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Use of bio-loggers to characterize red fox behavior with implications for studies of magnetic alignment responses in free-roaming animals

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Abstract

Background: Spontaneous magnetic alignment (SMA), in which animals position their body axis in fixed alignments relative to magnetic field lines, has been shown in several classes of vertebrates and invertebrates. Although these responses appear to be widespread, the functional significance and sensory mechanism(s) underlying SMA remain unclear. An intriguing example comes from observations of wild red foxes (*Vulpes vulpes*) that show a ~fourfold increase in hunting success when predatory ‘mousing’ attacks are directed toward magnetic north-northeast. This form of SMA is proposed to receive input from a photoreceptor-based magnetoreception mechanism perceived as a ‘visual pattern’ and used as a targeting system to increase the accuracy of mousing attempts targeting hidden prey. However, similar to previous observational studies of magnetic orientation in vertebrates, direct evidence for the use of magnetic cues, and field-based experiments designed to characterize the biophysical mechanisms of SMA are lacking. Here, we develop a new approach for studies of SMA using triaxial accelerometer and magnetometer bio-loggers attached to semidomesticated red foxes.

Results: Accelerometer data were recorded from 415 ground-truth events of three behaviors exhibited by an adult red fox. A 5-nearest neighbor classifier was developed for behavioral analysis and performed with an accuracy of 95.7% across all three behaviors. To evaluate the generalizability of the classifier, data from a second fox were tested yielding an accuracy of 66.7%, suggesting the classifier can extract behaviors across multiple foxes. A similar classification approach was used to identify the fox’s magnetic alignment using two 8-way classifiers with differing underlying assumptions to distinguish magnetic headings in eight equally spaced 45° sectors. The magnetic heading classifiers performed with 90.0 and 74.2% accuracy, suggesting a realistic performance range for a classifier based on an independent set of training events equal in size to our sample.

Conclusions: We report the development of ‘magnetic ethograms’ in which the behavior and magnetic alignment of foxes can be accurately extracted from raw sensor data. These techniques provide the basis for future studies of SMA where direct observation is not necessary and may allow for more sophisticated experimental designs aimed to characterize the sensory mechanisms mediating SMA behavior.

Keywords: Accelerometer, Magnetometer, Magnetoreception, Spontaneous magnetic alignment, Light-dependent, Compass, Red fox, Radio-frequency, *Vulpes vulpes*

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Background

Recent advances in bio-logging technology and its increasing deployment in studies of animal behavior and physiology are providing new approaches to investigating both large-scale and fine-scale properties of spatial behavior in domestic and free-roaming animals [1–3]. Among the many sensor options, triaxial accelerometers can yield a wealth of valuable information about movements in three-dimensional space, but need sophisticated analysis techniques to be properly interpreted [4]. There is no consensus on the statistical techniques to use in extracting behavioral data from accelerometer signatures; the available methods have been shown to have varying degrees of success in identifying differences in behavior and posture across a range of diverse kinematic patterns [5, 6]. In addition to accelerometers, bio-logging devices equipped with triaxial magnetometers are now available, providing researchers with a continuous record of the alignment of the sensor with respect to magnetic north. Therefore, if the alignment of the sensor is in a known and fixed alignment with respect to the animal, the magnetic heading of the individual can be identified. However, transforming raw magnetometer data into reliable directional headings of free-roaming subjects poses several challenges [4, and see “Methods”]. Yet despite the technical obstacles, developing behavioral classifiers that can reliably identify behaviors of interest from accelerometer signatures time-synched with magnetometer data has the potential to provide investigators with a record of spatiotemporal behavior in animals inhabiting diverse environments where direct observation may not be possible, or in animals where direct observation may influence behavior (i.e., observer bias).

In particular, ‘magnetic bio-loggers’ used in studies of animal navigation and orientation could provide important new evidence for behavioral responses dependent on the geomagnetic field [7–14]. For example, several studies have reported evidence of spontaneous magnetic alignment (SMA) behavior across a range of vertebrates that show a strong tendency to align the anteroposterior axis bimodally along the north–south magnetic axis [for reviews see 15, 16, and recently 17]. Although SMA appears to be widespread, exhibited by a wide range of taxa including both vertebrate [15, 16, 17, 18] and invertebrate groups [19–22], the functional significance and biophysical mechanism mediating this behavior remains poorly understood. Possibilities of the functional significance underlying SMA include that the magnetic field could provide a stable reference frame for coordinating movement in open landscapes [23], or help to coordinate group responses in social animals [24]. Furthermore, magnetic alignment has been proposed to be involved in retinotopic matching in honeybees, helping to recognize

familiar environments [25], and may simplify encoding the spatial relationships between landmarks and/or help to place local maps of space into register [26]. Given the utility of magnetic cues underlying diverse spatial behaviors, it is clear that the magnetic field is not used only as source of ‘simple’ directional information, but rather may play a more general role in organizing and structuring spatial behavior and cognition. Therefore, SMA may reflect a basic form of spatial positioning to optimize the use of magnetic input for more complex spatial behaviors. However, the majority of vertebrate SMA studies have relied on field observations, which are difficult to conduct following double-blind protocols, are subject to observer bias, and are not well suited for experimental manipulations needed to confirm that alignment responses are directly mediated by magnetic cues. Furthermore, field studies have not implemented experimental designs aimed to characterize the sensory mechanisms underlying SMA that will be critical for helping to determine the functional significance of this widespread behavior.

An intriguing example providing the only clear evidence for a fitness advantage of SMA in mammals comes from predatory behavior in red foxes (*Vulpes vulpes*). Visual observations of ‘mousing’ red foxes, in which a fox is attracted to the sounds produced by small rodents and then performs an arching leap (‘mousing’) to land on the prey from above, showed a strong tendency for mousing attacks to be directed toward magnetic north-northeast [27]. The north-northeast alignment of mousing behavior is consistent with SMA responses exhibited by a variety of terrestrial vertebrates [16]. Interestingly, when a direct view of the prey was obstructed by high vegetation or snow cover, foxes more accurately aligned their attacks to the north-northeast and were approximately 3–4 times more successful at capturing prey than when aligned in other magnetic directions [27]. The authors proposed that the alignment behavior observed in foxes could be mediated by a light-dependent magnetoreception mechanism, similar to that used by migratory birds, newts, and insects [7, 14, 28–33] where specialized photopigments undergo a photo-induced chemical reaction that is sensitive to the alignment of the magnetic field. In animals where these photopigments are located in the retina, the magnetic field may be perceived as a three-dimensional pattern of light intensity or color superimposed on the animal’s visual surrounding and fixed in alignment with respect to magnetic north [26, 34]. Such a pattern could appear like a visual ‘after image’ that moves with the animal yet, remains fixed with respect to magnetic north [26]. Similar to the light-dependent magnetic compasses of migratory birds [35], newts [36], and sea turtles [37], that are sensitive to the inclination, not polarity, of the

magnetic field lines, the inclination of the pattern of 'visual' input generated by a light-dependent magnetic mechanism could be used to estimate distance. Specifically, in the northern hemisphere, the magnetic field lines re-enter the Earth at increasing angles with increasing latitude, and therefore, some components of the three-dimensional visual pattern would be superimposed on the substrate below the level of the retina [for additional details and proposed three-dimensional patterns see 26, 34, 38–40]. The inclination of the magnetic field would not vary substantially (e.g., $\sim 0.1^\circ$) over a fox's home range [41], and therefore, the fox could approach the prey along a fixed compass heading until some component of the visual pattern generated by the light-dependent magnetic mechanism was superimposed on the sound source generated by a prey hidden beneath the substrate (analogous to centering the 'cross-hairs' of a gun sight), enabling the fox to initiate mousing attacks from a fixed distance. Given the indirect ('ballistic') trajectory of mousing attacks, the accuracy of such attacks may be greater if they are initiated from a fixed distance and performed using a stereotyped set of mousing mechanics. Interestingly, the small clustering of successful mousing attacks directed toward magnetic south [27] suggests that the opposite end of the magnetic axis may also guide mousing behavior, and is consistent with an axially symmetrical pattern of magnetic input as proposed by [26, 34, 40]. Therefore, SMA in red foxes is thought to result from the fox aligning components of the visual pattern, providing a targeting system helping to estimate the distance of the unseen prey, increasing the likelihood of successful prey capture [27].

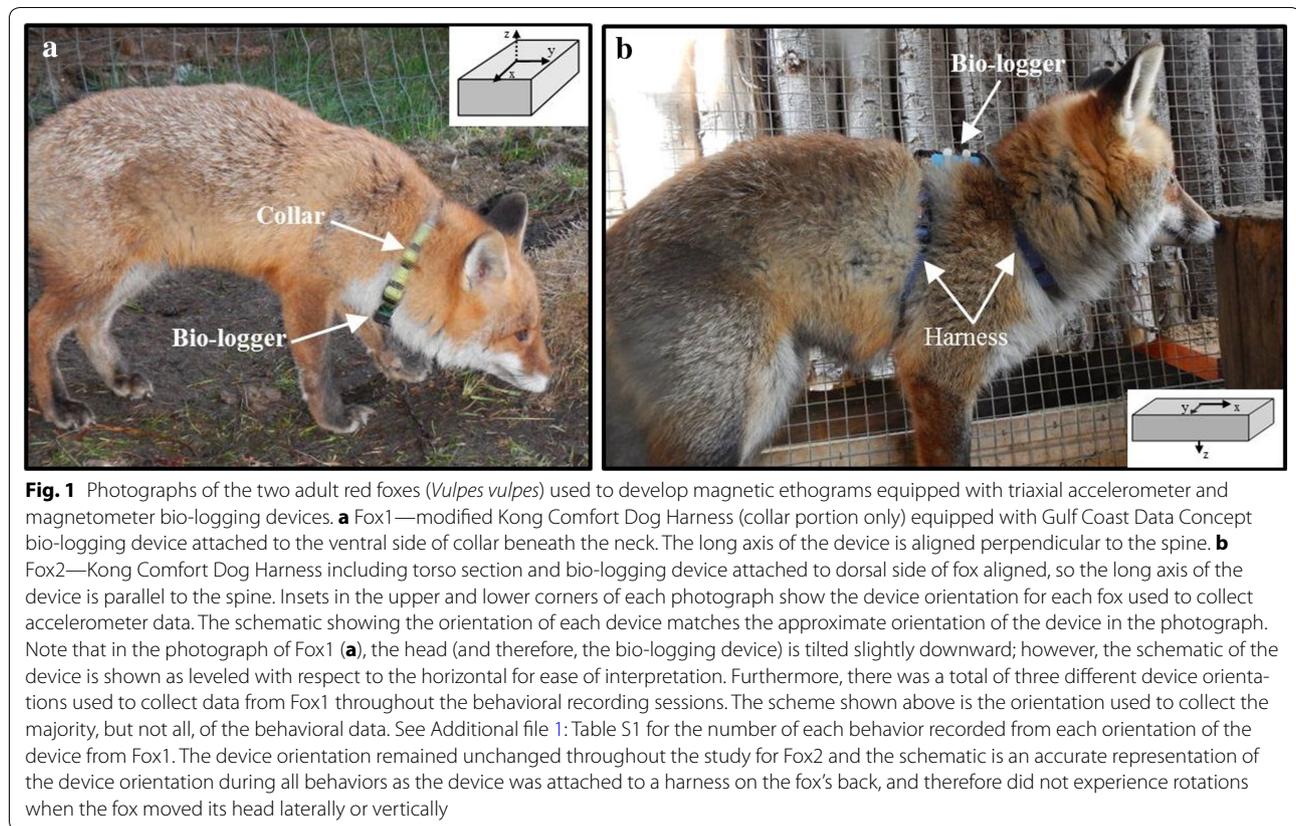
Here, we report the development of magnetic bio-logging techniques for future studies of SMA in free-roaming red foxes that will likely provide new approaches to studying other forms of magnetic behaviors across a range of terrestrial animals. Semi-tamed red foxes trained to exhibit 'mousing-like' leaps in outdoor enclosures were used to develop 'magnetic ethograms' to provide templates for data collection from free-roaming animals in the wild. Three predetermined behaviors performed in varying magnetic directions were videotaped to serve as ground-truth data. Raw accelerometer data were then used to establish classifiers 'trained' to identify these behaviors in unseen data sets recorded from the same individual and from a different individual exhibiting similar behavior. Using a similar machine learning algorithm approach, magnetic classifiers were developed using ground-truth video records and 'trained' to distinguish between eight magnetic directions corresponding to the cardinal (i.e., N, S, E, W) and anti-cardinal (i.e., NE, SE, SW, NW) magnetic axes. Taken together, the time-synchronized accelerometer and magnetometer data provide

magnetic directional headings for events identified by the behavioral classifier, demonstrating the potential for bio-logging applications in studies of SMA in free-roaming foxes that can be adapted for use in studies of magnetic responses in other mammals.

This work provides the basis for future SMA research in foxes and other free-living mammals equipped with miniaturized triaxial accelerometer and magnetometer devices and, more generally, makes it possible to improve the accuracy of directional observations for magnetic alignment studies of semidomesticated and wild animals and avoid potential biases inherent to observational studies. Such studies have been difficult to conduct following double-blind protocols, are not well suited for experimental manipulations, and have been criticized for failing to provide direct evidence for the involvement of magnetic cues underlying SMA responses. We discuss the potential use of magnetic bio-loggers in field-based studies of magnetic behavior in free-roaming animals and propose specific experiments to test for the involvement of light-dependent and magnetite-based magnetic mechanisms underlying magnetic targeting behavior of wild red foxes that would be possible using bio-logging technologies.

Methods

Two adult male red foxes (*Vulpes vulpes*) were used to develop magnetic ethograms, and all methods were approved by the Expert Commission of the Czech University of Life Sciences (SP506051228). Both foxes were rescued from the Bohemian Forest in the Czech Republic as pups and raised in captivity in separate cages. All data were collected within a 20-m-diameter circular outdoor enclosure located in Prášíly, Czech Republic (49.1033°N 13.3819° E), between February 15, 2014, and May 15, 2014. Both foxes were in good health verified by a veterinarian before the study and were fed commercial dog food supplemented with wild mice. An initial three-week period was required to acclimate both foxes to a modified adjustable domestic dog harness (Kong® Comfort Dog Harness). The subject used to collect the majority of behavioral and magnetic alignment data (Fox1) was fitted with a simplified version of the harness in which only the collar portion of the harness was used, securely fastened around the neck (Fig. 1a). The second fox (Fox2) wore the complete harness system in which the collar and torso portion of the harness was snugly secured around the fox's neck and torso (Fig. 1b). After the acclimation period, the collar was equipped with a bio-logging unit (Gulf Coast Data Concepts, Waveland, MS, USA <http://www.gcdadataconcepts.com/contact.html>, model: X8M-3 USB), designed to record simultaneous triaxial accelerometer and magnetometer data. On Fox1, the device was



secured with two plastic zip ties to the ventral side of the collar (Fig. 1a). The device was secured to the dorsal side of the harness on Fox2 (Fig. 1b). Two identical devices were used to collect behavioral and magnetometer data from each of the foxes and were switched every ~5 days to recharge the internal battery. The units recorded continuously until the battery was depleted or the device was removed from the animal. On average, two 30-min behavioral sessions were recorded each day taking place at various times between 7:00 and 19:00 local time.

Bio-logging Recording Specifications

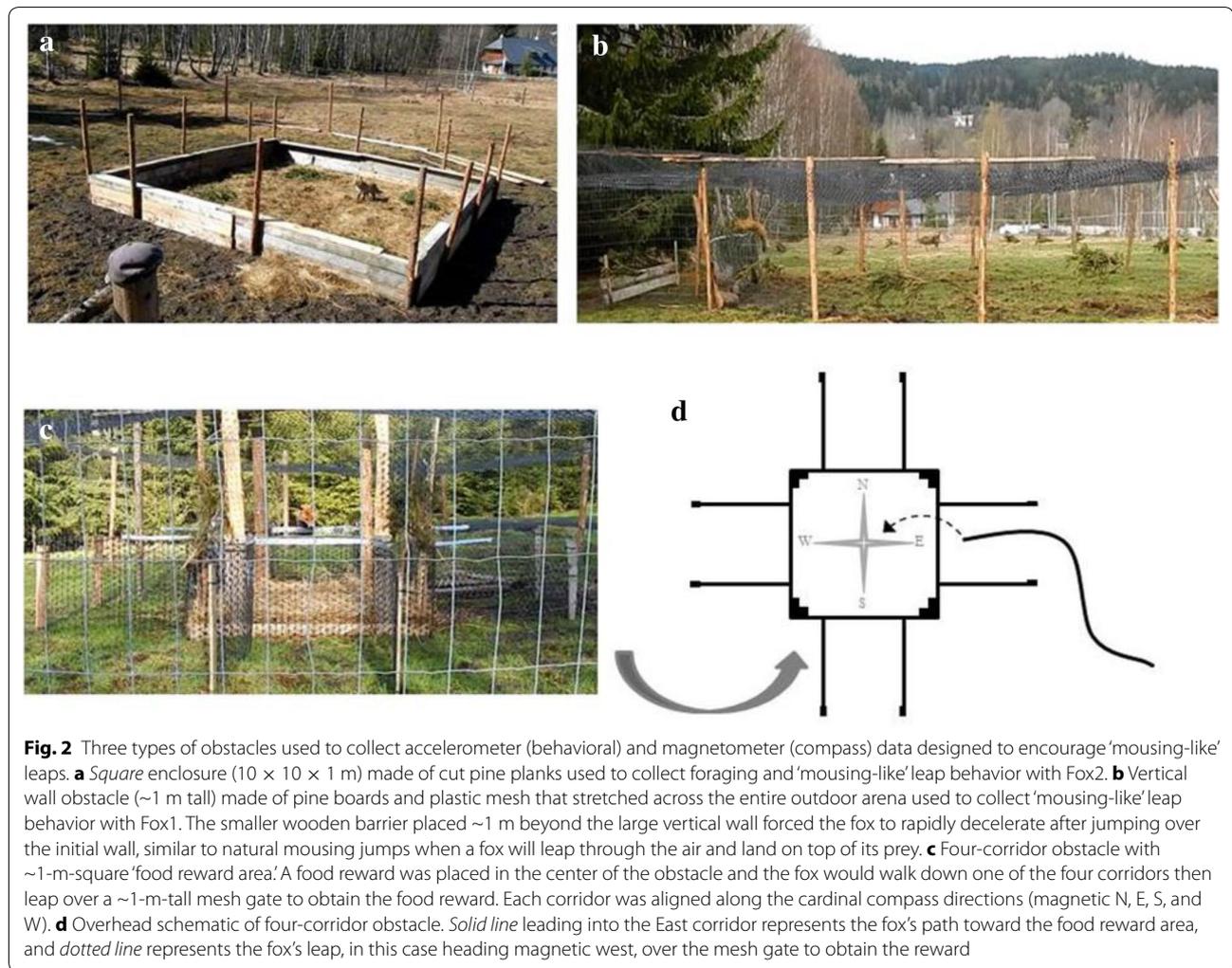
The X8M-3 USB accelerometer devices provided a continuous stream of time-stamped accelerometer and magnetometer data saved to a 2-GB internal flash memory drive. The devices included a ± 8 g accelerometer with a 14-bit analog-to-digital converter yielding 0.001 g measurement resolution on each axis. The magnetometer was programmed to operate over a ± 400 μ T range with measurements approximated to within ± 111 nT on the x - and y -axes and ± 125 nT on the z -axis. For all data collected, triaxial accelerometer data were recorded at a sampling rate of 6 Hz and the magnetometer data were recorded at 3 Hz. An internal lithium-ion polymer battery powered the device and when fully charged

would run continuously for ~130 h in these sample rate configurations.

Behavioral recording sessions

During the 30-min recording sessions, all behaviors were videotaped using a digital camera (Nikon Coolpix AW110) mounted on a tripod located approximately 1 m outside the observation arena. During each recording session, the fox was released from its home cage directly into a circular open field arena (~25 m diameter) where it was allowed to roam freely. At no point were the two foxes in the arena at the same time. The perimeter of the arena was secured using 1.5-m-tall aluminum mesh fencing, and the top of the arena was covered with plastic mesh to ensure the fox did not escape during trials. Additional structures were constructed inside the observational arena and used for behavioral and magnetic data collection (described immediately below).

Behavioral data were collected from Fox2 inside a 10 m \times 10 m square enclosure with walls 1-m-tall constructed of wooden boards located in the center of the observation arena (Fig. 2a and see Additional file 2: Video file S1, Additional file 3: Video file S2). The fox was placed inside the enclosure and allowed to forage for food pellets and live wood mice hidden under small brush piles



composed of straw and pine branches. After a foraging bout, typically less than 3 min, the fox would jump out of the square enclosure and return to the home cage. Unfortunately, Fox2 was stolen from its home cage shortly after beginning the behavioral recording sessions. Therefore, only a limited amount of behavioral data was obtained from this subject. As a result of the limited number of recording sessions and relatively primitive square enclosure used to collect behavioral data, the behaviors recorded from Fox2 were more variable and erratic relative to those of Fox1.

Most of the behavioral data were collected from Fox1. This fox was trained using two different obstacles built inside the observational arena, both designed to encourage the fox to perform 'mousing-like' leaps. One obstacle made of wooden boards and plastic netting stretched across the entire arena at a height of ~1 m. An observer inside the arena trained the fox to jump over the obstacle by tossing a food reward (one piece of dog food) on the

opposite side of the obstacle. In an attempt to make the jumps mimic natural mousing behavior, in which the fox performs a high vertical jump followed by a rapid deceleration as it lands on top of its prey, a second barrier was placed ~1 m beyond the first obstacle so that when the fox jumped over the first obstacle it had to stop immediately after landing to prevent a collision with the second wooden barrier (Fig. 2b and see Additional file 4: Video file S3).

A separate structure was introduced consisting of a 1-m central square area (the 'food reward area') enclosed with plastic fencing 1 m tall. Four corridors were attached to the four sides of the food reward area providing access to one wall of the enclosure from each of the four cardinal compass directions (i.e., north, south, east, west). Fox1 was trained to walk down one of the four corridors toward the food reward area and jump over the 1-m-tall fence barrier to receive a food reward (Fig. 2c, d and see Additional file 5: Video file S4, Additional file 6: Video

file S5). ‘Mousing-like’ leap data for Fox1 were collected using both of the obstacles described above. Magnetometer data were analyzed from Fox1 only using the arena with the attached corridors aligned along the cardinal compass directions to obtain more precise ground-truth magnetic data from time points when Fox1 was in cardinal (i.e., 0°, 90°, 180°, 270°) and anti-cardinal (i.e., 45°, 135°, 225°, 315°) magnetic alignments.

When the bio-logging unit was removed every ~5 days, the accelerometer and magnetometer data were downloaded. Each of the 30-min video recording sessions was partitioned into separate data files. The video was then replayed and manually analyzed for three discrete behaviors: trotting, foraging, and ‘mousing-like’ leaps over the barriers described above. These behaviors were chosen a priori with no knowledge about the acceleration signatures underlying each behavior. Mousing, trotting, and foraging behaviors are performed with distinct patterns of movement and have been observed in wild foxes (pers. observ.) and therefore were used to develop a behavioral classifier that would satisfy the focus of the current study (i.e., mousing behavior) and identify other functionally relevant behaviors for future bio-logging studies in red foxes. Trotting was defined as moving through the arena at a pace where two of the four paws were not in contact with the ground, but without the rapid horizontal accelerations and high rate of speed associated with sprinting behaviors (see Additional file 7: Video file S6) [6]. Foraging was defined as the fox slowly moving through the arena (slower pace than a walk) with its head and snout toward the ground searching for food using olfactory and visual cues (see Additional file 8: Video file S7). A ‘mousing-like’ leap was defined for Fox1 as jumping over the vertical barrier in either of the obstacles described above (Additional file 4: Video file S3, Additional file 5: Video file S4), or in the case of Fox2, out of the sides of the square enclosure (see Additional file 2: Video file S1, Additional file 3: Video file S2). Based on preliminary visual observations, a typical jump lasted ~2 s (~0.5 Hz) and the predominant oscillations in trotting and foraging behaviors did not exceed 3 Hz. Therefore, in order to fulfill the Nyquist sampling criterion which requires the sampling rate to be greater than twice the highest frequency of the signal components used to characterize the events of interest, the accelerometer sampling rate was programmed to record at 6 Hz after low-pass filtering to minimize aliasing [42].

Statistical analysis

Accelerometer data analysis

All accelerometer and magnetometer data were analyzed in MATLAB (MathWorks, Natick, MA). Cross-validation was used to estimate how the classifier would perform on

new, unseen accelerometer data. Prior to analysis, individual behaviors (i.e., ‘mousing-like’ leap, foraging, and trotting) were identified using raw video records. For each behavioral event, 15-sample segments, corresponding to 2.5 s of data given the accelerometer’s sampling rate of 6 Hz, were extracted and used for analysis. The 2.5-s window duration was chosen under the hypothesis that it would be long enough to characterize and distinguish, leaping, foraging, and trotting activity while short enough to minimize errors due to overlapping events and to maintain the practicability of real-time processing. In order to determine whether the preselected behaviors could be distinguished based on the acceleration signatures, a 5-nearest neighbor classifier was used, in which unknown (i.e., ‘test’) behaviors are classified by a ‘majority vote’ of their 5 nearest known (training) neighbors in a feature space. Expanded details of the behavioral classification method are provided in “*Accelerometer Feature Extraction*” and “*Behavioral Classification*” sections.

Accelerometer feature extraction

Each 15-sample data segment was zero-meaned prior to extracting the following four time-domain features: (1) the magnitude of the largest z-axis peak; (2) the time delay between the two largest z-axis peaks; (3) the energy in the output of a matched filter run on the z-axis; and (4) the energy in the output of a 2-Hz high-pass filter, averaged over the *x*- and *y*-axes. The matched filter used for feature 3 was a simple four-sample rectangular pulse with height 1 g. For feature 4, the data on both the *x*- and *y*-axes were first normalized to have unit energy; a third-order Butterworth high-pass filter [43] with a -3 dB point of 2 Hz, chosen for its maximally flat pass-band response, was then applied to each axis. Features 1 and 2 were designed to be selective for leaping events, which were expected to have large-magnitude vertical acceleration components; feature 3 was designed to be selective for foraging events, which were expected to have a relatively stable vertical axis acceleration profile; and feature 4 was designed to be selective for relatively high-frequency rhythmic trotting activity. The features were deliberately chosen to have some robustness to differences in accelerometer orientation, which can arise due to both movement and differences in mounting of the triaxial accelerometer, requiring only that one axis was known to be at least nominally aligned (at either 0° or 180°) with respect to vertical.

Behavioral classification

The four features described above were used as inputs to a 5-nearest neighbor classifier with the L1 norm as the distance metric (i.e., the distance between any two observations was the sum of the magnitudes of the differences

between corresponding feature values). K -nearest neighbor (k -NN) is a standard nonparametric technique for performing supervised learning tasks such as classification. It can be viewed as an approximation to the optimal but practically unrealizable Bayes classifier, which assigns an observation to the most likely of a predetermined set of classes given its feature measurements. In k -NN, the probability that an unknown observation belongs to a particular class is estimated by the fraction of its k closest training set neighbors belonging to that class. The predicted class label is then the class with the highest probability. Tenfold cross-validation was used to estimate test set error. That is, the entire data set was partitioned into ten randomly chosen disjoint subsets of approximately equal size, nine of which were used for training the classifier and the other for testing on each of ten iterations. Each subset served as the testing data exactly once. The overall estimate of test set error was obtained as an average of the test set errors achieved on each of the ten iterations.

Magnetometer data analysis

We defined the animal's magnetic heading (hereafter referred to as heading) as the angle between the posterior to anterior alignment of the spine, assumed to be rigid, and the direction of the Earth's magnetic field when both of these lines are projected onto the plane perpendicular to the gravity vector. A number of uncertainties associated with the free-roaming animal paradigm make it challenging to infer the animal's heading based upon triaxial magnetometer and accelerometer data. For example, due to the animal's normal movements, all of the following alignments are known only imprecisely: (1) alignment of the sensor relative to the fox's collar; (2) alignment of the collar relative to the fox's neck; and (3) alignment of the fox's neck relative to the rest of its spine. Additionally, it is reasonable to assume that a fox may slightly alter its heading on the timescale of a single magnetometer sample (0.33 s), leading to the potential measurement of sensor transient responses that add further noise to the data. Traditional tilt-compensation algorithms [e.g., 44] that attempt to use triaxial accelerometer data to sense device orientation and apply the appropriate trigonometric corrections to heading estimates are not reliable under these conditions because they assume an unchanging reference mounting orientation; in other words, they assume that the mapping (rotation matrix) between the coordinate frame of the sensor and the coordinate frame of the fox's body remains constant.

To deal with the uncertainties outlined above, we framed the heading estimation problem as a supervised learning task, and in particular, as a classification task. While in principle we could have treated this as a

regression, our video-labeling method of ground-truthing had limited resolution, and therefore, we used an 8-way classification task with the four cardinal and four anti-cardinal directions comprising the complete set of eight possible headings. This amounts to binning continuous headings using eight 45° sectors. Specifically, we sought to estimate the fox's heading with this degree of resolution given the acceleration and magnetic field strength measurements along the three orthogonal (x , y , z) axes of the sensor. Inputs to the classifier were thus the six-element vectors formed by concatenating the three accelerometer and three magnetometer measurements. We hypothesized that the optimal boundaries between each of the classes in this six-dimensional input space would be highly nonlinear due to the aforementioned sources of noise and therefore used random forest classification [45], which is capable of learning nonlinear boundaries while simultaneously mitigating overfitting.

Random forests offer improved predictive accuracy over single decision tree classifiers by smoothing predictions over an ensemble of related trees. Detailed reviews of tree-based classification, and random forests in particular, can be found in [46]. In brief, decision tree classifiers seek to optimally partition input space into a series of non-overlapping hyper-rectangles using labeled training data. Once the partitioning is learned, new observations are classified according to the region (hyper-rectangle) of input space into which they fall. Specifically, they are assigned the class label most frequently occurring among the training data points falling into the same region.

Because exhaustively searching over all possible unique partitionings of input space is generally computationally intractable, it is standard to use a greedy, top-down approach known as recursive binary splitting. At the start of the procedure (i.e., at the top (root) node of the tree), each training observation belongs to the same region. On each successive iteration, the algorithm chooses the single input feature and cut point (feature value), across all current regions, that leads to the greatest reduction in some objective function. It then forms two new regions by dividing the selected region in accordance with the chosen feature–cut point combination and continues the splitting procedure until a stopping criterion is met. In this work, we use the Gini index [47] in region m , G_m :

$$G_m = \sum_{k=1}^K \hat{p}_{mk}(1 - \hat{p}_{mk})$$

as the basis for the objective function, where K , the number of classes (headings), is 8 in our case, and \hat{p}_{mk} is an estimate of the probability that an observation in the m th hyper-rectangular region belongs to the k th class. The

latter is computed as the fraction of training observations within the m th region having the class label k . G_m can be interpreted as an estimate of what the misclassification rate for observations falling into region m would be if observations were randomly classified according to the distribution of classes in that region. It measures the class impurity of region m , being smallest (zero) when all observations in the region fall into a single class and largest when the distribution of observations across classes is constant.

The objective at each stage of the binary recursive splitting procedure is to find the region and split that leads to the smallest weighted sum of Gini indices across regions (where the weights are equal to the number of observations in each region). We stop the binary recursive splitting procedure when no split can be found that decreases the objective. Since this procedure is prone to growing deep trees that overfit the training data and therefore have poor generalization performance, it is typically augmented using some type of statistical method to reduce the variance of the learned model.

Random forests accomplish this variance reduction by learning separate trees on B (here $B = 1000$) bootstrapped data sets [48] of size equal to that of the original training data, formed by randomly sampling with replacement from the original training data. Furthermore, when considering each split for particular tree, only a random subset of j input features (here $j = 2$) are considered as potential candidates for the split. This has the effect of producing B trees that are less correlated than those that would be learned if the full set of input features were considered on each iteration, and hence a final classifier with lower variance and better generalization performance. Specifically, the final random forest classifier, C_{rf} , assigns its class labels according to:

$$C_{rf}(\vec{x}) = \text{mode}\{C_b(\vec{x})\}_{b=1}^B$$

where \vec{x} is the input vector and the right side of the equation denotes a plurality vote among the class predictions made for \vec{x} by each of the B single-tree classifiers learned on a separate bootstrap sample.

Magnetometer classifier training

To form a ground-truth data set for training the random forest classifier, a total of 381 samples recorded during 66 distinct heading events were assigned one of eight magnetic heading labels (N, NE, E, SE, S, SW, W, NW) by consensus between two human reviewers. Assignments were made based on reviewing video recordings of Fox1 behaving in the vicinity of the four-arm maze (Fig. 2c) aligned along the cardinal compass axes. Therefore, ground-truth heading classifications in the cardinal and anti-cardinal directions could be identified by

using the maze as a reference. Distinct heading events were identified as time-separated occasions on which the fox appeared to maintain a consistent heading for at least 0.5 s. The duration of the consistent-heading period determined how many samples were collected for each heading event.

Results

Behavioral classification

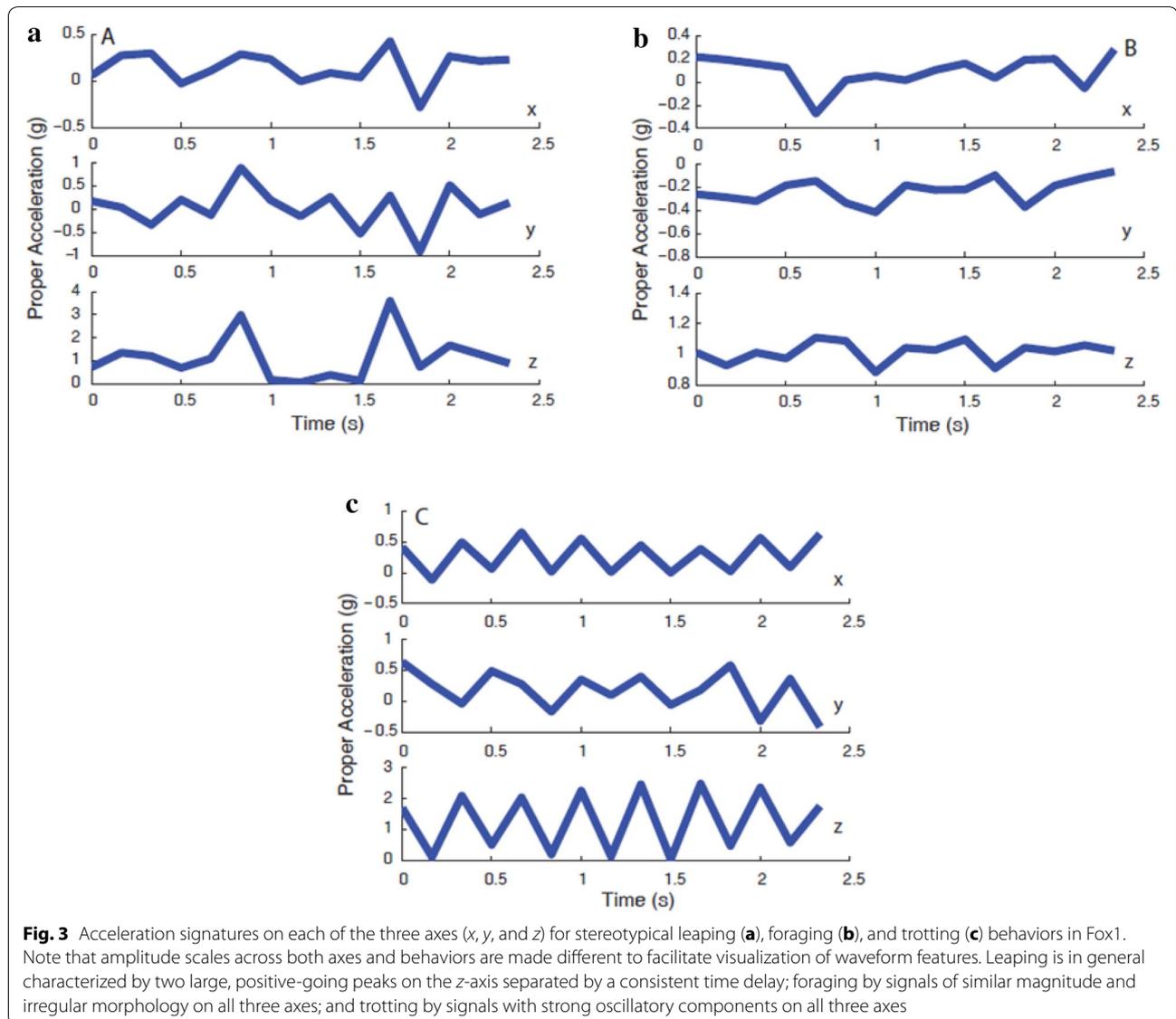
A total of 415 events from Fox1 trained to the two obstacles were manually identified using video records and input into the classifier. Of the 415 events, 265 (64%) were ‘mousing-like’ leaps, 100 (24%) were foraging, and 50 (12%) were trotting. Overall classification accuracy was 95.7% (chance performance with a null model of equiprobable classes is 33.3%; chance performance with a null model obtained by assigning each observation to the most frequent class is 63.9%; Table 1). The error rates for each class, leaping, foraging, and trotting, respectively, were 0.015, 0.00, and 0.28. Unsurprisingly, given its relative infrequency, trotting behavior was the most difficult to classify, with 11/50 trotting events misclassified as leaping and 3/50 as foraging (Table 1 and see “Discussion”). Accelerometer data showed stereotypical acceleration signatures recorded on each axis of the three behaviors used in the classifier (Fig. 3). Figure 4 shows the data plotted in the three-dimensional space given by the first three principal components projections after z scoring (i.e., subtracting the mean and dividing by the standard deviation of each feature), as well as in the two-dimensional spaces defined by all possible pairs of the first three principal components. Z scoring and PCA were employed only to facilitate viewing the four-dimensional feature data in three dimensions and were not used as processing steps prior to classification.

Table 1 Confusion matrix showing tenfold cross-validation performance of the 5-nearest neighbor classification algorithm run on Fox1

Accuracy (%)	95.7	True class			Precision (%)
		Leaping	Foraging	Trotting	
Cohen’s kappa:	0.92				
<i>Predicted class</i>	Leaping	261	0	11	96.0
	Foraging	0	100	3	97.1
	Trotting	4	0	36	90.0
	Recall (%)	98.5	100.0	72.0	

The results displayed in this matrix can be viewed as estimates of how well the classifier, trained on data from Fox1, would perform on new, unseen data from this same fox

Italicized values indicate cases where ground-truth behaviors (i.e. ‘true class’) match classifier prediction (i.e. ‘predicted class’)



Generalizability of the classifier to new foxes

To the extent that foxes perform mousing-like leaps, foraging, and trotting in a stereotyped way, one would expect our classifier to accurately distinguish these behaviors in new, free-living foxes. A small amount of data (33 events) from Fox2 were recorded prior to its removal from the study and used to test the classifier's ability to generalize across foxes not used to train the classifier. It should be noted that this fox had much less experience performing the behaviors of interest and was trained using a different type of obstacle compared to Fox1 (see "Methods"; "Behavioral recording sessions" and Additional file 2: Video file S1, Additional file 3: Video file S2, Additional file 4: Video file S3, Additional file 5: Video file S4, Additional file 6: Video file S5), and therefore, its 'mousing-like' leaps differed from Fox1 used to collect the majority of

behavioral data. However, even with the high degree of behavioral variability between Fox1 and Fox2, the accuracy of identifying the three types of behavior was 66.7% (Table 2). The error rates for mousing-like leaps, foraging, and trotting, respectively, were 0.3, 0.286, and 0.625. This accuracy is significantly better than chance ($\alpha = 0.05$) using a null model that assigns each observation to the most frequently occurring class in this fox's data set (binomial test, $p = 0.027$). We also note again that the sensor mounting scheme was different for Fox1 and Fox2. While the features we used were approximately invariant with respect to the 90° rotational difference, we did not have a way of quantifying other potential differences in accelerometer orientation between the two device mountings. Any such differences would most certainly negatively impact classification performance in Fox2, though

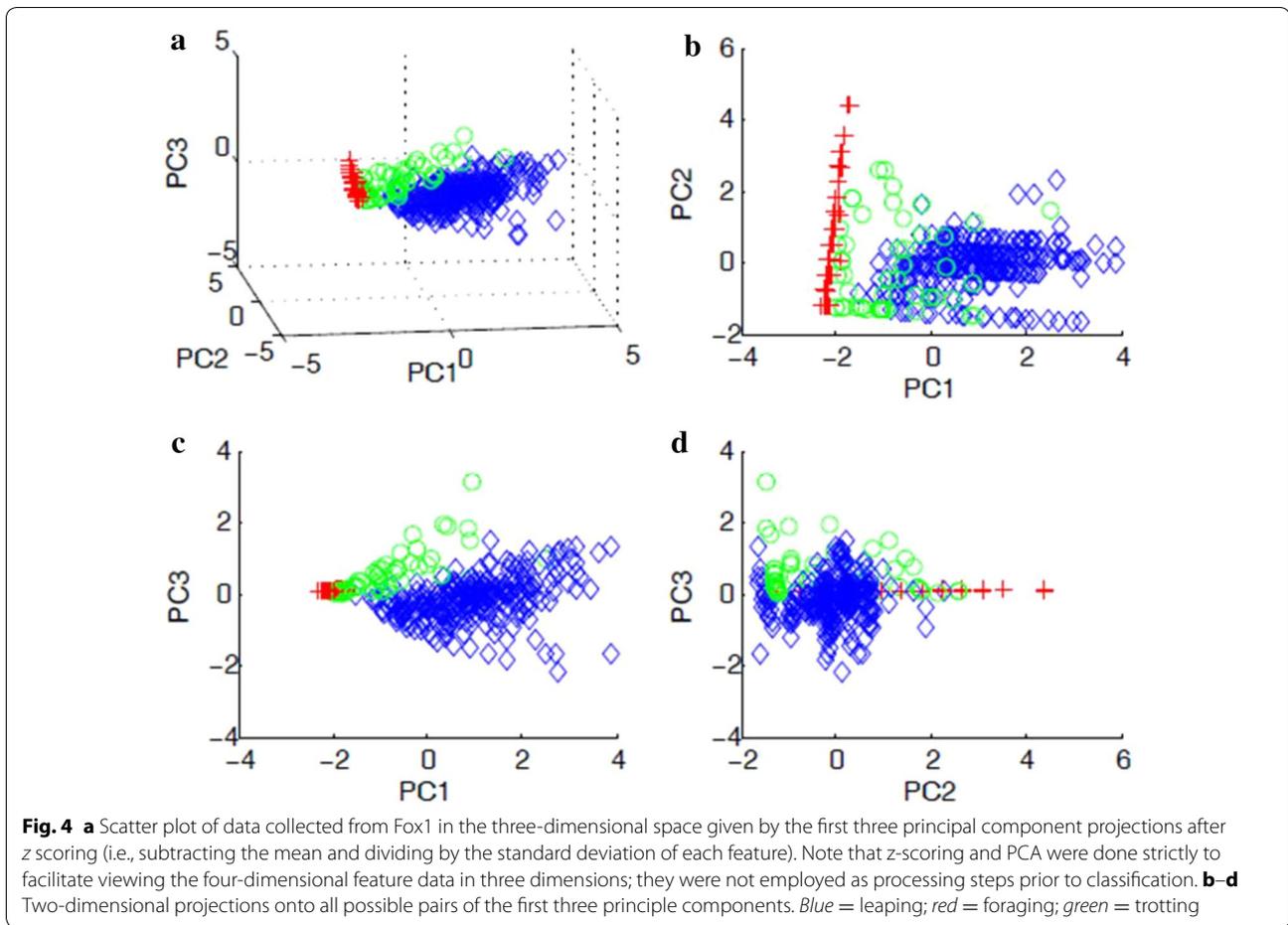


Table 2 Confusion matrix showing performance of the 5-nearest neighbor classification algorithm trained on data from Fox1 and tested on data from Fox2

Accuracy (%):	66.7	True class			Precision (%)	
		Leaping	Foraging	Trotting		
Cohen's kappa:	0.50					
	Predicted class	Leaping	7	0	0	100.0
		Foraging	3	5	6	35.7
		Trotting	0	2	10	83.3
	Recall (%)	70.0	71.4	62.5		

The results displayed in this matrix can be viewed as estimates of how well the classifier, trained on Fox1, would perform on new, unseen data from a different fox

Italicized values indicate cases where ground-truth behaviors (i.e. 'true class') match classifier prediction (i.e. 'predicted class')

the severity of the impact is difficult to estimate without a quantitative understanding of other orientation differences. No changes were made to the data processing chain to customize the classifier for Fox2 before testing; Fox2 was simply tested using a classifier trained exclusively on

data from Fox1. Results were particularly encouraging in light of the different mounting schemes and will likely improve if mounting can be standardized in future work. See "Discussion" for other factors that could contribute to decreased classifier performance across individuals and possible solutions to mitigate these sources of error for more accurate and reliable behavioral data in future studies involving wild foxes.

Magnetometer data
Classification results

As previously mentioned, the duration of the consistent-heading period determined how many samples were collected for each heading event. The number of samples per event ranged from 2 to 29 with a mean of 5.77 ± 5.16 (mean \pm 1 SD). Table 3 shows a detailed breakdown of events and samples for each of the eight heading directions.

We performed two separate classification analyses. The first implicitly assumed that all samples collected from a given heading were independent draws from the same unknown class-conditional probability density

Table 3 Summary of the data set used to train the random forest classifier

	N	NE	E	SE	S	SW	W	NW
Num. events	8	12	9	7	11	4	7	8
Samples/event (mean \pm 1 s.d.)	3.50 \pm 1.60	8.17 \pm 6.46	4.20 \pm 1.56	3.10 \pm 0.378	5.90 \pm 2.91	8.50 \pm 10.4	4.10 \pm 2.12	8.40 \pm 8.60
Total num. samples	28	98	38	22	65	34	29	67

An event corresponds to a time interval during which the fox was judged by human reviewers to be maintaining a consistent heading. Samples are six-element vectors of concatenated *x*, *y*, *z* accelerometer and magnetometer measurements, collected with a 3-Hz sampling rate during each event

function. Each sample was therefore treated as an individual observation (i.e., object for which a heading estimate is desired), giving a total number of observations of 381. Using tenfold cross-validation to estimate the generalization performance of the random forest classifier, the accuracy achieved was 90.0%. Table 4 lists the complete confusion matrix for the first classifier, which shows performance on a per-heading basis. A Rayleigh test was used to test for non-random clustering of magnetic headings, as predicted by the classifier, relative to ground-truth classifications determined by a human observer [49]. Therefore, data were pooled with respect to ground-truth predictions in each of the eight magnetic directions and plotted as the degree of error for each sample, regardless of the 'true' or absolute bearing for a given sample (Fig. 5). All circular statistics were performed using Oriana 4.0, Kovach Computing Services, and for each analysis, the data were treated as eight equal width groups, each width equal to 45°. Treating each sample as an independent point from the overall data set resulted in the following: sample size, $n = 381$, mean vector of the distribution, $\mu = 359^\circ$, r value = 0.95, where r represents the mean vector length of a circle with radius = 1, and $p < 1 \times 10^{-12}$ (Fig. 5a).

The second analysis replaced the assumption that all samples from a given heading were class-conditionally independent with the less-stringent assumption that only events within a given heading represented independent draws. This accounts for potential time correlations

among the samples within an event that might optimistically bias the measure of generalization performance. Individual observations in this second analysis were therefore heading events rather than samples and were computed by averaging the samples collected during each event. Collapsing the data set in this way yielded a training set with 66 observations, one for each of the heading events identified by the human reviewers. The tenfold cross-validated accuracy for the random forest classifier learned in this setting was 74.2%, and the complete confusion matrix is given in Table 5. Testing for non-random clustering of classifier predictions relative to ground-truth classifications when each event was treated as an independent point from the overall data set resulted in the following: sample size, $n = 66$, mean vector of the distribution, $\mu = 2^\circ$, r value = 0.87, where r represents the mean vector length of a circle with radius = 1, and $p < 1 \times 10^{-12}$ (Fig. 5b).

Discussion

The use of bio-logging technologies to help characterize spatial behavior and the underlying sensory mechanisms mediating magnetic alignment is a promising technique for future studies of free-roaming animals. In particular, the development of the behavioral ethogram coupled with time-synched recordings of magnetic headings provides a powerful tool to collect unbiased and noninvasive behavioral and magnetic data from wild foxes in the absence of direct observation. These techniques can also

Table 4 Confusion matrix for the random forest classifier treating each sample as an observation

Accuracy (%): 90.0		True class							
		N	NE	E	SE	S	SW	W	NW
Predicted class	N	20	0	0	0	0	0	0	2
	NE	2	97	1	2	0	0	0	0
	E	0	1	30	4	1	0	0	0
	SE	0	0	4	15	3	0	0	0
	S	0	0	2	1	58	3	0	0
	SW	0	0	0	0	2	30	1	0
	W	0	0	0	0	0	1	28	0
	NW	6	0	1	0	1	0	0	65

Italicized values indicate cases where ground-truth headings (i.e. 'true class') match classifier prediction (i.e. 'predicted class')

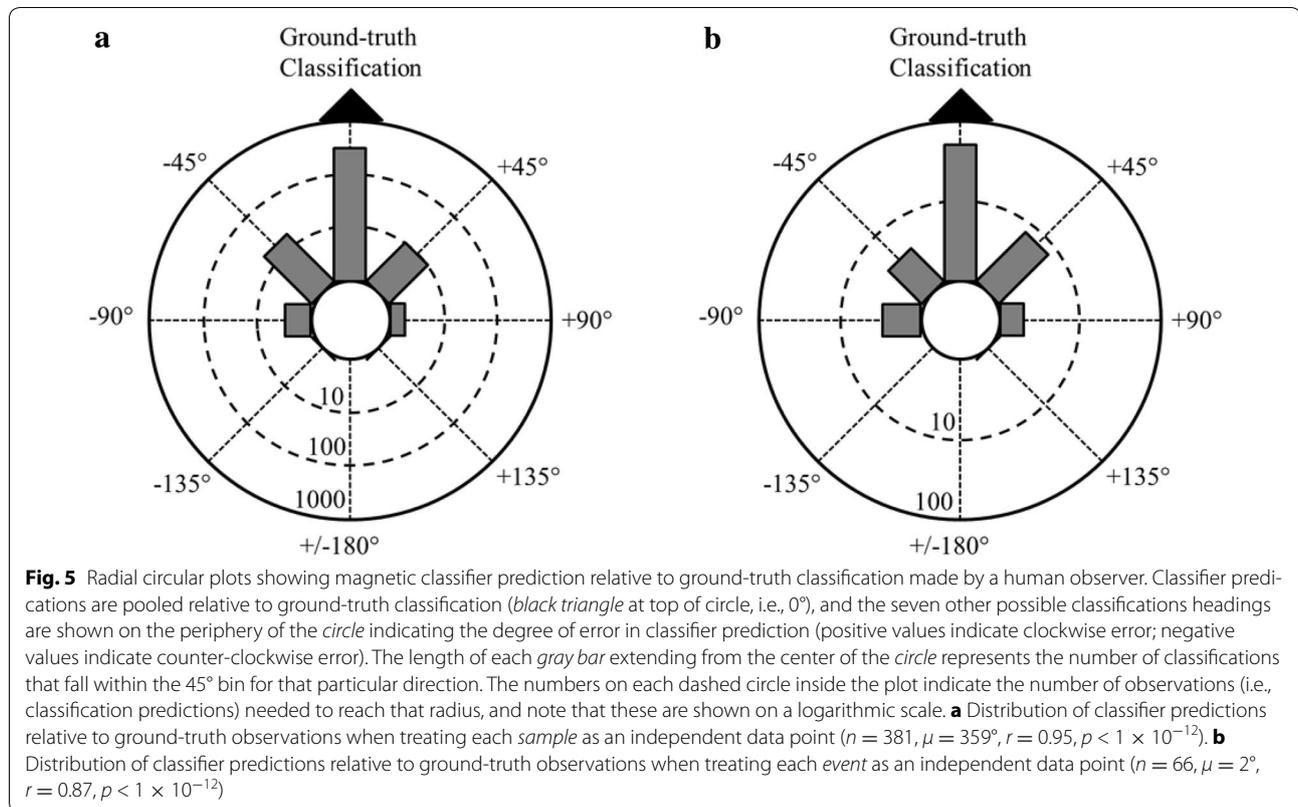


Table 5 Confusion matrix for the random forest classifier treating each event as an observation

Accuracy (%): 74.2		True class							
		N	NE	E	SE	S	SW	W	NW
Predicted class	N	7	0	0	0	0	1	0	2
	NE	0	9	1	1	0	0	0	1
	E	0	2	8	0	1	0	0	0
	SE	0	0	0	5	1	0	0	0
	S	0	0	0	1	9	1	0	0
	SW	0	0	0	0	0	0	0	0
	W	0	0	0	0	0	1	6	0
	NW	1	1	0	0	0	1	1	5

Italicized values indicate cases where ground-truth headings (i.e. ‘true class’) match classifier prediction (i.e. ‘predicted class’)

be adapted for deployment in magnetic studies of a variety of terrestrial vertebrates, an exciting potential given the growing evidence for SMA responses across diverse taxa [15–17, 24]. Although similar devices (i.e., accelerometer and magnetometer bio-loggers) have been used to characterize spatial behavior in free-roaming animals, extracting magnetic compass headings from animals moving in three-dimensional space is much more challenging and is rarely reported in behavioral studies. The first attempt to use magnetic sensors to record directional behavior in free-living animals monitored

changes in magnetic measurements over time using a fluid-filled ship’s compass equipped with Hall sensors to determine activity patterns in sea turtles [50]. A more recent study of flight paths in Andean condors equipped with bio-loggers was able to identify when birds were circulating within thermals using the sine wave signatures of compass measurement readouts, indicating the bird was rotating through 360° in the horizontal plane [51]. However, magnetic compass headings were not reported and only circular vs straight flight path trajectory could be derived from the information provided. Magnetic

heading data were reported in studies of pinnipeds that deployed triaxial sensors to measure changes in magnetic field intensity helping to reconstruct dive paths [52, 53]. However, the accuracy of the heading estimates was not reported or verified by ground-truth data, and it is unclear whether the devices were calibrated prior to deployment, as the magnetic output of each axis varies with device orientation and could lead to large errors when calculating magnetic headings [4].

Using a k-NN technique for behavioral classification, we have shown that three distinct behaviors can be identified with a 95.7% rate of accuracy when tested against unseen data from the same individual (Fox1) that was not used to train the classifier. As given in Table 1, trotting behavior was by far the most difficult to classify. However, the trots misclassified as leaping represented a false positive rate for leaping of only 4.0%. The rates of misclassification of the three behaviors in the current study are sensitive to relative sample sizes of each behavior and may not be representative of the relative occurrence of behaviors in wild foxes. Therefore, in future studies of free-living foxes, the percentages of misclassifications will be subject to the relative occurrence of each behavior. The low percentage of misclassifications of leaping events may be overly optimistic, and the algorithm's assignment of trotting events might be problematic depending upon the cost a particular researcher assigns to a missed trot in other types of studies. However, the precision for declaring a trot (i.e., the likelihood that a trot, as identified by the classifier, was indeed a trot) was 90.0%, and the recall for trotting (i.e., the percentage of total trots identified by the classifier) was 72%, much better than chance performance. Increasing the sample size for trots used to train the classifier would likely increase trotting precision and recall. In addition, implementing automated filters within the feature extraction process (e.g., *z*-axis max acceleration or peak-to-peak time interval thresholds) could be used to limit the influence of outlying training observations and further 'tune' the classifier for any behavior of interest, e.g., 'mousing'. Additional features that capture pre-mousing behavior (e.g., stalking and slow approach toward prey often accompanied by a brief pause prior to the mousing jump) could help to distinguish between mousing jumps and other non-predatory movements that may resemble mousing behavior. However, the foxes used in the current study did not display this type of pre-mousing behavior, and therefore, we were not able to incorporate such techniques into the algorithms.

Importantly, in the context of this study, the classifier was successful at identifying behaviors from data collected from a separate fox (Fox2) not used to train the classifier with an accuracy of 66.7%. These results are encouraging given the small sample size, the difference

in device mounting scheme on Fox2 (see "Methods"), and much higher behavioral variability relative to Fox1. This variability was due to differences in the design of the enclosure used to elicit mousing-like behavior, resulting in observable differences in jump approach, jump trajectory, and landing mechanics (see Additional file 2: Video file S1, Additional file 3: Video file S2, Additional file 4: Video file S3, Additional file 5: Video file S4, Additional file 6: Video file S5). However, even with the differences in sample size, device mounting orientations, and the behavior of Fox2, there were no false positives in identification by the classifier of 'mousing-like' leaps (Table 2), i.e., all mousing-like leaps identified in Fox2 were indeed mousing-like leaps.

The observed performance decline for Fox2 reflects not only differences in training and natural behavior between the experimental foxes but also potentially differences in device orientation, as mentioned above, as well as other sensor-related factors, such as temporal drift and calibration parameter differences between devices. All of these sources of error can be mitigated to some degree in future work by protocol standardization (e.g., of behavioral training and device mounting) and enlargement of the sample size of foxes and sensors used to build the training set. But classifier generalization performance will ultimately be governed by how representative any training data are of fox behavior in the wild. Since any wild fox experiment will necessarily involve first capturing subjects in order to instrument them, one possibility would be to use a protocol involving a brief classifier training period during which captive foxes are video-monitored under freely behaving conditions, perhaps in an enclosure similar to the one used in this study. Though it is unlikely that each fox would exhibit the full set of desired behaviors in this captive setting, data recorded from these sessions could be used to impart some degree of individualized tuning to the classifiers used for each fox, which would in turn result in improved performance for wild fox classifiers. Furthermore, this approach would help to confirm the generalizability of the current classifier by comparing ground-truth data from the semidomesticated foxes and wild foxes.

More generally, given the accuracy of the classifier within an individual and its above chance performance on a separate individual not used to train the classifier that exhibited a different set of kinematics, our methods show considerable promise for identifying leaping behaviors in wild foxes. To validate that accelerometer signatures recorded from mousing-like leaps are similar to those of functional mousing behavior in wild foxes, and to confirm the generalizability of the classifier across multiple individuals, ground-truth data from free-roaming foxes equipped with bio-logging devices will be critical

for future studies of SMA in red foxes. Moreover, the development of more sophisticated classifiers and using an expanded feature set could allow even greater precision of behavioral identification and provide opportunities to identify more complex behaviors, e.g., predatory from non-predatory leaps, and could be augmented with additional sensors such as jaw accelerometers or onboard video recording devices to identify behavioral outcomes, e.g., prey capture success or foraging habitat type.

In addition to the behavioral classification, we used a supervised learning task, specifically a classification task, to identify the magnetic compass headings of Fox1 during behaviors that parallel the behaviors preceding mousing attempts in wild foxes (e.g., slow, stalking approach). Due to resolution of ground-truth video records, we used an 8-way classification task with the four cardinal and four anti-cardinal directions, and therefore, the classifier could distinguish among eight magnetic directions with a 45° resolution.

We performed two separate random forest classification analyses, the first assumed each *sample* was an individual observation and therefore contained a total of 381 observations. Using tenfold cross-validation to estimate the generalization performance of the random forest classifier, the accuracy achieved was 90.0% (Table 4; Fig. 5a). Of note, all but 7 (1.8% of all observations) of the errors made by the classifier were 'off-by-one'; i.e., the classifier predicted either the correct heading or one of the headings adjacent to the true heading in 98.2% of all cases. However, we recognize that this analysis may be overly optimistic since this approach did not account for potential time correlations between samples drawn from a given heading. Therefore, the second analysis limited individual observations to heading *events*, defined in this study as periods of time when Fox1 was assigned to a consistent direction by a human reviewer. Heading events were then computed by averaging samples over each such time period. This resulted in a smaller sample size ($n = 66$ observations), and tenfold cross-validated accuracy for the random forest classifier learned in this setting was 74.2% (Table 5; Fig. 5b).

While it is tempting to conclude that the second analysis is more appropriate than the first, it should be noted that some of the observed performance decline is likely attributable to the dramatic reduction in the size of the training set used in the second analysis. It is reasonable to assume that if a larger number of events were available for training, accuracy on unseen data would fall somewhere between the bounds of 74.2 and 90.0% that was achieved (Fig. 5). Of course, even a pessimistic estimate of 74.2% accuracy far outstrips a chance classifier, which would perform at 12.5% on this problem.

Whether a digital compass (i.e., heading classifier) with the degree of resolution and accuracy we present here would yield conclusive data in a larger field study involving multiple wild foxes depends on the strength and scale of any effect being measured as well as the degree of behavioral similarity across animals. The latter can potentially be managed by training classifiers on individual foxes which participate in field-based studies, or across a representative group of wild and/or captive foxes. In addition, improvements in the sensor fixation and mounting technique, in the resolution of the apparatus and methodology used to collect and label ground-truth data, and in the size of the training data set would all likely yield improvements in the resolution and accuracy of the classifier. In future studies designed to characterize the biophysical mechanisms mediating SMA, treatment groups (e.g., foxes exposed to radio-frequency fields in the low-MHz range, see below) would be predicted to exhibit magnetic alignment responses indistinguishable from random, similar to the behavioral effects of radio-frequency exposure on the magnetic compass response in migratory birds [54]. The 8-way classifier developed in this study is well suited to distinguish between the magnetic headings of oriented and random mousing attempts, although the strength of the orientation in control group would determine the sample size needed to confirm any treatment effect. In conclusion, we are encouraged by the performance of the 8-way classifiers in light of the difficulty of the problem and believe the framework established here is promising that merits further study and development.

The development of these automated behavioral monitoring and classification techniques will make it possible to further investigate magnetic alignment responses exhibited by foraging red foxes whose prey capture success has been shown to be dependent on their orientation with respect to the Earth's magnetic field [27]. Červený et al.'s analysis of prey capture success in habitats where visual cues could not be used to guide mousing behavior (i.e., dense vegetation and snow cover) revealed that foxes were approximately four times more successful when attacks were directed toward magnetic north-northeast. One possible explanation for this type of alignment behavior is the involvement of a light-dependent magnetoreceptor mediated by the so-called radical pair mechanism that could be perceived as a visual pattern superimposed on the animal's visual surroundings [26, 34, 55]. As suggested by Červený et al. (and see "Background"), this visual pattern, fixed in alignment with respect to the magnetic field, could be used as a targeting system that may allow foxes to initiate their attacks from a fixed distance, increasing the accuracy of the mousing

attempt. Consistent with Červený et al.'s findings, the contribution of the proposed magnetic 'range-finder' (i.e., distance estimator) to the accuracy of predatory attacks could be especially pronounced when the fox's view of the prey is obstructed by dense vegetation or snow cover [27].

Although the red fox is considered a generalist species, several studies of central European red foxes suggest that a significant proportion of their diet comes from rodents that varies depending on elevation and season. For example, one study estimates that 65% of the fox's diet comes from rodents throughout the year [56], whereas more recent estimates suggest that >30% of their diet is composed of rodents (and hares), and during the winter months, small mammals make up approximately 40% of the fox's diet [57]. Although foxes will use sight to guide the majority of predatory behaviors in habitats with low vegetation and no snow cover, even just 2 weeks of tall grass or snow would require that foxes 'mouse' to catch prey burrowed under the substrate, and therefore, 'hard' evolutionary selection is considered to underlie the fox mousing phenotype. Given the importance of mousing to fox survival, it is not surprising that multiple cues and sensory systems are involved, helping to increase the success and efficiency of mousing behavior.

Magnetic bio-loggers can provide a valuable tool for future studies of magnetic alignment in free-roaming animals, and in particular foraging wild red foxes. The development of these devices along with well-developed behavioral classifiers and magnetic alignment data offers a noninvasive technique for collecting robust and unbiased behavioral data sets across multiple individuals. However, it must be confirmed that the bio-loggers themselves, the harness system, or any additional equipment secured to the fox do not introduce unintentional biases or effect the performance of functional behaviors in wild foxes. One advantage of the miniaturized bio-logger and collar use in the current study is that it offers a lightweight and durable approach for external data tag attachment. The mean body mass of western European adult red foxes ranges from roughly 5.5 to 6.5 kg (although individual, sexual, and seasonal factors introduce variation) [58], and therefore, the bio-logging equipment used in the current study (total mass = 55.2 g) accounts for less than 0.1% of the mean body mass, well below the suggested <10% body mass guidelines recommended by [59]. Since the collars were snugly secured around the fox's neck, it is unlikely that it would hamper the movements involved in wild mousing behavior, and indeed, no impairment in mousing-like leaps or decrease in frequency of leaps was observed in the semidomesticated

foxes when the harnesses were attached compared to when they were removed. However, this was based on general observations of the semidomesticated foxes inside the behavioral arena, and field-based observations comparing the behavior of free-roaming foxes with and without harnesses will be important to confirm that the equipment is not influencing the quality or frequency of natural behaviors. Furthermore, although the devices did contain small amounts of magnetic material causing a slight deflection (<5°) of a compass needle when placed in contact with the device, this effect was eliminated when the device was moved 2 cm from the compass. Therefore, it is unlikely that the small magnetic component inherent to the bio-logger would have an effect on perceiving the magnetic field, as the magnetic field strength falls off exponentially with distance, decreasing at a rate to the third power with distance (i.e., inverse cube law) [60], and therefore would produce a much weaker effect, if any, in the eyes and head region (i.e., the proposed site of magnetoreceptors [33, 61]). Also, magnetic compass responses in migratory birds exposed to field strengths differing by $\pm 30\%$ of the natural field strength showed no effect on compass orientation, suggesting a functional window of magnetic compass responses [62], and if SMA in red foxes is mediated by a similar sensory mechanism, weak magnetic fields produced by the bio-logging device would not be expected to disrupt magnetic alignment responses.

An exciting possibility is the use of additional technologies in combination with magnetic bio-loggers to perform field-based manipulative experiments on free-roaming animals, which have been difficult to implement in field studies of magnetic alignment to date. For instance, magnetic responses in a variety of animals (e.g., birds, mice, turtles, insects) [18, 54, 63, 64] have been shown to be affected by radio-frequency fields in the low-MHz range, presumably influencing the quantum spin states underlying the light-dependent radical pair mechanism, altering or disrupting the pattern of magnetic input. If a radical pair mechanism mediates the directional component of mousing attacks of red foxes, radio-frequency exposure should disrupt the accuracy (and, therefore, the success) of these attacks. We have developed radio-frequency emitting collars tuned to broadcast in the low-MHz range that have been shown to disrupt the magnetic compass orientation of amphibians and birds (sinusoidal frequency sweeps from ~1.0 to 1.8 MHz, at the rate of 10.0 kHz) [54, 65]. The collar is composed of two major units: the transmitter board and the multi-turn loop antenna, and the resulting signal is amplified using a Class-C power amplifier to provide greater power

efficiency and promote longer battery life for input to the multi-turn antenna. Because the physical size of the antenna was extremely small relative to the wavelength at 1.4 MHz (approximately 215 m), multiple loops of wire were stacked vertically to improve the antenna's efficiency and ensure sufficient energy was emitted to the fox. The resulting collar produces a radio-frequency stimulus with a maximal intensity of 88 nT, beyond intensities shown to disrupt migratory compass orientation in birds and SMA responses in hatchling snapping turtles [18, 66]. Integrating the radio-frequency collar with the bio-logging device and harness system will provide a powerful opportunity to test for the involvement of a light-dependent radical pair mechanism underlying magnetic alignment responses of mousing red foxes under otherwise natural conditions that can be conducted following double-blind protocols. Importantly, this system can provide further support for, or against, the impact of anthropogenic radio-frequency exposure on wild life [67–69]. Although field observations of wild foxes will be important to confirm that the radio-frequency collars do not affect natural behaviors, it is unlikely that they would impair the use of magnetic cues during mousing attempts. As discussed above, magnetic field strengths fall off exponentially as a function of distance [60], and the radio-frequency collars are only weakly magnetic, making it unlikely that the collar would appreciably affect the perception of magnetic fields. Furthermore, if foxes use a similar magnetoreception mechanism to that used by migratory birds, then magnetic field intensities would need to change by 30% or more to disrupt behaviors relying on magnetic cues [62]. Lastly, non-iron containing loops of wire, like those used in the current radio-frequency collar design, will not manipulate or distort the ambient magnetic field. The radio-frequency intensities proposed for use in future studies of red foxes are well below the guidelines for human exposure adopted by the World Health Organization [70] and are thought to only affect biological processes occurring at the quantum level. Therefore, we do not anticipate the animal to experience any discomfort or long-term effects from radio-frequency exposure.

If, however, mousing success is unaffected by radio-frequency exposure, alternative manipulations could be performed, aimed to test for the involvement of a magnetite-based mechanism similar to the one proposed to mediate spontaneous magnetic nest building behaviors in subterranean mole-rats [71–73]. For example, prior to attaching the harness system to wild-caught foxes, individuals could be exposed to a brief, high-intensity magnetic pulse that re-magnetizes particles of biogenic

magnetite. Similar to the effects of pulse re-magnetization on mole-rats that exhibited a 90° deviation of magnetic nest building orientation after pulse treatments [73], wild foxes would be predicted to exhibit shifted or abolished SMA responses while mousing if this behavior is mediated by a magnetite-based mechanism.

Conclusions

We report the development of 'magnetic ethograms' in which the behavior and magnetic alignment of red foxes can be accurately extracted from raw sensor data recorded from triaxial accelerometer and magnetometer bio-loggers. Three functionally relevant behaviors could be identified using a 5-nearest neighbor classifier that performed with an overall accuracy of 95.7% across 415 ground-truth events. To evaluate the generalizability of the classifier, similar behavioral data were recorded from a second fox and resulted in 66.7% performance accuracy when analyzed using identical techniques, suggesting the classifier can extract behaviors across multiple foxes. A similar classification approach was used to identify the fox's magnetic alignment using two 8-way classifiers with differing underlying assumptions to distinguish magnetic headings in eight equally spaced 45° sectors. The magnetic heading classifiers performed with 90.0 and 74.2% accuracy, suggesting a realistic performance range for a classifier based on an independent set of training events equal in size to our sample.

Given the performance of the behavioral and magnetic classifiers, we argue that 'magnetic bio-loggers' are well-suited for use in future studies of SMA in red foxes thought to use the magnetic field as a targeting system, increasing the accuracy of mousing attacks targeting small prey. The deployment of bio-loggers coupled with additional lightweight technologies, e.g., radio-frequency collars, provides an exciting opportunity to help characterize the adaptive significance and the biophysical mechanisms mediating SMA in free-roaming mammals, both of which remain enigmatic. More generally, these techniques will provide new opportunities for studies of SMA in free-roaming mammals and offer several advantages including the ability to collect large data sets autonomously across multiple individuals, recording data in habitats or locations that may otherwise be inaccessible by observers, observer biases can be avoided, experiments can be conducted following double-blind protocols, and these techniques can be adapted for studies across diverse animals and behaviors. Therefore, we hope that the current study inspires a new approach for future researcher of magnetic alignment in free-roaming animals.

Additional files

Additional file 1: Table S1. Bio-logging device orientations.

Additional file 2: Video file S1. Example of Fox2 exhibiting a 'mousing-like' leap out of the 10 × 10 × 1 m arena.

Additional file 3: Video file S2. Example of Fox2 exhibiting a 'mousing-like' leap out of the 10 × 10 × 1 m arena.

Additional file 4: Video file S3. Example of Fox1 trained to jump over a barrier and immediately come to a stop preventing a collision with a second, smaller barrier. This obstacle was designed to elicit 'mousing-like' leaps.

Additional file 5: Video file S4. Example of Fox1 trained to approach a ~1-m-tall barrier by walking down one of four corridors aligned in the four cardinal magnetic directions to receive a food reward in the central square of the arena.

Additional file 6: Video file S5. Example of Fox1 exhibiting 'mousing-like' behavior using the four-corridor arena described above.

Additional file 7: Video file S6. Example of Fox1 exhibiting behavior defined as 'trotting' for the behavioral analysis.

Additional file 8: Video file S7. Example of Fox1 exhibiting behaviors defined as 'foraging' for the behavioral analysis.

Abbreviation

SMA: spontaneous magnetic alignment.

Authors' contributions

MSP contributed to project conception, carried out experimental design, was responsible for data collection, data analysis and interpretation of data, and drafted and edited sections of the manuscript. JAB was responsible for data analysis and interpretation and drafted and edited sections of the manuscript. MSP and JAB contributed equally to the manuscript and should be considered co-first authors. EPM contributed to project conception, assisted with experimental design, data analysis and interpretation, and helped draft and edit sections of the manuscript. CRA assisted in experimental design, was responsible for radio-frequency collar conception and electrical design, data analysis and interpretation, and drafted and edited sections of the manuscript. DCS contributed to the experimental design, data collection methodology, contributed to data analysis and interpretation, and drafted and edited sections of the manuscript. CWH contributed to data analysis and interpretation. JC contributed to project conception, helped coordinate experiments with semidomesticated foxes, and provided logistical support throughout the course of the study. VH contributed to the project conception, assisted with experimental design, helped coordinate experiments with semidomesticated foxes, and provided logistical support throughout the course of the study. VT helped with construction and design of experimental arenas, gave permission for study of semidomesticated foxes, assisted with handling of semidomesticated foxes, and provided logistical support throughout the course of the study. EB provided logistical support throughout the course of the study and edited versions of the manuscript. HB contributed to project conception, assisted with experimental design, and provided logistical support throughout the study. JBP contributed to project conception, assisted with experimental design, assisted with data analysis and interpretation of data, provided logistical support throughout the course of the study, and drafted and edited sections of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data

The authors agree to make all of the raw accelerometer and magnetometer data publicly available.

Ethics approval

All methods were approved by the Expert Commission of the Czech University of Life Sciences (SP506051228).

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