BEHAVIORAL ECOLOGY −ORIGINAL RESEARCH

Prey species increase activity in refugia free of terrestrial predators

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Abstract

The decline of terrestrial predator populations across the globe is altering top-down pressures that drive predator–prey inter-actions. However, a knowledge gap remains in understanding how removing terrestrial predators affects prey behavior. Using a bifactorial playback experiment, we exposed fox squirrels to predator (red-tailed hawks, coyotes, dogs) and non-predator control (Carolina wren) calls inside terrestrial predator exclosures, accessible to avian predators, and in control areas subject to ambient predation risk. Fox squirrels increased their use of terrestrial predator exclosures, a pattern that corresponded with 3 years of camera trapping. Our findings suggest fox squirrels recognized that exclosures had predictably lower predation risk. However, exclosures had no effect on their immediate behavioral response towards any call, and fox squirrels responded most severely to hawk predator calls. This study shows that anthropogenically driven predator loss creates predictably safer areas (refugia) that prey respond to proactively with increased use. However, the persistence of a lethal avian predator is sufficient to retain a reactive antipredator response towards an immediate predation threat. Some prey may benefit from shifting predator–prey interactions by gaining refugia without sacrificing a sufficient response towards potential predators.

Keywords Antipredator response · Automated behavioral response · Playback experiment · Predation risk · Predator exclosure

Introduction

A defining feature of the Anthropocene is that humans are reducing and altering wildlife communities around the world (Dirzo et al. 2014; Pievani 2014). One of the most important changes is humanity's alteration of predator communities (Cardillo et al. 2005; Ripple et al. 2014; Worm 2015). Predators create top-down pressures on the environment, and reconfiguring predator communities can alter prey communities (Galetti and Dirzo 2013; Dorresteijn et al. 2015) and initiate trophic cascades (Estes et al. 2011; Suraci

et al. 2019a). These effects can be transmitted via numeric responses or changes in prey behavior alone (Cherry et al. 2016; Suraci et al. 2016; Winnie and Creel 2017; Zanette and Clinchy 2020; Allen et al. 2022).

Predators affect prey behavior by causing prey to initiate fear responses, prompting prey to engage in an antipredator behavioral response (Laundré et al. 2010) that is crucial to prey survival (Zanette and Clinchy 2020). However, engaging in these behaviors comes as a tradeoff to performing other behaviors, such as foraging (Lima and Dill 1990; Verdolin 2006). To optimize fitness, prey must balance their antipredator behavior in a way that minimizes predation risk while maximizing foraging opportunity. Failure to respond to an acute predation risk can result in prey being killed, while overreacting to predation risk causes animals to miss out on foraging opportunities unnecessarily (Brown 1999). To determine the optimal balance of antipredator behavior and foraging, prey rely on predation risk cues.

Predation risk cues can come directly from predators (e.g., predator vocalizations) or the environment (e.g., availability of cover; Thorson et al. 1998), and prey can respond proactively (e.g., avoiding risky areas) or reactively (e.g., fleeing or vigilance). According to the control

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of risk hypothesis when predation risk is spatially and temporally predictable, prey utilize proactive behaviors to reduce risk. In contrast, when predators pose an acute or unexpected threat prey rely on reactive behaviors (Creel 2018). Thus, when changes to predator communities result in more predictable patterns of risk, prey are likely to respond by altering their proactive behavior; when changes to the predator community result in less predictable patterns of risk, prey are likely to respond by altering their reactive behavior.

One of the key ways that humans alter predator communities is through predator extirpation (Estes et al. 2011; Atkins et al. 2019). Terrestrial predator extirpation is common in human-dominated landscapes (Ripple et al. 2014; Marneweck et al. 2021). This is especially true in urban green spaces where parks, campuses, and open spaces are often devoid of terrestrial predators (Crooks 2002) despite maintaining terrestrial prey species like squirrels (Family: *Sciuridae*; Riem et al. 2012). However, because terrestrial predator extirpation is often coupled with other marked environmental changes (e.g., habitat loss/landcover change, fragmentation, increased human presence, supplemental food, and altered vegetation structure), it can be difficult to isolate its influence on prey behavior (McCleery et al. 2020). One way to isolate the effects of human-induced terrestrial predator extirpation on prey behavior from other confounding facets of human disturbance is to recreate the disturbance of interest in an otherwise undisturbed area (e.g., Ware et al. 2015).

Prey may respond to changes in predator presence by altering their space use. Extirpation or reductions of terrestrial predators should convert formerly risky areas into safer ones, creating refugia for prey (Muhly et al. 2011; Conner et al. 2016; Epperly et al. 2021). This increased availability of refugia suggests predator extirpation may manifest in alteration of prey species use of space. Such changes should persist as long as the refugia remain less risky than adjacent areas where predators are more active (McNicol et al. 2020). The loss of predators may also alter the frequency and magnitude of prey's reactive antipredator behaviors by reducing overall risk. In situations when most risk has been eliminated, prey may become fearless and fail to engage in antipredator behavior (Suraci et al. 2017b).

On the other hand, non-terrestrial avian predators often benefit from human development and other anthropogenic disturbance (Berry et al. 1998; Chace and Walsh 2006; Kumar et al. 2018), and are less likely than terrestrial predators to become extirpated. It is, therefore, important to consider the possible synergistic effects of terrestrial predator extirpation and avian predator persistence on prey behavior. Species that are prey of both avian and terrestrial predators may, therefore, not change their use of space due to the loss of terrestrial predators because of the increased presence of avian ones. Similarly, the persistence of any predator might maintain the prey's reactive antipredator behaviors (Blumstein 2006).

In this paper, we use a bifactorial experiment to isolate the influence of terrestrial predator extirpation on the space use (proactive behavior) and antipredator response (reactive behavior) of a model prey species, the eastern fox squirrel (*Sciurus niger*) towards terrestrial and avian predators. We compare our short-term (approximately one month) experimental results to observations from a longer-term 3-year camera trapping study to assess changes in space use inside terrestrial predator exclosures across different temporal extents. Specifically, we test the prediction that prey alter proactive antipredator behavior by increasing use of areas where terrestrial predators are extirpated as these areas create predictable refugia and promote space use (Muhly et al. 2011; Conner et al. 2016). Second, we test the prediction that prey reduce the magnitude of their reactive antipredator response in areas of lower overall predation risk (Fowler et al. 2018), consistent with what might be expected when terrestrial predators are extirpated. Lastly, we predict that terrestrial predator extirpation will have no effect on prey's reactive antipredator response to perceived avian predation risk since avian predators should not be influenced by our terrestrial predator exclosures.

Methods

To understand the effects of anthropogenic alterations to terrestrial predator communities on prey proactive and reactive antipredator behaviors, we conducted a bifactorial experiment using terrestrial predator exclosures and predator playbacks on a model prey species, the eastern fox squirrel. To assess prey proactive antipredator behavior, we compared fox squirrel visitation at points in terrestrial predator exclosures and in control areas that maintained ambient terrestrial predation risk. We investigated fox squirrel reactive antipredator behavior by measuring two reactive antipredator behaviors of varying magnitude, time spent vigilant (lower magnitude response) and fleeing (higher magnitude response).

Study site and species

We conducted our experiment during February–March 2018 at The Jones Center at Ichauway (Ichauway), an approximately 12000 ha private research facility located near Newton, Georgia, USA (Fig. 1a). Ichauway is predominantly comprised of an upland longleaf pine (*Pinus palustris*) savanna, with interspersed oak (*Quercus* spp.) hardwoods occurring sporadically throughout the pine matrix. Longleaf pine savannas are characterized by an open canopy,
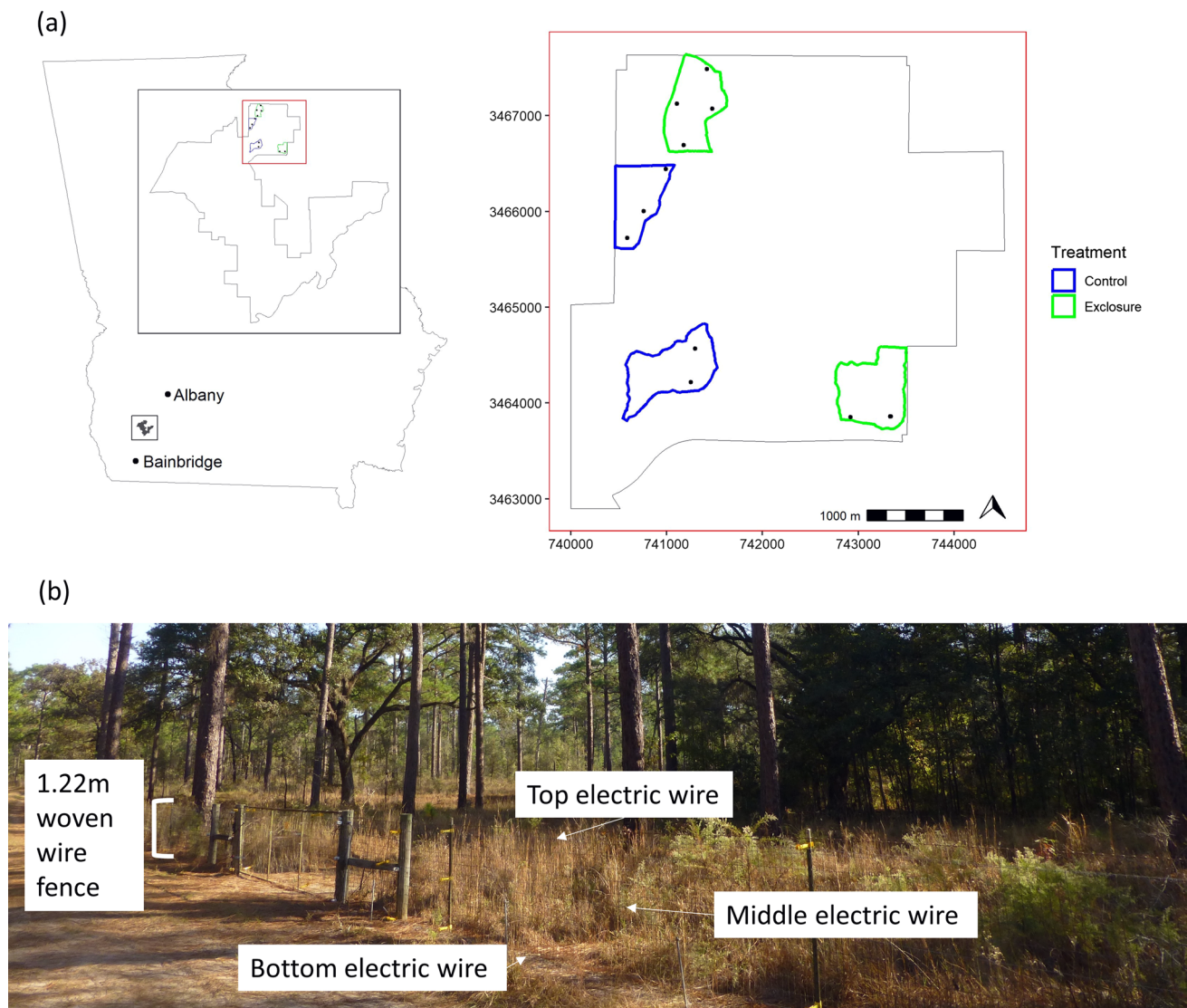
(a)

(b)

Fig. 1 a Map of Ichauway showing the location of ABR points (black dots) inside terrestrial predator exclosures and associated control areas. The exclosure **b** was composed of 1.22 m tall woven wire fence with an electrified wire running across the bottom, midpoint, and top of the fence to prevent access to terrestrial mammalian predators

non-existent midstory, and an open, park-like understory, dominated by wiregrass (*Aristida* spp.) and other forbs. Ichauway uses prescribed fire on a 2-year rotation to prevent hardwood encroachment and manage for longleaf pine savanna.

We conducted our research on the eastern fox squirrel, a highly mobile (Potash et al. 2018) diurnal tree squirrel found throughout most of the eastern United States and into southern Canada. Fox squirrels inhabit a wide variety of land cover types, including urban and suburban areas where humans have the greatest effect on native animal communities (McKinney 2002). Fox squirrels in the Southeastern US, including those at Ichauway, are among the largest North American tree squirrels, with adults weighing up to 1200 g

(Koprowski 1994). In the southeastern coastal plain, the fox squirrel is native to the longleaf pine savanna. Changing predator communities in human-dominated areas indirectly affects fox squirrel distribution (Van Der Merwe et al. 2005).

The predator community of interest at Ichauway included avian and terrestrial predators, notably the red-tailed hawk (*Buteo jamaicensis*), coyote (*Canis latrans*), and free-roaming dogs (*Canis lupus familiaris*). Bobcats (*Lynx rufus*), red and gray foxes (*Vulpes vulpes*, *Urocyon cinereoargentus*, respectively), and smaller mesopredators also occurred at Ichauway, but were considered unlikely to influence our study system. Hunting dogs were used during quail (*Colinus virginianus*) hunting season (November–February) at Ichauway, but quail hunting did not occur in or around the

study areas. Free-roaming dogs have been members of North American predator communities for several centuries (Leath-lobhair et al. 2018), and occur in low densities year-round at Ichauway across a wide range of habitat types (Smith et al. 2006). In general, fox squirrels show greater magnitude antipredator behaviors towards perceived risk from avian predators than from terrestrial predators (McCleery 2009; Kittendorf and Dantzer 2021).

Terrestrial predator exclosures

In 2003, four 40 ha terrestrial predator exclosures were created at Ichauway and the exclosures have been actively maintained since. Each exclosure was surrounded by a 1.22 m tall woven wire fence with three electrified wires running across the bottom, middle, and top of the fence (Fig. 1b). Each exclosure was paired with a nearby approximately 40 ha control where there were no barriers to terrestrial predators. We conducted our experiment in two of the terrestrial predator exclosures and in their two associated controls. The exclosures were highly effective at preventing access to medium and large-sized terrestrial mammalian predators commonly found in areas of increasing human development, including coyotes, free-roaming dogs, and other meso-mammals (Conner et al. 2010). Exclosures did not prevent access by avian predators or snakes. Small terrestrial mammals, including fox squirrels, could pass through exclosure boundaries through gaps in the fence (Potash et al. 2018). We established research points in exclosures and controls, and to maintain independence, we separated all concurrently operating points by a minimum of 500 m, the mean maximum distance moved by fox squirrels in the Southeastern US over a week (Greene and McCleery 2017a).

We established all research points in longleaf pine savannah, away from areas of dense canopy cover (hardwood hammocks) where competitively dominant gray squirrels (*Sciurus carolinensis*) excluded fox squirrels (Sovie et al. 2020). The landcover of each exclosure and control was over 75% pine and mixed forest (Online Resource 1) and there was minimal variation in forest composition and overhead cover between all exclosures and controls. To minimize variation in understory vegetation characteristics across points, all exclosures and controls were burned within 1 week of each other in January 2017.

Predator playbacks and behavior video (ABRs)

We used a playback experiment to manipulate perceived predation risk and assess reactive antipredator responses (Hettena et al. 2014). We implemented the playback experiment using an Automated Behavioral Response (ABR) system. An ABR pairs a motion-triggered wildlife camera (Browning Model BTC-8FHD-PX, Birmingham, AL, USA)

with a speaker, such that an animal triggering the camera initiates the speaker to play one of the calls (for a full review of the ABR method, see Suraci et al. 2017a). We contrasted the vocalizations of three different predator species against one non-predator control species. The predator species vocalizations included: (1) red-tailed hawk calls, (2) coyote barks, and (3) dog barks. For the non-predator control treatment, we played the songs of Carolina wrens (*Thryothorus ludovicianus*). We obtained raw audio files for vocalizations from Xeno-Canto (https://www.xeno-canto.org) and YouTube (https://www.youtube.com). We used Audacity version 2.2.2 (https://www.audacityteam.org/) to shorten the raw files into 10-s-long clips, reduce background noise, and standardize the volume of all calls to approximately 80 dB at 1 m in front of the ABR speaker. We created eight different exemplars for each species to reduce the likelihood of study specimens habituating to a repeated call.

Following a well-established protocol developed in prior ABR experiments (Epperly et al. 2021; Crawford et al. 2022; Widén et al. 2022; Rigoudy et al. 2022), the treatment broadcast if the ABR was triggered changed every 15 min. For example, if the ABR triggered between noon and 12:15 PM, one treatment would broadcast (e.g., coyotes), between 12:15 and 12:30 a different one would (e.g., dogs), and so on. We ensured the broadcast of treatments was balanced and randomized across the diel cycle by programming the ABR to broadcast each of the four treatments each hour, in a different random order each hour. Within the 15-min interval during which a given treatment was programmed to be broadcast, the specific exemplar played was randomly selected each time the ABR was triggered.

Field methods

We measured behavior at seven points within each treatment (exclosure or control), for a total of 14 points spread throughout the two exclosures and their paired controls. We selected points that we previously used to research fox squirrel fear (Potash et al. 2019), and selected additional points where we observed recent fox squirrel sign. Not all points were visited by fox squirrels. Points without fox squirrel activity provided no data, and were, therefore, excluded from our analysis, resulting in seven points for the exclosures and five points for the controls for a total of 12 points from which we collected data. At each point, we deployed an ABR. We set the camera to immediately begin recording a 30-s video when triggered. Nine seconds after the camera triggered, the speaker played one of the 10-s exemplars. The camera continued to record video for 11 s once the predator call finished playing.

We deployed all ABRs using a consistent setup to minimize variations in camera angle and the distance between squirrels and the playback speaker. We mounted each ABR

camera to a tree at a height of 120 cm and angled towards the ground at approximately 60°. To improve our video capture success and to standardize local vegetation conditions, which can affect predator-specific fox squirrel antipredator behavior (Potash et al. 2019), we cleared all ground vegetation in view of the camera. We mounted the speaker approximately 10 cm above the camera and angled it directly outward, facing the same direction as the camera. Between 200 and 230 cm in front of the camera, we centered four overturned rubber buckets (Fortiflex Inc., Durango, Puerto Rico) in a 2×2 pattern (Fig. 2) and baited each overturned bucket with a single whole pecan. One or two study personnel visited each point twice per day, once within 1 h of sunrise to replenish bait, and again within 1 h of sunset to retrieve SD cards. We checked cameras within 1 h of sunrise and sundown to avoid interfering with fox squirrels, which are most active in the late morning (Sovie et al. 2019). Although fox squirrels are diurnal, we kept the ABRs active 24 h per day. This ensured we captured all fox squirrel activity, including activity that occurred before or after we checked cameras around sunrise and sunset, respectively. We identified individual fox squirrels (Squirrel_ID) based on their unique pelage markings and other physical characteristics, which are highly variable between individuals (Tye et al. 2015; Potash et al. 2020).

To investigate the persistence of fox squirrel use of terrestrial predator exclosures for a 3-year period, we established two camera (HCO VH400, HCO Outdoors Inc., Norcross, GA) trap stations in each of the exclosures and controls in 2019 and operated them continuously until 2022. We used the default sensitivity settings and programmed a 30-s delay between consecutive triggers. Over the 3-year period, we visited camera traps weekly to retrieve data, replace batteries, and maintain the camera's field of view (i.e., clearing vegetation). All field methods were approved by the

Fig. 2 View of bait station used to attract fox squirrels to our Automated Behavioral Response systems to video-record their responses to playbacks of predator (coyote, dog, red-tailed hawk) or non-predator (Carolina wren) calls

University of Florida's Institutional Animal Care and Use Committee (IACUC# 201709906).

Behavior scoring

To avoid observer bias, we scored videos using a two-step process. First, one observer watched each video with the sound muted (i.e., blind to the call type and exposure), and recorded the proportion of time a fox squirrel spent with its head up (vigilant) and whether the fox squirrel fled. Once all videos and behaviors were scored by the first observer, a different observer reviewed each video to identify the call type and exposure. Both researchers scored videos using Solomon Coder version beta 19.08.02 (Péter 2011). Once videos had been scored, we considered each video to be a unique observation, and henceforth use the term observation to refer to the behaviors scored in a single video.

Data processing/cleaning

We scored videos in which the fox squirrel was in view of the camera before and after the call played. Using only videos in which fox squirrels were recorded before and after the call played reduced bias by ensuring that any observed behaviors were a response to the playback stimulus. Similarly, videos in which multiple fox squirrels interacted with one another were not scored to eliminate potentially confounding behavioral responses driven by intraspecific competition or other conspecific stimuli, rather than to perceived predation risk.

Repeated exposures of the same fox squirrel to the same treatment within an hour were not considered independent and we accordingly classified each exposure as to whether it was a "first" or "repeat" within an hour, as done in prior ABR experiments (Crawford et al. 2022; Rigoudy et al. 2022; Widen et al. 2022). The "first" and "repeat" exposures to the same treatment within an hour comprised an "independent exposure bout" (henceforth, bout; Rigoudy et al. 2022; Widen et al. 2022). We investigated the "first" and "bout" scales separately. For the behavioral event flight, the "first" dataset included only the first exposure observations from each bout. For the "bout" dataset, we collapsed all observations within a bout into a binary flight response (1 = fled, 0 = did not flee) depending on whether the fox squirrel fled at any time during the bout. For all observations in which a fox squirrel did not flee, we calculated the proportion of time the fox squirrel had its head up (vigilant) from the time the playback began until the end of the video, a maximum of 21 s. As with the flight response, we investigated vigilant behavior at two scales: "first" and "bout". The "first" dataset contained the proportion of each behavior

from only first exposures. For the "bout" dataset, we used the median value of the proportion of vigilant behavior from all observations in a bout.

Statistical methods

The programming of ABRs to balance and randomize which treatment was broadcast over the diel cycle meant there should be an equal probability of fox squirrels receiving each of the four treatments, but it was nonetheless important to verify this. We did so using a Chi-square test to compare the number of observations per call treatment. For the Chi-square test, we used only the "first" exposure observations. We used only the "first" observations because fox squirrels were naïve to the first call type of each bout, and therefore, each first observation was independent, unlike subsequent repeat exposures. For all statistical analyses, we used $\alpha < 0.05$ as a cutoff value for identifying significant differences in model outputs. We conducted all analyses using program R version 4.0.5 (R Core Team 2021).

To test our first prediction, that terrestrial predator exclusion created predator refugia, and therefore, caused prey to alter proactive antipredator behavior in both the short- and long-term, we compared fox squirrel visitation frequency at points in exclosures and controls. To look at broad aggregated changes in visitation in the short-term, we used a non-parametric Chi-square test to determine whether fox squirrel visitation frequency was significantly greater inside exclosures than controls. We selected the Chi-square test because all points were spatially independent and unpaired (McHugh 2013). Furthermore, the Chi-square test can be applied to datasets with unequal sizes of sample groups, as was the case in our experiment, by accounting for differences in sample size when calculating expected values (McHugh 2013). To investigate the long-term persistence of increased proactive behavior (i.e., increased use of exclosures), we used camera trap data from 2019 to 2022 and used a Chi-square test to determine whether the observed fox squirrel visitation at exclosures significantly exceeded the expected number of visits.

We used generalized linear mixed models (GLMMs) to test our second prediction, that in areas where terrestrial predators were extirpated, fox squirrels would exhibit reduced reactive antipredator behavior towards perceived predation risk because of lower overall predation risk (Fowler et al. 2018). In our models, we included Squirrel_ID nested within Point as a random intercept effect to account for variation between unique fox squirrels and unmeasured heterogeneity at points. To evaluate the binary response variable fleeing (i.e., fled or did not flee), we conducted a mixed logistic regression with an interaction between the fixed effects Call Type (hawk, coyote, dog, non-predator control) and Site Treatment (exclosure, control). If we found

no significant interaction between Call Type and Site Treatment, we refit the model using both fixed effects in an additive fashion. This analysis allowed us to compare the odds of a fox squirrel fleeing after hearing a terrestrial predator cue (coyote or dog) versus hearing an avian predator (red-tailed hawk) or non-threatening (Carolina wren) cue in exclosures and controls.

To evaluate variation in vigilance behavior, we excluded all observations in which a fox squirrel fled. We then converted time spent vigilant after the call played into proportion of time vigilant by dividing the total time vigilant after the call played by the length of time the fox squirrel was in sight of the camera after the call. In ecological studies, proportion data are often modeled using a beta distribution (Douma and Weedon 2019), which can incorporate data between, but not including, zero and one. For proportion data such as ours that include real instances of zero and one (i.e., no time spent vigilant or the entire time spent vigilant), it is common to transform the values to compress all values into a range of zero and one. We transformed our data following the recommendation of Smithson and Verkuilen (2006; Eq. 1):

$$y_i' = \frac{y_i(n-1) + 0.5}{n} \tag{1}$$

where y_i is the observed proportion of vigilance for the ith observation, and n is the total number of observations. This transformation minimally alters the observed values while compressing zero and one values into the 0–1 number space. Using the transformed proportion of time spent vigilant as a response variable, we fit the same models as described above for the flight response.

We fit all models using the package glmmTMB (Brooks et al. 2017), and tested parameter significance using a type II Wald Chi-square test implemented through the Anova function in package car (Fox and Weisberg 2019). We used the contrast function in package emmeans (Lenth 2021) to conduct pairwise comparisons between factor levels using a Sidak adjustment for multiple comparisons.

Results

We obtained 1457 fox squirrel videos across all points, of which we scored 1078. The remainder were not scored either because the fox squirrel was not in the camera's field of view both before and after the playback sounded (115 videos) or because conspecific interactions occurred (264 videos). Of the videos scored, 727 (67%) were recorded inside the exclosures and 351 were recorded in the controls. We observed 31 unique squirrels, of which 23 were observed inside exclosures. There were 291 unique bouts, and therefore, the same

number of first exposure observations. Among the first exposure observations, we found no significant difference in the number of observations from each call ($X^2 = 1.69$, df = 3, $p = 0.64$), verifying that call type was balanced across our study. For our statistical tests, results did not vary between the "first" and "bout" scales, so we only report results from first exposures for all analyses.

We found support for our first prediction, a proactive antipredator response to manipulated terrestrial predator extirpation in both short and long-term temporal scales. Fox squirrels disproportionately visited points in predator exclosures more than controls. Of 291 first exposure observations, 58.3% came from within exclosures, indicating a significant shift in proactive antipredator behavior in the short term ($X^2 = 13.8$, df = 1, $p < 0.001$). Between 2019 and 2022, long-term camera trapping recorded significantly more fox squirrel observations in exclosures ($n = 72$) compared to fox squirrel observations in controls ($n = 28$; $X^2 = 19.4$, df = 1, $p < 0.001$; Fig. 3).

We did not find support for our second prediction, that fox squirrels would show a reduced reactive antipredator response inside terrestrial predator exclosures. We observed fox squirrels fleeing 49 times ($n\_control = 15$; $n\_exclosure = 34$), resulting in 242 observations in which we were able to quantify vigilant behavior. We found no significant interaction between Predator Exclosure Treatment and Playback Type for either the probability of fleeing ($X^2 = 2.20$, df = 3, $p = 0.53$) or proportion of time vigilant ($X^2 = 2.86$, df = 3, $p = 0.41$), nor was there a significant Predator Exclosure Treatment effect for either response (fleeing: $X^2 = 0.02$, df = 1, $p = 0.88$; vigilant: $X^2 = 0.83$, df = 1, $p = 0.36$) in the

additive model. Playback Type was the sole influence on fox squirrel reactive antipredator behavior response (fleeing: $X^2 = 11.57$, df = 3, $p = 0.009$; vigilant: $X^2 = 12.31$, df = 3, $p = 0.006$), and fox squirrels were significantly more responsive to hawk calls than the non-predator control (wren) calls, irrespective of the exclosure. Fox squirrels fled 31.5% (95% CI 19.56–43.5%) of the time to hawks compared to only 13% (5.39–20.8%) to non-predator controls (odds ratio [Hawk/Wren] = 3.06, SE = 1.33, $p = 0.04$; Fig. 4a). Similarly, fox squirrels spent 71.1% (64.8–77.4%) of the time vigilant after hearing a hawk call compared to only 53.8% (46.9–60.1%) of the time after hearing a non-predator control call (odds ratio [Hawk/Wren] = 2.09, SE = 0.44, $p = 0.002$; Fig. 4b). Fox squirrels were not significantly more responsive to hearing coyote or dog calls than non-predator control calls, nor was there any difference in behavioral response towards dogs compared to coyotes (Table 1).

Discussion

We found that fox squirrels responded to terrestrial predator extirpation by adjusting their space use (i.e., proactive response) to take advantage of terrestrial predator-free refugia but did not alter their reactive antipredator responses in relation to whether they were inside or outside of these refugia. Our findings support predictions from the control of risk hypothesis, which suggests that predation risks that are predictable (i.e., decreased terrestrial predation risk in exclosures) and controllable (i.e., use of exclosures) result in prey altering their proactive behavior (Creel 2018). Fox squirrels likely increased their use of predator exclosures because they perceived exclosures as predictably safe areas with reduced predation risk across three years (Fig. 3). These findings suggest that fox squirrels persisted in greater densities inside exclosures relative to controls. Although it was possible that densities varied between exclosures and controls due to differences in demographic processes such as population growth rate (Allen et al. 2022) and offspring survival (Zanette et al. 2011), we believe this was unlikely since exclosures were permeable to fox squirrels (Potash et al. 2018). Fence permeability allowed fox squirrels to respond dynamically (i.e., proactively) to variations in spatial predation risk by increasing use of safer areas.

We expected but found no evidence that terrestrial predator extirpation created landscapes of minimal risk where squirrels optimized their behaviors by reducing the magnitude of their reactive antipredator behaviors (Brown 1999). While it is possible that the consistent behavioral response we observed across treatments simply reflected a generic startle reaction to an unexpected loud noise, significant differences between fox squirrel reactive responses to the wren and hawk calls indicate otherwise. We suggest that
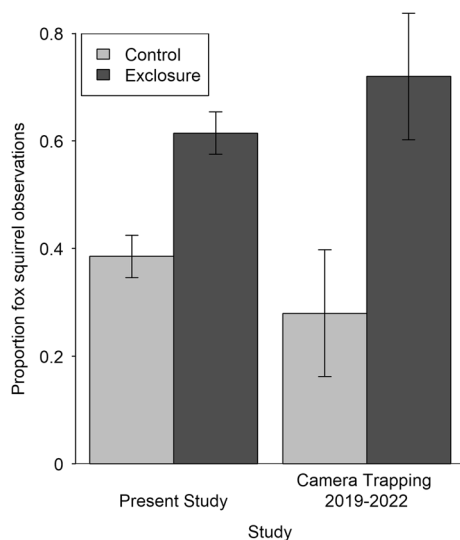

Fig. 3 Proportion of fox squirrel observations with 95% confidence intervals from terrestrial predator exclosures and controls at Ichauway in the present study (ABR experiment) and long-term camera trapping data

Fig. 4 Fox squirrel reactive antipredator responses after hearing predator or non-predator calls inside terrestrial predator exclosures and controls. Bars show the estimated mean and 95% confidence intervals for (**a**) probability of fleeing after hearing a call, and (**b**) proportion of time spent vigilant after hearing a call
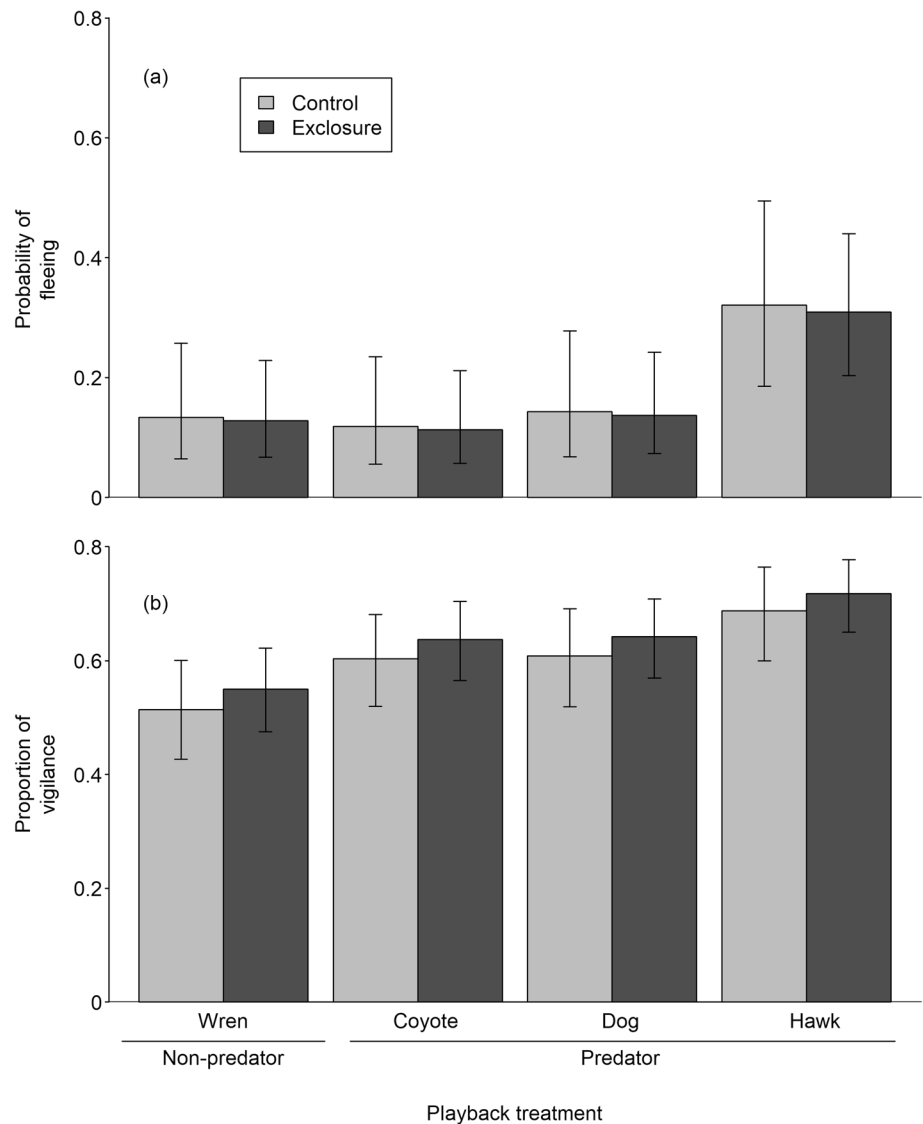

Table 1 Contrasts between fox squirrel behavioral response towards playbacks from predator (red-tailed hawk, coyote, and dog) and non-predator control (Carolina wren) calls

Behavior	Contrast	Odds ratio (SE)	P value
Flight	Hawk/wren	3.06 (1.33)	0.04
	Coyote/wren	0.87 (0.43)	0.99
	Dog/wren	1.08 (0.52)	0.99
	Dog/coyote	0.81 (0.40)	0.99
Vigilance	Hawk/wren	2.09 (0.44)	0.002
	Coyote/wren	1.44 (0.29)	0.25
	Dog/wren	1.47 (0.30)	0.19
	Dog/coyote	0.98 (0.20)	0.99

the indistinguishable reactive response between controls and exclosures was due to the consistent presence of avian predation risk in both treatments. Avian predators are commonly seen in green spaces (e.g., parks, neighborhoods, and sprawling campuses) embedded in developed landscapes (Berry et al. 1998; Chace and Walsh 2006; Kumar et al. 2018), where humans have extirpated terrestrial predators but prey, including fox squirrels, remain extant (McCleery 2010; Greene and McCleery 2017b). Terrestrial predator extirpation is only one facet of urbanization (McCleery 2010) and the process of urbanization comprises a variety of other factors, including the increased presence of humans, which may alter reactive antipredator behaviors not observed in our study (McCleery et al. 2008; McCleery 2009). These

contrasting findings highlight the need to better understand how wildlife behavior responds to the individual components of urbanization (e.g., terrestrial predator extirpation and human presence) rather than broadly attributing differences in behavior across a human disturbance gradient to urbanization in general. Further research using ABRs can help isolate and assess how the components of urbanization and predator-specific effects impact prey behavior by including human voices as a playback call (Clinchy et al. 2016; Suraci et al. 2019a; Crawford et al. 2022), and by varying the placement of the ABR speaker relative to bait sites to match each predator's hunting domain (i.e., avian or terrestrial).

Fox squirrel responses to coyote and dog calls inside and outside the exclosures were virtually identical and did not differ from the behavioral response to the control call (Fig. 4). Weak or non-significant reactive responses to dog playbacks have been reported in several recent experiments conducted on ungulates and carnivores in Europe and North America (Clinchy et al. 2016; Suraci et al. 2019b; Widén et al. 2022) including one conducted on white-tailed deer (*Odocoileus virginianus*) at the same study site as ours (Ichauway) which found even weaker reactive responses to coyote playbacks than dog playbacks (Crawford et al. 2022). These and other recent experiments (Epperly et al. 2021; Palmer and Packer 2021; Rigoudy et al. 2022) have begun revealing that prey demonstrate a "hierarchy of fear" toward their terrestrial predators, and the likelihood of interactive proactive–reactive responses can vary with predator identity. This hierarchy may be informed by prey responses towards predator-specific hunting modes and domains (Preisser et al. 2012), but may also reflect differences in the way that cover impacts prey risk perception (Potash et al. 2019). Thus, even in urbanized areas where some terrestrial predators remain extant, prey antipredator responses towards terrestrial predators may vary from behaviors observed in the wild due to differences in cover between urban and non-urban areas. Moreover, the persistence of terrestrial predators in urban areas may be inconsequential to diurnal prey species as human activity can shield prey from human-averse predators (Berger 2007). We recommend that future research should isolate and experimentally test for interactive effects of urbanization components and predator identity to better understand how terrestrial predator persistence in urban areas affects prey behavior. Further investigation into these predator-specific effects on prey behavior in urban and non-urban areas is an important avenue for future research as the ongoing loss of interspecific interactions poses a threat to ecosystem services and function (Valiente-Banuet et al. 2015).

Terrestrial predator extirpation is one facet of urbanization, and by replicating this anthropogenic disturbance apart from other confounding anthropogenic disturbance, we show that prey alter proactive behavior in response to terrestrial predator loss. Accordingly, we would expect proactive antipredator behavior is one possible explanation for increased squirrel density in human-dominated landscapes (Hadidian et al. 1987; Epperly et al. 2021). More broadly, the exclosures offer insight into the effects of anthropogenic disturbance that, through pathways such as predator extirpation, create novel ecosystems. Our research shows that prey are sensitive to anthropogenic predator community alteration, and alter their proactive antipredator behavior to a new optimal response. These new optimal proactive behavioral responses are likely to be increasingly common as humans continue to alter natural ecosystems at an unprecedented pace.

Declarations

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed. All field methods were approved by the University of Florida's Institutional Animal Care and Use Committee (IACUC# 201709906).

Consent to participate Not applicable.

Consent for publication Not applicable.

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