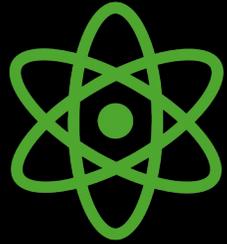


Discovering the secrets of the bats: stable isotope analysis for bat research



CJ Campbell, PhD
Bat Conservation International
ccampbell@batcon.org



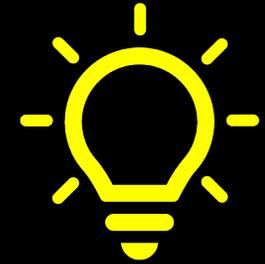
A GENTLE
INTRODUCTION TO
STABLE ISOTOPE
ECOLOGY



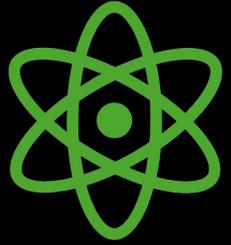
STABLE ISOTOPES TO
STUDY BAT
MOVEMENTS



STABLE ISOTOPES TO
REVEAL BAT
DIET

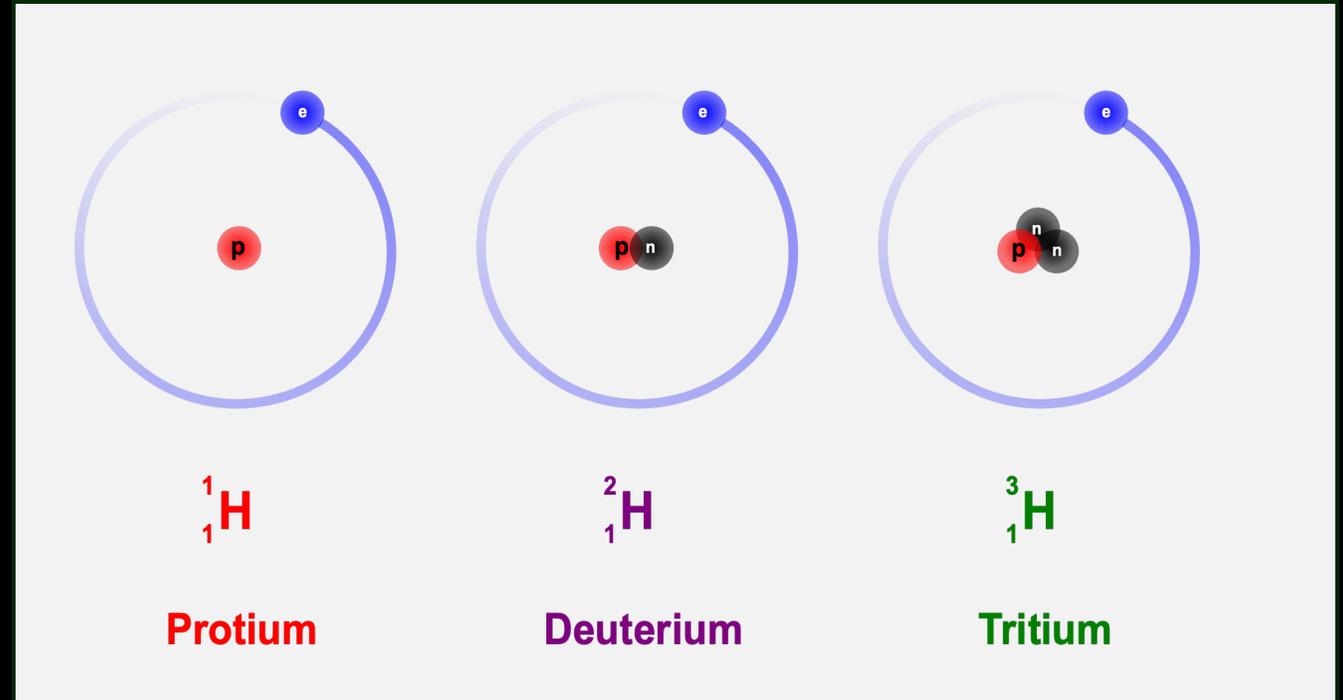


PURSUING
STABLE ISOTOPE
RESEARCH

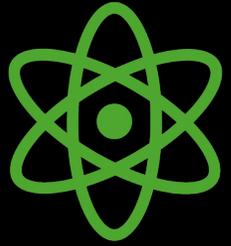


A gentle introduction to isotope chemistry

- Atoms are the same element when they share the same number of protons
- Isotopes of the same element might have different numbers of neutrons.

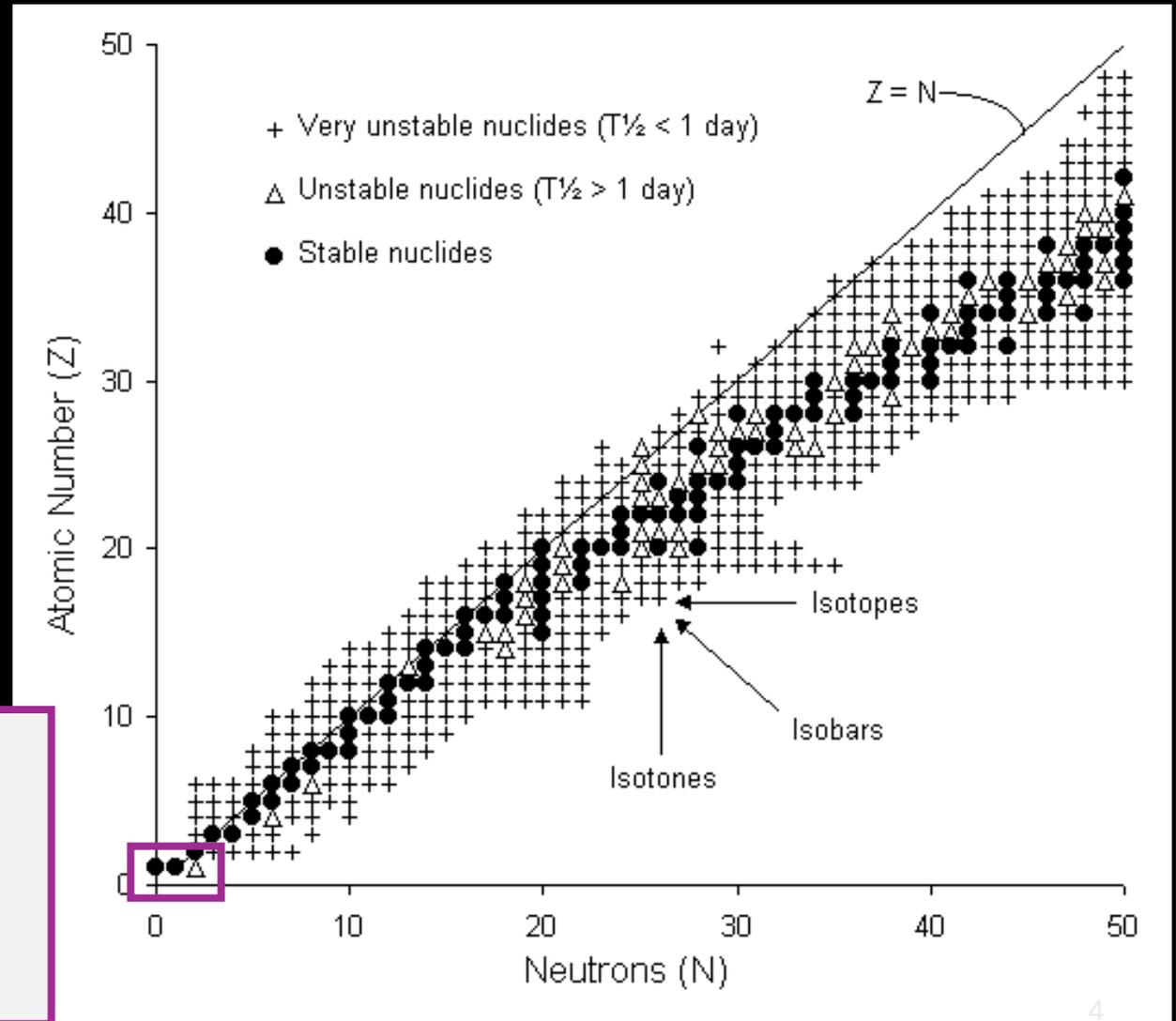
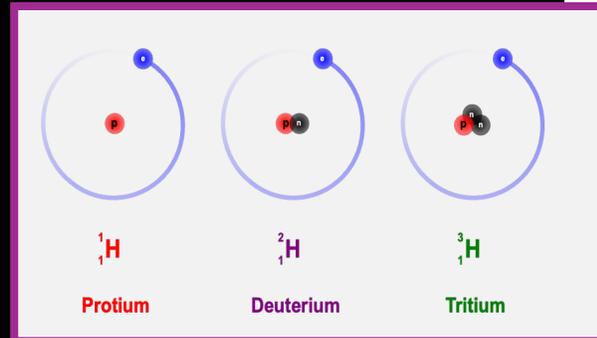


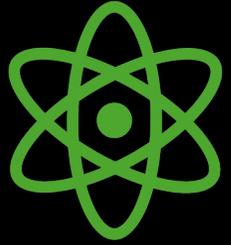
Hydrogen isotopes:
Same element, different atomic weights



A gentle introduction to isotope chemistry

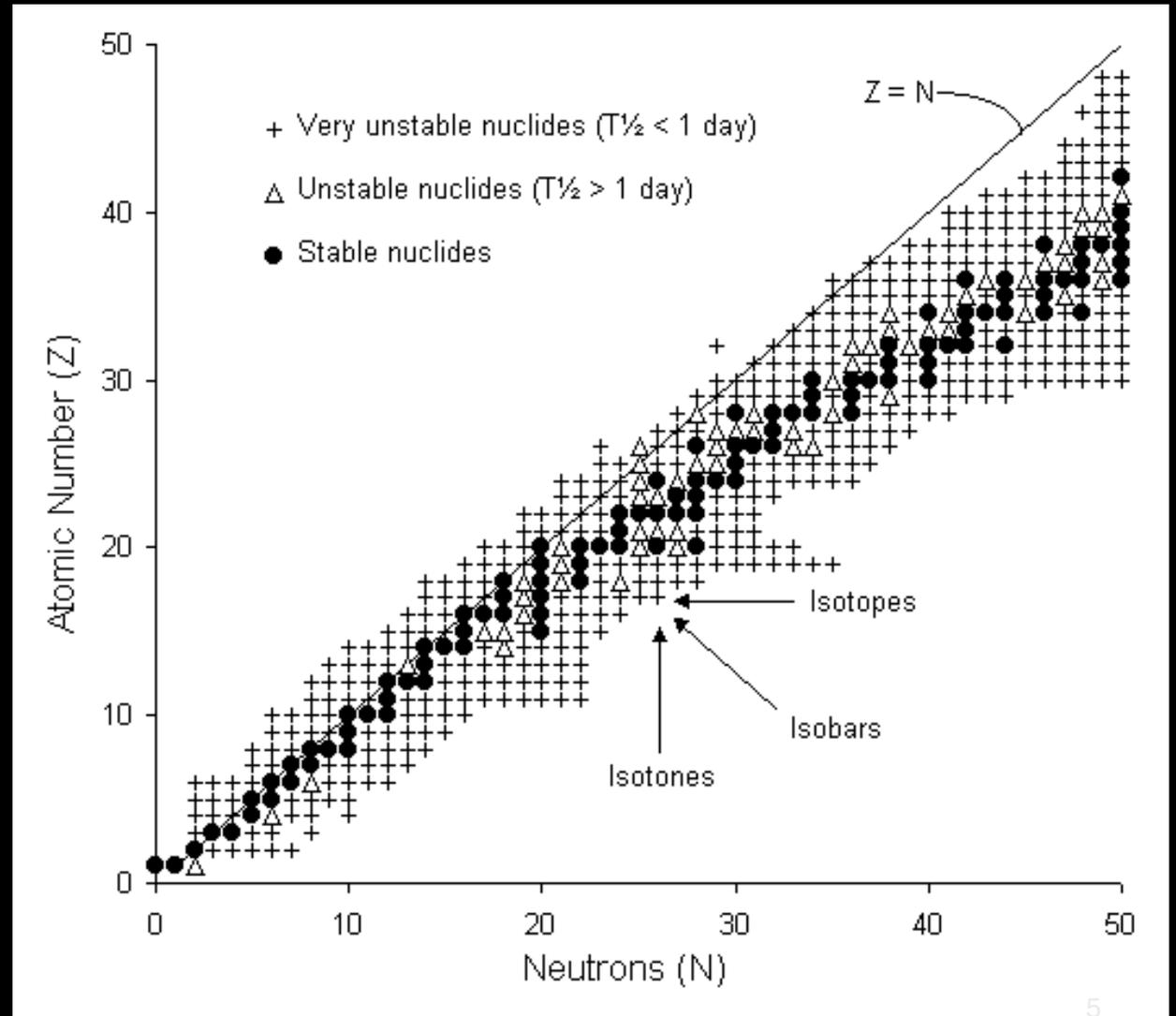
- Atoms are the same element when they share the same number of protons
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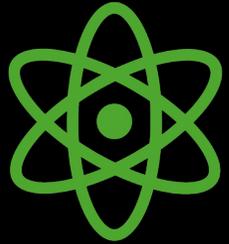




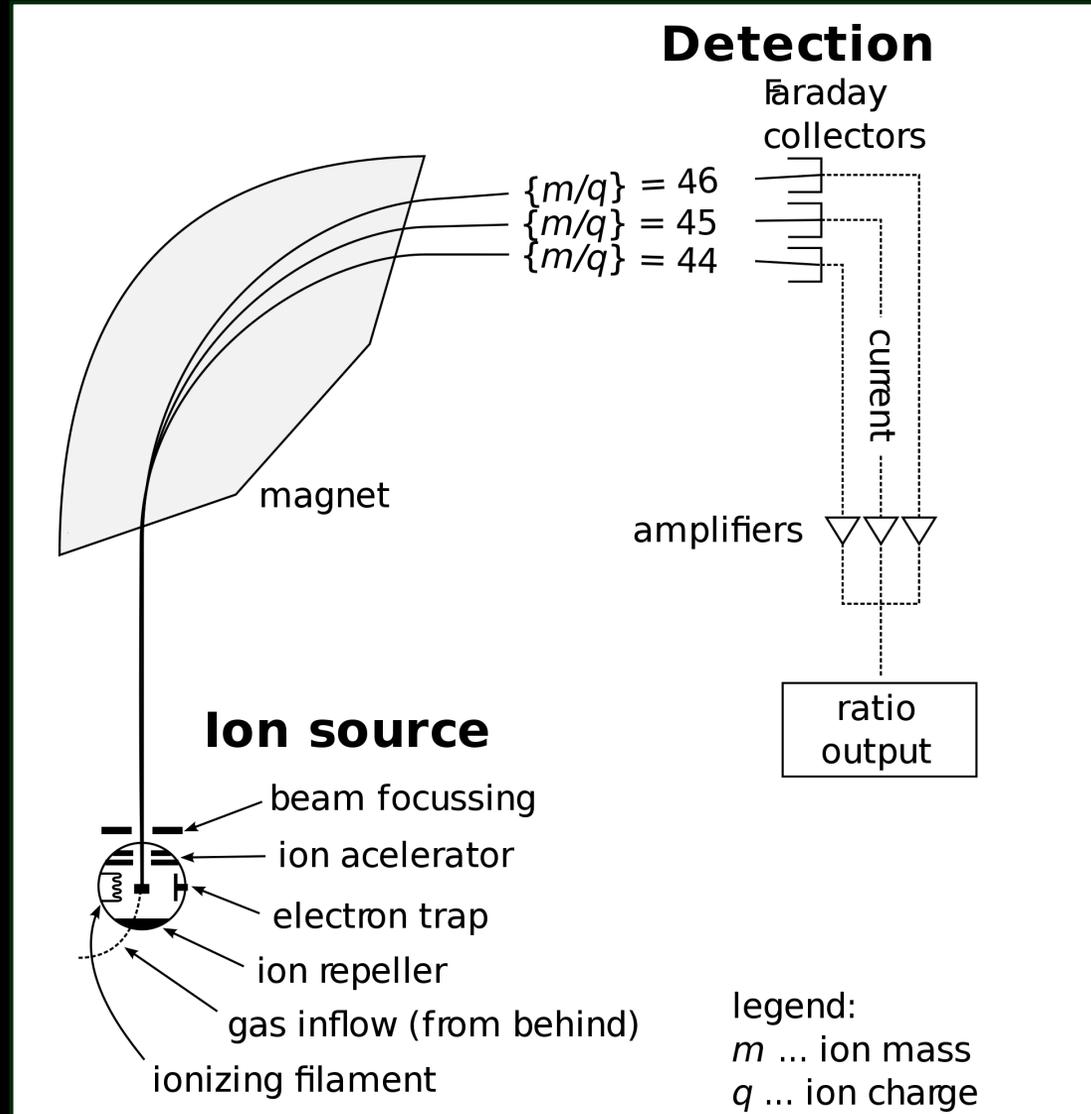
A gentle introduction to isotope chemistry

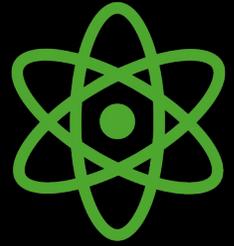
- Unstable isotopes are radioactive, break down over time
 - Radiocarbon dating
- Stable isotopes don't break down over time
 - Everywhere!





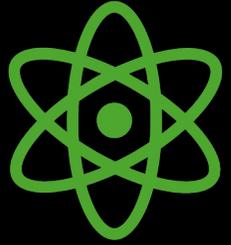
Measuring stable isotopes





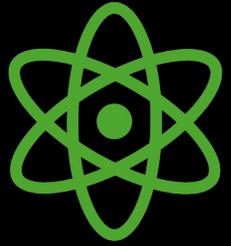
Measuring stable isotopes



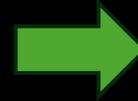


Measuring stable isotopes: delta notation

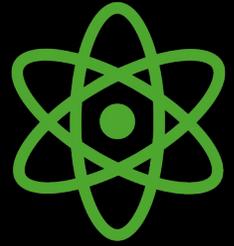
- We measure the ratio of abundance of heavy:light isotopes
 - e.g., $^2\text{H}/^1\text{H}$, $^{13}\text{C}/^{12}\text{C}$
- Report a 'delta' value δ
 - Measurements reported with respect to a standard measured value
 - e.g., hydrogen often reported on VSMOW-SLAP, mean ocean water - Antarctic snow
 - $$\delta = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \cdot 1000$$



Why measure stable isotopes for ecology? You are what you eat (and drink)!



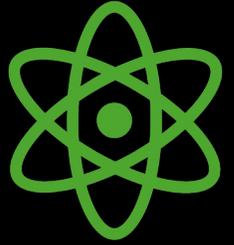
Rhinolophus ferrumequinum, Stephen Dalton of Minden Pictures, Bat Conservation International



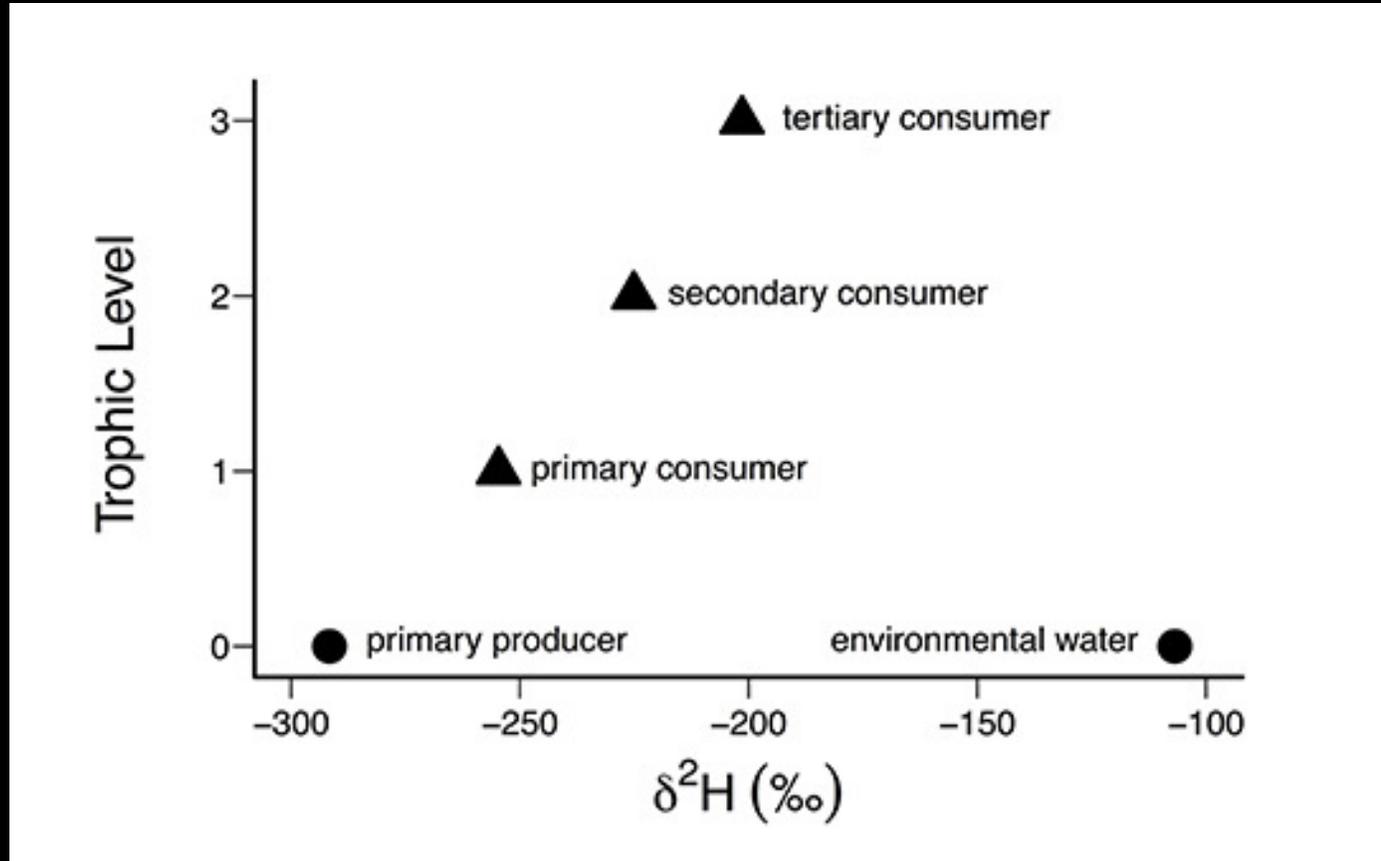
Why measure stable isotopes for ecology?

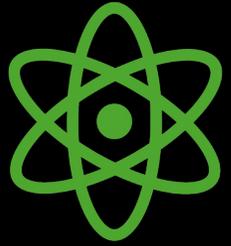


You are what you eat (and drink)!

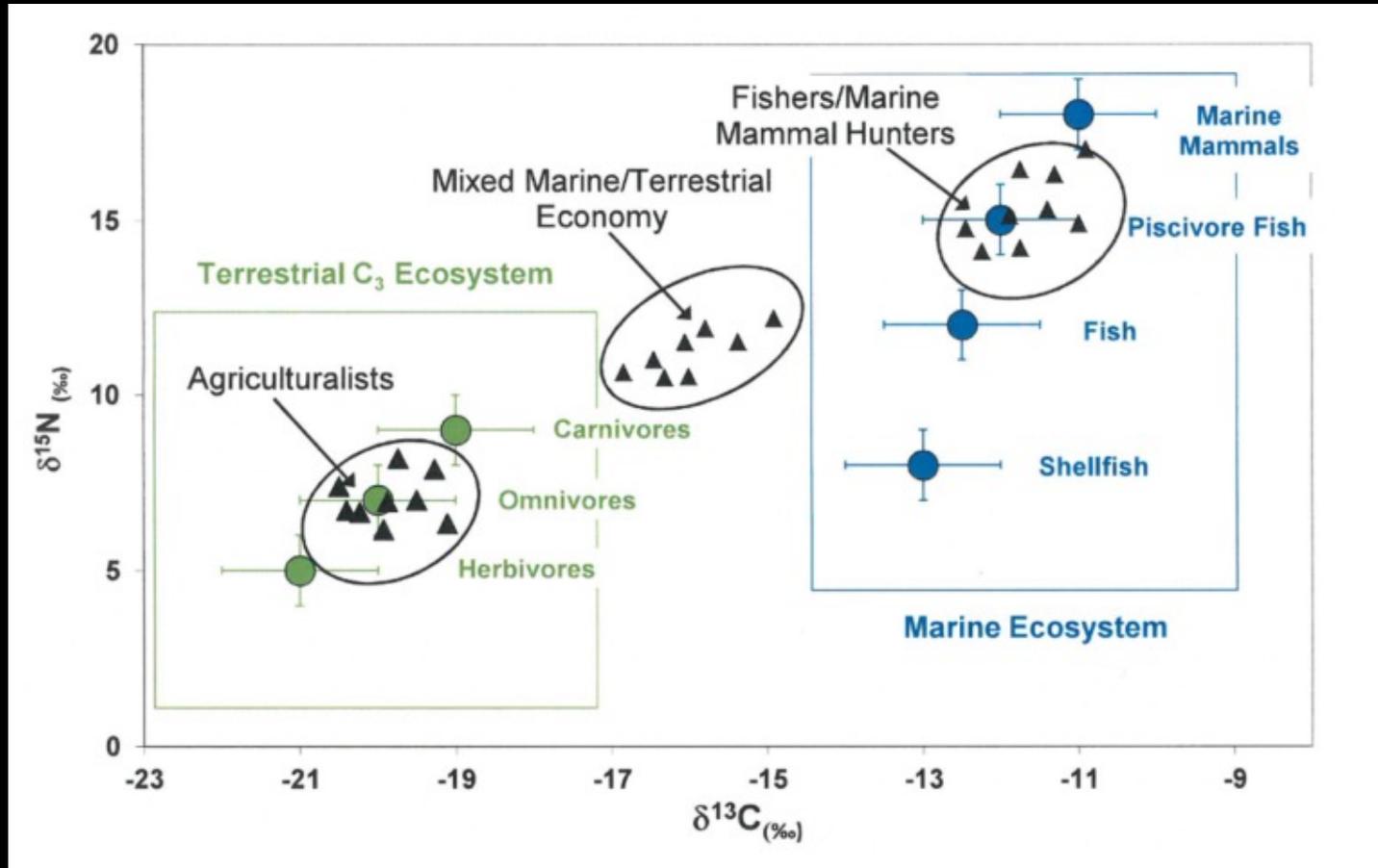


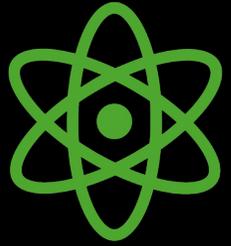
Stable isotopes vary within food webs





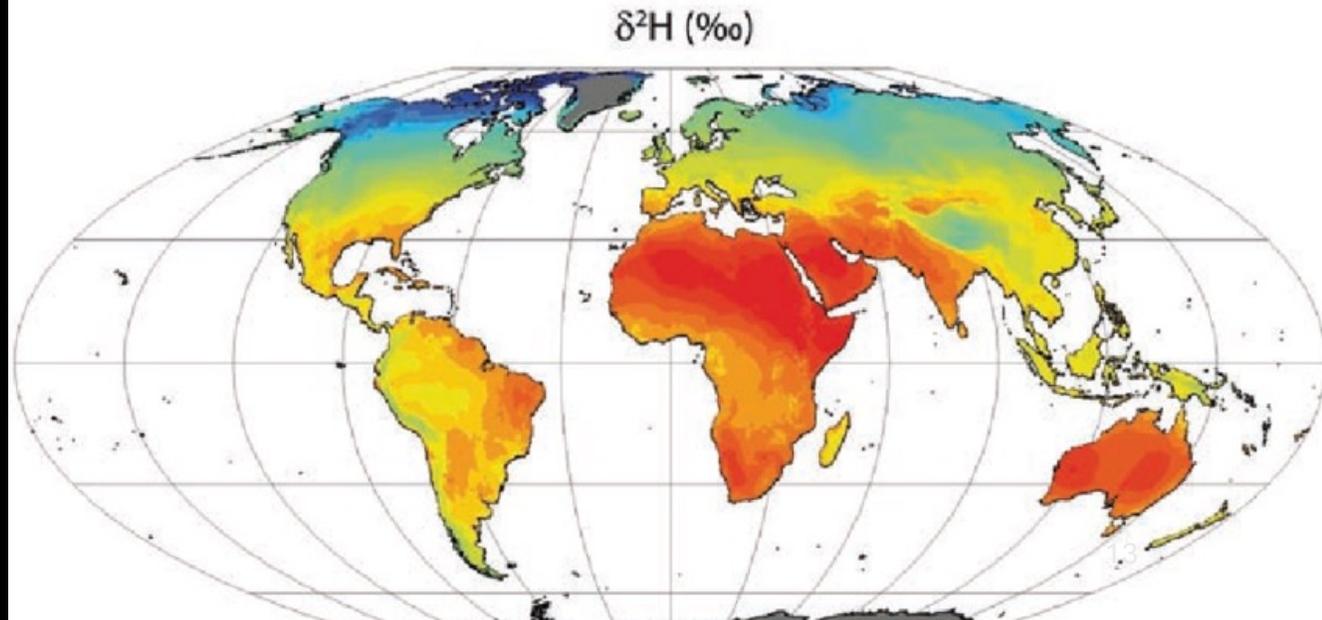
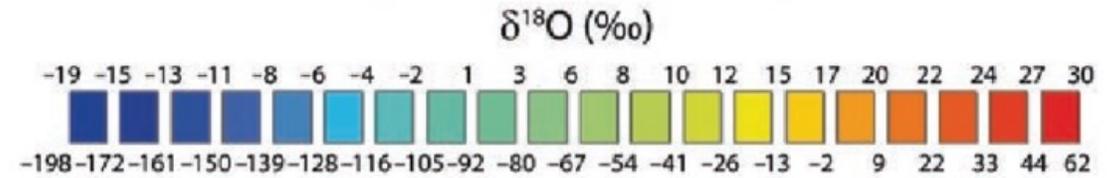
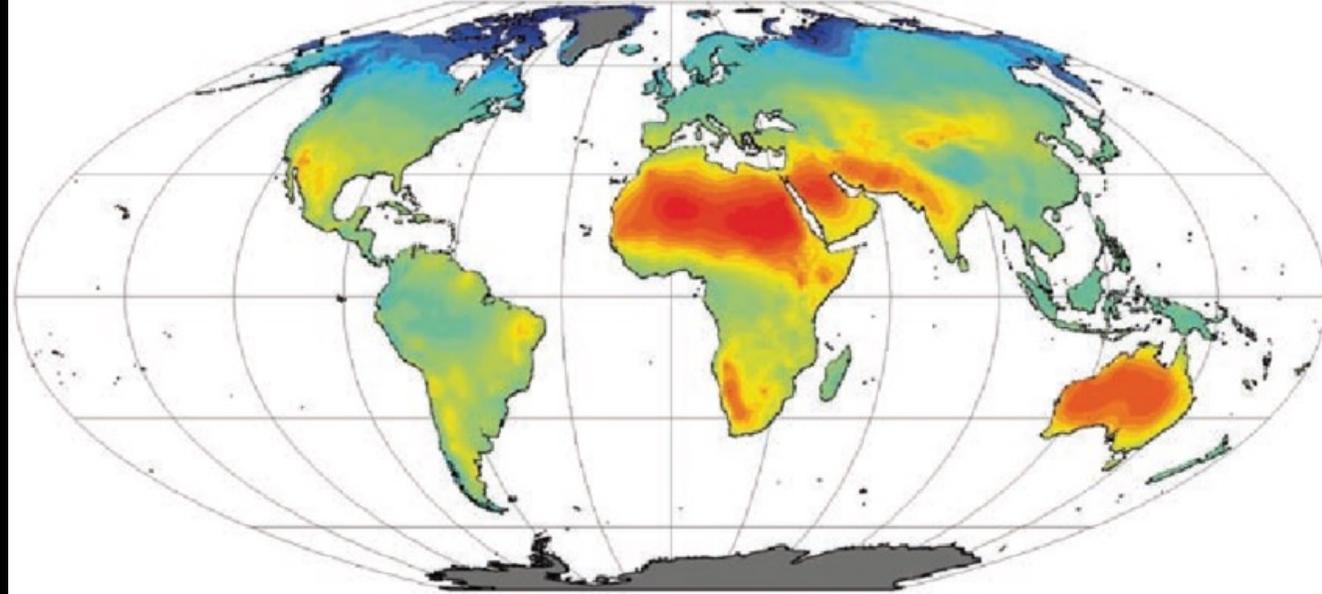
Stable isotopes vary within food webs

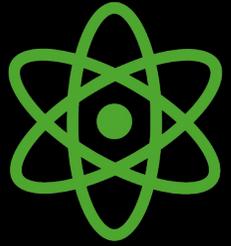




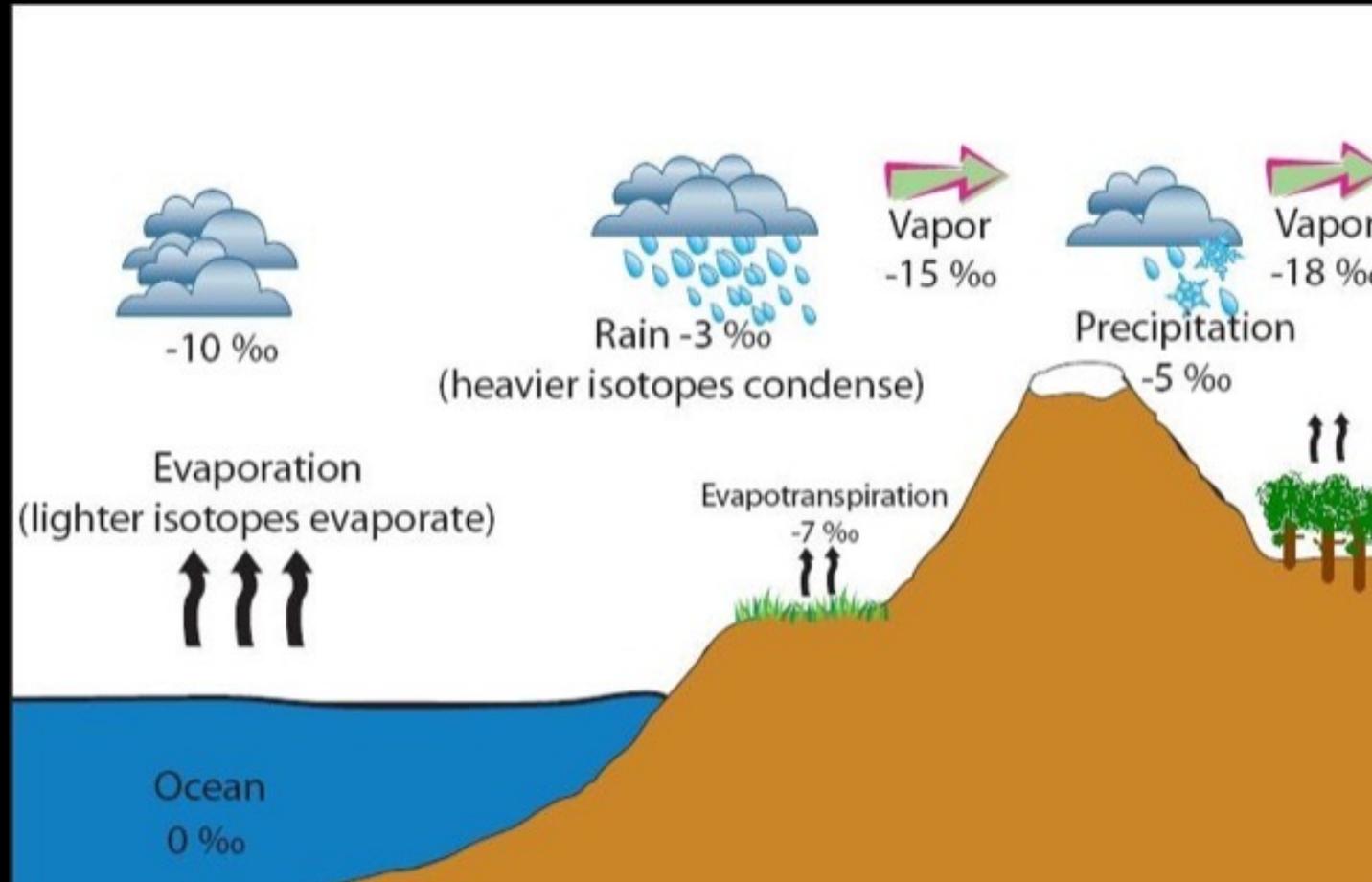
Stable isotopes that vary geographically

- Hydrogen (precipitation)
- Oxygen (precipitation)
- Nitrogen (plant communities)
- Carbon (plant communities)
- Strontium (groundwater)

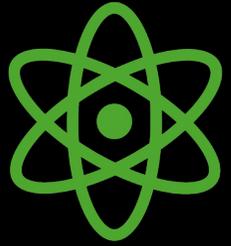




Hydrogen isotopes vary geographically



Processes driving geographic variation in stable hydrogen isotope ratios



Why use stable isotope analyses?

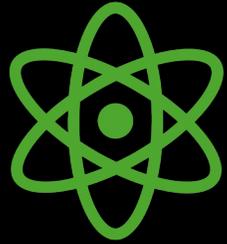
- Minimally-invasive sampling
 - Small amount of hair, claw, or tissue
- Can be applied to previously-collected specimens
- Single sampling event can be back-cast to conditions when tissue formed
- Can be applied to wide-ranging organisms
- Affordable



Volume of hair suitable for stable hydrogen analysis



Lasiurus cinereus carcass under wind turbine,
Photo by Michael Schirmacher



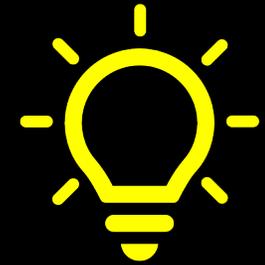
A GENTLE
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STABLE ISOTOPES TO
REVEAL BAT
DIET



PURSUING
STABLE ISOTOPE
RESEARCH



Mapping the origins of bats

1. Measured stable isotope composition of bat sample
2. Understanding of how and when environmental stable isotopes incorporated into that kind of sample
3. Map of environmental stable isotopes (isoscape)



4. Probability-of-origin mapping



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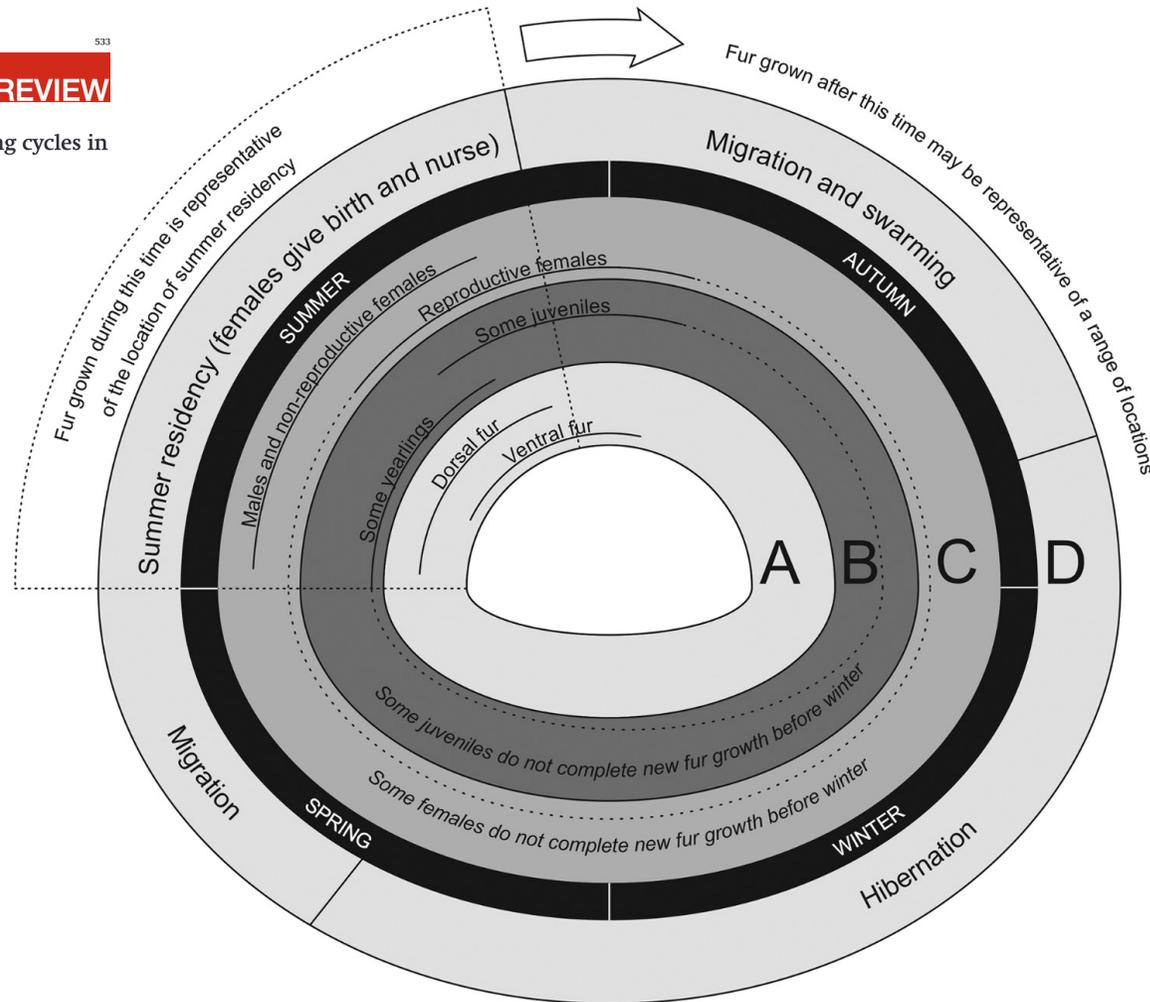
Understanding of how and when environmental stable isotopes incorporated into sample



533
REVIEW

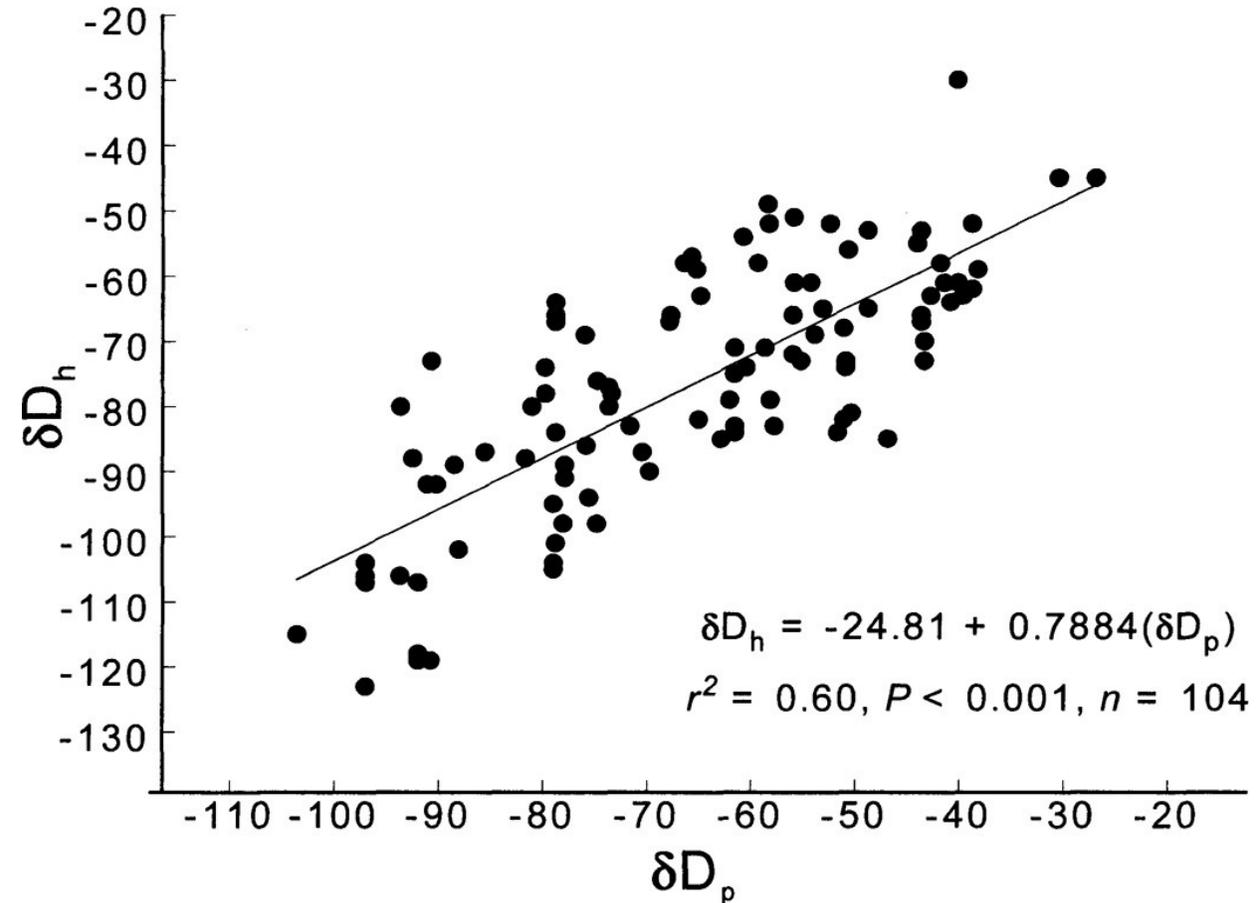
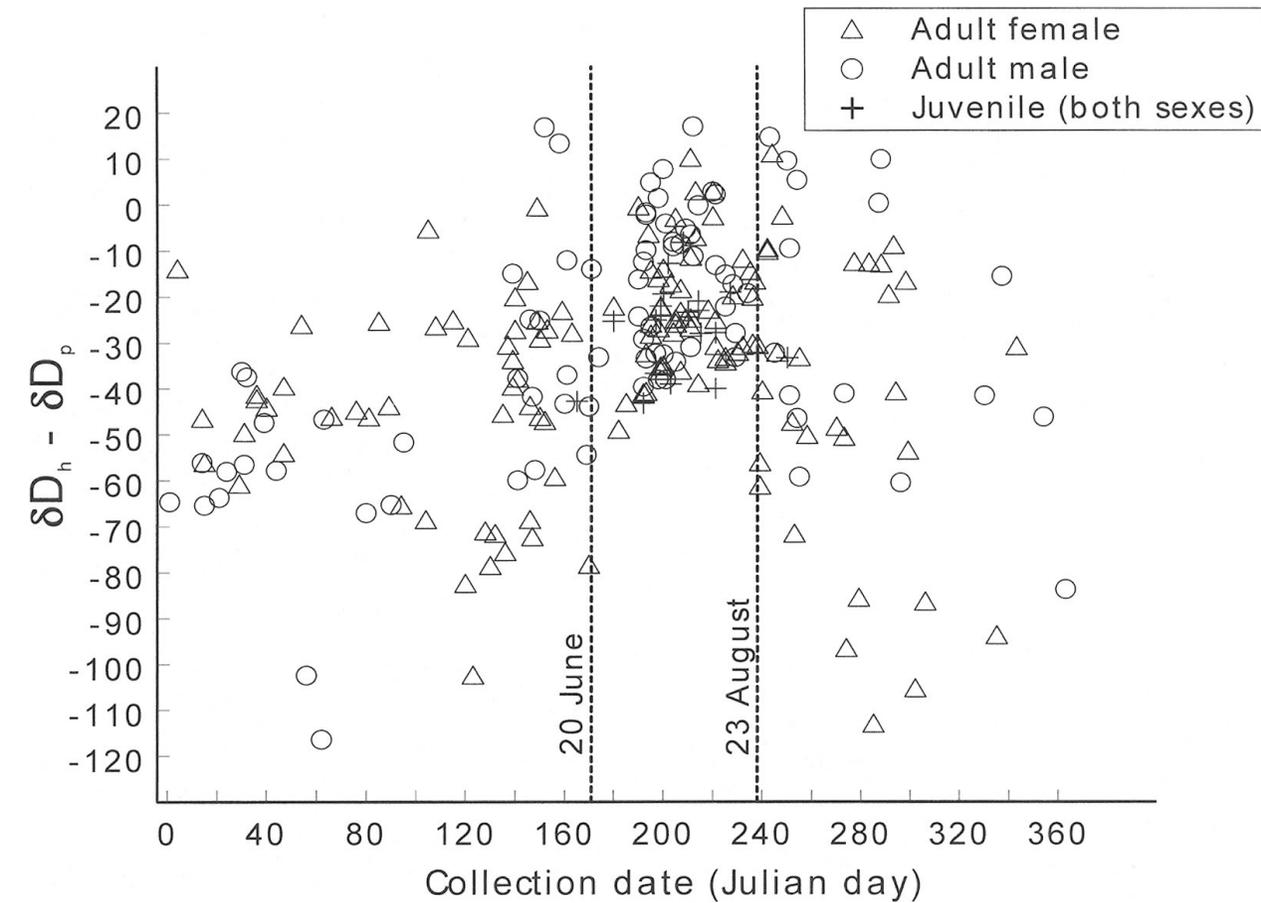
Moulting matters: the importance of understanding moulting cycles in bats when using fur for endogenous marker analysis

E.E. Fraser, F.J. Longstaffe, and M.B. Fenton





Understanding of how and when environmental stable isotopes incorporated into sample





Mapping the origins of bats

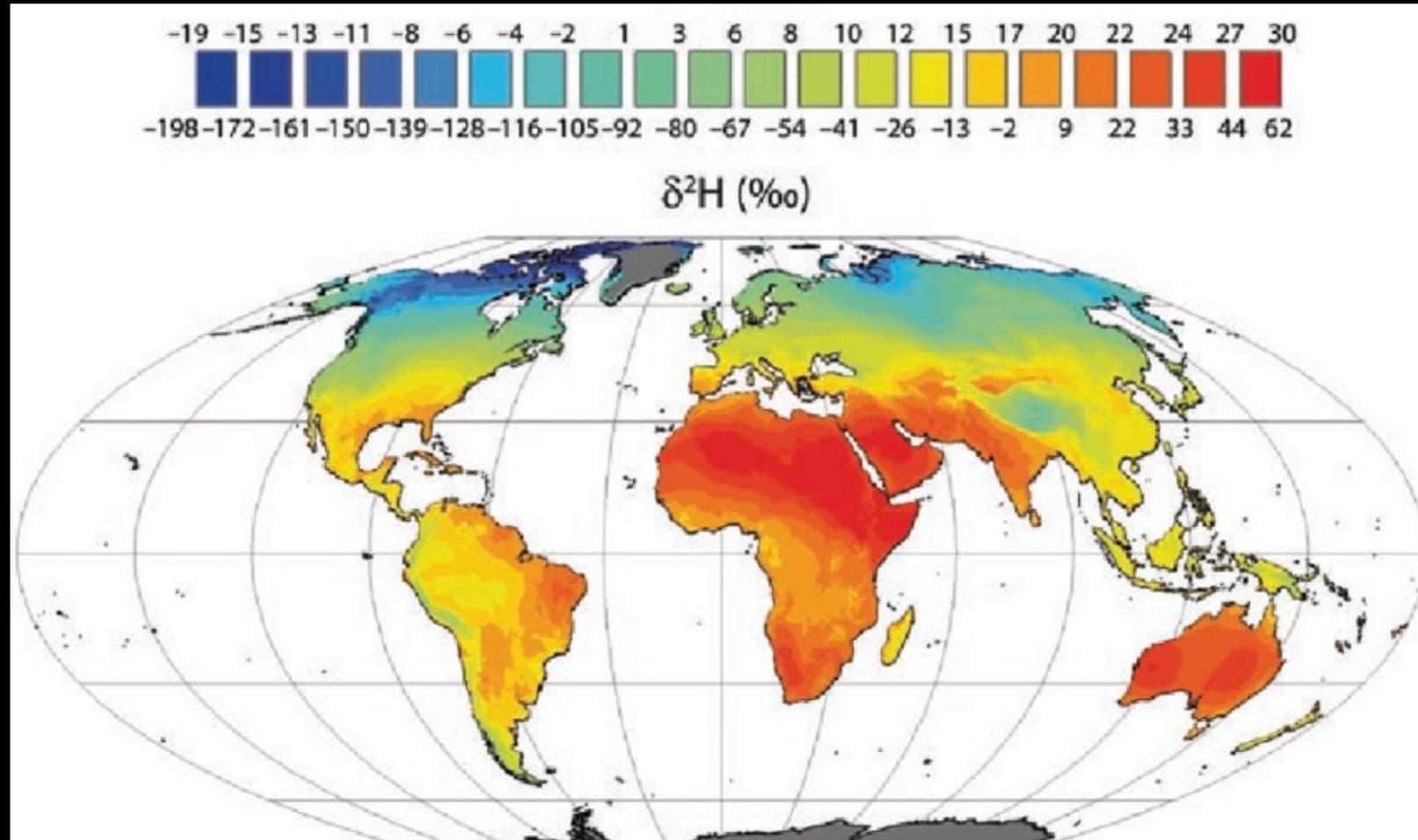
1. Measured stable isotope composition of bat sample
2. Understanding of how and when environmental stable isotopes incorporated into that kind of sample
3. Map of environmental stable isotopes (isoscape)



4. Probability-of-origin mapping



Mapping the origins of bats: isoscapes





Mapping the origins of bats

1. Measured stable isotope composition of bat sample
2. Understanding of how and when environmental stable isotopes incorporated into that kind of sample
3. Map of environmental stable isotopes (isoscape)



4. Probability-of-origin mapping



Mapping the origins of bats: Mapping

isocat: Isotope Origin Clustering and Assignment Tools

This resource provides tools to create, compare, and post-process spatial isotope assignment models of animal origin. It generates probability-of-origin maps for individuals based on user-provided tissue and environment isotope values (e.g., as generated by IsoMAP, Bowen et al. [2013] <[doi:10.1111/2041-210X.12147](https://doi.org/10.1111/2041-210X.12147)>) using the framework established in Bowen et al. (2010) <[doi:10.1146/annurev-earth-040809-152429](https://doi.org/10.1146/annurev-earth-040809-152429)>). The package 'isocat' can then quantitatively compare and cluster these maps to group individuals by similar origin. It also includes techniques for applying four approaches (cumulative sum, odds ratio, quantile only, and quantile simulation) with which users can summarize geographic origins and probable distance traveled by individuals. Campbell et al. [2020] establishes several of the functions included in this package <[doi:10.1515/ami-2020-0004](https://doi.org/10.1515/ami-2020-0004)>.

All: origin map making

isocat: clustering, post-processing transformations, isoscape averaging

assignR: Infer Geographic Origin from Isotopic Data

Routines for re-scaling isotope maps using known-origin tissue isotope data, assigning origin of unknown samples, and summarizing and assessing assignment results. Methods are adapted from Wunder (2010, in ISBN: 9789048133536) and Vander Zanden, H. B. et al. (2014) <[doi:10.1111/2041-210X.12229](https://doi.org/10.1111/2041-210X.12229)> as described in Ma, C. et al. (2020) <[doi:10.1111/2041-210X.13426](https://doi.org/10.1111/2041-210X.13426)>.

assignR: summarizing, known-origin data, data calibration across laboratories

IsoriX: Isoscape Computation and Inference of Spatial Origins using Mixed Models

Building isoscapes using mixed models and inferring the geographic origin of samples based on their isotopic ratios. This package is essentially a simplified interface to several other packages which implements a new statistical framework based on mixed models. It uses 'spaMM' for fitting and predicting isoscapes, and assigning an organism's origin depending on its isotopic ratio. 'IsoriX' also relies heavily on the package 'rasterVis' for plotting the maps produced with 'terra' using 'lattice'.

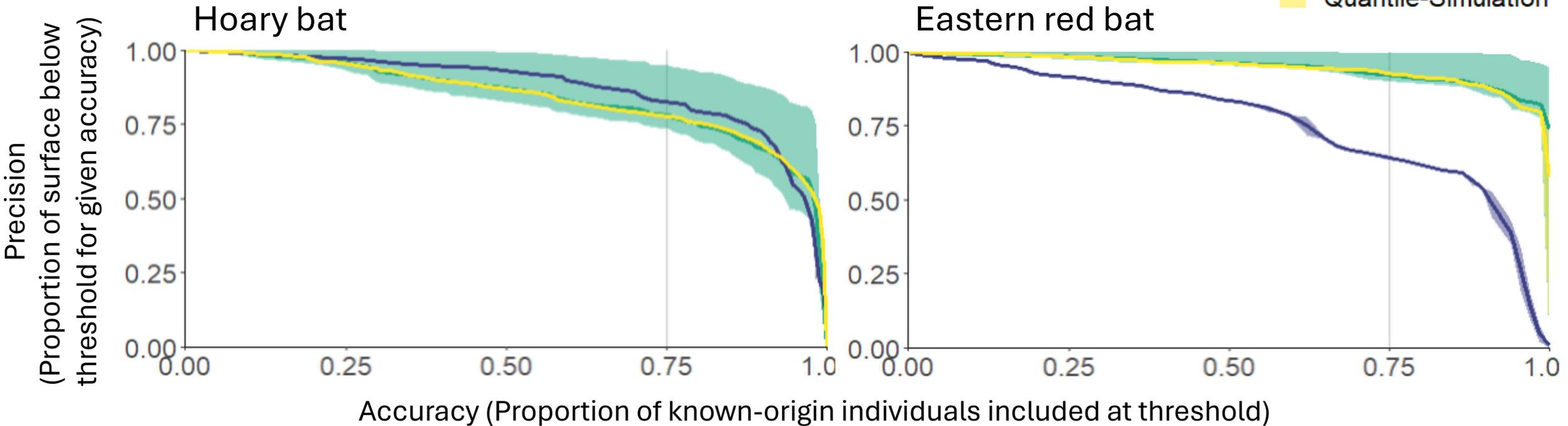
IsoriX: building isoscapes, callibrations



Mapping the origins of bats: Interpreting maps

Method

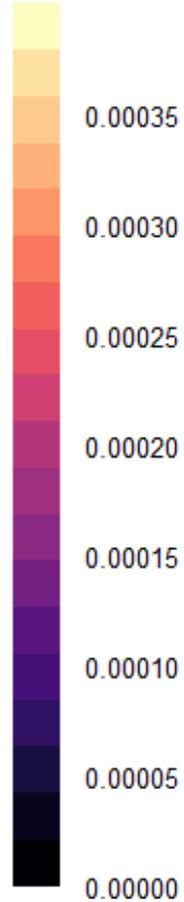
- Odds Ratio
- Quantile-Only
- Quantile-Simulation



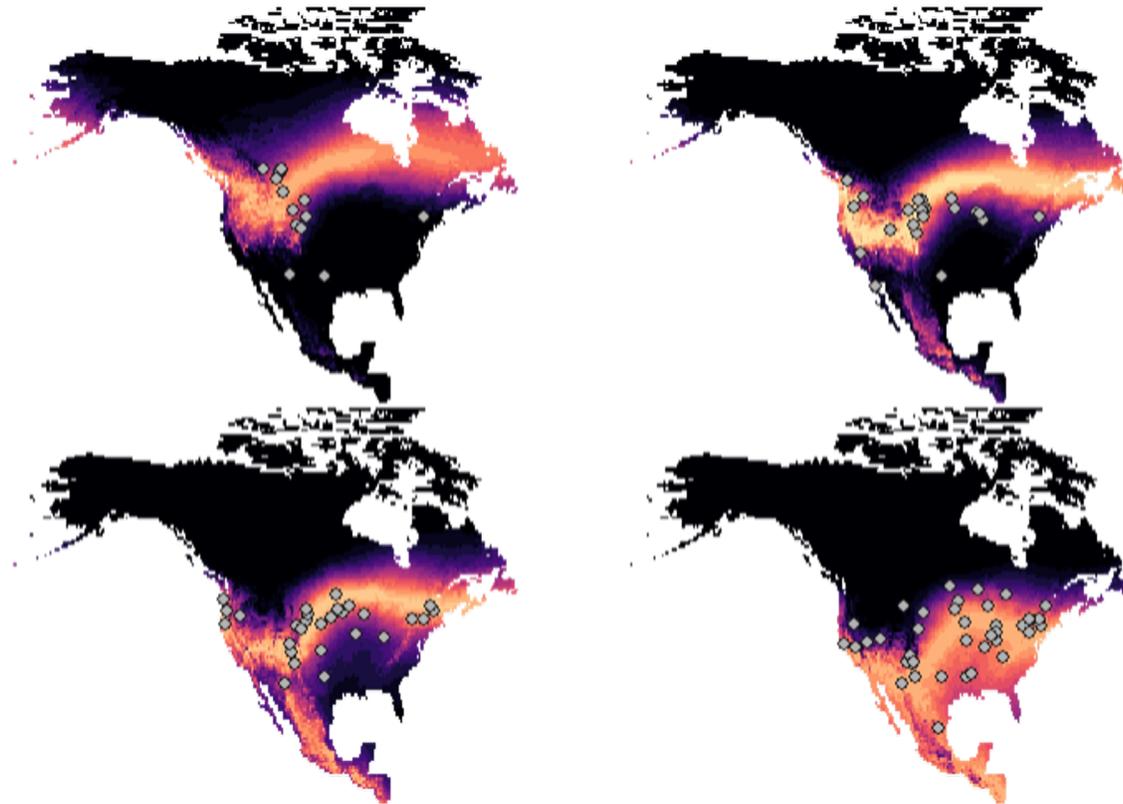


Mapping the origins of bats: Interpreting maps

Probability of Origin



Clustered Origins





Case study: A framework for understanding migration dynamics with a focus on North American tree-roosting bats



Silver-haired bat
(*Lasionycteris noctivagans*)
Photo: Juan Cruzado Cortés



Hoary bat
(*Lasiurus cinereus*)
Photo: Brock Fenton



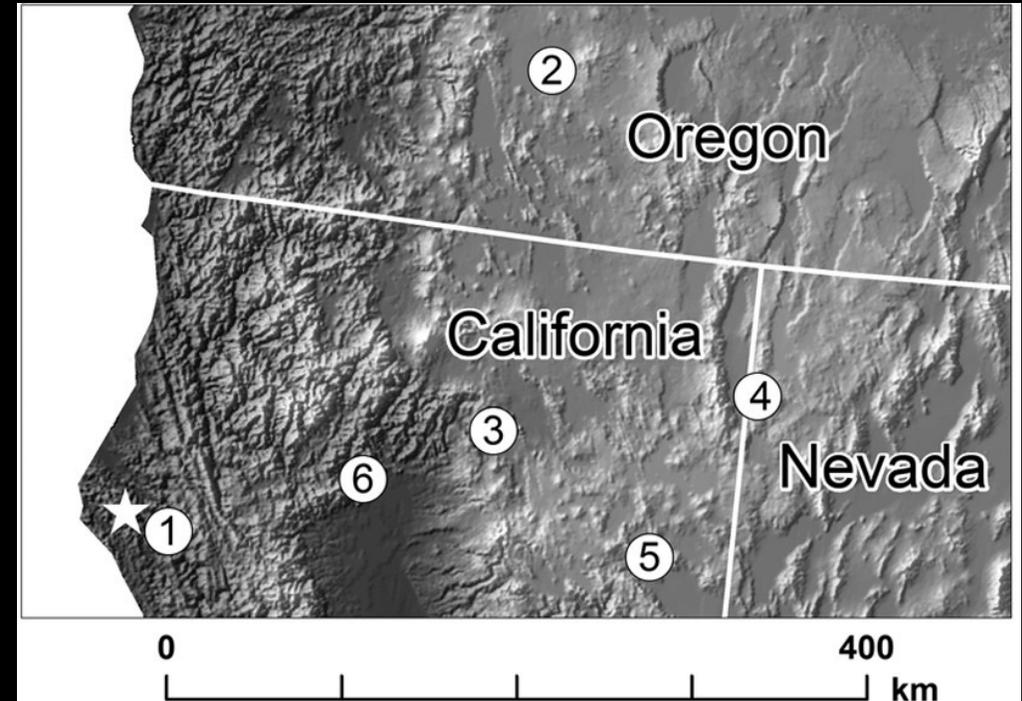
Eastern red bat
(*Lasiurus borealis*)
Photo: Brock Fenton



Stable isotopes to study bat movements

These bats defy most traditional and modern methods of studying animal migration

- Solitary, nocturnal, small, highly mobile
- Impressive capacity for long-distance movements
- Limited recapture data and location logging show long and circuitous migratory routes in a few individuals

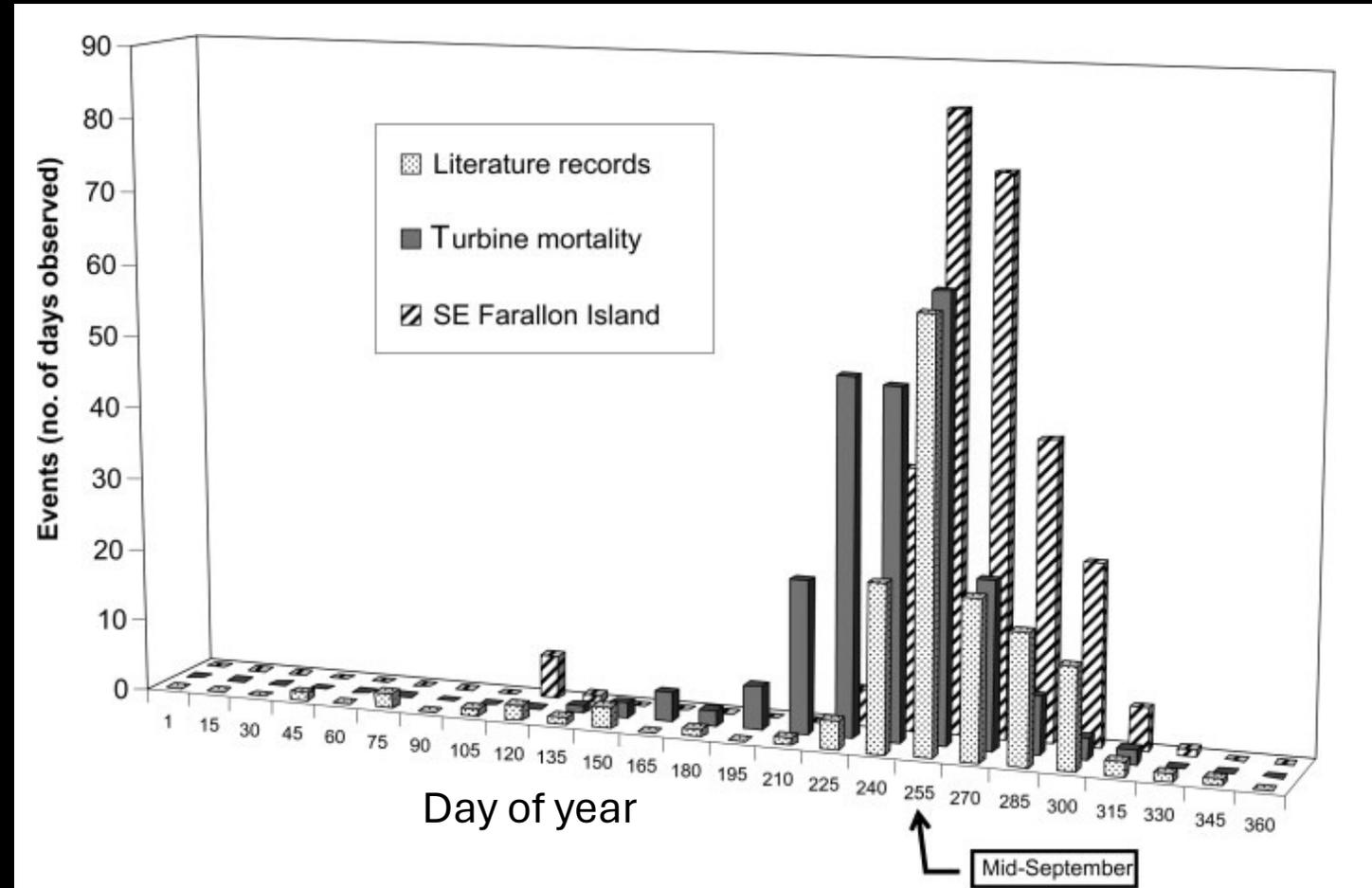


Weller et al. 2016



Circumstantial links between migration and wind energy impacts

- Bat migration and turbine mortalities temporally correlated, but no direct evidence of drivers
- Seasonal to-and-fro migration alone should correspond with spike in spring and fall– what is going on?





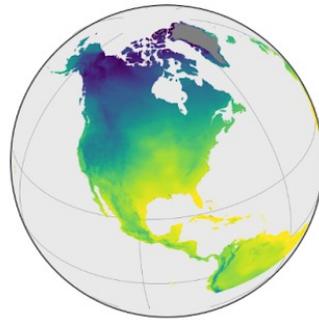
ENDOGENOUS MARKER MEASUREMENT



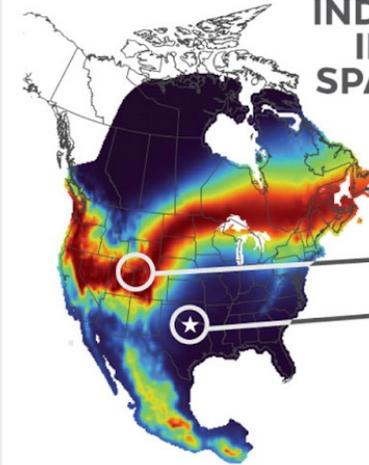
DATA SYNTHESIS



MACROECOLOGICAL-SCALE SAMPLING ACROSS GEOGRAPHIC AND TEMPORAL SPECIES RANGES



INDIVIDUAL-LEVEL INFERENCE OF SPATIOTEMPORAL LINKAGES



HIERARCHICAL MODELING FRAMEWORK

POPULATION-LEVEL MIGRATORY STRATEGY

Research Area	Habitat use and natural history	Migratory strategy and structure	Interactions and drivers of migration
Application	Sex-linked distributions and movements	Distance and direction of migration throughout annual cycle	Significance of migration and wind energy interactions



ENDOGENOUS MARKER MEASUREMENT



Sample acquisition through multiple methods



Individual

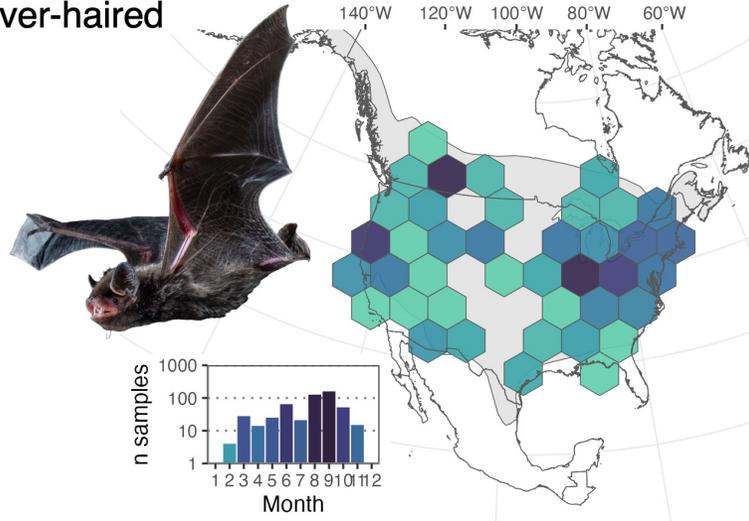


Tissue sample

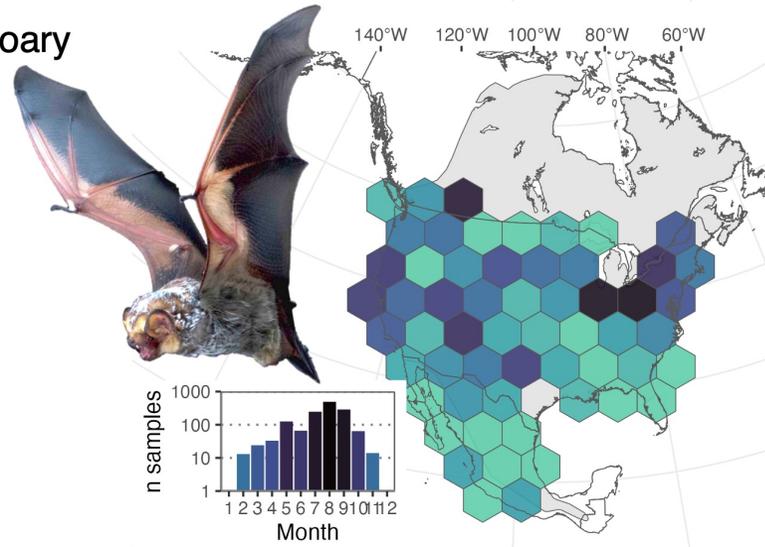


Measurement of endogenous marker

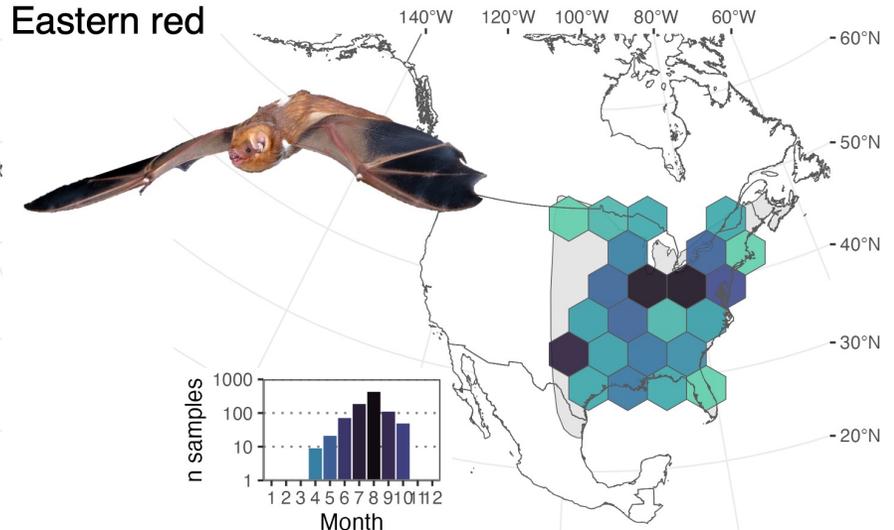
Silver-haired



Hoary

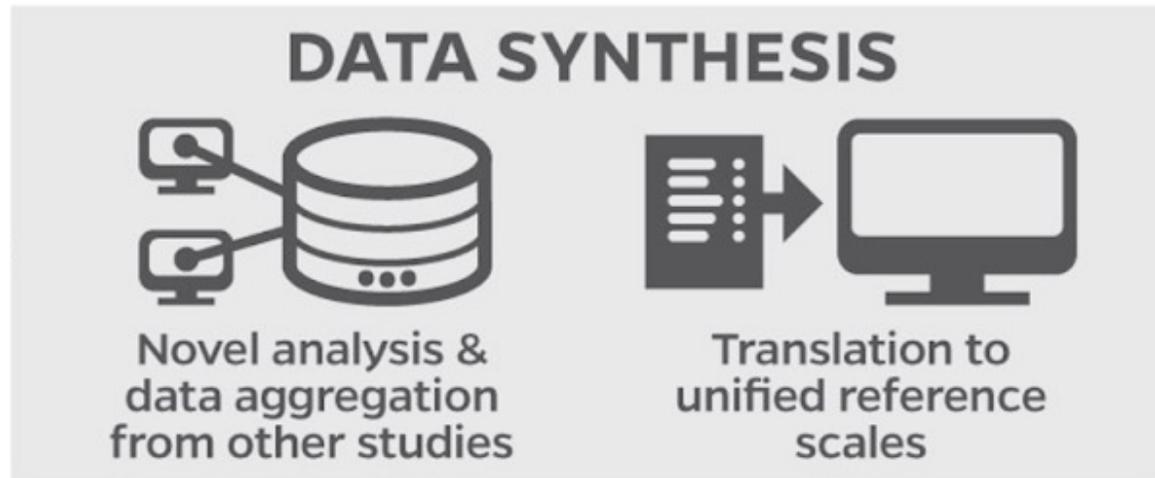


Eastern red

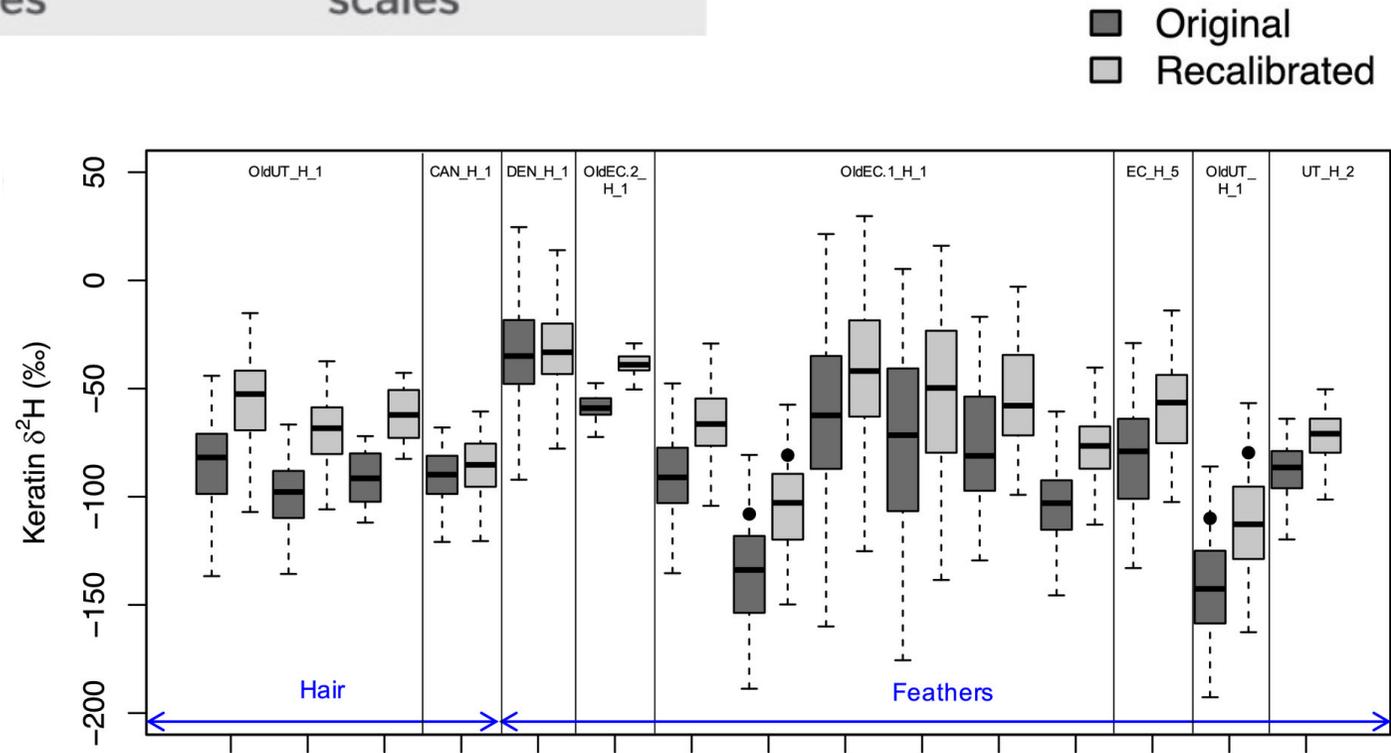


Sampling intensity (count individuals, log10 scale)



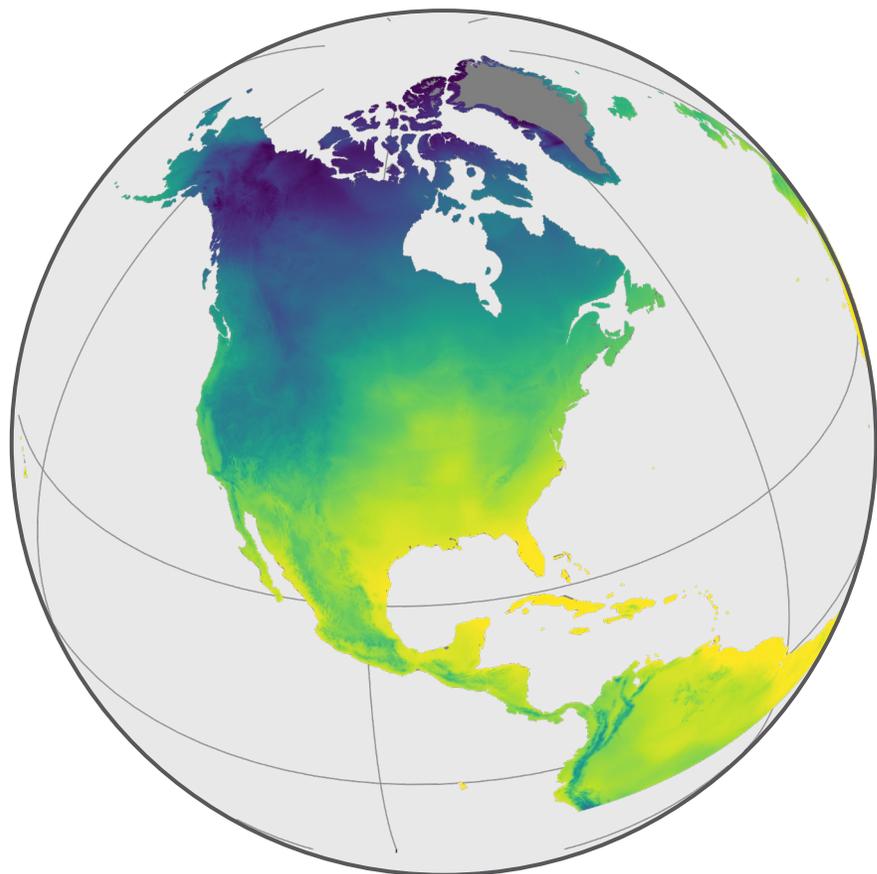


Data source	Hoary	Silver-haired	Eastern red	Total
This study	719	309	637	1665
Cryan et al. 2004	369	0	0	369
Baerwald et al. 2014	176	119	0	295
Pylant et al. 2014	0	0	112	112
Pylant et al. 2016	249	0	144	393
Fraser et al. 2017	0	117	0	117
Total	1513	545	893	2951

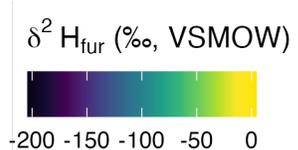




MACROECOLOGICAL-SCALE SAMPLING ACROSS GEOGRAPHIC AND TEMPORAL SPECIES RANGES



Sampling throughout
geographic range of
species, marker



Sampling throughout
annual cycle of
organism, marker