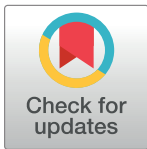RESEARCH ARTICLE

Tracking cats revisited: Placing terrestrial mammalian carnivores on δ^2H and δ^{18}O isoscapes

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Abstract

The relationship between hydrogen and oxygen stable isotopic compositions in environmental water and hair was investigated for both domestic cats (*Felis catus*) and dogs (*Canis lupus familiaris*). A strong, but different, covariance was measured between the hydrogen and oxygen stable isotopic compositions of local precipitation and hair keratin from both cats and dogs. These isotopic differences are most likely a result of the differences between the dietary and drinking water needs of cats compared to dogs. Moreover, the δ^2H and δ^{18}O values of hair from captive and wild felids and canids, such as cougars (*Puma concolor*), bobcats (*Lynx rufus*), and wolves (*Canis lupus*) are broadly consistent with these measurements. This evidence indicates that while the water budgets of terrestrial mammalian carnivores need to be considered in isotopic applications, it is clear that felids and canids may be placed on tissue–specific hydrogen and oxygen isotopic landscapes for ecological, provenance, or forensic studies.

Introduction

Global populations of terrestrial carnivores are increasingly threatened by habitat loss, reduction of prey, and direct hunting by humans for food, traditional curatives, predator control, and trophies [1, 2]. Of these, many species belonging to Felidae, Canidae, and Ursidae have suffered the largest and most severe population declines and range contraction [3, 4]. The numbers are shocking, with recent dramatic population and range declines being reported for most wild mammalian carnivores, including such charismatic and iconic species as lions (*Panthera leo*) [5, 6] and cheetahs (*Acinonyx jubatus*) [7]. Despite ongoing restorative conservation efforts, red wolves (*Canis rufus*) were declared extinct in the wild in 1980 [8] and tigers (*Panthera tigris*) are predicted to be functionally extinct within a few decades [9, 10]. Because of their large range, some carnivores, such as cougars (*Puma concolor*) and brown bears (*Ursus actos*), are listed as species of least concern on the IUCN (International Union for the

Conservation of Nature) Red List. However, it is evident that even these species have seen range contraction and population declines in recent decades [11].

As a consequence, many of these species, particularly the large African felids, are the focus of wildlife sanctuaries and thus protected from human activities such as hunting and habitat destruction. Despite these conservation endeavours, such species continue to be imperiled by human activities, many of which revolve around trophy hunting and illegal trade in wildlife and their parts. To combat this, forensic and ecological tools are required to trace tissues to potential geographic origins. In this respect, the hydrogen and oxygen stable isotopic compositions of animal tissues hold great potential for conservation in the form of forensic markers [12], provenance estimation [13], or determination of migration patterns of wildlife [14]. This is because the H and O stable isotopic compositions of metabolically inert animal tissues, such as hair, hooves, feathers, and horns, are related to those of body water, and thus ingested environmental waters [15]. Because the H and O stable isotopic compositions of water varies regionally, so do those of animal tissues. This is the main premise which links organisms with their environment through isotopic landscapes, or isoscapes [16], and has been used in many ecological studies [17, 18].

However, while a relationship exists between the H and O stable isotopic compositions of animal tissues and local water, it is complicated by the metabolic and physiological-chemical processes involved in the transfer of hydrogen and oxygen from environmental water to animal tissues [19]. This becomes particularly acute for carnivores who acquire a substantial portion of their body water from food, rather than from drinking water. This was the conclusion of Pietsch *et al.* [20] who reported that because of these physiological effects, the hydrogen and oxygen stable isotopic compositions of cougar and bobcat (*Lynx rufus*) hair in museum specimens had little relationship to those of environmental water and so are not valuable for forensic tracking. However, because their conclusion conflicts with other studies [21, 22] and because the use of isotopic methods to elucidate the provenance of carnivores is theoretically strong, we felt that a re-evaluation of the isotopic coupling between mammalian carnivores and environmental water was necessary but using modern unpreserved samples rather than museum specimens.

Ideally, to test this would require an inventory of hair or claw samples from a large number of wild mammalian carnivores from locations that have disparate stable isotopic compositions of environmental water. Obviously, this would be a large and expensive undertaking as wild carnivores tend to be found in remote locales and are, for the most part, reclusive and possibly dangerous. As a proxy, we collected hair from two common domesticated carnivores: cats (*Felis catus*) and dogs (*Canis lupus familiaris*) from urban animal shelters in different locations across North America. We assume that, isotopically speaking, domestic cats and dogs are physiologically close enough to their wild cousins that the processes responsible for isotopic coupling between hair and environmental water would be similar and would allow us to evaluate the application of these isotopic methods to wild mammalian carnivores.

Experimental

Samples

Adult cat (n = 112) and dog (n = 124) guard hair samples were collected into paper envelopes by participating animal shelters (Fig 1) and forwarded to the NHRC lab in Saskatoon. Shelters were asked to provide about 0.5g of hair and to sample approximately equal numbers of cats and dogs. Meta-data collected from each animal were: ID, species, gender, approximate age, food (wet or dry), approximate length of time the animal was resident in the shelter, and
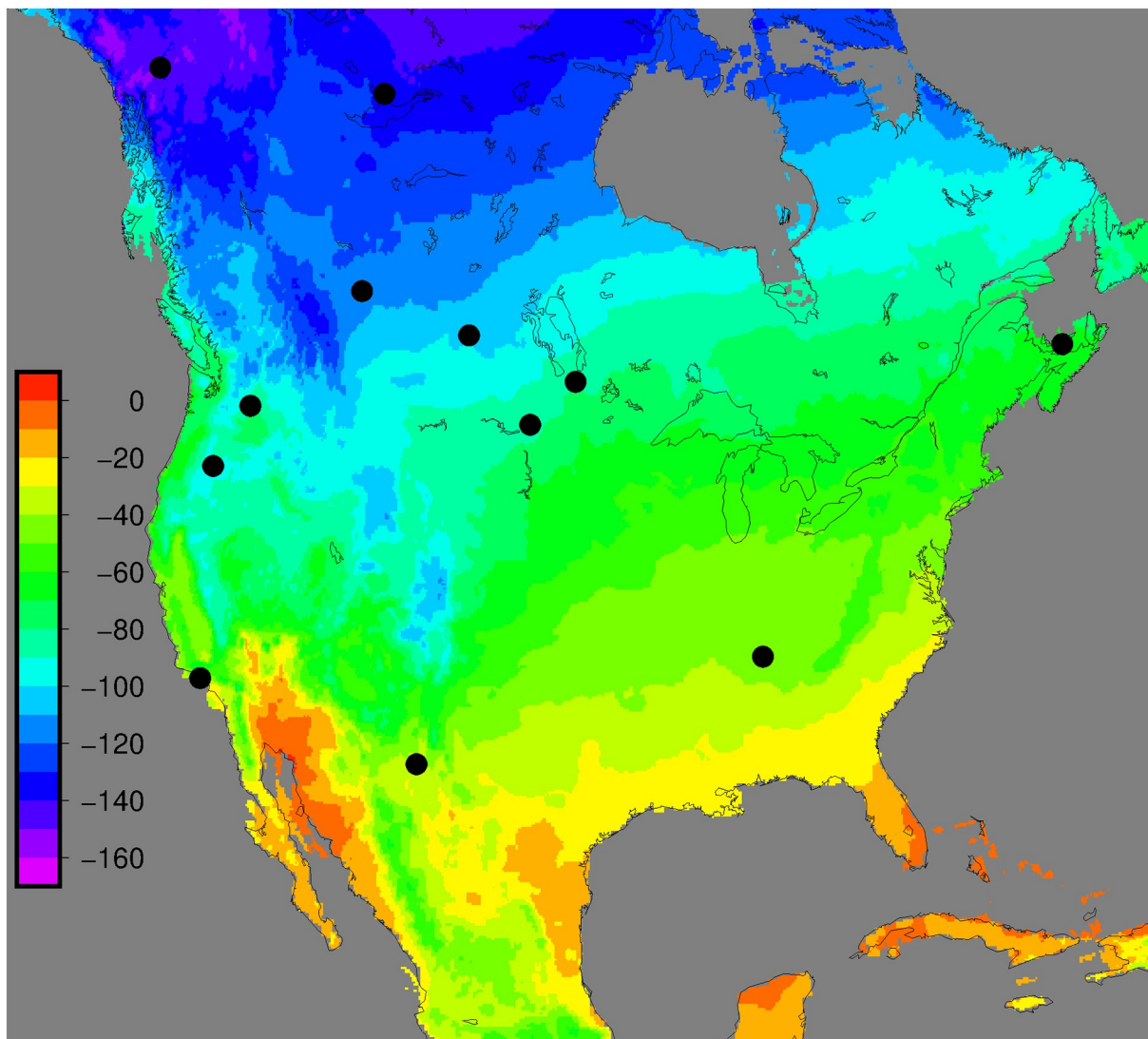
Fig 1. Locations of participating shelters. Coloured contours represent the hydrogen isotopic composition of growing-season precipitation from the RCWIP model program, IAEA [23].

general health or body condition. A sample of local water used in the shelter was also collected into supplied 2 mL bottles.

For comparison to these domestic animals, hair samples were also collected from both wild and captive cougars (n = 8), bobcats (n = 10), tigers (n = 1), wolves (*Canis lupus*, n = 2), coyotes (*Canis latrans*, n = 1), foxes (*Vulpes vulpes*, n = 2), and lions (n = 1) from participating zoos and also from existing collections within our laboratory. For zoos, hair was collected from the animal enclosure or directly from the animal during veterinary procedures. Untanned cougar hair and/or claw samples were also obtained from participating museums, where available.

Stable isotope analysis

Stable isotopic compositions of all hair and water samples were measured at the NHRC Stable Isotope Laboratory of Environment and Climate Change Canada in Saskatoon, Canada. Prior

to analyses, hair samples were cleaned of debris and any surface oils were removed by rinsing in 2:1 chloroform:methanol and finally homogenized to powder with a ball grinder (Retsch model MM-301, Haan, Germany). For hydrogen and oxygen, our approach involved the measurement of both δ^2H and δ^{18}O values with the same analytical run (*i.e.*, both H_2 and CO gases were analyzed from the same pyrolysis). Samples and standards were weighed to 350 ±20 μg in silver capsules and analysed using a IRMS system (Thermo Finnigan, Bremen, Germany) equipped with a Costech Zero-Blank autosampler. The helium carrier gas rate was set to 120 mL/min. We used a HTC 1.5 m 0.25 inch 5 Åmolecular sieve (80-100 mesh) GC column. The glassy carbon reactor was operated at a temperature of 1400˚C, and the GC column temperature was set to 90 ˚C. After separation, the gases were introduced into a Delta V Plus isotope–ratio mass spectrometer via a ConFlo IV interface (Thermo Finnigan, Bremen, Germany). The eluted N_2 was flushed to waste by withdrawing the CF capillary from the ConFlo interface. We used Environment Canada keratin reference standards CBS (Caribou hoof) and KHS (Kudu horn) to calibrate sample δ^2H (-197 and -54.1 per mil, respectively) and δ^{18}O values (+2.50 and +21.46 per mil, respectively [24]). This normalization with calibrated keratins also corrects for any hydrogen isotope measurement artefact caused by production of HCN [25] in the glassy carbon reactor as described by Soto *et al.*, 2017 [26]. Based on replicate (n = 5) within-run measurements of keratin standards and from historical analyses of an in-house QA/QC reference (SPK keratin), sample measurement error was estimated at ±2 per mil for δ^2H and ±0.4 per mil for δ^{18}O. All H results are reported for nonexchangeable H and for both H and O in the standard delta notation, normalized on the Vienna Standard Mean Ocean Water—Standard Light Antarctic Precipitation (VSMOW-SLAP) scale.

All water samples were measured for their stable isotopic compositions by Off Axis Integrated Cavity Output Spectroscopy (OA-ICOS) using a Los Gatos Research DLT-100 laser spectrometer. We used two calibrated reference waters (INV1 δ^2H = -217.7, δ^{18}O = -28.5 and ROD3 δ^2H = -3.9, δ^{18}O = -1.0 per mil, respectively) to normalize raw delta values to the VSMOW–SLAP scale. To minimize memory effects, samples and reference waters were injected nine times and the last five measurements were averaged to obtain the final raw delta values. Precisions as determined by replicate analyses of samples and reference waters were ± 1 and 0.1 per mil for hydrogen and oxygen, respectively. We used the R programming language for all statistical calculations [27].

Results and discussion

We observe a strong positive relationship between δ^2H and δ^{18}O values of amount–weighted growing season average precipitation and those measured in hair for both cats and dogs (Table 1, Figs 2 and 3), with a better fit for dogs than cats. It is also evident that isotopic coupling between hydrogen and oxygen isotopes in precipitation and hair keratin is weaker for cats than for dogs (Table 1). A slightly weaker fit but similar isotopic coupling for both cats

Table 1. RMA(orthogonal) linear regressions between hydrogen and oxygen isotopic compositions of growing season precipitation and hair keratin for cats and dogs. Subscripts h and p represent hair and precipitation, respectively.

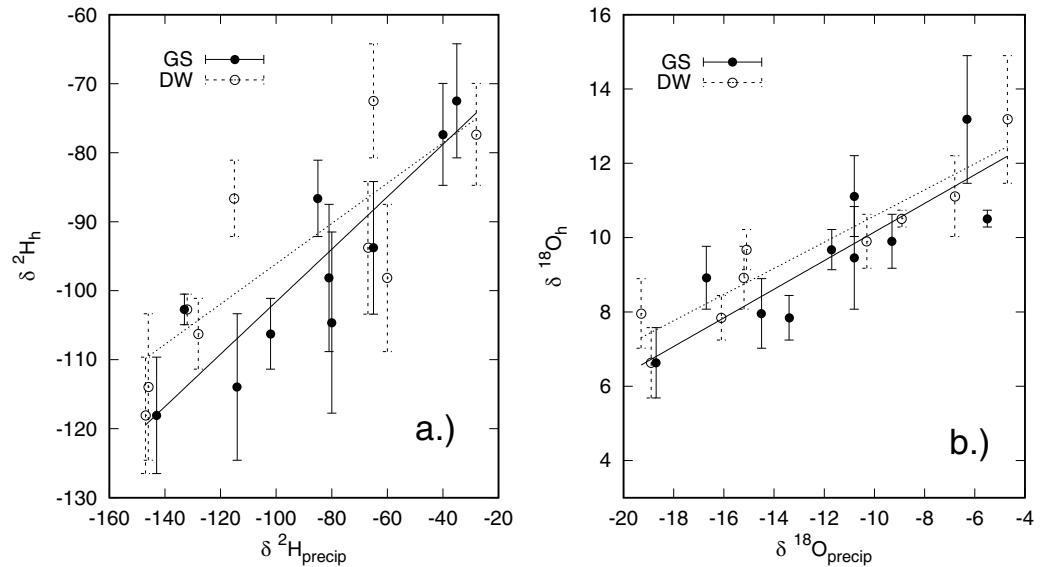| Hydrogen	fit	r^2	p
Cats	$\delta^2\text{H}_h = 0.380\delta^2\text{H}_p - 63.6$	0.769	< 0.001
Dogs	$\delta^2\text{H}_h = 0.511\delta^2\text{H}_p - 57.1$	0.953	<0.001
Oxygen			
Cats	$\delta^{18}\text{O}_h = 0.385\delta^{18}\text{O}_p + 14.0$	0.687	<0.001
Dogs	$\delta^{18}\text{O}_h = 0.583\delta^{18}\text{O}_p + 14.3$	0.814	<0.001

https://doi.org/10.1371/journal.pone.0221876.t001

Fig 2. Relationship between cat (*F. catus*) hair a) δ^2H values and b) δ^{18}O values and those of growing season precipitation for North America (GS) and of drinking water (DW).

and dogs was measured between the δ^2H and δ^{18}O values of drinking water supplied by the shelter and those of the hair (Figs 2 and 3). This was unexpected, however on closer examination it was evident that most of the animals in the shelters had been there only a short time (<1 month), were all local animals, and many were feral, especially in northern Canada. Therefore, it is likely that these animals integrate water in their local environment in a similar way to what would occur in the wild. Moreover, there were a few instances where the drinking water isotopic compositions were markedly different than the local precipitation. An example of this is for Winnipeg, Canada, where the local tap water is sourced from the Shoal Lake reservoir and has a different stable isotopic composition than modeled local precipitation. For these
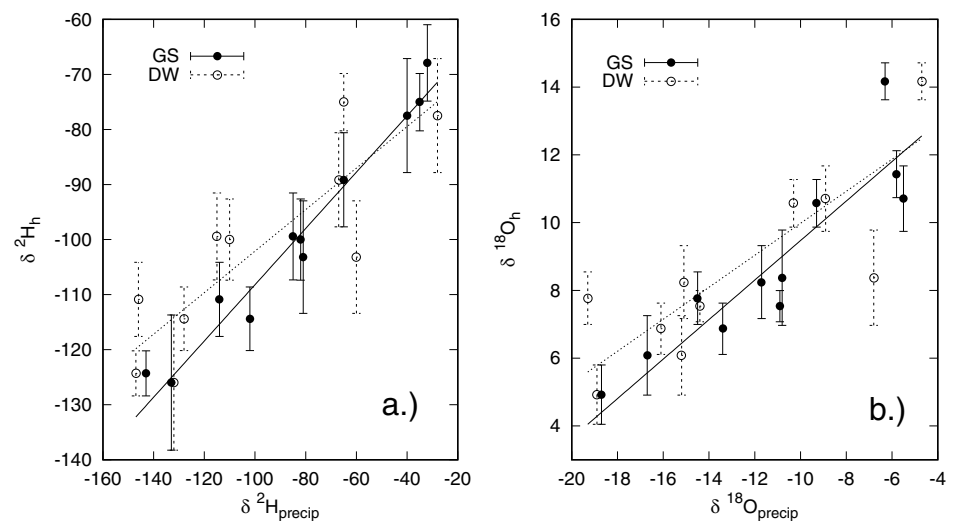

Fig 3. Relationship between dog (*C. lupus*) hair a) δ^2H values and b) δ^{18}O values and those of growing season precipitation for North America (GS) and of drinking water (DW).

reasons, we conclude that hair keratin hydrogen and oxygen isotopic compositions in this study are more closely correlated with growing season isotopic compositions of precipitation than of drinking water given to the shelter animals.

While the close relationship between the hydrogen and oxygen isotopic compositions of environmental water and hair keratin was expected for dogs, it contrasts sharply to previous work with cats. Pietsch *et al.* (2011) [20] did not measure any relationship between environmental water and hair keratin for cougars or bobcats, although a distinct relationship was found for the prey species, deer and cottontail rabbit. We have no explanation for this discrepancy, however we note that Pietsch *et al.* worked with museum specimens collected up to 142 years ago and which had been chemically preserved. Hair keratin is not affected by general tanning [28], however chemical treatments may vary, especially with historic samples. It is additionally possible that the hair may have been contaminated with other substances involved in the taxidermic process which resulted in erroneous isotopic compositions. Other rather remote possibilities include poorly constrained locations of museum specimens or that cougars and bobcats are sufficiently different from domestic cats that they are isotopically uncoupled from environmental water.

For hydrogen isotopes, our results are broadly consistent with the strong relationships found previously between H isotopes in environmental waters and those in human hair [29–32], bird feathers [33] and bat hair [34, 35]. For mammals, hydrogen in body water is obtained from drinking water, food water, and metabolized food. Because non-essential amino acids are synthesized *in vivo*, their hydrogen stable isotopic compositions reflect those of body water. Essential amino acids must be obtained from diet and therefore their δ^2H values are dependent on those of food sources. As a result, animals that biosynthesize more amino acids will have a higher contribution of environmentally derived hydrogen in their tissues, including keratin. Cats are obligate carnivores and need higher amounts of specific essential amino acids in their diet (taurine, arginine, methionine, and cysteine) than do facultative carnivores, such as dogs, or omnivores [36]. This is most likely because these essential amino acids are abundant in prey and *in vivo* synthesis is not required because it is redundant [37]. Carbohydrates, such as starches and plant material, can be tolerated by dogs but not by cats [38].

For oxygen isotopes, a similar dependence on body water is expected except that carboxyl bound O is fully available during peptide hydrolysis and therefore oxygen can exchange with body water for both essential and nonessential amino acids. This suggests that oxygen isotope ratios in keratins may be insensitive to the extent of *in vivo* synthesis [32] but more affected by the relative contribution of body water and the isotopic fractionation between water and carbonyl oxygen during amino acid synthesis [39]. Close isotopic coupling of oxygen isotopes between ambient water and wing material has been reported for aquatic emergent insects such as dragonflies [40], but this coupling is not as evident in birds or terrestrial insects [19, 41].

Many mammals vary in their relative use of drinking water versus metabolic water as a contribution to the body water pool available for H and O isotopic exchange. For example, polar bears (*Ursus maritimus*) do not drink fresh water. Instead, they produce body water by consumption of a high fat diet, for which the catabolic products are only water and carbon dioxide [42, 43]. By contrast, the water needs of cats reflect both their evolution in arid environments and their development as strict carnivores. They can obtain most of their water requirements from consumption of prey, but with their high protein intake, they do need some water because the end products of protein catabolism (urea, ammonia, uric acid, and creatinine) must be excreted in urine. Dogs, unlike cats, do not concentrate their urine and thus their water intake needs are higher still, as are humans and other non water–conserving mammals [44]. Other metabolic differences specific to felids include a higher metabolism [45] or relatively poor heat loss response involving increased internal evaporation (*i.e.* panting) [46, 47],

either of which may alter the hydrogen and oxygen stable isotopic compositions of body water, and thus hair.

In summary, the relative influence of H and O isotopes in environmental waters on hair keratin is expected to be affected by the extent of *in vivo* amino acid synthesis with more of an influence on hydrogen isotopes in cases of higher levels of synthesis. Because oxygen in body water is not only derived from drinking water and diet but also O_2 in air through respiration [48], it is possible that the isotopic coupling of oxygen isotopes between hair and body water will be dependent on the relative use of these two pools [49], or like hydrogen, may be influenced by the relative role of hydration in overall nutrition. It is worth noting, however, that the transfer of hydrogen and oxygen from environmental water into animal tissues is a complex process and is still poorly understood. For instance, δ^2H and δ^{18}O values of local food sources also likely vary with those of precipitation so that it is difficult to accurately asses the relative contributions between food sources and drinking water outside the laboratory environment. All things considered, however, we would expect a weaker relationship between the δ^2H and δ^{18}O values of hair keratin and those of local precipitation for cats than for dogs, which is consistent with our data.

The relationship between the δ^2H and δ^{18}O values of hair for cats and dogs are shown in Fig 4. For dogs, there is a good correlation ($r^2 = 0.785$) between δ^2H and δ^{18}O values of hair with a regressed slope that is similar to the relationship for meteoric water. The δ^2H and δ^{18}O values of cats, on the other hand, display a weaker correlation ($r^2 = 0.454$) and the slope of the regression line is shallower than we would expect for meteoric waters. This suggests that, on average, there is less relative transfer of hydrogen than oxygen isotopes from environmental waters into hair keratin. The reasons for this are unclear but most likely are the result of the dietary and metabolic differences inherent in the obligate carnivore physiology, as discussed previously.

Hydrogen and oxygen stable isotopic compositions from fur of both wild and captive large cats (tigers, cougars, bobcats, and lions) are broadly consistent with our data from domestic cats, although the sample size is small. Similarly, captive and wild canids (foxes, coyotes, and wolves) seem to follow the same trend as do domestic dogs (Fig 4). Moreover, our
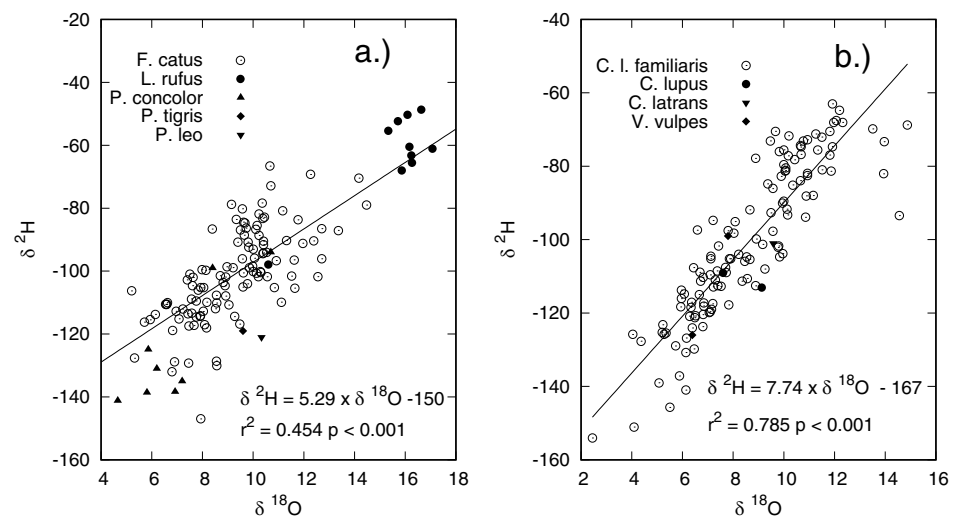

Fig 4. Relationship between a) cat (*F. catus*) and b) dog (*C. lupus familiaris*) hair δ^2H and δ^{18}O values. Also included are wild and captive bobcats, cougars, tigers, lions, coyotes, wolves, and foxes (*L. rufus, P. concolor, P. tigris, P. leo, C. latrans, C. lupus*, and *V. vulpes*).

measurements of the relative isotopic contribution of oxygen from environmental waters to body water of 0.385 (Table 1) agrees well with those measured in bone phosphate from wild bobcats and cougars (0.41 and 0.38, respectively) [22]. While not definitive, these data suggest that coupling of hydrogen and oxygen from environmental waters into tissues of wild felids and canids are likely similar to our observed measurements with domestic cats and dogs.

Conclusions

Our findings open up considerable new possibilities for determining origins or tracking migration of high level carnivores, such as felids and canids, using isotopic methods as has been demonstrated for birds, insects, and other taxa [17]. This is especially important because many terrestrial large mammals, particularly the carnivores, are currently under threat and isotopic methods for tracking and provenance have been investigated for only a few species, such as humans and bats [50].

Supporting information

S1 File. Excel streadsheet of δ^2H and δ^{18}O values of measured hair, water and predicted precipitation.
(XLSX)

Author Contributions

Data curation: Geoff Koehler.

Formal analysis: Geoff Koehler.

Investigation: Geoff Koehler.

Methodology: Geoff Koehler, Keith A. Hobson.

Resources: Keith A. Hobson.

Writing – original draft: Geoff Koehler.

Writing – review & editing: Geoff Koehler, Keith A. Hobson.

References

1. Ceballos G, Ehrlich PR, Soberón J, Salazar I, Fay JP. Global mammal conservation: what must we manage? Science. 2005; 309(5734):603–607. https://doi.org/10.1126/science.1114015 PMID: 16040704

2.   Barnosky AD, Matzke N, Tomiya S, Wogan GO, Swartz B, Quental TB, et al. Has the Earth's sixth mass extinction already arrived? Nature. 2011; 471(7336):51. https://doi.org/10.1038/nature09678 PMID: 21368823

3.   Schipper J, Chanson JS, Chiozza F, Cox NA, Hoffmann M, Katariya V, et al. The status of the world's land and marine mammals: diversity, threat, and knowledge. Science. 2008; 322(5899):225–230. https://doi.org/10.1126/science.1165115 PMID: 18845749

4.   Ripple WJ, Estes JA, Beschta RL, Wilmers CC, Ritchie EG, Hebblewhite M, et al. Status and ecological effects of the world's largest carnivores. Science. 2014; 343(6167):1241484. https://doi.org/10.1126/science.1241484 PMID: 24408439

5.   Macdonald DW, Loveridge AJ, Dickman A, Johnson PJ, Jacobsen KS, Du Preez B. Lions, trophy hunting and beyond: knowledge gaps and why they matter. Mammal Review. 2017; 47(4):247–253. https://doi.org/10.1111/mam.12096

6.   Bauer H, Chapron G, Nowell K, Henschel P, Funston P, Hunter LT, et al. Lion (Panthera leo) populations are declining rapidly across Africa, except in intensively managed areas. Proceedings of the National Academy of Sciences. 2015; 112(48):14894–14899. https://doi.org/10.1073/pnas.1500664112

7.   Durant SM, Mitchell N, Groom R, Pettorelli N, Ipavec A, Jacobson AP, et al. The global decline of cheetah Acinonyx jubatus and what it means for conservation. Proceedings of the National Academy of Sciences. 2017; 114(3):528–533. https://doi.org/10.1073/pnas.1611122114

8.   Hinton J, Chamberlain M, Rabon D. Red wolf (Canis rufus) recovery: a review with suggestions for future research. Animals. 2013; 3(3):722–744. https://doi.org/10.3390/ani3030722 PMID: 26479530

9.   Morell V. Can the wild tiger survive? Science. 2007; 317(5843):1312–1314. https://doi.org/10.1126/science.317.5843.1312 PMID: 17823325

10. Dinerstein E, Loucks C, Wikramanayake E, Ginsberg J, Sanderson E, Seidensticker J, et al. The fate of wild tigers. AIBS Bulletin. 2007; 57(6):508–514.

11. Wolf C, Ripple WJ. Range contractions of the world's large carnivores. Royal Society open science. 2017; 4(7):170052. https://doi.org/10.1098/rsos.170052 PMID: 28791136

12. Chesson LA, Barnette JE, Bowen GJ, Brooks JR, Casale JF, Cerling TE, et al. Applying the principles of isotope analysis in plant and animal ecology to forensic science in the Americas. Oecologia. 2018; 187(4):1077–1094. https://doi.org/10.1007/s00442-018-4188-1 PMID: 29955984

13. Meier-Augenstein W, Hobson KA, Wassenaar LI. Critique: measuring hydrogen stable isotope abundance of proteins to infer origins of wildlife, food and people. Bioanalysis. 2013; 5(7):751–767. https://doi.org/10.4155/bio.13.36 PMID: 23534421

14. Hobson KA, Wassenaar LI. Tracking animal migration with stable isotopes. vol. 2. Academic Press; 2008.

15. Hobson KA, Atwell L, Wassenaar LI. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. Proceedings of the National Academy of Sciences. 1999; 96(14):8003–8006. https://doi.org/10.1073/pnas.96.14.8003

16. West JB, Bowen GJ, Dawson TE, Tu KP. Isoscapes: understanding movement, pattern, and process on Earth through isotope mapping. Springer; 2009.

17. Hobson KA, Wassenaar LI. Tracking animal migration with stable isotopes. Academic Press; 2018.

18. Vander Zanden HB, Nelson DM, Wunder MB, Conkling TJ, Katzner T. Application of isoscapes to determine geographic origin of terrestrial wildlife for conservation and management. Biological Conservation. 2018; 228:268–280. https://doi.org/10.1016/j.biocon.2018.10.019

19. Hobson KA, Koehler G. On the use of stable oxygen isotope (δ18O) measurements for tracking avian movements in North America. Ecology and Evolution. 2015; 5(3):799–806. https://doi.org/10.1002/ece3.1383 PMID: 25691999

20. Pietsch SJ, Hobson KA, Wassenaar LI, Tütken T. Tracking cats: problems with placing feline carnivores on δ^{18}O, δD isoscapes. PLoS One. 2011; 6(9):e24601. https://doi.org/10.1371/journal.pone.0024601 PMID: 21931770

21. Henaux V, Powell LA, Hobson KA, Nielsen CK, LaRue MA. Tracking large carnivore dispersal using isotopic clues in claws: an application to cougars across the Great Plains. Methods in Ecology and Evolution. 2011; 2(5):489–499. https://doi.org/10.1111/j.2041-210X.2011.00107.x

22. Pietsch SJ, Tütken T. Oxygen isotope composition of North American bobcat (Lynx rufus) and puma (Puma concolor) bone phosphate: implications for provenance and climate reconstruction. Isotopes in environmental and health studies. 2016; 52(1-2):164–184. https://doi.org/10.1080/10256016.2015.1113957 PMID: 27002600

23. Terzer S, Wassenaar L, Araguás-Araguás L, Aggarwal P. Global isoscapes for [delta] 18O and [delta] 2H in precipitation: improved prediction using regionalized climatic regression models. Hydrology and Earth System Sciences. 2013; 17(11):4713. https://doi.org/10.5194/hess-17-4713-2013

24. Qi H, Coplen TB, Wassenaar LI. Improved online δ^{18}O measurements of nitrogen-and sulfur-bearing organic materials and a proposed analytical protocol. Rapid Communications in Mass Spectrometry. 2011; 25(14):2049–2058. https://doi.org/10.1002/rcm.5088 PMID: 21698688

25. Gehre M, Renpenning J, Gilevska T, Qi H, Coplen TB, Meijer HA, et al. On-line hydrogen-isotope measurements of organic samples using elemental chromium: An extension for high temperature elemental-analyzer techniques. Analytical chemistry. 2015; 87(10):5198–5205. https://doi.org/10.1021/acs.analchem.5b00085 PMID: 25874646

26. Soto DX, Koehler G, Wassenaar LI, Hobson KA. Re-evaluation of the hydrogen stable isotopic composition of keratin calibration standards for wildlife and forensic science applications. Rapid Communications in Mass Spectrometry. 2017; 31(14):1193–1203. https://doi.org/10.1002/rcm.7893 PMID: 28475227

27. R Core Team. R: A Language and Environment for Statistical Computing; 2015. Available from: https://www.R-project.org/.

28. Koehler G, Hobson KA. Effects of tanning on the stable isotopic compositions of hair. Forensic science international. 2018; 292:78–82. https://doi.org/10.1016/j.forsciint.2018.08.020 PMID: 30286339

29. Sharp ZD, Atudorei V, Panarello HO, Fernández J, Douthitt C. Hydrogen isotope systematics of hair: archeological and forensic applications. Journal of Archaeological Science. 2003; 30(12):1709–1716. https://doi.org/10.1016/S0305-4403(03)00071-2

30. Ehleringer JR, Bowen GJ, Chesson LA, West AG, Podlesak DW, Cerling TE. Hydrogen and oxygen isotope ratios in human hair are related to geography. Proceedings of the National Academy of Sciences. 2008; 105(8):2788–2793. https://doi.org/10.1073/pnas.0712228105

31. Bowen GJ, Ehleringer JR, Chesson LA, Thompson AH, Podlesak DW, Cerling TE. Dietary and physiological controls on the hydrogen and oxygen isotope ratios of hair from mid-20th century indigenous populations. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists. 2009; 139(4):494–504. https://doi.org/10.1002/ajpa.21008

32. Thompson AH, Chesson LA, Podlesak DW, Bowen GJ, Cerling TE, Ehleringer JR. Stable isotope analysis of modern human hair collected from Asia (China, India, Mongolia, and Pakistan). American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists. 2010; 141(3):440–451.

33. Hobson KA. Isotopic tracking of migrant wildlife. Stable isotopes in ecology and environmental science. 2008; p. 155.

34. Cryan PM, Bogan MA, Rye RO, Landis GP, Kester CL. Stable hydrogen isotope analysis of bat hair as evidence for seasonal molt and long-distance migration. Journal of Mammalogy. 2004; 85(5):995–1001. https://doi.org/10.1644/BRG-202

35. Popa-Lisseanu AG, Sörgel K, Luckner A, Wassenaar LI, Ibáñez C, Kramer-Schadt S, et al. A triple-isotope approach to predict the breeding origins of European bats. PLoS One. 2012; 7(1):e30388. https://doi.org/10.1371/journal.pone.0030388 PMID: 22291947

36. MacDonald M, Rogers Q, Morris J. Nutrition of the domestic cat, a mammalian carnivore. Annual review of nutrition. 1984; 4(1):521–562. https://doi.org/10.1146/annurev.nu.04.070184.002513 PMID: 6380542

37. Zoran D. The carnivore connection to nutrition in cats. Journal of the American Veterinary Medical Association. 2002; 221(11):1559. https://doi.org/10.2460/javma.2002.221.1559 PMID: 12479324

38. Legrand-Defretin V. Differences between cats and dogs: a nutritional view. Proceedings of the Nutrition Society. 1994; 53(1):15–24. https://doi.org/10.1079/PNS19940004 PMID: 8029223

39. O'Grady SP, Valenzuela LO, Remien CH, Enright LE, Jorgensen MJ, Kaplan JR, et al. Hydrogen and oxygen isotope ratios in body water and hair: modeling isotope dynamics in nonhuman primates. American journal of primatology. 2012; 74(7):651–660. https://doi.org/10.1002/ajp.22019 PMID: 22553163

40. Hobson KA, Soto DX, Paulson DR, Wassenaar LI, Matthews JH. A dragonfly (δ^2H) isoscape for North America: a new tool for determining natal origins of migratory aquatic emergent insects. Methods in ecology and evolution. 2012; 3(4):766–772. https://doi.org/10.1111/j.2041-210X.2012.00202.x

41. Hobson KA, Kardynal KJ, Koehler G. Expanding the isotopic toolbox to track Monarch Butterfly (*Danaus plexippus*) origins and migration: On the utility of stable oxygen isotope (δ^{18}O) measurements. Frontiers in Ecology And Evolution. 2019; 7(224):1–7.

42. Nelson RA. Black bears and polar bears—still metabolic marvels. In: Mayo Clinic Proceedings. vol. 62. Elsevier; 1987. p. 850–853.

43. Nelson RA, Folk GE Jr, Pfeiffer EW, Craighead JJ, Jonkel CJ, Steiger DL. Behavior, biochemistry, and hibernation in black, grizzly, and polar bears. Bears: their biology and management. 1983; p. 284–290.

44. Anderson R. Water balance in the dog and cat. Journal of Small Animal Practice. 1982; 23(9):588–598. https://doi.org/10.1111/j.1748-5827.1982.tb02519.x

45. McNab BK. The standard energetics of mammalian carnivores: Felidae and Hyaenidae. Canadian Journal of Zoology. 2000; 78(12):2227–2239. https://doi.org/10.1139/z00-167

46. Doris P, Baker M. Effects of dehydration on thermoregulation in cats exposed to high ambient temperatures. Journal of Applied Physiology. 1981; 51(1):46–54. https://doi.org/10.1152/jappl.1981.51.1.46 PMID: 7263423

47. Adams T. Characteristics of eccrine sweat gland activity in the footpad of the cat. Journal of Applied Physiology. 1966; 21(3):1004–1012. https://doi.org/10.1152/jappl.1966.21.3.1004 PMID: 5912716

48. Bryant JD, Froelich PN. A model of oxygen isotope fractionation in body water of large mammals. Geochimica et Cosmochimica Acta. 1995; 59(21):4523–4537. https://doi.org/10.1016/0016-7037(95)00250-4

49. Podlesak DW, Torregrossa AM, Ehleringer JR, Dearing MD, Passey BH, Cerling TE. Turnover of oxygen and hydrogen isotopes in the body water, CO2, hair, and enamel of a small mammal. Geochimica et Cosmochimica Acta. 2008; 72(1):19–35. https://doi.org/10.1016/j.gca.2007.10.003

50. Voigt CC, Lehnert LS. Tracking of Movements of Terrestrial Mammals Using Stable Isotopes. In: Tracking Animal Migration with Stable Isotopes. Elsevier; 2019. p. 117–135.