Ecological Informatics 79 (2024) 102431

Contents lists available at ScienceDirect

Ecological Informatics

journal homepage: www.elsevier.com/locate/ecolinf

Development of a non-invasive method for species and sex identification of rare forest carnivores using footprint identification technology

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ARTICLE INFO

Keywords:
Footprint
Track
Fisher
Marten
Wildlife biometrics
Non-invasive

ABSTRACT

Many wildlife species have sex specific habitat requirements. Due to the unique requirements for birthing and raising offspring, female reproductive habitat is often a limiting factor for a population and has been identified as a priority for conservation. Therefore, the ability to detect where females persist on the landscape and identify these potential reproductive areas is essential in creating effective conservation strategies. Here we describe the development of a non-invasive method to identify species and sex based on track images collected at track plate stations using footprint identification technology (FIT). We developed this technique using data from the southern Sierra Nevada fisher (*Pekania pennanti*) population and a co-occurring species of conservation concern the Pacific marten (*Martes caurina*). We coupled track plate footprints with non-invasive genetic samples and camera trap images to create a reference dataset of known species for fisher and marten and known sex for fisher. We used FIT to geo-reference 167 marten and 367 fisher tracks (34 males, 27 females) using 7 landmark points and then extracted 124 morphometric variables (distances, angles, and areas) for use in identifying species and sex for fisher using linear discriminant analyses. Using a single variable, we found species classification accuracy >99% in distinguishing fisher from marten. For fisher sex identification our most parsimonious model consisting of only 2 variables achieved an accuracy of 94.0% for the training set and 89.4% for the test set. We also report a method to quantify classification uncertainty for each track. This method provides a rapid, cost effective, entirely non-invasive method to accurately identify sex that can easily be implemented in field studies.

1. Introduction

Wildlife conservation needs are often sex-specific. Species may exhibit sex-based differences not only in morphology but also across a range of ecological and behavioral characteristics such as habitat use (Gantchoff et al., 2019; Oliveira et al., 2018), dispersal and connectivity (Maiorano et al., 2017; Tucker et al., 2017), behavioral response to disturbance (Ditmer et al., 2015) or prey selection (Pande and Dahanukar, 2012; White et al., 2011). Careful consideration of sex-specific needs is critical to effective conservation and management as many species require specialized conditions for successful reproduction such as habitat requirements for nesting sites of raptors (Blakey et al., 2019; Crandall et al., 2015) or denning locations of mammals (Aronsson et al., 2023; Green et al., 2019). Understanding patterns of sexual segregation is especially important for threatened or declining species, as there may be sex-based differences in habitat loss or other risk factors (Gantchoff et al., 2019; Spencer et al., 2016).

Collecting data on rare species that occur over large areas can be logistically difficult and expensive, especially at a population scale. In such circumstances biologists often rely on non-invasive sampling techniques such as camera traps, acoustic recorders, non-invasive genetic sampling (scat, hair, urine, feathers) or track collection. While these methods provide a cost-effective option for species detection, identifying sex can be challenging. For example, determining sex using non-invasive genetic samples can be cost-prohibitive, require access to a laboratory for analysis, or be limited by DNA quantity and quality. Methods to identify individual or sex from camera trap images have been developed for some species with unique pelage patterns (Rowland

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https://doi.org/10.1016/j.ecoinf.2023.102431
Received 9 June 2023; Received in revised form 11 December 2023; Accepted 11 December 2023
Available online 22 December 2023

et al., 2020) or easily identifiable sexually dimorphic characteristics (Brommer et al., 2021) but methods for cryptic species without these distinguishing characteristics for camera trap photos are complicated, time consuming, or currently insufficient (Dorning and Harris, 2019; Tucker et al., 2020).

The endangered southern Sierra Nevada fisher (*Pekania pennanti*) population is one such rare species that occurs at low density across a large and rugged landscape with ~300 animals across a ~ 12,000 km^2 area (Spencer et al., 2011; USFWS, 2020). Conservation concerns have amplified in recent years as the region has experienced extensive ecological changes including multiple periods of severe drought (Asner et al., 2016) that facilitated a massive bark beetle epidemic and killed over 100 million trees (Goulden and Bales, 2019). The tree mortality epidemic was followed by a series of large-scale wildfires that burned >2400 km^2 in the southern Sierra Nevada during 2020–2021 (Meyer et al., 2022; Steel et al., 2022; Stephens et al., 2022). These ecological changes have resulted in dramatic reduction of fisher habitat with an estimated ~40% of denning and foraging habitat lost prior to the 2020–2021 megafires, with a further 16–18% reduction in remaining reproductive habitat from high severity fire from 2017 to 2021 (USDA Forest Service, 2023). This habitat loss, coupled with past research showing negative short-term effects of fire on fisher (Green et al., 2022), has resulted in an urgent need to understand how fisher may be using this post-mortality/post-fire landscape in order to identify and conserve what habitat remains.

Female fisher have restrictive habitat requirements due to habitat and prey needs for raising kits (Green et al., 2018, 2019; Spencer et al., 2015). Therefore, identifying and preserving female reproductive habitat has been identified as a priority for sustaining and increasing the population (Spencer et al., 2011, 2016; Thompson et al., 2020). However, identifying active denning areas for fisher is difficult usually requiring capture studies and the use of telemetry or GPS collar data which is invasive, time intensive, and expensive such that that den site data is limited and often restricted to a small geographic subset of the population. Because of these data limitations conservation efforts have increasingly relied on non-invasive survey methods, which can be deployed more broadly across the entire population, to detect females and model female reproductive habitat as a proxy for reproductive habitat (Thompson et al., 2021). While this proxy is imperfect as not all females reproduce and they may be detected outside of reproductive habitat, it has been identified as the best available option to identify important conservation areas population wide given the large spatial extent and rapidly changing environmental conditions.

Fisher co-occur with the morphologically similar marten (*Martes* sp.) throughout their range (Pauli et al., 2022), and more specifically with the Pacific marten (*Martes caurina*) in the Sierra Nevada mountains. The development of sooted track plates as a non-invasive survey method for mesocarnivores in California, USA in the early 1990s facilitated widespread surveys across the state and resulted in a wealth of track based detection data on these species distributions (Zielinski and Kucera, 1995, Zielinski et al., 2005). One of the initial challenges was the inability to reliably distinguish marten from fisher tracks which was addressed when Zielinski and Truex (1995) developed a method to accurately identify fisher and marten tracks collected at track plates by using hand collected measurements based on assigning a cartesian grid to each track. Slauson et al. (2008) further applied the methods of Zielinski and Truex (1995) to develop a discriminant model to distinguish sex in marten and fisher which correctly classified 98% of training and 95% of test data using a model consisting of three measurement variables including interdigital pad height and width and total track length. While both of these methods provide robust outcomes with high levels of classification success, they involve a detailed and time-consuming hand measurement process for tracks that has prohibited widespread use.

Footprint identification technology (FIT) is an emerging non-invasive tool in wildlife conservation that has been adapted for a wide variety of species such as white rhino, *Ceratotherium simum* (Alibhai et al., 2008; Jewell et al., 2020); giant panda, *Ailuropoda melanoleuca* (Li et al., 2018); Eurasian otter, *Lutra lutra*, and other otter species (Kistner et al., 2022); cheetah, *Acinonyx jubatus* (Jewell et al., 2016); mountain lion, *Puma concolor* (Alibhai et al., 2017); and Amur tiger, *Panthera tigris altaica* (Alibhai et al., 2023). FIT develops a classification algorithm based on a training dataset of footprints of known species, sex, or individuals. In previous applications, FIT training datasets have been based on collecting sequences of footprints (trails) in natural substrates from captive or free ranging animals of known identity to develop a training dataset (Alibhai et al., 2008). However, for some species, ground conditions make collecting high quality tracks in natural substrate difficult (habitat in forest littler or heavy vegetation) and/or lack accessible captive populations. Here we report the development of a novel non-invasive application of FIT for species and sex discrimination where footprint images and genetic samples are collected non-invasively from free-ranging animals at track plates. We present this non-invasive based footprint identification method as a viable alternative to collecting tracks in natural substrates and thereby potentially opening the opportunity to develop FIT in a wider array of wildlife species and habitats.

2. Materials and methods

2.1. Collecting and identifying reference tracks

The majority of fisher and marten tracks and genetic samples were collected in conjunction with the U.S. Forest Service Sierra Nevada Carnivore Monitoring Program (Tucker et al., 2014; Zielinski et al., 2013). This monitoring program samples ~12,240 km^2 and encompasses the extant fisher population in the southern Sierra Nevada. Sampling was conducted from 2002 to 2021 at over 200 survey units that each consisted of an array of multiple non-invasive detection stations covering ~0.8 km^2 (Fig. 1). Survey units were comprised of either 6 track plate stations (2002–2009) or 3 paired camera and track plated stations (2011–2019) with genetic samples collected via hair snares at both device types (Fig. 2). Details regarding study area and non-invasive sampling methods are available in Zielinski et al. (2013, 2017) and Tucker et al. (2014, 2017).

Hair samples from stations that detected fisher or marten via tracks were genetically analyzed for species, individual, and sex identification. Species identification was conducted using the 16srRNA mitochondrial DNA region (Happe et al., 2020). Fisher samples were genotyped using a minimum of 10-microsatellites and marten samples using a minimum of 9 loci (Dallas and Piertney, 1998; Davis and Strobeck, 1998; Duffy et al., 1998; Fleming et al., 1999; Jordan et al., 2007). Sex was identified using the y-linked marker DBY-3 (Hedmark et al., 2004). A y-locus control sample from known individuals of each species was used to distinguish failed PCR reactions from true negatives (females).

Because the monitoring program study design and sampling techniques were designed to primarily target the southern Sierra Nevada fisher population, the number of marten genetic samples obtained was far lower than that for fisher. To increase the number of marten tracks available for use in species discrimination we selected additional track sheets based on other factors that provided high confidence in species identification. First, we generated a species detection history at survey units based on the overall long-term monitoring dataset (2002−2021) and selected units that had only detected marten and never detected fisher across all sampling years. From that list of units, we then identified stations with co-located camera and track plates (~100 m apart) that both detected marten during the same survey period and had a verifiable marten photograph at the camera adjacent to the track plate.

To increase the number of fisher tracks, we also included a small number of individuals of known sex from three past fisher live-capture research projects that had archived track sheets (4 females and 4 males). These individuals came from 3 capture studies conducted by the U.S. Forest Service Pacific Southwest Research Station on the Sequoia

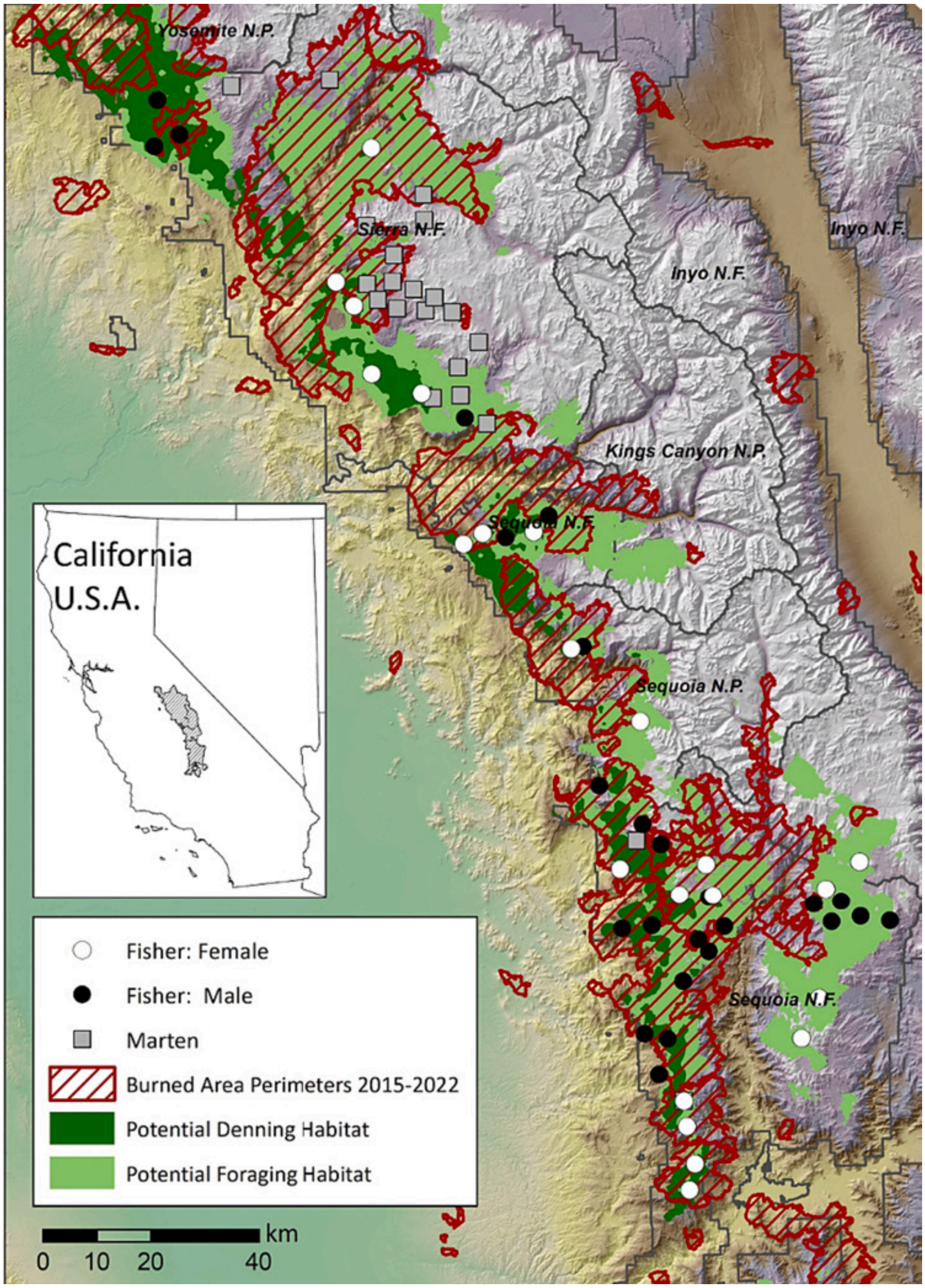

Fig. 1. Locations of fisher and marten tracks collected at track plate stations in the southern Sierra Nevada. Marten tracks are denoted with gray squares, and fisher tracks with circles (black = males, white = females).

National (1994–1996, Zielinski et al., 2004), the Six Rivers National Forest (1993–1997, Zielinski et al., 2004), and the Kings River Fisher Project on the Sierra National Forest (2018–2019, Green et al., 2018). During these capture studies an individual's sex was identified during immobilization and tracks collected during animal release via track plate enclosures attached to the live traps.

2.2. Footprint anatomy and geometric profiles

The FIT method is based on tracks from a specific foot (left/right and front/hind) and therefore requires reliable distinction of left from right and front from hind tracks. Determining left versus right is well established and relatively simple in fisher and marten due to the presence of a thumb pad on the inner side of the print and asymmetrical sizes and arrangement of the three palm (interdigital) pads. To determine front from rear we relied on the characteristics identified by Slauson et al., 2008 (e.g., wider front feet with larger interdigital pads and more rounded appearance to the interdigital pads on the hind feet) as well as collecting a reference set of front and hind feet from prints taken during a fisher live-capture and from a single captive fisher in local zoo collection. Initial trials to investigate the clarity of the prints on track sheets indicated that front foot impressions were more distinctive, detailed, and clearly outlined. This was likely due to a combination of what appears to be a slight rotation of the back foot and less fur on the front feet (Appendix A, Fig. A1). Front feet are more commonly recorded on track plates due to the way the animals move in the track plate box and so we proceeded with FIT development using tracks from front feet. We photographed each front footprint from directly above with a metric

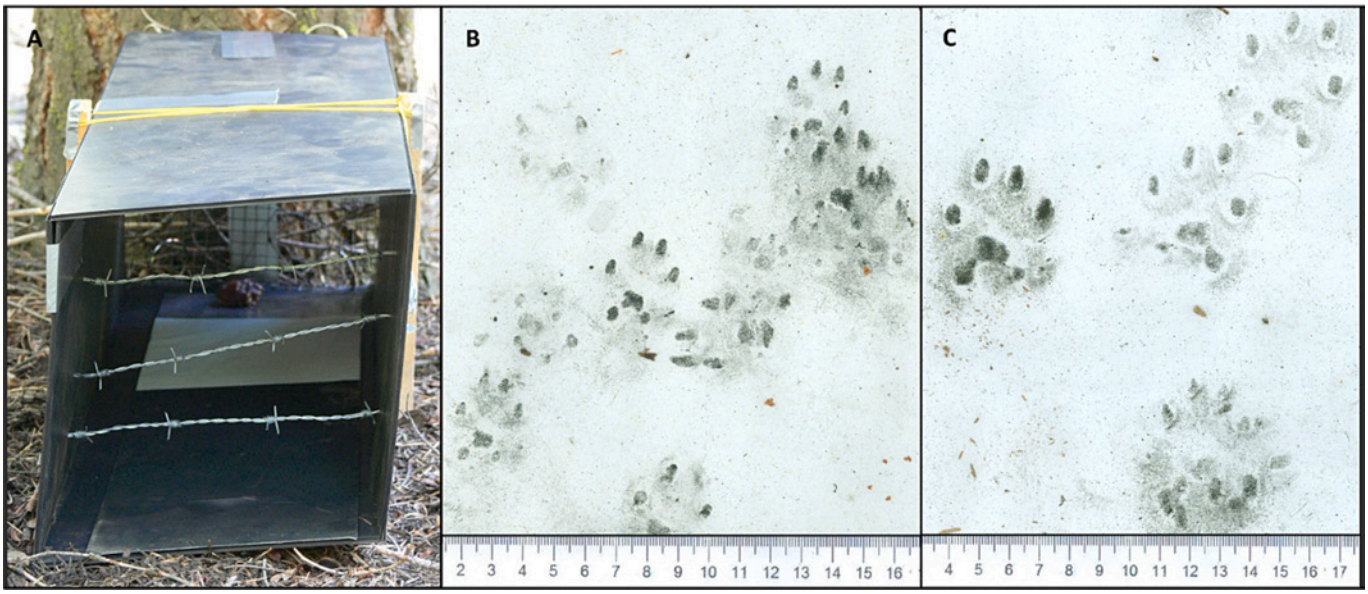

Fig. 2. A. Photo of a baited track plate station with a barbed wire hair snare installed in the front of the box. B-C: Examples of track plate detections of B = marten, and C = fisher. Rulers on the bottom of the example track plates are in cm.

size reference in the image according to the protocol described in Jewell et al. (2016), Alibhai et al. (2017) and Jewell et al. (2020).

Extraction of geometric profile and all statistical analyses detailed below were performed in JMP Statistical Software (SAS Institute Inc., Cary N.C.). Each digital footprint image was imported into a customized FIT add-in, resized, rotated for standardization, and scaled to a size reference in the imported image (Jewell et al., 2016). We then conducted the feature extraction process by manually placing seven landmark points to geo-reference each track. These landmark points were chosen because of their clarity and consistency across tracks. Points 1–4 were placed on the estimated centroid of the toe impressions and points 5–7 were placed on the centroids of the three consistently recorded interdigital pads. Based on these 7 landmark points, an additional 21 scripted derived points were added for a total of 28 points on

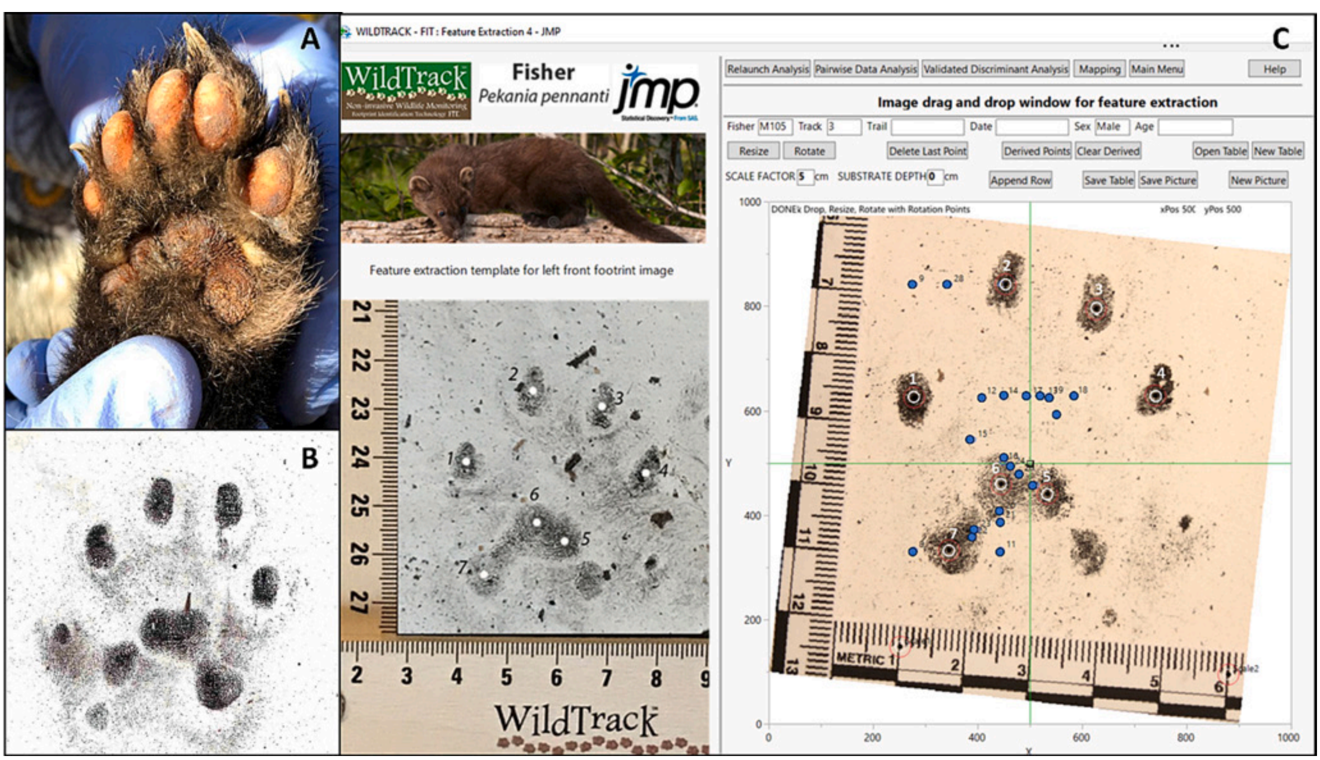

Fig. 3. A. Pads of a front right fisher foot, B. Right front fisher track obtained at a track plate, C. Footprint Identification Technology (FIT) user interface in JMP where an example left front footprint image with landmark points 1–7 indicated (left) that acts as a guide for implementing FIT (right) where each track is imported, resized, and rotated for standardization and a reference measurement scale is applied. Landmark points 1–7 are manually placed in the track pads by the user (white open circles) after which an additional 21 scripted derived points (blue circles) are automatically generated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

each footprint (Fig. 3). For each of the landmark and derived points we extracted a set of base X,Y coordinates and 124 calculated variables consisting of lengths, angles, and areas for use in species or sex discrimination. We arbitrarily chose the left front foot for the FIT model development. However, in order to increase the number of images for analyses, we also used right front images by flipping them on their vertical axis.

2.3. Screening predictor variables and bias

To identify variables with the greatest potential to discriminate between classes of objects (e.g., species or fisher sex) we used a predictor screening analysis that uses bootstrap forest partitioning to evaluate the contribution of predictors on the response object. For each response object we employed predictor screening using 1000 decision trees to evaluate all 124 potential FIT metrics. We then used the top predictor variables to evaluate the dataset for two potential sources of bias, 1) the use of mirrored right front tracks instead of only left front tracks (foot position bias) and 2) the potential for bias between observers when manually marking the landmark points in the FIT interface (observer bias) which is a somewhat subjective process. Foot position (left-front or right-front) of each track was recorded for each track during the feature extraction process. To evaluate observer bias we had 3 different observers repeat the feature extraction process for the same tracks from a subset of fisher individuals. We then conducted a multivariate ANOVA (MANOVA) of the 7 base landmark point coordinates, and five top predictor FIT variables against each factor (foot position or observer).

2.4. Identifying presence of multiple individuals on a single track plate

Because these footprints were not collected in a controlled field environment, there was potential that fisher of different sexes or different individuals of the same sex could visit the same track plate during a sampling period. However, only one of these individuals is likely to be detected genetically as the genotyping protocol is designed to detect a single individual per sample, and not every individual leaves a hair sample during a visit. If both sexes were present at that visit but unaccounted for genetically then attributing all tracks from that plate to a single individual/sex could introduce error into the fisher sex discrimination analysis.

To account for this potential source of error we conducted an analysis to identify and remove potential multi-individual track detections from the dataset. We based this analysis on past research on track collection from captive animals that demonstrated that variability across tracks for an individual was expected but was relatively small once an animal reaches maturity (Alibhai et al., 2023). Therefore, substantial variability within repeated tracks assigned to the same individual indicates the tracks are potentially from multiple individuals. During the initial screening of track measurements, we observed the top predictor variables for a few individuals had a bimodal distribution rather than a single continuous distribution as expected, indicating the possibility of multiple individuals (Fig. A2).

To systematically determine if a track plate collected tracks from more than one animal, we employed control charts which are a common industrial statistical tool used to determine if products are starting to vary outside of prescribed specifications. Applied to track measurements, control charts identify any individual whose measurements are outside of what would be considered routine variation within an individual's (variation in impression due to gait or measurement error). We created control charts of the standard deviation of the top five predictor variables for fisher sex with the control chart bounds defined using a sigma multiplier of 3 as defined in JMP 17 Quality and Process Methods (2022). Individuals whose track standard deviations fell above or below the control chart limits for the majority of variables (minimum of 3/5), indicative of the presence of >1 individual, were identified as outliers and removed from the dataset for subsequent analyses.

2.5. Discriminant analysis: species and sex identification

It can be difficult to distinguish marten and fisher tracks due to similarity in overall track characteristics, shape, and overlap in size between female fisher and male marten (Zielinski and Truex, 1995). Therefore, it was important to first develop a FIT method to clearly distinguish marten from fisher before conducting fisher sex identification. Furthermore, a FIT method for species identification would provide an additional tool to assist with species detection as both marten and fisher are species of conservation interest in the region. Due to an insufficient number of marten tracks of known sex in our dataset our sex identification analysis was limited to fisher. We used the same methodology to develop both fisher/marten species identification and fisher sex identification.

Our approach employed linear discriminant analysis, which identifies the linear combinations of variables that best distinguish categorical variables, to discriminate first species and then fisher sex. We conducted two analyses (1) species: marten/fisher and 2) fisher sex: male/female, using a hold-out validation approach by dividing the data into training, validation, and test sets with approximately 50% of the footprints in the training set and the remaining footprints roughly equally divided among the validation and test sets. The division was made randomly while attempting to maintain an equal ratio of species or sex (depending on the analysis) in each of the training/validation/test sets as in found in the overall dataset (Table 1).

We used a stepwise variable selection procedure with the *p*-value serving as the decisive factor (threshold of 0.05) to select the variables that had the best discriminating power based on their F-ratios. We used linear discriminant analysis to generate a discrimination line (2 variables) or hyperplane (>3 variables) depending on the number of variables in the resulting model to classify species or sex. To quantify uncertainty in classification, we then generated bands of uncertainty around this discrimination hyperplane. We assigned values that fall within these bands a probability value (p) indicating a level of certainty about the classification using a logistic regression model, as in Eq. (1)

$$p = \frac{1}{1 + e^{-\frac{1}{2}f(x)}} \quad (1)$$

where $f(x)$ represents the equation of the discrimination line. We assessed model performance using misclassification rates. To confirm our results, we also conducted a secondary analysis using principal components as the response variable. This alternate approached yielded comparable accuracy in fisher sex discrimination with a misclassification rate of 9.1% (Appendix B). However, in this paper we report the linear discriminant analysis using variables derived directly from landmark points due to its comparative simplicity in methodology and interpretation.

3. Results

We collected 367 fisher tracks from 61 genetically identified individuals of which 34 were males (177 tracks) and 27 were females (190 tracks), and 167 marten tracks from 61 different track plates spaced

Table 1
Sample sizes for training, test, and validation groups for discriminant analysis of species and sex.

	Species Discrimination		Fisher Sex Discrimination	
	Marten Tracks	Fisher Tracks	Individuals Total (Male/Female)	Tracks Total (Male/Female)
Training	82	184	29 (17/12)	169 (72/97)
Validation	43	92	19 (9/10)	99 (55/44)
Test	42	91	15 (7/8)	99 (50/49)
Total	**167**	**367**	**63 (33/30)**	**367 (177/190)**

across 27 different sample units. 41 marten tracks were genetically confirmed to species with the remainder classified based on location and associated trail camera station photographic evidence. The number of tracks suitable for FIT analysis per track plate varied from 1 to 8 useable tracks per track sheet for both fisher and marten. However, fisher females had a greater number of useable tracks per track plate than males (mean tracks/plate: female = 3.1, male = 2.5).

3.1. Predictor screening

We found similar variables to have the highest predictive power for both species and fisher sex discrimination. The 5 highest ranked predictor variables (V) for species discrimination were V15 (linear distance between landmark points 3 and 7) followed in order of importance by V16, V14, V13, and Area 1 (see Fig. 4 for visualization of these variables). These 5 variables accounting for 95.4% of the variation in species and V15 alone accounting for the majority of the variation (58.4%) (Appendix A, Fig. A3). The same 5 variables also ranked highest for fisher sex discrimination but combined only accounted for 50.5% of the variation between sexes, with variation spread out more diffusely across many variables. For fisher sex identification V16 (distance between landmark points 2 and 7) was the highest ranked predictor but only accounted for 16.8% of the variation.

3.2. Foot position and observer bias

To assess potential bias in foot position we analyzed 96 left and 69 right front tracks from marten and 200 left and 167 right front tracks from fisher. We did not find any statistically significant difference between measures of left and right front tracks difference in the top five predictive variables for either species (fisher $p = 0.85$, marten $p = 0.72$). Observer placement was a significant source of bias in our data set for producing the landmark coordinates ($p < 0.001$), but this was limited to points within the larger interdigital pads. However, we did not find significant bias in any of the top predictive variables (fisher $p = 0.44$, marten $p = 0.33$) (Appendix A, Fig. A4).

3.3. Identifying presence of multiple individuals on a single track plate

We identified 4 individuals with track measurements that had standard deviation above the control chart limit for at least 3 of the 5 top predictor variables for sex discrimination (Fig. 5: female F14, and males M17, M36, and M182; Appendix A Fig. A5). As this indicated the potential presence of a second individual at that track plate that was not genetically identifiable, we removed these 4 outliers from the dataset for subsequent analyses.

3.4. Discriminant analysis

3.4.1. Fisher and Marten species discrimination

We found the best model for species discrimination between fisher and marten consisted of 12 variables and had near perfect classification success, only misclassifying 1 out of 532 tracks as the wrong species. However, as this full species discrimination model was so complex, we used backward stepwise selection to try to identify an effective model with fewer variables. We found a univariable model consisting only of variable V16 had a species classification success of over 99% only misclassifying 4/532 tracks as the incorrect species. Based on this single variable a track measurement where V16 > 3.17 has a 90% probability of being a fisher. The discrimination line for the simple species discrimination model is given by the Eq. (2):

$$\text{Species Discrimination Model}: \ 28.853^{*}\text{V16} = 86.976 \tag{2}$$

Equation values >86.976 are classified as fisher and values less than this value are classified as marten. Statistical summaries of measurements for the most important variables for species identification from predictor screening and the final species discrimination model are detailed in Table 2.

3.4.2. Fisher sex discrimination

The best performing model for fisher sex discrimination consisted of 4 variables. However, similar to the best model for species discrimination, this model was also complex and difficult to interpret due to the inclusion of variables based on the derived points rather than the landmark points. Therefore, we decided to consider a second, more parsimonious model involving only the first two features selected in the stepwise procedure, V6 and V15 which are both linear measurements based solely on the landmark points (Fig. 4b). The discrimination line for the simple fisher sex discrimination model is given by the Eq. (3):

$$\text{Fisher Sex Discrimination Model}: 25.25^{*}\text{V15} + 27.09^{*}\text{V6} = 129.29 \tag{3}$$

Values above this line (>129.29) would classify the track as male and below the line as female. We found this simple sex discrimination model produced comparable classification accuracy to the more complex full model with some slight increase in misclassification for the training and validation sets (a difference of 1–2 individual tracks), but a modest improvement in the test set. (Table 3).

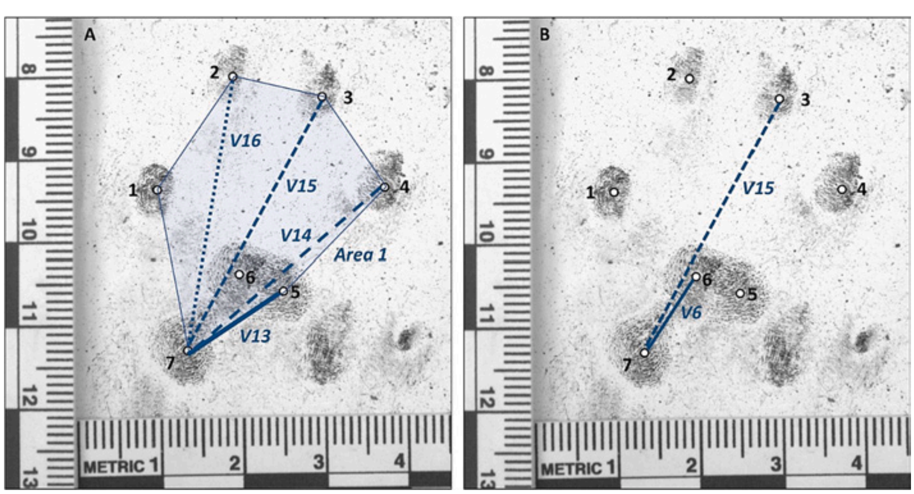

Fig. 4. Illustration of the FIT calculated variables important in species and fisher sex discrimination. These are 6 of the 124 calculated variables (lengths, angles, and areas) generated by FIT. White circles numbered 1 to 7 represent the landmark points placed in the approximate center of each track pad in the feature extraction process. Fig. A: 5 top predictor variables for both species and sex discrimination, Fig. B: Final 2 variables in the simple model for fisher sex discrimination.

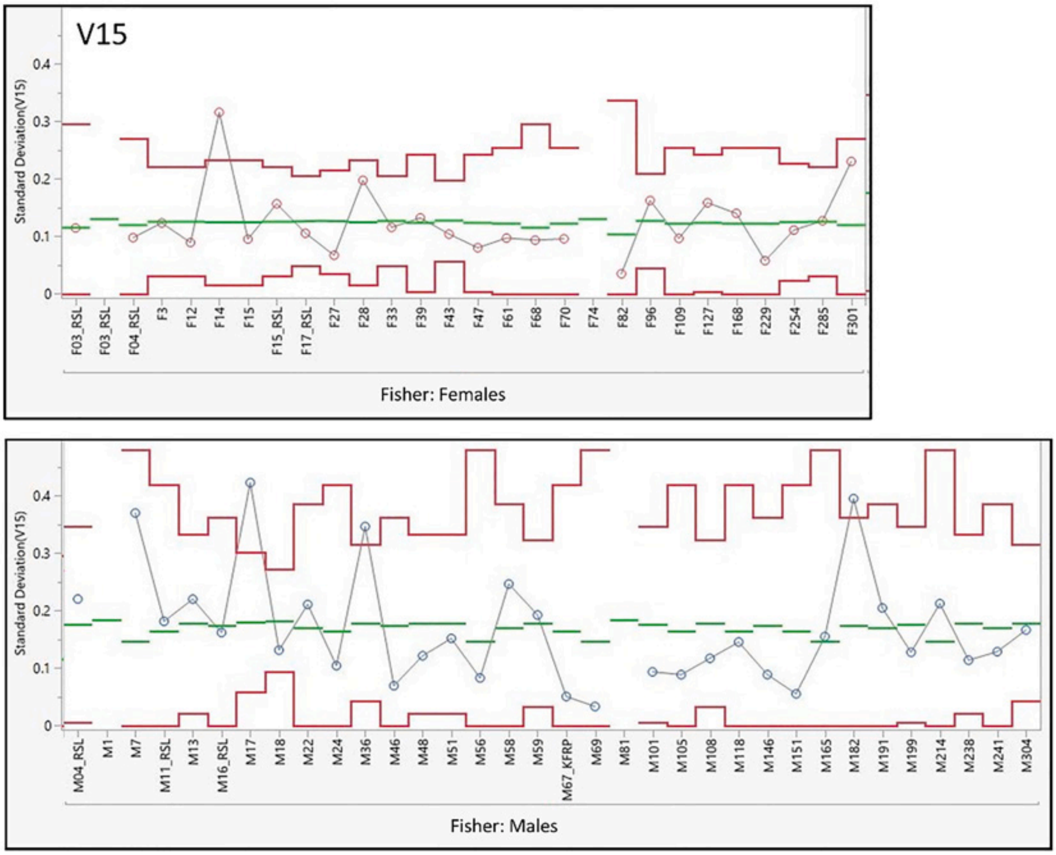

Fig. 5. Control charts for female (top) and male (bottom) fisher using the standard deviation of variable V15 (linear distance between landmark points 3 and 7, see Fig. 4). Fisher individual numbers are labeled on the x-axis. Green lines indicate the expected standard deviation for all tracks from an individual, and blue dots indicate the sample standard deviation for all tracks for each individual. Outliers are identified as individuals whose sample standard deviation values fall outside of the control chart limits (red lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Summary statistics (cm) for measurement variables found to be the most informative for species and fisher sex discrimination during predictor screening and in the final discrimination models based on 367 fisher tracks (177 male, 190 female) and 167 marten tracks. V15: linear distance between landmark points 3 and 7; V16: linear distance between landmark points 2 and 7; V6: linear distance between landmark points 6 and 7.

Variable	Species	Sex	Mean	Median	SD	Minimum	Maximum	Lower 95 CI	Upper 95 CI
V15	Marten	–	2.57	2.49	0.24	2.16	3.29	2.53	2.61
	Fisher	Female	3.57	3.57	0.17	3.13	4.07	3.55	3.59
	Fisher	Male	4.07	4.09	0.21	3.38	4.61	4.04	4.11
V16	Marten	–	2.44	2.37	0.24	1.97	3.13	2.41	2.48
	Fisher	Female	3.37	3.36	0.15	2.94	3.73	3.35	3.39
	Fisher	Male	3.81	3.83	0.20	3.13	4.34	3.78	3.84
V6	Marten	–	0.76	0.75	0.10	0.47	1.10	0.75	0.78
	Fisher	Female	1.10	1.10	0.09	0.87	1.48	1.09	1.12
	Fisher	Male	1.29	1.28	0.11	0.90	1.55	1.27	1.30

Table 3
Misclassification rates for linear discriminant analysis of fisher sex identification for both the full and simplified models.

Fisher Sex Discrimination		Full Model		Simple Model	
	N Tracks	N Misclassified	% Misclassified	N Misclassified	% Misclassified
Training	151	7	4.6	9	6.0
Validation	99	5	5.1	7	7.1
Test	85	11	12.9	9	10.6

The simple fisher sex identification model has a classification accuracy of 94.0% for the training data, and 89.4% for the test data. With this model, all of the inaccuracy in classification was in mis-classifying males as females (test set classification accuracy 75%). However, the majority of the misclassified male tracks (7 out of 9) were from a single individual (M108). Females were classified with >94% accuracy in the training data and 100% accuracy in the test data (Table 4).

Using Eq. (1) with the equation for the discriminant line this sex identification model (Eq. (3)) allows us to define bands of uncertainty based on selected probability values, as shown in Eq. (4)

Table 4
Results of predicted fisher sex identification from the simplified sex identification model compared to verified sex from genetic samples.

Actual Sex	Predicted Sex (%)					
	Training		Validation		Test	
	Female	Male	Female	Male	Female	Male
Female	0.94	0.06	0.96	0.05	1.00	0.00
Male	0.07	0.93	0.09	0.91	0.25	0.75

$$25.25^{*}V15 + 27.09^{*}V6 = 129.29 \pm lnln\left(\frac{p}{1-p}\right) \tag{4}$$

We report the resulting bands of uncertainty in Table 5. We found higher certainty in discrimination of female tracks compared to males, with 86% of female tracks classified as highly certain, compared to only 76% of male tracks (Fig. 6). Measurement summaries for the most informative fisher sex discrimination variables are detailed in Table 2.

4. Discussion

Our results show that footprint identification technology (FIT) is an effective tool for both species and sex discrimination from tracks collected at track plates with >99% accuracy in species identification, and > 90% accuracy in fisher sex identification. Our method builds upon prior track measurement-based methods for these species that successfully demonstrated track-based species and sex discrimination (Zielinski et al. 1995, Slauson et al., 2008) but provides a much more streamlined approach in comparison to past methods that required extensive hand based measurements from numerous track pads. In this FIT approach both species and sex identification only require a few simple measurements from at most 3 track pads providing a tool that can be implemented quickly and easily. Our use of centroid points in the track pads as the FIT landmark points (as opposed to marking the edges of pads, e.g., Alibhai et al., 2023) further simplifies the approach, increasing efficiency in the FIT process by requiring fewer landmark points, and also making it easier to employ on tracks of varying quality that may lack edge definition.

Our goals were to provide a new non-invasive tool to quickly, simply, and accurately distinguish between fisher and marten tracks and determine sex in fisher using tracks. We found species discrimination can be accomplished with over 99% accuracy with a single measurement. An accurate method for species detection is especially important in areas of fisher and marten sympatry as visual discrimination between the two species' tracks can be difficult due to similarities in track morphology. In areas with a high degree of sympatry we recommend measuring to confirm species prior to implementing the fisher sex identification technique. Mistakenly conducting sex identification measurement on a marten track would likely result in a mis-identifying that track as a female fisher.

This method can provide an alternative, cost effective method for reliable sex identification of fisher which, to date, has relied primarily on genetic analysis. Classification results can be obtained immediately thereby avoiding the time lag associated with genetic classification which can be a problem for time sensitive projects since female detections can be critical information for land management decisions and in the federal Endangered Species Act consultation process. Additionally, track plates are inexpensive, easy to deploy in the field, and the data management process is simple for a single track sheet compared to other non-invasive survey methods like camera traps which can generate tens of thousands of photos per camera and create a non-trivial data management workload.

This approach also provides a method to quantify uncertainty in sex determination to provide additional resolution to the data and prevent misclassification which could have significant conservation implications. The ability to classify certainty or uncertainty of detections is also important to prevent analytical bias that can be created by unaccounted for false positives in the data (McKibben et al., 2023). Furthermore, track based methods provide multiple options to verify detections. The FIT method assigns sex based on a single track so analyzing repeated tracks from the same track plate can provide a way to confirm species or sex if results are inconclusive or if further confirmation is desired. Additional confirmation could be obtained by applying the alternative track-based discriminant methods of Zielinski et al. (1995) or Slauson et al. (2008).

Non-invasive identification of females has become a recent focus for this population as an approximate surrogate for reproductive habitat due to difficulties in obtaining other types of data on den sites and reproductive habitat across the large spatial extent of the population. Given the aim is to identify and document the presence of female fisher then this method can be considered a conservative approach as most misclassifications were males misclassified as females. This is likely due to the presence of smaller juvenile males in the dataset as the misclassified males were detected in early summer when the young of the year are not yet fully grown. At this time of year juvenile males would still be associated with their mother's breeding territory, so misidentifying a juvenile male in this timeframe would still result in correctly identifying an area that supports reproduction. We caution that this association of juvenile males with females may not hold true at a different time of year after the juvenile males disperse from their natal range (fall/winter). However, as overall misclassification rates are very low, we do not feel that this is a major concern with the approach.

We assessed two potential sources of bias, foot position (left foot vs mirrored right foot) and observer. We found no bias in using mirrored right front instead of left front tracks which greatly increases the flexibility of this method in the diversity tracks it can successfully be used with. We did find some slight observer variation in the placement of the landmark points in the center of interdigital pads (palm pads) as the placement of these points is somewhat subjective (e.g., some observers placed these points slightly higher or lower than other observers). However, this observer variation did not create a significant bias in any of the measurement variables used in our discriminant models for either species or sex. Due to the potential for bias, we recommend caution in placing the landmark points if multiple observers will be used. Clear communication and consistent training across observers on where to place landmark points in the pad centroids could help minimize this potential source of bias.

It is important to note that the discriminant functions we describe may be locally specific to the southern Sierra Nevada fisher population which is smaller in body size than fisher in more northern populations (Lofroth et al., 2010). Further testing is needed to determine the accuracy of our FIT method in other fisher or marten populations. However, the FIT classification approach could be easily replicated to extend this method to other populations or species if there is a reference set of tracks

Table 5
Probability of certainty ranges for fisher sex identification from the linear discriminant analysis for Model 2.

Predicted Female					Predicted Male				
Highly Certain	Moderately Certain			Uncertain		Moderately Certain			Highly Certain
>90%	80–90%	70–80%	60–70%	50–60%	50–60%	60–70%	70–80%	80–90%	>90%
<124.9	124.9–126.5	126.5–127.6	127.6–128.5	128.5–129.3	129.3 –130.1	130.1–131.0	131.0–132.1	132.1–133.7	>133.7

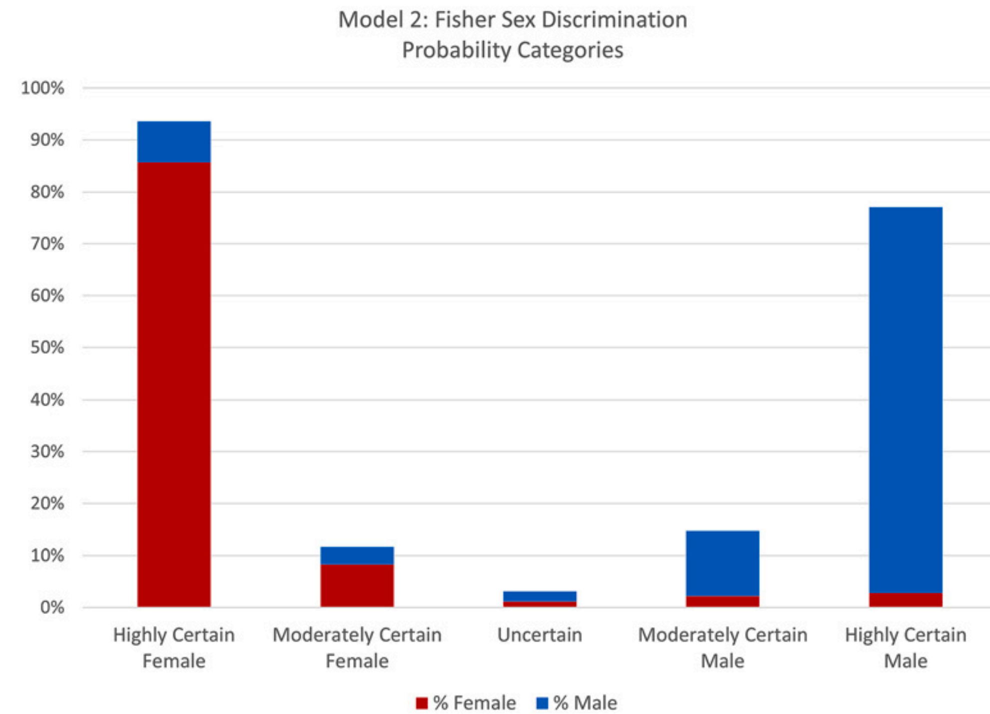

Fig. 6. Sex assignment results for all tracks from the FIT fisher sex identification model binned into categories reflecting certainty of sex assignment as compared to their genetic sex identification (colors, red = genetic female, blue = genetic male). High is defined by a track identification with >90% probability of certainty, moderate is defined as 60–90% certainty, and uncertain is assigned to any track with <60% certainty. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of known species or sex to train the model.

We developed a robust, cost effective, and streamlined method for distinguishing species and identifying sex using tracks with an FIT approach based on non-invasive survey methods. This work provides an example of how the FIT track identification method can be developed non-invasively without reliance on captive or captured animals or the ability to collect tracks from natural substrates. Our results advance previous track identification methods that had shown it was possible to determine sex and species but that were difficult to implement, by providing an alternative method that is simple and fast to execute. This evolution in methods strengthens support for the use of tracks to identify species and sex and builds confidence in the overall use of footprints for wildlife monitoring (Nichols et al., 2019). Effective wildlife conservation, especially for rare species in rapidly changing environments, requires up-to-date information on where and how these species are using the landscape. Development of non-invasive survey methods such as FIT, that can expand non-invasive survey data beyond just species presence/absence to sex identification, provide important new tools for the study of wildlife that can have immediate, on-the-ground conservation applications.

Funding

This work was supported by the USDA Forest Service, Pacific Southwest Region and Rocky Mountain Research Station.

Credit authorship contribution statement

Jody M. Tucker: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Caleb King:** Conceptualization, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ryan Lekivetz:** Conceptualization, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Remi Murdoch:** Data curation, Writing – review & editing, Methodology. **Zoe C. Jewell:** Conceptualization, Funding acquisition, Investigation, Methodology, Writing – review & editing. **Sky K. Alibhai:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank B. Smith, M. Bingaman, B. Murley, K. Gundermann, and R. Green for their work on track collection and assistance in the FIT process. We also would like to acknowledge the work of numerous field technicians from the Sierra Nevada Carnivore Monitoring Program for their hard work in deploying and collecting track plates used in this analysis. We thank the staff of the Charles Paddock Zoo and Tommy for their assistance in track collection and identification. We also thank JMP software (http://www.jmp.com) and the SAS Institute (USA) for the technical support provided by their software. Lastly, we thank the members of the WildTrack Specialist Group for their knowledge and support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoinf.2023.102431.

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