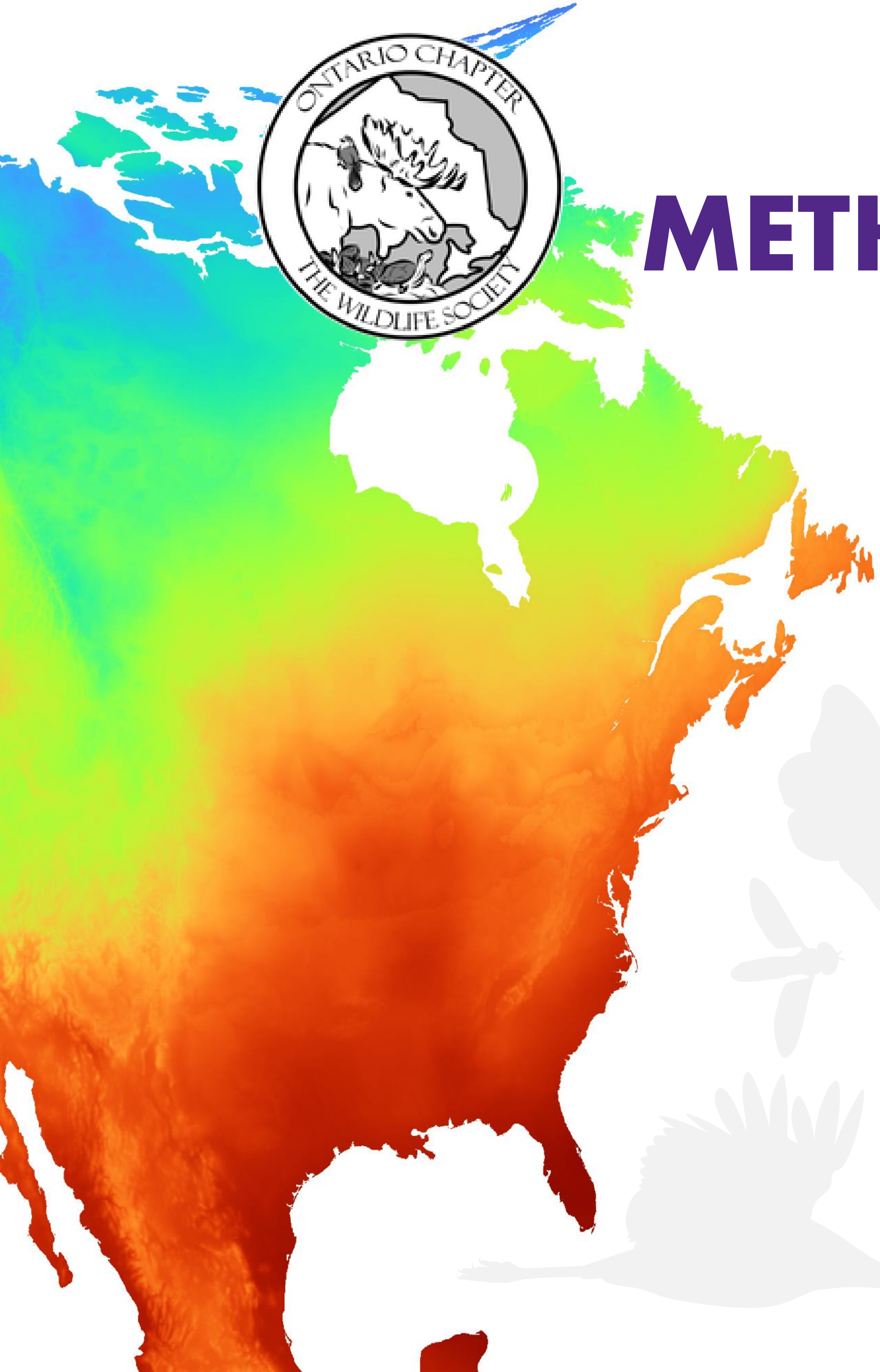


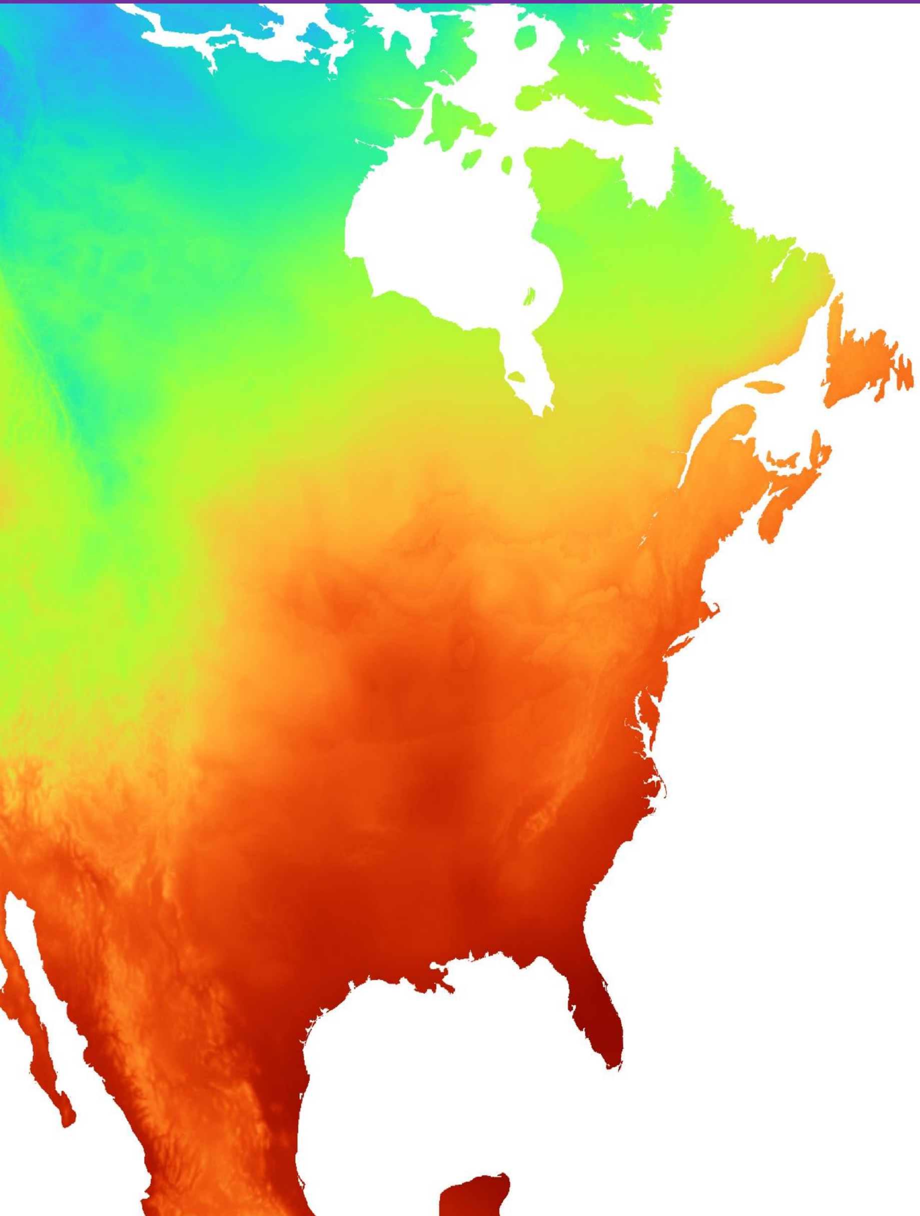
Ontario Chapter of the Wildlife Society

METHODS IN WILDLIFE RESEARCH

Introduction to Stable Isotope Assignment in R

Jackson W. Kusack (He/Him)
PhD Candidate
Western Centre for Animals on the Move

Overview

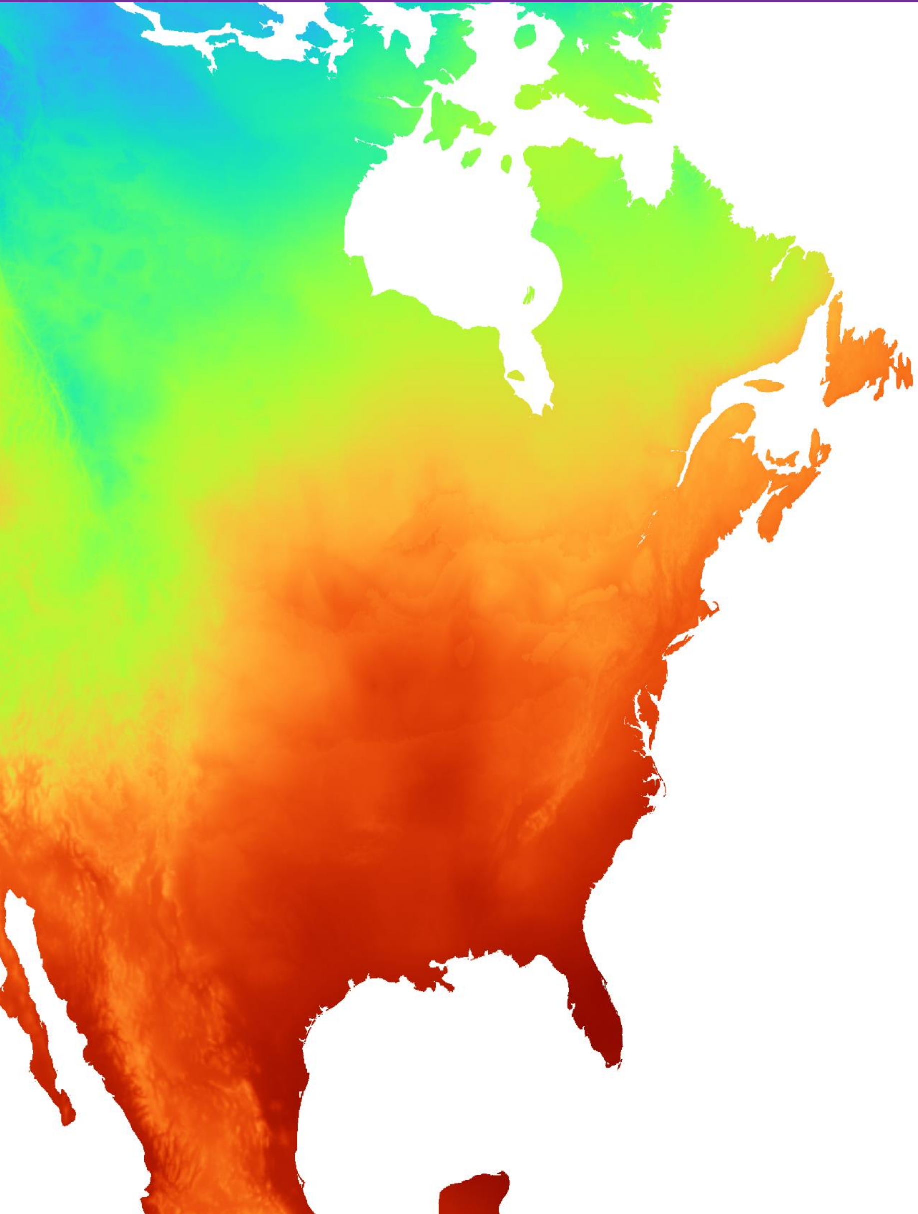


Can stable isotopes determine where my study species originates?

What data do I need?

How can I do the data analysis?

Overview



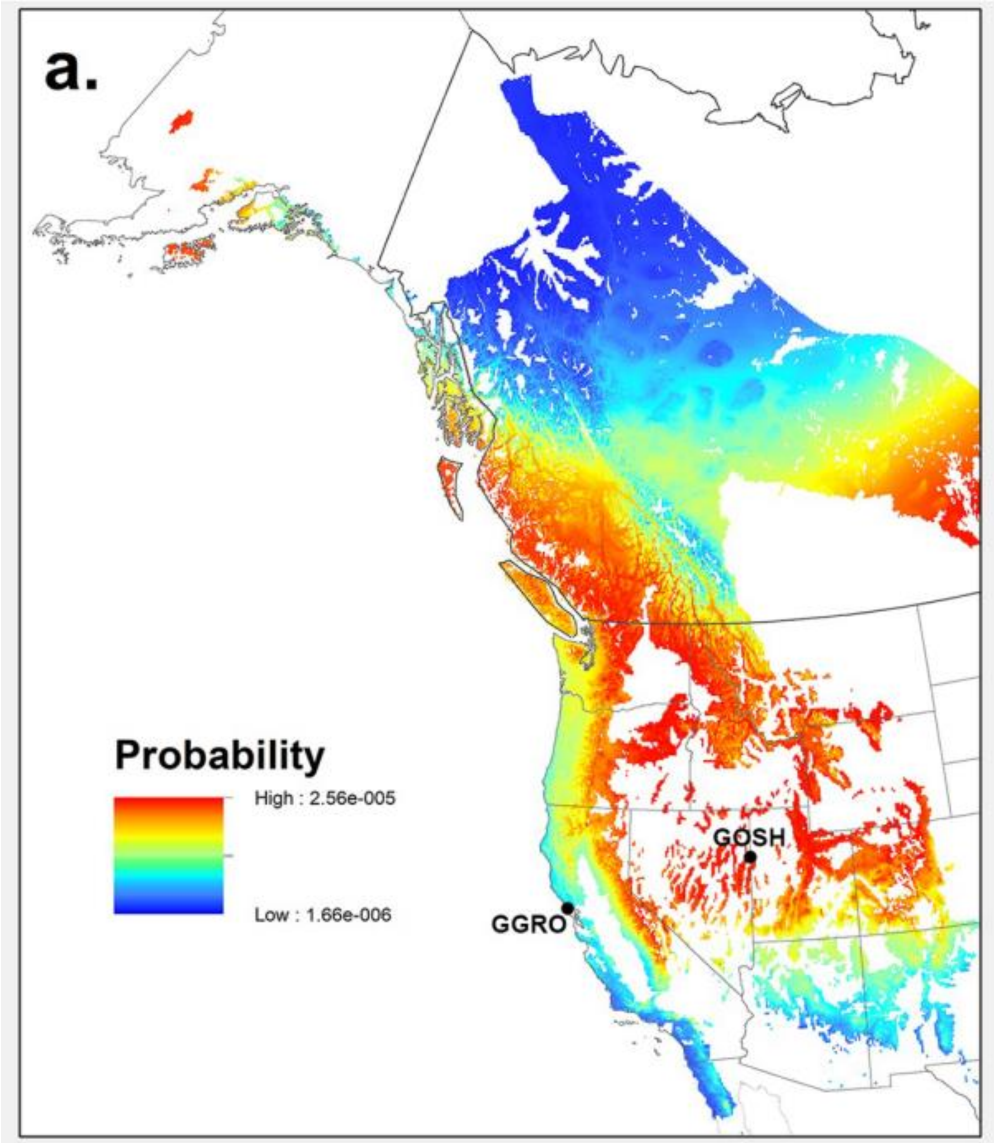
Can stable isotopes determine where my study species originates?

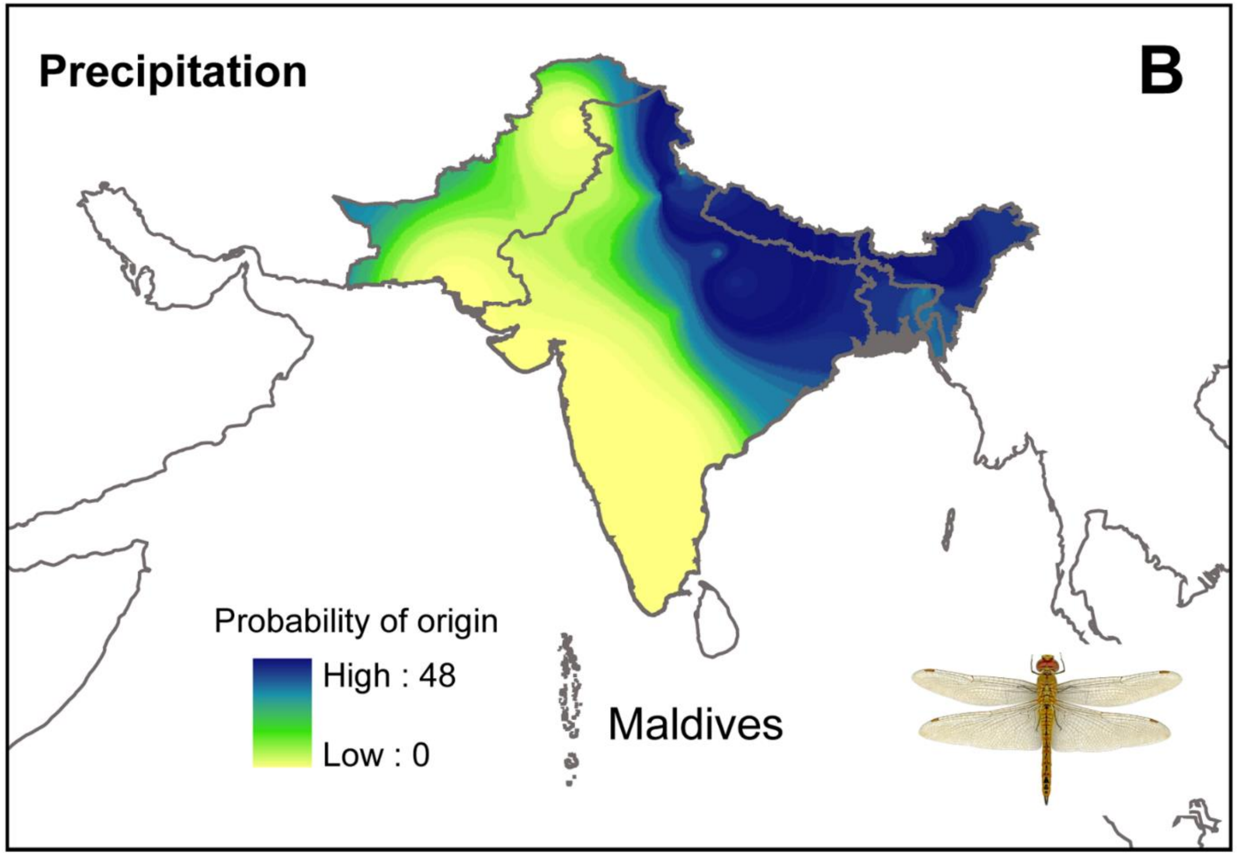
- Background on stable isotopes
- Depends on life-history

What data do I need?

How can I do the data analysis?

Likelihood-based Assignment





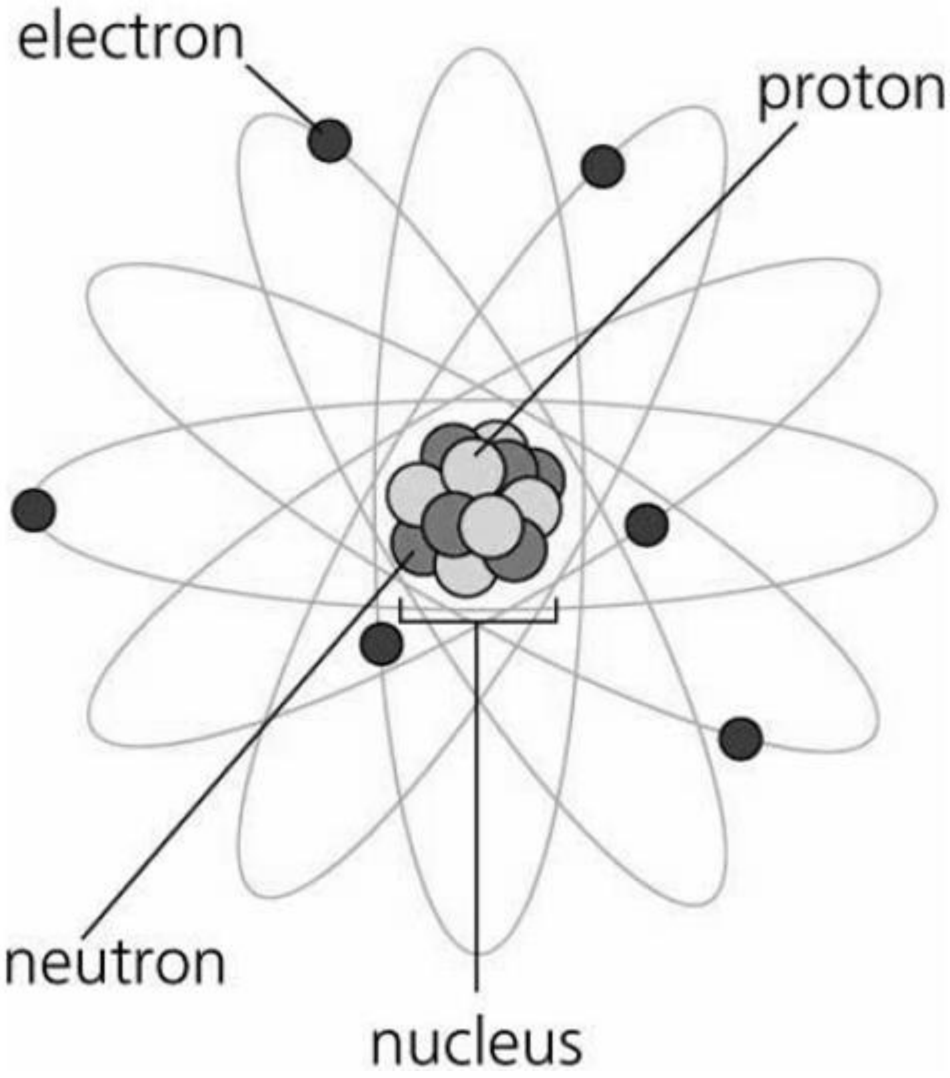
Wandering Glider *Pantala flavesens*

(Hobson et al. 2012)

Sharp-shinned Hawks *Accipiter striatus*

(Wommack et al. 2020)

Stable Isotopes



Stable

- Not radioactive – does not spontaneously change to another nuclide

Isotope

- Atoms of the same element, having different atomic weights (additional neutrons) but the same number of protons

Stable Isotopes

Ratio of **heavy** to **light** stable isotopes

$$^{H}R = \frac{^{H}X}{^{L}X}$$

^{H}X ← Less common

^{L}X ← Common

$$\delta^{H}X = \left(\frac{R\ sample - R\ standard}{R\ standard}\right) * 1000$$

Units = parts per thousand (‰)

Stable Isotopes

'Light' stable-isotopes

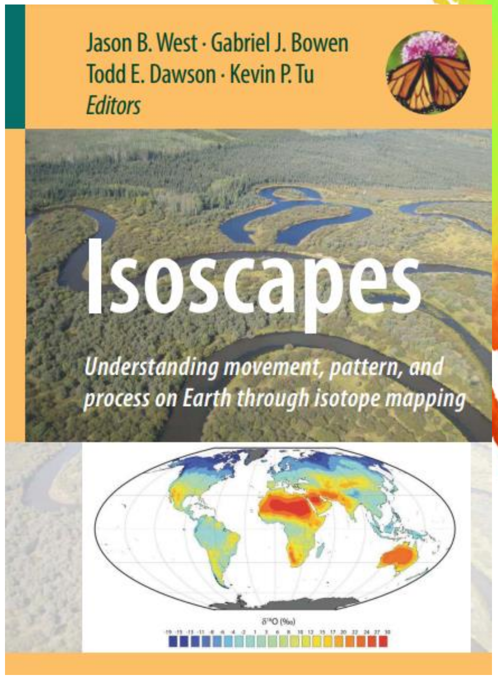
$$\boxed{{}^{2}H = \frac{{}^{2}H}{{}^{1}H}} \qquad {}^{13}C = \frac{{}^{13}C}{{}^{12}C} \qquad {}^{15}N = \frac{{}^{15}N}{{}^{14}N}$$

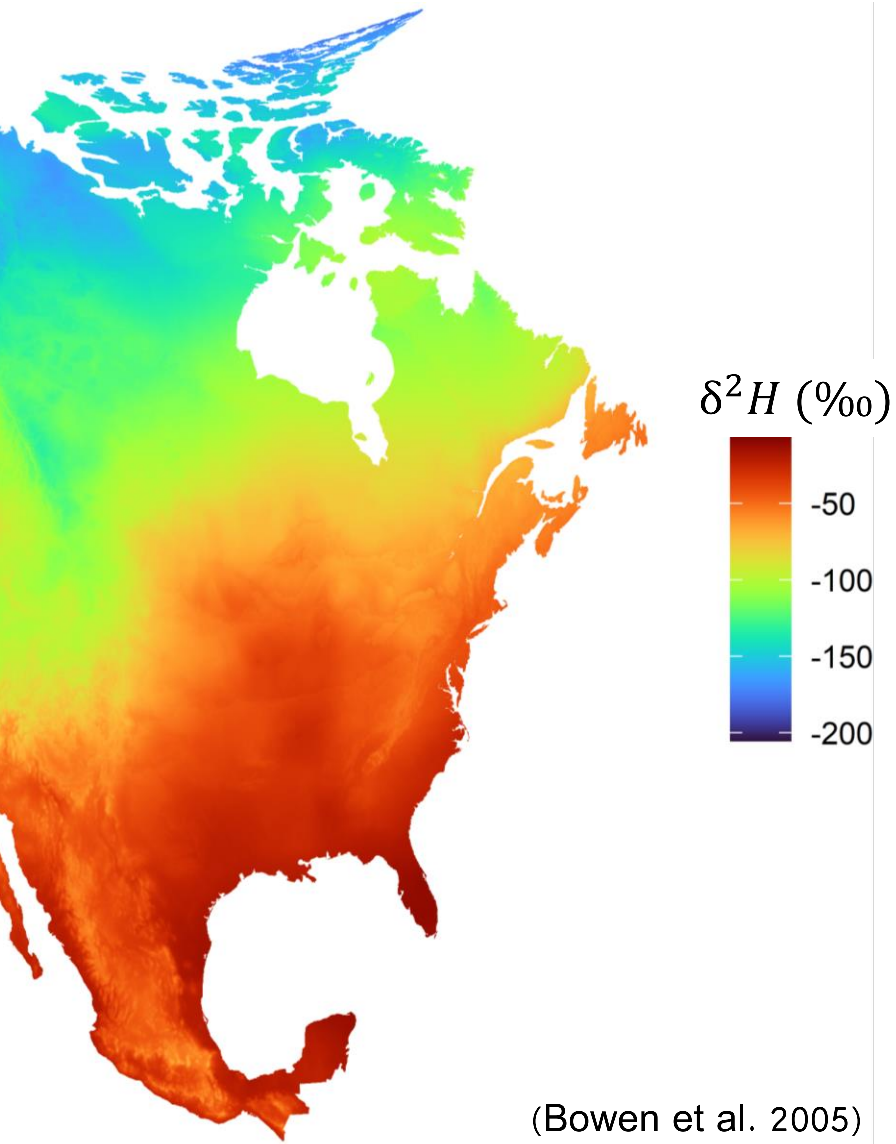
$${}^{18}O = \frac{{}^{18}O}{{}^{16}O} \qquad {}^{34}S = \frac{{}^{34}S}{{}^{32}S}$$

Isoscapes

Isoscape

- **‘iso’**tope land‘**scape’**
- Geospatial representation of predicted stable-isotope values
  - Plant, animal tissue
  - Precipitation, surface, and tap water
  - Bedrock





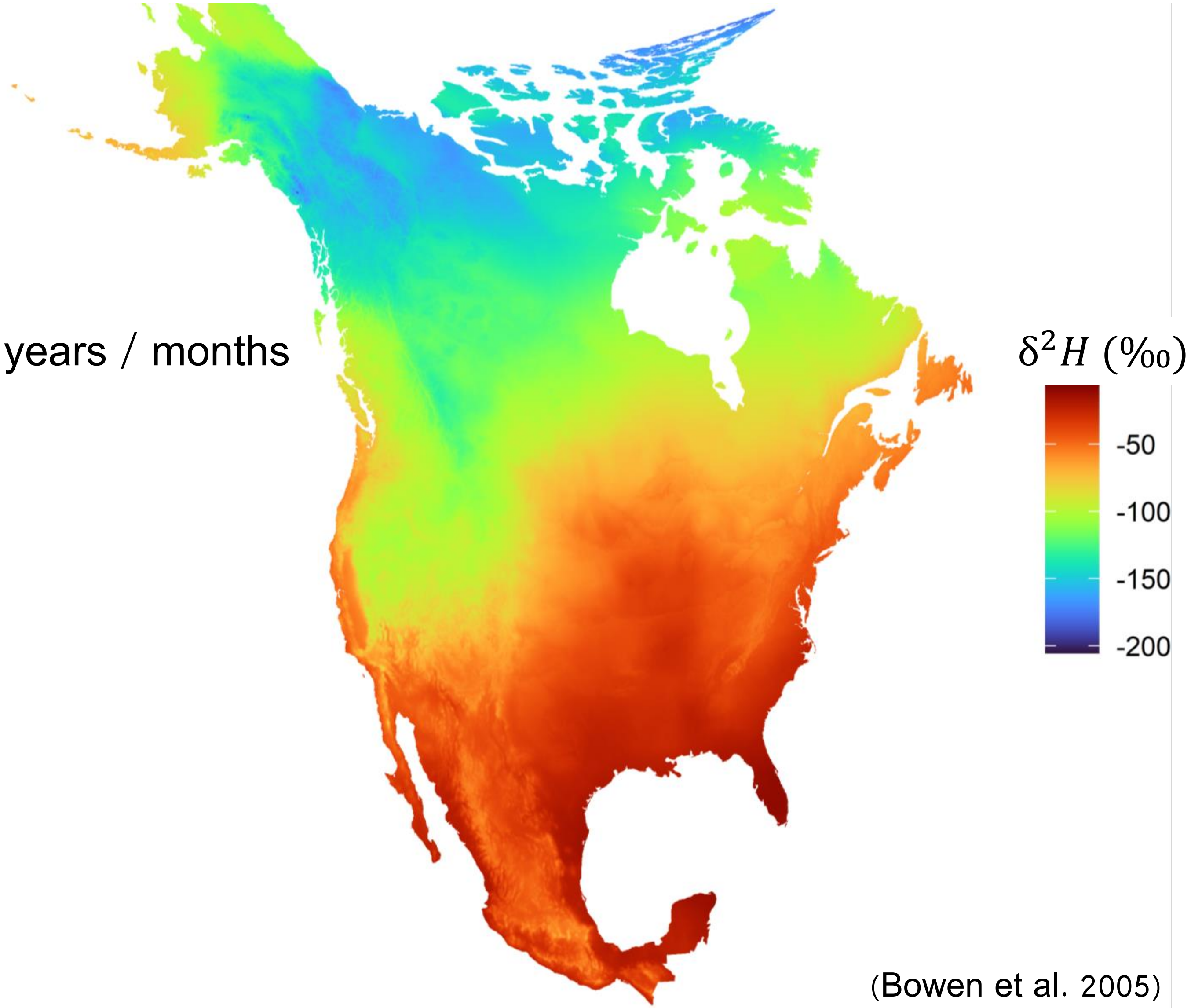
(Bowen et al. 2005)

Precipitation isotopes

Hydrogen (δ^2H)

Precipitation isoscape

- Amount-weighted average values over many years / months
  - Growing-season δ^2H
- Predictable latitudinal and altitudinal gradient
- δ^2H Incorporated into food web



(Bowen et al. 2005)

IAEA
International Atomic Energy Agency

Can stable isotopes determine where my study species originates?

Life-history

Movement patterns

- Migration
- Dispersal
- Nomadism
- Human-mediated
    - Trafficking

Diet

Isotopes within consumer tissues are derived from diet

- What are they eating?
- Where are they eating?

Development

- Where/when/how are tissues replaced?

Tissues

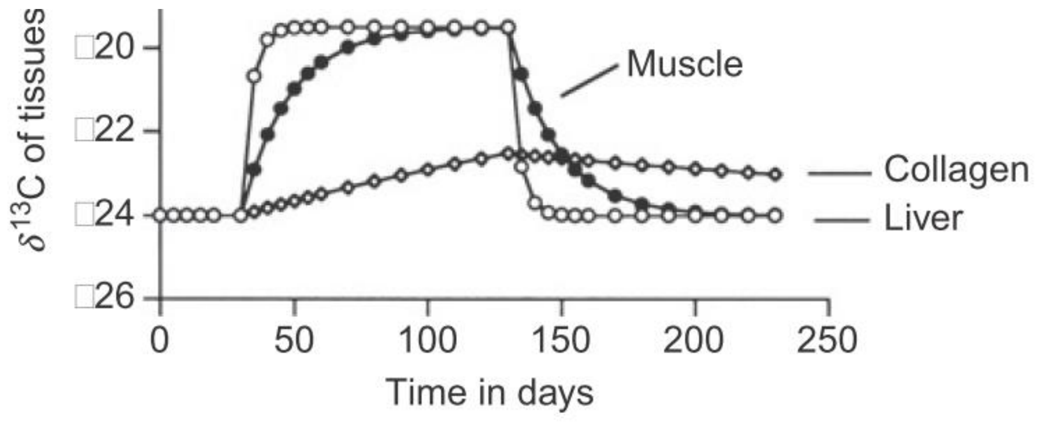
Isotopic turnover

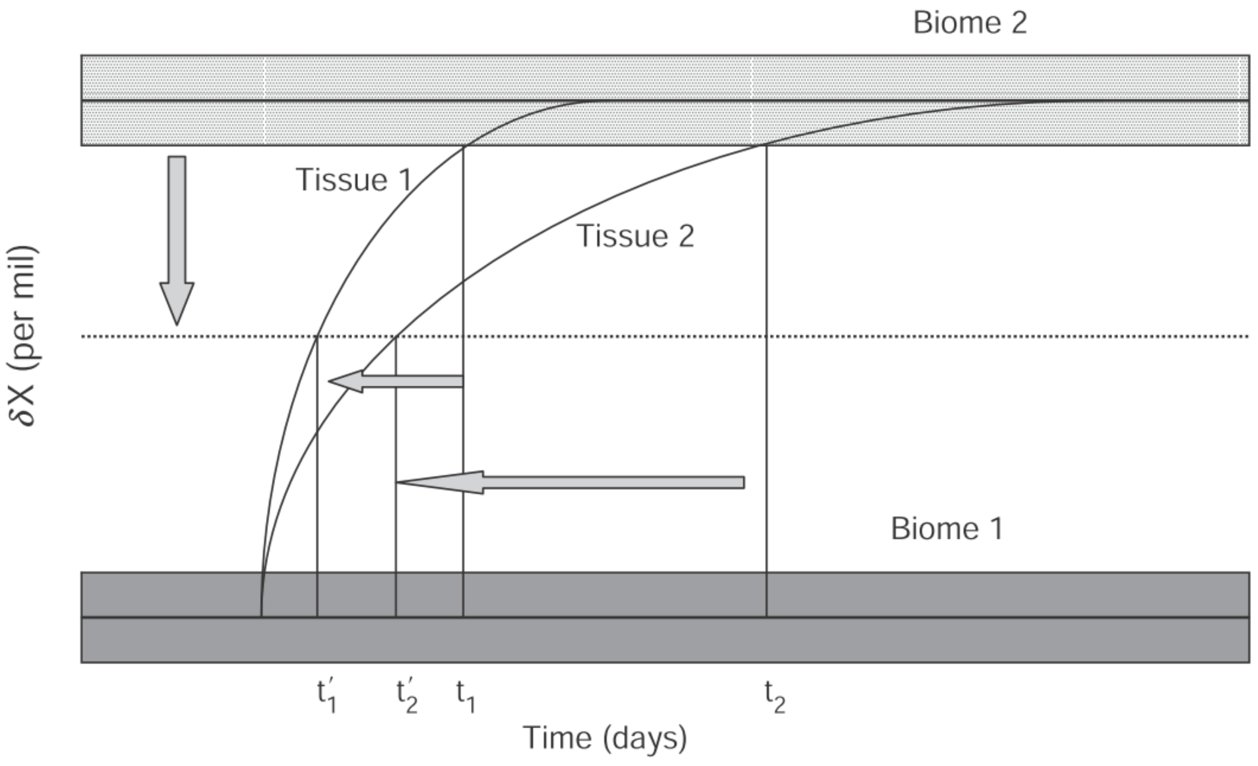
Tissues with constantly changing values:

- Half-life: Liver < Blood < Muscle < Bone

Tissues with fixed values:

- Keratin – Hair, Claw, Feathers, Chitin





Tissues

Inert tissue

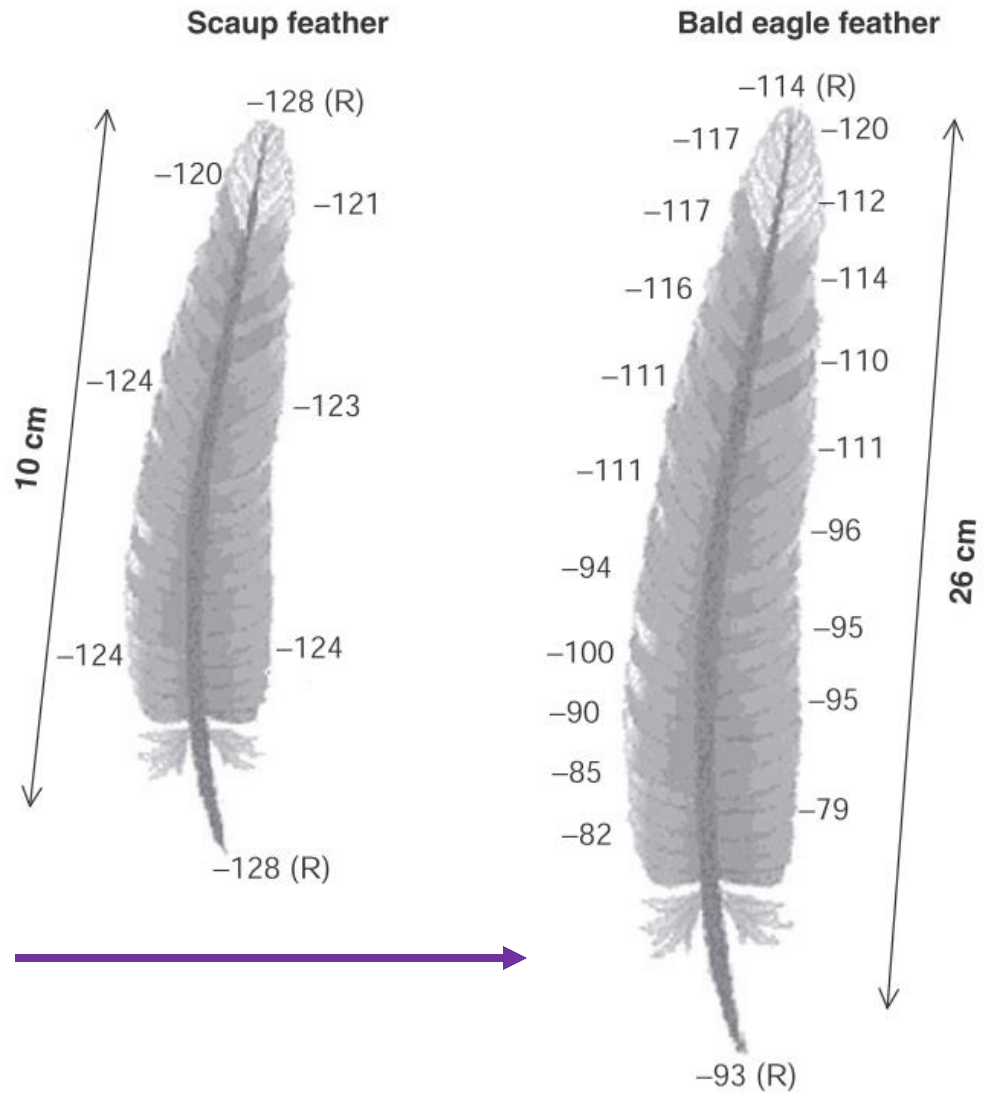
- Representative of diet during growth

Synchronous moult: all feathers are replaced simultaneously

- **Immature** – natal site
- **Adult** – moulting site

Asynchronous moult: feathers are replaced at different times

- **Adult** – initial moulting site + stopovers

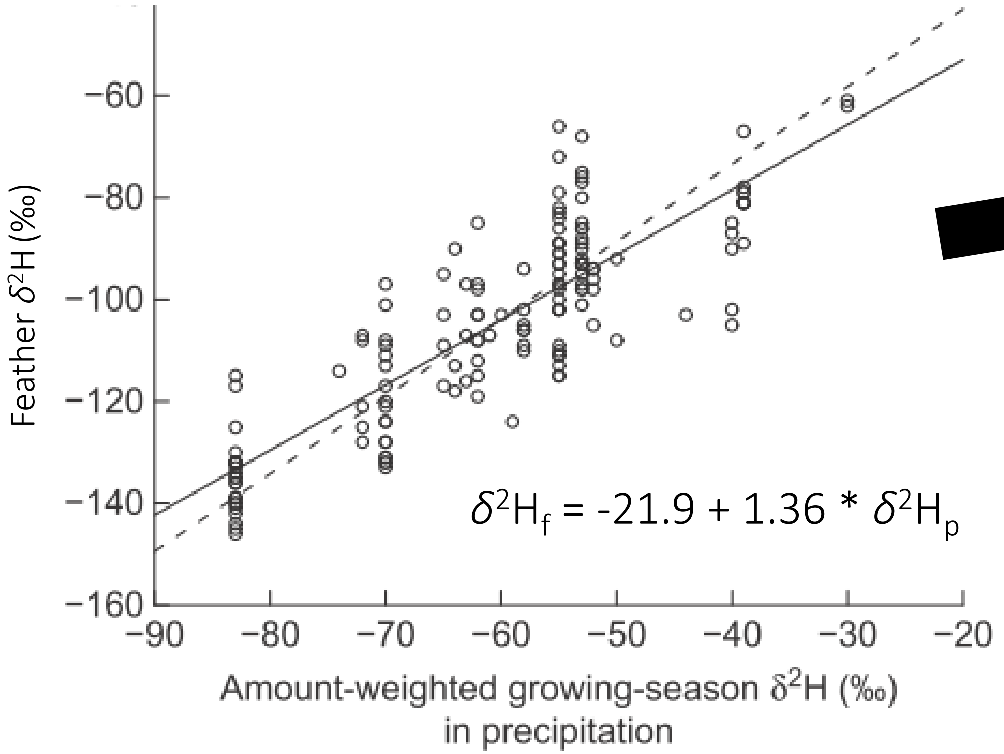


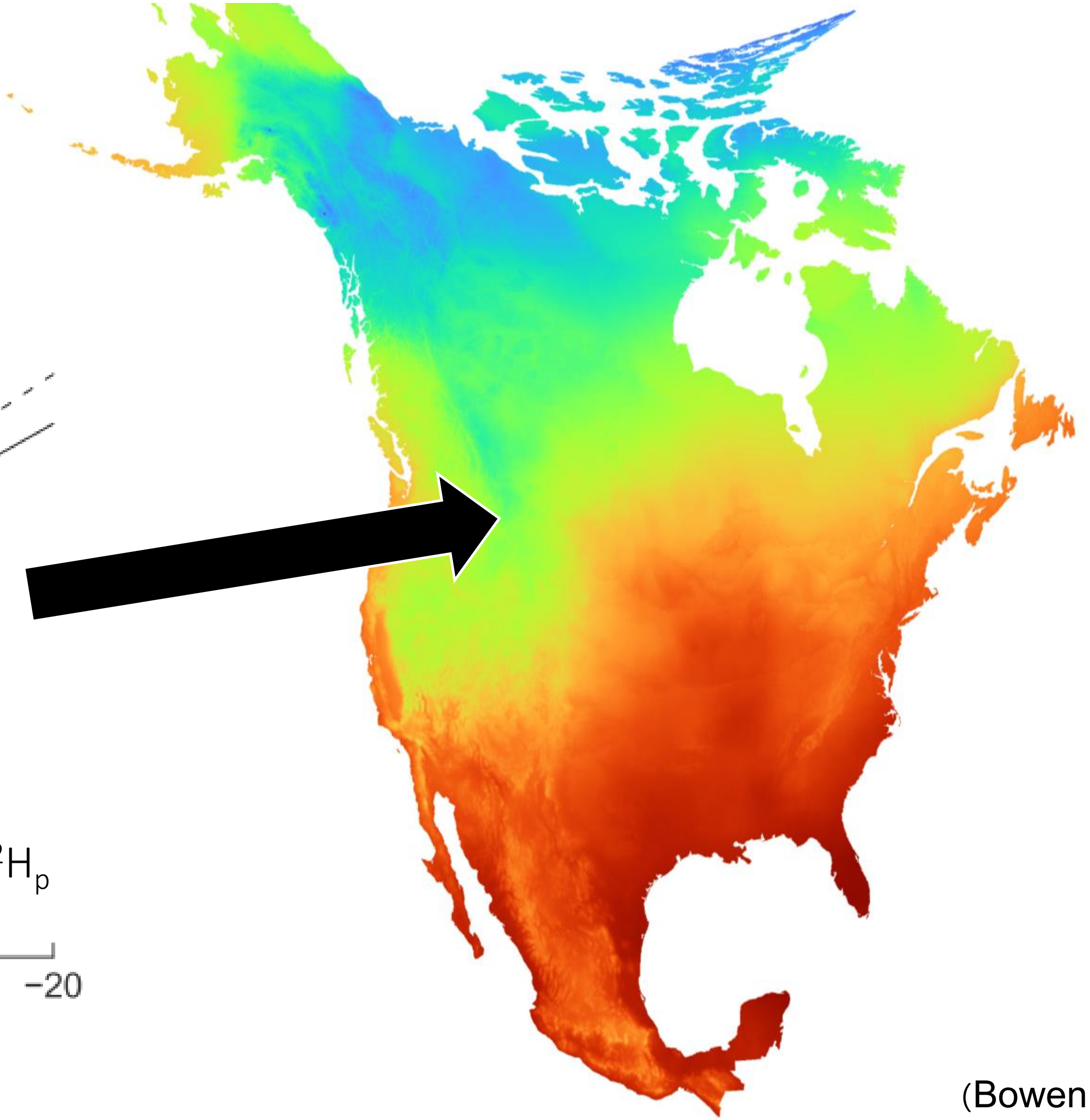
Calibration

Calibration equation

Relationship between $\delta^2H_p + \delta^2H_{tissue}$

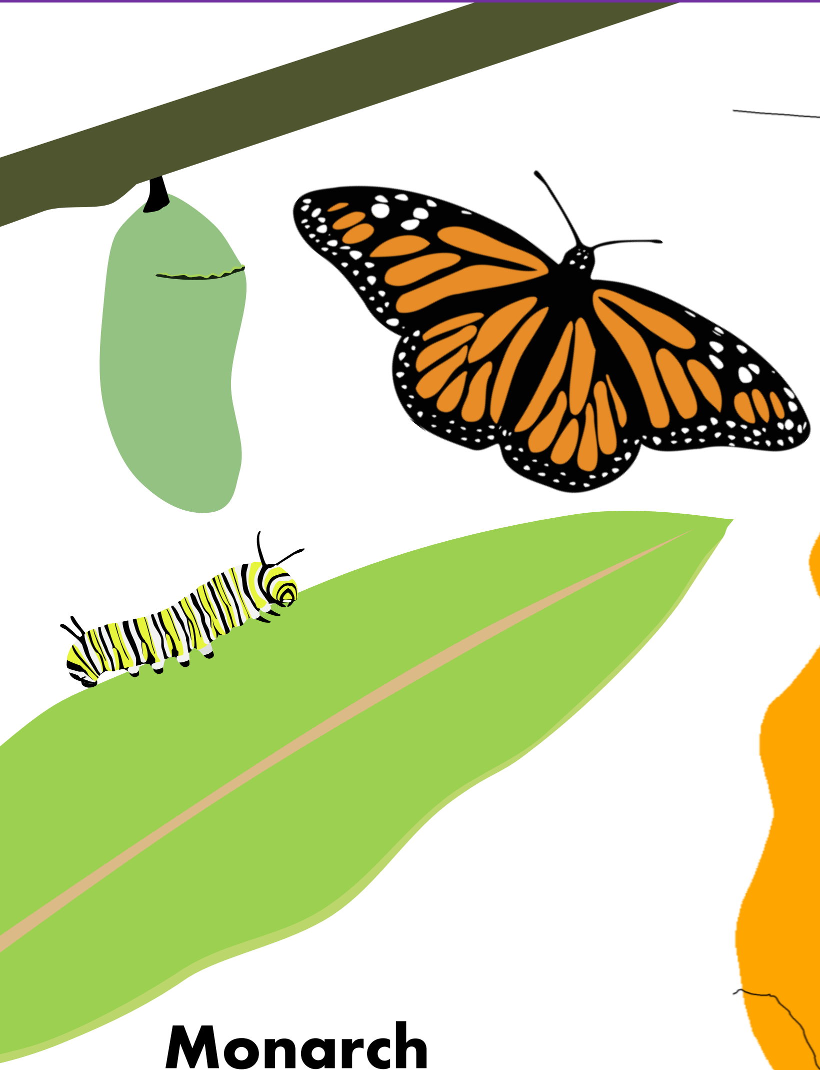
- Tissues grown at known locations

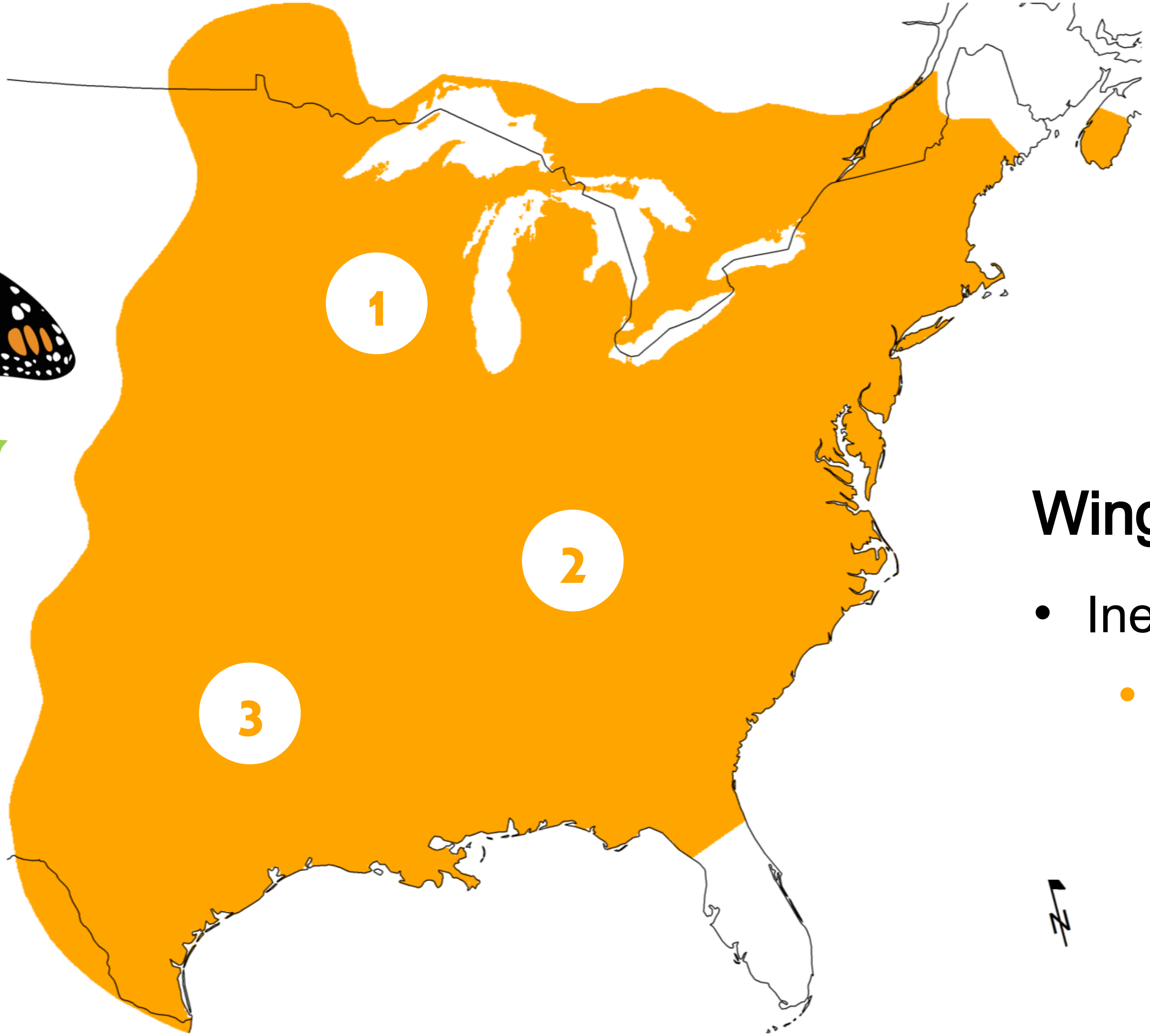




(Bowen et al. 2005)

Example





Wing Chitin δ^2H

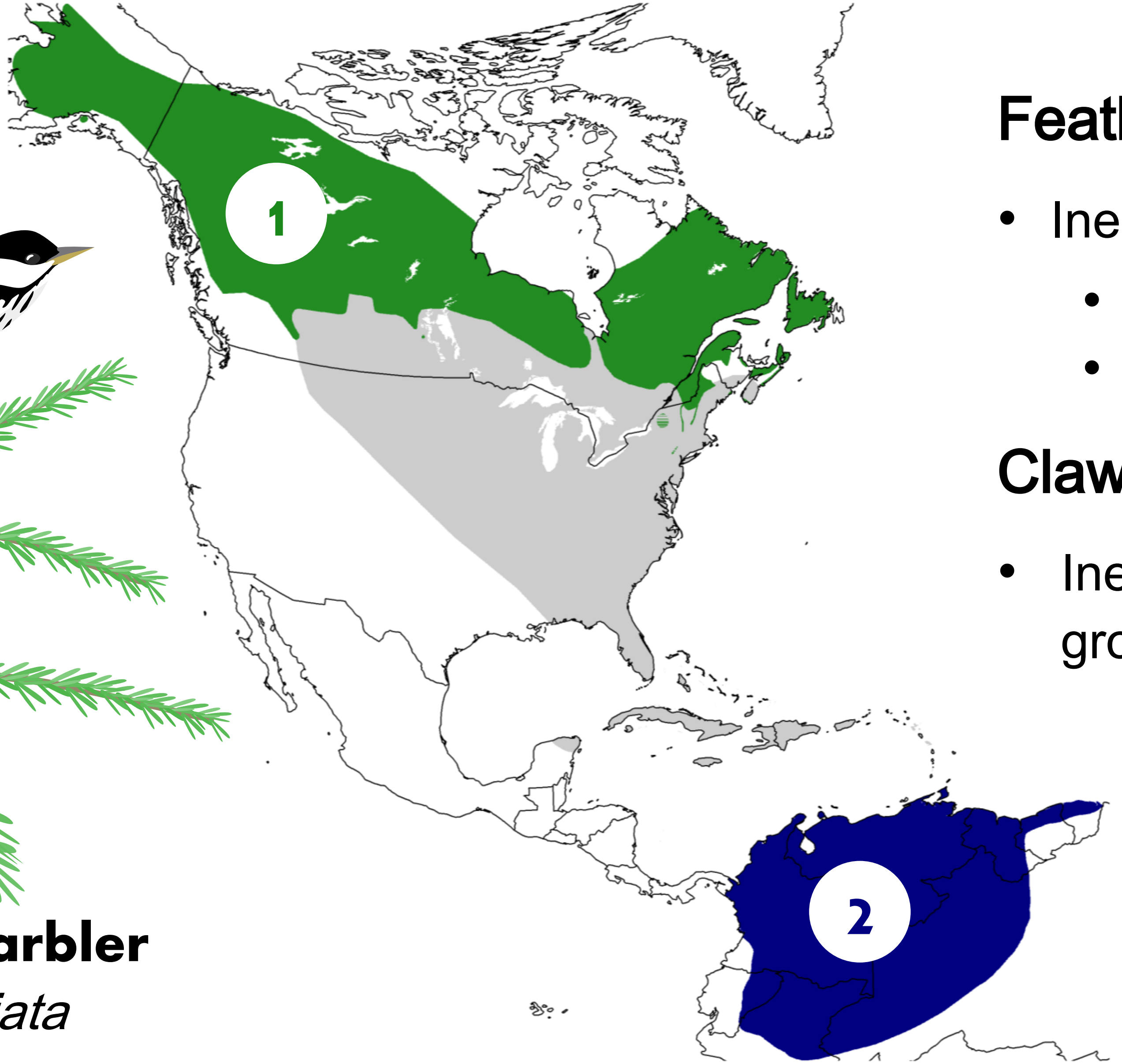
- Inert tissue
  - Natal site

Monarch

Danaus plexipplus

Example

Blackpoll Warbler
Setophaga striata



Feather δ^2H

- Inert tissue
  - 1. Head – Non-breeding
  - 2. Primary – Breeding

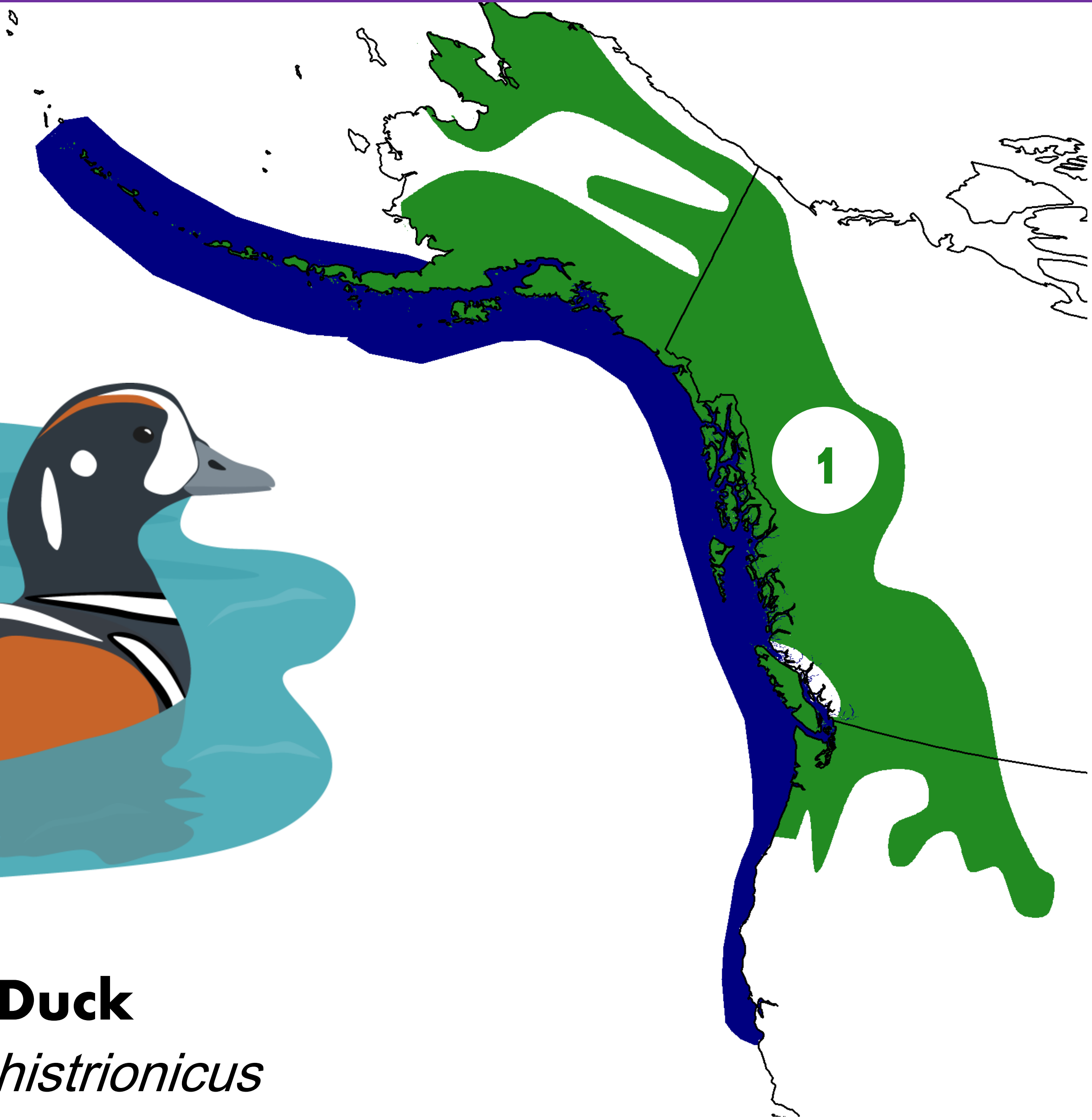
Claw δ^2H

- Inert tissue but continuously grows, depends on timing

Example

Harlequin Duck

Histrionicus histrionicus



Feather δ^2H

- Inert tissue
  - 1. Primary (Adult) – Non-breeding (Marine)
  - 2. Primary (Juvenile) – Breeding

What data do I need?

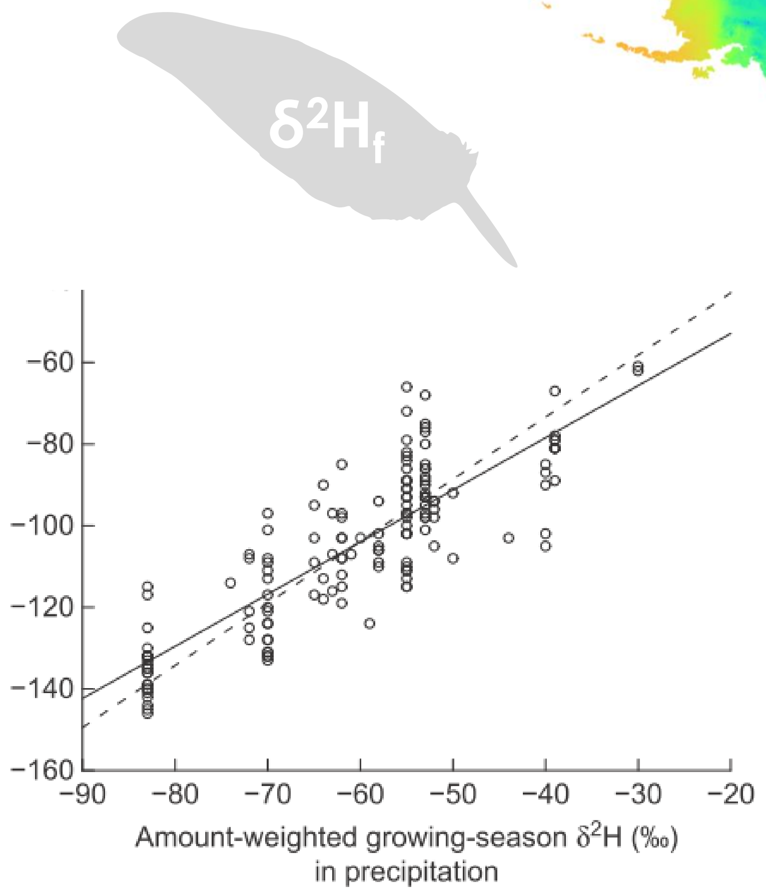
Stable Isotopes

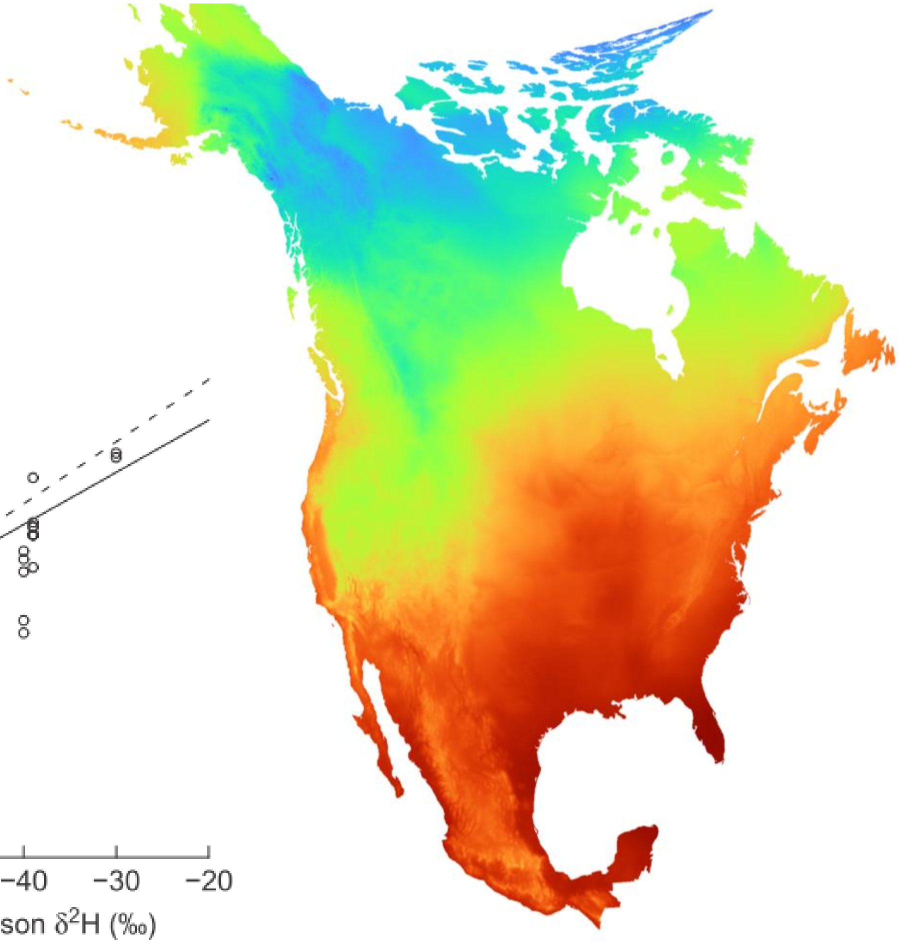
Tissue-isotopes

Isoscape

Calibration

*Priors

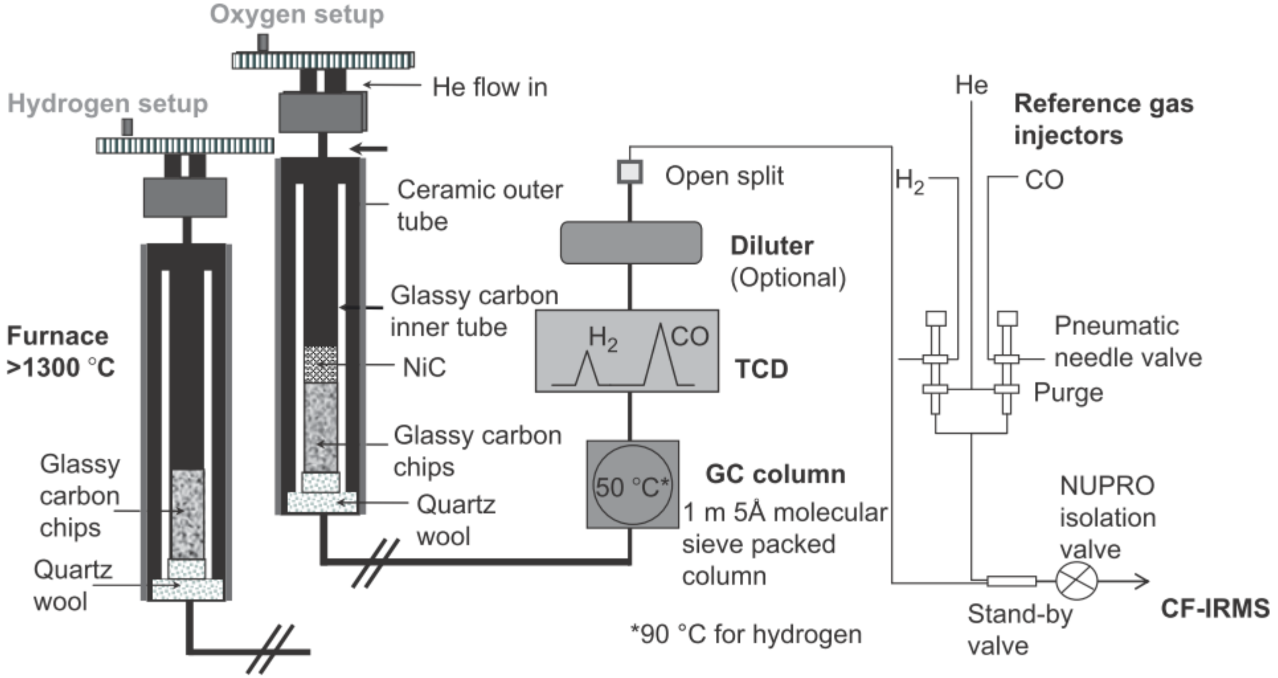


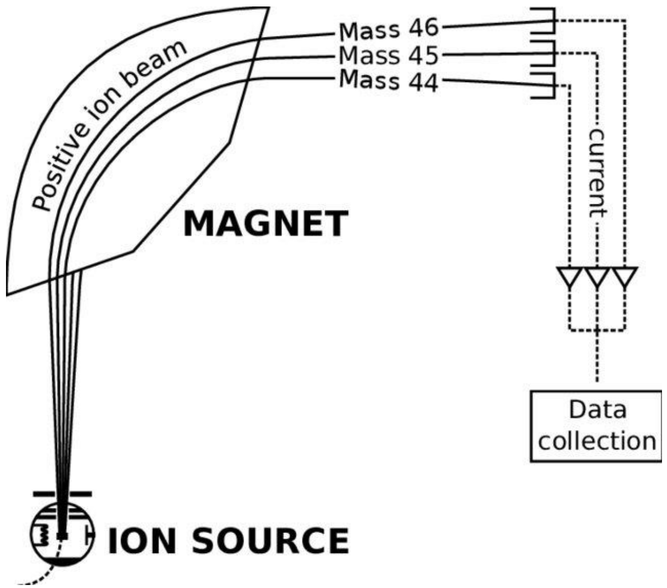


Tissue δ²H

Continuous-flow isotope-ratio mass spectrometry (CF-IRMS)

- Measure small differences in the abundances of isotopes

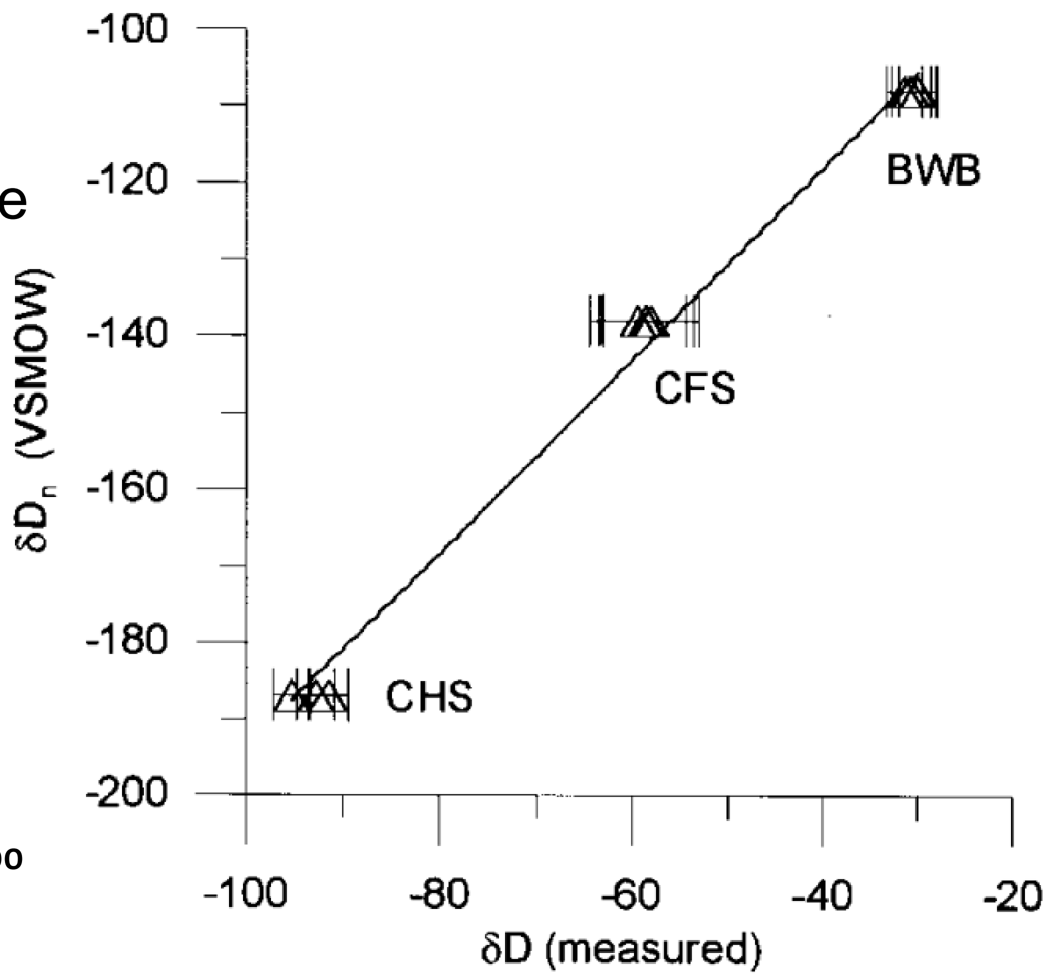




Tissue δ^{2}H

Lab methods to consider:

- **Exchangeable Hydrogen** – uncontrolled hydrogen exchange between ambient moisture and 'exchangeable' hydrogen in sample
  - Comparative equilibration
    - Air calibrate (~48h) keratin standards and samples with lab air
  - Keratin Standards
    - KHS (Kudu) – δ^2H_{VSMOW} = -35.3 ‰
    - CBS (Caribou) – δ^2H_{VSMOW} = -157.0 ‰



(Hobson and Wassenaar 2003)

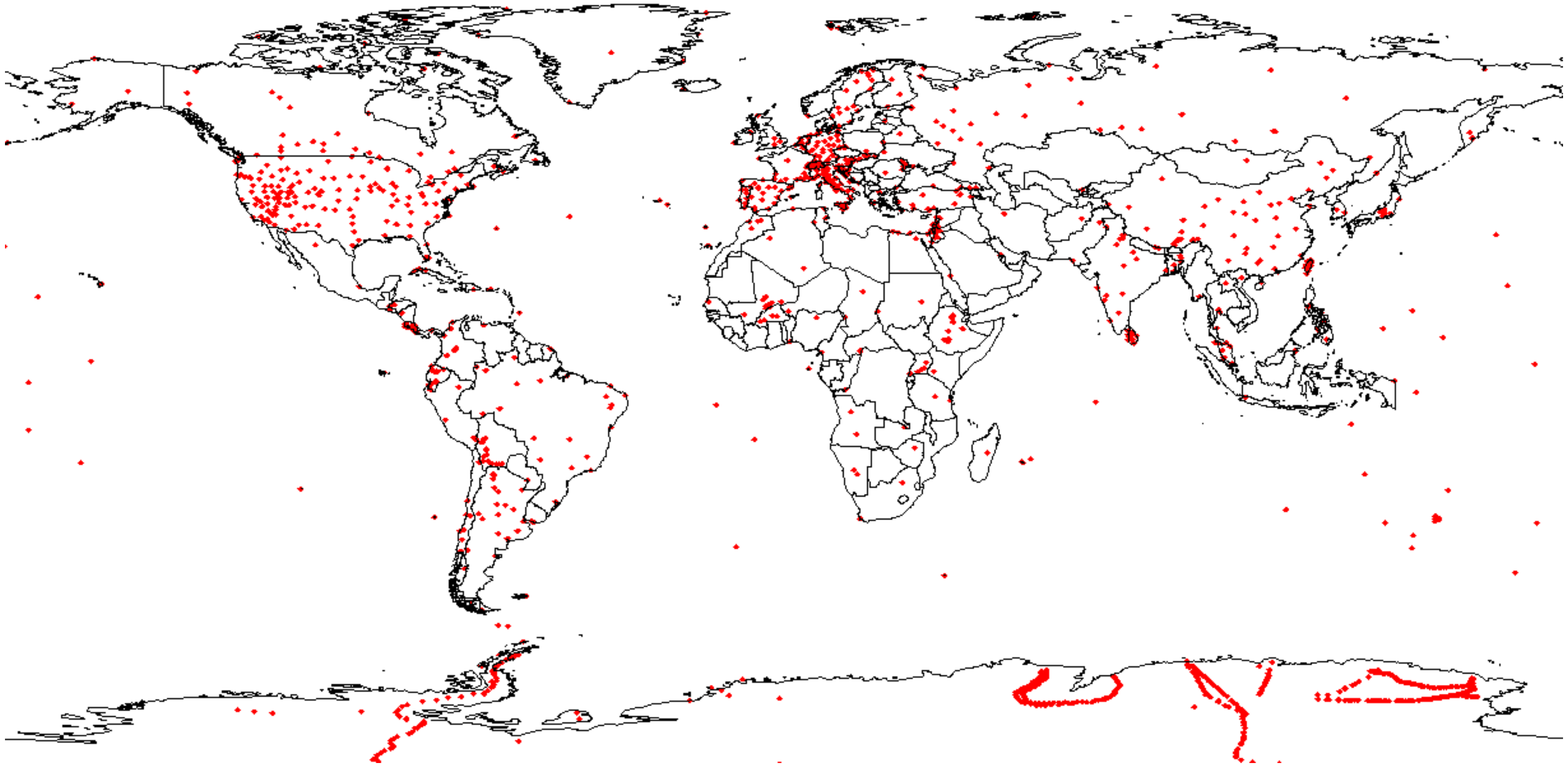
Isoscapes

Hydrogen (δ^2H) Isoscapes

Global Network of Isotopes in Precipitation (GNIP)

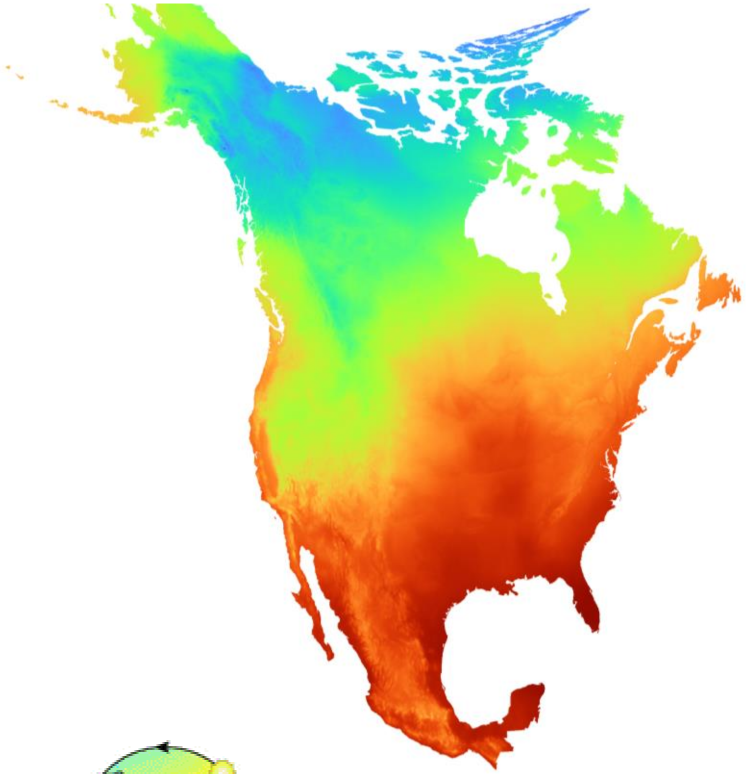
- Established 1961 (1491 stations)

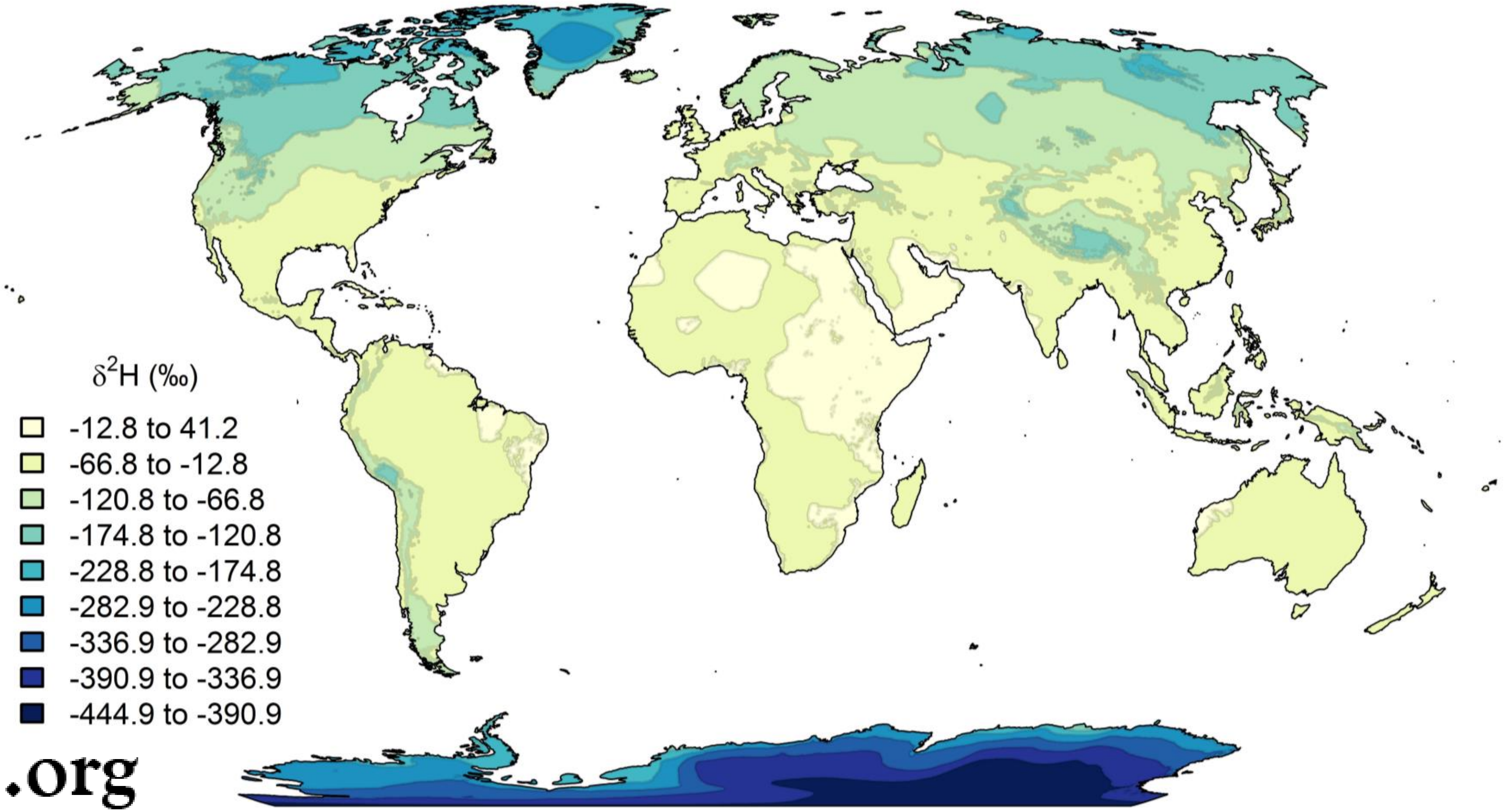
IAEA
International Atomic Energy Agency



Isoscapes

Hydrogen (δ^2H) Isoscapes



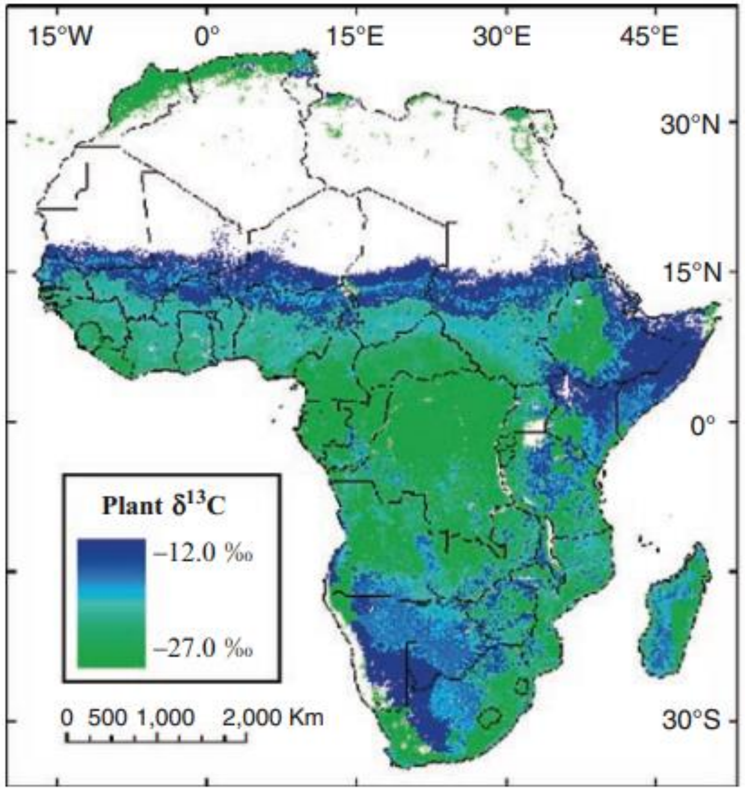


Waterisotopes.org

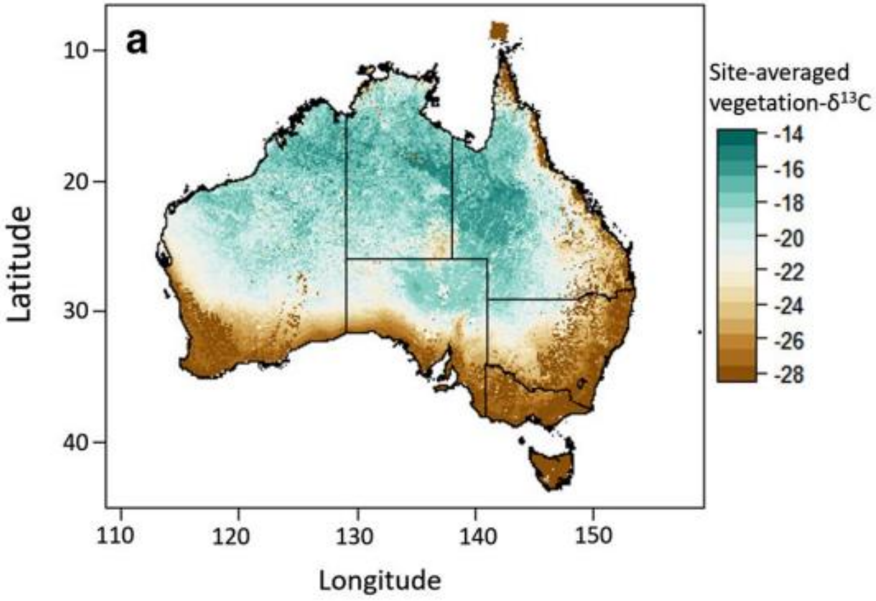
(Bowen et al. 2005)

Isoscapes

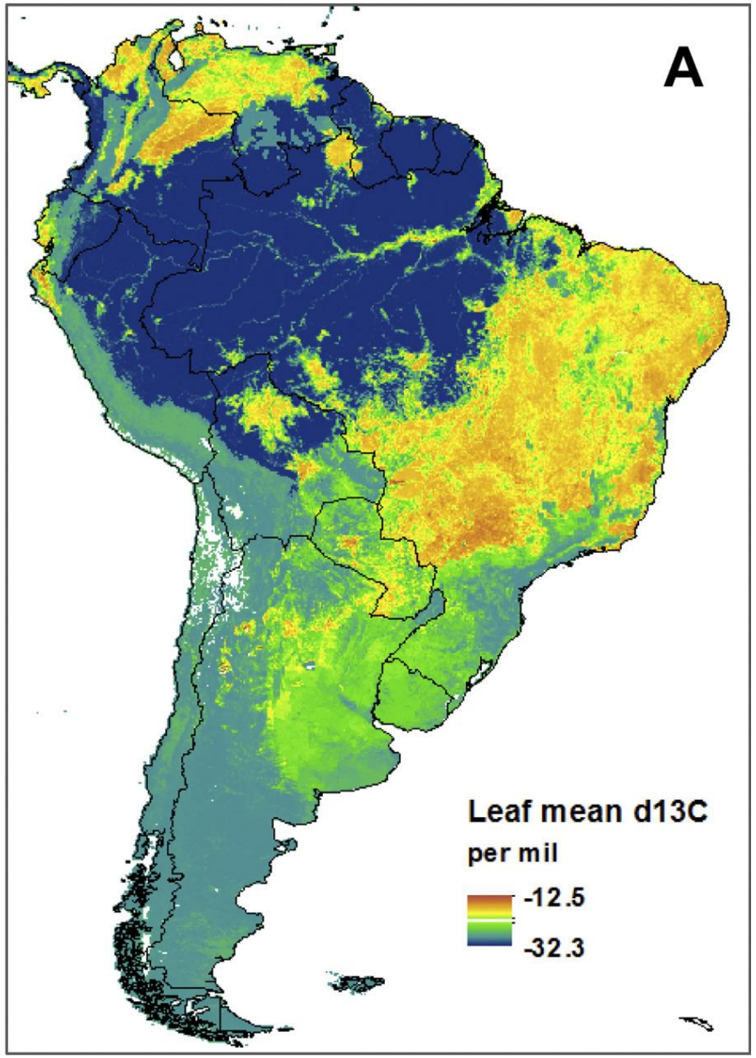
Carbon (δ^{13}C) Isoscapes



(Still and Powel 2010)

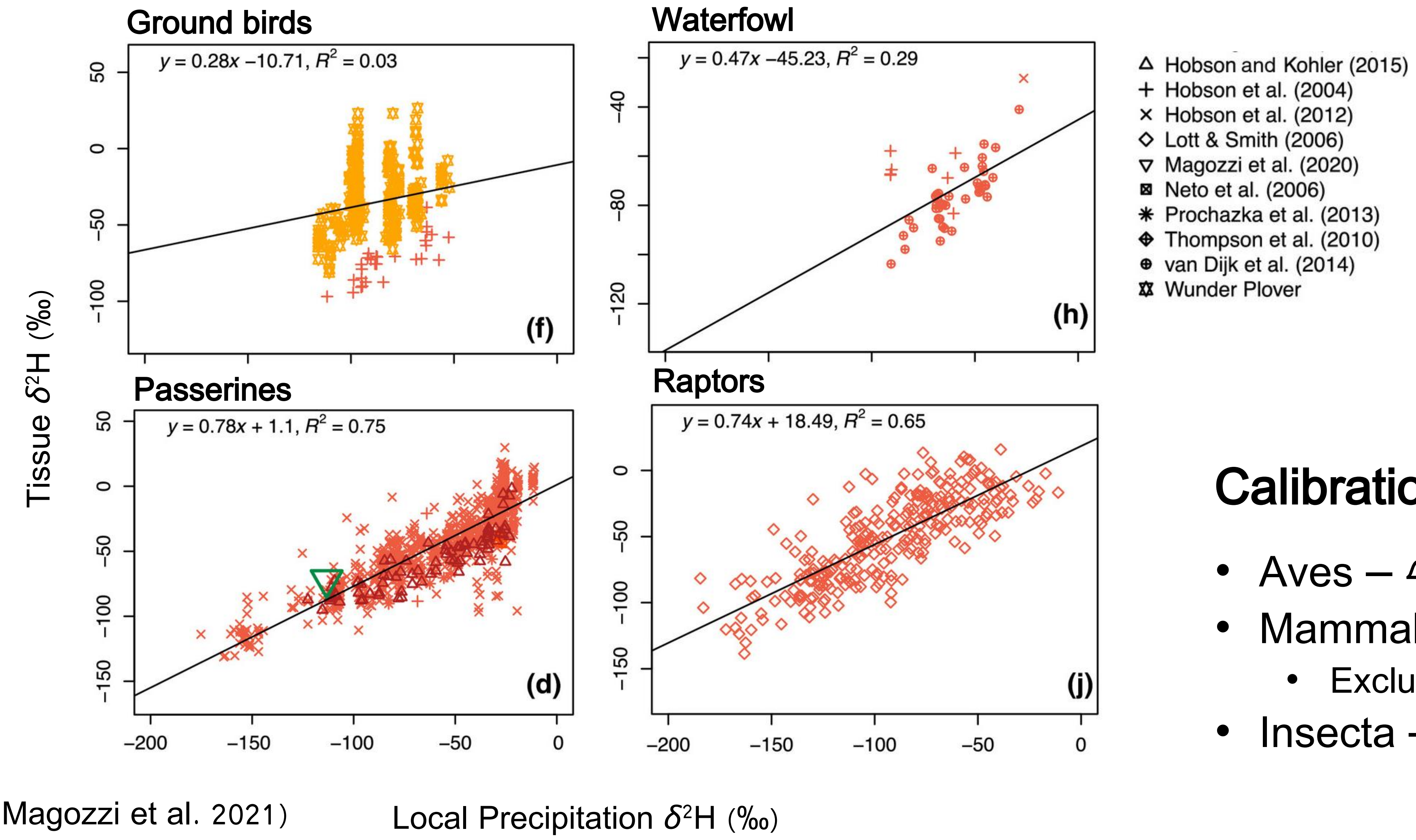


(Munroe et al. 2022)



(Powel et al. 2012)

Calibration data



(Magozzi et al. 2021)

Calibration Studies:

- Aves – 42
- Mammalia – 5
  - Excluding humans
- Insecta – 10

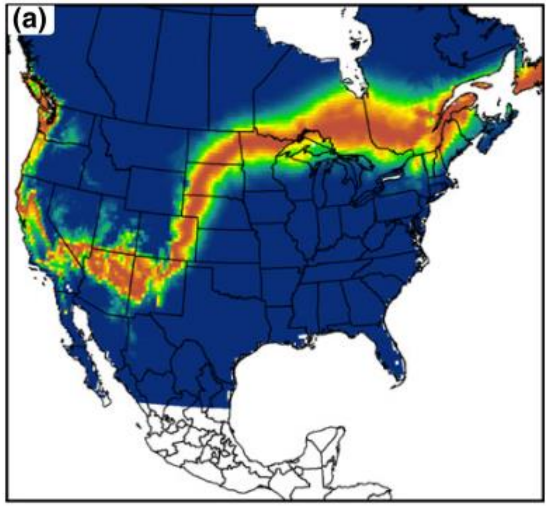
Priors

Prior probability of origin

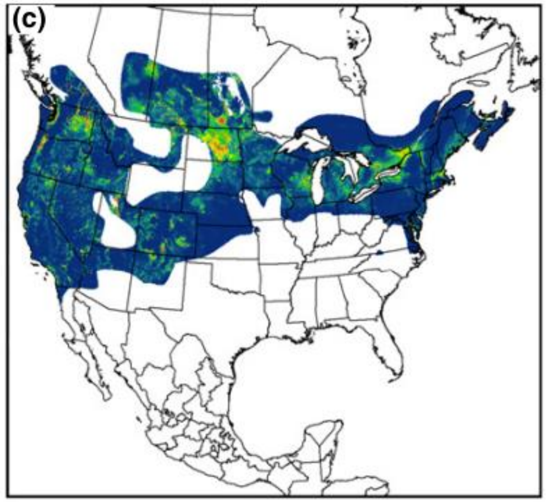
Refine assignment to origin using other sources of information

- **Banding data** (Palumbo et al. 2019)
- **Genetics** (Chabot et al. 2012)
- **Habitat composition** (Ruegg et al. 2017)
- **Relative abundance** (Fournier et al. 2017)

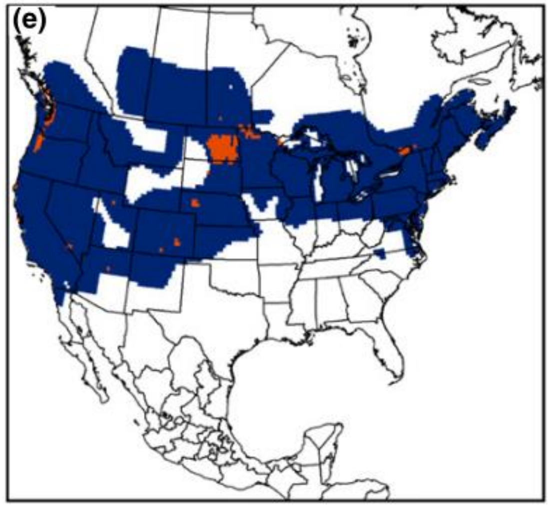
Stable-hydrogen isotopes



Relative abundance

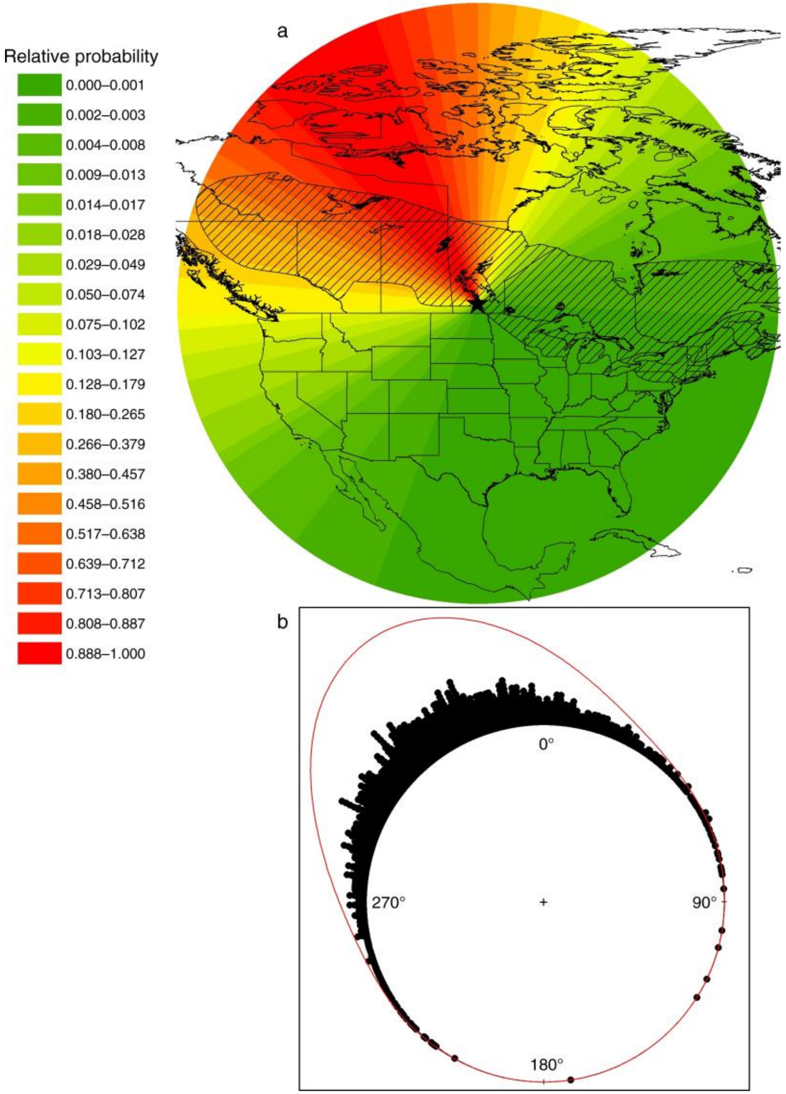


Combined

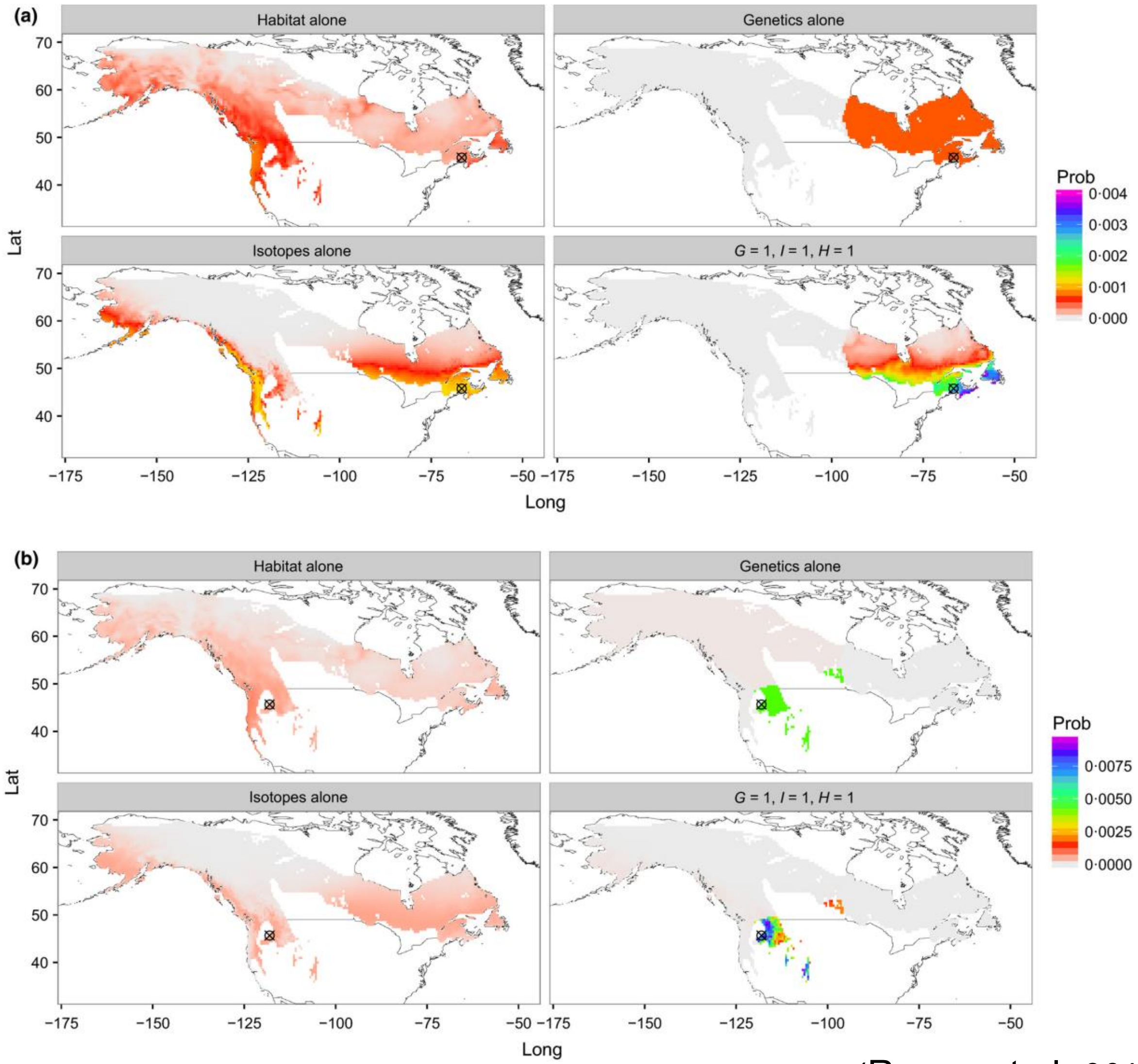


(Modified from Fournier et al. 2017)

Priors



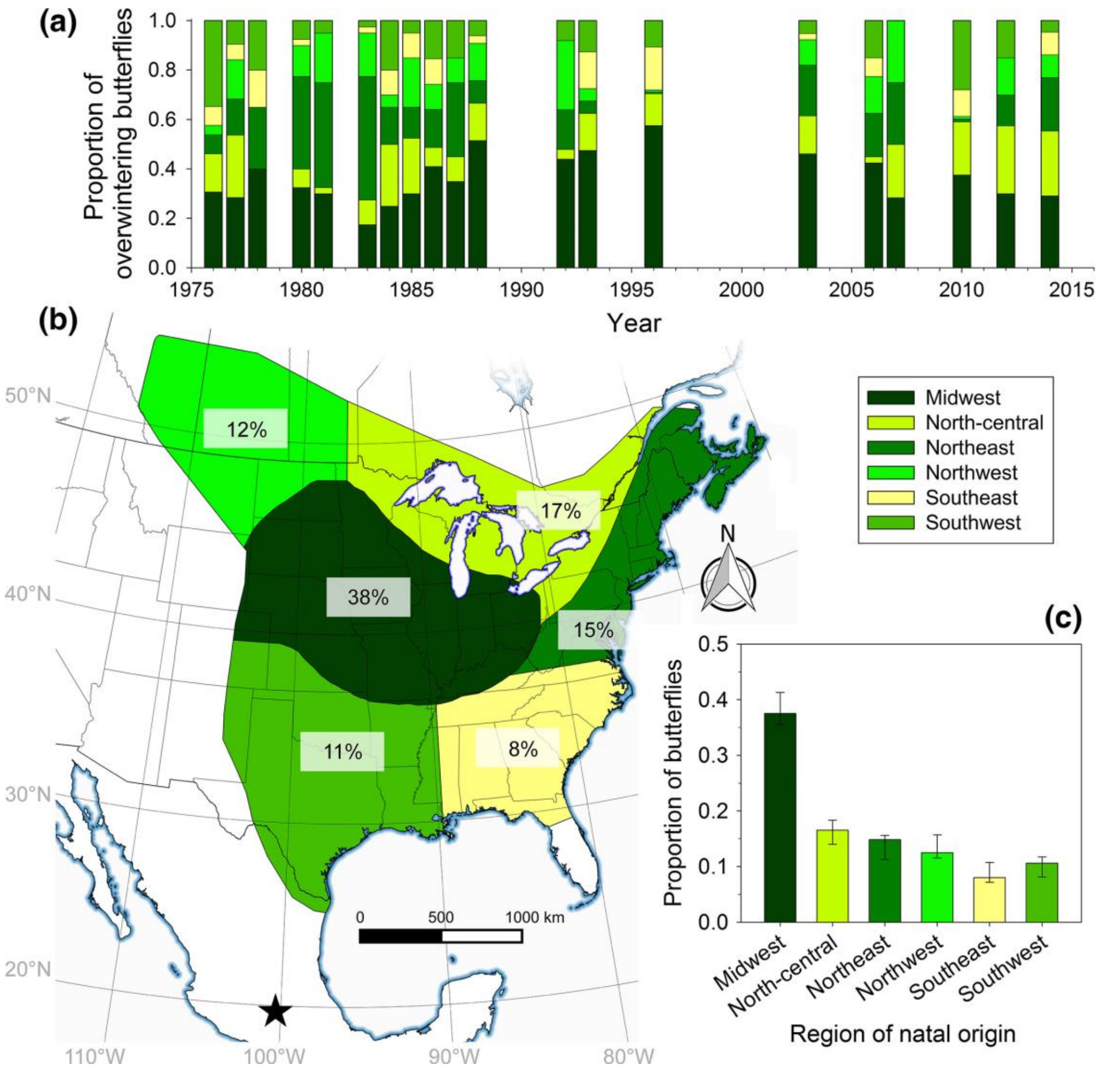
(van Wilgenburg and Hobson 2011)



(Ruegg et al. 2017)

How do I do the data analysis?

Categorical Assignment



Assignment to bins

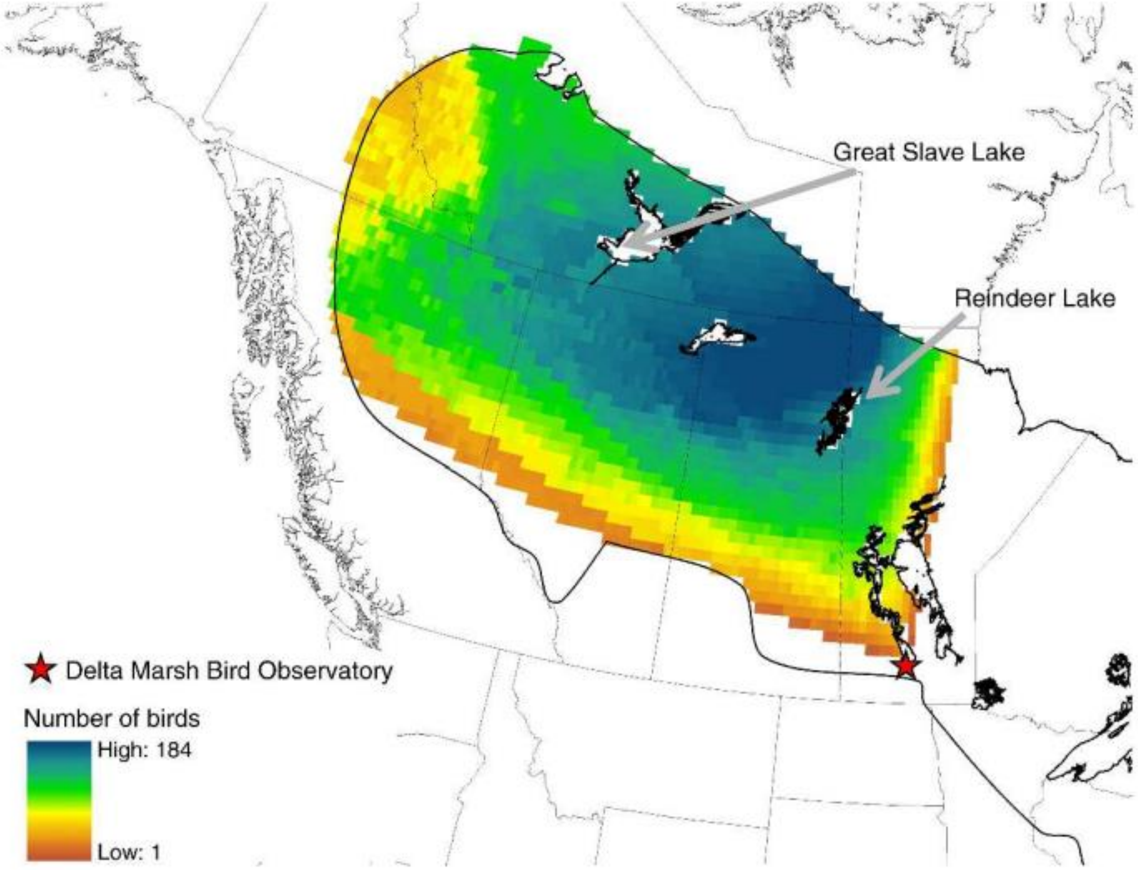
- Compare stable isotope values within feathers to mean values to determine likely origins
- Determine most likely bin of origin
  - Example: Monarchs captured at overwintering sites, assigned to natal areas using δ^2H and $\delta^{13}C$ values

(Flockhart et al. 2017)

Spatially-explicit Assignment

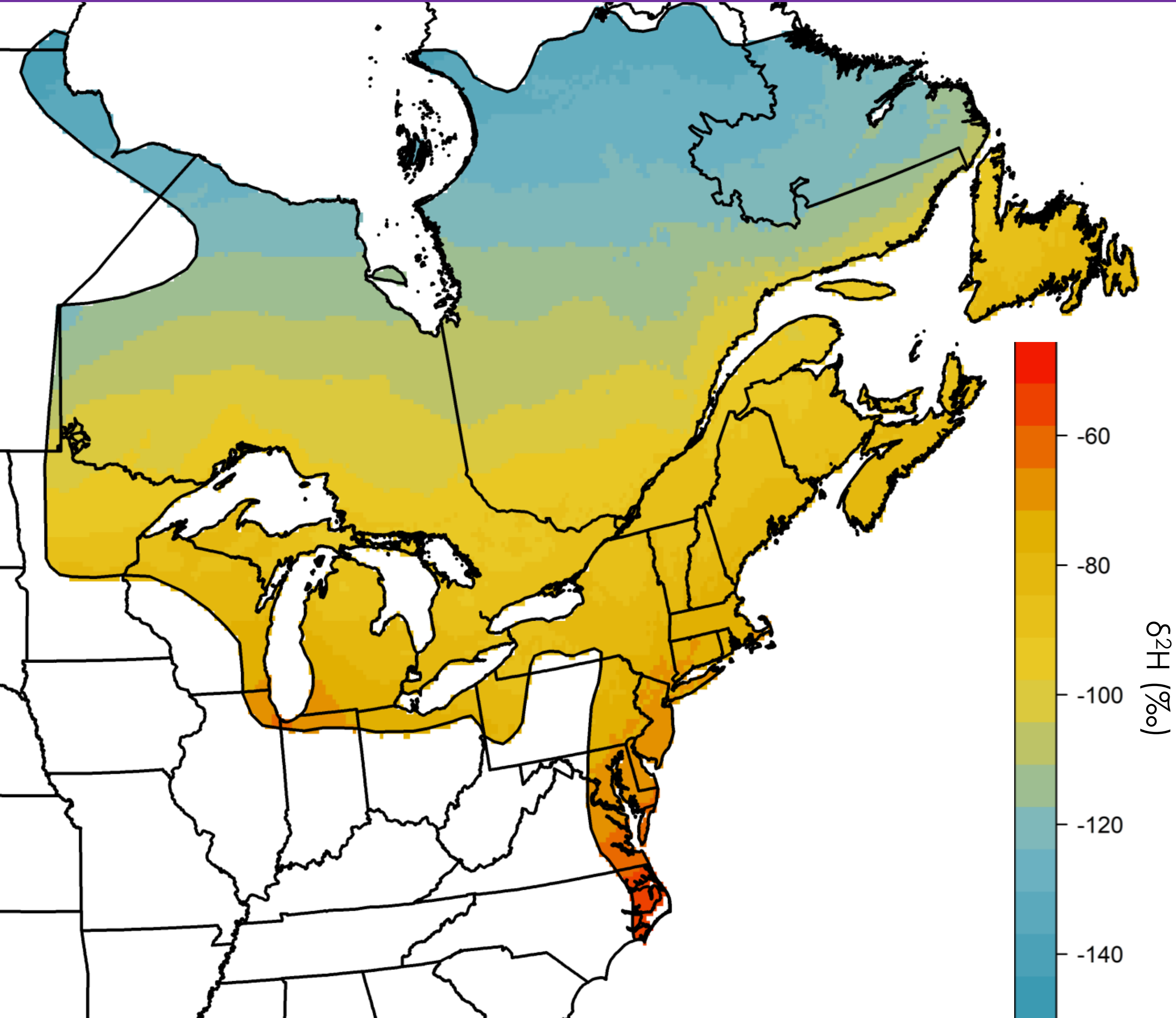
Likelihood-based assignment

- Compare stable isotope values within tissues to isoscapes to determine likely origins
- Color scale represents the number of individuals that were likely assigned at any pixel



(van Wilgenburg and Hobson 2011)

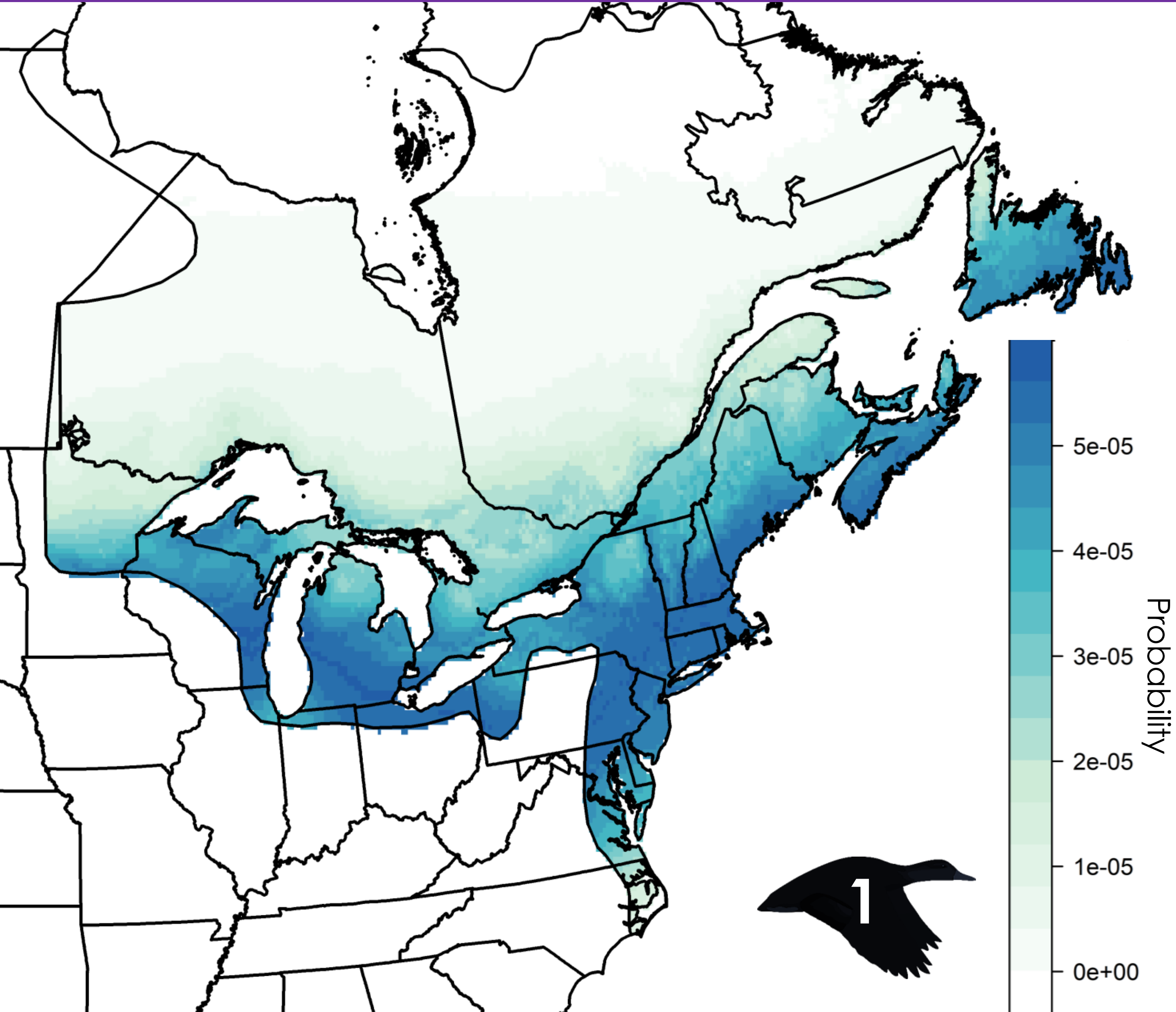
Spatially-explicit Assignment



Likelihood-based Assignment

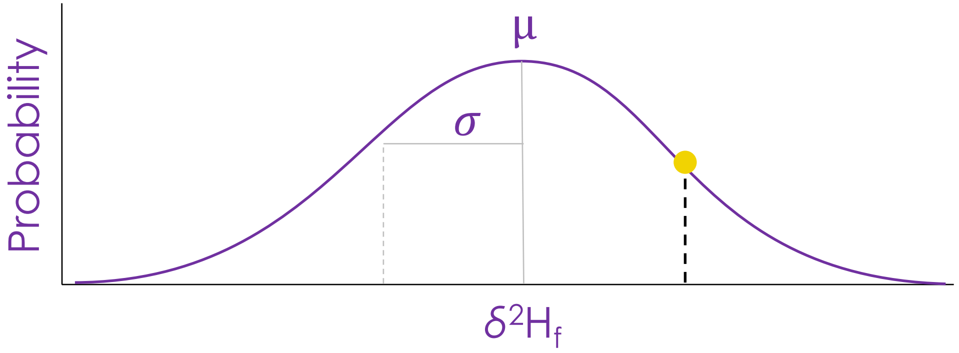
1. Restrict isoscape to breeding range
   - Only interested in the area where the individual could have originated from

Spatially-explicit Assignment



Likelihood-based Assignment

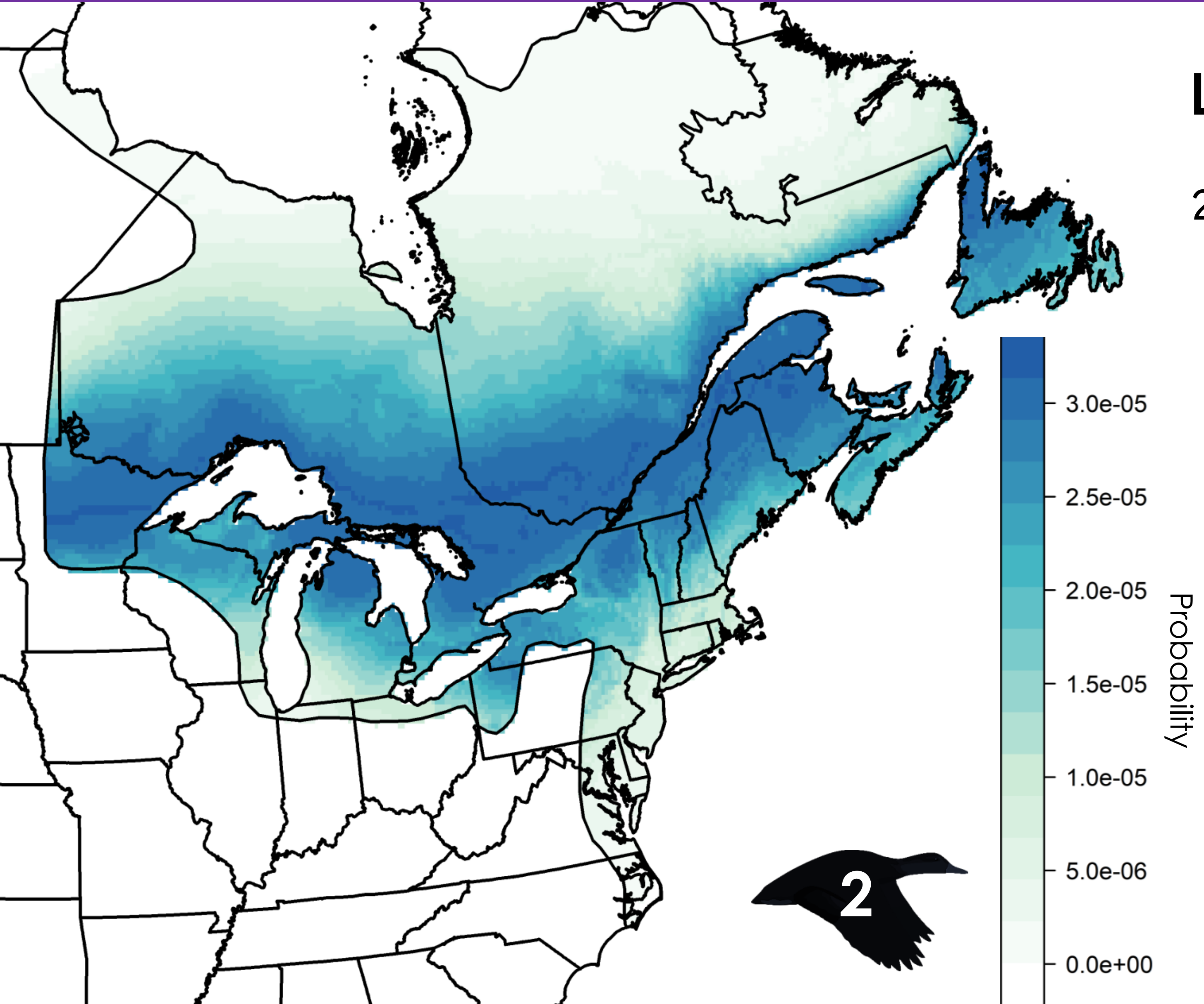
2. For every pixel, estimate probability that it is the origin, based on the ducks δ^2H_f and the pixel's δ^2H_f



$$f(y|\mu_c,\sigma_c) = \left(\frac{1}{\sigma_c\sqrt{2\pi}}\right) e\left[-\frac{(y-\mu_c)^2}{2{\sigma_c}^2}\right]$$

Normal probability density function

Spatially-explicit Assignment



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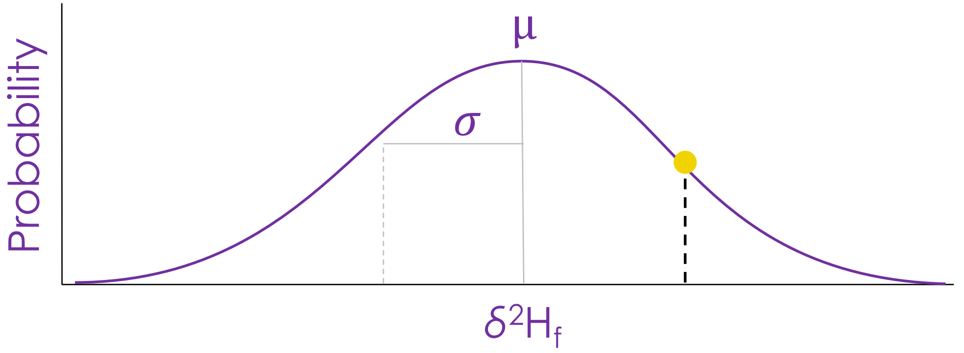
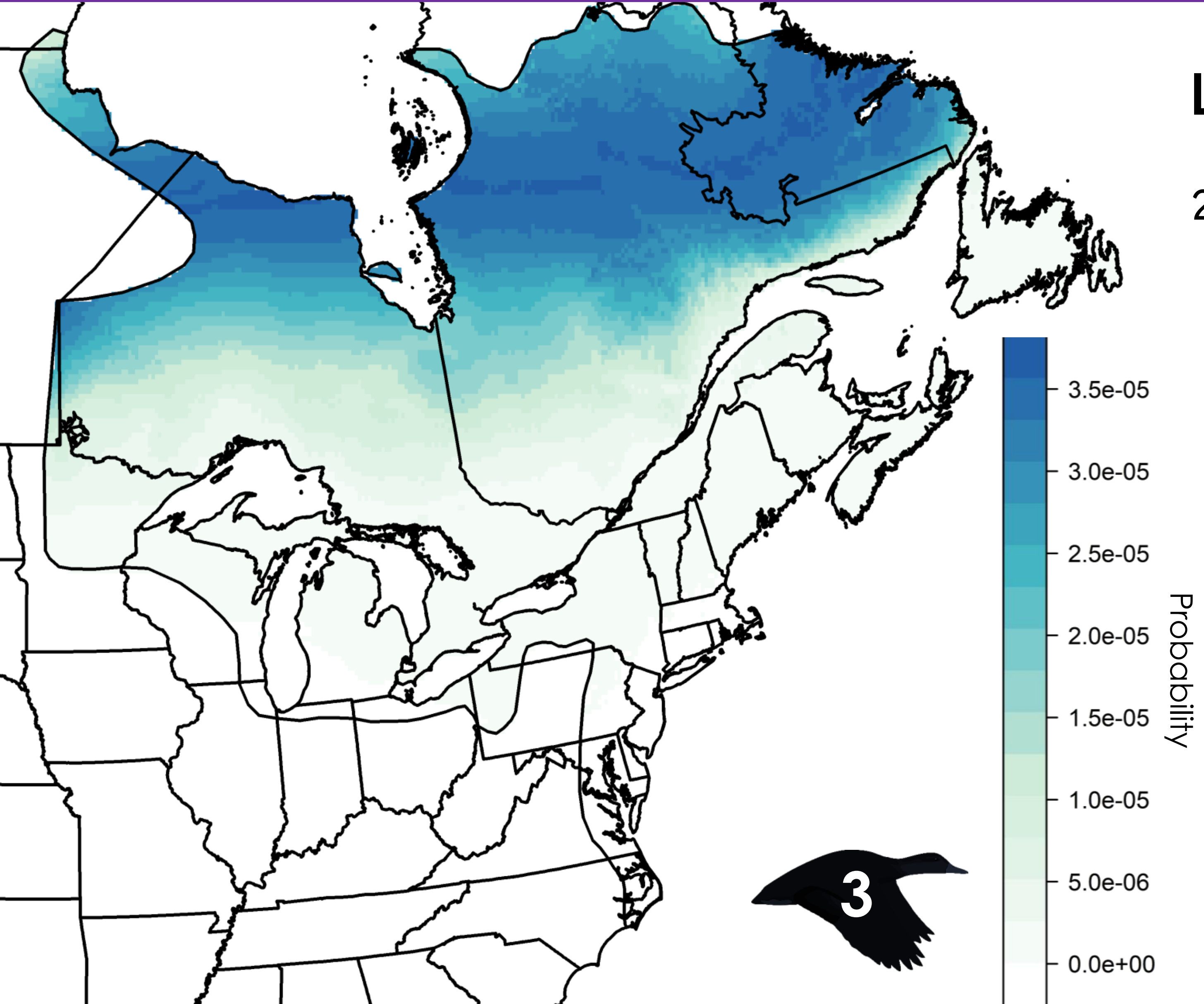


$$f(y|\mu_c, \sigma_c) = \left(\frac{1}{\sigma_c\sqrt{2\pi}}\right) e\left[-\frac{(y-\mu_c)^2}{2\sigma_c^{\ 2}}\right]$$

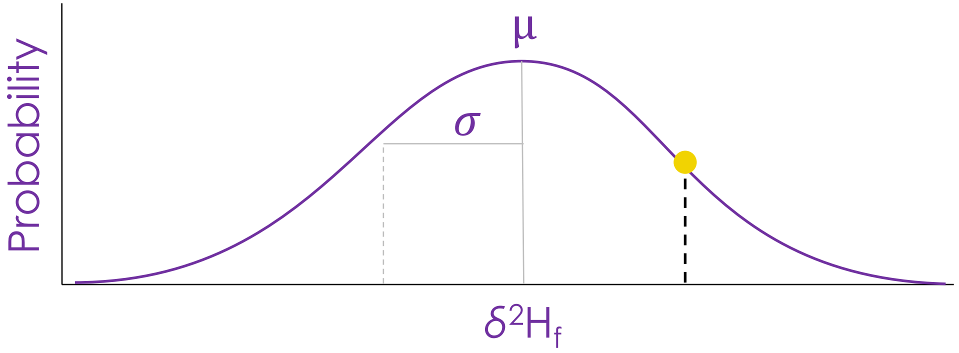
Normal probability density function

Assignment



Likelihood-based Assignment

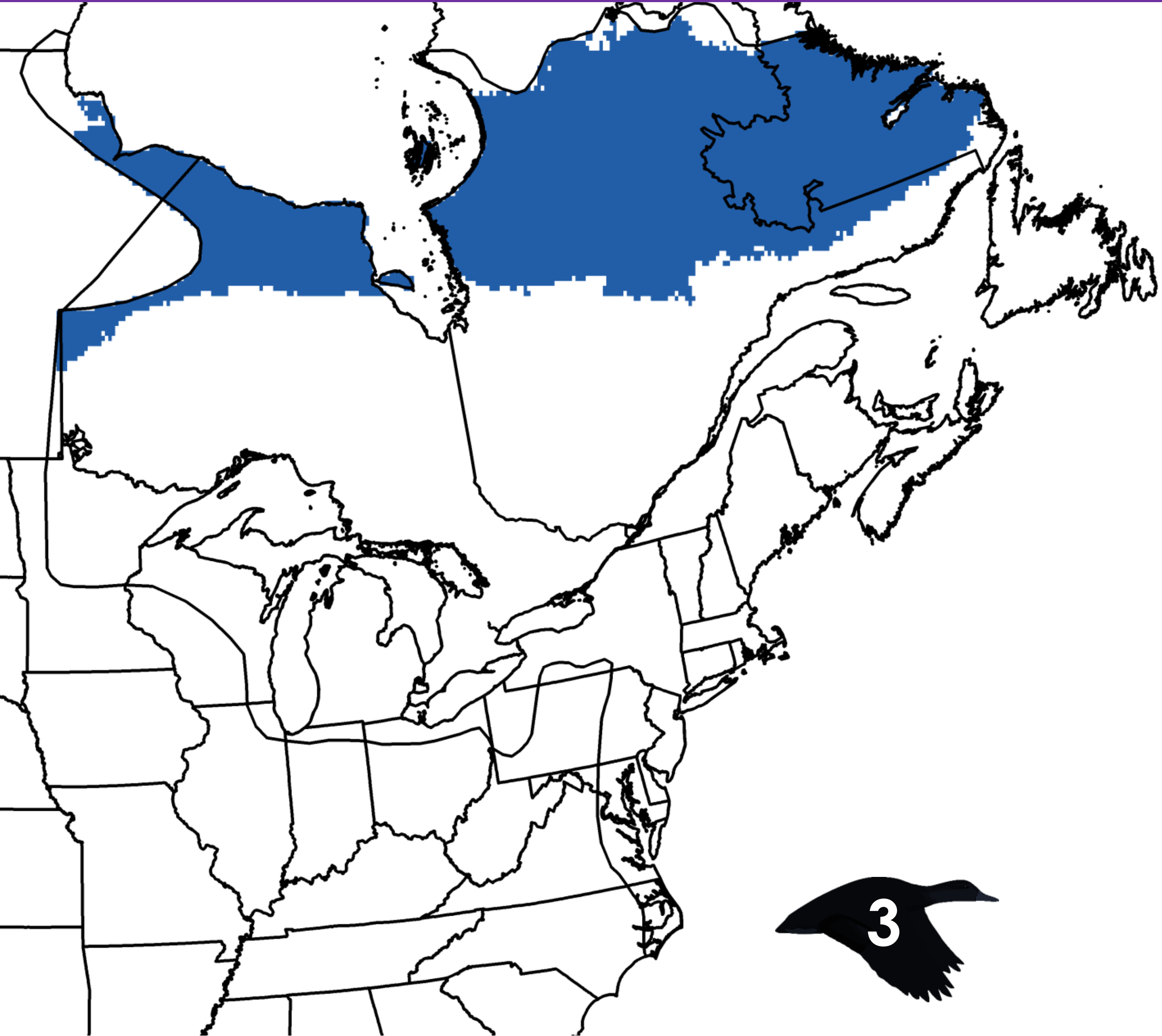
2. For every pixel, estimate probability that it is the origin, based on the ducks δ^2H_f and the pixel's δ^2H_f



$$f(y|\mu_c, \sigma_c) = \left(\frac{1}{\sigma_c\sqrt{2\pi}}\right) e\left[-\frac{(y-\mu_c)^2}{2\sigma_c{}^2}\right]$$

Normal probability density function

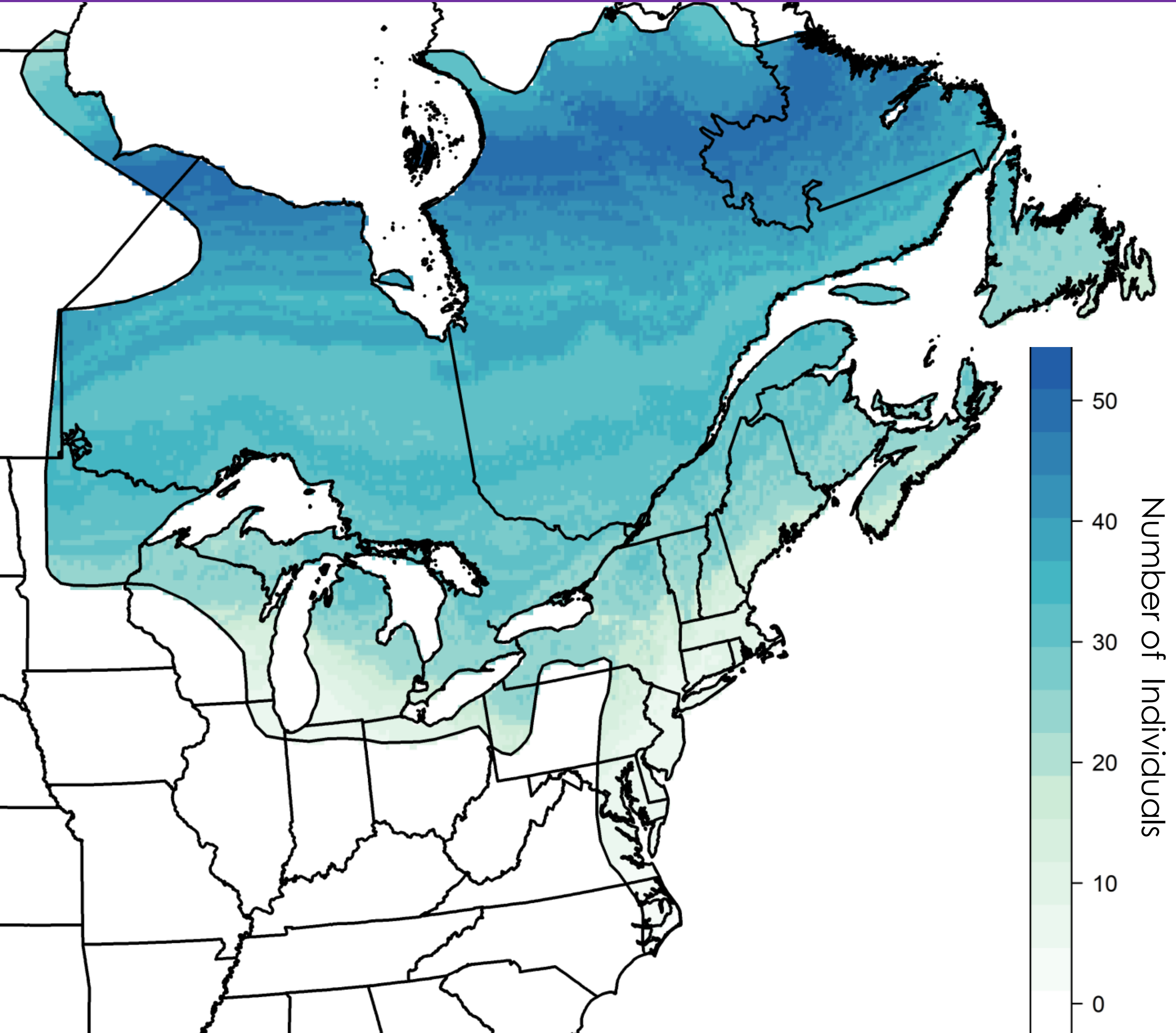
Spatially-explicit Assignment



Likelihood-based Assignment

3. Simplify the region into a binary ‘likely’ origins region
   - **Odds ratio** - Odds (2:1 here) that a given bird had truly originated from within the simplified range

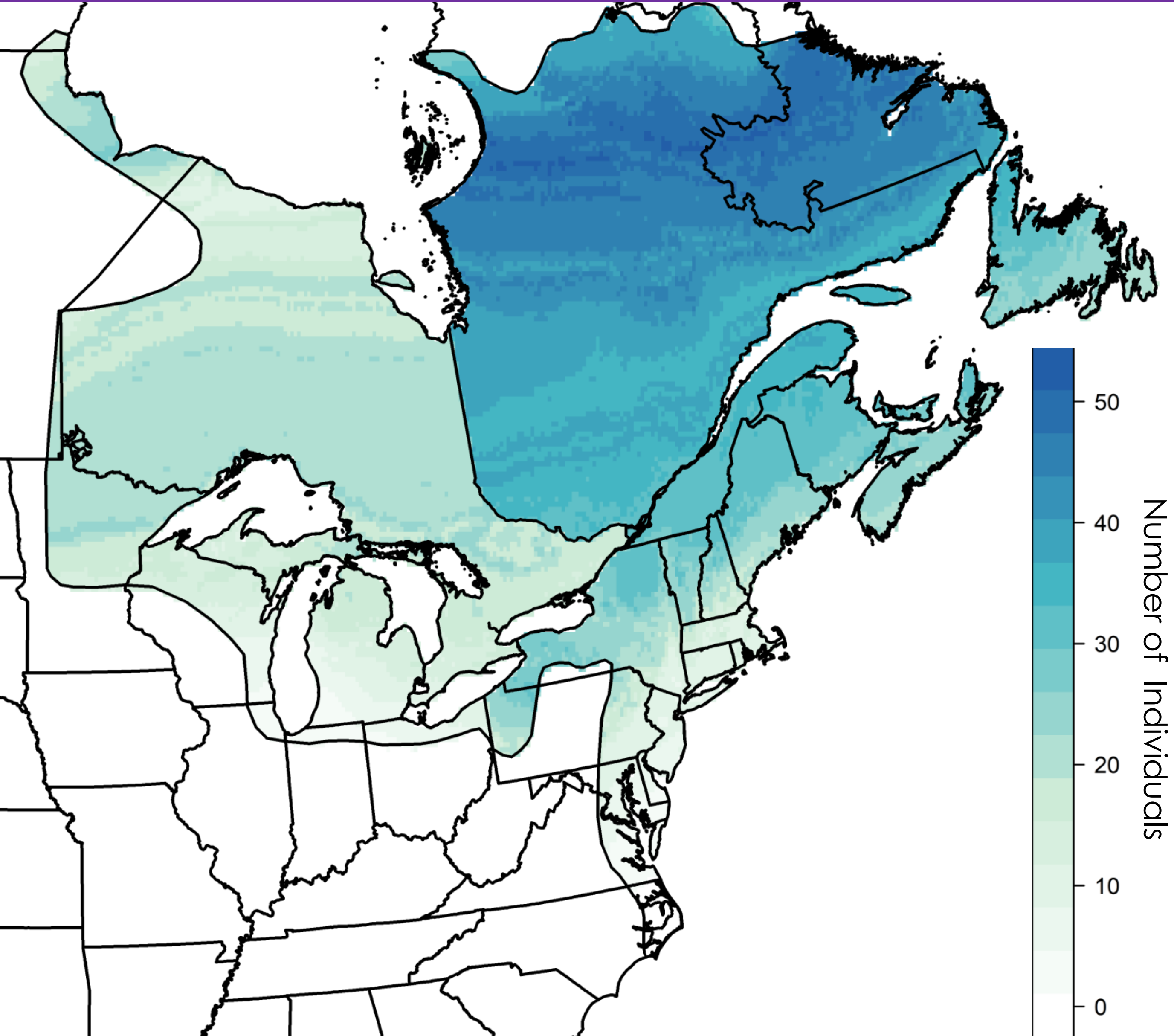
Spatially-explicit Assignment



Likelihood-based Assignment

4. Sum the surfaces across all individuals
    - The scale represents the number of individuals assigned to any pixel

Spatially-explicit Assignment



Likelihood-based assignment

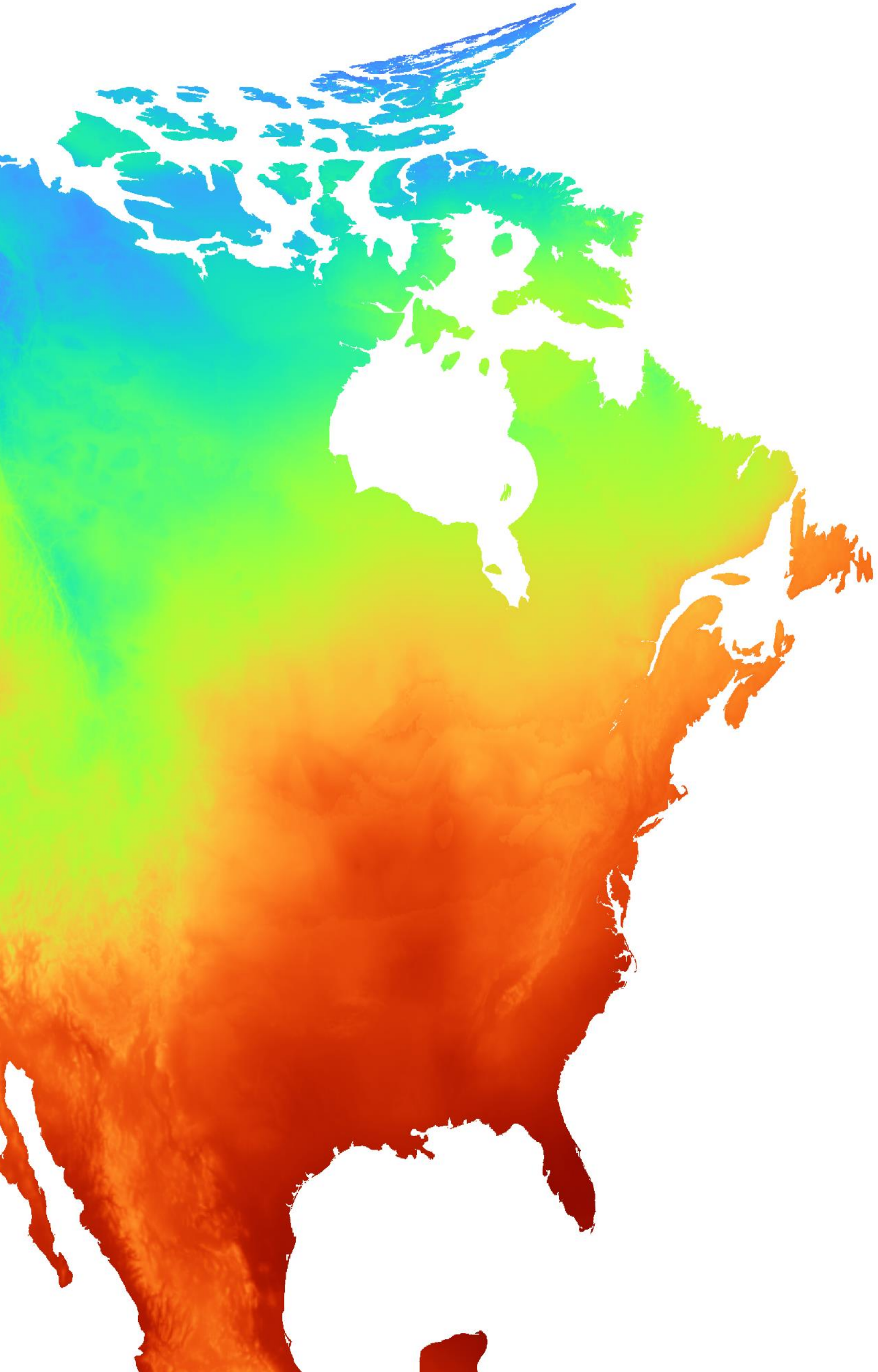
Posterior Probability - f_x

Prior Probability - f_b

- Prior probability of origin: band returns by flyway

Baye's Rule

$$f_x = \frac{f(y|\mu_c, \sigma_c)\, f_b}{\Sigma f(y|\mu_c, \sigma_c) f_b}$$



ANY QUESTIONS?

SHORT BREAK - THEN R!

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