

Changes in fox squirrel anti-predator behaviors across the urban–rural gradient

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Received: 3 March 2008 / Accepted: 15 January 2009 / Published online: 30 January 2009
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Abstract Predator stimuli created by humans in the urban environment may alter animals' anti-predator behaviors. I hypothesized that habituation would cause anti-predator behaviors to decrease in urban settings in response to humans. Additionally, I hypothesized that populations habituated to humans would show reduced responses to other predator stimuli. I observed three populations of squirrels (urban, suburban and rural) responses to human approaches, red-tailed hawk vocalizations (*Buteo jamaicensis*) and coyote (*Canis latrans*) vocalizations. Mahalanobis distances of anti-predator behaviors in response to human approaches were consistent with the urban–rural gradient. Flight initiation distances ($X^2 = 26.33$, $df = 2$, $P < 0.001$) and amount of time dedicated to anti-predator behavior ($X^2 = 10.94$, $df = 2$, $P = 0.004$) in response to human approaches were also consistent with the urban–rural gradient. Supporting the habituation hypothesis, naive juvenile squirrels increased flight initiation distances ($X^2 = 35.89$, $df = 1$, $P < 0.001$) and time dedicated to anti-predator behaviors ($X^2 = 9.46$, $df = 1$, $P = 0.002$) relative to adult squirrels in the same urban environment. Time dedicated to anti-predator behaviors differed among all

three sites in response to both coyote ($X^2 = 9.83$, $df = 2$, $P = 0.007$) and hawk ($X^2 = 6.50$, $df = 2$, $P = 0.035$) vocalizations. Responses to both vocalizations on rural sites (coyote = 45%, hawk = 55%) greater than twice that found on the urban sites (coyote = 11%, hawk = 20%). This is possibly the first case of a transfer of habituation demonstrated under field conditions.

Keywords Anti-predator behavior · Vigilance · Squirrel · Urban · Urban–rural gradient

Introduction

Urbanization of the planet's landscapes has created new environments that are novel to most wildlife species (Adams et al. 2005). Some wildlife species have been unable to utilize these new environments, while others have altered their population dynamics, physiology, movements, habitat selection, and behavior in response to urban surroundings (VanDruff et al. 1996; Adams et al. 2005; Ditchkoff et al. 2006). Understanding the factors and mechanisms responsible for behavioral changes of wildlife populations in urban settings can help in the management of urban wildlife. Additionally it can help identify reasons behind demographic population level changes and physiological changes that are common in urban populations (Bowers and Breland 1996; Metcalf et al. 2000; Ditchkoff et al. 2006).

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Urban areas can be identified as areas of high densities of humans resulting in major modification to the environment along with high concentration of food, water, energy, materials, sewage, pollution, and garbage (McDonnell et al. 1997). Urban landscapes are diverse and studies of urban wildlife should account for this diversity (Matson 1990). Fortunately, regardless of their structure, urban landscapes usually follow a consistent pattern of heavy human alteration and human presence in a city center that is gradually reduced as the city extends outward to less developed or rural areas (Adams 1994). This landscape transition is commonly referred to as the urban–rural gradient (Matson 1990; McDonnell and Pickett 1990). Instead of simply looking at anthropogenic effects on wildlife at the extremes (in city centers and rural areas) of the gradient, examining the influence of humans on wildlife populations and communities along the urban–rural gradient provides a better understanding of the spatial varying ecological effects radiating out from the city center (McDonnell and Pickett 1990).

Across the urban–rural gradient (and in all landscapes) animals make behavioral choices to balance the risks of predation with the risks of starvation (Brown 1999; Brown et al. 1999; Olsson et al. 2002). As landscape alterations, anthropogenic structures and human activities increase from rural areas to urban centers there are at least two characteristics that should theoretically influence the behavioral choices made by wildlife when balancing predation risks with foraging. First, as human activity increases toward the city center it creates an almost constant predator stimuli for animals in these environments. These disturbances even without the risk of predation may negatively alter an animal's behavior by increasing vigilance behaviors (Berger et al. 1983; Frid and Dill 2002). Animals use vigilance behaviors such as scanning and listening for predators and other anti-predator behaviors such as running, remaining still, alarm calls and threatening gestures to avoid predation (Brown 1999; Alcock 2001). Anti-predator behaviors and vigilance may reduce an animal's risk of predation (Lima and Dill 1990); however, when the risk of predation is reduced, these behaviors can have a negative effect. By limiting the amount of time available for foraging, vigilance and anti-predator behaviors can reduce an animal's energy intake, body condition, and reproductive success (Lima and Dill 1990; Brown 1999).

One well known way that researchers have attempted to measure the influence of human disturbances on wildlife populations is to measure the distance to which humans can approach, before an animal flees; this measure is commonly called the flight initiation distance (Lima and Dill 1990). In urban areas vertebrate species have generally (Knight et al. 1987; Labra and Leonard 1999; Magle et al. 2005) shown decreased flight initiation distances relative to their rural counterparts, indicating that they have a reduced wariness to humans. In spite of the reduced wariness, studies have suggested that the vigilance of urban species is still higher than their rural counterparts (Ward and Low 1997; Randler 2003) but no studies have quantified the differences in behaviors of animals' response to humans. Here I attempted to determine whether animals in urban environments not only reduce their flight initiation distances but, also, alter the amount and types of vigilance and anti-predator behaviors in response to humans.

It has been hypothesized that animals in urban areas that are continually exposed to human stimuli reduce their anti-predator responses and their vigilance behavior through a process of habituation (Knight et al. 1987; Metcalf et al. 2000; Reimers and Sigurd 2001; Magle et al. 2005). In other words, animals have developed a decreased responsiveness to the repeated exposure of the human stimulus (McFarland 1993). Nonetheless, Frid and Dill (2002) claim that habituation to non-lethal human disturbance is usually only partial (Burger and Gochfeld 1981; Burger and Gochfeld 1990) and in some cases animals can even show a stronger anti-predator response to non-lethal disturbances after frequent exposure to them (Dill 1974; Magle et al. 2005). Still, without some mechanisms to reduce behavioral responses to humans, animals would be unlikely to utilize urban environments. I contend that vertebrate species that have been successful in utilizing urban habitats will show a reduction in anti-predator responses and vigilance behaviors, and that the mechanism for the reduction of their anti-predator behaviors is habituation.

The second factor of more urbanized environments that should affect animals' behavioral choices is a reduction in the risk of predation along the urban–rural gradient due to the elimination and avoidance of predators from most urbanized areas (Blumstein

2002; Lopez et al. 2003; Adams et al. 2005). Coss (1999) hypothesized that a reduced or eliminated threat of predation (Anchor et al. 2002; Lopez et al. 2003) in areas may reduce the need for anti-predator behaviors. Moreover, Olsson et al. (2002) presented a model of predation risk that predicts populations with extremely low predation risk, like those found in urban areas, should be free to reduce their use of anti-predator behaviors (Brown 1999; Frid and Dill 2002; Fritz et al. 2002). Nonetheless, this hypothesis and model do not explain what mechanism might cause animals foraging in areas of minimal predation to reduce their vigilance and anti-predator behaviors. I believe that one mechanism that might allow animals to reduce their vigilance especially in urban areas is the transfer of habituation (Hinde 1954).

A potential artifact of habituation to human stimuli may be a reduced response toward other stimuli (Hinde 1954). This transfer of habituation has been shown in laboratory settings but is not known to occur in the wild (Curio 1993); however, researchers have not investigated the different circumstances under which the transfer of habituation may occur (Curio 1993). I believe one reason that field studies have failed was that field setting contained a high risk of predation (Labra and Leonard 1999). Although, the animals' were habituated to one predator stimuli (humans), other stimuli were eliciting useful anti-predator responses to potential predators. So, when presented with new stimuli the animal reacted as if it were responding to a predator.

I believe that in a lab setting or an urban setting with little or no real risk of predation to many species, animals should transfer their habituated response to humans toward other stimuli. As the risk of predation increases along the urban–rural gradient, I would expect to see a reduction in the transfer of habituation to predator stimuli.

To investigate the differences in anti-predator behaviors, habituation, and the transfer of habituation along the urban–rural gradient, I used fox squirrels (*Sciurus niger*). I chose squirrels because they are common and observable in urban and rural environments (Conover 1997; Adams et al. 2006) and have been commonly used in models to investigate hypotheses of vigilance behaviors (Lima and Valone 1986; Newman et al. 1988; Bowers and Breland 1996).

The purpose of this research was to understand the relationships between the urban environment and the

behavior of wildlife. I focused inquiries on the mechanisms and influences that affect animals' anti-predator behavior along the urban–rural gradient. Specifically, I used fox squirrels to test the following five hypotheses:

H1 Flight initiation distances will decrease in response to human approach as the prevalence of humans increase along the rural–urban gradient.

H2 The amount of anti-predator behaviors will decrease in response to human approach as the prevalence of humans increase along the rural–urban gradient.

H3 The types of anti-predator behaviors will change in response to human approach as the prevalence of humans increase along the rural–urban gradient.

H4 Reduction of flight initiation distances and anti-predator behaviors along the urban–rural gradient is due to habituation.

H5 Populations habituated to humans will show a reduced response to predator stimuli in environments of low predation risk.

Methods

Study areas

I conducted research on three sites chosen to represent urban, suburban and rural areas on an urban–rural gradient. The ecology of animals across the urban–rural gradient may be influenced by any number or combination of human associated components (McDonnell and Pickett 1990); however, for this study I choose sites along the gradient that had fox squirrel populations, varying risks of predation on fox squirrel populations and clear differences in amount of human activity.

The urban study site was located on 140 ha of the main campus of Texas A&M University, College Station, TX, USA. There were over 45,000 students, faculty, staff and visitors on the study site daily, and >9,000 students in residence. The study site was dominated by buildings, pavement and manicured exotic grasses. On the center of the site was a dense cluster of buildings, parking lots and streets. Extending

out from this aggregation of buildings was an area of buildings with open and sparsely treed lawns of exotic grasses. The areas surrounding the study site were characterized by dense residential and commercial development. Some of the dominant tree species found along roads and in front of buildings on the study site were live oak (*Quercus fusiformis* and *Q. virginiana*), post oak (*Q. stellata*), Bradford pear (*Pryus calleryana*), arborvitae (*Thuja* spp.) and elm (*Ulmus* spp.). Forty squirrel nest-boxes (Weigl et al. 1989) were placed on trees around the study area to aid in the monitoring of squirrel reproductive activities.

To represent the rural end of the urban–rural gradient, I conducted squirrel behavioral observations on a private ranch 18-km from the urban site. Rural lands can be classified as sparsely settled lands surrounded by agricultural and natural lands (Marzluff et al. 2001). The ranch was surrounded by other ranches and bordered by the Navasota River. The portion of the ranch used for research was ~240 ha of undisturbed bottomland hardwood forest and meadows that had little to no human activity on it. Squirrels had not been hunted or fed on this site for at least 30 years. Some of the dominant trees species on the site included post oak, water oak (*Q. nigra*), pecans (*Carya* spp.), and elms.

The suburban study site was chosen to represent the middle of the urban–rural gradient. Suburban areas are lands on the urban fringe that can be characterized moderate density of single family dwellings, lawns, gardens, and remnant habitat patches (Marzluff et al. 2001; Chapman and Reich 2007; Adams et al. 2006). The study site was comprised of 15 suburban parks (Central Park, Oaks Park, Bee-Creek Park, Anderson Park, Brison Park, Hensel Park, Brothers Pond Park, Luther Jones Park, Thomas Park, Henderson Park, Williamson Park, Sue Haswell Park, Tanglewood Park, Sadie Thomas Park and the Bryan Regional Athletic Complex) ranging in size from 10 to 112 ha. These parks were located on the periphery (5–10 km from the urban site) of the city of College Station, TX, USA and had a variety of landscapes, but were mostly dominated by oaks with open under stories and grass fields. The parks were all surround by single family homes and subdivisions and varied in their rates of human visitation (averaging 32 visitors daily). It is also important to note that unlike the urban site the parks were frequented by dogs and dog walkers. Nonetheless, all the parks

had considerably less human activity than the urban site with its >45,000 residence, visitors and employees and considerably more human activity than the rural study site with almost no human activity.

Independent observations

The urban site was divided into 14 blocks (10 ha), the rural site was divided into 12 (20 ha) blocks, and each of the 15 parks was considered an independent unit (a block). Each park was separated from the nearest park by >2 km. Blocks were created to ensure independent observation of squirrels and so that no squirrel was sampled twice. Although, squirrel ranges vary and squirrels are not know for territoriality (Steele and Koprowski 2001), telemetry data has shown squirrel ranges to be <10 ha in urban areas (R. R. Lopez, personal communication) and studies of fox squirrels in rural areas of Texas have shown ranges of <20 ha (Geeslin 1970).

Predation risk

A concurrent study of squirrel predation on the urban and rural sites showed that predation was the most significant source of squirrel mortality on the rural site, but was negligible on the urban site (McCleery et al. 2008). Predation by mammals or raptors accounted for >65% of the mortalities in a radio telemetry study of 50 squirrels on the rural site, an area with a high risk of predation (McCleery et al. 2008). On the contrary, squirrels on the urban site had a minimal risk of predation. During a 2-year study overlapping the current study, only one of 78 radio-collared squirrels was preyed upon (McCleery et al. 2008). I did not conduct a study of predation rates on suburban sites; however, for this study I assumed that the relative amount of predation on a suburban site was less than that on the rural site and more than that of the urban site. Assuming that the effects of human impacts on the ecology of the urban–rural gradient are scaled is reasonable and effective for measuring its results on target populations (Matson 1990; Bowers and Breland 1996).

Observations

To test each of the five hypotheses, I collected a minimum of 33 squirrel behavioral observations per

site (one observation of each three treatments per block, see independent observations section) from January to November 2005. When it was possible to obtain more observations on unused blocks, I did so to increase sample sizes. Observations were performed during periods of high squirrel activity (from 11:00 to 14:00 hours during fall and summer, and from 12:00 to 03:00 hours during spring and winter; Geeslin 1970). Focal squirrels were obtained within a block after the observer had been positioned under a tree for >4 min. To reduce the possibility of observing a squirrel's response to the observer or other squirrels, observations were not collected if a squirrel was <30 m away from the observer or if other squirrels were known to be present.

Response to human disturbance

To test my first three hypotheses, (1) that flight initiation distance, (2) the amount of anti-predator behaviors will decrease and (3) that the types of behaviors will change in response to human approach as the prevalence of humans increases along the rural–urban gradient, I recorded anti-predator behaviors in response to human approach. Squirrels were approached by an observer at a constant-paced walk from a distance >30 m. When the squirrel fled the observer recorded the distance (flight initiation distance, *approach*) between themselves and the spot recently vacated by the squirrel. To detect changes in squirrel anti-predator behaviors before and after the human approach, the observer recorded the number of specific anti-predator behaviors (Table 1) and the amount of time spent performing these behaviors for a 2-min interval before and a 2-min interval 20 s after the approach. The amount of time spent on anti-predator behaviors was classified into one of four categories (0–24, 25–49, 50–74, or 75–100%).

I examined the differences between flight initiation distances and changes in the amount anti-predator behavior for non-normal data using logistic regression (PROC LOGISTIC) (SAS Institute 1999) and examination of graphically represented means and standard errors. To determine overall differences in the types of behaviors displayed across the urban–rural gradient, I used non-parametric discriminate analysis (SAS Institute 1999; Fernandez 2003) to calculate and test differences among Mahalanobis distances.

I used non-parametric discriminate analysis because it eliminated the violated assumptions (equal variance, normality) common in the use of discriminate analysis on ecological data (Williams 1983). To select the variables used in the discriminate analysis I used a logistic stepwise regression (entry 0.3, exit 0.4) procedure (PROC LOGISTIC) to remove weak or redundant variables (Klecka 1980).

Habituation

Habituation is a decreased responsiveness caused by repeated exposure to a stimulus (McFarland 1993). Animals from the same habitat and population that have not yet experienced repeated exposure to a stimulus should not show the decreased responsiveness that is displayed by members of a population that have been exposed to the stimuli. To test my fourth hypothesis (that reduction in flight initiation distance and anti-predator behaviors along the urban–rural gradient are due to habituation), I examined the flight initiation distance and anti-predator behaviors of young and adult squirrels on the urban site. To obtain a sample of young squirrels that had not yet been repeatedly exposed to humans, I monitored 33 squirrel nest boxes on the urban site twice a week to determine when young squirrels were weaned and first ventured out of the nest. Within 2 weeks from the time that young squirrels left the nest boxes for the first time, I recorded flight initiation distances along with anti-predator behaviors (Table 1) before and after the approach of a human on foot, as specified in the previous section. In an effort to eliminate temporal bias I also attempted to conduct adult observations during the 2-week periods when the young left their boxes in April and October. At this time the size of young squirrels made them clearly distinguishable from adults. Only one young squirrel was observed per litter (nest box) to avoid the possibility of observing an individual twice. I used logistic regression (PROC LOGISTIC) to examine differences in flight initiation distance and the change in time dedicated to anti-predator behaviors of juvenile and adult squirrels before and after a human approach. In addition, I used stepwise logistic regression (PROC LOGISTIC, entry 0.3, stay 0.4) to identify relevant behaviors and performed non-parametric discriminate analysis (PROC DISCRIM). I calculated Mahalanobis distances and tests of

significance of the anti-predator behaviors among juvenile urban, adult urban, suburban and rural squirrels to determine differences in squirrels' anti-predator behaviors and to determine whether juvenile squirrels' behaviors more closely resembled squirrels with less human stimuli experience.

Transfer of habituation

To test my fifth hypothesis, that animals habituated to humans show a reduced response to predator stimuli in environments of low predation risk, I exposed squirrels to auditory predator stimuli. After 2 min of initial observations of squirrel anti-predator behaviors (procedures and behaviors measured were the same as in the previous section) and ensuring that the squirrel was >30<50-m away, I played 6 s of a recorded vocalization of a red-tailed hawk (*Buteo jamaicensis*) or coyote (*Canis latrans*) to represent the auditory stimuli (Berger et al. 2001) of potential predators (Steele and Koprowski 2001). I recorded the squirrels' response by measuring the number of seconds the squirrel froze (*resfree*) and the distance traveled in 20 s after the call (*dis*) (Table 1). After 20 s, I used the previously established protocol to

measure squirrel's anti-predator behaviors for an additional 2 min. I used logistic regression (PROC LOGISTIC) to compare the changes in time spent on vigilance behaviors before and after the hawk and coyote vocalizations and represented these changes graphically. Additionally, I explored the similarities and differences in behavioral responses to the hawk and coyote vocalizations across the urban–rural gradient. I used a stepwise logistic regression (PROC LOGISTIC, entry 0.3, stay 0.4) to identify relevant behaviors, conducted a non-parametric discriminate analysis to calculate Mahalanobis distances and tested for significance among all three sites.

Results

Response to human disturbance

Flight initiation distances were different among sites ($X^2 = 26.33$, $df = 2$, $P < 0.001$) and mean distances were ~ 7 times greater on rural sites (mean = 18.55 m) compared to urban sites (mean = 2.57 m). Graphical representations of means and standard errors suggest an increased trend in

Table 1 Description of squirrel anti-predator behaviors and response variables recorded to quantify the impact of human approaches, and hawk and coyote vocalizations across the urban–rural gradient in College Station and Bryan, TX, USA

Behavior/response	Variable	Description
Chattering teeth	Teeth	Squirrel will chatter teeth rapidly and then, slowing, create a distinct noise
Scan	Scan	On the way up or down a tree, or in the middle of a foraging bout the squirrels will find a vantage point (bench, tree limb) and in a frozen position stare or slowly swivel head from side to side
Freeze	Freeze	Squirrel comes to a stop with hind legs on ground, locking at least one of the two forelimbs on the ground or limb and placing the head above the shoulders. The head will be cocked to the side or face forward
Freeze upright	Freeup	Squirrel comes to a stop with hind legs on the ground or limb and back straight or slightly bent forward. The forelimbs are off the ground
Tail wag aggressive	Tail	The tail moves rapidly snapping back and forth over the head
Lay down	Lay	The squirrel become motionless (usually on a branch) with body and head resting on the same surface
Fight initiation distance	Approach	The distance from observer to squirrel when the squirrel fled in response to a human approach
Flush distance (human)	Flush	The distance from where the squirrel fled in response to a human approach to where it stopped moving or its distance traveled after 20 s
Flush distance (vocalization)	Dis	The distance from where the squirrel fled in response to a hawk or coyote call to where it stopped moving or its distance traveled after 20 s
Freeze response	Resfree	The amount of time the squirrel froze in response to a hawk or coyote call, not exceeding 20 s
Change in anti predator behavior	Anti	The change in the amount of time spent on anti-predator behaviors from before to after a human approach, hawk call, or coyote call

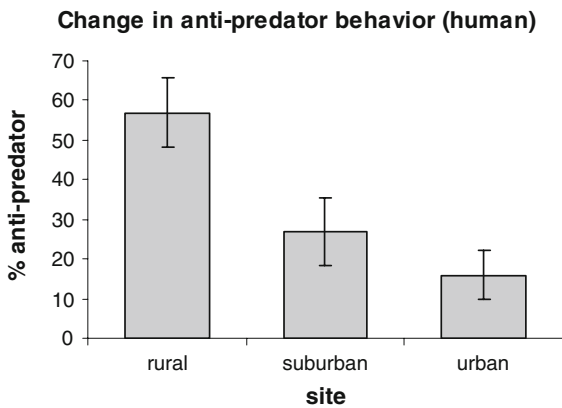


Fig. 1 Means and standard errors of flight initiation distances and changes in the amount of anti-predator behavior of squirrels in response to human approach across the urban–rural gradient on urban, suburban and rural sites in College Station and Bryan, TX, USA

average flight initiation distance across the urban gradient, increasing from urban to suburban and from suburban to rural (Fig. 1). Additionally, changes in the amount of time dedicated to anti-predator behavior in response to human approaches was significantly different among the sites ($X^2 = 10.94$, $df = 2$, $P = 0.004$), with graphical representations of means and standard errors (Fig. 1) suggesting an increase of about three times the amount of anti-predator behavior from the urban (16%) to the rural sites (56%). A stepwise logistic regression selected *approach*, *lay*, *anti*, and *scan* as relevant variables for differentiating between sites. Using these variables in non-parametric discriminate analysis showed significant differences in behaviors among all of sites ($P < 0.036$, Table 2). Mahalanobis distances of overall differences in anti-predator behavior among the sites were consistent with the urban–rural gradient, with the rural and urban sites being separated by the most distance (5.01 m) and rural and suburban sites separated by the least distance (2.01 m, Table 2).

Table 2 Mahalanobis distances and tests of significance of squirrel anti-predator behaviors in response to human approach among urban, suburban and rural sites in College Station and Bryan, TX, USA

Site	Distance	Urban (<i>F</i>)	<i>P</i>	Distance	Suburban (<i>F</i>)	<i>P</i>	Distance	Rural (<i>F</i>)	<i>P</i>
Urban	0	0	0	3.99	6.64	<0.001	5.01	7.09	<0.001
Suburban	3.99	6.64	<0.001	0	0	0	2.01	2.93	0.035
Rural	5.01	7.09	<0.001	2.01	2.93	0.035	0	0	0

Habituation

Flight initiation distances were significantly different between juvenile and adult squirrels on the urban site ($X^2 = 35.89$, $df = 1$, $P < 0.001$). Juveniles had a mean approach distance of 17.75 m while adults had an approach distance of 2.57 m. Changes in the amount of time dedicated to anti-predator behavior between the adults and juveniles was significant ($X^2 = 9.46$, $df = 1$, $P = 0.002$) with juveniles (46%) averaging almost three times greater changes in time spent on anti-predator behaviors after a human approach than adults (16%). A stepwise logistic regression selected approach, freeze, tail, teeth and scan as the most significant behaviors in differentiating among the juveniles, adults, suburban and rural squirrels. Using these variables in a non-parametric discriminate analysis I found a significant difference between urban and juvenile behaviors ($P < 0.001$) but failed to find a significant difference in anti-predator behaviors between juvenile and suburban, and juvenile and rural squirrels (Table 3).

Transfer of habituation

Overall changes in the amount of time spent on vigilance behaviors were significantly different among sites for both coyote ($X^2 = 9.83$, $df = 2$, $P = 0.007$) and hawk ($X^2 = 6.50$, $df = 2$, $P = 0.035$)

Table 3 Mahalanobis distances and tests of significance comparing anti-predator behaviors in response to human approach of juvenile urban squirrels to adult urban, suburban and rural squirrels in College Station and Bryan, TX, USA

Site	Distance	Juvenile (<i>F</i>)	<i>P</i>
Juvenile	0	0	0
Urban	5.98	7.08	<0.001
Suburban	1.18	1.44	0.23
Rural	1.67	1.76	0.14

vocalizations. Responses to both vocalizations on rural sites (coyote = 45%, hawk = 55%) measured by the change in time spent on anti-predator behaviors averaged at least twice that found on the urban sites (coyote = 11%, hawk = 20%). Figure 2 depicts the time dedicated to anti-predator behaviors in

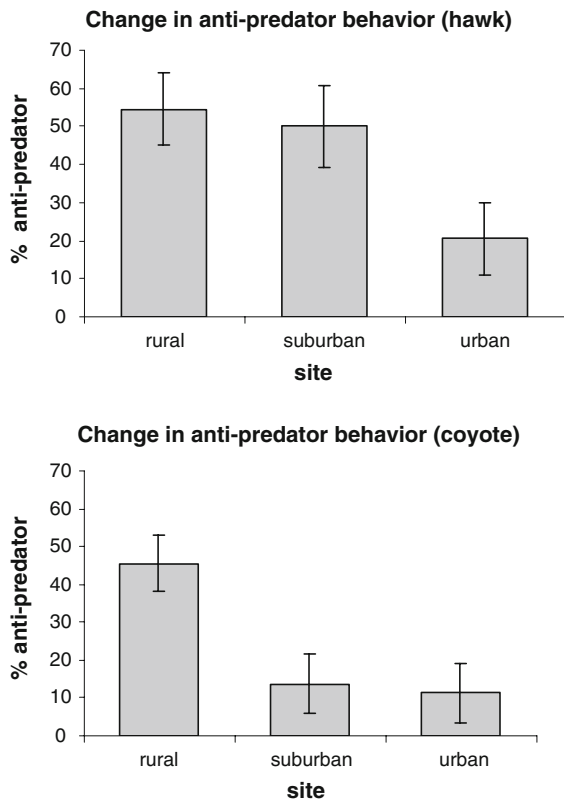


Fig. 2 Means and standard errors of changes in the amount of anti-predator behavior of squirrels in response to hawk and coyote vocalizations across the urban–rural gradient on urban, suburban and rural sites in College Station and Bryan, TX, USA

response to both hawk and coyote vocalization and suggests trends of increasing anti-predator behaviors across the urban–rural gradient, although this trend appears to be less pronounced in response to the coyote vocalization. Stepwise logistic regression indicated that *distance*, *anti* and *freeze* were relevant behaviors for determining differences in responses to the coyote vocalization, while *lay*, *freeze*, *scan*, *tail* and *resfree* were relevant variables in determining differences between the site in response to hawk vocalizations. Tests of Mahalanobis distances from non-parametric discriminate analysis of the relevant variables for response to hawk vocalizations indicated significant differences between urban and rural, and urban and suburban sites ($P < 0.04$) but not between suburban and rural sites (Table 4). Nonetheless, Mahalanobis distances did increase from the urban site to the suburban site and again to the rural sites. Urban and rural sites differed in response to coyote vocalization ($P < 0.001$) (Table 4), however, there was little overall difference (distance = 0.23, $P = 0.75$) between the anti-predator behaviors displayed by the squirrels on the urban and suburban site.

Discussion

Response to human disturbance

I found that squirrel flight initiation distances and the amount of time spent on vigilance behaviors increased while the types of anti-predator behaviors changed, when the numbers of humans present along the urban–rural gradient decreased. This suggests that squirrels have a mechanism to adjust their behavior and cope with the constant predator stimuli created

Table 4 Mahalanobis distances and tests of significance of squirrel anti-predator behaviors in response to hawk and coyote vocalizations between urban, suburban and rural sites in College Station and Bryan, TX, USA

Predator stimuli	Site	Distance	Urban (F)	P	Distance	Suburban (F)	P	Distance	Rural (F)	P
Hawk Call	Urban	0	0	0	3.32	3.17	0.023	3.98	3.8	0.01
	Suburban	3.32	3.17	0.023	0	0	0	1.33	1.27	0.31
	Rural	3.98	3.8	0.01	1.33	1.27	0.31	0	0	0
Coyote Call	Urban	0	0	0	0.23	0.4	0.75	4.09	7	<0.001
	Suburban	0.23	0.4	0.75	0	0	0	3.71	6.34	0.002
	Rural	4.09	7	<0.001	3.71	6.34	0.002	0	0	0

by humans in urban environments. If squirrels in urban environments had shown a response to humans similar to those in rural areas, they would have spent most of their time and energy on anti-predator behaviors, leaving little time for foraging and reproduction. For example, after an approach of a human within ~19 m, rural squirrels on average spent more than 60% of the next 2 min performing anti-predator behaviors and, on average, at least 50% of time the squirrel was laying flat and motionless on a branch. Squirrels on the urban site most likely spend a good portion of their life within 18.55 m of humans and did not flee from human approach until they were ~3 m away. This study suggests that squirrels have altered their behavior allowing them to better utilize the urban environment. It does not, however, contradict findings that urban wildlife spends more overall time on vigilance than their rural counterparts (Ward and Low 1997; Randler 2003). It is certainly possible that a multitude of lesser behavioral responses in reaction to numerous stimuli in urban areas outweigh fewer heightened behaviors in rural areas.

Habituation could explain the reduced response to humans by squirrels. Some studies have shown a reduction in flight initiation distances in animals with greater prior exposure to humans (Knight et al. 1987; Labra and Leonard 1999; Metcalf et al. 2000; Reimers and Sigurd 2001), and one of the most common and highly plausible explanations given for the phenomenon is a reduced response as a result of habituation (Knight et al. 1987; Labra and Leonard 1999; Metcalf et al. 2000; Reimers and Sigurd 2001). An alternative explanation is the minimal predation risk in the urban environment caused a general reduction in anti-predator behaviors. Researchers believe that animals are able to assess their predation risk and adjust their behaviors (Lima and Dill 1990; Blumstein 2002) and, in the absence of predators, might greatly reduce their use of anti-predator behaviors and recognition of predators (Blumstein 2002). However, the mechanism for such behavioral changes is not clearly specified.

Habituation

There were drastic differences in flight initiation distances and anti-predator behaviors to approaching humans in the responses of juvenile squirrels compared to adult squirrels in the same urban

environment. This supports my hypotheses that reduction of flight distance and vigilance behaviors along the urban–rural gradient are due to habituation. I assumed that the anti-predator response to humans was innate (Frid and Dill 2002) and that repeated exposure to humans caused a reduction in anti-predator behavior in response. This hypothesis also was supported by the analysis of anti-predator behaviors that showed juvenile squirrels' behavioral responses to be most similar to those of suburban and rural adult squirrels (Table 3) that were also presumably not habituated to humans. One flaw of the study design was a lack of a control; undoubtedly, the juvenile squirrels were exposed to humans prior to my observations.

There is a clear alternative explanation to account for differences in flight initiation distances and behaviors. It is possible that young squirrels show heightened predator responses while adults have more behavioral plasticity in response to predator stimuli. **Similar experiments on juvenile and sub-adult rural and suburban squirrels might help clarify this.** Nonetheless, I believe that the hypothesis of habituation is the most plausible hypothesis and this study provides a basis to begin understanding the mechanisms that influence the behavioral choices of animals on the urban–rural gradient.

Transfer of habituation

There was a clear reduction in anti-predator behaviors shown in squirrels from urban areas in response to hawk and coyote vocalizations in comparison to rural squirrels. In response to hawk vocalizations the reduction of anti-predator behaviors appeared to be consistent with the urban–rural gradient. However, suburban and urban squirrels showed little difference in their responses to the coyote vocalizations. Nonetheless, my findings do support the hypothesis that habituation to humans may cause a reduction in response to other predator stimuli when there is a minimal risk of predation. This is the first documented case of this phenomenon occurring in a field setting, although it has rarely been tested (Curio 1993; Labra and Leonard 1999), and to my knowledge never tested under the scope of predation risk.

A competing hypothesis presented in the previous response to human disturbance section might also be applicable here. The reduced response to hawk and

coyote vocalizations might have resulted solely from the reduced predation risk in the urban environment, rather than a requirement for the transfer of habituation. Nonetheless, this alternative hypothesis does little to explain the differences in response to humans shown between adult and juvenile squirrels in the same environment, nor provide an explanation as to how the squirrels might have assessed the risk and altered their behavior.

One explanation for the lack of differentiation between squirrel responses to coyote vocalizations on the urban and suburban sites was that the assumption of varying predation risk was violated. A competing explanation is that a prevalence of dogs found on the suburban site caused squirrels to habituate to canine vocalizations; both the urban and suburban squirrels showed little response to the coyote vocalizations. A final alternative hypothesis is that the squirrels on the urban and suburban sites are exposed to constant noise stimuli to which they have become habituated, and they reacted to the vocalization as they would any noise of the same volume. In such a hypothesis it is not habituation to humans that has caused a reduced predator response, but rather the anthropogenic noise from the urban environment. To test this, one would need to eliminate the possibility of habituation to humans but not the noise they create, which would be virtually impossible in a field setting.

A potential shortcoming of this study was that it was limited to one urban–rural gradient in College Station, TX. On this one gradient, the results of the study showed a reduction of anti-predator behaviors by squirrels in response to humans and other predator stimuli was in most circumstances consistent with the urban–rural gradient. The results also suggest the urban–rural gradient does affect squirrels anti-predator behaviors and provides support for the hypotheses that wildlife in urban areas can become habituated to humans and that this reduction in anti-predator behavior can be transferred to other stimuli under the right circumstances. These behaviors may allow individuals and population to better utilize the resources of urban environments but set the stage for increased confrontation of humans and wildlife. Additionally, if reduced anti-predator behaviors increase the fitness of individuals in urban environments and these traits are perpetuated in urban wildlife populations there is an obvious cost. Urban

populations would be highly susceptible to alterations of urban ecosystems that would allow for the introduction or invasion of predators.

Acknowledgments I would like to thank Roel R. Lopez and Nova J. Silvy for their support during this project. I am grateful to Jane Packard who helped me formulate the ideas for this project. I would also like to thank the anonymous reviewers of this manuscript whose constructive criticism and editing suggestions greatly improved it. Thanks are extended to the undergraduate students whose hard work and dedication made this project possible. I also want to make a special note of the contributions of S. Kahlich and L. Gallant, whose countless hours trapping, and tracking fox squirrels made them invaluable. Funding and support was provided by the Ed Rachel Foundation and the Texas Agricultural Experiment Station.

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