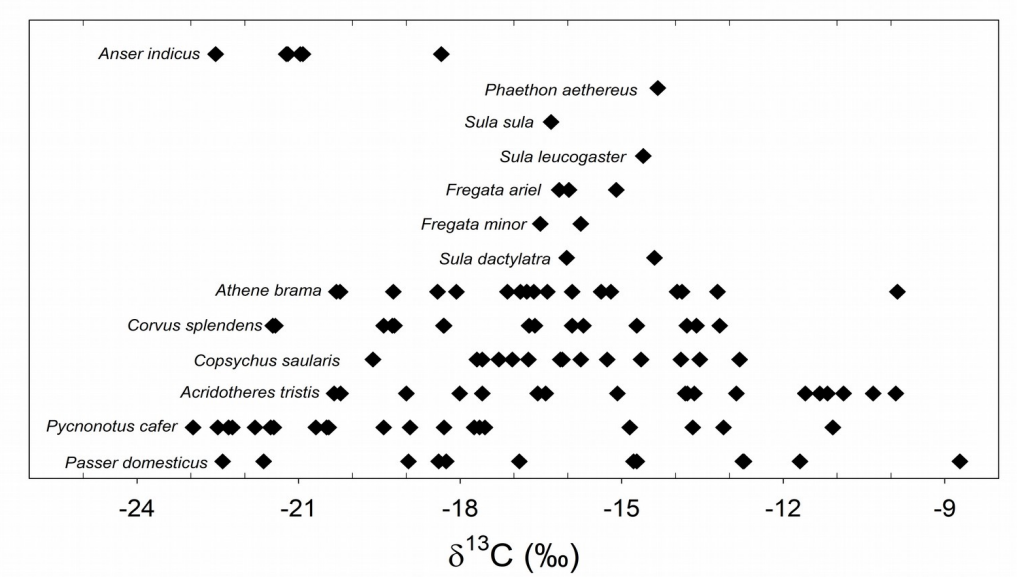


Figure.3.2. Carbon isotopic variation

Carbon isotopic composition also gives us idea about animal diet. $\delta^{13}C$ isotopic values are used to determine food source of birds. $\delta^{13}C$ variation occurs due

to variation in photosynthesis type. C3 plant range from -20‰ to -37‰ C4 plants isotopic range is from -12‰ to -16‰ while phytoplankton shows values in range -18‰ to -25‰ Species on higher tropic level are expected to show enriched $\delta^{13}C$ isotopic values(O'Leary 1988; Hayes 2001; Kohn 2010). Lowest value for $\delta^{13}C$ was observed for Red-vented Bulbul -23.0‰ a bird obtained from Surat. While highest value was for House Sparrow -8.7‰ obtained from Shimla.

Lower δ13C values in all species suggests that the inland resident species diet is dependent mostly on C3 food sources or on the C3-plant dependent insects. Seabird species are showing very low standard deviation suggesting all of them are having similar diet. Some species are showing enriched values at some location this implies their diet contains C4 food sources or more amount of insects.

Species(Carbon)	No	Minimum	Maximum	Mean ± Std
House Sparrow	12	-22.4	-8.7	-16.0 ± 4.1
Red-vented Bulbul	15	-23.0	-11.1	-18.6 ± 3.7
Common Myna	11	-20.3	-9.9	-14.9 ± 3.8
Indian Robin	14	-19.6	-12.8	-16.1 ± 1.8
Spotted Owlet	12	-20.3	-9.9	-16.3 ± 2.8
House Crow	9	-21.5	-13.2	-17.8 ± 2.9
Seabird species	9	-16.5	-14.3	-15.5 ± 0.9

Table 3.2. Carbon isotopic variation among analyzed species

3.1.3. $\delta^{34}S$ variations

Lowest $\delta^{34}S$ values is for Common Myna -1.0‰ specimen obtained from Shimla. While the highest $\delta^{34}S$ values were recorded in case of seabird species Great Frigetbird 23.3‰ for the specimen obtained from Valapattana, Kerala. $\delta^{34}S$ values in bird feather are dependent on Sulfur containing dietary proteins and rock

pattern of that place. Interpretation of $\delta^{34}S$ variations is discussed in the subsequent section (Section 3.5).

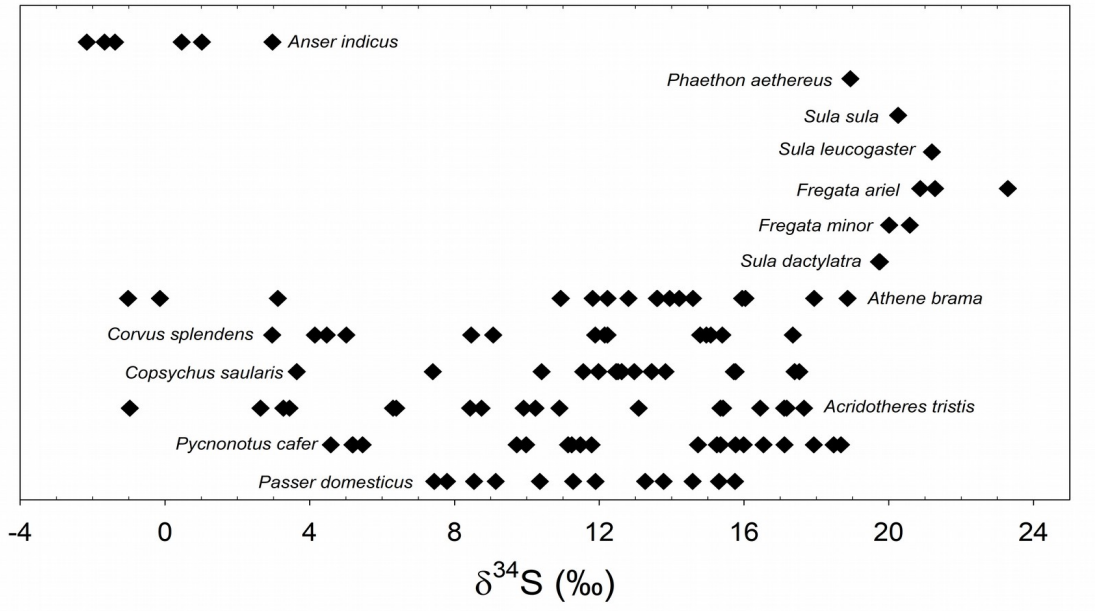


Figure.3.3. Sulfur isotopic variation

Species(Sulfur)	No	Minimum	Maximum	Mean ± Std
House Sparrow	12	7.4	15.8	11.6 ± 3.0
Red-vented Bulbul	15	4.6	18.7	12.6 ± 4.8
Common Myna	11	-1.0	17.7	9.6 ± 6.1
Indian Robin	14	3.6	17.5	12.6 ± 3.6
Spotted Owlet	12	-1.0	18.9	12.3 ± 5.7
House Crow	9	3.0	17.4	10.7 ± 4.9
Seabird species	9	18.9	23.3	20.4 ± 1.0

Table 3.3 Sulfur isotopic variation among species

3.1.4. $\delta^{18}O$ variations

Lowest $\delta^{18}O$ value was observed for Red-vented Bulbul 11.4‰ for sample obtained from Tirhut, Bihar. Highest $\delta^{18}O$ was observed for Spotted Owlet 21.8‰

obtained from Patan, North Gujrat. $\delta^{18}O$ values are mostly dependent on rainfall $\delta^{18}O$ values. Interpretation of $\delta^{18}O$ variations is discussed in the subsequent section (Section 3.5).

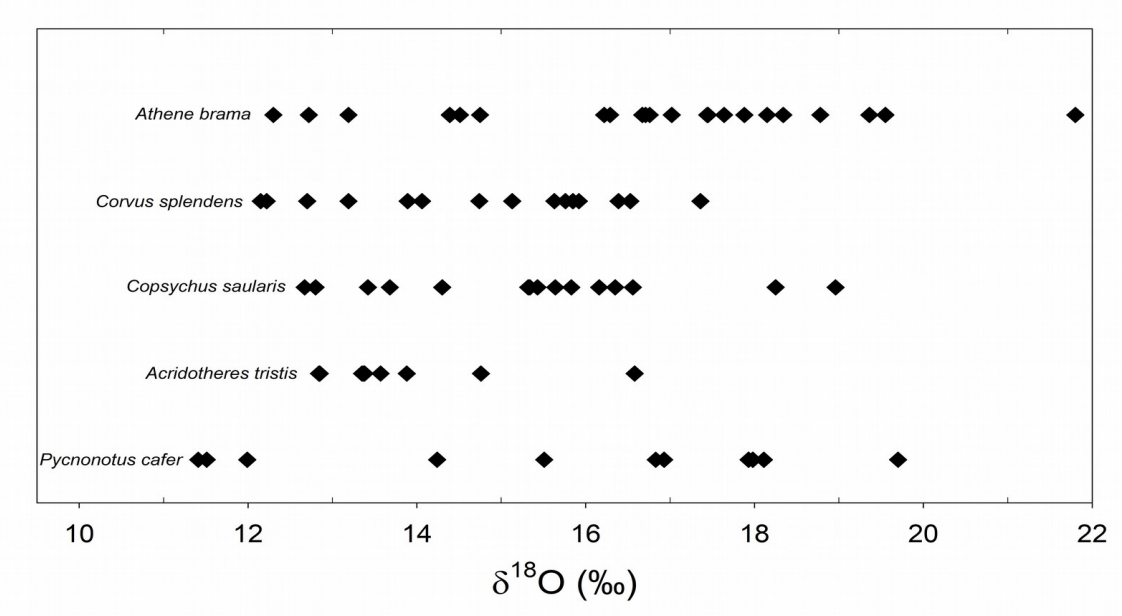


Fig.3.4. Oxygen isotopic variation

Species(Oxygen)	No	Minimum	Maximum	Mean ± Std
Red-vented Bulbul	8	11.4	19.7	15.7 ± 3.0
Common Myna	8	12.8	16.6	13.9 ± 1.2
Indian Robin	12	12.7	19.0	15.4 ± 2.0
Spotted Owlet	12	12.3	21.8	17.2 ± 1.6
House Crow	9	12.2	17.4	14.5 ± 1.6

Table 3.4 Oxygen isotopic variation among species

3.2 Within location variation

For our analysis we had multiple species from single location. Here we compared them to understand if there are any emerging information from. It was observed that there is large variation within single location for $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ values

across different species. This suggest that, as expected, carbon and nitrogen isotopic values are species specific and cannot be used very effectively to get the idea of a bird's location. It can be effectively used to decipher the diet. Sulfur values are surface rock/water dependent while comparing it across species there might be some effect related to diet. In case of oxygen isotopes as well there is some variation within the same location. This could be due to the fact that the samples were collected during different years.

Location	Species	$\delta^{18}O$
Kutch	Robin	18.3
	Crow	15.9
Mumbai	Crow	16.5
	Owlet	17.5
	Myna	12.8
Darbhanga	Crow	13.1
	Myna	13.6
Ambala	Owlet	16.4
	Myna	16.6
Delhi	Robin	12.7
	Bulbul	15.5
	Owlet	15.1
Shimla	Robin	15.7
	Bulbul	17.7
	Myna	13.9
Travancore	Bulbul	14.2
	Robin	16.6
Bharatpur	Bulbul	18.0
	Crow	12.2
Trivandrum	Owlet	17.0
	Myna	14.8
Tirhut	Bulbul	11.5
	Owlet	17.5

Table 3.5. Within location variation for $\delta^{18}O$

Location	Species	$\delta^{15}N$	$\delta^{13}C$	$\delta^{34}S$
Nashik	Sparrow	6.2	-12.8	11.9
	Sparrow	4.6	-14.8	13.3
	Robin	4.9	-16.1	12.5
	Robin	3.9	-12.8	12.5
Bharatpur	Sparrow	7.0	-18.3	10.4
	Bulbul	6.0	-13.1	9.7
	Myna	12.0	-18.0	10.9
	Crow	7.6	-14.5	12.2
Mumbai	Sparrow	5.6	-18.4	7.4
	Bulbul	7.5	-14.9	16.5
	Myna	10.3	-11.2	13.1
	Crow	9.7	-16.7	15
	Owlet	8.6	-17.1	14.2
	Fregata ariel	14.2	-15.1	20.9
	Sula dactylatra	13.3	-14.4	19.7
Shimla	Sparrow	3.2	-8.7	11.3
	Bulbul	4.3	-17.9	11.5
	Myna	12.4	-9.9	-1.0
	Robin	10.4	-17.3	11.6
Delhi	Sparrow	5.9	-19.0	7.8
	Bulbul	4.5	-19.4	11.1
	Robin	9.0	-15.3	17.5
	Owlet	10.4	-13.6	12.3
Khandala	Owlet	7.9	-16.2	16.0
	Owlet	8.1	-16.6	14.6
	Robin	4.0	-14.6	12.0
	Robin	5.7	-16.7	13.8
	Robin	5.6	-17.7	10.4

Table 3.6. Within location variation for $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$

3.3 Male female variation

We also checked if there are isotopic variations between the male and female of the same species across different locations. We find no gender specific systematic variation. We did not have the samples of both genders from the same location. Therefore, the absence of gender specific variations might reflect the geographical or time related variations.

3.4 Within specimen variation

Species/Isotope	Location	$\delta^{18}O$
Red-vented Bulbul	Shimla	16.9 17.9 18.1
	Tirhut	11.4 11.5
Spotted Owlet	Mumbai	17.4 17.5
	Kanpur	12.3 12.7 13.2 19.4
	Delhi	14.4 14.8 16.2
	Ambala	16.7 16.7
	Gorapur	17.6 14.5
Common Myna	Bharatpur	12.9 13.4
Indian Robin	Shimla	15.7 15.8
	Indore	15.3 16.2
House Crow	Mumbai	16.4 15.6 17.4
	Kutch	15.8 15.9
	Kolkata	15.1 14.7
	Srinagar	13.2 15.8
	Darbhanga	14.1 12.2

Table 3.7 Within species variation for $\delta^{18}O$ isotope

Species/Isotope	Location	$\delta^{15}N$	$\delta^{13}C$	$\delta^{34}S$
Red-vented Bulbul	Surat	6.4 5.9	-23.0 -20.7	18.5 18.7
	Sivoke	7.7 4.4	-22.3 -22.5	5.5 5.2
	Shimla	4.6 4.0 6.0	-17.6 -17.7 -18.3	11.5 11.2 11.8
	Jodhpur	8.5 8.5	-20.5 -18.9	15.8 15.3
Common Myna	Goalpora	6.2 8.3 7.6	-13.8 -19.0 -16.4	8.8 9.9 8.4
	Kutch	9.7 9.2 9.0	-12.9 -13.7 -13.8	16.5 15.4 15.4
	Nallamalai Range	5.4 8.1	-10.3 -10.9	17.2 17.7
	Ambala	10.9 8.2	-11.6 -11.3	3.3 2.6
	Kashmir	6.6 6.7	-17.6 -16.6	6.4 6.3
House Crow	Bharatpur	7.2 7.5 7.4	-13.2 -14.7 -15.7	12.2 12.2 12.2
	Darbhanga	9.0 9.1 8.6	-13.6 -15.9 -16.6	5.0 4.5 4.1
	Bastar	10.1 10.3 10.3	-19.4 -18.3 -18.3	14.8 15.1 15.4
Spotted Owlet	Delhi	10.7 10.1	-13.2 -13.9	12.8 11.8
	Trivandrum	7.1 8..7	-16.8 -15.4	13.6 12.2
	Tirhut	6.5 6.4	-20.3 -20.2	-1.0 -0.1
	Kalinpur	6.0 6.4	-16.9 -13.1	10.9 10.9
	Khandala	7.5 8.2	-16.4 -15.9	15.9 16.0

Table 3.8 Within species variation for $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$

Multiple feathers of a various species were used to check within specimen variation of the isotopic composition. Low variation in this case implies analyzing fewer samples can be sufficient. As we had very limited number of feathers this test could not be done for every species and every specimen. As chest feathers are shaded and regrown in a continuous manner higher variation in some cases can be explained by the feathers not grown together and reflecting different isotopic signature in them. Despite this, in many cases, within specimen variations are very less.

3.5 Geographical variation

The figures in this section illustrate geographical patters in $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ and $\delta^{18}O$ of feathers. In these, the $\delta^{13}C$ and $\delta^{15}N$ are diet dependent and do not have any geographical pattern.

Spatial patter of the $\delta^{18}O$ variations show similarities with the precipitation isoscape for $\delta^{18}O$ (shown in Figure 1.5). This isoscape was created using online website isomap.org this site contains global data for isotopic values. The year range chosen to make this isoscape was 1960-2000. We considered data from all the months and this data is collected at 86 stations located all over the region.

The lower precipitation isotopic values for $\delta^{18}O$ were observed in Sikkim and North Bihar which are reflected in bird obtained from this two states. They are showing lowest $\delta^{18}O$ value amongst all. Values showed in Multan, Pakistan and from Kutch, Gujrat are on higher side this is due to very high temperature and evaporation rate at this place which enriches the $\delta^{18}O$ values. Values for location that are in close proximity are showing similar color and are clustered. This shows that there is a geographical variation in oxygen isotopic value. Hydrogen isotopic composition of feathers would bring out the geographical variations more clearly.

$\delta^{18}O$ isoscape of India figure (1.5a) and House Crow $\delta^{18}O$ variability across India shown in figure(3.5) shows similar trends. This indicates that isotopic variability is determined by precipitation during Monsoon. Values in Western Ghats are highly enriched due to arrival of Monsoon first in this region. Heavier isotope of oxygen is

first to get removed from clouds. As cloud system proceeds further the $\delta^{18}O$ values in them get depleted.

$\delta^{34}S$ values are dependent on the underlying geology of the place and rock patterns. Sulfur values that are in close proximity are showing similar colors showing geographical variation. There are few anomalies that may be arising due to variation in the diet. Sulfur values in Srinagar (Kashmir), Tista valley (Sikkim), Ambala (Punjab), Tirhut, Darbhanga (Bihar) are on the lower side across all species. Sulfur values in Kerala and Karnataka are higher. Sulfur isotopic composition of the coastal birds reflect the isotopic composition of seawater sulfur.

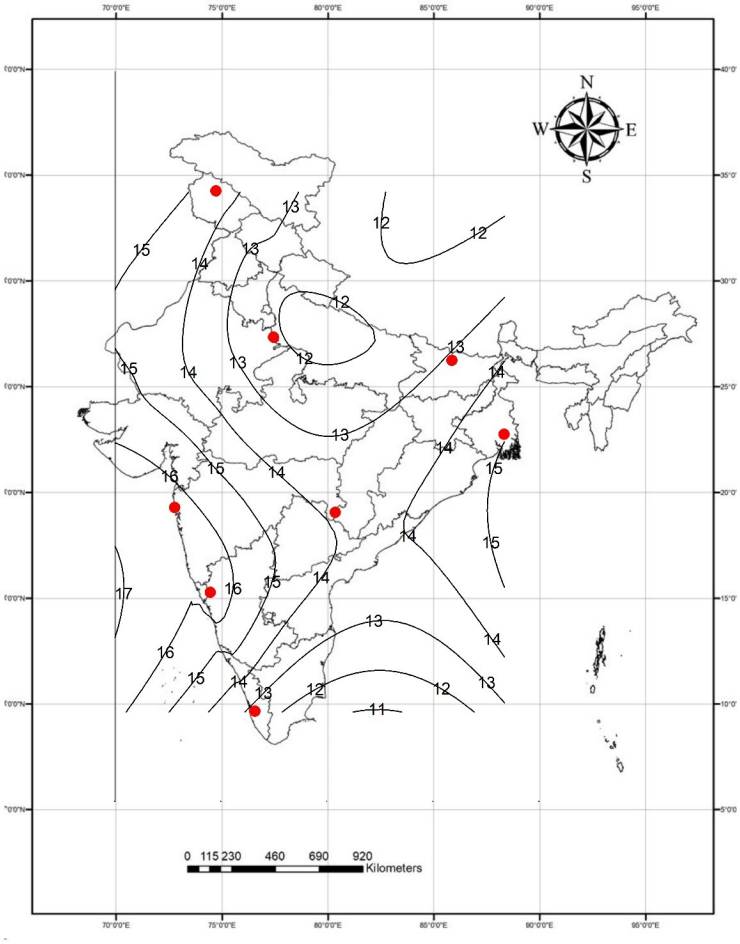
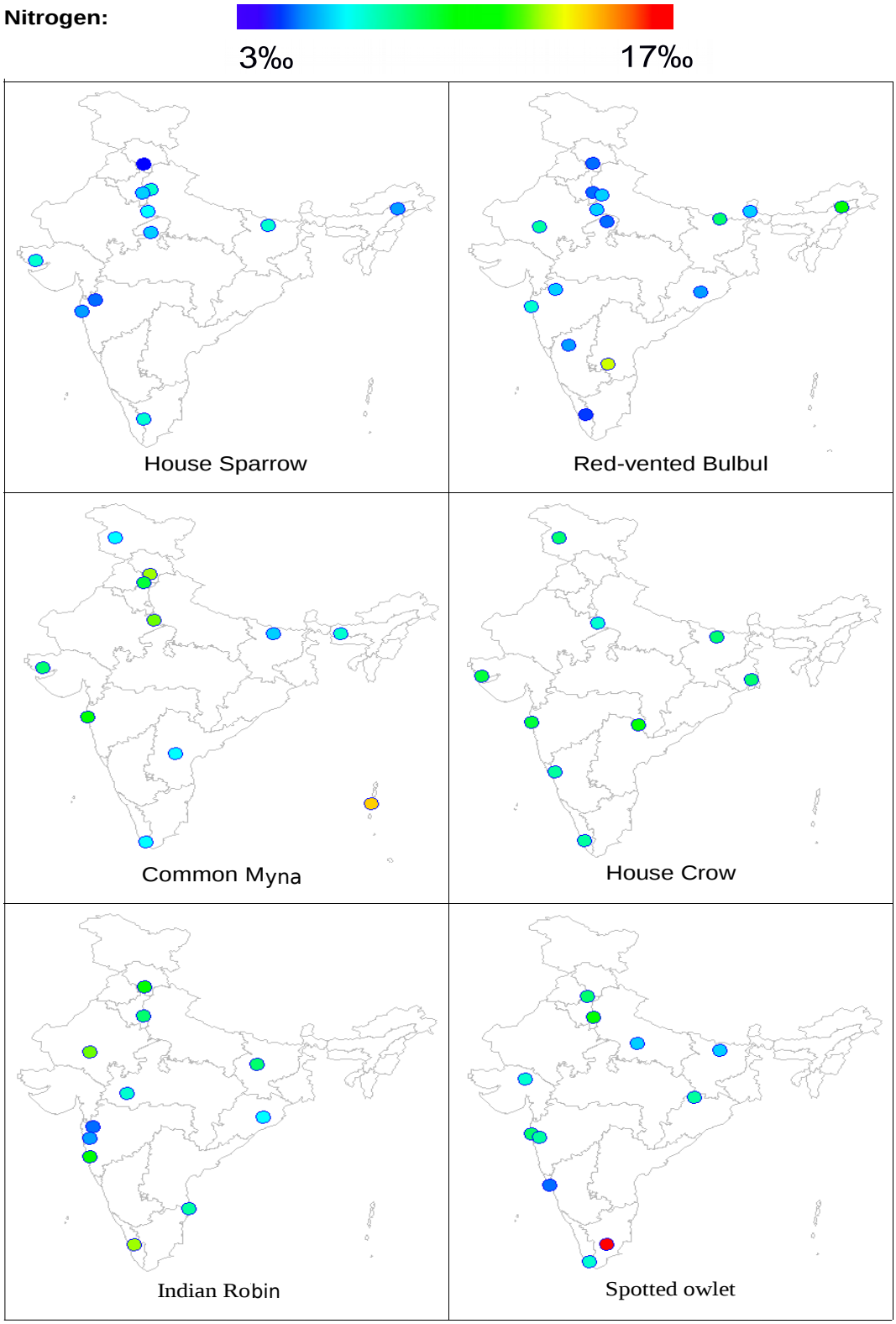


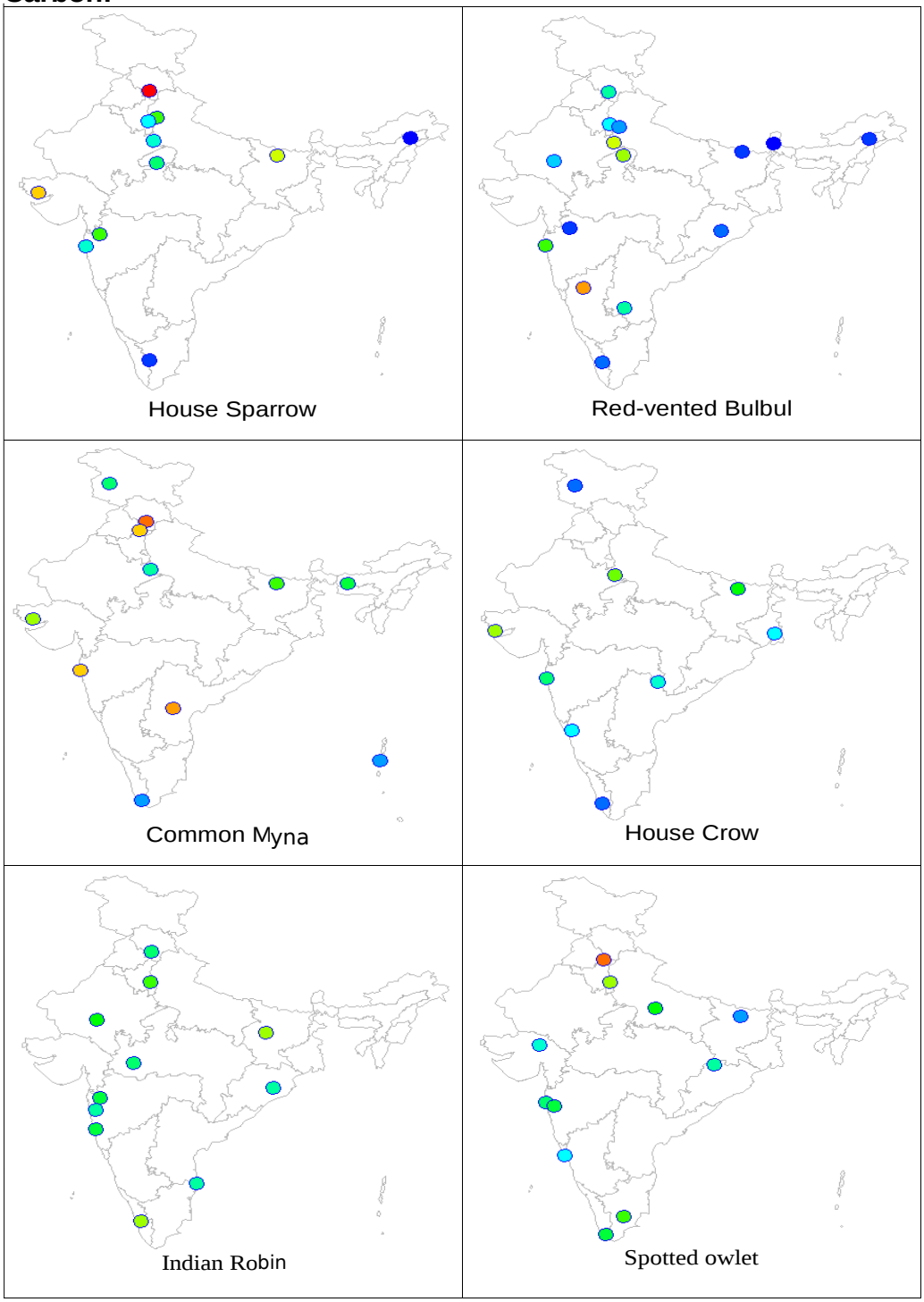
Figure 3.5 House Crow $\delta^{18}O$ variability


Nitrogen:
3‰
17‰
House Sparrow
Red-vented Bulbul
Common Myna
House Crow
Indian Robin
Spotted owlet

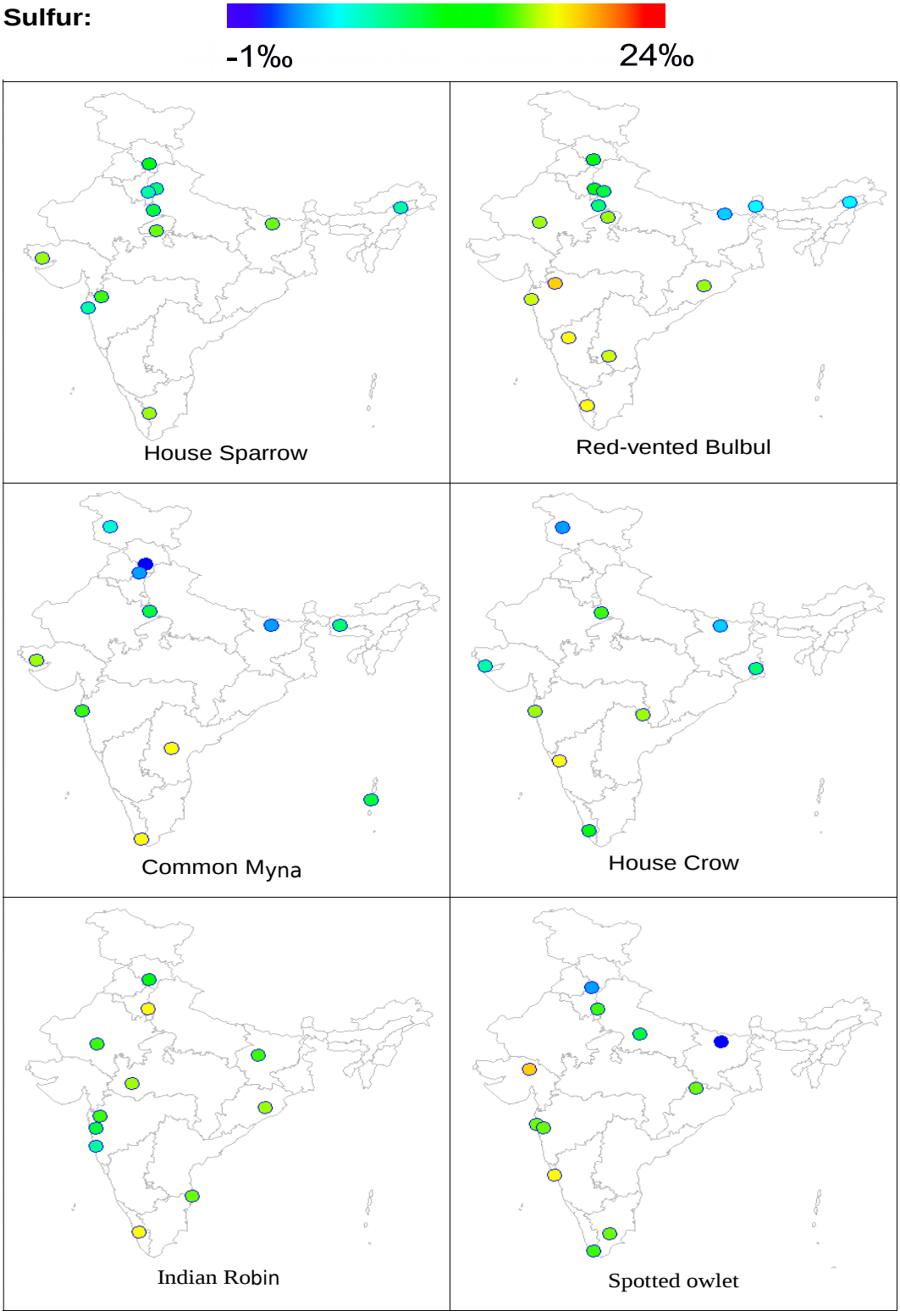
[a]

-23‰ -8.5‰

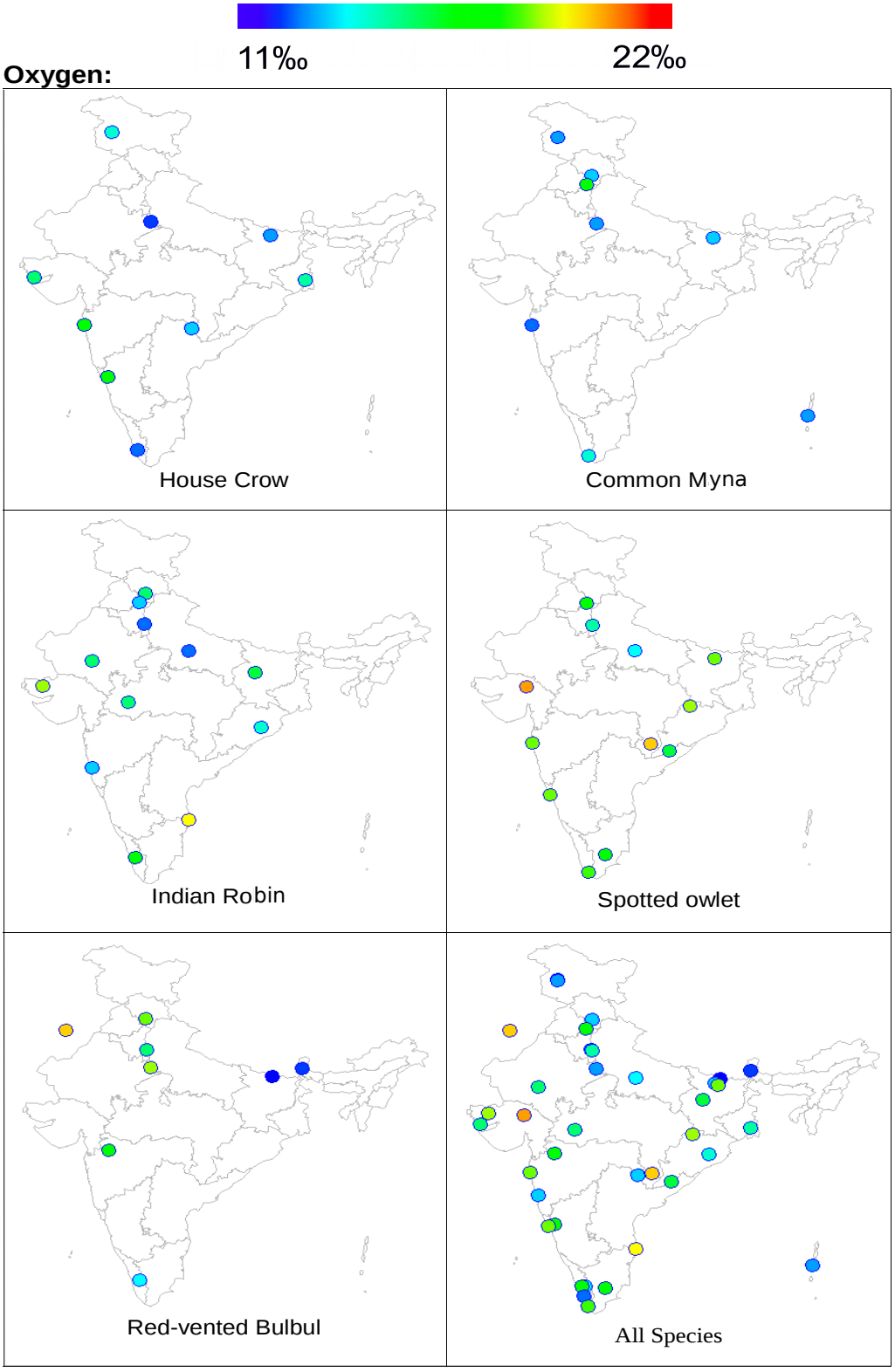
Carbon:



[b]


Sulfur:
-1‰
24‰
House Sparrow
Red-vented Bulbul
Common Myna
House Crow
Indian Robin
Spotted owlet

[c]



[d]

Figure 3.6 Geographical distribution of $\delta^{15}N$[a], $\delta^{13}C$[b], $\delta^{34}S$[c], $\delta^{18}O$[d] values.

3.6 Bar-Headed Goose migration:

Bar-headed Goose migration is extensively studied by (Zhang et al. 2011). Bar-headed Goose are found in central China, Mongolia, Bangladesh and India. They are known to fly in higher altitude and many of them cross Himalaya in migration between Tibet and India. Qinghai lake in China is largest known breeding colony for Bar-headed Goose. Most of the birds from here winter in the water shades of the Yarlung Zangbo River, the Lhasa River, and the Nianchu River in Tibet where there is moving water during the winter in the river, with available marshland and farmland habitat (Zhang et al. 2011). During March to April, migration happen between wintering ground to breeding ground. Reverse journey to wintering ground take place in September- November (Zhang et al. 2011). They moult their feather at their breeding ground. Available satellite data shows another possible migratory route for bird breeding in Qinghai Lake, they travell by way of Donggeicuona Lake, Eling Lake, and Hangcuo for a short stay in Tibet, then flew over the Himalayan Mountains to winter in India (Zhang et al. 2011). We analyzed a feather of Bar-headed Goose collected in the field. Its isotopic analysis suggests that the changes in the carbon and nitrogen values likely capture the change in during migration while sulfur values suggest route taken. Nitrogen and Carbon isotopic values are depleting from top to bottom along the feather. A similar trend is observed in case of sulfur isotope. Hydrogen and oxygen isotopic analyses for this feather are not done. It should help in identifying the migratory route for the bird.

$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
–20.9	9.9	3.0
–21.2	9.6	1.0
–21.0	8.9	0.5
–18.4	8.6	–1.7
–21.2	7.8	–2.2
–22.5	8.9	–1.4

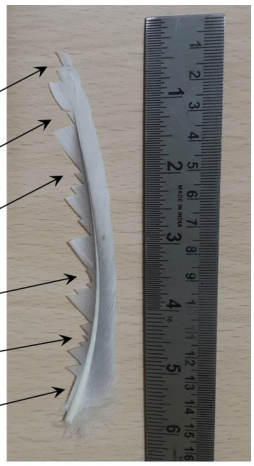


Figure 3.7 Bar-headed Goose feather's isotopic variation

Conclusion

Studying isotopic composition of bird feathers is an emerging field. It can yield important information regarding diet and provenance of the birds. If used in conjunction with traditional observations, it can be a great tool in avian research. Although many studies have been carried out in other countries, such studies have not been attempted in India. Given its different biomes and geology, it is important to have an idea about spatial patterns in the isotopic composition of feathers belonging to different tropic level and different geological settings. This is a first attempt in this direction.

Collecting feathers of different species from different parts of India is a challenging task. Given this situation, use of feathers from museums specimens offers great advantage as the information such as place and month of collection of individual specimens is available. In addition, accurate information about the gender of the specimen is also available. Museum specimens from the museum of Bombay Natural History Society (BNHS) was used in this context. Given the priceless nature of the specimens, only feathers coming out during cleaning of the specimens were used. As such feathers are typically chest feathers, this study mostly employs chest feathers. Chest feathers are shed and grown throughout the year. The collected feathers thus reflect isotopic composition of diet that lead to given feather. In addition, a feather of Bar-headed Goose which was collected from the field was used to check how isotopic compositions of evolve during the migration.

Here, carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), sulfur ($\delta^{34}S$) and oxygen ($\delta^{18}O$) isotopic compositions of feathers of 13 species were carried out. These species include pelagic birds (Red-billed Tropicbird, Masked Booby, Brown Booby, Red-footed Booby, Lesser frigatebird and Great Frigatebird), raptor (Spotted Owlet), graminivorous (Bar-headed Goose), frugivorous (Red-vented Bulbul), omnivores (Common Myna, House Crow), and grainivorous (Hose Sparrow) birds. A spread in the isotopic compositions of a given species was observed.

Analysis of isotopic composition of different feathers in the same specimen mostly showed consistent values.

Nitrogen isotopic composition mainly reflect the trophic level. With trophic level the mean value $\delta^{15}N$ was observed to increase. Slightly lower value in the case of Spotted Owlet can be explained by its diet which is independent of agricultural crops. Marine food sources are enriched in $\delta^{15}N$ which is reflected in feathers of pelagic species. $\delta^{15}N$ values can be used to distinguish between marine and terrestrial food sources.

$\delta^{13}C$ values are dependent on C3 and C4 plant sources in the diet. Lower $\delta^{13}C$ Values suggests that inland species are mostly dependent on C3 plants for their diet or on the C3 plant-dependent organism.

$\delta^{34}S$ values are dependent on the underlying rock and level of marine influence. Sulfur values that are in close proximity are showing similar values suggesting geographical variation. There are few anomalies that may be arising due to variation in the diet where sulfur is incorporated in the body from sulfur-bearing amino acids. Pelagic and coastal species have higher $\delta^{34}S$ around 20 ‰.

$\delta^{18}O$ shows strong geographical variation with $\delta^{18}O$ values becoming more negative when moving towards northern and northwestern India. It likely reflect variation in the isotopic composition of precipitation. It was not possible to measure $\delta^{18}O$ of every specimen.

$\delta^{18}O$ and $\delta^{34}S$ of bird feathers appears to have a spatial control and hence could be useful in studying migration of birds in the Indian context. More sample analysis would further refine the observed spatial pattern. Hydrogen Stable isotopic study might show more prominent geographic variations.

Analysis of Bar-headed Goose for which satellite data is available showed that during migratory route the diet of bird is changing. $\delta^{34}S$ variations likely reflect geographic control. Further, oxygen and hydrogen isotopic studies are yet to be done for this feather which may give us information about its migratory route. This can be cross-checked later with available satellite data.

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