Environmental Entomology, XX(XX), 2020, 1–12
doi: 10.1093/ee/nvaa019
Research

Employing Very High Frequency (VHF) Radio Telemetry to Recreate Monarch Butterfly Flight Paths

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Subject Editor: William Morrison

Received 19 December 2019; Editorial decision 10 February 2020

Abstract

The overwintering population of eastern North American monarch butterflies (*Danaus plexippus*) has declined significantly. Loss of milkweed (*Asclepias* sp.), the monarch's obligate host plant in the Midwest United States, is considered to be a major cause of the decline. Restoring breeding habitat is an actionable step towards population recovery. Monarch butterflies are highly vagile; therefore, the spatial arrangement of milkweed in the landscape influences movement patterns, habitat utilization, and reproductive output. Empirical studies of female movement patterns within and between habitat patches in representative agricultural landscapes support recommendations for habitat restoration. To track monarch movement at distances beyond human visual range, we employed very high frequency radio telemetry with handheld antennae to collect movement bearings on a biologically relevant time scale. Attachment of 220–300 mg transmitters did not significantly affect behavior and flight capability. Thirteen radio-tagged monarchs were released in a restored prairie, and locations were estimated every minute for up to 39 min by simultaneous triangulation from four operators. Monarchs that left the prairie were tracked and relocated at distances up to 250 m. Assuming straight flights between locations, the majority of steps within the prairie were below 50 m. Steps associated with exiting the prairie exceeded 50 m with high directionality. Because butterflies do not fly in straight lines between stationary points, we also illustrate how occurrence models can use location data obtained through radio telemetry to estimate movement within a prairie and over multiple land cover types.

Key words: ecology and behavior, landscape, insect VHF radio telemetry, habitat utilization, occurrence models

Since the early 1990s, the overwintering population of eastern North American monarch butterflies (*Danaus plexippus*) has declined significantly (Brower et al. 2012, Oberhauser et al. 2017). Many factors contribute to this decline; yet, loss of milkweed (*Asclepias* sp.), the monarch's obligate host plant, in the breeding range is generally considered to be a significant cause (Brower et al. 2012, Inamine et al. 2016, Thogmartin et al. 2017). Restoring breeding habitat in the Midwest United States, including milkweed and nectar sources, is an actionable step toward population recovery (Thogmartin et al. 2017, Pitman et al. 2018). To maximize the efficacy of conservation efforts, milkweed should be established in the landscape in spatial patterns that optimize female reproductive output. Improved understanding of how monarch females move through agricultural landscapes and distribute eggs is needed.

The monarch butterfly is a vagile species (Zalucki and Kitching 1984, Zalucki and Lammers 2010) that can traverse up to 15 km/d during the breeding season (Zalucki et al. 2016). During their lifetime, females produce 300–400 eggs (Urquhart 1960, Oberhauser 2004) that are typically laid singly per milkweed ramet (Zalucki and Kitching 1982a). Given these behaviors, the spatial arrangement of milkweed in the landscape is expected to influence movement patterns, habitat utilization, and reproductive output (Bergin et al. 2000, Misenhelter and Rotenberry 2000, Pitman et al. 2018, Grant and Bradbury 2019). Higher realized fecundity is predicted when habitat is distributed in many small patches as compared with a smaller number of large patches (Zalucki and Lammers 2010, Zalucki et al. 2016).

Typically, animal movement models are based on correlated random walk algorithms without incorporating behavioral factors (Siniff and Jessen 1969, Smouse et al. 2010, Zhao et al. 2015). These algorithms can be problematic in heterogeneous landscapes that require flight direction decisions at habitat edges in the mosaic of cropland, pastures, conservation land, and roadside rights of way in the monarch's summer breeding range (Grant et al. 2018). Recently, individual-based models were developed to explore the inclusion of female monarch behavioral factors in simulating landscape-scale movement and egg distribution (Zalucki et al. 2016, Grant et al. 2018). Behavioral factors were derived from expert opinion and limited empirical evidence (Zalucki 1983, McIntire et al. 2007, Zalucki et al. 2016, Grant et al. 2018). An uncertainty analysis indicated model predictions in a spatially explicit Iowa United States landscape are sensitive to assumptions concerning flight step lengths and directionality, in addition to perceptual range and spatial memory (Grant et al. 2018). Empirical studies of female monarch movement patterns within and between habitat patches in representative agricultural landscapes would reduce uncertainty in predicting landscape-scale egg densities for alternate habitat restoration plans.

Several approaches could be employed to quantify monarch movement patterns across agricultural landscapes. To date, insect flight behavior has been studied through visual observation or the use of technologies such as harmonic radar (Drake and Reynolds 2012), very high frequency (VHF) radio telemetry (Wikelski et al. 2006, Hagen et al. 2011, Levett and Walls 2011, Svensson et al. 2011, Fornoff et al. 2012, Kissling et al. 2014, Liegeois et al. 2016, Wang et al. 2019) , and radio frequency identification (RFID; Schneider et al. 2012). The strengths and limitations of each approach to quantify flight movement depend on the behaviors of interest within relevant spatial and temporal domains.

Visual observation, aided by binoculars (Merckx et al. 2003) or recording theodolites (Zalucki and Kitching 1982b), in tandem with Geospatial Positioning System (GPS) units (Schultz and Crone 2001; Schultz et al. 2012, Schultz et al. 2017; Kallioniemi et al. 2014; Fernandez et al. 2016) have been used to record locations of over 25 butterfly species up to 50 m, with one report of tracking a sliver-studded blue butterfly (*Plebejus argus*) 259 m. Flagging locations between flight steps and measuring distances and compass bearings between locations have been used to track the Fender's blue butterfly (*Icaricia icarioides fender*), alpine butterfly (*Parnassius smintheus*), and the endangered scarce large blue butterfly (*Phengaris teleius*) up to 37.5 m (Turchin et al. 1991, Schultz 1998, Fownes and Roland 2002, Skorka 2013). These approaches provide the means to recreate flight paths within a habitat patch, assuming the time between successive two- or three-dimensional locations can be quantified. Given limitations of the human visual range, these approaches have limited means to quantify movement patterns within and between habitat patches at landscape scales.

Studies that implement VHF radio telemetry have relocated migrating dragonflies (*Anax junius*) over 1,500,000,000 ha (Wikelski et al. 2006) and bumblebees (*Bombus* sp.) over 44 ha (Hagen et al. 2011), both with the aid of aircraft. Increased frequency of data collection can theoretically provide greater certainty of estimated flight paths; however, to date, insects have been relocated once or twice per day (Wikelski et al. 2006, Hagen et al. 2011, Levett and Walls 2011, Svensson et al. 2011, Fornoff et al. 2012, Kissling et al. 2014, Liegeois et al. 2016). Recently, Wang et al. (2019) employed VHF radio telemetry to collect data from migratory golden birdwing butterflies (*Troides aeacus*) every 30 min; however, successful location estimates were achieved in 38% of the observation periods. Thirty minutes to 24- or 48-h data collection intervals are insufficient to reconstruct monarch flight patterns with sufficient resolution to enhance individual-based model predictions of monarch movement at habitat edges in landscapes. New approaches with tracking technology need to be implemented to provide higher frequency estimates of locations and flight paths.

Based on Kissling et al.'s (2014) review of the advantages and limitations of a variety of technologies, we are applying VHF radio telemetry methods to reduce uncertainty in estimated flight paths of female monarch butterflies at distances beyond human visual limits. Here, we report: 1) a 220 mg transmitter does not significantly affect monarch butterfly behavior and flight capability, 2) a technique using handheld antennae to collect location data every minute, which is a time-scale relevant for monarch flight behavior, and 3) preliminary insights on monarch butterfly movement with a straight-line flight path analysis over distances larger than previously reported with visual data collection. Because monarch flight paths are not straight lines between stationary locations, we also illustrate how continuous-time movement models can use estimated locations to estimate flight patterns. Employing the VHF radio telemetry methods described here, combined with continuous-time movement models, will provide the means to estimate flight paths in landscapes with multiple types of land cover.

Methods

Field Sites

Studies were undertaken in restored prairie areas of at least four ha containing a diversity of grasses, blooming forbs, and *Asclepias sp.* in Boone, IA (UTM zone 15N, E: 427169.32, N: 4656779.47), Ames, IA (UTM zone 15N, E: 447691.83, N: 4653386.83), and Rochester, MN (UTM zone 15N, E: 541666.13, N: 4873350.54). All work was conducted with nonmigratory monarchs in late June to early August in 2016, 2017, and 2018. All studies occurred on clear or mostly clear days between 1,000 and 1,500 h with temperatures between 21 and 30°C and wind speeds below 16 kph.

Insects

We collected male and female monarch butterflies in and around field site locations. Butterflies were held in a soft-sided, mesh cage for 1–5 d before use in an experiment, in accordance with Zalucki and Kitching (1982b). Gatorade (Chicago, IL) was provided ad libitum as a sugar source.

Transmitter Attachment

Transmitters (LB-2X, 220 mg, Holohil Systems Ltd, Ontario, Canada) were attached to the ventral side of the monarch's abdomen with superglue by Loctite (Henkel Canada, Brampton, Ontario, Canada; Boiteau et al. 2009; Fig. 1a). Monarchs with a transmitter attached are referred to as 'radio-tagged'. Monarchs with attached watch batteries (300 mg; Energizer AZ10DP, St. Louis, MO) are referred to as 'sham-tagged' (Fig. 1b). Sham-tagged monarchs were used to assess the extent to which added weight altered behavior and flight ability. After transmitter or watch battery attachment, butterflies were anesthetized in a Styrofoam cooler containing dry ice wrapped in a cloth for one minute to prevent fright behavior (Schultz and Crone 2001; Schultz et al. 2012, 2017; Kallioniemi et al. 2014; Fernandez et al. 2016). Handling and attachment were completed in <3 min.

Effects of Transmitter Attachment

To determine the extent to which added weight affected behavior time allocation, we observed male and female butterflies with ('sham-tagged') and without ('untagged') an attached watch battery (sham-transmitter). Sham-tagged and untagged butterflies were released individually on either a blooming common milkweed ramet or a blooming forb in a restored prairie at one of the field sites. Monarchs were observed for 20 min or until they flew from the observation area. Using stopwatches, three observers stood approximately 5 m from the butterfly (Skorka 2013) and recorded the continuous-time intervals of various behaviors, including resting, feeding, and flying (oviposition was not observed). In 2016 and 2017,

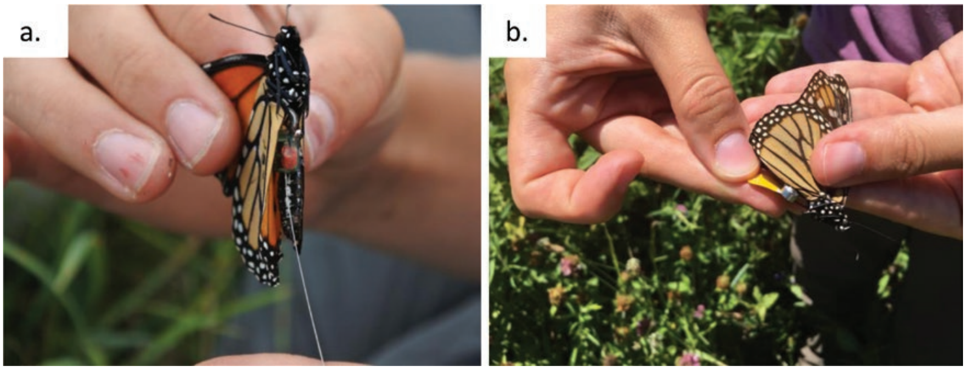

Fig. 1. Monarch butterflies with a 220 mg LB-2X radio telemetry transmitter (a) or sham-transmitter (b; 300-mg watch battery) attached to the ventral side of the abdomen with superglue

16 and 28 individual monarchs were observed (Table 1). Behaviors were subsequently classified as 'in-air' (flying) or 'on-plant' (resting and feeding). In 2018, observations of 49 individuals (Table 1) were classified as 'on-plant' or 'in-air'.

For statistical analyses, observations from all three years were combined into on-plant or in-air groups. RStudio version 1.2.1335 (R Studio Team 2016, R Core Team 2016) with the Estimated Marginal Means (emmeans) package (Lenth et al. 2020) was used to fit the proportion of time observed in-air to a binomial generalized linear model that accounted for tag attachment, monarch sex, and year. An analysis of the proportion of time observed resting while on-plant in 2016 and 2017 was conducted in a similar manner. There was no effect of monarch sex or year in either analysis.

Tracking Female Monarch Butterflies with VHF Radio Telemetry

Over 5 d in August 2016, radio-tagged female monarchs were released one at a time on vegetation in the center of the 4-ha Boone, IA site (Fig. 2) to assess the means to track female-specific movements and behaviors; e.g., oviposition which has a direct impact on population growth (Grant et al. 2018). One observer stood approximately 5 m from the butterfly to observe behavior, as described previously. To recreate a flight path, compass bearings in the direction of the transmitter were collected from multiple locations simultaneously and repeatedly to triangulate sequential estimated locations of the transmitter. Radio telemetry operators were stationed in the four corners of the prairie (Fig. 2; 50–135 m from the monarch release location). Each operator held a compass, and a three-element directional Yagi antenna (directional Yagi 3 element antenna for frequencies ranging from 164.000–166.000 MHz, Johnson's Telemetry, El Dorado Springs, MO) connected with a 0.9-m coax cable (Johnson's Telemetry, El Dorado Springs, MO) to a VHF radio telemetry receiver (Alinco DJ-X11; Toyama, Japan). Every minute after release, operators noted the compass bearing (Fig. 2) for the loudest signal with an iPhone tape recorder application. Signals were noted every minute until the butterfly crossed the edge of the prairie or was observed within the prairie a maximum of 39 min. At this point, butterfly capture was attempted. When capture was unsuccessful (n = 4), tracking with telemetry continued by foot or in vehicles until the butterfly was relocated. When monarchs were captured, their location was georeferenced (Trimble Geo7x; Sunnyvale, CA). Transmitters were removed from the radio-tagged monarch and re-used to track other monarchs. No transmitter was used >48 total hours (or >25% of their estimated battery life).

Table 1. Number of female and male wild-caught individuals observed with and without sham-transmitters (300-mg watch batteries) in 2016, 2017, and 2018 to determine whether there was an effect of weight attachment on behavior or flight ability

Year	Sham-tagged		Untagged	
	Female	Male	Female	Male
2016	5	1	9	1
2017	4	12	2	10
2018	10	13	16	10

Space-Use Analysis

Bearings from all four corners of the prairie with associated date and time were transcribed for 13 butterflies. Using Location of a Signal software (LOAS; Ecological Software Solutions LLC, www.ecostats.com), locations and error ellipses were estimated each minute based on triangulation of at least three of the four bearings with a maximum likelihood estimator. Locations collected with the Trimble Geo7x (i.e., release sites and recapture sites) were considered true locations without error ellipses (error was <30 cm). Step lengths (distance traveled in one minute) between two consecutive locations and turn angle created from three consecutive locations were calculated using the movement.pathmetrics package in Geospatial Modeling Environment (GME; Spatial Ecology LLC, www.spatialecology.com/gme/). All step lengths for an individual were summed to estimate total distance traveled; Euclidian distances from start to end were measured in ArcMap 10.3 to estimate true displaced distances.

Straight-line analyses are typically used to infer movement; however, this approach leads to overconfidence in movement inferences because 1) movement is likely not a straight line between two data collection points, and 2) no error is assumed in location estimates (Calabrese et al. 2016). Occurrence models created using the continuous-time movement model (ctmm) package (Flemming et al. 2019) in R Studio (R Studio Team 2016, R Core Team 2016) can estimate an animal's location during the tracking period, including times between data collection (Calabrese et al. 2016). As sampling frequency increases, the occurrence model output more closely resembles the true movement path (Fleming et al. 2016). In the present study, occurrence models were selected based on the most representative semivariogram estimates with the smallest AIC value. Output was a raster-image scaled with the proportion of time spent in each raster cell. Since each butterfly was observed for a short time period (i.e., up to 39 min), occurrence models for nine butterflies were summed to create one output. Four of the 13 monarchs were

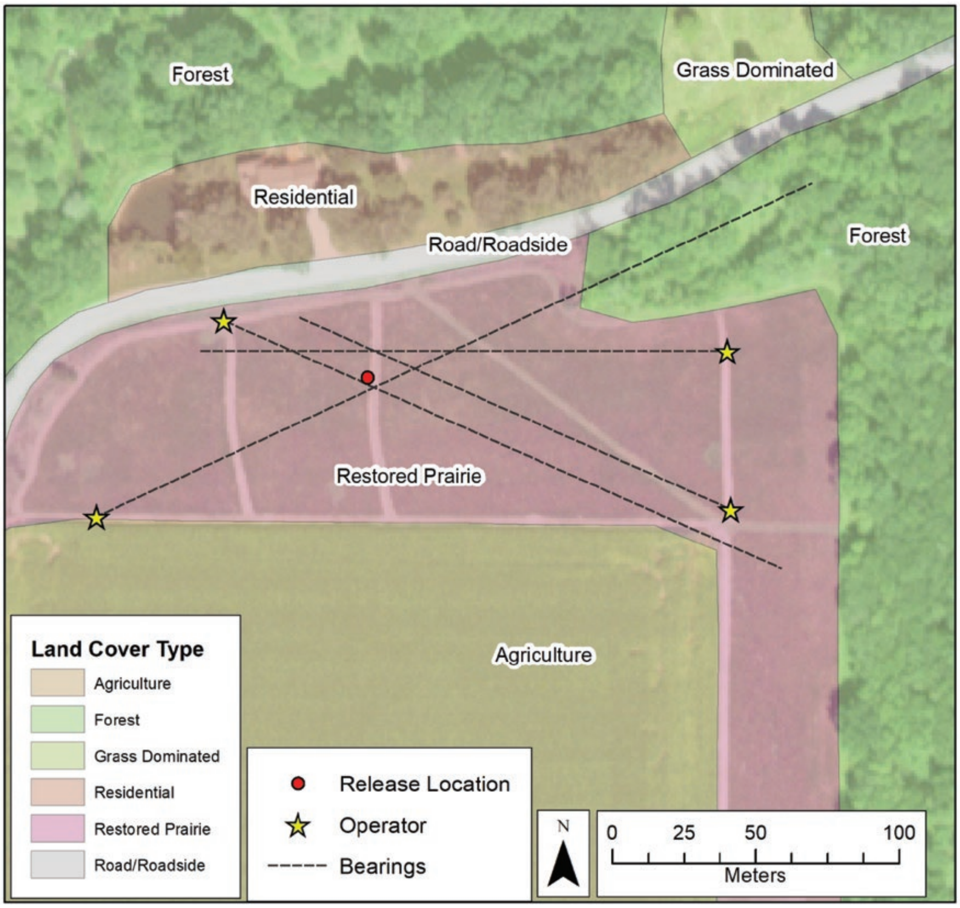

Fig. 2. Field site in Boone, IA, where flight paths of monarch butterflies were tracked with VHF radio telemetry. The circle is the release site for transmitter-tagged female monarch butterflies, and stars indicate the locations of four operators equipped with a compass, a 3-element Yagi antenna, and a VHF radio telemetry receiver. Dashed lines are example compass bearings collected by operators in the direction of the transmitter at the release site

removed from the occurrence analysis because too few location estimates were made or error ellipses were too large to employ the model properly. A GeoTIFF of the field site (Iowa Geographic Map Server 2015) was digitized by land use type and converted into a raster image in ArcMap 10.3. Land use types included: restored prairie, grass-dominated habitat, forest, agriculture, road and roadside, and residential. Area covered by intermediate to high-occurrence probability within the prairie was estimated. Percent of time in each land use type was calculated with raster calculation in ArcMap 10.3. Because our study was primarily designed to assess the feasibility of employing VHF radio telemetry to track high-resolution monarch flight behavior, individuals were typically captured before they left the prairie. Consequently, while our findings are relevant for assessing behavior within the prairie, the results of the occurrence models over multiple land-use types should not be considered representative of flight patterns across larger, more complex landscapes. This study does, however, illustrates a workflow that can be applied in future studies to analyze movement data within and across different land cover types without assuming straight-line flights.

Results

Effects of Transmitter Attachment

Of the monarchs observed with (n = 45) or without (n = 48) sham-transmitters, 88.9% of sham-tagged and 91.7% of untagged monarchs flew at least once during their observation period. There was no significant effect of sham-transmitter attachment on time spent in-air (Fig. 3a; F = 0.097; df = 1, 96; P = 0.7551). Sham-tagged monarchs were observed in-air for 27.5 ± 33.0% (SD) of the observation period, while untagged flew for 32.1 ± 36.4% of their observation period. Twenty-three sham-tagged (51.1%) and 25 untagged (52.1%) monarchs left the prairie before the 20-min observation period was completed and were not recaptured.

For those individuals with behavior observations on-plant (22 sham-tagged and 26 untagged), there was no significant difference in the percent of time spent resting with or without a sham-transmitter (Fig. 3b; F = 3.034; df = 1, 47; P = 0.0816). While not significant at the P = 0.05 level, there was an indication that sham-tagged monarchs rest more than untagged monarchs. Monarchs with sham-transmitters rested for 66.2 ± 35.6% of the observation period, while those without sham-transmitters rested for 36.9 ± 35.0% of the period.

Space-Use Analysis

Thirteen female monarch butterflies were tracked with radio telemetry for up to 39 min. Attempts were made to track additional monarchs for up to 90 min; however, bearing estimates were unreliable and occurred at inconsistent time intervals due to operator fatigue. Four radio-tagged monarchs were not captured at the edge of the prairie, but were successfully tracked and recaptured in surrounding habitat up to 250 m away from the prairie. Radio-tagged monarchs

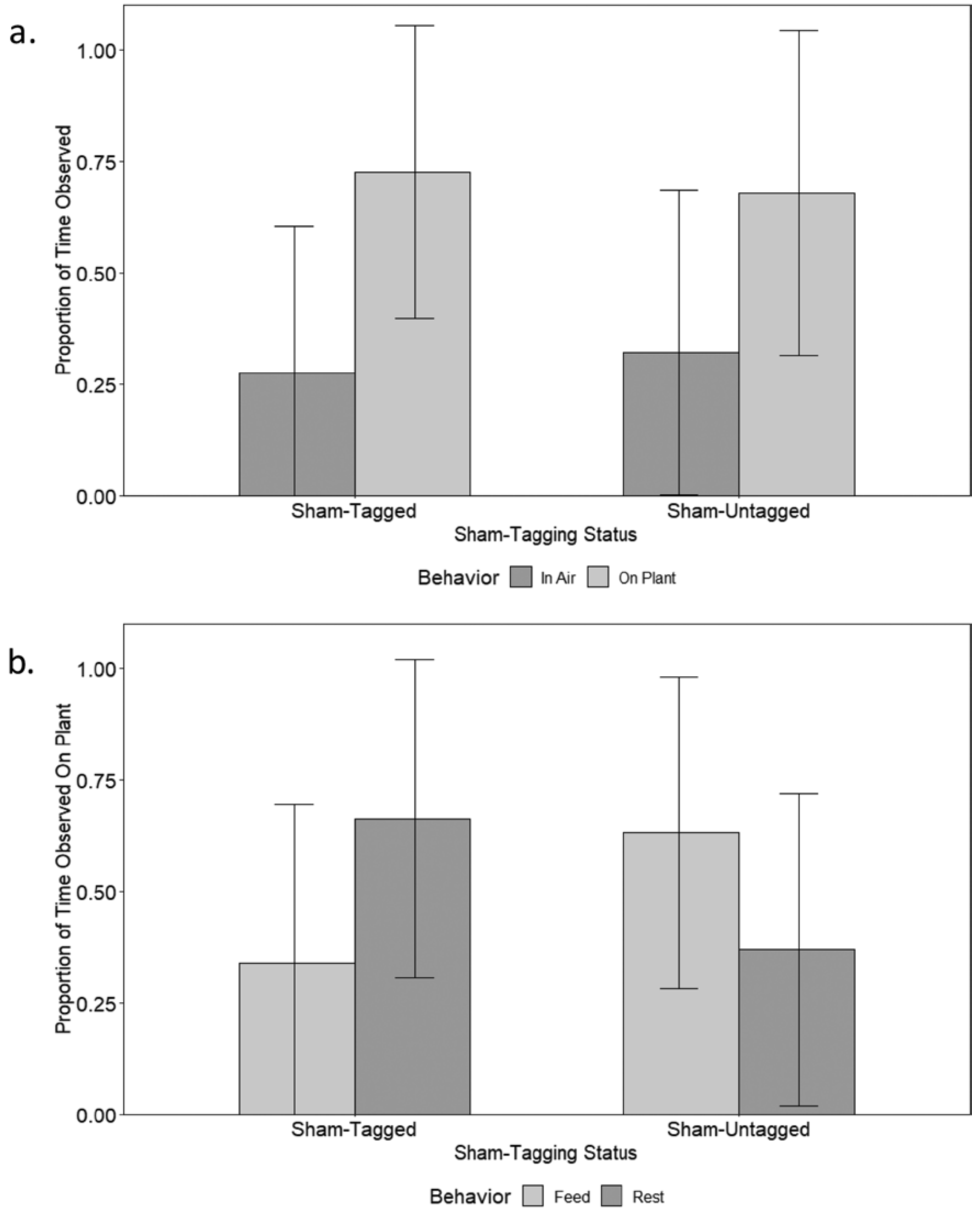

Fig. 3. Proportion of time sham-tagged (300-mg watch battery) and sham-untagged monarchs were observed engaging in various behavior classifications. There was no significant effect of sham-transmitter attachment on time spent in-air; sham-tagged monarchs were observed in-air for 27.5 ± 33.0% (SD) of the observation period, while those without sham-transmitters flew for 32.1 ± 36.4% of their observation period (a). For those individuals observed on-plant, there was no significant difference in the percent of time spent resting with or without a sham-transmitter (b). While not significant at the $P = 0.05$ level, there was an indication that monarchs with sham-transmitters rest more than sham-untagged monarchs. Monarchs with sham-transmitters rested for 66.2 ± 35.6% of the observation period, while those without sham-transmitters rested for 36.9 ± 35.0% of the period.

were recaptured on a variety of plants, including trees, maize, tall grass, and forbs.

Straight-line flight paths were constructed for the 13 butterflies with 4–39 one-minute separated sequential estimated locations (example flight path in Fig. 4; Supp Fig. 1 [online only] for all 13 straight-line flight paths). Within these flight paths, the majority of step-lengths were below 50 m (Fig. 5a) and generally associated with foraging, as noted by an observer 5 m from the butterfly. However, after 2–30 short steps below 50 m, four individuals took steps ranging from 75 to 213 m and exited the prairie (Fig. 5b). Summing the distance covered in the 1-min time-steps for each butterfly indicates individuals traversed 84.6–497.8 m during their observation periods. However, Euclidian distance between the start and end locations ranged from 1.6–238.9 m, suggesting that true displacement was much smaller than the total distance traversed. Consistent with these findings, although turn angles spanned 360 degrees, the majority of turn angles were approximately 180 degrees, which indicates butterflies turned completely around and moved in the opposite direction of where it was originally headed (Fig. 6).

The calculated error ellipse size was variable and ranged from 0.05–6560.22 m^2. Most of the error ellipses were over 100 m^2 (101 of 145 total). When error is large relative to movement distances, it should be included in space-use analyses to provide a more robust analysis of movement (Calabrese et al. 2016). Occurrence models were calculated for 9 of the 13 monarch butterflies, as four individuals showed too few points or too large of error ellipses to employ

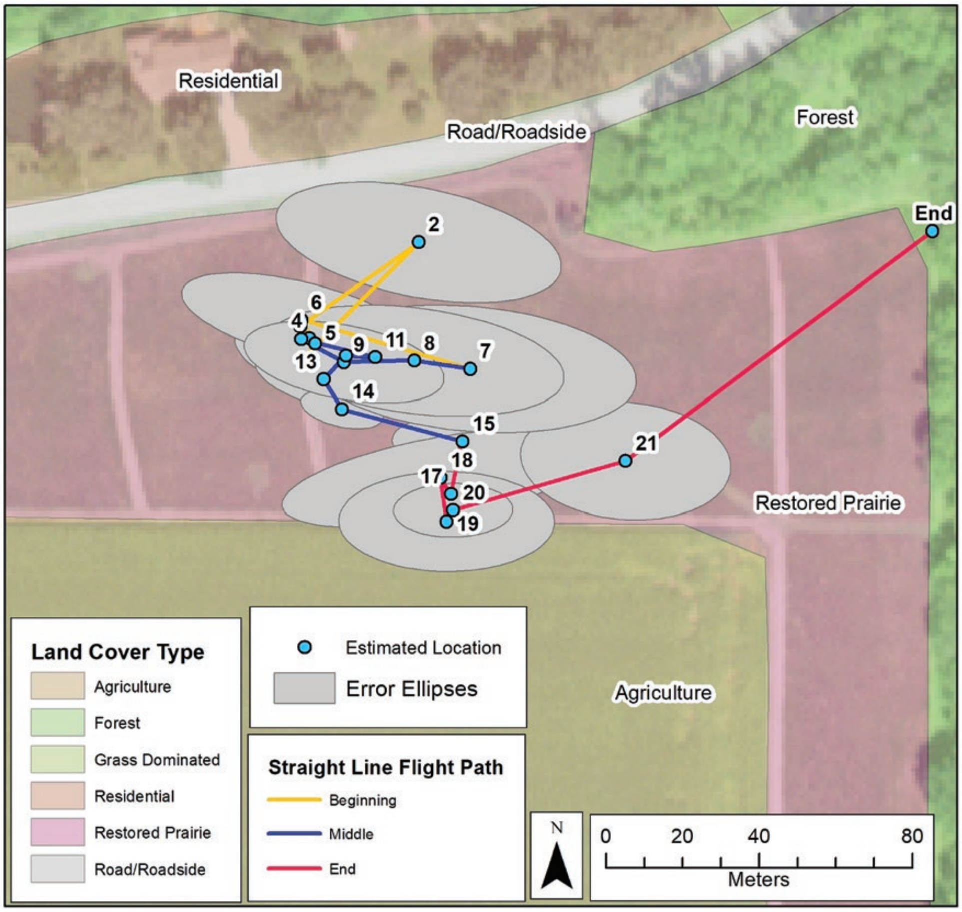

Fig. 4. Example recreated flight path of one monarch butterfly. Small circles indicate estimated locations, label next to the point represents the minute of observation. Lines connecting points represent the straight-line steps between the points. Large ovals represent the error ellipse calculated for each estimated location using Location of a Signal software (LOAS).

this technique. The nine occurrence models were combined to better estimate the utilization of the multiple habitat types (Fig. 7a). Areas of high probability of occurrence are associated with many estimated locations (Fig. 7b). Intermediate or high occurrence was observed over 1.5 ha (15,267.59 m^2) of the 4-ha prairie (37.5%). Though our dataset was biased for locations within the prairie, because error ellipses of the estimated locations spanned multiple land cover types, the occurrence model estimated that of the time observed, monarchs spent 98.95% of their time in the prairie, 1.00% in crop fields, 0.03% over the road, and 0.02% in the forest (Table 2).

Discussion

To date, insect movement studies within the range of visual observations provide reasonable estimates of movement at small scales (e.g., within a habitat patch; Zalucki and Kitching 1982b). Tracking technology adapted for flying insects creates an opportunity to quantify movement at larger spatial scales. Application of this technology can advance understanding of how butterflies move at habitat patch edges within landscape scales. Here, we report a new application of VHF radio telemetry to improve the means of quantifying monarch butterfly flight movement at distances beyond the line of sight in a landscape containing a diversity of land cover classes.

Typically, VHF radio telemetry tracking is used to study the movement of large, slow-moving animals. In these instances, a researcher can walk around the radio-tagged animal and calculate an estimated location (Garton et al. 2001); however, flying insects move too quickly for this approach. Recently, Wang et al. (2019) described a novel application of insect radio telemetry to track the migration of golden birdwing butterflies (*Troides aeacus*) over distances up to 4,314 m. Data were collected at 30-min intervals by a single operator with a 38% success rate for estimating locations. Errors associated with estimated locations were not explicitly reported. The approach of Wang et al. (2019) shows promise for tracking flights of a large-bodied butterflies over 4,000 m for up to 4 d; however, the 30-min collection interval precludes the means to quantify flight patterns of butterflies encountering habitat edges within a landscape over shorter periods of time; e.g., over 30–90 min. Our VHF radio telemetry approach, using the smaller-bodied, nonmigratory monarch, with four operators collecting simultaneous bearings at one-minute intervals, provided the means to quantify errors associated with estimated locations every minute for up to 39 min.

Attaching a transmitter to an insect may have an effect on its behavior or flight ability (Kissling et al. 2014). In studies of butterflies and moths with attached weight equaling up to 15% of their body weight, there was no effect on flight ability (Srygley and Kingsolver 2000, Liegeois et al. 2016). However, when the transmitter was 66–100% of bumblebees' (*Bombus* sp.) body weight, transmitter attachment caused increased resting, fewer flower visitations, and more time spent on a single flower (Hagen et al. 2011). To assess

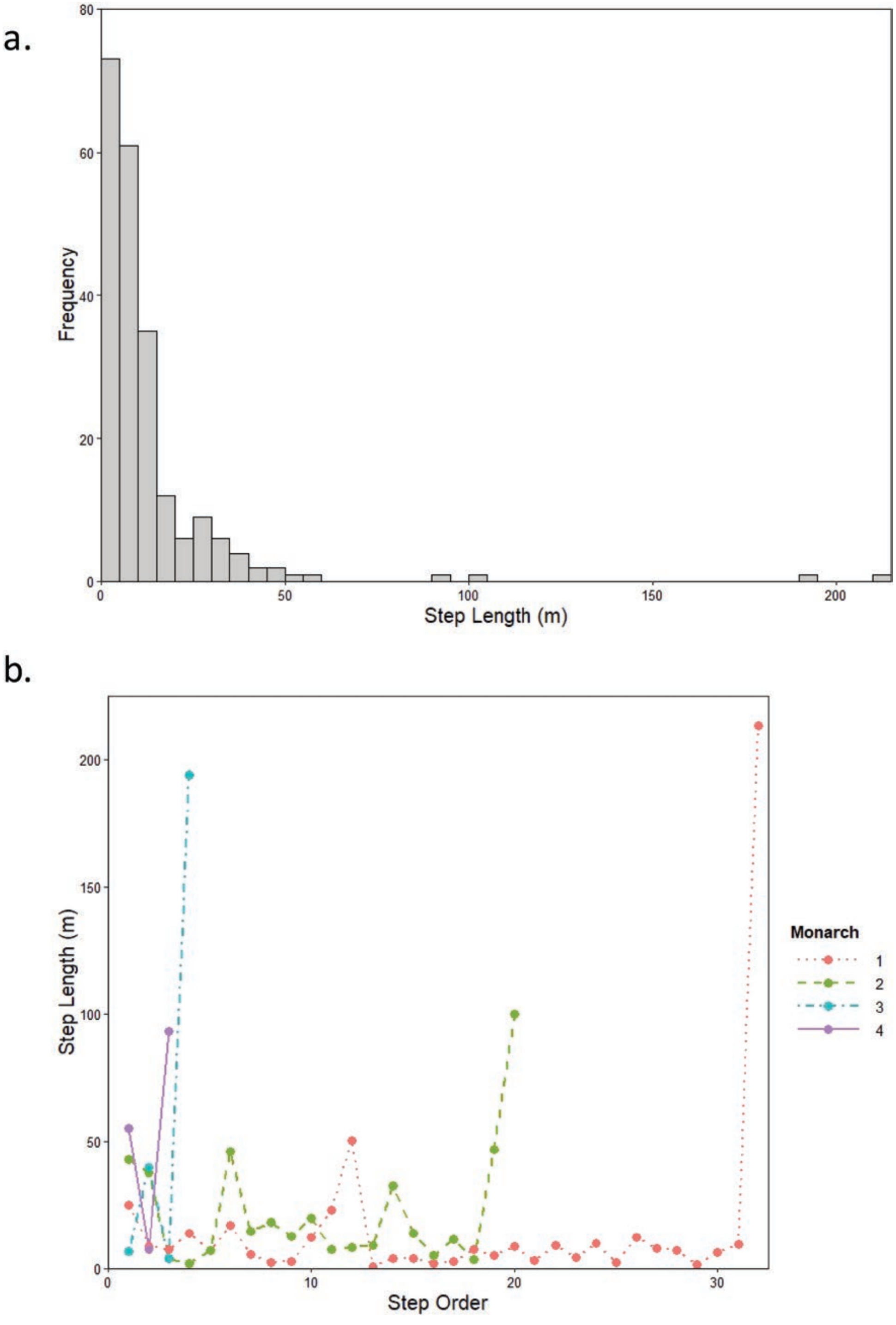

Fig. 5. Characterization of 13 female monarch butterfly flight steps (distances between two estimated locations) tracked with radio telemetry in a restored prairie. The majority of step lengths were <50 m (a). Four monarchs with steps larger than 75 m flew out of the prairie after 2–19 steps (b).

the extent to which our 220-mg transmitter could perturb the flight behavior of monarchs with a mass between 300 and 700 mg (transmitter weight 31–73% of body weight), we conducted experiments with sham-transmitters (300-mg watch batteries) to observe any effects on behavior and flying ability. Approximately ninety percent of our sham-tagged butterflies flew at least once during the observation periods. On average, monarchs flew for 30% of their observed time and approximately half of the monarchs flew from the prairie release point in excess of 100 m. In one trial, we attached a battery to a 300 mg female monarch, and it robustly flew away from the release point, and we were unable to recapture her. Consistent with Hagen et al. (2011), our findings suggest monarch butterflies with transmitters may rest more than those without transmitters. This observation, however, did not preclude the ability to use radio telemetry transmitters to track their flight behavior. Based on our observations, sham-tagged monarchs will likely respond to environmental stimuli and make movement decisions in a manner similar to untagged monarchs.

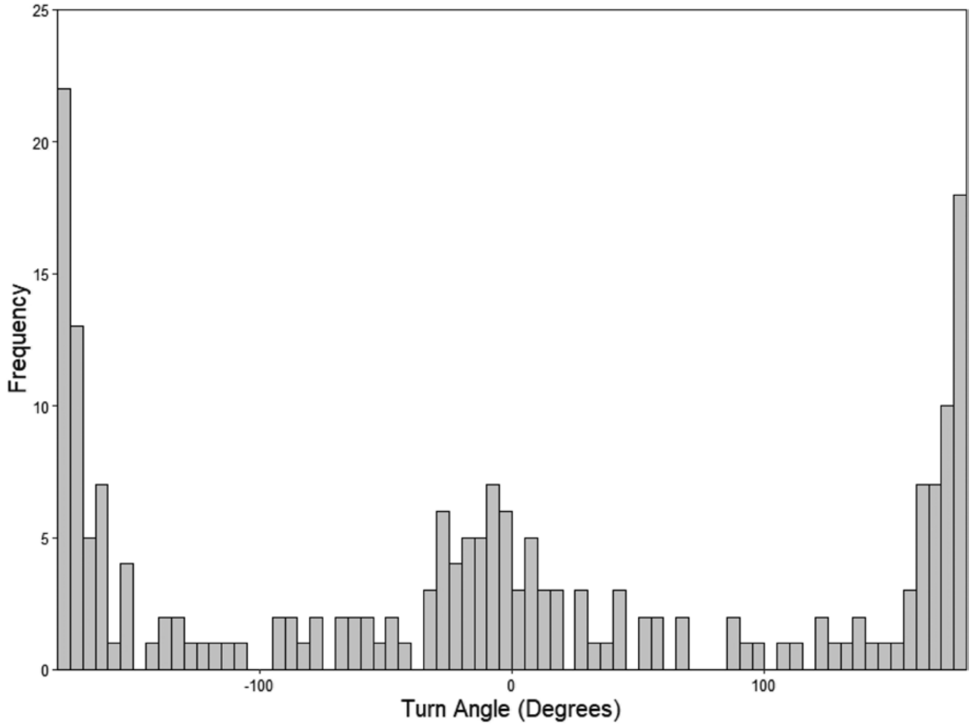

Fig. 6. Frequency of turn angles (angle created from three consecutive, estimated locations) for 13 female monarch butterflies tracking with radio telemetry in a restored prairie. Turn angles near 0 degrees indicate directional flight, while turn angles of ~180 degrees indicate pivoting in a more tortuous flight path.

Inputs of female monarch movement behavior within individual-based models (Zalucki 1983, Zalucki et al. 2016, Grant et al. 2018) are based primarily on an observational study of monarch butterflies using recording theodolites in a grass-dominated, 0.03-ha experimental plot with planted milkweed (Zalucki and Kitching 1982b). In that study, monarchs concentrated most of their activity and flights in areas of the plot that contained milkweed. Within the plot, monarch locations and step lengths were estimated every 1.25 min. In the 0.03-ha plot, all reported steps were under 5 m, and butterflies flew generally straight, with turn angles mostly around 0 degrees. While Zalucki and Kitching (1982b) could estimate the exit angle of monarchs flying out of their 0.03-ha plot, their methodology precluded the means to track flight patterns further.

Using radio telemetry, we expanded understanding of flight steps and directionality by tracking locations of radio-tagged monarchs every minute in a 4-ha restored prairie containing milkweed, nectar plants, and grasses at natural densities and distributions. Within the prairie, we quantified small, undirected steps, while steps associated with exiting the prairie were large with high directionality. These movement patterns are qualitatively consistent with simulation modeling (Grant et al. 2018). We found that the majority of steps within the prairie were below 50 m, and most were under 5 m, consistent with Zalucki and Kitching (1982b). In contrast to Zalucki and Kitching's (1982b) observation of turn angles centering on 0 degrees, we recorded wide turn angles that centered around 180 degrees suggesting that monarchs were pivoting. Within the prairie, these 180-degree turn angles were associated with foraging behavior and monarchs flying small distances from flower to flower in a pattern with low directionality. Consequently, the total distance traveled was much greater than the Euclidian distance displacement.

With the use of radio telemetry, we attained more information about monarch butterfly movement than was possible by visual observation only. Simple mark-recapture or mark-resight studies with flying insects have an estimated recovery rate of 52% (Ide 2002). When our operators lost sight of a butterfly, they were rarely able to relocate the individual with confidence, consistent with observations of sham-tagged monarchs. Four of our radio-tagged monarchs left the prairie, and although we could not detect them visually, each was relocated up to 250 m from the prairie using radio telemetry. When monarchs left the prairie, they took directional steps exceeding 50 m. To the best of our knowledge, quantitative measurements of flight steps of this length with nonmigratory monarchs have not previously been reported. Our findings indicate that by using radio telemetry with monarch butterflies, we can attain movement information at scales relevant for landscape analyses.

Our error ellipses were large, suggesting it is not appropriate to base space utilization on estimated locations between steps (Calabrese et al. 2016, Fleming et al. 2016). Therefore, we incorporated estimated locations and error into occurrence models for nine of the 13 monarchs. Based on the continuous-time movement model, we estimated monarchs spent 98.95% of their time within the prairie, and 1.00, 0.03, and 0.01% in agriculture, road and roadside, and forest, respectively, because error ellipses of the estimated locations included in the model spanned these land cover types. In this time, monarchs had intermediate to high probability of occurring in 37.5% of the 4-ha prairie. Since we captured the monarchs as they crossed the edge of the prairie, these results are not surprising and should not be considered representative of habitat utilization estimates based on data collected for longer time periods and/or across more complex settings. However, these results are encouraging and suggest with sufficient frequency of data collection, continuous-time movement models can be used to estimate habitat utilization when monarchs are tracked over multiple land cover types. These analyses are especially useful to understand how animals move as they encounter different landscape features and to identify corridors between resources (Calabrese et al. 2016, Fleming et al. 2016).

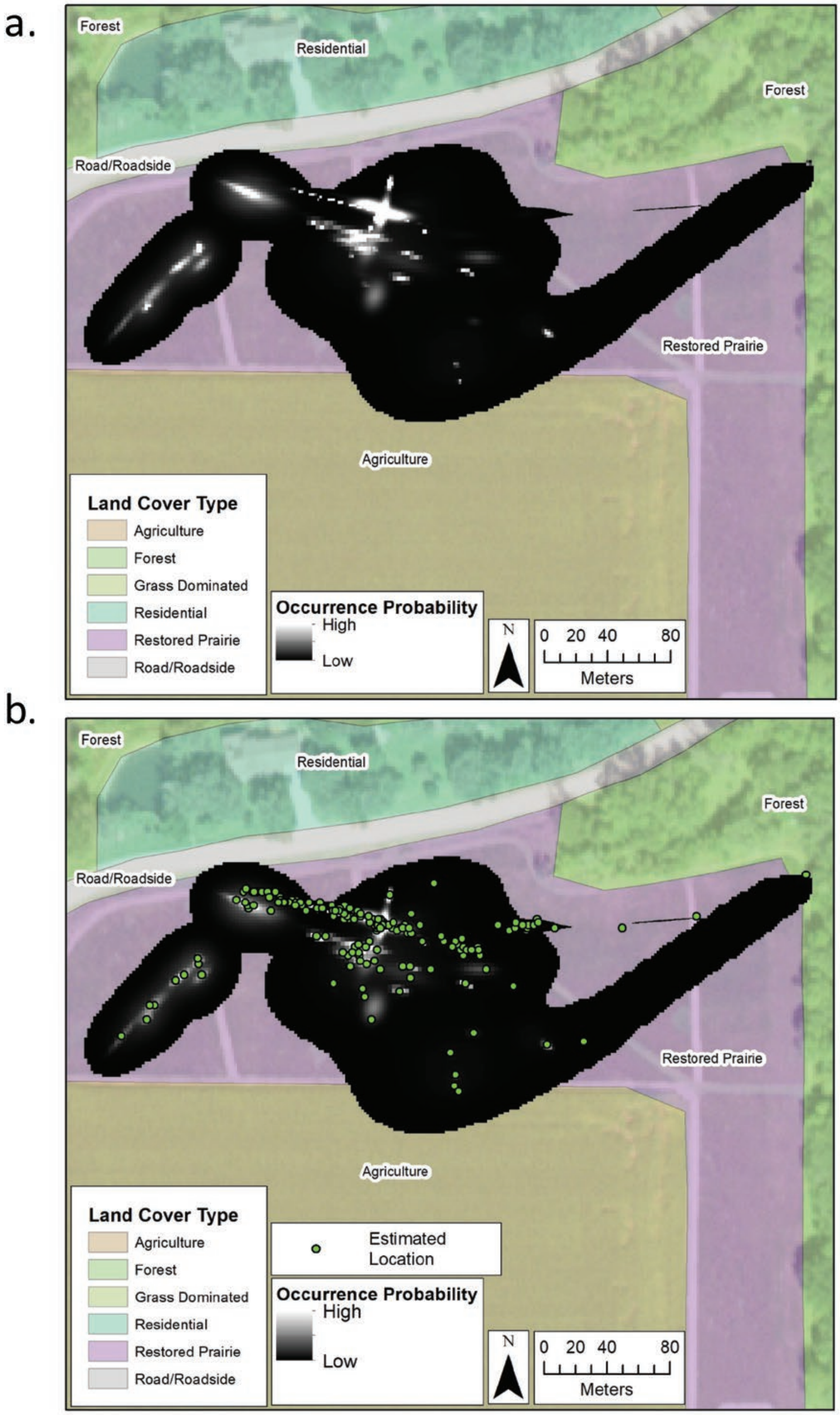

Fig. 7. Occurrence model created using the ctmm package in RStudio for nine radio-tagged monarch butterflies. Black and dark gray shading represent areas with a low probability of occurrence. Light gray and white shading represent areas with a high occurrence probability. Occurrence model of nine radio-tagged monarchs [see (a)] overlaid with estimated locations of the monarchs based on simultaneous triangulation (b).

Table 2. Percent of time observed monarchs occurred in various landcover types, as estimates with ctmm occurrence models

Land cover type	Percent
Restored Prairie	98.95
Agriculture	1.00
Road and Roadside	0.03
Forest	0.01
Grass Dominated	0.00
Residential	0.00

When employing a continuous-time movement model, biologically relevant data collection intervals for the study species is critical. Model estimates of locations and error ellipses are dependent on the elapsed time between data collection periods (Calabrese et al. 2016, Fleming et al. 2016). Improved precision in utilization estimates could be gained with increased frequency of location estimates (Calabrese et al. 2016). While wide time intervals for collecting location data may be appropriate for large, slow-moving animals, for small, quick-moving, vagile species, like the monarch butterfly, data collection intervals in the order of ≤1 min are required. Prior to our study, radio telemetry data sets for flying insects with the shortest collection interval was 30 min (Wang et al. 2019), which is too coarse to quantify adult monarch habitat utilization. By using simultaneous radio telemetry with a 1-min data collection interval, we could successfully track monarch butterflies and recreate flight paths.

Although our method helps increase the temporal resolution of radio telemetry for small, flying insects, there are limitations. Estimated locations were calculated with triangulation of simultaneously estimated bearings. Identifying the true bearing with this method can be difficult. Both errors in bearing estimates and timing of data collection correlated to an increased error in location estimation. Bearings were based on human auditory perception. There was interoperator variation in perceived signal strength when individuals were listening to the same signal. In addition, radio signals can reflect and occasionally produce loud signals in the wrong direction. This method also required four operators to collect bearing information at the same time consistently. To reach this operational objective, bearings were collected at the top of the minute. However, maintaining a 1-min collection interval rushed personnel in making bearing decisions, and occasionally collection times were missed. In these instances, we used three bearings instead of four to calculate locations. To improve bearing estimates, a receiver that reports signal strength would provide a more objective means to identify the bearing with the strongest signal (Wang et al. 2019).

Through the use of radio telemetry, we could track monarch movement in areas larger than reported in previously published studies based on human observation (up to 50 m; Turchin et al. 1991; Schultz 1998; Schultz and Crone 2001; Fownes and Roland 2002; Schultz et al. 2012, 2017; Skorka 2013; Kallioniemi et al. 2014; Fernandez et al. 2016). In our prairie, setting, monarchs fly distances well beyond human visual range, and some of the radio-tagged monarchs flew greater than 250 m in a few minutes. At these distances, estimating locations and flight paths by sight is difficult, if not impossible. While advancing the means to track monarchs at greater distances, our approach requires a team of five field technicians (1 to release the monarch and 4 to collect simultaneous bearings). To ensure data collection occurred simultaneously, and signal would be detected by the four operators at the same time, individuals needed to be stationed within 50–135 m of the release point.

To increase the area covered using this telemetry method, additional operators could be stationed further from the release site to create a grid. Alternatively, the use of automated systems has been suggested to increase scalability and data collection intervals while potentially reducing errors in location estimates (Kissling et al. 2014). A customized, automated system deployed in Panama successfully tracked 38 avian and mammalian species (Kays et al. 2011). Currently, our group is developing and employing an automated radio telemetry system from commercially available equipment to increase the frequency of data collection for flying insects, using the monarch as a model species. This system can complement operator-based, simultaneous radio telemetry reported here to track flying insects and quantify movement patterns and habitat utilization.

Data Accessibility

Data and metadata pertaining to this manuscript are publicly available through GitHub: https://github.com/kelseyefisher/employing-vhf-radio-telemetry-to-recreate-monarch-flight-paths

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

This work was supported by the Agriculture and Food Research Initiative Pollinator Health Program (Grant 2018-67013-27541) from the USDA National Institute of Food and Agriculture, Garden Club of America Board of Associates Centennial Pollinator Fellowship received in 2017, Holohil Grant Program received in 2018, ISU College of Agriculture and Life Sciences, and the Iowa Monarch Conservation Consortium. We thank Signey Hilby and Chloe Weingarten for collecting behavior allocation data in 2017 and 2018, respectively. Additional thanks to Jacqueline Appelhans, Alec Euken, Cory Haggard, Signey Hilby, Anthony Kerker, Riley Nylin, and Julia Pfeiffer for aid in monarch tracking. Dr. Philip Dixon and Audrey McCombs, ISU Statistics Department, provided statistical consulting. Appreciation to the property landowners, especially Barb Licklider, for allowing us to chase butterflies in their restored prairies, wooded areas, and agriculture fields. Special thanks to Justin Calabrese, Christen Fleming, Michael Noonan, and Joe Kolowski for leading the February 2019 AniMove workshop and use of the ctmm package in RStudio for analyzing tracking data. Finally, thank you to Myron Zalucki, Chip Taylor, Rick Hellmich, and USDA-ARS CICGRU for incredibly valuable insight and guidance when developing and planning this project.

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