Rapid, Effective Screening of Tar Seep Fossils for Radiocarbon and Stable Isotope Analysis

[1]Robin B. Trayler [1]Lauren E. Lopes [2]Patrica A. Holroyd
[1]Sora L. Kim [3]John R. Southon

[1]Department of Life and Environmental Sciences, University of California, Merced, CA

[2]Museum of Paleontology, University of California, Berkeley, CA, https://orcid.org/0000-0003-1292-6356

[3]Department of Earth System Science, University of California, Irvine, Irvine, CA, USA.

[*]Corresponding author: rtrayler@ucmerced.edu

Abstract

1 Introduction

Asphalt seep *lagerstätten*, colloquially known as "tar pits", have produced remarkable fossil assemblages, giving an unparalleled look into the ecology of floras and faunas during the late Pleistocene and Holocene (see reviews in Stock and Harris (1992) and McDonald et al. (2015)). Tar pits form when natural tar seeps through subsurface fractures to form a viscous sticky layer on the surface. This tar tends to trap animal and plant remains, leading to large accumulations of fossils. Importantly the rapid entrapment and later impregnation of tar into the remains can preserve fossils in environments where local conditions are otherwise not amenable to long term preservation (e.g., the neotropics; Lindsey and Seymour (2015)), and therefore act as an important source of information about these ecosystems. The remarkable morphological preservation of tar impregnated fossils comes at a cost however, for studies interested in applying geochronological ($^{14}C_{collagen}$) (Fox-Dobbs et al., 2014; Fuller et al., 2015; Fuller et al., 2014; O'Keefe et al., 2023) or stable isotope techniques ($\delta^{13}C_{collagen}$) (Coltrain et al., 2004; Fox-Dobbs et al., 2007, 2006; Fuller et al., 2020). Natural tar is 80-90% carbon by weight and is radiocarbon-dead. As a consequence, even a small amount of tar is a potent contaminant that can bias $^{14}C_{collagen}$ and $\delta^{13}C_{collagen}$ measurements, and it must be removed prior to geochemical analysis (Coltrain et al., 2004; Fox-Dobbs et al., 2006; Fuller et al., 2014). Briefly, the current best practice for extracting collagen from tar impregnated fossils involves repeatedly washing an aliquot of bone powder with organic solvents followed by acid digestion of the bone mineral, and finally collagen purification by ultrafiltration (Fuller et al., 2014). Taken together, these methods require multiple days of work, large sample sizes (~150 mg) and due to variations in collagen preservation, may not yield viable collagen for further analysis. Therefore, identifying fossils with well preserved collagen *prior* to tar removal and collagen extraction can reduce unnecessary damage to fossil collections, reduce analytical costs, and improve research outcomes for geochemical studies of these fossils.

In this study we investigate potential screening methods to identify tar impregnated fossils with a high likelihood of collagen preservation. We developed a training data set of fossils from two geographically distinct seep areas in California, ancho La Brea in Los Angeles County and McKittrick in Kern County, where collagen preservation state was known from previous studies. These data were used to test two potential screening methods, visual, non-destructive, taphonomic scoring (Behrensmeyer, 1978) and minimally-destructive Fourier Transform Infrared (FTIR) spectroscopy. We calculated several commonly used FTIR indices to assess diagenesis in bone as well as a new index to assess tar impregnation. We used this initial training data to identify the most useful predictors of collagen preservation. We then used these predictions to choose additional fossils for collagen extraction that address the limitations of the initial training data, resulting in a robust freely available reference data set that can be used to screen tar seep fossils for further analysis.

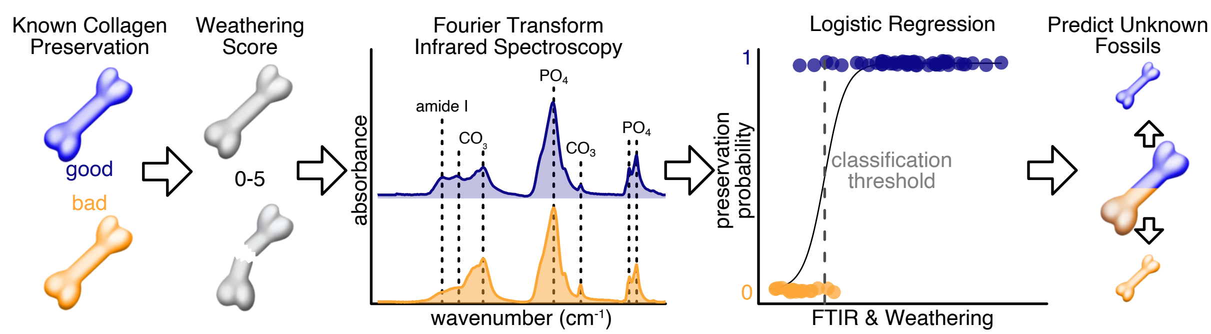

Figure 1: Workflow of model development.

2 Background

2.1 Depositional and Curatorial Context

2.1.1 McKittrick

The McKittrick tar seeps are located along the eastern edge of the Temblor Range in the Southern San Joaquin Valley of California, just outside the town of McKittrick. Here, faulting allows oil to migrate from the underlying shale through porous sandstones of the Etchegoin and Tulare formations to form layers of asphaltum in a NW-SE trending brea belt. The faunas appear to span from the late Pleistocene into the Holocene and the small number of radiocarbon dates for the locality support a late Pleistocene age for the majority of the fossils (Fox-Dobbs et al., 2014; France, 2008).

Specimens used in the current study are from collections made by University of California personnel and by Charles Sternberg for both the University of California and California Institute of Technology which primarily took place from 1921-1927 in several different pits.(Merriam and Stock, 1921; Schultz, 1938; Sternberg, 1932; Stock, 1928) As reported by Sternberg (1932), fossils from McKittrick were cleaned using kerosene on site, which removed most surface tar. Based on direct examination of the collections, plaster was also used in joining broken bones, and there is no indication that animal hide glues were used in preparation.

2.1.2 Rancho La Brea

The Rancho La Brea tar seeps occur in the northern part of the Los Angeles Basin, south of the Santa Monica Mountains, in urban Los Angeles that has produced a diverse fauna span the late Pleistocene to Holocene (see e.g., Stock and Harris (1992)). The tar seeps here are known to form pools of liquid asphaltum, that act as natural traps. The fossils included in this study are from University of California excavations conducted from 1908-

1912 (Merriam, 1911; Stoner, 1913) and include those with radiocarbon dates reported in O'Keefe et al. (2009) and stable isotope analysis from Fox-Dobbs et al. (2006).

2.2 Bone Collagen Preservation and Isolation

Bone is a composite material with organic and mineral component, both of which are common tissues for geochemical analysis. The mineral fraction is bioapatite [$Ca_4(PO_4)_3OH$] with carbonate (CO_3) substitutions in the hydroxyl and phosphate sites (Driessens and Verbeeck, 1990; Elliott, 2002). The organic phase is primarily collagen (Collins et al., 2002). Collagen is relatively insoluble and can persist in bone for tens of thousands of years (Clementz, 2012; Collins et al., 2002). The high collagen content of bone has made it a tissue of choice for many paleontologists and archaeologists interested in stable ($\delta^{13}C$, $\delta^{15}N$) and radioactive (^{14}C) isotope analysis of fossil specimens. However, collagen preservation can vary considerably between localities depending on a variety of taphonomic factors.

Collagen preservation in modern and fossil specimens is usually assessed using a combination of collagen yield, carbon and nitrogen content, and the atomic carbon-to-nitrogen ratio ($C{:}N_{atomic}$). The amino acid profile of collagen is relatively conservative across a variety of taxonomic groups (Szpak, 2011). Collagen content in fresh bone is ranges from 12 - 33% with an average of about 22% (Ambrose, 1990; Collins et al., 2002; Van Klinken, 1999). Low collagen yield in fossil specimens can indicate degradation and loss of amino acids (Van Klinken, 1999), potentially also indicating changes in stable and or radioactive isotope composition. Likewise low carbon and nitrogen contents can indicate alteration (Guiry and Szpak, 2020). Several $C{:}N_{atomic}$ thresholds have been proposed as indicative of well preserved collagen including 2.9–3.6 (Ambrose, 1990), 3.1–3.5 (Van Klinken, 1999), and a variety of of taxon specific ranges (Guiry and Szpak, 2021). Weathering is another potential pathway of collagen loss (Koch et al., 1999, 1999; Trueman et al., 2004), although it may be less pronounced in temperate climates (Fernández-Jalvo et al., 2010).

2.3 The Problem of Tar

Collagen is also susceptible to contamination from a variety of sources, including endogenous lipids, humic acids, and non-collagenous proteins (Guiry and Szpak, 2021). In particular, lipids and humics have high carbon, but low nitrogen, contents and lead to $C:N_{atomic}$ higher than the accepted thresholds. Tar, is a particularly potent contaminant; it has a very high carbon content, readily impregnates bone pore space, and is pernicious and difficult to completely remove. Several methods have been developed to purify and extract collagen from tar-impregnated fossils (Coltrain et al., 2004; Fox-Dobbs et al., 2006; Fuller et al., 2015; Fuller et al., 2014) that follow a similar set of steps. First bone (powder or chunks) is repeated washed with solvents (e.g., toluene, methanol, acetone) to extract the tar. Second, bone bioapatite is dissolved using either acid or chelating agents, leaving isolated collagen. While these methods differ in their specific details, in all cases the tar removal is a complex, time consuming process (~3 days, Coltrain et al. (2004); 5-6 days, Fox-Dobbs et al. (2006); 2-3 days, Fuller et al. (2014)). Finally, even after these steps, not all specimens will yield viable collagen. Collagen preservation can vary substantially between tar-pit deposits. Fore example, Coltrain et al. (2004) reported that 13 of 143 bones from Rancho La Brea failed to yield collagen (8% failure), whereas France (2008) (following the methods of Coltrain et al. (2004)) reported a 78% failure rate (22 of 28) for fossils from McKittrick. Similarly, preliminary attempts at collagen extraction for this study from randomly selected fossils yielded a success rate of 28%.

2.4 Infrared Spectroscopy

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR), is a minimally destructive vibrational spectroscopic technique that characterizes the molecular functional groups of a material by irradiating it with infrared light. Since molecular structure determines which infrared wavelengths are transmitted or absorbed, ATR-FTIR can be

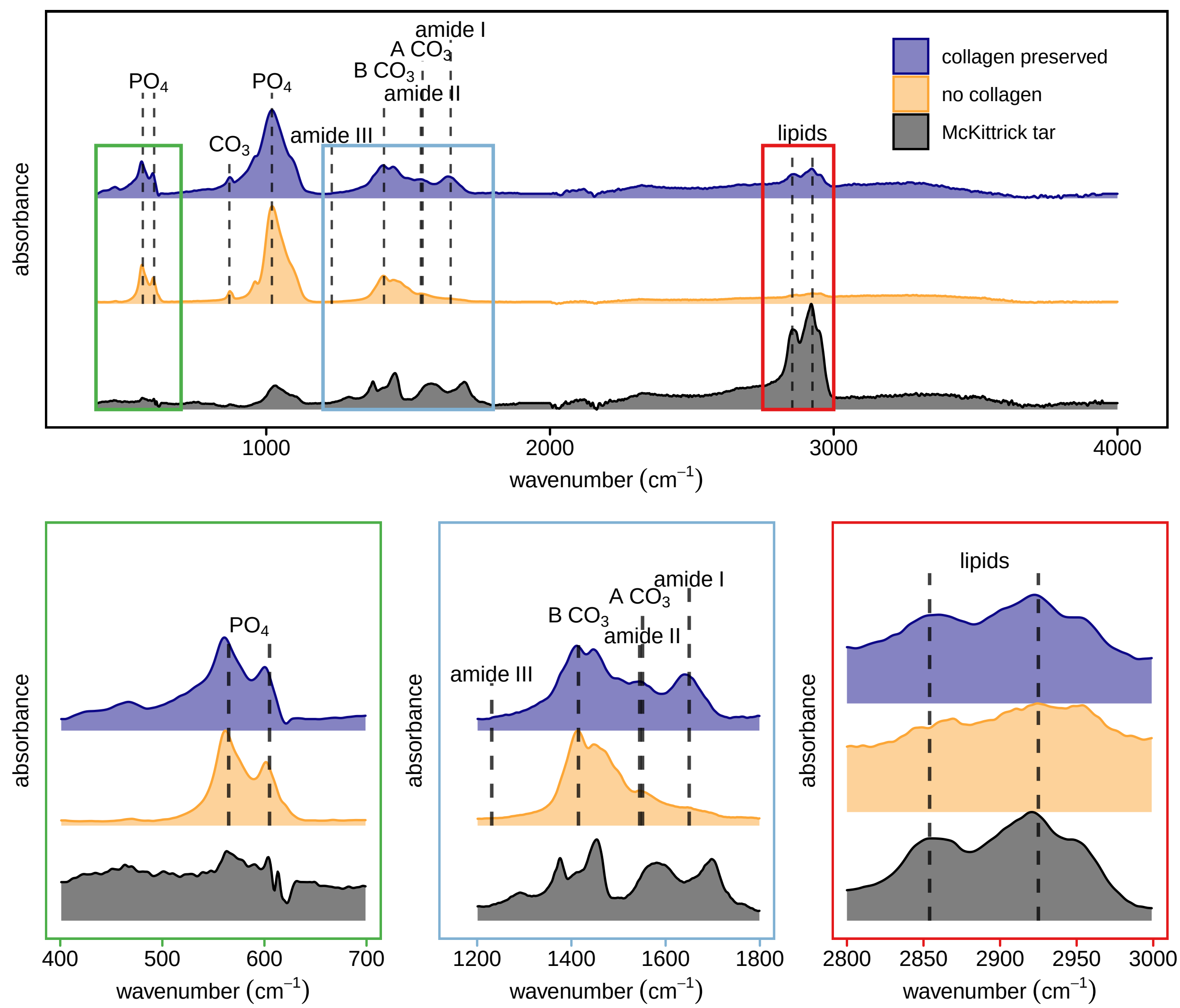

Figure 2: Representative example ATR-FTIR spectra for bone with/ without preserved collagen and McKittrick tar. Vertical dashed lines indicate band positions from Table 1. Carbonate, phosphate, and amide bands are only applicable to bone spectra while the lipid bands occur in in both materials. Top panel: full spectrum for all materials. Bottom panels: zoomed view of the phosphate, amide/ carbonate, and lipid regions highlighted by colored boxes.

used to attribute absorbance bands to different functional groups, and semi-quantitatively determine the chemical composition of a material (Stuart, 1991).

Within the context of paleontological and archaeological specimens, ATR-FTIR is often used to investigate the crystal-chemical properties of bone bioapatite and collagen, with a particular focus on diagenesis and alteration during fossilization (Chadefaux et al., 2009; Hassan et al., 1977; Roche et al., 2010; Sponheimer and Lee-Thorp, 1999). The absorbance band-positions of the major components of bone are know; including inorganic phosphate (PO_4) and carbonate (CO_3) (Fleet, 2009; Sponheimer and Lee-Thorp, 1999), and organic amides and lipids (Chadefaux et al., 2009; Lebon et al., 2016; Liden et al., 1995) (Table 1). Previous work has shown that FTIR is an effective tool at identifying organic preservation in archaeological contexts (Lebon et al., 2016), but to our knowledge, there have been no prior attempts to apply the methodology to tar impregnated fossils.

Table 1: Nominal FTIR band positions of several relevant chemical groups. Actual band positions may be shifted by several cm^{-1}.

band position (cm^{-1})	Functional Group
2925	Lipid
2854	Lipid
1650	Amide I
1551	Amide II
1545	A-Type Carbonate
1415	B-Type Carbonate
1231	Amide III
1020	Phosphate
880	Carbonate
605	Phosphate
565	Phosphate

3 Materials and Methods

3.1 Sample Selection

We selected specimens from the University of California, Museum of Paleontology (UCMP) collection where radiocarbon dating and/or isotopic analysis ($\delta^{13}C$, $\delta^{15}N$, ^{14}C) of the organic collagen fraction has previously been attempted (Table 2) as part of other research projects (Fox-Dobbs et al., 2014; Fox-Dobbs et al., 2006; O'Keefe et al., 2009) Ask J. Southon how to cite the unpublished UCMP data. These specimens fall into two groups: 1) fossils that produced viable, well-preserved collagen(n = 50) and 2) fossils that failed to yield viable collagen(n = 20). We considered collagen well preserved if it passed the standard metrics for collagen preservation (see reviews of (Guiry and Szpak, 2021, 2020)) as reported by the original study authors. We collected 5-10 mg of cortical bone powder using a handheld rotary tool and a dental drill bit. These powders were stored in 1.5 mL micro-centrifuge vials prior to analysis. The bone powders were not chemically treated to remove tar (Fuller et al., 2014) or otherwise treated to remove carbonates or organics (Koch et al., 1997) prior to FTIR analysis. All specimens are from either Rancho La Brea (n = 48) or McKittrick (n = 22) and cover a wide range of carnivore and herbivore taxa, and include mammals, birds, and reptiles.

Table 2: Number of specimens in the well preserved and poorly preserved groups for Rancho La Brea and McKittrick asphalt seeps.

Locality	Collagen	No Collagen	Collagen Preservation %
Rancho La Brea	43	5	90%
McKittrick	7	15	31%

3.2 FTIR Indices

ATR-FTIR spectra were collected for all fossil specimens using a *Bruker Vertex 70 Far-Infrared Fourier Transform Infrared Spectrometer* from the Nuclear Magnetic Resonance Facility at the University of California, Merced. The spectra were collected from 400 to 4000 cm-1 over 32 scans at a spectral resolution of 4 cm^{-1}. Each spectrum was background-corrected using several baseline points and slightly smoothed prior to index calculation using custom R scripts available in the supplementary material.

The resulting 70 spectra were used to calculate two FTIR indices commonly used to investigate organic content and diagenesis in fossil bone. The Water-Amide-on-Phosphate-Index (WAMPI) is the ratio of the Amide-I and v_2PO_4 phosphate absorbance bands ($\frac{B_{1650}}{B_{605}}$) tracks bone collagen content and higher WAMPI values should indicate better collagen preservation (Lebon et al., 2016; Roche et al., 2010; Trayler et al., 2023). The Phosphate-Crystalinity-Index (PCI) is the sum of the v_2PO_4 and v_4PO_4 phosphate absorbance band maxima, normalized by the depth of valley between these two peaks ($\frac{B_{605}+B_{565}}{V_{590}}$; (Sponheimer and Lee-Thorp, 1999)). Since increases in bone crystallinity sharpen the two phosphate peaks and deepen the valley, higher PCI reflect greater diagenetic alteration of the bone mineral (Sponheimer and Lee-Thorp, 1999). Furthermore, higher PCI values can also reflect heat-induced changes to crystal order and structure, resulting from deliberate (cultural) or natural burning (wildfires), which is also expected to remove organic material (Thompson et al., 2013, 2009).

We also calculated the ratio of two lipid absorbance bands (Liden et al., 1995) normalized to the v_2PO_4 phosphate absorbance band ($\frac{B_{2925}+B_{2854}}{B_{605}}$). Endogenous lipids exhibit prominent absorbance bands at about 2925 cm^{-1} and 2854 cm^{-1}, as does McKittrick and Rancho La Brea asphaltum (Figure 2). Thus this Lipid-on-Phosphate-Index (LPI) should reflect excess tar or lipid content in bone. While the loss of endogenous lipids is variable in the fossil record (Collins et al., 2002; Koch et al., 1999), tar seep fossils are impregnated with oils which has been proposed as a "preservative" (Stock and Harris, 1992), suggesting that higher LPI values could correspond to better collagen preservation.

3.3 Taphonomy and Weathering Scores

To assess if visual assessment of weathering was a reliable indicator of collagen preservation, we scored each specimen according to the set of weathering stages established by (Behrensmeyer, 1978) for large mammals and using images in Behrensmeyer and Miller (2012) and Fernández-Jalvo and Andrews (2016) as visual referents. These stages are numbered 0 to 5, with stage 0 showing no modification and stage 5 showing significant splintering, flaking, and being easily broken. If weathering is a significant factor, we would expect specimens with less collagen to have higher weathering scores.

3.4 Statistical Methods

3.4.1 Statistical Analyses

To determine the best predictors of collagen preservation, we used the weathering scores and FTIR indices from the fossils of known collagen preservation to develop a training dataset to assess which predictors are most strongly associated collagen presence or absence. We fit a logistic regression model in the form of:

$$ln(odds) = ln(\frac{p}{1-p}) = \beta \times x + \alpha \tag{1}$$

to each predictor (WAMPI, PCI, LPI, weathering score) separately, where p is the probability of collagen preservation (between 0 and 1), x is an individual predictor, and β and α are model coefficients. These logistic regression models were used to predict collagen presence for each fossil using a log-odds > 1 as a classification threshold, such that specimens where $p \geq 0.5$ were predicted to contain collagen and specimens where $p \leq 0.5$ were predicted to lack collagen.

Model performance was assessed by calculating the sensitivity and specificity of each logistic regression model to determine the most useful predictor(s) for collagen presence. Sensitivity is defined as:

$$sensitivity = \frac{true\,positives}{true\,positives + false\,negatives}$$

and specificity as:

$$specificity = \frac{true\,negatives}{true\,negatives + false\,positives}$$

Both sensitivity and specificity vary between 0 and 1, and in general, higher values for both indicate better model performance. A perfectly performing model that predicts zero false negatives and zero false positives would therefore have both a sensitivity and specificity of 1. However, a model that *always* predicts the presence of collagen would have a high sensitivity (~ 1), and low specificity (~ 0), with the opposite being true for a model that always predicts the absence of collagen. Therefore, an ideal model is both sensitive and specific, with both values close to one.

4 Results & Discussion

4.1 Training Data

Table 3: Summary of training data logistic regression results. Model coefficients (α, β) correspond to eq. 1. The *Threshold* column indicates the predictor value with an odds-ratio of 1. If β is positive then *Predictor* values higher than the *Threshold* predict collagen presence, whereas if β is negative, *Predictor* values lower than the *Threshold* predict collagen presence.

Predictor	α	β	p value	Threshold	Sensitivity	Specificity
WAMPI	-9.13	21.06	0.003	0.43	0.96	0.90

Predictor	α	β	*p* value	Threshold	Sensitivity	Specificity
PCI	13.80	-4.05	0.004	3.40	0.92	0.30
Weathering Score	2.06	-0.93	0.001	2	0.94	0.25
LPI	-0.49	1.47	0.057	0.34	1.00	0.05

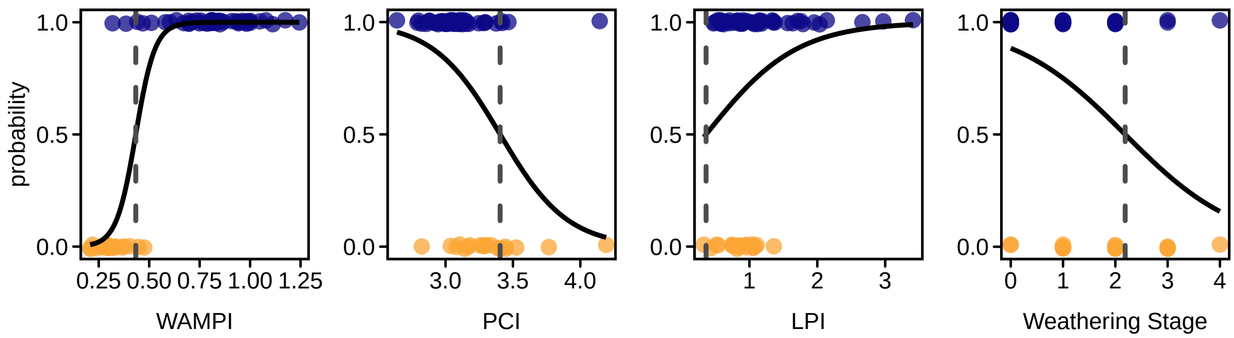

Figure 3: Training data logistic regression results for each of the four possible predictors of collagen presence. The vertical dashed line is the predictor value with an odds-ratio of 1 which we used as the prediction threshold (see Table 3 for details). Blue dots indicate samples with known collagen preservation and yellow dots indicate samples with known collagen absence. Points have been vertically-jittered slightly for clarity.

The WAMPI, PCI, and weathering score logistic regression model-fits were statistically significant, and the LPI was not. The Water-Amide-on-Phosphate Index was the best performing predictor and is both highly sensitive and specific when predicting collagen presence and absence (Table 3). The WAMPI is calculated using the Amide-I band height which directly correlates to bone nitrogen and organic content (Lebon et al., 2016; Roche et al., 2010). The Phosphate-Crystallinity Index and weathering scores are highly sensitive, but are only weakly specific. Both models have negative β coefficient, indicating increasing predictor values correspond to lower collagen preservation (Figure 4). Weathering scores and PCI track taphonomic processes; macro scale weathering and microscopic apatite recrystallization, respectively. In other words, high degrees of weathering (high PCI, high weathering score), is somewhat predictive of collagen absence, but less weathering is not necessarily predictive of collagen presence. The proposed Lipid-on-Phosphate Index performed poorly and was not able to distinguish collagen presence or absence.

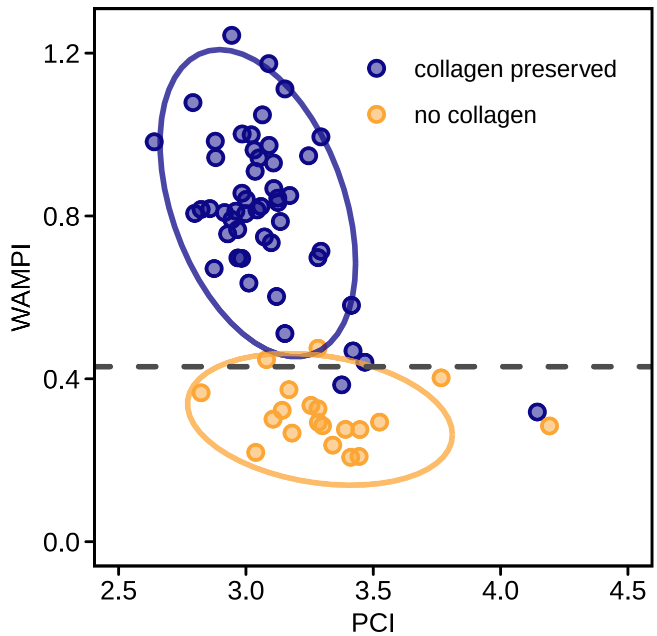

Figure 4: Plot of the PCI and WAMPI indices for the training data set. These two indices have the highest combined sensitivity and specificity. The colored ellipses contain 95% of the data in each group. The horizontal dashed line indicate the WAMPI classification threshold (Table 3).

4.2 Validation

To validate model performance, we collected FTIR spectra on 235 other UCMP McKittrick fossils for which collagen preservation was unknown. Using these data we calculated the best performing FTIR index (WAMPI) to predict collagen presence. A WAMPI threshold of 0.43 (Table 3) predicts that 75 of 235 fossils (31%) have preserved collagen, similar to the rate we observed from random selection of McKittrick fossils (28%). From these 235 fossils we selected 67 for attempted collagen extraction; 18 were predicted to contain no collagen (WAMPI < 0.43) and 49 were predicted to have well preserved collagen (WAMPI

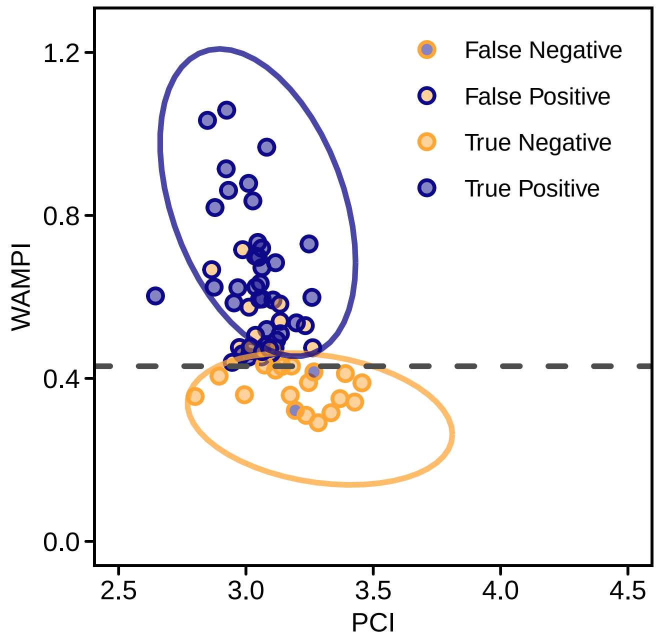

Figure 5: Model validation results using 67 fossils with unknown collagen preservation. The rim color of each point indicate the WAMPI based prediction of presence/absence and the fill color indicates actual collagen presence/ absence. The colored ellipses and horizontal dashed line are the same as shown in Figure 4.

> 0.43). Collagen was isolated at the UC Irvine Keck Carbon Cycle AMS Laboratory using the procedure of Fuller et al. (2014) for tar impregnated fossils. check with J. Southon on exact methods

Of the 67 fossils in the validation set, 34 produced well preserved collagen ($C{:}N_{atomic}$ = 3.30±0.08, mean±1S.D); 32 from the group predicted to contain collagen, and 2 from the group predicted to contain no collagen. Given that the underlying collagen preservation rate in McKittrick fossils appears to about 20-30%, a 65% chance of successfully extracting collagen is a substantial improvement. However, the validation dataset had a sensitivity of 0.94 and a specificity of 0.5. While the sensitivity of the validation data is similar to that of the training data (Table 3), the specificity is notably lower. This suggests that when applied to only McKittrick fossils, training classification threshold (WAMPI > 0.43) has a higher false positive rate. This is likely because of imbalances in the trading data set. About 80% of training samples with well preserved collagen are from Rancho La Brea, whereas 76% of the samples without preserved collagen are from McKittrick which reflects the underlying preservation rate of the two localities.

4.3 Combined Data and Further Use

Table 4: Logistic regression result for the Water-Amide-on-Phosphate Index using the combined, training and validation datasets.

Predictor	α	β	p value	Threshold	Sensitivity	Specificity
WAMPI	-6.94	13.9	1×10^{-8}	0.50	0.87	0.87

To address the mismatch in the training data set, we combined the training and validation data sets and recalculated the WAMPI logistic regression model (Table 4). The sensitivity and specificity for the combined dat are both 0.87, indicating a low false positive and low false negative rate. The threshold with a greater than 50% chance of collagen preservation is slightly higher (WAMPI > 0.5) than the training data threshold. Importantly, it should be noted however that our choice of an odds-ratio of 1 (> 50% preservation probability) is somewhat arbitrary, and that other thresholds could be calculated and used depending on the needs of a particular study (Figure 6). Rearranging eq. 1 to solve for the threshold and using the regressions coefficient in Table 4 gives:

$$WAMPI\ threshold = \frac{ln(odds) - \alpha}{\mathrm{B}} = \frac{ln(odds) + 6.94}{13.9}$$

Which can be used to calculate classification thresholds at arbitrary probabilities. For example, a higher odds-ratio of 3 (> 75% preservation probability) gives a WAMPI threshold of 0.58 and an odds-ratio of 19 (> 95% preservation probability) gives a WAMPI threshold of 0.78. These higher odds ratios come with a corresponding higher false-negative rate however, if fossil are abundant, then this trade off may be worthwhile to ensure only the best preserved specimens are chosen for further work.

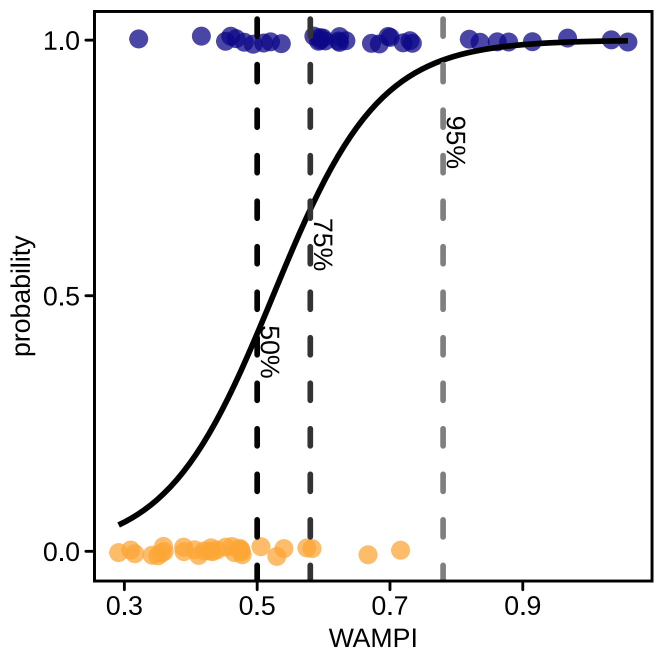

Figure 6: Final logistic regression model using the Water-Amide-on-Phosphate index for the combined data set. The vertical dashed lines indicate the 50%, 75%, and 95% preservation probability WAMPI thresholds.

5 Conclusions

Preparing tar seep fossils for isotope analysis ($\delta^{13}C$, $\delta^{15}N$, ^{14}C) is costly and time consuming and can be hampered by poor collagen preservation. Here we have presented methods and a reference data set for quickly identifying tar seep fossil with a high likelihood of collagen preservation. Completely non-destructive visual taphonomic indicators and the amount of impregnating tar are moderate to poor predictors of collagen preservation. In contrast minimally-destructive, FTIR analysis is highly sensitive and specific and a threshold of WAMPI > 0.5 is effective at identifying tar impregnated fossils with preserved collagen. Sample and data collection are both simple and rapid, and Fourier-Transform-Infrared spectrometers are available at most major research universities. Our combined reference data is robust (n = 137) and is broadly applicable to multiple tar pits. However, since the underlying data and code are freely available, it can be used as an initial screening tool for fossils from other tar pits (McDonald et al., 2015) and strengthened by the continued addition of data.

6 Supplementary Information

All infrared spectra and analysis code are available at github.com/robintrayler/mckittrick_FTIR

7 Acknowledgments

We thank HyeJoo Ro and Dr. Gina Palefsky for their help with sample collection, and Dr. David Rice for his assistance with FTIR data collection, and Dr. Elizabeth Black for her advice on statistical analyses. This work was supported by NSF-EAR-2138163 (RBT and SLK) and NSF-EAR-2138164 (PAH).

8 Author Contributions

RBT, SLK, and PAH conceived the project. RBT, LEL, and PAH collected the screening data. JRS extracted collagen. RBT, LEL, SLK, and PAH developed the statistical framework. RBT, LEL, and PAH wrote the manuscript with input from all authors.

References

Ambrose, S.H., 1990. Preparation And Characterization Of Bone And Tooth Collagen For Isotopic Analysis. Journal of Archaeological Science 17, 145.

Behrensmeyer, A.K., 1978. Taphonomic and ecologic information from bone weathering. Paleobiology 4, 150–162.

Behrensmeyer, A.K., Miller, J.H., 2012. Building links between ecology and paleontology using taphonomic studies of recent vertebrate communities. Paleontology in ecology and conservation 69–91.

Chadefaux, C., Le Hô, A.-S., Bellot-Gurlet, L., Reiche, I., 2009. Curve-fitting Micro-ATR-FTIR studies of the amide I and II bands of type I collagen in archaeological bone materials.

Clementz, M.T., 2012. New insight from old bones: Stable isotope analysis of fossil mammals. Journal of Mammalogy 93, 368–380.

Collins, M.J., Nielsen–Marsh, C.M., Hiller, J., Smith, C., Roberts, J., Prigodich, R., Wess, T.J., Csapo, J., Millard, A.R., Turner–Walker, G., 2002. The survival of organic matter in bone: A review. Archaeometry 44, 383–394.

Coltrain, J.B., Harris, J.M., Cerling, T.E., Ehleringer, J.R., Dearing, M.D., Ward, J., Allen, J., 2004. Rancho La Brea stable isotope biogeochemistry and its implications for the palaeoecology of late Pleistocene, coastal southern California. Palaeogeography, Palaeoclimatology, Palaeoecology 205, 199–219.

Driessens, F.C., Verbeeck, R.K., 1990. Biominerals. CRC Press, Boca Raton, FL.

Elliott, J.C., 2002. Calcium Phosphate Biominerals. Reviews in Mineralogy and Geochemistry 48, 427–453.

Fernández-Jalvo, Y., Andrews, P., 2016. Atlas of taphonomic identifications: 1001+ images of fossil and recent mammal bone modification. Springer.

Fernández-Jalvo, Y., Andrews, P., Pesquero, D., Smith, C., Marín-Monfort, D., Sánchez, B., Geigl, E.-M., Alonso, A., 2010. Early bone diagenesis in temperate environments: Part I: Surface features and histology. Palaeogeography, Palaeoclimatology, Palaeoecology 288, 62–81.

Fleet, M.E., 2009. Infrared spectra of carbonate apatites: $N2$ -Region bands. Biomaterials 30, 1473–1481.

Fox-Dobbs, K., Bump, J.K., Peterson, R.O., Fox, D.L., Koch, P.L., 2007. Carnivore-specific stable isotope variables and variation in the foraging ecology of modern and ancient wolf populations: Case studies from Isle Royale, Minnesota, and La Brea. Canadian Journal of Zoology 85, 458–471.

Fox-Dobbs, K., Dundas, R.G., Trayler, R.B., Holroyd, P.A., 2014. Paleoecological implications of new megafaunal ^{14}C ages from the McKittrick tar seeps, California. Journal of Vertebrate Paleontology 34, 220–223.

Fox-Dobbs, K., Stidham, T.A., Bowen, G.J., Emslie, S.D., Koch, P.L., 2006. Dietary controls on extinction versus survival among avian megafauna in the late Pleistocene. Geology 34, 685–688.

France, C., 2008. A Carbon And Nitrogen Isotopic Analysis Of Pleistocene Food Webs In North America: Implications For Paleoecology And Extinction.

Fuller, B.T., Fahrni, S.M., Harris, J.M., Farrell, A.B., Coltrain, J.B., Gerhart, L.M., Ward, J.K., Taylor, R., Southon, J.R., 2014. Ultrafiltration for asphalt removal from bone collagen for radiocarbon dating and isotopic analysis of Pleistocene fauna at the tar pits of Rancho La Brea, Los Angeles, California. Quaternary Geochronology 22, 85–98.

Fuller, B.T., Harris, J.M., Farrell, A.B., Takeuchi, G., Southon, J.R., 2015. Sample preparation for radiocarbon dating and isotopic analysis of bone from Rancho La Brea. La Brea and beyond: The paleontology of asphalt-preserved biotas, ed. JM Harris. Natural History Museum of Los Angeles County, Science Series 151–167.

Fuller, B.T., Southon, J.R., Fahrni, S.M., Farrell, A.B., Takeuchi, G.T., Nehlich, O., Guiry, E.J., Richards, M.P., Lindsey, E.L., Harris, J.M., 2020. Pleistocene paleoecology and feeding behavior of terrestrial vertebrates recorded in a pre-LGM asphaltic deposit at Rancho La Brea, California. Palaeogeography, Palaeoclimatology, Palaeoecology 537, 109383.

Guiry, E.J., Szpak, P., 2021. Improved quality control criteria for stable carbon and nitrogen isotope measurements of ancient bone collagen. Journal of Archaeological Science 132, 105416.

Guiry, E.J., Szpak, P., 2020. Quality control for modern bone collagen stable carbon and nitrogen isotope measurements. Methods in Ecology and Evolution 11, 1049–1060.

Hassan, A.A., Termine, J.D., Haynes, C.V., 1977. Mineralogical studies on bone apatite and their implications for radiocarbon dating. Radiocarbon 19, 364–374.

Koch, P., Behrensmeyer, A.K., Stott, A.W., Tuross, N., Evershed, R.P., Fogel, M.L., 1999. The effects of weathering on the stable isotope composition of bones. Ancient Biomolecules 3, 117–134.

Koch, P.L., Tuross, N., Fogel, M.L., 1997. The Effects Of Sample Treatment And Diagenesis On The Isotopic Integrity Of Carbonate In Biogenic Hydroxylapatite. Journal of Archaeological Science 24, 417–429.

Lebon, M., Reiche, I., Gallet, X., Bellot-Gurlet, L., Zazzo, A., 2016. Rapid quantification of bone collagen content by ATR-FTIR spectroscopy. Radiocarbon 58, 131–145.

Liden, K., Takahashi, C., Nelson, D.E., 1995. The effects of lipids in stable carbon isotope analysis and the effects of NaOH treatment on the composition of extracted bone collagen. Journal of archaeological science 22, 321–326.

Lindsey, E.L., Seymour, K.L., 2015. Tar Pits" of the western Neotropics: Paleoecology, taphonomy, and mammalian biogeography. Natural History Museum of Los Angeles County Science Series 42, 111–123.

McDonald, H.G., Harris, J.M., Lindsey, E.L., 2015. Introduction. La Brea and beyond: The paleontology of asphalt-preserved biotas, ed. JM Harris. Natural History Museum of Los Angeles County, Science Series 42, 1–4.

Merriam, J.C., 1911. The fauna of Rancho La Brea: Part 1: Occurrence. Memoirs of the University of California 1, 197–213.

Merriam, J.C., Stock, C., 1921. Occurrence of Pleistocene Vertebrates in an Asphalt Deposit Near McKittrick, California. Science 54, 566–567.

O'Keefe, F.R., Dunn, R.E., Weitzel, E.M., Waters, M.R., Martinez, L.N., Binder, W.J., Southon, J.R., Cohen, J.E., Meachen, J.A., DeSantis, L.R.G., Kirby, M.E., Ghezzo, E., Coltrain, J.B., Fuller, B.T., Farrell, A.B., Takeuchi, G.T., MacDonald, G., Davis, E.B., Emily L. Lindsey, 2023. Pre–Younger Dryas megafaunal extirpation at Rancho La Brea linked to fire-driven state shift. Science (New York, N.Y.) 381, eabo3594. https://doi.org/10.1126/science.abo3594

O'Keefe, F.R., Fet, E.V., Harris, J.M., 2009. Compilation, calibration, and synthesis of faunal and floral radiocarbon dates, Rancho La Brea, California.

Roche, D., Ségalen, L., Balan, E., Delattre, S., 2010. Preservation Assessment Of Miocene–Pliocene Tooth Enamel From Tugen Hills (Kenyan Rift Valley) Through FTIR, Chemical And Stable-Isotope Analyses. Journal of Archaeological Science 37, 1690–1699. https://doi.org/10.1016/j.jas.2010.01.029

Schultz, J.R., 1938. A late Quaternary mammal fauna from the tar seeps of McKittrick, California. Carnegie Instutution of Washington Publication 111–215.

Sponheimer, M., Lee-Thorp, J.A., 1999. Alteration of enamel carbonate environments during fossilization. Journal of Archaeological Science 26, 143–150.

Sternberg, C.H., 1932. The Pleistocene Fossil Bed at McKittrick, California, in: Hunting Dinosaurs in the Bad Lands of the Red Deer River, Alberta, Canada. NeWest Press, Edmonton, Alberta, Canada, pp. 214–221.

Stock, C., 1928. A peccary from the McKittrick Pleistocene, California.

Stock, C., Harris, J.M., 1992. Rancho La Brea: A record of Pleistocene life in California. Natural History Museum of Los Angeles.

Stoner, R.C., 1913. Recent observations on the mode of accumulation of the Pleistocene

bone deposits of Rancho La Brea. University of California Press.
Stuart, A.J., 1991. Mammalian extinctions in the late Pleistocene of northern Eurasia and North America. Biological Reviews 66, 453–562.
Szpak, P., 2011. Fish bone chemistry and ultrastructure: Implications for taphonomy and stable isotope analysis. Journal of Archaeological Science 38, 3358–3372.
Thompson, T., Gauthier, M., Islam, M., 2009. The application of a new method of Fourier Transform Infrared Spectroscopy to the analysis of burned bone. Journal of Archaeological Science 36, 910–914.
Thompson, T., Islam, M., Bonniere, M., 2013. A new statistical approach for determining the crystallinity of heat-altered bone mineral from FTIR spectra. Journal of Archaeological Science 40, 416–422.
Trayler, R.B., Landa, P.V., Kim, S.L., 2023. Evaluating the efficacy of collagen isolation using stable isotope analysis and infrared spectroscopy. Journal of Archaeological Science 151, 105727.
Trueman, C.N., Behrensmeyer, A.K., Tuross, N., Weiner, S., 2004. Mineralogical and compositional changes in bones exposed on soil surfaces in Amboseli National Park, Kenya: Diagenetic mechanisms and the role of sediment pore fluids. Journal of Archaeological Science 31, 721–739.
Van Klinken, G.J., 1999. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. Journal of Archaeological Science 26, 687–695.